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By Z. Mourhat, M. Elbir, S. Touzara, M. Berkani, R. Maallah, M. Mbarki, A. Barakat & A. Chtaini

*Université Sultan Moulay Slimane, Morocco*

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# Electrochemical Evaluation of the Antioxidant Capacity of Moroccan Olive Kernel

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## I. Introduction

Virgin olive oil is the primary source of lipids in the Mediterranean diet; it is obtained from the fruit of several olive cultivars, providing oils with different compositions and properties. The compositions of olive oil and its sensorial characteristics besides being strongly dependent on the cultivar are also influenced by several other factors namely climatic and agronomic conditions, time of harvest, and agricultural practices. The virgin olive oils represent a rich source of natural antioxidants such as cinnamic, tyrosol and phenolic compounds. Many of the benefits associated with consumption of phenolic compounds are associated with their antioxidants activities[1, 2], related to their molecular structure[3]. It is reported[13] . Those phenolics may prevent lipid peroxidation via hydrogen atom donation from the hydroxyl group(s) attached to the benzene ring.

Antioxidants are molecules that can delay or even almost prevent the development of oxidation by direct quenching of reactive oxygen species, formed during radical chain oxidation processes.

Reactive oxygen species (ROS) including superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide which is often classified, not as free radical but to reactive oxygen species group, and hydroxyl radical ($OH^{\cdot}$), are generated naturally *in vivo* during metabolic processes, and keeps in a balance level in normal living organisms[4]. However, when a body is subject to the environmental or behavioral stressors (pollution, sunlight exposure, cigarette smoking, excessive alcohol consumption, etc.,), excess ROS are generated[5]. If the excess ROS cannot be scavenged in time, they would attack and induce DNA, proteins and lipids damage, and impede normal cell functions[6]. Therefore, overproduction of ROS is associated with numerous diseases like cancer and Alzheimer's disease, as well as aging. In living systems, the deleterious effects of ROS can be neutralized by the endogenous and exogenous antioxidant systems[7]. Antioxidants are synthetic or natural substances that prevent or delay the oxidative damage by scavenging the free radicals. Fruits and vegetables are good sources of high amounts of known antioxidants.

Several analytical methods have been developed for olive oil total antioxidant capacity assessment. A number of these methods measure the inhibition of stable or an artificially generated radical upon olive oil addition. Spectrometric methods are mainly used in the analysis of antioxidant properties. However, these methods depend on many parameters, such as temperature, time of the analysis, character of a compound or mixture of compounds, concentration of antioxidants, and many other substances[8-10]. Electrochemical methods used for the determination of antioxidant capacity are still developing. These provide rapid, simple and sensitive alternative method in the analysis of bioactive compounds associated with the scavenging of the radicals as well as the antioxidant capacity itself[11-12].

This work aims to map the antioxidant capacity of Moroccan olive kernel according to production region and cultivar.

## II. Materials and Methods

### a) Apparatus

Electrochemical experiments were performed using a voltalab potentiostat (model PGSTAT 100, Eco Chemie B. V., Utrecht, The Netherlands) driven by the general purpose electrochemical systems data processing software (voltalab master 4 software).

All the electrochemical experiments were performed in a standard one-compartment three-electrode cell. The reference electrode was SCE and the counter electrode was platinum. All electrode potentials

*Author ρ ¥ ν: Equipe d'Electrochimie Moléculaire et Matériaux Inorganiques, Faculté des Sciences et Techniques, Université Sultan Moulay Slimane, Béni Mellal, Maroc. e-mail: a.chtaini@usms.ma*

*Author α σ ω §: Equipe Transdisciplinaire des Sciences Analytiques pour le Développement Durable, Faculté des Sciences et Techniques, Université Sultan Moulay Slimane, Béni Mellal, Maroc.*

*Author ¥ χ: Laboratoire Géoressources et Environnement, Faculté des Sciences et Techniques, Université Sultan Moulay Slimane, Béni Mellal, Maroc.*

were referred to this reference electrode. The working electrode was copper modified carbon paste electrode (Cu-CPE).

*b) Preparation of the electrochemical electrode*

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

*c) 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. $CuSO_4$ was obtained from Merck chemicals. Deionized water was used to prepare all solution.

*d) Sample preparation*

The fruit samples of Moroccan Picholine, Haouzia and Menara varieties, were collected from different localities in Tadla-Azilal region, at the beginning of January 2015, and were stored at -20 °C until use. After drying in the shade, the kernels were ground to powder in a grinder. The powders were extracted with n-hexane (1:4, w/v) by agitation for 48 h in the dark at room temperature. The solvent was evaporated to dryness in vacuo at 42 °C.

## III. Results and Discussion

*a) Electrochemical behaviour of prepared electrode*

The cyclic voltammograms (CVs) of the copper modified carbon paste electrode (Cu-CPE) and carbon paste electrode (CPE) were recorded in the supporting electrolyte ($Na_2SO_4$) (Figure 1). We can see that the shape of the cyclic voltammogram was modified in the presence of copper at CPE surface, suggesting that the carbon paste electrode was effectively modified by copper.

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 supporting electrolyte solution.

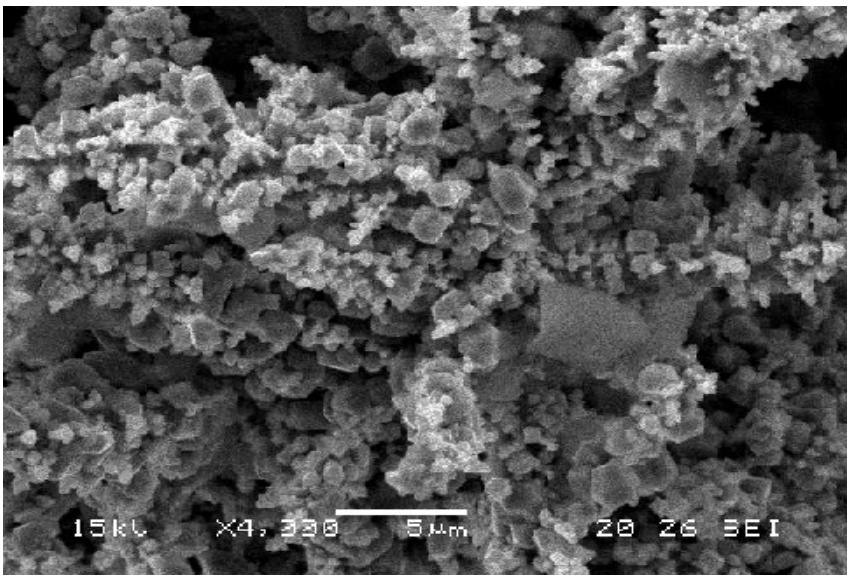


*Figure 2 :* Scanning electron micrograph of Cu-CPE

*b) Calibration graph*

The detection of $H_2O_2$, was examined by square wave voltammetry, in the electrochemical sensor. The electrode response was tested for different amounts of $H_2O_2$, in the range from 1µL/100mL to 100 µL/100mL (0.1M $Na_2SO_4$ solution). Figure 3 shows some typical square wave voltammetry curves recorded at Cu-CPE electrode. A calibration graph was then constructed from the observed peak currents. The square wave voltammetric response was almost linearly dependent on the concentration of $H_2O_2$ (Fig. 4). The linear regression analysis gave:

$$i_p = -0.0014\ [H_2O_2] + 1.2646$$

with a correlation coefficient of 0.9599.

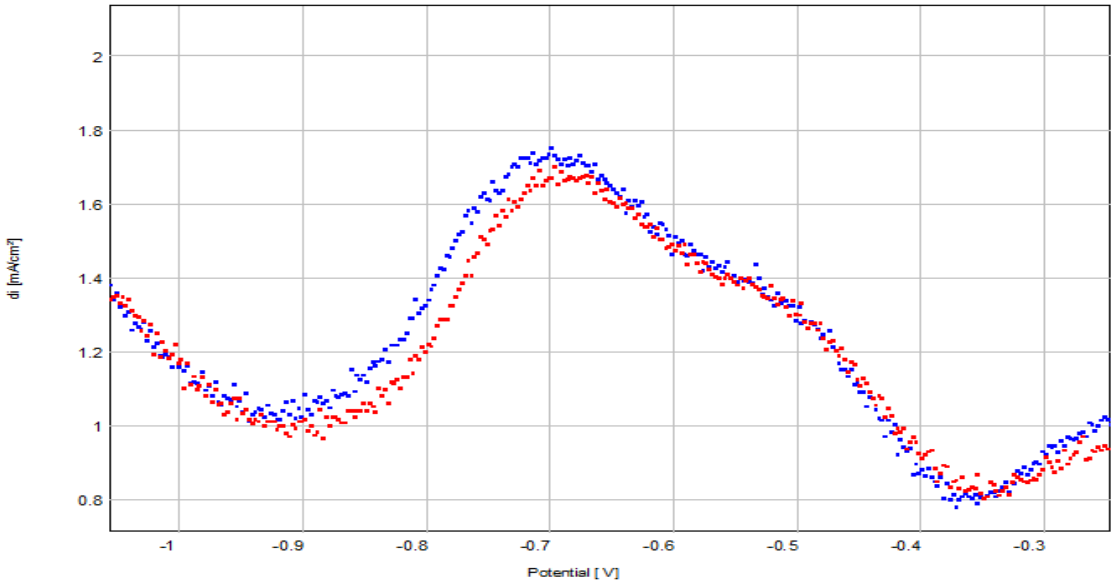


*Figure 3 :* Square wave voltammograms of $H_2O_2$, in 0.1M $Na_2SO_4$ solution pH ≈ 7.4 at Cu-CPE, scan rate 50 mV/s

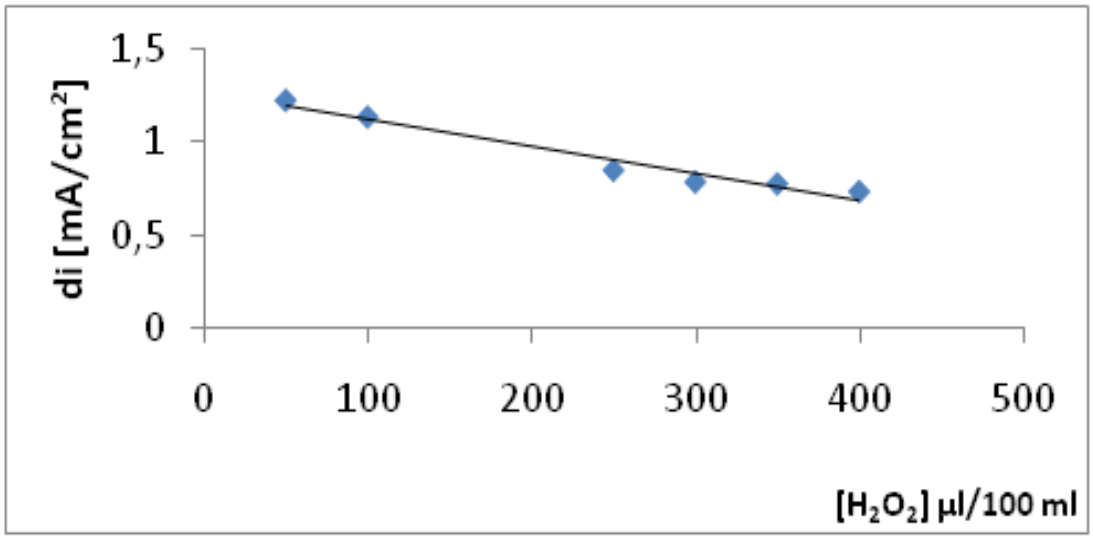


*Figure 4 :* Calibration graph

*c) Antioxidant capacity determination*

The anti-oxidant properties of the studied molecules were evaluated, investigating, cyclic voltammetry and square wave voltammetry, by comparing the reduction of $H_2O_2$.

Preliminary CV experiments were performed to investigate the effect of the addition of a specified quantity of each studied sample to the electrolytic solution containing (50 µL/100 mL) [$H_2O_2$]. Comparing the three voltammograms, that recorded by Cu-CPE electrode (Figs. 5, 6 and 7), we note that the $H_2O_2$ reduction peak decrease in the presence of the studied samples. The effect of inhibiting reduction of $H_2O_2$ varies as follows:

*Haouzia sample ≥ Picholine sample > Menara sample*

To obtain the results shown in Figure 7, we proceeded as follows:

- Progressive additions, of each the three samples tested, in an electrolytic solution containing $H_2O_2$,
- the $H_2O_2$ reduction current densities are determined by square wave voltametry method
- From the calibration graph already drawn (Fig. 4) we determine the amount of $H_2O_2$ inhibited by the addition of the sample.

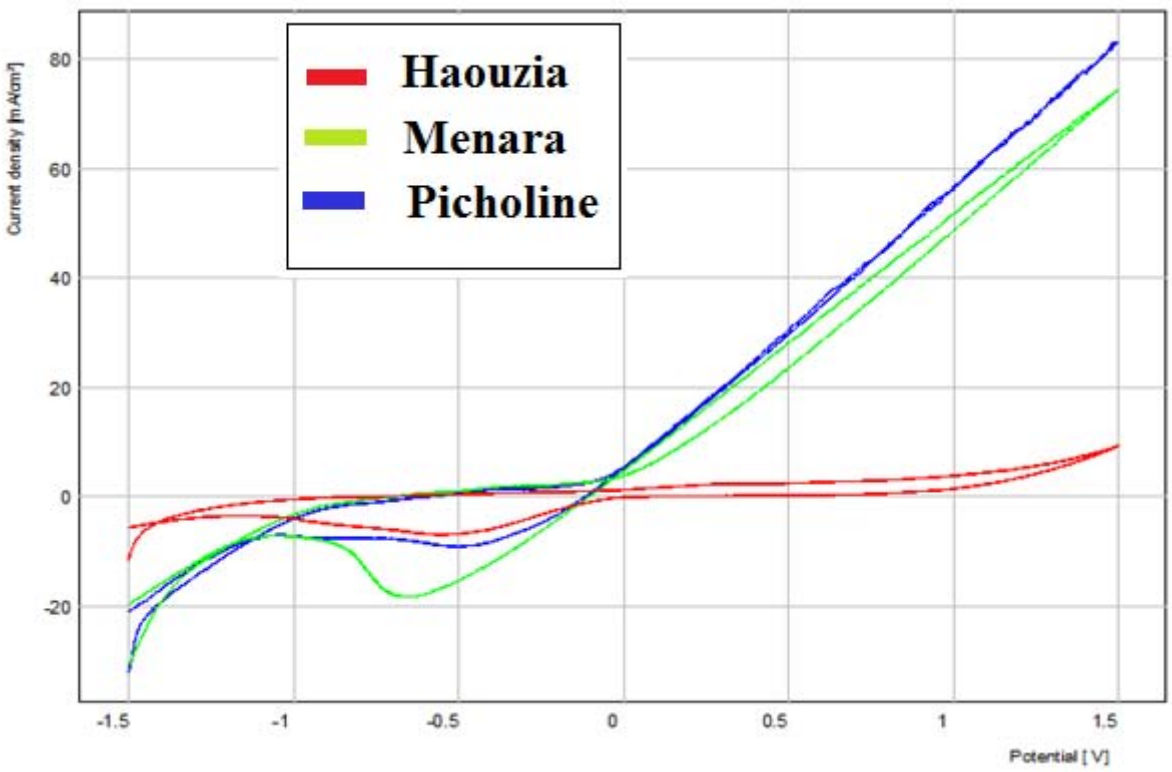


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*Figure 5 :* Cyclic voltammograms- recorded at Cu-CPE in 0.1M $Na_2SO_4$, containing 50 µl/100ml of $H_2O_2$ and 350µl/100ml

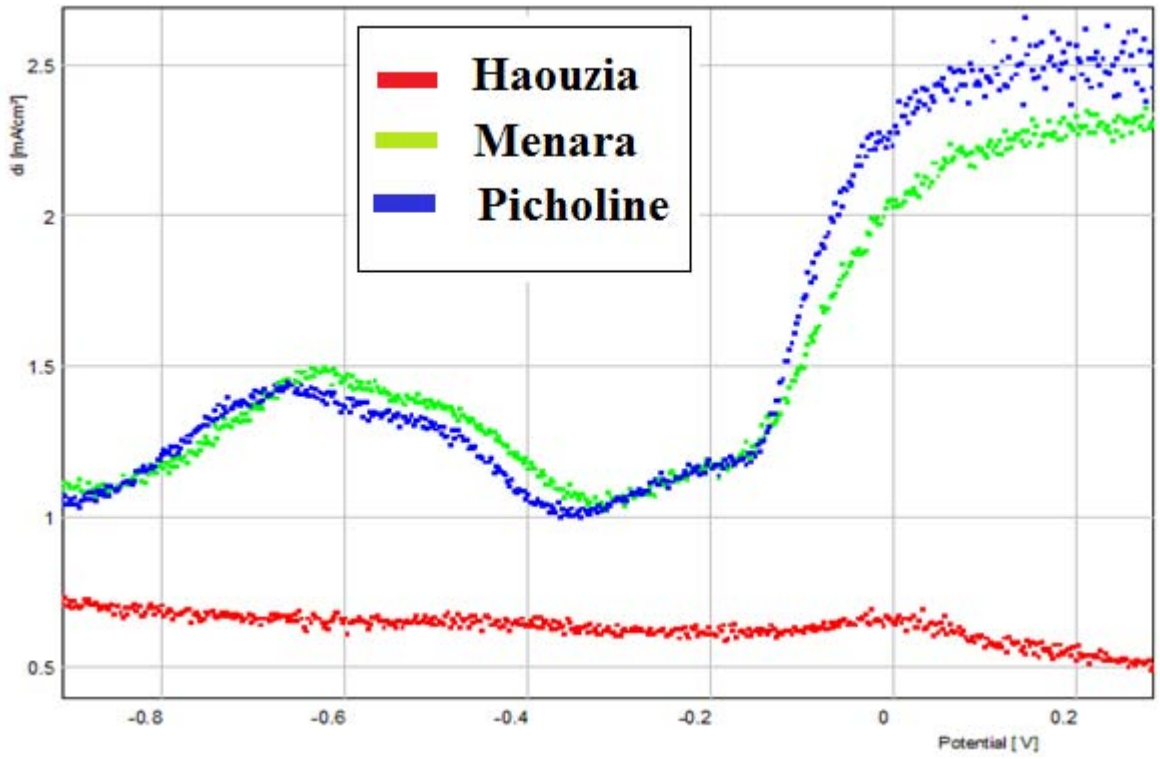


*Figure 6 :* Square wave voltammograms recorded at Cu-CPE in, 0.1M $Na_2SO_4$ solution, containing 50 µl/100ml of $H_2O_2$ and 350µl/100ml

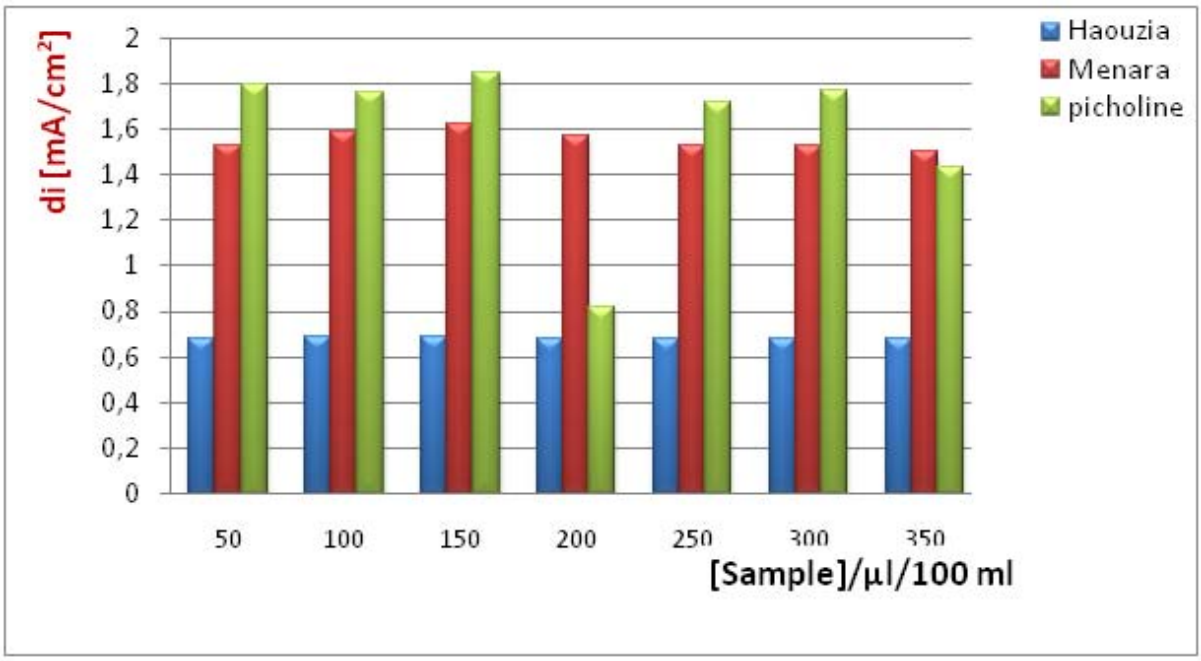


*Figure 7 :* Evolution of the $H_2O_2$ reduction current density with the concentration of different studied samples

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

$$AOC = \frac{I_{H2O2} - I_{H2O2\text{-antioxidant sample}}}{I_{H2O2}} \times 100$$

Where $I_{H2O2}$, is the current density due to $H_2O_2$ reduction, and, $I_{H2O2\text{-antioxidant sample}}$, represent the current density due to antioxidant sample addition. The results are summarized in Figure 8.1. The Antioxidant capacity (AOC) of the studied samples varies in the following sense:

*Haouzia sample ≥ Picholine sample > Menara sample*

Except for a sample concentration near 200 µl, the picholine kernel presents the best antioxidant capacity.

## IV. Conclusion

An electrochemical analytical system for the evaluation of the antioxidant capacity has been developed. The main advantage of the new approach is based on coupling the radical analysis and the reduction inhibition of $H_2O_2$ by the studied kernel oils. The results obtained show that the proposed system is fast, sensitive and better suited than conventional methods.

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