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Electrochemical Sensor Based on a Cyclodextrin Modified Carbon Paste Electrode for *Trans*-Resveratrol Analysis

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Resveratrol, a phenolic phytoalexin, shows a variety of biological activities such as protection against cardiovascular diseases and atherosclerosis. In recent years it has gained an upswing in its popularity due to the so called "French paradox". In this connection a novel electrochemical sensor based on cyclodextrin modified carbon paste electrodes was designed and investigated for detecting trans-resveratrol in grape samples. The cyclodextrin modified electrodes show significant activity in the presence of resveratrol when applying differential pulse voltammetry. Under optimized conditions using α-cyclodextrin as modifier a well defined voltammetric peak appeared at about +450 mV vs. Ag/AgCl in a methanolic Britton-Robinson buffer solution (1:1; v/v). The peak height of the resveratrol current signal increased linearly in the concentration range of 30 – 1000 µg/L (R= 0.998). The limit of detection (LOD) was determined to be 12 µg/L. The modified electrode was successfully employed to determine resveratrol in complex grape material.

Keywords: resveratrol; carbon paste electrode; cyclodextrins; grape extracts; voltammetry

1. INTRODUCTION

Resveratrol is a polyphenolic compound that is formed by several plants as a response to stress attack like fungal infection and UV-light exposure. It exists in two isomeric forms as *trans*- and *cis*-resveratrol, whereas the *trans*-form is the most abundant one. However *trans*-resveratrol can be easily converted into its *cis*-isomer under the influence of heat and UV-light [1]. *Trans*-resveratrol is found at high levels especially in the skin of red grape berries as well as in red wine. The increasing interest of *trans*-resveratrol is based on investigations concerning the so called "French paradox". This is a

phenomenon found in the Southern French and known as a low incidence of cardiovascular diseases coexisting with the intake of high-fat diet and moderate consumption of red wine [2-4].

Thus intensive studies with resveratrol were carried out with respect to its biological activities and have been reported in several review articles. In these articles a wide spectrum of biological activities for resveratrol is reported such as protection against cardio-vascular diseases, chemopreventive and anticarcinogenic properties as well as anti-inflammatory activities [5-8].

Due to the above mentioned interest of resveratrol, several analytical methods for its quantitative determination in grapes and wine were developed. Liquid chromatography with various detection modes (UV-, electrochemical-, fluorometric- and MS-detection) is thereby the most frequently applied analytical system [9-10]. In addition gas chromatography (GC/MS) and capillary electrophoresis (CE/ED; CE/UV) were also suggested for its analysis [11-12]. Though these methods have been well approved, they have however some limitations as they are rather complex, time-consuming and use high priced equipment. Resveratrol as phenolic compound shows electrochemical activity and can be analysed by voltammetric and amperometric methods too [13-14]. In this context few electrochemical sensors e.g. based on indium tin oxide electrode and/or biosensors with peroxidase as bioactive component have been already reported [15-16].

Due to the advantages of sensor systems being simple, economic and cheap, the present work is focused on the development of a new electrochemical sensor for the determination of resveratrol in complex plant extracts of red grape berries. For the construction of the sensor, carbon paste as electrode material has been selected as it offers a simple renewable surface, low cost and a very low background current. Particularly the application of appropriate modifiers as molecular recognition elements in carbon paste electrodes has attracted enormous attention for improving selectivity and sensitivity of the electrochemical sensor. In case of resveratrol, cyclodextrins (α-, β- or γ-) seemed to be suitable as molecular receptors as they can form, due to the cage-like structures, stable host-guest inclusions with phenolic substances. Therefore carbon paste electrodes with cyclodextrins as modifiers for the oxidation of resveratrol were investigated in order to develop a sensitive and selective analytical tool for the detection of resveratrol in plant material.

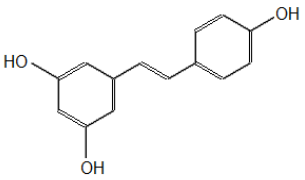
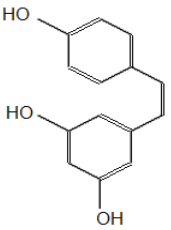
2. EXPERIMENTAL PART

2.1 Chemicals and materials

The *trans*-resveratrol reference was purchased from Sigma-Aldrich GmbH (Vienna, Austria) and stored frozen (Table 1). For the preparation of the cyclodextrins standard solution the cyclodextrin powder (α-, β- or γ-) 98% from Sigma-Aldrich GmbH (Vienna, Austria) was used. The carbon paste was made out of glassy carbon powder, 2-12 micron, from Sigma-Aldrich GmbH (Vienna, Austria) and paraffin oil Uvasol® from Merck KGaA (Vienna, Austria) or Adeps neutralis from Herba Chemosan (Graz, Austria) as binding agents. The nanotube carbon powder was purchased from Sigma-Aldrich GmbH (Vienna, Austria) and the spectral carbon powder was from Ringsdorff-Werke GmbH

(RW-B type, Bad Godesberg, Germany). The used water was deionized and finally purified using a Millipore® water purification system.

Table 1. Characteristics of the resveratrol molecule

IUPAC Name	Molecular Formula	Molar Mass	*trans*-Resveratrol	*cis*-Resveratrol
5-[2-(4-Hydroxyphenyl) ethenyl]benzene -1,3-diol	$C_{14}H_{12}O_3$	228.24 g/mol		

Following solutions were used as supporting electrolytes: Phosphate buffer (KH_2PO_4/K_2HPO_4; pH 6), acetate buffer (HAc/Ac; pH 6) and Britton Robinson (BR) buffer ($CH_3COOH/H_3BO_3/H_3PO_4$; pH 4-8) each with concentrations of 0.1 M. The proper pH value was obtained with the addition of 0.5 M NaOH solution. Each buffer was also mixed in ratio of 3:7 or 1:1 (v/v) with methanol, ethanol or acetonitrile.

Resveratrol stock solution (30 mg/L) was prepared using ethanol as solvent and the cyclodextrin stock solutions (500 mg/L) were prepared using BR buffer (0.1 M, pH 6).

The grapes were collected and donated by Dr. M. Wurglics from Johann Wolfgang Goethe-University of Frankfurt am Main. This red grapes were breed “Blaufränkisch”, collected in years 2006, 2007 and 2008.

2.2 Apparatus

Voltammetric measurements were made with a 797 VA Computrace (Metrohm AG, Herisau, Switzerland) in a three electrode arrangement. A carbon-paste electrode with/without modification, Ag/AgCl (3M KCl), and a platinum wire were used as working electrode, reference electrode and auxiliary electrode respectively. For the differential pulse voltammetry (DPV) analysis of resveratrol the analyser was operated under following parameters: start potential: + 0.10 V; end potential: + 0.60 V; pulse amplitude: + 50.0 mV; pulse time: 0.10 s; voltage step: 0.006 V; voltage step time: 0.40 s; sweep rate: 0.015 V/s. All measurements were carried out at room temperature.

2.3 Construction of carbon paste electrode and cyclodextrin modified electrode

A pure carbon paste electrode **(**CPE) was prepared by thoroughly mixing carbon powder and binding agent in ratio of 7:3 (m/m) in a mortar with pestle. Cyclodextrin modified electrodes (CD-CPE) were prepared by taking the unmodified paste and adding 1 mL of the cyclodextrin stock solution (500 mg/L). The mixture was dried in the oven for one hour at 45 °C until the buffer evaporated.

A portion of CPE/CD-CPE was packed into one end of a Teflon tube of 1.5 mm in diameter and the surface of the electrode was polished on a smooth paper sheet. Because the resveratrol oxidation products adsorbed very strong to the electrode surface and could not be removed by polishing or cleaning of the electrode the sensing area was renewed after every measurement.

2.4 Measuring procedure

When using plane CPE, 5 mL of the appropriate supporting electrolyte was transferred into the measuring cell. After degasing with nitrogen the blank value was determined following the above described instrumental parameters. A volume of 50 µL of the resveratrol stock solution was added for DPV analysis.

For plotting calibration curves including linearity and range using the α-cyclodextrin modified carbon paste electrode (α-CD-CPE), 5 mL BR buffer (0.1 M, pH 6) as methanolic solution (1:1; v/v) was used as supporting electrolyte. After degasing and the determination of the blank value, 5 aliquots (40, 80, 120, 150, 170 µL) of the resveratrol stock solution were added. By applying the aforementioned instrumental parameters, the voltammogram was then recorded.

The obtained DVP data was evaluated using the tangential method.

2.5 Extraction and analysis of the plant ("Blaufränkisch" grape)

The grapes were extracted according to the procedure of Romero-Perez *et al*. [17]. Briefly, 25 g of grape was extracted with 40 mL methanol using the ultrasonic bath for 10 min. 40 mL of the extract were mixed with 10 mL water and then filtered to get a clear solution. After the solution was compressed on the rotavapor it was washed three times with ether and dried with Na_2SO_4. To remove the ether the extract was degased with nitrogen. The residue was solubilised in 2 mL methanol and used for the analysis. Therefore 5 mL of a methanolic BR buffer (0.1 M, pH 6, 1:1; v/v) were transferred to the voltammetric cell. After determination of the blank value, 50 µL of the plant extract (mean linearity range) was added. The voltammogram was then recorded using the instrumental parameters described above. The content of resveratrol was analysed using the standard evaluation method.

3. RESULTS AND DISCUSSION

3.1 Optimization of the operating conditions

CPEs as electrochemical sensors are very attractive in electroanalysis when preparing an inexpensive versatile electrode, both in research and in commercial use. The high interest of these heterogeneous electrodes resulted in the physical property of themselves as well as in the immense potential of modifications [18-19].

Due to these advantages this type of electrode was selected for the development of the electrochemical resveratrol sensor. In order to improve the performance of the electrode for the analysis of resveratrol some optimization steps were carried out concerning carbon particles, binding agents as well as the mixing ratio of both components. In this connection as electrically conductive particles glassy carbon powder, spectral carbon powder and carbon nanotubes powder were tested in combination with paraffin oil or adeps neutralis as binding agents. These initial investigations were done in BR buffer as this buffer is applicable in a wide pH-range. As shown in figure 1, best results concerning peak height, peak form and background current were achieved when applying glassy carbon powder with paraffin oil in a ratio of 7:3 (m/m). At this plane electrode resveratrol is oxidized at + 450 mV and + 620 mV due to former reports [20].

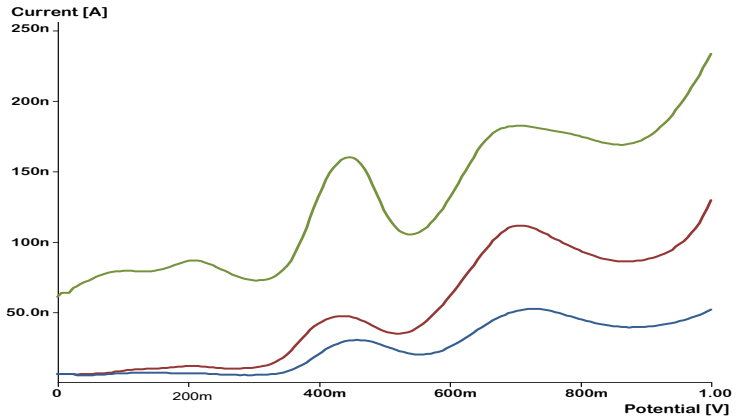


Figure 1. DP-Voltammograms of resveratrol (300 µg/L) in BR buffer (0.1 M, pH 6) using CPEs prepared with • Glassy carbon, • Spectral carbon, • Carbon nanotubes and paraffin oil as binding agent.

Additionally the influence of the electrolyte medium and its pH on the resveratrol signal was studied. Acetate, phosphate and BR buffer were tested as supporting electrolytes. Although phosphate buffer induces the highest current, measurements in this, as well as in acetic media have not shown good results as far as linearity is concerned (R=0.880). BR buffer leads in this context to the best results and was therefore chosen as supporting electrolyte. Concerning its pH, BR buffer showed the highest peak current at pH 6 (Figure 2). This behavior can be explained as following. The oxidation of the analyte takes place at the phenol groups and releases protons, therefore an increase in pH will favor

this process. On the other hand resveratrol as phenol may be deprotonated (pKa1 = 8.8) yielding phenolate which is more difficult to oxidize due to its additional stabilization by the delocalized negative charge [21].

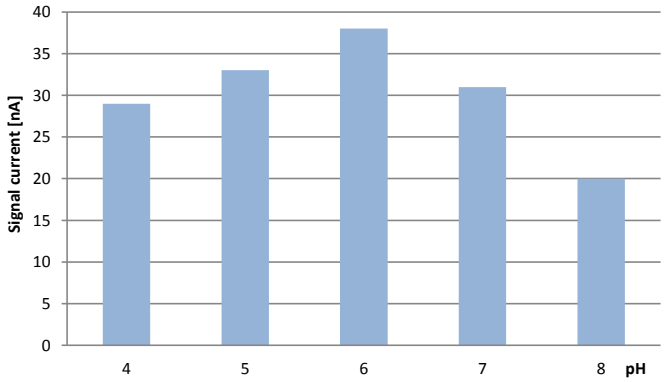


Figure 2. Influence of pH on the current peak of resveratrol (300 µg/L) using BR buffer of different pH (4-8).

As the precision still was not satisfying (R.S.D. 30%) under the aforementioned conditions, the addition of different solvents to the electrolyte medium was investigated. Methanol, ethanol and acetonitrile were mixed in ratios of 3:7 or 1:1 (v/v) with the BR buffer and DPV measurements were performed. The mixture of 50 % methanol with the BR buffer increased the precision and was therefore used for all measurements to follow.

In summary a carbon paste working electrode, prepared of glassy carbon and paraffin oil was chosen as working electrode. The electrolyte medium for the electrochemical determination of resveratrol was selected to be BR buffer (0.1 M, pH 6) in combination with 50 % methanol.

3.2 Modification steps and the refining of the CD-CPE sensor system

After these basic experiments modification steps were carried out in order to develop the resveratrol sensor with an appropriate sensitivity and selectivity. In literature an enormous number of substances are suggested as molecular recognition elements for phenolic compounds but however especially for resveratrol no appropriate mediator could be found [22-23]. A high variety of analyte - modifier interactions are described as e.g. catalytic effects, complex formations or adsorption processes. For resveratrol cyclodextrins (α-, β- and γ-) are a very interesting class of modifiers for designing sensors as they can form, due to the cage-like structure, stable host-guest inclusions. So α-, β- and γ- cyclodextrins were tested by adding an appropriate amount to the carbon paste. When comparing plane CPE with CD-CPEs under the same experimental conditions a markedly increase of peak height was found.

α-Cyclodextrin increased the peak current in comparison to the unmodified electrode up to 70 %, while β-cyclodextrin increased it to 60 % and γ-cyclodextrin to 50 % (Figure 3). These results

clearly indicate that cyclodextrins show activity towards resveratrol and are therefore attractive modifiers for its analysis.

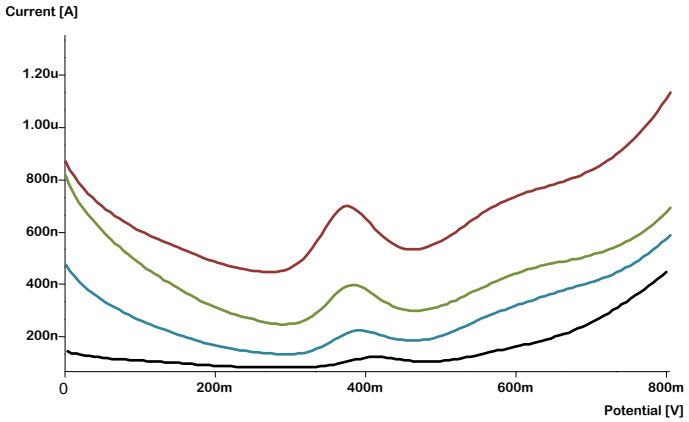


Figure 3. DP-Voltammograms of resveratrol (300 µg/L) in 50 % methanolic BR buffer (0.1 M, pH 6) using CPE • plane and CPE modified with • γ-cyclodextrin**,** • β-cyclodextrin**,** • α-cyclodextrin.

The excellent effect of resveratrol on α-cyclodextrin carbon paste electrodes (α-CD-CPE) is probably influenced by the size and structure of α-cyclodextrin in order to form the best host-guest inclusion [23]. Finally it can be concluded that α-cyclodextrin is the most appropriate modifier for designing the resveratrol sensor. Various modification procedures like entrapping of the α-cyclodextrin directly into the carbon bulk or drop-coating of a mediator containing film on the electrodes were studied. Best results were obtained when mixing an appropriate solution of cyclodextrin into the paste. After evaporating the solvent in the oven, the α-CD-CPE was then applicable for the determination of resveratrol. Additionally the influence of the modifier concentration on the resveratrol signal was investigated in the range between 50 and 5000 mg/L. Best results were achieved with a 500 mg/L cyclodextrin solution.

In order to optimize the analytical system and to check if the chosen operating conditions fit the α-cyclodextrin modified sensor, the same experiments as described above, including carbon material, electrolyte source and pH were performed. Glassy carbon and paraffin oil as electrode material and BR buffer (0.1M, pH 6) with 50% methanol as electrolyte media suit the α-CD-CPE sensor.

3.3 Validation of analytical parameters

Under optimized conditions linearity of the α-CD-CPE sensor was estimated to be in the 30 – 1000 µg/L concentration range with a mean regression coefficient of R=0.998. The calibration curve resulted in the following mean linear equation: i[nA] = 310.78*conc.[µg/L] + 0,484.

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined as 12 µg/L and 30 µg/L respectively, based on the visual evaluation method according to the ICH Q2(R1)

guidelines. Typical differential pulse voltammograms of resveratrol measured on α-CD-CPE are represented in figure 4.

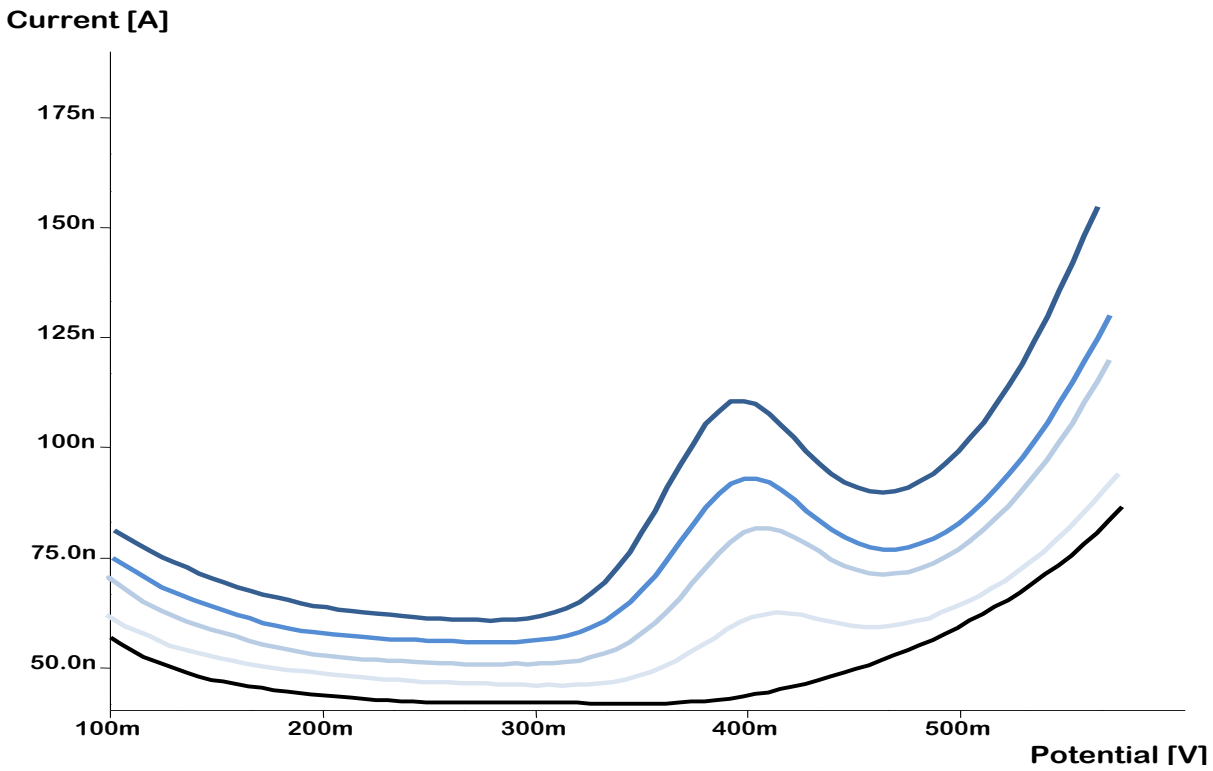


Figure 4. DP-Voltammograms of resveratrol in 50 % methanolic BR-buffer (0.1M, pH 6) on α-CD-CPE. Concentrations of resveratrol employed were: • blank, • 120**,** • 240**,** • 355 and • 470 µg/L**.**

Intra-day precision experiments were carried out in solutions of 300, 590 and 870 µg/L by measuring each concentration four times in one day. Coefficient of variation (CV %) was estimated to be < 4 % and the recovery rate, evaluated under same conditions, was found to be between 98.2 and 99.2 %. In addition, interday precision, analysed by measuring 250 µg/L of resveratrol at six different days also resulted in a CV of < 4 %.

3.4 Selectivity

As far as the sensor was designed for the application in complex grape matrix, interference tests were carried out to investigate the selectivity. Therefore standard interferences as there were acetylsalicylic acid and uric acid were tested but also substances that were expected in grape material like rutin, quercetin and catechin. Concentrations up to 100-fold of resveratrol were checked but only an insignificant influence on the analyte signal was found (Figure 5).

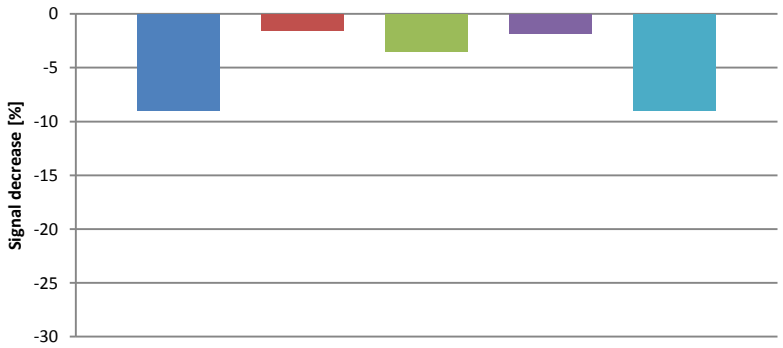


Figure 5. Influence of • quercetin, • uric acid, • acetysalicylic acid, • rutin, • catechin on the resveratrol signal using the α-CD-CPE sensor. Employed concentrations were 100-fold of the resveratrol concentration.

3.5 Application of the sensor for the analysis of resveratrol in grape extracts

The utilization of the α-CD-CPE sensor as analytical system for the determination of resveratrol was tested in grape extracts prepared from red grapes breed "Blaufränkisch" collected in different years.

The extraction of resveratrol from grapes was carried out according to Romero-Perez *et al.* [17]. An appropriate amount of the resulted methanolic extract was then analysed under the above described and optimized experimental conditions using the α-CD-CPE sensor. The measured resveratrol concentrations were between 870 and 1100 ng ± 39 ng/g grape (Table 2). As far as the results are in fairly good agreement with former reports based on HPLC analysis [24] and could be reproduced using standard addition method, it may be specified that the method is applicable for the determination of resveratrol in complex plant samples without interferences when used in pre-treated form e.g. extracts.

Table 2. Summary of the grape extract analysis*

Collection year	Resveratrol concentrations [ng/g grape]
2006	1150 ± 46
2007	940 ± 37
2008	872 ± 34

* n = 4

4. CONCLUSION

These investigations reveal that the developed α-CD-CPE sensor offers marked increase of peak height and hence of sensitivity for the determination of resveratrol. The designed sensor emerged as a cheap, easy to prepare/modify, non-toxic and highly reproducible analytical tool. Furthermore

surfaces can simply be renewed. As the results show the α-CD-CPE sensor is feasible for matrix extracts, therefore, the electrode might be an attractive analytical system for the assay of resveratrol in plant materials.

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References

1. J. Lopez-Hernández, P. Paseiro-Losada, A.T. Sanches-Silva, M.A. Lange-Yusty, *Eur. Food Res. Technol.*, 225 (2007) 789.
2. J. Constant, *Clin.Cardiol.*, 20 (1997) 420.
3. E.H. Siemann, L.L. Creasy *American Journal of Enology and Viticulture*, 43 (1992) 49.
4. B. Simini, *The Lancet*, 355 (2000) 48.
5. J. Lekekis, L.S. Rellidis, I. Andreacadou, G. Vamvakou, G. Kazantzoglou, P. Magiatis, A.L. Skaltsounis, D.T. Kremastinos, *Eur. J. Cardiovasc. Prev. Cardiology*, 12 (2005) 596-600.
6. M. Jang, L. Cai, G. O. Udeani, K. V. Slowing, C. F. Thomas, C.W. W. Beecher, H. H. S. Fong, N. R. Farnsworth, A. D. Kinghorn, R. G. Mehta, R. C. Moon, J. M. Pezzuto, *Science*, 275 (1997) 218.
7. B. B. Aggarwal, A. Bhardwaj, R.S. Aggarwall, N.S. Seeram, S. Shishodia, Y. Takada, *Anticancer Res.*, 24 (2004) 2783.
8. G. Chen, W. Shan, Y. Wu, L. Ren, J. Dong, Z. Ji, *Chem.Pharm.Bull.*, 53 (2005) 1587.
9. I. Kolouchová-Hanzlikova, K. Melzoch, V. Filip, Smidrkal, *J. Food Chemistry*, 87 (2004) 151.
10. P. Jeandet, A.C. Breuil, M. Adrian, L.A. Weston, S. Debord, P. Meuier, G. Maume, R. Bessis, *Analytical Chemistry*, 69 (1997) 5172.
11. P. Viñas, N. Campillo, N. Martinez-Castillo, M. Hernández-Córdoba, *Journal of Chromatography A*, 1216 (2009) 1279.
12. X. Gu, Q. Chu, M. O´Dwyer, M. Zeece, *Journal of Chromatography A*, 881(2000) 471.
13. J.X. Liu, Y.J. Wu, F. Wang, l. Gao, B.X. Ye, *Journal of the Chinese Chemical Society*, 55 (2008) 264.
14. L. Gao, Q. Chu, J. Ye, *Food. Chem.*, 78 (2002) 255.
15. H.Y. Xiang, W.G. Li, *Electroanalysis*, 21 (2009) 1207.
16. A.M., Granero, H. Ferandez, E. Agostini, M.A. Zon, *Electroanalysis*, 20 (2008) 858.
17. A.I. Romero-Peréz, R.M. Lamuela-Raventós, C. Andrs-Lacueva, M.C. de la Torre-Bornat, *J. Agric. Food Chem.*, 49 (2001) 210.
18. S.A. Jaffari, J.C. Pickup, *Biosensors & Bioelectronics*, 11 (1996) 1167.
19. J. Wang, T. Tangkuaram, S. Loyprasert, T. Vazquez-Alvarez, W. Veerasai, P. Knatharana, P. Thavarungkul, *Analytica Chimica Acta*, 581 (2007) 1.
20. J.X. Liu, Y.J. Wu, F. Wang, l. Gao, B.X. Ye, *Journal of the Chinese Chemical Society*, 55 (2008) 264.
21. J.M. Lopez-Nicolas, F. Garcia-Carmona, *J. Agric. Food Chem.*,56 (2008) 7600.
22. I. Naranjo Rodriguez, J. Munoz Leyva, J.L. Hildago de Cisneros, *Analyst*, 122 (1997) 601.
23. D. El-Hady, N. El-Maali, *Microchim. Acta*, 161 (2008) 225.
24. Y. Wang, F. Catana, Y. Yang, R. Roderick, R.B. von Breemen *J. Agric. Food Chem.*, 50 (2002) 431.