Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

Antioxidant capacity assays: classical vs. nanomaterial based methods

Measurement of phenolic content of foods via Folin, ABTS, gold and silver nanoparticles based assays; amperometric detection and AOC assay

Michele Del Carlo
Flavio Della Pelle

UNIVERSITÁ
DEGLI STUDI
DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species.

They can be naturally present in a commodity or to be added to enhance its stability

They are used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals.

UNIVERSITÁ
DEGLI STUDI
DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

Because of their activity against free radicals they act as a protector of those biochemical substrate that are keen to be oxidized by a variety of chemical stressors

Antioxidants are involved in the defense mechanism of the organism against the pathologies associated to the attack of free radicals.

UNIVERSITÁ
DEGLI STUDI
DI TERAMO

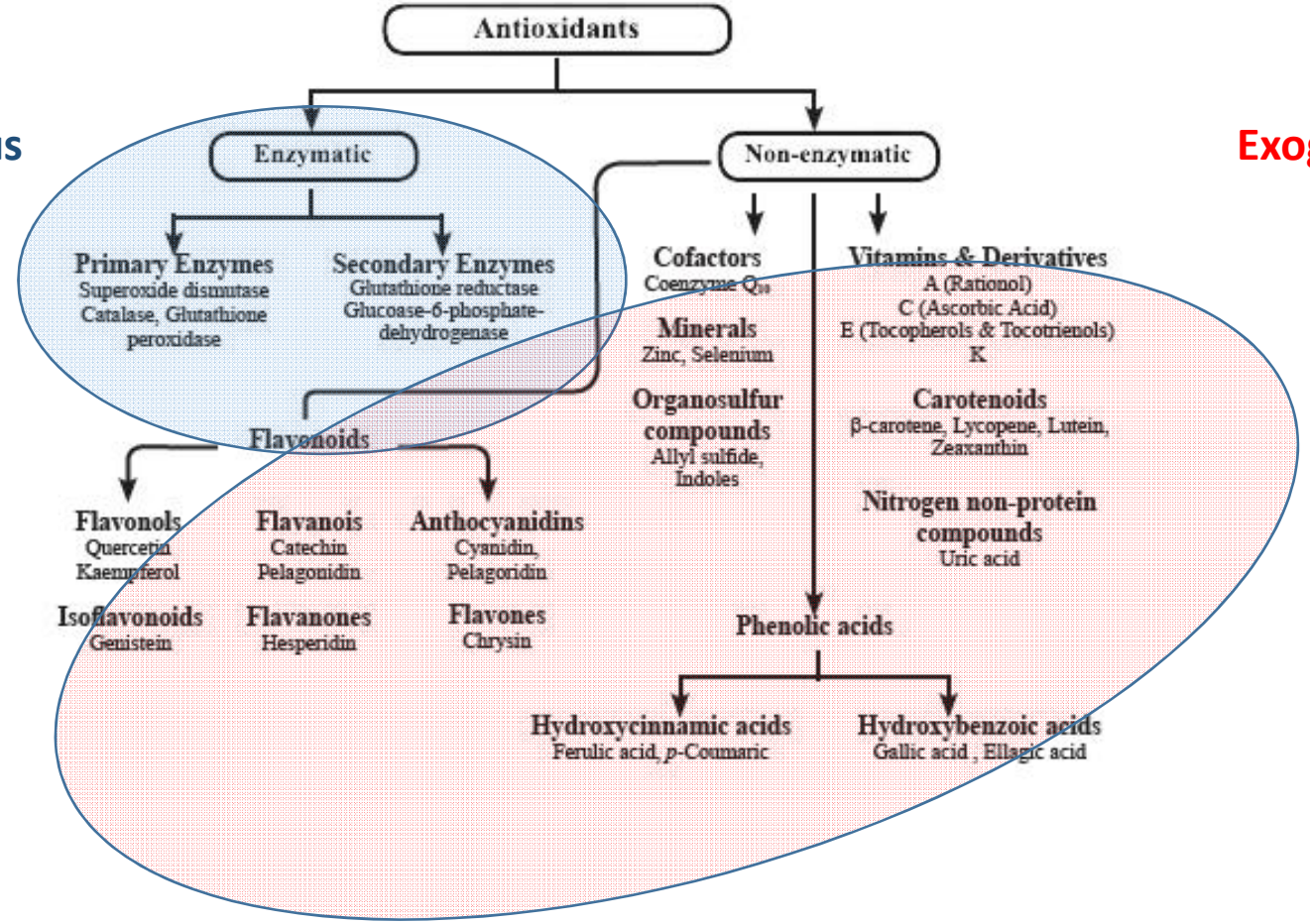
Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

Antioxidants can be divided into: enzymatic and non-enzymatic

Endogenous

Exogenous



UNIVERSITÁ DEGLI STUDI DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

Food science interest in antioxidants understanding is double:

Food Stability (“*green*” shelf life enhancer)

Health enhancer due to prevention of oxidative process

Triple claim: no “chemical preservative”, enhanced stability, nutraceutical

UNIVERSITÁ
DEGLI STUDI
DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

Health Benefits of Antioxidants

Antioxidants have attracted considerable attention in relation to radicals and oxidative stress, cancer prophylaxis and therapy, and longevity.

Antioxidants are demonstrated on epidemiological data, an effective protection against the development of other diseases caused by oxidative stress, such as cancer, coronary heart disease, obesity, type 2 diabetes, hypertension and eyes desease (e.g cataract).

UNIVERSITÁ
DEGLI STUDI
DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

There are numerous antioxidants in dietary plants:

carotenoids, phenolic compounds, benzoic acid derivatives, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans, and lignins, vitamin C, vitamin E, Maillard reaction products, β-carotene, and lycopene.

Some of these are hydrophilic whereas some other are hydrophobic

Methods to determine antioxidant activity should take this into account.

UNIVERSITÁ
DEGLI STUDI
DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

This chemical diversity also reflects on the cooperative effect on antagonistic effect that individual molecules may exhibit when present in a mixture.

Therefore… levels of single antioxidants in foodstuffs do not necessarily reflect their total antioxidant potential (TAP)

the total antioxidant potential depends on the synergic and redox interaction among the different molecules present in food

UNIVERSITÁ DEGLI STUDI DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

Thus… the estimation of total antioxant capacity is the tool to characterize the health features of a food commodity, rather than the chemical characterization of the pattern of antioxidants molecules present in that food.

UNIVERSITÁ
DEGLI STUDI
DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

The Mechanism of Action of Antioxidants

Excess **free radicals** circulating in the body oxidize the low density lipoproteins (LDL), making them potentially a potential hazard;

The excess free radicals can also accelerate aging processes and have been linked to other very serious pathologies (e.g brain stroke, diabetes mellitus, rheumatoid arthritis, Parkinson's disease, Alzheimer's disease and cancer).

Physiologically, the oxygenated free radicals are among the most important radical species. Reactive oxygen species (ROS) comprise species with a strong oxidizing tendency, both of a **radical nature** (the superoxide radical, the radical) and a **non-radical nature** (ozone, hydrogen peroxide).

UNIVERSITÁ DEGLI STUDI DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

A number of chemical and physical phenomena can initiate oxidation, which proceeds continuously in the presence of a suitable substrate, until a blocking defence mechanism occurs.

Target substances include polyunsaturated fatty acids, phospholipids, cholesterol and DNA.

The essential features of oxidation via a free radical-mediated chain reaction are initiation, propagation, branching and termination steps.

The process may be initiated by the action of external agents such as heat, light or ionizing radiation or by chemical initiation involving metal ions or metalloproteins.

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

Generally the oxidation of a biological substrate by ROS follows this path:

Initiation

Propagation

Branching

Propagation

UNIVERSITÁ
DEGLI STUDI
DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

Initiation

$$LH + R\cdot \rightarrow L\cdot + RH$$

where LH represents the substrate molecule, for example, a lipid, with R· as the initiating oxidizing radical. The oxidation of the lipid generates a highly reactive allyl radical (L·) that can rapidly react with oxygen to form a lipid peroxyl radical (LOO·).

Branching

$$LOOH \rightarrow LO\cdot + HO\cdot$$
$$2\,LOOH \rightarrow LOO\cdot + LO\cdot + H_2O$$

The breakdown of lipid hydroperoxides often involves transition metal ion catalysis, in reactions similar to those involving hydrogen peroxide, yielding lipid peroxyl and lipid alkoxyl radicals.

Propagation

$$L\cdot + O_2 \rightarrow LOO\cdot$$
$$LOO\cdot + LH \rightarrow L\cdot + LOOH$$

The peroxyl radicals are the chain carriers of the reaction; they can further oxidize the lipid, producing lipid hydroperoxides (LOOH), which in turn break down to a wide range of compounds [69], including alcohols, aldehydes, alkyl formates, ketones and hydrocarbons, and radicals, including the alkoxyl radical (LO·).

Termination

Termination reactions involve the combination of radicals to form non-radical products:

$$LO\cdot + LO\cdot$$
$$LOO\cdot + LOO\cdot$$
$$LO\cdot + LOO\cdot$$

Primary antioxidants, AH, when present in trace amounts, may either delay or inhibit the initiation step by reacting with a lipid radical or inhibit the propagation step by reacting with peroxyl or alkoxyl radicals [70].

$$L\cdot + AH \rightarrow LH + A\cdot$$
$$LOO\cdot + AH \rightarrow LOOH + A\cdot$$
$$LO\cdot + AH \rightarrow LOH + A\cdot$$

Secondary or preventative antioxidants are compounds that retard the rate of oxidation. This may be achieved in a number of ways, including removal of substrate or singlet oxygen quenching [66, 71].

Co-funded by the
Erasmus+ Programme
of the European Union

Methods of Total Antioxidant Capacity Assessment

ERASMUS + PROJECT : AG-LAB

Antioxidant capacity assay	Principle of the method	End-product determination
	Spectrometry	
DPPH	Antioxidant reaction with an organic radical	Colorimetry
ABTS	Antioxidant reaction with an organic cation radical	Colorimetry
FRAP	Antioxidant reaction with a Fe(III) complex	Colorimetry
PFRAP	Potassium ferricyanide reduction by antioxidants and subsequent reaction of potassium ferrocyanide with Fe3+	Colorimetry
CUPRAC	Cu (II) reduction to Cu (I) by antioxidants	Colorimetry
ORAC	Antioxidant reaction with peroxyl radicals, induced by AAPH (2,2'-azobis-2-amidino-propane)	Loss of fluorescence of fluorescein
HORAC	Antioxidant capacity to quench OH radicals generated by a Co(II) based Fenton-like system	Loss of fluorescence of fluorescein
TRAP	Antioxidant capacity to scavenge luminol-derived radicals, generated from AAPH decomposition	Chemiluminescence quenching
Fluorimetry	Emission of light by a substance that has absorbed light or other electromagnetic radiation of a different wavelength	Recording of fluorescence excitation/ emission spectra

UNIVERSITÁ DEGLI STUDI DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

The ABTS method: The ABTS cation radical (ABTS•+) which absorbs at 743 nm (giving a bluish-green colour) is formed by the loss of an electron by the nitrogen atom of ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)). In the presence of Trolox (or of another hydrogen donating antioxidant), the nitrogen atom quenches the hydrogen atom, yielding the solution decolorization.

ABTS can be oxidized by potassium persulphate or manganese dioxide giving rise to the ABTS cation radical (ABTS•+) whose absorbance diminution at 743 nm was monitored in the presence of Trolox, chosen as standard antioxidant.

UNIVERSITÁ
DEGLI STUDI
DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

Electrochemical Techniques		
Cyclic voltammetry	The potential of a working electrode is linearly varied from an initial value to a final value and back, and the respective current intensity is recorded	Measurement of the intensity of the cathodic/ anodic peak
Amperometry	The potential of the working electrode is set at a fixed value with respect to a reference electrode	Measurement of the intensity of the current generated by the oxidation/reduction of an electroactive analyte

UNIVERSITÁ
DEGLI STUDI
DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

Electrochemical techniques

Amperometry

Voltammetry

Both methods are based on the concept that lower is the potential required to electrochemically oxidize a compound the higher is its capacity to donate electrons, that can be used to quench a radical or to reduce an oxidant.

UNIVERSITÁ
DEGLI STUDI
DI TERAMO

Most of the molecules able to act as primary or secondary antioxidants are chemically classified as **polyphenols**.

In our research group we developed techniques for the detection of **polyphenols** based on:

- Direct electrochemistry (chemically modified electrode or un-modified electrode)
- Optical sensing using metal nanoparticle
- Selective artificial binding receptors coupled with fluorescence and electrochemistry

UNIVERSITÁ DEGLI STUDI DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

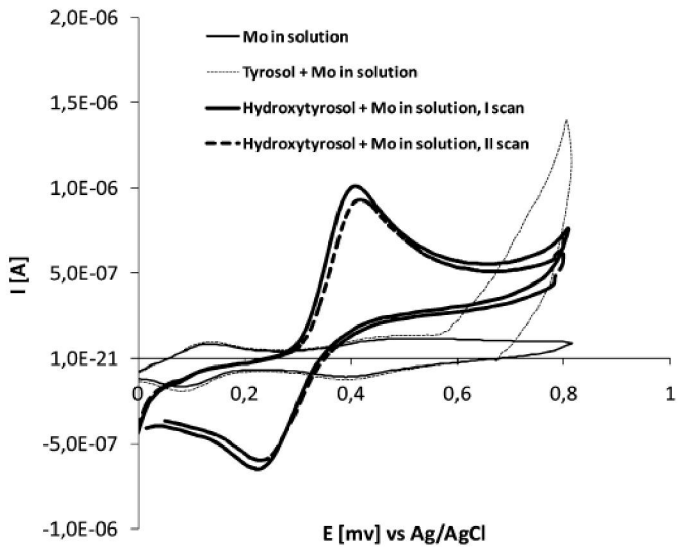
Full Paper **ELECTROANALYSIS**

Selective Voltammetric Analysis of *o*-Diphenols from Olive Oil Using Na_2MoO_4 as Electrochemical Mediator

M. Del Carlo,[*a] *A. Amine,*[b] *M. Haddam,*[b] *F. della Pelle,*[a] *G. C. Fusella,*[a] *D. Compagnone*[*a]

[a] Dipartimento di Scienze degli Alimenti, Università degli Studi di Teramo, Italia
[b] Faculté de Science, Université Hassan II, Mohammedia, Morocco
*e-mail: mdelcarlo@unite.it; dcompagnone@unite.it

Received: October 20,
Accepted: December 2



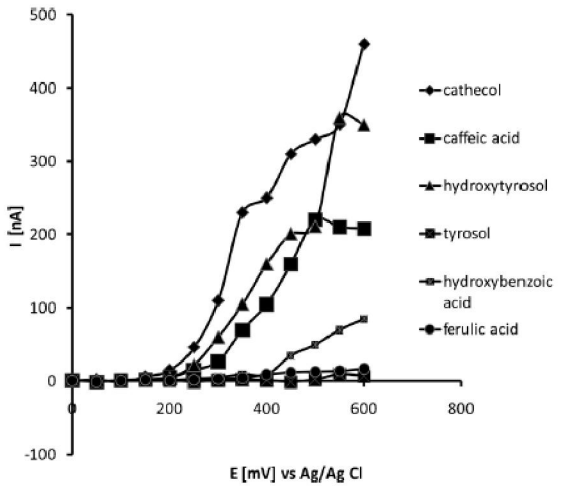
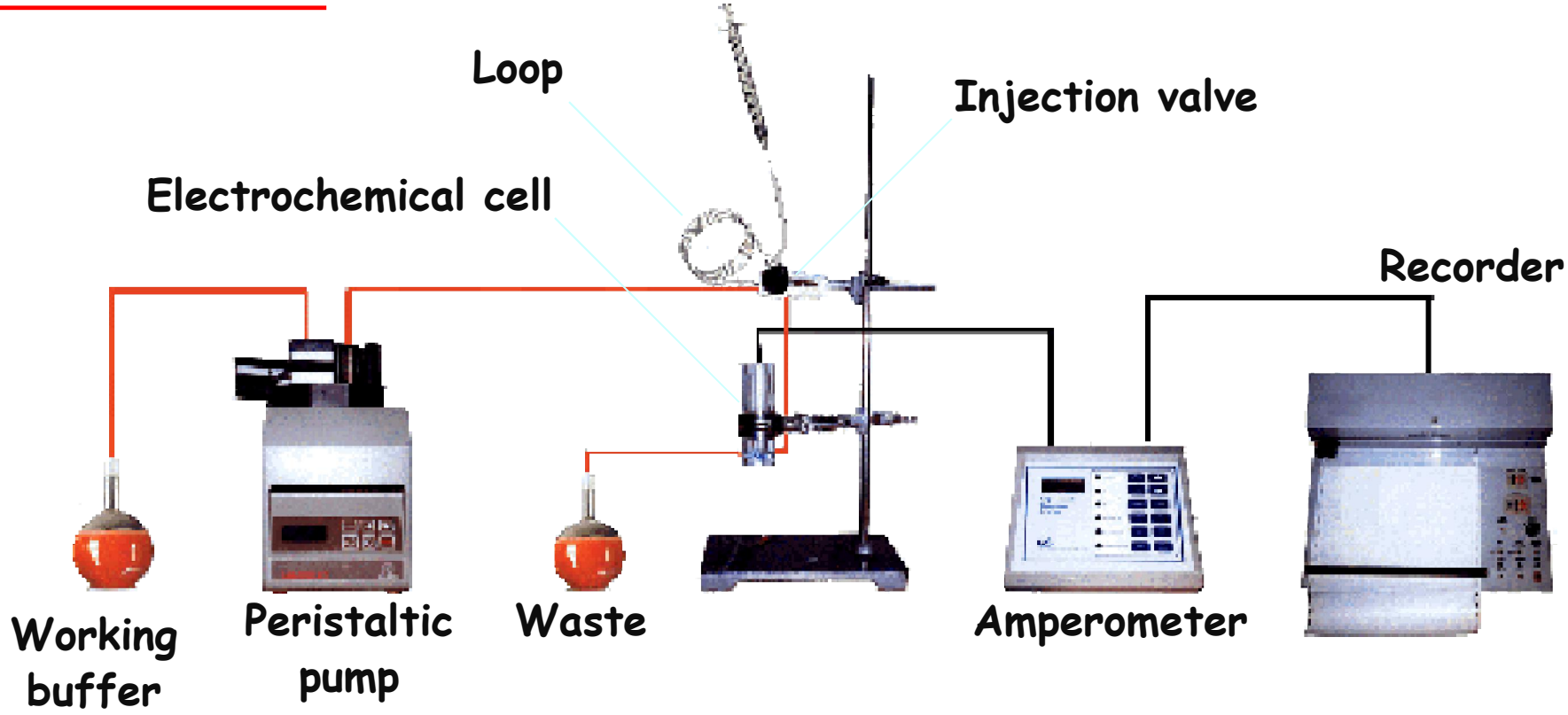
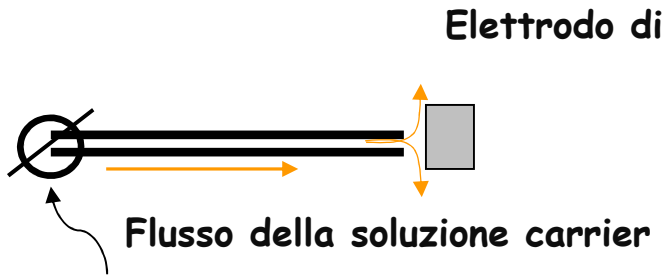


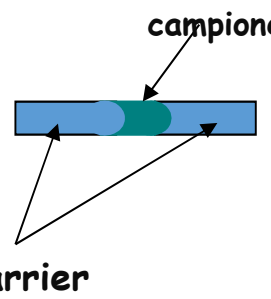
Fig. 5. Hydrodynamic voltammogram of catechol, caffeic acid, hydroxytyrosol, tyrosol, hydroxybenzoic acid, ferulic acid. Comparison of the electrochemical behavior in presence of Na_2MoO_4.

UNIVERSITÁ DEGLI STUDI DI TERAMO

FIA-AMPEROMETRY







UNIVERSITÁ DEGLI STUDI DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

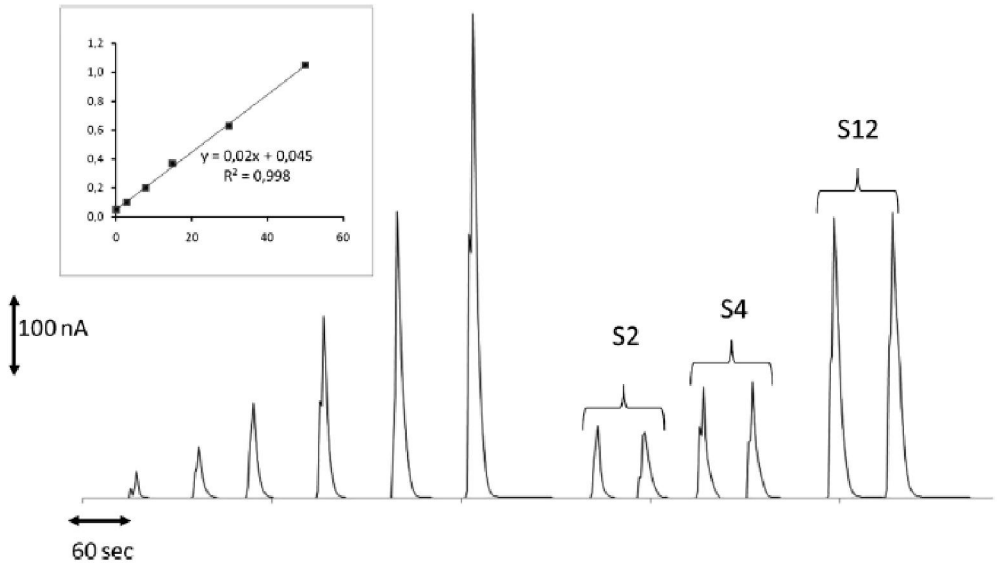


Fig. 7. Typical amperometric flow injection analysis records of catechol standards and two replicates of selected samples (S2, S4 and S12). In the inset the calibration curve of catechol is shown (concentration range 0.2–50 ppm), the current is recorded as μA.

Table 1. Comparison between the spectrophotometric and the amperometric method for the detection of *o*-diphenols in SPE extracts. All samples were measured in triplicate with a maximum $CV < 8\%$.

Sample ID	Catechol equivalent (mg L^{-1})	
	Spectrophotometric	Amperometric
S1	10.0	4.3
S2	9.4	5.0
S3	35.0	16.0
S4	68.0	30.0
S5	18.0	12.0
S6	34.4	17.2
S7	12.9	4.2
S8	37.0	23.0
S9	68.2	36.0
S10	21.2	10.0
S11	45.5	21.0
S12	34.7	13.0
S13	12.8	12.0

UNIVERSITÁ
DEGLI STUDI
DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

Optical techniques based on metal nanoparticle synthesis

Optical (Colorimetric) methods have a number of interesting features:

Widely spread instrumentation in any laboratory (no need to buy new instrument)

Personnel is generally confident on spectrophotometric techniques

Transferable to non instrumental assays (visual assays)

Measurable concentration range at ppm level

UNIVERSITÁ
DEGLI STUDI
DI TERAMO

Colorimetric method for the direct determination of total polyphenols in the matrix: application to virgin, extra virgin olive oil

Flavio Della Pelle*[a,b], Roberta Pasquarelli[a], Alberto Escarpa[b], Dario Compagnone[a], Michele Del Carlo[a]

[a]Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, 64023, Teramo, Italy

[b]Departamento de Química Analítica, Química-Física e Ingeniería Química. Facultad de Química. Universidad de Alcalá, 28871 Alcalá de Henares, Madrid, España

Co-funded by the Erasmus+ Programme of the European Union

AIM OF THE WORK

ERASMUS + PROJECT : AG-LAB

-Development of a simple colorimetric assay for the total polyphenols content determination based on gold nanoparticles formation

-Desired analytical features of the assay:

a) working in aqueous-organic solvent

b) application to fat food matrix, without extraction

c) fast, ease to use, cheap and environmental friendly (minimal solvent use)

analytical chemistry

Article

pubs.acs.org/ac

Gold Nanoparticles-based Extraction-Free Colorimetric Assay in Organic Media: An Optical Index for Determination of Total Polyphenols in Fat-Rich Samples

Flavio Della Pelle,[†,‡] María Cristina González,[‡] Manuel Sergi,[†] Michele Del Carlo,[†] Dario Compagnone,*[,†] and Alberto Escarpa*[,‡]

[†]Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, 64023 Teramo, Italy

[‡]Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, Faculty of Biology, Environmental Sciences and Chemistry, University of Alcalá, E-28871 Alcalá de Henares, Madrid, Spain

Supporting Information

UNIVERSITÁ DEGLI STUDI DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

- Nanomaterials are widely used in various fields of analytical chemistry, thanks to their unique properties

- In particular, gold nanoparticles (AuNPs) show:

  -high S/V, catalytic property, stability

  -produced by various types of synthesis with different

  diameter

  - are easily functionalizable (with different molecules)

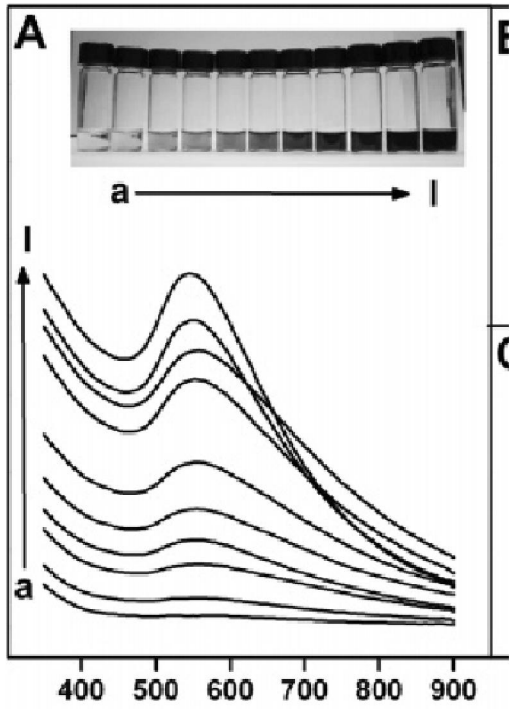
  - shown unique optical properties

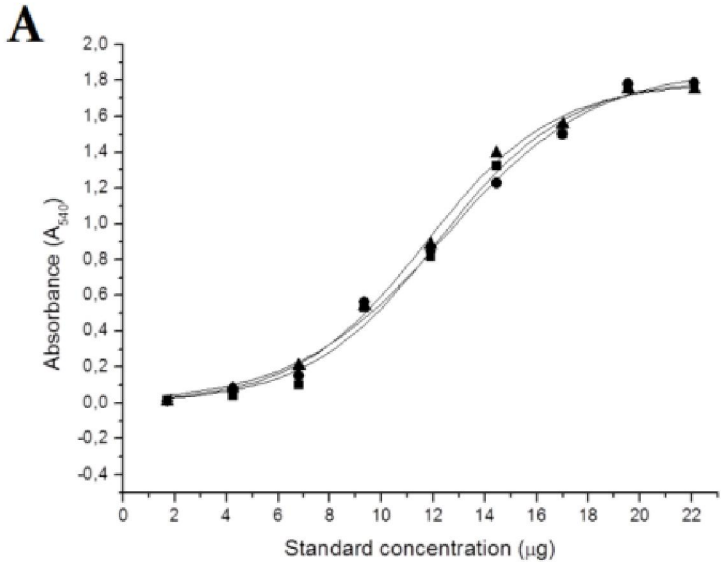
UNIVERSITÁ
DEGLI STUDI
DI TERAMO

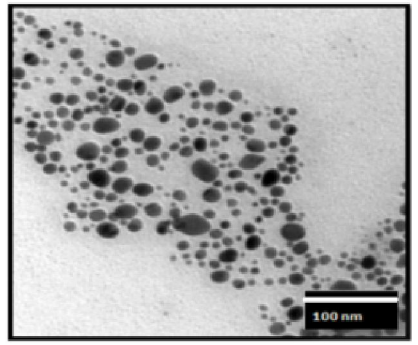
Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

Polyphenols are able to drive the synthesis of AuNPs (diameter ≤ 25 nm), reducing the gold (III) to gold (0)







UNIVERSITÁ
DEGLI STUDI
DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

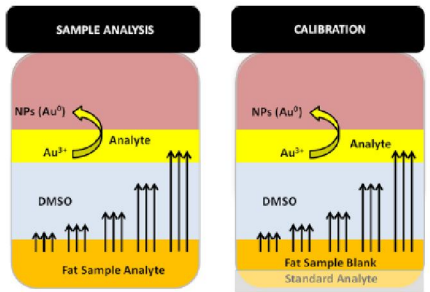
ERASMUS + PROJECT : AG-LAB

AuNPs synthesis driven by polyphenols, in organic-aqueous solvent : elimination of the extraction step

DMSO / Fat Matrix make possible the synthesis / stabilization of AuNPs

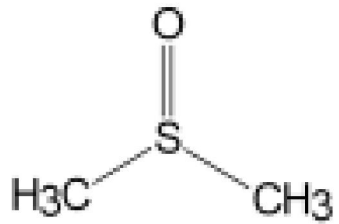
The proposed AuNPs synthesis does not require any surfactant (stabilizing agent) to facilitate the formation / stabilization of colloidal gold solution (with CTAC emulsion formation)

Scheme 1. Analytical Strategy for Sample Analysis and Calibration Using the AuNPs Synthesis Free-Extraction and Free-Surfactant Approach for Determination of Total Polyphenols in Fat-Enriched Samples



DMSO functional solvent:

- interfacing fat matrix / aqueous fraction
- cryogenic protector
- stabilizing agent and solvent for the nanoparticle systems



Fatty matrix (Sample or refined oil)

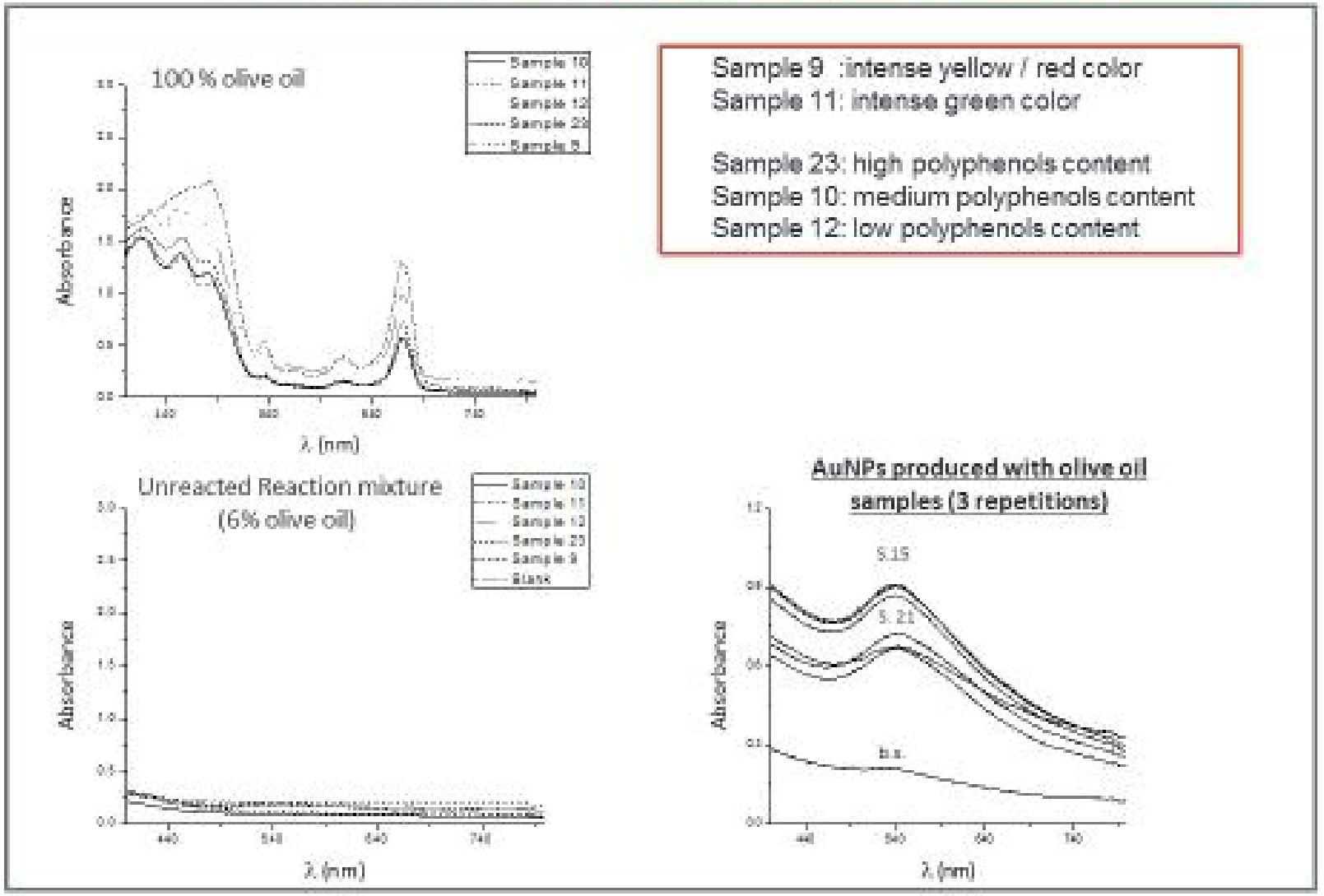
- Surfactants themselves : fatty acids and the carboxylic groups (especially at alkaline pH) can help the stabilization / formation of nanoparticles

UNIVERSITÁ DEGLI STUDI DI TERAMO

Co-funded by the
Erasmus+ Programme
of the European Union

ERASMUS + PROJECT : AG-LAB

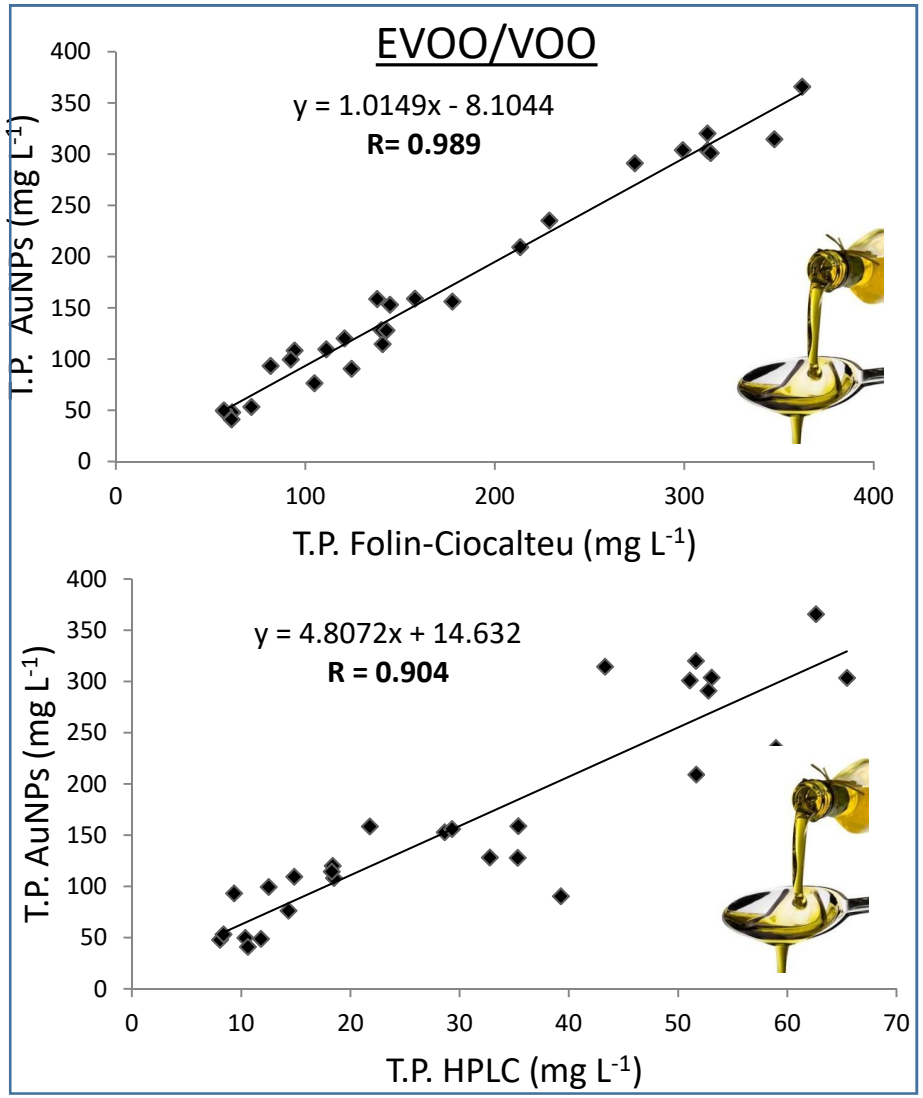
Olive oil (EVOO/VOO) samples spectra

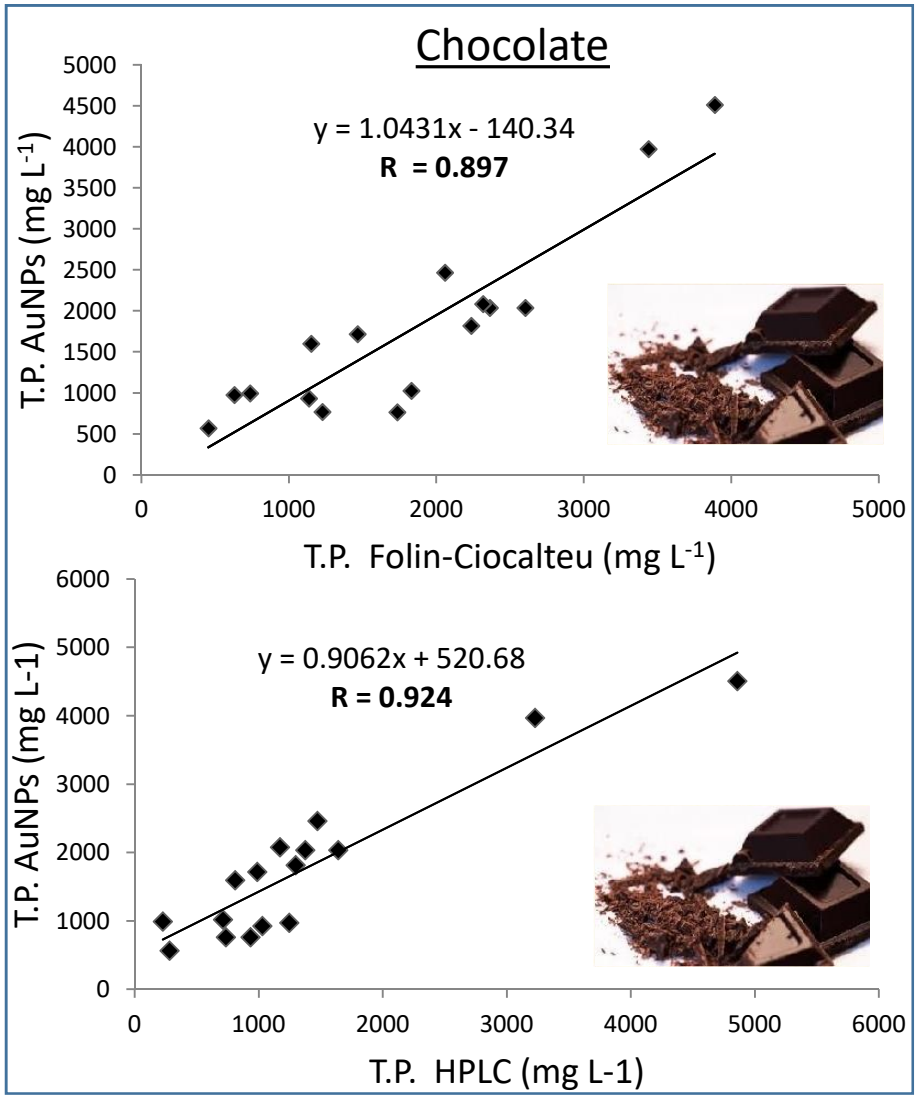


UNIVERSITÀ DEGLI STUDI DI TERAMO

Co-funded by the Erasmus+ Programme of the European Union

Proposed method vs Folin-Ciocalteu and the chromatographic method (HPLC-UV/VIS)





Underestimation HPLC oil samples: Escarpa A. et al., Anal. Chim. Acta, 427 (2001) 119-27; Weingerl V. et al., Acta Chim. Slov., 56 (2009) 698-703.

Co-funded by the Erasmus+ Programme of the European Union

ACS SENSORS

ERASMUS + PROJECT : AG-LAB

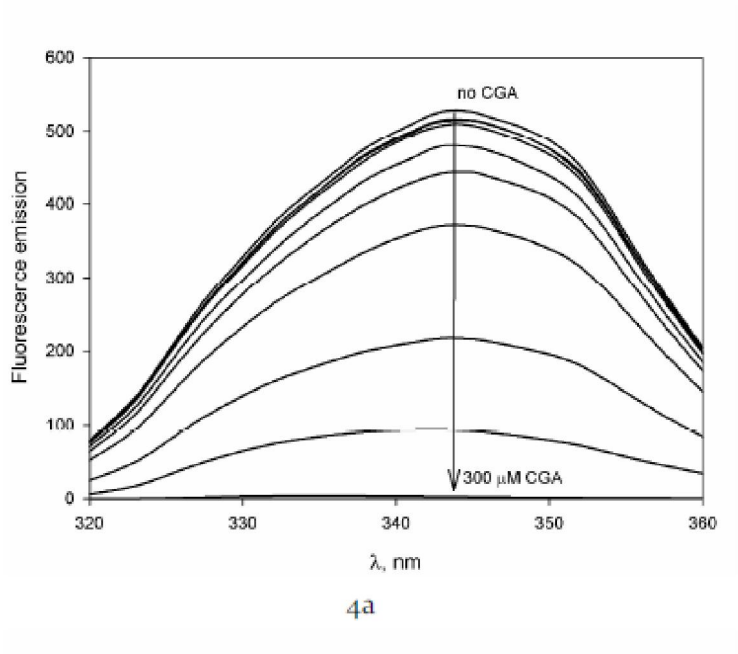
Subscriber access provided by GAZI UNIV

Article

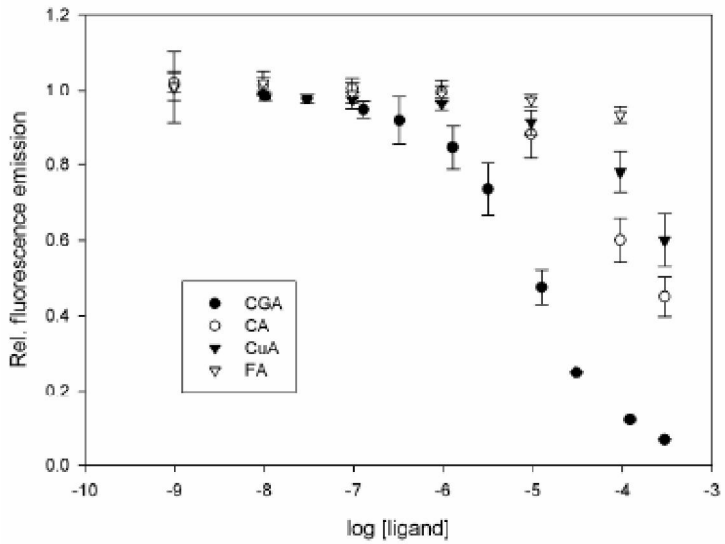
In silico design of short peptides as sensing elements for phenolic compounds

Michele Del Carlo, Denise Capoferri, Ivan Gladich, Filomena Guida, Cristina Forzato, Luciano Navarini, Dario Compagnone, Alessandro Laio, and Federico Berti

ACS Sens., **Just Accepted Manuscript** • DOI: 10.1021/acssensors.5b00225 • Publication Date (Web): 11 Jan 2016



4a



UNIVERSITÁ DEGLI STUDI DI TERAMO