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DETERMINATION OF ANTIOXIDANT PARAMETERS OF PLEUROTUS MUSHROOMS GROWING ON DIFFERENT WOOD SUBSTRATES

Strapáč, I.[1], Kuruc, M.[1], Baranová, M.[2]

[1]Department of Chemistry, Biochemistry and Biophysics, Institute of pharmaceutical chemistry,
[2]Department of Food Hygiene and Technology, Institute of milk hygiene and technology
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
Slovakia

imrich.strapac@uvlf.sk

ABSTRACT

Extracts of the fruiting bodies of the Oyster mushroom (*Pleurotus ostreatus*) grown on wood substrates (beech, oak, linden, walnut, poplar) and extracts of the fruiting bodies of the Oyster mushroom (*Pleurotus pulmonarius*) grown in nature on aspen wood were used to determine the total phenols, total flavonoids, lycopene and β-carotene. The content of individual antioxidants varies considerably depending, not only on the substrate, but also on the extracting agents. The highest content of total phenols and total flavonoids was found in methanol and water extracts of the fruiting bodies of the Oyster mushrooms grown on oak and linden substrates. The maximum content of lycopene and β-carotene was determined in acetone and n-hexane (ratio 4 : 6) extracts of the fruiting bodies of the Oyster mushroom grown on an oak block. The results obtained in this study demonstrated that the quantitative and also probably the qualitative composition of the antioxidants in the fruiting bodies of Oyster mushrooms depended considerably on the substrate composition.

Key words: antioxidant activity; β-carotene; flavonoids; lycopene; *Pleurotus ostreatus*; *Pleurotus pulmonarius*; polyphenols; wood substrates

INTRODUCTION

Today's world is full of opposites. Hunger and poverty on the one hand and incredible wealth on the other; bad way of living, large energy intake and its low use create conditions for the development of diseases of civilization. It has been assumed that daily stress and increased free radicals in the diet are the starters for these diseases, the occurrence of which is increasing. Currently researchers are looking for natural sources of substances, which have the ability to capture free radicals, to stimulate the immune system and bring many other health benefits [6]. Oyster mushrooms (*Pleurotus ostreatus*) contain a large spectrum of medicinal substances [1, 8, 15], chemical elements, vitamins [10, 17] and nutrients [4, 5]. Because of the presence of numerous nutritional components and various biologically active substances, the Oyster mushrooms have found a wide

potential medicinal usage as a part of treatment and prevention of diseases induced as a result of modern lifestyle or malnutrition [3, 9]. Many studies have drawn attention to the extreme variability of the composition of the fruit mushrooms of the Oyster (*Pleurotus ostreatus*), starting with the content of chemical elements up to the polysaccharides [12, 14]. The Oyster mushrooms growing on substrates consisting of residues of cereal crops (e. g. corn, rice bran and others) are most often examined. Only a few studies have been published about Oyster mushrooms growing on their natural substrate — wood [10].

The aim of this study was to compare the antioxidant properties of the fruiting bodies of the Oyster mushrooms grown on various wood substrates originating from one specific location. This way we tried, as much as possible, to eliminate differences in soil composition and the effects of climate conditions on the mycelium and the wooden substrate used. We presumed that by this approach we could obtain more reliable results and more precisely identify differences caused by growing Oyster mushrooms on different substrates. The Oyster mushrooms were cultivated on blocks of trees typical for the Slovak territory (beech, oak, linden, walnut, poplar and aspen).

MATERIALS AND METHODS

Chemicals

All chemicals and water — Folin-Ciocalteu reagent (Sigma-Aldrich Co., USA), gallic acid (Fisher Scientific, UK), n-hexane (Centralchem, s. r. o., Slovakia), aluminum chloride hexahydrate (LACHEMA BRNO, Czechia), distilled water (Reg Pur, s. r. o., Košice, Slovakia) and other (MIKROCHEM s. r. o., Slovakia) were of an analytical grade and p. a. purity.

Materials

For our analysis we used fruiting bodies of the Oyster mushrooms (*Pleurotus ostreatus*) cultivated on varies wood substrates (beech, oak, linden, walnut, poplar) on a private plot in Lemešany (Slovakia) and fruiting bodies of the Oyster mushrooms (*Pleurotus pulmonarius*) growing on their natural substrate – aspen wood. All Oyster mushrooms were cultivated by the same method (Fig. 1), which ensures the most natural conditions for the growth of mycelium and the development of fruiting bodies. The inoculated wood and growing fruiting bodies have to be protected against birds, snails and insects that can destroy the whole crop of Oyster mushrooms. Sufficiently large fruiting bodies (4 cm on average) were picked from blocks, weighed and dried at 40 °C in a dryer (Thermo scientific, Thermo electron led

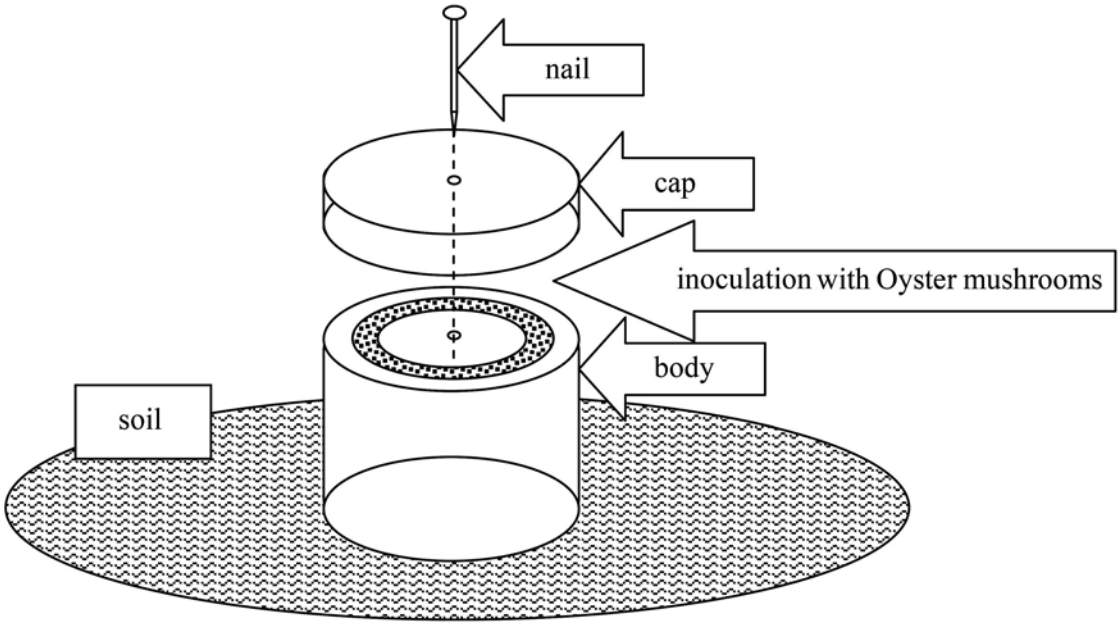

Fig. 1. Wooden block imbedded in soil and inoculated with Oyster mushrooms under garden conditions
Source: Self-made picture

GmbH, Germany) to a constant weight and homogenized with a homogenizer Straume (Ukraine). The obtained homogeneous powder was used for extraction.

Preparation of extracts

Extracts were obtained by extracting 100 mg of dried and homogenized fruiting bodies of the Oyster mushrooms in 2 cm^3 of water or methanol, or a mixture of acetone and n-hexane (4:6), with vigorous stirring for 30 minutes. The extraction mixtures were then filtered through a filter paper (Whatman No. 4) and the filtrates were used to determine the content of total phenols, total flavonoids, lycopene and β-carotene.

Determination of total phenols, total flavonoids, lycopene and β-carotene

The total phenolic compounds were determined by a micromethod, using Folin-Ciocalteu reagent (Sigma, USA) according to the method described by Waterhouse [16]. Freshly prepared methanol and water extracts (20 µl) of dried fruiting bodies of the Oyster mushrooms were used in the process.

The determination of the total flavonoids was carried out according to the method published by Konczak et al. [7] in freshly prepared methanolic and water extracts of dried fruiting bodies of Oyster mushrooms.

The content of the total phenolic substances and total flavonoids were expressed in gallic acid equivalents (GAE) that were read from the respective calibration curve.

The content of lycopene and β-carotene was determined by a spectrophotometric method of Nagata and Yamashita [11] and Dasgupta et al. [2]. Filtered extracts of dried fruiting bodies of Oyster mushrooms prepared in a mixture of acetone and n-hexane (4:6) were tested by UV-VIS spectrometry at wavelengths of 663 nm, 505 nm and 453 nm. The content of lycopene and β-carotene was calculated by means of the following [11]:

lycopene (mg.100 cm^{-3}) =

= –0.0458*A663 + 0.372*A505 – 0.0806*A453

β-carotene (mg.100 cm^{-3}) =

= 0.216 *A663 – 0.304 *A505 + 0.452*A453

Spectrophotometric measurements were carried out using a UV VIS spectrophotometer (Biochrom Libra S12, England). The wavelengths used for measurements are stated in the cited methods. The results are reported as means of three measurements with corresponding standard deviation (SD).

All samples for determination of the total phenolic compounds, the total flavonoids and the content of lycopene and β carotene were examined in triplicate.

RESULTS AND DISCUSSION

The mean contents ± SD of the total phenols (TP), total flavonoids (TF) in methanol and water extracts of dried fruiting bodies of Oyster mushrooms cultivated on various wood substrates (beech, oak, linden, walnut, poplar and aspen) are presented in Table 1.

The mean contents ± SD of lycopene and β-carotene in the mixture of acetone and n-hexane (4:6) extracts of the dried fruiting bodies of the Oyster mushrooms cultivated on various wood substrates (beech, oak, linden, walnut, poplar and aspen) are presented in Table 2.

The ratios of evaluated substances in dried fruiting bodies of Oyster mushrooms cultivated on various wood substrates (beech, oak, linden, walnut, poplar and aspen) are summarised in Tables 2 and 3.

All samples of Oyster mushrooms contained biologically active phenolic compounds. It is evident, that the total content of phenolic substances depended on both wood substrates used to cultivate Oyster mushrooms (*Pleurotus ostreatus*) and the extracting agent. We observed that water extracts of fruiting bodies of Oyster mushroom had higher total phenolic content in comparison with the total phenolic content in the methanolic extracts (Table 1.). The highest levels of phenolic substances were detected in Oyster mushrooms cultivated on linden (10.014 mg GAE.g^{-1} DW) and oak (9.306 mg GAE.g^{-1} DW) in water extracts of dried fruiting bodies and also in their methanolic extracts (linden 2.057 mg GAE.g^{-1} DW; oak 2.115 mg GAE.g^{-1} DW). High levels of phenolic substances were detected also in water extracts of dried fruiting bodies of Oyster mushrooms cultivated on walnut (9.477 mg GAE.g^{-1} DW). The lowest content of these substances was recorded in both types of extracts of dried fruiting bodies of Oyster mushrooms cultivated on poplar blocks (water: 8.667 mg GAE.g^{-1} DW; methanol: 1.293 mg GAE.g^{-1} DW).

Methanol extracts of dried fruiting bodies of *Pleurotus pulmonarius* growing on their natural substrate, aspen wood, exhibited extremely low levels of phenolic substances (0.937 mg GAE.g^{-1} DW).

The comparison of the content of phenolic substances in

Table 1. The mean content ± SD of total phenols (TP) and total flavonoids (TF) in methanolic and water extracts of dried fruiting bodies of Oyster mushrooms cultivated on various wood substrates (beech, oak, linden, walnut, poplar and aspen)

Substrate	Total phenolic substances				Total flavonoids			
	Methanolic extract		Water extract		Methanolic extract		Water extract	
	mg GAE.g^{-1} DW	SD	mg GAE.g^{-1} DW	SD	mg GAE.g^{-1} DW	SD	mg GAE.g^{-1}″DW	SD
beech	1.503	0.005	8.827	0.007	2.418	0.007	3.002	0.012
oak	2.115	0.003	9.306	0.005	2.955	0.009	6.368	0.015
linden	2.057	0.003	10.014	0.005	2.659	0.013	5.820	0.013
walnut	1.294	0.005	9.477	0.005	2.608	0.011	3.176	0.012
poplar	1.293	0.007	8.667	0.009	2.154	0.013	2.632	0.013
aspen	0.937	0.005	8.838	0.007	1.833	0.011	4.016	0.014

SD — standard deviation; GAE — gallic acid equivalent; DW — dry weight

Table 2. The mean content ± SD of β-carotene and lycopene in the mixture of acetone and n-hexane (4:6) extracts of dried fruiting bodies of Oyster mushrooms cultivated on various wood substrates (beech, oak, linden, walnut, poplar and aspen) and the ratios of β-carotene : lycopene

Substrate	β-carotene		Lycopene		Ratio of
	mg.g^{-1} DW	SD	mg.g^{-1} DW	SD	β-carotene : lycopene
beech	0.188	0.003	0.171	0.001	1.099
oak	0.231	0.002	0.153	0.002	1.510
linden	0.107	0.002	0.061	0.003	1.754
walnut	0.487	0.001	0.336	0.002	1.449
poplar	0.253	0.003	0.149	0.002	1.698
aspen	0.349	0.003	0.230	0.003	1.517

SD – standard deviation; DW – dry weight

Table 3. The ratios of the content of total phenolic compounds and total flavonoids in water and methanolic extracts of dried fruiting bodies of Oyster mushrooms cultivated on various wood substrates (beech, oak, linden, walnut, poplar and aspen)

Substrate	Ratios of the content of the evaluated substances (H2O : CH3OH extracts)	
	Total phenolic compounds	Total flavonoids
beech	5.873	1.242
oak	4.400	2.156
linden	4.868	2.189
walnut	7.324	1.218
poplar	6.703	1.222
aspen	9.443	2.191

water and methanolic extracts of our experimental samples of dried fruiting bodies of Oyster mushrooms showed interesting information. The ratio of the content of phenolic substances in water and methanolic extracts varied depending on the wood substrate (Table 3). The greatest difference in the content of phenolic substances in water and methanol extracts was in samples of dried fruiting bodies of *Pleurotus pulmonarius* growing on their natural substrate – aspen wood. Water extract contained 9.443-fold higher content of phenolic substances than the methanolic extract. High ratios of phenolic substances in water and methanolic extracts were found in dried fruiting bodies of Oyster mushrooms cultivated on walnut (7.324) and poplar (6.703) while low ratios were detected in dried fruiting bodies of Oyster mushrooms cultivated on linden (4.868) and oak (4.400).

The fruiting bodies of Oyster mushrooms contain remarkable quantities of different phenolic acids such as gallic acid, homogentisic acid, ferulic acid, p-coumaric acid, p-hydroxybenzoic acid, protocatechuic acid and chlorogenic acids, respectively [1, 13]. The ratio of the content of phenolic substances in water and methanolic extracts can demonstrate also the different qualitative and quantitative composition of fruiting bodies of Oyster mushrooms depending upon the wooden substrate used for their cultivation. It is evident, that the total content of phenolic substances depended on the wood substrates used for cultivation of the Oyster mushrooms (*Pleurotus ostreatus*) and on the extracting agents (Table 1). To draw a final conclusion about the dependence of the content of individual phenolic substances on the composition of the wood substrate, more extensive and detailed investigations are needed.

Water extracts of the fruiting bodies of the Oyster mushroom contained higher levels of total flavonoids in comparison with levels in methanolic extracts (Table 1). The highest levels of flavonoids were determined in Oyster mushrooms cultivated on oak blocks (in methanolic extract 2.955 mg GAE.g^{-1} DW and in water extract 6.368 mg GAE.g^{-1} DW) and linden block (2.659 mg GAE.g^{-1} DW in methanolic extract and 5.820 mg GAE.g^{-1} DW in water extract). The lowest content of these substances was detected in extracts of dried fruiting bodies of Oyster mushrooms cultivated on poplar blocks (2.154 mg GAE.g^{-1} DW in methanolic extract and 2.632 mg GAE.g^{-1} DW in water extract) and in methanolic extract (1.833 mg GAE.g^{-1} DW) of Oyster mushrooms (*Pleurotus pulmonarius*) grown on their natural substrate – aspen wood.

The water extracts of the fruiting bodies of the Oyster mushroom grown on oak, linden and aspen contained more than a 2-fold higher level of flavonoids (2.156 to 2.191 times) than the methanolic extracts (Table 3). The water extracts of fruiting bodies of Oyster mushrooms grown on beech, walnut and poplar blocks showed only 1.218 to 1.242-fold higher levels of flavonoids than the methanolic extracts. The Oyster mushrooms contain several kinds of flavonoids, especially rutin and chrysin but also catechin and myricetin [1, 13]. The different ratio of the content of flavonoids in water and methanolic extracts can also be caused by different qualitative and quantitative composition of fruiting bodies of the Oyster mushrooms cultivated on different wood substrates. Additional detailed examinations are needed to explain this relationship and significance of differences between the types of wood used to cultivate Oyster mushrooms.

Table 2 presents biologically active β-carotene and lycopene in the mixture of acetone and n-hexane (4:6) extract of dried fruiting bodies of the Oyster mushrooms cultivated on various wood substrates (beech, oak, linden, walnut, poplar and aspen). These compounds are well known for their antioxidant properties. However, cultivation on different wood substrates resulted in different amounts of these substances in acetone — n-hexane extracts. The highest content of lycopene was found in an Oyster mushroom sample grown on a walnut block (0.336 mg.g^{-1} DW) and the lowest in a sample grown on a linden block (0.061 mg.g^{-1} DW). Low levels of lycopene were also found in extracts of the dried fruiting bodies of the Oyster mushrooms cultivated on beech and poplar blocks (0.153 mg.g^{-1} DW and 0.149 mg.g^{-1} DW, respectively).

All extracts of the dried fruiting bodies of the Oyster mushrooms contained higher amounts of β-carotene than lycopene (Table 2). Similar to lycopene, different levels of β-carotene were detected in the samples of the Oyster mushrooms grown on different wood substrates. The highest content of β-carotene was found in a sample of the Oyster mushrooms grown on a walnut block (0.487 mg.g^{-1} DW). Low levels of β-carotene were found in extracts of the dried fruiting bodies of the Oyster mushrooms cultivated on linden and beech blocks (0.107 mg.g^{-1} DW and 0.181 mg.g^{-1} DW, respectively). Table 2 presents also the ratio of the content of β-carotene to lycopene. The content of β-carotene in extracts of fruiting bodies of Oyster mushrooms was 1.099 to 1.754-fold higher than the content of lycopene.

CONCLUSIONS

Oyster mushrooms contain large amounts of substances with antioxidant properties. Our experiments showed that the content of phenolic compounds, flavonoids, β-carotene and lycopene in the fruiting bodies of the Oyster mushrooms can be influenced by different wood substrates used to cultivate these mushrooms. The ratio of the content of the flavonoids and phenolic substances in water and methanolic extracts demonstrated the different qualitative and quantitative composition of the fruiting bodies of the Oyster mushrooms dependent on wooden substrate used for their cultivation. Such a variable composition of the fruiting bodies of the Oyster mushrooms with their different antioxidant potential may distort clinical studies based on diets containing dried mushrooms. In our study the oak and linden blocks appeared to be the most suitable wood substrates for the cultivation of Oyster mushrooms. We also hope that this study may stimulate interest in the mycological field of research in Central Europe, which was for many centuries known for rich tradition in picking and consumption of mushrooms.

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