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IMPACT OF GRAPE POMACE AS A CULTIVATION SUBSTRATE ON THE *PLEUROTUS OSTREATUS* CHEMICAL AND BIOLOGICAL PROPERTIES

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The objective of this study was to develop a single quality index of chemical characteristics of Pleurotus ostreatus extracts on 7th and 14th day of its shelf life, derived from the mushroom fruiting bodies. P. ostreatus was cultivated on four substrates containing different ratio of wine industry waste-grape pomace (P) and wheat straw (S): 100P, 80P20S, 50P50S, 20P80S. Four quality parameters of P. ostreatus mushroom extracts, i.e. antioxidative parameters: $ABTS^{+}$ and $DPPH^{\bullet}$ free radical scavenging capability, total phenolic compounds (TPC) and total polysaccharides (TPS) were used to define the final extract quality index. Analysis indicated 100P and 80P20S as the samples cultivated on the substrate with higher percent of grape pomace, as the best quality at the 7th day of its shelf life. On the other hand, final quality score indicated 50P50S and 20P80S, cultivated on a substrate with a lower percent of grape pomace, as the best quality samples at the 14th day of its shelf life. According to the results, samples cultivated on a higher pomace content substrate are of better quality in a shorter storage time period.

Keywords: *Pleurotus ostreatus*, food waste, quality index, grape pomace, mushroom.

INTRODUCTION

One of the most commonly cultivated and consumed mushrooms worldwide is *Pleurotus ostreatus* (Oyster mushroom). According to the Food and Agriculture Organisation of the United Nations data (FAOSTAT), total world production of mushrooms in 2018 was 9 million metric tons, with *P. ostreatus* holding the second place in industrial world production (1). Besides its specific pleasant taste and adaptibility to various cellulose substrates, scientific interest considering this mushroom species is also directed to its various bioactive compounds that improve defense mechanisms for cell oxidative damage (2). These components are also related to mushroom antioxidant, antimicrobial, antitumor, antiviral and immunomodulatory properties (1,3).

Apart from these positive properties, *P. ostreatus* belongs to "white-rot fungi" category. This is related to its ability to produce lignolytic enzymes, beside to its cellulose degradation capability. This feature gives the opportunity of using the wide range of agroindustrial and food production wastes for mushroom cultivation, instead of the ones ordinary used in global production (4). Considering *P. ostreatus*, it is usually cultivated on wheat straw (5), but the use of alternative substrates could be of great importance for increasing productivity and improving the properties of mushroom. Except the benefits of mushroom production,

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agroindustrial waste recycling has the other adventages, such as solving of environmental global issues (6,7).

In this study quality of *P. ostreatus*, cultivated on grape pomace derived from the wine industry as a waste, was estimated by analyzing its chemical and biological properties at 7th and 14th day of its shelf life. *P. ostreatus* was cultivated on four substrates with different ratio of grape pomace and wheat straw. Four quality parameters, namely free radical scavenging capability determined in $ABTS^{+}$ and DPPH·assays and totals of phenolic compounds and polysaccharides, were involved in calculation of a single total quality index (TQI), in order to compare the quality of four mushroom extracts samples in two shelf life stages.

EXPERIMENTAL

SUBSTRATE PREPARATION

P. ostreatus extracts were derived from the fruiting bodies cultivated on four substrates containing different ratio of grape pomace and wheat straw, described in Table 1.

Table 1. Substrate composition used for *P. ostreatus* mycelium inoculation

No.	Substrate codes	Composition of substrate content
1	100S	100% wheat straw
2	100P	100% grape pomace
3	80P20S	80% grape pomace: 20% wheat straw
4	50P50S	50% grape pomace: 50% wheat straw
5	20P80S	20% grape pomace: 80% wheat straw

Grape pomace was collected during the summer 2018 from the grape variety Prokupac, originating from Aleksandrovačka Župa, Serbia. The tissue culture inoculation method described by Mondal et al. (8) was used, by isolation from fresh mushroom fruiting bodies of commercial strain A15.

Each substrate mixture was prepared in triplicates. The mixture was filled into polypropilene bags with filters (Microsac, PP75/BEH6/X37-53, Nevele, Belgium) and sterilized in autoclave for 2 h at 121 °C with 1.5 kg cm^{-2} pressure. Mushroom mother culture was added afterwards in sterile conditions to each substrate bag, and placed in an incubation room at 25 °C under 85% relative humidity. Mycelial growth was completed in approximately 20 days. Upon completion, each bag was opened and placed in a room with 80-85% relative humidity and the mycelium was in contact with external atmosphere, which enabled the growth of fruiting bodies in the next 5-7 days. Fresh mushrooms, harvested from the first flush, were selected to be of uniform size and shape. 50 g of mushrooms were individually packed in 85 μm thick (PA/PE/PE) bags (200mm x 300 mm) with transmission rates of 60 mL O_2, 12 mL N_2 and 180 mL CO_2 m^{-2} day^{-1} atm^{-1}, using HVC-510T/2A packaging machine; they were refrigerated under controlled conditions at 4 °C for 7 and 14 days.

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EXTRACT PREPARATION

In order to prepare mushroom extracts for further analysis, mushroom fruiting bodies were air-dried at 55 °C and powdered. The crude hot water extracts were prepared by adding 40 mg mL^{-1} of distilled water and heated at 75-85 °C for 2h. The samples were centrifuged for 10 min and supernatant was decanted and stored for further analysis.

CHEMICAL CHARACTERIZATION - TOTAL PHENOLIC AND POLYSACCHARIDE CONTENTS

Total phenolic content (TPC) was investigated by adapted Folin-Ciocalteu reaction method, previously described by Djekic et al. (9), with the same concentration range (0.625-40 mg mL^{-1}), using 96-well microplate reader (microplate reader ELx808, BioTek Instruments, Inc., SAD). Results were expressed as mg gallic acid equivalent (GAE) g^{-1} of the extract.

Determination of total polysaccharide content (TPS) was done by the phenol-sulfuric acid method with D-glucose [10], in a concentration range from 0.625-40 mg mL^{-1}.

ANTIOXIDATIVE POTENTIAL

Antioxidative potential was expressed by DPPH·and $ABTS^+$ free radical scavenging capability, expressed as EC_{50} (mg mL^{-1}) values, being defined as "the effective concentration of mushroom extract required to neutralize 50% of $ABTS^+$/DPPH·radicals". Higher antioxidant ability corresponds to lower EC_{50} value (2). DPPH·free radical scavenging activity assay was performed by the method of Ekanayake et al. (11), with catechin and Trolox as positive controls, in a concentration range of 0.03125 – 2 mg mL^{-1}. $ABTS^+$ free radical scavenging activity assay was performed by the method of [12], with catechin and BHA as positive controls, in the same concentration range of 0.03125 – 2 mg mL^{-1}. Concentrations ranging from 0.625 – 40 mg mL^{-1} for each extract were analyzed.

TOTAL QUALITY INDEX APPROACH AND STATISTICAL ANALYSIS

For calculating TQI of mushroom extracts, the following rules were applied for selected quality parameters: (a) rule "the smaller the value, the better the quality" has been applied for the EC_{50} values determined in ABTS+ and DPPH·assays; (b) rule "the higher the value, the better the quality" was applied for the TPC and TPS values. The final TQI score was calculated as previously described in the literature (13, 14). Interpretation of the final score is "the lower value of TQI, the better overall quality". Statistical analysis was obtained by two-way analysis of variance (ANOVA) and *Tukey's post hoc test* with statistical significance at the level $p<0.05$. SPSS Statistics 17.0 and Microsoft Excel 2010 were used for statistical data analysis.

RESULTS AND DISCUSSION

All obtained results are shown in Table 2 and 3. Our results indicate the growing values of TPC with prolonging of shelf life period. Namely, TPC of each extract was higher at the 14^{th} day compared to the 7^{th} day. Moreover, the growing trend was more extensive for the extracts

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with higher grape pomace substrate content. However, the highest value of TPC was shown for the 80P20S extract, not for the 100P, with the highest value determined after 14 days of growth, as well. Concerning TPS values, our results showed that they were approximately uniform for all the values determined at each day of shelf life (with exception of the value for the 100P extract determined at 7th day), but was slightly decreased in 14th day.

Table 2. The effects of grape pomace as a substrate on the *P. ostreatus* chemical properties

Chemical properties					
Day	**100P**	**80P20S**	**50P50S**	**20P80S**	**100S***
TPC (mg/g GAE)					
7th	19.83 ± 0.32^{A}	27.09 ± 0.56^{B}	21.10 ± 0.84^{A}	20.26 ± 1.01^{A}	19.40 ± 1.07
14th	25.11 ± 0.28^{A}	37.39 ± 2.42^{B}	24.9 ± 0.39^{A}	20.73 ± 0.59^{C}	
TPS (mg/g GLU)					
7th	110.06 ± 8.67	90.84 ± 1.05	114.93 ± 9.90	111.70 ± 2.64	107.44 ± 10.21
14th	69.27 ± 9.21^{A}	89.62 ± 2.60^{AB}	88.03 ± 2.70^{AB}	94.71 ± 3.40^{B}	

Means of ten replications ± standard deviation. Means in the same row with different capital letters are significantly different ($p<0.05$). Legend: 100P - 100% grape pomace substrate content; 80P20S - 80% grape pomace: 20% straw substrate content; 50P50S - 50% grape pomace: 50% straw substrate content; 20P80S - 20% grape pomace: 80% straw substrate content; 100S* - 100% wheat straw substrate content (control sample, day 0).
TPC - total phenolic compounds; TPS - total polysaccharides.

Mushrooms are well known for their antioxidant properties, being directly related to all tested extracts were characterized by some antioxidative activity, but generally higher potential was observed in $ABTS^{+}$ assay (Table 3.).

Table 3. The effects of grape pomace as a substrate on the *P. ostreatus* antioxidant properties

Antioxidant properties					
Day	**100P**	**80P20S**	**50P50S**	**20P80S**	**100S***
$ABTS^{+}$ EC_{50} (mg/mL)					
7	< 0.625^{A}	4.41 ± 0.05^{C}	4.96 ± 0.16^{C}	7.49 ± 0.01^{B}	8.42 ± 0.20
14	3.91 ± 0.11	3.68 ± 0.05	4.27 ± 0.21	3.59 ± 0.04	
DPPH·EC_{50} (mg/mL)					
7	2.66 ± 0.14^{A}	8.15 ± 0.21^{B}	13.21 ± 0.33^{C}	10.73 ± 0.04^{BC}	15.04 ± 0.08
14	8.08 ± 0.13^{A}	> 40^{B}	8.90 ± 0.23^{A}	8.11 ± 0.36^{A}	

Means of ten replications ± standard deviation. Means in the same row with different capital letters are significantly different ($p<0.05$). Legend: 100P - 100% grape pomace substrate content; 80P20S - 80% grape pomace: 20% straw substrate content; 50P50S - 50% grape pomace: 50% straw substrate content; 20P80S - 20% grape pomace: 80% straw substrate content; 100S* - 100% wheat straw substrate content (control sample, day 0).

The highest potential of both radicals neutralization was detected for the 100P extract prepared from 7th day of mushroom storage. On the other hand, control sample have shown the lowest potential of both radicals neutralization.

Figure 1. derived from TQI of four quality parameters of mushroom extracts analysis, shows that 100P was the extract with the best quality, followed by 80P20S, but only at the 7th day of their shelf life.

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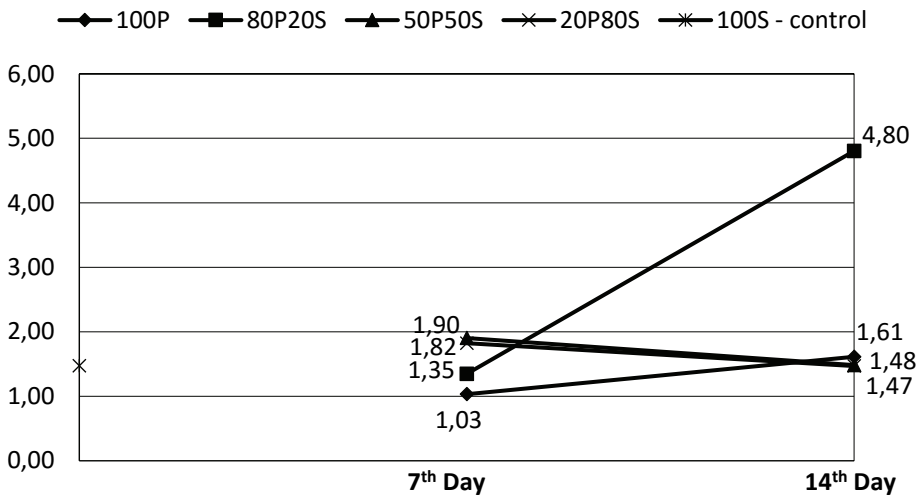

Legend: 100P - 100% grape pomace substrate content; 80P20S - 80% grape pomace: 20% straw substrate content; 50P50S - 50% grape pomace: 50% straw substrate content; 20P80S - 20% grape pomace: 80% straw substrate content

Figure 1. Total quality index (TQI) of parameters derived from measured values of mushroom extracts of the 7th and 14th day of its shelf life

The fact is that the chemical properties of the mushrooms are directly related to the substrate used for cultivation (15), and for that reason the variability of TPC and TPS values obtained for different extracts was expectable. With regard to the control sample, 100S, all other samples cultivated on the grape pomace substrate have shown higher TPC values, which can be related to the polyphenol content derived from the pomace. The growing trend of TPC values during shelf life period is in opposite with regard to Jiang T. et al. (16) and Jafri M. et al. (17), who reported decrease of phenolic content during mushroom storage, and explained this phenomenon as the enzymatic oxidation of phenolic compounds via polyphenol oxidase, which could be also associated with mushroom browning. Liu Z. and Wang X. (18) reported that the amount of polyphenolics increases with the influence of high oxygen conditions, but according to Gao M. et al. (19) additional phenolics in fruits could be formed as a defence mechanism. Vacuum-packaging applied in this study, as shelf life prolonging method, provides an anaerobic environment that suits to facultatively anaerobic bacteria: lactic acid bacteria, *Enterobacteriaceae* spp., etc. (20), with CO_2 present in the package as a metabolic by-product of microbial metabolism (21). Our results, indicating an increase of TPC during mushrooms storage, are in agreement with Karowe and Grubb (22), who reported that elevated CO_2 in the atmosphere may cause the increased levels of simple, complex and total phenolics of *Brassica rapa* (oilseed rape) under elevated CO_2.

Taking into account that Gąsecka et al. (23) reported 9.64 ± 0.33 mg g^{-1} polyphenols in *P. ostreatus* extract, being noticeable lower then the results of each analyzed extract, one could note that cultivation method that was used in our research favoured polyphenols

production. Namely, Jiang et al. (16) used beech sawdust and flax shives, supplemented with wheat bran, corn flour, gypsum and wheat straw enriched with Se and Zn.

On the other hand, general trend was the drop of the TPS with the prolonging of shelf life, while substrate used (except the 100P sample) did not affect significantly this feature. Study by Li et al. (24) reported decrease of water-soluble crude polysaccharide content in shiitake mushroom (*Lentinus edodes*) after storage, which is justified by the sugar consumption caused by respiration of the mushrooms during the storage.

Scavenging of free radical in biological systems has a preventive role against arising of oxidative damage of DNA, cellular proteins and lipids and consequently could protect from many chronic diseases and carcinogenesis (25). Mushrooms are well known for their antioxidant properties, being directly related to phenolic compounds (26, 27). However, the correlation between phenolic compounds and antioxidant activity of the extracts is not noticed in this research, which concurs to the following finding (28). According to Antonić et al. (29), grape pomace is rich in mineral content, such as Fe and Zn, which could be directly related to mineral composition of the mushroom cultivated on grape pomace substrate (15). Namely, phenolic compounds have ability to make complexes with bioavailable minerals, such as Fe, Zn and Li, which could be present in mushroom chemical structure. This complexation process reduces scavenging activity of phenolic compounds and consequently leads to limited antioxidant ability.

Finally, in order to quantify the overall quality and to point out the best cultivation method, all obtained results were summarized in TQIs of all tested samples. The outcomes were changed during the shelf life period, especially for the 80P20S. However, this time-dependent changes in the quality of two extracts with the lower share of grape pomace in substrate (50P50S and 20P80S) were less pronounced. According to this feature, these two substrate modes can be considered as those that favor quality stability over extended storage times.

CONCLUSION

This investigation was meant to point out the benefits of using wine industry waste as a substrate for growing mushrooms, in the form of a total quality index (TQI). The advantage of this type of production is exploitation of food waste into the value-added new product. Additionally, the kind of waste used in the mushroom production distinctly affect nutritional profile of mushroom fruiting bodies, which concurs to this investigation outcome. Results indicated grape pomace as a valuable substrate for *P. ostreatus* cultivation. Lastly, this investigation pointed out the use of quality index approach as a useful tool for applied quality evaluation.

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