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Determination of Trolox Equivalent Antioxidant Capacity in Berries Using Amperometric Tyrosinase Biosensor Based on Multi-Walled Carbon Nanotubes

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Abstract: In this contribution, Trolox equivalent antioxidant capacity (TEAC) was determined in various berries using carbon paste tyrosinase biosensor with multi-walled carbon nanotubes (MWCNTs), coated with Nafion® layer. Electrochemical behaviour of the biosensor and influence of MWCNTs on carbon paste surface were studied with respect to the sensitive amperometric detection of total content of phenolic compounds in berries, expressed as concentration equivalent of Trolox. After optimization of key instrumental and electroanalytical parameters, the biosensor was used for determination of TEAC in blackberries, blueberries, cranberries, raspberries and strawberries by method of multiple standard additions. Electrochemical TEAC assays corresponded well with results obtained by spectrophotometric 1,1-diphenyl-2-picrylhydrazyl radical method, known as DPPH assay. Obtained values were compared with those listed in the National Nutrient Database for additional antioxidant capacity assays as well.

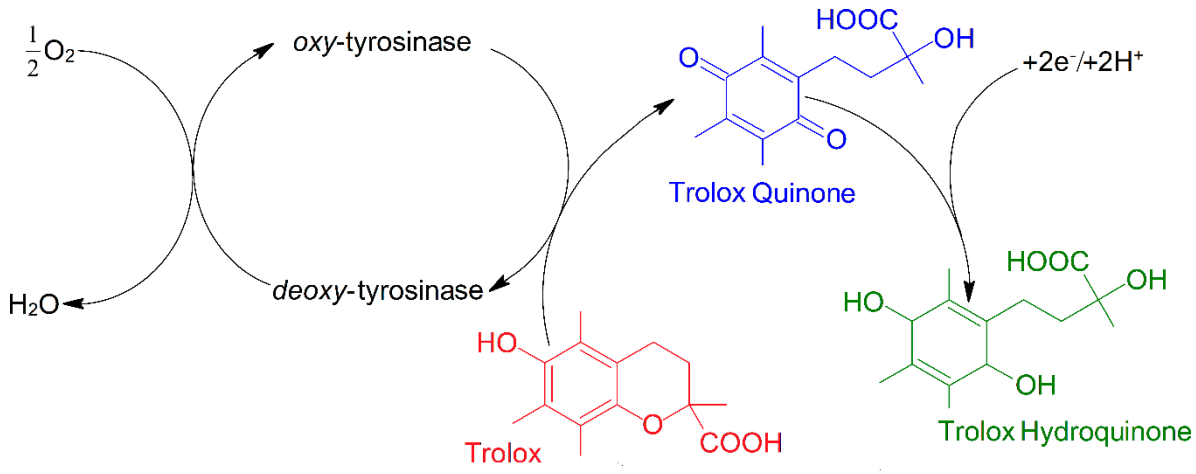
Keywords: amperometry; ascorbic acid; berries; biosensor; multi-wall carbon nanotubes; carbon paste electrode; tyrosinase; Trolox; antioxidant capacity

1. Introduction

The antioxidant properties of the phenolic compounds in berries (such as cranberry or blueberry) play a major role in their ability to decrease damages related to cardiovascular diseases and aging, as well as some of their reported antitumor activities [1–3]. Trolox is a synthetic water-soluble analogue of α-tocopherol, known as the most active form of vitamin E, which is used as standard chemical substance for comparison of antiradical activity of food expressed as Trolox equivalent antioxidant capacity (TEAC) [4]. The solubility of Trolox at neutral values of the pH is around 3 mg·mL^{-1}, which is high enough for common antioxidant assays. For the determination of TEAC,

several spectrophotometric methods based on reductive properties of present antioxidants were developed. One of the most frequently performed method, 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, is based on 1,1-diphenyl-2-picrylhydrazyl radical [5]. Some methods, including an oxygen radical absorbance capacity (ORAC) [6], ferric reducing antioxidant power (FRAP) [7], and total radical trapping antioxidant potential (TRAP) [8], were also employed in the analysis of berries. L-ascorbic acid (AA), known as vitamin C, is a non-phenolic chemical compound, contained also in berries with high antioxidant activity [9]. It occurs commonly together with polyphenols in foodstuffs. AA has an interfering effect during electrochemical analysis of polyphenols due to its reduction properties (reduction of resulting quinones back to polyphenols). The interfering effect of AA on the determination of hydroquinone (HQ), which has a similar chemical structure to α-tocohydroquinone [10], is known from previous studies [11]. The mechanism of HQ reduction by AA, which was proposed by Isaacs and Eldik, is shown in Scheme S1 [11]. However, it was found earlier that the presence of AA is not interfering up to the molar ratio of AA to HQ 1:1, in the case of the amperometric biosensor based on a carbon paste electrode modified with tyrosinase (TYR) [12].

Mushroom TYR enzyme from *Agaricus bisporus* (E.C. 1.14.18.1) is an oxidase enzyme which contains copper. It possesses a catalytic activity toward the oxidation of both catechols and cresols [13]. In the presence of TYR enzyme, the conversion of phenolic compounds to their quinone derivatives is catalyzed. Resultant products can then be detected by amperometric methods at constant reductive potential in phosphate buffer media of pH~7, where the enzyme exhibits optimum activity [14,15]. TYR is capable of catalyzing an oxidation reaction of Trolox by oxygen, resulting in the formation of Trolox quinone [12], which can be electrochemically reduced by 2 e^-/2 H^+ to Trolox hydroquinone [16]. These reactions simply describe the principle of amperometric tyrosinase biosensor behavior (Scheme 1).



Scheme 1. Principle of amperometric tyrosinase biosensor for evaluation of TEAC.

As reported, the biosensor, prepared by covering the bare carbon paste electrode (CPE) with a thin layer of Nafion® and the TYR enzyme (CPE/Tyrosinase/Nafion®), provides a low sensitivity against Trolox in comparison to *o*-substituted polyphenols [12]. Hence, carbon nanotubes (CNTs) were used to improve this parameter, due to their high catalytic effect and large surface area. When considering the type of CNTs, multi-walled carbon nanotubes (MWCNTs) exhibited similar behavior to single-walled carbon nanotubes (SWCNTs), as shown in previously reported electrochemical study with Trolox [17]. Later, the CPE/MWCNTs/Tyrosinase/Nafion® biosensor was tested in analysis of polyphenols in various Moravian wines [18]. In this contribution, the previously introduced biosensor is employed in the determination of TEAC, in comparable matrices such as berries.

2. Materials and Methods

2.1. Reagents and Chemicals

Spectroscopic graphite (with an average particle size of 2 μm) from Graphite Týn s. r. o., Týn nad Vltavou, Czech Republic and paraffin oil for spectrometry from Merck, Darmstadt, Germany were used for the preparation of CPE. Multi-walled carbon nanotubes (diameter 10–30 nm, length 5–15 μm and special surface area 40–300 $m^2 \cdot g^{-1}$) from Shenzhen Nanotech Port Co., Ltd., Shenzhen, China were used for CPE surface coverage. *N*,*N*-dimethylformamide (DMF), mushroom (ex. *Agaricus bisporus*) tyrosinase (E.C. 1.14.18.1; 3130 $U \cdot mg^{-1}$ solid), Nafion® (5% in ethanolic solution), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 1,1-diphenyl-2-picrylhydrazyl radical were obtained from Sigma Aldrich, Prague, Czech Republic. The other chemicals used in this work were of analytical grade purity. The solutions were also prepared using the ultrapure water (ρ = 18.3 MΩ·cm; Milli-Q system, Millipore, Burlington, MA, USA).

2.2. Preparation of Electric Transducer

To obtain a homogenous paste, an amount of 0.500 g of carbon powder and 0.125 g of paraffin oil were finely blended in a ceramic mortar for 30 min. Then, the freshly prepared homogeneous carbon paste was firmly packed into the cavity of piston-driven Teflon® holder (3 mm in diameter) [19]. Measured ohmic resistance of prepared carbon paste electrodes was 20 Ω maximum, which corresponds with already published values [20]. Laboratory conditions were used to store the bare CPEs. A suspension of MWCNTs (~2.0 $mg \cdot mL^{-1}$) in DMF was homogenized in an ultrasonic bath for 30 min. Afterwards, 20 μL of resultant dispersion was applied on the surface of bare CPE and left to dry for one day under laboratory conditions.

2.3. Preparation of Tyrosinase Biosensor

For the immobilization of the TYR enzyme on surface of amperometric transducer, a sulfonated tetrafluoroethylene-based fluoropolymer-copolymer (Nafion®) was used. The solution for casting the enzyme-entrapping membrane was prepared by mixing of 40 μL Nafion® (neutralized by 8% ammonia), 60 μL of redistilled water and 150 μL enzyme solution (500 $\mu g \cdot mL^{-1}$ dissolved in 0.01 $mol \cdot L^{-1}$ phosphate buffer of pH 7.0). In the next step, 10 μL of the mixture was drop-casted onto the surface of CPE/CNTs and left for drying under laboratory conditions for one hour. If not in use, the prepared biosensors were stored in solution of phosphate buffer (PB) in a fridge at 5 °C, as reported previously [21].

2.4. Apparatus and Methods

All amperometric measurements were performed in a three-electrodes system, consisting of CPE/MWCNTs/Tyrosinase/Nafion® as a working electrode, Ag/AgCl/3.0 $mol \cdot L^{-1}$ KCl (Metrohm, Czech Republic) as a reference electrode and platinum wire as a counter electrode connected to potentiostat EmStat (Ivium Technologies B.V., Eindhoven, The Netherlands). Then, 0.01 $mol \cdot L^{-1}$ PB (pH 7.0) was chosen as supporting electrolyte and every measurement was repeated minimally five times. Batch injection analysis (BIA) with amperometric detection was carried out in a voltammetric glass cell under constant stirring speed 400 rpm and at constant working potential of −0.25 V. For analysis, usually 1.0 mL of berries extract was injected into the 9.0 mL of the above-mentioned electrolyte. After that, three additions of standard Trolox solution (0.5 mL 0.01 $mol \cdot L^{-1}$) were successively added.

Drier (Memmert, Schwabach, Germany) and Helios Delta UV-VIS spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) have been used for other experiments. Images of electrode surface structure were obtained by scanning electron microscope (SEM) JEOL JSM7500F (Tokyo, Japan). The DPPH assay was selected as a reference method. The procedure consisted of several steps: (i) 100 μL of ethanolic extract of individual berries was added into 5.0 mL of 25 $\mu g \cdot mL^{-1}$ DPPH methanolic solution and kept for reaction in darkness for 10 min; (ii) the colour of DPPH radical

solution changed from violet to yellow, causing a decrease in absorbance, which was measured at λ = 517 nm.

2.5. Preparation of Berries for Analysis

Five different kinds of berries, such as blackberries *Rubus fruticosus* (Spain), blueberries *Vaccinium corymbosum* (Chile), cranberries *Vaccinium erythrocarpum* (USA), raspberries *Rubus idaeus* (Morocco) and strawberries *Flagaria magna* (Spain) purchased from local stores were selected for analysis of TEAC. Approx. 40 g of berries were firstly dried to constant weight and then pulverized in a mortar for 20 min. Optimum temperature for drying these kinds of fruits ranges from 40 to 60 °C for 3 to 5 days [22]. Each berry powder (2.0 g) was transferred into the 50.0 mL volumetric flask and filled with 50% water ethanolic solution up to mark and kept in ultrasonic bath at laboratory temperature for 60 min. Afterwards, the sample suspension was filtered using regular filter paper. Filtration of strawberry suspension had to be performed using vacuum pump due to the high content of insoluble fiber, which caused clogging of filter paper pores.

3. Results and Discussion

Prior to the analysis of berries using previously introduced tyrosinase amperometric biosensor [17, 18], it was necessary to optimize several conditions such as influence of stirring speed, working potential and pH value of supporting electrolyte, as described in the following chapters. Moreover, some additional characterisation information related to transducer structure were added. From previous study [18] resulted that the transducer consisting of CPE/MWCNTs exhibits higher sensitivity rather than bare CPE due to higher electrode surface area, fast electron transfer and also due to high enzyme affinity towards hydrophobic CNTs, leading to enhanced enzyme loading at the electrode surface. Furthermore, enzyme/Nafion® mixture shows higher mechanical stability on CNTs layer of transducer due to the fibrous structure of polymers fitting into bundles of CNTs. The characterisation of transducer structures was performed using SEM (Figure 1) and differences between both types of CPEs can be clearly observed.

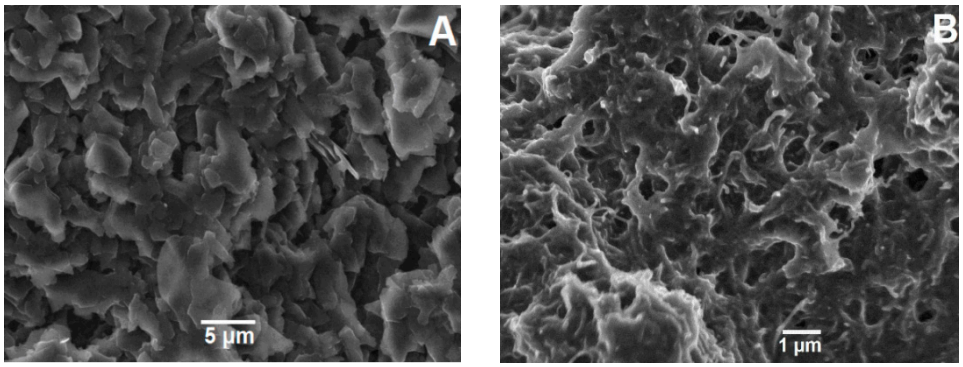


Figure 1. SEM images of bare CPE (**A**) and CPE/MWCNTs (**B**) surfaces.

3.1. Construction of Tyrosinase Biosensor

Expected catalytic activity of TYR enzyme towards Trolox in comparison with 1,2-dihydroxybenzenes was not sufficient to construct a simple bioelectroanalytical device [12]. This means that the amount of Trolox oxidation product formed during enzymatic conversion is low and, therefore, a more sensitive amperometric transducer is required for the detection in the given time frame. Generally, current response increases with larger electrode surface area. For that reason, MWCNTs were used to enlarge the surface of amperometric transducer. Furthermore, MWCNTs provide fast electron transfer, which positively affects the sensitivity of the detection.

Six different amounts (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg·mL^{-1}) of MWCNTs in DMF were prepared and a volume of 20 µL was applied for immobilization of MWCNTs on CPE surface. Obtained CPE/MWCNTs electrodes were compared using cyclic voltammetry of 500 µmol L^{-1} Trolox. As shown in Figure S1 (Supplementary Materials), a significant shift of the cathodic peak potential (E_p^c) to lower values ("easier reduction") was observed for the amount of 2.0 mg·mL^{-1} MWCNTs, therefore, this amount was considered as optimum.

After that, a volume of 5, 10, 15, 20, 25, and 30 µL, with a constant concentration of 2.0 mg·mL^{-1} MWCNTs of fresh homogenized dispersion was applied on polished surfaces of CPE to immobilize MWCNTs. For volumes from 15 to 25 µL, a significant increase of current response for cathodic peak (I_p^c) was observed. From Figure S2, it is evident that the measured peak currents decreased for volumes higher than 20 µL, probably due to the aggregation of MWCNTs and unfavorable morphology at electrode surface. As the optimum volume for immobilization, the value of 20 µL MWCNTs (2.0 mg·mL^{-1}) dispersion was selected.

An amount of TYR enzyme in the biorecognition layer was optimised to achieve the highest current response. With increasing content of the TYR in the Nafion® membrane, the height of the anodic peak of Trolox decreased, while the corresponding cathodic peak increased. Higher amounts than 3.0 µg enzyme did not cause any significant improvement, and therefore it was further used to obtain sufficiently high current responses.

3.2. Characterisation of Tyrosinase Biosensor

Electrochemical behaviour of Trolox was investigated at bare CPE, CPE/MWCNTs, and CPE/MWCNTs/TYR/Nafion® (TYR biosensor), to find out an effect of each component. Trolox provided a distinct oxidation signal with cathodic counterpart (ΔE_p = 65 mV) in 0.1 mol·L^{-1} phosphate buffer pH 7.0 at bare CPE, attributed to one-electron oxidation to radical cation with subsequent proton transfer to form phenoxy radical and then immediate oxidation by one electron to phenoxonium cation (ECE mechanism), similarly to α-tocopherol [16]. After repetitive cycles, another redox couple was observed (ΔE = 280 mV). It is assumed that after the formation of phenoxonium cation, a nucleophilic addition of water starts in aqueous solution with concomitant formation of Trolox quinone via hemiketal intermediate. Trolox quinone is then reduced to corresponding hydroquinone, which can be oxidized to quinone in subsequent scan to positive potentials [10,16]. Increasing current responses of the second redox couple with each cycle is caused by gradual buildup of Trolox quinone by oxidation of the original compound (Figure 2A).

The ratio of the peak currents (I_p^a/I_p^c) was equal to 1.544 at bare CPE, unlike ratio values of 0.615 and 0.320 obtained for CPE/MWCNTs and CPE/MWCNTs/TYR/Nafion®, respectively. Several times, a higher reduction peak of Trolox was observed at the TYR biosensor at peak potential (E_p^c) of +0.049 V (compare with values of +0.079 and +0.099 V for CPE/MWCNTs and bare CPE, respectively), whereas its oxidation signal markedly decreased owing to catalytical activity of the TYR (Figure 2B) towards Trolox. According to these findings, working potential with values lower than +0.050 V should be investigated for amperometric detection in batch configuration. Based on these experimental data, a principle of amperometric tyrosinase biosensor for evaluation of TEAC (Scheme 1) was established, which will enable electrochemical detection of Trolox at low potentials with markedly improved sensitivity.

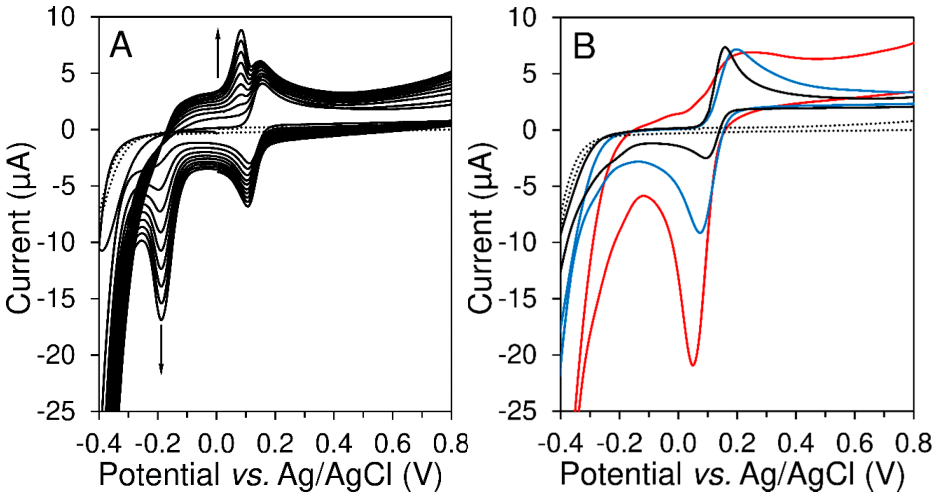


Figure 2. Repetitive (n = 10) cyclic voltammograms of 500 μmol L^{-1} Trolox, obtained at bare CPE (**A**) and cyclic voltammograms of 500 μmol·L^{-1} Trolox obtained at bare CPE (black), CPE/MWCNTs (blue), and CPE/MWCNTs/TYR/Nafion® (red curve) (**B**) in 0.1 mol·L^{-1} PB of pH 7.0, E_{start} = 0 V, $E_{vertex1}$ = −0.5 V, $E_{vertex2}$ = +1.3 V, E_{step} = 5 mV, v = 10 mV·s^{-1}. Dotted black lines represent voltammograms obtained at CPE in 0.1 mol L^{-1} PB (pH 7.0).

3.3. Tyrosinase Activity towards Trolox

Previous voltammetry experiments may suggest that mushroom TYR enzyme is able to catalyze the oxidation of Trolox to corresponding Trolox quinone (*p*-quinone with side chain). This claim had to be confirmed by further experiments. Trolox provides a wide band in the UV spectrum with a maximum at wavelength 291 nm (red line in Figure 3). If TYR enzyme is present, another overlapping band with maximum at wavelength 278 nm will be established. The width of this new band increases with the time of enzyme presence, as shown in Figure 4. This phenomenon can be considered as additional confirmation that Trolox probably does not inhibit catalytic activity of TYR enzyme but represents a substrate. A polymeric reaction of Trolox catalyzed by TYR enzyme cannot be excluded though.

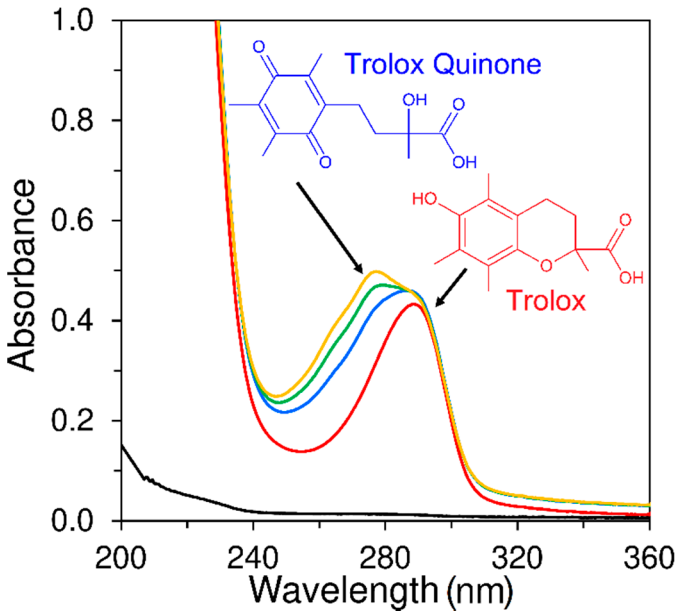


Figure 3. Ultraviolet spectra of 50 μg mL^{-1} TYR (black) and 100 μmol L^{-1} Trolox obtained in 0.1 mol L^{-1} PB (pH 7.0) for 0 (red), 10 (blue), 20 (green), and 30 min (yellow line).

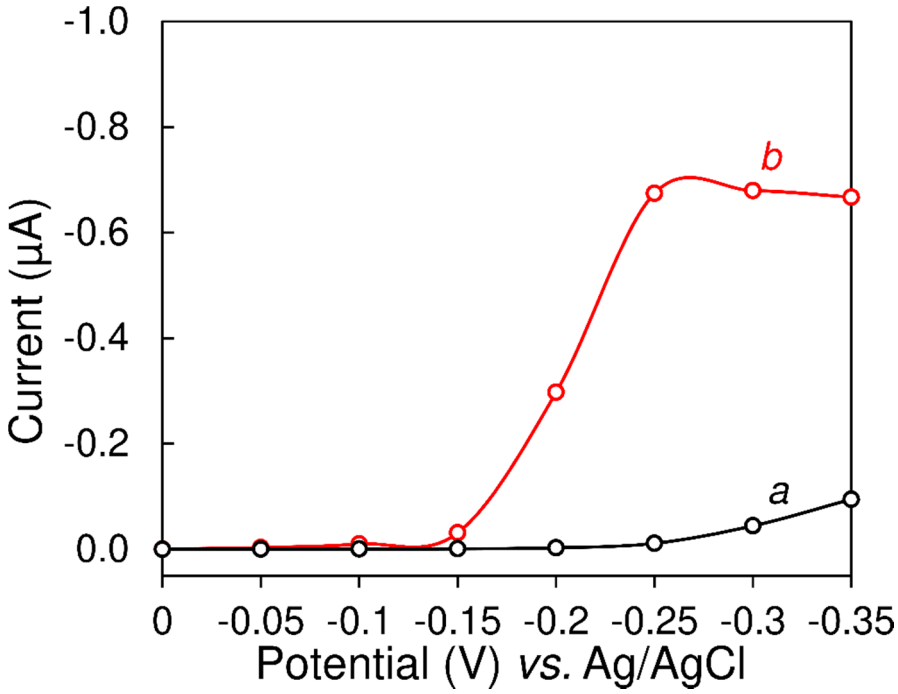


Figure 4. The dependency of the amperometric response on the magnitude of the working potential applied at the CPE/MWCNTs (curve *a*) compared to CPE/MWCNTs/TYR/Nafion® (curve *b*); analyte 500 μmol·L^{-1} Trolox; supporting electrolyte 0.01 mol·L^{-1} PB of pH 7.0; stirring speed of 400 rpm.

3.4. Effect of Stirring Speed

In BIA with amperometric detection, the rate of convective analyte transport (phenolic compounds and Trolox) to the biorecognition layer is affected dramatically by the stirring speed, therefore it has an effect on sensitivity of the TYR biosensor. The stirring speed was varied from 100 to 500 rpm. Finally, the stirring rate of 400 rpm was selected as the optimum value, because no significant increase was observed by further increasing the stirring rate.

3.5. Selection of Optimal pH Value of Working Medium

A pH range of the supporting electrolytes is limited in case of TYR enzyme use due to its lower activity in acidic or alkaline media. For that reason, it was found that neutral PB is the most convenient for following experiments. It was experimentally confirmed that the highest current response of Trolox at the developed CPE/CNTs/TYR/Nafion® biosensor was obtained at pH 7.0, which coincides with the previously obtained results [15,23].

3.6. Effect of Working Potential

If the electrochemical detection of the products of enzymatic reaction takes place at a constant value of the applied potential, the magnitude of the working potential will play the most important role in every amperometric biosensor application. The dependence of height of reduction current on working potential is shown in Figure 4. The resulting curve has the highest reduction response at −0.25 V. Therefore, it was selected as the optimum, which corresponds to the previously reported value (−0.24 V) for amperometric detection (reduction) of *p*-quinone [17]. Moreover, the negative working potentials more than −0.30 V have a negative influence on stability of baseline and background noise of amperometric signal due to oxygen reduction.

3.7. Water Content in Berries

The content of water in berries was determined during the drying of berries to a constant weight at 60 °C. The highest content of water was observed for strawberries (93%), whereas the lowest content of water was found in raspberries (82%). For other examined berries the content of water was 85%. TEAC values were determined in 100 g of dry berries, so it was very important to know the water content in individual berries in order to calculate the TEAC in the pulp.

3.8. Analysis of Berries

In order to eliminate the matrix effect, the multiple standard addition method was used in analysis with the developed TYR biosensor at optimal conditions, where a volume of 1.0 mL of berries extract was added to 9.0 mL of supporting electrolyte and subsequently 3 or 4 standard additions of 0.01 mol·L^{-1} Trolox (each of 0.5 mL) were applied. A representative amperogram of raspberries analysis with standard addition curve I (μA) = −2.0082 c (mmol·L^{-1}) − 1.5137 (μA) and R^2 = 0.9978 is shown in inset of Figure 5. As reference method, DPPH assay was used with these parameters of calibration curve for standard compound Trolox A = −0.0103 c (mmol L^{-1}) + 0.3516 and R^2 = 0.9943.

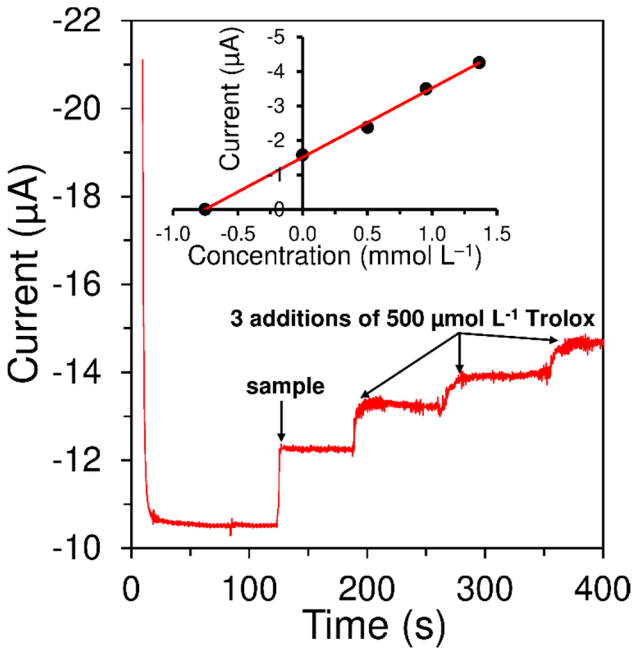


Figure 5. Representative TEAC analysis of raspberries using multiple standard additions method with CPE/MWCNTs/TYR/Nafion® biosensor at constant working potential -0.25 V and stirring speed of 400 rpm.

The biosensor provided constant current response with stable base line after 120 s. In amperometric techniques, response time is one of the most important parameters describing the performance and quality of biosensors, because it reflects kinetics of electrode reaction. The response time differed with each kind of berries. It is interesting to note that a shorter response time was observed for raspberries and cranberries (<10 s) than for standard addition of Trolox (>25 s). It can be attributed to polyphenolic compounds present in berries, which are more suitable for TYR active site (simpler substrate) in comparison to Trolox.

In the case of lifetime, the long-term stability of the biosensor (stored dry in a fridge at 5 °C) was tested for up to two weeks by monitoring of 150 μmol L^{-1} Trolox solution (see Figure S3) every 2 days (results not shown). TYR biosensor usually provides a constant current response for one week (signal drop by 19%). The fabrication method based on Nafion® is relatively simple, but potential leaching of

the enzyme should be taken in account. Moreover, a probable polymerisation of Trolox could shorten the lifetime of TYR biosensor.

Final TEAC values of individual dried berries obtained at the TYR biosensor and DPPH assay are shown in Table 1. It is obvious that TEAC values are different for the both used analytical methods. Generally, measurements with DPPH radical offer satisfactory repeatability of more than 95%. The repeatability of obtained results at TYR biosensor was comparable with DPPH assay for all studied berries except strawberries (90%). It can be caused by the high content of nonphenolic antioxidant e.g., AA, carotenoids, minerals with reduction properties, etc. and by presence of insoluble fiber. For example, the AA content in strawberries pulp is up to 90 mg·100 g^{-1} depending on variety, which is at a similar level as in citrus fruits. A presence of AA in pulp of berries is known and its content taken from the National Nutrient Database (USDA) (U.S. Department of Agriculture, 2010); for standard reference, the release available can be found in Table 1. Presented data are also comparable with information from the literature [24,25].

Moreover, the studied amperometric CPE/MWCNTs/TYR/Nafion® biosensor can be useful for determination of total phenolic content (TPC) [26] and also total antioxidant capacity (TAC) [27], as Trolox equivalent for samples with low concentration levels of other nonphenolic antioxidants. For dried berries (blueberries, cranberries and raspberries), the values of TEAC for the presented biosensor are in good correlation with reference method of DPPH assay as shown in Table 1. One of the discrepancies can be observed in the case of blueberries where, especially in pulp, the value of TEAC for the biosensor is lower than the value from the reference method. This is probably due to the presence of a higher amount of anthocyanins which exhibit anti-TYR activity [28].

Table 1. TEAC and AA content in various berries.

Berries	TEAC in Dry Matter (in Pulp) (mg·100 g^{-1})		AA in Pulp [b] [mg·100 g^{-1}]
	Tyrosinase Biosensor [a]	DPPH [a] Assay	
Strawberries	1569 (133)	6735 (570)	59
Blackberries	2593 (337)	3868 (503)	30
Raspberries	5317 (793)	5968 (890)	32
Blueberries	1917 (270)	4878 (687)	14
Cranberries	6738 (935)	8027 (1114)	13

[a] Values given as arithmetic mean for five repetitions. [b] Data of AA content in berries are taken from U.S. Department of Agriculture [29] and Ref. [24,25].

Another difference can be observed in the analysis of strawberries which contain the highest concentration level of AA (according to literature). The enzyme TYR catalyzes the oxidation of polyphenolic compounds in berries only, therefore the lower TEAC values of strawberries at biosensor in comparison to reference method was found. This phenomenon corresponds to higher TEAC values obtained by DPPH assay compared to that of biosensor, because AA discolors the methanolic solution of DPPH radical, which tends to show a positive error. However, the developed biosensor could be applied in the direct determination of TPC as TEAC, in samples where phenolic compounds are major antioxidants. The comparison with other spectrophotometric assays such as ORAC, FRAP and TRAP intended for the determination of total antioxidant capacity in pulps of berries is shown in Table 2. Corresponding TEAC values in berries obtained at individual assays were found or calculated from data presented in literature [8,30–33].

The TYR biosensor provides a similar sensitivity to DPPH and TRAP assays. In comparison to ORAC assay, the sensitivity of biosensor is twice as low. From all of the analytical methods, the lowest ability to eliminate the negative influence of free radicals was observed for strawberries and oppositely the highest for cranberries. If we want to compare antioxidant capacities of individual berries, TYR biosensor provides similar information as DPPH, ORAC and TRAP assays in this sequence: cranberries > raspberries > blackberries > blueberries > strawberries. A correlation

coefficient $R^2 = 0.8409$ was found during the comparison of the TYR biosensor with the DPPH assay in the analysis of TEAC in each berry's pulp. The better value of the correlation coefficient ($R^2 = 0.9374$) was obtained, when the TEAC of blackberries was excluded as an outlier. The same situation can be found for the comparison of TYR biosensor with TRAP assay ($R^2 = 0.9622$). Corresponding correlation graphs are shown in Figure 6.

Table 2. Survey of TEAC values in pulp of berries determined by other assays.

Berries	TEAC (mg·100 g^{-1})		
	ORAC [a]	FRAP [b]	TRAP [c]
Strawberries	1077	543	214
Blackberries	1478	1269	526
Raspberries	1268	994	262
Blueberries	1169	911	233
Cranberries	2275	1259	678

Data of TEAC content in berries are taken from Ref. [a] [6,8], [b] [30,31] and [c] [32].

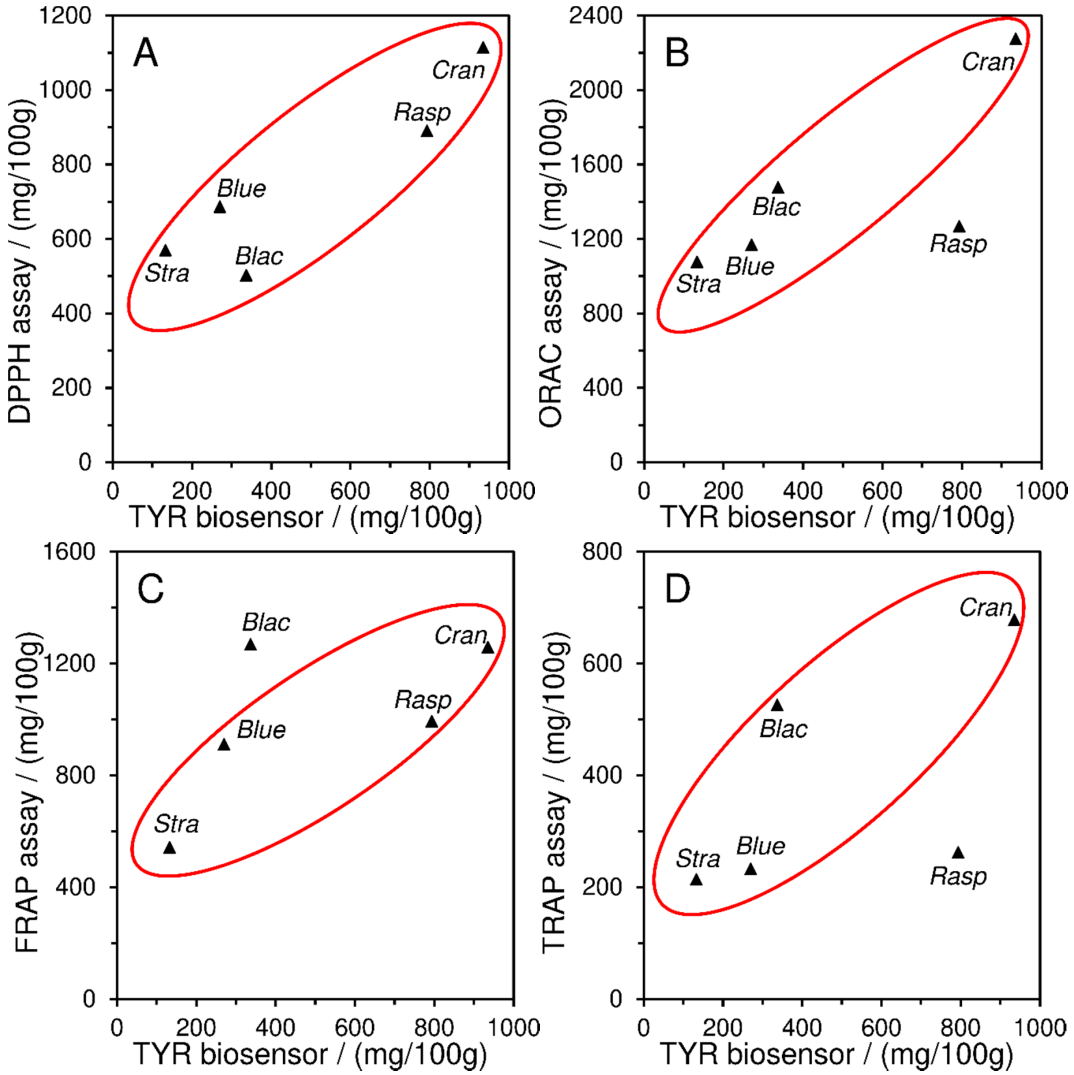


Figure 6. Comparison of TYR biosensor with other analytical assays (**A**) DPPH, (**B**) ORAC, (**C**) FRAP and (**D**) TRAP for analysis of TEAC in pulps of strawberries (*Stra*), blackberries (*Blac*), raspberries (*Rasp*), blueberries (*Blue*) and cranberries (*Cran*).

Moreover, it seems that ORAC and TRAP assays provide lower TEAC values than the TYR biosensor and two other spectrophotometric assays. An explanation could be found in different representations of phenolic substances in raspberries and cranberries, which probably contain a higher amount of phenolic substance, having catechol group in their chemical structures. It is also necessary to mention that spectrophotometric methods do not provide identical TEAC values for certain samples and their mutual correlations can have values from 0.6 to 0.9 [34].

4. Conclusions

In this contribution, the amperometric CPE/MWCNTs/TYR/Nafion® biosensor behavior is demonstrated in the analysis of various kinds of berries. After several steps of optimization, the determination of TEAC in strawberries, blackberries, raspberries, blueberries, and cranberries was performed. The highest content of polyphenolic compounds expressed as TEAC values was found for cranberries (6378 mg/100 g) and raspberries (5317 mg/100 g) in dry matter and furthermore their pulp; cranberries (935 mg/100 g) and raspberries (793 mg/100 g). Contrary to this, the lowest TEAC value was found for strawberries in dry matter (1569 mg/100 g) and in pulp (133 mg/100 g). The obtained results were compared to the reference method of DPPH assay, as well as to the data of other methods (ORAC, TRAP, FRAP), based on the spectrophotometry taken from literature. It can be stated that our results from the presented TYR biosensor are in satisfactory correlation with above mentioned DPPH assay. For ORAC and TRAP assays, values of correlation coefficient higher than 0.9 can be achieved, excluding raspberries. It has to be mentioned that the results obtained by the biosensor exhibit a negative error, due to the presence of nonphenolic compounds, mainly AA. Therefore, TYR biosensor is suitable for determination of "total phenolic content" and "total antioxidant capacity" for samples with low content of non-polyphenolic antioxidants. It has also one disadvantage, namely its stability, which results from lifetime of present TYR enzyme; hence, the current response starts to decrease approximately after one week. The developed biosensor offers a portable device for rapid quality control of the foodstuff in terms of their freshness and nutritional value. It could bring some favorable features, like low costs, simple instrumentation, measuring of turbid samples, working with less hazardous chemicals, etc. It is by no means a replacement of spectrophotometric assays, but an alternative to them. The electrochemical approach could also serve as a complementary method to already established ones, which could bring additional information about analyzed samples, reflecting their electrochemical properties directly.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/7/2497/s1, Figure S1: Effect of carbon nanotubes content in DMF dispersion on the anodic and cathodic peak potentials, Figure S2: Dependency of peak current on different volumes of MWCNTs, Figure S3: Typical amperometric responses of the CPE/MWCNTs/TYR/Nafion® biosensor, Scheme SI: Proposed mechanism of p-quinone reduction by ascorbic acid.

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