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Detection of Benzoic Acid by an Amperometric Inhibitor Biosensor Based on Mushroom Tissue Homogenate

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Summary

An amperometric benzoic acid-sensing inhibitor biosensor was prepared by immobilizing mushroom (*Agaricus bisporus*) tissue homogenate on a Clark-type oxygen electrode. The effects of the quantity of mushroom tissue homogenate, the quantity of gelatin and the effect of the crosslinking agent glutaraldehyde percent on the biosensor were studied. The optimum concentration of phenol used as substrate was 200 μM. The bioanalytical properties of the proposed biosensor, such as dependence of the biosensor response on the pH value and the temperature, were investigated. The biosensor responded linearly to benzoic acid in a concentration range of 25–100 μM. Standard deviation (s.d.) was ±0.49 μM for 7 successive determinations at a concentration of 75 μM. The inhibitor biosensor based on mushroom tissue homogenate was applied for the determination of benzoic acid in fizzy lemonade, some fruits and groundwater samples. Results were compared to those obtained using AOAC method, showing a good agreement.

Key words: benzoic acid, inhibitor biosensors, polyphenol oxidases, food additives, Clark-type oxygen electrode

Introduction

Benzoic acid is a white solid that is slightly soluble in water. Sodium benzoate is about 200 times more soluble in water. Sodium benzoate is primarily used as a preservative and corrosion inhibitor (*e.g.* in technical systems as an additive to automotive engine antifreeze coolants). Benzoic acid and sodium benzoate are used as food preservatives and are most suitable for foods, fruit juices and soft drinks that are naturally in acidic pH range. Their use as preservatives in food, beverages, toothpastes, mouthwashes, dentifrices, cosmetics, and pharmaceuticals is regulated. The estimated global production capacity for benzoic acid is about 600 000 tons per year (*1,2*).

Benzoic acid occurs naturally in many plants and animals. It is therefore a natural constituent of many foods including milk products. Anthropogenic releases of benzoic acid and sodium benzoate into the environment are primarily emissions into water and soil from their uses as preservatives. Concentrations of naturally occurring benzoic acid in several foods did not exceed average values of 40 mg/kg of food. Maximum concentrations reported for benzoic acid or sodium benzoate added to food for preservation purposes were in the range of 2000 mg/kg of food (*1–4*).

Cases of urticaria, asthma, rhinitis, or anaphylactic shock have been reported following oral, dermal, or inhalation exposure to benzoic acid and sodium benzoate. The symptoms appear shortly after the exposure even to low doses, and disappear within a few hours (*4–8*). The information concerning skin reactions caused by benzoic acid or sodium benzoate in general population is limited. In a study with 2045 patients of dermatological clinics, only 5 persons (approximately 0.2 %) showed a positive reaction in patch tests, while 34 of 5202 patients (approximately 0.7 %) with contact urticaria reacted positively (*9–11*). From these data, it can be concluded that

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skin reactions caused by benzoic acid or sodium benzoate in the healthy general population are rare. Moreover, sodium benzoate is used in the treatment of patients with urea cycle enzymopathies (*i.e.* hyperammonaemia due to inborn errors of urea synthesis) in order to facilitate an alternative pathway of nitrogen excretion (*6*,*12*, *13*).

Analytical methods for the determination of benzoic acid include spectrophotometric methods such as UV spectroscopy, high performance liquid chromatography and gas chromatographic methods (*14–21*). HPLC is the most common analytical method for the quantification of benzoic acid.

This paper reports the study of immobilized mushroom tissue homogenate on the Clark-type oxygen electrodes as a biosensor for determination of benzoic acid. When phenol was used as a substrate, the consumption of oxygen by polyphenol oxidase was retarded due to the inhibition effect of benzoic acid injected to the reaction cell. Principle of the measurements was based on this basis of inhibition effect of benzoic acid.

Materials and Methods

Chemicals

All chemicals used in the experiments were obtained from Merck (Germany) as the analytical grades. Mushrooms for bioactive material were purchased from a local grocery.

Apparatus

YSI 54 A model oxygen meter and YSI 5700 series dissolved oxygen (DO) probes (YSI Co. Inc., Yellow Springs, Ohio, USA) were used. A water bath was used for preparation of bioactive material (Stuart scientific linear shaker bath SBS 35, UK). All the measurements were carried out at constant temperature using a thermostat (Haake, Germany). Magnetic stirrer (IKA-Combimag, RCO) and pH meter with electrode (WTW pH 538, Germany) were used for preparing buffer solutions. The temperature in the reaction cell was maintained constant by circulating water at appropriate temperature around the cell compartment during the experiment.

Dissolved oxygen probe

To construct the biosensor a dissolved oxygen probe was covered with highly sensitive teflon membrane by using an O-ring and then the teflon membrane, which is selective for oxygen, was pretreated with 0.5 % sodium dodecylsulphate in phosphate buffer (50 mM, pH=7.5) to reduce the tension on the membrane surface.

Preparation of the mushroom tissue homogenate based on bioactive material

A mass of 100 mg of mushroom was weighed and homogenized with 400 μL of working buffer (50 mM, pH=7.5, phosphate buffer) by a manual glass homogenizer. Then, 10 mg of gelatin was weighed and added to a test tube. A volume of 300 μL of mushroom tissue homogenate was pipetted into the test tube. The mixture of mushroom tissue homogenate and gelatin was incubated at 38 °C for 10–15 min to dissolve gelatin.

Biosensor preparation

A volume of 200 μL of gelatin-mushroom tissue homogenate mixture was dispersed over the dissolved oxygen probe membrane surface and allowed to dry at 4 °C for 15–30 min. For crosslinking with glutaraldehyde, the probe carrying bioactive layer was immersed into 2.5 % glutaraldehyde solution and was allowed to wait for 5 min. Then, the biosensor was washed with distilled water to remove excess of glutaraldehyde.

For getting moisture medium, the biosensor was stored in a flask that contained some distilled water but it was not in contact with it. This moisture medium was needed to prevent drying of the bioactive layer of the biosensor through the storage period.

Principle of the measurements

Polyphenol oxidase (E.C. 1.14.18.1 and E.C. 1.10.3.1, PPO) is a copper containing enzyme, which catalyzes both the oxygen-dependent hydroxylation of monophenols to their corresponding *o*-diphenols and the oxidation of *o*-diphenols to their cognate *o*-quinones (*22*). Mushroom contains polyphenol oxidase enzyme abundantly. Benzoic acid shows competitive inhibitory kinetics on polyphenol oxidase (*23*). The principle of the measurements was based on the determination of the decrease in the differentiation of oxygen level which had been caused by the inhibition of polyphenol oxidase in the biological material by benzoic acid. Measurements were carried out by standard curves that were obtained by the determination of decrease in the consumed oxygen level related to benzoic acid concentration.

Biosensor assay procedure

The biosensor based on mushroom tissue homogenate was put into the thermostatic reaction cell containing working buffer and the magnetic stirrer was fixed at a constant speed. A few minutes later, dissolved oxygen concentration was equilibrated because of the diffusion of dissolved oxygen between the working buffer and the dissolved oxygen probe. At this time, phenol was injected into the thermostatic reaction cell. The dissolved oxygen concentration started to decrease and a few minutes later it reached constant dissolved oxygen concentration due to the enzymatic reaction described above.

At this moment, dissolved oxygen concentration was recorded and then benzoic acid standard or sample was injected into the cell. DO concentration started to increase because of the inhibition of polyphenol oxidase by benzoic acid. Measurements were carried out by the change of dissolved oxygen concentration related to benzoic acid concentration added to the reaction cell.

Results and Discussion

Immobilization conditions of the mushroom tissue homogenate

In the first set of experiments, the effects of the quantity of mushroom tissue homogenate (22.12, 44.25

Table 1. The results of optimization studies of the bioactive layer and biosensor performances

	Biosensor compositions			Linear range	R^2	y	Response time
	Mushroom tissue	Gelatin	Glutaraldehyde	μM			min
	mg/cm^2	mg/cm^2	%				
1	22.12	5.9	2.5	50–100	0.9643	0.0060x–0.1667	20
2	44.25	5.9	2.5	25–100	0.9885	0.0063x–0.0375	20
3	66.37	5.9	2.5	50–100	0.9805	0.0045x–0.0658	20
4	44.25	2.95	2.5	50–100	0.9758	0.0055x–0.1625	20
5	44.25	4.43	2.5	25–100	0.9741	0.0047x+0.005	20
6	44.25	8.85	2.5	100–200	0.9806	0.0026x–0.1071	20
7	44.25	5.9	1.25	50–100	0.9868	0.0050x–0.1417	20
8	44.25	5.9	5	100–200	0.9776	0.0028x–0.1171	20

and 66.37 mg/cm^2) and the quantity of gelatin (2.95, 4.43, 5.9 and 8.85 mg/cm^2) on the biosensor, and the effect of the percentage of crosslinking agent glutaraldehyde (1.25, 2.5 and 5.0 %) on the biosensor were investigated. Measurements were accomplished by using each of the standard curves obtained under these conditions. The optimization studies of the immobilization revealed that optimum mushroom tissue homogenate quantity, optimum gelatin quantity and glutaraldehyde percentage were 44.25 mg/cm^2, 5.9 mg/cm^2, and 2.5 %, respectively. Through all experiments, mushroom tissue homogenate quantity, gelatin quantity, and glutaraldehyde percentage were kept constant at optimum as mentioned above. Bioactive layer compositions and the corresponding biosensor parameters are summarized in Table 1.

pH dependence

Fig. 1 shows the effect of the pH value of the working buffer on the response behaviour of the present biosensor. In this figure, the maximum response can be observed at pH=7 (50 mM, phosphate buffer).

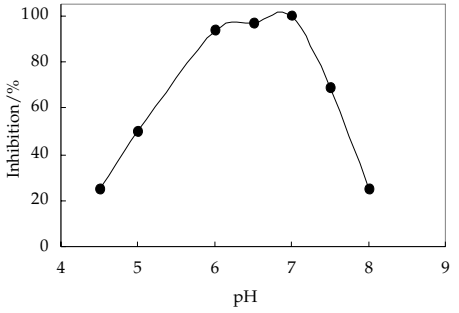

Fig. 1. The effect of pH on the biosensor response
Citrate buffers: 50 mM and pH=4.5, 5.0 and 6.0; sodium-phosphate buffers; 50 mM and pH=6.5, 7.0, 7.5, 8.0. Phenol concentration used as substrate in the experiments was 200 μM, benzoic acid concentration was 75 μM and *T*=35 °C

Temperature effect and thermal stability

The working temperature was changed from 20 to 45 °C, and the corresponding response to 75 μM of benzoic acid (with the presence of 200 μM of phenol) (Fig. 2). From this figure it can be concluded that the biosensor response increased with the increase of working temperature. At temperature greater than 40 °C, the biosensor response decreased rapidly. This result is in agreement with enzymology principles.

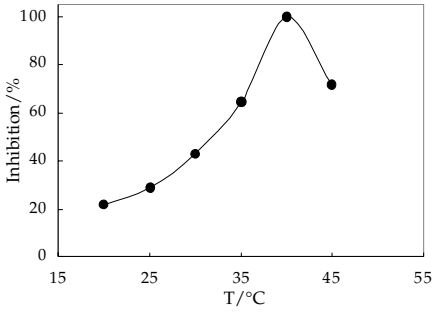

Fig. 2. Temperature effect
Working conditions: phosphate buffer 50 mM, pH=7. Phenol concentration used as substrate in the experiments was 200 μM, benzoic acid concentration was 75 μM and *T*=35 °C

In order to determine the working temperature correctly, thermal stability of the biosensor at the temperatures of 35 and 40 °C was characterized. The results showed that the biosensor lost part of its initial activity at the end of the 3rd hour by working at 40 °C. However, at 35 °C, the biosensor response did not change 3 hours later. Consequently, all experiments were done at 35 °C.

Substrate concentration

For an inhibitor biosensor, substrate and inhibitor concentrations have to be adjusted carefully in order to obtain correct results. The calibration curves in Fig. 3 have all been recorded under the same conditions but in the presence of different substrate concentrations.

As can be seen in the figure, the best results were obtained by using 200 μM of phenol, so in all experiments phenol concentration was kept constant at 200 μM.

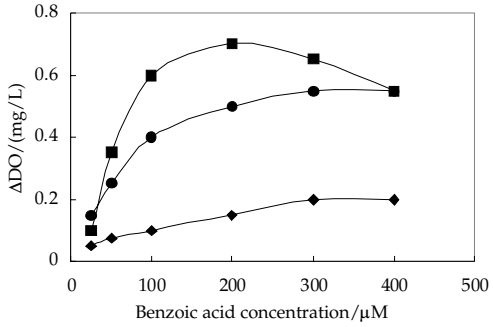

Fig. 3. Substrate concentration dependency of the biosensor
Working conditions: phosphate buffer 50 mM, pH=7
–■–■– 300 μM, –●–●–200 μM, –♦–♦– 100 μM phenol concentrations were used for this purpose, *T*=35 °C

Benzoic acid determination by the biosensor

Fig. 4 illustrates the response of the biosensor based mushroom tissue homogenate as an inhibitor electrode. The typical calibration curve is linear with benzoic acid concentration over the entire 25 to 100 μM range and then with a curvature at a higher level.

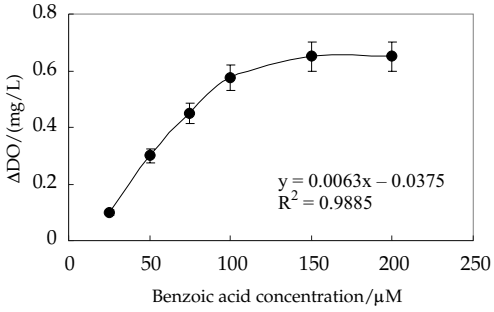

Fig. 4. Calibration graph for benzoic acid
Working conditions: phosphate buffer 50 mM, pH=7. Phenol concentration used as substrate in the experiments was 200 μM, *T*=35 °C

Repeatability

Repeatability is a measure of biosensor stability over time. Typically, sample to sample repeatability can be lower. As the sample concentration is lowered, repeatability can be improved but this does not continue indefinitely. Repeatability studies of the present biosensor were carried out by making seven measurements with 75 μM of benzoic acid standard. The average value (x), standard deviation (s.d.) and variation coefficient (CV) were calculated as (75.1±0.49) μM and 8.54 %, respectively. The results showed that the proposed biosensor worked without the decrease in activity after 10 measurements. The cost of one biosensor based on mushroom tissue homogenate was very low. Because of this, ten measurements without the activity loss were a sufficient performance for an inhibitor biosensor.

Sample analysis

Fizzy lemonade samples were assayed to demonstrate the practical use of the biosensor based on mushroom tissue homogenate. Moreover, the samples were analyzed with the reference method AOAC (Association of Official Analytical Chemists, Procedure 963.19) to validate the new biosensor (*24*). These results are given in Table 2.

Table 2. Quantification of benzoic acid in fizzy lemonade (FL) and fruit samples by the proposed biosensor and comparison with AOAC method. Benzoic acid is expressed as g/100 mL of lemonade or g/kg of fruit

Sample	AOAC method	Biosensor	Relative error/%
FL1	$36.4 \cdot 10^{-3}$	$33.5 \cdot 10^{-3}$	–8.0
FL2	$35.4 \cdot 10^{-3}$	$33.0 \cdot 10^{-3}$	–6.7
FL3	$34.4 \cdot 10^{-3}$	$34.0 \cdot 10^{-3}$	–1.0
Apple	$58.2 \cdot 10^{-3}$	$55.4 \cdot 10^{-3}$	–4.8
Peach	$28.4 \cdot 10^{-3}$	$26.2 \cdot 10^{-3}$	–7.8
Groundwater	$98.2 \cdot 10^{-3}$	$94.4 \cdot 10^{-3}$	–3.9

In the literature, a lot of biosensors that utilize tyrosinase or PPO have widely been cited. Most of these biosensors were developed for determination of phenolic compounds (*25–32*). Besides, biosensors based on tyrosinase or PPO inhibition effect have also been reported. For example, Stanca and Popescu (*33*) reported an amperometric study of inhibitory effect of carboxylic acids, such as benzoic acid, on tyrosinase. Moreover, Wang *et al*. (*34*) reported a mushroom-carbon paste tissue electrode for amperometric measurements of inhibitors of tyrosinase. In our previous work we reported a biosensor based on mushroom tissue homogenate for detecting some phenolic compounds and usage of the biosensor for quantifying certain substances that inhibit the PPO activity in the tissue (*35*). The detection limit of the biosensor for benzoic acid was 25 μM. The detection limit was not better than in the study of Tfouni and Toledo (*36*). The detection limits of the biosensor and the method by Ferreira *et al*. were close to each other (*37*). However, both of these studies were based on high performance liquid chromatography. Unfortunately, this made the methods more difficult to work. On the other hand, the biosensor was based on a traditional Clark-type oxygen electrode. In order to construct a biosensor and to work with it was neither difficult nor expensive. This was the most important advantage of the biosensor to the methods above.

Besides, an enzyme electrode for the control of the benzoic acid content in food was reported (*38*). A limit of detection of $9 \cdot 10^{-7}$ M of benzoic acid was obtained for the electrode. This limit was better than that by us, however, in this system the construction of the enzyme electrode needed time consuming steps. In our system, homogenization of mushroom tissue, and immobilization of the mushroom tissue homogenate via gelatin and glutaraldehyde on the Clark-type oxygen electrode took just 45 min. That is to say that the construction of the biosensor was very easy and its cost was lower than of the other methods. Nevertheless, the reference method

for the determination of benzoic acid in food also recommended tedious methodologies, with extensive extraction procedures, involving large amounts of reagents and time incurring considerable costs.

On the other hand, the biosensor had an important disadvantage. Because PPOs were not specific enzymes, they catalyzed the oxidation of various phenolic compounds. In this respect, the presence of a phenolic compound in the sample could alter the response of the biosensor. We concluded that when a natural sample was analyzed, it was needed to pretreat the sample to oxidize phenolic compounds.

Conclusion

Although practical application of inhibitor biosensors seems much more complicated than that of substrate biosensors, their potentials are far from being exhausted. In this point of view, we developed an inhibitor biosensor for determination of benzoic acid in soft drinks such as fizzy lemonade. The construction of a Clark-type oxygen electrode based on mushroom tissue homogenate resulted in an amperometric benzoic acid--sensing inhibitor biosensor with good performance characteristics, such as low detection limit and response time which was only about 20 min. Moreover, usage of mushroom tissue instead of pure PPO enzyme helped to reduce the cost of the biosensor considerably. Further, the biosensor was considered to be useful to determine benzoic acid in natural samples.

References

1. B.F. Goodrich: *Benzoic Acid. Product Information Bulletin*, Kalama Inc., Kalama (1999).
2. J.R. Chipley: Sodium Benzoate and Benzoic Acid. In: *Antimicrobials in Foods*, A.L. Branen, P.M. Davidson (Eds.), M. Decker, New York (1983) pp. 11–35.
3. A. Aguirre, R. Izu, J. Gardeazabal, J.L. Diaz-Perez, Edematous allergic contact cheilitis from a toothpaste, *Contact Dermatitis, 28* (1993) 28–42.
4. C. Bindslev-Jensen, ABC of allergies, *Br. Med. J. 316* (1998) 1299–1302.
5. M.L. Batshaw, S.W. Brusilow, Evidence of lack of toxicity sodium pehnylacetate and sodium benzoate in treating urea cycle enzymopathies, *J. Inherit Metab. Dis. 4* (1981) 231.
6. M.L. Batshaw, P.S. Monahan, Treatment of urea cycle disorders, *Enzyme*, *38* (1987) 242–250.
7. J. Coverly, L. Peters, E. Whittle, D.A. Basketter, Susceptibility to skin stinging, non-immunologic contact urticaria and skin irritation – Is there a relationship?, *Contact Dermatitis, 38* (1998) 90–95.
8. B.J. Freedman, Asthma induced by sulphur dioxide, benzoate and tartrazine contained in orange drinks, *Clin. Allerg. 7* (1977) 407–415.
9. M. Petrus, S. Bonaz, E. Causse, M. Rhabbour, N. Moulie, J.C. Netter, G. Bildstein, Asthme et intolérance aux benzoates (Benzoate intolerance in asthma), *Archives de pédiatrie*, *3* (1996) 984–987 (in French).
10. W. Broeckx, A. Blondeel, A. Do-Go, G. Achten, Cosmetic intolerance, *Contact Dermatitis*, *16* (1987) 189–194.
11. J. Brasch, T. Henseler, P. Frosch, Patch–test reactions to a preliminary preservative series – A retrospective study based on data collected by the Information network of Dermatological Clinics (IVDK) in Germany, *Dermatosen*, *41* (1993) 71–76.
12. F. Feillet, J.V. Leonard, Alternative pathway therapy for urea cycle disorders, *J. Inherit. Metab. Dis. Suppl. 21* (1998) 101–111.
13. G.C. Tremblay, I.A. Qureshi, The biochemistry and toxicology of benzoic acid metabolism and its relationship to the elimination of waste nitrogen, *Pharmacol. Ther. 60* (1993) 63–90.
14. G. Burini, P. Damiani, Determination of sorbic acid in margarine and butter by high-performance liquid chromatography with fluorescence detection, *J. Chromatogr. 543* (1991) 69–80.
15. B.H. Chen, S.C. Fu, Simultaneous determination of preservatives, sweeteners and antioxidants in foods by paired--ion liquid chromatography, *Chromatographia*, *14* (1995) 43–50.
16. S. Manning, M.S. Cosio, Determination of benzoic and sorbic acids in food by microdialysis sampling coupled with HPLC and UV detection, *Int. J. Food Sci. 8* (1996) 311–316.
17. A. Montano, A.H. Sanchez, L. Rejano, Determination of benzoic and sorbic acids in packaged vegetable products. Comparative evaluation of methods, *Analyst*, *120* (1995) 2483–2487.
18. M.C. Bennett, D.R. Petrus, Quantitative determination of sorbic acid and sodium benzoate in citrus juice, *J. Food Sci. 42* (1977) 1220–1221.
19. J.C. Castro, M.A. Delgado, M.J. Sánchez, F.G. Montelongo, Simultaneous 2nd order derivative spectrophotometric determination of sorbic and benzoic acids in soft drinks, *Anal. Lett. 25* (1992) 2357–2376.
20. K. Kubota, Y. Horai, K. Kushida, T. Ishizaki, Determination of benzoic acid and hippuric acid in human plasma and urine by high performance liquid chromatography, *J. Chromatogr. 425* (1988) 67–75.
21. A. Sioufi, F. Pommier, Gas chromatographic determination of low concentrations of benzoic acid in human plasma and urine, *J. Chromatogr. 181* (1980) 161–168.
22. A.M. Mayer, E. Harel: Phenoloxidases and Their Significance in Fruit and Vegetables. In: *Food Enzymology, Vol. 1*, P.F. Fox (Ed.), Elsevier, New York (1991) pp. 373–398.
23. L. Bubacco, E. Vijgenboom, C. Gobin, A. W.J.W. Tepper, J. Salgado, G.W. Canters, Kinetic and paramagnetic NMR investigations of the inhibition of *Streptomyces antibioticus* tyrosinase, *J. Mol. Catal. B–Enzym. 8* (2000) 27–35.
24. Official Methods of Analysis, AOAC, 15th ed., Arlington (1990) section 963.19.
25. S.Q. Zhang, H.J. Zhao, R. John, Oxygen dependence in a dual-phase electrochemical biosensing system, *Electroanalysis*, *17* (2005) 239–245.
26. V.C. Dall'Orto, J.M. Vago, R.R. Carballo, I.N. Rezzano, Comparison of tyrosinase biosensor and colorimetric method for polyphenol analysis in different kinds of teas, *Anal. Lett. 38* (2005) 19–33.
27. S. Cosnier, C. Mousty, J. de Melo, A. Lepellec, A. Novoa, B. Polyak, R.S. Marks, Organic phase PPO biosensors prepared by multilayer deposition of enzyme and alginate through avidin-biotin interactions, *Electroanalysis, 16* (2004) 2022–2029.
28. R. Solna, S. Sapelnikova, P. Skladal, M. Winther-Nielsen, C. Carlsson, J. Emneus, T. Ruzgas, Multienzyme electrochemical array sensor for determination of phenols and pesticides, *Talanta*, *65* (2005) 349–357.
29. D. Quan, Y. Kim, W. Shin, Sensing characteristics of tyrosinase immobilized and tyrosinase, laccase co-immobilized platinum electrodes, *Bull. Korean Chem. Soc. 25* (2004) 1195–1201.

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

31. S.E. Stanca, I.C. Popescu, L. Oniciu, Biosensors for phenol derivatives using biochemical signal amplification, *Talanta, 61* (2003) 501–507.

32. T. Yao, C.O. Ho, Analytical evaluation of catecholamine biosensors based on tyrosinase from mushroom and mushroom tissue, *Bunseki Kagaku, 51* (2002) 469–472.

33. S.E. Stanca, I.C. Popescu, Amperometric study of the inhibitory effect of carboxylic acids on tyrosinase, *J. Mol. Catal. B-Enzym. 27* (2004) 221–225.

34. J. Wang, S.A. Kane, J. Liu, M.R. Smyth, K. Rogers, Mushroom tissue-based biosensor for inhibitior monitoring, *Food Technol. Biotechnol. 34* (1996) 51–55.

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

36. S.A.V. Tfouni, M.C.F. Toledo, Determination of benzoic and sorbic acids in Brazilian food, *Food Control, 13* (2002) 117–123.

37. I.M.P.L.V.O. Ferreira, E. Mendes, P. Brito, M.A. Ferreria, Simultaneous determination of benzoic and sorbic acids in quince jam by HPLC, *Food Res. Int. 33* (2000) 113–117.

38. M.D. Morales, S. Morante, A. Escarpa, M.C. Gonzalez, A.J. Reviejo, J.M. Pingarron, Design of a composite amperometric enzyme electrode for the control of the benzoic acid content in food, *Talanta, 57* (2002) 1189–1198.

Određivanje benzojeve kiseline amperometrijskim inhibitorskim biosenzorom pripravljenim od homogenata tkiva pečurke

Sažetak

Amperometrijski inhibitorski biosenzor za benzojevu kiselinu pripravljen je imobilizacijom homogenata tkiva pečurke na kisikovu elektrodu tipa Clark. Ispitan je učinak količine homogenata tkiva pečurke i želatine na biosenzoru, a i utjecaj postotka glutaraldehida upotrijebljenog za unakrsno povezivanje. Optimalna količina fenola što se koristio kao supstrat iznosila je 200 μM. Od bioanalitičkih svojstava biosenzora utvrđena je njegova ovisnost o pH-vrijednosti i temperaturi. Biosenzor je reagirao linearno na količinu benzojeve kiseline u području od 25 do 100 μM. Pri koncentraciji od 75 μM standardna devijacija iznosila je ±0,49 μM tijekom 7 uzastopnih određivanja. Inhibicijski biosenzor, pripravljen od homogenata tkiva pečurke, upotrijebljen je za određivanje benzojeve kiseline u gaziranoj limunadi, voću i podzemnoj vodi. Rezultati su se dobro poklapali s onima dobivenim AOAC postupkom.