

EFFECTS OF NON-THERMAL POSTHARVEST IRRADIATION OF DRIED MUSHROOMS ON THEIR ANTIOXIDANT CONTENT AND ACTIVITY

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Abstract. This study presents the effects of non-thermal physical treatment of *Boletus* mushroom on its antioxidants. A slight increase of their content was noticed after 30 min of UV-C postharvest exposure at 20 cm. Instead, a low polyphenols degradation occurred at shorter exposure distance, characterized by kinetic parameters ($k = 2.80 \times 10^{-3} \text{ min}^{-1}$, $t_{1/2} = 247.50 \text{ min}$). The UV-C had a significant positive effect on the antioxidant activity. The ATR-FTIR spectra confirmed the results on the changes induced by UV-C light. A weak positive effect on antioxidants was found in dried mushrooms exposed to 50 Hz magnetic field of 3 mT.

Key words: antioxidant activity, *Boletus edulis*, magnetic field, phenolics, tannins, UV-C.

1. INTRODUCTION

Edible mushrooms, wild and cultivated, are macroscopic fungi consumed all over the world both fresh and preserved, for their well established nutritional benefits and taste [1]. *Boletus edulis* is a well-known wild-growing European mushroom (porcini) high in valuable components with antioxidant, anti-inflammatory and profibrinolytic effects [2].

Because fresh mushrooms rapidly deteriorate, different types of primary processing are required, among them drying being preferred [3]. A secondary non-thermal processing based on physical treatments such as high-pressure, UV, pulsed light or ionizing radiation offers promising alternatives to thermal treatments in terms of preserving bioactive compounds, and applications in food engineering. UV irradiation have been used because of its strong bactericidal and germicidal effects, without producing undesirable by-products or residual radioactivity compared to ionizing radiation [4]. The efficiency of UV irradiation against microorganisms depends on the exposure time, intensity and temperature, the most accepted mechanism of action being the degradation of bacterial cell walls causing disruption

of biomolecules [5]. It has been shown that UV light did not highly impact on food characteristics and content, even more, an increase of the content of flavonoids and phenolic compounds was noted in several cases [6]. A small number of studies reported the application of another type of non-thermal irradiation, static or oscillating magnetic field (MF) at frequencies of 5–50 Hz and various strengths, for the inactivation of microorganisms contaminating foods, but with modest use [5]. No such studies on dried *Boletus edulis* mushrooms have been identified in the literature. Most of the experimental studies focused on the *in vivo* effects produced in plants by exposure to extremely low frequency MF, at the cellular and sub-cellular level, on enzyme activities, plant growth, seed germination, cell division and differentiation [7–8].

When using non-thermal preservation technologies to food products or ingredients, in addition to the study of the impact of physical treatment on the nutritional, microbiological and sensorial food characteristics [9], the evaluation of the content of micronutrients and the effect on biological properties after irradiation are equally important.

Based on the limited information about the effects of non-thermal postharvest irradiation on the phenolic content and antioxidant activity of dried *Boletus edulis* mushrooms, the present study aimed to investigate the influence of UV-C at 254 nm and extremely low frequency MF exposure, as function of different process parameters, on the antioxidant content and activity of *Boletus edulis* mushrooms. The ATR-FTIR analysis was used to provide additional information.

2. MATERIALS AND METHODS

2.1. MATERIALS AND CHEMICAL REAGENTS

Edible mushrooms (*Boletus edulis* L.) were collected from the Râul-Sadului forest, Romania. The fruit body cut into 2 cm pieces was dried at 57°C for 8 h using the convection oven (UFE 400 with forced air circulation, Memmert, Germany) at a fan speed of 60%, until the moisture content was ~3%. The moisture content was determined at 105°C using the moisture analyser (MAC 210 Radwag, Poland). The samples were further ground using the knife mill (Grindomix GM 200, Retsch, Germany) and kept at –18°C until analysis. All chemical reagents were of analytical grade.

2.2. EXPOSURE TO UV-C LIGHT

Samples (5 g) of dried mushroom powder were exposed to UV-C light at 254 nm, using a low pressure UV lamp with an illuminating intensity of 14 $\mu\text{W}\cdot\text{cm}^{-2}$ (6 KLU 254 + 366 nm, NeoLab, Germany), in a closed box. Samples were irradiated using different exposure times (15 and 30 min) and exposure distances

(10, 15 and 20 cm under the UV lamp). After treatments, all irradiated samples and untreated sample (control) were stored at 4°C until the evaluation of total phenolic and tannins content, and antioxidant activity.

The degradation of phenolic compounds was evaluated by calculating the degradation rate according to the following formula [10]:

$$\text{degradation [\%]} = \frac{C_{\text{control}} - C_t}{C_{\text{control}}} \times 100 \quad (1)$$

where C_{control} is the total phenolic content (TPC) in the untreated sample and C_t is the TPC at time t .

The degradation rate constant (k) and half-life time ($t_{1/2}$) were calculated using the equations:

$$\ln \frac{C}{C_0} = -kt \quad (2)$$

$$t_{1/2} = \frac{\ln 2}{k} \quad (3)$$

2.3. EXPOSURE TO EXTREMELY LOW FREQUENCY MF

Samples (3 g) of dried mushroom powder were placed on 9 cm diameter Petri dishes and exposed to homogenous 50 Hz magnetic field with magnetic flux density of 3 mT, using a laboratory Helmholtz coil system, for different exposure times (15, 30, 60, 120 and 240 min). The exposure system previously described by our group [11] is presented in Fig. 1.

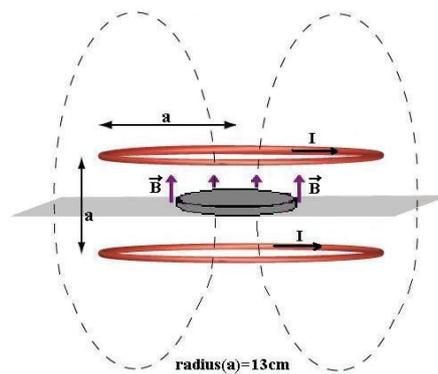


Fig. 1 – Schematic drawing of the MF exposure system of mushroom samples; B – magnetic flux density; I – current intensity; a – radius of coil from Helmholtz coil system.

A homogeneous vertical extremely low frequency MF was generated in the central area engaged by each sample within the coils system. No temperature gradient was recorded in the central zone of the exposure system as measured using the temperature device (Luxtron One), in the absence of sample. Longer MF exposure times were not investigated, based on the results of a previously paper published by our group which showed that phenolic content and antioxidant activity of blackberries increased up to 6 h of exposure [12]. Untreated sample was used as control.

2.4. PREPARATION OF EXTRACTS

An amount of 1 g of dried mushrooms was mixed with 10 ml 70% ethanol (V/V). Extraction of compounds of polyphenolic structure was performed overnight at room temperature. The mixture was centrifugated at 4°C, $7369 \times g$ for 10 min using the refrigerated centrifuge (Universal 320, Hettich, Germany).

2.5. DETERMINATION OF PHENOLICS AND TANNINS

Total phenolic content (TPC) and total tannins content (TTC) were determined spectrophotometrically according to the Folin-Ciocalteu method [13] and method described by Price *et al.* [14], respectively. The Specord 200Plus UV-Vis spectrophotometer (Analytik Jena, Germany) was used. The results were expressed as mg gallic acid equivalents (GAE) per 100 g dry weight (DW) and as mg catechin equivalents (CE) per 100 g dry weight (DW), respectively.

2.6. DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY

The antioxidant activity of physically treated samples was determined spectrophotometrically by the Ferric Reducing Antioxidant Power (FRAP) assay [15]. The results were expressed as mg ascorbic acid equivalents (AAE) per 100 g dry weight (DW).

2.7. ATR-FTIR ANALYSIS

Fourier transform infrared (FTIR) analysis was carried out using an ALPHA FTIR spectrometer (Bruker, Germany) with the combined QuickSnap™ sampling modules and ZnSe ATR (attenuated total reflectance). Spectra were collected from an average of 32 scans recorded in the ATR mode in the spectral range of $4000\text{--}600\text{ cm}^{-1}$ at 4 cm^{-1} spectral resolution.

2.8. STATISTICAL ANALYSIS

Data were expressed as mean values \pm standard deviation (SD) of duplicate experiments. Differences between the samples were tested using paired t-test by

Excel Analysis ToolPack version 2010. Statistical correlation between variables was performed by calculating the Pearson correlation coefficient. The results were considered statistically significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. THE EFFECT OF UV-C EXPOSURE ON TPC, TTC AND ANTIOXIDANT ACTIVITY OF DRIED MUSHROOMS

The results regarding the evolution of the phenolic and tannins content, as well as the antioxidant activity of dried mushrooms before and after the UV-C treatment are presented in Table 1.

The TPC mean value of irradiated samples group was slightly higher than that of control, but not statistically significant at $p < 0.05$.

Table 1

TPC, TTC and antioxidant activity of control and UV-C treated mushroom samples as function of irradiation time and distance

Parameters	Control	UV-C treated samples			
		Exposure time (min)	Exposure distance (cm)		
			10	15	20
Phenolics (mg GAE 100 g ⁻¹ DW)	588.21 ± 7.53	15 30	576.33 ± 7.20 554.68 ± 6.93	591.31 ± 7.39 584.20 ± 7.30	589.43 ± 9.91 689.65 ± 8.62
Tannins (mg catechin 100 g ⁻¹ DW)	85.98 ± 0.12	15 30	86.95 ± 0.25 86.93 ± 0.17	94.29 ± 0.08 99.53 ± 0.19	84.05 ± 0.17 94.17 ± 0.14
Antioxidant activity (mg AAE 100 g ⁻¹ DW)	434.98 ± 2.10	15 30	458.25 ± 1.11 467.81 ± 1.06	467.82 ± 1.46 470.85 ± 2.04	455.19 ± 2.04 467.24 ± 2.21

Note: results represent average values of duplicate determinations ± standard deviation.

The TPC declined to a small extent during the UV-C treatment at the lowest distance (10 cm) indicating a degradation process of polyphenols but at a low rate. The degradation rate and kinetic parameters as presented in Table 2, was very low to an exposure time of 15 and 30 min. The calculated rate constant (k) of the degradation reactions following the first-order kinetic model was $2.80 \times 10^{-3} \text{ min}^{-1}$, and the half-life time $t_{1/2}$ was 247.50 min. Some authors reported k values ranging from $2.10 \times 10^{-3} \text{ min}^{-1}$ for catechin to $5.40 \times 10^{-3} \text{ min}^{-1}$ for gallic acid, in plant extracts exposed to UV-C light using a low-pressure mercury lamp [16].

Mushroom powder irradiated for 30 min at the highest investigated distance (20 cm) had the greatest TPC value ($689.65 \pm 8.62 \text{ mg GAE } 100 \text{ g}^{-1} \text{ DW}$), 17% higher than in the control group, which points out the potential use of these experimental conditions for the enhancement of the TPC in dried *Boletus edulis* powder. There are few studies on the influence of UV-C treatment on polyphenols from dried samples, because most studies have usually focused on fresh samples,

such as fresh-cut faba beans, in which the TPC increased by 15% after exposure to UV-C light [17]. A published work on dried lemon pomace reported significantly higher TPC and proanthocyanidins compared to untreated samples [18].

Table 2

First-order kinetic parameters fitted for phenolics degradation under UV-C exposure at 10 cm

Irradiated samples	Exposure time	Degradation
	(min)	(%)
	15	2.02
	30	3.76
<i>Kinetic parameters</i>		
	$10^{-3} k$ (min ⁻¹)	2.80
	$t_{1/2}$ (min)	247.50

The increase of the TPC is related to the better release of biocompounds from the cell wall as induced by UV light, which determines an improved extractability [19], and to the activity of the enzymes involved in the synthesis of polyphenols [20], enzymes which may resist to some extent in dried samples.

Regarding the content of tannins, the TTC mean value of irradiated samples group was slightly higher than that of control, but the difference was not statistically significant at $p < 0.05$. Generally, higher TTC values were obtained for samples situated at longer distances from the UV lamp (15–20 cm). The highest content was found in the sample irradiated for 30 min at a distance of 15 cm (99.53 ± 0.19 mg CE 100 g^{-1} DW), ~16% higher than in control.

The effect of UV-C treatment of dried samples on the antioxidant activity of their aqueous ethanol extracts was evaluated using the FRAP assay. UV-C light had a significant effect, the difference between control and treated groups being highly significant ($p < 0.001$). Regarding the influence of time (15, 30 min), significant differences between control and treated groups were found at both times ($p < 0.05$). The influence of irradiation distance was significant at 15 cm compared to control ($p < 0.05$), while no significant differences were found at 10 and 20 cm. Among treated samples, the highest antioxidant activity was registered at 15 cm irradiation distance for 30 min exposure time (470.85 ± 2.04 mg AAE 100 g^{-1} DW), while the lowest was at 20 cm for 15 min (455.19 ± 2.04 mg AAE 100 g^{-1} DW). The significant increase of FRAP values of mushrooms powder after UV-C exposure is in agreement with other described results. Thus, positive effects of UV-C treatments of dried lemon pomace have been reported for FRAP, in particular at lower irradiation of $4 \text{ kJ} \cdot \text{m}^{-2}$ compared to untreated samples [18]. Similar results have been reported by other studies on fresh bananas (30 min exposure, 10 cm) and blueberries ($1 \text{ kJ} \cdot \text{m}^{-2}$) [19].

We found a moderate positive correlation between FRAP and TTC ($R = 0.6344$) but not significant at $p < 0.05$, while no correlation between FRAP and TPC. Beside tannins, other antioxidants *e.g.* sterols in the extracts, may contribute to an enhanced total antioxidant activity. We do not exclude the synthesis of phenolic and/or

non-phenolic compounds with antioxidant activity by photochemical reactions under UV-C light (254 nm), such as chalcone derivatives [21]. Additional investigations are required to elucidate the mechanism by which UV light of various types and intensities influences the content of bioactive compounds in dried mushrooms.

To our knowledge, no published studies have been identified dealing with the effect of UV-C irradiation on total phenolics, tannins and antioxidant activity of dried *Boletus edulis* mushrooms.

3.2. EFFECT OF EXTREMELY LOW FREQUENCY MAGNETIC FIELD ON ANTIOXIDANT CONTENT AND ACTIVITY OF DRIED MUSHROOMS

Table 3 presents a comparative view of the results regarding the content of polyphenolic antioxidant compounds and antioxidant activity of *Boletus edulis* extracts obtained from dried samples, untreated (control) and exposed to oscillating extremely low frequency MF (3 mT, 50 Hz).

Table 3

TPC, TTC and antioxidant activity of control and mushrooms exposed to 50 Hz magnetic field, as function of exposure time

Parameters	Control	MF treated samples (3 mT, 50 Hz)				
		Exposure time (min)				
		15	30	60	120	240
Phenolics (mg 100 g ⁻¹ DW)	588.21 ± 7.53	573.51 ± 6.80	577.73 ± 7.88	570.96 ± 7.76	592.40 ± 7.56	602.78 ± 6.55
Tannins (mg 100 g ⁻¹ DW)	85.98 ± 0.12	85.05 ± 0.07	87.18 ± 0.10	87.93 ± 0.11	88.39 ± 0.12	89.45 ± 0.12
Antioxidant activity (mg 100 g ⁻¹ DW)	434.98 ± 2.10	430.08 ± 1.22	432.86 ± 1.21	434.53 ± 1.13	435.92 ± 0.98	435.82 ± 0.95

Note: The results represent average values of duplicate determinations ± standard deviation.

A slight increase of TPC, TTC and antioxidant activity by FRAP was noted in relation to the exposure time to MF, but differences were not statistically significant compared to the control sample at $p < 0.05$. The weak effect of 50 Hz MF could be related to the dehydration previously carried out to samples, since most studies showed that MF mainly influences the physical-chemical properties and structure of water [22–23], which may indirectly favour extractability of bioactive compounds in fresh samples or samples with higher content of water. There are few reports in the literature regarding the influence of MF on the antioxidant content and activity of dried food samples. No studies on dried *Boletus edulis* mushrooms have been identified in the literature. A study investigating chokeberry fruits showed that low MF (150 μ T, 100 Hz) did not significantly changed the level of TPC [24]. Authors indicated that longer exposure time (60 min) determined an increase compared to lower exposure time (30 min), both being

slightly lower than control. Another study dealing with the growth of oyster mushroom (*Pleurotus* spp.) confirmed the positive influence of low MF (5 mT, 50 Hz, 15 min) exposure on the mushroom growth and yield [25].

3.3. ATR-FTIR ANALYSIS

By recording the ATR-FTIR spectra of control and UV-C irradiated samples of *Boletus edulis* dried mushrooms, several differences have been found between control and samples treated as follow: 30 min at 15 cm, 15 min at 15 cm and 30 min at 20 cm. The ATR-FTIR spectra are displayed in Figs. 2–3.

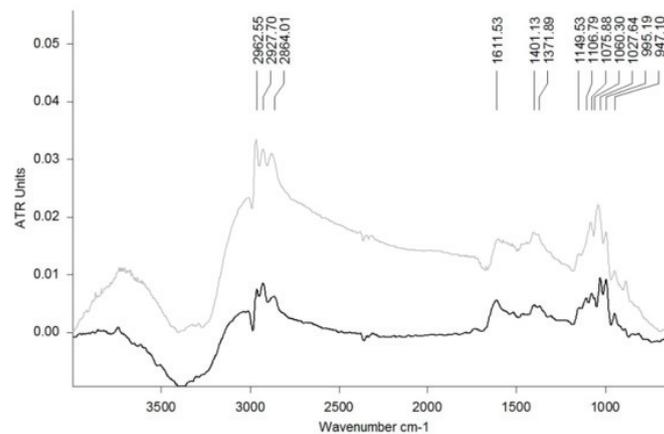


Fig. 2 – ATR-FTIR spectra of dried mushroom samples, control (–) and UV-C treated for 30 min at 15 cm (–).

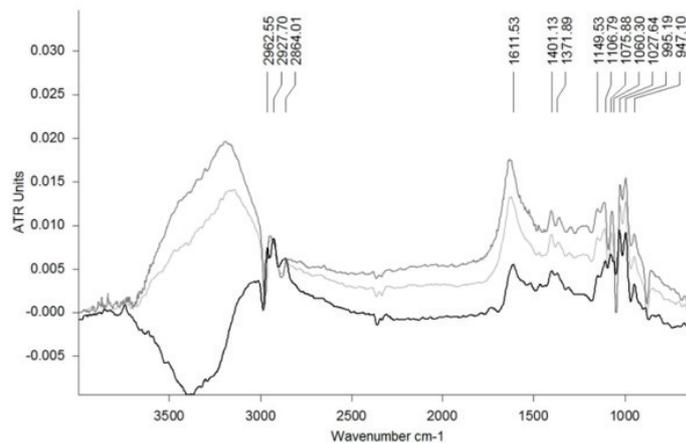


Fig. 3 – ATR-FTIR spectra of dried mushroom samples, control (–) and UV-C treated for 15 min at 15 cm (–) and 30 min at 20 cm (–).

Based on the literature reports [26], the absorption peaks in FTIR spectra of untreated samples may be attributed as follow: 3000–3400 cm^{-1} O–H stretching of water and N–H vibrations, 2962 cm^{-1} asymmetric C–H stretching vibration, 2927 cm^{-1} C–H stretching of lipid CH_2 or aromatic CH_3 , 2864 cm^{-1} symmetric C–H stretching of CH_2 (pyranose ring, lipids) or methoxy CH_3 (polyphenols), 1611 cm^{-1} C=O (phenolic acids, protein amide), C=N stretchings and N–H bending of proteins, 1401 cm^{-1} alcohol O–H and C–H bendings of pyranose ring of carbohydrates, 1371 cm^{-1} phenol O–H, 1075 cm^{-1} ether =C–O–C group of phenolics/ flavonoids or C=S of ergothioneine, peaks 1149, 1106, 1060, 1027 and 995 cm^{-1} alcohol C–O, C–O–C and C–C skeleton stretchings of polysaccharides, 947 cm^{-1} glycosidic bonds of α -glucans.

Several changes in FTIR spectra of irradiated samples may be observed. Thus, peaks at 1106 and 1060 cm^{-1} attributed to polysaccharides groups, no longer occur in spectra of the irradiated sample, probably due to the hydrolysis of polyphenols attached to cell-wall polysaccharides, in particular tannins. This is correlated to our results showing an increased TTC for the irradiated sample (30 min, 15 cm) (Table 1). The strong broad peaks at 3000–3250 cm^{-1} and the more intense strong peak at 1075 cm^{-1} (Fig. 3) might be attributed to N–H and C=S stretching of ergothioneine, a sulphur-containing histidine derivative, which may have been increased in amounts during the UV-C exposure [27]. The peak at 1075 cm^{-1} could be attributed to the ether =C–O–C group of phenolics/ flavonoids. These findings confirm the high antioxidant activity and TPC of irradiated samples (15 min at 15 cm and 30 min at 20 cm) (Table 1). Some minor changes occurred in the region of medium peaks at 2962 and 2927 cm^{-1} attributed to C–H stretching, and at 1060 cm^{-1} attributed to polysaccharides.

Regarding the ATR-FTIR analysis of dried mushrooms exposed to MF (3 mT, 50 Hz), the obtained spectra were similar to those of untreated mushrooms, confirming that no significant structural changes occurred, in accordance with the results described in Table 3.

4. CONCLUSIONS

The UV-C exposure of dried mushrooms for 30 min at 20 cm exposure distance led to a 17% increase of phenolics content and to ~16% increase of tannins content, compared to untreated sample. At lower exposure distance (10 cm), a low degradation of polyphenols occurred, with a rate constant (k) of the first-order kinetic model of $2.80 \times 10^{-3} \text{ min}^{-1}$, and a half-time $t_{1/2}$ of 247.50 min. Significant positive effects of UV-C on antioxidant activity of mushroom extracts were found, in particular at 15 cm irradiation distance for 30 min exposure time. A weak positive effect of MF treatment on the antioxidant content and activity was observed. The ATR-FTIR spectra of irradiated and non-irradiated samples confirmed

the results on the changes in phenolics and tannins content and on the antioxidant activity of dried mushroom powder.

Our results point out the potential use of secondary non-thermal mushroom processing, in particular UV-C treatment for improving the extraction of antioxidant compounds from *Boletus edulis* powder. We conclude that the two types of physical treatments, besides their established role in food sterilization, do not affect its composition, but improve the quality of the extracts and reduce the extraction time. These findings reveal the practical use of non-thermal technology without affecting the mushroom quality.

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