

Electrochemical Evaluation of the Antioxidant Capacity of Phenolic Compounds in Virgin Olive Oil

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Abstract

Cyclic voltametry (CV) and Square wave voltametry (SWV) were used to study the electrochemical behavior and evaluate the antioxidant capacity of virgin olive oil. Firstly, in this work we illustrate the electrochemical methods based on the preparation and characterization of H_2O_2 amperometric sensor, then the antioxidant capacity of the virgin olive oil was evaluated by measuring of the of virgin olive oil effect on the H_2O_2 reduction.

Keywords: Olive oil; Antioxidant capacity; Cyclic voltammetry; Square wave voltammetry

Introduction

Research Article

The major components of olive oil are: oleic acid, phenolic constituents and squalene. The main phenolics include hydroxytyrosol, tyrosol, and oleuropein, which occur in highest levels in virgin olive oil and have demonstrated antioxidant activity. Oxidative stress caused by production of reactive oxygen species is linked to various pathological conditions [1]. Several authors have shown that free radicals lead to the pathogenesis of a various disease including diabetes, hypertension, inflammation and cancer [2]. Reactive oxygen species (ROS), naturally generated during the metabolism, can damage biological structures such as proteins, lipids or DNA. Normally, cells defend themselves against ROS damage with enzymes, but sometimes the natural defenses are overwhelmed by an excessive generation of ROS and a situation of oxidative stress occurs. In this case, cellular and extracellular macromolecules (proteins, lipids, and nucleic acids) can suffer oxidative damage, causing tissue injury [3,4].

A wide variety of electrochemical sensors and biosensors have already been proposed for antioxidant capacity evaluation [5-14]. Most of them are based on the amperometric detection of H_2O_2 , resulting from the catalyzed dismutation of superoxide radicals (O_2 .) in presence of superoxide dismutase. The purpose of this work was to investigate the electrochemical properties of antioxidants contained in virgin olive oil, using copper modified carbon paste electrode, site of oxidative reduction reactions.

Experiment Section

Apparatus

Electrochemical measurements were recorded on a Voltalab potentiostat instrument (model PGSTAT 100, Eco Chemie B. V., Utrecht, The Netherlands) driven by the general purpose electrochemical systems data processing software (voltalab master 4 software). The cell was equipped with a copper modified carbon paste electrode (Cu-CPE), a platinum electrode of a larger area and an Ag/AgCl reference electrode.

Reagents and solutions

All chemicals were of the highest quality. Graphite powder (spectroscopic grade RWB, Ringsdorff-Werke GmbH, Bonn-Bad Godesberg, Germany) was obtained from Aldrich and was used without further purification. CuSO₄ was obtained from Merck chemicals.

Deionised water was used to prepare all solution.

Preparation of the Cu-CPE

The carbon paste unmodified was prepared by adding paraffin oil to carbon powder and thoroughly hand-mixing in a mortar. The resulting paste was packed into the electrode and the surface was smoothed. The modified electrode was developed by depositing the copper at fixed potential (0.1 V for 1 h) onto the carbon paste electrode surface.

Results and Discussion

Cyclic voltammograms of Cu-CPE is recorded in the electolytical solution $0.1 \text{ M Na}_2\text{SO}_4$ at scan rate of 100 mV/s is shown in Figure 1. It is a typical voltammogram of copper, which shows two redox peaks, the first one in the direction of the cathodic scan, at about -0.9 V, and the second one, in the opposite direction at -0.2 V.

The EIS experiments were carried out in 0.1 mol L⁻¹ Na₂SO₄ in order to confirm the mechanisms suggested in the voltammetric part of this work regarding the Cu-CPE interaction. Figure 2 shows the Nyquist plot for Cu-modified electrode included a semicircle at higher frequencies corresponding to the electrontransfer limited process and the linear part at lower frequencies corresponding to the diffusion process.

The surface structure of copper modified carbon paste surface was observed using scanning electron microscopy (Figure 3). The film layer of copper was formed on the surface of carbon paste electrode; it was not disintegrated or detached from the surface when immersed in the electrolytic solution. The surface has a porous shape and constitutes by clusters of copper of different dimensions, forming a rough film.

First, we plotted the calibration curve, of H_2O_2 from the SWV recorded at Cu-CPE in 0.1M Na₂SO₄, containing various concentrations

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Figure 1: Cyclic voltammograms recorded at Cu-CPE in 0.1M $\rm Na_2SO_4$ solution, scan rate 100 mV/s.







Figure 3: Scanning Electron Micrograph of Cu-CPE.

of H_2O_2 . The square wave voltammetric response was almost linear dependent on the radical oxygen concentration (Figure 4).

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After the addition of 350 μ l/100 ml of the sample (Virgin olive oil –VOO-), the H₂O₂ reduction current density decreases (Figure 5), subsequently leading to a decrease in free radical concentration in the solution, which leads to the conclusion that VOO inhibits Oxidizing capacity of H₂O₂.

Figure 6 shows the performance of the copper modified carbon paste electrode in the presence of antioxidant (350 μ L of virgin olive oil) and the hydroxyl radical (50 μ l). To perform the electrochemical study all current peaks were compared with the signal current obtained with the H₂O₂. The electrochemical reduction of radical is manifested in square wave volatammetry by a defined peak at around -0.6 V. The addition of the antioxidant sample to the solution containing the free radical causes the decrease in the current density of this peak [14-18].

The H_2O_2 reduction current density decreases with increasing the amount of the antioxidant sample (VOO) (Figure 7). The square wave voltammetric response was almost linear dependent on the concentration of sample (VOO) (Figure 8). The linear regression analysis gave:

I (mA/cm²)= -0.003 [sample]+2.306 with a correlation coefficient of 0.988.

The corresponding antioxidant capacity values, was calculated using the relation:

$$AOC = \frac{I_{H2o2} - I_{H2o2} \text{ presence of antioxidant sample}}{I_{H2o2}} \times 100$$

Where $\rm I_{\rm H2O2}$, is the current density due to $\rm H_2O_2$ reduction and $\rm I_{\rm H2O2}$ antioxidant sample represent the current density due to antioxidant sample addition.

In this work we calculated and compared the antioxidant capacities of several samples of virgin olive oil, collected on different sites of the Tadla Azilal region, Morocco. The results are shown in the following histogram (Figure 9)







Figure 6: Square wave voltammograms recorded at Cu-CPE, in CPE in 0.1 M Na $_2$ SO $_4$ solution containing H $_2$ O $_2$, a- in absence of VOO, b- in presence of VOO.



Conclusion

A copper modified copper electrode for the AOC quantification of a variety of virgin olive oil was prepared and characterized in electrolytic



Figure 8: Evolution of the $\rm H_2O_2$ reduction current density with the concentration of VOO.



media. First, a calibration curve is determined from the relationship between the reduction current density of H_2O_2 and $[H_2O_2]$. The virgin olive oil had the capacity to inhibit the superoxide radical reduction which is responsible for the oxidation of the cells, causing subsequent oxidative stress. Several virgin olive varieties have been tested and their AOCs have been determined, these samples harvested at different sites in the Tadla Azilal region, Morocco. The largest AOC has exceeded the lowest of the order of 20%.

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