Comparison among Differential Pulse Voltammetry, Amperometric Biosensor, and HPLC/DAD Analysis for Polyphenol Determination

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Polyphenols are widespread in vegetables and fruits. They can play an important role in human diet and health, and they influence the sensorial properties of many foods, and act as natural antioxidants. This study was conducted using HPLC/DAD, tyrosinase biosensor, and differential pulse voltammetry (DPV) analyses to detect polyphenolic compounds in natural complex matrices. The analyses were applied to a series of both standards and natural extracts derived from grape, olives, and green tea. The pure compounds include phenolic acids, flavones, flavonols, catechins, tannins, and oleuropein. HPLC/DAD, DPV, and the biosensor approach were used as independent analytical techniques. Bare graphite screen-printed electrodes were employed in DPV and in the biosensor analysis. The most accurate data were obtained by HPLC/DAD analysis, while the DPV approach using screen-printed electrodes could represent a quick screening method for the determination of polyphenols in natural extracts. Use of the biosensor for the analysis of complex matrices needs further study in order to improve its performance.

Keywords: *Polyphenols; anthocyanins; green tea; EGCG; olives; grapes*

INTRODUCTION

Polyphenols are natural molecules containing one or more hydroxylated rings. They can be divided into different subclasses such as phenylethanol derivatives, phenolic acids, flavones and flavonols, catechins, procyanidins, and anthocyanins.

Polyphenols are widespread in vegetables and fruits and play an important role in human diet and health. They also contribute to the taste of some foods, influencing the sensorial properties (i.e., olive oil and wine). Polyphenols act mainly as antioxidants and radical scavengers (Ghiselli et al., 1998), and recently, some of them have shown hypolypidaemic (Visioli and Galli, 1994), hypocholesterolemic (Ficarra et al., 1991), and anticarcinogenic properties (Hirose et al., 1994; Troll et al., 1994).

Among polyphenols, the anthocyanins are used as natural red colorants for acid foods such as soft drinks, jams, and red wines. The potential for various food plants as a commercial source of anthocyanins is limited either by their chemical stability or availability of raw material related to economic considerations (Jackman and Smith, 1996). Interest in polyphenols is related also to their use as taxonomic markers in phytochemistry and for their impact on fruit quality (Vlahov et al., 1992; Amiot et al., 1989).

In the literature, several analytical methods are proposed for the quali-/quantitative evaluation of polyphenols in different matrices. A recent paper provides an excellent overview of analytical techniques applied for bioflavonoid detection (Robards and Antholovich, 1997). HPLC/DAD has been widely applied to polyphenol analysis. Electrochemical detection methods, coupled with chromatographic methods, have also been used for polyphenol determination, particularly HPLC with coulometric detection (Achilli et al., 1993) and liquid chromatoghaphy with voltammetric detection (Roston and Kissinger, 1991). Madigan and co-workers developed a dual-channel electrochemical detection system coupled to HPLC that is particularly suited for direct determination of flavanols in beer samples and for the analysis of acetone extracts of barley samples (Madigan et al., 1994).

Among electrochemical techniques, direct oxidation of phenolic compounds on graphite electrodes has also been applied. The main drawback of phenol electrochemical oxidation is the deactivation of the electrode surface. This is due to the formation of a passivating-polymeric film produced by the coupling of electrogenerated phenoxy radical (Wang et al., 1991). Experimentally, this phenomenon can be observed as a decrease in the oxidation current and an increase of the oxidation potential when consecutive cycles are performed on the same electrode. A negative effect of this behavior is the low reproducibility of the measurement.

Among electrochemical approaches, the development of an amperometric biosensor for phenolic compounds has been reported by several authors. Burested and coworkers coupled on-line a solid-phase extraction and fractionation with the biosensor detection for phenolic compounds in surface waters (Burested et al., 1995).

Behavior of the tyrosinase enzyme electrode has also been investigated under different experimental condi-

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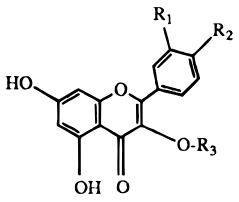
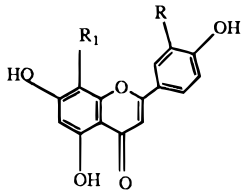
10.1021/jf990767e CCC: $19.00
Published on Web 03/07/2000

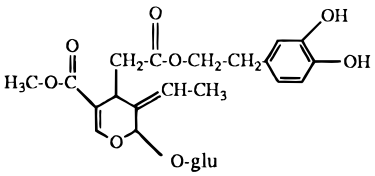
Table 1. Chemical Structures of Some Tested Polyphenols



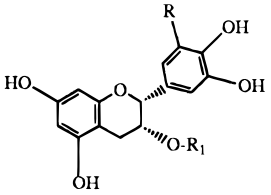
R_1	R_2	R_3	
H	OH	H	Kaempferol
OH	OH	H	Quercetin
OH	OH	rutinose	Rutin



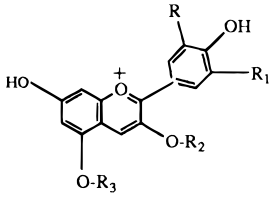
R	R_1	
OH	H	Luteolin
H	glucose	Vitexin



Oleuropein



R	R_1	
H	H	(-) epicatechin
OH	galloyl	(-) epigallocatechin gallate



R	R_1	R_2	R_3	
OCH_3	OCH_3	glucose	glucose	Malvin
H	H	glucose	H	Callistephin
H	OH	rutinose	H	Keracyanin

tions (Campanella et al., 1994). Several authors (Iwuoha et al., 1995; Stancick et al., 1995; Wang et al., 1992; Campanella et al., 1994b) have tested the performance of the tyrosinase electrode in different organic solvents, and the effect of different additives has also been investigated (Lutz, 1995). The electrodes used in the electrochemical detection of polyphenols are carbon paste electrodes and, additionally, Pt or glassy carbon electrodes.

Recently, the use of screen-printed electrodes has also been proposed to evaluate the polyphenol content, and they can represent an alternative device for the detection of these compounds. Screen-printing technology is used for the production of disposable sensors. These sensors are very attractive for the electrochemical detection of phenolic compounds because during the oxidation process a polymeric film is formed on the electrode surface leading to electrode surface "inactivation". Therefore, the disposable aspect of these sensors is especially important in order to overcome the described phenomenon referred to as "electrode fouling", which is one of the main drawbacks of common graphite-based electrodes.

The present paper reports a study on both a series of standard polyphenols and natural polyphenolic extracts. The pure compounds include phenolic acids, flavones, flavonols, catechins, anthocyanins, and oleuropein. The natural matrices were grape and olive extracts containing anthocyanins and green tea extract with catechines. HPLC/DAD, differential pulse voltammetry (DPV), and the biosensor approach were used as independent detection systems for the analysis of all these samples.

In the present paper, results obtained from both electrochemical and HPLC/DAD techniques are compared. The aims of this study were to test quick electrochemical screening methods for determination of polyphenols in natural matrices, to evaluate selectivity and sensitivity of the different methods employed, and to investigate the possibility of new applications of sensor devices to on-line/off-line quality process control.

MATERIALS AND METHODS

Materials. Pure compounds used as standards were purchased from Extrasynthese s.a., Lyon, Nord-Genay, France; the main structures are shown in Table 1.

The tested compounds were 4-OH-phenylethanol (tyrosol), gallic acid, caffeic acid, chlorogenic acid, luteolin, kaempferol, quercetin, quercetin 3-*O*-rutinoside (rutin), malvidin 3,5-*O*-diglucoside (malvin), cyanidin 3-*O*-rutinoside (keracyanin), pelargonidin 3-*O*-glucoside (callistephin), (+)-catechin, (−)-

epicatechin 3-*O*-gallate (ECG), (−)-epigallocatechin 3-*O*-gallate (EGCG), and oleuropein.

Sample Preparation. *Green Tea Extract*. Three milligrams of a commercially available sample (Indena Milan, Italy) was dissolved in 1 mL of phosphate buffer pH 7.4 (100 mmol L^{-1}). This sample was analyzed as such by HPLC/DAD and suitably diluted for DPV and biosensor analysis.

Grape Extract. The sample extract was obtained from berries of *Vitis vinifera L*. Fresh peels (100 g) were manually separated from the fruit pulp, dipped in liquid N_2, and then blended in an Osterizer. The frozen powder was then extracted more times (300 mL × 4) with H_2O acidified to pH 2.5 with tartaric acid 0.75% at 25 °C. The aqueous extract was concentrated under vacuum (T = 28−30 °C) up to 250 mL.

Olive Extract. The olive fruits were from the Tuscan cultivar Scarlinese. A 40 g portion of the frozen pulp was ground and then extracted with 4 × 400 mL of EtOH/H_2O (pH 2.0 by formic acid) 80:20. A volume of 500 mL of the aqueous ethanolic extract was concentrated under reduced pressure (T = 28 °C). The aqueous solution was then extracted various times with *n*-hexane, evaporated, and rinsed with acid water (pH 2.5 by formic acid). The aqueous solution was extracted with *n*-hexane to completely remove lipophilic compounds, and the residual aqueous phase was concentrated (T = 26 °C) to 20 mL and fractionated by liquid−solid extraction on the Extrelut cartridge (Merck, Darmstadt, Germany). The following elution steps were performed 20 min after the deposition: *n*-hexane, EtOAc, and acid MeOH (pH 2.5 by HCOOH) up to a colorless eluate.

The MeOH fraction, containing the anthocyanin compounds, was concentrated to 20 mL and directly analyzed by HPLC/DAD. An appropriate dilution was performed for DPV and biosensor analyses. To ensure the stability of the anthocyanic extracts, the pH value was maintained below 2.0 and the samples were stored at −18 °C.

Apparatus and Measurements. *HPLC/DAD Analysis*. The HPLC/DAD analyses were performed on an HPLC HP1090 L liquid chromatograph equipped with an HP1040A DAD detector and managed by an HP 9000 workstation (all from Hewlett-Packard, Palo Alto, CA).

The green tea extract was analyzed by the previously reported method (Romani et al., 1996). The anthocyanic fractions of olives and grapes were analyzed according to Baldi and co-workers (Baldi et al., 1993, 1995).

The HPLC/DAD quantitative analyses were performed by applying four-point calibration curves for each standard. The regression coefficient values (R) were in the range 0.971−1.00. The reference compounds were (−)-epigallocatechin gallate (EGCG) for the green tea extract, malvin for grape skin extract, and keracyanin for anthocyanic olive extract.

Electrochemical Techniques. *DPV and Biosensor Analyses*. Both electrochemical techniques employed screen-printed electrodes. These electrodes were printed in our laboratory following an optimized procedure (Cagnini et al., 1995**)** using a Model 245/Screen printer obtained from DEK (Weimouth, UK) and employing different inks from Acheson Italiana (Milan, Italy). A graphite-based ink (Electrodag 433), a silver ink (Electrodag 477 SS RFU), and an insulating ink were used. The substrate was a flexible polyester film (Autostat HT5) obtained from Autotype Italia (Milan, Italy).

The electrochemical device consists of three independent electrodes placed one next to another to form a rectangle 3 cm high and 1.5 cm wide comprising a screen-printed graphite working electrode, a silver reference, and a counter-electrode. The distance between the electrodes is 3 mm. Each printing process results in a sheet containing 40 devices. Therefore, these electrodes are considered inexpensive and designed to be for a single use. Compared with devices obtained from different printed sheets, the reproducibility among our screen-printed electrodes is more than 90%. The system, without any modification, was used for the differential pulse voltammetry (DPV). For the development of the biosensor, the graphite-working electrode was modified by a chemical coupling with the tyrosinase (see biosensor).

Differential Pulse Voltammetry (DPV). DPV was performed using the screen-printed device, consisting of a bare graphite working electrode, a silver reference electrode, and a silver counter electrode. To perform DPV analysis, an AMEL polarographic analyzer model 433/W (Amel, Milan, Italy) was used. The system for data acquisition and evaluation was computer controlled (Software Amel 433/A). The potential range was 0/+800 mV, the scan speed was 20 mV/s, the pulse repetition was 0.1 s, the pulse amplitude was 20 mV, the pulse width was 50 ms, and the sampling time was 8 ms. The standard concentrations tested were in the range 10−100 μmol L^{-1}. A 100 μL portion of the sample solution was deposited on the three-electrode system in order to complete the electrochemical cell, and the measurement was performed. Calibrations with standards were performed in the range 10−100 μmol L^{-1} at specific peak potential values (Table 2). To consider the matrix effect, the single standard addition method was employed for the polyphenol evaluation in green tea, grape, and olive extracts.

Table 2. DPV Analysis[a]

compound	E_i (mV)	linear regression (y)	correlation coefficient (R)
gallic acid	434	6.4 × 0.1	0.99
caffeic acid	474	3.1 × −6.3	0.93
chlorogenic acid	470	49.3 × −36.8	0.99
luteolin	517	22.0 × −2.3	0.99
kaempferol	435	2.9 × −4.4	0.96
callistephin	590	2.2 × 1.5	0.99
(+)-catechin	450	32.9 × 61.1	0.99
(−)-EGCG	120	0.025 × −0.01	0.99
rutin	383	14.2 × 77.6	0.99
malvin	595	40.0 × 131.8	0.99
keracyanin	384	13.3 × 47.7	0.99
oleuropein	410	73.1 × −26.4	0.99

[a] Peak potential (E_i), linear regressions, and correlation coefficient (R) calculated from calibration curves of the pure standards (10−100 μmol L^{-1}). All the measurements were performed at pH 2.0 except for EGCG, which was performed at pH 6.7.

Biosensor Analysis. The biosensor was developed using tyrosinase (Sigma, St. Louis) as a biological component immobilized on graphite screen-printed electrodes. A solution of tyrosinase 0.5 mg mL^{-1} buffer (100 mmol L^{-1} phosphate buffer solution pH 7.4) with glutaraldehyde (0.25% v/v) was prepared. Tyrosinase is a tetrameric protein (MW 128.000 D) with two active sites/molecule. The enzyme catalyzes the oxidation of the phenolic substrate to a quinonic form that is reduced at the electrode polarized at a fixed potential. The working potential was −200 mV, except for EGCG and rutin, which had a working potential of −400 mV. The specificity of the system relies on the immobilized enzyme, which selectively oxidizes phenolic compounds. During measurement, the amount of oxidized substrate by the enzyme is negligible so this technique is considered noninvasive.

Assembly of the Sensor. A 10 μL portion of the enzyme solution was deposited on the working electrode surface and allowed to dry for 1 h. The enzyme adsorption, with a chemical binding to the electrode surface, was ensured by glutaraldehyde before the biosensor was ready for use. All measurements were performed in the batch mode at room temperature by adding a standard solution to the buffer. The signal was taken in steady-state conditions at the plateau of the electrochemical response, and the current signal was controlled performing the appropriate calibration curves.There is a linear correlation between the substrate present in the matrix and the current signal.

The biosensor, when not used immediately, was stored at 4 °C. Calibrations were performed in the range 10−100 μmol L^{-1}, and the single standard addition method was employed for analyte evaluation in the vegetal matrices of green tea, grape, and olive extracts.

For amperometric measurements, a potentiostat from Methrom, Switzerland,was used in combination with a potentiometer Amel model 336 (Amel, Milano, Italy). The current signal was transferred to a BD single channel Kipp Zone recorder (Carlo-Erba, Genoa, Italy). The current was recorded at −200

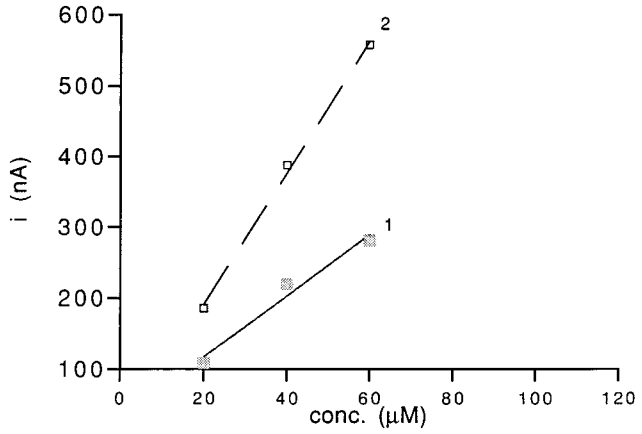


Figure 1. Response curves for EGCG (**1** and **2**) recorded with the tyrosinase biosensor at −200 mV (**1**) and −400 mV (**2**).

mV for all the analyzed compounds except for (−)-epigallocatechin 3-gallate (EGCG) and rutin, where −400 mV was applied. The concentrations tested were in the range 10−100 μmol mL^{-1}.

RESULTS AND DISCUSSION

Pure Compounds. The pure compounds were tested with the DPV and biosensor methods to choose the most suitable potential for their quali-/quantitative determination. The anthocyanic compounds were not tested with the biosensor because the optimal pH value of the tyrosinase (7.0) is not compatible with the chemical stability of these pigments. The degradation of these molecules can be easily observed with the disappearance of their characteristic red color when the pH values go over 4.0. The red color, due to the presence of the flavilium ion, is maintained only at pH values below 2.0 (Brouillard, 1988).

HPLC/DAD analyses were also performed to acquire the retention times as well as UV−vis spectra of the different standards. In Table 1, some chemical structures of the analyzed compounds are reported.

DPV Analysis. The peak potentials (E_i), the linear regressions, and the correlation coefficients (R) obtained with different standards by DPV analysis are shown in Table 2. For all the pure compounds, the R values were 0.99, except for kaempferol (0.96) and for caffeic acid (0.93). Observing the slope values of the linear regressions, the highest sensitivity is shown by oleuropein followed by chlorogenic acid, malvin, catechin, and luteolin.

Biosensor. For all the compounds, the optimal working potential was estimated by hydrodynamic voltammogram; the applied range was −400/+100 mV. For most of them, −200 mV was the optimal potential, as previously reported in the literature (Madigan et al., 1994; Burested et al., 1995; Campanella et al., 1994; Wang et al., 1992).

In the case of EGCG and rutin, the working potential was −400 mV since at this potential better results were obtained. The intensity response (nA) for EGCG is reported in Figure 1 and it was analogously observed for rutin. Table 3 shows the linear regressions obtained with the pure standards. The R values of the regression curves are 0.99 for all the compounds, indicating very good performance of the biosensor with the tested standards. In addition, it is interesting to note how the slope values are quite different: the highest sensitivity belongs to EGCG followed by tyrosol and oleuropein.

Table 3. Tyrosinase Biosensor Analysis: Linear Regressions and Correlation Coefficient (R) of Regression Line Calculated from Calibration Curve of Pure Standards (10−100 μmol L^{-1})[a]

compound	linear regression (y) (pH 6, 7)	correlation coefficient (R)
tyrosol	3.08 × 9.28	0.99
gallic acid	0.05 × 0.42	0.99
caffeic acid	0.74 × 0.62	0.99
chlorogenic acid	0.13 × 0.99	0.99
luteolin	0.71 × 6.03	0.99
quercetin	0.77 × 5.45	0.99
(−)-EGCG	9.3 × 5.33	0.99
oleuropein	2.01 × 12.19	0.99

[a] The working potential was −200 mV, while for EGCG and rutin it was −400 mV.

Table 4. Estimated Concentration of Total Polyphenols in Natural Extracts. Comparison among Results Obtained by HPLC/DAD, DPV, and Biosensor Analysis: (a) Green Tea Extract, (b) Grape Skin Extract, and (c) Olive Extract[a]

(a) green tea extract	HPLC/DAD (mmol L^{-1})	DPV (mmol L^{-1})	biosensor (mmol L^{-1})
polyphenolic content expressed in EGCG	4.7 ± 0.09	4.5 ± 0.16	3.0 ± 0.42

(b) grape skin extract	HPLC/DAD (mmol L^{-1})	DPV (mmol L^{-1})
anthocyanic content expressed in Ma	2.8 ± 0.02	2.6 ± 0.12

(c) olive extract	HPLC/DAD (mmol L^{-1})	DPV (mmol L^{-1})
anthocyanic content expressed in Cy-3R	3.9 ± 0.06	2.5 ± 0.32

[a] The reported data are the means ± SD of three determinations.

Natural Extracts. Olive, grape skin, and green tea polyphenolic extracts were tested by applying the two electrochemical methods and using HPLC/DAD as the reference technique because it allows identification and quantification of each single phenol. To indirectly evaluate the matrix effect, the data obtained by HPLC/DAD were considered the most accurate results as compared to findings obtained by applying electrochemical methods.

For green tea, all three techniques were applied while for the anthocyanic fractions (olive and grape skin extracts) only HPLC/DAD and DPV were employed. In fact, these compounds are stable only at low pH values, and this condition is not compatible with biosensor use.

Green Tea Extract. The main polyphenolic compounds present in the commercial extract are catechins and gallic acid (Ho et al., 1994). The analyses were performed at pH 6.7 by phosphate buffer with all three techniques. The stability of these compounds at pH 6.7 was checked by HPLC analysis, and the relative chromatogram at 280 nm is shown in Figure 2 where the main compounds are EGCG (**5**) and ECG (**6**). The quantitative findings obtained by HPLC/DAD, DPV, and biosensor are expressed in EGCG, which represents 55% of the total polyphenolic content. The quantitative HPLC data were calculated at 280 nm as the sum of all the compounds indicated in Figure 2 (gallic acid, catechin and its derivatives), taking into account their relative molecular weights. The DPV quantitative valuation was carried out at 120 mV corresponding to the peak potential of EGCG. The obtained results, reported in Table 4a, show that DPV and the biosensor under-

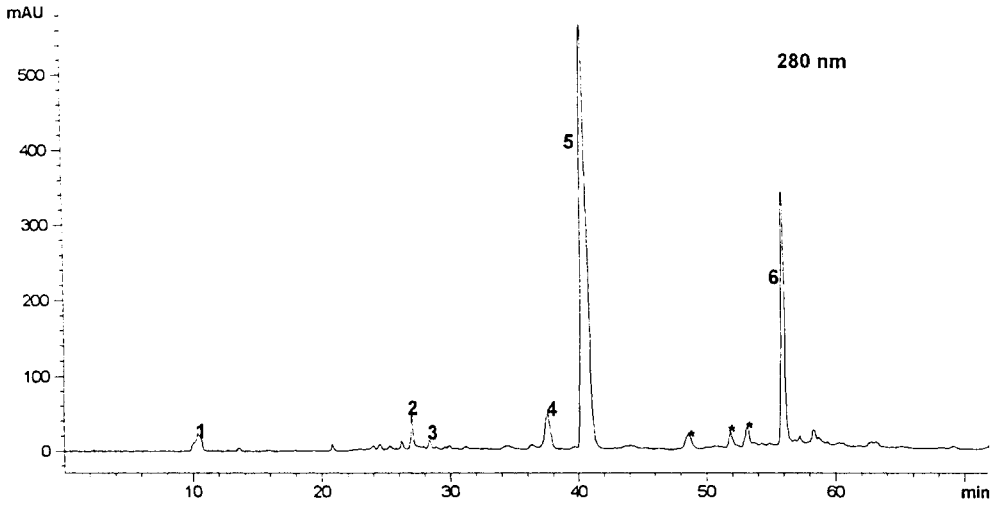


Figure 2. Chromatographic profile at 280 nm of the polyphenols of green tea extract: **1** = gallic acid; **2** = (−)-epigallocatechin; **3** = (+)-catechin; **4** = (−)-epicatechin; **5** = (−)-epigallocatechin 3-*O*-gallate (EGCG); **6** = (−)-epicatechin 3-*O*-gallate (ECG); * = catechin derivatives.

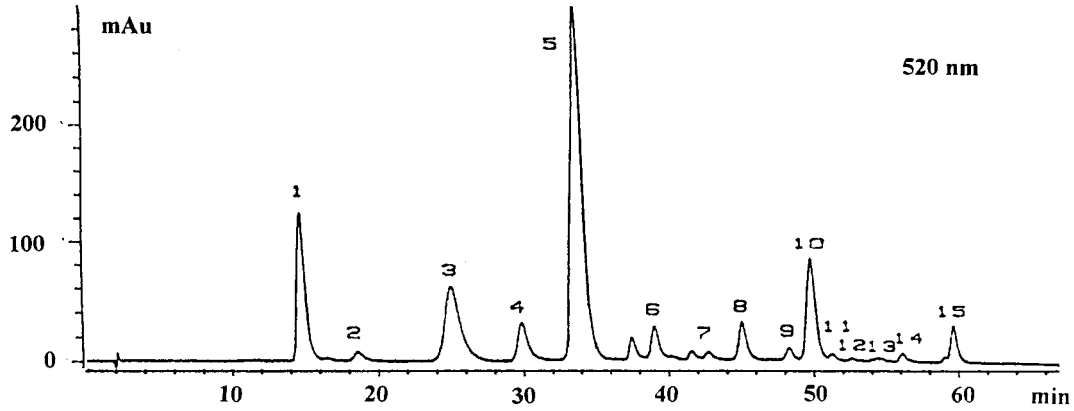


Figure 3. Chromatographic profile, at 520 nm, of the anthocyanic fraction of grape skin: **1** = delphinidin 3-*O*-glucoside; **2** = cyanidin 3-*O*-glucoside; **3** = petunidin 3-*O*-glucoside; **4** = peonidin 3-*O*-glucoside; **5** = malvidin 3-*O*-glucoside; **6** = delphinidin 3-*O*-(6-*O*-acetyl) glucoside; **7** = cyanidin 3-*O*-(6-*O*-acetyl)glucoside; **8** = petunidin 3-*O*-(6-*O*-acetyl)glucoside; **9** = peonidin 3-*O*-(6-*O*-acetyl)glucoside; **10** = malvidin 3-*O*-(6-*O*-acetyl)glucoside; **11** = delphinidin 3-*O*-(6-*O*-*p*-cumaroyl)glucoside; **12** = cyanidin 3-*O*-(6-*O*-*p*-cumaroyl)glucoside **13** = petunidin 3-*O*-(6-*O*-*p*-cumaroyl)glucoside; **14** = peonidin 3-*O*-(6-*O*-*p*-cumaroyl)glucoside; **15** = malvidin 3-*O*-(6-*O*-*p*-cumaroyl) glucoside.

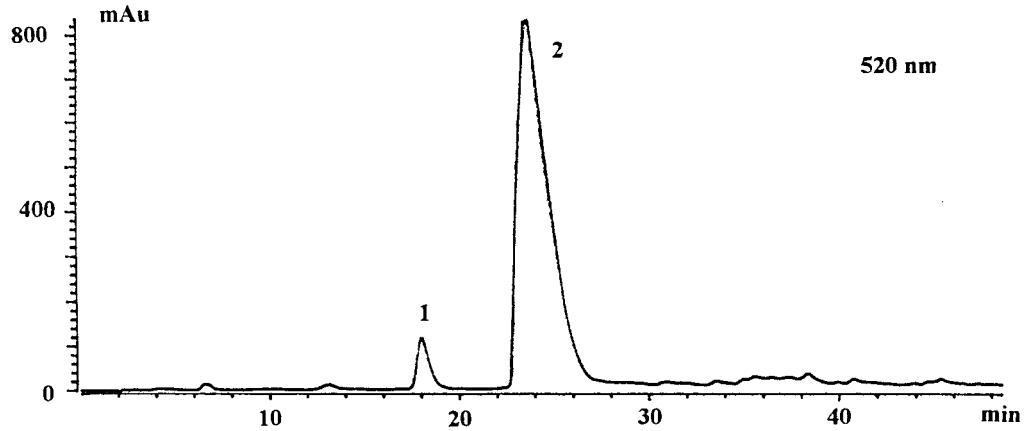


Figure 4. Chromatographic profile, at 520 nm, of the polyphenols of olive extract. **1** = cyanidin 3-*O*-glucoside; **2** = cyanidin 3-*O*-rutinoside.

estimate, respectively, 4.2% and 36% of the value obtained by HPLC/DAD. The best agreement between DPV and HPLC/DAD findings can be correlated with the oxidation potential value of EGCG because at this low potential there is a small number of possible interfering compounds. On the other hand, the biosensor response indicates that some problems occur at the electrode surface. The decay in current response can be attributed to a mass transfer phenomenon if a film is formed (Haslam, 1989; Wang et al., 1991) at the enzyme surface preventing the access of substrate to the enzyme, or more likely poisoning the enzyme itself.

Grape Skin Extract. The complexity of this anthocyanic fraction is evidenced in the chromatographic profile at 520 nm (Figure 3) for the 15 identified compounds.

The anthocyanic content evaluated by HPLC/DAD at 520 nm is expressed in malvin since the malvidin derivatives represent 64% of the total anthocyanic compounds. DPV measurement was performed at 595 mV corresponding to the peak potential of malvin. This latter method underestimates the total anthocyanic content content by 7.1% with respect to HPLC/DAD findings as shown in Table 4b. Therefore, considering the complexity of the matrix, this finding represents a good result. In fact, DPV has two major advantages: a higher sensitivity compared to HPLC/DAD analysis (micromolar vs millimolar concentration) and a short analysis time associated with simple operating conditions. These two peculiarities contribute to making DPV an attractive method for quick screening control of in-line processes because for this application error values ranging between 5 and 10% are therefore acceptable.

Olive Extract. The sample used for this analysis comes from a Tuscan cultivar with high pigmentation, and thus, it is very rich in anthocyanic compounds. For the olive extract, the quantitative results, both for HPLC/DAD and DPV, were expressed in cyanidin 3-*O*-rutinoside (Cy-3R), which is about 95% of the total anthocyanic amount. The HPLC/DAD profile at 520 nm is reported in Figure 4. The DPV measurement, performed at 384 mV, relative to the peak potential of Cy-3R, surprisingly underestimates the anthocyanic content by 36% with respect to HPLC/DAD analysis (Table 4c). Presumably, the copresence of small amounts of rutin in this olive extract interferes with the response of the detector because the peak potential value of this flavonol (383 mV) is very close to that of Cy-3R.

CONCLUSIONS

HPLC/DAD analysis allows both the final confirmation of the quali-quantitative amount of each polyphenol and avoids false positive results. However, the findings obtained with this technique, even if more accurate, required much more time with respect to the electrochemical analyses, which are performed in a few minutes.

This tyrosinase-biosensor was characterized by its ability to detect different polyphenols, but improvement for its use in complex matrices is needed. In fact, two main limitations occur with this device in real matrices: the pH working conditions and the risk of enzyme inactivation.

For DPV, the reported peak potential for various polyphenols might be used for simultaneous analysis of two or more polyphenolic molecules in complex matrices. A good agreement between the reference method HPLC/DAD and DPV is obtained for the grape extract, which is a very complex matrix. The analysis time is from a few seconds to a few minutes for each sample depending on the time consumed to reach the plateau of the electrochemical response. As pointed out, DPV, using screen-printed graphite electrodes, seems to be a suitable and inexpensive method for quick polyphenol detection in natural matrices. In addition, the sensitivity of the electrochemical techniques is greater than the HPLC/DAD method; in fact, the working range for DPV and the biosensor techniques was on the order of micromoles, while for HPLC it was in millimoles.

Future work will be directed toward optimization of DPV performance for quantitative subclass analysis of polyphenol compounds and to study in more detail the relationships between DPV behavior and the structural characteristics of these compounds.

ACKNOWLEDGMENT

We thank MURST (Ministero dell'Università e della Ricerca Scientifica e Tecnologica) and the CNR for their financial support.

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Received for review July 13, 1999. Revised manuscript received December 7, 1999. Accepted December 14, 1999.

JF990767E