ASPECTS REGARDING ELECTROCHEMICAL DETECTION OF THE ANTIOXIDANT ACTIVITY FOR SUBCRITICAL EXTRACTS FROM PLEUROTUS OSTREATUS

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Abstract: *The paper presents a comparative study of antioxidant activity of extracts from Pleurotus Ostreatus, measured through electrochemical detection by using a biosensor. The extract from Pleurotus was obtained by using subcritical extraction with R 134a. Extraction at subcritical pressures of bioactive compounds from plants in soft extraction conditions represents an alternative to replace classical methods of extraction with different solvents, or on enzyme basis. The most important advantage that the HFC extractors with liquefied gas at subcritical pressure offer is that they may extract oils in pure estate, at room temperature and in the absence of air, which allows to create new categories of products with a wide range of bioactive substances.*

Keywords: *antioxidant activity, subcritical extraction, Pleurotus Ostreatus.*

1. Introduction

The genus Pleurotus comprises about 40 species and they are commonly referred to as "oyster mushroom", grow widely in tropical and subtropical areas and easily artificially cultivated. Pleurotus genus includes **P. ostreatus**, P. sajorcaju, P. florida, P. flabellatus, P. highbing 51, P. cystidiosus, P. sapidus, P. eryngii, P. tuberegium, P. ulmarium, P. pulmonarius, P. citrinopileatus, P. geesteranus and other some of which are of a special consideration due to their high nutritional values and medicinal importance [1, 3, 7].

Generally, Pleurotus mushrooms are rich in vitamin and selenium content which are the important naturalantioxidants in biological systems. Some researchers reported that, an extract of P. Ostreatus enhanced the Catalase gene expression and decreased the incidence of free radical-induced protein oxidation in aged rats, thereby protecting the occurrence of age-associated disorders that involve free radicals.

The ethanolic extract of the oyster mushroom P. ostreatus are reported to have potent antioxidant activity in both in vitro and in vivo. The ethanolic extract exhibit in vitro antioxidant activity by virtue of its scavenging hydroxyl and superoxide radicals, inhibiting lipid peroxidation, reducing power on ferric ions, chelating ferrous ions and quenching 2,3-diazabicyclo[2,2,2]oct-2-ene (DBO). It also exhibits as a good in-vivo antioxidant activity by reducing the intensity of lipid peroxidation and by enhancing the activities of enzymatic and non-enzymatic antioxidants [12].

The scope of the project is to find the best extraction method for the bioactive compounds, such antioxidants, by using subcritical extraction with HFC 134a. Preliminary results show that the quality and total amount o bio-compounds is higher than classical extraction [8].

Recent researches was done by combining the two methods. In the first stage lipids were extracted on HFC extractor (FC 100, Timatic, Italy), then second phase extracts hydrosoluble compunds in SLE extractor (MiniTimatic, Italy). In this case, extraction eficiency was higher 20-30%-and time was reduced between 32-35 %, also depending on temperature (which was varying between 30-40C degrees). Better results were obtained by applying ultrasound waves in extraction liquid in the second phase.

Various methods for characterization or analytical evaluation of preservatives and antioxidants have been explored and applied.

Alongside chromatographic or spectrophotometric alternatives, electrochemistry of various natural antioxidants is the subject of a active research as is the electrochemical study of the phenolic compounds or their derivatives [6, 9].

2. Materials and methods

The raw material studied was Pleurotus Ostreatus (fig.1). The mushrooms were procured from a specialized farm in Harman, Brasov County, Romania. After drying 8 hour in a convective dryer at 35degree temperature, the material was milled with a hammer milling machine. Extraction from Pleurotus was done with FC 100 extractor (Timatic, Italy) by using HFC 134a (1,1,1,2-tetrafloretan) at pressure 5-8 bar and temperature 5-35 C degrees. The extracts were diluted at 3 different concentration: 20, 40, 60 µl in pH7 ws.

Fig. 1. *Pleurotus Ostreatus*

Antioxidant activity of samples was measured by an electrochemical biosensor, EDEL meter (Edel Therapeutics, Lausanne, Switzerland). The biosensor used in this study was based on the electrochemical measure of potential to determine concentration of analytes or to characterize the chemical reactivity of a compound. Differential Pulse Voltammetry (DPV) has been used for quantification, since it is suitable to measure the redox properties of chemical compounds having low molecular weights. Applying a potential, a redox reaction occurs on working electrode surface; electrons involved in the reaction modify the current applied in the cell, and this modification is elaborated by a signal transducer. 3 different solution with different concentration of 20, 40, 60 µl were used for the experiments.

Each sample, standard solutions or diluted extract samples, was transferred in an aluminium-wrapped becker under magnetic stirring. For each concentration was used a new sensor. 14 experiments were done with the same sensor, for the same concentration.

deacreasing with the increasing of concentration level (20, 40, 60 µl).

The next experiments, done with the same sensors for each concentration, show, for distinct case the saturation of the sensors after imersing in solutions.

In figure 2, 3, 4 can be observed the comparative results of the antioxidant activity of *Vitis semen*, *Mustard*, *Polygonum Cuspidatum***,** evaluated through the intensity of the current between electrodes [nA].

3. Results and discussions

Figures 2, 3, 4 show the class of courves obtained from all 42 experiments. The intensity of the current was recorded during 11 sec, and each time, a maximum value was found between 4 and 6 sec. The first experiment (series1) was done with the sensors unaffected by the extract. It can be seen that the peak value of series 1 is

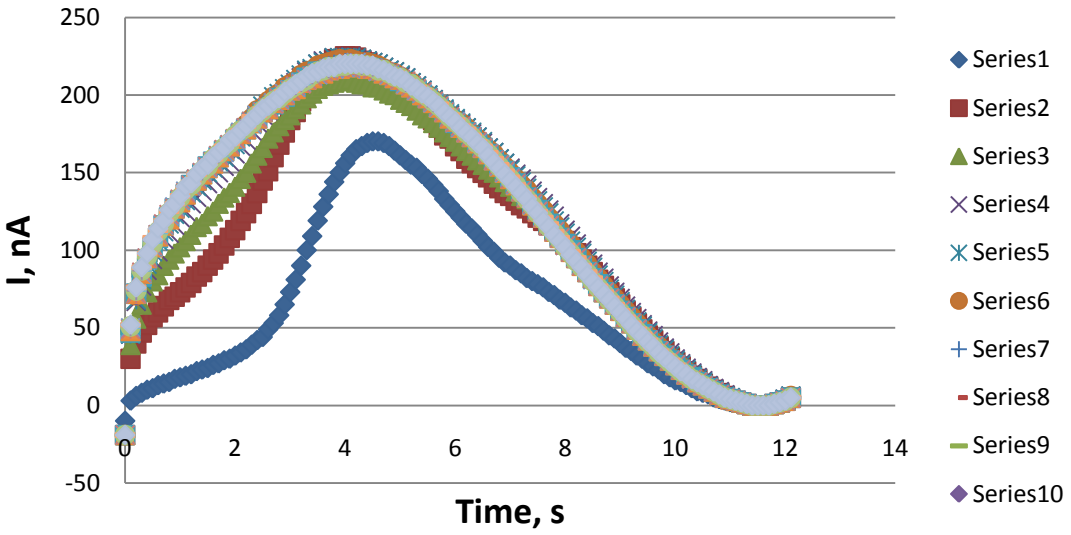

Fig. 2 *Antioxidant activity of Pleurotus Ostreatus, 20 µl concentration, evaluated through intensity of the current between electrodes [nA]*

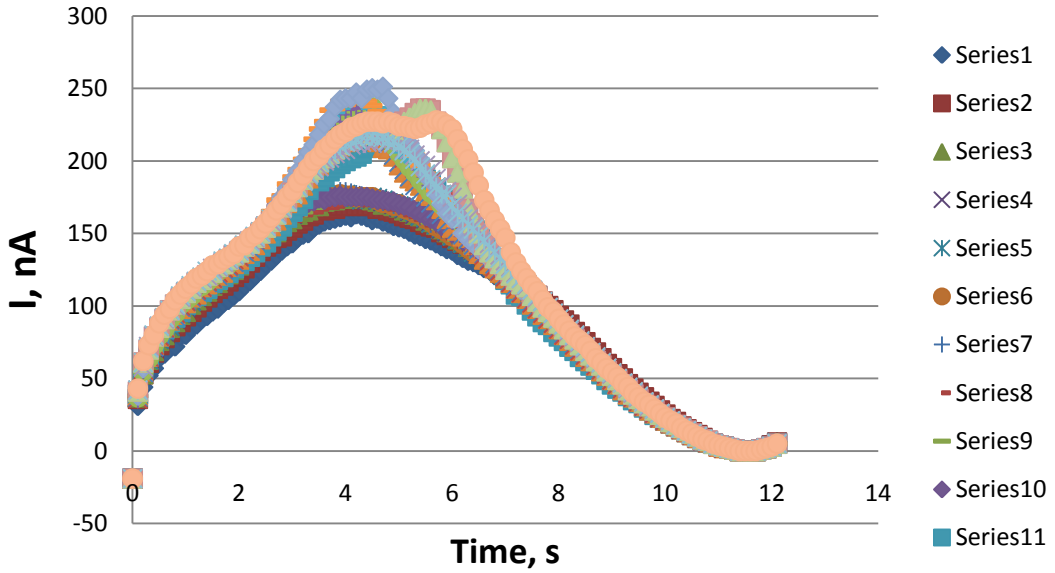

Fig. 3 *Antioxidant activity of Pleurotus Ostreatus, 40 µl concentration, evaluated through intensity of the current between electrodes [nA]*

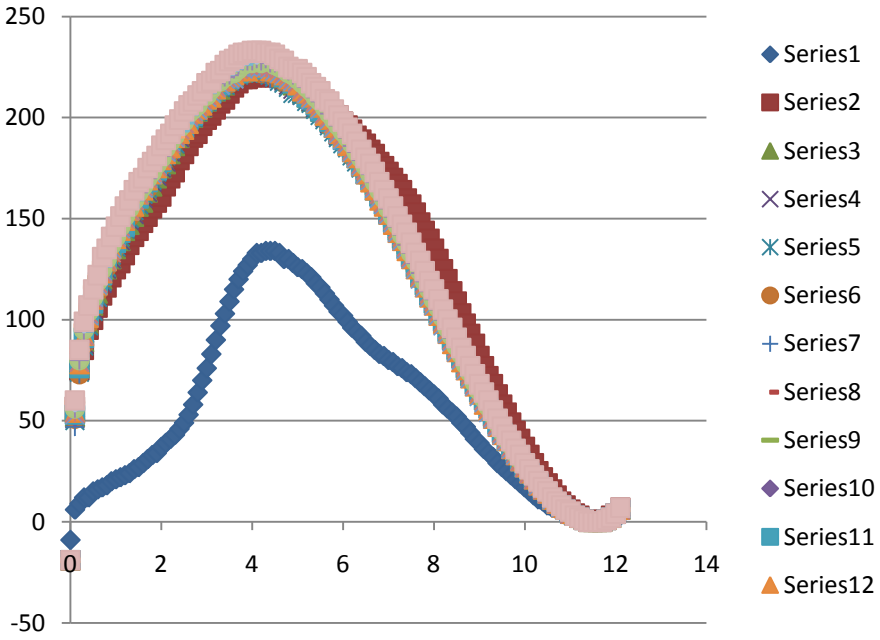

Fig. 4 *Antioxidant activity of Pleurotus Ostreatus, 60 µl concentration, evaluated through intensity of the current between electrodes [nA]*

Conclusions

This paper signified the role of electrochemical method for determination of antioxidant activity in the biological samples of plant origin. Electrochemical techniques represent because its sensitivity, a usefull tool for the determination, under the low concentrations of antioxidants.

The preliminary data obtained with this method showed a high variability in the antioxidant activity of samples, in particular for *Pleurotus ostreatus.*

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