Uniwersytet Rolniczy im. H. Kołłątaja w Krakowie

Wydział Biotechnologii i Ogrodnictwa

Piotr Zięba

Nr albumu: 1061

Zawartość substancji biologicznie aktywnych w mycelium i owocnikach grzybów *Pleurotus* spp. uzyskanych z podłoży suplementowanych

Autoreferat pracy doktorskiej

Praca wykonana pod kierunkiem:

dr hab. Agnieszki Sękary prof. URK
Katedra Ogrodnictwa
Wydział Biotechnologii i Ogrodnictwa
Uniwersytet Rolniczy w Krakowie

prof. dr hab. Bożeny Muszyńskiej
Katedra i Zakład Botaniki Farmaceutycznej
Wydział Farmaceutyczny
Uniwersytet Jagielloński Collegium Medicum

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Spis treści

1. PUBLIKACJE STANOWIĄCE ROZPRAWĘ DOKTORSKĄ ORAZ DOROBEK NAUKOWY

1.1. Wykaz publikacji będących podstawą rozprawy doktorskiej

Poniższe prace zostały ponumerowane, a numeracji tej użyto w rozprawie doktorskiej. Publikacja nr 1 jest pracą przeglądową związaną z tematyką pracy doktorskiej natomiast publikacje 2-4 są pracami eksperymentami.

1. **Zięba, P.,** Sękara, A., Sułkowska-Ziaja, K., Muszyńska, B. (2020). Culinary and medicinal mushrooms: Insight into growing technologies. Acta Mycologica, 55(2).

Punkty wg listy czasopism punktowanych MEiN = 20 pkt.

2. Krakowska, A., **Zięba, P.,** Włodarczyk, A., Kała, K., Sułkowska-Ziaja, K., Bernaś, E., Sękara A., Ostachowicz B., Muszyńska, B. (2020). Selected edible medicinal mushrooms from *Pleurotus* genus as an answer for human civilization diseases. Food Chemistry, 327, 127084.

IF (wg bazy Web of Science, 2021) = 9.231; Punkty wg listy czasopism punktowanych MEiN = 200 pkt.

3. **Zięba, P.,** Kała, K., Włodarczyk, A., Szewczyk, A., Kunicki, E., Sękara, A., Muszyńska, B. (2020). Selenium and zinc biofortification of *Pleurotus eryngii* mycelium and fruiting bodies as a tool for controlling their biological activity. Molecules, 25(4), 889.

IF (wg bazy Web of Science, 2021) = 4.927; Punkty wg listy czasopism punktowanych MEiN = 140 pkt.

4. **Zięba, P.,** Sękara, A., Bernaś, E., Krakowska, A., Sułkowska-Ziaja, K., Kunicki, E., Suchanek, M., Muszyńska, B. (2021). Supplementation with magnesium salts–a strategy to increase nutraceutical value of *Pleurotus djamor* fruiting bodies. Molecules, 26(11), 3273.

IF (wg bazy Web of Science, 2021) = 4.927; Punkty wg listy czasopism punktowanych MEiN = 140 pkt.

Współczynnik oddziaływania IF prac wchodzących w skład cyklu prac = 19,085
Punkty MEiN = 500 pkt.

1.2. Pozostałe prace wchodzące w skład dorobku naukowego

1. **Zięba, P.,** Kała, K., Smoleń, Z., Lazur, J., Sułkowska-Ziaja, K., Sękara, A., Muszyńska, B. (2018). Działanie biologiczne grzybów nadrewnowych: *Laetiporus sulphureus*, *Fomitopsis betulina* i *Trametes versicolor*. Medicina Internacia Revuo, 28(2).
2. Fijałkowska, A., Krakowska, A., Lazur, J., Włodarczyk, A., **Zięba, P.,** Suchanek, M., Sułkowska-Ziaja, K., Muszynska, B. (2021). Fortified Mycelium of *Fomitopsis officinalis* (Agaricomycetes) as a Source of Biologically Active Substances Effective in the Prevention of Civilization Diseases. International Journal of Medicinal Mushrooms, 23(9).
3. Rząsa-Duran, E., Kryczyk-Poprawa, A., Drabicki, D., Podkowa, A., Sułkowska-Ziaja, K., Szewczyk, A., Kała, K., Opoka, W., **Zięba, P.,** Muszyńska, B. (2022). Yerba Mate as a Source of Elements and Bioactive Compounds with Antioxidant Activity. Antioxidants, 11(2), 371.
4. Kała, K., Krakowska, A., **Zięba, P.,** Opoka, W., Muszyńska, B. (2021). Effect of conservation methods on the bioaccessibility of bioelements from *in vitro* digested edible mushrooms. Journal of the Science of Food and Agriculture, 101(8), 3481-3488.
5. Włodarczyk, A., Krakowska, A., Sułkowska-Ziaja, K., Suchanek, M., **Zięba, P**., Opoka, W., Muszyńska, B. (2020). *Pleurotus* spp. Mycelia enriched in magnesium and zinc Salts as a potential functional food. Molecules, 26(1), 162.
6. Włodarczyk, A., Fijałkowska, A., Jędrejko, K., **Zięba, P.,** Lazur, J., Sułkowska–Ziaja, K., & Muszyńska, B. (2020). Edible and medicinal mushroom *Hericium erinaceus* as a potential natural material with influence on brain functions. Medicina Internacia Revuo-International Medicine Review, 29(114), 4-10.

Łączny IF prac wchodzących w skład całego dorobku naukowego = 37,733
Punkty MEiN = 880 pkt.
Indeks H wg bazy Google Schoolar = 4
Łączną ilość cytowań wg bazy Google Schoolar = 75

1.3. Czynny udział w wybranych konferencjach naukowych krajowych i zagranicznych

1. **Piotr Zięba,** Agnieszka Sękara. 2017. Alternatywne techniki w uprawie boczniaka, Ogólnouczelniana Sesja Kół Naukowych, Kraków, **referat**
2. **Piotr Zięba**, Agnieszka Sękara. 2017. Podłoże a jakość biologiczna grzybów. Ziemia, Roślina, Człowiek, Kraków, **poster**
3. **Piotr Zięba**, Agnieszka Sękara. 2018. The effect of supplementation of substrates for the oyster mushroom cultivation with residues from Apiaceae crops. 3rd International Scientific Conference „Human ecology", Lublin, **poster**
4. **Piotr Zięba,** Agnieszka Sękara. 2018. Medicinal and cultivated mushrooms – chosen applications in human health management. 3rd International Scientific Conference „Human ecology", Lublin, **referat**
5. **Piotr Zięba,** Katarzyna Kała, Agnieszka Sękara, Bożena Muszyńska. 2019. Właściwości antyoksydacyjne owocników i grzybni wybranych gatunków boczniaka. MycoRise UP! Młodzi w Mykologii, Spała, **referat**
6. **Piotr Zięba,** Krystian Marzec, Bartłomiej Pawelec, Daniel Łukawski, Agnieszka Sękara. 2022. Ozonation of the mushroom substrate - an innovative and ecological technology. MycoRise UP! Młodzi w Mykologii, Warszawa, **referat**

1.4. Granty

1. Badania młodych, projekt wewnętrzny UR Kraków, 2018. Suplementacja podłoża do uprawowy boczniaka mikołajkowego *Pleurotus eryngii* mikroelementami deficytowymi w diecie człowieka. **Kierownik projektu.**
2. Projekt w ramach programu pod nazwą „Studenckie koła naukowe tworzą innowacje" nr rejestracyjny: SKN/SP/496634/20215, Umowa SKN/SP/496634/20212 z dnia 24.06.2021, pt. Nowatorskie technologie w uprawie jadalnych i leczniczych grzybów nadrewnowych. **Wykonawca projektu.**
3. Program grantowy Podkarpackiego Centrum Innowacji na prace B+R jednostek naukowych, nabór III, nr konkursu 1/2021, Nr projektu: N3_567. Metoda identyfikacji składników suplementów diety oparta o barkodowanie DNA. **Wykonawca projektu.**

1.5. Inne osiągniecia

Pomoc przy realizacji prac dyplomowych – planowanie i realizacja doświadczeń uprawowych

Rok 2017
Ocena wartości biologicznej wybranych gatunków grzybów leczniczych w zależności od rodzaju podłoża – **Praca magisterska WBiO**
Rok 2018
Analiza zawartości związków biologicznie czynnych w lakownicy lśniącej (*Ganoderma lucidum*) w zależności od rodzaju podłoża – **Praca magisterska WBiO**
Rok 2019
Wpływ składu pożywki płynnej na masę i właściwości prozdrowotne grzybni soplówki jeżowatej *Hericium erinaceus* (Bull.) Pers. – **Praca inżynierska WBiO**
Rok 2020
Wpływ suplementacji podłoża do uprawy boczniaka różowego (*Pleurotus djamor*) siarczanem cynku na plon, zawartość polifenoli oraz wybranych makro- i mikroelementów w owocnikach – **Praca inżynierska WBiO**
Wpływ metody produkcji na jakość kiszonych owocników boczniaka – **Praca magisterska WTŻ**
Analiza właściwości prozdrowotnych kiszonych owocników boczniaka – **Praca magisterska WTŻ**
Rok 2021
Porównanie wartości biologicznej maczużnika bojowego w zależności od rodzaju surowca lub preparatu oraz metody produkcji – **Praca inżynierska WBiO**

Właściwości lecznicze i prozdrowotne grzybów z rodzaju *Auricularia* – **Praca inżynierska WBiO**
Wpływ rodzaju podłoża na zawartość wybranych składników bioaktywnych w boczniaku królewskim – **Praca magisterska WTŻ**
Wpływ rodzaju podłoża na zawartość wybranych składników bioaktywnych w boczniaku florydzkim – **Praca magisterska WTŻ**
Rok 2022
Wpływ rodzaju podłoża na wartość odżywczą owocników boczniaka – **Praca inżynierska WTŻ**
Wpływ rodzaju podłoża na aktywność przeciwutleniającą owocników boczniaka – **Praca inżynierska WTŻ**
Wpływ rodzaju podłoża na zawartość polifenoli w owocnikach boczniaka – **Praca inżynierska WTŻ**
Porównanie zawartości witaminy C w owocnikach *Pleurotus* spp., w zależności od warunków uprawy – **Praca inżynierska WTŻ**
Wpływ przechowywania wybranych gatunków boczniaka *Pleurotus* spp. na ich wartością prozdrowotną – **Praca inżynierska WTŻ**

Tematy prac naukowych prezentowanych przez studentów w ramach opieki nad kołem naukowym – sekcja „Grzybów Jadalnych i Leczniczych” Koła Naukowego Ogrodników na Wydziale Biotechnologii i Ogrodnictwa.

Rok 2018
Właściwości antyoksydacyjne wybranych gatunków grzybów uprawianych w kulturach wodnych
Uprawa boczniaka ostrygowatego (*Pleurotus ostreatus*) – porównanie szczepu dzikiego z rasą uprawną bezzarodnikową
Wpływ składu pożywki na wzrost grzybni soplówki jeżowatej *Hericium erinaceus*
Suplementacja podłoży do uprawy boczniaka mikołajkowego *Pleurotus eryngii* warzywami korzeniowymi z rodziny *Apiaceae*
Rok 2019
Porównanie zawartości wybranych makro- i mikroelementów w owocnikach wybranych gatunków boczniaka – **Praca wyróżniona – 2 Miejsce na Wydziałowej Sesji Kół Naukowych**
Rok 2021
Zastosowanie młóta browarniczego w uprawie grzybów **Praca wyróżniona – 3 Miejsce na Wydziałowej Sesji Kół Naukowych**
Zastosowanie słomy konopnej oraz wytłoków konopnych w uprawie boczniaków
Analiza ilości wybranych związków indolowych w owocnikach, mycelium z kultur in vitro i komercyjnych preparatach maczużnika bojowego
Nowatorskie technologie w uprawie jadalnych i leczniczych grzybów nadrewnownych – raport z projektu ministerialnego

2. STRESZCZENIE

Rodzaj boczniak *Pleurotus* spp. to jeden z najważniejszych gospodarczo grzybów uprawnych na świecie. Boczniak ostrygowaty *Pleurotus ostreatus* a także inne gatunki jak b. mikołajkowy *P. eryngii* czy b. różowy *P. djamor* to cenione kulinarnie grzyby o potwierdzonych naukowo właściwościach leczniczych i prozdrowotnych. Zawierają szereg substancji bioaktywnych np. związki indolowe, sterole czy biopierwiastki, które powodują, że są cennym składnikiem diety. Nie tylko owocniki grzybów mają te właściwości, ale również mycelium z biotechnologicznych kultur *in vitro*. Celem pracy doktorskiej była ocena zawartości substancji bioaktywnych w owocnikach i mycelium 6 gatunków *Pleurotus* spp.: boczniaka cytrynowego (*Pleurotus citrinopileatus*), boczniaka różowego (*P. djamor*), boczniaka mikołajkowego (*P. eryngii*), boczniaka florydzkiego (*P. florida*), boczniaka ostrygowatego (*P. ostreatus*) i boczniaka łyżkowatego (*P. pulmonarius*). Wyniki tych badań zostały opublikowane w publikacji nr 2. Na podstawie uzyskanych wyników wybrano *P. eryngii* i *P. djamor* to dalszych badań związanych z suplementacją biopierwiastkami. W Publikacji 3 dodano związki selenu i cynku do podłóż standardowych oraz pożywek *in vitro* i oceniono ich wpływ na uzyskaną biomasę owocników i mycelium. Dodane związki modyfikowały biochemię uzyskanego materiału a zawartość Se i Zn zwiększała się w badanym materiale względem kontroli. W publikacji 4 zastosowano suplementację siarczanem i chlorkiem magnezu w 3 różnych stężeniach podłoża do uprawy *P. djamor*. Zaobserwowano zwiększenie zawartości magnezu w uzyskanych owocnikach a także zwiększenie poziomu niektórych substancji bioaktywnych po zastosowaniu suplementacji Mg.

3. SUMMARY

The genus *Pleurotus* spp. oyster mushrooms is one of the most economically important cultivated mushrooms in the world. The oyster mushroom *Pleurotus ostreatus* as well as other species such as *P. eryngii* or *P. djamor* are culinary valued mushrooms with scientifically proven health-promoting properties. They contain many bioactive substances, e.g. indole compounds, sterols or bioelements, which make them a valuable component of the human diet. Not only the fruiting bodies have these properties, but also mycelium from *in vitro* cultures. The aim of the doctoral thesis was to assess the content of bioactive substances in fruiting bodies and mycelium of 6 species of *Pleurotus* spp: *P. citrinopileatus*, *P. djamor*, *P. eryngii*, *P. ostreatus* and *P. pulmonarius*. The results of these studies were published in publication no. 2. On the basis of the obtained results, *P. eryngii* and *P. djamor* were selected for further research related to the supplementation of bioelements. In Publication 3, selenium and zinc compounds were added to standard media and *in vitro* cultures and their influence on the obtained fruiting body and mycelium biomass was assessed. The added compounds modified the biochemistry of the obtained material, and the content of Se and Zn increased in the tested material in relation to the control. In publication 4, supplementation with magnesium sulfate and magnesium chloride was used in 3 different concentrations – it was added to the cultivation substrate for the *P. djamor*. An increase in the magnesium content in the obtained fruiting bodies as well as an increase in the level of some bioactive substances after the use of Mg supplementation was observed.

4. WSTĘP

Poszukiwanie nowych źródeł pokarmu jak i substancji o działaniu leczniczym, które mogą być produkowane w sposób zrównoważony, z minimalnym wpływem na środowisko naturalne, jest jednym z największych wyzwań XXI wieku [Garcia i in. 2020]. Potencjalnych rozwiązaniem wybranych aspektów tego problemu jest uprawa grzybów dla pozyskania nie tylko owocników, ale także grzybni, o wysokiej zawartości pierwiastków i związków chemicznych, pełniących rolę dietetyczną i leczniczą. Owocniki grzybów spożywane są przez wiele ssaków, w tym naczelnych, więc można wnioskować, że towarzyszą człowiekowi od początku ewolucji [Hanson i in. 2003]. Grzyby zostały wprowadzone do uprawy znacznie później niż rośliny, a pierwsze półnaturalne plantacje były zakładane od X wieku w Chinach. Technologie upraw grzybów w skali wielkotowarowej zostały opracowane dopiero w drugiej połowie XX wieku, natomiast obecnie można zaobserwować dynamiczny rozwój tego sektora ogrodnictwa, dotyczący nie tylko powierzchni upraw, ale także różnorodności gatunkowej i odmianowej. Według danych FAO, w 2020 roku światowa produkcja grzybów wyniosła ponad 40 mln ton, a wartość ta podwoiła się w ostatnich latach [FAO 2020]. Większość upraw zlokalizowana jest w Chinach, dlatego dokładnie oszacowanie ich powierzchni oraz składu gatunkowego jest trudne, ze względu na brak precyzyjnego monitoringu. Royse i in. [2017] podali, że w 2013 roku najpowszechniej uprawianym gatunkiem grzyba był twardnik japoński (*Lentinula edodes*) - 22% globalnej produkcji, kolejno boczniak (*Pleurotus* spp.) - 19% globalnej produkcji), następnie uszak (*Auricularia* spp.) – 18% globalnej produkcji, natomiast najbardziej znana w Europie i Ameryce Północnej pieczarka dwuzarodnikowa (*Agaricus bisporus*) znalazła się dopiero na 4 miejscu (18% globalnej produkcji). Jednak mając na uwadze dynamiczny wzrost produkcji grzybów, wartości te uległy zmianie, ale aktualne dane nie są dostępne w literaturze naukowej.

W kulturze kulinarnej Europy Zachodniej grzyby uważane były za pokarm mało wartościowy, o niskich walorach odżywczych, nawet współcześnie rozpowszechniona jest opinia o ich ciężkostrawności. Rosnąca świadomość konsumentów wpływa na stopniową zmianę zwyczajów kulinarnych, a szereg badań naukowych potwierdza, że grzyby są cennym źródłem wielu substancji potrzebnych do prawidłowego funkcjonowania organizmu i powinny być częścią zróżnicowanej diety [Cheung 2010, Rhizzo i in. 2021, Rahman 2021]. Owocniki grzybów są źródłem witamin z grupy B oraz D, zawierają również dużo błonnika pokarmowego. Białko grzybów zawiera wszystkie aminokwasy egzogenne, stąd jego wartość

dietetyczna oceniana jest wyżej w porównaniu do białka roślinnego [Siwulski i in. 2014, Singh 2017]. Owocniki oraz mycelium zawierają szereg makro i mikroelementów, ważnych w diecie człowieka, takich jak potas, wapń, cynk oraz selen [Podkowa i in. 2021]. Dodatkowo, owocniki zawierają szereg substancji o działaniu leczniczym i prozdrowotnym, dlatego grzyby powinny być stałym elementem diety człowieka.

Wykorzystanie grzybów w celach leczniczych przez długi okres czasu było ignorowane w krajach Europy. Natomiast w krajach azjatyckich, w szczególności w Chinach oraz Japonii, grzyby były powszechnie stosowane w lecznictwie. Przykładem może być lakownica żółtawa (*Ganoderma lucidum*), zwana Ling Zhi, co można przetłumaczyć jako „grzyb wiecznego życia". Lakownica znana jest w medycynie chińskiej od ponad 4 tysięcy lat, a obecnie jest używana w profilaktyce i leczeniu wielu chorób cywilizacyjnych [Grys i in. 2011]. Rosnące zainteresowanie naukowców wykorzystaniem grzybów w celach leczniczych przyczyniło się do pogłębienia i usystematyzowania wiedzy w tym zakresie. Wykorzystanie grzybów w leczeniu ludzi jest udokumentowane badaniami, których wyniki są publikowane w renomowanych czasopismach naukowych, dla przykładu, do roku 2017 ukazało się ponad 600 publikacji zawierających wyniki klinicznych badaniach z wykorzystaniem grzybów [Wasser 2017]. Na rynku pojawia się coraz więcej suplementów diety zawierających w składzie grzyby lub wyizolowane z nich związki chemiczne o działaniu prozdrowotnym, a temat ten jest również widoczny w mediach.

Najważniejszą grupą związków aktywnych w grzybach są polisacharydy, głównie β-glukany oraz związane z nimi kompleksy białkowo-cukrowe. Ich główne działanie polega na aktywacji układu immunologicznego człowieka, przez co wykazano ich skuteczność w zapobieganiu i leczeniu wielu chorób, od nowotworów po infekcje bakteryjne i wirusowe [Wasser 2014, Venturella i in. 2021]. β-Glukany są nieskrobiowymi sacharydami zbudowanymi z reszt D-glukopiranozowych. Stanowią część ścian strzępek wszystkich gatunków grzybów wielkoowocnikowych. Ich zróżnicowana budowa, a w konsekwencji biologiczna aktywność, jest związana nie tylko z gatunkiem grzyba, ale także metodami uprawy oraz surowcem – grzybnią, owocnikami lub zarodnikami [Erletti i in. 2021, Venturella i in. 2021]. Najlepiej przebadane β-glukany to lentinan z twardnika japońskiego (*Lentinula edodes*), schizofilan z rozszczepki pospolitej (*Schizophyllum commune*), pleuran z boczniaka ostrygowatego (*Pleurotus ostreatus*) oraz krestin z wrośniaka różnobarwnego (*Trametes versicolor*). Ich działanie zostało potwierdzone wieloma badaniami *in vitro* na liniach komórkowych nowotworów, na modelach zwierzęcych a także w badaniach klinicznych u ludzi

[Vetvicka i in. 2019, Steimbach i in. 2021]. Substancje aktywne wyizolowane z owocników, mycelium a także zarodników grzybów są różnorodne. Ważną grupą są związki indolowe, takie jak 5-hydroksytryptofan, tryptamina, a także hormony, jak melatonina oraz serotonina. Pozytywnie wpływają one na działanie mózgu, podejrzewa się także, że suplementacja nimi pomaga zapobiegać chorobom wieku starczego, takim jak demencja czy choroba Alzheimera [Muszyńska i in. 2012, Salinaro i in. 2018]. Grzyby są również bogate w różnego rodzaju substancje o działaniu antyoksydacyjnym, od witamin, przez związki fenolowe po specyficzne substancje izolowane z poszczególnych gatunków [Kozarski i in. 2015].

Dynamiczny rozwój technologii upraw grzybów w ostatnich dziesięcioleciach pozwolił na całoroczną podaż świeżych owocników na rynek. W Polsce najpopularniejsze są świeże pieczarki, coraz powszechniej też pojawiają się w sprzedaży inne gatunki, takie jak boczniaki, czy typowy dla kuchni azjatyckiej twardnik japoński – shiitake. Technologie upraw grzybów są ciągle udoskonalane. Szeroko badane jest zastosowanie alternatywnych podłoży/substratów – w tym zawierających produkty uboczne przemysłu rolnego, leśnego i spożywczego, dzięki temu uprawa grzybów wpisuje się w zrównoważoną produkcję żywności [Barshteyn i Krupodorova 2016]. Jednym z największych problemów w uprawie grzybów jest natomiast odkażanie podłoża, ponieważ standardowe metody termicznej pasteryzacji oraz sterylizacji są energochłonne. Alternatywne technologie, jak np. ozonowanie czy zastosowanie radiacji, w znaczącym stopniu mogą obniżyć koszty produkcji i są uznawane za proekologiczne [Buntat i in. 2013]. Możliwość łatwego modyfikowania składu podłoża do uprawy grzybów powoduje, że można do niego dodawać np. biopierwiastki, dzięki czemu uzyskane owocniki będą się charakteryzowały zwiększoną ich zawartością i biodostępnością dla człowieka [Niedzielski in. 2014, Carrasco i in. 2018, Włodarczyk i in. 2020]. Temat ten został podjęty w niniejszej rozprawie doktorskiej.

Boczniak (*Pleurotus* spp.) to jeden z najważniejszych rodzajów grzybów uprawnych – szacuje się, że obecnie gatunki z tego taksonu mogą być najczęściej uprawianymi grzybami na świecie. W Polsce produkuje się ich około 100 tys. ton rocznie, co stawia je na drugim miejscu po pieczarce dwuzarodnikowej – ok. 350 tys. ton [Golak-Siwulska i in. 2018]. Spośród ponad 200 opisanych gatunków w obrębie tego rodzaju, największe znaczenie gospodarcze mają boczniak ostrygowaty (*Pleurotus ostreatus*), boczniak mikołajkowy (*P. eryngii*), boczniak różowy (*P. djamor*), boczniak cytrynowy (*P. citrinopileatus*) i boczniak łyżkowaty (*P. pulmonarius*) [Krakowska i in. 2020] (Zdjęcie 1-6). Gatunki te różnią się wymaganiami środowiskowymi oraz kolorem kształtem i składem chemicznym owocników. Technologie ich

uprawy są zróżnicowane, a jako składniki podłoży wykorzystywane są łatwo dostępne i tanie produkty uboczne przemysłu rolniczego, leśnego i spożywczego, w tym słoma, trociny, czy też np. fusy od kawy, które są odkażane różnymi metodami, głównie pasteryzacją lub sterylizacją termiczną. Wykorzystanie takich odpadów wpisuje się w ekologiczne trendy produkcji żywności [Barshteyn i Krupodorova 2016]. Owocniki boczniaka są pełnowartościowym pożywieniem, zawierającym wszystkie egzogenne aminokwasy, są także bogate w witaminy z grupy B (B_1, B_2, B_6 i B_{12}) oraz szereg makro i mikroelementów (K, Ca, Mg, Zn, Na, Se, Fe) [Bano i in. 1988, Krakowska i in. 2020]. Boczniaki są również surowcem farmaceutycznym, w tym przypadku wykorzystywane są nie tylko owocniki, ale także grzybnia pozyskiwana metodami biotechnologicznymi. Zawarte w nich substancje mają działanie regulujące metabolizm organizmu człowieka, w tym obniżające poziom egzogennego cholesterolu a także zwiększające odporność, co zostało udokumentowane licznymi badaniami klinicznymi [Rogers 2020].

Zdjęcie 1. Owocniki boczniaka mikołajkowego (fot. P. Zięba)

Zdjęcie 2. Owocniki boczniaka różowego (fot. P. Zięba)

Zdjęcie 3. Owocniki boczniaka łyżkowatego (fot. P. Zięba)

Zdjęcie 4. Owocniki boczniaka cytrynowego (fot. P. Zięba)

Zdjęcie 5. Owocniki boczniaka florydzkiego (fot. P. Zięba)

Zdjęcie 6. Owocniki boczniaka ostrygowatego (fot. P. Zięba)

5. CEL PRACY

Celem pracy było otrzymanie kultur płynnych *in vitro* sześciu gatunków boczniaków: boczniaka cytrynowego (*Pleurotus citrinopileatus*), boczniaka różowego (*P. djamor*), boczniaka mikołajkowego (*P. eryngii*), boczniaka florydzkiego (*P. florida*), boczniaka ostrygowatego (*P. ostreatus*) i boczniaka łyżkowatego (*P. pulmonarius*) oraz opracowanie optymalnej metody uprawy tych gatunków w celu uzyskania surowca o najwyższej jakości prozdrowotnej. W celu uzyskania wystarczającej ilości biomasy mycelium do eksperymentu namnożono też grzybnię w bioreaktorach o pojemności 10 L, z systemem air–lift.

Równolegle uzyskano owocniki wymienionych powyżej gatunków boczniaka w uprawach prowadzonych w substracie o autorsko opracowanym składzie i w zoptymalizowanych warunkach mikroklimatycznych (stała wymiana powietrza, wilgotność, temperatura).

Następnie otrzymaną biomasę mycelium z kultur *in vitro* i owocniki wymienionych powyżej gatunków boczniaka z uprawy w substracie porównano pod względem składu jakościowego i ilościowego substancji biologicznie aktywnych w celu określenia ich przydatności, jako surowców zawierających pierwiastki i związki chemiczne o znaczeniu dietetycznym oraz leczniczym, determinującego ich potencjalne zastosowanie zwłaszcza w profilaktyce chorób cywilizacyjnych (Publikacja 2).

Ważnym celem prowadzonych badań było też określenie, czy możliwe jest fortyfikowanie kultur *in vitro* oraz substratu uprawowego w biopierwiastki (cynk, selen, magnez), które mogą wpływać na zwiększenie właściwości prozdrowotnych surowca grzybowego. Obiektem badań były *P. eryngii* (Publikacja 3) i *P. djamor* (Publikacja 4), wytypowane na podstawie najkorzystniejszych parametrów biochemicznych oznaczanych w wyżej opisanym doświadczeniu. Na tym etapie badań dokonano oceny wpływu suplementacji pożywki i podłoża związkami selenu i cynku na ich zawartość w mycelium i owocnikach *P. eryngii* (Publikacja 3). Następnie zanalizowano wpływ suplementacji solami magnezu substratu w uprawie *P. djamor* i oceniono jakość pozyskanych owocników (Publikacja 4).

6. METODYKA BADAŃ

6.1. Kultury grzybów wybrane do badań w cyklu publikacji

Kultury macierzyste boczniaków użytych do badań w pracy doktorskiej zostały otrzymane dzięki uprzejmości prof. dr hab. Marka Siwulskiego z Uniwersytetu Przyrodniczego w Poznaniu. Do badań wybrano 6 gatunków boczniaka: boczniaka cytrynowego (*Pleurotus citrinopileatus*), boczniaka różowego (*P. djamor*), boczniaka mikołajkowego (*P. eryngii*), boczniaka florydzkiego (*P. florida*), boczniaka ostrygowatego (*P. ostreatus*) i boczniaka łyżkowatego (*P. pulmonarius*). Żywe kultury grzybni są przechowywane w banku genów Katedry Botaniki Farmaceutycznej, Wydział Farmaceutyczny Uniwersytetu Jagielińskiego w Krakowie oraz w banku genów Katedry Ogrodnictwa, Wydział Biotechnologii i Ogrodnictwa Uniwersytetu Rolniczego w Krakowie.

6.2. Otrzymywanie grzybni z kultur *in vitro Pleurotus* spp.

Mycelium boczniaka do badan opisanych w Publikacji 2 i Publikacji 3 uzyskano w Katedrze Botaniki Farmaceutycznej Wydziału Farmaceutycznego CM Uniwersytetu Jagiellońskiego według procedur opracowanych przez prof. dr hab. Bożenę Muszyńską [Muszyńska i in. 2016]. Stosowano pożywkę Oddoux wg składu zamieszonego w Tabeli 1. Pożywkę tę zmodyfikowano w eksperymencie opisanym szczegółowo w Publikacji 3 o dodatkową zawartość soli cynku oraz selenu. Opracowana metodyka pozwoliła na otrzymanie powtarzalnego materiału wykorzystywanego w dalszych analizach, jak również potencjalnego surowca do komercyjnej produkcji dużej ilości biomasy mycelium. W kolbach Erlenmeyera o pojemności 500 mL umieszczano 250 mL pożywki, sterylizowano parowo, po schłodzeniu zaszczepiono fragmentami grzybni kultur matecznych boczniaka utrzymywanych na pożywce agarowej. Kolby były wytrząsane w cyklu całodobowym, 140 obrotów na minutę, w temperaturze 25±2ºC i stałym naświetleniu. Po 2 tygodniach otrzymane mycelium było odsączane, mrożone i liofilizowane. Stosowano również napowietrzane hodowle *in vitro* w bioreaktorach o pojemności 10 L, które pozwalały na otrzymanie większej ilości biomasy w krótszym okresie, mianowicie 7 dni. Szczegółowe metodyki upraw są opisane w Publikacji 2 oraz 3.

Tabela 1. Skład pożywki do hodowli *in vitro*

Składnik	Zawartość
Ekstrakt maltozowy	7,5 g
Glukoza	10 g
NH_4Cl	0,5 g
L-asparagina	1,0 g
KH_2PO_4	0,5 g
$MgSO_4 \cdot 7\ H_2O$	0,5 g
$FeCl_3$ [1%]	10 kropli
$MnSO_4 \cdot H_2O$ [0,5%]	1,5 mL
$ZnSO_4 \cdot 6\ H_2O$ [0,3%]	1,5 mL
$CaCl_2 \cdot 6\ H_2O$ [2%]	5,0 mL
Hydrolizat kazeiny	0,2 g
Ekstrakt drożdżowy	0,03 g
Adenina	0,012 g
Woda destylowana	ad 1000 mL

Zdjęcie 7. Kultury napowietrzane 10 L w Katedrze Botaniki Farmaceutycznej CMUJ

6.3. Metoda pozyskania owocników *Pleurotus* spp.

Owocniki wybranych gatunków boczniaka uzyskano z upraw prowadzonych w pracowni Katedry Ogrodnictwa, Wydziału Biotechnologii i Ogrodnictwa, Uniwersytetu Rolniczego w Krakowie, wyposażonej w urządzenia do przygotowania i sterylizacji substratu oraz pomieszczenia uprawowe z możliwością precyzyjnego sterowania parametrami mikroklimatycznymi. Pracownia ta została zaprojektowana, wykonana i wyposażona przez autora tej pracy w celu umożliwienia produkcji dowolnych podłoży uprawowych oraz utrzymania odpowiednich warunków do inkubacji oraz plonowania grzybów wielkoowocnikowych – zmiany w pracowni zaprezentowano na zdjęciu 8 i 9. Opracowano metodyki upraw wybranych gatunków boczniaków, a także poza tematem doktoratu, przetestowano uprawę innych gatunków grzybów, w tym gatunków do tej pory nieuprawianych np. twardzioszka czosnkowego (*Mycetinis alliaceus*). Do analiz biochemicznych opisanych w Publikacji 2 przeznaczono owocniki sześciu gatunków *Pleurotus*: *P. citrinopileatus, P. djamor, P. eryngii, P. florida, P. ostreatus, P. pulmonarius*, wyprodukowane w substracie sporządzonym na bazie trocin bukowych, słomy pszennej oraz otrębów pszennych, umieszczonym w workach polipropylenowych z mikroflitrem i wysterylizowanym parowo. Do analiz opisanych w Publikacji 3 przeznaczono owocniki z uprawy prowadzonej w słoikach szklanych o objętości 3 litrów, przy czym podłożem były trociny bukowe oraz słoma pszenna. Metoda uprawy w słoikach została opracowana i zmodyfikowana na bazie komercyjnej uprawy butelkowej stosowanej w krajach azjatyckich. W Publikacji 4 zamieszczono wyniki doświadczenia z zastosowaniem substratu na bazie pelletu bukowego z dodatkiem otrębów pszennych, umieszone w workach polipropylenowych z mikro filtrem. We wszystkich doświadczeniach przeprowadzono pełen cykl uprawowy, obejmujący przygotowanie grzybni ziarnistej na bazie sterylizowanej, nawodnionej pszenicy zaszczepianej fragmentem kultury matecznej utrzymywanej na agarze. Szczegółowe metodyki zostały opisane w Publikacjach 2, 3 oraz 4 natomiast w Publikacji 1 przedstawiono przegląd literatury naukowej, która pozwoliła na modyfikację i dostosowanie technologii upraw i technologii analitycznych wykorzystanych w doświadczeniach.

Zdjęcie 8. Pierwsze pomieszczenia do uprawy grzybów (2016-2019), Katedra Ogrodnictwa, Wydział Biotechnologii i Ogrodnictwa UR w Krakowie

Zdjęcie 9. Pomieszczenie do uprawy grzybów (2020-2022), Katedra Ogrodnictwa, Wydział Biotechnologii i Ogrodnictwa UR w Krakowie

6.4. Analizy biochemiczne wykonane w cyklu prac badawczych

Analizy uzyskanego materiału badawczego (mycelium i owocniki poszczególnych gatunków *Pleurotus* spp.) przeprowadzano w wieloosobowym zespole badawczym pracowników i doktorantów krakowskich uczelni wyższych. Wiodącą część badań przeprowadzano w Katedrze Botaniki Farmaceutycznej Wydziału Farmaceutycznego Uniwersytetu Jagiellońskiego Collegium Medicum w Krakowie. Badania przeprowadzano w Katedrze Ogrodnictwa Wydziału Biotechnologii i Ogrodnictwa Uniwersytetu Rolniczego w Krakowie jak i również na Wydziale Technologii Żywności Uniwersytetu Rolniczego w Krakowie. Analizy biopierwiastków wykonano na Wydziale Fizyki i Informatyki Stosowanej Akademii Górniczo-Hutniczej w Krakowie. Metodyki badań przedstawiono krótko w kolejnych podrozdziałach niniejszej rozprawy, przy czym szczegółowy opis zamieszczono w Publikacjach 2, 3 i 4.

6.5. Metodyka oznaczenia biopierwiastków w materiale grzybowym

W celu określenia składu biopierwiastków w materiale grzybowym próbki grzybów mineralizowano na mokro w mineralizatorze mikrofalowym w mieszaninie kwasu azotowego HNO_3 (65%) oraz perhydrolu H_2O_2 (30%), a otrzymane roztwory przenoszono do kolb o objętości 10 mL. W Publikacjach 2 i 3, do oznaczania pierwiastków zastosowano metodę spektrometrii rentgenowskiej całkowitego odbicia (TXRF) natomiast w Publikacji 4 metodę spektrometrii absorpcji atomowej (AAS).

6.6. Metoda oznaczeń substancji organicznych

W celu oznaczenia związków organicznych wykonano ekstrakty z suchego materiału mycelium oraz owocników badanych gatunków *Pleurotus* spp. – metody ekstrakcji zostały opisane w publikacjach 2, 3 i 4 w zależności od badanej grupy związków. Związki organiczne, w tym związki indolowe, związki fenolowe, sterole, lowastatyna, ergotioneina i witaminy z grup B oznaczano metodą wysokosprawnej chromatografii cieczowej (HPLC). W celu określenia potencjału antyoksydacyjnego, zawartości glutationu oraz całkowitej zawartości cukrów użyto metody spektrometrii kolorymetrycznej. Do oznaczenia witaminy C użyto

metody miareczkowej. Szczegółowe metodyki tych analiz zostały opisane w Publikacjach 2, 3 i 4.

6.7 Analizy statystyczne

Uzyskane wyniki opracowano statystycznie używając programu Satistica 13.0. Przeprowadzono szereg analiz statystycznych opisanych w Publikacjach 2, 3 i 4.

7. STRESZCZENIE ZAŁĄCZONYCH PUBLIKACJI

W Publikacji 1 dokonano przeglądu literatury związanej z szeroko pojętą uprawą grzybów. Opisano najważniejsze gospodarczo gatunki uprawiane na świecie, różne technologie upraw a także historie ich powstawania. Dokonano również przeglądu literatury związanej z właściwościami leczniczymi i prozdrowotnymi grzybów.

W Publikacji 2 zamieszczono wyniki badań, których celem była jakościowa i ilościowa analiza mycelium i owocników sześciu gatunków boczniaka uprawianych na pożywce wg. Oddoux oraz w standardowym substracie uprawnym. Uzyskane wyniki pozwoliły przede wszystkim na porównanie zawartości substancji bioaktywnych w mycelium i owocnikach *Pleurotus* spp. oraz na zanalizowanie różnic międzygatunkowych. Najważniejsze rezultaty przedstawiono w kolejnym rozdziale rozprawy doktorskiej. Na podstawie tych badań wybrano *P. eryngii* i *P. djamor*, jako obiekty kolejnych doświadczeń związanych z suplementacją pożywki i substratu biopierwiastkami (Publikacja 3 i 4).

W Publikacji 3 opisano wyniki badań związanych z wpływem suplementacji solami cynku oraz selenu pożywki i substratu na skład chemiczny mycelium i owocników *P. eryngii*. Użyto wodoroasparaginianu cynku, jako organicznej formy tego pierwiastka, siarczanu cynku, jako formy nieorganicznej oraz selenianu sodu, jako formy przyswajalnej. Stężenia zostały dobrane w ramach eksperymentów poprzedzających doświadczenie główne, jako najwyższe możliwe stężenia niehamujące wzrostu grzybni. Hodowlę mycelium *P. eryngii* prowadzono na pożywce Oddoux'a, do której dodano wyżej wymienione związki selenu i cynku. Uprawę *P. eryngii* w celu otrzymania owocników przeprowadzono na mieszaninie słomy pszennej oraz trocin bukowych, umieszczonej w szklanych, 3 litrowych słojach, do których dodawano związki selenu i cynku. Podłoże wysterylizowano i zaszczepiono wcześniej przygotowaną grzybnią ziarnistą. Doświadczenie wykazało, że dodatek soli cynku przyczynił się do uzyskania większej biomasy grzybni z kultur *in vitro* i nie wpływał negatywnie na plon owocników. W przypadku selenu, jego dodatek prawie dwukrotnie zmniejszył plon owocników w uprawie w substracie, nie wpłynął natomiast negatywnie na biomasę mycelium z kultur *in vitro*. Suplementacja zmodyfikowała zawartość związków organicznych wymienionych w metodyce we wszystkich badanych obiektach. Dodatek siarczanu cynku oraz selenianu sodu zwiększył zawartość związków o działaniu antyoksydacyjnym, co potwierdził wzrost aktywności antyoksydacyjnej oznaczonej w owocnikach *P. eryngii*. W przypadku wodoroasparaginianu cynku nie zaobserwowano tej zależności. W przypadku mycelium – najwyższą aktywność

antyoksydacyjną wykazano w materiale grzybowym z obiektu kontrolnego oraz z obiektu, w którym zastosowano suplementację selenem. Najniższą aktywność antyoksydacyjną stwierdzono w materiale grzybowym z obiektów suplementowanych związkami cynku. Wpływ suplementacji pożywki i podłoża na zawartość pozostałych związków chemicznych był zróżnicowany, zaobserwowano istotne zmniejszenie syntezy lowastatyny pod wpływem działania badanych soli. Analiza zawartości selenu i cynku w owocnikach i mycelium *P. eryngii* wykazała, że suplementacja pożywki i substratu okazała się skuteczną metodą zwiększenia zawartości tych pierwiastków, zwłaszcza w mycelium.

W Publikacji 4 opisano wyniki dotyczące wpływu suplementacji substratu uprawowego *P. djamor* siarczanem magnezu oraz chlorkiem magnezu w trzech stężeniach na plon i jakość owocników. Przeprowadzano uprawę w mieszaninie pelletu bukowego i otrębów pszennych umieszczonych w workach polipropylenowych z mikro filtrem, sole magnezu dodano przed sterylizacją i dokładnie wymieszano z podłożem. Badania wykazały, że suplementacja substratu pozwoliła zwiększyć zawartość magnezu w owocnikach w porównaniu do kontroli, jednak wiązało się to ze spadkiem plonu. Równocześnie suplementacja substratu spowodowała stres antyoksydacyjny – wykazano zwiększenie całkowitej zawartości związków fenolowych oraz zwiększenie potencjału antyoksydacyjnego ekstraktów z owocników. W przypadku pozostałych badanych związków zaobserwowano zróżnicowany wpływ suplementacji substratu na ich zawartość w owocnikach *P. djamor*.

8. WYNIKI

8.1. Analiza zawartości biopierwiastków w mycelium i owocnikach *Pleurotus* spp. (Publikacja 2)

W przedstawionej pracy, w pierwszym etapie przeprowadzono badania, których celem było porównanie zawartości makroelementów (Ca, K i Mg) i mikroelementów (Cu, Cr, Fe, Mn, Ni, Rb, Se, Sr i Zn) w mycelium uzyskanym z kultur *in vitro* w oraz w owocnikach z upraw w substracie sześciu gatunków *Pleurotus* spp. (Publikacja 2).

Analizę stężenia metali wykonano za pomocą metody TXRF oraz F-AAS. Zoptymalizowane procedury preparatyki uzyskanego materiału badawczego w połączeniu z zastosowanymi metodami analizy, cechującymi się precyzją i dokładnością, pozwoliły na efektywną analizę biopierwiastków w badanych gatunkach grzybów.

Na podstawie przeprowadzonej analizy stwierdzono, że najwyższe poziom wśród makroelementów analizowanych w owocnikach oznaczono w przypadku potasu u *P. djamor* (2516 mg 100 g^{-1} s.m.) z kolei najniższe stężenie odnotowano w przypadku *P. florida* odnośnie zawartości wapnia (388 mg 100 g^{-1} s.m.). Badania zawartości makroelementów takich jak potas, magnez i wapń w mycelium z kultur *in vitro* wykazały istotnie niższą ich zawartość w odniesieniu do owocników w przypadku wszystkich gatunków (Tabela 2). Najwyższe stężenie magnezu w mycelium z kultur *in vitro* oznaczono w przypadku *P. citrinopileatus* (1354 mg 100 g^{-1} s.m.), natomiast najniższym stężeniem wapnia charakteryzowało się mycelium *P. pulmonarius* (290 mg 100 g^{-1} s.m.). Analiza zawartości mikroelementów wykazała ich wyższy poziom w mycelium z kultur *in vitro* niż w owocnikach badanych gatunków *Pleurotus* spp. W mycelium najwyższą zawartość żelaza stwierdzono u *P. ostreatus* (34,82 mg 100 g^{-1} s.m.) a najniższy poziom chromu odnotowano u *P. djamor* i *P. ostreatus* (0,01 mg 100 g^{-1} s.m.). W przypadku owocników, najwięcej żelaza zawierał *P. djamor* (9,41 mg 100^{-1} g s.m.).

Tabela 2. Zawartość wybranych pierwiastków i substancji aktywnych zawartych w badanych gatunkach *Pleurotus* spp. (Publikacja 2).

	Mg	Zn	Se	5-HTP	Fenyloalanina	Ergotioneina
Owocniki						
P. citrinopileatus	1235 ±91	4,79 ±0,77	0,08 ±0,01	368,67±23,53	294,58±8,41	129,89±9,25
P. djamor	851 ±74	9,41 ±0,11	0,56 ±0,32	703,56±37,79	247,03±18,15	71,29±4,48
P. eryngii	1110 ±104	4,74 ±0,0	0,84 ±0,01	120,11±20,12	143,41±2,17	70,52±0,04
P. florida	890 ±64	6,01 ±0,5	1,29 ±0,01	553,87±2,62	154,16±22,11	49,01±2,12
P. ostreatus	956 ±78	8,82 ±0,09	0,12 ±0,01	215,53±4,82	169,26±13,74	60,43±2,87
P. pulmonarius	1280 ±113	4,38 ±0,04	0,27 ±0,01	221,51±5,86	168,41±19,53	58,06±6,08
Mycelium						
P. citrinopileatus	1354 ±81	12,39 ±0,03	1,44 ±0,01	368,67±23,53	331,65±21,07	20,05±1,77
P. djamor	979 ±47	11,81 ±0,03	1,60 ±0,01	703,56±37,79	n.d.	128,35±7,15
P. eryngii	844 ±66	14,96 ±0,09	2,37 ±0,06	120,11±20,12	n.d.	46,71±5,69
P. florida	759 ±50	7,18 ±0,06	1,88 ±0,02	553,87±2,62	n.d.	96,32±3,81
P. ostreatus	1101 ±68	11,19 ±0,03	0,30 ±0,01	215,53±24,82	n.d.	66,69±4,64
P. pulmonarius	1260 ±63	8,30 ±0,04	1,03 ±0,01	221,51±5,86	n.d.	47,65±2,92

5-HTP: 5-hydroksy-L-tryptofan. Zawartość podana w mg na 100 g^{-1} s.m.

8.2. Analiza zawartości związków organicznych w mycelium i owocnikach *Pleurotus* spp. (Publikacja 2)

W opisywanym eksperymencie przeprowadzono szczegółową analizę zawartości w myceliach z kultur *in vitro*, uzyskanych owocników i myceliów z hodowli kultur *in vitro Pleurotus* spp. wybranych związków organicznych o właściwościach leczniczych. Na podstawie uzyskanych wyników stwierdzono, że zawartość substancji bioaktywnych, takich jak lowastatyna, związki indolowe, związki fenolowe i witaminy z grupy B była zróżnicowana w zależności od gatunku oraz surowca grzybowego (mycelium i owocniki) Analiza zawartości związków fenolowych wykazała, że mycelium *P. eryngii* i *P. djamor* zawiera wykrywalne ilości tylko niektórych z analizowanych związków, m.in. kwas benzoesowy. Bogatsze w związki fenolowe okazały się owocniki, w których u wszystkich analizowanych gatunków oznaczono fenyloalaninę (zakres 143,41–294,58 mg 100 g^{-1} s.m.), w przeciwieństwie do mycelium gdzie związek ten oznaczono tylko w przypadku *P. citrinopileatus* (331 mg 100 g^{-1} s.m.). Jest to istotna zależność, ponieważ wysoka zawartość związków fenolowych jest bezpośrednio związana z potencjałem antyoksydacyjnym surowca. Wynika to z faktu, iż związki fenolowe wykazują właściwości redukujące wolne rodniki, przez co pełnią rolę

antyoksydantów. Stąd też poszczególnym gatunkom boczniaków, a w szczególności boczniakowi cytrynowemu można przypisać silne działanie antyoksydacyjne, co warunkuje jego wartość prozdrowotną. W przypadku analizy związków indolowych na uwagę zasługuje *P. eryngii*. Zarówno owocniki tego gatunku, jak również mycelium z kultur *in vitro* charakteryzowała wysoka zawartość L-tryptofanu, 5-CH_3-tryptofanu i 5-hydroxytryptofanu. Należy podkreślić, iż w tym przypadku mycelium pozyskane z hodowli *in vitro* odznaczało się istotnie większą, w porównaniu do owocników, zawartością wymienionych powyżej związków (L-tryptofan 35,28 mg 100 g^{-1} s.m., 5-CH_3-tryptofan 14,49 mg 100 g^{-1} s.m., 5-hydroksytryptofan 221,51 mg 100 g^{-1} s.m.). Analiza zawartości w pozostałych związków organicznych wykazała, że mycelium *Pleurotus* spp. jest lepszym źródłem specyficznych dla grzybów związków chemicznych w porównaniu do owocników. m.in. lowastatyny i ergotioneiny, W mycelium *P. eryngii* oznaczono 124,18 mg 100 g^{-1} s.m. lowastatyny, natomiast w mycelium *P. djamor* 128,35 mg 100 g^{-1} s.m. ergotioneiny (Tabela 2).

Podsumowując wykazano, że owocniki *P. eryngii* i *P. djamor* charakteryzują się najwyższą zawartością związków indolowych spośród analizowanych sześciu gatunków *Pleurotus* spp. W przypadku mycelium, wyróżniającym się gatunkiem, okazał się *P. eryngii*, zawierający istotnie najwięcej lowastatyny, związku o działaniu regulującym poziom cholesterolu we krwi człowieka. Wykazano również zróżnicowaną zawartość pierwiastków w owocnikach oraz mycelium badanych gatunków *Pleurotus* spp. Na podstawie uzyskanych wyników związanych z efektywnością upraw i jakością owocników i mycelium, do dalszych badań mających na celu wzbogacanie związkami cynku oraz selenu wybrano *P. eryngii*, a związkami magnezu *P. djamor*.

8.3. Analiza zawartości biopierwiastków i związków organicznych w mycelium i owocnikach *Pleurotus eryngii* uzyskanych z podłoży suplementowanych Zn i Se (Publikacja 3)

Podłoże do uprawy *P. eryngii* zmodyfikowano względem podłoża opisanego w Publikacji 2, stosując inne składniki oraz dodając związki cynku i selenu. Optymalne stężenie soli określono na podstawie badań wstępnych na kulturach agarowych, oceniając poziom niewpływający negatywnie na wzrost grzybni. Oceniono plon owocników *P. eryngii*, który wynosił między 13-15 g suchej masy na 100 g^{-1} podłoża dla kontroli i suplementacji siarczanem Zn oraz hydroasparaginianem Zn, jednakże suplementacja Se podłoża spowodowała dwukrotne

zmniejszenie plonów. Dodatkowo zaobserwowano deformacje owocników w przypadku zastosowania selenu, co może świadczyć zbyt wysokim jego poziomie w podłożu. W przypadku mycelium uzyskano średnio 6,09 g s.m. na 1 dm^3 płynnej pożywki Oddoux. Zastosowanie siarczanu Zn i hydroasparaginian Zn istotnie zwiększyło plon mycelium w porównaniu z kontrolą, podczas gdy Se nie wpływał istotnie na plon mycelium *P. eryngii*. Dodatek związków Zn i Se wpłynął na wzrost tych substancji w owocnikach jak i mycelium a także powodował zmianę zawartości innych oznaczanych pierwiastków. Zawartość Se wzrosła w największym stopniu w mycelium *P. eryngii* wyniosła 18,79 mg na 100 g^{-1} s.m. – w kontroli poziom był 9 krotnie mniejszy (Tabela 3). W przypadku owocników, dodatek Se pozwolił na otrzymanie grzybów zawierających 1,36 mg tego pierwiastka na 100 g^{-1} s.m. – w przypadku pozostałych obiektów poziom Se był na granicy oznaczalności. Suplementacja związkami cynku również miała największy wpływ na poziom tego pierwiastka w przypadku mycelium – dodatek hydroasparaginianu Zn zwiększył zawartość tego pierwiastka do poziomu 17,3 a siarczanu cynku 289,5 względem zawartości 15,1 mg na 100 g^{-1} s.m. w kontroli. W przypadku owocników jedynie suplementacja siarczanem Zn zwiększyła nieznacznie zawartość względem kontroli – 5,39 względem 4,94 na 100 g^{-1} s.m. (Tabela 3). Zaobserwowano bardzo zróżnicowany wpływ zawartości innych pierwiastków, często różny w mycelium i owocnikach np. suplementacja Se zwiększyła ponad dwukrotnie akumulację Mg w owocnikach a dwukrotnie zmniejszyła w przypadku mycelium. Dodatek cynku zmniejszył akumulację K w owocnikach i mycelium, w przypadku mycelium również zmniejszył zawartość Fe. Suplementacja Zn jak i Se miała zróżnicowany wpływ na zawartość oznaczanych związków organicznych. W przypadku lowastatyny oznaczonej jedynie w mycelium *P. eryngii*, dodatek Se zmniejszył jej zawartość do poziomu 2,26 mg w stosunku do kontroli gdzie oznaczono 27,02 mg tej substancji na 100 g^{-1} s.m. a suplementacja Zn obniżyła zawartość lowastatyny do poziomu 0,11 i 0,69 mg na 100 g^{-1} s.m. (Tabela 3). Zawartość związków fenolowych również była bardzo zmienna – całkowita zawartość związków fenolowych w kontrolnych owocnikach *P. eryngii* wynosiła 212,76 mg, najmniej oznaczono w przypadku dodatku hydroasparaginianu Zn 183,72 a najwięcej w przypadku siarczanu Zn 263,04 mg na 100 g^{-1} s.m. – zaobserwowano również dodatnią korelację z aktywnością antyoksydacyjną oznaczoną metodą rodnika DPPH. W przypadku mycelium całkowita zawartość związków fenolowych była najniższa w obiekcie suplementowanym Se – 257,16 mg a najwyższa w przypadku suplementacji Zn 317,52–318,06 mg na 100 g^{-1} s.m. (Tabela 3). Jednakże w przypadku dodatku Se aktywność antyoksydacyjna była najwyższa 56,5% względem 39,9% przy suplementacji siarczanem Zn.

Tabela 3. Zawartość wybranych pierwiastków i substancji aktywnych zawartych w suplementowanych *Pleurotus eryngii* (Publikacja 3).

		BE	Se	Zn	Lowastatyna	DPPH	Z.F.
Owocniki *Pleurotus eryngii*	Kontrola	15,29 ±0,79	*	4,94 ±0,14	*	78,20 ±0,36	212,76 ±9,36
	Se(IV)	7,00 ±0,87	1,36 ±0,07	4,48 ±0,35	*	69,55 ±0,72	226,44 ±19,44
	$ZnSO_4$	12,96 ±0,73	*	5,39 ±0,33	*	91,05 ±0,44	263,04 ±7,52
	HAZn	13,87 ±0,89	*	4,39 ±0,32	*	81,86 ±0,51	183,72 ±10,72
Mycelium *Pleurotus eryngii*	Kontrola	4,90 ±0,73	0,23 ±0,08	15,2 ±1,19	27,02 ±0,07	93,33 ±0,24	283,68 ±8,64
	Se(IV)	4,31 ±0,44	18,88 ±0,66	10,6 ±0,49	2,26 ±0,09	88,29 ±2,76	257,16 ±2,24
	$ZnSO_4$	7,41 ±0,21	0,36 ±0,07	177,4 ±2,76	0,11 ±0,03	77,48 ±0,48	318,06 ±2,28
	HAZn	7,44 ±0,29	0,21 ±0,09	289,8 ±1,67	0,69 ±0,04	76,82 ±0,28	317,52 ±2,88

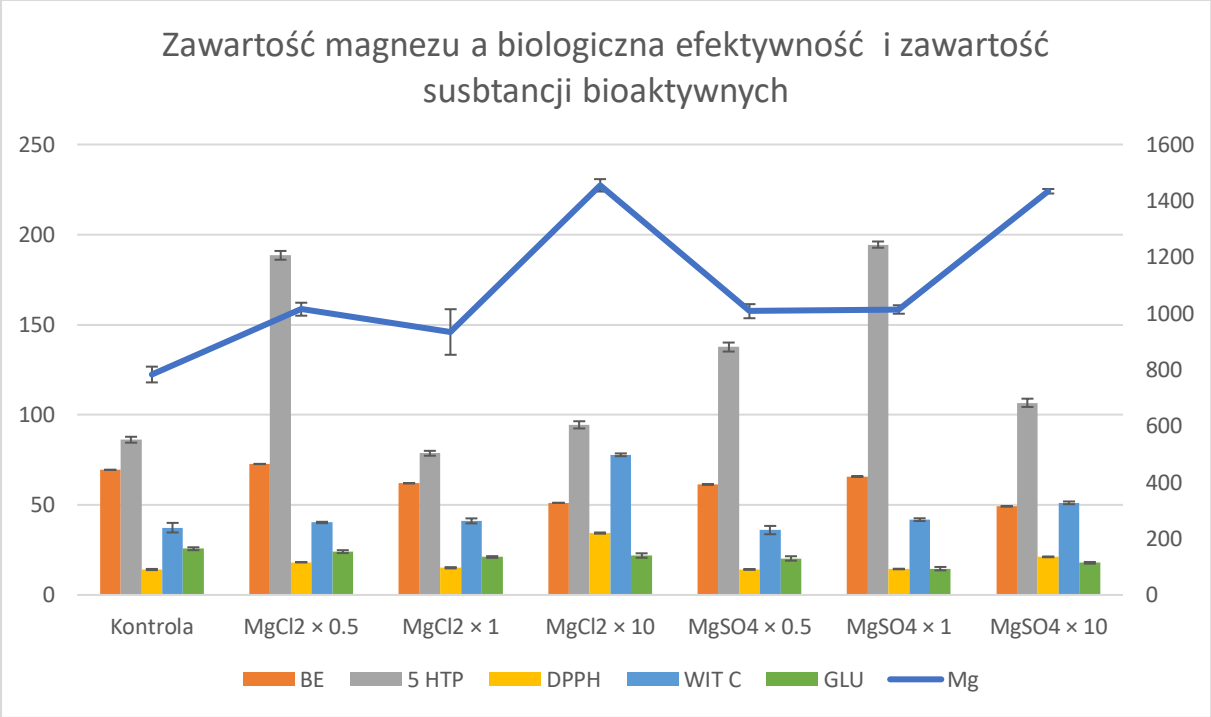
BE – biologiczna efektywność w %, DPPH – aktywność antyoksydacyjna w %, Se – selen w mg na 100 g^{-1} s.m., Zn – cynk w mg na 100 g^{-1} s.m., Z.F. – całkowita zawartość związków fenolowych oznaczana metodą Folina-Ciocâlteu w mg na 100 g^{-1} s.m.

8.4. Analiza zawartości biopierwiastków i związków organicznych w owocnikach *Pleurotus djamor* uzyskanych z podłoży suplementowanych Mg (Publikacja 4)

W publikacji 4 uprawiano *P. djamor* na podłożu wzbogaconym chlorkiem i siarczanem Mg w 3 różnych stężeniach. Najlepszy efekt uzyskano w przypadku najwyższych stężeń soli dodanych do podłoża – 4200 mg Mg w formie siarczanu i chlorku zwiększył zawartość tego pierwiastka w owocnikach o średnio 185% względem kontroli, równocześnie każde stężenie soli istotnie zwiększało akumulację Mg w owocnikach (Wykres 1). Nie zaobserwowano różnic między formami soli Mg, jednakże różne formy wpływały na akumulację innych biopierwiastków oznaczonych w owocnikach – większość przypadków zmniejszając ich zawartość w materiale grzybowym. Biologiczna efektywność uprawy została obniżona przez wysokie dawki Mg, najmniejsza była w przypadku najwyższego stężenia soli – dla siarczanu Mg 49% i dla chlorku Mg 51%, najwyższa w przypadku kontroli 69% i najmniejszego stężenia chlorku Mg 73% (Wykres 1). Dodatek soli Mg miał bardzo zróżnicowany wpływ na zawartość oznaczanych substancji aktywnych – w przypadku 5-hydroksy-L-tryptofanu suplementacja Mg zwiększyła ponad 2-krotnie zawartość tej substancji względem kontroli w przypadku niskich i średnich dawek – do poziomu 194,43 mg na 100 g^{-1} s.m. Suplementacja Mg wpłynęła na wzrost zawartości kwasu askorbinowego o 108% oraz aktywności antyoksydacyjnej o 143% w przypadku chlorku Mg w najwyższym stężeniu względem kontroli. Wykazano również wzrost

całkowitej zawartości związków fenolowych we wszystkich suplementowanych Mg obiektach – od 11% do 50% względem kontroli. Zaobserwowano spadek zawartości glutationu w suplementowanych owocnikach *P. djamor* – w kontroli oznaczono 25,65 µg tej substancji na 1 g^{-1} ś.m. a w innych obiektach od 14,59 do 21,87 µg (Wykres 1). W przypadku innych związków suplementacja miała bardzo zmienny wpływ na ich zawartość – różne dawki soli Mg wpływały bardzo zmiennie na zawartość badanych substancji aktywnych.

Wykres 1. Zawartość magnezu i wybranych substancji aktywnych zawartych w suplementowanych *Pleurotus djamor* (Publikacja 4).



Oznaczenia przy słupkach – $MgCl_2/MgSO_4$ x 0.5 210 mg magnezu na worek doświadczalny, $MgCl_2/MgSO_4$ x 1 – 420 mg magnezu na worek doświadczalny, $MgCl_2/MgSO_4$ x 10 4200 mg magnezu na worek doświadczalny, BE – biologiczna efektywność w %, 5 HTP – 5-hydroksy-L-tryptofanu w mg na 100 g^{-1} s.m. DPPH – aktywność antyoksydacyjna w %, WIT C – kwas askorbinowy w ma na 100 g^{-1} ś.m. GLU – glutation w µg na 1 g^{-1} s.m. Mg – magnez w mg na 100 g^{-1} s.m.

9. PODSUMOWANIE

Opublikowane badania wykazały, że mycelium i owocniki *Pleurotus* spp. są wartościowym produktem spożywczym jak i potencjalnym surowcem do produkcji suplementów diety i leków. Oznaczona w badanych gatunkach zawartość lowastatyny wskazuje na to, że substancja ta może być potencjalne pozyskiwana z mycelium i grzybni wybranych gatunków *Pleurotus* spp., po opracowaniu odpowiednich metod ekstrakcji i oczyszczania. Badania nad suplementacją wykazały, że jest możliwe uzyskanie surowca wzbogaconego w deficytowe pierwiastki, np. selen, w celu zwiększenia ich podaży w diecie człowieka. Celem kolejnych badań powinna być optymalizacja technologii suplementacji a także dokładniejsze zbadanie modyfikacji metabolizmu grzybów po aplikacji związków chemicznych do pożywki lub substratu. Opublikowane badania sugerują bardzo duże zróżnicowanie wpływu suplementacji na poziom analizowanych związków biochemicznych w grzybach dokładniejsze zbadanie tych zależności potencjalnie pozwala na kilkukrotne zwiększanie zawartości danej substancji biochemicznej w surowcu. Jest to łatwiejszy i bezpieczniejszy sposób produkcji surowca bogatego np. w witaminy z grupy B niż stosowanie zmodyfikowanych organizmów genetycznych. Taka produkcja może być bardzo efektywna w kulturach *in vitro* – przy doborze odpowiednich technologii możliwe jest uzyskanie dużej ilości biomasy o kontrolowanym składzie.

W przypadku upraw w substracie wykazano duże zróżnicowanie zawartości badanych pierwiastków i związków chemicznych – mimo zastosowania podobnych metodyk wyniki różniły się w obiektach kontrolnych, w kolejnych doświadczeniach. Było to wynikiem stosowania zmodyfikowanych receptur substratu, w celu udoskonalenia technologii uprawy dla poszczególnych gatunków. W kolejnych doświadczeniach modyfikowano skład substratu, opierając się na wynikach doświadczeń pilotażowych. Podłoże bazowe do uprawy grzybów jest bardzo różnorodne i jego wpływ na biochemię owocników wydaje się być większy niż wpływ suplementacji, co można wnioskować z wyników zamieszczonych w Publikacjach 2, 3 i 4. Inne aspekty uprawowe też mogą mieć znaczący wpływ na skład chemiczny owocników, np. sposób i czas trwania inkubacji, warunki mikroklimatyczne, czy stopień dojrzałości owocnika podczas zbioru. Tym samym uzyskanie pożądanej zawartości związków o charakterze leczniczym jest trudniejsze w przypadku owocników niż w kulturach mycelianych. Z drugiej strony, łatwiejsza i tańsza uprawa oraz możliwość wykorzystania surowców odpadowych powoduje, że tradycyjna uprawa w substracie jest bardziej atrakcyjna dla producentów, a przy

standaryzowanych technologiach, suplementacja może być efektownym sposobem uzyskiwania spożywczych grzybów zawierających zwiększoną ilość biopierwiastków i związków chemicznych o działaniu prozdrowotnym.

10. WNIOSKI

1. Mycelium i owocniki *Pleurotus* spp. charakteryzuje zmienny skład biochemiczny, zdeterminowany gatunkowo.
2. Zawartość biopierwiastków jest zmienna w owocnikach i mycelium *Pleurotus* spp. mimo zastosowania jednorodnego podłoża badawczego.
3. Najwięcej 5-hydroksy-L-tryptofanu zawierają owocniki *P. djamor* oraz jego mycelium, w tym ten drugi surowiec zakumulował prawie trzykrotnie więcej tego związku.
4. Najwięcej lowastatyny zawiera mycelium *P. eryngii* 124,18 mg na 100 g^{-1} s.m. W przypadku owocników najwyższą zawartość tego związku stwierdzono w *P. citrinopileatus* – 7,66 18 mg na 100 g^{-1} s.m.
5. Suplementacja związkami selenu podłoża do upraw tradycyjnych *P. eryngii* pozwala na zwiększenie zawartości tego biopierwiastka jednak negatywnie wpływa na plon.
6. Suplementacja związkami selenu i cynku pożywki w kulturach *in vitro* pozwala na znaczne zwiększenie poziomu tych pierwiastków w biomasie z kultur *in vitro.*
7. Wpływ suplementacji związkami selenu i cynku jest bardzo zmienny na metabolizm grzybni niezależnie od zastosowanego medium – w większości przypadków zaobserwowano spadek zawartości badanych związków bioaktywnych.
8. Suplementacja solami magnezu podłoża do upraw *P. djamor* pozwala na zwiększenie zawartości tego pierwiastka prawie dwukrotnie, jednak przy prawie 20% spadku plonu.
9. Każdy dodatek soli magnezu, niezależnie od stężenia, wpływa na metabolizm *P. djamor* a w konsekwencji na zawartość substancji bioaktywnych.
10. Dodatek soli magnezu pozwala na uzyskanie owocników *P. djamor* zawierających więcej 5-hydroksy-L-tryptofanu.
11. Możliwe jest sterowanie metabolizmem grzybów i składem biochemicznym surowca przez dodanie do pożywki lub substratu soli i związków pierwiastków.
12. Potrzebne są bardziej szczegółowe badania by określić optymalne dawki suplementacji związkami selenu, magnezu oraz cynku, które nie obniżają zawartości substancji bioaktywnych.
13. Równie istotne, co dawka jest również rodzaj soli czy związku organicznego zawierającego dany biopierwiastek.
14. Mycelium jak i owocniki *Pleurotus* spp. to cenne surowce, które mogą być wykorzystane, jako pożywienie czy suplement diety o działaniu prozdrowotnym.

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Wojciech Pusz; Wrocław University of Environmental and Life Sciences, Poland; https://orcid.org/0000-0003-1531-2739

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REVIEW in MYCOLOGY

Culinary and Medicinal Mushrooms: Insight into Growing Technologies

Piotr Zięba[1], Agnieszka Sękara[1], Katarzyna Sułkowska-Ziaja[2], Bożena Muszyńska[2,*]

[1]Department of Horticulture, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Krakow, 31-425, Poland
[2]Department of Pharmaceutical Botany, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, Krakow, 30-688, Poland

*To whom correspondence should be addressed. Email: muchon@poczta.fm

Abstract

Humans have used mushrooms from the beginning of their history. However, during the last few decades, the market demand for these fruiting bodies has increased significantly owing to the spread in the capabilities of culinary and pharmacological exploitation. Natural mushroom resources have become insufficient to meet the support needs. Therefore, traditional methods of extensive cultivation as well as modern technologies have been exploited to develop effective growing recommendations for dozens of economically important mushroom species. Mushrooms can decompose a wide range of organic materials, including organic waste. They play a fundamental role in nutrient cycling and exchange in the environment. The challenge is a proper substrate composition, including bio-fortified essential elements, and the application of growing conditions to enable a continuous supply of fruiting bodies of market quality and stabilized chemical composition. Many mushroom species are used for food preparation. Moreover, they are treated as functional foods, because they have health benefits beyond their nutritional value, and are used as natural medicines in many countries. Owing to the rapid development of mushroom farming, we reviewed the growing technologies used worldwide for mushroom species developed for food, processing, and pharmacological industries.

Keywords

mushroom cultivation; *Agaricus bisporus*; *Auricularia* spp.; *Ganoderma lucidum*; *Lentinula edodes*; *Pleurotus* spp.

1. Introduction

The first mushrooms appeared on the earth's surface as early as 800 million years ago, much earlier than the first representatives of the animal and plant kingdoms (Bonneville et al., 2020). Currently, it is estimated that there may be more than 5 million species of mushrooms; to date, more than 100,000 species have been described (Bruns, 2006). The development of molecular genetics in the twenty-first century made it possible to determine the relationship between different groups of mushrooms. However, many taxonomic groups still have inaccurate connections. Mushroom taxonomy is still evolving, and changes are taking place at the family as well as phylum and division levels. One major international study involving detailed phylogenetic analyses has divided mushrooms into the Dikarya subkingdom, which comprises the two most numerous divisions, Ascomycota and Basidiomycota, and various other divisions and subdivisions (Hibbett et al., 2007). The term "mushroom" is itself not a taxonomic category but is used to refer to fruiting bodies that appear below (hypogeous) or above (epigeous) the ground of the phyla Basidiomycota and Ascomycota from the Dikarya subkingdom (Ganeshpurkar et al., 2010).

Mushroom fruiting bodies have been part of the human diet at least since the Neolithic period. This is indicated by archeological excavations dating back to 8000 BC, whereas the first documented therapeutic use can be associated with the finding of a frozen body of a Paleolithic man from 3300 BC, which was discovered in the alpine glacier in the 1990s. The fruiting bodies of the tinder fungus (*Fomes fomentarius*) and the birch polypore (*Fomitopsis betulina*), which can be used as provisional wound dressings owing to their lymphatic, anti-inflammatory, and antibacterial properties, were found by the body (Schlegel et al., 2000; Wangun et al., 2004).

More than 2,000 species of mushrooms are considered edible, and the medicinal or health-promoting properties of approximately 700 species of mushrooms have been documented. The most studied pro-health substances contained in mushroom fruiting bodies, mycelia, and spores are polysaccharides, mainly β-glucans. Their effectiveness in the prevention and treatment of diseases has been proven; they are immuno-stimulants, owing to which they have a positive effect on the health of patients with cancer and viral or bacterial diseases (Manzi & Pizzoferrato, 2000). To date, a number of β-glucans extracted from Basidiomycota fungi have been analyzed; the best known include shizophyllan (*Schizophyllum commune*), pleuran (*Pleurotus ostreatus*), lentinan (*Lentinula edodes*), and krestin (*Trametes versicolor*), which is a polysaccharide–protein complex (Villares et al., 2012) (Table 1, Figure 1).

Table 1 Structural features of main biological active polysaccharides isolated from medicinal and culinary mushrooms.

Species and reference in Figure 1		β-glucan structure	Reference
Agaricus bisporus	Figure 1A	(1→6) Linking β-D-glucose as the main backbone with (1→4)-linked α-D-mannose units	He et al., 2014
Auricularia auricula-judae	Figure 1B	β-(1→3)-D-Glucan with two β-(1→6)-D-glucosyl	S. Xu et al., 2012
Flammulina velutipes	Figure 1C	3-*O*-D-Mannopyranosyl-L-fucopyranosyl, α-D-mannopyranosyl, and α-L-fucopyranosyl	Smiderle et al., 2008
Ganoderma lucidum	Figure 1D	β-(1→3)-Linked D-glucan; (1→6)-glucan with (1→4) branches at O-4; α-(1→4)-D-glucopyranosyl and β-(1→6)-D-galactopyranosyl with branches at O-6 of glucose and O-2 of galactose	Bao et al., 2002; Dong et al., 2012; Wang & Zhang, 2009
Lentinula edodes	Figure 1E	(1→3),(1→6)-D-Polysaccharide; fucomannogalactan of (1→6)-linked α-D-galactopyranoses branched at O-2	Carbonero et al., 2008; Palacios, Guillamón, et al., 2012
Pleurotus ostreatus	Figure 1F	(1→3),(1→6)-D-Polysaccharide; α-(1→3)-D-glucan	Palacios, García-Lafuente, et al., 2012; Synytsya et al., 2009
Schizophyllum commune	Figure 1G	(1→3),(1→6)-D-Glucan	Numata et al., 2006
Trametes versicolor	Figure 1H	α-(1–4) And β-(1–3) glucosidic linkages in their polysaccharide moieties	Awadasseid et al., 2017

Another large group of pro-health substances in mushrooms is phenolic compounds, which have antioxidant effects (Asatiani et al., 2011). They exhibit strong anti-inflammatory and anticancer properties; moreover, ethanol extracts from over 100 different mushroom species show strong antioxidant properties. Phenolic acids such as caffeic acid, hydroxybenzoic acid, and protocatechuic acid have been extracted from the fruiting bodies of the most popular culinary species (Siwulski et al., 2014).

Terpenoids, characterized by strong bioactivity, are important compounds contained in the fruiting bodies of Basidiomycota mushrooms. A number of compounds from this group have been isolated. In most cases, they are sesquiterpenoids, and the best known and studied are those extracted from the fruiting bodies and mycelia

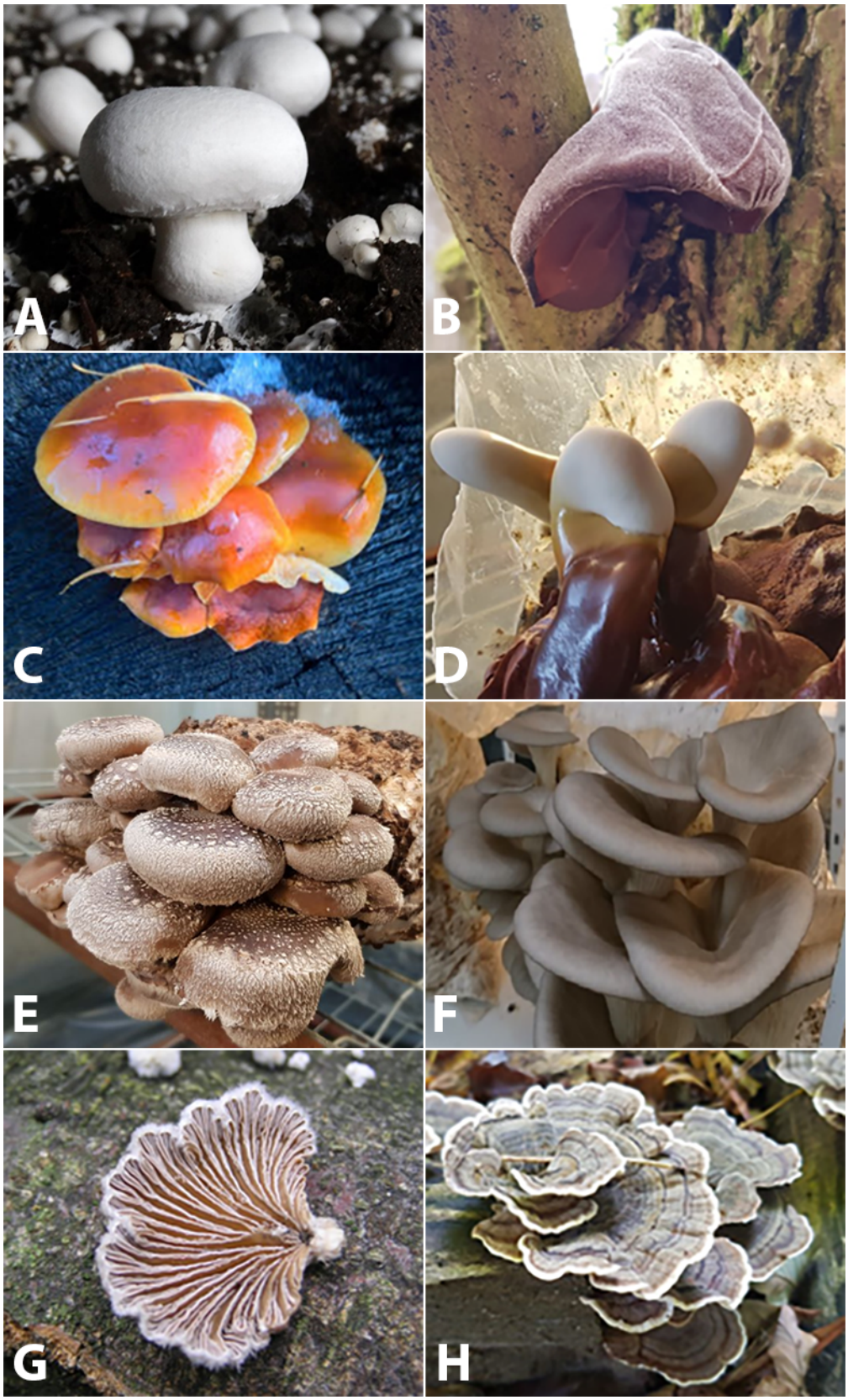

Figure 1 Medicinal and culinary mushrooms as a source of polisaccharides. (**A**) *Agaricus bisporus*; (**B**) *Auricularia auricula-judae*; (**C**) *Flammulina velutipes*; (**D**) *Ganoderma lucidum*; (**E**) *Lentinula edodes*; (**F**) *Pleurotus ostreatus*; (**G**) *Schizophyllum commune*; (**H**) *Trametes versicolor*. Photographs by B. Muszyńska, A. Sękara, K. Sułkowska-Ziaja, and P. Zięba.

of the Lingzhi mushroom (*Ganoderma lucidum*) (Liu et al., 2007). These substances, similar to β-glucans, exhibit a number of pro-health properties; their anticancer, antibacterial, and antiviral activity has been proven (Mothana et al., 2000).

Lovastatin, which can be found in many culinary mushrooms, especially in oyster mushrooms, has an inhibitory effect on the synthesis of endogenous cholesterol, which is harmful to health. Synthetic equivalents of this substance are used in the treatment of hypercholesterolemia, cardiovascular diseases, or strokes (Muszyńska et al., 2010).

More than 100 species of mushrooms are grown worldwide, and cultivation technologies are very diverse, even for the same species. Different growing media are used; however, the base is typically easily accessible and cheap lignin–cellulose waste and different kinds of sawdust and straw, often enriched with protein additives such as bran. The use of atypical and problematic waste, e.g., coffee grounds or cartons, on which some species of mushrooms are successfully grown commercially, is becoming increasingly popular (Stamets, 2011).

According to Food and Agriculture Organization, mushroom cultivation worldwide in 2018 was approximately 9 million tons. Since 2006, i.e., in just 10 years, there has been a doubling of the volume of fruiting body harvests as well as an increase in the species diversity of cultivated edible mushrooms (Food and Agriculture Organization, 2020). It can therefore be concluded that mushroom production is currently the fastest growing branch of horticulture. The dominant species in cultivation in Europe is the white button mushroom (*Agaricus bisporus*), the production of which, in 2015, was over 1.1 million tons, accounting for 80% of the total mushroom production on the old continent (Kayzer, 2017). More than a third of the white button mushrooms consumed in Europe are produced in Poland, which is considered the world leader in the export of this species. Currently, approximately 90% of the companies producing edible and medicinal mushrooms operate in China, and the species most commonly grown on a commercial scale belong to the *Lentinula*, *Pleurotus*, *Auricularia*, *Agaricus*, and *Flammulina* genera, and account for 22%, 19%, 18%, 15%, and 11% of the world production, respectively (Royse et al., 2017) (Figure 2).

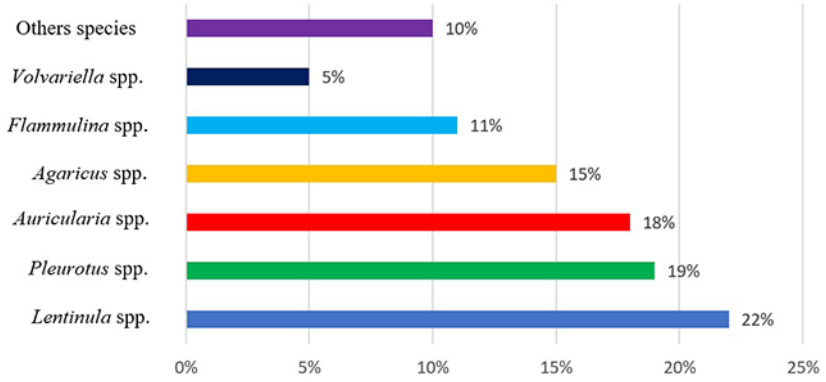

Figure 2 World edible mushroom production by genus in 2013 (Royse et al., 2017).

The nutritional and therapeutic use of mushrooms in Asian countries results from centuries of tradition and is much better documented than in Europe.

The technologies for growing these mushrooms have been developed independently in Europe and Asia, with Asian countries having a longer cultivation tradition (using different technologies) and a different production profile compared to Europe and North America. The first attempts to grow mushrooms were made in China in the twelfth century, and they consisted of combining freshly cut tree trunks with those on which the fruiting bodies of the shiitake (*Lentinula edodes*) already grew (Siwulski et al., 2011). However, the breakthrough was not made until the end of the nineteenth century, when the first pure mycelium culture was achieved by

pasteurization. Firstly, composted horse manure was grafted with the obtained clean mycelium of white button mushroom, initiating intensive mushroom cultivation (Szudyga, 1998).

In general, mushroom growing consists of several operations, viz., pure mycelium maintenance, inoculum preparation, substrate preparation, inoculation, incubation, and fruiting. The conditions and technology for each of these operations are specific for mushroom species or even strains (Sánchez, 2010). The spores or clones from wild mushroom fruiting bodies are the source of pure mycelia of the specific mushroom strain, which is produced in specialized laboratories in a sterile environment, and maintained and provided by germplasm suppliers. The commercial inoculum is obtained through the development of selected strain mycelia on cereal grains, e.g., wheat, rye, millet, or synthetic speed spawn. Spawn quality is crucial for the success of mushroom production. Therefore, many studies have focused on modern techniques of best-quality spawn production (Elhami & Ansari, 2008; Nwanze et al., 2005; Pathmashini et al., 2009; Sainos et al., 2006). The optimal composition of the growing substrate for a particular mushroom strain is inoculated and maintained under optimal temperature, moisture, and other conditions for mycelium growth, and then under the conditions that initiate fruiting (Kirbag & Akyuz, 2008; Onuoha et al., 2009).

The agri-food industry produces wastes that are not utilized worldwide, and their disposal is a threat to the environment and public health. Most of these are lignocellulosic materials, which can be suitable substrates for solid-state fermentation processes operated using mushrooms (Ritota & Manzi, 2019). Currently, for 1 kg of edible processed mushrooms, the industry produces 5 kg of spent mycelium substrate and byproducts of mushroom production (Schimpf & Schulz, 2016). Spent mushroom substrate can be used as an organic fertilizer or animal feed. In this context, the valorization of mushroom byproducts into highly valuable compounds can be developed by integrating the potential of these bioactive compounds and their high availability (Buruleanu et al., 2018).

2. *Lentinula edodes*

Lentinula edodes is currently the most widely cultivated mushroom in the world. Modern cultivation technologies and traditional methods of cultivation on wood are used in parallel, both in a mass and on a commercial scale. The name "shiitake" refers to the Japanese name of the tree "shii" on which *L. edodes* is naturally present (*Castanopsis cuspidata*, Fagaceae) and "take," which means "mushroom" (Wasser, 2005).

This species is one of the best-researched mushrooms in terms of its pro-health properties, and its effectiveness in treating or supporting the treatment of a number of diseases has been confirmed. The strong immuno-stimulatory effect of the extracts from its mycelium and fruiting bodies is a prerequisite for the effective support of cancer treatment, as well as for combating various bacterial and viral infections. Its most potent active substances are polysaccharides, viz., LEM, isolated only from the mycelium, lentinan, registered as a dietary supplement, and specific substances such as eritadenine or lentysine, which exhibit cardio-protective effects (Bisen et al., 2010). *Lentinula edodes* is also a valuable source of wholesome protein and B vitamins, as well as macro- and microelements, especially potassium, calcium, zinc, and selenium (Muszyńska, Pazdur, et al., 2017).

The first report on the consumption of *L. edodes* comes from Japan in 199 BC; the mushroom was a gift to Emperor Chuai. In China, the cultivation of these mushrooms began during the Song Dynasty (960–1127 AD), and in 1313, Chinese writer Wang Cheng described the technology of shiitake cultivation in his *Book of Agriculture*, paying attention to the choice of the right place and trees (Ito, 1978). During the Ming Dynasty (1368–1644 AD), the physician Wu-Rui wrote in the book *Ri Youg Pen Tsao* that *L. edodes* should also be used to treat respiratory diseases, poor blood circulation, and liver problems, as well as to increase life energy. He also wrote about the role of *L. edodes* in delaying aging. "Shiitake increases life energy, distracts hunger, heals cold and overcomes unstable body energy" (as cited in Rahman & Choudhury, 2012).

The biggest breakthrough in shiitake cultivation was achieved in 1936 in Japan, when K. Kitajima successively produced mycelium of *L. edodes* on a sterile substrate of straw and then used it for grafting wood. This accelerated the overgrowth of the wood logs and significantly improved the entire production (Mori, 1987). Traditional methods of growing *L. edodes* on wood logs are still used worldwide, but all-year-round intensive methods that employ artificial logs prepared with heat-treated substrates based on sawdust enclosed in plastic bags are becoming increasingly popular. The so-called "bag-log cultivation" has resulted in a shortening of the growing cycle and an increase in yields, although the final results are highly dependent on the strain, substrate composition, and conditions of incubation and fruiting (Sánchez, 2004). The substrate is a mix of sawdust, grain bran, and $CaCO_3$ with a moisture content of 60%, sterilized at 121 °C for 1 hr (Miles & Chang, 2004). However, intensive cultivation of *L. edodes* is longer and more complicated than that of other cultivated species. It takes more than 3 months from the creation and inoculation of artificial logs to the first harvest, depending on the strain, which is almost 3 times longer than that of the oyster mushroom. Hence, shiitake mushrooms are much more expensive than oyster or white button mushrooms (Siwulski et al., 2007). Similar to that of all cultivated mushrooms, the yield of *L. edodes* is related to many factors. The substrate, strain, and proper growing conditions are most important to obtain satisfactory yields for commercial growers (Stamets, 2011). The best way to exhibit the mushroom crop is by indicating the biological efficiency (BE%), which is the weight (kg) of fresh mushrooms harvested from 1 kg weight of dry substrate, expressed in percentages. The acceptable BE% for shiitake commercial growers starts at 40%. However, it is possible to have BE% above 100%. This corresponds to 350–800 g of fresh shiitake mushrooms from a typical 2.5 kg mushroom block, sometimes in more than four flushes (Siwulski et al., 2007).

3. *Pleurotus* spp.

There are more than 200 species of oyster mushrooms in the world (Catalog of Life, 2020), and eight species, viz., *Pleurotus ostreatus*, *P. eryngii*, *P. pulmonarius*, *P. djamor*, *P. sajor-caju*, *P. cystidiosus*, *P. citrinopileatus*, and *P. cornucopiae*, are commercially grown worldwide (Bellettini et al., 2019). Moreover, there have been many studies on new naturally occurring *Pleurotus* strains to improve yields. For example, *P. albidus* is recognized as a novel species for commercial production owing to its high BE% (Lechner & Albertó, 2011). There are a number of technologies for intensive cultivation of these mushrooms, differing in the type of substrate or containers. Oyster mushrooms are one of the easiest to grow because of their rapid growth of mycelia, short production cycle, resistance to diseases, high adaptability to growing conditions, and in consequence, low production costs (Sulistiany et al., 2016).

The first cultivation methods were developed in Germany during World War I and then successfully applied on a large scale, as a result of the search for new sources of food, owing to the hunger problem in Germany. Cultivation consists of inoculating pieces of wood and then harvesting fruiting bodies within a few years until the wood is completely decomposed (Spahr, 2009). However, at the beginning of the 1970s, the cultivation of oyster mushrooms on a commercial scale began, mainly in Asian countries. Several different cultivation technologies have been developed, and new strains have been selected, mainly those with different optimal fruiting temperatures (Sánchez, 2010). The BE% of *Pleurotus* spp. is highly related to the genus and growing technology. In Poland, most commercial growers use pasteurized wheat or rye straw, which is formed into cubes wrapped in foil, with a weight from 10 to 20 kg. This technology is used for *P. ostreatus* or *P. pulmonarius*, and the BE% ranges from 60% to 80%; however, supplementation with bran can result in a BE% above 100% (Gapiński et al., 2001). In bottle technology, used mostly for *P. eryngii* cultivation in Asia, the substrate contains sawdust of deciduous trees as the main component supplemented with 50% rice bran. The BE% can be above 90%. However, to obtain the highest quality of mushrooms, only two mushrooms are grown from a bottle with one flush; therefore, the BE% is less

than 50% (Peng et al., 2000; Yamanaka, 2017). A popular technology in western countries is the cultivation of oyster mushrooms on sterilized substrates made with sawdust (sometimes hardwood pellets) supplemented with bran, placed in a special polypropylene bag with a microfilter, having a weight of wet substrate of approximately 3 kg. The BE% for this type of growing technology is easily above 100% with three flushes (Stamets, 2011).

Pleurotus spp. have a set of enzymes, including carboxymethyl cellulase, xylanase, laccase, and manganese-dependent peroxidase, that decompose a wide variety of lignocellulosic waste (Zhai & Han, 2018). Generally, they can be grown on pasteurized wheat or rice straw; straw of bazar, ragi, sorghum, maize, weed plants, wood, cotton stalks, cotton seed hulls, poplar sawdust, beer grain, coffee grounds, waste paper, walnut and hazelnut shells, palm fruit bunch, etc. can also be used (Das & Mukherjee, 2007; Sözbir et al., 2015; Sulistiany et al., 2016; Yildiz et al., 2002). *Pleurotus ostreatus*, *P. pulmonarius*, *P. citrinopileatus*, *P. sajor-caju*, and *P. cistidiosus* are suitable for cultivation with these kinds of substrates (Miles & Chang, 2004).

The growth substrate is commonly a mixture of the aforementioned raw materials, and its composition affects the cultivation time, BE%, yield quantity, and quality (Alananbeh et al., 2014). A recent study revealed that bacteria isolated from fruiting bodies promoted the growth of *P. ostreatus* (Suarez et al., 2020).

The growth substrate is thermally decontaminated, and spawned with the desired strain in amounts up to 5% of the wet weight of the substrate (Royse, 2002). *Pleurotus* spp. yield increases with the spawn rate because of faster substrate decomposition and more effective competition with weed molds and bacteria (Sánchez, 2010). The photoperiod of mycelia stimulation for fruiting should be approximately 200–640 lux 8–12 hr a day (M. Ahmed et al., 2013). During fruiting, the strains of *Pleurotus* spp. produce spores, causing an allergy in some workers; therefore, there is an increase in interest in developing spore-less strains (Obatake et al., 2003). The most popular species for the commercial growth of *P. ostreatus* can produce 660 million spores per gram tissue per day, which is dangerous for growers, and damages climate systems. The spore-less strain "SPOPPO" of *P. ostreatus*, protected by CPV Rights and patented by Sylvan Inc., the largest mushroom spawn producer in the world, is now the most popular strain grown worldwide. It was obtained by protoplast fusion, using a wild spore-less mutant that produced deformed fruiting bodies of the commercial strain (Baars et al., 2004).

The nutritional value of *Pleurotus* spp. depends on the substrate composition. Ali et al. (2007) determined that *P. sajor-caju* showed maximum fat content when grown on luckrine razing, whereas *P. ostreatus* showed the same when grown on chimney gutter. Maximum protein was found in the fruiting bodies of *P. cornucopiae* grown on blow gutter. S. A. Ahmed et al. (2009) showed that a substrate composed of soybean straw produced the highest yield, crude protein, and maximum phosphorus content. The use of paddy straw resulted in higher fiber content, whereas a combination of these substrates produced significantly higher fat, calcium, and iron content. *Pleurotus* spp. production on wheat bran is recommended to support the market with quality edible mushroom products as well as enable innovative applications of the fermented substrate in animal feeds (Wanzenböck et al., 2017).

4. *Auricularia* spp.

The genus *Auricularia* comprises 10 to 15 species, which are saprophytic, with gelatinous, ear- to shell-shaped fruiting bodies that are recognized worldwide, and with intercontinental to cosmopolitan distributions (Looney et al., 2013). Cultivated species of the genus *Auricularia* (*A. auricula-judae*, *A. polytricha*, *A. fuscosuccinea*) are of higher economic importance, especially in Asian countries, because of their culinary value and pro-health properties, including antitumor, cholesterol-lowering, anticoagulant, antioxidant, immunomodulatory, anti-inflammatory, and antimicrobial effects (Sękara et al., 2015). In European countries, *Auricularia* spp. were not seen as edible species; however, they were used in folk medicine as early as the seventeenth century. Herbalist John Gerard described, in 1597, a very detailed use of the wood ear, although he briefly treated the therapeutic value of

other mushrooms. John Gerard recommended the wood ear for sore throats, in the form of liquid extract obtained by boiling the fruiting bodies in milk or leaving them immersed in beer, which should then be drunk slowly. The resulting infusion was probably similar to the Chinese soup obtained from the cloud ear fungus, which was also used to treat sore throat. In 1601, Carolus Clusius wrote that *Auricularia* spp. can be used to treat sore throats, and John Parkinson, in 1640, reported that cooking in milk or soaking in vinegar were the only methods the author knew (Roupas et al., 2012).

Called "Mun" mushrooms in China, the wood ear is the basic ingredient of many Asian dishes, and was probably the first cultivated mushroom in China, based on records in the *Chinese Materia Medica* from the Tang Dynasty (600 AD), which shows the oldest method of wood ear cultivation. In Asian countries, *Auricularia* spp. were cultivated as early as the Tang Dynasty (618–907 AD). Chinese pharmacist from the sixteenth century, Li Shih-chen, advised simple technic to cultivate wood ear "… plough the logs with boiled bran, cover them with straw, and the wood ear will grow…" (as cited in Chang, 1977). As with other cultivated mushrooms, the greatest breakthrough was achieved in the 1970s, when intensive cultivation began on sterilized sawdust placed in plastic bags. In 2010, approximately 2 million tons of these mushrooms were produced in China; unlike in Asia, *Auricularia* spp. crops are marginal compared to other cultivated mushrooms (R. Y. Zhang et al., 2012). *Auricularia auricula* and *A. polytricha* are commonly grown on natural logs of deciduous trees, or on a substrate made of 90% sawdust, 9% rice bran, and 1% $CaCO_3$ with a moisture content of approximately 65%, sterilized for 60 min at 121 °C (Stamets, 2011; Wu et al., 2017). The detailed recommendations are dependent on available raw materials, growing methods, and growing conditions. For example, Onyango et al. (2011) determined that the best *A. auricula* yield and quality were obtained with the use of a substrate made of maize cobs and wheat straw supplemented with wheat bran, and such combinations were recommended to wood ear mushroom growers. Devi et al. (2013) stated that mixing wheat and rice bran with paddy straw at a 3:1 ratio induced faster growth of *A. polytricha* in beds. Liang et al. (2019) cultivated *A. polytricha* on a sawdust basal substrate supplemented with different proportions of stalks of *Panicum repens*, *Pennisetum purpureum*, and *Zea mays* to determine the most effective substrate. The ash content of *A. polytricha* cultivated on a substrate containing *Z. mays* stalk was higher than that of the control; on the other hand, the protein content of mushrooms cultivated on a substrate containing *P. repens* stalk was higher than that of the control. An incubation temperature of 25–26 °C and relative humidity of 85%–90% is recommended for a spawn run for 20–25 days. The yield is approximately 1.0–1.4 kg fresh mushroom per kg dry straw in three–four flushes (Stamets, 2011). Recently, X. Y. Zhang et al. (2018) performed domestication experiments of *A. villosula*, and proposed conditions of spawning, manufacturing of cultural bags, spawn running, inducement of primordia, and fruiting period management to collect fruiting bodies with satisfactory performance. Similar experiments were performed by Bandara et al. (2017) with *A. thailandica*. In China, the major problems faced by growers of *A. auricula* and another species of this genus are the random labeling of strains and their introduction into different regions of identical strains under different designations (Tang et al., 2010). The precise identification and classification of commercial *A. auricula* cultivars is of major importance for Chinese and overseas markets.

5. *Agaricus bisporus*

Agaricus bisporus of the family Agaricaceae is grown in at least 70 countries worldwide. In the early 1990s, its global production was 1.5 million tons. Poland and the Netherlands are the largest white button mushroom producers in Europe (330 thousand tons per year) and the largest exporters of these mushrooms in the world (Kayzer, 2017). In the sixteenth century, the French were the first to cultivate white button mushrooms. This was owing to the French botanist Nicolas Marchant. The first white button mushrooms cultivated in France were considered a great rarity and were very expensive. The famous expert on cuisine, Anthelme Brillat-Savarin, valued

them almost as much as truffles. This lawyer, judge, and kitchen-lover in one person in his book, *Physiology of Taste*, published in 1825, gave examples highlighting the taste and smell of white mushrooms, which were then served with such specialties as crayfish, capers, or the best kinds of wine and cognac (Smith, 1993). In the USA, by coincidence of manipulation with the substrate, a white button mushroom that was less aromatic but with better market worth was bred; fortunately, the original, i.e., brown mushroom, is being increasingly appreciated (Muszyńska, Kała, et al., 2017).

Agaricus bisporus, *A. bitorquis*, or *A. subrufescens* commercial cultivation is performed on compost containing lignocellulosic material as a source of carbon and nutrients for mushroom production. The compost is produced from fermented wheat straw, chicken, swine manure, and gypsum as the main raw materials (Jurak et al., 2015). Recently, many white button mushroom growers and substrate producers have been concerned about the hygiene and safety of workers, owing to their exposure to the avian influenza virus and other pathogens found in animal manure. Furthermore, composting substrate in the traditional way, in an open area, causes smell problems not only for workers but to all local society; hence, increasing attention is being devoted to finding alternative sources of nitrogen (other than those of animal origin) for mushroom substrate preparation (Altieri et al., 2009). Owing to the animal breeding industry, manure is still the cheapest and most accessible substrate. Modern white button compost producers conduct every part of production indoors, in special bunkers. With air and water recycling reducing environmental pollution, microbe inoculation to raw manure accelerates the process (Sharma et al., 2000). Phase I (fermentation) is carried out in tunnels with controlled airflow. Microbial activity increases the compost mass temperature up to 80 °C, turning carbohydrates and proteins into heat and ammonia. Phase II consists of a pasteurization process (56 °C for 5–6 hr) followed by further high-temperature fermentation (45 °C for 4–5 days). Then, the compost is spawned, and mycelium can develop in either the same or another tunnel. Phase III compost is fully colonized by mycelium, which grows in compost mass at 25 °C for 16–18 days (Kabel et al., 2017; Van Griensven & Van Roestel, 2004). The major indicators of compost quality are moisture content, C/N ratio, pH, and absence of mites, nematodes, and competitor molds (Zied et al., 2011). Sugars other than hexoses provide energy for growth and maintenance of the vegetative growth of mycelia or are metabolically converted in the mycelium and transported to the fruiting body (Patyshakuliyeva et al., 2013). The casing layer is necessary to promote fruiting; it is commonly composed of peat and limestone to correct the pH to approximately 7.8, which is optimal (Zied et al., 2011). The casing layer provides the proper conditions for the physiological change in the mycelium behavior from the vegetative to the reproductive stage. A casing layer should support microbiota-stimulating fruiting processes but must be free of mites, nematodes, and competitor molds. The high water-holding capacity and porosity of the casing layer enable water liberation without changing its structure (Martos et al., 2017). Intensively cultivated *Agaricus* spp. need 16–20 °C during the pinning and cropping periods. This requirement restricts white button mushroom cultivation to the temperate zone, with the best economy and quality resulting from the use of proper air-conditioning during summer months (Geösel & Győrfi, 2008).

The yield of white button mushrooms is typically expressed as kg of fresh mushrooms obtained from 1 m^2 of cultivation rack. The BE% is also indicated, and for commercial profitable scale, needs to be above 80%, which corresponds to approximately 35 kg of fresh white button mushrooms from 1 m^2 in three flushes (Royse & Chalupa, 2009). Under the best conditions, some commercial growers obtain a yield of more than 40 kg/m^2 from three flushes. However, many white button mushroom farms are picking mushrooms from two flushes because the third flush is rarely more than 10% of the total yield, and there is a higher possibility of pest and disease that can infect other growing chambers (Sakson, 2017).

Werner & Beelman (2002) demonstrated that the addition of sodium selenite to a commercial compost in deep bags resulted in a significant increase in Se content in white button mushrooms (*A. bisporus*). Another issue is the high respiration rate of the fruiting bodies, leading to rapid senescence, browning, decay, and microbial

infection after harvest (Song et al., 2019). The application of new packages, e.g., chitosan/zein/α-tocopherol films, can improve the antioxidant properties and maintain the quality of the mushrooms during the transport, storage, and shelf life stages (L. Zhang et al., 2020).

6. *Ganoderma lucidum*

Ganoderma lucidum from the family Ganodermataceae is one of the best-known medicinal mushrooms. It is referred to as the "mushroom of immortality" and has been used in traditional Eastern medicine for over 4,000 years. *Ganoderma* spp. are distributed worldwide, growing as facultative parasites or as saprobes on decaying wood (Pilotti, 2005). The mushrooms of the *Ganoderma* genus can form two types of fruiting bodies: a laccate fruiting body with a shiny upper surface, or a nonlaccate fruiting body with a dull upper surface (Pilotti et al., 2004). Fruiting bodies are not used as food because of their bitter taste and hardness, but have been valued for millennia as traditional medicine in China, Japan, Korea, and other Asian countries, for maintaining vivacity and longevity (Dai et al., 2009). The Chinese names, "Lingzhi," "Chi–zhi," or "Rui–zhi" (auspicious herb), and the Japanese names, "Reishi" (divine mushroom), "Munnertake" (10,000-year-old mushroom), or "Sachitake," are mainly applied to *G. lucidum* was recorded in the first herbal manuscript "Shen Nong Ben Cao Jing" and in a poem written during the Han Dynasty 2,000 years ago (Paterson, 2006). The first *Chinese Pharmacopoeia*, which was written in the sixteenth century, contains a record on Lingzhi (Wachtel-Galor et al., 2005). Lingzhi is commonly found in paintings, carvings, furniture, jewelry, handicrafts, and many other artworks (Wasser & Weis, 1999). Currently, *G. lucidum* is not the most important species grown for a commercial purpose worldwide; *Ganoderma applanatum*, *G. capense*, *G. sinense*, *G. tsugae*, and *G. neojaponicum* (Chen & Chen, 2004; Tan et al., 2015) are cultivated as well. Many novel studies have been undertaken to isolate secondary metabolites of *Ganoderma* spp. to identify new drugs or leading compounds. Therefore, a number of bioactive constituents have been analyzed, including polysaccharides, proteins, enzymes, and polysaccharide–protein complexes (J. W. Xu et al., 2010; X. Xu et al., 2011). *Ganoderma* spp. extracts perform various pharmacological functions in the absence of side effects; therefore, they are widely used as the ultimate herbal substances. *Ganoderma* spp. have been included in the *Chinese Pharmacopoeia* (X. Zhou et al., 2007) and the *American Herbal Pharmacopoeia and Therapeutic Compendium* (Sanodiya et al., 2009).

Cultivation of *Ganoderma* spp. was attempted in 1937, but the first trial was performed in 1969 using spore separation. Traditional cultivation was based on the inoculation of natural logs of deciduous hardwoods without sterilization. Fruiting started after 6–24 months and continued for 5 years (Hapuarachchi et al., 2018). Natural log cultivation enables the production of fruiting bodies of superior quality that obtain the best prices in the markets of Southeast Asia. However, the growing cycle is long, and the yield is lower than that of sawdust synthetic log cultivation. It is important to conserve the forests where logs are collected, and this is a significant environmental concern (Chen & Chen, 2004). The current methods for the commercial production of *Ganoderma* use wood logs, short basswood segments, tree stumps, sawdust bags, and bottle procedures. *Ganoderma* spp. have been cultivated intensively on short wood logs and solid substrates, including grain, sawdust, tea waste, cotton seed husk, cork residues, sunflower seed hull, corn cobs, olive oil press cakes, and wheat straw (González-Matute et al., 2002; Gregori & Pohleven, 2015; Peksen & Yakupoglu, 2009; Riu et al., 1997; Ueitele et al., 2014). The most popular substrate is a mix of 75%–77% sawdust, 2% $CaCO_3$, 5%–9% corn bran, 5%–9% oak bran, and 3.5%–4% wheat bran sterilized under standard conditions (121 °C, 1–2 hr), whereas the moisture level is maintained at 65% (Pérez-Clavijo et al., 2016). Veena and Pandey (2011) determined that the highest BE% of *G. lucidum* was produced with a combination of sawdust:paddy straw:rice bran of 22.5:67.5:10. The optimum temperature ranges for mycelium growth and primordial initiation are 25–30 °C and 18–25 °C, respectively. The relative humidity ranges for mycelium growth, primordial initiation, and fruiting are 60%–70%, 85%–90%,

and 85%–95%, respectively. The concentration of CO_2 should be above 0.1%. The light intensity should be 500–1,000 lux during primordia initiation and 3,000–50,000 lux for fruit body development (Miles & Chang, 2004; X. W. Zhou et al., 2012). Over the past few decades, the *Ganoderma* spp. industry has developed greatly and currently offers thousands of products to markets (Hapuarachchi et al., 2018). Some problems with *Ganoderma*-based products have been reported because seasonal variations, different substrate conditions, and stages of fruiting body development affect the product quality. The qualitative and quantitative differences in the chemical composition of *Ganoderma* spp. products are dependent on the strain, cultivation conditions, and extraction procedures (X. W. Zhou et al., 2012). Hence, it is important to develop acceptable and reproducible protocols for manufacturing processes to ensure high quality, standard, and safety of *Ganoderma* products (Hapuarachchi et al., 2018). Breeding of new *Ganoderma* strains with higher yield and resistance to diseases can increase productivity and reduce the use of chemicals for pest control. The main breeding strategies include identification, purification, and construction of gene pools, followed by conventional breeding, radiating and mutant breeding, cell and gene engineering breeding, and the evaluation of agronomic trials and biochemical characteristics (X. W. Zhou et al., 2012).

7. Other Cultivated Mushrooms

The enormous number of macrofungi, including a large number of species on which very little or no research has been conducted, allows us to put forward a thesis that the development of crops will continue to grow rapidly. Apart from mycorrhizal species, whose intensive cultivation under artificial conditions seems to be impossible based on current knowledge, all saprophytic species can potentially be cultivated on a massive scale. In addition to fruiting bodies, a valuable raw material for the production of dietary supplements, and potentially, medicines, is the mycelium from in vitro cultures. The mycelium obtained from bioreactors using special media has a homogeneous, constant composition of various substances with pro-health and medicinal properties, which can be easily modified by changing the conditions of cultivation (Zięba et al., 2020).

The number of new species of mushrooms that are commercially cultivated grows yearly owing to the search for new kinds of food as well as potentially new medicinal substances. Although Asian countries continue to be at the forefront of new technologies, there is a growing interest in mushrooms other than *Agaricus* or *Pleurotus* spp. in the USA and Europe. Mushrooms of the *Pholiota*, *Flammulina*, *Tremella*, *Hypsizygus*, *Cordyceps*, and *Hericium* genera are only a few types of species increasingly sought-after not only for food but also medicinal and cosmetic use (Stamets, 2011).

Calocybe indica is cultivated in polypropylene bags filled with a mix of coconut coir, kash, paddy straw, maize stalks, rice straw, sorghum stalks, sugarcane leaf, wheat bran, or waste cotton, and sterilized at 121 °C for 1 hr (Amin et al., 2010; Lakshmipathy et al., 2017).

Flammulina velutipes is produced on sawdust and rice bran contained in polypropylene bottles that are sterilized (4 hr at 95 °C and 1 hr at 121 °C), mechanically inoculated, and incubated (25 days at 20 °C). During fruiting, the temperature is lowered to 3–8 °C to obtain a better-quality yield. Mushrooms grow in a plastic collar holding the fruiting bodies in place and slow down CO_2 evaporation to receive long stripes and small caps (Miles & Chang, 2004; Stamets, 2011).

Grifola frondosa cultivation is performed in polypropylene bags of 2.5 kg weight. The substrate is composed of sawdust powder, 15% rice bran, and 5% wheat bran, and 65% humidity is used. Sterilization is performed at 121 °C for 2 hr or at 110 °C for 7 hr (Mayuzumi & Mizuno, 1997).

Hericium erinaceus is commonly cultivated in polypropylene bags containing sugarcane bagasse, sawdust, cottonseed hulls, corncobs, and chopped up paddy straw mixed with rice or wheat bran, sucrose, and gypsum, and sterilized at 121 °C for 1 hr (Miles & Chang, 2004).

Hypsizygus tessulatus is cultivated in bags or bottles. The substrate is a mix of sawdust (18.2%), rice bran (8.8%), soybean shell (5.3%), corncob meal (4.7%), and $CaCO_3$ (0.5%), and 63%–70% humidity is used. Sterilization is performed at 121 °C for 1 hr (Harada et al., 2004; Stamets, 2011).

Pholiota nameko is grown in polypropylene bags. The substrate is a mix of hardwood sawdust (90%) and corn bran, wheat bran, and dried tofu refuse (10%); a humidity of 64%–65% is used, and sterilization is performed under standard conditions of 121 °C for 1 hr (Yamanaka, 2017).

Volvariella volvácea is grown in polypropylene bags filled with substrate produced by soaking cotton wastes with a particle size of 2–3 cm in water for 24 hr and mixing with wheat bran (10%) and $CaCO_3$ (2%), and sterilized at 121 °C for 2 hr (Philippoussis et al., 2001).

8. Conclusions

Commercial cultivation of medicinal and culinary mushrooms has been introduced worldwide to meet the gradually increasing demand of the food, processing, and pharmacological industries. Cultivation of edible mushrooms combines the production of protein-rich food with lignocellulosic organic waste recycling, thus meeting the pro-ecological strategies of modern agriculture. With regard to growing technologies, progress has been made in the following areas: high yield of conversion of raw ingredients to foods, limited use of pesticides for controlling pests and pathogens by using biocontrol and resistant strains, decrease in the consumption of energy by cultivating strains adapted to various climatic conditions, and greater access to technological innovations and genetic progress. The main prospects are the breeding of new strains with high yield and resistance to diseases, increasing productivity, and diminishing the use of chemicals for pest control. In addition, the improvement and development of modern engineering technologies, such as computerized control of environmental parameters, utilization of new substrates, methods for their sterilization, and spawn preparation, can increase the productivity. All these aspects will be crucial in the production of mushrooms with better appearance, texture, nutritional qualities, and medicinal value. Good laboratory, agriculture, manufacturing, production, and clinical practices are essential to achieve quality mushroom products, which can be used as new mineral sources in dietary supplements or as value-added ingredients for the formulation of functional foods or nutraceuticals.

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mgr inż. Piotr Zięba

Piotr Zięba

31-120 Kraków, Al. Mickiewicza 21
tel. +48 (12) 662 53 03, e-mail: ko@urk.edu.pl

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Dr hab. Agnieszka Sękara prof. URK

31-120 Kraków, Al. Mickiewicza 21
tel. +48 (12) 662 53 03, e-mail: ko@urk.edu.pl

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Selected edible medicinal mushrooms from *Pleurotus* genus as an answer for human civilization diseases

Agata Krakowska[a], Piotr Zięba[b], Anna Włodarczyk[c], Katarzyna Kała[c], Katarzyna Sułkowska-Ziaja[c], Emilia Bernaś[d], Agnieszka Sękara[b], Beata Ostachowicz[e], Bożena Muszyńska[c,*]

[a] Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland
[b] Department of Horticulture, Faculty of Biotechnology and Horticulture, University of Agriculture in Kraków, 29 Listopada Street, 31-425 Kraków, Poland
[c] Department of Pharmaceutical Botany, Faculty of Pharmacy, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland
[d] University of Agriculture in Krakow, Department of Plant Product Technology and Nutrition Hygiene, 122 Balicka Street, 30-149 Kraków, Poland
[e] Faculty of Physics and Applied Computer Sciences, AGH University of Science and Technology, 30 Mickiewicza Street, 30-059 Kraków, Poland

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ABSTRACT

Edible mushrooms are classified as a functional food. The study aimed to initiate *in vitro* cultures of selected species of *Pleurotus*: *P. citrinopileatus*, *P. djamor*, *P. eryngii*, *P. florida*, *P. ostreatus*, and *P. pulmonarius* and to optimize the method of cultivation of these species to obtain raw materials characterized by pro-health properties. Another objective was to analyze the biologically active substances in the obtained mushroom materials. By determining the organic compounds and bioelements present in the species selected for the study, it was found that the fruiting bodies obtained by cultivation were characterized by a high content of phenolic and indole compounds. Similarly, the mycelia obtained from *in vitro* culture were found to contain significant amounts of organic compounds exhibiting biological effects such as lovastatin. The study revealed that the composition of biologically active substances varied between the mycelia obtained from *in vitro* cultures and the fruiting bodies obtained by cultivation.

1. Introduction

According to the FAO data, global mushroom cultivation amounted almost 9 million tons in 2018, indicating that it was one of the fastest growing branches of horticulture. In the last 10 years, the harvest of fruiting bodies has doubled together with an increase in species diversity (FAO, 2018). A good example of this phenomenon can be found in the Japanese market, where the production of *Pleurotus eryngii* has increased more than five times in less than 10 years, with the yield reaching over 37,000 tons in 2009 (Yamanaka, 2011). Currently, the species that are most often commercially cultivated are those of the genera *Lentinula*, *Pleurotus*, *Auricularia*, *Agaricus*, and *Flammulina*, representing 22%, 19%, 18%, 15%, and 11% of the world production, respectively. The nutritional and therapeutic use of mushrooms has been better documented in Asian countries over many centuries compared to Europe. The dominant species of cultivated mushroom consumed in Europe is *Agaricus bisporus*, but *Pleurotus* spp. and *Lentinula edodes* become more and more popular (Royse, Baars & Tan, 2017) *Pleurotus* genus is represented by 205 species (Catalogue of Life, 2020). A significant number of these species are edible and harvested from the natural habitats, but the following species are popular commercially cultivated: *P. citrinopileatus*, *P. djamor*, *P. eryngii*, *P. florida*, *P. ostreatus*, and *P. pulmonarius* (Bellettini et al., 2019). Cultivation technologies are very diverse, and easily accessible agro wastes, mainly originating from the local agricultural practices and forestry (e.g., straw or sawdust), are used as the substrates (Stamets, 2011). *Pleurotus* mushrooms contain numerous therapeutic substances, and different species have been proven as effective in the treatment of bacterial, viral, and cardiovascular diseases and even cancers (Golak-Siwulska, Kałużewicz, Spiżewski, Siwulski, & Sobieralski, 2018). The β-glucans contained in oyster mushrooms have been clinically identified to possess immunostimulating properties; among them, the best tested is pleuran isolated from *P. ostreatus* (Patel, Naraian, & Singh, 2012). The most important active substances contained in oyster mushrooms include phenolic and indole compounds and bioelements. Phenolic compounds are strong antioxidants and possess anti-inflammatory and anticancer properties (Asatiani, Elisashvili, Songulashvili, Reznick, & Wasser, 2011). *Pleurotus* genus is also a recognized source of lovastatin, which

* Corresponding author.
E-mail address: muchon@poczta.fm (B. Muszyńska).

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inhibits the synthesis of endogenous cholesterol known to be harmful to health. The synthetic equivalent of this substance is a prescription drug used to treat hypercholesterolemia (Atlı, Yamaç, Yıldız, & Şőlener, 2019). *Pleurotus* spp. also contains B complex vitamins, as well as fat-soluble ones (e.g., vitamin D) (Furlani & Godoy, 2008). Not only fruiting bodies of *Pleurotus* spp. are used; also, mycelium obtained from *in vitro* cultures have potential to become a raw material for diet supplements or even medicines because of its stable active substances content and easy controllable production (Cardoso et al., 2017; Li et al., 2019).

Nowadays, functional food is of great interest, which, apart from providing the basic nutritional benefits, has a positive impact on human health. Mushrooms are also currently considered as functional food due to their natural ability to accumulate various types of substances that allow improving their health-promoting properties and can supplement the human diet (Gąsecka, Mleczek, Siwulski, & Niedzielski, 2016; Kała, Muszyńska, Zając, Krężałek, & Opoka, 2016; Poniedziałek et al., 2017).

As far as we know, the content of biologically active substances in the fruiting bodies and mycelia originating from *in vitro* cultures of various species of oyster mushrooms has not been compared in the scientific literature. Therefore, our research aimed to initiate *in vitro* cultures of six selected species of the genus *Pleurotus*—*P. citrinopileatus*, *P. djamor*, *P. eryngii*, *P. florida*, *P. ostreatus*, and *P. pulmonarius*—and to obtain cultures of these species from mycelia to obtain raw material of the highest cultivation quality and characterized by high dietary and pro-health properties. Another aim was to analyze the composition of biologically active substances and bioelements in the mushroom material obtained from *in vitro* cultures and from standard cultivation methods.

2. Materials and methods

2.1. Mushroom materials

Strains of *Pleurotus* spp. (*P. citrinopileatus*, *P. djamor*, *P. eryngii*, *P. florida*, *P. ostreatus*, *P. pulmonarius*) used for the generation of *in vitro* cultures were provided courtesy of Prof. Marek Siwulski from Poznań University of Life Sciences. Representative voucher specimens were deposited at the Department of Pharmaceutical Botany of Jagiellonian University Medical College (Kraków, Poland).

2.2. Mycelial cultures

The mycelia of *in vitro* cultures grown on the solid medium were transferred to 250 mL of the modified liquid Oddoux medium in order to obtain the highest possible amount of biomass for use in further analyses (Oddoux, 1957). The Oddoux medium contains as a source of carbon: glucose, maltose extract; as a nitrogen source: casein hydrolysate, L-asparagine, adenine; macroelements in the form of aqueous solutions of NH_4Cl, KH_2PO_4, $MgSO_4{\cdot}7H_2O$, $CaCl_2{\cdot}6H_2O$, while microelements in the form of aqueous solutions of the $FeCl_3$, $MnSO_4{\cdot}H_2O$, $ZnSO_4$. Also, the medium contains yeast extract. All reagents were from Sigma Aldrich (St. Louis, MO, USA).

The obtained liquid cultures were shaken at 140 rpm (Altel, Łódź) at 25 ± 2 °C under a cycle resembling natural light conditions. These liquid *in vitro* cultures were maintained for 4 weeks to obtain a sufficient amount of biomass for the study.

After the *in vitro* cultures of the tested mushroom species were established on the liquid medium for 4 weeks, the biomass was separated from the medium and rinsed several times with four-time-distilled water. The obtained biomass was frozen and then lyophilized. The lyophilized samples were weighed and used for analysis.

2.3. Cultivation of Pleurotus spp.

Mycelia from the *in vitro* cultures of the six species of oyster mushroom selected for the study were used for cultivation. The first stage of cultivation was to obtain grain mycelium. For this purpose, moistened wheat grains were placed in polypropylene bags with a microfilter. The bags were sterilized at 121 °C for 1 h, cooled down, and then inoculated with *in vitro* cultures. The next step was preparing cultivation substrate, containing homogeneous beech sawdust, ground wheat straw, and wheat bran in a 6:1:3 ratios and 1% horticultural gypsum. The substrate was thoroughly mixed, moistened to a relative humidity of 65%, and placed in polypropylene bags with a microfilter. Then, 2.5 kg of the substrate was placed in the bags and sterilized. After cooling down, 3% of the previously prepared grain mycelium was passaged on the substrate, the mixture was stirred, and cultivation cubes were formed. The cubes were incubated at 24 ± 1 °C. After the substrate was completely overgrown, the upper part of the foil was removed and the cubes were placed in the cultivation chambers where a humidity of 90 ± 3%, a temperature of 18 ± 2 °C, and a light intensity of 900 lx per 12 h a day. These conditions supported the growth of all the *Pleurotus* spp. studied. Fruiting bodies were harvested when they reached the maturity stage characteristic of the species. Only the first-flush, homogeneous fruiting bodies showing typical appearance were used, and the nonfood fragments were not intended for further analysis. After selection, the fruiting bodies were frozen, lyophilized, and ground to be used for chemical analyses.

2.4. Mineralization process for TXRF and F-AAS analysis

For mineralization, 0.2 g samples of lyophilized materials (mycelia from *in vitro* cultures and fruiting bodies) were weighed, with an accuracy of 0.01 g, and transferred to Teflon vessels, to which 2 mL of 30% H_2O_2 solution (Merck, Darmstadt, Germany) and 4 mL of concentrated 65% HNO_3 solution (Merck, Darmstadt, Germany) were added. Mineralization was carried out in a Magnum II microwave apparatus (ERTEC) in three stages, each for 10 min, at a power of 70% and 100%, respectively, maintaining the temperature of the device at 290 °C. After mineralization, the solutions were transferred to quartz evaporators and evaporated on a heating plate at 150 °C to remove excess reagents and water. The residue was quantitatively transferred to 10 mL volumetric flasks with four-time-distilled water.

2.5. TXRF analysis of selected bioelements and F-AAS analysis of mg

The composition of bioelements, such as K, Ca, Zn, Fe, Rb, Cu, Se, Mn, Ni, Cr, and Sr, in the prepared samples was analyzed using a total reflection X-ray fluorescence (TXRF) spectrometer (Nanohunter II, Rigaku) equipped with an X-ray tube containing a molybdenum anode at 50 kV for 1000 s. For this analysis, 1000 ppm gallium was used as an internal standard. The results are expressed in mg/100 g dry weight (d.w.) (Table 1).

To analyze the composition of Mg, standard solutions were used. Bioelement analysis was carried out using flame atomic absorption spectroscopy (F-AAS) with an AAS iCE3300 Thermo Scientific™ spectrophotometer (UK).

2.6. HPLC analysis of indole and phenolic compounds, cinnamic acid, phenylalanine, lovastatin and ergothioneine

Lyophilized material (mycelia and fruiting bodies) were powdered in a mortar. Samples weighing 5 g were extracted with methanol in an ultrasonic bath at 49 kHz for 30 min (Sonic-2, Polsonic). Extraction was repeated three times for each sample. The obtained extracts (300 mL) were combined and concentrated to dryness using a rotary vacuum evaporator at 22 ± 2 °C. The extracts were quantitatively dissolved in HPLC-grade methanol and then purified using membrane filters. These extracts were used for the examination of organic compounds.

Table 1
Bioelements, organic compounds contents in fruiting bodies, and mycelia from *in vitro* liquid cultures of *Pleurotus* spp. (mg/100 g d.w.) and antioxidant activity.

Bioelements							
	Substrate	*P. citrinopileatus*	*P. djamor*	*P. eryngii*	*P. florida*	*P. ostreatus*	*P. pulmonarius*
Fruiting bodies							
K	849 ± 19	1774 ± 154^{a}	1879 ± 154^{ab}	1700 ± 23^{a}	2154 ± 91^{b}	2516 ± 73^{c}	1839 ± 191^{ab}
Mg	69.8 ± 1.6	123.5 ± 9^{c}	85.1 ± 7.4^{a}	111 ± 10.4^{bc}	89 ± 6.4^{ab}	95.6 ± 7.8^{ab}	12.8 ± 113^{c}
Ca	153.6 ± 2.3	623 ± 39^{c}	460 ± 25^{ab}	619 ± 42^{c}	388 ± 17^{a}	527 ± 29^{b}	421 ± 31^{a}
Zn	3.43 ± 0.08	4.79 ± 0.77^{a}	9.41 ± 0.11^{c}	4.74 ± 0.02^{a}	6.01 ± 0.5^{b}	8.82 ± 0.09^{c}	4.38 ± 0.04^{a}
Fe	13.6 ± 0.46	4.38 ± 0.79^{b}	6.90 ± 0.04^{c}	5.09 ± 0.04^{b}	7.89 ± 0.03^{d}	8.41 ± 0.02^{d}	3.15 ± 0.01^{a}
Rb	0.33 ± 0.04	0.77 ± 0.04^{b}	1.59 ± 0.02^{e}	0.45 ± 0.01^{a}	1.06 ± 0.02^{c}	0.76 ± 0.02^{b}	1.15 ± 0.03^{d}
Cu	0.27 ± 0.08	0.77 ± 0.32^{bc}	0.58 ± 0.01^{abc}	0.56 ± 0.01^{ab}	0.92 ± 0.01^{c}	2.69 ± 0.02^{d}	0.34 ± 0.02^{a}
Se	0.1 ± 0.01	0.08 ± 0.01^{a}	0.56 ± 0.32^{bc}	0.84 ± 0.01^{c}	1.29 ± 0.01^{d}	0.12 ± 0.01^{a}	0.27 ± 0.01^{ab}
Mn	9.27 ± 0.11	0.92 ± 0.05^{b}	1.13 ± 0.01^{e}	0.75 ± 0.03^{a}	1.07 ± 0.01^{de}	0.96 ± 0.02^{bc}	0.99 ± 0.02^{cd}
Ni	6.24 ± 0.15	0.22 ± 0.02^{b}	0.22 ± 0.01^{b}	0.17 ± 0.01^{ab}	0.36 ± 0.01^{c}	0.18 ± 0.04^{ab}	0.16 ± 0.01^{a}
Cr	1.40 ± 0.25	0.04 ± 0.01^{a}	0.08 ± 0.01^{c}	0.07 ± 0.01^{bc}	0.15 ± 0.01^{d}	0.04 ± 0.01^{a}	0.06 ± 0.02^{bc}
Sr	0.84 ± 0.06	0.03 ± 0.01^{a}	0.08 ± 0.01^{c}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.05 ± 0.01^{b}	0.02 ± 0.01^{a}
Mycelia from in vitro cultures on Oddoux medium							
K	–	984 ± 2.5^{b}	1245 ± 8.5^{d}	963 ± 2.3^{c}	595 ± 1.3^{b}	461 ± 4.7^{a}	981 ± 35^{c}
Mg	–	135.4 ± 8.1^{e}	97.9 ± 4.7^{bc}	84.4 ± 6.6^{ab}	75.9 ± 5.0^{a}	110.1 ± 68^{cd}	126.0 ± 6.3^{de}
Ca	–	757 ± 35^{c}	520 ± 39^{b}	429 ± 50^{b}	293 ± 26^{a}	321 ± 19^{a}	290 ± 21^{a}
Zn	–	12.39 ± 0.03^{e}	11.81 ± 0.03^{d}	14.96 ± 0.09^{f}	7.18 ± 0.06^{a}	11.19 ± 0.03^{c}	8.30 ± 0.04^{b}
Fe	–	22.77 ± 0.11^{b}	24.94 ± 0.08^{c}	30.97 ± 0.25^{e}	22.13 ± 0.04^{a}	34.82 ± 0.21^{f}	26.12 ± 0.09^{d}
Rb	–	0.21 ± 0.01^{a}	0.53 ± 0.01^{b}	0.51 ± 0.02^{b}	0.19 ± 0.03^{a}	0.23 ± 0.01^{a}	0.26 ± 0.01^{a}
Cu	–	0.19 ± 0.01^{c}	0.13 ± 0.01^{b}	0.07 ± 0.01^{a}	0.20 ± 0.01^{c}	0.11 ± 0.01^{b}	0.18 ± 0.03^{c}
Se	–	1.44 ± 0.01^{c}	1.60 ± 0.01^{d}	2.37 ± 0.06^{f}	1.88 ± 0.02^{e}	0.30 ± 0.01^{a}	1.03 ± 0.01^{b}
Mn	–	7.92 ± 0.02^{a}	13.71 ± 0.06^{e}	10.37 ± 0.12^{d}	9.96 ± 0.06^{c}	21.41 ± 0.13^{f}	8.89 ± 0.02^{b}
Ni	–	0.20 ± 0.01^{c}	0.15 ± 0.01^{b}	0.24 ± 0.03^{c}	0.09 ± 0.00^{a}	0.16 ± 0.02^{b}	0.13 ± 0.01^{ab}
Cr	–	0.03 ± 0.01^{a}	0.01 ± 0.01^{a}	0.13 ± 0.05^{b}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.03 ± 0.01^{a}
Sr		0.10 ± 0.01^{b}	0.22 ± 0.01^{d}	0.05 ± 0.01^{a}	0.17 ± 0.03^{c}	0.56 ± 0.02^{e}	0.15 ± 0.02^{c}

Organic compounds						
	P. citrinopileatus	*P. djamor*	*P. eryngii*	*P. florida*	*P. ostreatus*	*P. pulmonarius*
Fruiting bodies						
Phenolic compounds						
Protocatechuic acid	1.29 ± 0.16^{b}	n.d.	0.45 ± 0.03^{a}	n.d.	0.63 ± 0.05^{a}	n.d.
p-Hydroxybenzoic acid	0.23 ± 0.02^{a}	1.76 ± 0.09^{c}	3.64 ± 0.02^{d}	0.53 ± 0.02^{b}	n.d.	n.d.
Vanillic acid	1.17 ± 0.03^{a}	n.d.	n.d.	n.d.	n.d.	n.d.
Indole compounds						
L-tryptophan	13.84 ± 1.01^{c}	24.84 ± 0.97^{d}	35.28 ± 1.57^{e}	10.84 ± 0.41^{b}	5.79 ± 0.06^{a}	11.85 ± 1.18^{bc}
5-CH_3-tryptophan	5.14 ± 0.01^{b}	6.43 ± 0.01^{c}	5.45 ± 0.41^{b}	n.d.	5.16 ± 0.05^{b}	4.47 ± 0.05^{a}
Melatonin	n.d.	n.d.	0.13 ± 0.11^{a}	n.d.	0.33 ± 0.01^{b}	*
Serotonin	n.d.	7.68 ± 0.39^{b}	13.18 ± 0.88^{c}	3.31 ± 0.32^{a}	n.d.	n.d.
6-CH_3-tryptophan	0.89 ± 0.03^{b}	n.d.	8.13 ± 0.13^{e}	0.23 ± 0.01^{a}	3.34 ± 0.07^{d}	2.95 ± 0.00^{c}
L-tryptamine	1.29 ± 0.08^{a}	3.54 ± 0.18^{b}	17.84 ± 1.36^{c}	1.52 ± 0.03^{a}	1.04 ± 0.15^{a}	n.d.
5-Hydroxy-L-tryptophan	128.89 ± 10.67^{bc}	193.95 ± 17.69^{d}	149.73 ± 2.87^{c}	95.21 ± 11.99^{ab}	67.45 ± 7.94^{a}	117.02 ± 18.23^{bc}
Other organic compounds						
Cinnamic acid	n.d.	0.16 ± 0.01^{a}	0.48 ± 0.01^{c}	0.25 ± 0.02^{b}	n.d.	n.d.
Lovastatin	7.66 ± 0.19^{d}	n.d.	0.39 ± 0.01^{a}	1.18 ± 0.02^{b}	1.14 ± 0.11^{b}	1.67 ± 0.01^{c}
Ergosterol	31.04 ± 0.42^{b}	64.56 ± 0.29^{f}	60.07 ± 0.15^{e}	23.47 ± 0.14^{a}	33.98 ± 0.14^{c}	41.37 ± 0.21^{d}
Thiamine	0.58 ± 0.03^{b}	0.57 ± 0.05^{b}	0.15 ± 0.01^{a}	1.08 ± 0.03^{e}	0.64 ± 0.02^{b}	0.79 ± 0.03^{c}
Riboflavin	2.31 ± 0.07^{cd}	2.43 ± 0.08^{d}	1.55 ± 0.13^{a}	2.12 ± 0.03^{c}	1.53 ± 0.02^{a}	1.80 ± 0.09^{b}
Ergothioneine	129.89 ± 9.25^{c}	71.29 ± 4.48^{b}	58.06 ± 6.08^{ab}	60.43 ± 2.87^{ab}	70.52 ± 0.04^{b}	49.01 ± 2.12^{a}
Phenylalanine	294.58 ± 8.41^{c}	247.03 ± 18.15^{b}	168.41 ± 19.53^{a}	169.26 ± 13.74^{a}	143.41 ± 2.17^{a}	154.16 ± 22.11^{a}
Antioxidant activity						
% DPPH**	91.55 ± 0.85^{e}	87.15 ± 0.93^{d}	91.84 ± 0.66^{e}	83.82 ± 1.39^{c}	61.98 ± 1.21^{a}	78.79 ± 1.46^{b}
Mycelia from in vitro cultures on Oddoux medium						
Phenolic compounds						
Protocatechuic acid	8.01 ± 1.12^{d}	n.d.	1.98 ± 0.24^{a}	n.d.	n.d.	7.18 ± 0.28^{b}
p-Hydroxybenzoic acid	0.34 ± 0.01^{ab}	1.81 ± 0.15^{d}	0.51 ± 0.01^{b}	0.51 ± 0.01^{b}	0.21 ± 0.01^{a}	1.33 ± 0.02^{c}
Vanillic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Indole compounds						
L-tryptophan	7.82 ± 0.31^{b}	24.34 ± 1.44^{d}	7.60 ± 2.34^{b}	n.d.	1.89 ± 0.04^{a}	17.29 ± 1.60^{c}
5-CH_3-tryptophan	n.d.	n.d.	14.49 ± 0.01^{b}	n.d.	4.81 ± 0.01^{a}	n.d.
Melatonin	*	n.d.	0.08 ± 0.01^{a}	0.09 ± 0.01^{a}	4.45 ± 0.24^{b}	*
Serotonin	n.d.	n.d.	8.54 ± 0.08^{a}	n.d.	n.d.	n.d.
6-CH_3-tryptophan	1.19 ± 0.16^{c}	3.44 ± 0.12^{d}	0.57 ± 0.01^{a}	1.35 ± 0.03^{c}	0.83 ± 0.02^{b}	n.d.
L-tryptamine	3.71 ± 0.34^{c}	n.d.	2.67 ± 0.02^{b}	n.d.	1.03 ± 0.15^{a}	n.d.
5-Hydroxy-L-tryptophan	368.67 ± 23.53^{c}	703.56 ± 37.79^{e}	221.51 ± 5.86^{b}	215.53 ± 24.82^{b}	120.11 ± 20.12^{a}	553.87 ± 2.62^{d}
Other organic compounds						
Cinnamic acid	n.d.	n.d.	n.d.	0.56 ± 0.01^{a}	0.71 ± 0.05^{b}	n.d.
Lovastatin	28.84 ± 0.62^{c}	20.07 ± 0.14^{b}	124.18 ± 5.21^{d}	17.27 ± 0.02^{b}	2.61 ± 0.07^{a}	1.68 ± 0.02^{a}
Ergosterol	8.43 ± 0.07^{a}	37.96 ± 0.42^{f}	34.12 ± 0.01^{e}	13.32 ± 0.41^{c}	12.12 ± 0.01^{b}	18.44 ± 0.05^{d}

(*continued on next page*)

Table 1 (*continued*)

Bioelements							
	Substrate	*P. citrinopileatus*	*P. djamor*	*P. eryngii*	*P. florida*	*P. ostreatus*	*P. pulmonarius*
Thiamine	2.01 ± 0.12^{e}	1.12 ± 0.05^{d}	0.17 ± 0.02^{a}	0.34 ± 0.01^{b}	0.72 ± 0.01^{c}	0.43 ± 0.01^{b}	
Riboflavin	3.26 ± 0.09^{c}	1.73 ± 0.04^{a}	2.67 ± 0.02^{b}	1.53 ± 0.12^{a}	2.54 ± 0.13^{b}	2.53 ± 0.01^{b}	
Ergothioneine	20.05 ± 1.77^{a}	128.35 ± 7.15^{e}	47.65 ± 2.92^{b}	66.69 ± 4.64^{c}	46.71 ± 5.69^{b}	96.32 ± 3.81^{d}	
Phenylalanine	331.65 ± 21.07^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	
Antioxidant activity							
% DPPH**	72.44 ± 1.88^{c}	85.95 ± 2.01^{d}	53.19 ± 0.22^{a}	69.01 ± 1.38^{c}	60.23 ± 1.12^{b}	49.68 ± 0.58^{a}	

N = 6; values followed by a different letter within the same row are significantly different ($p < 0.05$). n.d.—not detected; *—under detection limit; ** represents % of DPPH reduction.

2.6.1. Indole compounds

For analyzing the content of indole compounds, the extracts were subjected to separation by reversed-phase high performance liquid chromatography (RP-HPLC), using a Hitachi HPLC system (Merck, Japan) equipped with a type L-7100 pump. The Purospher® RP-18 (4 mm × 200 mm, 5 μm) column was kept at 25 °C, and the UV detector was operated at λ = 280 nm. A mixture of methanol, water, and ammonium acetate (15:14:1, v/v/v) was used as the liquid phase at a flow rate of 1 mL/min. The quantitative analyses of indole compounds were performed using a calibration curve with the assumption of the linear size of the area under the peak and the concentration of the reference standard.

2.6.2. Phenolic compounds

The resultant extracts were analyzed for their contents of phenolic acids using RP-HPLC according to the procedure described by Muszyńska et al. (2016), with modifications. The HPLC analyses were conducted using an HPLC VWR Hitachi-Merck apparatus, equipped with L-2200 autosampler, L-2130 pump, RP-18e LiChrospher (4 mm × 250 mm, 5 μm) column thermostated at 25 °C, L-2350 column oven, and L-2455 diode array detector operating at the UV range of 200–400 nm. The mobile phase consisted of solvent A, containing methanol and 0.5% acetic acid (1:4, v/v), and solvent B, containing methanol. The gradient was as follows: 100:0 for 0–25 min; 70:30 for 35 min; 50:50 for 45 min; 0:100 for 50–55 min; and 100:0 for 57–67 min. The comparison of the UV spectra and retention times with those of the standard enabled the identification of phenolic compounds present in the tested samples. The quantitative analysis of free phenolic acids was performed using a calibration curve with the assumption of the linear size of the area under the peak and the concentration of the reference standard. This method was also used to determine cinnamic acid and phenylalanine.

2.6.3. Lovastatin

To determine the content of lovastatin the RP-HPLC method was used according to the method described by Pansuriya and Singhal (2009). The process was carried out in an isocratic system with a mobile phase of constant composition. The apparatus was equipped with a UV detector (λ = 238 nm), a column (Purospher® RP-18, 14 mm × 200 mm, 5 μm), and a lamp (L-7100). All the measurements were carried out using a previously prepared developing system (acetonitrile and 0.1% phosphoric acid at a ratio of 60:40 (v/v)).

2.6.4. Ergothioneine

The content of ergothioneine was analyzed using RP-HPLC according to the method described by Zhou et al. (2014). A Hitachi HPLC system equipped with a type L-7100 pump was used for the analysis. The Purospher® RP-18 (4 mm × 200 mm, 5 μm) column was heated at 25 °C, and the UV detector was operated at λ = 257 nm. A mixture of 1% methanol containing boric acid adjusted to a pH of 5.0 was used as the liquid phase at a flow rate of 0.5 mL/min. The quantitative analysis of ergothioneine was performed using a calibration curve with the assumption of the linear size of the area under the peak and the concentration of the reference standard.

2.7. Extraction and RP-HPLC analysis of sterols

Five grams of mushroom materials under study were extracted with a mixture of methanol and dichloromethane (75:25, v/v). The sample was sonicated at 40 kHz for 10 min. After 2 h, the extract was centrifuged at 12,000 rpm for 5 min and decanted. The extraction procedure was repeated twice, and the obtained extracts were mixed and evaporated under reduced pressure.

The filtered sample (Millipore PTFE membrane, 0.45 μm) was injected (20 μL) in the HPLC column. The chromatographic analyses were conducted using an HPLC Hitachi liquid chromatograph described above.

HPLC method was carried out according to the procedure developed by Yuan et al. (2008) with own modifications (Sułkowska-Ziaja, Szewczyk, Galanty, Gdula-Argasińska, & Muszyńska, 2018). The mobile phase used for the analysis consisted of solvent A, containing methanol and water (80:20, v/v), and solvent B, containing methanol and dichloromethane (75:25, v/v). The gradient program was as follows: 80:20 for 0–10 min; 40:60 for 10–35 min; 0:100 for 35–50 min; 80:20 for 50–55 min; and a hold time of 15 min at 30 °C. The flow rate was 1.0 mL/min. The chromatographic peaks were recorded at a wavelength of 280 nm.

2.8. Determination of vitamin B_1 (thiamine) and B_2 (riboflavin) content in *Pleurotus* materials using the HPLC method

The content of vitamins in the mycelia obtained from the *in vitro* cultures and fruiting bodies of *Pleurotus* spp. selected for the study was determined by the HPLC method. Vitamins such as thiamine and riboflavin were analyzed in the lyophilized material. Samples for the HPLC analyses were prepared according to the methodology included in the PN-EN 14122:2004/AC:2006 and PN-EN 14152:2004/AC:2006 standards. The abovementioned vitamins were determined after the oxidation reaction before the column. For this purpose, 0.04% solution of potassium hexacyanoferrate(III) in a 15% solution of sodium hydroxide was added to the sample and shaken, after which the mixture was set aside for 2 min. After pH adjustment to a value of 7 with an orthophosphoric acid solution, the sample was centrifuged. The extracts were cleaned on solid phase extraction (SPE), centrifuged again, and analyzed using HPLC. An HPLC chromatograph (Merck Hitachi) equipped with L-7612 on-line degasser, L-7250 Programmable Autosampler, L-7100 pump, L-7480 fluorescent detector, L-7360 thermostat Oven Columns (Merck), and D-7000 Interface, with D-7000 HPLC System Manager (HSM) software, was used for the detection of vitamins. The analysis was performed on a Bionacom Velocity C18 PLMX column (4.6 mm × 250 mm, 5 μm) obtained from Bionacom LTD (UK) together with the precolumn from the same company. The measurement was made at the excitation and emission wavelengths of 360 and 503 nm, enabling the simultaneous determination of thiamine and

riboflavin. The mobile phase used was a mixture of water and acetonitrile. Gradient elution was performed as follows: t = 0, water/acetonitrile ratio of 88:12; t = 12, water/acetonitrile ratio of 0:100 at 22 °C. The external standards of thiamine and riboflavin were used for the identification of these compounds and their quantitative analysis, respectively, in hydrochloric and acetic acid.

2.9. DPPH analysis

The antioxidant activity of the analyzed samples was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical (Sigma Aldrich, St. Louis, MO, USA). On the analytical scale, 0.01 g of homogenized mycelia from the *in vitro* cultures and the fruiting bodies of the oyster mushroom species tested were weighed in four repetitions. Then, 5 mL of 0.1 mM DPPH solution in 80% methanol was added to each mushroom material. The mixture was stirred for 1 h in a centrifuge (ELMI shaker DOS-20L) at 120 rpm in the dark at room temperature. The resulting solution was then filtered through laboratory filters, and its absorbance (λ = 517 nm) was measured with a Helios Beta UV–VIS spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The DPPH radical reduction was calculated using the formula AA (%) = ((A0–A1)/A0) × 100, where AA is the antioxidant activity expressed in %, A0 is the blank/zero sample absorbance, and A1 is the absorbance of the examined concentration.

3. Statistical analysis

The data were analyzed using standard methods of statistical analysis, including one-way Student's test for multiple comparisons, and the results are expressed as mean ± standard deviation. Each sample was analyzed in independent repetitions. To facilitate the analysis and interpretation of data obtained in the experiments, chemometric tools, such as cluster analysis (CA) and principal component analysis (PCA), were used. The calculations were performed using Statgraphics Centurion XVIII. Statistical significance was established at a level of $p < 0.05$.

4. Results and discussion

The yield of the *Pleurotus* spp. biomass growth on the Oddoux medium averaged about 9–11 g d.w./L (the highest for *P. djamor*). The dynamics of mycelium growth on the same medium did not differ from that reported in the earlier studies (Kała et al., 2016; Muszyńska et al., 2020).

4.1. Bioelements analysis

In the first stage of the study, the content of macro- (Ca, K, and Mg) and microelements (Cu, Cr, Fe, Mn, Ni, Rb, Se, Sr, and Zn) present in the mycelia obtained from the *in vitro* cultures and fruiting bodies of *Pleurotus* spp. obtained through cultivation was analyzed, and the results are presented in Table 1.

The concentration of metals was evaluated using TXRF and F-AAS analysis. The optimized procedures of preparation of the study material in combination with the methods of analysis applied, which are characterized by high precision and accuracy, allowed for an effective analysis of the bioelements present in the examined mushroom species. The results of the analysis are presented in Fig. 1 a) and b).

From the results of the analysis, it was found that among the macroelements present in the fruiting bodies obtained by cultivation, the highest concentration was determined for K in *P. ostreatus* (2516 mg/100 g d.w.), while the lowest concentration was found for Ca in *P. florida* (388 mg/100 g d.w.). In turn, the analysis of macroelements in the mycelia obtained from the *in vitro* cultures showed their content to be significantly lower than that in the fruiting bodies obtained through cultivation. Among the species studied, the highest concentration was determined for Mg in the mycelia of *P. citrinopileatus* (135.4 mg/100 g d.w.), whereas the lowest concentration was found for Ca in the mycelia of *P. pulmonarius* (290 mg/100 g d.w.). A different trend was found when the content of microelements was analyzed, as higher concentrations were found in the tested mycelia obtained from *in vitro* cultures than the corresponding fruiting bodies of the cultivated species. With respect to the mycelia from *in vitro* cultures, the highest concentrations were determined for Fe in *P. ostreatus* (34.82 mg/100 g d.w.) and the lowest concentration was found for Cr in *P. djamor*, *P. florida* and *P. ostreatus* (0.01 mg/100 g d.w.). In the case of fruiting bodies, the highest concentration was determined for Fe (9.41 mg/100 g d.w.) in *P. djamor*. It should be noted that the conducted analyses showed that both the fruiting bodies obtained through cultivation of *Pleurotus* spp. and the corresponding mycelia from *in vitro* cultures contained different amounts of macro- and microelements than the other mushroom species, for example, *Cantharellus cibarius* and *Boletus badius*, in which the highest concentration was determined for Mg (108.5 mg/100 g d.w.) in the case of macroelements and for Fe (148.6 mg/100 g d.w.) in the case of microelements (Reczyński et al., 2013).

4.2. Organic compounds analysis

In the second stage of the study, a detailed analysis of methanol extracts and dichloromethane–methanol extract of mycelia obtained from the *in vitro* cultures of *Pleurotus* spp. and the fruiting bodies obtained by cultivation was carried out to determine the content of selected organic compounds with medicinal properties. The content of phenolic compounds, indole compounds, sterols, lovastatin, ergothioneine, and B-group vitamins was examined, and the corresponding results for fruiting bodies and mycelia from the *in vitro* cultures of selected *Pleurotus* spp. are presented in Table 1, respectively.

The analysis of the content of other organic compounds showed that mycelia from the *in vitro* cultures of *Pleurotus* spp. contained only a few of these compounds, mainly *p*-hydroxybenzoic acid. On the other hand, the fruiting bodies of *Pleurotus* mushrooms turned out to be richer in phenylalanine which was found to be present in all the fruiting bodies (at a concentration ranging from 143.41 to 294.58 mg/100 g d.w.), in contrast to the mycelia from *in vitro* cultures, in which this compound was found only in *P. citrinopileatus*, but at the highest amount (331 mg/100 g d.w.). This information indicated that a significant content of phenolic compounds and phenylalanine detected in a species may explain that it has strong antioxidant properties. This is due to the fact that phenolic compounds exhibit redox properties and thus act as antioxidants. With respect to indole compounds, *P. eryngii* species deserves a special mention. Both the fruiting bodies and the mycelia from the *in vitro* cultures were characterized by high contents of L-tryptophan and 5-hydroxy-L-tryptophan. It should be emphasized that in comparison, the mycelia obtained from the *in vitro* cultures were characterized by a significantly higher content of the abovementioned compounds: 5-CH_3-tryptophan, 14.49 mg/100 g d.w.; melatonin 4.45 mg/100 g d.w. and 5-hydroxy-L-tryptophan, 703.56 mg/100 g d.w.

Ergosterol was determined in both the fruiting bodies and biomass from *in vitro* cultures. Its content in the examined fruiting bodies was in the range from 23.47 (*P. florida*) to 64.56 mg/100 g d.w. (*P. djamor*). On the other hand, in the case of the biomass from the *in vitro* cultures, the highest content of 37.96 mg/100 g d.w. was found in *P. djamor*.

Ergosterol, a component of the cell walls of mushrooms, is converted into vitamin D_2 in the presence of sunlight or other sources of ultraviolet rays, which plays an extremely important role in the prevention of cancer as it increases phagocytosis (absorption and destruction of cancer cells) and facilitates other immunomodulatory functions. It is also responsible for the absorption of calcium and phosphorus, which aids in proper bone growth and prevents osteoporosis (Sułkowska-Ziaja, Hałaszuk, Mastej, Piechaczek, & Muszyńska, 2016).

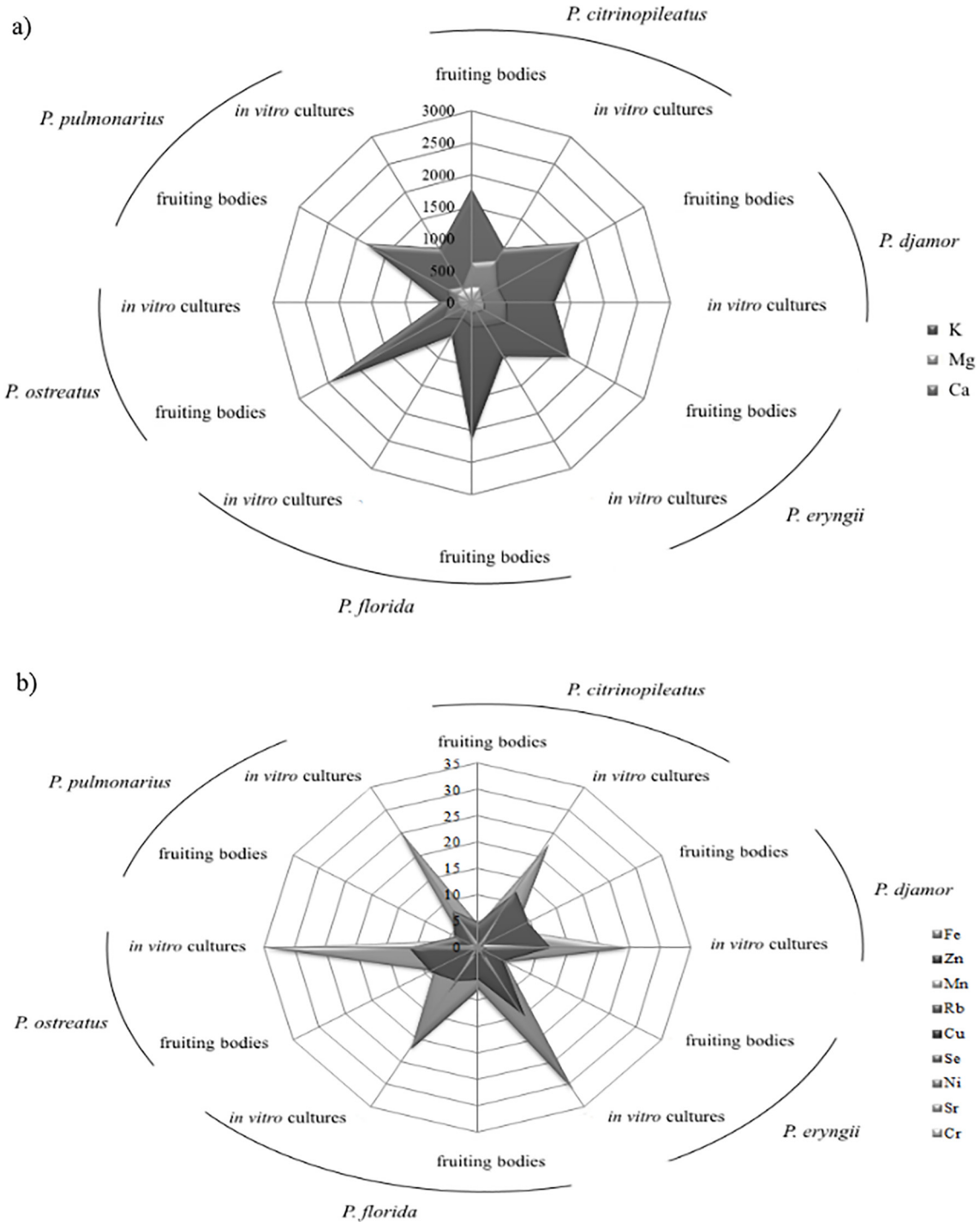

Fig. 1. Concentration of a) macroelements and b) micronutrients (mg/100 g d.w.) in mycelia from *in vitro* cultures and fruiting bodies of the selected *Pleurotus* spp. obtained from them by cultivation.

Examination of other organic compounds demonstrated that not all of them are present in the largest quantities in the fruiting bodies of the studied *Pleurotus* spp. Some of these compounds, for example, lovastatin or ergothioneine, were also found at high concentrations in the mycelia of *P. eryngii* (lovastatin content, 124.18 mg/100 g d.w.) and *P. djamor* (ergothioneine content, 128.35 mg/100 g d.w.). Among the fruiting bodies, the highest content of lovastatin and ergothioneine was found in *P. citrinopileatus* (7.66 and 129.89 mg/100 g d.w., respectively) (Table 1). It should be noted that the lovastatin content determined in the mycelium of *P. eryngii* was many times higher than that described by Chen et al. (4.45 mg/100 g d.w.), whereas the ergothioneine content was almost four times lower than that described by the same group (151.46 mg/100 g d.w.) (Chen, Ho, Hsieh, Wang, & Mau, 2012). By contrast, in the fruiting bodies the contents of lovastatin and ergothioneine were higher and ranged from 11.99 (*P. eryngii*) to 60.7 mg/100 g d.w. (*P. ostreatus*), and from 84.04 (*P. eryngii*) to 285.07 mg/100 g d.w. (*P. citrinopileatus*), respectively, which may highlight the differences in the content of biologically active substances in the fruiting bodies obtained from different regions of the world using different substrates (Chen et al., 2012). The content of vitamins in edible mushrooms is of great value, since they perform important functions in humans and animals. Mushrooms could be a good source of vitamins

such as thiamine, riboflavin, biotin, niacin, and vitamin C (Furlani & Godoy, 2008). When analyzing the content of B-group vitamins, it turned out that their best source was *P. citrinopileatus* as it contained 2.01 mg/100 g d.w. of thiamine and 3.26 mg/100 g d.w. of riboflavin. This content of thiamine was higher in comparison with 100 g of fresh fruiting bodies of *A. bisporus* (1.24 mg), while that of riboflavin was lower (5.06 mg) (Bernaś & Jaworska, 2016).

4.3. Chemometric analysis

The extensive amount of data analyzed (six species of mushrooms, two methods of culturing—*in vitro* culture and cultivation, content of metals and organic compounds) made the unambiguous, detailed interpretation of the obtained results complicated. Therefore, the study was supplemented by chemometric analysis.

Two methods were used for the chemometric analysis of data: Cluster analysis (CA) and Principal Component Analysis (PCA). In the analysis, the objects studied were different species of *Pleurotus* (*P. citrinopileatus*, *P. djamor*, *P. ostreatus*, *P. pulmonarius*, *P. florida*, *P. eryngii*), which were used in the form of fruiting bodies obtained by cultivation and as mycelia from *in vitro* cultures. The measured parameters—concentration of macro- (Ca, K, and Mg) and microelements (Cu, Cr, Fe, Mn, Ni, Rb, Se, Sr, and Zn) and numerous organic compounds—were assigned to the analyzed objects. The extensive data set as well as the wide variability of the results fully justified the use of chemometric analysis in the present study. This analysis allowed obtaining additional information concerning mutual correlations in the studied data set, for example, the relationships between the objects (mushroom species) and the contents of bioelements determined in them, as well as the influence of the form of mushrooms (mycelia obtained from *in vitro* cultures and their fruiting bodies by cultivation) on the degree of metal accumulation in them.

In the first stage, the CA method was used to determine the similarity between the objects under the study (different *Pleurotus* spp.). In terms of the applied method, the similarity of the objects was determined by their relatively close location in the analyzed multidimensional space. The result of the analysis is presented in the form of a dendrogram (Fig. 2)—commonly called the tree of similarities. The objects on a dendrogram that are characterized by mutual similarity form clusters. The x- and y-axis shown in the dendrogram do not correspond to the numerical axes in the Cartesian system (Mazerski, 2000; Miller & Miller, 1999). In the dendrogram presented in Fig. 2, the x-axis represents the analyzed objects (mushroom species), while the y-axis shows the distances between the objects. The distance was calculated using the Ward agglomeration method. On the basis of the CA method applied (Fig. 2), two main clusters were distinguished.

Table 2
Values of loads assigned to the three main components (PC1, PC2, and PC3).

	PC1	PC2	PC3
K	0.4132	–0.1469	0.0987
Mg	0.0516	0.1266	–0.7539
Ca	0.1735	0.1694	–0.3087
Zn	–0.3226	–0.0773	0.0949
Fe	–0.4374	0.0329	–0.0087
Rb	0.3007	–0.2690	0.2036
Cu	0.2842	–0.1820	0.0600
Se	–0.2713	0.0645	0.4279
Mn	–0.4143	0.0636	–0.0827
Ni	0.2176	0.4966	0.1960
Cr	0.1902	0.5185	0.2088
Sr	–0.0291	0.5452	0.0140

The first cluster (continuous line) is formed by the fruiting bodies of *Pleurotus* spp. obtained by cultivation. The second cluster (dashed line) includes the mycelia obtained from the *in vitro* cultures. The fact that certain objects belong to particular clusters proves their considerable similarity within the group in question. Due to the fact that CA was performed on the basis of the measured values of metal concentration in mushrooms, similarity within the groups can be identified with the amount of macro- and microelements found in the fruiting bodies and mycelia from *in vitro* cultures. The different ability of the studied species to accumulate metals may result from the form in which these were tested (fruiting bodies and mycelia from *in vitro* cultures), as they have differences in the development of the absorption surface. In turn, the amount and type of accumulated metal are influenced by its ionic beam. The short arms of the dendrogram within the cluster corresponding to the mycelium obtained from *in vitro* culture show the highest correlation—similarity—between these samples (Miller & Miller, 1999; Mazerski, 2000). In the case of *in vitro* culture, the similarity between mycelia is related to the possibility of culturing them under strictly controlled conditions, which is directly reflected by the acquisition of repeatable material with very similar properties.

The CA method described above was complemented by PCA in the present study. This method made it possible to reduce the large set of

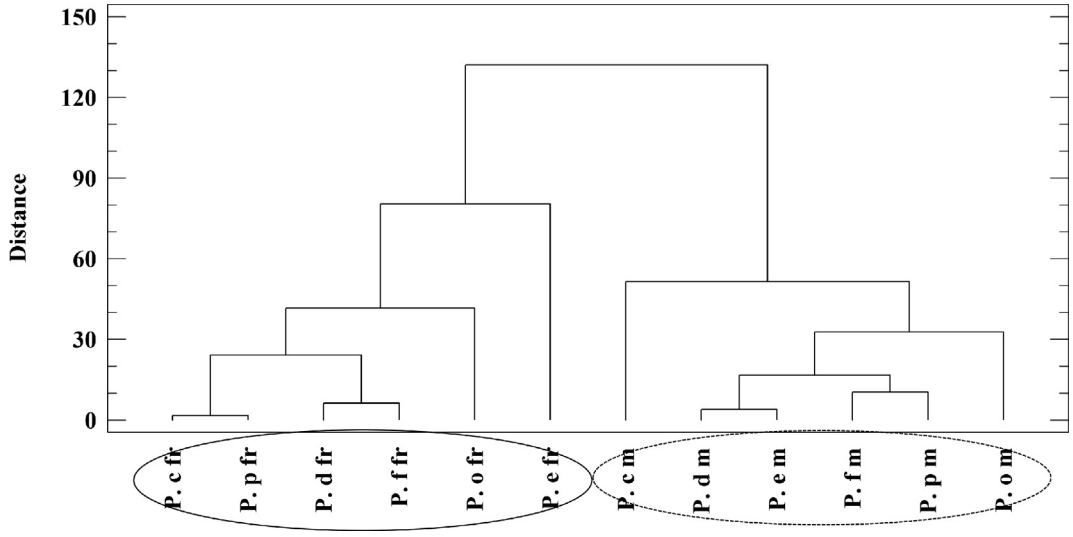

Fig. 2. Dendrogram presenting the similarity between the objects (*Pleurotus* spp. material) in relation to the way they were cultivated (**fr**—cultivated fruiting bodies, **m**—mycelia from *in vitro* culture, **P. c**—*P. citrinopileatus*, **P. d**—*P. djamor*, **P. o**—*P. ostreatus*, **P. p**—*P. pulmonarius*, **P. f**—*P. florida*, and **P. e**—*P. eryngii*) (Euclidean, Ward's algorithm).

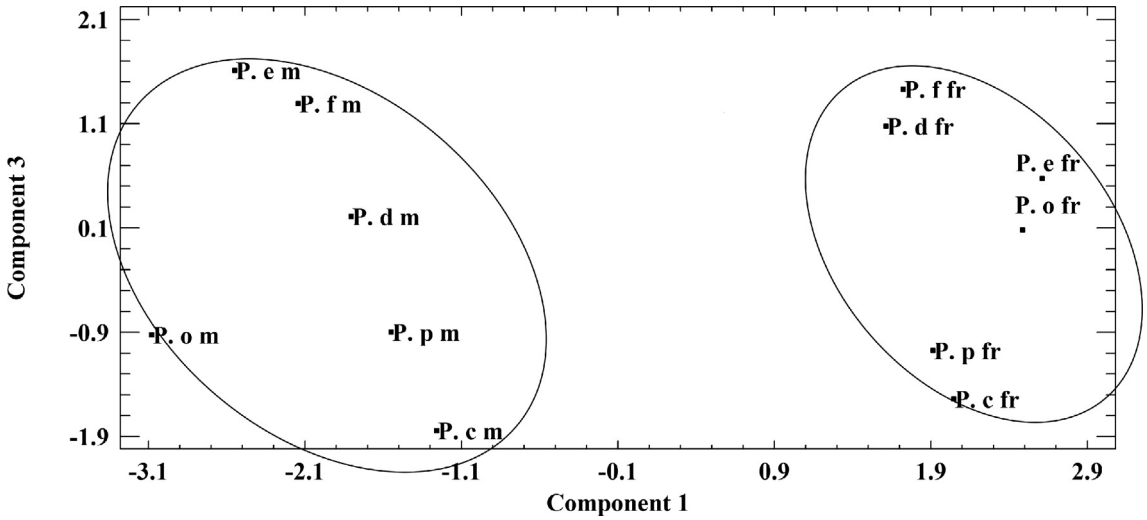

Fig. 3. Scatterplot of similarity between the analyzed mushroom species (**fr**—cultivated fruiting bodies, **m**—mycelia from *in vitro* cultures, **P. c**—*P. citrinopileatus*, **P. d**—*P. djamor*, **P. o**—*P. ostreatus*, **P. p**—*P. pulmonarius*, **P. f**—*P. florida*, and **P. e**—*P. eryngii*).

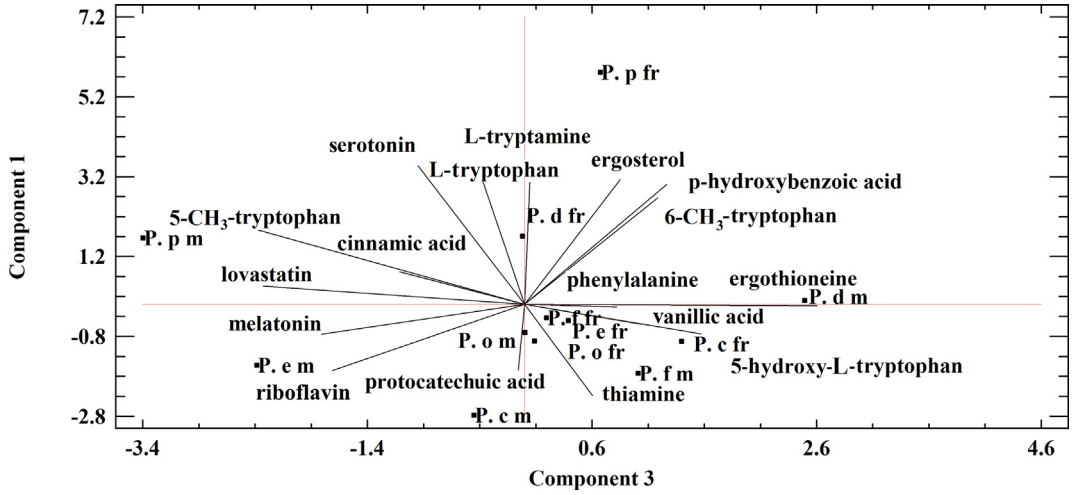

Fig. 4. Biplot presenting the correlation between the tested organic compounds found in *Pleurotus* mushrooms and their form—fruiting bodies and mycelia from *in vitro* culture (**fr**—cultivated fruiting bodies, **m**—mycelia from *in vitro* cultures, **P. c**—*P. citrinopileatus*, **P. d**—*P. djamor*, **P. o**—*P. ostreatus*, **P. p**—*P. pulmonarius*, **P. f**—*P. florida*, and **P. e**—*P. eryngii*).

measurement data under consideration to a minimum that was necessary to describe the correlations occurring between the objects. The considered extensive set of data (macro- and microelements) describing the analyzed objects (*Pleurotus* mushrooms) limited their unambiguous interpretation and visualization in a multidimensional space. Therefore, the number of data was minimized by correlating them. New factors called main components were created. From PCA, it was found that 76.4% of the variability in the set of results presented can be described by means of three main components. Therefore, only three components—PC1, PC2, and PC3—were considered for further analysis. These components were linear combinations of input variables multiplied by the corresponding charge values (Table 2).

The size of the assigned load is equal to the correlation coefficient of the component with the input variable. On this basis, it was concluded that the measured K value had the most significant influence on the PC1 component. The analysis of the other main components was carried out analogously. Reducing the area of the input data to three main components allowed presenting the results of the analysis on a two-dimensional space (Fig. 3).

By analyzing the set of objects (mushrooms) in relation to the form of their occurrence (Fig. 3), two distinct clusters were distinguished. The degree of metal accumulation in mushrooms differs in the fruiting bodies and mycelia from *in vitro* culture, which confirms that they belong to separate groups, as presented in Fig. 3. In turn, a similarity was observed in metal absorption in the area of a cluster under consideration, indicating that they have similar accumulation mechanisms, but these mechanisms occur differently in the mycelia from *in vitro* cultures and in the fruiting bodies.

In the next part of the study, the relationships between the forms of the examined object (fruiting bodies and mycelia from *in vitro* cultures) and the examined organic compounds were determined. For this analysis, a set of indole compounds, phenolic compounds, and other biologically active substances were considered (Fig. 4).

The analysis of organic compounds present in *Pleurotus* mushrooms (Fig. 4) showed that the mushrooms obtained in the form of cultivated fruiting bodies were characterized by a high content of phenylalanine, vanillic acid, cinnamic acid, and indole compounds (L-tryptophan, 5-CH_3-tryptophan, and 5-hydroxy-L-tryptophan). On the other hand, mycelia from *in vitro* cultures were characterized by a high content of other organic compounds (e.g., valuable lovastatin), which confirms the direction of arms in the biplot diagram (Fig. 4). Thus, the study confirms that the content of metals, as well as individual organic

compounds, is strictly dependent on the form of mushrooms—cultivated fruiting bodies or mycelia obtained *in vitro*.

5. Conclusion

The analysis of bioelements and organic compounds in six species of the genus *Pleurotus* selected for the study showed for the first time that both the biomass from *in vitro* cultures and the fruiting bodies derived from these *in vitro* cultures by cultivation were characterized by a high content of compounds (such as phenolic compounds, cinnamic acid, phenylalanine, indole derivatives, sterols, lovastatin, ergothioneine, B-group vitamins, and bioelements) with prohealth effects. *In vitro* cultures of *P. eryngii* species demonstrated the best antiatherosclerotic properties (lovastatin content was 124.18 mg/100 g d.w.). In the case of fruiting bodies, the highest content of lovastatin as well as ergothioneine, which is a strong antioxidant, was determined in the species of *P. citrinopileatus*. Based on the content of indole compounds, especially 5-hydroxy-L-tryptophan, it can be proved that mycelia obtained from *in vitro* cultures can have a greater impact on improving the cognitive functions of the brain than fruiting bodies of the same species. In the case of B-group vitamins, also mycelial cultures were the most beneficial source, especially *P. citrinopileatus* species. The experiments carried out in the study also demonstrated that the content of metals and organic compounds depends on the conditions under which the mushroom materials were obtained and their form—fruiting bodies or mycelia from *in vitro* cultures. It has been proved that the fruiting bodies of medicinal mushrooms of the genus *Pleurotus* can be successfully used as functional foods. In addition, their mycelial *in vitro* cultures can also be a source of supplementation of bioelements and organic compounds for the human body.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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mgr inż. Piotr Zięba

Piotr Zięba

31-120 Kraków, Al. Mickiewicza 21
tel. +48 (12) 662 53 03, e-mail: ko@urk.edu.pl

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Dr hab. Agnieszka Sękara prof. URK

31-120 Kraków, Al. Mickiewicza 21
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- Współudziałem w opracowaniu metodologii doświadczenia,
- Nadzorem nad wykonywaniem analiz laboratoryjnych,
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- Współudziałem w przygotowaniu wstępnej i ostatecznej wersji manuskryptu,
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Selenium and Zinc Biofortification of *Pleurotus eryngii* Mycelium and Fruiting Bodies as a Tool for Controlling Their Biological Activity

Piotr Zięba [1,*], Katarzyna Kała [2], Anna Włodarczyk [2], Agnieszka Szewczyk [2], Edward Kunicki [1], Agnieszka Sękara [1,*] and Bożena Muszyńska [2]

[1] Department of Horticulture, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, 31-425 Krakow, Poland; e.kunicki@urk.edu.pl

[2] Department of Pharmaceutical Botany, Faculty of Pharmacy, Jagiellonian University Medical College, 30-688 Krakow, Poland; kat3kala@gmail.com (K.K.); annawlodarczyk1966@gmail.com (A.W.); agnieszka.szewczyk@uj.edu.pl (A.S.); muchon@poczta.fm (B.M.)

* Correspondence: p.zieba90@gmail.com (P.Z.); agnieszka.sekara@urk.edu.pl (A.S.)

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Abstract: *Pleurotus eryngii* (DC:Fr.) Quel. is a cultivated mushroom of high culinary value and medicinal properties. Mycelium of *P. eryngii* is characterized by the ability of effective bio-elements absorption from growth media so it could be biofortified with trace elements with a functional activity in the human body. In this study, the ability of *P. eryngii* mycelia from in vitro cultures as well as fruiting bodies were investigated in terms of their effectiveness in zinc and selenium accumulation. The effect of Se and Zn biofortification on productivity, chemical compounds, and bio-elements content of *P. eryngii* was determined as well. To enhance Se and Zn content in *P. eryngii* fruiting bodies and mycelia, substrates were supplemented with sodium selenite, at a concentration of 50 mg L^{-1}, zinc sulfate, and zinc hydro-aspartate at a concentration of 87.2 and 100.0 mg L^{-1}, respectively. Mentioned Zn concentrations contained the same amount of zinc(II) ions, namely 20 mg L^{-1}. The content of organic compounds include phenolic compounds and lovastatin, which were determined by a high-performance liquid chromatography with diode-array detector (HPLC-DAD) and reverse phase high-performance liquid chromatography (RP-HPLC) method with UV detection. The ability of *P. eryngii* to accumulate zinc and selenium from the culture medium was demonstrated. The degree of accumulation of zinc turned out to be different depending on the type of salt used. The present study also showed that conducting mycelium of *P. eryngii* in in vitro culture, with a higher content of zinc ions, can result in obtaining the materials with better antioxidant ability. The results of this study can be used to develop the composition of growing media, which ensures the production of biomass with the desired composition of elements.

Keywords: biofortification; lovastatin; phenoloic compounds; *Pleurotus eryngii*; selenium; zinc

1. Introduction

Polypore mushrooms from Basidomycota and *Pleurotus* genus are species with wide medicinal and cosmetological activity because of their immunostimulatory, anti-cancer, and anti-aging properties. King oyster mushroom, *Pleurotus eryngii* (DC.) Quél, is one of the best known species from this genus because of culinary value and pharmacological activity [1–4]. It is closely related to the oyster mushroom (*Pleurotus ostreatus* (Jacq. ex Fr.) P.Kumm), but it definitely differs concerning the fruiting body appearance. The sporocarp reaches a size of up to 15 cm. It is composed of cream-colored to a light brown hat with a lamellar type hymenophore going down and the thick, soft stalk, which is edible in contrast to other *Pleurotus* spp. [2]. *P. eryngii* is native to the regions ranging from the Atlantic coast

of Europe through the Mediterranean area to Kazakhstan and India. In Poland, it is a rare species with a protected status. The distinguishing feature of *P. eryngii* is the parasitic behaviour. In nature, it occurs as an optional parasite of *Eryngium campestre* L., as well as on other Apiaceae, such as *Elaeoselinum asclepium* (L.) Bertol and *Ferula communis* L. [5,6].

Pleurotus eryngii is cultivated in organic substrates similar to those used for oyster mushroom, supplemented high-protein waste, e.g., rice bran. It is characterized by great potential of absorption nutrients from lignocellulose residues through an effective ligninolytic enzyme system (laccase, Mn-oxidizing peroxidases, and aryl-alcohol oxidase). Due to this ability, *P. eryngii* is used in many biotechnological processes including food production, biotransformation of raw plant materials to feed, bio-pulping, and bio-bleaching of paper pulp, or bioremediation of soil and industrial waters [7]. It reacts with extreme sensitivity to changes in growth conditions so some research were performed to modify the substrate composition to increase the level of chemical compounds. Generally, substrate additives, especially supplements rich in nitrogen and carbohydrates, are widely recognized and accepted in agricultural practice because of a substantial impact on mushroom yield and quality [8,9]. The mycelium produced in the liquid culture media have become a viable alternative for receiving a raw material for biomedical use, food, and enzyme production. The liquid culture technology was also adapted to the production of mycelia used for substrate spawning in cultivating fruit bodies [9]. The use of in vitro cultures leads to an increase in biomass, a reduction of production duration, and a stable production of raw material with a predictable amount functional metabolites [10]. The liquid medium composition, pH, temperature, time of cultivation, etc., have an important influence on biomass of the mycelium and its physicochemical and nutritional properties, but generally mycelia maintain the functional components of the parent mushroom, such as phenolic acids and ergosterol. The anti-inflammatory properties could be used in nutraceutical or pharmaceutical formulations [11,12]. Such formulations have been developed based on the in vitro mycelium cultures of the most important species like *P. ostreatus*, *P. diamor*, and *Agaricus bisporus* [10–13]. A relatively new approach is supplementation of growing medium with bio-elements employed for the cultivated mushrooms not only to improve their agronomic potential in terms of yield and quality but also to functional food and diet supplements production [14–17].

The *P. eryngii* fruiting body of this species has a nice flavor and rich taste, similar to *Boletus edulis* Bull. Sporocarp contains almost 20% of protein in dry matter and up to 4% fat, which makes it more nutritious when compared to most of the cultivated mushrooms [3,4]. Generally, mushroom species contain from 3 to 15 phenolic compounds, and gallic, and protocatechuic acids are the most commonly reported [18]. All pohenolics are used as indicators of the antioxidant capacity, which is linked to their hydroxyl groups [19]. Gallic acid is known to activate diverse pharmacological and biochemical effects in humans including strong anti-cancer, antioxidant, and anti-inflammatory factors [20]. In addition, it elevated the levels of enzymatic and nonenzymatic antioxidants [21]. *Pleurotus eryngii* is a significant source of lovastatin, also known as monocline k, which is a naturally occurring compound, and the hypolipidemic agent acts through the inhibition of the HMG-CoA reductase [22]. Lovastatin not only reduces blood cholesterol, but it also has anti-inflammatory, anticoagulation, antioxidative, anti-fungal, and anti-carcinogenic effects [23,24]. Acidic and alkalic-extractable zinc polysaccharides extracted from *P. eryngii* mycelia have demonstrated hepatoprotective and antioxidant effects [25]. *P. eryngii* contains the highest total glucan concentrations among *Pleurotus* spp., concentrated mainly in stalk and the highest α-glucans proportion. Another compound, immunostimulatory β-glucan—pleuran has antioxidant properties, which, combined with high taste values, makes the fruiting bodies of this species one of the most valuable cultivated mushrooms [1]. Generally, mushrooms contain higher amounts of bio-elements than agricultural crops, vegetables, and fruits because of the presence of effective mechanisms of their active uptake [26]. Bio-elements accumulation in *Pleurotus* mycelium should be considered as a potential alternative to produce non-animal food sources of essential elements.

Selenium is an essential component of several major metabolic pathways in human cells, such as thyroid hormone metabolism, antioxidant defense, immune function, reproduction, and viral

inhibition. Selenium is involved in biosynthesis of selenoenzymes and selenoproteins, as glutathione peroxidases, iodothyronine 5'-deiodases, thioredoxin reductases, selenoprotein P, and selenoprotein W. Selenoprotein P is a transport protein maintaining Se homeostasis while selenoprotein W is involved in the antioxidant defense of cardiac and skeletal muscle as well as in cell cycle progression [27,28]. Zinc is a strong antioxidant because it acts as a cofactor of superoxide dismutase and many other enzymes [29]. Zn stabilizes the molecular structure of subcellular organelles and their membranes and it is required for metabolism of nucleic acids, proteins, carbohydrates, lipids, and secondary metabolites, which affects cell division, growth, and repair [30]. Zn and Se deficiency resulted in decreased antioxidant capacity, whereas supplementation with these elements significantly improved the antioxidant status in animal testes [31]. The mushrooms, including *Pleurotus* spp., have the capacity to absorb, accumulate, and transform Se inorganic compounds into organic compounds [32], but the chemical forms of metal salts used for supplementation can affect the mycelial growth and fruit bodies production, and its chemical composition as well.

The aim of the presented work was to determine the content of bio-elements and organic compounds in *P. eryngii* mycelium from in vitro cultures and fruiting bodies grown in media/substrates fortified with zinc and selenium salts. We hypothesized that (i) Zn and Se affect the yield and chemical composition of mycelium/fruiting body in different ways, (ii) mycelium from in vitro culture could be used as a comparative or alternative model to obtain raw material with medicinal properties, and (iii) diversified biofortification allow us to obtain raw material with chemical compounds targeted for utilization by food, pharmaceutical, veterinary, or cosmetology industries.

2. Results

2.1. Organic Compounds Total Phenolics and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity Analysis

The yield of *P. eryngii* fruiting bodies was of 12.28 g dry weight (d.w.) per 100 g^{-1} substrate. Fortification with Zn sulfate and Zn hydroaspartate did not cause a decrease of the yield, but Se application affected in two-fold yield reduction was compared to the remaining trials. The mycelium yield was, on average, 6.09 g d.w. per 1 dm^3 of liquid Oddoux medium. Application of Zn sulfate and Zn hydroaspartate significantly increased the mycelium yield as compared to the control while selenite did not affect *P. eryngii* mycelium productivity (Figure 1).

Pleurotus eryngii mycelium and fruiting bodies were differentiated with respect to organic compounds levels and showed a different reaction against Zn and Se biofortification (Table 1). Supplementation of growth substrate with Zn sulfate caused a significant increase of *p*-hydroxybenzoic acid and cinnamic acid in *P. eryngii* fruiting bodies when compared to the control. Supplementation with selenite resulted in a decrease of phenylalanine, 3, 4-dihydroxyphenylacetic acid, and *p*-hydroxybenzoic acid, whereas Zn hydroaspartate implementation caused a decrease in all investigated organic acid contents when compared to the control. Supplementation of in vitro growth medium with Se and Zn significantly increased phenylalanine and gallic acid contents, whereas Zn sulfate and Zn hydroaspartate positively affected 3, 4-dihydroxyphenylacetic acid content in mycelium as compared to the control. Protocatechuic, syringic, and cinnamic acids were not detected in mycelium while gallic acid was not detected in fruiting bodies of *P. eryngii*. Generally, phenylalanine content in mycelium was 102% higher than those determined in fruiting bodies, but 3, 4-dihydroxyphenylacetic and *p*-hydroxybenzoic acid contents were lower at 88% and 91%, respectively.

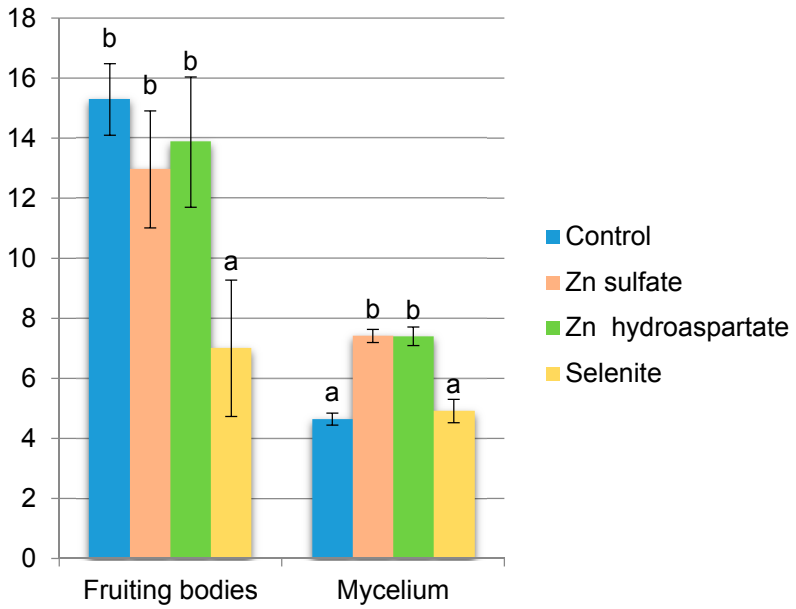

Figure 1. The biomass of *Pleurotus eryngii* fruiting bodies (g of fruiting bodies in d.w. per 100 g^{-1} of d.w. substrate) and mycelium (g of mycelium in d.w. per 1 dm^3 of liquid Oddoux medium) as dependent on Zn and Se biofortification. Means followed by different letters are significantly different at $p \leq 0.05$, $n = 6$. Comparisons performed using Tukey's honestly significant difference test. Error bars represent ± standard deviation (SD).

Lovastatin was determined only in *P. eryngii* mycelium. Supplementation with Se and Zn strongly decreased lovastatin content in *P. eryngii* mycelium. We determined 27.02 mg 100 g^{-1} d.w. of lovastatin in control treatment, while 1.02 mg 100 g^{-1} d.w. in average in Se and Zn supplemented treatments. Total phenolics content was a little higher in mycelium 3.07 mg 100 g d.w., average for treatments) than in fruiting bodies 2.21 mg 100 g^{-1} d.w. Significant differences between treatments were determined only for fruiting bodies. Zn sulfate fortification caused a significant increase of these compounds. 2,2-Diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) scavenging activity was 36.7% and 48.0%, on average, for fruiting bodies and mycelium, respectively. Supplementation with selenite and Zn sulfate of growth substrate significantly increased $DPPH^{\cdot}$ scavenging activity in fruiting bodies. Supplementation of in vitro medium with Zn sulfate and Zn hydroaspartate decreased antioxidant activity in mycelium.

Pearson's correlation coefficients between Zn and Se and organic compounds in fruiting bodies showed a positive effect of Zn on phenylalanine, *p*-hydroxybenzoic acid, and cinnamic acid content and a negative effect on total phenolics and $DPPH^{\cdot}$ scavenging activity (Table 2). Selenium was positively correlated with syringic acid. Correlations determined for mycelium were positive in the case of Zn for phenylalanine, gallic, protocatechuic, and *p*-hydroxybenzoic acids, while being negative for lovastatin and $DPPH^{\cdot}$ scavenging activity. In the case of Se, positive correlation was determined only for $DPPH^{\cdot}$ scavenging activity, while a negative correlation was determined for gallic, 3,4-dihydroxyphenylacetic, and *p*-hydroxybenzoic acids as well as for lovastatin.

The principal component analysis (PCA) was performed to compare mycelium and fruiting bodies in organic compounds' composition. The first two principal components (PCs) explained 90.95% of the total variation in the data for fruiting bodies and 94.87% for mycelium (Figure 2), which reflects the complexity of the relationships among the treatments and the organic acids' content. All traits' vector lengths are of almost maximum length. Therefore, their effects are significant. Acute angles (<90 °) between vectors representing syringic, protocatechuic, 3,4-dihydroxyphenylacetic acids, and phenylalanine as well as cinnamic and *p*-hydroxybenzoic acids depict the positive correlation between these characteristics for fruiting bodies. A positive correlation was also shown between all organic compounds determined in the mycelium.

Table 1. The content of organic acids (mg 100 g^{-1} d.w.), lovastatine (mg 100 g^{-1} d.w.), total phenolics (mg 100 g^{-1} d.w.), and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (%$DPPH^{\cdot}$) in fruiting bodies and mycelium of *P. eryngii* grown in media supplemented with selenite, Zn sulfate, and Zn hydroaspartate.

Treatment	Phenylalanine	Gallic Acid	Protocatechuic Acid	3,4-Dihydroxy Phenylacetic Acid	*p*-Hydroxybenzoic Acid	Syringic Acid	Cinnamic Acid	Lovastatin	Total Phenolics	$DPPH^{\cdot}$ Scavenging Activity
					Fruiting Bodies					
Control	176 ± 0.85 ^{d1}	n.d.	0.42 ± 0.01 ^{b}	99.30 ± 0.38 ^{d}	2.44 ± 0.03 ^{b}	0.09 ± 0.01 ^{b}	0.92 ± 0.01 ^{b}	n.d.	212.76 ± 14.04 ^{a,b}	33.15 ± 1.03 ^{a}
Selenite	141 ± 0.69 ^{c}	n.d.	0.40 ± 0.02 ^{b}	97.01 ± 0.27 ^{c}	2.35 ± 0.03 ^{a}	0.09 ± 0.01 ^{b}	0.94 ± 0.07 ^{a}	n.d.	226.44 ± 25.03 ^{b}	39.22 ± 0.47 ^{b}
Zn sulfate	137 ± 1.28 ^{b}	n.d.	0.36 ± 0.02 ^{a}	92.11 ± 0.89 ^{b}	3.34 ± 0.03 ^{c}	0.06 ± 0.01 ^{a}	1.61 ± 0.05 ^{d}	n.d.	263.04 ± 10.29 ^{c}	41.56 ± 0.75 ^{b}
Zn hydroaspartate	121 ± 0.46 ^{a}	n.d.	0.36 ± 0.01 ^{a}	84.20 ± 0.29 ^{a}	2.31 ± 0.02 ^{a}	0.07 ± 0.01 ^{a}	0.82 ± 0.01 ^{a}	n.d.	183.72 ± 14.04 ^{a}	33.03 ± 2.21 ^{a}
					Mycelium					
Control	257 ± 10.4 ^{a}	0.58 ± 0.09 ^{a}	n.d.	6.75 ± 0.10 ^{a}	0.26 ± 0.02 ^{b}	n.d.	n.d.	27.02 ± 0.07 ^{d}	283.68 ± 12.96 ^{a}	54.6 ± 0.25 ^{b}
Selenite	622 ± 13.4 ^{b}	1.14 ± 0.09 ^{b}	n.d.	n.d	0.17 ± 0.01 ^{a}	n.d.	n.d.	2.26 ± 0.09 ^{c}	257.16 ± 3.11 ^{a}	56.5 ± 1.53 ^{b}
Zn sulfate	2233 ± 88.9 ^{d}	10.42 ± 0.22 ^{d}	n.d.	15.78 ± 0.26 ^{c}	0.25 ± 0.02 ^{b}	n.d.	n.d.	0.11 ± 0.03 ^{a}	318.06 ± 3.42 ^{a}	39.9 ± 1.37 ^{a}
Zn hydroaspartate	1531 ± 45.1 ^{c}	8.87 ± 0.26 ^{c}	n.d.	8.34 ± 0.07 ^{b}	0.28 ± 0.05 ^{b}	n.d.	n.d.	0.69 ± 0.04 ^{b}	317.52 ± 3.81 ^{a}	41.6 ± 0.15 ^{a}

[1] Means in a column followed by different superscript letters, separately for fruiting bodies and mycelium, are significantly different at $p \leq 0.05$ according to Tukey's *t* test, $n = 6$. Each value represents the mean ± standard deviation.

Table 2. Pearson's correlation coefficients between Zn and Se (used as supplements for growing media) and organic compounds in fruiting bodies and mycelium of *P. eryngii*.

Organic Compounds	Fruiting Bodies		Mycelium	
	Zn	Se	Zn	Se
Phenylalanine	0.301 *	−0.045	0.766 **	−0.479
Gallic acid	nd	nd	0.890 ***	−0.612 *
Protocatechuic acid	−0.123	0.357	nd	nd
3,4-Dihydroxyphenylacetic acid	0.223	0.397	0.575 *	−0.824 ***
p-Hydroxybenzoic acid	0.772 ***	−0.349	0.564 *	−0.842 ***
Syringic acid	−0.273	0.526 *	nd	nd
Cinnamic acid	0.567 *	−0.322	nd	nd
Lovastatin	nd	nd	−0.578 *	−0.176
Total phenolics	−0.738 ***	0.0896	0.195	−0.513 *
2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity	−0.708 **	0.3555	−0.957 ***	0.711 ***

***, **, * significant at $p \leq 0.001$, 0.01, and 0.05, respectively. nd—compound not detected.

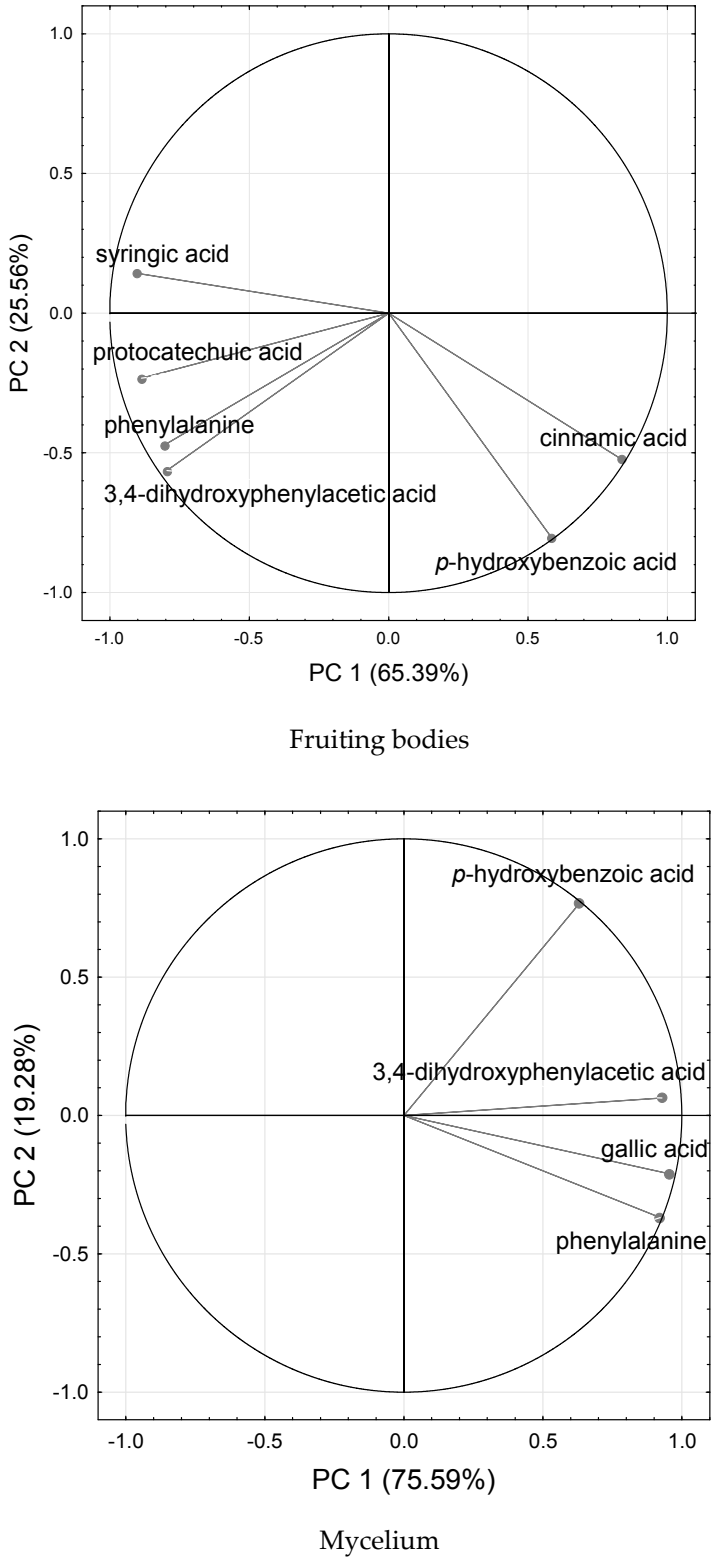

Figure 2. Integrated comparative bi-plot based on principal component analysis (PCA). The position of the organic acids was determined by the first two principal axes (87.9% explained variance for fruit bodies and 94.9% explained variance for mycelium from in vitro cultures).

2.2. Bioelements Analysis

Bioelement content was differentiated depending on source of *P. eryngii* material as well as Se and Zn supplementation of substrate and growth medium (Table 3). Potassium content was three-fold higher in fruiting bodies than in mycelium. Biofortification with Zn sulfate and Zn hydro-aspartate decreased K accumulation in fruiting bodies, while supplementation with Se, followed by Zn, decreased significantly K content in mycelium. The mycelium supplemented with Se contained a two-fold lower level of calcium than the control. *Pleurotus eryngii* fruiting bodies grown in substrate fortified with Se and Zn hydroaspartate accumulated more magnesium than in the control. Mycelium supplemented with Zn sulfate and Zn hydro-aspartate also contained a significantly higher Mg level, but those biofortified with Se contained a lower Mg level as compared to the control. Supplementation with Se and Zn increased Ca content in fruiting bodies, while enrichment of liquid in vitro medium with Se decreased Ca content in mycelium as compared to the control. Zinc-content in fruiting bodies increased significantly only as an effect of Zn hydro-aspartate supplementation of growth medium while Zn content in mycelium was 19-fold and 12-fold higher than in the control after addition of Zn sulfate and Zn hydroaspartate, respectively, to liquid medium.

Application of Se and Zn to growth substrates significantly increased iron content in fruiting bodies of *P. eryngii*, while application of Se to liquid solution increased, and Zn salts decreased Fe content in mycelium as compared to the control. Mycelium contained 27.1 mg 100 g^{-1} d.w., while fruiting bodies had 4.8 mg 100 g^{-1} d.w., on average for treatments. The chlorine level was not diversified in fruiting bodies from experimental treatment, while Se application to liquid medium caused an increase of its level in mycelium as compared to the control. Mycelium contained about 11-folds higher level of Cl when compared to fruiting bodies. Rubidium content in *P. eryngii* fruiting bodies and mycelium decreased as a result of biofortification of growth substrate and liquid medium. The mean for treatments' content of Rb in fruiting bodies was 1.41, while, in mycelium, it was −0.22 mg 100 g^{-1} d.w. Biofortification decreased copper content in fruiting bodies, but increased in mycelium but only in the case of Zn salts. Generally, fruiting bodies contained three-fold more Cu than mycelium. Se biofortification was effective in the case of fruiting bodies and mycelium. Both tissues were significantly enriched in this element even though mycelium accumulated a 15-fold higher amount of this element (mean for treatments). Manganese content in *P. eryngii* fruiting bodies was not affected by substrate supplementation, while it decreased in mycelium grown in liquid medium enriched with Se and Zn. Fruiting bodies contained a mean of 0.88 mg 100 g^{-1} d.w, and mycelium from 4.46 to 9.98 mg 100 g^{-1} d.w. of Mn. Supplementation of substrate with Zn sulfate decreased nickel content in fruiting bodies. There were no noted significant differences between the remaining treatments for Ni content in fruiting bodies and mycelium. No statistical differences were also noted for *P. eryngii* fruiting bodies and mycelium treatments regarding chrome, bromine, and strontium content.

The principal component analysis (PCA) was performed to compare mycelium and fruiting bodies in bioelements' composition. The first two principal components (PCs) explained only 55.05% of the total variation in the data for fruiting bodies, but 72.7% for mycelium. Significant relationships among the bioelements analyzed were found, although they varied in *P. eryngii* fruiting bodies and mycelium (Figure 3). The analysis of fruiting bodies showed inverse correlations between Se and Cu, and positive correlations for Se and Ca, Mg, Cr, and Sr. The Zn was positively correlated with K and Rb, but negatively correlated with Ca and Cr. In the case of mycelium, Se was positively correlated with the contents of Fe and Br, whereas negative associations occurred for K, Ca, Mg, Mn, Zn, Rb, and Sr. The Zn enrichment had a significantly negative effect on Fe, Ni, Se, and Br content, but had a positive effect on Cu and Mg levels.

Table 3. The content of bio-elements in cultivation substrate (mg 100 g^{-1} d.w.), fruiting bodies, and mycelium of *P. eryngii* (mg 100 g^{-1} d.w.) grown in media supplemented with selenite, Zn sulfate, and Zn hydro-aspartate.

Treatment	K	Mg	Ca	Zn	Fe	Cl	Rb	Cu	Se	Mn	Ni	Cr	Br	Sr
	Growth Substrate													
	449 ± 19	49.8 ± 1.6	153.6 ± 2.3	3.43 ± 0.08	13.6 ± 0.46	1.22 ± 0.33	0.33 ± 0.04	0.27 ± 0.08	0.02 ± 0.02	4.27 ± 0.11	1.24 ± 0.15	1.40 ± 0.25	0.14 ± 0.06	0.84 ± 0.06
	Fruiting Bodies													
Control	2328 ± 235 ^{c1}	117 ± 7 ^{a,b}	17.4 ± 1.6 ^{a}	4.94 ± 0.12 ^{b}	3.85 ± 0.22 ^{a}	1.98 ± 0.36 ^{a}	1.64 ± 0.13 ^{c}	1.42 ± 0.06 ^{c}	0.05 ± 0.05 ^{a}	0.90 ± 0.04 ^{a}	0.49 ± 0.05 ^{b}	0.12 ± 0.02 ^{a}	0.10 ± 0.03 ^{a}	0.08 ± 0.02 ^{a}
Selenite	2070 ± 129 ^{b,c}	235 ± 11 ^{c}	52.8 ± 4.7 ^{d}	4.48 ± 0.35 ^{a}	5.11 ± 0.97 ^{b}	1.89 ± 0.39 ^{a}	1.34 ± 0.06 ^{a,b}	0.92 ± 0.03 ^{a}	1.36 ± 0.08 ^{b}	0.92 ± 0.08 ^{a}	0.50 ± 0.11 ^{b}	0.16 ± 0.07 ^{a}	0.12 ± 0.06 ^{a}	0.14 ± 0.09 ^{a}
Zn sulfate	1753 ± 240 ^{a}	110 ± 2 ^{a}	40.1 ± 0.3 ^{c}	4.39 ± 0.29 ^{a}	4.98 ± 0.37 ^{b}	1.85 ± 0.29 ^{a}	1.24 ± 0.14 ^{a}	1.23 ± 0.23 ^{b}	0.03 ± 0.03 ^{a}	0.86 ± 0.11 ^{a}	0.38 ± 0.05 ^{a}	0.13 ± 0.03 ^{a}	0.08 ± 0.03 ^{a}	0.07 ± 0.02 ^{a}
Zn hydroaspartate	2034 ± 122 ^{b}	121 ± 3 ^{b}	30.5 ± 6.7 ^{b}	5.45 ± 0.25 ^{c}	5.56 ± 1.06 ^{b}	1.86 ± 0.18 ^{a}	1.44 ± 0.08 ^{b}	1.14 ± 0.09 ^{b}	0.03 ± 0.03 ^{a}	0.87 ± 0.04 ^{a}	0.43 ± 0.06 ^{b}	0.11 ± 0.04 ^{a}	0.13 ± 0.03 ^{a}	0.09 ± 0.03 ^{a}
	Mycelium													
Control	872 ± 62 ^{c}	175 ± 7.1 ^{b}	61.8 ± 7.1 ^{b}	15.1 ± 0.88 ^{b}	31.4 ± 0.36 ^{c}	17.46 ± 4.75 ^{a,b}	0.29 ± 0.05 ^{c}	0.29 ± 0.07 ^{a}	2.1 ± 0.03 ^{b}	9.98 ± 0.41 ^{d}	0.63 ± 0.13 ^{b}	0.14 ± 0.06 ^{a}	0.26 ± 0.09 ^{a}	0.20 ± 0.00 ^{a}
Selenite	432 ± 9 ^{a}	79 ± 1.5 ^{a}	29.1 ± 6.5 ^{a}	10.9 ± 0.54 ^{a}	33.46 ± 0.88 ^{d}	31.64 ± 2.62 ^{c}	0.13 ± 0.03 ^{a}	0.35 ± 0.07 ^{a,b}	18.79 ± 0.44 ^{b}	4.46 ± 0.17 ^{a}	0.46 ± 0.05 ^{a}	0.14 ± 0.02 ^{a}	0.24 ± 0.04 ^{a}	0.14 ± 0.05 ^{a}
Zn sulfate	739 ± 46 ^{b}	192 ± 8.2 ^{c}	54.7 ± 5.9 ^{b}	289.5 ± 1.80 ^{d}	22.81 ± 0.74 ^{b}	21.95 ± 5.50 ^{b}	0.24 ± 0.03 ^{b}	0.46 ± 0.06 ^{c}	0.23 ± 0.08 ^{a}	7.82 ± 0.13 ^{b}	0.45 ± 0.08 ^{a}	0.17 ± 0.07 ^{a}	0.19 ± 0.02 ^{a}	0.18 ± 0.03 ^{a}
Zn hydroaspartate	729 ± 29 ^{b}	193 ± 0.4 ^{c}	61.8 ± 6.7 ^{b}	178.3 ± 2.43 ^{d}	20.74 ± 1.28 ^{a}	16.09 ± 1.46 ^{a}	0.25 ± 0.00 ^{b,c}	0.41 ± 0.03 ^{b,c}	0.36 ± 0.05 ^{a}	8.48 ± 0.21 ^{c}	0.41 ± 0.07 ^{a}	0.12 ± 0.06 ^{a}	0.19 ± 0.02 ^{a}	0.18 ± 0.03 ^{a}

[1] Means in a column followed by different superscript letters, separately for fruiting bodies and mycelium, are significantly different at $p \leq 0.05$ according to Tukey's *t* test, n = 6. Each value represents the mean ± standard deviation.

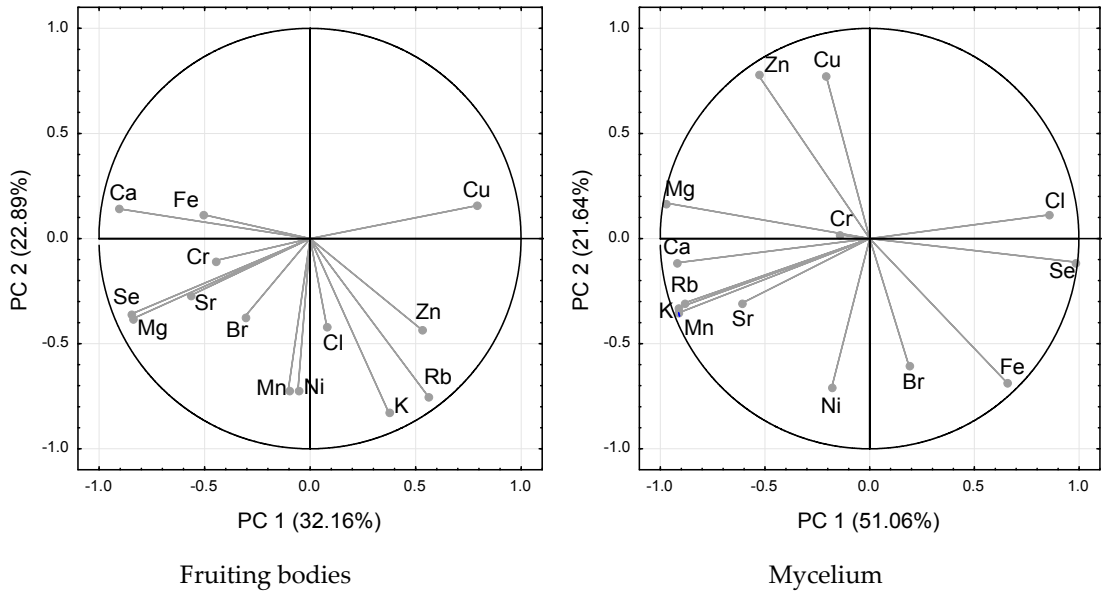

Figure 3. Integrated comparative bi-plot based on principal component analysis (PCA). The position of the elements was determined by the first two principal axes (55.1% explained variance for fruit bodies, 72.7% explained variance for mycelium from in vitro cultures).

3. Discussion

The results of this study significantly contribute to the rise of information on the species consumed as fresh mushrooms and the possibility of bio-elements enriched mycelium and fruiting bodies production as a source of bioactive compounds. Munoz et al. [33] determined that Se specifically binds to chitin in the cell walls of *P. ostreatus*. Moreover, lower Se concentration (2.5 mg L^{-1}) stimulated the growth of the mushroom, while higher concentration (5 mg L^{-1}) had an inhibitory effect. Kim et al. [34] investigated the growth and enzyme activity in *P. eryngii* grown in medium supplemented with 1, 10, 100, 1000, and 10,000 µM of sodium selenite. Mycelial growth was increased at lower Se levels, but declined significantly at 1000 and 10,000 µM of Se as a result of Se toxicity. In the present study, Se did not affect the in vitro mycelium yield, but significantly decreased fruiting bodies' weight despite the concentration of this element being higher in supplemented mycelium. Se-mediated stimulating or toxic response in *P. eryngii* growth could be differentiated during vegetative (mycelium) and generative (fruiting bodies) stage of development as well as growth conditions (liquid medium vs. growth substrate). As a mechanism of a metal mediated growth response, Stajic et al. [7] and Kim et al. [34] demonstrated increased laccase activity at low levels of Se, Fe, and Zn and reduced activity at high levels of these elements in *P. eryngii*, *P. ostreatus*, and *P. pulmonarius*. Fungal laccases control various functions as degradation of lignin and many xenobiotic compounds, morphogenesis, stress, and pathogen defense indirectly affect fungal growth and development. They could be involved in Se and Zn response in *P. eryngii* yielding in the present research. In terms of mushroom productivity, Zn supplementation seems to be the most promising approach in liquid media cultures. Enrichment of liquid medium with Se leads to stable mycelium production with respect to dry weight while Se application to a growing substrate causes a decrease of fruit bodies' production.

3.1. Organic Compound—Total Phenolics, Lovastatin, and DPPH· Scavenging Activity Analysis

In the present study, gallic acid content in *P. eryngii* mycelium was negatively correlated with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity ($r = -0.962$; $p = 0.000$) even though it does not exclude its positive action in the human body. Moreover, zinc and, in a lesser degree, selenium supplementation positively affected gallic acid content in *P. eryngii* mycelium, while this acid was

not detected in fruiting bodies. According to Carrasco-Gonzalez et al. [21], coumaric and ferulic acid contents in *P. ostreatus* fruiting bodies were positively affected through Se biofortification.

We determined seven phenolic compounds with phenylalanine as a dominating agent in fruiting bodies and mycelium of *P. eryngii*. In turn, 12 phenolic acids, namely gallic, protocatechuic, chlorogenic, caffeic, vanillin, ferulic, naringin, resveratrol, naringenin, hesperetin, formononetin, and biochanin-A were detected in fruiting bodies of *P. citrinopileatus* [18]. Gallic acid, protocatechuic acid, chlorogenic acid, vanillin, ferulic acid, naringin, naringenin, hesperetin, formononetin, and biochanin-A were detected from acetonitrile and 0.1 N hydrochloric acid solvent extract of *P. eryngii* fruiting bodies, and protocatechuic acid was determined in the highest concentration [35]. We performed principal component analysis (PCA) analyses to compare mycelium and fruiting bodies in organic compounds' composition. Because the first two PCs explained more than 90% of the total variation in the data for fruiting bodies and mycelium, we can conclude that the Se and Zn supplementation and the organic acids' content have complex relationships. According to Yan and Chang [31], the boxplot model fits well if the first two PC's should reflect more than 60% of the total variation. Basing on the cited and presented results, it can be stated that the phenolic acids show great differentiation among *Pleurotus* species even within the same species because of genetic differences, substrate composition, growing conditions, or mushroom raw material. Se and Zn supplementation can be used as a tool to control phenolic acids' level in *P. eryngii* mycelium and fruiting bodies. In the present study, this relationship was clearly demonstrated for gallic acid accumulation in *P. eryngii* mycelium, which is positively affected by Zn and Se addition to liquid solutions.

Phenolic compounds have been reported to be the major antioxidants determined in mushrooms. Moreover, radical scavenging activity has a strong correlation with the phenolic content in *Pleurotus* spp. [36]. Our results confirmed this relationship for fruiting bodies with a correlation coefficient value of r = 0.751, $p = 0.001$, while, for mycelium, it was non-significant (r = 0.234, $p = 0.383$, even though total phenolics content was higher in mycelium as compared to fruiting bodies. It can be explained that the slight effect of Zn and Se supplementation on total phenolics in mycelium and a significant decrease of mycelium DPPH radical scavenging activity by Zn salts' supplementation. In the present study, total phenolics in *P. eryngii* samples determined by the Folin-Ciocalteu assay was higher than reported by Li and Shah [35], namely 1090.42 ± 13.77 μg GAE g^{-1} d.w., but lower than the results by Reis et al. [37] for methanolic extracts from fruiting bodies (7.14 ± 2.01 mg GAE g^{-1}) as well as mycelium (9.11 ± 0.23 mg GAE g^{-1}). This may be due to differences in growth medium, growing conditions, extraction solvents, and the conditions used.

Atli and Yamac [38] screened 136 macro-fungi isolates from the Basidiomycetes for lovastatin production and only six of them were found to be lovastatin producers, *Omphalotus olearius*, and *P. ostreatus*, which is the most effective. Lovastatin seems to be an uncommon compound in higher mushrooms, so the demonstration of *P. eryngii* non-supplemented mycelium effectiveness in lovastatin synthesis is an important achievement of the present research. In addition, in our research, we proved that modification of Oddoux medium by adding extra Zn and Se salts, intensely decreased lovastin production of mycelium, but mechanisms of this phenomenon need future investigation. Anyway, the decision on Zn and Se supplementation should be taken with the awareness that it could negatively affect lovastatin content in mycelium from in vitro cultures. There is also a need of future investigations on the liquid media formulations leading to increased lovastatin content in *P. eryngii* mycelium.

Generally, Se is considered to be an essential nutritional trace element of strong antioxidant function [39]. According to Kim et al. [40], Se at concentrations above 100 μM leads to decreases of antioxidative functions of peroxidase and laccase in the *Pleurotus eryngii*. *Pleurotus ostreatus* enrichment with Se also affected the antioxidant capacity, and, particularly, the methanolic extract obtained from the second flush of fruiting bodies had the best antioxidant activity [21]. In this study, the $DPPH^{\cdot}$ scavenging activity of fruiting bodies was significantly increased by Se addition to the growth substrate, which indicates the potential use of Se-enriched *P. eryngii* as antioxidants in

diet. Fasoranti et al. [17] determined that antioxidant activity of the ethanol extracts of Se-enriched mushrooms was significantly higher than the non-enriched *Pleurotus* species. Selenium has strong antioxidant activity. Moreover, the mechanism of $DPPH^{\cdot}$ scavenging power may be an incorporation of selenyl group (SeH) or seleno-acid ester to three-dimensional structure of polysaccharide that changed the hydrogen atom-donating capacity [41]. In general, it can be suspected that the significantly improved antioxidant profile of mushrooms was enriched with Se, which was also stated by Kaur et al. [42].

3.2. Bioelements Analysis

Mycelia of *P. eryngii* show the ability of trace elements' accumulation in amounts depending on the metal source in medium [43]. Some of them are essential for fungal metabolism as enzyme components or enzyme activity modulators. According to Gogavekar et al. [44], the mean metal concentration in *P. ostreatus* fruiting bodies was in the order: Ca > Fe > Mg > Na >K > Zn > P > Ni > Mn > Pb > Cu > Cr > Co. *Pleurotus* spp. could be an important source of nutritionally valuable minerals because they contained high amounts of K, Mg, and Ca [45]. Moreover, enrichment of growth medium with Se and Zn was effective in biofortification in this element including both mycelium and fruiting bodies. In the present research, the increase of Se concentration in the liquid medium led to an increase of its content in mycelium of *P. eryngii*. Poursaeid et al. [46] determined that the ability of Zn bioaccumulation in the mycelia of *P. florida* was much higher than in the fruiting bodies. The enrichment of growth substrate in minerals, namely Zn, Li, and Fe, promoted a decrease in the content of Fe in *P. ostreatus* fruiting bodies. Moreover, *P. ostreatus* enriched with Fe, Zn, or Li provided minerals such as K, P, Fe, Zn, Li, and Cu. In addition, no heavy metals such as Ni, Cr, and Cd were detected, and only low levels of Pb and Al were observed [47]. Bioelements are involved in all metabolic reactions, transmission of nerve impulses, regulation of water and salt balance, and many other processes crucial for proper functioning of the human body [48]. *P. eryngii* is a good source of minerals including potassium, phosphorus, magnesium, manganese, zinc, and calcium. Moreover, the content of crucial elements could be modified through growth medium enrichment. We can recommend that addition of Zn salts to growth substrate lead to increased K and Rb content, while supplementation with Se increases Br, Sr, Mg, Cr, Ca, and Fe. We can also formulate the recommendations for controlling minerals in mycelium through Zn and Se supplementation. Essentially, the addition of Zn to liquid medium can increase accumulation of Cu in mycelium, while the addition of Se salt can increase Br, Fe, and Cl. These results show complex bio-elements' interactions in mushroom fruit bodies and mycelium, which is reported by Siwulski et al. [49]. The amount of nutrients recommended per day for Americans four years of age or older by FDA [50] are for Ca 1000 mg, Cr 120 μg, Cu 2 mg, Fe 18 mg, Mg 400 mg, Mn 2 mg, P 1000 mg, K 3500 mg, Se 70 μg, Na 2400 mg, and Zn 5 mg. The present study confirmed that *P. eryngii* has beneficial effects from a nutritional point of view K:Na ratio such as low Na and high K content. K affects the reduction of blood pressure, lowers the risk of cardiovascular disease, stroke, and coronary heart disease as well [51]. We demonstrated that 1 g of dry *P. eryngii* mycelium supplemented with Se can provide 268% of recommended daily intake, while it was supplemented with Zn sulfate 58% of recommended daily intake. Therefore, supplemented mycelium could be used as a diet supplement providing bioelements as Zn and Se with additional biological active compounds that can be find in mycelium. In our growing experiment, the effect of Zn supplementation was vestigial and only a slightly increased amount of it in fruiting bodies of *P. eryngii.* Supplementation fruiting bodies with Se was more effective, but still 20 times less effective than mycelium and a negatively affected yield of mushrooms. Despite this supplementation, other elements' absorption substantially change organic compounds in fruiting bodies and mycelium. More specific research is needed to understand these phenomena. According to Estrada et al. [52], selenium-enriched mushrooms would supply more than 20% of the daily intake. They could be considered an excellent source of selenium. Our results demonstrated that both fruiting bodies and mycelium of *P. eryngii* can be considered as functional food or raw material enriched in essential nutrients, which can be widely used in food supplementation.

Despite total bioelements content, the amount provided by mushroom consumption is not always enough to meet nutritional requirements of humans if the bioavailability is low, as for non-heme Fe or Zn. According to Kalac and Svoboda [48], the bioavailability of iron in mushrooms is considerably high and human body can absorb up to 90% of the available form.

4. Materials and Methods

4.1. Reagents and Standards

Zinc hydroaspartate ($C_8H_{12}N_2O_8Zn$) was obtained from Farmapol (Poznań, Poland), zinc sulfate ($ZnSO_4 \cdot 7H_2O$) from OUM-7, (Łódź, Poland) and sodium selenite (Na_2SeO_3) from Sigma-Aldrich (St. Louis, MO, USA). Water (four times distilled) with a conductivity of less than 1 μS cm^{-1} was obtained using an S2-97A2 distillation apparatus (ChemLand, Stargard Szczecin, Poland).

Standards of organic compounds: *p*-hydroxybenzoic acids were obtained from Fluka (Chemie AG, Germany), and gallic acid, 3-4-dihydroxyphenylacetic acid, syringc acid, cinnamic acid, protocatechuic acid, lovastatin, 2,2-Diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) radical, Folin & Ciocalteu's phenol reagent from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol, phosphoric acid, acetic acid, and acetonitrile from Merck (Darmstadt, Germany). Concentrated HNO_3 Suprapur®, and H_2O_2 Suprapur® from Merck (Darmstad, Germany).

Chemicals for Oddoux medium: glucose, maltose extract, casein hydrolysate, L-asparagine, adenine, and yeast extract were purchased from Sigma-Aldrich (St. Louis, MO, USA). NH_4Cl, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 6H_2O$, $FeCl_3$, $MnSO_4 \cdot H_2O$, from PPH Golpharm (Kraków, Poland). Growing medium, namely wheat straw and beech sawdust, were bought from a local producer as a homogeneous batch.

HClO solution was manufactured by Unilever (Nyírbátor, Hungary), n-hexane, chloroform, were purchased from Merck (Darmstad, Germany).

4.2. Pleurotus Eryngii Materials

The *P. eryngii* explants for the generation of the in vitro cultures were provided courtesy of the prof. Marek Siwulski, Poznań University of Life Sciences. Representative voucher specimens were deposited at the Department of Pharmaceutical Botany, Jagiellonian University Medical College, Kraków, Poland. In order to conduct the experiment, in vitro cultures of *P. eryngii* were grown on a modified liquid medium with composition according to Oddoux.

4.3. In Vitro Cultures of Pleurotus eryngii

To achieve a maximum efficiency in mushroom biomass growth, the mushroom cultures were transferred to the modified liquid Oddoux medium (starting inoculum transplanted from the solid medium culture was 0.1 g). To prepare the experimental cultures, the obtained biomass was passed into Erlenmeyer's flasks (500 mL) containing the liquid medium (250 mL) and conducted at 25 ± 2 °C under 16 h of lighting (11.5 μmol s^{-1} m^{-2}) and 8 h of darkness. The biomass was obtained from the cultures grown on the Oddoux medium (control), and on the same medium, but with the addition of sodium selenite at a concentration of 50 mg L^{-1} (0.00029 mol L^{-1}), zinc hydroaspartate at a concentration of 100 mg L^{-1} (0.00027 mol/L), and zinc sulfate (0.000304 mol L^{-1}) at a concentration of 87.23 mg L^{-1}. The applied concentrations of both compounds contained the same content of zinc (II) ions (20 mg L^{-1}). After four weeks, since the initiation of in vitro cultures on liquid medium, the biomass was separated from the medium and rinsed three times with four-fold distilled water. The resulting biomass was frozen and then dried via lyophilization (lyophilizer Freezone 4.5, Labconco, temperature: −40 °C).

4.4. Fruiting Bodies of Pleurotus eryngii

Mushrooms were grown in 3-L glass jars, on beech sawdust, and grinded wheat straw (1:1 *v*/*v*) moisturized with distilled water to the content of 65% ± 1%. Zinc and selenium compounds'

concentration was the same per liter as in in vitro cultures. Jars were filled with the mix of salts and growing medium, and sterilized at 121 °C for 1.5 h. The cooling substrate was inoculated with previously prepared wheat grain spawn of *P. eryngii* (3% of substrate weight) in a cavity made in the center of a jar. Jars were incubated at 24 ± 2 °C in dark for four weeks until mycelium fully colonized the substrate. After then, jars were put in a growing room with a temperature of 18 ± 1 °C, humidity of 95 ± 3%, and light intensity of 11.5 μmol s^{-1} m^{-2} with a 12 h photoperiod. Each treatment consisted of four jars. Fruit bodies were harvested in a stage of market maturity and dried in a laboratory dryer at 40 °C.

4.5. Mushroom Extracts

For the preparation of *P. eryngii* methanol extracts, the lyophilized mushroom materials such as the fruiting bodies and biomass from the in vitro culture were portioned and weighed (5 g of each sample), then ground in a mortar, and subjected to extraction with petroleum ether in percolators in order to remove the lipid fraction, according to the procedure developed by Kała et al. [53]. The remaining degreased biomass was dried and again subjected to extraction with methanol in a percolator for 24 h (kept in the dark). The obtained extracts were concentrated by distillation in a vacuum evaporator (Büchi, Germany) under reduced pressure (200 mbar) at 40 °C. The resultant extracts were dissolved in methanol (1 g of dry extract to 1 mL of methanol) and then filtered through bacteriological 0.2 μm syringe filters. Then, they were diluted to desired concentrations. The prepared extracts were stored at 4 °C until use.

4.6. Organic Compounds Analysis—Lovastatin

Chromatographic separation was performed using a high-performance liquid chromatography (HPLC) analyzer (Merck Hitachi). The process was carried out in an isocratic system with a mobile phase of constant composition. The apparatus was equipped with a ultraviolet (UV) detector (λ = 238 nm), a column (Purospher RP18 14 × 200 mm, 5 μm), and a lamp (L7100). During each measurement, 20 μL of the analyzed sample was injected, and the measurement was performed within 15 min. All measurements were carried out using a previously prepared developing system (acetonitrile and 0.1% phosphoric acid in the ratio of 60:40 (*v*/*v*)). The retention time of the standard substance was 12.75 min.

4.7. Organic Compounds Analysis—Phenolic Compounds

Methanolic extract was evaporated to dryness (Buchi evaporator, Germany) under a pressure of 200 mBa at 40 °C. The residues were quantitatively dissolved in methanol (1.5 mL) and filtered through a Millipore Millex–GP, 0.22 μm.

The resultant extracts were analyzed for their contents of phenolic acids by the high-performance liquid chromatography with diode-array detector (HPLC-DAD) method. These analyses were carried out according to the procedure developed by Kała et al. [53], where methanolic extracts from 12 species of fruiting bodies of edible mushrooms were analyzed using the HPLC method with some modifications. HPLC analyses were conducted using an HPLC VWR Hitachi-Merck apparatus: L-2200 autosampler, L-2130 pump, RP-18e LiChrospher (4 mm × 250 mm, 5 μm) column thermo-stated at 25 °C, L-2350 column oven, and L-2455 diode array detector at the UV range of 200–400 nm. The mobile phase consisted of solvent A: methanol/0.5% acetic acid 1:4 (*v*/*v*) and solvent B: methanol. The gradient was as follows: 100:0 for 0–25 min, 70:30 for 35 min, 50:50 for 45 min, 0:100 for 50–55 min, and 100:0 for 57–67 min. The comparison of UV spectra and retention times with standard compounds enabled the identification of phenolic acids present in analyzed samples. The quantitative analysis of free phenolic acids was performed with the use of a calibration curve with the assumption of the linear size of the area under the peak and the concentration of the reference standard.

4.8. Scavenging Activity Analysis (%DPPH˙)

0.1 mL of mushrooms methanolic extracts was mixed with 0.1 mM 2,2-Diphenyl-1-picrylhydrazyl (DPPH) dissolved with 4.9 mL of 100% methanol. The mixture was shaken and kept in dark for 45 min. The absorbance was measured at 517 nm using a Ultraviolet-Visible Spectroscopy UV-VIS Helios Beta spectrophotometer (Termo Fisher Scientifc Inc., Waltham, MA, USA). DPPH radical scavenging activity was calculated using the formula: AA (%) = [(A0−A1)/A0] × 100, where AA is the antioxidant activity, A0 is the absorbance of the reference solution, and A1 is the absorbance of the test solution.

4.9. Total Phenol Content

Total phenolic content was estimated using the modified Folin-Ciocalteu colorimetric method. Additionally, 0.1 mL of mushrooms' methanolic extracts was mixed with 2 mL of sodium carbonate. After the next 2 min, 0.1 mL of Folin-Ciocalteu's reagent, mixed with deionised water (1:1 *v/v*), was added. The absorbance of the resulting blue colour was measured at 750 nm using the UV-VIS Helios Beta spectrophotometer against a reference solution. The results are expressed as gallic acid equivalents (GAE).

4.10. Bioelements Analysis

The amount of elements in the fruiting bodies and mycelium of *P. eryngii* was determined using the total reflection x-ray fluorescence spectrometry (TXRF) method. The TXRF method was chosen for this study due to several significant aspects such as high sensitivity, precision, accuracy, and repeatability of analyses as well as the possibility to achieve a faster measurement with a very large number of samples. The optimization of mineralization conditions for analysis of samples in combination with the TXRF method enabled optimal analysis of bioelements in the fruiting bodies, and mycelia cultures of *P. eryngii.*

For this analysis, 0.2 g of samples of lyophilized mushroom material were weighed, with an accuracy of 0.01 g, and were transferred to Teflon vessels to which 2 mL of H_2O_2 solution (30%) and 4 mL of concentrated HNO_3 solution (65%) were added. Mineralization was carried out in Magnum II microwave apparatus (ERTEC) in three stages of 10 min each at a power of 70% and 100%, respectively, while maintaining the temperature of the device at 290 °C. After mineralization, the solutions were transferred to quartz evaporators and evaporated on a heating plate at 150 °C to remove excess reagents and water. The residue obtained after evaporation was quantitatively transferred to 10 mL of volumetric flasks with four-times-distilled water. To analyze the composition of bioelements such as K, Ca, Mn, Fe, Cu, Zn, and Se in the prepared test samples, 1000 ppm gallium was used as an internal standard. The composition of elements was measured using a TXRF spectrometer Nanohunter II (Rigaku) equipped with an X-ray tube containing a molybdenum anode at 50 kV for 1000 s.

The highest detection limits (LOD) are obtained for heavy elements: potassium and calcium above 1 mg/kg for light elements. The remaining LOD values oscillate below 1 mg/kg (0, 0.09, 012, 0.22, 0.29, 0.38, 0.38, 0.45, 046, and 0.99 for Rb, Se, Sr, Ni, Cu, Zn, Fe, Mn, Cr, and Mg respectively).

4.11. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) followed by Tukey's HSD tests using the Statistica 12.0 software package (StatSoft Inc., Tulsa, OK, USA). Correlation coefficient r was calculated to determine the relation between Zn, Se, and each of the chemical traits. Experimental data were also processed for a principal component analysis (PCA) in order to evaluate the existing relationships with original variables.

5. Conclusions

P. eryngii is found to be a rich source of important major minerals like K, Mg, Ca, Fe, and some minor minerals. This species contains a significant amount of secondary metabolites like phenolic

acids, polyphenols, and lovastatin being considered as compounds having a wide influence on human body functions. Therefore, *P. eryngii* fruiting bodies and mycelium can be used as a raw material for diet supplements production and for pharmaceutical industry. *P. eryngii* fruiting bodies consumption provides a wide range of major and minor nutrients, so it can be treated as a functional food to combat various deficiency diseases and malnutrition. Enrichment of growing media in Zn and Se salts leads to higher content of these metals in the mycelium and fruiting bodies with a diverse effect regarding other chemical constituents. Due to the fact that fruiting bodies' production takes a long time to complete, and the production is difficult to control due to the use of different agricultural residues, the mycelium may be an economic and safe alternative for the production of raw material for different industries. Careful selection of dose, form, and the way of Zn and Se application enable us to produce *P. eryngii* fruiting bodies or mycelium with predictable amounts of these elements. Considering the complex effect of supplementation on mushrooms' chemical composition, precise formulations for growing media composition should be formulated to produce raw material standarised with respect to particular elements or chemical compounds.

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mgr inż. Piotr Zięba

Piotr Zięba

31-120 Kraków, Al. Mickiewicza 21
tel. +48 (12) 662 53 03, e-mail: ko@urk.edu.pl

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Katedra i Zakład
Botaniki Farmaceutycznej UJ CM
dr Agnieszka Szewczyk
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prof. dr hab. inż. Edward Kunicki

31-120 Kraków, Al. Mickiewicza 21
tel. +48 (12) 662 53 03, e-mail: ko@urk.edu.pl

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Dr hab. Agnieszka Sękara prof. URK

31-120 Kraków, Al. Mickiewicza 21
tel. +48 (12) 662 53 03, e-mail: ko@urk.edu.pl

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Supplementation with Magnesium Salts—A Strategy to Increase Nutraceutical Value of *Pleurotus djamor* Fruiting Bodies

Piotr Zięba [1], Agnieszka Sękara [1,*], Emilia Bernaś [2], Agata Krakowska [3], Katarzyna Sułkowska-Ziaja [4], Edward Kunicki [1], Małgorzata Suchanek [5] and Bożena Muszyńska [4]

1 Department of Horticulture, Faculty of Biotechnology and Horticulture, University of Agriculture in Kraków, 29 Listopada 54, 31-425 Kraków, Poland; p.zieba90@gmail.com (P.Z.); edward.kunicki@urk.edu.pl (E.K.)
2 Department of Plant Product Technology and Nutrition Hygiene, Faculty of Food Technology, University of Agriculture in Krakow, 122 Balicka Street, 30-149 Kraków, Poland; emilia.bernas@urk.edu.pl
3 Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland; agata.krakowska@uj.edu.pl
4 Department of Pharmaceutical Botany, Faculty of Pharmacy, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland; katarzyna.sulkowska-ziaja@uj.edu.pl (K.S.-Z.); muchon@poczta.fm (B.M.)
5 Department of Analytical Chemistry and Biochemistry, Faculty of Materials Science and Ceramics, AGH University of Science and Technology, Al. Mickiewicza 30, 30-059 Kraków, Poland; msuchanek@agh.edu.pl
* Correspondence: agnieszka.sekara@urk.edu.pl

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Abstract: The use of substrates supplemented with minerals is a promising strategy for increasing the nutraceutical value of *Pleurotus* spp. The current research was performed to analyze the effect of substrate supplementation with magnesium (Mg) salts on the Mg content, biomass, and chemical composition of pink oyster mushroom (*Pleurotus djamor*) fruiting bodies. Before inoculation, substrate was supplemented with $MgCl_2 \times 6\ H_2O$ and $MgSO_4$, both salts were applied at three concentrations: 210, 420, and 4200 mg of Mg per 2 kg of substrate. The harvest period included three flushes. Substrate supplementation with 4200 mg of Mg caused the most significant decrease in mushroom productivity, of about 28% for both Mg salts. The dry matter content in fruiting bodies was significantly lower in the treatment in which 210 mg of Mg was applied as $MgSO_4$ in comparison to the control. Supplementation effectively increased the Mg content in fruiting bodies of *P. djamor* by 19–85% depending on the treatment, and significantly affected the level of remaining bioelements and anions. One hundred grams of pink oyster fruiting bodies, supplemented with Mg salts, provides more than 20% of the Mg dietary value recommended by the Food and Drug Administration (FDA); thus, supplementation can be an effective technique for producing mushrooms that are rich in dietary Mg. Although *P. djamor* grown in supplemented substrate showed lower productivity, this was evident only in the fresh weight because the differences in dry weight were negligible. Mg supplementation increased the antioxidant activity of the fruiting bodies, phenolic compounds, and some amino acids, including L-tryptophan, and vitamins (thiamine and L-ascorbic acid).

Keywords: pink oyster; *Pleurotus djamor*; functional food; biofortification; magnesium

1. Introduction

The species of the genus *Pleurotus*, commonly known as oyster mushrooms, are distributed in tropical and temperate regions of the world. Some of these, including *P. citrinopileatus*, *P. djamor*, *P. eryngii*, *P. flabellatus*, *P. florida*, and *P. ostreatus* have been used as both food and medicine since the time of ancient civilizations. They have now gained wide popularity as nutraceuticals due to their high nutritional and medicinal value and their ability to grow on commonly available agricultural wastes [1,2]. Nutritionally, oyster

mushrooms are valued as sources of protein, fiber, carbohydrates, vitamins, and minerals. Nagy et al. [3] reported 17.9–30.3% dry weight (DW) of crude proteins, 1.10–2.2% DW of lipids and 57.6–62.4% DW carbohydrates in *Pleurotus ostreatus* fruiting bodies collected from natural habitats. The major amino acids were lysine and valine, and fatty acids determined at the highest amounts were linoleic and oleic acids. Oyster mushrooms contain biologically active substances—immuno-modulating polysaccharides, indole compounds, and numerous antioxidants, such as phenolic acids or ergothioneine [4,5]. The amino acid score of *P. ostreatus* meets the nutritional requirements of all essential amino acids for adults, with the one limiting amino acid, leucine, which can be easily acquired by supplementing with cereals [6,7]. Thus, oyster mushrooms are considered to be a functional food, which should be a standard component of the human diet [8]. Many medicinal effects have been confirmed for *Pleurotus* spp.; as a result, so mycelia and fruiting bodies are used as raw materials by the pharmaceutical and cosmetics industries [5,9]. Researches has also confirmed positive health and potential medicinal properties of basidiocarps and mycelium obtained from in vitro cultures of *P. djamor*. β-glucans isolated from pink oyster mushroom have a cytotoxic effect against ovarian carcinoma cells in in vitro models [10]. The β-glucans complex with zinc obtained from *P. djamor* mycelium has an antioxidative impact on liver and kidney damage [11–13]. Acetylated polysaccharides obtained from in vitro mycelium have anti-aging effects in D-galactose-induced aging mice, suggesting potential application to age-related diseases in human [14].

Pleurotus djamor (Rumph. ex Fr.) Boedijn is a tropical species of the oyster mushrooms group native to South East Asia and Central America [15]. In tropical regions, pink oyster mushrooms are commonly collected in the wild and are readily available in rural markets. These mushrooms are appreciated and eagerly bought by customers as a substitute for soybean or egg [16]. Although this species is less important economically than the grey oyster mushroom (*P. ostreatus*), it is becoming more popular due to its unusual pink color and specific texture that resembles fried meat. *P. djamor* can be easily grown on various agriculture and food residues [17,18]. Furthermore, it is more disease-resistant than the grey oyster mushroom and can be cultivated at higher temperatures, even exceeding 35 °C [19]. The main challenge of pink oyster mushroom cultivation and trade is the mushroom's short shelf life. Basidiocarps have to be picked when they are young; before spores' release, they cannot be stored for more than a few days [15].

Production of the mushrooms is based on agricultural and agro-industrial wastes. The residual substrate after mushroom harvesting can be used as a valuable organic fertilizer or a complement for animal feed. Therefore, mushroom production meets the requirements of modern, sustainable agriculture, while simultaneously providing a functional food [20–22]. Mushrooms produce several enzymes that degrade lignin, hemicellulose, and cellulose. Lignin–cellulose substrates are widely used in mushroom cultivation [23]. Mushroom mycelium converts lignocellulosic residues into a food product in oxygen conditions and at a specific pH, gaining all macroelements (C, N, P, K, and Mg), and microelements (Fe, Se, Zn, Mn, Cu, and Mo) from a substrate, which is sometimes supplemented with an additional source of N [24,25]. In addition to supplementing the growing substrate using protein, several authors have underlined the role of supplements containing fats, carbohydrates, fibers, and ashes to increase mushroom yields [26]. Alternatively, substrate supplementation in mineral salts was recently recognized as an effective method of enriching the bioelements of mycelia and fruiting bodies. Oliveira and Naozuka [27] demonstrated that *P. djamor* accumulates selenium and Se-proteins, which were reported to be highly bio-accessible. Włodarczyk et al. [28] stated that the addition of Zn and Mg salts' to the media increased the biomass production of *Pleurotus* spp. mycelium and the accumulation of inorganic salts. Mg is an essential macroelement in the human diet, with a vital role in numerous metabolic processes. Its deficit is often encountered in many countries, particularly among older people [29–31]. Considering the above, the biofortification of oyster mushrooms with Mg could be an alternative means of producing a functional food.

This research aimed to determine the impact of fortification of the substrate with magnesium salts the productivity and chemical composition of *P. djamor* fruiting bodies. To the best of our knowledge, this issue has not been sufficiently described in the scientific literature, despite its potential scientific importance and applicability to the horticulture and food production sectors. From a practical perspective, this research contributes to developing sustainable horticulture, in addition to improving competitiveness and increasing the incomes of the mushroom production sector. The hypotheses verified within this research include: (i) the dose-dependent increase in Mg content in fruiting bodies of *P. djamor* as a result of the addition of Mg salts to the substrate; (ii) the differentiated effect of supplementation with Mg chloride and sulfide on the Mg level in mushroom fruiting bodies; (iii) dose- and salt-dependent effects of Mg supplementation on the biomass of fruiting bodies; and (iv) salt-dependent effects of Mg supplementation on the chemical composition of fruiting bodies.

2. Materials and Methods

2.1. Mushroom Materials and Experiments Design

The material used in this study was *Pleurotus djamor* (Rumph. ex Fr.) Boedijn maintained in agar culture, from the deposit of the Department of Horticulture, University of Agriculture in Krakow, Poland.

The substrate prepared for *P. djamor* cultivation was supplemented with magnesium salts: magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) and magnesium sulfate ($MgSO_4$) both from Warchem (Warszawa, Poland). These magnesium salts ($MgCl_2 \cdot 6H_2O$ and $MgSO_4$) we applied to the substrate at three concentrations (i) 50% of dietary value (DV) for Mg, namely 210 mg of Mg in a form of chloride ($MgCl_2 \times 0.5$) and sulfate ($MgSO_4 \times 0.5$) per 2 kg of substrate, (ii) 100% of DV for Mg, namely 420 mg of Mg ($MgCl_2 \times 1$ and $MgSO_4 \times 1$); and (iii) 1000% of DV for Mg, namely 4200 mg of Mg ($MgCl_2 \times 10$ and $MgSO_4 \times 10$). The DV used was based on the 2015–2020 Dietary Guidelines for Americans for Mg, equal to 420 mg per day for adult men [32]. Thus, the experimental layout was as follows: two Mg salts (first source of variation) and three concentrations of both Mg salts (additional source of variation).

2.2. Substrate Preparation

In the first stage of the experiment, rye grain (from local farmer) mycelium was prepared. Hydrated rye was placed in polypropylene bags with a microfilter and sterilized at 121 °C for 1.5 h at 1 atm pressure in an ASVE steam sterilizer (SMS, Poland). After cooling, grain was inoculated with fresh *P. djamor* mycelium, and incubated at 23 °C, in darkness until the rye grain was fully colonized with mycelium. The cultivation substrate was prepared from hardwood beech pellets (Biomasa Magdalena Małaczyńska, Trześń, Poland) and wheat bran (from local farmer) in a ratio of 5:1 (*v*:*v*) with the addition of 1% dry weight (DW) of gypsum, which is the standard cultivation medium used in our laboratory. Substrate was hydrated to obtain a moisture level of 65%, mixed, and placed in polypropylene bags with a microfilter in an amount of 2 kg of substrate per bag. The magnesium salts were added to each bag in the concentrations described above and mixed with substrate (three bags per combination of salts). Substrate in bags was sterilized for 1.5 h at 121 °C and pressure of 1 atm in an ASVE steam sterilizer (SMS, Poland). The cooled bags were inoculated with 3% rye grain mycelium obtained in the first stage, and the entire mix was molded into cubes, which were incubated at 23 ± 1 °C. After being fully colonized by mycelium, the cultivation cubes were placed in a cultivation chamber, in which constant growing conditions were maintained: 90 ± 3% humidity, 18 ± 2 °C, and a photoperiod of 12 h of light intensity of 11 μmol s^{-1} m^{-2}. Fruiting bodies were collected when they reached the harvesting maturity, which meant before the spores' release. Mushrooms were harvested in 3 flushes—the first flush was used for fresh material analyzed; all homogenized flushes were used for dry weight analysis.

2.3. Dry Weight and Biological Efficiency

Mushrooms were weighed with a Sartorius A120S balance (Sartorius AG, Göttingen, Germany) to determine the fresh weight (FW) and oven dried at 65 °C until a constant weight was obtained to determine the dry weight. Then, the difference in weight was calculated and expressed as the FW percentage. Biological efficiency was calculated by relating the total harvest from 3 flushes to 1000 g of dry substrate (weight of harvest/weight of dry substrate) × 100%.

2.4. Determination of Total Phenols Content

The concentration of total phenols in mushroom extracts was estimated by the Folin–Ciocalteu colorimetric procedure described by Djeridane et al. [33] with modifications. Two grams of fresh mushroom material was mixed with 10 mL of 80% methanol (Chempur, Gliwice, Poland) and centrifuged for 10 min at 3492 g. Next, mushroom extracts (0.1 mL) were mixed with 2 mL of 2% sodium carbonate (Na_2CO_3) (Warchem, Warszawa, Poland); after 2 min, 0.1 mL of Folin–Ciocalteu reagent (Sigma-Aldrich, Darmstadt, Germany), mixed with deionized water (1:1 v/v), was added to the test tubes. The final mixture was shaken and then incubated for 45 min in the dark at room temperature before the absorbance was measured at 750 nm using an ultraviolet–visible (UV-VIS) Helios Beta spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA USA) against a reference solution containing 0.1 mL of methanol instead of 0.1 mL of mushroom extract. The results were determined from a standard gallic acid curve and are expressed as milligrams of gallic acid equivalents (GAE) per gram FW (mg GAE g^{-1} FW).

2.5. Determination of Antioxidant Activity Using DPPH• (2,2-Diphenyl-1-picrylhydrazyl) Method

The antioxidant activity (AA) was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH•) (Sigma-Aldrich, Darmstadt, Germany) as a free radical [34]. The decrease in absorbance was measured at 517 nm with an UV-VIS Helios Beta spectrophotometer. Mushroom extracts used in total phenols content were used. The test tubes contained 0.1 mL of supernatant and 4.9 mL of 0.1 mM DPPH• dissolved with 80% methanol. The reaction mixture was shaken and incubated in the dark at 20 °C for 15 min. The following formula was used to calculate DPPH• radical scavenging activity: AA (%) = ((A0 − A1)/A0) × 100; AA—antioxidant activity, A0—absorbance of the reference solution, and A1—absorbance of the test solution. AA was expressed as the percentage of DPPH• free radical scavenging

2.6. Total Soluble Sugars

Total soluble sugars were determined using the anthrone method described by Yemm and Willis [35]. Fresh mushroom material (10 g) was mixed with 80% ethanol (Warchem, Warszawa, Poland) and anthrone reagent (Sigma-Aldrich, Darmstadt, Germany), then the absorbance was measured at 625 nm with a Helios Beta spectrophotometer.

2.7. L-Ascorbic Acid Content

The content of L-ascorbic acid was determined by Tillman's titration method as described by Krełowska-Kułas [36]. Fresh mushroom material (12.5 g) was homogenized with 50 mL acetic acid (Warchem, Warszawa, Poland) applied as an acidity regulator. After 30 min, the mixture was titrated with Tillman's reagent (2,6-dichlorophenol-indophenol) (Sigma-Aldrich, Darmstadt, Germany). Excessive dye in an acidic environment gives a pink color and marks the end point of the titration. Then, the content of L-ascorbic acid in the sample was calculated based on the amount of the changed solution of 2,6-dichlorophenol-indophenol used for titration.

2.8. Glutathione Content

The reduced form of glutathione (GSH) was determined according to the method described by Guri [37] with some modifications. Fresh mushroom material (2.5 g) was homogenized in an ice bath (4 °C) with the addition of 6 mL of 0.5 mM ethylenedinitrilotetraacetic acid (EDTA) and 3% trichloroacetic acid (TCA) both from Sigma-Aldrich (Darmstadt, Germany), and the homogenate was centrifuged at 4 °C for 10 min at 6208 g. To bring the pH of the solution to ca. 7.0, K-phosphate buffer (Pol-Aura, Dywity, Poland) was used. The content of reduced GSH was assessed using Ellman's reagent (5,5-dithiobis-2-nitrobenzoic acid, DTNB) (Sigma-Aldrich, Darmstadt, Germany) on a Helios Beta spectrophotometer. The solution extinction was measured at the wavelength of λ = 412 nm. The absorbance of a mixture of 2.0 mL of mushroom homogenate and 1 mL of 0.2 M K-phosphate buffer, which absorbed part of the radiation was measured as a blind sample. The concentration of GSH was calculated from the standard curve and expressed as μg g^{-1} FW.

2.9. Analysis of Bioelements

Dried fruiting bodies were powdered in an Pulverisette 14 ball mill (Fritsch GmbH, Idar-Oberstein, Germany; 0.5 mm sieve). Samples were analyzed for the content of Mg, K, Ca, Fe, Zn, and Cu. Three independent samples (0.2 g) were weighed from each of the dried mushroom materials and were transferred into Teflon vessels containing 2 mL of 30% H_2O_2 and 6 mL 65% HNO_3 both from Merck (Darmstad, Germany). Then, the samples were subjected to wet mineralization in a closed system in a Magnum II mineralizer (ERTEC, Wrocław, Poland). The mineralized solution obtained was heated on a hot-plate for 60 min at 120 °C to remove excess reagents. Subsequently, all the samples were quantitatively transferred to 10 mL flasks and topped with quadruple distilled water. To determine elements, flame atomic absorption spectrometry (FAAS) was used. For all the measurements, an atomic absorption spectrometer by Thermo Scientific (Model iCE 3500, Cambridge, UK) was used.

2.10. Determination of Chloride and Sulfate Ions

Cl^- and $SO_4{}^{2-}$ were determined using spectrophotometry with a Spectroquant Nova 60 spectrophotometer (Merck KGaA, Darmstadt, Germany). We used validated tests to determine $SO_4{}^{2-}$ (Cat. No. 101812, Merck KGaA, Darmstadt, Germany) and Cl^- (Cat. No. 114897, Merck KGaA, Darmstadt, Germany). Spectrophotometric determinations were performed in quartz cuvettes. The results of the determination of the content of Cl^- and $SO_4{}^{2-}$ anions are presented as mean values from three independent measurements.

2.11. Analysis of Organic Compounds—Preparation of the Extract

Dried fruiting bodies were powdered in an Pulverisette 14 ball mill. Three grams of mushroom material was extracted with methanol by ultrasound (49 kHz for 30 min; Sonic-2, Polsonic, Warszawa, Poland). The extraction was repeated thrice for each sample. The obtained extracts were combined (300 mL) and evaporated to dryness. Subsequently, the extracts were quantitatively dissolved in high-performance liquid chromatography (HPLC)-grade methanol and filtered using membrane filters.

2.11.1. Determination of Phenylalanine and Phenolic Acids

The analysis of the content of phenylalanine and phenolic acids in the tested samples was carried out according to the procedure proposed by Sułkowska-Ziaja et al. [38]. The analysis was performed using reversed-phase high-performance liquid chromatography (RP-HPLC VWR, Hitachi-Merck, Tokyo, Japan) equipped with a DAD (diode array detector) (L-2455) (λ = 254 nm) and autosampler (L-2200), pump (L-2130), and RP-18e LiChrospher column (4 × 250 mm, 5 μm) kept at 25 °C. The mobile phase was prepared as follows: solvent A: methanol/0.5% acetic acid 1:4 (v/v) and solvent B: methanol. The gradient was set as follows: 100:0 time 0–25 min; 70:30 time 35 min; 50:50 time 45 min; 0:100 time 50–55 min; 100:0 time 57–67 min. The identification of the compounds was undertaken by

comparing the obtained spectra with the spectra of the standard compounds (purity ≥99.0% from Sigma-Aldrich) and the compounds of the spectrum were compared. Quantitative analysis was performed using a calibration curve. The content of compounds is expressed in mg/100 g dry weight.

2.11.2. Analysis of Indole Compounds

The extracts were analyzed for the content of indole compounds using the RP-HPLC method with UV detection [39]. The prepared extracts were quantitatively dissolved in 1.5 mL of the solvent mixture and the components were separated via RP-HPLC method (Hitachi RP-HPLC with UV detection, Merck, Tokyo, Japan) equipped with an L-7100 type pump. The Purospher® RP–18 column (4 × 200 mm, 5 µm) was maintained at 25 °C and equipped with a UV detector (λ = 280 nm). The applied liquid phase consisted of a mixture of methanol/water/ammonium acetate (Chempur, Gliwice, Poland) (15:14:1 v/v). The flow rate was established at 1 mL min^{-1}. Indole compounds were quantitatively analyzed with the help of a calibration curve and with the assumption of linearity of the size of the area tested under the peak relative to the concentration of the standard used (purity ≥99.0% from Sigma-Aldrich, St. Louis, MO, USA).

2.11.3. Analysis of Lovastatin

To determine the content of lovastatin the RP-HPLC method was used according to the method described by Pansuriya and Singhal [40]. The process was carried out in an isocratic system with a mobile phase of constant composition. The apparatus was equipped with a UV detector (λ = 238 nm), a column (Purospher® RP-18, 14 × 200 mm, 5 µm), and a lamp (L-7100). All the measurements were carried out using a previously prepared developing system (acetonitrile and 0.1% phosphoric acid at a ratio of 60:40 v:v) (Chempur, Gliwice, Poland). Lovastatin was quantitatively analyzed with the help of a calibration curve and with the assumption of linearity of the size of the area tested under the peak relative to the concentration of the compound standard (purity ≥99.0% from Sigma-Aldrich, St. Louis, MO, USA) used.

2.12. Determination of Vitamin B_1 (Thiamine) and B_2 (Riboflavin) Content

The content of vitamins B_1 and B_2 in freeze-dried *P. djamor* was determined by the HPLC method [41,42]. The aforementioned vitamins were determined after the oxidation reaction before the column using potassium hexacyanoferrate(III) solution, purity ≥99.0% from Sigma-Aldrich, Merck KGaA, Darmstadt, Germany. Later, reaction and centrifugation extracts were cleaned on solid phase extraction (SPE) using Chromabond C18 columns (3 mL/200 mg). Finally, 20 µL of sample was injected into the HPLC system. An HPLC chromatograph (LaChrome, Merck, Hitachi, Tokyo, JP) equipped, inter alia, with an autosampler, fluorescent detector, and thermostat oven columns (Merck), was used for the detection of vitamins. The analysis was performed on a Bionacom Velocity C18 PLMX column (4.6 × 250 mm, 5 µm), together with the precolumn (Bionacom LTD (London, UK). The measurement was made at the excitation and emission wavelengths of 360 and 503 nm, respectively, enabling the simultaneous determination of thiamine and riboflavin. The mobile phase used was a mixture of water (W) and acetonitrile (A) (acetonitrile for HPLC, POCH, Avantor Performance Materials Poland S.A., Gliwice, Poland). Gradient elution was performed as follows: t = 0, ratio 88:12 W/A; t = 12, ratio 0:100 W/A, temperature 22 °C. The external standards of thiamine (thiamine hydrochloride in HCl, purity ≥99.0% from Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) and riboflavin (riboflavin in CH_3CO_2H, purity ≥98.0% from Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) were used for the identification of these compounds and their quantitative analysis.

2.13. Data Analysis

The experiment was established using 3 repetitions per treatment, and the results are presented as the mean of 3 repetitions ± standard deviation (SD). Statistical analysis

was performed with the Statistica 13.3 package (TIBCO Software Inc., Palo Alto, CA, USA). Differences between particular parameters were analyzed using one-way analysis of variance (ANOVA) and Tukey's test, and *p*-values of less than or equal to 0.05 were considered to be statistically significant. The results were also examined for Pearson's correlation coefficient (*r*) between analyzed parameters. Principal component analysis (PCA) was performed, and the first two components (PC1 and PC2) were used to derive biplots.

3. Results

3.1. Bioelements in Pleurotus Djamor Fruiting Bodies and Growing Substrate

The content of elements in the fruiting bodies of *P. djamor* was differentiated depending on the type and concentration of magnesium salts used in the experiment (Table 1). The highest content of magnesium was determined in mushrooms collected from substrate supplemented with the highest concentration of $MgCl_2 \times 10$ and $MgSO_4 \times 10$. These supplementation variants increased Mg content in fruiting bodies by 185% on average, compared to the control. The remaining supplementation treatments increased the content of Mg by 127% on average, and no significant differences were noted according to the salts used and their concentrations. Supplementation with $MgCl_2 \times 0.5$ resulted in the highest contents of potassium, iron, and copper in *P. djamor* fruiting bodies, whereas the lowest contents of these elements were found in samples from $MgSO_4 \times 1$ (K), $MgSO_4 \times 10$ (Fe), and $MgSO_4 \times 0.5$ (Cu) treatments. Moreover, K content was positively correlated with Fe ($r = 0.542$, $p \leq 0.05$) and Fe with Cu ($r = 0.506$, $p \leq 0.05$). The content of Ca and Zn was the highest in fruit bodies collected from control treatments. The antagonistic relation between Mg and Zn was confirmed by the correlation coefficient between these elements in *P. djamor* fruiting bodies ($r = -0.537$, $p \leq 0.05$). In general, the content of all elements was significantly lower in treatments supplemented with $MgSO_4$ in comparison to $MgCl_2$. The content of SO_4^{2-} and Cl^- was higher in fruiting bodies supplemented with $MgSO_4$ and $MgCl_2$, respectively, as compared to the control. The correlation matrix for all the elements is demonstrated in subchapter 3.9.

Table 1. The content of elements and anions in fruiting bodies and growing substrate of *P. djamor* depending on magnesium salts supplementation (mg 100 g^{-1} DW).

Treatment	Mg	K	Ca	Fe	Zn	Cu	Cl^-	SO_4^{2-}
Control	783 ± 28 [a1]	658 ± 6 [b]	27.2 ± 2.9 [c]	6.63 ± 0.44 [c]	43.3 ± 7.2 [b]	4.03 ± 0.25 [c]	81 ± 11 [a]	428 ± 25 [a]
$MgCl_2 \times 0.5$ [2]	1015 ± 23 [b]	759 ± 20 [c]	25.1 ± 0.9 [bc]	6.52 ± 0.81 [c]	18.6 ± 3.1 [a]	4.69 ± 0.03 [d]	192 ± 10 [b]	n.a. [3]
$MgCl_2 \times 1$	934 ± 81 [b]	682 ± 29 [bc]	20.9 ± 0.8 [b]	5.95 ± 0.01 [bc]	15.2 ± 1.5 [a]	5.56 ± 0.02 [e]	275 ±13 [c]	n.a.
$MgCl_2 \times 10$	1455 ± 22 [c]	624 ± 28 [ab]	24.3 ± 1.9 [bc]	6.42 ± 0.69 [c]	17.1 ± 2.2 [a]	5.99 ± 0.26 [e]	351 ± 11 [d]	n.a.
$MgSO_4 \times 0.5$	1008 ± 25 [b]	614 ± 23 [ab]	11.9 ± 1.3 [a]	5.71 ± 0.07 [abc]	13.9 ± 0.1 [a]	2.28 ± 0.28 [a]	n.a.	694 ± 25 [b]
$MgSO_4 \times 1$	1014 ± 15 [b]	564 ± 55 [a]	15.6 ± 1.4 [a]	4.73 ± 0.21 [ab]	12.2 ± 0.9 [a]	3.51 ± 0.26 [bc]	n.a.	765 ± 12 [b]
$MgSO_4 \times 10$	1434 ± 8 [c]	640 ± 9 [ab]	13.4 ± 0.9 [a]	4.64 ± 0.14 [a]	11.3 ± 0.9 [a]	3.05 ± 0.31 [b]	n.a.	1043 ± 76 [c]
Growing substrate	921 ± 23	47 ± 3	37.7 ± 2.6	1.77 ± 0.21	3.5 ± 0.6	4.25 ± 0.31	64 ± 9	* [4]

[1] Means in a column followed by different superscript letters (a,b,c,d,e) are significantly different $p \leq 0.05$ according to Tukey's test, N = 6. Each value represents the mean ± standard deviation. [2] $MgCl_2 \times 0.5$—210 mg of Mg, $MgCl_2 \times 1$—420 mg of Mg, $MgCl_2 \times 10$—4200 mg of Mg (in a form of $MgCl_2$ per 2 kg of substrate); $MgSO_4 \times 0.5$—210 mg of Mg, $MgSO_4 \times 1$—420 mg of Mg, $MgSO_4 \times 10$—4200 mg (in a form of $MgSO_4$ per 2 kg of substrate). [3] n.a. = Not analyzed in a sample. [4] * = Below the detection level.

3.2. Biological Efficiency and Dry Weight Content

The highest biological efficiency was obtained for treatments supplemented with $MgCl_2 \times 0.5$ (73%) and the control (69%) (Figure 1). Fortification with the remaining concentrations of $MgCl_2$ and $MgSO_4$ decreased the biological efficiency of *P. djamor*; the lowest was noted for the maximum dose of Mg salts used in the experiment. The correlation coefficient between Mg content and biological efficiency was $r = -0.769$, $p \leq 0.001$ as it was demonstrated in subchapter 3.9. DW of fruiting bodies varied slightly but significantly between treatments (11.7–13.1 g 100 g^{-1} FW). The highest DW was determined in fruiting

bodies collected from substrate supplemented with $MgSO_4 \times 10$, whereas the lowest related to the substrate supplemented with $MgSO_4 \times 0.5$. Fruit bodies from remaining treatments were not differentiated significantly concerning dry matter content. Contrary to biological efficiency, the dry weight of fruiting bodies was positively correlated with substrate supplementation with Mg ($r = 0.547$, $p \leq 0.01$) as it was demonstrated in Section 3.9.

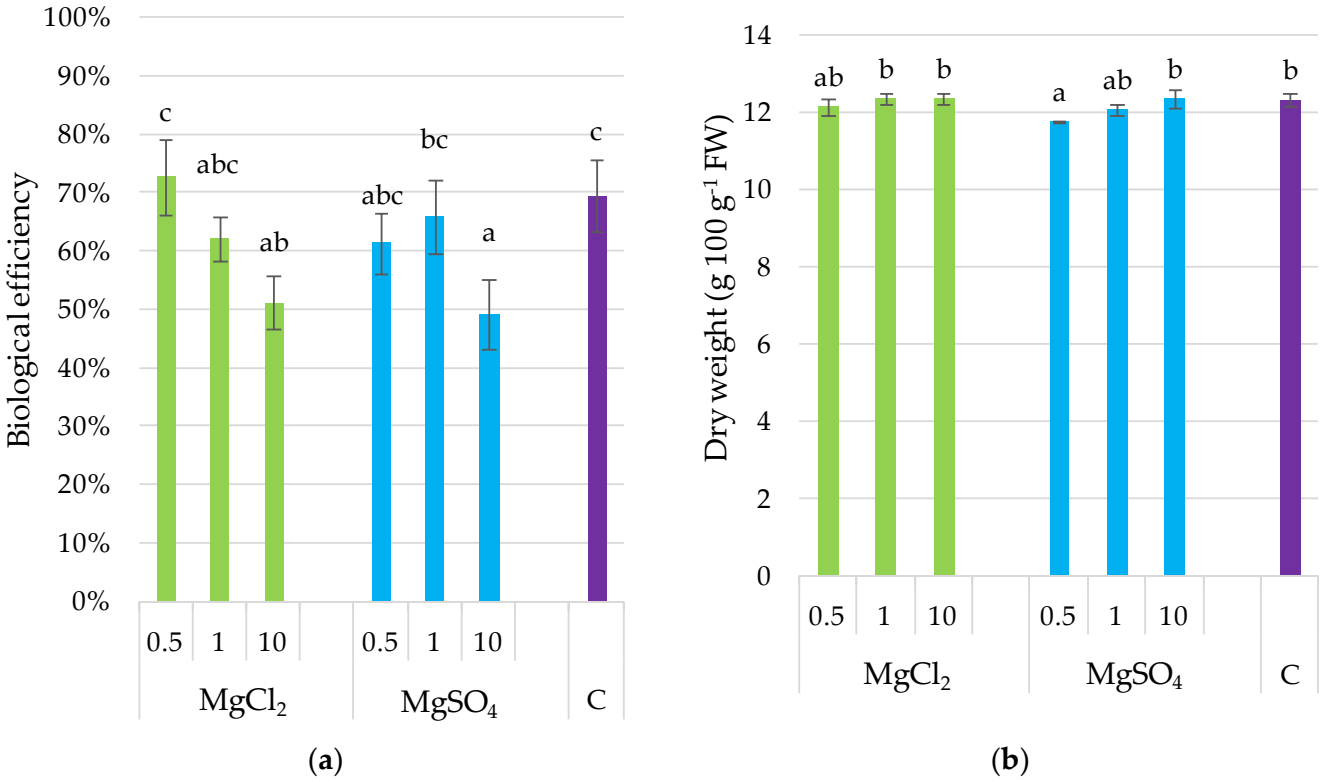

Figure 1. Biological efficiency (**a**) and dry weight (**b**) content in *P. djamor* fruiting bodies depending on Mg salts supplementation, Bars marked with different letters (a,b,c) are significantly different $p \leq 0.05$ according to Tukey's test N = 6. $MgCl_2 \times 0.5$—210 mg of Mg; $MgCl_2 \times 1$—420 mg of Mg; $MgCl_2 \times 10$—4200 mg of Mg (in the form of $MgCl_2$ per 2 kg of substrate); $MgSO_4 \times 0.5$—210 mg of Mg; $MgSO_4 \times 1$—420 mg of Mg; $MgSO_4 \times 10$—4200 mg (in the form of $MgSO_4$ per 2 kg of substrate); C—standard growing medium.

3.3. Total Phenolics and DPPH• Scavenging Activity

Total phenolic content and DPPH• scavenging activity were statistically differentiated; the highest was in fruiting bodies collected from a substrate supplemented with $MgCl_2 \times 10$ (Figure 2), which was 50% higher than that of the control. Generally, supplementation with magnesium increased both parameters, with the exception of the $MgSO_4 \times 0.5$ and $MgSO_4 \times 1$ treatments for DPPH•. The stimulative effect of the Mg supplementation of the substrate on the phenolic content and DPPH• scavenging activity in *P. djamor* fruiting bodies was supported by the coefficients of correlation between these parameters ($r = 0.888$, $p \leq 0.001$ for Mg and phenolics; $r = 0.818$, $p \leq 0.001$ for Mg and DPPH• scavenging activity) as it was demonstrated in Section 3.9.

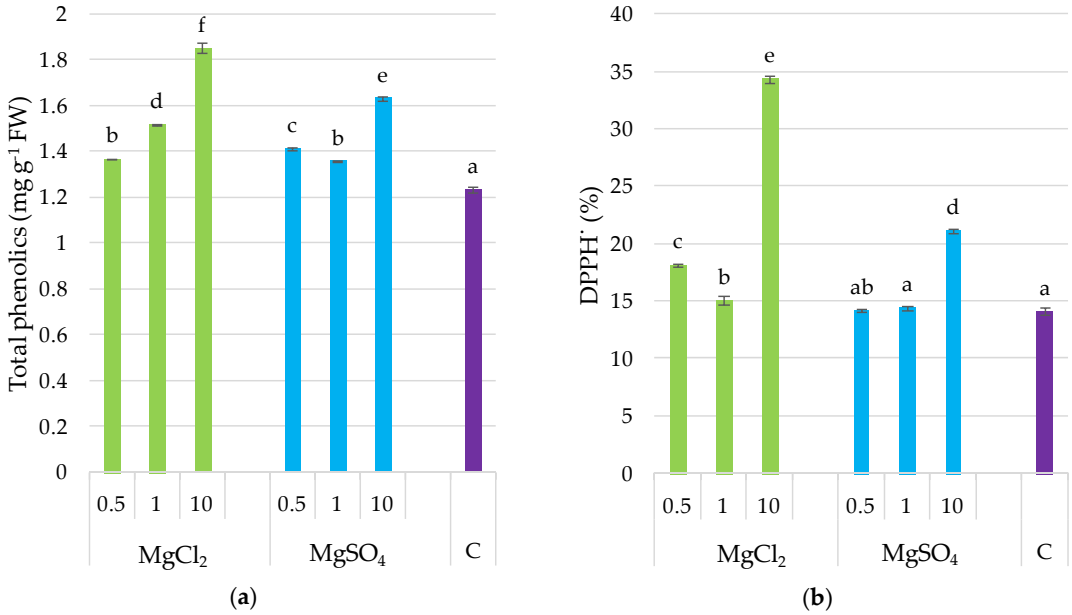

Figure 2. Total phenolics content and DPPH• scavenging activity in *P. djamor* fruiting bodies depending on Mg salts supplementation. (**a**) Total phenolics content; (**b**) DPPH• scavenging activity. Each bar represents mean value ± standard deviation. Bars marked with different letters (a,b,c,d,e,f) are significantly different $p \leq 0.05$ according to Tukey's test, N = 6. $MgCl_2 \times 0.5$—210 mg of Mg; $MgCl_2 \times 1$—420 mg of Mg; $MgCl_2 \times 10$—4200 mg of Mg (in the form of $MgCl_2$ per 2 kg of substrate); $MgSO_4 \times 0.5$—210 mg of Mg; $MgSO_4 \times 1$—420 mg of Mg; $MgSO_4 \times 10$—4200 mg (in the form of $MgSO_4$ per 2 kg of substrate); C—standard growing medium.

3.4. Soluble Sugar and L-Ascorbic Acid

Supplementation of the growing substrate with Mg chlorides and sulfides affected in different ways the soluble sugar content in *P. djamor* fruiting bodies. $MgCl_2 \times 1$ and $MgCl_2 \times 10$ caused a significant increase in soluble sugar in the investigated mushroom species, whereas $MgSO_4 \times 1$ application caused a decrease in soluble sugars, in contrast to $MgCl_2 \times 10$. This dose application resulted in a significant increase in soluble sugar content (Figure 3). L-ascorbic acid was the highest for fruit bodies sampled from the $MgCl_2 \times 10$ treatment. Moreover, the determined value was 48% higher than that of other samples. Lower $MgCl_2$ doses did not significantly affect L-ascorbic acid content in *P. djamor*, similarly to the $MgSO_4 \times 0.5$ treatment. Higher $MgSO_4$ doses increased L-ascorbic acid content in fruiting bodies of *P. djamor*. In general, a positive correlation was noted for Mg and L-ascorbic acid content ($r = 0.817$, $p \leq 0.001$) as it was demonstrated in Section 3.9.

3.5. Thiamine and Riboflavin

The supplementation of *P. djamor* substrate with $MgSO_4$ at all doses significantly increased the thiamine content in fruiting bodies (by 21% on average for the doses) and riboflavin (with the exception of $MgSO_4 \times 1$) (Figure 4). Moreover, thiamine content was positively correlated with Mg ($r = 0.545$, $p \leq 0.05$), but negatively correlated with the other elements, including Ca ($r = -0.786$, $p \leq 0.001$), Fe ($r = -0.793$, $p \leq 0.001$), Zn ($p = -0.548$, $p \leq 0.001$), and Cu ($r = -0.604$, $p \leq 0.01$) as it was demonstrated in subchapter 3.9. $MgCl_2$ application to the growing substrate had no significant effect on the thiamine content in fruiting bodies, but caused a decrease in riboflavin, when Mg salts were applied in doses of $MgCl_2 \times 0.5$ and $MgCl_2 \times 10$. Riboflavin content was negatively correlated with K ($r = -0.621$, $p \leq 0.01$), Ca ($p = -0.753$, $p \leq 0.001$), Fe ($r = -0.507$, $p \leq 0.01$), and Cu ($r = -0.712$, $p \leq 0.001$). Both vitamins of group B ware negatively correlated with glutathione content in *P. djamor* fruiting bodies ($r = -0.627$, $p \leq 0.05$, and $r = -0.442$, $p \leq 0.05$, respectively) as it was demonstrated in Section 3.9.

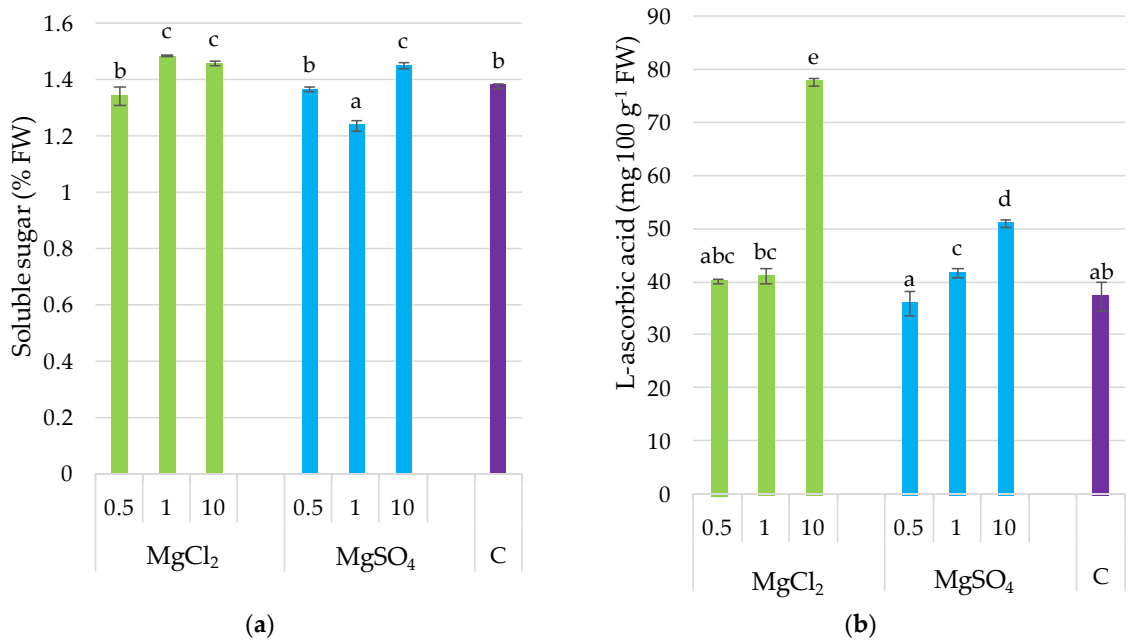

Figure 3. Soluble sugar and L-ascorbic acid content in *P. djamor* fruiting bodies depending on Mg salts supplementation. (**a**) Soluble sugar content; (**b**) L-ascorbic acid content. Each bar represents mean value ± standard deviation. Bars marked with different letters (a,b,c,d,e) are significantly different $p \leq 0.05$ according to Tukey's test, N = 6. $MgCl_2$ × 0.5—210 mg of Mg; $MgCl_2$ × 1—420 mg of Mg; $MgCl_2$ × 10—4200 mg of Mg (in the form of $MgCl_2$ per 2 kg of substrate); $MgSO_4$ × 0.5—210 mg of Mg; $MgSO_4$ × 1—420 mg of Mg; $MgSO_4$ × 10—4200 mg (in the form of $MgSO_4$ per 2 kg of substrate); C—standard growing medium.

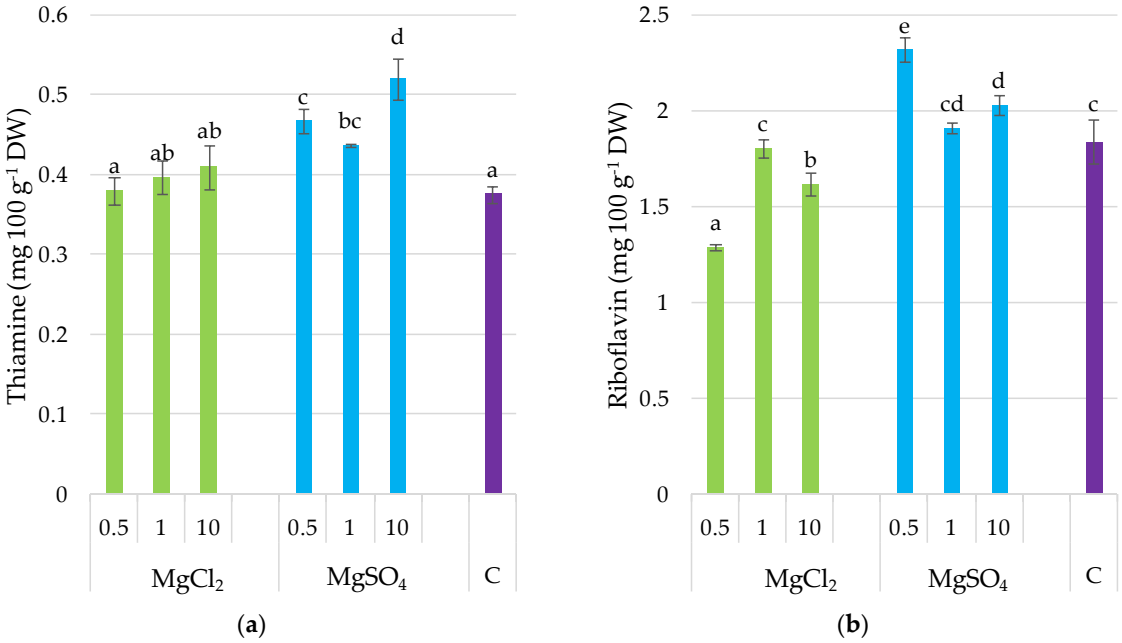

Figure 4. Thiamine and riboflavin content in *P. djamor* fruiting bodies depending on Mg salts supplementation. (**a**) Thiamine content; (**b**) Riboflavin content. Each bar represents mean value ± standard deviation. Bars marked with different letters (a,b,c,d,e) are significantly different $p \leq 0.05$ according to Tukey's test, N = 6. $MgCl_2$ × 0.5—210 mg of Mg; $MgCl_2$ × 1—420 mg of Mg; $MgCl_2$ × 10—4200 mg of Mg (in the form of $MgCl_2$ per 2 kg of substrate); $MgSO_4$ × 0.5—210 mg of Mg; $MgSO_4$ × 1—420 mg of Mg; $MgSO_4$ × 10—4200 mg (in the form of $MgSO_4$ per 2 kg of substrate); C—standard growing medium.

3.6. p-Hydroxybenzoic Acid and Phenylalanine

The application of $MgSO_4$ salts to the growing substrate caused an increase in *p*-hydroxybenzoic acid in *P. djamor* fruiting bodies, from 9% ($MgSO_4 \times 10$) to 46% ($MgSO_4 \times 1$) (Figure 5). Concerning the supplementation with chlorides, a significant effect of *p*-hydroxybenzoic acid in fruiting bodies was observed only for $MgCl_2 \times 0.5$ treatment. Substrate supplementation with $MgCl_2 \times 0.5$ and $MgSO_4 \times 0.5$ and $MgSO_4 \times 1$ significantly increased phenylalanine content in *P. djamor* fruiting bodies by eight, ten-, and nine-fold, respectively, relative to the control.

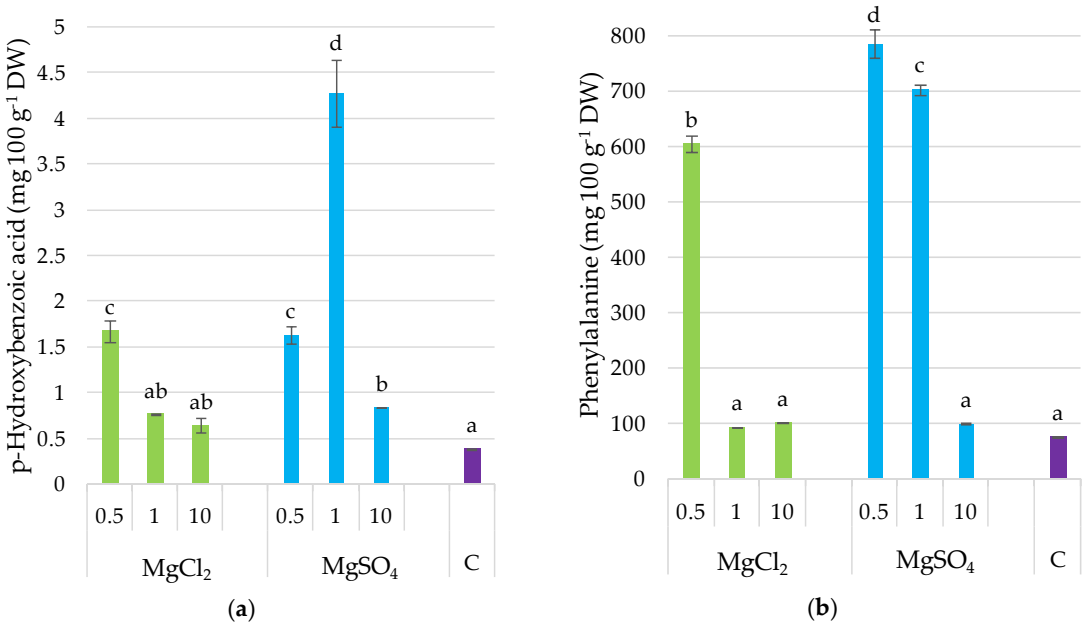

Figure 5. *p*-Hydroxybenzoic acid and phenylalanine content in *P. djamor* fruiting bodies depending on Mg salts supplementation. (**a**) *p*-Hydroxybenzoic acid content; (**b**) Phenylalanine content. Each bar represents mean value ± standard deviation. Bars marked with different letters (a,b,c,d) are significantly different $p \leq 0.05$ according to Tukey's test, N = 6. $MgCl_2 \times 0.5$—210 mg of Mg; $MgCl_2 \times 1$—420 mg of Mg; $MgCl_2 \times 10$—4200 mg of Mg (in the form of $MgCl_2$ per 2 kg of substrate); $MgSO_4 \times 0.5$—210 mg of Mg; $MgSO_4 \times 1$—420 mg of Mg; $MgSO_4 \times 10$—4200 mg (in the form of $MgSO_4$ per 2 kg of substrate); C—standard growing medium.

3.7. L-Tryptophan and 5-Hydroxy-L-tryptophan

The supplementation of the substrate with both chlorides and sulfates of Mg caused a significant increase in L-tryptophan content in *P. djamor* fruiting bodies. A similar observation was noted for 5-hydroxy-L-tryptophan, with the exception of the $MgCl_2 \times 1$ treatment. L-tryptophan was positively correlated with Mg content ($r = 0.643$, $p \leq 0.05$) (Figure 6) as it was demonstrated in Section 3.9.

3.8. Glutathione and Lovastatin

The glutathione content was the highest in the control. Generally, supplementation decreased glutathione content, with the lowest value noted for the treatment $MgSO_4 \times 1$ (Figure 7). Generally, supplementation with $MgCl_2$ reduced glutathione content to a lower extent (by 15%, average for doses), whereas $MgSO_4$ increased it to a higher extent (by 46%, average for doses), compared to the control. It is interesting that glutathione content was positively correlated with crucial mineral compounds, including K ($r = 0.680$), Ca ($r = 0.751$), Fe ($r = 0.826$), and Zn ($r = 0.688$), with $p \leq 0.001$ in all cases (Figure 8). Lovastatin content was differentiated without any regular trend for experimental treatments. Only substrate supplementation with $MgSO_4 \times 0.5$ significantly increased the content of this compound in *P. djamor* fruiting bodies by 48% compared to the control.

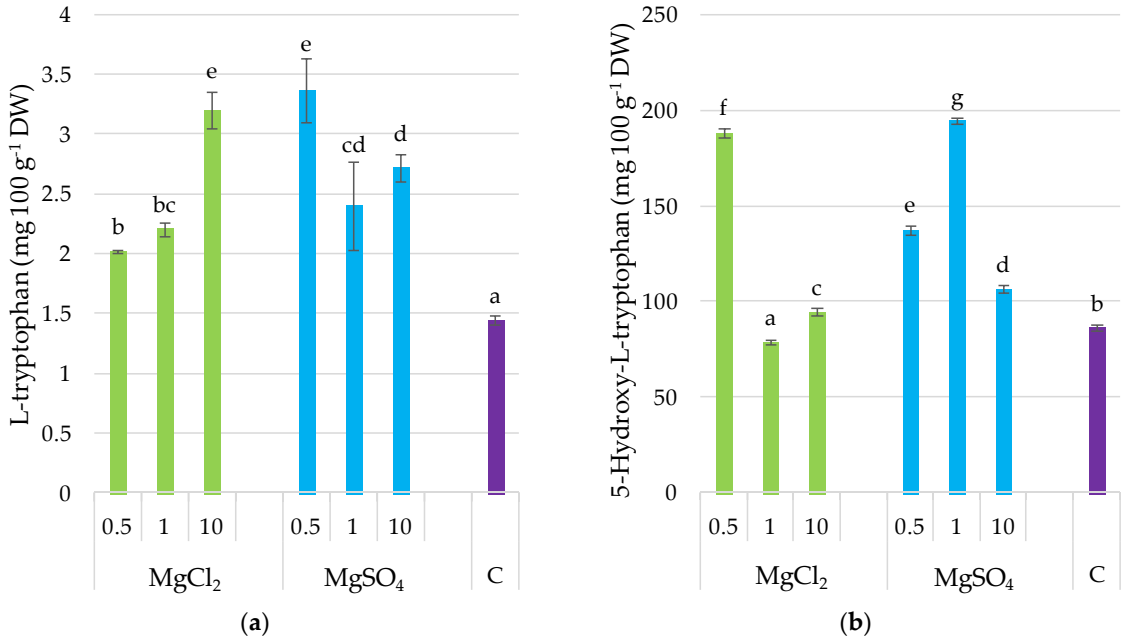

Figure 6. L-Tryptophan and 5-hydroxy-L-tryptophan content in *P. djamor* fruiting bodies depending on Mg salts supplementation. (**a**) L-Tryptophan content; (**b**) 5-hydroxy-L-tryptophan content. Each bar represents mean value ± standard deviation. Bars marked with different letters (a,b,c,d,e,f,g) are significantly different $p \leq 0.05$ according to Tukey's test, N = 6. $MgCl_2$ × 0.5—210 mg of Mg; $MgCl_2$ × 1—420 mg of Mg; $MgCl_2$ × 10—4200 mg of Mg (in the form of $MgCl_2$ per 2 kg of substrate); $MgSO_4$ × 0.5—210 mg of Mg; $MgSO_4$ × 1—420 mg of Mg; $MgSO_4$ × 10—4200 mg (in the form of $MgSO_4$ per 2 kg of substrate); C—standard growing medium.

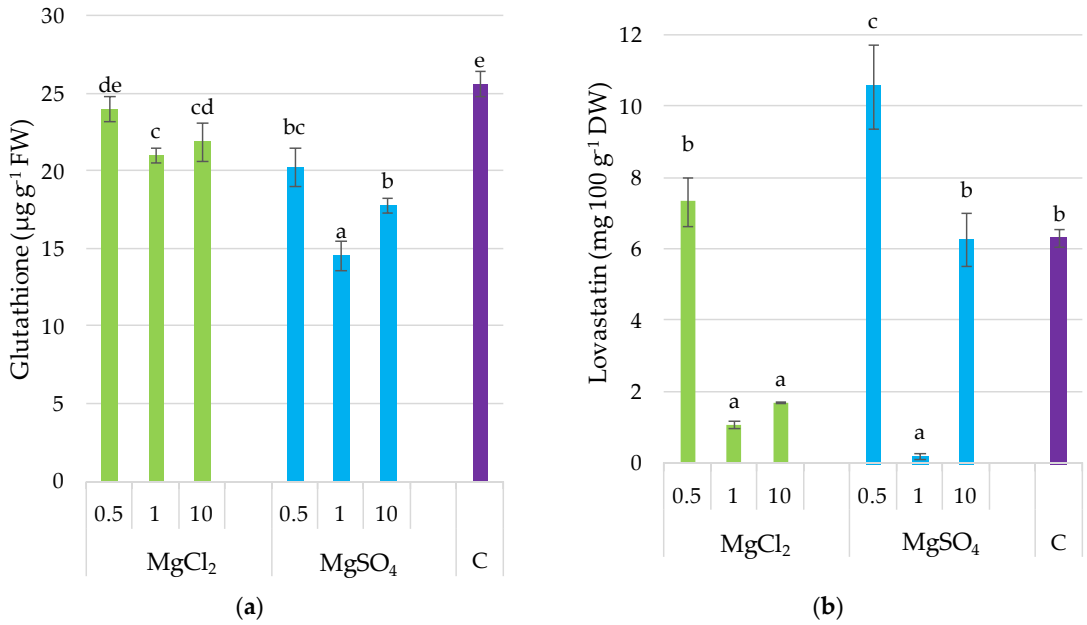

Figure 7. Glutathione and lovastatin content in *P. djamor* fruiting bodies depending on Mg salts supplementation. (**a**) Glutathione content; (**b**) Lovastatin content. Each bar represents mean value ± standard deviation. Bars marked with different letters (a,b,c,d,e) are significantly different $p \leq 0.05$ according to Tukey's test, N = 6. $MgCl_2$ × 0.5—210 mg of Mg; $MgCl_2$ × 1—420 mg of Mg; $MgCl_2$ × 10—4200 mg of Mg (in the form of $MgCl_2$ per 2 kg of substrate); $MgSO_4$ × 0.5—210 mg of Mg; $MgSO_4$ × 1—420 mg of Mg; $MgSO_4$ × 10—4200 mg (in the form of $MgSO_4$ per 2 kg of substrate); C—standard growing medium.

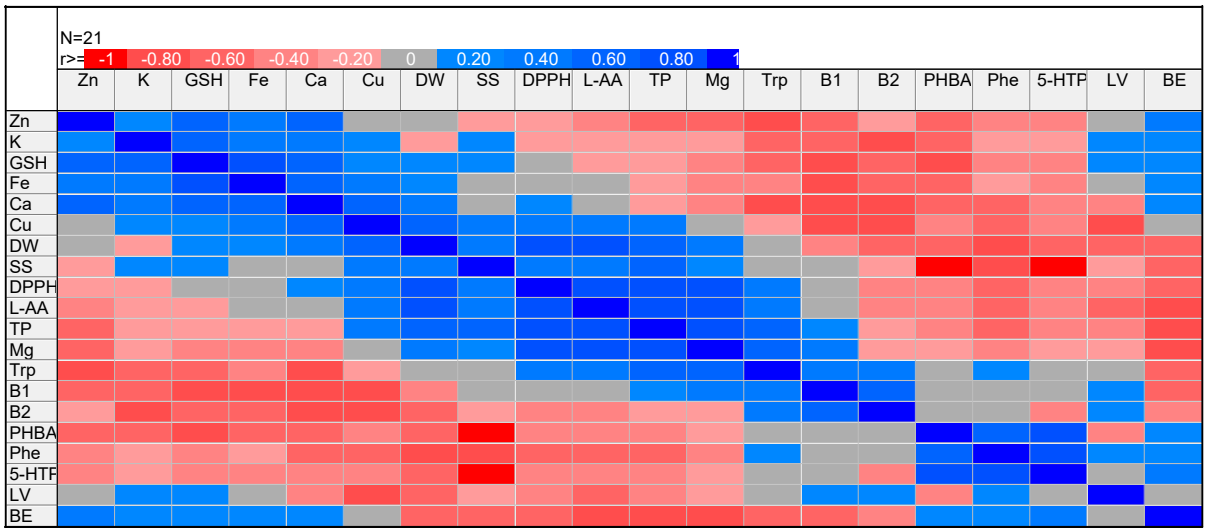

Figure 8. Correlation matrix of *P. djamor* productivity and quality parameters. Abbreviations: Zn—zinc, K—potassium, GSH—glutathione, Fe—iron, Ca—calcium, Cu—copper, DW—dry weight, SS—soluble sugar, DPPH—DPPH• scavenging activity, L-AA—L-ascorbic acid, TP—total phenolics, Mg—magnesium, Trp—L-tryptophan, B_1—thiamine, B_2—riboflavin, PHBA—*p*-hydroxybenzoic acid Phe—phenylalanine, 5-HTP—5-hydroxy-L-tryptophan, LV—lovastatin, BE—biological efficiency.

3.9. Correlation Matrix and Bi-Plot Presentation of Correlations

PCA illustrated that $MgSO_4 \times 0.5$ and $MgSO_4 \times 1$ treatments contributed significantly and negatively to PC 1, whereas $MgSO_4 \times 10$ contributed significantly and negatively to PC 2. $MgCl_2 \times 10$ treatment contributed significantly and negatively to PC 2 but positively to PC 1. $MgCl_2 \times 0.5$ contributed significantly and positively to PC 2 (Figure 9). Based on the PCA analysis (Figure 10), magnesium results were in contrast to those of biological efficiency and most of the other bioelements analyzed (Figure 5a,b). Concerning organic compounds, magnesium was located in a separate group, consisting of parameters responsible for antioxidant activity, as total phenols, L-ascorbic acid, and L-tryptophan.

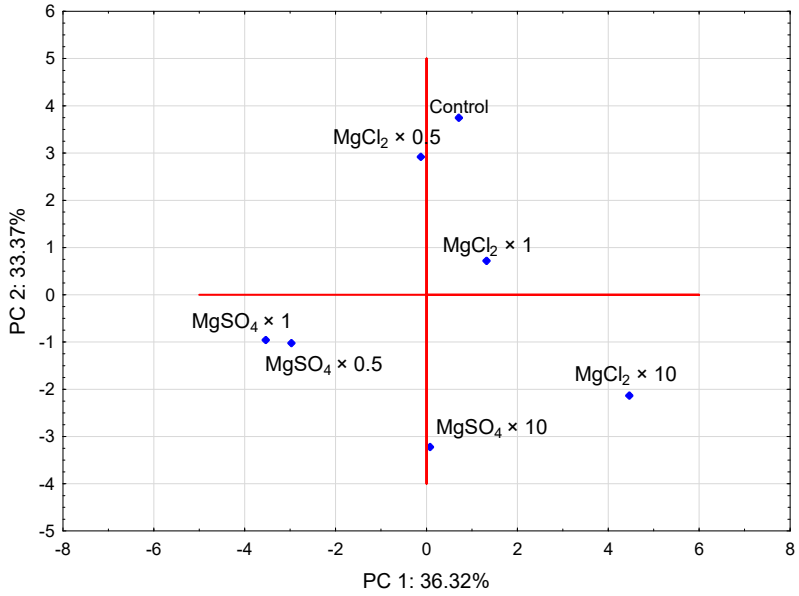

Figure 9. Bi-plot presenting the correlation between the tested treatments. $MgCl_2 \times 0.5$—210 mg of Mg, $MgCl_2 \times 1$—420 mg of Mg, $MgCl_2 \times 10$—4200 mg of Mg (in a form of $MgCl_2$ per 2 kg of substrate); $MgSO_4 \times 0.5$—210 mg of Mg, $MgSO_4 \times 1$—420 mg of Mg, $MgSO_4 \times 10$—4200 mg (in a form of $MgSO_4$ per 2 kg of substrate).

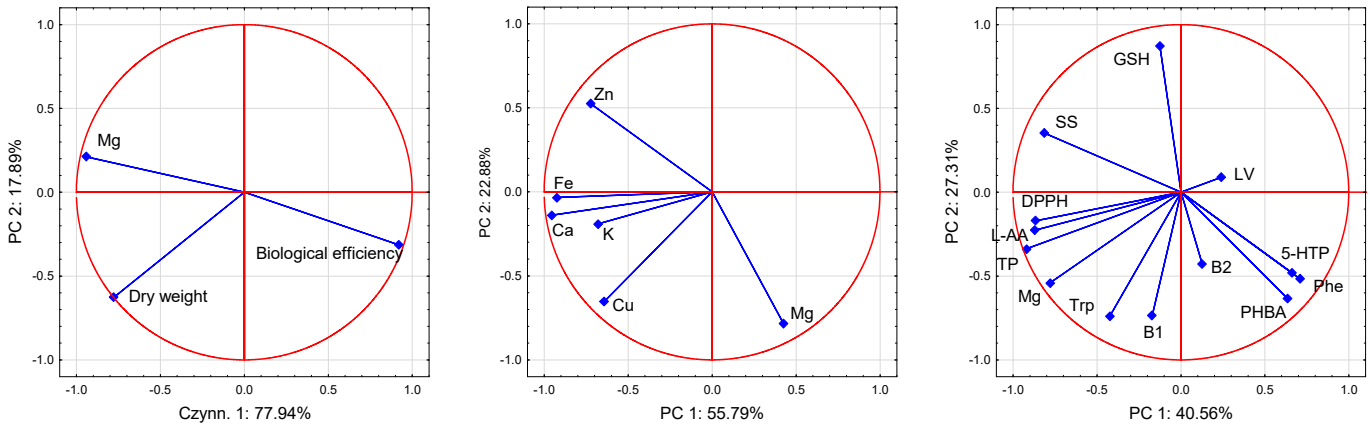

Figure 10. Bi-plot presenting the correlation between the tested magnesium (Mg) and yield quantity (**a**); macro- and micronutrients (**b**), and organic compounds and antioxidant efficiency (**c**). Abbreviations: Zn—zinc, K—potassium, GSH—glutathione, Fe—iron, Ca—calcium, Cu—copper, DPPH—DPPH• scavenging activity, 5-HTP—5-hydroxy-L-tryptophan, B_1—thiamine, B_2—riboflavin, L-AA—L-ascorbic acid, LV—lovastatin, PHBA—*p*-hydroxybenzoic acid, Phe—phenylalanine, SS—soluble sugar, TP—total phenolics, Trp—L-tryptophan.

4. Discussion

4.1. Bioelements in P. djamor Fruiting Bodies and Growing Substrate

The substrate supplementation with magnesium caused a significant increase of its concentration in the fruiting bodies of *P. djamor*; both Mg salts, $MgSO_4$ and $MgCl_2$, showed similar effectiveness when applied in the same doses. The remaining bioelement content was significantly lower in fruiting bodies collected from treatments supplemented with $MgSO_4$ in comparison to $MgCl_2$. The percentage of the dietary value (% DV) is used to define a food serving as high or low in an individual nutrient. In general, 5% DV or less of a nutrient per serving is considered low, whereas 20% DV or more of a nutrient per serving is considered high. The allowance for Mg from the 2015–2020 Dietary Guidelines for Americans is equal to 420 mg per day per adult [32]. A quantity of 100 g of fresh *P. djamor* fruiting bodies supplemented with Mg salts in the present research contained from 27% DV ($MgCl_2 \times 1$) to 41% DV ($MgCl_2 \times 10$); thus, supplementation can be an effective technique to produce mushrooms rich in Mg as a dietary component. Although *P. djamor* grown in supplemented substrate showed lower productivity, this was only evident in the fresh weight. Differences in dry weight were negligible and, in most treatments, comparable with the control.

In the present research fruiting bodies of *P. djamor*, not supplemented with Mg, contained Mg—738, K—658, Ca—27, Fe—6.6, Zn—0.1, and Cu—0.1 mg 100 g^{-1} DW. The analysis of the concentration of these elements showed both similarities and differences compared to the data from the literature [43,44]. For example, in the recent research of Mleczek et al. [25] the most abundant minerals found in *P. djamor* fruiting bodies were K 2000–2390, Ca 108–191, Mg 52–71, Fe 0.2–0.6, Zn 5,8, and Cu 1,3 mg 100 g^{-1} DW, depending on the substrate composition. Siwulski et al. [45] classified minerals detected in mushroom fruiting bodies into five groups; three of these comprising the most abundant elements are as follows: (i) exceeding 1000 mg kg^{-1} DW (K, P, Mg, and Ca); (ii) ranging from 100 to 1000 mg kg^{-1} (Fe, Na, Zn); (iii) ranging from 10 to 100 mg kg^{-1} (Cu, Al, Mn, B). Potassium and magnesium were reported as the most abundant elements in *Pleurotus* species and many other edible mushrooms, both cultivated and collected from natural ecosystems [46,47]. Ca, Fe, Zn, and Cu content in *P. djamor* fruiting bodies in the present research was lower than values classified by Siwulski et al. [45], and Krakowska et al. [5], but comparable to the values mentioned by Vieira et al. [48] for *P. ostreatus*. Interestingly, magnesium in the fruiting bodies of *P. djamor* was determined at a higher level than can be found in

the literature. Considering that mushrooms' mineral composition is highly related to the substrate, growing conditions, flux, etc., supplementation of fruiting bodies with lower initial magnesium content can be more effective. The present research showed that *P. djamor* supplementation with Mg affected the fruit body's composition concerning the other macro- and micronutrients. The most significant was the antagonism between Mg and Zn, which was confirmed with a negative correlation coefficient.

4.2. Biological Efficiency and Dry Weight Content

The biological efficiency of the specific substrates is an essential factor that decides on their suitability to cultivate a particular species or strain of mushrooms. The substrates can be processed either by composition or pasteurization, and further additions that affect yield quality and quantity [49,50]. The supplementation of *Pleurotus* spp. has not always resulted in a higher yield of fruit bodies [25]. However, the main target of modern mushroom production is not always accelerated biological efficiency, but rather a high level of bioactive compounds, which determine the market quality of the raw material. Moreover, the decrease in biological efficiency of *P. djamor* following Mg supplementation was caused by the decrease in water content, with dry matter yield slightly differentiated between experimental treatments. It appears that the biological efficiency and dry weight production is related to the salt used as a supplement. In selenium-fortified oyster mushrooms, de Oliveira and Naozuka [27] did not notice alterations in the moisture of fruiting bodies with the increase in Se concentration in the culture medium. Furthermore, the biological efficiency showed that Se enrichment did not alter the potential of the fungus to biodegrade the organic substrate.

4.3. Organic Compounds and Antioxidant Activity

Fungal major bioactive compounds, known as mycochemicals, are naturally found in the *Pleurotus* spp. fruiting bodies and their concentration may be increased by modifying the substrate composition, culture, or postharvest conditions [51]. Some of these are phenolic compounds that could be extracted and included in formulations to prevent oxidative stress [52]. Generally, supplementation with Mg in the present research increased the phenolic content and DPPH$^{\bullet}$ scavenging activity, with the exception of two treatments with lower $MgSO_4$ content for DPPH$^{\bullet}$. Moreover, the stimulative effect of Mg supplementation of substrate on *P. djamor* fruiting bodies was supported by close eigenvectors of PC analysis and a positive correlation coefficient between these parameters, which was also reported by Puttaraju et al. [53]. By contrast, Vieira et al. [48] demonstrated that supplementation with iron, zinc or lithium reduced antioxidant activity in *P. ostreatus* fruiting bodies because polyphenol groups formed complexes with metal ions such as Fe and Zn, manifesting a reduced availability for free radicals' donation and lower antioxidant activity. Thus, it appears clear that the antagonism, which in the present research was statistically significant for Mg and Zn, and slightly notable for Mg and Fe, affects the manifestation of polyphenols' antioxidant activity. However, it cannot be ruled out that the total antioxidant activity may also relate to other compounds present in *P. djamor* fruiting bodies, such as L-tryptophan and L-ascorbic acid.

Carbohydrates in mushrooms are involved in structural composition, but are essential in maintaining the high osmotic concentration and providing the source of energy. Due to their wide range of celluloid substances, including dietary fiber, mushrooms can be part of a low-calorie diet with higher therapeutic value [18]. Glucose, mannitol, and trehalose are abundant sugars in cultivated edible mushrooms, but fructose and sucrose are found in low amounts [54]. Soluble sugars can contribute to positive health characteristics of *P. djamor* fruiting bodies. Recently, Maity et al. [55] isolated from pink oyster mushroom fruiting bodies a soluble galactoglucan of moderate DPPH$^{\bullet}$ scavenging activity that increased in a dose-dependent manner. The authors suggested that the isolated compound can be used as natural antioxidant. The positive correlation between soluble sugars determined in the present research contributes to the general definition of mushroom carbohydrates as a

biologically active molecules that can be active components in functional products due to their antioxidant advantages.

Mushrooms are also a good source of vitamins, with high riboflavin, niacin, folates, and traces of vitamin C, B_1, B_{12}, D, and E [54]. One hundred grams of fresh *P. ostreatus* fruiting bodies provides 15% of the recommended daily intake of L-ascorbic acid, and 40% of niacin, riboflavin, and thiamin [56]. Supplementation of *P. djamor* substrate with magnesium sulfate in all doses significantly increased thiamine content in fruiting bodies, and riboflavin in most cases; thiamine content was positively correlated with Mg, confirming the effectiveness of Mg supplementation concerning thiamine content. $MgCl_2$ application to the growing substrate had no significant effect on thiamine content in fruiting bodies but caused the decrease in riboflavin. The thiamine and riboflavin contents determined by Goyal et al. [57] for *P. sajor-caju* were 4.13 and 3.71 mg 100 g^{-1} DW, respectively. By comparison, in the present study, the values were 0.48 and 0.38 mg 100 g^{-1} DW for thiamine, and 2.1 and 1.6 mg 100 g^{-1} DW for riboflavin, in *P. djamor* fruiting bodies supplemented with $MgSO_4$ and $MgCl_2$, respectively. On a fresh weight basis, L-ascorbic acid content was 4.34 mg 100 g^{-1} in *P. sajor-caju* mushrooms in the study of Goyal et al. [57], whereas *P. djamor* contained 43 and 53 mg 100 g^{-1} FW, depending on substrate supplementation with Mg sulfides or chlorides, respectively. Based on the present results, pink oyster mushrooms can significantly contribute to functional food composition as an excellent source of the aforementioned vitamins.

Pleurotus spp. are a good source of proteins, comprising all of the essential amino acids with excellent digestibility. Non-protein nitrogen compounds include amino acids, chitin, and nucleic acids. Some amino acids contribute to the taste of mushrooms, which is highly valued by consumers [58]. Phenylalanine, L-tryptophan, and 5-hydroxy-L-tryptophan, determined in the *P. djamor* fruiting bodies in the present research, are essential aromatic amino acids, and act as precursors for neurotransmitters, such as serotonin, and catecholamines. L-tryptophan is the precursor of vitamin B_3, which is a stimulator of insulin secretion and growth hormone [59]. The present research proved the possibility of linking Mg supplementation with an increased L-tryptophan level in *P. djamor* fruiting bodies. Some experimental treatments also increased the level of p-hydroxybenzoic acid, 5-hydroxy-L-tryptophan, and phenylalanine. However, the correlation between magnesium and L-tryptophan was the most notable, and was confirmed statistically with a significant and positive correlation coefficient. L-Tryptophan also contributed significantly to the total antioxidant activity of *P. djamor* fruiting bodies supplemented with Mg. The biological role of L-tryptophan in free radical scavenging in *Pleurotus* spp. was reported by Jegadeesh et al. [1]. Consumption of *P. ostreatus* may contribute to the cysteine pool. The cysteine which is a precursor in glutathione synthesis can affect the functions of glutathione [60]. In the present research, the glutathione content was the highest in the control and, generally, supplementation decreased the content of glutathione.

Lovastatin content was differentiated without any regular trend for experimental treatments and varied from 0.20 to 10.5 mg 100 g^{-1} DW. Only substrate supplementation with the lowest dose significantly increased lovastatin content in *P. djamor* fruiting bodies. Lovastatin is characterized by a high degree of differentiation in its content in mushrooms, depending on the species, location, growing conditions, technologies of cultivation, etc. [56]. For example, Krakowska et al. [5] determined 7.76, 1.18, 1.14, and 0.39 mg 100 g^{-1} DW of lovastatin in the fruiting bodies of *P. citrinopileatus*, *P. florida*, *P. ostreatus*, and *P. eryngi*, respectively.

5. Conclusions

The cultivation of pink oyster mushrooms in a medium enriched with magnesium salts proved to be an effective technique for producing fruiting bodies with a chemical profile that allows them to be classified as a functional food. Mg sulfates and chlorides applied to the substrate to grow *P. djamor* effectively increased Mg content in fruiting bodies and significantly altered the content of all analyzed elements, albeit in different

ways. Based on the results of the present experiment, and the cited literature, significant differentiation in the mineral composition of mushroom fruiting bodies was confirmed. Mg supplementation was proven to be effective in the increase in organic compounds that contribute to the antioxidant activity of pink oyster mushrooms. Based on these results, the supplementation of a substrate with major and trace elements, which is crucial for the utilization of mushrooms as a functional food, appears to be a promising technique with which to forecast and standardize the chemical composition of fruiting bodies.

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mgr inż. Piotr Zięba

Piotr Zięba

31-120 Kraków, Al. Mickiewicza 21
tel. +48 (12) 662 53 03, e-mail: ko@urk.edu.pl

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31-120 Kraków, Al. Mickiewicza 21
tel. +48 (12) 662 53 03, e-mail: ko@urk.edu.pl

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Katarzyna Sułkowska-Ziaja

Dr hab. Katarzyna Sułkowska-Ziaja

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- z pozyskaniem funduszy na badania i opublikowanie artykułu.

prof. dr hab. inż. Edward Kunicki

31-120 Kraków, Al. Mickiewicza 21
tel. +48 (12) 662 53 03, e-mail: ko@urk.edu.pl

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