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ORIGINAL ARTICLE

QUANTITATIVE EVALUATION OF 1,3-1,6-β-D-GLUCAN CONTENTS IN WILD–GROWING SPECIES OF EDIBLE POLISH MUSHROOMS

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ABSTRACT

Background. Macrofungal β-glucans are mainly represented by compounds with β-1,3- and β-1,6 glycosidic bonds. They have been shown to have immunomodulatory, anticancer, and antioxidant properties. Although there are many reports on the bioactivity and structure of fungal glucans, studies on the quantitative assessment of these compounds are sparse.

Objective. The aim of the study was to determine total β-glucans and 1,3-1,6-β-D-glucan contents in selected species of wild-growing edible Polish mushrooms.

Material and methods. Eight species of wild-growing edible mushrooms *Boletus pinophilus*, *Hydnum repandum*, *Craterellus cornucopioides*, *Suillus variegatus*, *Suillus granulatus, Gyroporus cyanescens, Tricholomopsis rutilans*, and *Auricularia auricula-judae* and one species of cultivated mushroom for comparison purposes *Agaricus bisporus*, were analyzed. Quantitative analysis of 1,3-1,6-β-D-glucans was done using a colorimetric method in accordance with *Nitschke* et al.

Result. Mean total β-glucan content varied from 13.5 g/100 g dry mass in *A. bisporus* (portobello variety) to 40.9 g/100 g dry mass in *T. rutilans*. Mean 1,3-1,6-β-D-glucan content in the analyzed fruiting bodies ranged from 3.9 g/100 g dry mass in *Agaricus bisporus* (cremini) to 16.8 g/100 g dry mass in *Auricularia auricula-judae* (wood ear). The following mushrooms demonstrated the greatest percentage of 1,3-1,6-β-D-glucan contents in relation to the total β-glucan content: *Gyroporus cyanescens* (54%), *Suillus granulatus* (49.8%), *Auricularia auricula-judae* (47.9%), and *Suillus variegatus* (40.6%).

Conclusions. Among the analyzed species, wild-growing mushrooms had a generally higher average 1,3-1,6-β-D-glucan content compared with cultivated mushrooms such as *A. bisporus*. The highest average content of these polysaccharides was observed in medicinal mushroom *Auricularia auricula-judae*. Comparable 1,3-1,6-β-D-glucan content, in relation to this mushroom species, was found in Gyroporus cyanescens, Suillus granulatus and Suillus variegatus, which points to the possibility of the use of these species of mushrooms as medicinal foods.

Key words: *beta-glucans, edible mushrooms, medicinal mashrooms*

STRESZCZENIE

Wprowadzenie. β-glukany grzybów wielkoowocnikowych występują głównie w postaci związków o wiązaniach β-1,3 oraz β-1,6 glikozydowych. Wykazano, że posiadają one właściwości immunomodulacyjne, przeciwnowotworowe i przeciwutleniające. Pomimo, że istnieje wiele doniesień na temat bioaktywności i struktury glukanów grzybowych, badania dotyczące ilościowej oceny tych związków są rzadkie.

Cel. Celem badań była ocena i oznaczenie całkowitej zawartości β-glukanów oraz 1,3-1,6-β-D-glukanów w wybranych jadalnych gatunkach polskich grzybów dziko rosnących.

Materiał i metody. Przebadano osiem gatunków grzybów jadalnych dziko rosnących: borowika sosnowego (*Boletus pinophilus*), kolczaka obłączastego (*Hydnum repandum*) lejkowca dętego (*Craterellus cornucopioides*) maślaka pstrego (*Suillus variegatus*) maślaka ziarnistego (*Suillus granulatus*) piaskowca modrzaka (*Gyroporus cyanescens*), rycerzyka czerwonozłotego (*Tricholomopsis rutilans*) *i* uszka bzowego (*Auricularia auricula-judae*) oraz jeden uprawny – pieczarkę dwuzarodnikową (*Agaricus bisporus*) w celach porównawczych. Ilościową ocenę 1,3-1,6-β-D-glukanów przeprowadzono metodą kolorymetryczną według *Nitschke* i wsp.

Wyniki. Średnia całkowita zawartość β-glukanów wahała się od 13,5 g/100 g suchej masy w pieczarce dwuzarodnikowej (*A. bisporus*, odmiany portobello) do 40,9 g/100 g suchej masy w rycerzyku czerwonozłotym (*T. rutilans*). Średnia zawartość 1,3-1,6-β-D-glukanów w analizowanych owocnikach grzybów wynosiła od 3.9 g/100 g s.m. w pieczarce dwuzarodnikowej (*A. bisporus* odmiany cremini) do 16,8 g /100 g s.m. w uszaku bzowym (*Auricularia auricula-judae*). Największy udział 1,3-1,6-β-D-glukanów w stosunku do całkowitej zawartości β-glukanów wyka-

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zywały następujące gatunki grzybów: piaskowiec modrzak (*Gyroporus cyanescens*) (54%), maślak ziarnisty (*Suillus granulatus*) (49,8%), uszak bzowy (*Auricularia auricula-judae*) (47,9%) oraz maślak pstry (*Suillus variegatus*) (40,6%).
Wnioski. Pośród analizowanych grzybów, gatunki dziko rosnące charakteryzowały się wyższą średnią zawartością 1,3-1,6-β-D-glukanów w porównaniu do uprawnych – pieczarki dwuzarodnikowej (*A. bisporus*). Najwyższą średnią zawartość tych polisacharydów stwierdzono w owocnikach grzybów leczniczych – uszak bzowy (*Auricularia auricula-judae*). Porównywalną zawartość 1,3-1,6-β-D-glukanów, w stosunku do uszaka bzowego, stwierdzono w owocnikach piaskowca modrzaka, maślaka ziarnistego oraz maślaka pstrego, co wskazuje na możliwość wykorzystania tych gatunków grzybów jako żywności funkcjonalnej.

Słowa kluczowe: *beta-glukany, grzyby jadalne*

INTRODUCTION

Beta-glucans are polymers of β-D-glucose, present in the cell walls of cereals, bacteria, microscopic fungi and macrofungi [18]. Macrofungal β-glucans are mainly represented by compounds with β-1,3- and β-1,6 glycosidic bonds. Their structure can vary from a single unbranched chain to branched structures with side glycosidic chains or non-sugar substances, such as proteins. The side branches of polysaccharides can be chains other than glucose, comprised of xylose, mannose or other sugars. The chains of β-glucans can form triple helices, single helices or random coils [8]. 1,3-1,6-β-D-glucans, which are believed to have a number of preventive and therapeutic properties, represent a valuable type of β-glucans found in macrofungi. They have been shown to have immunomodulatory [9], anticancer [16] and antioxidant properties [12].

1,3-1,6-β-D-glucans include, among others, lentinan isolated from *Lentinus edodes*, schizophyllan from *Schizophyllum commune*, and pleuran from *Pleurotus ostreatus* [2]. Polysaccharide extracts from *Auricularia auricula-judae* have proven anti-inflammatory, antioxidative, and cardioprotective properties [4, 11]. These species have been considered medicinal due to their significant amounts of total β-glucans as well as 1,3-1,6-β-D-glucans [2]. Medicinal fungi of significant medical importance include species such as: *Ganoderma lucidum* (M.A. Curtis), *Grifola frondosa* (Dicks.) Gray), *Hericium erinaceus* (Bull.) Pers.), *Lentinula edodes* (Berk.) Pegler), *Pleurotus ostreatus* (Jacq.) Kumm.), *Auricularia auricula-judae* (Bull.) Quél), *Armillaria mellea* (Vahl) P. Kumm), and *Sparassis crispa* (Wulf.: Fr.) [13, 17].

The assessed mushrooms included are mycorrhizal species, such as: *Boletus pinophilus*, *Hydnum repandum*, *Craterellus cornucopioides*, *Suillus variegatus, Suillus granulatus*, *Gyroporus cyanescens*, and *Tricholomopsis rutilans;* as well as saprophytes showing some parasitic characteristics: *Auricularia auricula-judae;* and saprophytes, including *Agaricus bisporus*: cremini and portobello varieties. Saprophytic fungi are easier to grow; therefore they can be cultivated on an industrial scale [6] and are more commonly used for fungi-derived formulations. Growing mycelial *in vitro* cultures is another method for obtaining secondary fungal metabolites [20]. Basic tests used to determine the content of health promoting components in different mushroom species enable selecting species with significant amounts of β-glucans, for example, and thus identify species whose mycelial growth is important for commercial (technological and pharmacological) purposes. Although there are many reports on the bioactivity and structure of fungal glucans [5, 13, 24], studies on the quantitative assessment of these compounds are sparse [13, 19]. The fungal contents of β-glucans varies considerably and depend on factors such as species, growth environment, and maturity of the fruiting body [14].

The aim of the study was to determine total β-glucans and 1,3-1,6-β-D-glucan contents in selected species of wild-growing edible Polish mushrooms, which were not so far tested for these compounds.

MATERIAL AND METHODS

The study material consisted of nine edible mushroom species (3 samples for each species), including 8 wild-growing species: *Boletus pinophilus* (Pilát & Dermek), *Hydnum repandum* (L.), *Craterellus cornucopioides* (L.) Pers.), *Suillus variegatus* (Sw.) Kuntze), *Suillus granulatus* (L.) Roussel), *Gyroporus cyanescens* (Bull.) Quél), *Tricholomopsis rutilans* (Schaeff.) Singer) and *Auricularia auricula-judae* (Bull.) Quél); as well as two cultivated varieties of *Agaricus bisporus*: cremini and portobello. Mushroom samples were collected in 2012-2013. The fruiting bodies were obtained from natural stands located within the area of six municipalities of Podlaskie Province, Poland. Commercially cultivated mushrooms were purchased in local food markets. The obtained fruiting bodies were fully developed (of a typical size for each species), without reddening. The mushrooms harvested from natural stands were identified using atlases and fungi identification keys [6, 7]. Fresh, whole fruiting bodies (stems and pilei) of approximately 50-100 g were used as test samples. They were weighed, cut into pieces using disposable plastic knives (LDPE) and stored frozen (-70ºC) in grip bags until freeze-drying. Next, the samples were freeze-dried at -50ºC under reduced pressure of 0.027 mBar using the FreeZone Freeze System (Labconco, USA).

Dry mass determination

Dry mass contents of mushroom samples were determined by freeze-drying method. Mushroom samples were weighted before and after the drying process on an analytical balance with an accuracy up to 0.01 g. The freeze-drying process was conducted until the solid mass was achieved (about 24 h). The freeze-dried mushrooms were ground in a ceramic mortar and stored in a desiccator until analysis in sealed grip bags without access of air.

Total β-glucan content

The assay of the total β-glucan content in the evaluated mushrooms was performed using the K-YBGL 09/13 kit (Megazyme, Ireland). Extraction, laboratory analysis and mathematics calculation were performed in accordance with the manufacturer's instructions [1]. β-glucan content were calculated from the difference between total glucan content and alfa-glucan content. The obtained values were converted into g/100 g dry mass, in accordance with the formulas provided with the assay kit (K-YBGL 09/13).

Total glucan (α-glucan and β-glucan) and D-glucose in oligosaccharides, sucrose and free D-glucose extraction procedure

Weighed amounts of 100 mg were prepared from the freeze-dried mushroom powders. Next, 1.5 cm^3 of concentrated hydrochloric acid (37% v/v) was added to each test tube and the samples were thoroughly mixed in a shaker and placed in a 30°C water bath for 45 minutes. The samples were stirred on a shaker every 15 minutes. Then, 10 cm^3 of distilled water was added to each tube and the stirring continued. Thus prepared samples were placed in an uncovered 100ºC water bath for 5 minutes. Next, the test tubes were capped and incubated for 2 hrs. The samples were then cooled at room temperature and 10 cm^3 of 2 mol/L potassium hydroxide (KOH) was added. Each sample was subsequently transferred to a 100 cm^3 volumetric flask. The flasks were filled up to the mark with 0.2 mol/L acetate buffer at pH=5 and stirred thoroughly. Next, the contents of the flasks were centrifuged for 10 minutes at 3000 rpm. Thus obtained supernatant was used to determine the total glucan (α-glucan and β-glucan) and oligomers etc. content.

Determination of total glucan (α-glucan and β-glucan) and oligomers etc. content by spectrophotometric method (a)

Portions of 0.1 cm^3 of the obtained supernatant were transferred into test tubes. Next, 0.1 cm^3 of a mixture of 1,3-β-glucanase (20 U/cm^3) and β-glucosidase (4 U/cm^3) dissolved in 0.2 mol/L acetate buffer at pH=5 was added to each test tube. The contents of the test tubes were stirred with a vortex and incubated for 60 minutes at 40ºC. Then, 3 cm^3 of GOPOD reagent (glucose oxidase/peroxidase) was added. Absorbance was measured against blank samples, at a wavelength of 510 nm using a UV-1800 spectrophotometer (Shimadzu, Japan).

Solubilisation, hydrolysis and measurement of α-glucan (phytoglycogen and starch), and D-glucose from sucrose and free D-glucose (b)

100 mg of milled sample were added to the tube with 2 mol/L KOH and stirred for approximately 20 min in ice/water bath. Then, 8 cm^3 of 1.2 mol/L sodium acetate buffer and the amyloglucosidase and invertase was added to each tube. Thus prepared samples were mixed well and placed in a water bath at 40ºC for 30 min. Next, the contents of the tubes were centrifuged for 10 minutes at 3000 rpm. Thus obtained supernatant was used to determine the α-glucans (phytoglycogen and starch) and D-glucose in oligosaccharides, sucrose, and free D-glucose. Portions of 0.1 cm^3 of the obtained supernatant were transferred into glass test tubes. Next the 0.1 cm^3 of the sodium acetate buffer 0.2 mol/L and 3 cm^3 of GOPOD reagent and incubate for 20 min. at 40ºC. Absorbance was measured against blank samples, at a wavelength of 510 nm using UV-1800 spectrophotometer.

Results obtained from procedure named as a and b were subtracted to eliminate the interference induced by the other compounds such as free D-glucose, sucrose, oligosaccharides etc.

The obtained absorbance values were converted into g/100 g dry mass, in accordance with the formulas provided with the assay kit (K-YBGL 09/13, Megazyme, Ireland).

1,3-1,6-β-D-glucan extraction procedure

A 500 mg of dry, powdered mushroom sample was used for extraction. Each sample was subjected to triple extraction in accordance with the method by Nitschke *et al.* [14]. The first phase of extraction was performed in 1 mol/L solution of potassium hydroxide (KOH) in a water bath with shaking, at 60ºC for 20 minutes. The suspension was then centrifuged for 15 minutes at 3000 rpm. The supernatant was collected into a 50 cm^3 volumetric flask and designated as "KOH fraction." The sediment remaining in the tube was quantitatively transferred into a 100 cm^3 flask by rinsing it with hydrochloric acid (HCl) solution at two concentrations: 0.55 mol/L and 1 mol/L, and again subjected to shaking in a water bath at 100ºC for 60 minutes. The obtained suspension was cooled and again centrifuged. The supernatant was collected into a 50 cm^3 volumetric flask and designated as "HCl fraction." The remaining sediment was suspended in 1 mol/L sodium hydroxide solution and placed in a water bath with shaking, at 60ºC for 20 minutes. Again, the supernatant was collected, and the fraction was designated as "NaOH fraction." Each of the collected fractions was neutralized to pH=7.0.

Determination of 1,3-1,6-β-D-glucans by spectrophotometric method

The 1,3-1,6-β-D-glucan content was determined spectrophotometrically (UV-1800 spectrophotometer, Shimadzu, Japan) in accordance with the method by Nitschke *et al.* The new quantitative colorimetric method using Congo red allows to determine the amount of 1,3-1,6-β-D-glucans in the form of triple helices [14, 19]. The sample concentration of β-glucans was read from a calibration curve plotted from the known concentration of schizophylane (Contipro Group, Czech Rep.). The analysis of each extract was done in triplicate.

Statistical analysis

Statistical analysis was performed using the Statistica 10 software (StatSoft, Inc., USA). The results were not normally distributed therefore a nonparametric ANOVA Kruskal-Wallis test with the post-hoc analysis was used. Differences between the samples were analyzed using the Spearman's rank correlation test. The differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The methods of extraction and determination of total glucan content we used in the study were also used by Synytsya *et al*. [21]. The 1,3-1,6-β-D-glucan triple extraction method using sodium and potassium hydroxide solutions as well as hydrochloric acid solution was used by Nitschke *et al.* and Semedo *et al*. [14, 19]. The validity of using the colorimetric method to determine 1,3-1,6-β-D-glucans with Congo red, developed by Nitschke *et al*. [14], is confirmed by reports of Ogawa *et al.* and Semedo *et al.* [15, 19]. Total content of glucans and β-glucans in the form of triple helix (1,3-1,6-β-D-glucans) was determined by spectrophotometric method, in accordance with the guidelines set forth by the authors of these methods [1, 14]. The spectrophotometric method is recommended for quantitative analysis of β-glucans due to its low cost, speed and ease of determination, without the need for additional cleaning of extracts [14, 19, 22]. Among the analyzed mushroom species, there were species commonly harvested by mushroom pickers but rarely studied thus far.

The dry mass of the analyzed samples was from 6.56 % to 23.70 % (Table 1). Average dry mass in the mushrooms was 11.06 %. These values are in accordance with other authors' reports [10]. *Tricholomopsis rutilans* had the lowest dry mass content (6.56%), and *Auricularia auricula-judae* had the highest (23.70%).

Figure 1 presents the total β-glucan content in the analyzed mushroom samples. The lowest average β-glucan content was found in: portobello mushrooms (13.5 g/100 g dry mass) and *Craterellus cornucopioides* (16.2 g/100 g dry mass); while the highest in *Tricholomopsis rutilans* (40.9 g/100 g dry mass) and *Auricularia auricula judae* (35.0 g/100 g dry mass). Other authors obtained similar β-glucan values [13].

Table 1. 1,3-1,6-β-D-glucan content in the analyzed wild-growing and cultivated mushrooms in different fractions

No.	Species	n	Dry mass (%)	KOH	HCl	NaOH	Total 1,3-1,6-β-D-glucan
				X ±SD			
				g/100 g dry mass	g/100 g dry mass	g/100 g dry mass	g/100 g dry mass
1.	*Boletus pinophilus*	3	9.42-9.92	6.06±1.27	0.801±0.02	2.95±0.44	9.81±0.86
2.	*Suillus granulatus*	3	6.89-7.80	7.58±0.08	1.70±0.29	4.02±0.21	13.3±0.89
3.	*Craterellus cornucopioides*	3	10.00-11.12	0.81±0.24	2.72±0,35	0.95±0.13	4.48±0.25
4.	*Suillus variegatus*	3	7.55-10.55	8.29±0,62	1.79±0,17	3.6±0.04	13.69±0.41
5.	*Hydnum repandum*	3	12.89-16.31	1.01±0.22	1.82±0.03	1.35±0.09	4.18±0.10
6.	*Gyroporus cyanescens*	3	9.28-9.28	9.70±1.09	1.18±0.05	3.45±0.51	14.33±0.63
7.	*Tricholomopsis rutilans*	3	6.56-8.10	3.67±0.28	1.03±0.11	5.49±0.61	10.20±0.73
8.	*Agaricus bisporus* (portobello)	3	8.75-10.12	0.72±0.11	0.844±0.02	2.39±0.51	3.96±0.64
9.	*Agaricus bisporus* (cremini)	3	9.97-10.65	0.65±0.10	1.32±0.04	1.95±0.04	3.94±0.08
10.	*Auricularia auricular-judae*	3	20.22-23.70	2.54±0.68	1.73±0.21	12.49±0.84	16.76±0.67

X – mean 1,3-1,6-β-D-glucan content; SD – standard deviation

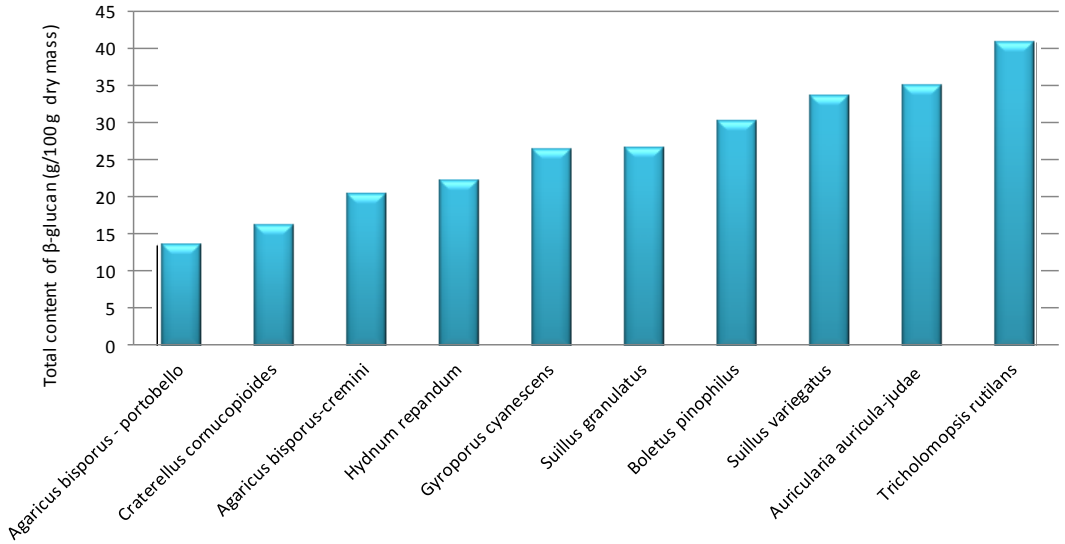

Figure 1. Total β-glucan content in the analyzed edible mushrooms

The average content of 1,3-1,6-β-D-glucans in individual fractions (KOH, HCL, and NaOH) for the analyzed species of edible mushrooms is presented in Table 1. The highest average content of 1,3-1,6-β-D-glucans in the KOH fraction was extracted from the fruiting bodies of *Gyroporus cyanescens* (9.7 g/100 g dry mass); in the HCl fraction, from the fruiting bodies of *Craterellus cornucopioides* (2.7 g/100 g dry mass); and in the NaOH fraction, from *Auricularia auricula-judae* (12.5 g/100 g dry mass).

We compared the 1,3-1,6-β-D-glucan content in each fraction, using the nonparametric ANOVA *Kruskal-Wallis* test with post-hoc analysis of multiple comparisons of mean ranks for all samples in the case of many groups.

Post-hoc analysis showed statistically significant differences in the 1,3-1,6-β-D-glucan content in the KOH fraction and the HCl fraction (p=0.0004), and in the NaOH fraction and the HCl fraction (p=0.005). Median 1,3-1,6-β-D glucan content in the KOH fraction was 3.59 g/100 g dry mass (Q1=0.98; Q3=7.69), in the HCl fraction was 1.53 g/100 g dry mass (Q1=1.14; Q3=1.84), and in the NaOH fraction was 3.27 g/100 g dry mass (Q1= 2.03; Q3=4.27). The differences are presented in a box and whisker plot.

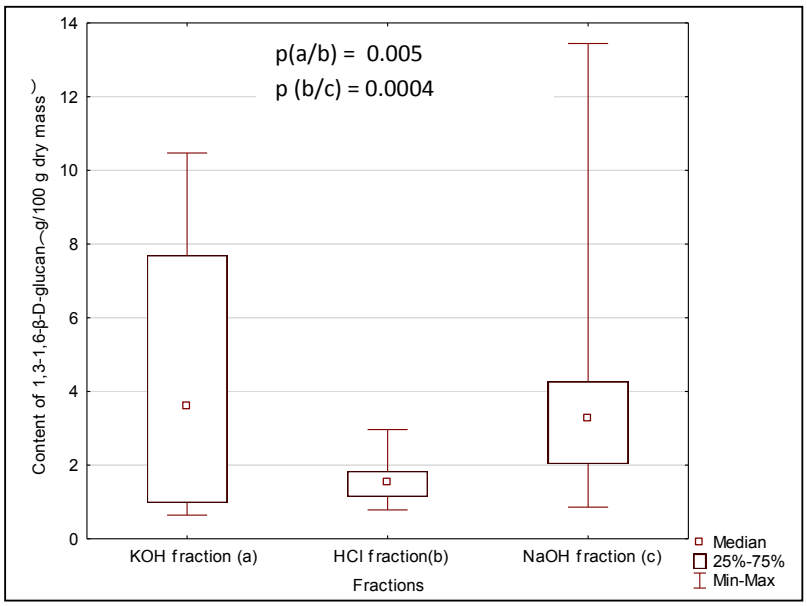

Figure 2. Statistically significant differences between the content of 1,3-1,6-β-D-glucans in the KOH, HCl, and NaOH fractions

Our analysis shows that extraction using potassium hydroxide and sodium hydroxide solutions was more effective compared with extraction using hydrochloric acid solution; other authors observed a similar tendency [14, 25].

We calculated total 1,3-1,6-β-D-glucan content by summing 1,3-1,6-β-D-glucans extracted in each fraction (KOH, HCl, NaOH), in accordance with the method by Nitschke *et al*. [14]. Figure 3 presents total 1,3-1,6-β-D-glucan content in the analyzed wild-

growing and cultivated mushrooms. Their average content ranged from 3.9 g/100 g dry mass (cremini mushroom) to 16.8 g/100 g dry mass (*Auricularia auricula-judae*). This is confirmed by prior findings of the health-promoting properties of *Auricularia auricula-judae* resulting from its 1,3-1,6-β-D-glucan content [23]. In addition to *Auricularia auricula-judae*, the following mushrooms had a high 1,3-1,6-β-D-glucan content: *Gyroporus cyanescens* (14.3 g/100 g dry mass), *Suillus variegatus* (13.7 g/100 g s.m.), and *Suillus granulatus* (13.3 g/100 g dry mass)

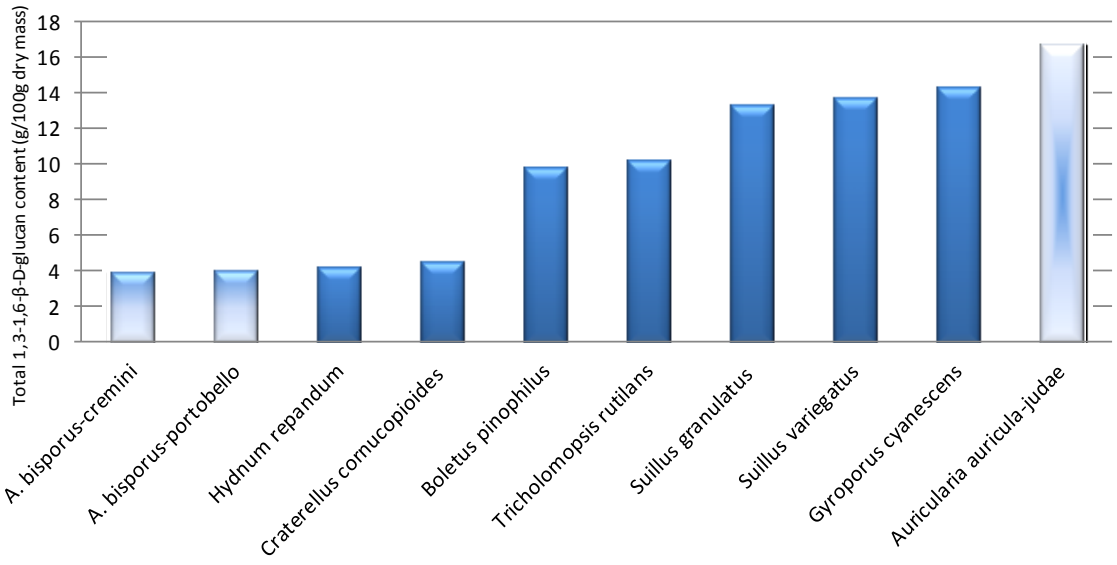

Figure 3. Total 1,3-1,6- β-D-glucan content in the analyzed mushrooms.

The 1,3-1,6-β-D-glucan content in *Suillus variegatus*, *Suillus granulatus*, *Gyroporus cyanescens*, and *Auricularia auricula-judae* was similar to the content obtained in other authors' studies [14]. The 1,3-1,6-β-D-glucan content in mushrooms with therapeutic properties in the study by *Nitschke* et al. was approximately 10 g/100g dry mass in *Lentinula edodes* and approx. 8 g/100g dry mass in *Pleurotus ostreatus* [14]. The β-glucan content in *Lentinula edodes* in the study of *Bak* et al. [3] was in the range of 25 to 44 g/100 g dry mass. The thus far conducted quantitative analyses of 1,3-1,6-β-D-glucan content pertained a small group of macrofungi, therefore there are few reports on this topic [3, 14].

Spearman's rank correlation coefficient was used to analyze the correlation between the total β-glucan content and the 1,3-1,6-β-D-glucan content in the analyzed mushrooms. Results with a level of $p < 0.05$ were considered statistically significant. We observed a strong correlation (r=0.7) between the total glucan content and the 1,3-1,6-β-D-glucan content in the analyzed mushrooms. Figure 4 illustrates this correlation.

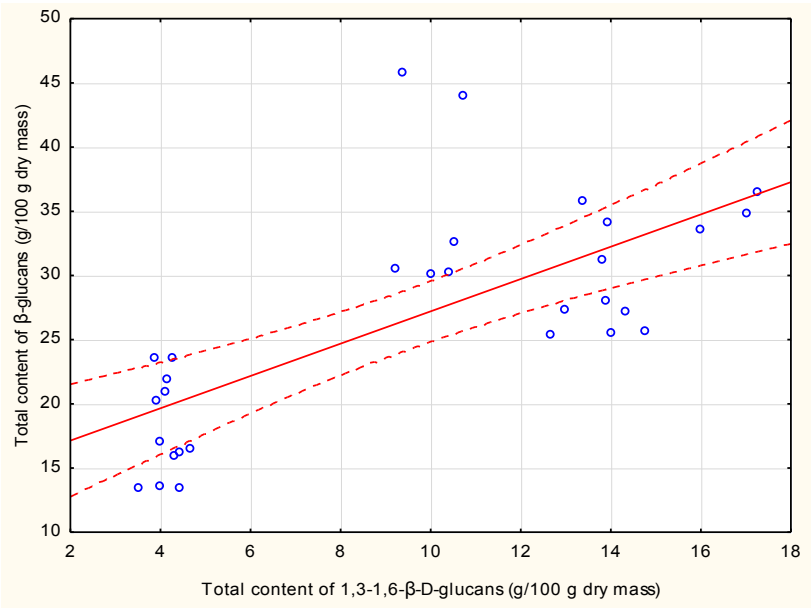

Figure 4. Correlation between total β-glucan content and 1,3-1,6-β-D-glucan content in the analyzed mushrooms

Figure 5 presents the percentage value of 1,3-1,6-β-D-glucan content in relation to total β-glucan content. The mushrooms with the lowest percentage of 1,3-1,6-β-D-glucan content in relation to total β-glucan content were: *Hydnum repandum* (18.8 %) and *Agaricus bisporus* (cremini variety) (19.2 %) and; and the highest were: *Gyroporus cyanescens* (54 %), *Suillus granulatus* (49.8 %) and *Auricularia auricula-judae* (47.9 %).

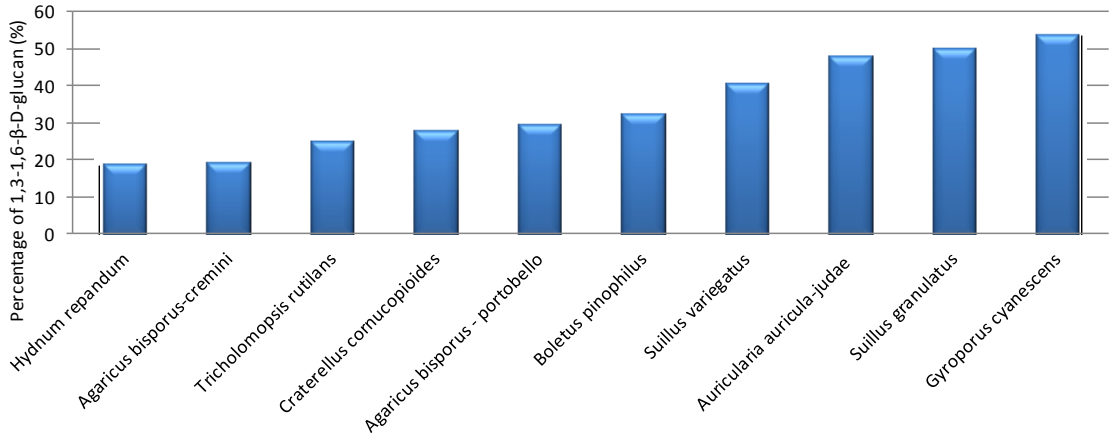

Figure 5. Percentage of 1,3-1,6- β-D-glucan content in relation to total β-glucan content in the analyzed mushrooms.

CONCLUSIONS

Among the analyzed species, wild-growing mushrooms had a generally higher average 1,3-1,6-β-D-glucan content compared with cultivated mushrooms such as *A. bisporus*. The highest average content of these polysaccharides was observed in medicinal mushroom *Auricularia auricula-judae*. Comparable 1,3-1,6-β-D-glucan content, in relation to this mushroom species, was found in *Gyroporus cyanescens, Suillus granulatus* and *Suillus variegatus*, which points to the possibility of the use of these species of mushrooms as medicinal foods.

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Conflict of interest

The authors declare no conflict of interest.

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