Determination of Antioxidant Capacity and Total Phenolic and Ascorbic Acid Contents of Some Fruits and Vegetables with an Electrochemical Approach

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Abstract

The determination of antioxidant capacity, total phenolic and ascorbic acid contents with high accuracy and efficiency, low cost, and fast methods has great analytical importance. The antioxidant capacity, total phenolic and ascorbic acid contents of apricot, arugula, banana, cranberry, spinach, and strawberry were investigated with an electrochemical approach and their compatibility with conventional methods. Antioxidant activity was determined by ABTS and DPPH assays, total phenolic content was determined by the Folin Ciocalteu method, which are spectrophotometric techniques, and ascorbic acid was quantified by HPLC technique. In order to perform more sensitive and simultaneous measurements in electrochemical measurements, the surface of the glassy carbon electrode was modified by electropolymerization of *p*-toluene sulfonic acid (TSA/GCE). The TSA/GCE modified sensor was used for the first time for the determination of antioxidant capacity and total phenolic content. The surface of the modified sensor was characterized by cyclic voltammetry and scanning electron microscopy. The TSA/GCE sensor was linearly correlated with the DPV technique for Trolox and gallic acid in 0.1 M NaNO3 and ascorbic acid in phosphate buffer solution pH 7.4 supporting electrolyte solution. Electrochemical methods have offered a promising alternative for the determination of antioxidant capacity and total phenolic content due to their simplicity, rapid response, low cost, sensitivity, and reproducibility.

Introduction

Antioxidants represent a wide class of chemical compounds that fight against oxidative processes, prevent possible damage caused by reactive oxygen species formed, and provide detoxification, take place in the body's defense system, and are found in biological environments and foods (Pisoschi et al. 2015; Ames 1983; Kaur and Kapoor 2001; Diaz et al. 1997; Güleşci and Aygül 2016). The human body is equipped with an effective defense system including high and low molecular weight antioxidants and various enzymes. Antioxidants neutralize the free radical by giving their electron to it, thus ending the chain oxidation reaction that proceeds based on electron transfer. Since the antioxidant has a stable structure under all conditions, it does not turn into a free radical in the position where it donates its electron. They minimize the possibility of experiencing possible damage by scavenging free radicals, so they can also be described as stabilizing or quenching free radicals (Kaur and Kapoor 2001; Arshiya 2013). This situation has increased the interest in investigating the effectiveness of natural compounds with antioxidant properties. Plants have been tested against reactive oxygen compounds in biological systems, and very successful results have been obtained, and it has been emphasized that they have strong antioxidant potentials (Bonnely et al. 2000). Thus, plant foods such as vegetables and fruits attract attention due to their safety, antioxidant effect, nutritional value, and therapeutic effects (Kaur and Kapoor 2001; Prior and Cao 2000). Since fruits and vegetables have formulations rich in phytochemicals and essential nutrients that reduce or eliminate the risk of various chronic diseases such as diabetes, cardiovascular diseases, various types of cancer, inflammation, and septic shock, they also have very positive effects on human health (Doğan Cömert et al. 2019; Ravimannan and Nisansala 2017).

Considering the role of antioxidants in human health, giving priority to the consumption of foods with high antioxidant content (especially vegetables and fruits) in the diet is the easiest and best way to get antioxidants into the body (Ravimannan and Nisansala 2017). The World Health Organization recommends daily consumption of at least 400 g of fruit (Singh et al. 2016). It has been reported as a common result of studies on the subject that there is a negative relationship between fruit and vegetable consumption and the rate of developing heart diseases, cancer, and other degenerative diseases (Prior and Cao 2000; Garcia-Closas et al. 1999; Wargovich 2000). The variety and concentration of bioactive components, including ascorbic acid, tocopherols, carotenoids, and phenolic compounds, are effective on the antioxidant capacity of fruits and vegetables (Mártìnez-Sánchez et al. 2008). Phenolic compounds have a direct role in antioxidant activity, as well as in the defense mechanisms of plants against pathogens and radiation (Ravimannan and Nisansala 2017). Ascorbic acid (vitamin C) is a water-soluble antioxidant. The antioxidant activity of ascorbic acid is due to its easy electron loss. Because it is an electron donor, it works as a reducing agent for many reactive oxidant species. It offers a protective effect for the water-soluble parts of cells and tissues, and reduces tocopherol radicals from the cellular membrane to their active form. However, it can also exhibit an oxidant behavior by converting Fe^{+3} to Fe^{+2} (Kaur and Kapoor 2001). The best sources of ascorbic acid are fresh fruits and vegetables. Strawberry and cranberry are very rich in ascorbic acid content (Güleşci and Aygül 2016).

Spectrophotometric methods are widely used to determine the antioxidant activities of fruits and vegetables, but the results are evaluated by ignoring the various negative features (solvent, pH dependence, lipophilic/hydrophilic system incompatibility) of the methods. In order to eliminate the deficiencies of in-vivo tests and to increase the reliability of scientific evidence, scientific data should be strengthened by using common analysis techniques (spectrophotometric, chromatographic, and electrochemical) in different research areas. Recently, different electrochemical methods have been used to determine antioxidant activity (Śeruga et al. 2011). Alternative methods are presented using electrochemical techniques to determine the total reducing power of especially low molecular weight antioxidants in foods and beverages (Piljac-Žegarac et al. 2010). An advantage of these electrochemical methods is that they allow fast, simple, and inexpensive determinations. Moreover, in some cases, measurements are easily performed even in the presence of interfering compounds such as ascorbic acid in fruits compared to other methods (Caramês et al. 2020; Kevers et al. 2014; Cacique et al. 2021; Rodríguez-Bonilla et al. 2017; Barba et al. 2013; Berker et al. 2010; Agcam 2022; Horst et al. 2016; Široká et al. 2013; Velde et al. 2012; Rigolon et al. 2022; Lin et al. 2011; López-Froilán et al. 2018).

This study aimed to measure the antioxidant capacities, total phenolic and ascorbic acid contents of the main fruits and vegetables (apricot, arugula, banana, cranberry, spinach, and strawberries) grown in different seasons and considered to have superior antioxidant quality, as well as using an electrochemical approach. No study was found that electrochemically examined the aforementioned fruits and vegetables in terms of antioxidant capacity, total phenolic, and ascorbic acid contents. In this study, the suitability of the electrochemical detection to be applied was evaluated with the results obtained by conventional techniques, and it was observed that the results were in agreement. In this way, a fast, reliable, and economical application was developed about their antioxidant properties.

Material And Methods

Samples and chemicals

Apricot (*Prunus armeniaca* L.), arugula (*Eruca sativa* L.), banana (*Musa acuminata*), cranberry (*Cornus mas*), spinach (*Spinacia oleracea* L.), and strawberry (*Fragaria x ananassa Duch*) were purchased from a local greengrocer. Non-edible parts (leaves, stalk, peel, root, etc.) were discarded. The samples were washed with tap water and homogenized using a T18 Ultra Turrax (Ika, Staufen, Germany). The samples were freeze-dried and stored in polyethylene bags at -18°C until the extractions were carried out.

Ascorbic acid, 2.2'-Azino-bis (3-ethylbenzenothiazoline6-sulfonic acid) (ABTS), butylated hydroxytoluene (BHT), 1,1- diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, methanol (HPLC grade), metaphosphoric acid, potassium persulfate, sodium carbonate, tetrahydrofuran, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (USA). Acetone, acetonitrile, chloroform, ethanol, hexane, methanol sodium nitrate, and *p*-toluene sulfonic acid were purchased from Merck (Darmstadt, Germany).

Extraction, antioxidants capacity assays, and Total Phenolic Contents

The freeze-dried samples (2 g) were extracted with 50 mL of solvent mixture (methanol/water/hydrochloric acid, 80:19:1) in an ultrasonic water bath at 20°C for 30 min. After that samples were centrifuged at 5580 *g* at 4°C for 6 min and the supernatant was collected. The pellet was extracted twice with the same solvent. Supernatants were combined, filtered using a 0.45 µm syringe filter, and used for antioxidants capacity, total phenolic content assays and electrochemical measurements.

The free radical scavenging capacity of the extracts was determined by DPPH and ABTS assays. ABTS+ cationic radical was produced by reaction between 7 mM ABTS and 2.45 mM ammonium persulfate in the dark for 12-16 h, diluted with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm, and used as stock ABTS solution for ABTS assay (Re et al. 1999). The ABTS stock solution (3.8 mL) was added to the extract (200 µL), incubated for 60 min in the dark, and absorbance was measured at 734 nm using a UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). DPPH radical scavenging assay was applied according to the procedure as reported by Brand-Williams et al. (1995). The methanolic solution of DPPH (1 g/mL) was mixed with the extract, incubated for 60 min in the dark, and absorbance was measured at 520 nm. Calibration graphs were constructed by plotting the absorbance against the known concentrations of Trolox for both assays. The results were expressed as µmol Trolox equivalent antioxidant capacity (TEAC)/100 g dry weight (DW).

The total phenolic content (TPC) was determined by the Folin-Ciocalteu method as described elsewhere (Kraujalyte et al. 2013). In brief, extract (100 µL), 1 mL of 0.2 N Folin–Ciocalteu reagent, 400 µL of deionized water, and 1 mL of 7% sodium carbonate were added sequentially. The mixture was placed in the dark for 90 min at room temperature and the absorbance was measured at 725 nm. The results were expressed as a gallic acid equivalent (GAE) and mg/100g of the DW.

Extraction and HPLC analysis

Ascorbic acid extraction was performed according to the procedure applied by Fecka et al. (2021). Prior to the homogenization of the freeze-dried sample (10 g), 10 mL of methanolic BHT solution (1%) was added and homogenized using a blender. Approximately 5 g of homogenized sample (1 g of sample was used for electrochemical measurement) was extracted with 30 mL of aqueous metaphosphoric acid (2%, m/v) at room temperature and in the dark. The obtained solution was centrifuged (5580 *g*) for 10 min at 4°C and then filtered through a membrane filter (0.45 µm) and stored at 4°C before HPLC analysis.

Analysis of ascorbic acid was performed in triplicate on a Shimadzu HPLC system equipped with an auto-sampler (SIL-20A HT), a column oven (CTO-10AS VP), a degasser system (DGU2A 5R), a gradient pump (LC-20AR), a diode-array detector (DAD, SPDM20A), and a software package for system control and data acquisition (LC solution). The extract (20 µL) was injected into a Rezex ROA column (300 ´ 37.8 mm; Phenomenex, Torrance, CA), which was held at 20°C. The flow rate of the mobile phase was set at 1 mL/min. The mobile phases consisted of water containing 0.1% (v/v) metaphosphoric acid. The detection was carried out at 245 nm.

Voltammetric procedures

All voltammetric determinations were carried out by a Gamry Interface 1010B potentiostat (Gamry, USA) instrument. Voltammetric analyzes were performed in a three-electrode system (BASi C3 Cell Stand). The triple electrode system consisted of a platinum wire auxiliary electrode, a silver-silver chloride reference electrode, and a poly (p-TSA) modified glassy carbon electrode. Nitrogen was bubbled through each solution for 2 minutes before all voltammetric measurements were carried out.

The cyclic voltammetry (CV) technique was used to investigate the electrochemical behavior of Trolox, gallic acid, and ascorbic acid, and the differential pulse voltammetry (DPV) technique was used for quantitative detection of Trolox, gallic acid, and ascorbic acid. The Trolox standard solution was solubilized with 80% methanol (20:80, ultrapure water: methanol (V/V)). Gallic acid and ascorbic acid standard solutions were prepared in 0.1 M $NaNO_3$ supporting electrolyte solution. 0.1 M $NaNO_3$ supporting electrolyte solution was prepared with ultrapure water. The same extracts used for spectroscopic and chromatographic measurements were also used for voltammetric measurements. It was diluted tenfold with a supporting electrolyte solution to

perform voltammetric analyzes of fruit and vegetable extracts. Voltammetric current responses and calibration graphs were drawn for Trolox, gallic acid, and ascorbic acid, and their amounts in the extracts were calculated using these graphs.

In order to find the limit of detection (LOD) and limit of quantitative detection, DPV responses of increasing concentrations of Trolox, gallic acid, and ascorbic acid were measured at the TSA/GCE modified sensor. Oxidative peak current responses corresponding to the concentrations were plotted. LOD (*3 x SD/m*) and LOQ (*10 x SD/m*) values were calculated by dividing the standard deviation (SD) of the ten measurements for the lowest signal concentration by the slope of this curve (*m*).

Preparation of poly (p-TSA) modified sensor

The GCE was mechanically cleaned on emery paper with 0.3 and 0.05 µm Al_2O_3 slurry. After that, it was cleaned electrochemically by CV from -0.7 to +1.7 V at 0.1 V s^{-1} in 0.5 M H_2SO_4 with 10 cycling before each modification. It was then passed through ultrapure water and immersed in a 0.1 M NaCl solution containing 1.0 mM *p*-toluene sulfonic acid. It was modified by electropolymerization with a potential range of -0.2 to +2.5 V, scanning speed of 0.05 V s^{-1}, applying 5 cycles of CV technique (Fig. S1). After the modified sensor (TSA/GCE) was prepared, it was conditioned by performing a measurement DPV analysis in 0.1 M $NaNO_3$ solution, the supporting electrolyte solution, before the measurement was performed.

Results And Discussion

Spectrophotometric measurements and HPLC analysis

The antioxidant capacities, total phenolic and ascorbic acid contents of the samples evaluated by conventional spectrophotometric and HPLC techniques are shown in Table 1. The results showed good agreement between total phenolic and ascorbic acid contents and antioxidant capacity, except for the banana sample. Despite the lowest total phenolic and ascorbic acid contents, the banana sample has the highest antioxidant activity values. This finding corresponds well with that of a previous study. It is well known that phytochemicals such as phenolic substances and ascorbic acid as well as flavonoids, tocopherols, carotenoids, and even fibers may be responsible for scavenging of DPPH and ABTS radicals (Li et al. 2014; Tiveron et al. 2012). The results are also in accordance with the studies carried out with different fruits and vegetables (Li et al. 2014; Velioglu et al. 1998; Maduwanthi and Marapana 2021).

Voltammetric measurements

In order to get the highest analytical response in voltammetric studies, optimization parameters such as electrode modification, the film thickness in modification, and supporting electrolyte solution effect were investigated. First, thin films of the electro polymerized TSA/GCE modified sensor were prepared using the CV technique with one to ten cycles, and their DPV responses for 100 ppm Trolox were investigated (Fig S2). When the DPV responses were examined, the highest peak current was obtained at the TSA/GCE modified sensor electro polymerized with 5 cycles. Thus, the sensor with this film thickness was used in subsequent studies. Then, the supporting electrolyte solution environment was investigated in order to obtain a high voltammetric response. DPV responses at TSA/GCE modified sensor in 0.1 M KCl, $LiClO_4$, $NaClO_4$, NaCl, $NaNO_3$, Na_2SO_4, and PBS (pH 7.4) electrolyte solutions of 100 ppm Trolox were investigated (Fig S3). The best DPV response was obtained in 0.1 M $NaNO_3$ solution, and this solution medium was used in subsequent studies.

Surface analysis of the electro polymerized TSA/GCE modified sensor was carried out by SEM technique (Figure 1). A thin film structure was obtained with a film thickness of approximately 20.31 µm. Its surface was highly porous and many porous structures were seen. Such porous and rough structure increased the sensitivity of the modified sensor during detection.

Differential pulse voltammetric analyzes were performed in 0.1 M $NaNO_3$ at a TSA/GCE modified sensor to determine the voltammetric oxidation behavior and detection limits of Trolox, gallic acid, and ascorbic acid. The concentrations of Trolox were increased as 0.083, 0.48, 2.05, 2.60, 3.15, 4.08, 4.41, 5.68, 6.82, 7.38, 8.68, 10.29, 10.94, 12.00 ppm from the Trolox standard solution to the electrolyte solution. A linear curve was obtained with the equation I_p *(µA) = 0.8464 C (ppm) + 1.0954* in this concentration range (Figure 2). Concentrations of gallic acid were 0.30, 0.31, 0.45, 1.54, 2.08, 2.61, 3.13, 3.65, 5.17, 7.60, 8.54, 10.34, 12.96, 14.61, 15.41, 16.20, 19.20, 23.33, 26.46, 32.01, 34.48, 37.98, 41.45, 48.32, 55.10, 61.77, 68.36, 74.85, 82.25, 87.57, 93.79, 99.94 ppm in 0.1 M $NaNO_3$. Two oxidation peaks were observed for gallic acid at approximately 350 mV (p_1) and 750 mV (p_2) at the TSA/GCE modified sensor (Figure 3). Linear curves in the concentration range of 10.34 – 99.94 ppm were obtained with the equations I_{p1} *(µA) = 0.1099 C (ppm) + 4.*9467 for the first oxidation peak and I_{p2} *(µA) = 0.0254 C (ppm) + 0.4719* for the second peak (Figure 3). The concentrations of ascorbic acid were increased to 10.0, 13.6, 24.4, 31.4, 38.4, 45.2, 52.0, 58.6, 62.7, 71.7, 78.0, 84.3, 91.9, 96.7, 102.7 ppm from the ascorbic acid standard solution to 0.1 M $NaNO_3$. In this concentration range, a linear curve was obtained with the equation I_p *(µA) = 0.0391 C (ppm) + 0.048* (Figure 4). In addition, the LOD values obtained for Trolox, gallic acid, and ascorbic acid were calculated as 0.23, 0.40, 0.75 ppm, respectively, and the LOQ values were calculated as 0.77, 1.32, 2.51 ppm, respectively.

Simultaneous determinations of Trolox, gallic acid, and ascorbic acid were performed to test the selectivity of the method and the designed modified sensor. (Fig S4). Figure 5 shows the DPV responses at the poly (*p*-TSA) modified sensor as a result of increasing Trolox concentrations in the presence of constant concentrations of gallic acid and ascorbic acid. The anodic peak potentials for ascorbic acid, Trolox and gallic acid at the modified sensor were observed at +0.14, +0.25, and +0.41 V, respectively. Results of simultaneous voltammetric analysis for solutions with increased gallic acid and ascorbic acid concentrations and constant concentrations of other analytes were shown in Figure S5, Figure S6. These results showed that there was no

interaction in the electrochemical determination of Trolox, gallic acid and ascorbic acid. Three analytes were detected sensitively, stable and with good anodic peak resolution at the poly (*p*-TSA) modified sensor. It can be said that simultaneous determination of three analytes in real sample extracts at the poly (*p*-TSA) modified sensor is possible.

Antioxidant capacity, total phenolic, and ascorbic acid contents in apricot, arugula, banana, cranberry, spinach and strawberry extracts used in spectrophotometric and chromatographic methods were determined electrochemically by DPV technique at a poly (*p*-TSA) modified sensor. Voltammetric calibration curves were generated for Trolox, gallic acid and ascorbic acid. Antioxidant capacity, total phenolic and ascorbic acid contents were calculated by finding the concentrations corresponding to the current values of the extracts from these calibration charts. The average of the data (µmol TE/100g DW, mg GAE/ 100g DW and µg/g DW respectively) calculated as a result of three voltammetric analyzes for each extract was shown in Table 2. Very low concentrations of Trolox, gallic acid, and ascorbic acid could be calculated in the extracts by electrochemical measurement.

Regression analysis was performed to correlate the results obtained by conventional methods with electrochemical measurements. The Pearson's correlation coefficients between conventional techniques (spectrophotometric and HPLC) and voltammetric methods are shown in Table 3. High correlation was observed between voltammetric TEAC value and spectrophotometric DPPH (R^2 = 0.985, p<0.01) and ABTS (R^2 = 0.983, p<0.01) assays. The voltammetric total phenolic and ascorbic acid contents also signified strong correlations with those of spectrophotometric (R^2 = 0.992, p<0.01) and HPLC (R^2 = 0.995, p<0.01) results, respectively. Good compatibility between analytical techniques used in this study, voltammetric technique is promising as an alternative technique in terms of antioxidant capacity, total phenolic and ascorbic acid contents determination.

Conclusion

In recent years, electrochemical methods have been frequently used to determine the antioxidant capacity and total phenolic content of food and beverage samples. It is an alternative method to determine the antioxidant capacity and total phenolic contents in a fast, easy and economical way. Additionally, due to the high sensitivity achieved with these techniques, this approach has been attractive to researchers. The basis of electrochemical measurement is based on measuring the current due to an oxidation/reduction on the electrode surface and applying a potential in the electrochemical cell.

The surface of glassy carbon electrodes was modified by electropolymerization of *p*-TSA to increase the sensitivity of the detection. With this sensor, the responses of the antioxidant compounds were investigated electrochemically and the optimized conditions were selected where the best response was obtained. In this study, electrochemical analyzes were performed on fruit and vegetable extracts such as apricot, arugula, banana, cranberry, spinach and strawberry with a poly (*p*-TSA) modified sensor. Antioxidant capacity and total phenolic content were calculated from calibration curves for Trolox and gallic acid generated using the DPV technique. Eventually, simultaneous determinations of Trolox, gallic acid and ascorbic acid were performed on poly (*p*-TSA) modified sensor with DPV with excellent resolution. Voltammetric technique, one of the electrochemical methods, has become an important alternative method by proving that they have an excellent relationship with other analytical approaches.

Declarations

Author Contribution

T.B.K.: sampling, research concept, chemical analysis, E.K.S.: electrochemical experiment, interpretation of the results, preparation of manuscript, I.K.: supervisation of study**,** interpretation of the results, statistics, preparation of manuscript.

Data Availability All data generated or analyzed during this study are included in this published article and in its supplementary information files

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Ethics Approval This article does not contain any studies with human participants or animals.

Consent to Participate Not applicable.

Conflict of Interest Tugca Bilenler Koc declares that she has no confict of interest. Ebru Kuyumcu Savan declares that she has no confict of interest. Ihsan Karabulut declares that he has no confict of interest.

References

1. Agcam E (2022) A Kinetic Approach to Explain Hydroxymethylfurfural and Furfural Formations Induced by Maillard, Caramelization, and Ascorbic Acid Degradation Reactions in Fruit Juice-Based Mediums. Food Anal Methods 15:1286–1299. https://doi.org/10.1007/s12161-021-02214-x
2. Ames BN (1983) Dietary carcinogens and anticarcinogens oxygen radicals and degenerative diseases. Science 221:1256-1264. https://doi: 10.1126/science.6351251.
3. Arshiya S (2013) The antioxidant effect of certain fruits: a-review. J Pharm Sci 5:265-268.
4. Barba FJ, Esteve MJ, Tedeschi P, Brandolini V, Frígola A (2013) A Comparative Study of the Analysis of Antioxidant Activities of Liquid Foods Employing Spectrophotometric, Fluorometric, and Chemiluminescent Methods Food Anal Methods 6:317–327. https://doi.org/10.1007/s12161-012-

9441-3

5. Berker KI, Güçlü K, Tor İ, Demirata B, Apak R (2010) Total Antioxidant Capacity Assay Using Optimized Ferricyanide/Prussian Blue Method. Food Anal Methods 3:154–168. https://doi.org/10.1007/s12161-009-9117-9
6. Bonnely S, Peyrat-Maillard MN, Rondini L, Masy D, Berset C (2000) Antioxidant activity of malt rootlet extracts. J Agric Food Chem 48:2785-2792. https://doi.org/10.1021/jf990793c.
7. Brand-William W, Cuvelier ME, Berset C (1995) Use of free radical method to evaluate antioxidant activity. LWT – Food Sci Technol 28: 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5.
8. Cacique AP, Barbosa ES, Pinho GP, Silvério FO (2021) Miniaturized Methodologies for Determining the Total Phenol and Flavonoid Concentrations and the Antioxidant Activity Food Anal Methods 14:1110-1120. https://doi.org/10.1007/s12161-020-01934-w
9. Caramês ETS, Alamar PD, Pallone JAL (2020) Bioactive Compounds and Antioxidant Capacity in Freeze-Dried Red Cabbage by FT-NIR and MIR Spectroscopy and Chemometric Tools. Food Anal Methods 13:78-85. https://doi.org/10.1007/s12161-019-01523-6.
10. Diaz MN, Frei B, Vita JA, Keaney JF (1997) Antioxidants and atherosclerotic heart disease. New England J Medicine 337:408-416.
11. Doğan Cömert E, Ataç Mogol B, Gökmen V (2019) Relationship between color and antioxidant capacity of fruits and vegetables. Curr Res Food Sci 2:1-10. https://doi.org/10.1016/j.crfs.2019.11.001.
12. Fecka I, Nowicka A, Kucharska AZ, Soklół-Łętowska A (2021) The effect of strawberry ripeness on the content of polyphenols, cinnamates, L-ascorbic and carboxylic acids. J Food Comp Anal 95:103669. https://doi.org/10.1016/j.jfca.2020.103669.
13. Garcia-Closas R, Gonzalez CA, Agudo A, Riboli E (1999) Intake of specific carotenoids and avonoids and the risk of gastric cancer in Spain. Cancer Causes and Control 10:71-75.
14. Güleşci N, Aygül İ (2016) Beslenmede yer alan antioksidan ve fenolik madde içerikli çerezler. Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi. 5:1-21.
15. Horst C, Silwana B, Iwuoha E, Gil E, Somerset V (2016) Improved Detection of Ascorbic Acid with a Bismuth-Silver Nanosensor. Food Anal Methods 9:2560–2566. https://doi.org/10.1007/s12161-016-0444-3
16. Kaur C, Kapoor HC (2001) Antioxidants in fruit and vegetables-the millennium's health. Int J Food Sci Technol 36:703-725. https://doi.org/10.1111/j.1365-2621.2001.00513.
17. Kevers C, Sipel A, Pincemail J, Dommes J (2014) Antioxidant Capacity of Hydrophilic Food Matrices: Optimization and Validation of ORAC Assay. Food Anal Methods 7:409-416. https://doi.org/10.1007/s12161-013-9640-6
18. Kraujalyte V, Venskuonis PR, Pukalskas A, Cesoniene Daubaras T (2013) Antioxidant properties and polyphenolic compositions of fruits from different European cranberrybush (*Viburnum opulus* L.) genotypes. Food Chem 141: 3695-3702. https://doi.org/10.1016/j.foodchem.2013.06.054.
19. Lin JT, Liu SC, Shen YC, Yang AJ (2011) Comparison of Various Preparation Methods for Determination of Organic Acids in Fruit Vinegars with a Simple Ion-Exclusion Liquid Chromatography. Food Anal Methods 4:531–539. https://doi.org/10.1007/s12161-011-9204-6.
20. Liu C, Zhao Y, Li X, Jia J, Chen Y, Hua Z (2014) Antioxidant capacities and main reducing substance contents in 110 fruits and vegetables eaten in China. Food Nutr Sci 5:293-307. https://doi.org/10.4236/fns.2014.54036.
21. López-Froilán R, Hernández-Ledesma B, Cámara M, Pérez-Rodríguez ML (2018) Evaluation of the Antioxidant Potential of Mixed Fruit-Based Beverages: a New Insight on the Folin-Ciocalteu Method. Food Anal Methods 11:2897-2906. https://doi.org/10.1007/s12161-018-1259-1.
22. Maduwanthi SDT, Marapan RAUJ (2021) Total phenolics, flavonoids and antioxidant activity following simulated gastro-intestinal digestion and dialysis of banana (*Musa acuminata*, AAB) as affected by induced ripening agents. Food Chem 339:127909. https://doi: 10.1016/j.foodchem.2020.127909.
23. Mártìnez-Sánchez A, Gıl-Izquıerdo A, Gıl MI, Ferreres FA (2008) Comparative study of flavonoid compounds vitamin C, antioxidant properties of baby leaf Brassicaceae Species. J Agric Food Chem 56:2330-2340. https://doi.org/10.1021/jf072975+.
24. Piljac-Zˇegarac J, Valek L, Stipcˇevic´ T, Martinez S (2010) Electrochemical determination of antioxidant capacity of fruit tea infusions. Food Chem 121:820–825. https://doi.org/10.1016/j.foodchem.2009.12.090.
25. Prior RL, Cao G (2000) Antioxidant phytochemicals in fruits and vegetables: diet and health implications. Hortic Sci 35:588-592. https://doi.org/10.21273/HORTSCI.35.4.588.
26. Ravimannan N, Nisansala A (2017) Study on antioxidant activity in fruits and vegetables-a review. Int J Adv Res Biol Sci 4:1-9. http://dx.doi.org/10.22192/ijarbs.2017.04.03.010.
27. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved abts radical cation decolorization assay. Free Radic Biol Med 26:1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.
28. Rigolon TCB, Barros FAR, Silva LHM, Stringheta PC (2022) Study of the Infuence of Some Compounds on the rediction Equations of Total Phenolic Content and Antioxidant Capacity by Colorimetric Parameters. Food Anal Methods https://doi.org/10.1007/s12161-022-02262-x
29. Rodríguez-Bonilla P, Gandía-Herrero F, Matencio A, García-Carmona F, López-Nicolás JM (2017) Comparative Study of the Antioxidant Capacity of Four Stilbenes Using ORAC, ABTS+ , and FRAP Techniques. Food Anal Methods 10:2994-3000. https://doi.org/10.1007/s12161-017-0871-9
30. Śeruga M, Novak I, Jakobek L (2011) Determination of polyphenols content and antioxidant activity of some red wines by differential pulse voltammetry, HPLC and spectrophotometric methods. Food Chem 124:1208-1216. https://doi.org/10.1016/j.foodchem.2010.07.047.
31. Singh B, Singh JP, Kaur A, Singh N (2016) Bioactive compounds in banana and their associated health benefits-a review. Food Chem 206:1-11. https://doi.org/10.1016/j.foodchem.2016.03.033.

32. Široká J, Martincová A, Pospíšilová M, Polášek M (2013) Assay of Citrus Flavonoids, Troxerutin, and Ascorbic Acid in Food Supplements and Pharmaceuticals by Capillary Zone Electrophoresis. Food Anal Methods 6:1561–1567
33. https://doi.org/10.1007/s12161-013-9573-0.
34. Tiveron AP, Melo PS, Bergamaschi KB, Vieira TMFS, Regitano-d'Arce MAB, Alencar SM (2012) Antioxidant activity of Brazilian vegetables and its relation with phenolic composition. Int J Mol Sci 13:8943–8957. https://doi: 10.3390/ijms13078943.
35. Velde F, Pirovani ME, Cámara MS, Güemes DR, Bernardi CMH (2012) Optimization and Validation of a UV–HPLC Method for Vitamin C Determination in Strawberries (Fragaria ananassa Duch.), Using Experimental Designs. Food Anal Methods 5:1097–1104. https://doi.org/10.1007/s12161-011-9347-5.
36. Velioglu YS, Mazza G, Gao L, Oomah BD (1998) Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J Agric Food Chem 46:4113–4117. https://doi.org/10.1021/jf9801973.
37. Wargovich MJ (2000) Anticancer properties of fruits and vegetables. Hortic Sci 35:573- 575.

Tables

Table 1. Antioxidant activity, total phenolic (spectrophotometric) and ascorbic acid (HPLC) contents.

Sample	DPPH		ABTS		TPC		Ascorbic acid	
	(µmol TE/100g DW)		(µmol TE/100g DW)		(mg GAE/ 100g DW)		(µg/g DW)	
Apricot	810.28	±41.33	677.20	±11.33	206.51	±7.04	592.85	±1.77
Arugula	731.53	±7.04	721.80	±4344	1114.47	±190.46	1037.03	±4.73
Banana	7562.94	±96.44	6614.47	±11.90	5.20	±1.01	90.68	±0.10
Cranberry	1916.21	±143.00	1043.79	±4.12	51.20	±5.48	674.46	±2.08
Spinach	5068.03	±38.61	4545.42	±189.06	1135.79	±20.87	905.41	±9.27
Strawberry	2312.86	±2.87	1762.66	±61.98	588.27	±3.47	1504.94	±32.75

Table 2. Antioxidant activity, total phenolic and ascorbic acid contents (electrochemical, TSA/GCE).

Sample	Antioxidan		TPC		Ascorbic acid	
	(µmol TE/100g DW)		(mg GAE/ 100g DW)		(µg/g DW)	
Apricot	507.02	±0.00	160.02	±2.43	452.90	±14.59
Arugula	654.97	±5.80	928.16	±22.21	964.22	±20.90
Banana	6381.48	±435.57	3.03	±0.18	67.87	±5.15
Cranberry	1225.86	±114.53	40.25	±9.57	621.57	±42.03
Spinach	3493.68	±358.60	886.01	±7.89	790.86	±31.21
Strawberry	1230.72	±140.26	433.28	±27.12	1303.51	±8.38

Table 3. Pearson's correlation coefficients between conventional techniques (spectrophotometric and HPLC) and voltammetric method.

	TEAC voltammetric	TPC voltammetric	Ascorbic acid voltammetric	DPPH spectrophotometric	ABTS spectrophotometric	TPC spectrophotometric	Ascorbic acid HPLC
TEAC voltammetric	1	-0.236	-0.618**	0.985**	0.983**	0.229	-0.625**
TPC voltammetric	-0.236	1	0.612**	-0.199	-0.126	0.992**	0.585*
Ascorbic acid voltammetric	-0.618**	0.612**	1	-0.534*	-0.526*	0.632**	0.995**
DPPH spectrophotometric	0.985**	-0.199	-0.534*	1	0.993**	-0.183	-0.537*
ABTS spectrophotometric	0.983**	-0.126	-0.526*	0.993**	1	-0.111	-0.528*
TPC spectrophotometric	0.229	0.992**	0.632**	-0.183	-0.111	1	0.606*
Ascorbic acid HPLC	-0.625**	0.585*	0.995**	-0.537*	-0.528*	0.606**	1

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

Figures

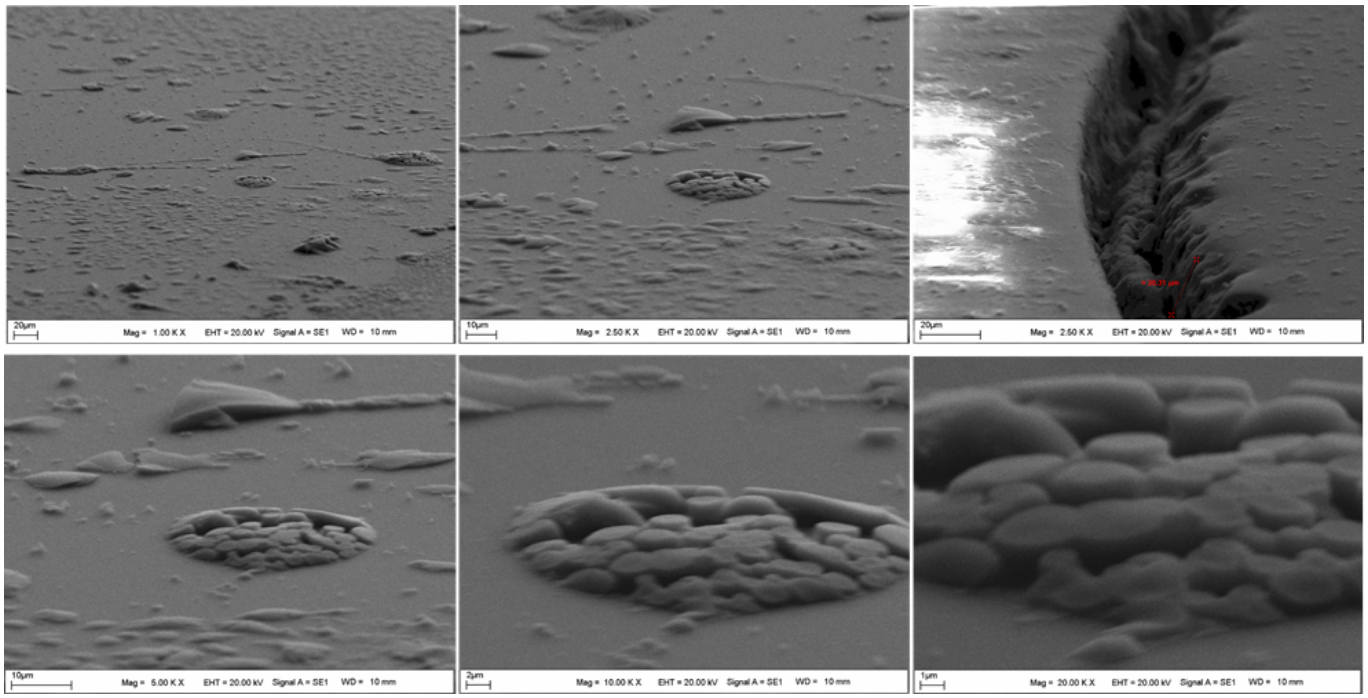

Figure 1

SEM analysis of the electropolymerized TSA/GCE modified sensor

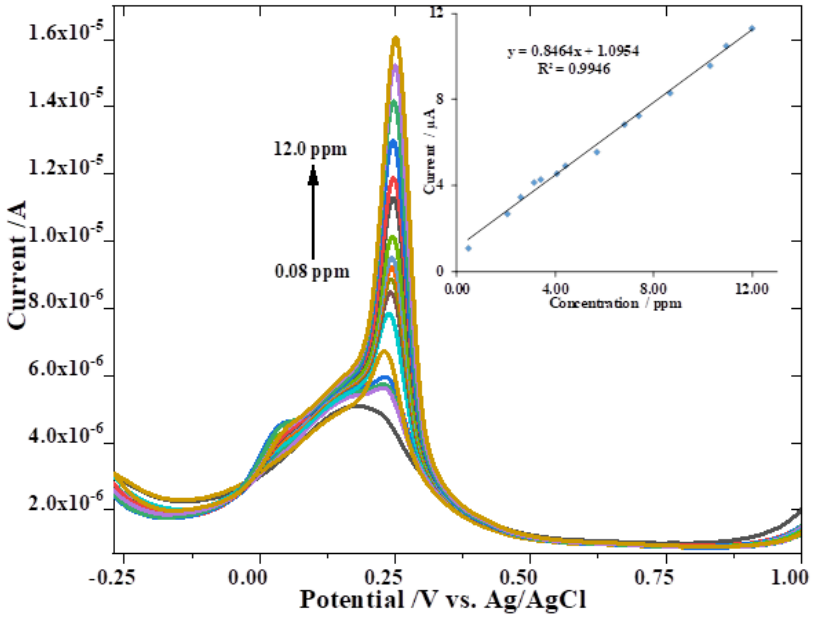

Figure 2

DPV responses in a 0.1 M NaNO3 solution of Trolox (0.083 ppm – 12.0 ppm) at the poly (*p*-TSA) modified sensor

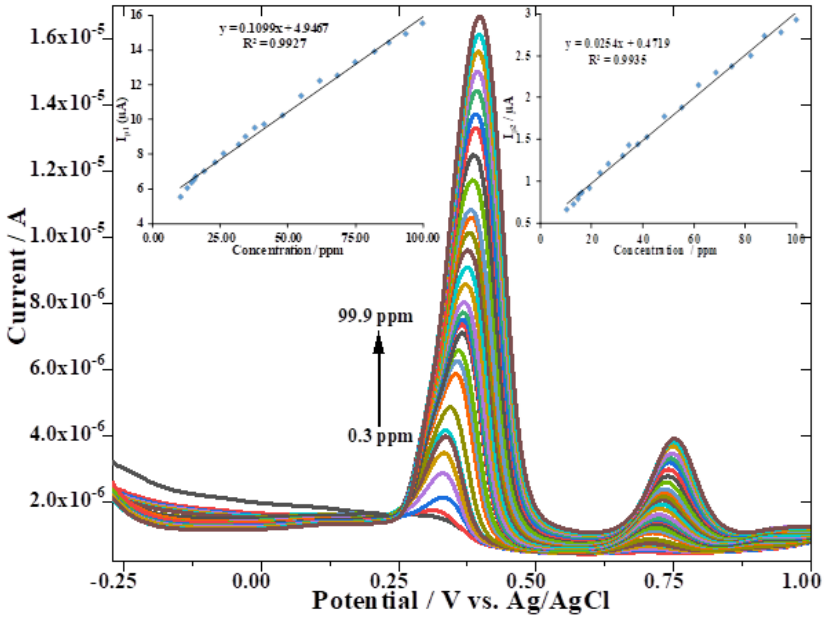

Figure 3

DPV responses in 0.1 M NaNO3 solution of gallic acid (0.3 ppm – 99.9 ppm) at the poly (*p*-TSA) modified sensor

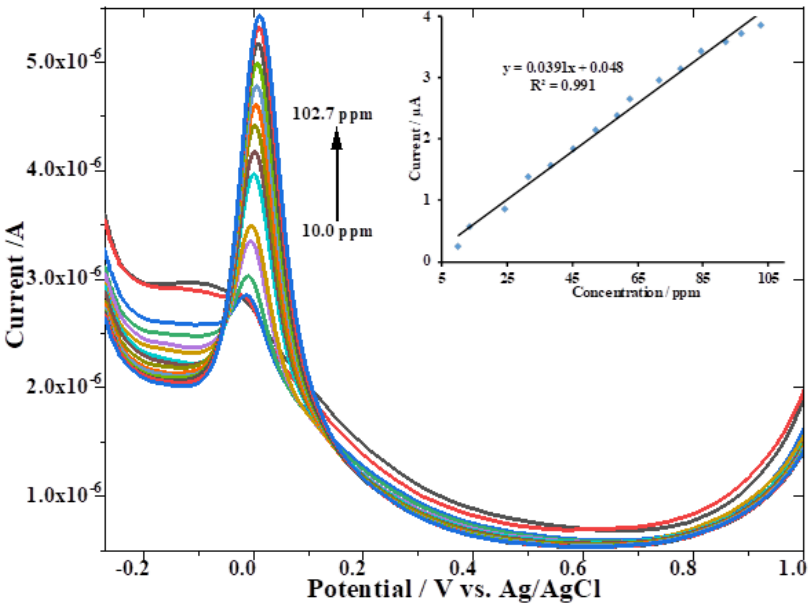

Figure 4

DPV responses of ascorbic acid (10.0 ppm – 102.7 ppm) at a poly (*p*-TSA) modified sensor in 0.1 M NaNO3

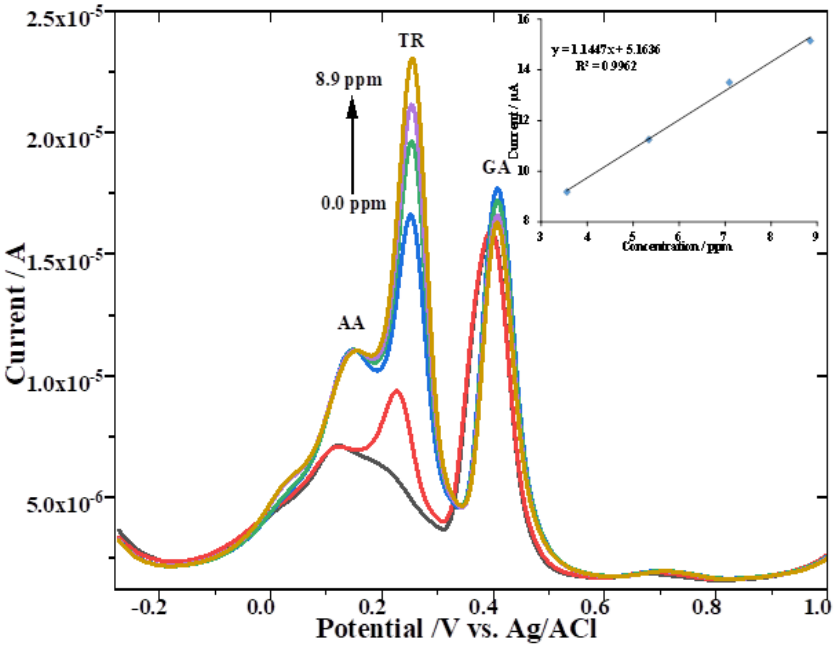

Figure 5

DPV responses of (0.0, 1.9, 3.6, 5.3, 7.1, 8.9 ppm) Trolox (TR), 19.2 ppm gallic acid (GA) and 89.1 ppm ascorbic acid (AA) in 0.1 M NaNO3 solution at the poly (*p*-TSA) modified sensor. The inner graph represents the curve showing the relationship between the measured current values versus increasing Trolox concentration

Supplementary Files

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- SupplementaryInformation.docx