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CHARACTERISATION OF TRUFFLES USING ELECTROCHEMICAL AND ANALYTICAL METHODS

A.E. SEGNEANU[a*], P. SFIRLOAGA[a], I. DAVID[b], I.BALCU[a], I.GROZESCU [a,c]
[a]*National Institute of Research &Development for Electrochemistry and Condensed MatterINCEMC Timisoara, Romania, 144 Dr. A. P. Podeanu, 300569 Timisoara, Timis, Romania,*
[b]*Banat University of Agricultural Sciences and Veterinary Medicine Timisoara, Calea Araduluinr.119, 300645 Timisoara, jud. Timis, Romania, fax. 0256200296*
[c]*Aurel Vlaicu University Arad, B-dul Revoluţiei nr. 77, 310130 Arad, Romania,*

This paper aims to investigate a comparative study on two types of truffles: *Tuber magnatum pico* and *Tuber melanosporum* using different analytical methods. In this investigation we have determined the antioxidant activity of truffles by an electrochemical method (cyclic voltammetry), the content of total organic carbon and heavy metals. Moreover, it was comparative examined the morphology and elemental composition by scanning electron microscopy and EDAX to estimate the diversity of this two different types of truffles.

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Keywords: Truffle, Cyclic voltammograms, Electrochemical methods, Scanning electron microscopy

1. Introduction

Truffles (*Tuber spp*.) are hypogean fungus, which form symbiotic relationships with compatible host tree species (beech, poplar, oak, birch, hornbeam, hazel, and pine) (1). Although it is one of the world's most expensive foods, truffles are very popular in French and Italian cuisine, due to their unique aroma and flavour, delicious taste and highly nutritional value. Mushrooms, and especially edible mushroom species were also among the healthy food because are the best sources of other essential nutrients (protein, amino acids, fatty acids, minerals and carbohydrates). Truffles are known for their antioxidant, immuno-modulating effects and antitumor activity in humans (2-3).

Growth of truffles depends on many factors such as rainy season and its timing, soil characteristics, water availability, and climatic conditions. Truffles grow in temperate, moist climates throughout the world; they like warm, dry summers, cool, wet winters and alkaline (limestone) soil (1).

The white truffle *(Tuber magnatum pico)* is a hypogean fungus, living entirely underground, ensconced among the roots trees, and those among the oaks are the most sought. It is found from October 1 through December 31, in the vicinity of oaks, lime trees and poplars. It is known for its luscious, heady aroma. The black truffle (*Tuber melanosporum*) commonly known as the ‘‘black diamond of cuisine” is considered the most aromatic, while white truffle (Tuber magnatum) from Italy is considered the finest because of its complex aroma, and it is also the rarest and the most expensive. White truffles are softer and more perishable than black truffles, and are almost always served raw (they cannot tolerate the heat of cooking) (5).

For this reason, there are necessary to develop a method of conservation to preserve intact the unique aroma and the flavor of truffles.

* Corresponding author: s_adinaelena@yahoo.com

2. Experimental section

Reagents and Analytes

The truffles were harvested from Romanian natural population only. The fruiting bodies of truffles were cleaned and preserved in pressure nitrogen atmosphere. Small sample of truffles were analyzed.

- *Cyclic voltammetry*

Examined material: sample of preserved truffles (*Tuber magnatum pico)* and (*Tuber melanosporum*) substances: solvents (methylic alcohol); equipment: Radiometer PGZ-402 Universal Dynamic Pulse Voltammetry EIS equipped withVoltaMaster software 4. The working parameters were experimental established (the base electrolyte and pH). The base electrolyte is composed of 0.1 M phosphate buffer solutions (PBS) at different pH´s (2.8 and 8).

For voltammetric studies were used 8.83 g of white truffle extracted in 20 mL MeOH with stirring for 3 hours at room temperature. A volume of 3 ml of truffles extract were dissolved in 10 ml of base electrolyte (PBS solution) and then were recorded cyclic voltammograms.

The antioxidant activity of *Tuber magnatum pico and Tuber melanosporum* was analyzed by cyclic voltammetry using the following parameters:

- two working electrodes were used: nickel and platinum plate
- The cyclic voltammograms for nickel electrode were recorded in the potential range of -250 ÷ 1200mV and for platinum plate electrode in the potential range of -250÷1400mV/s at different polarization rate: 25mV/s, 50mV/s, 100mV/s, at room temperature.

Electrochemical cell was equipped with three electrodes, namely:

- reference electrode:Ag/AgCl;
- auxiliary electrode: platinum wire with 0.25cm^2 active surface;
- working electrode: nickel with surface of 0.2 cm^2 and platinum plate with surface of 0.8 cm^2;

- *Atomic absorption spectrometry (AAS)*

The tests for determination of heavy metals contents from truffles were conducted under international standard ISO 15586:2003 (E), on equipment: Analytik Jena novAA 400G - apparatus, with a graphite furnace, equipped with autosampler MPE60 and software WinAAS 3.17.0.

Examined materials: samples of truffles (0.2541 g) weighed on a Sartorius analytical balance, with an accuracy of ± 0.0001 g.; Substances: nitric acid, ultrapure water. The samples is treated with 5.5 mL HNO_3 65% and subjected to digestion in a Berghof microwave oven MWS 2, using a three stages program: T_1=160°C, t_1= 15 min, p_1= 80%. After digestion, the sample is brought to a volume of 100 mL with ultrapure water.

- *Total organic carbon content*

The TOC analyzer used in this study was a SHIMADZU TOC-V_{CPN} equipped with a 94 – position auto sampler. The TC principle analysis was catalytic combustion at high temperature (900^{o}C) and for IC –acidification at 200^{o}C.

Examined materials: samples of truffles (0.301 g) weighed on a Sartorius analytical balance, with an accuracy of ± 0.0001 g. Substances: phosphoric acid, 1:1 v/v (Merck).

- *Scanning electron microscopy (SEM/EDAX)*

To highlight the morphology and elemental composition of two types of truffles were analyzed by scanning electron microscopy (SEM) using Inspect S PANalytical model coupled with the energy dispersive X-ray analysis detector (EDX).

3. Results and disscution

Studies for determination of natural sources of antioxidants compounds (phenolic and flavonoids) are very important due to the positive biological effects on human health and welfare.

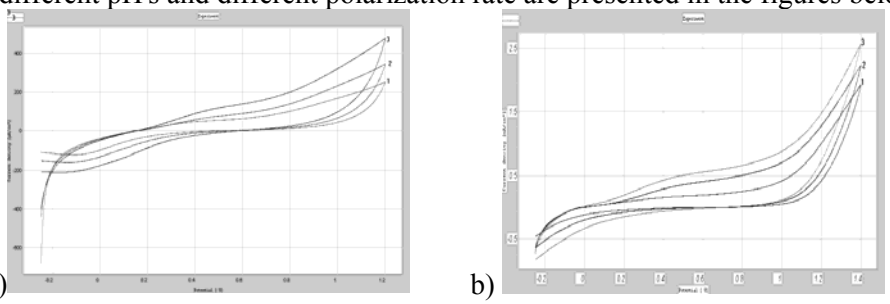
These compounds function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation (6-10).

Previous research demonstrate that there is a direct corelation between electrochemical activity and antioxidant activity (11).

The present paper aims to investigate the electrochemical activity of the two type of truffles.

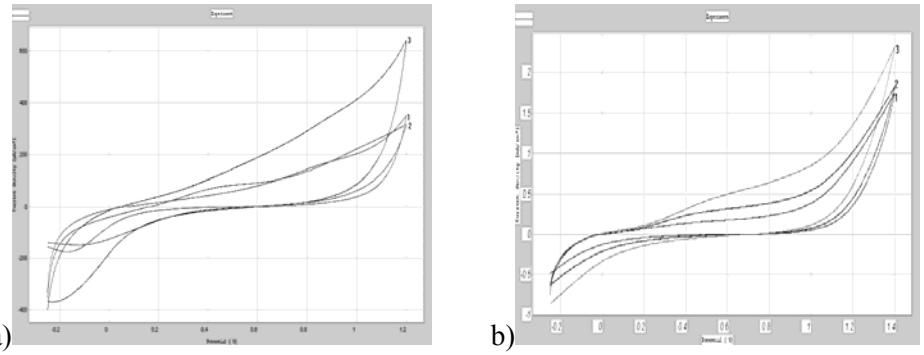
- *Comparative evaluation of Tuber magnatum pico and Tuber melanosporum antioxidant activity of through electrochemical methods*

The cyclic voltammograms indicate the influence of pH and reversible oxidation process of *Tuber magnatum pico*.The cyclic voltammograms of *Tuber magnatum pico* in phosphate buffer solutions at different pH's and different polarization rate are presented in the figures below.



a) b)

Fig. 1. Cyclic voltammograms of: a) Tuber melanosporum , b) Tuber magnatum pico on platinum plate electrode; pH=2.8; scan rate of: 1- 25mV/s; 2- 50 mV/s; 3- 100 mV/s



a) b)

Fig. 2. Cyclic voltammograms of: a) Tuber melanosporum, b) Tuber magnatum pico on platinum plate electrode; pH= 8; scan rate of: 1- 25mV/s; 2- 50 mV/s; 3- 100 mV/s

Table 1. Tuber melanosporum - cyclic voltammograms results on platinum plate electrode

pH	2.8			8		
Scan rate	25mV/s	50mV/s	100mV/s	25mV/s	50mV/s	100mV/s
$\mathbf{i^{\rightarrow}_{pic}}$ **(mA/cm^2)**	46.48	75.87	120.3	16.7	23.7	34.8
	-	-	-	-170.2	-275.5	-371.4
$\mathbf{\varepsilon^{\leftarrow}_{pic}}$ **(V)**	0.5	0.5	0.5	0.9	0.9	0.9
	-	-	-	0.16	0.16	0.16

*Table 2. Tuber magnatum pico - cyclic voltammograms results on platinum plate electrod*e

pH	2.8			8		
Scan rate	25mV/s	50mV/s	100mV/s	25mV/s	50mV/s	100mV/s
$\mathbf{i^{\rightarrow}_{pic}}$ **(mA/cm^2)**	18.05	39.16	520	16	29.36	49.59
$\mathbf{\varepsilon^{\leftarrow}_{pic}}$ **(V)**	0.5	0.5	0.5	0.5	0.5	0.5

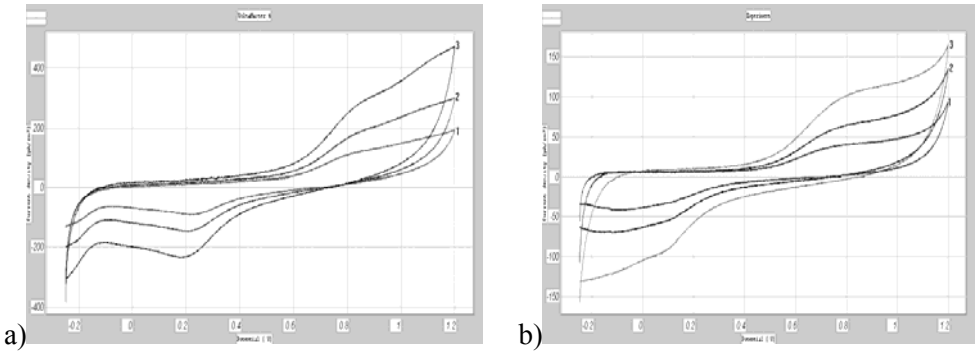


Fig. 3. Cyclic voltammograms of: a) Tuber melanosporum, b) Tuber magnatum pico on nickel electrode at pH=2.8; scan rate of: 1 - 25mV/s; 2- 50 mV/s; 3 - 100 mV/s

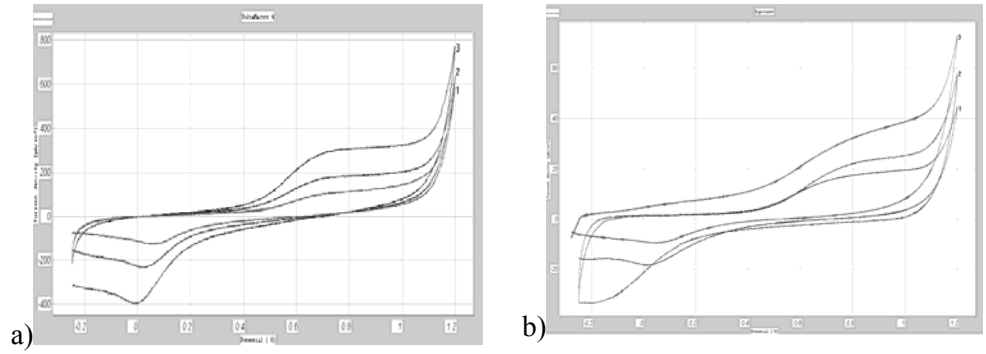


Fig. 4. Cyclic voltammograms of: a) Tuber melanosporum; b) Tuber magnatum pico on nickel electrode at pH= 8; scan rate of: 1- 25mV/s; 2- 50 mV/s; 3- 100 mV/s

Table 3. Tuber melanosporum - cyclic Voltammograms results on nickel electrode

pH	2.8			8		
Scan rate	25mV/s	50mV/s	100mV/s	25mV/s	50mV/s	100mV/s
$\mathbf{i^{\rightarrow}_{pic}}$ **(mA/cm^2)**	113.9	166	268.8	103.7	168.3	281
	-88.61	-137.7	-221.5	-123	-228	-397.8
$\mathbf{\varepsilon^{\leftarrow}_{pic}}$ **(V)**	0.8	0.8	0.8	0.8	0.8	0.8
	0.23	0.23	0.23	0.06	0.03	0.003

Table 4. Tuber magnatum pico - cyclic Voltammograms results on nickel electrode

pH	2.8			8		
Scan rate	25mV/s	50mV/s	100mV/s	25mV/s	50mV/s	100mV/s
$\mathbf{i^{\rightarrow}_{pic}}$ **(mA/cm^2)**	40.57	65	103.7	32.56	22.6	18.07
	-40.84	-68.03	-92.61	-9.32	-18.07	-32.06
$\mathbf{\varepsilon^{\leftarrow}_{pic}}$ **(V)**	0.8	0.8	0.8	0.8	0.8	0.8
	0.07	0.07	0.07	0.07	0.04	0.01

Comparative study of the both types of truffles releases that the cyclic voltammograms of recorded on platinum plate electrode, in acid media show that a single irreversible process occurs and the intensity of anodic peak increases with the scan rate.

In basic media, cyclic voltammograms recorded for *Tuber melanosporum* present a reversible process occurs, and only minor differences appear on the anodic current density with increasing the scan rate of both types of truffles. The reduction peak it is more pronounced with decreasing the scan rate. Instead for the cyclic voltammograms of both types of truffles recorded on the nickel electrode in both reaction media, a reversible process can be noticed in which the anodic peak intensity is much higher for the *Tuber melanosporum* at all three scan rates and is increasing with the increasing of scan rate while for the current density of the cathodic peak it is much higher with the decrease of rate. From the cyclic voltammograms on nickel electrode at pH = 2.8 it appears that a reversible oxidation process occurs for all three polarization rate: 25, 50 and

100 mV/s, with the appearance of anodic peak around 0.8V. Instead it can see a slight shift of cathodic peak potential to positive values with increasing polarization rate. When a pH=8 solution was used, the reversible oxidation process appears for all three different polarization rate, but here the reduction peak is more pronounced with decreasing the polarization rate. For the cyclic voltammograms recorded on platinum electrode for all three pH only one irreversible process occurs, where the oxidation peak intensity increases with the polarization rate increasing.

4. Characterization of *truffles samples* through analytical methods

Atomic absorption spectrometry analysis

The actual state of atomic absorption spectrometry of two types of truffles as the method of determining As, Cu, Pb, Zn, Mn, Fe and Ni is described on the basis of literature data.

Table 5. The metals content (mg/g) from the truffles samples:

No	Sample	As	Cu	Pb	Zn	Mn	Fe	Ni
1.	*Tuber magnatum pico*	*	*	*	39.67	2.32	68.67	0.581
2.	*Tuber melanosporum*	*	7.242	*	34.08	2.414	523.58	0.569

* below the detection device

From the results obtained it can see that in *Tuber magnatum pico* and *Tuber melanosporum* metals such Zn, Mn, Fe, and small amounts of Ni were found.

- ***Total organic carbon analysis***

Table 6. Results of the TOC analysis

Sample no.	Area	CNV	Abs C (µg)	Conc (mg)	Weight (mg)	Volume
1	200.1	200.1	2420	2.420	35.80	35 µL
2	347.1	347.1	4201	4.201	34.10	34 µL

Sample 1. *Tuber magnatum pico;* Sample 2.- *Tuber melanosporum*

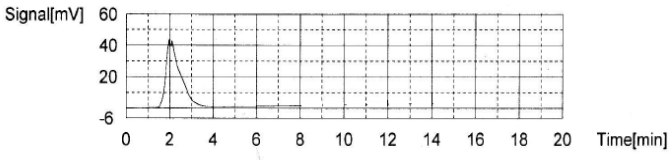


Fig. 5. Tuber magnatum pico - TOC measurement

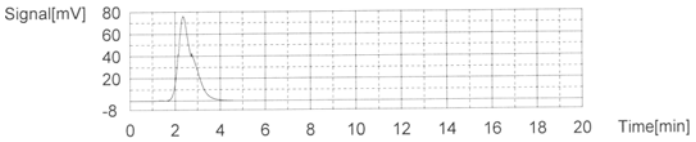


Fig. 6. Tuber Tuber melanosporum - TOC measurement

Total Carbon analysis

Table 7. Results of the TC analysis.

Sample no.	Dilution.	Density (mg/µl)	Result (mg)
1	1.000	1.000	TOC:2.420; TC:2.420; IC:0.000
2	1.000	1.000	TOC:4.188; TC:4.201; IC: 0.01265

From TOC analysis results that, the sample 2, *Tuber melanosporum* contain practically much more carbon than the sample of *Tuber magnatum pico.*

- ***Scanning electron microscopy (SEM/EDAX)***

To highlight the morphology and elemental composition of two types of truffles were analyzed by scanning electron microscopy and EDAX. We made a comparative study between the *Tuber magnatum pico* and *Tuber melanosporum*. The results are shown follow figures.

From the SEM images (Figure 7. (a)) it can be observed the surface topography on the *Tuber magnatum pico*. This shows a fibrous structure with a thickness about few μm. EDAX analysis provided a semi quantitative elemental analysis of the surface indicating the elements of the study material. Thus, it can be observed that for sample of *Tuber melanosporum* (Figure 8. (b)) the corresponding carbon peak is much higher than in the *Tuber magnatum pico*, keeping most of the components of this material.

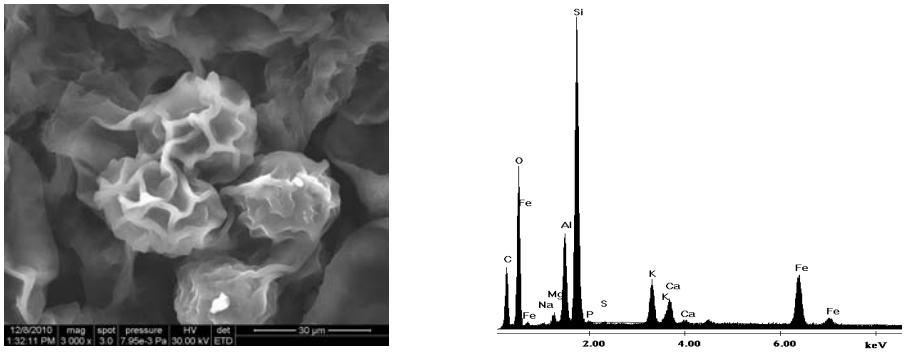


Fig. 7. (a) Tuber magnatum pico - SEM morphology; (b) EDX spectra for elemental analysis

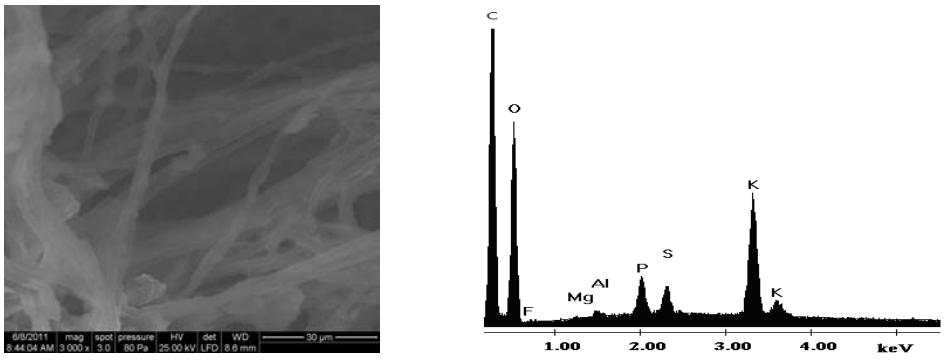


Fig. 8. (a) Tuber melanosporum - SEM morphology; (b) EDX spectra for elemental analysis.

5. Conclusion

The results of the present investigation indicated that the electrochemical evaluation of antioxidant activity of both types of truffles shows an dependence of the oxidation process by the pH and the nature of electrode. Reversible oxidation process of both types of truffles samples occurs better in acid medium reaction and the oxidation peak is more pronounced on the nickel electrode than for the platinum plate electrode.

The comparative electrochemical study of both truffles in different conditions (medium reactions, electrodes, different scan rates) demonstrates that the sample of *Tuber melanosporum* shows a higher antioxidant activity than *Tuber magnatum pico.*

Further studies should be performed on the isolation and identification of the antioxidant components in two types of truffles.

The analytical methods used to characterize the samples revealed that the two types of truffles shows different morphologies and composition. The sample of *Tuber melanosporum* contains a high amount of carbon and Fe than *Tuber magnatum pico.* The content of the other metals in both truffles samples determinate by atomic absorption spectrometry and EDX analysis is approximately the same.

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