TOTAL ANTIOXIDANT CAPACITY WITH ELECTROGENERATED Br_2 FOR THE DETERMINATION OF EXTRA-VIRGIN OLIVE OIL BIOPHENOLS

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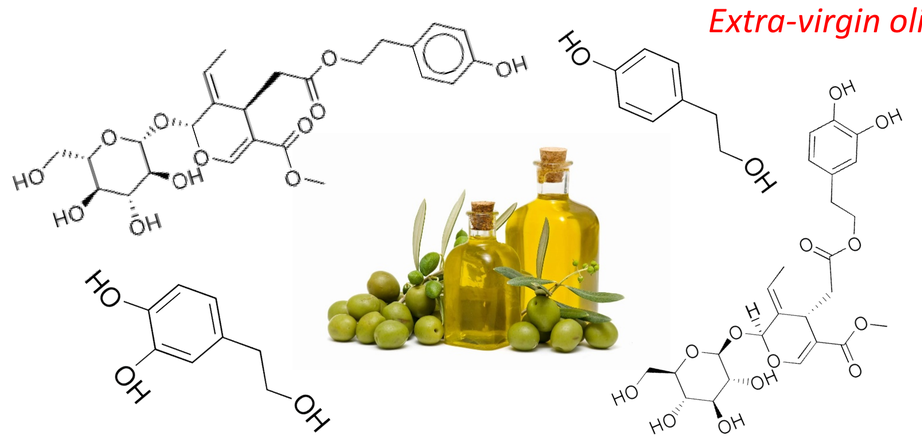
Introduction: Extra Virgin Olive Oil (EVOO) containing more than 250 mg kg^{-1} of polyphenols can be labelled with the specific **health claim**: "Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress"[1]. This health claim is largely underutilized, due to analytical drawbacks related to the simultaneous determination of many structurally heterogeneous compounds. The International Olive Council (IOC) has approved only the HPLC-UV (λ = 280 nm)-based determination of hydroalcoholic EVOO extracts[2], which is rarely adopted for routine determinations.

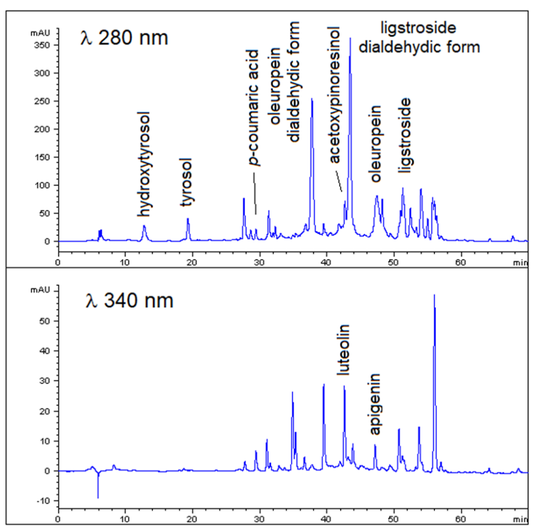
Extra-virgin olive oil biophenols



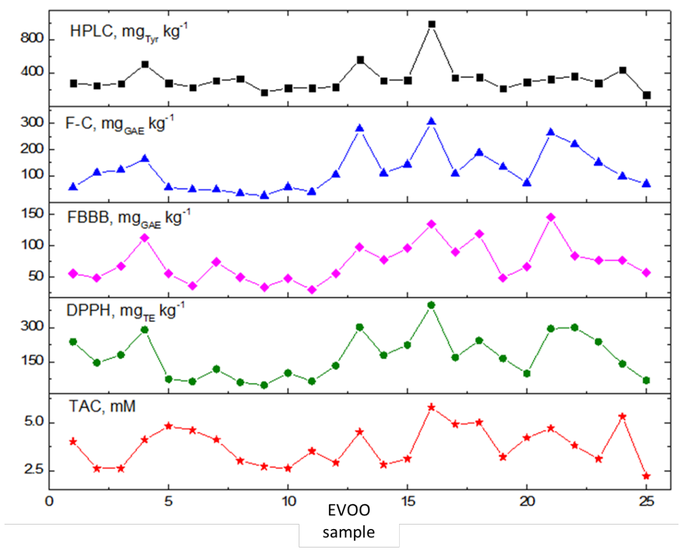
Aims: To establish novel quantification strategies or classification methods with robustness comparable to HPLC, improving both cost- and time-effectiveness as well as rapidity and easiness, also for real time and on-site (at the oil mill) classification of EVOO.

Materials & Methods: In parallel with the HPLC-UV and other spectrophotometric determinations, TAC of 25 EVOO polyphenol extracts was determined. Br_2 was produced coulometrically on a Pt anode; the circuit was completed with a twin Pt cathode, separated from the solution through a porous glass frit. The biamperometric system consisted in a couple of 1 cm^2 Pt foils at a constant 50 mV potential difference for the detection of Br_2 excess.

TAC determinations were compared with those obtained with several other methods, including RP-HPLC, Folin-Ciocalteu DPPH, Fast Blue BB.[3,4]

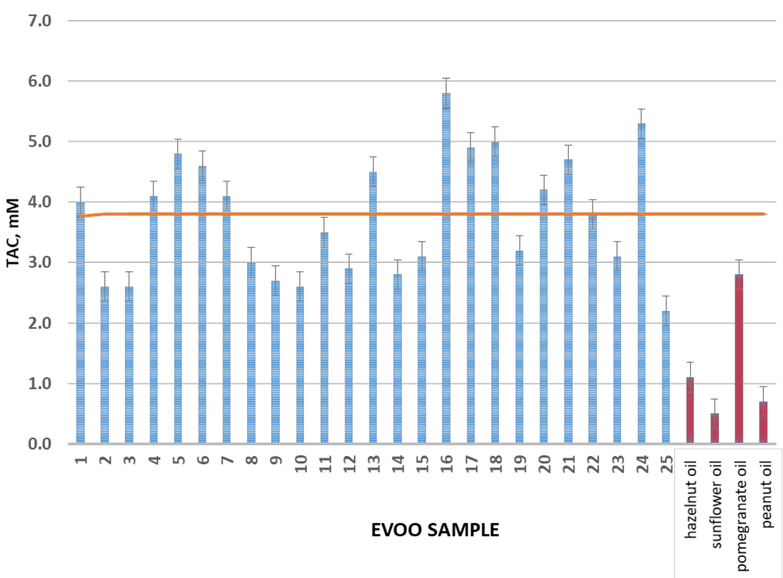


Typical HPLC chromatograms of EVOO hydroalcoholic extracts (λ 280 and 340 nm)



Comparative direct or indirect (antioxidant activity) determinations of biophenols in 25 EVOO samples

Results: TAC of EVOO is averagely higher than extracts from other edible oils. The average TAC of oils with polyphenols > 250 mg kg^{-1} (4.0 ± 1.0 µmol cm^{-3}) was higher than the average TAC of EVOO with total polyphenols < 250 mg kg^{-1} (3.1 ± 0.8 µmol cm^{-3}).



EVOO with >250 mg/Kg biophenols*:
TAC_{mean} 4.0 ± 1.0

TAC_{Mean} EVOO: 3.8

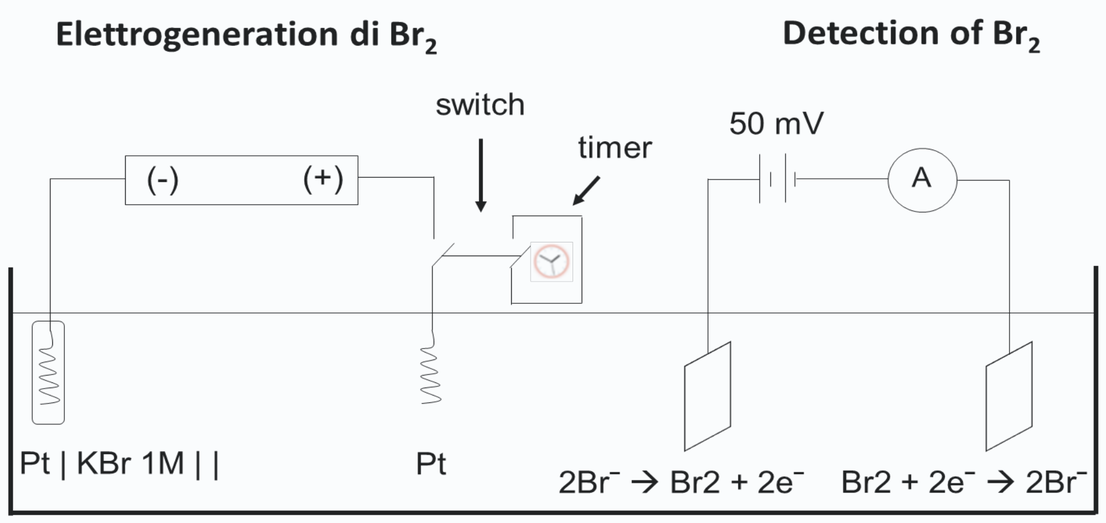
EVOO with <250 mg/Kg biophenols*:
TAC_{mean} 3.1 ± 0.8

TAC of 25 EVOO samples and comparison with TAC of other edible seed oils

** Determined by RP-HPLC-DAD*

Determination of TAC

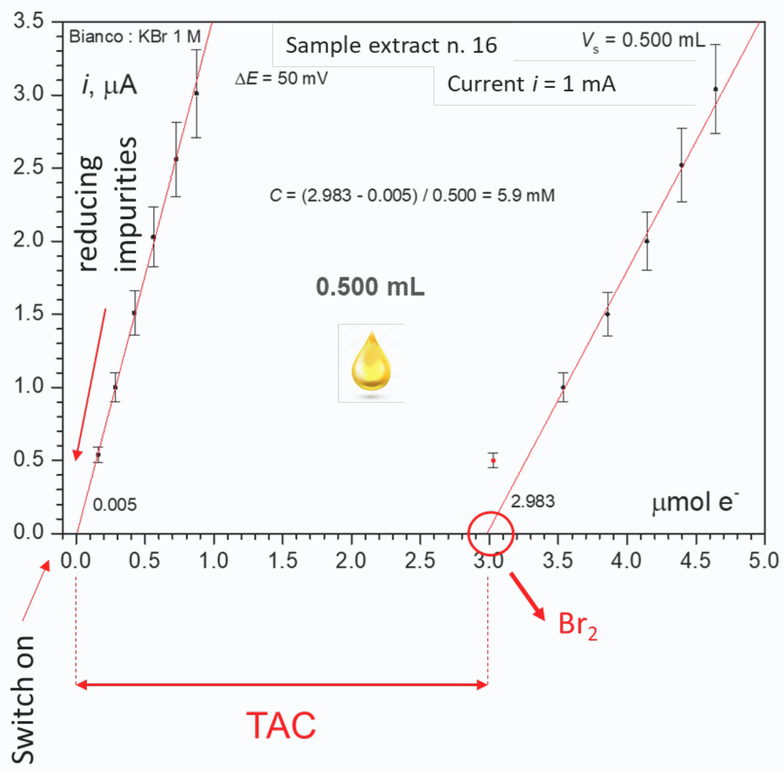
Total Antioxidant Capacity (TAC) determined with electrogenerated Br_2 as the titrating agent and biamperometric end-point detection could be an alternative easy and cost-effective strategy to classify EVOO (also practicable at the oil mill), considered that biophenols are primary contributors to TAC.



Schematic of the devise for electrogeneration of Br_2 and bioamperometric detection

$$\mu mol\ Br_2 = \frac{1}{2}\mu mol\ e^- = \frac{1}{2}\frac{i(mA) \times t(s)}{96.487}$$

µmol of Br2 utilized to oxidize polyphenols are determined with high accuracy



Typical graphical representation of the TAC measurement

Conclusions: **Although not strictly correlated to HPLC,** TAC has potential to be an easy, robust, cheap and rapid test and it might be a candidate method for a **novel alternative classification of EVOO**, based on the antioxidant capacity rather than on the HPLC-determined content of polyphenols.

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References

1. *EU regulation n. 432/2012*; 2. *International Olive Council (2009). Determination of biophenols in olive oils by HPLC. COI/T.20/Doc No 29*; 3. *Medina BM, J Funct Foods 2011*; 4. *Medina BM, J Agric Food Chem 2011*