1. Experimental

1.1. Reagents

Quercetin was purchased from Fluka. The reagents used as supporting electrolytes such as potassium chloride and chemicals for preparation of Britton-Robinson phosphate buffers (0.04 M stock solutions of H_3PO_4, CH_3COOH, H_3BO_3 and 0.2 M NaOH) were of reagent grade. Phosphate buffers (pH 1.9 – 11.4) were prepared at constant ionic strength. The solutions were prepared with ultrapure water (Millipore). Methanol and acetonitrile were HPLC grade (Carlo Erba, Milan, Italy). Ethyl acetate (AcOEt) was purchased from Anal R (BDH). Hexadecane and 2,4-dihydroxybenzophenone, used as internal standards, and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane were purchased from Sigma (Milan, Italy). Standard solutions of analytes in methanol were prepared from quercetin from Fluka.

All reagents and chemicals were used without any further purification.

1.2. Methods

Electrochemical measurements were done using an electrochemical system for cyclic voltammetry. It consisted of a fast rise-time potentiostat interfaced to a personal computer via an IEEE–interface card (AdvanTech, model PCL–848) and a data acquisition card (PCL–818) using 12-bit precision. Cyclic voltammetry was also conducted using a PGSTAT 12 AUTOLAB potentiostat. A three-electrode electrochemical cell was used with an Ag|AgCl|1M LiCl reference electrode separated from the test solution by a salt bridge. The working electrodes were platinum electrode (0.8 mm) and glassy carbon electrode (0.7 mm). The auxiliary electrode was cylindrical platinum net. Oxygen was removed from the solution by passing a stream of argon. The oxidation products of quercetin were prepared by exhaustive electrolysis of its 0.7×10^{-3} M – 2×10^{-3} M solutions on carbon paste electrode.

Spectrophotometry

Spectroelectrochemistry was performed using an optically transparent thin-layer electrode (OTTLE) cell [M. Krejčik, M. Danek, F. Hartl, J. Electroanal. Chem. Interfacial Electrochem. 317 (1991), 179] with a three electrode system (platinum working and auxiliary electrode, silver quasi reference electrode) mounted in a thin layer (thickness 1.7 mm) between optical windows. Sufficiently optically transparent platinum gauze (80 mesh) of the size 5x5 mm served as the working electrode. The response of the cell allows completing electrolysis within time of several tens of seconds (20 s when tested with ferrocene in acetonitrile). The potential scan rate was 5 $mV \times s^{-1}$. Spectral changes in the course of the electrolysis were registered using Agilent 8453 diode-array UV-Vis spectrometer or Philips PU9800 FTIR spectrometer. The 1.0 cm quartz cuvettes were used for recording the absorption spectra during the bulk electrolysis.

Gas chromatography

The products were identified using a Trace GC gas chromatograph (Thermo Electron Corporation, USA) equipped with a PTV injection port and a mass spectrometric detector based on an ion trap analyzer (Polaris Q, Thermo Electron Corporation, USA). The PTV injector was in the CT 'splitless with surge' mode at 280 ºC with a surge pressure of 100 kPa, and the mass spectrometer parameters were: electronic impact ionization (70 eV), ion source temperature 230 ºC, scan range m/z 50-700 and interface temperature 280 ºC. Chromatographic separation was performed on a DB-5MS chemically bonded fused silica capillary column (J & W Scientific, Agilent Technologies) with stationary phase 5%phenyl-95%methylpolysiloxane, and of dimensions 0.25 mm i.d., 0.1 µm film thickness, 25 m and 30 m length. The gas chromatographic conditions were as follows: initial temperature 57 ºC, 2 min isothermal, then ramped at 10 ºC/min up to 200 ºC, 3 min isothermal, then ramped at 20 ºC/min up to 300 ºC and then isothermal for 20 min. The carrier gas was He (purity 99.9995 %), at a constant flow rate of 1.2 mL/min. The peak assignment was based on comparison

with analytical reference compounds and materials, with library mass spectra (NIST 1.7) and with mass spectra reported in the literature.

In order to perform GC-MS analysis, electrolysis products were derivatised with a silylating agent N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Derivatisation conditions are: 10 µL of 2,4-dihydroxybenophenone (solution in isopropanol; internal standard IS1) is added to the sample; the solution is dried and 30 µL of the derivatisation agent BSTFA in 50 µL of AcOEt is added; the reaction takes place at 60 °C for 30 min in closed glass vials. Just before injection, 10 µL of hexadecane (solution in isooctane; internal standard IS2) and 150 µL of AcOEt is added; 2 µL of the final solution is injected in the GC system.

Liquid Chromatography with photodiode array detector

An high-pressure liquid chromatography (HPLC) consisting of a PU-2089 Quaternary Gradient Pump with a degasser (Jasco International Co., Japan), equipped with a Rheodyne Model 7125 injection valve and coupled to a spectrophotometric diode array detector MD-2010 (Jasco International Co., Japan) was used. The data were processed by ChromNav® software. The chromatographic separation was performed on analytical reverse phase TC-C18 column (Agilent, 4,6 x 250 mm, 5 µm). The eluents were (A): aqueous solution of 0.1% trifluoroacetic acid (TFA) and (B): acetonitrile with 0.1% TFA. The gradient was: 0-5 min, 85% A; 5 – 30 min, linear gradient to 50% A; 30 – 40 min, linear gradient to 30% A ; 40 – 50 min, 10% A; hold for 10 min. The flow rate was 1 mL/min, injection volume 20 µl. DAD acquisition parameters were: acquisition range 200-650 nm, 4 nm step.

Liquid Chromatography with Mass Spectrometer

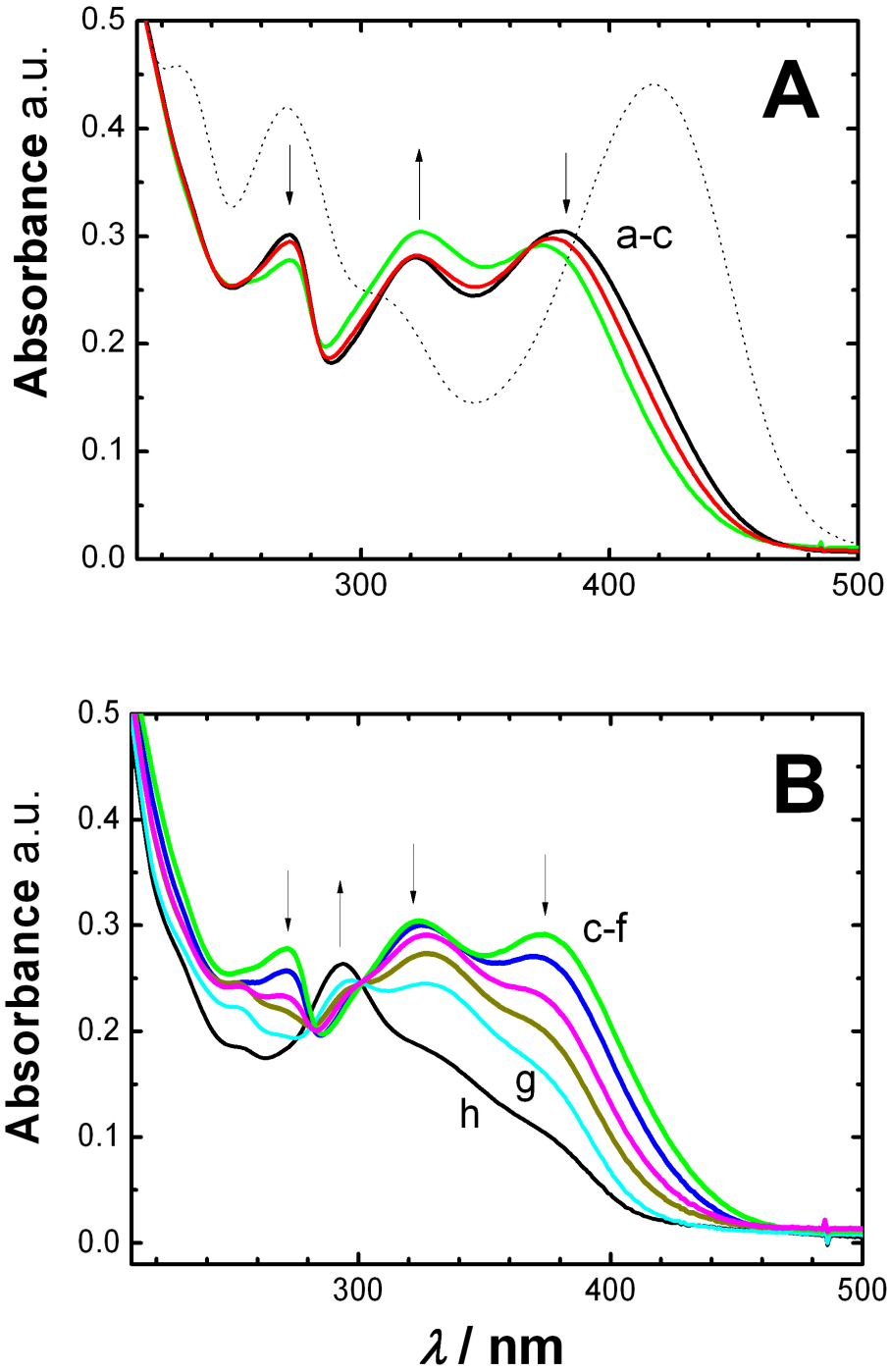
The chromatographic separation was performed on analytical reverse phase C-8 column (HyPurity C8, 150x3 mm, 5 µm, Thermo Scientific). The gradient elution program used

eluents (A): aqueous solution of 0.1% H_3PO_4 and (B): acetonitrile. The gradient was: 0-2 min, 95% A; 2 – 30 min, linear gradient to 40% A; 30 – 35 min, linear gradient to 0% A and 100% B; 35 – 40 min, 100% B. The flow rate was 0.2 mL/min, the injection volume was 5 µl. The Waters 1525µ Binary HPLC pump with detector Waters 2487 Dual λ Absorbance Detector (250 nm, 280 nm) and the mass spectrometer detector Waters® Quattro PremierTM (Waters, Watford, Great Britain) was used. The ESI probe and ion source were operated in negative mode with parameters: electrospray capillary voltage at 2.7 kV, cone voltage at 40 V, extractor voltage was 4V and RF lens voltage 0.0 V, collision energy 20 eV. Desolvation gas flow and temperature were maintained at 50 L/h and 150◦C respectively and the source temperature was 100 ºC, desolvation temperature 300 ºC. The data were processed by MassLynx V4.1 software.

Tandem mass spectrometry

Electro-spray ionisation (ESI) tandem mass spectrometry was carried out on a Waters® Quattro PremierTM tandem quadruple mass spectrometer (Waters, Watford, UK) instrument operating in negative ion mode. The sample is ionized at atmospheric pressure in the source. The ions enter the vacuum system through a sampling cone, then pass through the source travelling wave ion guide into the first quadrupole, where they are filtered according to their mass-to-charge ratio. The mass-separated ions pass into the T-Wave collision cell where they either undergo collision-induced decomposition (CID) or pass to second quadrupole. Any fragment ions are then mass-analyzed by the second quadrupole. The instrument conditions were as follows: electrospray capillary voltage 3.0 kV, sample cone voltage 21V, extractor voltage was 5V and RF lens voltage 0.0 V and collision energy 20 eV. Desolvation gas flow and temperature were maintained at 50 L/h and 150◦C respectively and the source

temperature was 100 ◦C. Resolution was 15.0 for MS1 and 15.0 for MS2, the photomultiplier energy was 645 V.



SuppFig1. The ex-situ absorption spectra of 2×10-5 M quercetin in 0.1 M KCl and 3.6×10-3 M KOH during the exhaustive electrolysis at carbon paste electrode at potential 1.0 V measured at a) 0, b) 82, c) 130, d) 182, e) 221, f) 280, g) 410, h) 600 min. Dotted line represents the absorption spectrum of quercetin in degassed solution under inert atmosphere of argon.