Artificial Cells, Nanomedicine, and Biotechnology, 2013; Early Online: 1–6

ISSN: 2169-1401 print / 2169-141X online
DOI: 10.3109/21691401.2013.764313

informa
healthcare

Fresh broad (*Vicia faba*) tissue homogenate-based biosensor for determination of phenolic compounds

Hakki Mevlut Ozcan & Ayten Sagiroglu

Faculty of Science, Department of Chemistry, Trakya University, Section of Biochemistry, Edirne, Turkey

Abstract

In this study, a novel fresh broad (*Vicia faba*) tissue homogenate-based biosensor for determination of phenolic compounds was developed. The biosensor was constructed by immobilizing tissue homogenate of fresh broad (*Vicia faba*) on to glassy carbon electrode. For the stability of the biosensor, general immobilization techniques were used to secure the fresh broad tissue homogenate in gelatin-glutaraldehyde cross-linking matrix. In the optimization and characterization studies, the amount of fresh broad tissue homogenate and gelatin, glutaraldehyde percentage, optimum pH, optimum temperature and optimum buffer concentration, thermal stability, interference effects, linear range, storage stability, repeatability and sample applications (Wine, beer, fruit juices) were also investigated. Besides, the detection ranges of thirteen phenolic compounds were obtained with the help of the calibration graphs. A typical calibration curve for the sensor revealed a linear range of 5–60 μM catechol. In reproducibility studies, variation coefficient (CV) and standard deviation (SD) were calculated as 1.59%, 0.64×10^{-3} μM, respectively.

Keywords: amperometric biosensor, fresh broad, phenolic compound, tissue biosensor, *Vicia faba*

Introduction

Phenolic compounds (PCs) are produced as secondary metabolites by most plants, in which they probably act as natural antimicrobial agents, as natural deterrents to grazing animals or as inhibitors of pre-harvested seed germination (Haslam and Lilley 1988). Antioxidant compounds as quercetin, catechin, and rutin, which exist in fruits, have been claimed to protect low density lipoproteins and have anticancer effects (Haslam 1998). Polyphenols are also important for their organoleptic properties, astringency, odors and savour to tea, wine and other fruits (Bravo 1998). Furthermore, PCs are also formed during the natural decomposition of humic substances, tannins and photolytic or metabolic degradation of insecticides and herbicides (Masque et al. 1998, Narang et al. 1983). More recently, the significance of PCs as anticarcinogens, antioxidants, antimutagens and agents in the treatment of Rh-factor-threatened pregnancies, problems encountered during parturition and AIDS has received a lot of research attention (Singleton 1981, Mukhtar et al. 1992, Chung et al. 1998). These compounds also affect cell-to-cell signaling, receptor sensitivity, inflammatory enzyme activity and gene regulation (Virgili and Marino 2008). Therefore, a rapid, sensitive and precise determination of PCs has created growing interest in the clinical, biomedical, environmental, industrial and pharmaceutical analysis (Rajesh et al. 2004, Yu et al. 2003). Colorimetric and ultraviolet spectrophotometric analyses (Townshend 1995, Vogel 1989), high performance liquid chromatography (Fiehn and Jekel 1997), gas chromatography (Kishi et al. 1998) and capillary electrophoresis (Morales and Cela 2000) are now commonly used for the determination of PCs as standard methods. However, these schemes do not easily allow continuous on-site monitoring, as they involve high costs, are time-consuming, and need skilled operators, in addition to requiring preconcentration and extraction steps at times. To solve this problem, a simple, effective and fast alternative method for the determination of PCs is desirable.

Amperometric biosensors for PCs based on polyphenol oxidase proved to be promising for this purpose (Ozsoz et al. 1996). Biosensor based on pure enzymes may provide a promising method for a simple, fast and sensitive detection of PCs. But many of them have limited lifetime due to enzyme inactivation by the biocatalytically generated quinine products. Thus, development of good immobilization methods and materials to improve the biosensor stability is very significant (Mokower et al. 1996, Rogers 1995). For determination of PCs, biosensors modified with tyrosinase (V´edrine et al. 2003, Ozoner et al. 2010, Yu and Ju 2004) peroxidase (Imabayashi et al. 2001, Graneroa et al. 2010, Yang et al. 2006) laccase (Portaccio et al. 2006, Chawla et al. 2012, Shimomura et al. 2011), Laccase–Tyrosinase (Kochana et al. 2008, Montereali et al. 2010) have been reported. Plant and animal tissues have been successfully employed as biocatalytic components in the construction of biosensors for about two decades. When biosensors are compared

Correspondence: Hakki Mevlut Ozcan, Faculty of Science, Department of Chemistry, University of Trakya, Section of Biochemistry, 22030, Edirne, Turkey. Tel: + 90-284-235 95 92. Fax: + 90-284-235 1198. E-mail: ozcanhakki@gmail.com

(Received 19 November 2012; accepted 3 January 2013)

with immobilized isolated and pure enzymes, tissue-based biosensors show potential advantages of low cost, high stability, longer life time and high level activity. Since 1981, when the first plant tissue-based electrode was reported, immobilization of Polyphenol oxidase (PPO) with some plant tissue homogenate in order to use for determination of PCs was performed by dissolved oxygen probe (Odacı et al. 2004, Topcu et al. 2004). However, in this article, PPO within fresh broad tissue homogenate was immobilized on Glassy carbon electrode (GCE) like our previous manuscript (Ozcan and Sagıroglu 2010).

In this study, we described a new amperometric biosensor based on fresh broad (*Vicia faba*) plant tissue which has a better performance than banana peel-based biosensor for the determination of PCs . We have described enzyme PPO in the banana peel (*Musa cavendish*) in our previous study but the enzyme PPO in the fresh broad (*Vicia faba*) has not been characterized in literature yet (Ozcan and Sagıroglu 2010). Optimization and characterization studies of the new biosensor based on fresh broad were carried out. For each phenolic substrate, the calibration graphs were drawn and detection limits were determined by using the biosensor. Inhibitor studies and application for determination of the PCs in some drinks were also performed.

Materials and methods

Reagents

All reagents used were of analytical grade. Glutaraldehyde (25%), sodium sulfite, sodium bisulfide, cysteine, gelatin (225 Bloom), glycine, L-Dopa, p-cresol, gallic acid, caffeic acid, rutin hydrate, 4 hydroxy cinnamic acid, resorcin and quercetin were purchased from Sigma (St. Louis, USA). The fresh broad used was purchased from Edirne grocery. KH_2PO_4,,K_2HPO_4, phenol, Na_2HPO_4, NaH_2PO_4, hydroquinone, pyrogallol, catechol, ascorbic acid, thiourea, benzoic acid, $CuSO_4$, $ZnSO_4$ and $HgCl_2$ were purchased from E. Merck (Germany).

Apparatus

In this study a potentiostat (Palmsense, Netherlands), glassy carbon-working electrode, Pt counter electrode and Ag/AgCl reference electrodes (Basi, W. Lefayette,USA) were used for determination of the flowing current level. To prepare buffer solution magnetic stirrer (RCA, UK) and pH meter with an electrode (Schott handlylab, Spain) were used. The temperature in the reaction cell was maintained unchanged by circulating water at appropriate temperature around the cell compartment during the experiment. All the measurements were carried out at a constant temperature using a thermostat (Nüve BM 302, UK).

Procedure

Preparation of the bioactive layer material

200 mg of fresh broads were weighed and homogenized with 500 µL working phosphate buffer (66 mM, pH 7) by a manual glass homogenizer. Freshly prepared tissue samples were used daily.

Schema 1. Concept of process.

Process steps	Process
1	200 mg of fresh broads were weighed and homogenized with 500 µL working phosphate buffer (66 mM, pH 7). The mixture of 270 µl fresh broad homogenate and 10 mg gelatin was incubated at 38°C for 15 min to dissolve gelatin.
2	30 µL Gelatin-fresh broad tissue homogenate was dispersed over the cleaned GCE surface.
3	It was allowed to dry at 4 •C for 30 min.
4	For cross linking with glutaraldehyde, the probe carrying bioactive layer was immersed into 1.25% (v/v) glutaraldehyde solution and was allowed to stay for 5 min.
5	The biosensor was washed with distilled water to remove excess glutaraldehyde and it was ready to use.

Then, gelatin was weighed and added to a test tube (10 mg). 270 µL fresh broad tissue homogenate was added into the test tube. The mixture of fresh broad homogenate and gelatin was incubated at 38°C for 15 min to dissolve gelatin.

Preparation of the biosensor

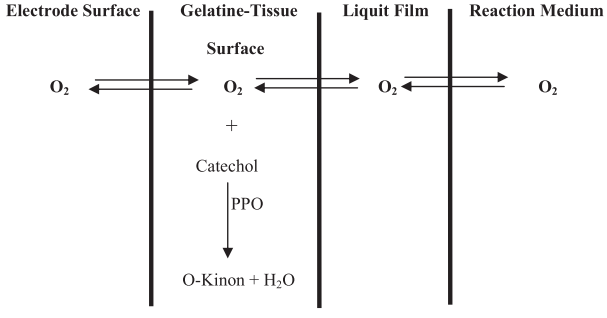
30 µL Gelatin-fresh broad tissue homogenate was dispersed over the cleaned GCE surface and allowed to dry at 4 °C for 30 min. For cross linking with glutaraldehyde, the probe carrying bioactive layer was immersed into 1.25% (v/v) glutaraldehyde solution and was allowed to stay for 5 min. Then the biosensor was washed with distilled water to remove excess glutaraldehyde and it was ready to use.

After it is used, the biosensor was stored at 4°C in a flask which contains distilled water with cotton. This condition provided a moisturizing medium; therefore, the dryness of the bioactive layer was prevented.

The concept of process is given in Schema 1.

Measurement procedure

The GCE based on fresh broad tissue homogenate, Pt counter electrode and Ag/AgCl reference electrode were put into the thermostatic reaction cell containing working buffer (pH 7.0, 66 mM sodium phosphate buffer) and the magnetic stirrer was fixed at a constant speed. Then − 0.700 mV potential was applied for the reduction of the oxygen on the tissue modified GCE surface. A few minutes later, the throughout current of the system was equilibrated because of the diffusion of dissolved oxygen between working buffer and tissue-modified GCE. At this time, phenolic compound was injected into the thermostatic reaction cell. The flowing current started to decrease and a few minutes later, it reached to the constant value due to the enzymatic reaction equilibration below.



At this moment, constant current was recorded. Electrical response, which constituted the output signal from the biosensor, was acquired using the PalmsensePC software package, purchased from the Palm Instrument PV (Ivium Technologies, Netherlands). Measurements were carried out by noting the decrease of current in relation to phenolic substrate concentration added into the reaction cell.

Results and discussion

Effect of the fresh broad tissue amount on biosensor response

The amount of tissue on GCE surface changed as shown in Figure 1 for the determination of the effect of tissue amount on the biosensor response. As it is obvious from the figure, the optimum amount of the tissue was 10,60 mg/cm^2 because of having better linear range and higher response than 8.75 mg/cm^2 and 12.45 mg/cm^2 tissue.

Effect of the gelatin amount on biosensor response

Experiments were carried out by keeping the amount of fresh broad tissue and percentage of glutaraldehyde constant, in order to investigate the effect of differing gelatin amounts on the GCE surface. Results obtained are given in Figure 2.

As it can be seen in Figure 2, the highest biosensor response is observed when 0.98 mg/cm^2 gelatin is used.

Effect of the glutaraldehyde percentage on biosensor response

For the determination of the effect of glutaraldehyde percentage on the biosensor response, fresh broad tissue amount and gelatin amount were kept constant and we changed the glutaraldehyde percentage in the experiments. As it can be seen in Figure 3, the optimum glutaraldehyde percentage is 1.25%.

pH effect on the biosensor activity

The activity of the biosensor as a function of pH when catechol is used as a substrate is shown in Figure 4. Citric acid buffer used was pH 4, phosphate buffer used was in the pH range from 5–8 and glycine buffer used was pH 9. It is seen that there are decreases in the activity of the biosensor in the pH range from 4–7 and 7.5–9. The highest activity was obtained at pH 7.0.

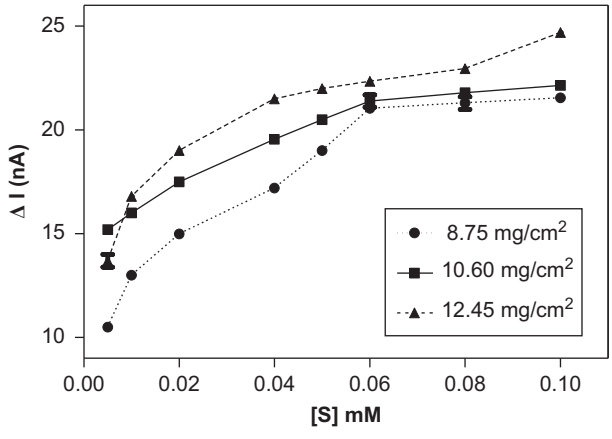


Figure 1. Effect of the fresh broad amount on the biosensor response.

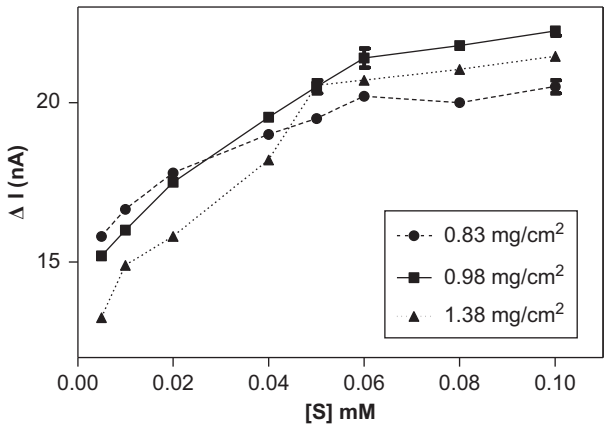


Figure 2. Effect of the gelatin amount on the biosensor response.

Effect of the temperature on the biosensor activity

The effect of assay temperature (20–50°C) was examined by using catechol. As it is shown in Figure 5, maximum sensor response is found at 37.5°C. Therefore, this temperature was accepted as optimum temperature for all the following experiments.

The effect of the buffer concentration on the biosensor response

The increase of buffer concentration contributes to the reproducibility of biosensor response together with the decay of the response value and the lifetime of the immobilized enzyme or tissue. Because of this, we also investigated optimum concentration of the working buffer. According to the results obtained by testing three different buffer concentrations, 33, 66, 99 and 132 mM, the highest biosensor response was obtained in 66 mM sodium phosphate buffer.

Linear range

Figure 6 shows the calibration curve of the biosensor based on fresh broad tissue for catechol. Linear graph, defined by the equation y = 108.9 × + 15.02, (R^2 = 0.999) was obtained.

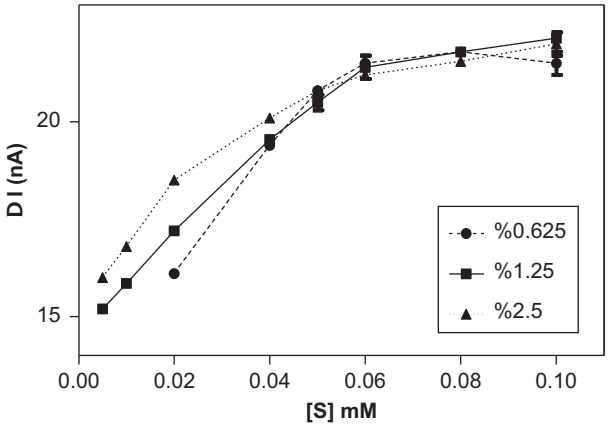


Figure 3. Effect of the glutaraldehyde percent on the biosensor response.

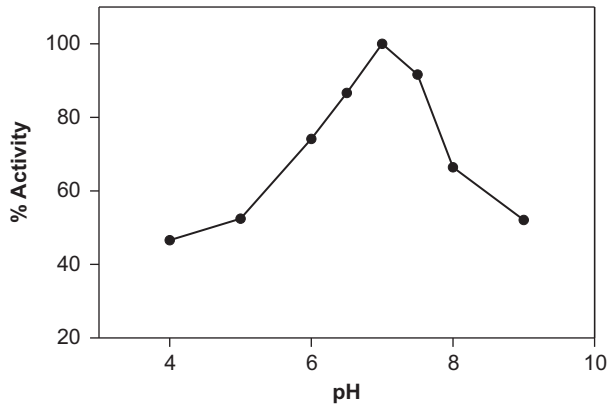


Figure 4. Effect of the pH on the biosensor activity.

Linearity was found in the range of 5–60 μM. At higher concentrations, standard curve showed a deviation from linearity. The deviation was due to the limited amount of the polyphenol oxidase in the bioactive material or insufficient dissolved oxygen which is a co-substrate of the enzyme. The minimum detectable concentration of the catechol was estimated to be 5 μM.

Reproducibility

The reproducibility of the biosensor was tested by 6 average standard solutions containing equal amount of catechol (40 μM). The standard deviation (SD), variation coefficient (cv) and average value (X) were calculated as 0.64×10^{-3} μM, 1.59% (n = 6) and 40.20 μM, respectively.

Storage stability

For this purpose, the long term performance of the biosensor was evaluated intermittently over a period of 24 days by monitoring its response to standard catechol solution. The biosensor was stored as described in the biosensor preparation section above. Although this condition provides a moisturizing medium for the biosensor, the bioactive layer of the biosensor may get dry after a few weeks. In this condition after 4 days storage period, the biosensor did not lose any activity at the end of the 5th, 10th, 17th, 20th and 24th days, the remaining activities of the biosensor were 99.0, 84.6, 80.0, 78.3 and 76.9%, respectively.

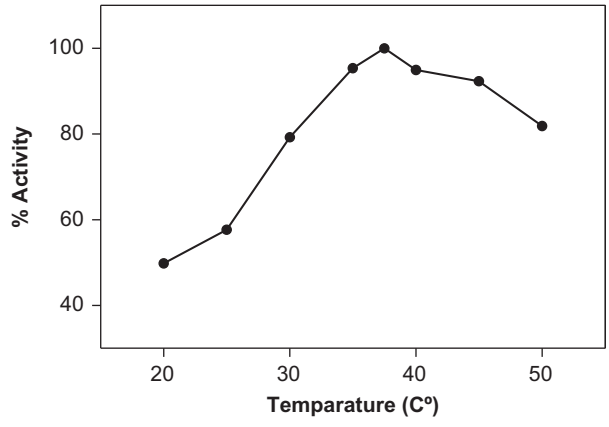


Figure 5. Effect of the temperature on the biosensor activity.

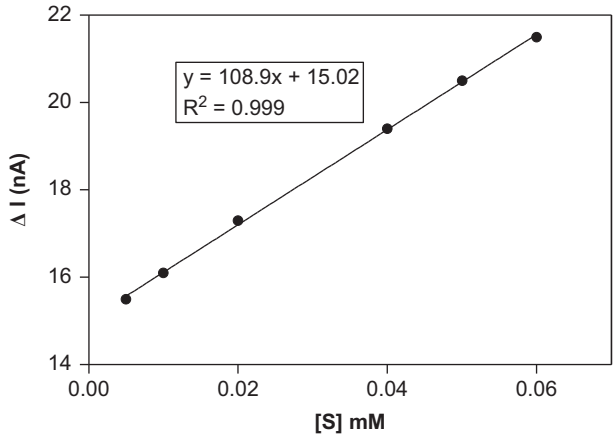


Figure 6. Calibration curve for catechol.

Inhibitory effect

In order to show the inhibitor effect on the biosensor-based fresh broad, some metal ions and certain substances were tested. Catechol is used as a substrate and inhibitors tested were of 200 μM and 100 μM, respectively. After electrodes were placed in the thermostatic reaction cell and the inhibitor solution was added, − 0.700 mV potential was applied. When the flowing current reached a constant value, catechol was injected to reaction cell. The flowing current started to decrease and a few minutes later it reached the constant value but not as much as the measurement which had been done without an inhibitor. Inhibition studies were carried out by the change of flowing current. The results of the inhibition studies for two biosensors are shown in Table I. As it is seen in Table I, the fresh broad-based biosensor is affected less than banana peel-based biosensor by all inhibits.

Determination of some PCs

The biosensor was applied to eleven PCs and ascorbic acid. Analytical results for each compound which is obtained by the experiments, are summarized in Table II.

As seen in Table II P-Cresol showed the highest activity. The activity of Caffeic Acid was 15.9% higher than the activity of catechol. Caffeic Acid activity was followed by hydroquinone, cinnamic acid, catechol, ascorbic acid, pyrogallol, resorcin, phenol, L-dopa, gallic acid, quercetin and rutin.

Table I. Inhibition studies.

Substances[a,d]	Inhıbition (%)[e]	Inhıbition (%)[f]
Cysteine	12.9	10.7
Hg^{2+}[b]	21.2	18.7
Sulfide	9.3	5.2
Bisulfide	9.5	6.2
Cu^{2+}[c]	1.3	0.9
Zn^{2+}[c]	1.1	1.0
Benzoic acid	4.3	4.2
Thiourea	3.5	3.0

[a]Concentrations of substances tested and catechol used as a substrate were 100 and 200 μM, respectively.
[b]$HgCl_2$ was used for Hg^{2+} ions.
[c]$CuSO_4$ and $ZnSO_4$ were used for Cu^{2+} and Zn^{2+} ions.
[d]Mean of three determination.
[e]Inhibition values for banana peel-based biosensor (our previous study (Ozcan and Sagıroglu 2010)).
[f]Inhibition values for fresh broad-based biosensor.

Table II. Results obtained from the measurements of some phenolic compounds.

Phenolic compound	Activity(%)	Linear Range (μM)	R^2	Y
Catechol	100.00	5–60	0.999	108.9x + 15.02
Pyrogallol	87.63	10–60	0.996	209.1x + 8.71
P-Cresol	136.08	10–50	0.990	224x + 16.06
Hydroqinone	121.13	10–60	0.997	157.2x + 16.96
phenol	68.56	10–40	0.996	80x + 10.05
L-Dopa	68.04	20–60	0.997	105x + 8.94
Gallic Acid	65.98	10–70	0.998	122.3x + 7.97
Caffeic Acid	133.50	10–60	0.993	120.4x + 20.82
Rutin	59.28	10–40	0.995	70x + 8.65
Cinnamic Acid	110.10	10–50	0.998	172x + 25.76
Resorsine	69.59	10–70	0.999	242.3x + 3.75
Quersetine	65.46	20–60	0.988	31x + 11.4
Ascorbic Acid	98	10–60	0.998	241.5x + 5.63

Although ascorbic acid was not a phenolic compound, the response obtained by the biosensor for ascorbic acid was 2%, which is less than the response of catechol. Probably, this resulted from the universal interference effect of ascorbic acid. Moreover, it may result from the cyclic form of ascorbic acid in an aqueous solution.

As seen in Table III the fresh broad-based biosensor was more effective than the banana biosensor for determination of PCs. Fresh broad-based biosensor has shown higher activity than banana peel-based biosensor to all PCs without Pyrogallol.

Sample applications

The tissue biosensor based on fresh broad was applied by using standard addition method in some drink samples. The samples which included known amount of catechol (100 μM), were used as stock substrate solutions with different dilution ratios derived by working buffer and were added into the reaction cell, was equilibrated and then the change in current was measured. The signals derived from the drink samples were found to be very similar with the standard catechol solutions having the same concentration. Results are expressed as mean ± S.D, (n = 5). The results of the measurements of drink samples are shown in Table IV. As it is seen in Table IV, the biosensor response is not influenced by alcohol and sugar during the measurements of PCs in beer, wine and fruit juice.

Table III. Comparison of the performance of banana peel and fresh broad biosensor for determination of different phenolic compounds.

Phenolic compound	Activity(%)[a]	Activity(%)[b]	Linear Range (μM)[a]	Linear Range (μM)[b]
Catechol	100	100.00	20–80	5–60
Pyrogallol	117.7	87.63	10–60	10–60
P-Cresol	96	136.08	10–50	10–50
Hydroqinone	106.7	121.13	10–70	10–60
phenol	63.6	68.56	10–40	10–40
L-Dopa	50	68.04	20–60	20–60
Gallic Acid	83.3	65.98	10–80	10–70
Caffeic Acid	139.9	133.50	20–70	10–60
Rutin	55.6	59.28	20–50	10–40
Cinnamic Acid	108.1	110.10	10–60	10–50
Resorsine	65.1	69.59	10–40	10–70
Quersetine	55.1	65.46	20–60	20–60
Ascorbic Acid	96	98	20–80	10–60

[a]Banana peel- based biosensor (Ozcan and Sagıroglu 2010).
[b]Fresh broad-based biosensor.

Table IV. Phenolic compound measurements in some drink samples.

Substances[a]	Included amount (μM)	Calculated amount of calibration curve (μM)	Recovery (%)
Apple Juice	22	22.08 ± 0.46	100.36
(Pınar	50	50.68 ± 0.82	101.36
%100)			
CocaCola	32	32.32 ± 0.50	101.00
	54	54.18 ± 0.77	100.34
Fanta	27	27.36 ± 0.65	101.34
	50	70.09 ± 0.46	100.18
Grape Juice	22	22.58 ± 0.77	104.55
(Tamek	54	54.54 ± 0.53	101.10
%100)			
Beer (Efes	12	11.93 ± 0.41	99.42
Pilsen)	50	49.58 ± 0.81	99.18
White Wine	16	15.98 ± 0.49	99.87
(Guzel	46	46.10 ± 0.82	100.22
Marmara)			

[a]Mean of five determimation.

Conclusion

Our observations showed that the biosensor based on fresh broad tissue developed in this study could be a good alternative detection method without requiring sample pretreatment. Moreover, sugar containing or alcoholic nature of the samples did not interfere in our measurements. The biosensor is able to measure PCs, such as caffeic acid, pyrogallol, cinnamic acid, hydroquinone, catechol, p-cresol, ascorbic acid, gallic acid, resorcin, phenol, rutin, quercetin and L-dopa; and the biosensor will be useful for studies seeking to understand the total level of these compounds in some products. All the measurements showed that the biosensor obtained could be used simply and rapidly, as well as having an advantage of being inexpensive. The total analysis time of a measurement takes 10–12 min. Also as seen in the analysis of different PCs and inhibits the biosensor is more effective than banana peel-based biosensor (Ozcan and Sagıroglu 2010). The proposed biosensor can analyse thirteen kinds of PCs by using the standard curves of them. However, in a real drink sample such as wine and fruit juice, it is more difficult to detect PCs, separately. In these samples it is possible to analyse total PCs by the banana peel tissue-based biosensor. Moreover, we anticipate that this biosensor can be used not only for the analysis of PCs but also for monitoring certain substances which inhibit the PPO in the bioactive material. This study also demonstrates the successful application of a plant tissue on GCE for construction of a biosensor by using uncharacterized tissue (fresh broad) in literature.

Acknowledgements

We would like to thank Dr. E. Dinckaya, Dr. A. Telefoncu, Dr. M.K. Sezginturk and Dr. D. Odacı (Biochemistry Department, Faculty of Science, Ege University, •zmir/Turkey) for their support.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

Financial support for this work was provided by Trakya University (Edirne, Turkey), throughout the projects TUBAB-843.

References

Bravo L. 1998. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. Nut Rev. 56:317–333.

Chawla S, Rawal R, Kumar D, Pundir CS. 2012. Amperometric determination of total phenolic content in wine by laccase immobilized onto silver nanoparticles/zinc oxide nanoparticles modified gold electrode. Anal Biochem. 430:16–23.

Chung KT, Wei CI, Johnson MG. 1998. Are tannins a double-edged sword in biology and health. Trends Food Sci Technol. 9:168–175.

Fiehn O, Jekel M. 1997. Analysis of phenolic compounds in industrial wastewater with high-performance liquid chromatography and post-column reaction detection. J Chromatogr A. 769: 189–200.

Graneroa AM, Fernándeza H, Agostinib E, Zón MA. 2010. An amperometric biosensor based on peroxidases from Brassica napus for the determination of the total polyphenolic content in wine and tea samples. Talanta. 83:249–255.

Haslam E, Lilley TH. 1988. Natural astringency in foodstuffs- A molecular interpretation. CRC Crit Rev Food Sci Nut. 27:1–40.

Haslam E. 1998. Practical Polyphenols: From Structure to Molecular Recognition and Physiological Action. Cambridge: University Press.

Imabayashi S, Kong Y, Watanabe M. 2001. Amperometric biosensor for polyphenol based on horseradish peroxidase immobilized on gold electrodes. Electroanalysis. 13:408–412.

Kishi H, Arimoto H, Fujii T. 1998. Analysis of alcohols and phenols with a newly designed gas chromatographic detector. Anal Chem. 70:3488–3492.

Kochana J, Nowak P, Jarosz-Wilkołazka A, Biero• M. 2008. Tyrosinase/laccase bienzyme biosensor for amperometric determination of phenolic compounds. Microchem J. 89:171–174.

Masque N, Pocurull E, Marce RM, Borrull F. 1998. Determination of eleven priority EPA phenolics at ng L^{-1} levels by on-line solid-phase extraction and liquid chromatography with UV and electrochemical detection. Chromatographia. 47:176–182.

Mokower A, Eremenko AV, Streffer K, Wollenberger U, Scheller FW. 1996. Tyrosinase-glucose dehydroenase substrate-recycling biosensor: a highly sensitive measurement of phenolic compounds. J Chem Tech Biotechnol. 65:39–44.

Montereali MR, Della Seta L, Vastarella W, Pilloton R. 2010. A disposable Laccase–Tyrosinase based biosensor for amperometric detection of phenolic compounds in must and wine. J Mol Catal B Enzym. 64:189–194.

Morales S, Cela R. 2000. Highly selective and efficient determination of US Environmental Protection Agency priority phenols employing solid-phase extraction and non-aqueous capillary electrophoresis. J Chromatogr A. 896:95–104.

Mukhtar H, Wang ZY, Katiyar SK, Agarwal R. 1992. Tea components: antimutagenic and anticarcinogenic effect. Preventative Med. 21:351–360.

Narang AS, Vernoy CA, Eadon GA. 1983. Evaluation of Nellson–Kryger steam distillation technique for recovery of phenols from soil. J Assoc Off Anal Chem. 66:1330.

Odacı D, Timur S, Telefoncu A. 2004. Immobilized jarusalem artichoke *(Helianthus tuberosus)* tissue electrode for phenol detection. Artif Cells Blood Substit Immobil Biotechnol. 32:315–323.

Ozcan HM, Sagiroglu A. 2010. A novel amperometric biosensor based on banana peel *(Musa cavendish)* tissue homogenate for determination of phenolic compounds. Artif Cells Blood Substitutes Biotechnol. 38:208–214.

Ozoner SK, Yalvac M, Erhan E. 2010. Flow injection determination of catechol based on polypyrrole–carbon nanotube–tyrosinase biocomposite detector. Curr Appl Phys. 10:323–328.

Ozsoz M, Erdem A, Kilinc E, Gokgunnec L. 1996. Mushroom based cobalt phthalocyanine dispersed amperometric biosensor for the determination of phenolic compounds. Electroanal. 8:147–152.

Portaccio M, Di Martino S, Maiuri P, Durante D, De Luca P, Lepore M, et al. 2006. Biosensor for phenolic compouns: the catechol as a substrate model. J Mol Catal B Enzym. 41:97–102.

Rajesh, Takashima W, Kaneto K. 2004. Amperometric phenol biosensor based on covalent immobilization of tyrosinase onto an electrochemically prepared novel copolymer poly(*N*-3-aminopropyl pyrroleco-pyrrole) film. Sens. Actuators B Chem. 102:271–277.

Rogers KR. 1995. Biosensors for environmental applications. Biosens Bioelectron. 10:533–541.

Shimomura T, Itoh T, Sumiya T, Hanaoka T, Mizukami F, Ono M. 2011. Amperometric detection of phenolic compounds with enzyme immobilized in mesoporous silica prepared by electrophoretic deposition. Sensors Actuators B. 153:361–368.

Singleton VL. 1981. Naturally occuring food toxicants: phenolic substances of plant origin common in foods. Adv Food Res. 27:149–242.

Topçu S, Sezgintürk MK, Dinçkaya E. 2004. Evaluation of a new biosensor-based mushroom (*Agaricus bisporus*) tissue homogenate: investigation of certain phenolic compounds and some inhibitor effects. Biosens Bioelect. 20:592–597.

Townshend A. 1995. Encyclopedia of Analytical Science, vol.8. London: Academic Press, p. 3928.

V´edrine C, Fabiano S, Tran-Minh C. 2003. Amperometric tyrosinase based biosensor using an electrogenerated polythiophene film as an entrapment support. Talanta. 59:535–544.

Virgili F, Marino M. 2008. Regulation of cellular signals from nutritional molecules: a specific role for phytochemicals beyond antioxidant activity. Free Radic Biol Med. 45:1205–1216.

Vogel AI. 1989. Textbook of Quantitative Chemical Analysis. London: Longman, p. 707.

Yang S, Li Y, Jiang X, Chen Z, Lin X. 2006. Horseradish peroxidase biosensor based on layer-by-layer technique for the determination of phenolic compounds. Sens Act B. 114:774–780.

Yu J, Ju H. 2004. Pure organic phase phenol biosensor based on tyrosinase entrapped in a vapor deposited titania sol–gel membrane. Electroanalysis. 16:1305–1310.

Yu J, Liu S, Ju H. 2003. Mediator-free phenol sensor based on titania sol–gel encapsulation matrix for immobilization of tyrosinase by a vapor deposition method. Biosens Bioelectron. 19:509–514.