Water soluble polysaccharides content in three species of edible and medicinal mushrooms: *Lentinula edodes, Pleurotus ostreatus, Agaricus blazei*

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S u m m a r y

Mushrooms (*Basidiomyceteae*) are potent source of biologically active substances which have beneficial effect on human health. Many, if not all, mushroom species contain polysaccharides which may boost human immune system. The purpose of this research was assessing the content of crude water soluble polysaccharides (WSP) in three species of cultivated edible and medicinal mushrooms: *Lentinula edodes, Pleurotus ostreatus* and *Agaricus blazei* Murill. The content was determined both in caps and stems and the isolated WSP fractions were analyzed using FTIR spectroscopy. The results demonstrate that analyzed mushrooms species differ greatly in the WSP content. The largest amount was found in the fruiting bodies of *Pleurotus ostreatus*. On the contrary, the fruiting bodies of *L. edodes* contained the lowest amount of WSP. Furthermore, WSP in caps and steps are synthesized at a slightly different level. FTIR analyses suggest that there are no significant differences between chemical composition of crude WSP isolated from the stems and caps of the same species. The FTIR spectra of crude WSP also reveal polysaccharides and proteins patterns.

Key words: *mushrooms, polysaccharides, FTIR spectroscopy, fruiting bodies*

INTRODUCTION

Mushrooms have attracted human attention for many ages due to their nutritional and medicinal value [1, 2]. In recent decades much research have

been done on biologically active substances originated from *Basidiomyceteae* which have beneficial effect on health and help in the treatment of many diseases [3-5]. Among these compounds, polysaccharides (especially β-glucans) seem to play the most important role due to their anticancer and immunomodulating activities. They are regarded as biologically response modifier (BRM) because they do not attack cancer cells directly but their activity is mediated through thymus-dependent immune mechanism [6-8]. Since the late sixties, when the first antitumor polysaccharides were isolated from a few species of *Basidiomyceteae*, numerous mushroom-derived polysaccharide were discovered and purified from fruiting bodies, mycelium, or culture broth [1,9]. In general, they are high molecular weight glucans or glycans and some of them are linked to proteins. It is believed that high molecular weight, triple helix and branching are necessary for these macromolecules to retain antitumor activity [1,10]. All the mushroom species examined in this study (*i.e. Lentinula edodes, Pleurotus ostreatus, Agaricus blazei* Murill) are commercially cultivated, edible and are reported to synthesize water soluble antitumor and immunomodulating polysaccharides [1].

Lentinula edodes (commonly known as shiitake) originates from Asia and is the second worldwide cultivated mushroom after the button mushroom (*Agaricus bisporus*) [3]. It is a good source of many bioactive polysaccharides among which the most known is lentinan – water soluble high molecular (1-3) β-D-glucan with β-(1-6) branches [11]. *Pleurotus ostreatus*, known as oyster mushroom, is also commercially important. Several biologically active proteoglycans which demonstrate anticancer properties have been isolated from this species [12, 13]. Both shiitake and oyster mushroom belong to the group of so-called primary decomposers. These are organisms which cause white rot wood decay, as they developed enzymatic systems which enable them to degrade ligno cellullosic compounds – the main constituent of wood [14]. On the contrary, *Agaricus blazei* occurs naturally in meadows, rich soils and along forest edges and it originates from Brazil [15]. In recent years this species has been extensively cultivated, especially in Japan, due to its high antitumor potential resulting from the presence of proteoglycans with predominant β-glycosidic linkages [16].

The aim of this research was to compare the content of crude WSP in fruiting bodies of three cultivated species of higher fungi. Crude WSP were isolated both from stems and caps, according to the same isolation method developed in Department of Fruits, Vegetables and Mushrooms Technology laboratory. Chemical properties of the obtained polysaccharides fractions were characterized using FTIR spectroscopy.

MATERIALS AND METHODS

Extraction of crude WSP

Mushrooms of different species (*L. edodes, P. ostreatus, A. blazei* Murill) were cultivated in the laboratory of Department of Fruits, Vegetables and Mushrooms Technology. The fruiting bodies of each species were collected in the state of maturity and dried soon after harvest in a convection dryer. Subsequently, caps were separated from stems and all parts were ground in a mill to obtain fine powder.

Five grams of powdered caps and stems were boiled in 80% ethanol to remove low molecular substances. The ethanol extract was removed by centrifugation and insoluble residue was suspended in distilled water (ratio 1:50 w/v). Water extraction of polysaccharides was conducted under the reflux for three hours in 100°C. The obtained slurry was cooled and centrifuged. Supernatant containing water soluble polysaccharides (WSP) was filtered through paper filter and concentrated using rotary evaporator. Then, 96% ethanol was added (1:3 v/v) to precipitate the WSP. The precipitates were collected by centrifugation, washed in acetone twice, centrifuged and dried to constant mass. After drying, they were ground in a mortar (with acetone) to obtain a fine brownish powder. The extraction was repeated three times and the results are presented as a dry mass of crude WSP ± SD. The results were statistically tested using the analysis of variance with a level of significance set at $p<0.05$.

FTIR ANALYSES

Infrared spectra of the dried crude WSP were recorded on Perkin Elmer in the range of 400–4000 cm^{-1}. The samples were prepared in form of KBr disks.

RESULTS AND DISCUSSION

Crude WSP content

The objective of this research was to estimate and compare the content of crude WSP in three medicinal mushroom species. As presented in figure 1, mushrooms species analyzed in this study differs significantly in the crude WSP content. Furthermore, its amount depends on part of fruiting bodies it was isolated from. The highest amount of WSP was found in *P. ostreatus* and was measured to be 799.20±30.83 mg in stems and 669.10±32.67 mg in caps. The lowest amount was in *L. edodes* (241.48±32.49 mg in stems and 376.5± 37.43 mg in caps). The *Agaricus blazei* species presented lower levels of WSP than *P. ostreatus* but higher

than *L. edodes*. Its level in stems was found to be 562.33±26.83 mg while in caps 376.54±24.70 mg. As stated previously, WSP are not distributed equally in fruiting bodies. In *P. ostreatus* and *A. blazei* its level was higher in stems than in caps. On the contrary, *L. edodes* contained higher amount of WSP in caps than in stems.

Chemical analysis of crude WSP

In order to examine chemical composition of isolated crude WSP, FTIR spectroscopy was employed. As shown in figure 2, the FTIR spectra taken from different species were found to be alike. All six samples had characteristic peaks which indicate the presence of polysaccharides and protein. However, the certain bands may differ in relative strength. The broad band between approximately 3600 and 3200 cm^{-1} is the result of stretching vibrations of O-H in the sugar residue, while the C–H stretching occurred as a peak at 2980–2840 cm^{-1}. The intense absorption at 1200–900 cm^{-1} is also characteristic of carbohydrates and corresponds to stretching vibrations of C–C, C–O–C and C–O occurring in glucopyranose structure. The peaks which indicate the presence of protein were observed with absorption at approximately 1640 cm^{-1} (amide I) and 1530 cm^{-1} (amide II). The presence of these bands in the spectra results from proteins which are covalently bound to polysaccharides but also may results from protein impurities occurring in crude WSP fraction [10, 16, 17]. The amide II band which is due to bending vibrations of N–H groups is much weaker in *P. ostreatus.* Because this band is best used for estimation of protein content [18, 19] it may be concluded that there is much less protein in crude WSP, as compared to the other species. The spectra of WSP

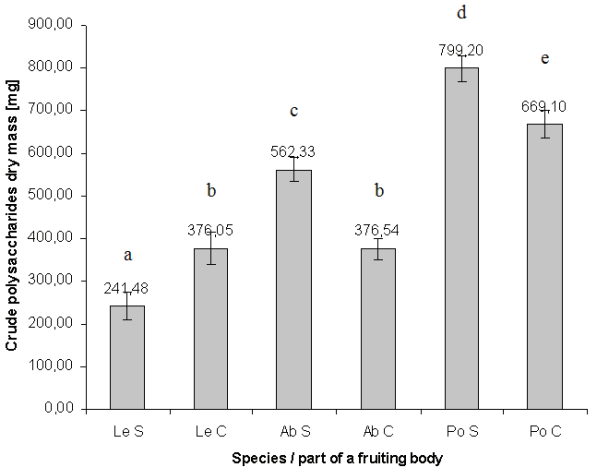

Figure 1.
Content of crude WSP isolated from stems and caps of different mushrooms edible species isolated from five grams of dried mushrooms expressed as dry mass ±SD (abbreviations: Le – *L. edodes*; Ab – *A. blazei*; Po – *P. ostreatus*; S – stems; C – caps). The letters indicate significant differences according to LSD test ($p<0.05$)

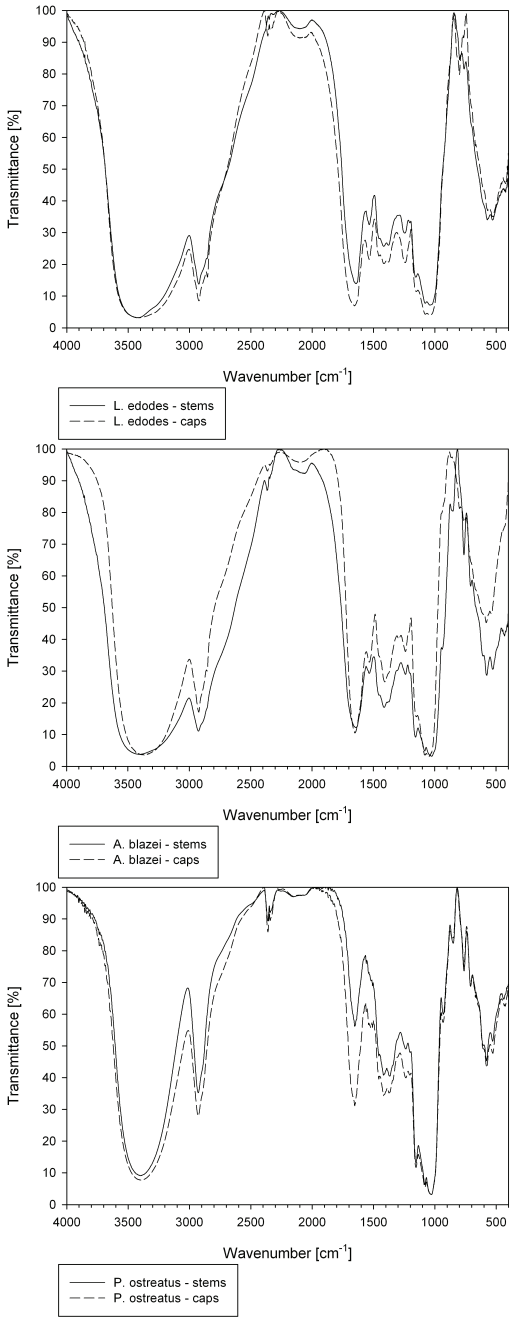

Figure 2.
The FTIR spectra of crude WSP extracted from caps and stems of *L. edodes*, *A. blazei* and *P. ostreatus*

isolated from caps and stems of the same species reveal very strong similarity which suggests that there is no significant difference in chemical composition of these fractions. However, weaker amide I band and no amide II band was observed in the spectrum of WSP extracted from *P. ostreatus* stems which may lead us to conclude that protein content is much smaller there.

CONCLUSIONS

1. All mushroom species examined in this study synthesize water soluble polysaccharides at varying level. The highest amount was found in *P. ostreatus* fruiting bodies, while the lowest amount in *L. edodes* fruiting bodies.

2. The significant difference in the amount of crude WSP isolated from stems and caps was observed. The most notable difference was observed in case of *A. blazei*, where the amount of crude WSP was higher in stems than in caps. Similarly, fruiting bodies of *P. ostreatus* contain more WSP in stems than in caps, although, the difference is less crucial. To the contrary, caps of *L. edodes* contained higher amount of crude WSP, as compared to stems.

3. The FTIR analyses suggest that there is no significant difference between chemical composition of crude WSP isolated from the stems and caps of the same species. The exception is WSP isolated from stems of *P. ostreatus* where weak protein bands were observed, as compared to caps.

4. The FTIR spectra of crude WSP extracted from all three species revealed polysaccharides and proteins patterns.

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ZAWARTOŚĆ POLISACHARYDÓW ROZPUSZCZALNYCH W WODZIE W TRZECH GATUNKACH GRZYBÓW JADALNYCH: *LENTINULA EDODES, PLEUROTUS OSTREATUS, AGARICUS BLAZEI*

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Streszczenie

Grzyby należące do *Basidiomyceteae* są cennym źródłem substancji aktywnych biologicznie, wywierających korzystny wpływ na ludzkie zdrowie. Znaczna część gatunków grzybów, jeśli nie wszystkie, syntetyzują polisacharydy mające zdolność do stymulowania układu odpornościowego. Celem niniejszej pracy było określenie zawartości surowej frakcji polisacharydów rozpuszczalnych w wodzie (WSP) w trzech gatunkach uprawianych grzybów wyższych: *Lentinula edodes, Pleurotus ostreatus,* i *Agaricus blazei* Murill. Zawartość WSP oznaczona była zarówno w trzonach jak i kapeluszach, a wyizolowane polisacharydy poddane zostały analizie z zastosowaniem spektroskopii FTIR. Badania wykazały znaczne różnice w zawartości WSP pomiędzy gatunkami. Najwięcej WSP wyizolowano z owocników *P.*

ostreatus. Z kolei owocniki *L. edodes* cechowała najmniejsza zawartość surowych polisacharydów. Ponadto zaobserwowano różnice w ilości syntetyzowanych WSP w zależności od części owocnika. Na podstawie analiz FTIR można przypuszczać, że nie ma istotnych różnic w składzie surowej frakcji WSP wyizolowanych z trzonów i kapeluszy tego samego gatunku. W widmach FTIR oprócz pasm absorpcji charakterystycznych dla polisacharydów, obecne są także pasma świadczące o obecności białek.

Słowa kluczowe: *grzyby, polisacharydy, spektroskopia FTIR, owocniki*