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Electrochemical sensor and biosensor for polyphenols detection in olive oils

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Abstract

The aim of the work was to compare different techniques, in evaluating the phenolic content of an extra-virgin olive oil with varying storage time and storage conditions. A disposable screen-printed sensor (SPE) was coupled with differential pulse voltammetry (DPV) to determine the phenolic fractions after extraction with a glycine buffer; DPV parameters were chosen in order to study the oxidation peak of oleuropein, which was used as reference compound. A calibration curve of oleuropein was performed in glycine buffer 10 mM, pH = 2, NaCl 10 mM (D.L. = 0.25 ppm oleuropein, RSD = 7%). Moreover a tyrosinase based biosensor operating in organic solvent (hexane) was also assembled, using an amperometric oxygen probe as transducer. The calibration curves were realised using flow injection analysis (FIA) with phenol as the substrate (D.L. = 4.0 ppm phenol, RSD = 2%). Both of these methods are easy to operate, require no extraction (biosensor) or rapid extraction procedure (SPE), and the analysis time is short (min). The results obtained with these two innovative procedures were compared with a classical spectrophotometric assay using Folin–Ciocalteau reagent and HPLC analysis. Other extra-virgin olive oil quality parameters were investigated using classical methods in order to better define the alteration process and results are reported.

Keywords: Polyphenols; Screen-printed electrodes; Tyrosinase biosensor; HPLC; Folin–Ciocalteau reagent

1. Introduction

Phenolic compounds are natural antioxidants and are important factors to be considered in order to evaluate the quality of an extra-virgin olive oil since they are partly responsible for its auto-oxidation stability and organoleptic characteristics (Robards & Antolovich, 1997; Robards, Prenzler, Tucker, Swatsing & Glover, 1999; Ryan & Robards, 1998). The phenolic content is correlated with many quality parameters (also regulated by EU laws) such as the oxidation level (monitored by the peroxide numbers or UV specific absorbance) or free fatty acidity. Free fatty acids provide an index of the degree of lipase activity and can produce undesirable aromas in the oil; a high value of free fatty acid content indicates a high degree of lipase activity and hence a reduced antioxidants content. Moreover, the oxidation level is dependent upon the composition of the oil and therefore upon the degree of unsaturation and the presence of antioxidants such as phenols (Robards & Antolovich, 1997; Robards et al., 1999).

The phenolic content of a virgin olive oil is influenced by the cultivar, location, degree of ripeness, storage conditions of olive fruits as well as the type of oil extraction procedure used and olive oil storage conditions (Cinquanta, Esti & La Notte, 1997; Cinquanta & La Notte, 1998; Ranalli, De Mattia & Ferrante, 1997). It is known that there is a reduction in the content of glycosidic and flavonoid compounds in olive oil compared to olive pulp. This reduction may be attributed to glycosidic modification or degradation as a result of oil extraction, which is due to the oil production process (Ryan & Robards, 1998).

The innovative idea of this work is to monitor the variation with the time of the polyphenol content in an extra-virgin olive oil using two rapid procedures based on disposable sensors for differential pulse voltammetric analysis and on an amperometric tyrosinase based biosensor operating in organic solvent (Campanella, Favero, Sammartino & Tomassetti, 1994; Campanella,

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Fortuney, Sammartino & Tomassetti, 1994; Campanella, Favero, Pestorino & Tomassetti, 1999; Campo Dall'Orto, Danilowicz, Rezzano, Del Carlo & Mascini, 1999; Romani, Minnuni, Vincieri, Del Carlo & Mascini, 1999). Another important aim is to compare the results with that obtained by classical analysis.

The voltammetric sensor is realised with screen-printing technology; this technique permits mass-production of low cost, disposable, easy to use and reproducible electrodes. The disposability of the electrodes eliminates electrode surface regeneration step, which is often considered a time consuming step.

Analysis of olive oil samples with this device is performed after a liquid–liquid extraction that requires minutes rather than hours such as with classical phenol analytical methods (Ryan & Robards, 1998) (HPLC or spectrophotometry with the Folin–Ciocalteau reagent).

The biosensor analysis is based on the catalytic activity of the tyrosinase. The main advantage in the use of this device is that prior extraction is not necessary, as the biosensor is able to work in an organic solvent. Thus, pre-treatment of the sample is eliminated and the analysis time is decreased. Moreover, the experiments with the biosensor were performed in flow injection analysis, this achieves semi-automization of the entire procedure saving time and minimising the exposure of personnel to solvent vapors.

The two proposed sensors are relatively inexpensive, easy to operate, and require short analysis times compared with standard methods (HPLC and Folin–Ciocalteau reaction) for polyphenol analysis.

The interesting experimental results are that with the two innovative methods proposed we are able to monitor the degradation reaction of some polyphenol molecules present in oil, whilst with classical method only the final products of these degradation reactions are measured.

Extra-virgin olive oil samples were also analysed to evaluate quality parameters and to correlate the influence on these parametr of storage time and storage conditions with the influence on phenolic content.

2. Materials and methods

2.1. Reagents and apparatus

Isooctane, *n*-hexane, alumina, sodium carbonate and methanol were obtained from Carlo Erba (Milan, Italy); Uv–Vis spectrophotometer was from Varian, Milan, Italy; 1 cm cuvette thickness was used. Folin–Ciocalteau reagent, NaCl and H_3PO_4 was obtained from Merck (Darmstadt, Germany), the gallic acid, tyrosinase (EC 1.14.18.1., CAS number 9002-10-2, 50,000U) and glycine buffer was purchased from Sigma. Acetonitrile, methanol and HPLC water were purchased from Baker (The Netherlands), syringic acid was from Fluka (Milan, Italy). For the HPLC analysis a LC 410 Perkin-Elmer mod. 410 LC Pump was used with a Diode Array UV detector. Reverse-phase column Lichrosob RP-18 25 cm×0.46 cm was obtained from Supelco (Milan, Italy). Oleuropein was purchased from Extrasynhese Genay France. DPV experiments were carried out using an Amel polarographic analyzer model 433/W (Amel, Milan, Italy). The system for data acquisition and evaluation was computer controlled (software Amel 433/W). Screen-printed strips were prepared with a DEK Model 245 screen-printer (Weymouth, UK), using different inks obtained from Acheson Italiana (Milan, Italy): a graphite based ink (Elettrodag 423), a silver ink (Elettrodag 477 ss rfu) and an insulating ink (Elettrodag 6018 ss) were used. Flow injection amperometric analysis was performed with the tyrosinase biosensor, using a Oxyliquid potentiostat (Idronaut, Milan, Italy), a peristaltic pump Gilson mod. miniplus 3 (Villiers, France), a Rheodyne (USA) type 50 teflon rotary valve, a Teflon wall-jet cell, and Elkay (UK) solvent resistant tubing. Immobilon and polyethylene membrane were purchased from Millipore, USA.

2.2. UV spectrophotometric indices of the oxidation level

The method described in the EEC Regulation no. 2568/91 was used.

2.3. Pigments spectrophotometric determination

Ten g of extra-virgin olive oil was diluted to 25 ml with *n*-hexane. The spectrophotometric analysis was performed at $\lambda = 676$ nm. The content in chlorophyll has been calculated using the following formula: $E = E_0 C$, where $E = E^{1\%}_{1\,cm}$ referred to a concentration of 1000 mg/100 ml; C = concentration mg/100 ml, $E_0 = 0.613$ at $\lambda = 676$ nm for chlorophyll.

2.4. Spectrophotometric determination of polyphenols with the Folin–Ciocalteau reagent

A calibration curve of gallic acid in methanol was performed in concentration range 0.04–0.7 mg/ml. The solutions for the spectrophotometric analysis were performed as follows: in a 50 ml volumetric flask 1 ml of a standard solution of gallic acid, 6 ml of methanol, 2.5 ml of the Folin–Ciocalteau reagent, 5 ml of 7.5% Na_2CO_3 were added, reaching the final volume with purified water. The solutions were stored overnight and the spectrophotometric analysis was performed at $\lambda = 765$ nm. The determination of polyphenols was performed as follows: 2.5 g of extra-virgin olive oil was diluted with 2.5 ml of *n*-hexane and extracted three times by 5 min centrifugation (5000 rpm) with CH_3OH:H_2O 80:20 v/v. The extract was added to 2.5 ml Folin–Ciocalteau reagent, 5 ml of Na_2CO3 (7.5%), in a

50 ml volumetric flask reaching the final volume with purified water. The samples were stored overnight, and the spectrophotometric analysis was performed at $\lambda = 765$ nm.

2.5. HPLC determination of polyphenols

The analysis was performed as follows: 2 g of extra-virgin olive oil was diluted in a 10 ml centrifuge tube with 2.5 ml of *n*-hexane and 2 ml of 0.01 mg/ml syringic acid, the extraction was performed three times with 2.5 ml of CH_3OH:H_2O 80:20 v/v centrifuging for 5 min at 5000 rpm. The aqueous alcoholic extract was washed with 2×2.5 ml *n*-hexane and then concentrated to dryness under reduced pressure ($T = 35°C$). The residual product was diluted with 100 μl of CH_3OH and analysed by HPLC (injection volume 20 μl, mobile phase flow rate 1.0 ml/min). A ternary mobile phase was used: (a) water with H_3PO_4 0.5%, (b) acetonitrile, (c) mehanol, with the following gradient:

Time	A%	B%	C%
15	96	2	2
1	96	2	2
25	70	15	15
10	40	30	30
30	2	49	49

Syringic acid was used as the internal standard. A fixed wave length value (280 nm) was used for all experiments. Using the reported conditions many peaks are generally present in the chromatogram (Fig. 1) with a retention time lower than 50 min, and these were assigned and evaluated according to Cortesi, Azzolini, Rovellini and Fedeli (1995). The concentration of the different polyphenol compounds, expressed as ppm, was evaluated by the ratio of the peak area of the polyphenol investigated (A_p) versus the peak area of the internal standard ($A_{s.i.}$), using the following formula:

$$[P] = (A_p/A_{s.i.}) \times \text{r.f.}$$

where $[P]$ is the polyphenol concentration expressed in ppm, A_p is the peak area of the polyphenol, $A_{s.i.}$ is the peak area of the internal standard, r.f. is the response factor of tyrosol calculated using solutions of different concentration of tyrosol and a fixed concentration of syringic acid. This value was then used for quantitative analysis of each phenolic compound.

2.6. Differential pulse voltammetry (DPV) analysis

DPV conditions were: potential range 100–700 mV vs. screen-printed reference electrode, pulse amplitude 50 mV, scan rate 50 mV/s, pulse width 60 ms. Screen-printed three-electrode strips (a carbon working electrode and silver counter and reference electrodes) were employed for all experiments. The printing procedure is reported elsewhere (Cagnini, Palchetti, Lionti, Mascini & Turner, 1995). A scheme of the system is illustrated in Fig. 2.

The calibration curve of oleuropein was performed in glycine buffer 10 mM, pH 2, and NaCl 10 mM. DPV experiments were carried out using 100 μl of a solution dropped directly onto the sensor surface. To perform the analysis with real samples a prior extraction step was necessary with glycine buffer 10 mM pH2, NaCl 10 mM (oil:buffer = 1:10). The extract was diluted 1:10 with glycine buffer, 100 μl of this solution was deposited on the sensor surface and the DPV analysis was carried out. For each sample a new electrode was employed in order to eliminate surface fouling of the working electrode. Quantitative analysis of samples was performed using a calibration curve. The detection limit (LOD) was calculated as 3 times the standard deviation of the blank.

2.7. Tyrosinase biosensor analysis

The biosensor was developed using tyrosinase as the biological component immobilised onto a pre-activated membrane (Immobilon). The enzymatic membrane was placed on the head of an amperometric gas diffusion electrode for oxygen between the gas permeable membrane of the electrode and a dialysis membrane; the whole system was fixed on the metallic cap of the sensor by means of a Teflon O-ring. N-Hexane was used as carrier with a flow rate of 2 ml/min. The loop volume was 75 μl. In Fig. 3, the scheme of the biosensor and of the apparatus are reported.

The enzymatic membrane was prepared by dissolving 2 mg of enzyme and 3 mg of bovine serum albumin (BSA) in 60 μl of phosphate buffer 0.06 M, pH 6.6, KCl 0.1 M; to this solution 20 μl of a glutaraldehyde solution 2.5% were added. Seventy μl of this solution was deposited onto 2×2 cm membrane pieces. The membrane was then cut into pieces of 0.5×0.5mm. The enzymatic membranes were then washed with glycine buffer 0.5 M pH 10 for 10 min and are dried stored at −16°C. They were washed again in phosphate buffer for another 10 min before use. Quantitative analysis of samples was performed using a calibration curve.

The oxygen decrement signal was calculated using the following formula: $-[(L_1 - L_0)/L_0] \times 100$ where L_0 is the oxygen probe signal (in arbitrary unit) before adding the substrate and L_1 is the signal following the addition of the substrate. LOD was calculated as three times the standard deviation of the blank. All chemicals were purchased from Sigma.

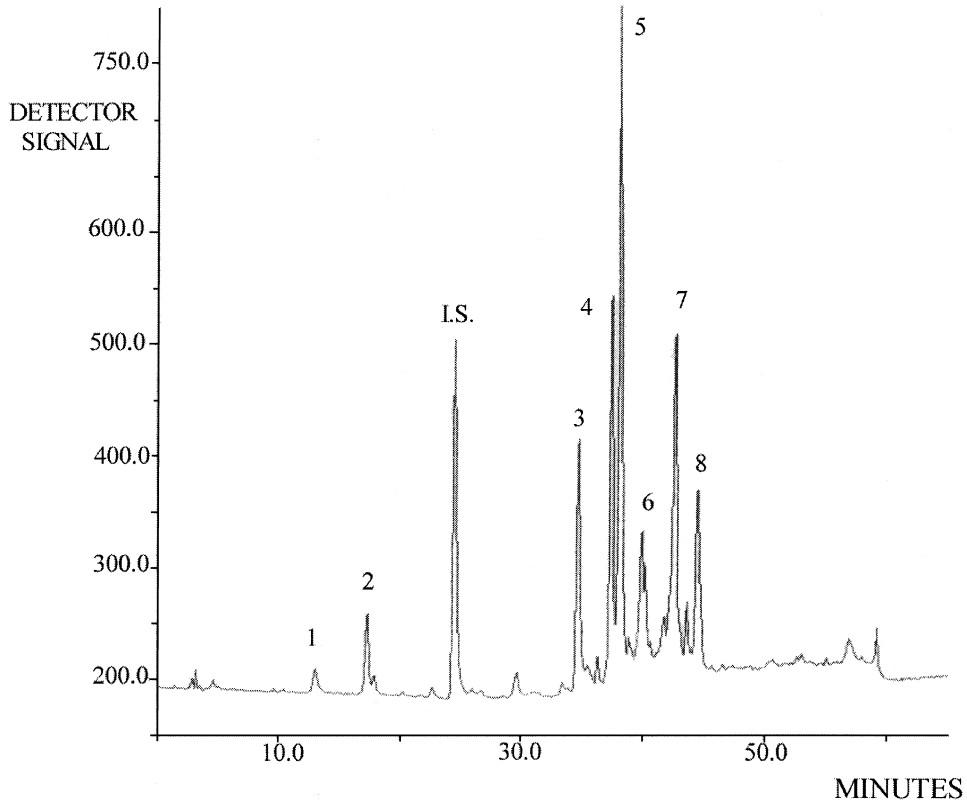

Fig. 1. HPLC chromatogram using a UV detector (280 nm): (1) tyrosol, 3 ppm; (2) hydroxytyrosol, 5 ppm; (3) deacetoxy oleuropein aglycone, 30 ppm; (4) deacetoxy ligstroside aglyc., 23 ppm; (5) ligstroside aglyc., 44 ppm; (6) oleuropein aglyc., 42 ppm; (7) ligstroside aglyc. dealdehyd., 18 ppm; (8) flavone quercitin, 4 ppm.

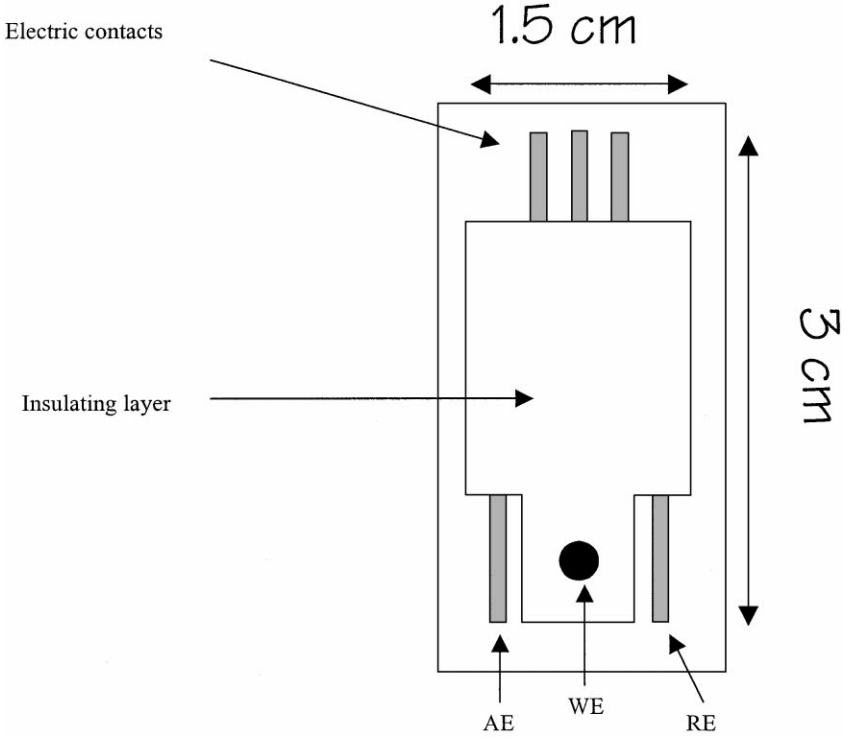

Fig. 2. Scheme of the screen-printed electrodes (SPE). The device consists of a graphite working electrode (WE), and silver counter (AE) and reference (RE) electrodes. All the conductive layers are printed with a silver ink. The surface of the working electrode is defined by the insulating layer.

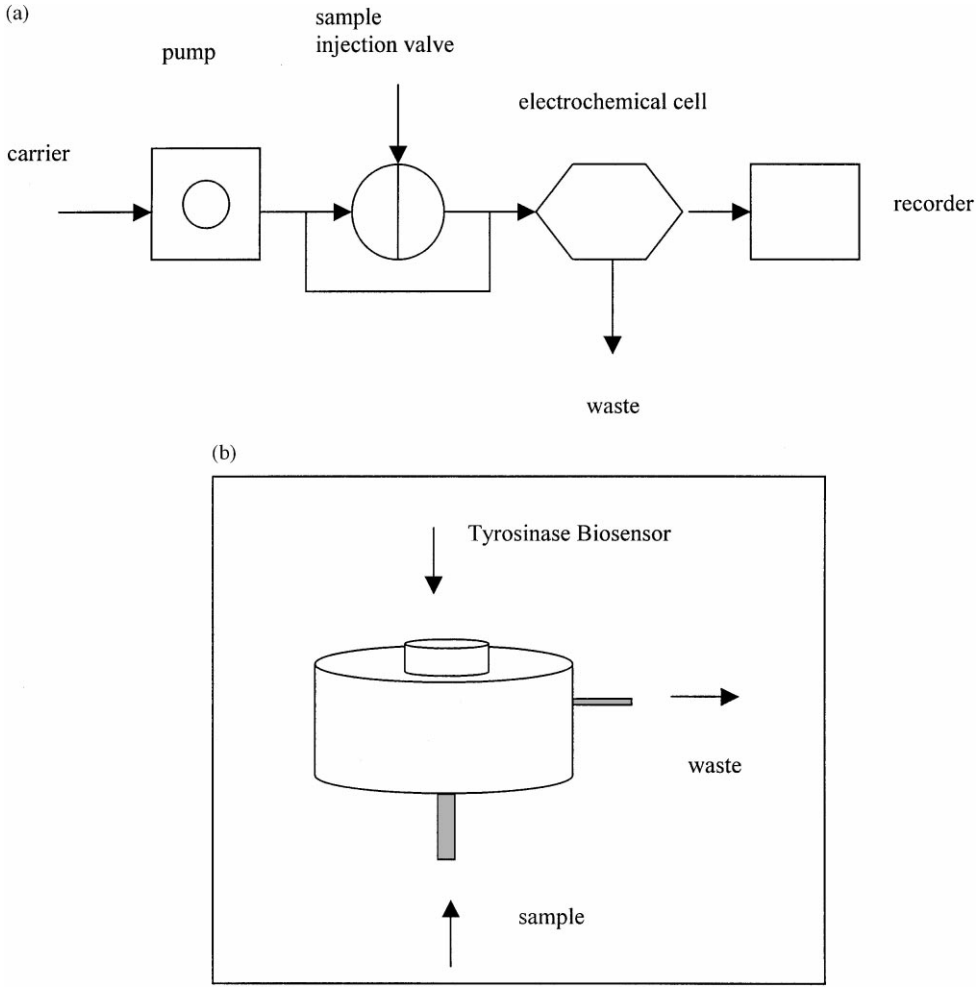

Fig. 3. Scheme of the apparatus (a) and of the wall-jet electrochemical cell (b) used for biosensor analysis.

3. Results and discussion

3.1. Biosensor analysis

The biosensor is based on the activity of the enzyme tyrosinase that catalyses the oxidation of the phenolic compounds, following the reaction scheme:

$$\text{phenol} + O_2 \rightarrow \text{hydroquinone} + H_2O$$

The oxygen consumption is amperometrically monitored by the oxygen probe. Some optimisation measurements were performed by just dipping the biosensor into the analyte solution. Preliminary experiments were carried out both in organic solution (*n*-hexane) and in phosphate buffer, pH 6.6, KCl 0.1 M. Fig. 4 shows that, even if in both solutions there is a linear increase of the signal with concentration, the signal value (expressed as the percentage of O_2 decrement) of the biosensor was higher in an organic solvent than in water. This is a particularly interesting result, since it shows that oil samples can be directly analysed in organic solvent without any extraction or further pre-treatment. Phenol was used as standard; in Table 1, sensitivity and reproducibility are reported and compared to that obtained with catechol, another frequently used substrate of tyrosinase. The enzymatic membranes were stored dried at $-16^{\circ}C$ and were stable for weeks.

A comparison of F.I.A. results with those obtained in a beaker (Table 2) show that even if in beaker analysis (that is dipping the biosensor in a stirred solution) it is possible to obtain a better sensitivity, but with F.I.A. the results are more reproducible. Nevertheless, since

Table 1
Comparison between different substrates: the measurements were carried out dipping the biosensor into the stirred *n*-hexane solution and adding the substrate[a]

Substrate	Sensitivity (ppm/A.U.%)	RSD%	Linearity range (ppm)
Phenol	0.2	10	0.5–6
Cathecol	0.1	20	0.5–5

[a] The current variation related to oxygen consumption were recorded after 3 min.

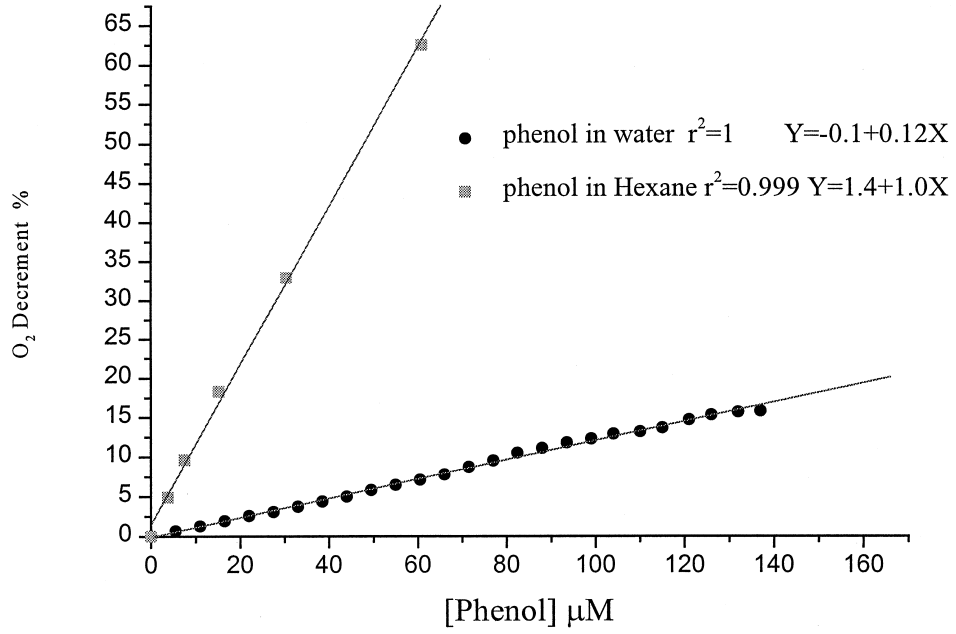

Fig. 4. Calibration curve for phenol in water and in *n*-hexane solution; the measurements were carried out dipping the biosensor (Clark electrode with tyrosinase immobilized on the electrode surface) into a stirred *n*-hexane solution and adding the substrate; the current variation related to oxygen decrement was recorded after 3 min (the same electrode is used for all curves in such kind of measurement).

the concentration range of polyphenols in olive oil is 50–500 ppm, both of these techniques can be used for monitoring the polyphenols in oil samples.

F.I.A. is used for real samples analysis also because it is easier to semi-automize, allows to minimise the exposure of personnel to solvent inhalation, and can be use in *field* experiments.

3.2. DPV experiments

In Fig. 5, a calibration curve of oleuropein used as standard compound is reported ($y = -33 + 201x$ (y = current, μA; x = oleuropein, ppm], $r^2 = 0.996$, LOD = 0.25 ppm oleuropein, RSD = 7%). The concentration range examined corresponds to 1/100 of the concentration found in extra-virgin olive oil. Therefore, the real samples were extracted and diluted with a suitable buffer to match the calibration curve (see DPV analysis in Materials and Methods). To perform these measurements the screen-printed sensors were used as disposable in order to eliminate the fouling of the working electrode surface due to the formation of an insulating polyphenolic film, that reduce sensor sensitivity and reproducibility. DPV analysis was carried out to eliminate the working electrode polarisation step which can be as long as 10–15 min; this time consuming step affects classical amperometric measurements while using a fast electrochemical technique such as DPV the analysis time is drastically reduced (few seconds).

Analysis of real samples were performed after a liquid–liquid extraction with glycine buffer.

Table 2
Comparison of results obtained with F.I.A. and in batch analysis[a]

Technique	LOD (ppm)	RSD% (n = 10, phenol 10 ppm)	Linearity range (ppm)
Batch analysis	0.04	10	0.5–6
F.I.A.	4	2	10–500

[a] Experimental conditions are reported in the text.

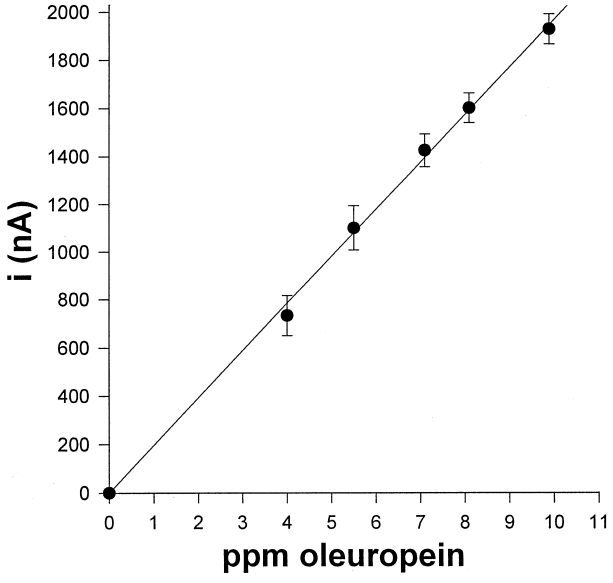

Fig. 5. Calibration curve of oleuropein using DPV; parameters are reported in the text; SPE strips are used as disposable. (each point for each electrode). 100 μl of glycine buffer with the standard oleuropein concentration is added to the electrode strip and DPV is carried out.

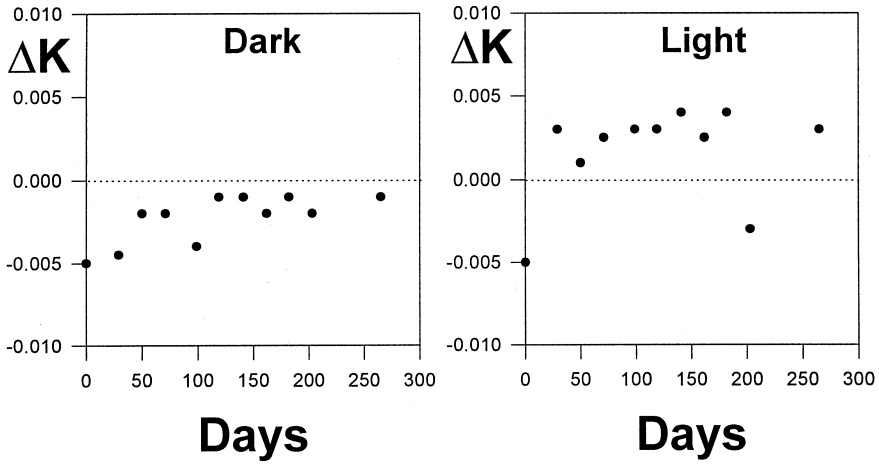

Fig. 6. Change in the oxidation level with storage conditions and time: K value is the specific absorbance of an oil, and ΔK value was calculated using the following formula: $\Delta K = (K_{270} - K_{266} + K_{274})/2$ (s. Appendix 9, Reg. EU 2568/91).

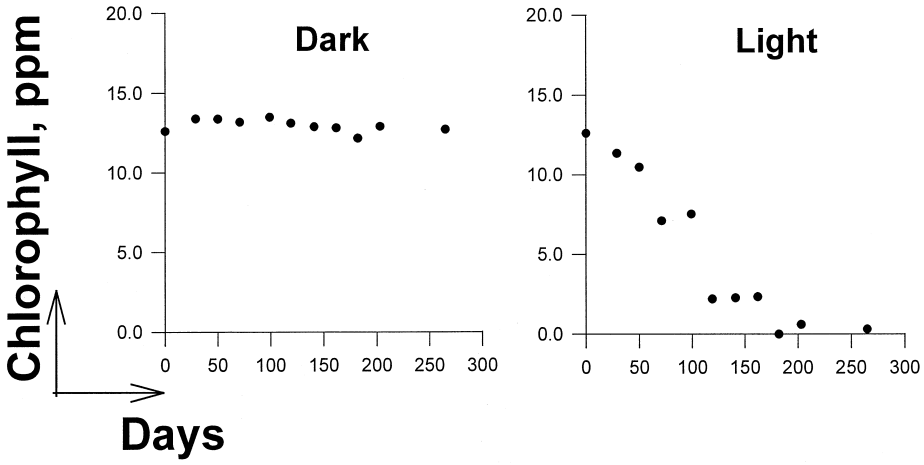

Fig. 7. Change of pigment content with storage conditions and time.

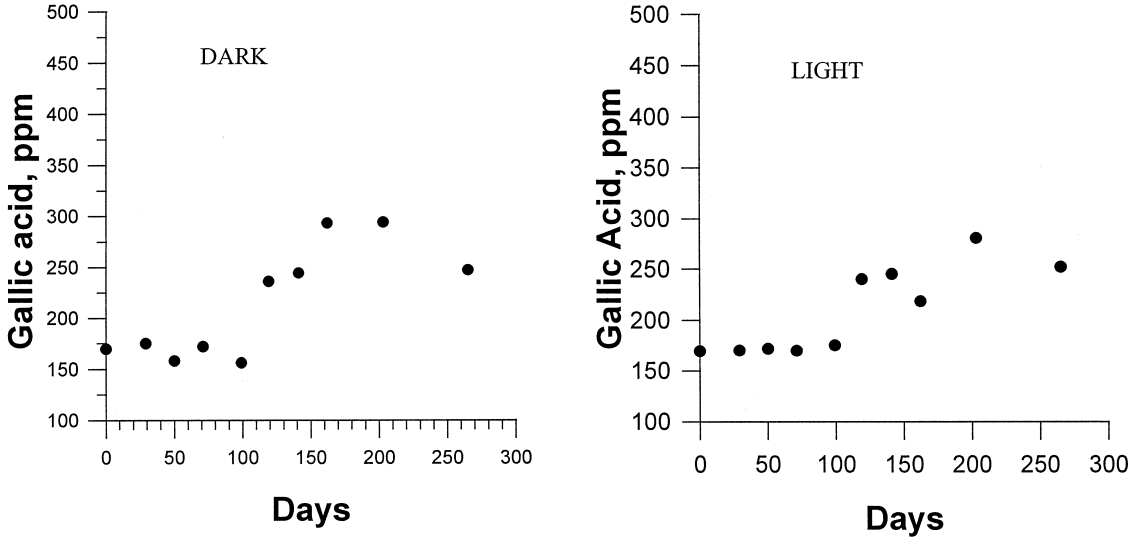

Fig. 8. Spectrophotometric analysis of the polyphenol content with Folin–Ciocalteau reagent.

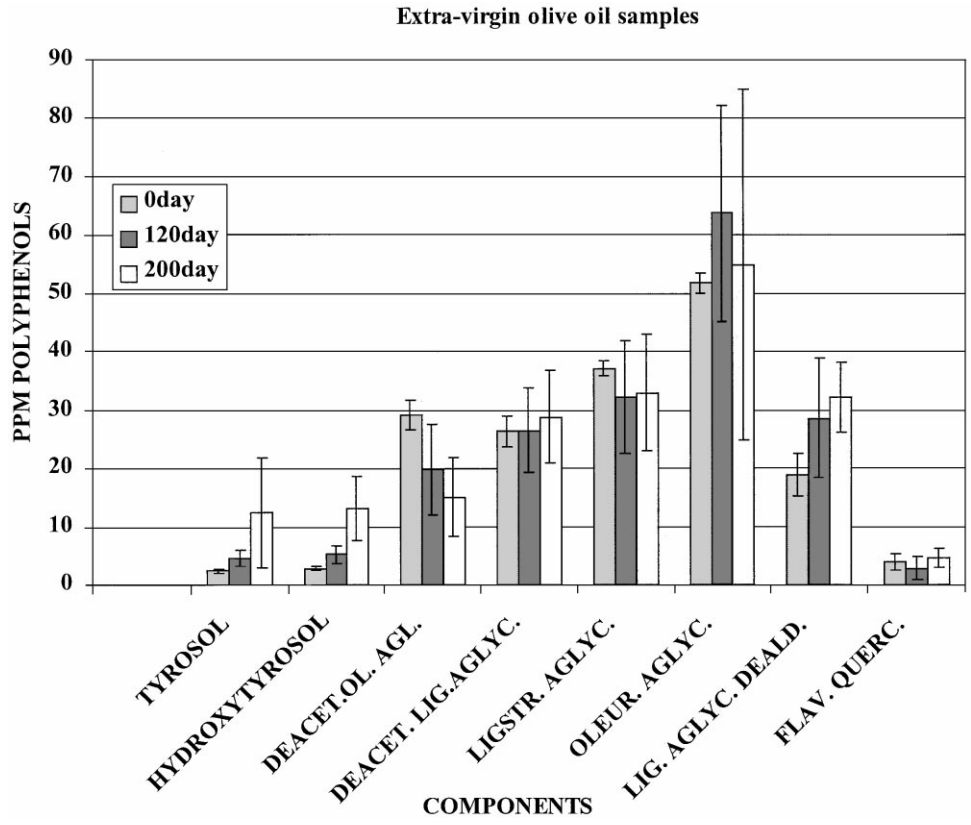

Fig. 9. Comparison of the change in concentration of various compounds in storage obtained by HPLC analysis.

3.3. Extra-virgin olive oil samples

The extra-virgin olive oil samples were collected at the same time and stored for 250 days in different conditions. Some of them were permanently exposed to artificial light and the others were stored in absence of light. During the storage time different analysis were performed, such as the UV spectrophotometric determination of the oxidation level as reported in Fig. 6. The ΔK value (oil specific absorbance) has to be in the range -0.01 to 0.01 for the EU regulations, and the increase of this value reflects the increase in the oxidation state of the sample. As expected, the sample stored at light exhibits a higher level of ΔK and then this appears soon and then remain constant. Therefore, this can be assumed as general index of extra-virgin olive oil.

The pigment content (expressed as chlorophyll content) was also influenced by the storage conditions. The pigments are continuously degraded by the light as reported in Fig. 7. They are considered as acting as prooxidants as reported in the literature (Cimato, Cantini, Sani & Marranci, 1997).

The classical spectrophotometric analysis of the polyphenols content with the Folin–Ciocalteau reagent (Fig. 8) reveals an increase in the total phenolic content (reported as ppm of gallic acid, a commonly occurring polyphenol molecule) during the examined time. Moreover no differences between the two series (light and dark) of samples are reported. The Folin–Ciocalteau reagent, although widely used, is not specific and detects all phenolic groups; thus the increase in the phenolic fraction of the examined samples, is probably due to the fact that during the storage time degradation reactions occur, and that they determine the fractionation of larger phenolic molecules with the formation of smaller compounds that can react with the Folin–Ciocalteau reagent.

Fig. 9 reports the variations of the different identified compounds in HPLC during the storage. Light and dark do not change the trend. Tyrosol and hydroxytyrosol, considered as final products of degradation reactions, are present in low quantities at the beginning but their concentrations increase with time; on the contrary for other compounds such as deacetoxyoleuropein aglycone the concentration trend is the opposite. This can explain the constant value of the ΔK over time (Fig. 6); some phenols decrease and others increase. The behaviour explains also the Folin–Ciocalteau trend (Fig. 8), phenols with low molecular weight increase with time and are able to react with such reagents.

The analysis performed with the DPV-SPE sensor is reported in Fig. 10a. No differences are observed between the two series of samples (light and the dark), but a general trend of decreasing in the polyphenols content is evident. This behaviour is also confirmed by the biosensor analysis (Fig. 10b).

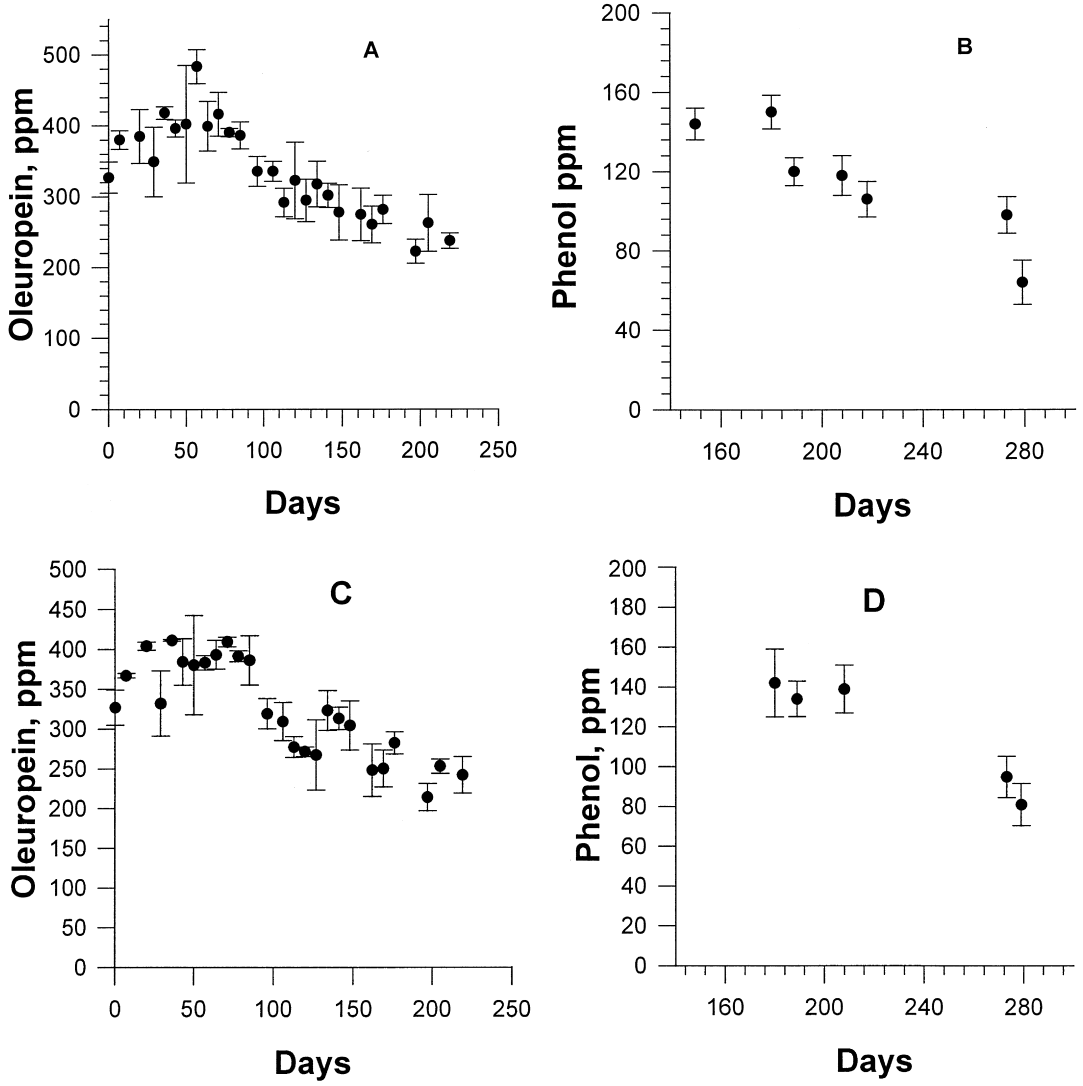

Fig. 10. Analysis of extra-virgin olive oil samples with DPV-SPE (dark 10a, light 10c) and with a biosensor (dark 10b, light 10d).

From the reported results, it seems that the DPV analysis measures polyphenol compounds that have a structure similar to the oleuropein molecule, which are degraded with time (Fig. 9) and thus a decreasing trend is found. A similar explanation can be used for the biosensor results.

Thus, the two proposed methods monitor the degradation reactions of oleuropein derivatives occurring in an extra-virgin olive oil during storage, while the spectrophotometric analysis reveals the final products of these degradation reactions.

HPLC analysis performed using the reported conditions confirms such hypothesis.

4. Conclusions

All the methods reported monitor the variation in the content of polyphenols in extra-virgin olive oil; nevertheless, they describe two different aspects of the same phenomena. The two innovative methods are correlated and detect the degradation reaction of large molecules (such as oleuropein derivatives) in smaller ones. Classical methods analyse and quantify the final products of this degradation reaction. The innovative and classical methods can be correlated until alteration reactions occur.

The two proposed methods are more rapid and inexpensive in comparison with the classical methods for polyphenols analysis, and can be considered promising systems for the evaluation of this class of compounds in oil samples.

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References

Cagnini, A., Palchetti, I., Lionti, I., Mascini, M., & Turner, A. P. F. (1995). *Sensor and Actuators B, 24–25,* 85–89.

Campanella, L., Favero, G., Pastorino, M., & Tomassetti, M. (1999). *Biosensor & Bioelectronics, 14,* 179–186.

Campanella, L., Favero, G., Sammartino, M. P., & Tomassetti, M. (1994). *Talanta, 41*(6), 1015–1023.

Campanella, L., Fortuney, A., Sammartino, M. P., & Tomassetti, M. (1994). *Talanta, 41,* 1397–1404.

Campo Dall'Orto, V., Danilowicz, C., Rezzano, I., Del Carlo, M., & Mascini, M. (1999). *Anal. Letters, 32*(10), 1981–1990.

Cimato A., Cantini C., Sani G., & Marranci M. (1997). *Il germoplasma dell'olivo in toscana* (2nd ed.). Regione Toscana, 1997.

Cinquanta, L., Esti, M., & La Notte, E. (1997). *JAOCS, 74*(10), 1259–1264.

Cortesi N., Azzolini M., Rovellini P. & Fedeli E. (1995). *La Rivista Italiana delle Sostanze Grasse* (Vol. LXXII) (pp. 241–251) June 1995.

Esti, M., Cinquanta, L., & La Notte, E. (1998). *Journal of Agricultural and Food Chemistry, 46,* 32–35.

Ranalli, A., De Mattia, G., & Ferrante, M. L. (1997). *International Journal of Food Science and Technology, 32,* 289–297.

Robards, K., & Antolovich, M. (1997). *Analyst, 122,* 11R–34R.

Robards, K., Prenzler, P. D., Tucker, G., Swatsing, P., & Glover, W. (1999). *Food Chemistry, 66,* 401–436.

Romani A., Minunni M., Vincieri F. F., Del Carlo M., & Mascini M. (in press). *Journal of Agricultural and Food Chemistry*.

Ryan, D., & Robards, K. (1998). *Analyst, 123,* 31R–44R.