

IMPACT OF FREEZE DRYING ON THE CONTENTS OF BIOLOGICALLY  
ACTIVE SUBSTANCES IN THE FRUITING BODIES OF SELECTED  
EDIBLE MUSHROOM SPECIES

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**Abstract.** Water and alcohol extracts were obtained from the Oyster Mushroom and White Button Pizza Mushroom, which were then freeze dried. Using two methods: FRAP and FTC, the anti-oxidation effectiveness of the biological material was determined. It was determined as a result of the analysis that the freeze drying process does not significantly lower the anti-oxidation effectiveness of the extracts. Antioxidation activity depended to a large degree on the type of dissolvent used. Both species in question were characterized by anti-oxidation activity. The greatest effectiveness was observed in extracts from raw mushrooms.

**Keywords:** freeze drying, mushrooms, *Pleurotus ostreatus*, *Agaricus bisporus*, anti-oxidation activity

INTRODUCTION

In the official health services of western European countries, sixty to seventy percent of the drugs currently used are products originating from plants or fungi. The ability of fungi to fruit repeatedly and to synthesize secondary metabolites is closely related with the biological properties of the species and its commercial varieties. The nutritional, toning and curative properties of fungi have been known for a long time [5,9].

The anti-oxidant effectiveness of fungi powders and extracts is their most important property. Anti-oxidants are defined as substances which, when present in low concentrations compared with those of an oxidize substrate significantly delay or prevent the oxidation of that substrate. Examination of the anti-biological

activity of 150 fungi species proved that one third had antioxidant properties. Among them, 27 species showed extremely strong activity. From the taxonomic point of view, most of these last species belonged to the *Boletaceae*, and the *Suillus* type of fungi and showed the strongest activity [7,8].

Also polysaccharide extracts from fungi show a free-radical scavenging activity. Polysaccharide extracts from *Ganoderma lucidum* showed scavenging activity in relation to peroxy and hydroxyl radicals. Peroxy radicals are also scavenged in the presence of PSK, a polysaccharide bound to protein obtained from the fruit of the *Coriolus versicolor*. While examining abilities for the scavenging of peroxy and hydroxyl polysaccharide, extracts were taken from eight fungi species with anti-carcinogenic properties using various methods. It was proved that part of these extracts showed abilities to scavenge  $O_2^{\cdot-}$  and  $OH^{\cdot}$  free radicals. Extracts from *Ganoderma lucidum* and *Grifola frondosa* had the highest activity with respect to  $O_2^{\cdot-}$  radicals, and PSK and extracts from *Ganoderma lucidum* against  $OH^{\cdot}$  radicals. Lentinan and extracts from *Schizophyllum commune* showed the lowest anti-radical activity. The antioxidant activity of the above mentioned extracts was measured by determining the malonaldehyde (MDA) content formed from the disintegration of polysaturated, fatty acids in the liver [2,3,4,6].

#### MATERIALS AND METHODS

The fructification of the following edible mushrooms was used for the present experiment: oyster mushroom (*Pleurotus ostreatus*) and the white /cup/ mushroom (*Agaricus bisporus* Sing.) Sublimation drying was carried out in a Labconco type lyophiliser; the initial process temperature was  $-50^{\circ}C$ . The duration of the drying process was 48 hours. Sixteen experimental combinations of extracts and their respective substrates after lyophilisation were prepared.

##### 1. Hydrous extract of raw fructification

One hundred grams of fresh mushroom fructification were prepared; the preparation process included washing, crumbling and mixing with 100 ml of distilled water in order to homogenise the mixture. The substance obtained was centrifuged for 10 min at 4,000 rpm and the supernatant was removed from the sediment [1,2].

##### 2. Protein fraction of the hydrous extract

A hydrous extract from the raw fructification of the species studied, was divided in a chromatographic column using Sphadex G-25 gel; 2 ml was extracted and introduced and a 7.3 ml of the protein fraction was obtained [1,2].

### 3. Ethanol extract of fructification

One hundred grams of fresh fructification of the mushrooms studied were prepared. After washing, the fructification was crumbled and mixed to obtain a homogenizate with the addition of 100 ml of ethanol; the homogenizate was centrifuged for 10 min at 4,000 rpm, and the supernatant was then removed from above the sediment. After extracting the ethanol, the sample was distilled at a temperature of +45°C under decreased pressure to remove the solvent. Samples for chemical analysis and to determine the antioxidant activity were taken from the extracts; they were then submitted to lyophilization. The following were determined from the raw material, extracts and lyophilizates [1,2]:

- Determination of antioxidant properties.

1. Method for Measuring the Reducing Power (antioxidant FRAP) – In the FRAP assay, the reducing agents present in the sample reduce the complex of Fe (III) tri-pyridyltriazine which is present in excess amounts, to obtain a blue-stained Fe (II) form with a change in absorbance ( $\Delta A$ ) at 593  $\mu\text{m}$ . The value of  $\Delta A$  is proportional to the total reducing (antioxidant) power of the antioxidants present in the sample [1,2].

FRAP assays were carried out on the following samples:

Extracts:

- water extracts of raw (PS) and boiled (PG) white (cup) mushroom in two dilutions: 1:4 – a 10% extract and 1:1 – a 50% extract;
- freshly obtained protein fraction and low molecular fraction of a hydrous white mushroom extract (P. protein) and (P. salts);
- hydrous extracts of raw (BS) and boiled (BG) oyster mushroom in a dilution of 1:1, i.e. a 50% solution;
- a freshly obtained protein and low molecular fraction of a hydrous oyster mushroom extract (B. protein) and (B. salts).

The following were determined from the fresh material, extracts and lyophilizates:

- protein determination by the Bradford method,
- phenol compound assay by the DASA method,
- phenol compound assay by the Folin-Ciocalteu method,
- sugar content assay by the Somogyj-Nelson method,
- re-evaluation of the antioxidant properties in the extracts and lyophilizates.

## 2. FTC (Ferric thiocyanate) method

The second of the methods applied in the present study was the FTC method with ferric thiocyanate which allows the total antioxidant activity of the biological material tested to be determined. The antioxidant properties of the sample lyophilizates were determined against linolenic acid, the oxidation of which was to be inhibited by the addition of the lyophilizate used as the antioxidant. Lyophilizate samples were prepared in a water and ethanol solution and in two concentrations, i.e. 0.25% and 0.05%. The antioxidant activity of the lyophilizates from the fructification of the mushrooms studied was compared to BHA (0.01% and 0.05%) activity and Trolox (0.25% and 0.05%). The reference in the study was a sample without an antioxidant (control). The results for the samples with identical concentrations and comparative antioxidants were then subjected to a one-factor analysis of variance.

The ferric thiocyanate method (FTC) allows the total antioxidant activity of mushroom lyophilizates to be determined. The above activity was tested on linolenic acid since it is a substance which is susceptible to oxidation. The efficiency of the antioxidants originating from the mushrooms was compared to a synthetic BHA antioxidant and Trolox and related to a control sample without anti-oxidants.

Solutions from mushroom lyophilizates were prepared in two concentrations, i.e. 0.025% and 0.05% with the application of two solvents, i.e. distilled water and ethanol (99.5%). Moreover, ethanol (99.5%) BHA solutions were prepared in two concentrations, i.e. 0.01% and 0.05% and Trolox solutions in two concentrations of 0.25% and 0.05% [1,2].

## RESULTS AND DISCUSSION

In the present study, two methods were used to determine the content of phenol agents, i.e. DASA and Folin-Ciocalteu. The authors of the Folin-Ciocalteu method stated that the relative values are usually different to other methods. It was found that the DASA assay yields a result which is 3-4 times lower than the other method. According to the DASA assay, the phenol content in the extract from the raw fructification of the white mushroom was 48 mg in the extract dry mass, and the salt fraction was 36.4 mg in the extract dry mass; whereas in the boiled extract it was 36 mg; the protein fraction contained 39 mg in the dry mass and the salt fraction – 36.4 mg in the dry mass. The method showed a higher content of phenols in the oyster mushroom fructification than in the white mushroom fructification. The extract from the raw fructification of the oyster

mushroom contained 73 mg (%)<sup>-1</sup> of phenol compounds in the dry mass, and the boiled extract – 44 mg in the dry mass.

The protein fraction contained 23 mg in the dry mass and the salt fraction 77 mg in the extract dry mass. In the white mushroom lyophilizates, the phenol content according to the DASA determinations was from 2.74 to 0.05 mg g<sup>-1</sup> of lyophilizate, and in the oyster mushroom lyophilizates it was 3.00-0.04 mg g<sup>-1</sup> of the lyophilizate. In the white mushroom lyophilizates, the content of phenols according to Folin-Ciocalteu, was from 13.6 to 0.72 mg g<sup>-1</sup> of the lyophilizate. A higher content of the phenol compound was found in the oyster mushroom, i.e. 14.2 to 0.18 mg g<sup>-1</sup> of the lyophilizate.

**Table 1.** Content of phenol compounds

Pos.	Type of sample	mg of phenols / g lyophilizate	
		DASA method	FTC method
1	Raw white mushroom (H <sub>2</sub> O;0.5%)	2.660	13.16
2	Raw white mushroom (et.;2.5%)	0.025	0.72
3	Cooked white mushroom (H <sub>2</sub> O;0.5%)	2,560	12.56
4	Cooked white mushroom (et.;2.5%)	0.124	1.11
5	Ethanol white mushroom (H <sub>2</sub> O;0.5%)	2.740	13.16
6	Ethanol white mushroom (et.;2.5%)	0.368	1.58
7	Raw oyster mushroom (H <sub>2</sub> O;0.5%)	2.960	14.20
8	Raw oyster mushroom (et.;2.5%)	0.040	0.18
9	Cooked oyster mushroom (H <sub>2</sub> O;0.5%)	2.900	11.28
10	Cooked oyster mushroom (et.;2.5%)	0.044	0.53
11	Ethanol oyster mushroom (H <sub>2</sub> O;0.5%)	3.000	12.08
12	Ethanol oyster mushroom (et.;2.5%)	0.468	1.14

The above discrepancies may result from the fact that the method used to determine the phenol compounds with a Folin-Ciocalteu reagent, i.e. phenols, [mainly nitrophenols], and to a lesser degree purines and pirimidines – together with uric acid – react with the Folin-Ciocalteu phenol reagent and increase color intensity. Both the choice of the method and the external factors (temperature, reagents used) may have influenced the results of the studies and the differences between them.

It was found that in the case of the oyster mushroom, phenol compounds occur in the ethanol extracts of fructification in higher amounts than in the spawn. The phenol content was as high as  $1660 \text{ mg } (\%)^{-1}$ . Studies by Kasuga confirm also the occurrence of phenol compounds in the fructification of edible mushrooms. The above author studied several species of edible vegetables and mushrooms and confirmed that natural antioxidants were present in them; the extracts of *Suilus bovinus* were especially considered to have strong antioxidant activity.

In the studies by Kasuga on the activity of antioxidant compounds in the extracts of *Suilus bovinus*, two compounds with anti-oxidant activity were isolated; it was proved that the activity of one of them was higher than that of tocopherol and almost the same as BHA.

Results of the present study cannot be directly related to the studies by Kasuga as they were carried out on purified substances isolated from mushroom extracts, whereas in this study, extracts contained several other substances such as proteins and sugars beside phenol compounds.

In the present author's studies, the efficiency of antioxidants originating from mushrooms was determined by two methods. The FRAP method – the method for determining reduction /antioxidant/ power, evaluates the power of ferrum reduction by the reducing agents that are present in the sample. The total antioxidant ability can be considered as analogous to the total reducing ability since antioxidant agents as electron donors can be considered reductors and the inactivation of oxidants by these reductors can be described as a redox reaction in which one compound is reduced and the other oxidized. In the FRAP test, the reducing agents (antioxidants) present in the sample, reduce the Fe (III) – tri-pyridyltriazine complex present in excess to the Fe (II) form.

FRAP measurements were carried out on extracts (supernatants) and lyophilizate solutions prepared with distilled water (concentration of 0.5% and 1.25% for the protein and salt fractions) and ethanol (concentration of 2.5%)

Whilst comparing the reducing properties of mushroom supernatants, it was found that the white mushroom was characterized by a higher reduction than the oyster mushroom. According to the FRAP, 50% of the extract of the raw white mushroom showed the highest reduction efficiency (2027.1  $\mu\text{M}$  FRAP). On the other hand, the low- and high molecular fraction from the oyster mushroom fructification did not show any activity in the last test which was probably due to too high a dilution of the samples subjected to testing. The lyophilisation process did not significantly diminish the reducing properties of the supernatants subjected to it. Each of the samples studied showed antioxidant activity.

Lyophilizates from the white mushroom fructification were characterized by a higher reduction power than the extracts from the oyster mushroom fructification. The highest FRAP value had the lyophilizate from the fructification of raw white mushroom dissolved in distilled water in a concentration of 0.05% (92  $\mu\text{M}$  FRAP – as indeed did all the extracts). The lowest activity was observed in the case of the lyophilizate of the salt fraction of the oyster mushroom dissolved in ethanol at a concentration of 1.25% (3.08  $\mu\text{M}$  FRAP). All ethanol solutions of lyophilizates showed low efficiency in the FRAP test which was caused by low solubility in ethanol.

The FRAP method can be carried out for various samples. Due to the lack of sources relating to studies on mushrooms, it was not possible to compare the present results with other studies [2]. The propagation maximum of the formation of peroxides was observed between the sixth and the eighth day of the experiment. On the seventh day, absorbance of the control and the remaining samples was highest. The relative antioxidant activity as a percentage of inhibition in relation to control was calculated from the results obtained on that day. Lyophilizates dissolved in water prepared with a 0.05% concentration showed high efficiency. These last samples did not show any significant differences among themselves and BHA for both concentration levels. Linolenic acid in the presence of ethanol solutions of lyophilizates underwent oxidation similar to or to a higher degree than the control. The low solubility of lyophilizates in ethanol probably decreased the concentration and composition of the active compounds. In all the mushroom samples, the best ability to inhibit the auto-oxidation of linolenic acids was shown by a hydrous extract of the oyster mushroom protein fraction. Comparing all antioxidants applied, it was found that the synthetic antioxidant BHA at a concentration of 0.01% was most effective. However, statistical calculations showed that an increase of the BHA dose did not influence its activity significantly. Trolox, as compared to BHA and samples of hydrous lyophilizates proved to be much worse; however, it showed a higher activity in relation to ethanol solutions. The FTC method showed the better efficiency of lyophilizates from the white mushroom fructification which could have resulted from the presence of fatty acids occurring naturally in the mushroom. The main fatty acid in the white mushroom is linolenic acid, the higher presence of which could have had a significant influence on the results obtained. What is more, natural tocopherols and other antioxidants present in the raw material studied could have acted synergistically or antagonistically with the substances added during the experiment. Moreover, differences in the fatty acid composition of the white mushroom and oyster mushroom could have influenced the changeable behavior of the same phenol compounds in various fats. Ramarathnam et al. stated that the activity of natural phenol antioxidants was difficult to evaluate in the multi-component solutions present in food products [9].

**Table 2.** Antioxidant activity of mushroom lyophilizates according to the FRAP test and FTC method

Pos.	Lyophilizates	Antioxidant activity		Inhibition in relation to control (%)	
		Absorbance	$\mu\text{M FRAP g}^{-1}$ of lyophilizate	0.25% x	0.05 % y
1	Raw white mushroom H <sub>2</sub> O	0.322	92 <sup>d</sup>	69.1 <sup>de</sup>	20.9 <sup>a</sup>
2	Raw white mushroom et.	0.141	8.05 <sup>a</sup>	105.8 <sup>f</sup>	110.8 <sup>c</sup>
3	Cooked white mushroom H <sub>2</sub> O	0.301	86 <sup>d</sup>	47.3 <sup>bcd</sup>	30.9 <sup>ab</sup>
4	Cooked white mushroom et.	0.105	6 <sup>a</sup>	121.8 <sup>f</sup>	107.6 <sup>c</sup>
5	Protein white mushroom H <sub>2</sub> O	0.589	67.3 <sup>d</sup>	54.4 <sup>cd</sup>	30.1 <sup>ab</sup>
6	Saltz white mushroom et.	0.045	5.1 <sup>a</sup>	124.6 <sup>f</sup>	110.5 <sup>c</sup>
7	Ethanol white mushroom et.	0.176	10.05 <sup>a</sup>	127.3 <sup>f</sup>	120.8 <sup>c</sup>
8	Raw oyster mushroom H <sub>2</sub> O	0.217	62 <sup>cd</sup>	31.4 <sup>abc</sup>	21.7 <sup>a</sup>
9	Raw oyster mushroom et.	0.118	6.7 <sup>a</sup>	114.8 <sup>f</sup>	114.9 <sup>c</sup>
10	Cooked oyster mushroom H <sub>2</sub> O	0.178	50.8 <sup>c</sup>	31.8 <sup>abc</sup>	22.9 <sup>a</sup>
11	Cooked oyster mushroom et.	0.125	6.8 <sup>a</sup>	109.3 <sup>f</sup>	112.1 <sup>c</sup>
12	Protein oyster mushroom, H <sub>2</sub> O	0.110	12.6 <sup>a</sup>	16.9 <sup>db</sup>	15.7 <sup>a</sup>
13	Saltz oyster mushroom et.	0.027	3.08 <sup>a</sup>	121.7 <sup>f</sup>	106.5 <sup>c</sup>
14	Ethanol oyster mushroom et.	0.061	3.5 <sup>a</sup>	119.0 <sup>f</sup>	107.6 <sup>c</sup>
15	Trolox			67.1 <sup>d</sup>	60.8 <sup>b</sup>
16	BHA			4.4 <sup>a</sup>	13.8 <sup>a</sup>

NIR for 0.25% = 31.56, NIR for 0.05% = 36.68,

a, b, c, d, e, f – letters denote significant statistical differences,

Lyophilizate samples were marked as described in the chapter "Methodology".

I met a similar methodology of examination in the work of Wołosiak and Worbiej [10] who studied the antioxidant properties of isolates and hydrolysates of pea proteins and observed a similar efficiency in the inhibition of the oxidation of linolenic acid in the presence of the samples added. It was stated that protein antioxidation activity increases after enzymatic hydrolysis. Moreover, the above authors are of the opinion that the contribution of the individual amino acids and their sequence in the peptides influences antioxidant properties. They assigned a special role to histidine and proline.

Studies by the present author can be partially related to the above results by comparing protein activity in mushroom fructification to pea proteins. The FTC method proved that proteins isolated from the extracts of the fructification of the white mushroom and the oyster mushroom had a very high antioxidant activity, comparable to that of BHA and twice as high as Trolox.



## CONCLUSIONS

The studies carried out made it possible to draw the following conclusions:

1. White mushroom (*Agaricus bisporus* (Lange) Sing) and oyster mushroom (*Pleurotus ostreatus*) contain substances with antioxidant properties in their fructification.

2. The FRAP method evaluating the ferrum reduction power, showed that the sample taken from the fructification of the raw white mushroom had the highest efficiency both as a fresh extract and the lyophilizate obtained from it.

3. An increase of the antioxidant agent concentration to 0.25% showed a lower efficiency compared to the concentration level of 0.05% which was observed in the case of mushroom samples and synthetic antioxidant agents.

4. The solvent used to dissolve lyophilizates had a considerable influence on their antioxidant efficiency. The ethanol extracts had a far lower activity than did the hydrous solutions.

No relation between the phenol content in the lyophilizates and their antioxidant efficiency was observed.

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WPŁYW LIOFILIZACJI NA ZAWARTOŚĆ SUBSTANCJI AKTYWNYCH  
BIOLOGICZNIE W OWOCNIKACH WYBRANYCH GATUNKÓW  
GRZYBÓW JADALNYCH

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**Streszczenie.** Z owocników *Boczniaka ostrygowatego* i *Pieczarki dwuzarodnikowej* otrzymano ekstrakty wodne i alkoholowe, które następnie liofilizowano. Przy użyciu dwu metod: metody pomiaru siły redukującej (FRAP) i metody określającej całkowitą aktywność antyoksydacyjną (FTC), określono efektywność przeciwutleniającą materiału biologicznego. W wyniku analizy ustalono, że proces liofilizacji nie obniża istotnie efektywności przeciwutleniającej ekstraktów. W większym stopniu aktywność przeciwutleniająca zależna była od rodzaju stosowanego rozpuszczalnika. Oba badane gatunki odznaczały się aktywnością przeciwutleniającą. Najwyższą efektywność uzyskały ekstrakty z pieczarki surowej.

**Słowa kluczowe:** liofilizacja, grzyby, *Pleurotus ostreatus*, *Agaricus bisporus*, aktywność przeciwutleniająca