*Studii şi Cercetări Ştiinţifice* 2022, **23** (3), pp. 243– 254
*Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară*

*Scientific Study & Research*
*Chemistry & Chemical Engineering, Biotechnology, Food Industry* *ISSN 1582-540X*

**ORIGINAL RESEARCH PAPER**

# THE INFLUENCE OF MICROWAVE EXPOSURE OF *BOLETUS* MUSHROOM-SOLVENT MIXTURES ON THE EXTRACTABILITY OF PHENOLICS AND ANTIOXIDANT ACTIVITY

**Miruna F. Popa[1], Maria Denisa Cocîrlea[1], Simona Miclăuș[2], Simona Oancea[1*]**

[1] *"Lucian Blaga" University of Sibiu, Department of Agricultural Sciences and Food Engineering, Dr. I. Ratiu Street, no. 7-9, 550012 Sibiu, Romania*
[2] *"Nicolae Bălcescu" Land Forces Academy, Department of Communication and IT, 3-5 Revoluției Street, Sibiu, Romania*

*Corresponding author: simona.oancea@ulbsibiu.ro

Received: April, 06, 2022
Accepted: September, 21, 2022

**Abstract:** Microwaves have been frequently applied for extraction of bioactive compounds of different origin, using systems operating at 200 - 1000 W power, the efficiency being related to the heating of molecules. This study investigated the effects of non-thermal microwaves at 1.7 and 2.5 GHz for different exposure time, on the phenolic content and antioxidant activity of *Boletus edulis* extracts. The irradiated mixtures of fresh samples in ethanol solution showed increased phenolic content compared to control and in contrast to those of dried samples. The ATR-FTIR analysis indicates no structural changes after irradiation, confirming that the effect is due to the cell membrane disruption facilitating the compounds' release. The antioxidant activity of extracts from fresh samples significantly increased after MW exposure at 1.7 GHz for 3 h, 775 $V \cdot m^{-1}$. These results suggest that microwaves at investigated frequencies and electric field strengths can contribute to an efficient extraction of antioxidants from fresh mushrooms.

***Keywords:*** *antioxidant activity, ATR-FTIR, Boletus edulis, microwaves, phenolics*

## INTRODUCTION

*Boletus edulis*, also known as porcini, is a delicious wild edible mushroom widely spread and freshly consumed during autumn, particularly in Central and Eastern Europe, including Romania [1, 2]. The high interest due to its nutritional value and taste has been increasing worldwide in the last years [3 – 5] based on the recent consumer awareness on the health significance of food products rich in nutrients and bioactive compounds [6]. There is also a current interest in medicinal mushrooms, known for their remarkable properties such as anticancer, antioxidant, anti-diabetic and anti-inflammatory [7].

Different types of extraction based on organic solvents are used to isolate valuable antioxidant compounds, such as polyphenols from edible mushrooms. The efficiency of the applied extractive technologies is strongly related to process parameters and material types, being expressed in terms of the yield and content of bioactive compounds. Besides traditional usually time-consuming extraction techniques (maceration, solvent heating), new alternative methods of polyphenol extraction are required. In recent years, modern methods, such as microwave assisted extraction (MAE) gained major attention of the food and pharmaceutical industry due to the benefits of reducing extraction time and increasing the antioxidant content and activity [8]. The efficiency of MAE is given by the heat generation, in particular when polar compounds and solvents have been involved [9]. Regarding the applied microwave (MW) frequencies, few are allowed for industrial, scientific and medical uses, ranging from 0.915 to 2.45 GHz, most equipment operating at 2.45 GHz and 100 W [10]. MAE energy and time are key factors in the extraction efficiency. On the other hand, heating may drastically influence the stability of thermolabile compounds, so that low or medium MW power is recommended.

In the present study, we investigated the extraction of phenolics from wild *Boletus edulis* mushrooms using a MW generator that provides an incident electric field strength between 370 and 775 $V \cdot m^{-1}$, in order to minimize the degradation of polyphenols. The paper describes the effects of MW exposure of sample-solvent mixtures, under constant environmental parameters (temperature, humidity and pressure), by varying the process parameters, frequency and exposure duration, on the phenolic content and antioxidant activity of bioextracts. Additionally, Fourier-Transform Infrared (ATR-FTIR) analysis was conducted.

## MATERIALS AND METHODS

### Materials and chemical reagents

Wild edible *Boletus edulis* mushrooms (fruiting bodies) were collected from Sibiu forests, Romania. Both fresh and dried samples (at 60 °C for 5 h using the convection oven, UFE 400 with following characteristics: 53 L volume, 1400 W, digital temperature and overtemperature monitor, fan speed 100 % - forced air circulation, Memmert, Germany) have been investigated. The final moisture content of dried samples, as determined at 105 °C using the moisture analyzer (MAC 210 Radwag, Poland), was ~6 %.

All reagents were of analytical grade: ethanol (> 96 %, Chimreactiv, Romania), Folin-Ciocâlteu reagent (Merck KGaA, Germany), sodium carbonate anhydrous (Reactiv srl, Romania), gallic acid (Fluka, Germany), sodium acetate (Chimreactiv, Romania), glacial acetic acid (Adrachim, Romania)), TPTZ (2,4,6-tri-pyridyl-*s*-triazine, Sigma-Aldrich), ferric chloride hexahydrate (Scharlau, Spain), L-ascorbic acid (Sigma-Aldrich). Buffer solutions were prepared in distilled water.

**Preparation of mushroom ethanol extracts**

An amount of 1 g sample either fresh or previously dried, was mixed with 10 mL 70 % aqueous ethanol. Part of the prepared sample-solvent mixtures was MW irradiated under different conditions, as described in the next section. Another part was kept as control, consisting in fresh or dried mushrooms mixed with ethanol solution and let at room temperature for 3 h. After extraction, all mixtures, samples and control, were centrifuged at 8000 rpm, 4 °C, for 10 minutes using the refrigerated centrifuge (Universal 320, Hettich, Germany). The obtained extracts were subjected to compositional analysis.

**Electromagnetic MW exposure of mushroom-ethanol mixtures**

An experimental exposure system described in Figure 1 was designed, consisting in a MW generator, a power amplifier, a horn antenna and a shielded enclosure where the samples were individually exposed. The environmental parameters *e.g.* temperature, humidity, pressure, were kept constant and identical during all the experimental runs. Each sample was placed in front of the directive antenna in near-field conditions of irradiation. The applied exposure variables were: (a) frequency, 1.7 GHz and 2.5 GHz; (b) exposure duration, 0.5, 1 and 3 h; (c) incident electric field strength, 370 -750 $V \cdot m^{-1}$. The incident electric field strength was measured using a tri-axial laser powered field probe with optical fibers connected to the measurement centre. The absorbed power levels were computed by means of simulations. Most of the exposures were at very low thermal interaction level, so that sample heating increase did not exceed 1 - 2 °C.

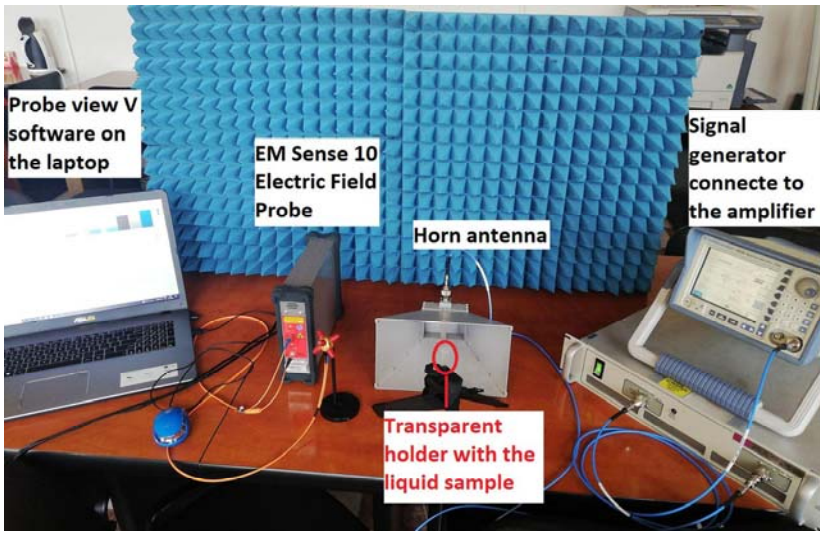


***Figure 1.*** *The experimental exposure system used for MW irradiation of mushroom-solvent mixtures, comprising of emitting and field-measurement components*

**Analysis of the total phenolic content (TPC)**

The total phenolic content of irradiated and non-irradiated mushroom extracts was evaluated by Folin-Ciocâlteu method [11] using the spectrophotometer Specord 200 Plus UV-Vis (Analytik Jena, Germany). The results were expressed as mg of gallic acid equivalents (GAE) per 100 g dry weight (DW).

**Analysis of the total antioxidant activity (TAA)**

The total antioxidant activity of irradiated and non-irradiated mushroom extracts was measured using the Ferric Reducing Antioxidant Power (FRAP) assay [12]. The results were expressed as mg ascorbic acid equivalents (AAE) per 100 g dry weight (DW).

**ATR-FTIR analysis**

Fourier transform infrared (FTIR) analysis was carried out using the ALPHA FTIR spectrometer (Bruker, Germany) with the combined QuickSnapTM sampling modules and ZnSe ATR (Attenuated Total Reflectance) with a resolution of 4 $cm^{-1}$. An average of 32 scans was recorded in the ATR mode.

**Statistical analysis**

Duplicate experiments were performed and results were expressed as mean values ± standard deviation (SD). The statistical analysis was performed by Kruskal-Wallis H test, Dunn's Test and Generalized Linear Model (GLM) using the R x 64 4.0.3. statistical software.

## RESULTS AND DISCUSSION

The flowchart of the experimental processes for the development of valuable final extracts from *Boletus edulis* mushrooms, under MW exposure, is presented in Figure 2.

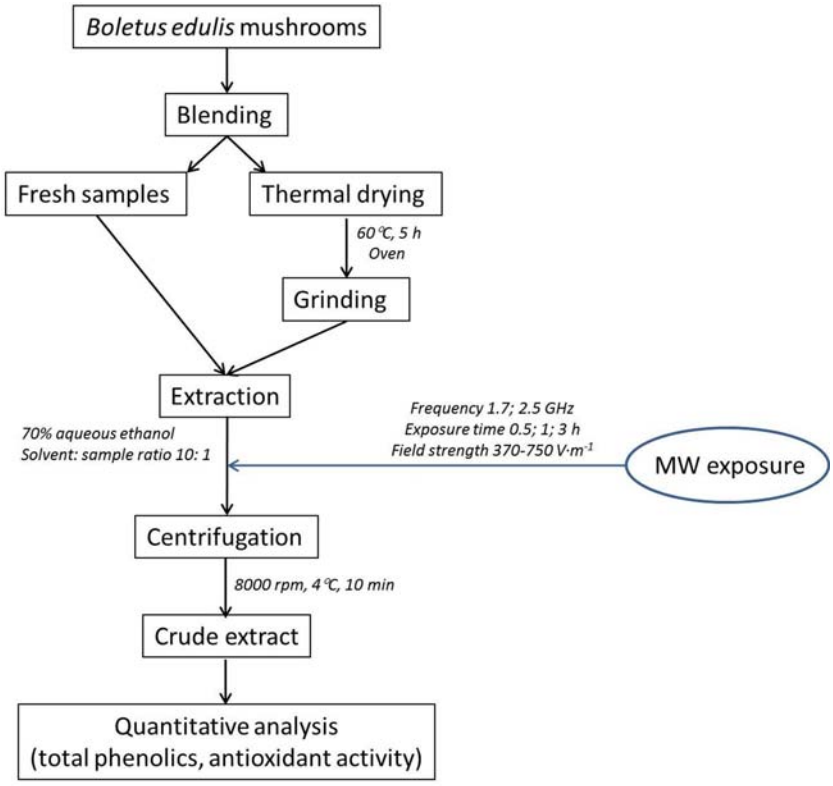


***Figure 2.*** *The experimental flowchart for production of bioactive extracts from Boletus edulis mushrooms*

### The effects of MW exposure of fresh *Boletus edulis* mushrooms-ethanol mixtures on their antioxidant content and activity

The results on the evolution of the TPC and TAA of control and MW irradiated ethanol mixture of fresh *Boletus edulis* mushrooms are presented in Figure 3.

According to the obtained results, the mean values of both phenolic content and antioxidant activity of the samples exposed to MW radiation, irrespective of the hereby investigated time, frequency or incident electric field, were higher than those of the control sample.

The TPC of extracts obtained from fresh samples increased by 27.5 % after the treatment with MW at the highest frequency (2.5 GHz) and exposure time (3 h), compared to the control sample, which has been not irradiated, but compounds were conventionally extracted (macerated) at room temperature. The results indicate that the MW radiation improved the extraction of compounds of phenolic structure, most probably due to the disruption of cell membrane which facilitates the release of such

compounds [13]. Regarding the influence of the exposure time, longer time increased the content of polyphenols and the antioxidant activity, irrespective of the radiation frequency. The highest TAA was recovered in samples exposed to 1.7 GHz for 3 h, showing an increase of 34.2 % compared to control. MW treatment of extracts from fresh mushrooms improved the yield of investigated compounds and antioxidant activity, compared to conventional extraction for the same extraction time.

Because literature is scarce in reports on low thermal MW extraction at low input power, our results could not be compared to other reported studies. However, a different MW experimental set was reported by other authors [14] describing a thermal method of MAE of bioactive compounds from lyophilized mushrooms in methanol solution by using a microwave digestion system with MW power of 0 - 1500 W adjusted to a temperature increase from 30 °C to 130 °C. Their results showed that the highest total antioxidant activity by CUPRAC method (122.4 ± 1.3 μmol trolox·g$^{-1}$ extract) of *Boletus edulis* extract was obtained after 5 min of MW irradiation at 80 °C.

Using the present method of exposure of bioextracts provides convenience, as follow: a) flexibility in choosing the proper frequency to be applied in function of the dielectric parameters of the exposed samples (allowing adjustments of the frequency to correspond to maximum electromagnetic absorption at minimum input power per each electric permittivity and conductivity of one specific sample); b) possibility of using not only continuous waves as incident stimulus, but also modulated signals, which are impossible to be applied with classical irradiation systems.

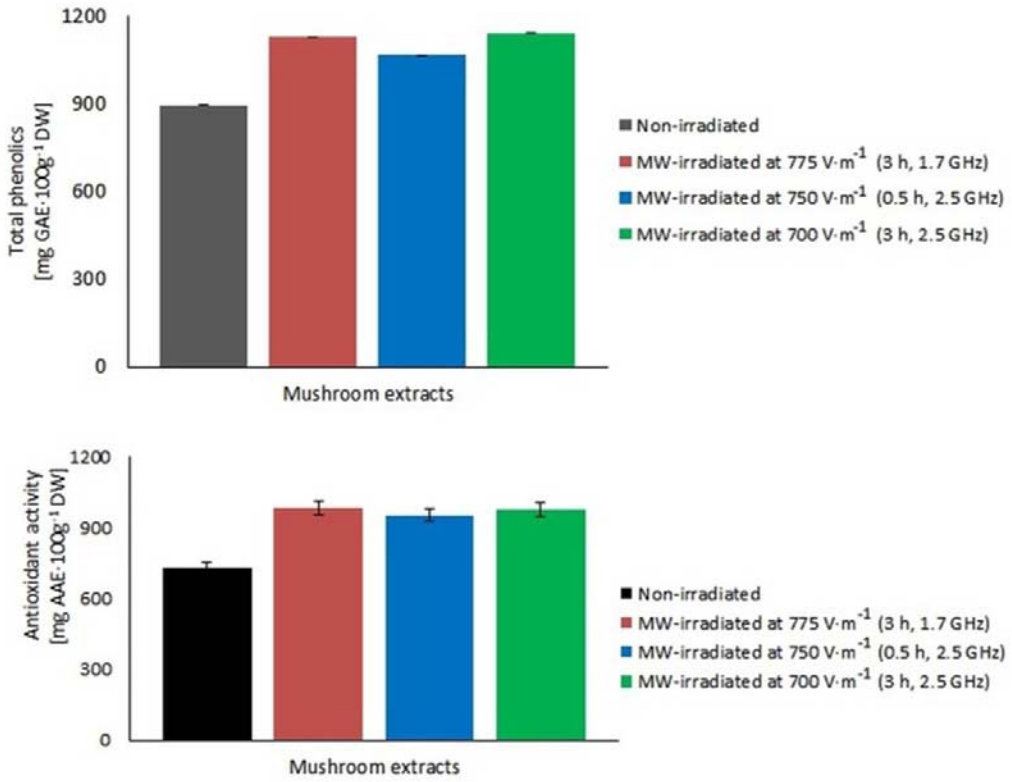


***Figure 3***. *The TPC and TAA of control and MW treated fresh mushroom samples in ethanol solution as function of incident electric field strength and exposure parameters*

**ATR-FTIR analysis of ethanol extracts of fresh *Boletus edulis* mushrooms**

The ATR-FTIR spectra recorded for the extracts obtained from the *Boletus edulis* fresh samples, control and MW irradiated are presented in Figure 4. The irradiated investigated samples were obtained by exposure for 3 h at 1.7 GHz and 775 V·m$^{-1}$, for 0.5 h at 2.5 GHz and 750 V·m$^{-1}$and for 3 h at 2.5 GHz and 700 V·m$^{-1}$, respectively.

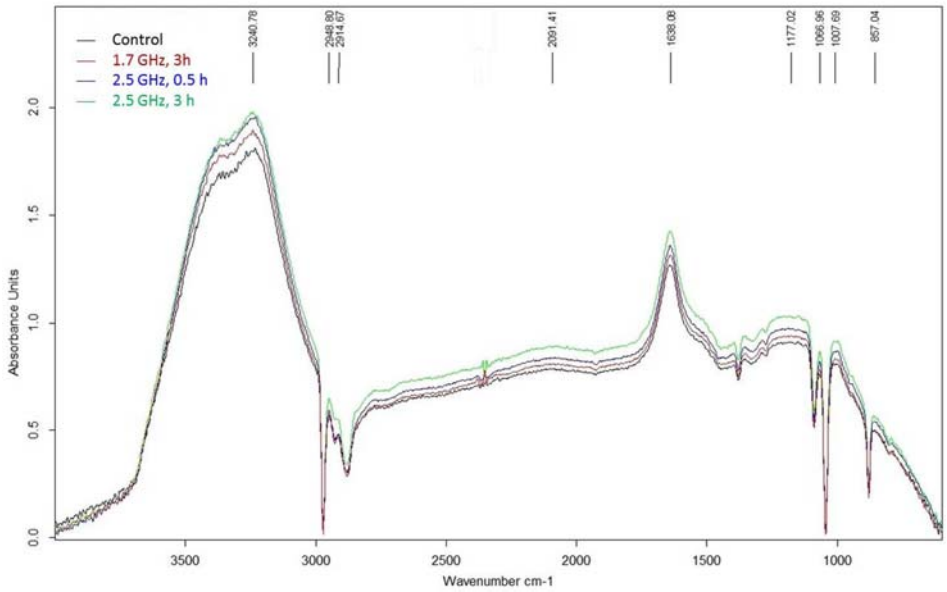


***Figure 4.*** *The ATR-FTIR spectra of fresh mushrooms extracts, control and MW irradiated*

The main absorption peaks in the FTIR spectra of control samples were assigned based on the reports from the literature [15, 16], as follow: the broad peaks at 3000 -3600 cm$^{-1}$ were attributed to O-H stretching (alcohol, phenol) and N-H stretching of amine/ amide; the peaks at 2948 cm$^{-1}$ and 2914 cm$^{-1}$ were due to asymmetrical and symmetrical C-H stretching vibration, of $CH_2$ (lipids) and aromatic $CH_3$. According to the literature, the 1700 - 1000 cm$^{-1}$ spectral region is characteristic for macrofungi [15, 17]. Thus, the medium sharp peak at 1638 cm$^{-1}$ was assigned to C=O stretching (lipid ester, phenolic acids, protein amide), C= N stretching and N-H bending of protein and chitin amide band; the peak at 1177 cm$^{-1}$ was attributed to alcohol O-H and carbohydrate pyranose ring bendings; the peak at 1066 cm$^{-1}$ was due to phenol C-OH stretching; the peak at 1007 cm$^{-1}$ was due to ether C-O-C stretching (phenolic group / flavonoids) or C=S of ergothioneine. The 1638 - 1177 cm$^{-1}$ region is relevant for proteins [16]. The peaks at 1177, 1066 and 1007 cm$^{-1}$ were attributed to alcoholic C-O, C-O-C and C-C stretching, which indicate the presence of polysaccharides, *e.g.* chitin and β-glucans [18, 19]. The peak at 857 cm$^{-1}$ may indicate the glycosidic bonds of α-glucans.
As resulted from Figure 4, no major changes occurred in irradiated samples compared to control, indicating that the MW did not influence the chemical structures of compounds, but facilitated the extraction due to mechanical events (cell membrane disruption).

**The effects of MW exposure of dried *Boletus edulis* mushrooms-ethanol mixtures on their antioxidant content and activity**

The results regarding the evolution of the TPC and TAA in ethanol extracts obtained from oven-dried mushroom samples (at 60 °C) subjected to the MW treatment under different exposure conditions, at 1.7 and 2.5 GHz, 370 - 700 $V \cdot m^{-1}$ are given in Table 1. Different field strengths ranging from 370 to 700 $V \cdot m^{-1}$ were intentionally used with the aim of fast observing more significant biochemical effects, if any, when power is different at the same frequency, given the fact that samples have been dried before extraction and exposure.

***Table 1***. *The TPC and TAA of control and MW treated dried mushroom samples in ethanol solution as function of irradiation time and frequency*

| Characteristics | Control sample | Exposure time [h] | Freq. [GHz] | Incident electric field strengths [$V \cdot m^{-1}$] | MW treated sample |
|---|---|---|---|---|---|
| **Total phenolics [mg GAE·100$g^{-1}$ DW]** | 1141.741±2.090 | 0.5 | 2.5 | 450 | 1122.085±2.892 |
| | | 1 | 2.5 | 430 | 1129.365±2.941 |
| | | 3 | 2.5 | 700 | 1103.233±2.460 |
| | | 0.5 | 1.7 | 450 | 1357.398±3.338 |
| | | 1 | 1.7 | 430 | 1192.517±3.113 |
| | | 3 | 1.7 | 370 | 1107.645±2.972 |
| **Antioxidant activity [mg AAE·100$g^{-1}$ DW]** | 594.365±2.783 | 0.5 | 2.5 | 450 | 594.224±11.977 |
| | | 1 | 2.5 | 430 | 589.897±14.654 |
| | | 3 | 2.5 | 700 | 582.727±1.906 |
| | | 0.5 | 1.7 | 450 | 581.510±10.878 |
| | | 1 | 1.7 | 430 | 591.945±1.889 |
| | | 3 | 1.7 | 370 | 560.972±0.619 |

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

No significant TPC differences were found between control and MW irradiated samples, with the exception of the mixture of mushroom powder and ethanol solution exposed to MW at 1.7 GHz for 0.5 h exposure time, which registered the highest phenolic content, 1357.398 ± 3.338 mg GAE·100$g^{-1}$ DW. The TPC value for this sample was 18.8 % higher than that of the control sample. The lowest phenolic content was obtained for higher exposure time (3 h) at 2.5 GHz (1103.233 ± 2.460 mg GAE·100$g^{-1}$ DW). This is different from the results on the TPC evolution in extracts obtained from fresh samples, indicating that the presence of water inside the samples may influence the extraction efficiency. Regarding the TAA, the obtained results showed no significant changes after MW exposure. However, most of MW irradiated samples showed slightly decreased values of the antioxidant activity as measured by FRAP assay. A weak positive correlation using the Pearson coefficient was found ($r = 0.0392$) between TPC and TAA, the correlation being not statistically significant ($p < 0.05$).

The statistical analysis using the Kruskal-Wallis test showed that there was no significant difference of TPC average values considering all samples, between fresh and dried mushroom samples ($p = 0.1859$), as shown in Figure 5. Instead, significant statistical differences regarding the TAA were found between fresh and dried mushroom samples, as shown in Figure 6. The Dunn test indicated that the fresh mushroom samples had stronger antioxidant activity (chi-squared = 7, df = 1, p-value = 0.01). The GLM test showed that there is a relation between the TAA and the incident electric field strength, the TAA increasing with the increase of this parameter, while the TPC level has not been significantly influenced.

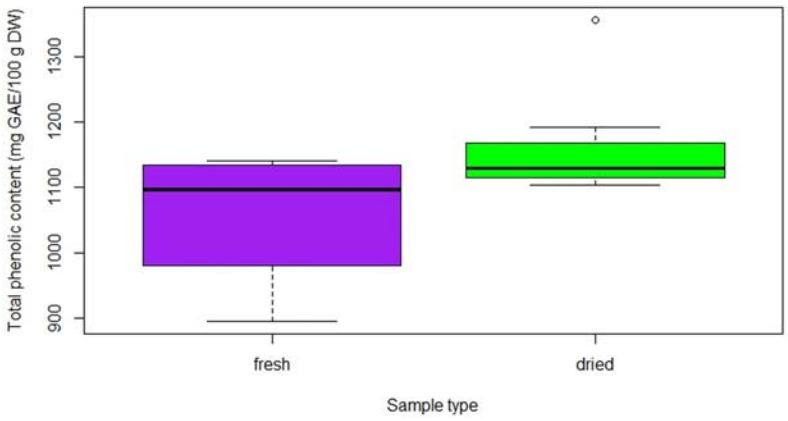


***Figure 5.*** *Boxplot of the TPC of fresh and dried Boletus edulis mushrooms*

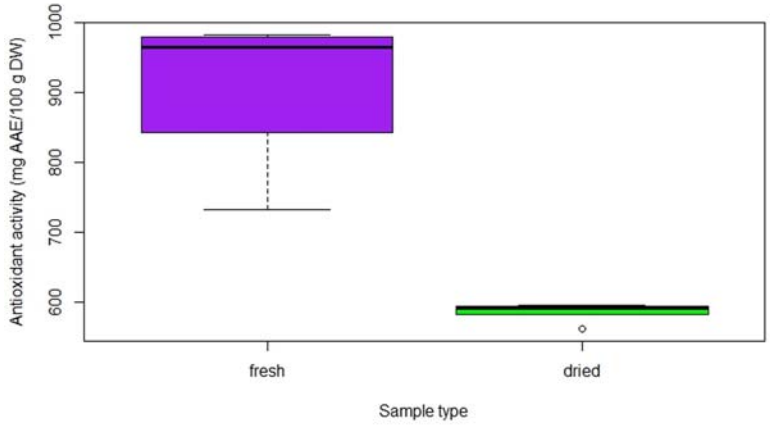


***Figure 6.*** *Boxplot of the TAA of fresh and dried Boletus edulis mushrooms*

The dielectric properties of investigated samples have a great impact on the amount of electromagnetic power deposited inside. Generally, higher water-content samples absorb greater electromagnetic energy than lower water-content or dried samples, emphasizing on the requirement for the presence of the water dipole. Polar solvents with high dielectric constants, such as water and ethanol, can absorb more MW energy compared to nonpolar ones [10]. On the other hand, the direct interaction of radiation at

the level of the cell membrane triggers several phenomena [20 – 22]. No heating was reported with frequencies > 2.45 GHz and rapid changes of the electrical component, or < 2.45 GHz and changes of the electrical component at a much lower speed [23, 24]. The following issues were relevant from our experiments aiming to study the non-thermal effects of MW [22 – 24]: a) the effects are dependent on the frequency (frequency windows with resonant effects were observed); b) a threshold of the incident power density of the radiation exists, below which no effect appears, but above which the effects depend weakly on the power density over several orders of magnitude (a sigmoid or S-shaped dependence); c) the effects depend on the duration of the exposure and on the type of sample.

To our knowledge, literature studies on MW exposure of ethanol/ mushroom mixtures at specific frequencies and exposure time are scarce, most of the published studies reporting heat effects during MAE using laboratory systems with time and power controls. A published study on the thermal MAE of phenolics from natural dried *Coriolus versicolor* medicinal mushrooms using ethanol solution [25], showed that efficient extraction was achieved at 125 W power and 3.8 h extraction time (470 mg GAE·100 $g^{-1}$ DW), but the value was not significantly different from conventional extracts by reflux (434 mg GAE·100 $g^{-1}$ DW). Instead, authors reported significant higher FRAP antioxidant activity by MAE (1710 μM TE·$g^{-1}$ DW) compared to reflux extraction at 95 °C for 4 h (1206 μM TE·$g^{-1}$ DW).

## CONCLUSIONS

The MW exposure of mixtures of *Boletus edulis* mushroom (fresh and oven-dried) in ethanol aqueous solution showed a significant increase of the phenolic content in fresh samples, in particular at higher exposure time (3 h compared to 0.5 h) at both investigated frequencies (1.7 and 2.5 GHz) under incident electric fields of 700-775 V·$m^{-1}$. The total antioxidant activity of mushroom extracts increased after MW exposure, in particular in the sample irradiated for 3 h at 1.7 GHz (775 V·$m^{-1}$). No significant impact on the total phenolic content and total antioxidant activity was found by MW exposure of ethanol mushroom extracts obtained from dried samples.

The statistical analysis using the Kruskal-Wallis test indicated significant differences regarding the TAA of fresh and dried samples and no significant TPC differences between fresh and dried mushrooms.

The ATR-FTIR analysis of the ethanol extracts obtained from fresh samples, indicates no structural changes after irradiation, confirming that the effect is due to cell membrane disruption which facilitates compounds' release.

These results highlight the potential of MW under irradiation conditions of 2.5 GHz and 1.7 GHz, respectively, for 3 h, using a laboratory exposure system, to positively influence the total phenolic content, and the antioxidant activity, respectively, of extracts prepared from fresh *Boletus edulis* mushrooms.

## ACKNOWLEDGEMENTS

This work is supported by the Project: POCU/993/6/13/153310 „Dezvoltarea competențelor de cercetare avansată şi aplicată în logica STEAM + Health"

## REFERENCES

1. Malinowski, R., Sotek, Z., Stasińska, M., Malinowska, K., Radke, P., Malinowska, A.: Bioaccumulation of macronutrients in edible mushrooms in various habitat conditions of NW Poland - Role in the human diet, *International Journal of Environmental Research and Public Health*, **2021**, **18** (16), 8881;
2. Fogarasi, M., Diaconeasa, Z.M., Pop, C.R., Fogarasi, S., Semeniuc, C.A., Fărcaş, A.C., Țibulcă, D., Sălăgean, C.-D., Tofană, M., Socaci, S.A.: Elemental composition, antioxidant and antibacterial properties of some wild edible mushrooms from Romania, *Agronomy*, **2020**, **10** (12), 1972;
3. Cheung, P.C.K.: The nutritional and health benefits of mushrooms, *Nutrition Bulletin*, **2010**, **35** (4), 292-299;
4. Siwulski, M., Sobieralski, K., Sas-Golak, I.: Nutritional and health-promoting value of mushrooms in Polish forests, *Sylwan*, **2014**, **158** (2), 151-160;
5. Agrahar-Murugkar, D., Subbulakshmi, G.J.F.C.: Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya, *Food Chemistry*, **2005**, **89** (4), 599-603;
6. Martins, N., Ferreira, I.C.: Mountain food products: A broad spectrum of market potential to be exploited, *Trends in Food Science & Technology*, **2017**, **67**, 12-18;
7. Chaturvedi, V.K., Agarwal, S., Gupta, K.K., Ramteke, P.W., Singh, M.P.: Medicinal mushroom: boon for therapeutic applications, *3 Biotech*, **2018**, **8** (8), 1-20;
8. Ballard, T.S., Mallikarjunan, P., Zhou, K., O'Keefe, S.: Microwave-assisted extraction of phenolic antioxidant compounds from peanut skins, *Food chemistry*, **2010**, **120** (4), 1185-1192;
9. Gujar, J.G., Wagh, S.J., Gaikar, V.G.: Experimental and modeling studies on microwave-assisted extraction of thymol from seeds of Trachyspermum ammi (TA), *Separation and Purification Technology*, **2010**, **70** (3), 257-264;
10. Leonelli, C., Veronesi, P., Cravotto, G.: Microwave-Assisted Extraction: An Introduction to Dielectric Heating in: *Microwave-assisted Extraction for Bioactive Compounds* (Editors: Chemat, F., Cravotto, G.), Springer, New York, **2013**, 1-14;
11. Singleton, V.L., Rossi, J.A.: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *American journal of Enology and Viticulture*, **1965**, **16** (3), 144-158;
12. Benzie, I.F., Strain, J.J.: The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, *Analytical biochemistry*, **1996**, **239** (1), 70-76;
13. Delazar, A., Nahar, L., Hamedeyazdan, S., Sarker, S. D.: Microwave-assisted extraction in natural products isolation, *Natural products isolation*, **2012**, 89-115;
14. Özyürek, M., Bener, M., Güçlü, K., Apak, R.: Antioxidant/antiradical properties of microwave-assisted extracts of three wild edible mushrooms, *Food chemistry*, **2014**, **157**, 323-331;
15. Mohaček-Grošev, V., Božac, R., Puppels, G.J.: Vibrational spectroscopic characterization of wild growing mushrooms and toadstools, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **2001**, **57** (14), 2815-2829;
16. Qi, L.M., Zhang, J., Liu, H.G., Li, T., Wang, Y.Z.: Fourier transform mid-infrared spectroscopy and chemometrics to identify and discriminate *Boletus edulis* and *Boletus tomentipes* mushrooms, *International Journal of Food Properties*, **2017**, **20** (sup1), S56-S68;
17. Nie, M., Luo, J., Xiao, M., Chen, J., Bao, K., Zhang, W., Chen, J., Li, B.: Structural differences between Fusarium strains investigated by FT-IR spectroscopy, *Biochemistry (moscow)*, **2007**, **72** (1), 61-67;
18. O'Gorman, A., Downey, G., Gowen, A.A., Barry-Ryan, C., Frias, J.M.: Use of fourier transform infrared spectroscopy and chemometric data analysis to evaluate damage and age in mushrooms

(agaricus bisporus) Grown in Ireland, *Journal of Agricultural and Food Chemistry*, **2010**, **58**, 7770–7776;
19. Mellado-Mojica, E., López, M.G.: Identification, classification, and discrimination of agave syrups from natural sweeteners by infrared spectroscopy and HPAEC-PAD, *Food Chemistry*, **2015**, **167**, 349-357;
20. Belyaev, I.: Dependence of non-thermal biological effects of microwaves on physical and biological variables: implications for reproducibility and safety standards, in: *Non-thermal effects and mechanisms of interaction between electromagnetic fields and living matter* (Giuliani, L., Soffritti, M.), ICEMS Monograph, Ramazzini Institute, Italy, **2010**, 187-218;
21. Belyaev, I., Markov, M.S.: Biophysical mechanisms for nonthermal microwave effects in: *Electromagnetic fields in biology and medicine*, Boca Raton, London, New York: CRC Press, **2015**, 49-68;
22. Belyaev, I.: Main Regularities and Health Risks from Exposure to Non-Thermal Microwaves of Mobile Communication, in *2019 14th International Conference on Advanced Technologies, Systems and Services in Telecommunications (TELSIKS)* **2019**, October, (pp. 111-116), IEEE;
23. Collier, R.J.: Transmission Lines, In: *Microwave Measurements*, (Editor Bailey, A.E.), Peregrinus Publisher, London, **1985**;
24. Corney, A.: *Atomic and Laser Spectroscopy*, Clarendon Publisher, Oxford University Press, New York, **2006**;
25. Maeng, J.H., Shahbaz, H.M., Ameer, K., Jo, Y., Kwon, J.-H.: Optimization of microwave-assisted extraction of bioactive compounds from *Coriolus versicolor* mushroom using response surface methodology, *Journal of Food Process Engineering*, **2017**, **40** (2), e12421;