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THE INFLUENCE OF EXTRACTION PARAMETERS ON PHYSICOCHEMICAL PROPERTIES OF SPECIAL GRAIN BRANDIES WITH *Ganoderma lucidum*

Article Highlights

- *Ganoderma lucidum* is an interesting raw material in the production of special grain brandy
- The extraction parameter had important influence on the content of identified triterpenoid acids
- The special grain brandy with *G. lucidum* shows a considerable antioxidant potential
- The addition of G. lucidum can be an alternative to long-time aging in wooden casks

Abstract

Ganoderma lucidum is one of the five major medicinal mushrooms. In Asian countries, alcoholic beverages with Ganoderma are traditionally produced and sold in local markets as a symbol of healthy products. The aim of this study was to examine the possibility of producing brandy enhanced with this mushroom and to investigate the influence of extraction parameters (time, concentration) on color, total phenolic content, antioxidant capacity, sensory characteristics and the composition and content of triterpenoid acids within the brandy. HPLC-DAD/ESI-ToF-MS analysis was used to identify triterpenoid acids. In brandy samples, 15 triterpenoid acids were determined, with the total content in the range of 2.63-4.06 mg/100 mg. In these samples, the most commonly detected triterpenoid acid was ganoderic acid A. In our study, the total phenolic content of analyzed samples ranged from 34.07 to 118.1 mg/L GAE. The color and sensory characteristics of analyzed brandies were significantly improved in comparison with samples without G. lucidum. The obtained samples represent an interesting new product for market worldwide with improved antioxidant capacity.

Keywords: Ganoderma lucidum, special grain brandy, triterpenoid acids, antioxidant capacity, color.

Traditional medicine of the far east countries is based on using wide diversity of medicinal mushrooms. *Ganoderma lucidum* (Lingzhi) is one of the five major medicinal mushrooms, which was marked as an upper class herb in Sheng Pen Tsao Ching [1]. In nature, the fruit body of this mushroom was very rare and during the ancient times in the far east it was available only for high-ranking officials and royal family [2]. In recent decades, the successful artificial cultivation provided sufficient amount of fungi for commercial exploitations and production of the different drugs and food supplements. Therefore, the interest for this brilliant mushroom has expanded from Eastern countries to all around the world, especially in the Western countries.

The fruit bodies of *G. lucidum* have woody texture and in food industry are used in different forms, such as alcohol or water extract, powder, syrup and liquors [3]. The world observes the continued growth in usage of all kinds of products made from this mushroom for the promotion of health, but it has also been used to prevent and treat various diseases, including some widespread and deadly diseases like cancer, HIV, hypertension and hepatitis [4]. According to pre-

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vious research, most of its pharmaceutical activities were assumed to correlate with its antioxidant activity [5]. Based on many scientific reports, many compounds from Lingzhi have proven the antioxidant activity *in vitro* assays and the most important components of Lingzhi with antioxidant effect are phenolics, triterpenoids and polysaccharides [6,7].

Triterpenoid components isolated from *G. lucidum* are one of the most important group of bioactive compounds and they show important medicinal effects, including anticancer, anti-HIV-1, anti-inflammatory and antioxidant properties [8-13]. The extracts of Lingzhi's fruit bodies, mycelia and spores contain more than 150 highly oxygenated lanostane-type triterpenoids [14]. These oxidized species can be easily extracted by any organic solvent [15]. Extraction of Lingzhi's wooden fruit bodies in alcohol-water solution can be more successful than extraction in water, because in some cases more bioactive compounds can be dissolved [16].

The important characteristic of terpenoids is their bitter taste of different intensity. Based on the intensity of bitterness, terpenoids are divided into three groups: intensely bitter (ganoderic acid A, C1, J; lucidenic acid A, D1; lucidon A, C), slightly bitter (ganoderic acid B, C2, K) and very slightly bitter (no bitter) (ganoderic acid D; lucidenic acid B,C, E1, G, H; ganolucidic acid C, D; lucidon B) [17].

Spirits are alcoholic beverages with content of ethanol over 15-20 vol.%, produced by distillation from fermented agricultural products containing carbohydrates [18]. According to the Regulation on categories, quality and labeling of brandy and other alcohol spirits of the Republic of Serbia, grain brandy is produced by the distillation of a fermented mash of cereals; these spirits have to contain at least 37.5 vol. % of ethanol [19]. In the process of spirits production, distillation is the main step by which the volatile compounds are partially separated. The new product is colorless and often characterized by a raw, unharmonious taste and odor [20]. Distilled beverages have a negligible amount of biologically active compounds. Their composition and biological activity could be improved by maturation in wooden barrels or by the addition of herbs.

In Asian countries, *G. lucidum* is traditionally used as a raw material for the production of alcohol beverages which are sold in local markets as a symbol of healthy products. The main aim of the addition of *G. lucidum* is to improve the functional properties of beverages, but moreover to have additional important effects on sensory characteristics. The Japanese sake beverage is manufactured with the addition of *Ganoderma* extract or its flavor [21]. *G. lucidum* have been used as raw material in the production of bitter liqueur "Bitter 55" (*Ganoderma* bitter) [22]. A study has found that the addition of *G. lucidum* improved both sensory and functional characteristics of traditional Korean rice wine yakja, and also affected its color [23].

The aim of this study was to examine the possibility of producing the special brandy with *G. lucidum* and to investigate the influence of extraction parameters (time, concentration) on color, total phenol content, antioxidant capacity, sensory characteristic and the composition and content of triterpenoid acids of obtained brandy.

EXPERIMENTAL

G. lucidum was isolated from the collection of the Department of Microbiology, Faculty of Agriculture, University of Belgrade, Serbia. Grain brandy used in experiment for the production of the special brandies with *G. lucidum* was obtained from local homemade manufacture. Air-dried fruit bodies of fungi *G. lucidum* were cut in to pieces (about 1 cm) and mixed with 45 vol.% alcohol medium (grain alcohol). Extraction was performed using shaker in dark place at room temperature for 7, 21, and 60 days with three different concentrations of mushroom: 10, 25 and 40 g/L (Table 1). After the extractions, the solutions were filtered and the samples of special brandies were stored in glass bottles in dark place at room temperature. All samples were made in triplicate.

HPLC-DAD/ESI-ToF-MS analysis of special grain brandy samples

The analyzed samples (100 mL) were vacuum--evaporated (45 °C) to obtain the desired volume (~10 mL) and then lyophilizates were dissolved in methanol to the concentration of 10000 mg/mL.

HPLC-DAD/ESI-ToF-MS analyses were carried out on an Agilent 1200 series HPLC system (Agilent

Table 1. The labels for various combinations of special brandy samples with G. lucidum

Factor	Sample								
	1	2	3	4	5	6	7	8	9
Concentration of *Ganoderma lucidum*, g/L	10	10	10	25	25	25	40	40	40
Time of extraction, days	7	21	60	7	21	60	7	21	60

Technologies, Waldbronn, Germany) equipped with a degasser, a binary pump, an autosampler, a termostated column compartment and a diode array detector (DAD) coupled with a 6210 time-of-flight LC/MS system (Agilent Technologies, Santa Clara, CA, USA) *via* an electrospray ionization (ESI) interface. The chromatographic separation was achieved on a Zorbax EclipsePlus C18 column (100 mm×2.1 mm i. d.; 1.8 μm). The mobile phase consisted of water containing 0.2% formic acid (A) and acetonitrile (B). A combination of isocratic and linear gradient modes of elution was applied as follows: 0-2 min 20% B, 2-30 min 20-95% B, 30-35 min 95% B, 35-36 min 95-20% B, 36-40 min 20% B. The mobile phase flow-rate was 0.40 mL/min, the column temperature was set at 40 °C, and the injection volume was 2 μL. The spectral data were accumulated in the range of 190-450 nm, and representative chromatograms were recorded at 254 nm. The HPLC effluent was directed into the atmospheric pressure ESI ion source of the mass spectrometer. The eluted compounds were mixed with nitrogen in the heated nebulizer interface and negatively charged ions were obtained by applying following ES parameters: capillary voltage, 4000 V; gas temperature, 350 °C, drying gas (N_2) flow, 12 L/min, nebulizer pressure, 45 psig (310.26 Pa), fragmentor voltage 140 V, and masses were measured in the range of 100-1500 *m*/*z*. A personal computer system running MassHunter Workstation software was used for data acquisition and processing. The molecular feature extractor of MassHunter Workstation was used to predict chemical formulas.

To confirm the identity of compounds for which molecular formula was calculated from measured high-accurate masses, an HPLC-DAD/ESI-MS-MS experiment was performed on a Waters TQ (Tandem Quadrupole) instrument coupled with a Waters Acquity UPLC H-Class HPLC system. The HPLC system consisted of a quaternary pump (Waters Quaternary Solvent Manager), an injector (Waters Sample Manager-FTN), and a photodiode array detector (Waters 2998 PDA). The HPLC conditions were the same as those for HPLC-DAD/ESI-ToF-MS. The HPLC effluent was introduced into the ESI ion source of the mass spectrometer without splitting. The detector was operated at low resolution in the full scan mode under the following MS conditions: negative ion mode; capillary voltage, 3000 V; cone voltage, 25 V; source temperature 120 °C; desolvation temperature, 250 °C; drying gas (N_2) flow, 500 L/h; scan range, 100-1500 *m*/*z*. The ultrahigh purity argon was used as the collision gas for collision induced dissociation (CID) experiments, and the collision energy was set at 20 eV. In the MRM (multiple reaction monitoring) mode, the characteristic transitions of the deprotonated ($[M-H]^-$) and/or $[M-H-H_2O]^-$ molecular ions of components (Table 2) were measured. The data acquisition and analysis were performed by MassLynx V4.1 software.

Above mentioned HPLC-DAD/ESI-MS-MS experiment in scan mode was used for estimation of amounts of components. Namely, the amounts of compounds were estimated by comparing the peak areas obtained for the particular component (1-15) with the peak area obtained for the internal standard (cholic acid).

Determination of total phenolics

Determination of total phenolic content (TPC) in the samples of grain brandy with *G. lucidum* was conducted by the Folin-Ciocalteu method described by Singleton and Rossi [24].

DPPH radical scavenging activity

DPPH-reducing activity was evaluated following the modified procedure described by Kaneda *et al.* [25]. The analyzed samples (0.2 mL, diluted in different ration with 96 vol.% ethanol) were added to the DPPH working solution (2.8 mL) (mixture of 0.186×10^{-4} mmol/L DPPH in ethanol and 0.1 M acetate buffer (pH 4.3) in ratio 2:1). The absorbance at 525 nm was measured after 90 min of incubation in the dark. DPPH reagent and distilled water were used as a blank reference. The Trolox calibration curve was plotted as a function of the inhibition percentage of DPPH radical. The results were expressed as mM of Trolox equivalents per liter of brandy. The percentage of DPPH inhibition was calculated by following equation:

$$\text{Activity (\% of DPPH reduction)} = 100\frac{A - As}{A} \qquad (1)$$

where A is the absorbance of DPPH solution with ethanol and As-absorbance of a DPPH solution with sample. All experiments were performed in triplicate.

FRAP assay

FRAP assay was performed according to the procedure by Benzie and Strain [26].

Color determination

Color determination of distillate beverages was performed according to the AOAC method [27]. The standard curve was produced with solutions of $K_2Cr_2O_7$ (0.05-0.5 g/L) in 0.005 M H_2SO_4, whose absorbance was determined at 430 nm and expressed in color units (CU), ranging from 1 to 10.

Table 2. The contents of triterpenoid acids in special grain brandy samples (mg/100mg)

No t_R / min DAD/MS	Content of components in the liophilizate of the sample, mg/100 mg									λ_{max}	Molecular formula and mass	Mass and *m/z* (measured)	MRM Transition	Compound
	G1	G2	G3	G4	G5	G6	G7	G8	G9	nm				
1 8.00/8.08	0.2379	0.1893	0.2115	0.2115	0.2621	0.2586	0.2483	0.0690	0.2345	256	$C_{30}H_{46}O_7$ (518.3087)	518.3244; 517.3168; 563.3232; 1035.6402	517→499	Ganoderic acid C2
2 8.36/8.45	0.1212	0.1275	0.0866	0.1113	0.1545	0.1841	0.1427	0.2004	0.1033	256	$C_{27}H_{40}O_6$ (460.2825)	460.2824; 459.2750; 505.2812; 919.5583	459→441 459→385	Lucidenic acid LM1
3 8.51/8.59	0.0890	0.1210	0.1062	0.0938	0.1157	0.1333	0.0993	0.1010	0.0897	254	$C_{30}H_{42}O_8$ (530.2879)	530.2879 529.2806 575.2862	529→511 511→467	Ganoderic acid C 6
4 8.83/8.92	0.3525	0.3836	0.4092	0.3495	0.4258	0.4551	0.4382	0.0877	0.4316	256	$C_{30}H_{44}O_8$ (532.3036)	532.2927; 531.2851; 1063.583	531→513 513→469	Ganoderic acid G
5 9.10/9.19	0.2155	0.2070	0.2951	0.2576	0.3214	0.2948	0.2991	0.2160	0.2829	254	$C_{30}H_{44}O_7$ (516.3087)	516.3087; 515.3016; 561.3054; 1031.6068	515→497 497→453	Ganoderic acid B
6 9.24/9.33	0.2441	0.2789	0.2553	0.2246	0.3034	0.3306	0.2586	0.2258	0.2431	258	$C_{29}H_{40}O_8$ (516.2723)	516.2810; 515.2738	515→473	Lucidenic acid E
7 9.55/9.65	0.2540	0.2525	0.2407	0.2966	0.3205	0.3212	0.3070	0.2330	0.0330	254	$C_{30}H_{40}O_8$ (528.2723)	528.2723 527.2651 573.2757	527→509 509→465	Elfvingic acid A
8 9.93/10.04	0.5480	0.5433	0.6177	0.5852	0.7813	0.6589	0.6664	0.5285	0.6340	256	$C_{30}H_{44}O_7$ (516.3087)	516.3088; 515.3015; 561.3073; 1031.6099	515→497	Ganoderic acid A
9 10.52/10.63	0.2195	0.1226	0.1384	0.2448	0.2490	0.2466	0.2497	0.1722	0.2375	254	$C_{27}H_{38}O_6$ (458.2668)	458.2669; 457.2595; 503.2652; 915.5264	457→439 457→287	Lucidenic acid A
10 10.63/10.74	0.0896	0.0677	0.0639	0.0913	0.0887	0.1128	0.1047	0.0238	0.0870	254	$C_{30}H_{42}O_8$ (530.2879)	530.2877; 529.2804; 575.2854; 1059.5595	529→511 511→467	12-Hydroxy--ganoderic acid D
11 11.11/11.22	0.2141	0.1874	0.2181	0.2959	0.3049	0.2682	0.3051	0.2186	0.3075	254	$C_{30}H_{42}O_7$ (514.2930)	514.2930; 513.2854; 559.2932; 1027.5754	513→495 495→451	Ganoderic acid D
12 11.29/11.40	0.2211	0.1538	0.1820	0.1882	0.2106	0.2264	0.2227	0.1733	0.1960	254	$C_{29}H_{38}O_8$ (514.2566)	514.2627; 513.2554	513→471	Lucidenic acid D2
13 11.40/11.52	0.0844	0.0888	0.0886	0.1029	0.0832	0.1011	0.1228	0.0848	0.1298	254	$C_{30}H_{40}O_7$ (512.2774)	512.2773; 511.2697; 557.2763; 1023.5455	511→493 493→449	Ganoderenic acid D
14 11.94/12.06	0.2613	0.2527	0.3089	0.3291	0.3510	0.3847	0.3277	0.2935	0.3845	254	$C_{32}H_{42}O_9$ (570.2829)	570.2829; 569.2757; 1139.5577	569→551 551→509	Ganoderic acid F

Table 2. Continued

No t_R / min DAD/MS	Content of components in the liophilizate of the sample, mg/100 mg									λ_{max}	Molecular formula and mass	Mass and *m/z* (measured)	MRM Transition	Compound
	G1	G2	G3	G4	G5	G6	G7	G8	G9	nm				
15 12.10/12.23	0.0505	0.0420	0.0477	0.0667	0.0846	0.0722	0.0810	0.0666	0.0715	254	$C_{30}H_{42}O_7$ (514.2930)	514.2930; 513.2854; 559.2932; 1027.5754	513→451 513→437	Ganoderic acid J
Σ	0.8180	0.7079	0.8038	0.8967	1.1149	0.9777	0.9971	0.7673	0.9430	-	-	-	-	Bitter triterpenoid acids
Σ	3.2027	3.0181	3.2699	3.449	4.0567	4.40487	3.873	2.6942	3.4659	-	-	-	-	Total triterpenoid acids

CIEL**a***b** chromatic parameters

Color measurements were performed on brandy samples using a portable tristimulus Chroma Meter model CR-400 (Konica Minolta, Osaka, Japan). Results were expressed in Commission Internationaled'Eclairage *L**, *a** and *b** color space coordinates. The following parameters were measured: *L** (lightness), *a** (+*a** = redness, -*a** = greenness), *b** (+*b** = yellowness, -*b** = blueness), *C** (chroma or saturation) and *h* (hue angle). CIEL**a***b** parameters were read using CIE illuminant D_{65} and the observer angle at 2°.

Sensory analyses

Sensory characteristics of the brandies enriched with mushroom were determined using a modified Buxbaum model of positive ranking. The common quality parameters were evaluated: clearness, color, distinction, odor and taste. In this evaluation a brandy sample may have a maximal score of 20 points [28]. The analysis was conducted by evaluation panel, made of 5 sensory experts. All evaluation experts had long tradition in evaluation of alcohol beverages. Samples were diluted with distilled water to reach an alcohol proof of 45° (vol.%).

Statistical analyses

The determination of polyphenol, antioxidant capacity, sensory characteristics and color were done in triplicate, and data were expressed as mean value ± standard deviation (*SD*). The experimental data were subjected to the analysis of variance (ANOVA). Analysis was conducted in a factorial arrangement where time extraction and concentration of added *G. lucidum* were analyzed factors. Tukey's test was used to determine difference ($p \leq 0.01$) between the mean values. Statistical analyses were performed with the statistical program Statistica 12 [29].

RESULTS AND DISCUSSION

The study was conducted to identify and compare the composition and concentration of triterpenoid acids in 9 samples of special grain brandies. In the research following, 15 triterpenoid acids were determined in all samples (Table 2): ganoderic acid (A, B, C2, C6, D, F, G and J), ganoderenic acid (D), lucidenic acid (A, E, D2 and LM1), 12-hydroxyganoderic acid D and elfingenic acid A. Based on these results, the extraction parameters did not effect on the composition of identified triterpenoid acids in special grain brandies. Previous research reported that the chemical composition of fungi *G. lucidum,* including triterpenoid acids, depends on the geographical distributions, growth conditions and substrates [30].

However, the extraction parameters had an important effect on the content of total triterpenoid acids in analyzed brandy samples (2.63-4.06 mg/100 mg, Table 2). According to the content of terpenoid acids, the analyzed samples can be ranged as: G5 > G6 > G7 > G4 > G9 > G3 > G1 > G2 > G8.

Wang *et al.* [31] estimated the quantity of six major triterpenoids in 36 *Ganoderma* samples. The average content of total triterpenoids in *G. lucidum* samples was 0.18-1.15 mg/100 mg. Hence, the content of triterpenoids in brandy samples was higher than in the analyzed *G. lucidum* samples.

Ganoderic acid A was the most abundant ganoderic acid in analyzed samples, which has been confirmed in some previous research [32]. The extraction time influenced ganoderic acid A content; with increasing concentration of added mushroom, the extraction of this compound completed faster. Addition of the higher amount of mushroom in brandy samples had limited effect on increasing the ganoderic acid A content and the highest value was found in sample G5 with 25 g/L mushroom extracted after 21 days.

Total phenolic content and antioxidant properties

TPC and antioxidant properties were presented in Table 3. The results of ANOVA showed that the concentration of mushroom and its extraction time had very significant influence on TPC of these special grain brandies ($p < 0.01$). The interaction fungi concentration×extraction time did not affect the phenolic content of analyzed samples, which indicates that these factors affect independently.

Grain brandy used as an alcohol medium for the production of special grain brandy (5.1 mg/L) had a low TPC content, which is consistent with all distillated unaged beverages. The phenolic components in the distilled beverages originated from the wooden barrels in which they are stored after distillation. According to the research of Ziyatdinova *et al.* [33], regular brandy shows TPC in the range of 59-334 mg/L gallic acid equivalents (GAE) depending on the type and origin of brandy. In our study, the TPC content of analyzed samples ranged from 34.07 to 118.1 mg/L GAE. It can be concluded that the TPC of these analyzed samples were also significantly increased by adding fungi to the alcoholic beverage. The regular brandies aged for at least two years (3 stars) did not have a significantly higher TPC then special brandies G7, G8, G9 made with 40 g/L *G. lucidum* [33]. Based on the results, the fungi concentration had a strong influence on the TPC. Also, extraction time did not have a considerable effect on the parameters investigated in the following samples: 25 (G4, G5, G6) and 40 g/L (G7, G8, G9). This outcome indicates that the extraction process was completed within seven days. The only exception of this behavior was the samples G1, G2 and G3 with 10 g/L of mushroom. Based on the results, extraction of phenolics was not finished after 60 days for these samples.

Two antioxidant assays were carried out to evaluate the antioxidant characteristics of analyzed brandy samples: *DPPH* and *FRAP* assay. Our results showed a considerable antioxidant potential of analyzed brandy samples, which strongly depends on the concentration of added fungi. The correlation between TPC and antioxidant capacity was very high and presented in Table 4, with values $r_{(TPC\text{-}FRAP)} = 0.9702$ and $r_{(TFC\text{-}DPPH)} = 0.9618$. Phenolic compounds of fungi significantly improve the antioxidant capacity of grain brandy, which is in correlation with previous research conducted by Kim *et al.* [3].

Table 4. Correlation between TPC, antioxidant characteristics and color intensity

	FRAP		DPPH		Color	
	r	p	r	p	r	p
TPC	0.9702	0.000	0.9618	0.000	0.9618	0.000
FRAP		0.000	0.9422	0.000	0.9291	0.000
Color					0.9231	0.000

Color measurement

Color is the most important visual feature, which creates the first and very important impression among consumers. Hence, the color of a distillate beverage is an important characteristic, especially for beverages matured in wooden casks in which the dark golden color signifies the highest quality of beverage. According to this research, the compounds extracted from this mushroom also affect the color of the alcoholic beverage.

The color intensity of grain brandy used as basic alcohol medium was 1.25 CU. After extraction of components from *G. lucidum*, the color of the brandy changed. Based on the results, it can be concluded that compounds of fungi significantly increased the color intensity of special brandy samples. The differ-

Table 3. Total phenol content, antioxidant activity and color of special brandy with Ganoderma lucidum; TPC – Total phenol content, expressed as milligram of gallic acid equivalents per liter of brandy; DPPH – DPPH radical scavenging activity expressed as mmol of Trolox equivalent; different letters in same row denote a not significant difference according Tuckey's test, at p < 0.01

Sample	Parameter			
	TPC / mg GAE/L	*FRAP*	*DPPH* / mmol TE	*CUI* / Color units
G1	35.13 ± 0.42^{a}	0.148 ± 0.008^{a}	0.338 ± 0.012^{a}	4.93±0.02
G2	34.07 ± 0.64^{a}	0.138 ± 0.001^{ab}	0.330 ± 0.005^{ab}	4.84±0.00
G3	51.40±6.20	0.151 ± 0.006^{ab}	0.405 ± 0.034^{ab}	8.99 ± 0.00^{a}
G4	77.13 ± 3.01^{b}	0.296 ± 0.000^{c}	0.867 ± 0.009^{c}	8.99 ± 0.01^{a}
G5	84.60 ± 8.23^{b}	0.311 ± 0.004^{cd}	1.014 ± 0.115^{cd}	9.32±0.00
G6	88.07 ± 7.64^{b}	0.321 ± 0.012^{cd}	0.924 ± 0.026^{cdf}	8.48±0.00
G7	114.60 ± 0.80^{c}	0.432 ± 0.015^{f}	1.318±0.037	14.18±0.00
G8	110.90 ± 1.90^{c}	0.417 ± 0.013^{fg}	0.976 ± 0.008^{cdfg}	13.11±0.00
G9	118.10 ± 2.30^{c}	0.438 ± 0.005^{fg}	1.043 ± 0.031^{dfg}	12.62±0.00

ent extraction time did not significantly increase the color intensity of samples with equal concentration of fungi. Hence, addition of higher amount of fungi makes the color more intensive for samples with the same extraction time. The correlation of TPC and color was very high (r = 0.9618). It can be concluded that the color intensity of samples was significantly correlated with phenolic content.

According to previous research, the color of plum brandies after 11 years of maturation in sessile oak and 18 year and in mulberry cask [28] was not significantly higher than the color value for the sample of special brandy G7. Therefore, the addition of this mushroom can be regarded as an innovative process and can replace the long period of aging in wooden casks.

The results of CIEL*a*b* method are presented in Table 5. The L* value decreased with increasing concentration of added mushroom. The value of parameter a* for samples with 10 g/L (G1, G2, G3) was significantly different from the samples with higher concentration (G4-G9), because it was defined with the light tone of green color, and the other samples had light tone of red color. The parameter b* for all samples describes the different intensity of the yellow color. According to the results for the hue angle, and parameters a* and b*, it can be concluded that all samples had yellow color, with very small proportion of red or green color. Based on these results, it can be concluded that with addition of the higher amount of *G. lucidum*, the lightness of samples is reduced. Also, the addition of higher amount of mushroom affected the values of a* and b* parameters and therefore increased the proportion of yellow color and red color of samples.

Sensory evaluation

The results of sensory evaluation were between 17.78 and 18.12 (Table 6). It is obvious that the brandy samples enriched with *G. lucidum* were well accepted by sensory experts. The results of factorial ANOVA indicate that the extraction parameters and their interaction did not have statistically significant effect on the sensory scores of special brandy samples. Therefore, the sensory characteristics of analyzed samples were improved with extracted components of fungi, but the quantity of extracted compounds did not have significant influence on the final sensory impression.

The triterpenoid acids were the most important compounds of fungi which define the taste of the brandy samples. Although the lowest content of total

Table 5. CIElab chromatic parameters of special brandy samples with Ganoderma lucidum; values represent means of triplicate determinations ± standard deviation

Sample	L*(D65)	a*(D65)	b*(D65)	C*(D65)	h(D65)
G1	54.84±0.01	-0.55±0.00	20.59±0.02	20.60±0.02	91.54±0.02
G2	54.98±0.01	-0.54±0.02	19.90±0.02	19.91±0.01	91.56±0.06
G3	54.55±0.01	-0.78±0.02	23.41±0.01	23.43±0.01	91.91±0.08
G4	50.07±0.01	2.35±0.01	34.16±0.00	34.24±0.00	86.07±0.01
G5	51.93±0.01	0.86±0.01	28.90±0.02	28.91±0.02	88.29±0.02
G6	50.06±0.00	2.57±0.01	35.76±0.01	35.85±0.01	85.89±0.02
G7	48.22±0.01	4.67±0.03	36.52±0.02	36.82±0.02	82.72±0.05
G8	49.47±0.01	3.46±0.03	35.42±0.01	35.59±0.01	84.42±0.05
G9	47.72±0.01	5.39±0.04	39.18±0.04	39.55±0.03	82.16±0.07

Table 6. Sensory characteristic of special brandies; values represent means of triplicate determinations ± standard deviation. G-grain brandy

Sample	Assessments characteristics				
	Color	Clearness	Distinction	Odor	Taste
G1	1	1	2	5.66±0.11	8.48±0.08
G2	1	1	2	5.64±0.11	8.36±0.11
G3	1	1	2	5.50±0.16	8.40±0.07
G4	1	1	2	5.68±0.22	8.34±0.05
G5	1	1	2	5.70±0.16	8.26±0.05
G6	1	1	2	5.62±0.13	8.48±0.08
G7	1	1	2	5.48±0.08	8.54±0.05
G8	1	1	2	5.62±0.13	8.50±0.07
G9	1	1	2	5.38±0.08	8.40±0.07

terpenoinds acids was found in the sample G8, the share of bitter triterpenoids was higher than in other samples. It can be concluded that the content of bitter acids had the more important influence on the sensory marks than the content of total triterpenoid acids. As reported in the previous research, the specific triterpenoid acid had a different medical effect. The higher content of bitter terpenoids, particularly ganoderic acid A, also improved the antioxidant capacity, antinociceptive, anti-inflammatory and antitumor activity of samples [11].

CONCLUSION

Chemical composition and sensory characteristics of grain brandy were changed by the maceration of *Ganoderma lucidum* due to the transition of soluble mushroom compounds in an alcohol-water mixture. Based on the analysis of the content of total phenolics, antioxidant properties and sensory characteristics, it can be concluded that extracted mushroom compounds refined the chemical complex. In the production process, the optimal extraction parameters have to be defined depending on the desired character of products. With the increase of fungi concentration the antioxidant capacity is enhanced, and improves the health characteristics of special brandies, but the higher amount of fungi did not have an important effect on the sensory evaluation. The extracted component colored the samples, and therefore the addition of this mushroom can be regarded as an innovative process and can be an alternative to the process of aging in wooden casks over a long period. The medicinal mushroom *G. lucidum* may be an interesting raw material in the production of special brandy with a bitter taste.

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NAUČNI RAD

UTICAJ PARAMETARA EKSTRAKCIJE NA FIZIČKOHEMIJSKE KARAKTERISTIKE SPECIJALNIH ŽITNIH RAKIJA SA DODATKOM GLJIVE *Ganoderma lucidum*

Ganoderma lucidum spada u pet najznačajnijih medicinskih gljiva. U Azijskim zemljama, alkoholna pića sa dodatkom G. lucidum se tradicionalno proizvode i prodaju u lokalnim marketima kao simbol zdravih proizvoda. Cilj ove studije bio je ispitivanje mogućnosti proizvodnje rakija obogaćenih ovom gljivom, kao i proučavanje uticaja parametara ekstrakcije (vremena ekstrakcije i koncentracije gljive) na boju, sadržaj ukupnih fenolnih jedinjenja, antioksidativni kapacitet, senzorne karakteristike i sadržaj triterpenskih kiselina specijalnih rakija. HPLC-DAD/ESI-ToF-MS metoda je korišćena za identifikaciju triterpenskih kiselina. U uzorcima specijalnih rakija, detektovano je 15 triterpenskih kiselina sa ukupnim sadržajem od 2,63 do 4,06 mg/100 mg. Ganoderinska kiselina A je bila najzastupljenija triterpenska kiselina u analiziranim uzorcima. Ukupan sadržaj fenola u analiziranim uzorcima se kretao od 34,07 do 118,1 mg/L GAE. Boja i senzorne karakteristike analiziranih uzoraka specijalnih rakija su značajno poboljšani u poređenju sa uzorcima bez dodatka G. lucidum. Dobijeni uzorci predstavljaju interesantan novi proizvod sa povećanim antioksidativnim kapacitetom za tržišta širom sveta.

Ključne reči: Ganoderma lucidum, specijalne žitne rakije, triterpenske kiseline, antioksidativni kapacitet, boja.