THE INFLUENCE OF MUSHROOM *CORIOLUS VERSICOLOR* AND HAZELNUTS ENRICHMENT ON ANTIOXIDANT ACTIVITIES AND BIOACTIVE CONTENT OF DARK CHOCOLATE

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ABSTRACT: Oxidative stress is among the main culprits for the progression of chronic diseases such as cardiovascular diseases, diabetes, and neurodegenerative disorders. It is well known that dark chocolate possesses polyphenols as major constituents which dietary consumption has been associated to health beneficial effects. Consequently, this study aimed to analyze dark chocolate (DC) and dark chocolate enriched with medicinal mushroom *Coriolus versicolor* (DCC) and hazelnuts (DCH) regarding antioxidant potential and total polyphenol (TPC), flavonoid (TFC), flavan-3-ol (FLA) and proanthocyanidin (PCA) content. DPPH$^\bullet$, CUPRAC and ABTS$^{\bullet+}$ assays were applied for measuring antioxidant capacity. The average of all antioxidant tests for each product was used for calculating the antioxidant potency composite index (ACI). The mean values of all antioxidant assays indicated that all chocolate products contained potent antioxidants. The addition of hazelnuts to dark chocolate significantly affected its total flavonoid content. Regression analysis among results obtained with antioxidant assays revealed that dark chocolate and dark chocolate products may act in mixed mode, by direct reduction via electron transfer or by radical quenching via H atom transfer. Flavan-3-ols had the most significant impact on the ability of the analyzed samples to reduce metals, while proanthocyanidins primarily acted as radical scavengers. The obtained results provided additional information regarding the value-added dark chocolates enriched with bioactive compounds of medicinal mushroom and plant origin.

Key words: *antioxidant potency composite index, dark chocolate, enriched dark chocolate products*

INTRODUCTION

Chocolate and chocolate products have been consumed throughout human history, but the healthy/unhealthy effects of chocolate have not always been equally recognized. Recently, the determination of secondary metabolites in cocoa and chocolate has changed the long lasting fallacies about the adverse effects of chocolate which has been presented over the last century. Nowadays, chocolate is not only an energy source of sugar and fat mixture, but is also a rich source of polyphenols and methylxanthines which show an important role in protecting human health (Todorovic et al., 2015).

The main polyphenols in chocolate and its products are flavan-3-ols and proanthocyanidins (Laličić-Petronijević et al., 2016). The most biological active are monomeric flavan-3-ols, catechin and epicatechin, and their oligomeric derivatives-procyanidins, members of the proanthocyanidin class of flavonoids (Katz et al., 2011). Flavan-3-ols and procyanidins may contribute to cardioprotective effects via

antioxidant and antiplatelet activity. They have immunoregulatory properties and beneficial effects on the endothelium (Katz et al., 2011). The epicatechin content of chocolate is primarily responsible for its favorable impact on vascular endothelium, which is the result of both acute and chronic upregulation of nitric oxide production (Galleano et al., 2009). Dark chocolate flavonoids can protect nerves from injury and inflammation and have beneficial effects on satiety, cognitive function, and mood (Katz et al., 2011). In addition to polyphenols, dark chocolate contains methylxanthine compounds, predominantly theobromine and caffeine in small amounts (Todorovic et al., 2015). Theobromine has antioxidant activity similar to caffeine and relatively little stimulating effect on the central nervous system (Todorovic et al., 2015).

Not all chocolates are equally rich in polyphenols and methylxanthines. Nowadays, there are numberless sorts and types of chocolate, starting from different cocoa solids content, the presence of milk or a great variety of additions, fillings and aromatizing agents (Komes et al., 2013). However, consumers are becoming more opting in the food market and they would like to have not just a delicious sweet but also product that can provide sufficient vitality and protection. Therefore, chocolate industry focuses to broaden their product ranges such as having high-cocoa polyphenols rich chocolate, probiotic chocolate, and prebiotic chocolate rather than ordinary chocolate (Gültekin-Özgüven et al., 2016).

Consequently, this study aimed to enrich dark chocolate with medicinal mushroom *Coriolus versicolor* and hazelnuts, and to compare antioxidant potential of the products. Over the past decade knowledge concerning the health benefits and functioning mechanisms of mushroom supplementation gained much interest in the food nutrition area (Ma et al., 2018). Mushrooms have been consumed for many centuries due to the alluring sensory characteristics, optimal nutritional compositions and for the treatment and prevention of several diseases such as arthritis, rheumatism, bronchitis, gastritis, cancer,

as well as in health and longevity maintenance (Soccol et al., 2014). Chocolates with whole nuts or coarse nuts are popular chocolate products and accordingly we used hazelnuts enriched chocolate as the referent standard. Total content of polyphenols (TPC), flavonoids (TFC), flavan-3-ols (FLA) and proantho-cyanidins (PCA) was evaluated. Antioxi-dant potency composite index (ACI) was finally used for the evaluation of antioxidant potential.

MATERIALS AND METHODS

Three samples of each analyzed product: dark chocolate (DC), dark chocolate with *C. versicolor* (DCC) and dark chocolate with hazelnuts (DCH) were analyzed in order to determine complete antioxidant activity. TPC, TFC, FLA and PCA content of chocolate products were analyzed using spectrophotometric methods.

Sample preparation

Commercial DC (Selection Noir, Belgium) was used to manufacture chocolate products with *C. versicolor* dried fruiting bodies powder and hazelnuts purchased at a local market specialized for organic food. DC was made from 58 g/100 g of cocoa mass, 38 g/100 g of sugar and 4 g/100 g of cocoa butter. Its nutritional composition and the energetic value were presented in Table 1.

The content of added mushroom and plant material was 5% in each chocolate product (Komes et al., 2013). Fresh wild-growing fruiting bodies of autochthonous mushroom species *C. versicolor* were collected from Fruška gora, a large forest area and National park in Vojvodina, Northern Serbia. Carpophores were identified according to the methods of classical herbarium taxonomy to confirm the correct species (Phillips, 1979).

The representative voucher specimens were deposited in the herbarium of the Department for Industrial Microbiology at the Faculty of Agriculture-University of Belgrade (No. CVR-1) together with their mycelial cultures. For further analysis, mushroom samples were soaked in ethanol to remove impurities, lyophilized (Telstar LyoAlfa 15-85, Terrassa, Spain) and powdered.

Table 1.

Nutritional composition and energetic value of the commercial dark chocolate (DC)

Nutritional composition	Content (g/100 g)
Moisture	0.87 ± 0.01[*]
Total fat	34.4 ± 1.2
Crude protein	6.7 ± 0.5
Carbohydrates	43.1 ± 2.0
-starch	4.6 ± 0.3
-sugars	38.5 ± 1.8
Fibers	9.8 ± 0.5
Energy	
535 kcal/100 g	
2215 kJ/100 g	

[*]*All parameters except moisture were presented on dry weight*
Nutritional composition was analyzed according to the Official Methods of Analysis- AOAC procedures (1995)
Total energy was calculated according to Barros et al. (2007)

Raw hazelnuts were manually cracked, then ground in a coffee mill and lyophilized.

Extracts preparation

The chocolate extracts were prepared as described by Todorovic et al. (2015). Chocolate samples were frozen and manually grated. Exactly 5.0 g of each cocoa product was extracted three times with 20 mL of n-hexane in order to eliminate lipids from the samples. Then defatted samples were air-dried during 24 h to evaporate residues of organic solvent. According to Komes et al. (2013), aqueous acetone was the most efficient to extract the polyphenolic compounds from chocolate products.

Accordingly, the components of interest were extracted from defatted products two times with 35 mL of solvent extraction mixture (acetone + Milli-Q water (MQ) + acetic acid (70 + 29.8 + 0.2 v/v/v) for 30 min in an ultrasonic bath (Labsonic LBS2-10, FALC, Treviglio, Italy). The mixture was centrifuged (Sigma 2-16P, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) for 10 min at 3000 rpm after each extraction and the supernatant was decanted.

After filtration to remove the residual particles, the supernatants were combined in a flask and filled up to obtain 100 mL of the extract. *C. versicolor* and hazelnuts were extracted according to the same procedure, in order to enable the comparison of the polyphenolic content and antioxidant capacity of chocolate products with mushroom and hazelnuts extracts.

Determination of total polyphenols (TPC), flavonoids (TFC), flavan-3-ols (FLA) and proanthocyanidins (PAC) content

The Folin-Ciocalteu reaction method adapted for a 96-well microplate reader (Hanna Instruments EC 215 Conductivity Meter, Chelmsford, UK) was used to determine the TPC according to Djekic et al. (2016). Gallic acid was used as the standard (0.015–0.25 mg/mL) and results were expressed as mg gallic acid equivalents (mg GAE/g of sample, defatted dark chocolate product or dry weight of the *C. versicolor* and hazelnuts extract).

Quantification of TFC was done based on a colorimetric microplate assay (Gajula et al., 2009). Catechin standard solutions (0.0625–2 mg/mL) were used for the construction of a standard curve. The results are expressed as mg catechin equivalents (mg CE/g of sample, defatted dark chocolate product or dry weight of the *C. versicolor* and hazelnuts extract).

PCA were analyzed using a butanol/HCl assay described by Todorovic et al. (2015). The quantity of PCA was determined from a standard curve of cyanidin chloride treated with the BuOH-HCl-Fe III mixture and expressed as mg cyanidin chloride equivalents (mg CyE/g of sample, de-fatted dark chocolate product or dry weight of the *C. versicolor* and hazelnuts extract). The range of standard solutions (0.05–0.3 mg/mL) was used for obtaining the calibration curve.

The content of FLA was estimated using the 4–dimethylaminocinnamaldeh (DMAC)

reagent according to Mraihi et al. (2015). The mentioned method was chosen because of its selectivity, sensitivity, and rapidity to flavan-3-ols quantification compared to other spectrophotometric protocols (Payne et al., 2010). The DMAC react specifically to monomeric flavanols such as catechin and epicatechin of cocoa and chocolate. Little or no reaction occurs with cyanidins and phenolic acids, flavornes, flavanones, flavonols, anthocyanidins, isoflavones, and stilbenes (Payne et al., 2010). The DMAC reagent was prepared immediately before uses, by dissolving 20 mg of DMAC in a cold mixture (1/1 v/v) of methanol and 1.5 M H_2SO_4. Extracts (50 µl) were added into the wells with 150 µL of DMAC and allowed to react at dark for 10 min, at room temperature. The absorbance at 640 nm was measured using an absorbance microplate reader (ELx808, BioTek Instruments, Inc., USA). Catechin standard solutions (0.0125–0.2 mg/mL) were used for the construction of a standard curve. The results are expressed as mg catechin equivalents (mg CE/g of sample, defatted dark chocolate product or dry weight of the *C. versicolor* and hazelnuts extract).

Antioxidant activity determination

Three methods were used to evaluate radical-blocking capacities: 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH$^\cdot$) scavenging ability, cupric reducing antioxidant capacity (CUPRAC) assay, and 2,2$^{'}$-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS$^{\cdot+}$) scavenging ability.

DPPH$^\cdot$ scavenging ability was evaluated by colorimetric assay (Vunduk et al., 2016) with slight modifications. Briefly, 100 µL of aliquoted samples were added to 50 µL of freshly prepared dimethyl sulfoxide (DMSO) solution of 0.2 mM DPPH in a 96 well plate (Sarstedt, Nümbrecht, Germany). The absorbance was measured at 490 nm using a 96-well micro-plate reader (ELx808, BioTek Instruments, Inc., USA). The calibration curve (0.015–1 mmol Trolox/mL) was used for the quantification of DPPH$^\cdot$ scavenging ability. The CUPRAC method was done according to Ozturk et al. (2011) with slight modifica-

tions. To each well, in a 96 well plate, 50 µL 10 mM Cu (II), 50 µL 7.5 mM neocuproine, and 60 µL NH_4Ac buffer (1 M, pH 7.0) solutions were added. 30 µL of aliquoted samples were added to the initial mixture to make the final volume of 190 µL. After 1 h, the absorbance was re-corded at 450 nm against a reagent blank using a 96-well microplate reader. The reagent blank was prepared using the conditions selected in this study, replacing 50 µL of 10 mM Cu (II) with the same volume of MQ water. The CUPRAC was calculated from the calibration curve (0.1–0.8 mmol Trolox/mL). ABTS$^{\cdot+}$ scavenging ability method was slightly modified and adapted for microplate reader by Petrović et al., (2016). Each extract (50 µL) was mixed with 200 µL of previously activated ABTS$^{\cdot+}$ water solution. The mixture was kept 6 min in the dark and its absorbance was read at 630 nm. The control was prepared by mixing 10 µL of MQ water with 200 µL of ABTS$^{\cdot+}$ solution. The calibration curve (0.2 mmol–1.5 mmol Trolox/mL) was used for the quantification of ABTS$^{\cdot+}$ scavenging ability.

The results of all assays were expressed as µmol Trolox equivalents (µmol TE/g of sample, defatted dark chocolate product or dry weight of the *C. versicolor* and hazelnuts extract.

Antioxidant potency composite index (ACI)

An overall antioxidant potency composite index was determined by assigning all assays (DPPH$^\cdot$, CUPRAC, ABTS$^{\cdot+}$) an equal weight, assigning an index value of 100 to the best score for each test, and then calculating an index score for all other samples within the test as follows: antioxidant index score = sample score/ best score x100 (Seeram et al., 2008). The average of all three tests for each chocolate product was then taken for the value of antioxidant potency composite index.

Statistical analyses

All experiments were carried out in triplicate and expressed as the mean ± standard deviation (SD). Statistical analyses were performed with the Statistica 12.0 software package (Statistica, Tulsa, OK, USA), using a one-way analysis of va-

riance (ANOVA) for all data collected. Differences between the means for each treatment were determined using Duncan's multiple range tests (*P*<0.05).

RESULTS AND DISCUSSION

Total TPC, TFC and PAC content

There is no uniform method for the extraction of all phenolic compounds with a single solvent system from chocolates and their products (Komes et al., 2013).

Polarities of different phenolic compounds may vary significantly due to their conjugation status and their association with the sample matrix. According to the results of Komes et al. (2013), aqueous acetone was the most efficient to extract polyphenolic compounds from chocolates and

dried fruit. Therefore, acetone was selected for the extraction and quantification of polyphenols in this study. TPC, TFC, PCA and FLA contents of dark chocolate, dark chocolate products, *C. versicolor* and hazelnuts extracts were shown in Table 2. Phenols were found in considerable quantities in all extracts. Dark chocolate samples with or without *C. versicolor* and hazelnuts were similar regarding TPC (Table 2). There was no statistical difference observed at *P*<0.05. Flavonoid contents were ranked in the order of DCH>DC≈DCC. TFC in DCH was statistically different and approximately 11% higher than in pure DC.

Using the same method for the determination of total phenols and the same extraction solvent, Todorovic et al. (2015)

Table 2.
Polyphenol, flavonoid, proanthocyanidin and flavan-3-ol contents in acetone extracts of dark chocolate, dark chocolate products, *C. versicolor* and hazelnuts

Sample	TPC[†] (mg GAE/ g)	TFC[‡] (mg CE/g)	PAC[‖] (mg CyE/g)	FLA[‡] (mg CE/g)
DC	12.3 ± 0.7^a	6.1 ± 0.3^b	2.0 ± 0.3^a	0.70 ± 0.04^a
DCC	11.5 ± 0.4^a	5.8 ± 0.6^b	1.5 ± 0.3^a	0.66 ± 0.08^a
DCH	12.6 ± 0.8^a	6.8 ± 0.3^a	1.8 ± 0.2^a	0.76 ± 0.04^a
CV	3.9 ± 0.3^c	0.3 ± 0.0^d	0.09 ± 0.003^c	0.02 ± 0.002^c
HA	7.0 ± 0.4^b	3.2 ± 0.5^c	0.5 ± 0.0^b	0.47 ± 0.03^b

[†]*gallic acid equivalents (GAE);* [‡]*catechin equivalents(CE);* [‖]*cyanidin chloride equivalents (CyE); all values are expressed per defatted dark chocolate product or dry weight of C. versicolor and hazelnuts extract; results are given as mean ± standard deviation (n = 3); within the same column, means followed by different letters are significantly different at P<0.05; DC – dark chocolate; DCC – dark chocolate with C. versicolor; DCH – dark chocolate with hazelnuts; CV – C. versicolor extract; HA – hazelnuts extract*

Table 3.
Antioxidant capacity of dark chocolate, dark chocolate products, *C. versicolor* and hazelnuts extracts

Sample	DPPH[•] scavenging ability (µmol TE/g)	CUPRAC (µmol TE/g)	ABTS[+•] scavenging ability (µmol TE/g)
DC	79.7 ± 2.1^a	392.5 ± 6.7^b	256.4 ± 7.1^a
DCC	79.0 ± 1.6^a	402.7 ± 10.5^b	249.0 ± 5.6^a
DCH	81.7 ± 2.4^a	407.5 ± 8.8^b	255.7 ± 6.5^a
CV	52.2 ± 2.2^c	69.5 ± 2.0^c	143.1 ± 5.5^c
HA	70.1 ± 3.2^b	460 ± 11.2^a	220.3 ± 3.9^b

Results are given as mean ± standard deviation (n = 3); within the same column, means followed by different letters are significantly different at P<0.05; DC – dark chocolate; DCC – dark chocolate with C. versicolor; DCH – dark chocolate with hazelnuts; CV – C. versicolor extract; HA – hazelnuts extract; TE – Trolox equivalents

Table 4.
Antioxidant potency composite index of dark chocolate and dark chocolate products

Sample	DPPH[•] index	CUPRAC index	ABTS[+•] index	ACI (%)
DC	97.6	96.3	100	97.9
DCC	96.7	98.8	97.1	97.5
DCH	100	100	99.7	99.9

DC – dark chocolate; DCC – dark chocolate with C. versicolor; DCH – dark chocolate with hazelnuts; ACI – antioxidant potency composite index

found an average of 11.99 mg GAE/g of dark chocolate, which was comparable to our study. Flavonoid content found in dark chocolate samples by the same group of authors was in average 20.1 µmol CE/g. In order to analyze dark chocolate products, polyphenol contents of CV and HA were determined. Comparing to the dark chocolate and dark chocolate products, polyphenols and flavonoids were found in significantly lower content in *C. versicolor* and hazelnuts acetone extracts (Table 2). TPC in HA was almost 45% higher than in CV. TFC of CV and HA were being about 8% and 46% of the total phenolics, respectively. As proanthocyanidins are the main flavonoids of cocoa products next to flavan-3-ols, their content was determined. PAC in dark chocolate products varied between 1.5 mg CyE/g in DCC and 2.0 mg CyE/g in DC (Table 2). Significant differences were not found between analyzed products ($P<0.05$), i.e. PAC was not influenced by the presence of *C. versicolor* and hazelnuts extracts (Table 2). Like-wise, significant differences in flavan-3-ol contents were not found between analyzed chocolate products (Table 2). Contrary, FLA were observed an almost twenty four–fold higher content in HA compared to CV. High flavanols content in hazelnuts extract in this study was in agreement with the research of Slatnar et al. (2014). They confirmed that the main polyphenolic subclass in hazelnut kernels comprised of mono- and oligomeric flavan 3-ols.

Structurally, flavonoids are classified into 12 major subclasses based on chemical structures, six of which, namely anthocyanidins, flavan-3-ols, flavonols, flavornes, flavanones, and isoflavones are of dietary significance (Manach et al., 2004; Kumar and Pandey, 2013). This structural diversity results in large variability of the physico-chemical properties influencing the extraction of polyphenols (Koffi, 2010).

Dark chocolate, hazelnuts and *C. versicolor* fruiting bodies are compositionally different substrates and maximum extraction efficiency of polyphenolic compounds could not be achieved with the same solvent, since they contain, to a certain ex-

tent, different types of polyphenolic compounds (Hammerstone et al., 1999; Natsume et al., 2000; Jakopic et al., 2011; Slatnar eta al., 2014; Janjušević et al., 2017; Cerulli et al., 2018). Additionally dark chocolate with hazelnuts and *C. versicolor* combined together can produce a complex matrix, and more extraction solvents of different polarity would be appropriate to compare the contents of polyphenols in the final products (Mojzer-Brglez et al., 2016). Consequently, further investigations are necessary to verify maximum quantities and qualitative profile of polyphenols that can be obtained by consuming these products.

Antioxidant capacity of dark chocolate products

Three different antioxidant assays (ABTS$^{\bullet+}$, DPPH$^{\bullet}$, and CUPRAC) were applied for the evaluation of the antioxidant capacity of chocolate extracts and the obtained results were shown in Table 3.

The mean values of all antioxidant assays indicated that all chocolate extracts contained potent antioxidants. Significant differences were not observed between dark chocolate extracts in DPPH$^{\bullet}$, CUPRAC and ABTS$^{\bullet+}$ assay. Increased TFC content in DCH was not followed by the increasing of the antioxidant potential. CA and HA had lower DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging abilities in the comparison of the dark chocolate products and it was in agreement with lower contents of polyphenolic, flavornoid, proanthocyanidin and flavan-3-ols observed (Table 2).

However, HA showed the highest potential in CUPRAC assay and reduction of Cu^{2+} to Cu^{+} among all analyzed extracts (Table 3) which may indicate that the qualitative composition of the extracts is of more importance than the quantity. Mira et al. (2002) showed that the Cu reducing activity seems to depend largely on the number of hydroxyl groups present in the polyphenols molecules, having their basic structures a small influence.

Significantly lower reduction ability of DCH compared to HA (Table 3) could be explained by the interaction of matrix components of the new chocolate products.

Correlation between the antioxidant potential and components of chocolate extracts

In order to better understand mechanism of antioxidant action, the correlation coefficients (r) among results obtained with antioxidant assays, and antioxidant assays with the content of bioactive components of chocolate extracts were determined.

Correlations among results obtained with antioxidant assays were positively strong for DPPH$^{\bullet}$ and CUPRAC (r=0.81), as well as DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ (r=0.89). Moderate, positive correlation was observed between ABTS$^{\bullet+}$ and CUPRAC (r=0.52).

It has been established that antioxidants can demonstrate their protective properties at different stages of the oxidation process and by different mechanisms (Gordon, 1990). There are two main types of antioxidants, namely, primary (chain breaking, free radical scavengers) and secondary or preventive (Gordon, 1990). Secondary antioxidants are the consequence of deactivation of metals, inhibition or breakdown of lipid hydroperoxides, regeneration of primary antioxidants, singlet oxygen ($^{1}O2$) quenching, etc.

The assays for measuring antioxidant capacity used in this study are based on different mechanisms. CUPRAC measures the ability of a sample to reduce metals while ABTS and DPPH measure a sample's free radical scavenging capacity (Gordon, 1990; Prior et al., 2005). Besides in FRAP, there is a SET (Single Electron Transfer) reaction mechanism, while DPPH and ABTS combine both, a HAT (Hydrogen Atom Transfer) reaction and SET reaction mechanism (Prior et al., 2005).

From the results obtained from correlation analysis among the antioxidant assays it could be concluded that dark chocolate and dark chocolate products may act in mixed mode, by direct reduction via electron transfers or by radical quenching via H atom transfer.

As a confirmation, the correlations between antioxidant assays and the content of bioactive components inferred: 1. TPC - very strong and positive correlation with DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ (r=0.94 and r=0.96), and moderate with CUPRAC (r=0.55); 2. TFC - very strong positive correlation with DPPH$^{\bullet}$ (r=0.90), and strong with ABTS$^{\bullet+}$ and CUPRAC (r=0.77 and r=0.73); 3. PCA - very strong positive correlation with ABTS$^{\bullet+}$ (r=0.94), strong with DPPH$^{\bullet}$ (r=0.70) and weak with CUPRAC (r=0.21); 4. FLA - very strong and positive correlation with DPPH$^{\bullet}$ (r=0.92), and strong with ABTS$^{\bullet+}$ (r=0.84) and CUPRAC (r=0.84).

Results of regression analysis indicated that flavan-3-ols exerted the most significant impact on the ability of analyzed samples to reduce metals, while proanthocyanidins primarily acted as radical scavengers.

Antioxidant potency composite index (ACI)

We used three different methods to determine antioxidant capacity. Each of these methods for antioxidant activity has certain advantages and limitations and differs in terms of the assay principles (Prior et al., 2005). Consequently, in order to give equal weight to these entire methods ACI was calculated. Although *in vitro* antioxidant capacity cannot be directly translated to biological systems, ACI presents a useful tool for ranking different commercial products (Seeram et al., 2008). The ACI values are shown in Table 3. DCH had the highest average ACI value of 99.9% among tested samples (Table 4).

CONCLUSIONS

The results of the present study confirmed the antioxidant potential of dark chocolate and dark chocolate enriched with *C. versicolor* mushroom and hazelnuts.

However, the addition of 5% of *C. versicolor* and hazelnuts to dark chocolate had no influence on antioxidant potential and total polyphenols, flavan-3-ols and proanthocyanidins contents of the enriched products. Contrary to this observation, changes in total flavonoid content were observed in the dark chocolate sample enriched with hazelnuts. Regression

analysis among results obtained with antioxidant assays revealed that dark chocolate and dark chocolate products may act in mixed mode, by direct reduction via electron transfer or by radical quenching via H atom transfer. Flavan-3-ols had the most significant impact on the ability of analyzed samples to reduce metals, while proanthocyanidins primarily acted as radical scavengers.

The obtained results provided additional information regarding the high valuable dark chocolates enriched with bioactive compounds of medicinal mushroom and plant origin. Further studies are needed to investigate the qualitative and quantitative diversity of polyphenols subclasses which are present in analyzed samples and subclasses impact on antioxidant mechanisms.

ACKNOWLEDGEMENTS

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УТИЦАЈ ДОДАТКА ЛЕКОВИТЕ ГЉИВЕ *CORIOLUS VERSICOLOR* И ЛЕШНИКА НА АНТИОКСИДАТИВНИ ПОТЕНЦИЈАЛ И БИОАКТИВНИ САДРЖАЈ ЦРНЕ ЧОКОЛАДЕ

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Сажетак: Оксидативни стрес је одговоран за напредовање хроничних обољења као што су кардиоваскуларне болести, дијабетес и неуродегенеративни поремећаји. Познато је да црна чоколада садржи полифеноле чији унос остварује позитиван утицај на здравље. Сходно томе, циљ овог истраживања био је да се анализира антиоксидативни потенцијал и укупни садржај полифенола (TPC), флавоноида (TFC), флаван-3-ола (FLA) и проантоцијанидина (PCA) црне чоколаде (DC) и црне чоколаде са додатком гљиве *Coriolus versicolor* (DCC) и лешника (DCH). Антиоксидативни потенцијал поређен је применом DPPH·, CUPRAC и ABTS·+ тестова за мерење антиоксидативних способности *in vitro*. Средња вредност свих антиоксидативних тестова за сваки анализирани производ коришћена је за израчунавање антиоксидативног композитног индекса (ACI). Резултати истраживања су показали да DC, DCC и DCH имају изражени антиоксидативни потенцијал. Додавање лешника тамној чоколади значајно је утицало на њен укупни садржај флавоноида. Регресиона анализа резултата добијених антиоксидативним тестовима је потврдила да DC, DCC и DCH испољавају антиоксидативни потенцијал различитим реакционим механизмима тј. директном редукцијом преношењем електрона и хватањем слободних радикала преношењем водоникових атома. Потврђено је да флаван-3-оли имају значајан утицај на способност анализираних узорака да редукују метале, док проантоцијанидини углавном делују као хватачи слободних радикала. Добијени резултати овог истраживања пружају додатне информације о биолошком потенцијалу црне чоколаде са додатком биоактивних компонената биљног порекла и лековитих гљива.

Кључне речи: *антиоксидативни композитни индекс, црна чоколада, црна чоколада са додацима лековите гљиве и лешника*