



## Beta-glucan content and antioxidant activities of mushroom-derived food supplements

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**Abstract:** Due to the presence of numerous bioactive compounds, including polysaccharides and polyphenols, mushroom-based food supplements are claimed to have many beneficial health effects. Despite their popularity, concerns have been raised in recent years over the quality of mushroom products, particularly regarding their non-standardized chemical composition. In this study, the  $\beta$ -glucan and total phenolic contents, as well as the antioxidant potential of mushroom-derived supplements available on the Serbian market were analyzed. The obtained results, revealing considerable differences in  $\beta$ -glucan and total polyphenol contents among these products, reflect variations in mushroom species, forms and recommended dosing regimens. A correlation between the total phenolic content and antioxidant activity was observed. The presence of other active ingredients, such as vitamin C, has contributed to antioxidant variability among the analyzed products. The obtained results indicate the need for the standardization of mushroom-derived food supplements to ensure their claiming effects.

**Keywords:** polysaccharides; polyphenols; powders; extracts; standardization.

### INTRODUCTION

Mushrooms are widely consumed in many parts of the world due to their nutrient contents, as well as the consequently recognized health benefits. Their important nutritional value is based on low calories, fat and sodium while being quite rich in protein, dietary fiber, vitamins and minerals.<sup>1</sup> In addition, mushrooms are rich in bioactive secondary metabolites, including polysaccharides, phenolic compounds, glycopeptides, polyketides, terpenoids and saponins.<sup>2</sup> Among these compounds, the most distinguishable are polysaccharides, which have been

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extensively studied,<sup>3</sup> especially  $\beta$ -glucans with a range of dry matter content of 15–22 %.<sup>4</sup> Unlike the  $\beta$ -glucans present in cereals, which have  $\beta$ -(1→3) (1→4) linkages, the  $\beta$ -glucans in the fungal cell wall, mainly contain  $\beta$ -(1→3) (1→6) linkages.<sup>5</sup> Opposed to  $\beta$ -glucans from yeast, mushroom-derived  $\beta$ -glucans usually have shorter side chains.<sup>6</sup> Beside the degree of branching, other factor such as molecular weight, conformation and solubility are factors that have significant impacts on its biological activity.<sup>7</sup>

Due to the  $\beta$ -glucans content, mushrooms have been shown to have prominent immunomodulatory activities.<sup>8</sup> Since these compounds cannot be synthesized endogenously, they are recognized by the immune system initiating both adaptive and innate immune responses.<sup>9</sup> Some mushrooms  $\beta$ -glucans have shown potent anticancer properties.<sup>10</sup> In addition, there is an increasing body of evidence that mushroom  $\beta$ -glucans possess antidiabetic, hypocholesterolemic, antiobesity, anti-hypertensive, wound healing, hepatoprotective and other beneficial effects.<sup>11,12</sup>

Besides polysaccharides, mushrooms contain a wide range of bioactive compounds, including phenolics, tocopherols, carotenoids, glycosides, ergothioneine and ascorbic acid,<sup>13</sup> which altogether contribute to mushrooms having a higher antioxidant potential than most vegetables and fruits have.<sup>14</sup>

Therefore, the growing evidence of health-promoting effects of mushroom and their compounds has led to increased number of products in the fungi-based supplements market.<sup>15</sup> These products contain refined, or partially refined extracts, dried fruiting bodies or mycelium, from reishi (*Ganoderma lucidum*), shiitake (*Lentinus edodes*), maitake (*Grifola frondosa*), cordyceps (*Cordyceps sinensis*) and Chaga (*Inonotus obliquus*) in the form of capsules, tablets, powder or liquids.<sup>16</sup>

In parallel with their popularity, due to the lack of standardization requirements, there is a rising concern regarding the effectiveness of these products. This in particular could be contributed to economically motivated adulteration and intentional substitution of mushroom-derived materials by some less expensive or lower quality ones.<sup>16,17</sup> Therefore, the requirement for chemical insights into the quality of mushroom supplements, based on the content of bioactive compounds, such as polysaccharides, has been recognized.<sup>18</sup> However, the quality of such products has not been the subject of extensive studies. Apart from acting as natural immunity enhancers, these products are also often claimed to be antioxidant capacity boosters,<sup>19</sup> without giving information related to the antioxidant capacity effectiveness.

Therefore, the purpose of this study was to evaluate the  $\beta$ -glucan content, as well as polyphenol content and antioxidant capacity of mushroom-derived supplements present on the Serbian market. Moreover, the obtained results in the recommended daily dose (dd) for each analyzed supplement were compared with

serving size of shiitake mushrooms, selected on the evidence for the effectiveness of its regular consumption.<sup>20</sup>

## EXPERIMENTAL

### *Standards and reagents*

Trolox (97 %), TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,20-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) were obtained from Sigma-Aldrich (St. Luis, MO, USA). Gallic acid (98 % purity) was purchased from Acros Organics (Morris Plains, NJ, USA). Folin-Ciocalteu reagent (FC), sodium carbonate, ferric chloride hexahydrate, potassium peroxodisulfate, neocuproine and copper(II) chloride were supplied by Merck (Darmstadt, Germany). All the reagents were of analytical grade.

### *Samples and sample preparations*

Samples ( $n = 16$ ) were food supplements commercially available in Serbian pharmacies and health food stores. Their selection was based on the content of mushroom-derived preparations (powder, extract or isolated compounds), either as a single active component or in various combinations. Detailed characteristics of the analyzed supplements, labeled by the producer, are presented in Table I.

Samples, or the contents of the capsules, were weighed (1 g of each) and 10 ml of distilled water was added. The mixtures were vortexed and after 1 h of extraction in an ultrasonic bath (FALC Instruments, Italy), the samples were centrifuged at 3000 rcf for 15 min (Janetzki T32C, Wallhausen, Germany). The supernatants were transferred to 50 ml flasks and filled to the mark with distilled water. The obtained extracts were immediately analyzed to obtain the total phenolic content and antioxidant activities.

Fresh fruiting bodies of shiitake mushrooms (*Lentinula edodes*) were purchased at a local market. Mushrooms (50 g) were cleaned, mashed and then macerated with 100 ml of distilled water at room temperature for 24 h. The mixture was centrifuged at 3000 rcf for 15 min, and the supernatant was removed and filtered through Whatman No. 1 filter paper. For phenolic content and antioxidant activities evaluation, the supernatant was filled to the mark obtain 200 ml in a flask.

Weighed samples of original mushroom supplements (or the content of capsules) and shiitake mushroom dried at room temperature were used for determination of  $\beta$ -glucan content.

### *Determination of the total phenolic content (TPC)*

The total phenolic content was determined using the rapid Folin-Ciocalteu microassay.<sup>21</sup> Reaction mixtures (extracts (10  $\mu$ L)), ten times diluted Folin-Ciocalteu's reagent (100  $\mu$ L) and 1M Na<sub>2</sub>CO<sub>3</sub> (80  $\mu$ L) were incubated in 96-well microplate for 1 h in the dark. The absorbance of the solutions was read at 630 nm on an MTP reader (Biotek, USA, ELx800 118 absorbance microplate reader). Gallic acid was used to obtain the standard curve and the results are expressed as mg of gallic acid equivalents (mg GAE) per g of the weighed samples and recommended daily doses for the mushroom-derived supplements and as mg of GAE per serving size (72.5 g) for shiitake mushrooms.<sup>22</sup>

### *Determination of antioxidant activity*

*Trolox equivalent antioxidant capacity (ABTS/TEAC) radical scavenging microassay.* The ABTS test was performed using the method proposed by Pastoriza *et al.*<sup>23</sup> Stock solutions of ABTS (14 mM) and potassium peroxodisulfate (4.9 mM) in phosphate buffer (pH 7.4) were

prepared and mixed in equal volumes. The mixture was left over night at room temperature and then diluted with phosphate buffer to achieve an absorbance of  $0.7 \pm 0.02$  at 734 nm. In order to determine the scavenging activity, 20 µl aliquots of extracts were mixed with 280 µl of the ABTS solution in a 96-well microplate. The absorbance was measured after 6 min at 630 nm using an MTP reader. A Trolox calibration curve was used for quantification of antioxidant activity. The results are expressed as µM Trolox equivalents (TE) per recommended daily doses for the mushroom-derived supplements and as µM TE per serving size for the shiitake mushrooms.

TABLE I. Characteristics of the commercially available mushroom-derived food supplements; C – capsule; S – syrup; P – powder

Code	Composition according to the producer	Recommended daily dose
C1	Reishi extract 300 mg (90 mg polysaccharide); vitamin C (acerola extract) 6.75 mg	2 capsules twice
C2	Cordyceps extract 300 mg (90 mg polysaccharide); vitamin C (acerola extract) 6.75 mg	2 capsules twice
C3	Shiitake extract 300 mg (90 mg polysaccharide); vitamin C (acerola extract) 6.75 mg	2 capsules twice
C4	Chaga extract (4:1) 300 mg; vitamin C 100 mg	1 capsule 2–3 times
C5	Patented mixture ( <i>Aphanizomenon flos conc.</i> , Cordyceps extract, <i>Undaria pinnatifida</i> extract, <i>Polygonum multiflorum</i> extract) 550 mg	2 capsules 1–2 times
C6	Reishi extract 180 mg; royal jelly 160 mg, grape seed extract (resveratrol) 100 mg; tomato extract (lycopene) 35 mg; broccoli extract (sulforaphane) 25 mg	1 capsule
C7	$\beta$ -(1→3) glucan 30 mg; vitamin C 100 mg; vitamin E 30 IU; zinc 20 mg; selenium 200 µg; arabinogalactan 1500 mg; Proprietary Blend® ( <i>Astragalus</i> (root), <i>Echinacea purpurea</i> (leaf and stem), <i>Elderberry</i> Fruit) 150 mg	1 capsule
C8	Reishi extract 96 mg; chlorophyll 50 mg; lutein 16 mg; lycopene 7 mg; grapefruit extract 30 mg; blueberry extract 30 mg	1 capsule
C9	Reishi extract 600 mg	2 capsules
C10	Reishi extract powder (10:1) 45 mg; Reishi powder 150 mg; Shiitake extract powder (4:1) 75 mg	2 capsules twice
S1	$\beta$ -(1→3) (1→6)-D-glucan 10 mg ml <sup>-1</sup> ; vitamin C 10 mg ml <sup>-1</sup>	1–2 ml/5kg
S2	$\beta$ -(1→3) (1→6)-D-glucan 29.5 mg ml <sup>-1</sup> ; vitamin C 10.25 mg ml <sup>-1</sup> ; zinc 0.75 mg ml <sup>-1</sup>	15 ml
S3	Chinese cordyceps 75 %; Linchzhi ( <i>Ganoderma lucidum</i> ) 6 %; Shiitake 6 %; young bamboo shoots 5 %; honey 2 %	3–5 ml daily
P1	Maitake mushroom powder	300 mg three times
P2	Reishi spore powder	300 mg three times
P3	Chaga powder 100 %	1 teaspoon per 2 dl of water 3–5 times

*Diphenylpicrylhydrazyl (DPPH) radical scavenging microassay.* The DPPH microassay was performed as described previously.<sup>24</sup> Briefly, 7 µl of diluted extracts were mixed with 193 µl of the DPPH radical solution (60 µM) in microplate wells. The absorbance readings were taken after 1 h of reaction at 490 nm on a MTP reader. Trolox was used in order to cons-

truct the standard curve, and hence the results were expressed as  $\mu\text{M TE}$  per recommended daily doses for the mushroom-derived supplements and as  $\mu\text{M TE}$  per serving size for the shiitake mushrooms.

*Ferric ion reducing antioxidant power (FRAP) microassay.* The antioxidant activity was determined using the FRAP microassay according to Bolanos *et al.* with some modifications.<sup>25</sup> The FRAP reagent was prepared by mixing acetate buffer (pH 3.6), TPTZ solution (2,4,6-tripyridyl-s-triazine in 40 mM HCl) and 20 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in a 10:1:1 volume ratio. Aliquots of the extracts (20  $\mu\text{l}$ ) were allowed to react with 280  $\mu\text{l}$  of FRAP reagent in a 96-well microplate. After 30 min at 37 °C, the absorbance readings were taken at 630 nm on a MTP reader. A standard curve was constructed by Trolox and the results are expressed as  $\mu\text{mol TE}$  per recommended daily doses for the mushroom-derived supplements and as  $\mu\text{M TE}$  per serving size for the shiitake mushrooms.

*Cupric ion reducing antioxidant capacity (CUPRAC) microassay.* This method was performed according to Zengin *et al.* with modifications.<sup>26</sup> After placing 67  $\mu\text{l}$  of diluted extracts in a 96-well microplate, 61  $\mu\text{l}$  of 0.01 M  $\text{CuCl}_2$ , 61  $\mu\text{l}$  of 7.5  $\mu\text{M}$  neocuproine and 61  $\mu\text{l}$  of ammonium acetate buffer (pH 7.0) were added. After 30 min of incubation, the absorbance was read against a reagent blank at 450 nm. Trolox was used to obtain a standard curve and the results are expressed as  $\mu\text{M TE}$  per recommended daily dose for the mushroom-derived supplements and as  $\mu\text{M TE}$  per serving size for the shiitake mushrooms.

*Antioxidant potency composite index (ACI).* The results obtained from the *in vitro* antioxidant assays (ABTS, DPPH, FRAP and CUPRAC) allowed the antioxidant composite index (ACI) to be calculated.<sup>27</sup> An index value of 100 is assigned to the best score for each assay.

An index score for all samples was calculated as follows:

$$\text{Antioxidant index score} = 100 \frac{\text{Sample score}}{\text{Best score}} \quad (1)$$

The ACI values were created by averaging all four assays for each sample.

#### Determination of $\beta$ -glucan content

The  $\beta$ -glucan content was quantified by a spectrophotometric method using the enzymatic assay kit K-YBGL (Megazyme, Bray, Wicklow County, Ireland) according to the manufacturer's instructions. This assay combines hydrolysis steps with enzymatic incubation and determines total glucan as well as  $\alpha$ -glucan content. The  $\beta$ -glucan content was calculated by subtracting the  $\alpha$ -glucan content from the total glucan content. The total glucan fragments were quantitatively hydrolyzed to glucose using a mixture of exo-1,3- $\beta$ -glucanase and  $\alpha$ -glucosidase. Afterwards, the  $\alpha$ -glucans were specifically determined, by hydrolysis of the glucans to glucose, using amyloglucosidase plus amylase and later by glucose-oxidase-peroxidase determination (GOPOD) reagent. All measurements were performed at 510 nm. The results are expressed as grams of  $\beta$ -glucan per 100 g of the weighed samples and as milligrams of  $\beta$ -glucan per recommended daily doses for the mushroom-derived supplements, as well as per serving size for the shiitake mushrooms.

#### Statistical analysis

All analyses were performed in triplicate and the obtained results are presented as the mean values and the standard deviations (*SD*). The Spearman correlation coefficient was used to calculate the correlations between data. Statistical analyses were completed using the computer program SPSS (version 20, Chicago, IL, USA) and a *p* value <0.05 was considered statistically significant.

Principal component analysis (PCA) was performed to identify and visualize relationships between *ACI* of mushroom-derived supplements and their respective *TPC*,  $\beta$ -glucan and labeled content of vitamin C, per daily doses. PCA was performed with the software Past 3.25.<sup>28</sup>

#### RESULTS AND DISCUSSION

The results of the  $\beta$ -glucan content in the mushroom-derived food supplements are summarized in Table II. They varied from 4.54 g (100 g)<sup>-1</sup> (P3) to 37.49 g (100 g)<sup>-1</sup> (C8). These values are in accordance with those of a previous study,<sup>29</sup> which revealed considerable differences in  $\beta$ -glucan content among commercial mushroom products. Namely, not only mushroom species and the type of mushroom materials, but also the manner of cultivation and the extraction procedures probably contribute to the observed variability.<sup>30</sup>

TABLE II.  $\beta$ -Glucan and total polyphenol contents in food supplements and shiitake mushrooms

Code	$\beta$ -glucan content g/(100 g)	$\beta$ -glucan content mg/dd <sup>a</sup>	<i>TPC</i> mg GAE/g	<i>TPC</i> mg GAE / dd <sup>a</sup>
C1	19.12±0.16	334.13±2.84	12.94±1.91	22.61±3.34
C2	25.47±1.24	347.41±8.49	4.08±0.07	5.57±0.09
C3	26.23±0.10	403.94±1.52	13.04±0.47	20.09±0.72
C4	13.24±0.17	165.24±2.12	127.43±7.52	159.03±9.38
C5	10.32±0.44	224.98±9.56	2.51±0.17	5.46±0.38
C6	8.44±0.20	42.20±0.99	8.17±0.16	4.09±0.08
C7	5.70±0.24	39.90±1.68	31.16±0.39	21.87±0.27
C8	33.25±0.13	156.92±0.63	6.72±0.11	3.17±0.05
C9	15.22±0.16	194.45±2.08	4.10±0.08	5.24±0.10
C10	37.49±2.17	653.74±37.86	2.74±0.53	4.77±0.93
S1	13.25±0.07	66.25±0.35	2.51±0.38	1.25±0.19
S2	7.80±0.59	327.60±24.95	2.44±0.05	10.22±0.22
S3	18.21±0.04	764.82±1.78	0.45±0.01	1.89±0.01
P1	15.18±0.30	136.62±2.67	2.38±0.08	2.15±0.07
P2	5.63±0.58	50.67±5.22	0.81±0.07	0.73±0.076
P3	4.54±0.44	953.40±92.07	3.68±0.07	77.35±1.37
shiitake	2.87±0.09	2078.21±64.08 <sup>b</sup>	4.23±0.44	306.34±31.54 <sup>b</sup>

<sup>a</sup>Daily dose; <sup>b</sup>serving size (72.5 g) instead of daily dose

*Ganoderma lucidum* (reishi) is known to have more than 200 different polysaccharides that are isolated from fruiting bodies, mycelium, spores or liquid cultures.<sup>31</sup> In recent years, there has been an increasing concern about the quality of reishi products.<sup>32</sup> In an analysis of polysaccharide and triterpene profiles, Wu *et al.* found that only 26.3 % of the *Ganoderma lucidum* supplements were in accordance with their labels.<sup>18</sup> This study also found significant variations in the  $\beta$ -glucan content among reishi supplements. The lowest  $\beta$ -glucan content was observed for sample P2 in the form of reishi spores powder. Within samples based on reishi fruiting bodies extracts, the  $\beta$ -glucan content ranged between 8.44

$\text{g (100 g)}^{-1}$  (C6) and  $33.25 \text{ g (100 g)}^{-1}$  (C8), reflecting differences in the qualitative and quantitative composition among them.

The dose of mushroom-based products in traditional medicine should correspond to 100–150 g of raw material. However, the consensus for the effective dosage for mushroom supplements has not been established. According to clinical trials, the accepted daily dose is 3000–6000 mg of biomass or mushroom extracts.<sup>16</sup> When the obtained results were expressed per maximum daily doses established by manufacturers, the content of  $\beta$ -glucan was up to 1000 mg with an average concentration of 293 mg (Table II). Chaga powder supplements (P3) provide the highest amount of  $\beta$ -glucan ( $953.40 \text{ mg dd}^{-1}$ ), followed by mixed mushroom supplements S3 ( $764.82 \text{ mg dd}^{-1}$ ) and C10 ( $653.74 \text{ mg dd}^{-1}$ ), respectively. These results indicate that mushroom supplements provide less  $\beta$ -glucans intake than one serving size of shiitake (2078.21 mg).

Interestingly, the  $\alpha$ -glucan contents in this study were low and ranged from 0.13 to  $2.32 \text{ g (100 g)}^{-1}$  (data not shown in Table II). In contrast to earlier findings,<sup>18,28</sup> these results indicate the absence of starch-based excipients, such as starch or maltodextrins, as possible adulterants in the analyzed mushroom supplements.

Mushrooms are recognized as good sources of dietary polyphenols.<sup>33</sup> Depending on the mushroom species, the total polyphenols content range from 1 to 6  $\text{mg g}^{-1}$  dried weight.<sup>34</sup> In addition to antioxidative properties, numerous studies have revealed that mushroom phenolic compounds exert anti-inflammatory, antimicrobial, anticancer, antiviral, neuroprotective and other health-promoting effects.<sup>35</sup> The total phenolic contents ranged from  $0.45 \text{ mg GAE g}^{-1}$  (S3) to  $127.43 \text{ GAE g}^{-1}$  (C4, Table II). Since many other, non-phenolic reducing compounds may interfere in the Folin–Ciocalteu assay, by far the highest value of TPC in the extract of sample C4, in comparison with other extracts, could be explained by the high-labeled content of vitamin C. As expected, higher levels of TPC were noticed among mushroom extracts mixed with herbal materials (C1, C3 and C7).

Similar to the  $\beta$ -glucan content, the difference in the polyphenol content among mushroom supplements could be due to different mushroom species, type of the mushroom product, as well as various processing factors, which makes comparison difficult. Stilinovic *et al.*<sup>36</sup> studied the total phenolics of commercial preparations of edible mushrooms and showed that the methanol extract from the powder of *Cordyceps sinensis* contained three times more total phenolics than from *Ganoderma lucidum*. In this study, opposite results were obtained for supplements from the same producers (C1 and C2), which contained the same quantity of extracts of these mushroom species. When the recommended daily doses were taken into account, the average TPC in the analyzed mushroom supplements was  $21 \text{ mg GAE dd}^{-1}$ . The content of polyphenols in the sample C4 (159

mg GAE dd<sup>-1</sup>) corresponds to only half of the intake that can be obtained from a single serving of shiitake mushrooms.

According to the prevalent consumption, it was estimated that food supplements contribute 25 % to the total daily antioxidant intake.<sup>37</sup> However, due to different origin, preparation processes, formulations and concentrations of their active ingredients, great variability in antioxidant capacity was revealed among the supplements.<sup>38</sup> In order to extensively evaluate the antioxidant potential of mushroom-derived supplements, different assays based on the transfer of electrons (DPPH, ABTS) and reducing power ability ferrous (*FRAP*) and cupric ions (*CUPRAC*) were employed. Taken into account the differences in the total unit mass and dosage regimen among different forms of supplements, the antioxidant activity was expressed per maximum daily dose recommended by the producers (Table III). Namely, based on the obtained results, the antioxidant activity was mathematically recalculated by one unit of products (gram or milliliter) and then multiplied by the maximum daily amount labeled on the product package.

TABLE III. Antioxidant activity (μM TE) in maximum daily dose of food supplements and one serving of shiitake mushrooms

Code	<i>FRAP</i>	DPPH	ABTS	<i>CUPRAC</i>
C1	112.41±3.12	92.40±1.82	47.64±0.98	265.91±9.21
C2	276.96±27.17	294.04±21.87	159.83±3.88	517.71±56.94
C3	89.04±0.50	86.75±1.54	43.37±0.12	211.81±2.25
C4	5380.86±136.93	4112.83±160.88	1766.32±1.99	8718.12±125.77
C5	13.23±1.99	53.00±0.53	21.07±0.01	73.12±1.39
C6	26.020.70	31.36±1.49	14.72±0.02	66.48±9.19
C7	671.72±2.50	273.37±20.79	189.00±0.61	945.77±23.95
C8	18.64±0.34	15.92±0.43	7.09±0.00	43.62±6.02
C9	20.99±0.36	33.68±0.42	17.48±0.10	60.44±4.97
C10	26.16±0.46	39.49±0.82	19.57±0.14	73.54±1.09
S1	5.80±0.22	13.03±0.30	1.88±0.11	23.93±3.46
S2	49.90±2.59	235.03±4.77	22.82±0.87	139.82±3.56
S3	5.64±0.72	101.23±1.87	2.97±0.34	20.87±0.67
P1	7.10±0.14	23.84±0.20	10.83±0.04	41.07±3.22
P2	2.42±0.24	46.31±0.23	0.10±0.00	4.22±2.03
P3	448.84±13.00	645.19±13.57	290.50±2.66	1905.96±18.83
Shiitake <sup>d</sup>	3102.28±452.16	1714.26±210.70	551.73±78.95	nd <sup>a</sup>

<sup>a</sup>Not determined; <sup>b</sup>serving size (72.5 g) instead of daily dose

Since the results of antioxidant activity obtained by different assays are difficult to compare with one other,<sup>39</sup> *ACI* values were calculated and presented in Fig. 1.

Although the *in vitro* antioxidant capacity cannot be directly translated to biological systems, the *ACI* value presents a useful tool for ranking different commercial products.<sup>27</sup> As could be seen, sample C4 had the highest *ACI* value,

followed by sample P3 and C7. A significant correlation was found between the *TPC* and *ACI* values ( $p < 0.001$ ). However, no significant correlation between the  $\beta$ -glucan content and *ACI*, or with any of the antioxidant activities, was observed. Since that majority of the analyzed products are based on mushroom extracts, these results could be explained by the fact that purified polysaccharides, free of phenolic compounds and proteins, have a low antioxidant activity.<sup>40</sup> Except for sample C4, one serving of shiitake provides better antioxidant potency than mushroom-based supplements.

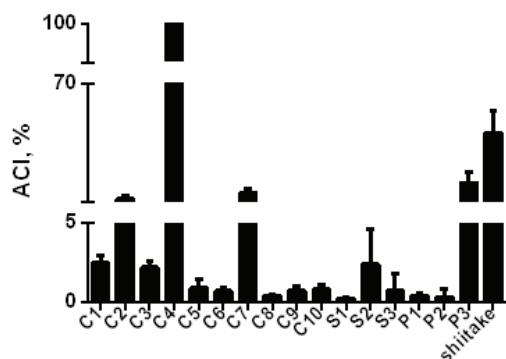


Fig. 1. Antioxidant composite indexes of mushroom-derived food supplements.

According to the PCA results, two principal components were sufficient to capture the variability of the sample properties. The first component, represented as the *x*-axis, describes 71.06 % of the variability, whereas the second component, represented as the *y*-axis, describes 26.77 % of the variability. For further analysis of the variability sources, scores and loadings were plotted and the bi-plot is represented in Fig. 2. The first component describes the majority of the variation between the samples and it is represented by a combination of the *ACI* value, total phenolic content and vitamin C per daily dose, since these three variables have a positive first component (*i.e.*, *x*-axis) coordinates and, according to the values of the loadings, contribute to the first component. This is indicative of the positive correlation between these three variables, and it is demonstrative of the fact that the product C4 with the highest *ACI* had the highest *TPC* and labeled vitamin C content per daily dose. All products with positive *x*-axis coordinates have high *ACI* values. On the other hand, the remaining variability of the samples is described by the second component (*i.e.*, *y*-axis), which is predominantly affected by the content of  $\beta$ -glucans per daily dose. Product samples that are projected on the positive side of the *y*-axis have higher  $\beta$ -glucans contents per daily dose. Sample P3, therefore, has a high amount of  $\beta$ -glucans and high *ACI* since it has positive values for both coordinates. Other samples are projected and grouped in the bi-plot according to their *ACI* and  $\beta$ -glucans content. With the increase of *x*-coordinate values, the content of *ACI* increases, whereas with increas-

ing values of the  $y$ -coordinate, the content of  $\beta$ -glucans increases. The relative contribution of  $ACI$ ,  $TPC$ ,  $\beta$ -glucans and vitamin C content on the principal components is represented in terms of their loadings values in Table IV.

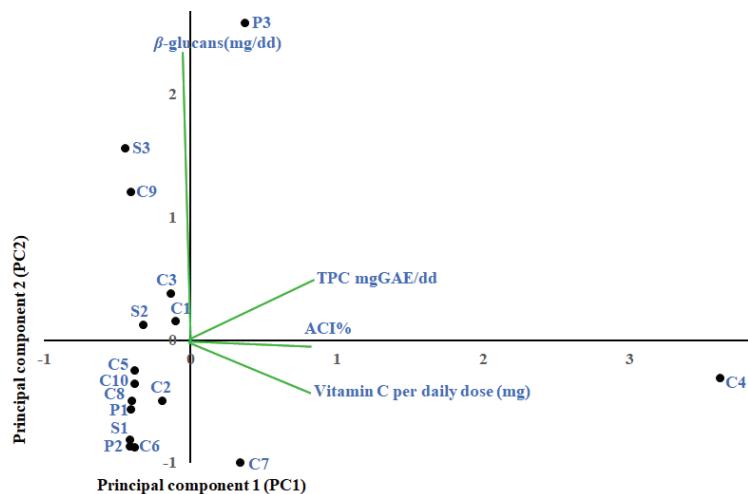


Fig. 2. Scores and loadings bi-plot.

TABLE IV. Principal components (PC1 and PC2) loadings values

Parameter	PC1	PC2
$ACI / \%$	0.5892	-0.0060
$TPC / \text{mg GAE dd}^{-1}$	0.5679	0.2163
$\beta\text{-glucans, mg/dd}$	-0.0282	0.9630
Vitamin C, mg/dd	0.5741	-0.1605

## CONCLUSIONS

Mushroom-derived food supplements available on the Serbian market were analyzed for their  $\beta$ -glucan and phenolic contents, as well as antioxidant capacities. Using PCA, the variability of daily doses in  $\beta$ -glucan content was less than in their antioxidant capacity. The addition of other active ingredients, such as vitamin C, has an impact on the antioxidant variability. In addition to the need for standardization for commercial mushroom-based products, the obtained results did not support the fact that usage of these products could contribute more  $\beta$ -glucan and dietary antioxidant intake than regular consumption of shiitake mushrooms as part of an optimal balanced diet.

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## ИЗВОД

АНАЛИЗА САДРЖАЈА БЕТА-ГЛУКАНА И АНТИОКСИДАТИВНА АКТИВНОСТ У  
ДОДАЦИМА ИСХРАНИ НА БАЗИ ГЉИВАМИЛИЦА ЗРНИЋ ЂИРИЋ<sup>1</sup>, НЕВЕНА ДАБЕТИЋ<sup>1</sup>, ВАЊА ТОДОРОВИЋ<sup>1</sup>, ЈЕЛЕНА ЂУРИШ<sup>2</sup> И БОЈАНА ВИДОВИЋ<sup>1</sup><sup>1</sup>Универзитет у Београду, Фармацеутски факултет, Капелера за Бромајолоџију, Београд и<sup>2</sup>Универзитет у Београду, Фармацеутски факултет, Капелера за Фармацеутску инженерологију и  
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Због присуства бројних биоактивних састојака, укључујући полисахариде и полифеноле, додаци исхрани на бази гљива имају бројне позитивне здравствене ефекте. Поред популарности, последњих година се велика пажња посвећује и квалитету производа од гљива, посебно у погледу њиховог нестандардизованог хемијског састава. У овом раду анализиран је садржај  $\beta$ -глукана и укупних полифенола, као и антиоксидативни потенцијал додатака исхрани на бази гљива који су доступни на тржишту Србије. Добијени резултати су показали велике разлике у садржају  $\beta$ -глукана и укупних полифенола што се може објаснити различитим врстама гљива, облицима и режимима дозирања. Уочена је корелација између укупног садржаја полифенола и антиоксидативне активности. Присуство других биолошки активних састојака, као што је витамин C, доприносило је варијабилности антиоксидантне активности између анализираних производа. Добијени резултати указују на потребу за стандардизацијом додатака исхрани на бази гљива, како би се осигурао њихов ефекат.

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