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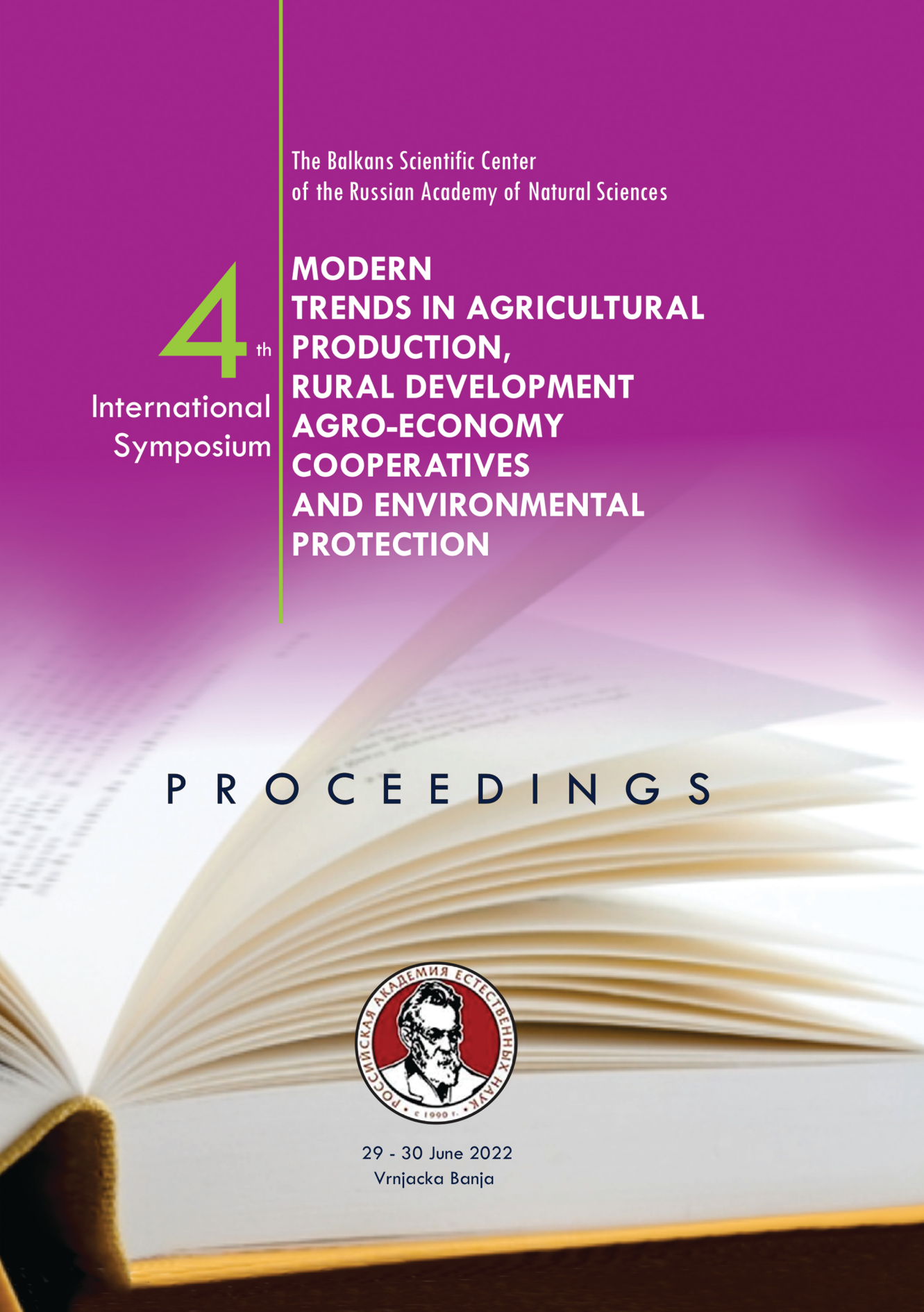
of the Russian Academy of Natural Sciences

4th International Symposium

MODERN TRENDS IN AGRICULTURAL PRODUCTION, RURAL DEVELOPMENT AGRO-ECONOMY COOPERATIVES AND ENVIRONMENTAL PROTECTION



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INVITED PAPERS

DETERMINATION OF POLYSACCHARIDE CONTENT OF *AGARICUS MACROSPORUS* AND *RUSSULA VESCA* MUSHROOM EXTRACTS

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ABSTRACT

The aim of this research was to determine the polysaccharide content of aqueous and ethanolic extracts from Agaricus macrosporus and Russula vesca mushrooms. Generally, aqueous extracts were characterized with higher ($p<0.05$) glucan content compared to the ethanolic extracts. From the point of the same extract from different mushrooms, can be seen that the extract from R. vesca was characterized with higher values ($p<0.05$) for most of the analysed parameters, compared to the extract from A. macrosporus. In accordance, both α- and β-glucan had higher ($p<0.05$) values in the aqueous (3.79%; 15.19%, respectively) as well as in the ethanolic extract (1.61%; 13.23%, respectively) from R. vesca compared to aqueous and ethanolic extract from A. macsorporus. On the other hand, in the aqueous extract from R. vesca was determined higher ($p<0.05$) monosaccharide content (18.46%) compared to the aqueous extract from A. macsorporus (16.27%). Therefore, it can be concluded that water extraction of mushrooms, especially of Russula vesca, is a successful method by which the most important bioactive polysaccharides (total, α- and β-glucan) can be extracted, as well as a high percentage of monosaccharides. This opens up new possibilities for further use of the extracts in various industries.

Keywords: *wild mushrooms, extracts, polysaccharide.*

INTRODUCTION

Mushrooms have long been considered to have medicinal value. The early herbalists were more interested in the medicinal properties of mushrooms than in their basic value as a source of food (Chang and Miles, 2004).

Several important compounds including bioactive polysaccharides (lentinan), dietary fiber, ergosterol, vitamins B_1, B_2, C, phenols, flavonoids and minerals have been isolated from the fruiting body, mycelia, culture medium of the mushrooms, as well as their extracts (Gursoy et al., 2010).

The study of biologically active compounds (phenolic compounds, flavonoids, carbohydrates) that are part of the mushroom or plants composition occupies an increasingly important place in terms of the medical effect these compounds have on consumers' health (Stojanova et al. 2021; Stojanova et al., 2022).

Among the bioactive compounds in mushrooms, polysaccharides are those that show most antitumoral, antiviral and immunomodulatory activity. In particular the polysaccharides that are found on the cellular wall are those that show most bioactivity. These polysaccharides are: chitin, cellulose and β-glucans (Mizuno and Nishitani, 2013).

Agaricus macrosporus (F.H. Muller and Jul. Schäff.), commonly known as the white button mushroom, is one of the most economically important edible mushrooms. It is considered as a valuable health food with high contents of polyphenols, ergothioneine, vitamins, minerals and polysaccharides (Dubost et al., 2007; Tian et al., 2012). Moreover, this mushroom has been demonstrated to possess various valuable biological properties including antitumor, anti-aromatase, antimicrobial, immunomodulatory, anti-inflammatory as well as antioxidant activities.

Russula vesca (Fr.) is a common and widespread edible mushroom on mainland Europe and North America. This mushroom appears in summer or autumn and grows primarily in deciduous forests. *Russula vesca* is considered edible and good, with a mild nutty flavour. In some countries, including Russia, Ukraine and Finland it is considered entirely edible even in the raw state (Dahlberg, 2019).

Numerous studies have shown that regular consumption of certain mushroom species as either a regular food or as extracted compounds is effective in both preventing and treating specific diseases, mainly through immunopotentiation and antioxidant activity. Thus, the intake of mushrooms and their extractable bioactive compounds appears to be effective in cancer

prevention and growth inhibition. Another important fact is the certainty that mushroom extracts, compared with other drugs, show a very low toxicity when regularly consumed, even in high dosages (Reis et al., 2014).

The aim of this research was to determinate the polysaccharide content of aqueous and ethanolic extracts of the wild mushroom species *Agaricus macrosporus* and *Russula vesca*.

MATERIAL AND METHOD

In this research, as a work material two types of mushrooms collected from the territory of the Republic of North Macedonia were used: *Agaricus macrosporus* and *Russula vesca*. The collected fresh mushrooms were chopped into thin slices. The mushroom pieces were dried in a chamber dryer with hot air at a temperature of 40 ºC for 6–7 h. Dried mushrooms were first ground to a fine powder and then, extracted in two ways, with water and ethyl alcohol as extragens.

Preparation of aqueous extract

Aqueous extract was prepared by Sławińska et al. (2013) and Ribeiro et al. (2015) method. The measured mass of dried and finely powdered mushroom sample (10 g) was poured with about 200 mL of distilled water, and after that was extracted on a boiling water bath for 1 h. To determine the yield of the extract, the mass of empty evaporation flask while it is empty was measured, and then with the evaporated sample. From the difference of these two values, the extract yield was obtained.

Preparation of ethanolic extract

Ethanol extract was prepared by Vidović et al. (2011) method. The measured mass of dried and finely powdered mushroom sample (10 g) was poured with 100 mL of 50% ethanol and extract was covered for 40 minutes on an ultrasonic bath at 45 °C. To determine the yield of the extract, the mass of the evaporation flask while it is empty was measured, and then with the evaporated sample. From the difference of these two values, the extract yield was obtained.

Determination of total, α and β-glucan content

The content of total glucan and α-glucan in aqueous and ethanolic extracts was determined using specific kits Mushroom and Yeast Beta-glucan Assay Procedure, K-YBGL 11, 2019 (Megazyme Co. Wicklow, Ireland) according to the manufacturer's instructions.

The β-glucan content was calculated as the difference between the total glucan content and the α-glucan content.

HPLC analysis of the monosaccharide composition of the extracts

Solutions of sugar standard 0.1g/10mL for fructose, glucose, rhamnose, ribose, mannose and galactose were prepared in water and stored at -20 °C. HPLC analysis equipment included an integrated system with "Wellchrom HPLC pump" K-10-01, detector RI-71, Shodek-RI detector. Efficient chromatographic separation was achieved by isocratic elution with a Lichrospher column (4.6 mm x 250 mm x 5 mm, MERCK) at 35 °C (Column thermostat 5–85 °C, injection value A 1365 included). The mobile phase contained a mixture of acetonitrile and deionized water in a ratio of 75:25 (v/v), the volume of the injected sample was 20 μL, and the flow rate was 1 mL/min. Sugars were identified by comparing the retention time of RT samples with standards, and the obtained values were expressed in % dry weight of the extract.

Statistical analysis

The obtained results were statistically processed using the software package SPSS 20. To determine the statistical significant differences of the obtained results was used the Independent Sample t-test ($p = 0.05$).

RESULTS AND DISCUSSION

Mushrooms are known to be a good source of various types of carbohydrates. They are divided into two groups, digestible and indigestible, ie. carbohydrates that the human body cannot digest. Of the digestible carbohydrates, the most common in mushrooms are mannitol with 0.30–5.50% (Vaz et al., 2011), glucose with 0.50–3.60% (Kim et al., 2009) and glycogen with 1 –1.60% (Díez and Álvarez, 2001). The most indigestible carbohydrates are oligosaccharides, chitin, β-glucan and mannan.

Table 1. Glucan content in aqueous extract (% dry matter extract)

Aqueous extracts	n	Total glucan	α-glucan	β-glucan
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Agaricus macrosporus	3	15.83 ± 0.05^{aA}	2.57 ± 0.07^{aA}	13.26 ± 0.01^{aA}
Russula vesca	3	18.98 ± 0.01^{bA}	3.79 ± 0.11^{bA}	15.19 ± 0.02^{bA}

$^{a, b}$ - values of the same extract of different fungal species marked with different letters, have a statistically significant difference (p<0.05), T-test.

$^{A, B}$ - values of different extract of the same fungus, marked with different letters, have a statistically significant difference (p<0.05), T-test.

Table 2. Glucan content in ethanolic extract (% dry matter extract)

Ethanolic extracts	n	Total glucan	α-glucan	β-glucan
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Agaricus macrosporus	3	12.20 ± 0.01^{aB}	1.03 ± 0.06^{aB}	11.17 ± 0.09^{aB}
Russula vesca	3	14.84 ± 0.02^{bB}	1.61 ± 0.02^{aB}	13.23 ± 0.07^{bB}

$^{a, b}$ - values of the same extract of different fungal species marked with different letters, have a statistically significant difference (p<0.05), T-test.

$^{A, B}$ - values of different extract of the same fungus, marked with different letters, have a statistically significant difference (p<0.05), T-test.

According to data presented in Table 1 and Table 2, can be seen that, generally, aqueous extracts were characterized with higher (p<0.05) glucan content compared to the ethanolic extracts. From the point of the same extract from different mushrooms, can be seen that the extract from *R. vesca* was characterized with higher values (p<0.05) for most of the analysed parameters, compared to the extract from *A. macrosporus*. Moreover, the higher total glucan content (p<0.05) was determined in aqueous extract from *R. vesca* (18.98%), while in the ethanolic extract this value was found to be significantly (p<0.05)

lower 14.84%. Furthermore, both α-glucan and β-glucan had higher (p<0.05) values in the aqueous (3.79%; 15.19%, respectively) as well as in the ethanolic extract (1.61%; 13.23%, respectively) from *R. vesca* compared to aqueous and ethanolic extract from *A. macrosporus*.

In their study, Özcan and Ertan (2018) reported that highest β-glucan content from wild mushrooms was found in *B. edulis* (13.93 %), while *A. bisporus* (cultivated mushroom), which was cultivated on compost and used for commercial purposes, had the highest overall β-glucan content at 14.57 %. Bulam et al. (2018) pointed out that the β-glucan contents of the mushrooms vary between 0.22 and 0.53 g/100 g on dry weight basis. According to Manzi and Pizzoferrato (2000), *Pleurotus pulmunarius* seemed to be the richest source of fungal β-glucans and it has been reported that *L. edodes* contains high levels of β-glucans in the soluble fraction. Camelini et al. (2005) found that *Agaricus brasiliensis* had higher (1→6)-β-glucan ratio and (1→3)-β-glucan increased with the maturation of fruiting bodies.

Table 3. HPLC analysis of the monosaccharide content from tested mushroom extracts (%)

Monosaccharide content	**n**	**Aqueous extract**		**Ethanolic extract**	
		A.macrosporus	*R. vesca*	*A. macrosporus*	*R. vesca*
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Fructose	3	2.51	2.99	1.73	2.26
Glucose	3	3.67	10.02	2.61	8.17
Galactose	3	5.34	/	4.15	1.09
Ramnose	3	0.78	1.01	/	0.30
Ribose	3	/	3.89	0.99	/
Mannose	3	3.97	0.56	2.14	0.79
Total	3	16.27 ± 0.09^{aA}	18.46 ± 0.15^{bA}	11.62 ± 0.10^{aB}	12.61 ± 0.07^{bB}

$^{a, b}$ - values of the same extract of different fungal species marked with different letters, have a statistically significant difference (p<0.05), T-test.

$^{A, B}$ - values of different extract of the same fungus, marked with different letters, have a statistically significant difference (p<0.05), T-test.

According to data presented in Table 3, can be seen that once again aqueous extracts had higher (p<0.05) monosaccharide content compared to both ethanolic extracts. On the other hand, extracts from *R. vesca* are characterised with better (p<0.05) monosaccharide content compared to both *A. macrosporus* extracts. Thus, the in the aqueous extract from *R. vesca* was determined statistically significant (p<0.05) higher monosaccharide content (18.46%) compared to the aqueous extract from *A. macrosporus* (16.27%). However, ethanolic extract from *R. vesca* had higher (p<0.05) total monosaccharide content (12.61%) compared to the ethanolic extract from *A. macrosporus* (11.62).

In addition, water as a polar extraction agent probably proved to be better due to the higher value of most of the polysaccharide components that are thought to be responsible for biological activity (Stojanova et al., 2021).

The difference in the composition of monosaccharides mainly depends not only on the type of fungus, but also on the conditions in which they grow (Kalogeropoulos et al., 2013). In this case, since these are species from natural habitats, their composition is conditioned only by the natural presence of moisture, sunlight, favorable air temperature and the like (Giri et al., 2012).

From the aspect of immunomodulatory and anticancer effects of mushrooms, the presence of glucans, especially β-glucans, is of the great importance. This component is thought to have the power to regulate the immune system, lower total cholesterol levels and LDL levels, and exhibit a number of other immunomodulatory effects (Ko and Lin, 2004).

Anticancer polysaccharides from the mushrooms are most often soluble in water, such as β-D-glucan, β-D-glucan linked to the heterosaccharide chain of xylose, mannose, galactose or β-D-glucan protein complexes, which is in accordance with this research (Mizuno, 1999).

CONCLUSION

According to the presented data can be conclude that both aqueous and ethanolic extracts from *Agaricus macrosporus* and *Russula vesca* are characterized with good polysaccharide content. Nevertheless, both aqueous extracts are characterized with statistically significant higher content of total, α- and β-glucan compared to ethanolic extracts. Even that, both extracts from *Russula vesca* showed significantly higher glucan content compared to those from *Agaricus macrosporus*. On the other hand, based on the HPLC analysis, can be pointed out that hot water extraction showed better results expressed through tested monosaccharide compared to the ethanol as less polar extragen.

Therefore, it can be concluded that water extraction of mushrooms, especially of *Russula vesca*, is a successful method by which the most important bioactive polysaccharides (total, α- and β-glucan) can be extracted, as well as a high percentage of monosaccharides. This opens up new possibilities for further use of the extracts in various industries, such as food, cosmetics and pharmaceuticals.

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