Electrochemical Methodologies for Plant and Fruit Extracts

Subjects: Chemistry, Organic

Contributor: Mir Waqas Alam, Jawayria Najeeb, Sumaira Naeem, Sheikh Muhammad Usman, Insha Nahvi, Fai Alismail, Alaaedeen Abuzir, Mohd Farhan, Allah Nawaz

The growing research interests in the applications of plant and fruit extracts (synthetic/stabilization materials for the nanomaterials, medicinal applications, functional foods, and nutraceuticals) have led to the development of new analytical techniques to be utilized for identifying numerous properties of these extracts. One of the main properties essential for the applicability of these plant extracts is the antioxidant capacity (AOC) that is conventionally determined by spectrophotometric techniques. Nowadays, electrochemical methodologies are emerging as alternative tools for quantifying this particular property of the extract. These methodologies address numerous drawbacks of the conventional spectroscopic approach, such as the utilization of expensive and hazardous solvents, extensive sample pre-treatment requirements, long reaction times, low sensitivity, etc.

Keywords: antioxidant capacity ; antioxidants ; plant extract ; spectrophotometric technique

1. Introduction

During the metabolic conversions of food into the energy, excess amounts of the free radicals are constantly generated in the human body. These unstable and highly reactive free radical species are a constant threat to the constituents of the cells (particularly the genetic materials) and destroy the constituents via numerous destructive mechanisms [1]. Therefore, the modulation of the concentration of these free radical species is an essential task [2]. Though oxidative metabolism plays an essential role in the survival of the cells, the production of free radicals (along with some other reactive oxygen species) during this process still causes various oxidative changes [3]. However, researchers are not defenseless against these free radicals and their relentless attack. This is attributed to the presence of certain molecules in the bodies called antioxidants. Antioxidants operate in two ways in order to address the issue of the oxidative stress in the human body: (1) the antioxidants scavenge/quench these reactive oxygen species (ROS) or reactive nitrogen species (RNS) by chain-breaking mechanisms (these antioxidants are called primary antioxidants) [4]; and (2) the antioxidants that suppress the generation of the oxidation promoters (such as singlet oxygen, metal ions, and pro-oxidation enzymes, etc.) by chelation mechanisms (these antioxidants are called secondary or preventive antioxidants) [5]. The most familiar out of thousands of such antioxidant substances are Vitamin C, Vitamin E, beta carotenes, many related carotenoids, and minerals like selenium and manganese, etc. [6]. Antioxidants hold great interest for pharmacists and biochemists because of their capability to moderate the damages caused by RNS, ROS, or even chlorine-like reactive species [7].

Growing interest has been recently observed in developing cost-effective and reliable techniques for screening and quantifying the antioxidants present in various biological/biogenic samples. The quantification of the antioxidant potential is done by using the parameter of the antioxidant capacity (AOC), which represents the concentration or number of moles of the specific free radical species scavenged by a particular antioxidant present in the sample [8]. The AOC parameter is a thermodynamic parameter and is found to be connected with the equilibrium constant of the process (scavenging reaction of antioxidant) [9]. Though a number of methodologies have been reported for estimating the AOC of the antioxidants, identifying the direct-action mechanism of antioxidants still remains a challenge in the field of free radical chemistry [9]. Antioxidant behavior is quantified using the two broad-term methodologies that reflect the focus on activity in foods (first category) and bioactivity in humans (second category). The category focusing on food systems involves the assessment of antioxidant efficacy, especially in fruits, vegetables and beverages so that a clear observation of their dietary burden and in vivo activity could be reported. As far as the antioxidant status in humans is concerned, there has been reported an obvious observation regarding an imbalance between the reactive oxygen species and defense/repair mechanisms in vivo [3][10]. Understanding both quantification methodologies (usually termed as antioxidant assays) is essential in developing a clear picture of the working of antioxidants.

The antioxidant assays can be broadly categorized into two classes of direct antioxidant assays and indirect antioxidant assays [11]. The first category of direct assays is the competitive technique, and the added probe, as well as the antioxidants present in the sample both competes with each other to attack the free radical/free radical initiator. This makes these direct assays a little less accurate technique to detect the AOC [10]. The indirect antioxidant assays are non-competitive tests where an artificial probe (an oxidant) is added to the sample to observe the impact of the antioxidant action on this probe. The attack of antioxidant molecules generates structural changes in the added probe, which is measured by spectroscopic, fluorescence, electrochemical, or other methodologies. The indirect assays are more common and provide more accurate results in comparison to the direct assays owing to their non-competitive nature [11].

2. Electrochemical Methodologies

In recent years, electrochemical methodologies have emerged as the competing technique against the antioxidants for the determination of AOC in biogenic samples [12]. The increase in the applicability of these techniques for AOC determination is attributed to the fact that extremely low molecular weight anti-oxidants can be easily detected and screened by using these techniques. The electrochemical methods operate by calculating the total reducing power of the sample, which represents the ability of certain antioxidants to donate/accept electrons in a redox (oxidation–reduction) environment [13]. Electrochemical methods are considered very sensitive and rapid methodologies for the determination of antioxidant potential in both stationary as well as dynamic flow systems [14]. Moreover, it should be made clear that most electrochemical techniques provide the information regarding the total antioxidant capacity (TAC) values of the antioxidants, which represents the cumulative impact of all the antioxidants present in the sample in contrast to the determination of the individual antioxidant potentials measured via a parameter of AOC. Electrochemical techniques involved in evaluating the antioxidant potential of biogenic samples are discussed in detail in the subsection [15].

Electrochemical techniques address a few major drawbacks associated with chromatographic and spectroscopic techniques, such as long sample preparation times, use of hazardous solvents, long analysis time, undefined reaction time and expensive setups. Furthermore, the sensitivity and reproducibility of the electrochemical methods is also quite high in comparison to the above-mentioned conventional antioxidant assays. Stationary systems are known for their suitability in quantifying only a limited amount of analytes, but the study of simple as well as more complicated biomolecules in dynamic systems can also be easily conducted by using these methodologies [16]. A carbon working electrode can be used in the case of both fluid and solid systems. Carbon paste electrodes, printed carbon electrodes, paraffin, and silicon oils modified carbonaceous materials (carbon pastes) are also used for a similar purpose [13]. Sometimes, the addition of inorganic/organic nanomaterials is also done to increase the selectivity or specificity of these electrodes. These modified electrodes perform quite well when used in voltammetry, amperometry and cyclic voltammetry techniques [13][17].

3. Cyclic Voltammetry (CV)

The CV is one of the most exploited electrochemical techniques for the determination of TAC in biogenic extracts. The CV comprises a stationary working electrode (WE) which is linearly ramped with respect to the time in a triangular waveform (i.e., the potential is increased from the lowest to the maximum value and after attaining the maximum potential, the potential is reversed to again attain the lowest value) [18]. Besides the WE, two other electrodes called reference electrodes (RE) and auxiliary/counter electrode (CE) is also part of the CV setup. The galvanostat/potentiostat is attached with the CV assembly that records the current values attained owing to the redox reactions occurring in the medium and the current versus potential graph is plotted for the understudy sample [19].

The reversible system is represented by the equation $O+ne \leftrightarrow R$ where O represents the oxidized form and R represents the reduced form. As discussed previously, antioxidant scavenges the superoxide radicals, which results in a decrease in the current values observed in the presence of chrysin and quercetin. This is a technique used to collect qualitative information while measuring the current response of any redox-active solution, such as the presence of intermediates in redox reactions to linearly cycled potential sweep between two or more set values [19]. Furthermore, this gives information about the characterization of redox systems, the number of redox states, stability of these states, and electron transfer kinetics of the redox reactions taking place in the medium [19][20]. The variation in the parameters of peak current (I_p) and peak potential (E_p) are the main tools utilized as a means to calculate the TAC value of antioxidants. It is used for determining the antioxidant activity of food, polyphenolic compounds [21], clinical samples [22], and pathological processes and infectious diseases [23][24].

4. Differential Pulse Voltammetry (DPV)

The quantitative chemical analysis, kinetics, thermodynamics and mechanisms of chemical reactions have also been studied by another electrochemical technique of DPV [25]. The DPV technique superimposes the fixed-magnitude pulses on a linear potential ramp. The potential-time curve acquired in the DPV technique is generated by recording the response current, which was sampled twice and the current difference is plotted against the potential to acquire the DPV voltammogram [26]. These measurements are advantageous because the effect of the charging current can be minimized, resulting in increased sensitivity in current measurements [27]. This reduction is utilized as a tool to measure the TAC of the antioxidants present in the tea extract [28]. Another important point is the extracted faradaic current which results in analyzing the electrode reactions more precisely [29]. It is also used effectively to measure the antioxidant capacity of food [30], plant extracts [31], and polyphenolic compounds such as procyanides and catechins from cone extracts which showed significant ability to reduce oxidative stress, scavenge free radicals, and transitions metal ions as well [32][33][34].

5. Square Wave Voltammetry (SWV)

This pulsed voltammetry technique uses a potential waveform (where the entire symmetrical potential square wave is superimposed on the staircase potential waveform) that is implemented on the WE [35]. As observed in the case of an electrochemical technique, the voltammograms of the understudy compound exhibit peak/peaks that correspond to the numerous redox processes that analytes experiences in the medium. The peak potential/potentials (potential at the maxima of the peak) also provide essential information regarding the tendency of the antioxidants in order to perform the ET reactions. Moreover, the antioxidants display the peaks at the lower values of oxidation potential, which displays the tendency of the antioxidants to be oxidized [36]. In comparison to the linear sweep voltammetry, the SWV is more sensitive and has a more extended dynamic range with lower detection limits. This technique was used to identify and quantify the synthetic antioxidants tert-butylhydroquinone (TBHQ) and butylated hydroxy anisole (BHA) in the presence of the cationic surfactant CPB using a carbon black electrode and analysis of food samples and biodiesel, and also in tea samples [37].

6. Amperometric Measurements (Chronoamperometry)

Chronoamperometry (CA) technique utilizes the stepping of the potential from the potential value at which there is no faradic reaction to the potential value and where the dose of electroactive species at the WE is essentially zero [38]. The variation in the response current is documented with respect to time to generate the CA potential-time graph. The typical chronoamperogram acquired in the case of the extracts of the tea leaves was performed by using the WE of glassy carbon electrode (GCE) that is modified with carbon nanotubes (MWCNTs). Various antioxidant compounds have been analyzed for their antioxidant activity using amperometric techniques [39]. Disposable polyester screen-printed graphite macroelectrodes have been used coupled with a batch injection cell for measurement of consumption of DPPH. This technique measures electric current from oxidation of a substance such as food samples when studied on the surface of a working electrode at some fixed voltage potential using amperometric detectors [40].

References

1. Munteanu, I.G.; Apetrei, C. Analytical methods used in determining antioxidant activity: A review. Int. J. Mol. Sci. 2021, 22, 3380.
2. Masek, A.; Latos-Brozio, M.; Chrzescijanska, E.; Podsedek, A. Polyphenolic profile and antioxidant activity of Juglans regia L. leaves and husk extracts. Forests 2019, 10, 988.
3. Antolovich, M.; Prenzler, P.D.; Patsalides, E.; McDonald, S.; Robards, K. Methods for testing antioxidant activity. Analyst 2002, 127, 183–198.
4. Apak, R.; Özyürek, M.; Güçlü, K.; Çapanoğlu, E. Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. J. Agric. Food Chem. 2016, 64, 997–1027.
5. Sadeer, B.N.; Montesano, D.; Albrizio, S.; Zengin, G.; Mahomoodally, M.F. The versatility of antioxidant assays in food science and safety—Chemistry, applications, strengths, and limitations. Antioxidants 2020, 9, 709.
6. Bunaciu, A.A.; Danet, A.F.; Fleschin, Ş.; Aboul-Enein, H.Y. Recent applications for in vitro antioxidant activity assay. Crit. Rev. Anal. Chem. 2016, 46, 389–399.
7. Shahidi, F.; Zhong, Y. Measurement of antioxidant activity. J. Funct. Foods 2015, 18, 757–781.

8. Ácsová, A.; Martiniaková, S.; Hojerová, J. Selected in vitro methods to determine antioxidant activity of hydrophilic/lipophilic substances. Acta Chim. Slov. 2019, 12, 200–211.
9. Dżugan, M.; Tomczyk, M.; Sowa, P.; Grabek-Lejko, D. Antioxidant activity as biomarker of honey variety. Molecules 2018, 23, 2069.
10. Siddeeg, A.; AlKehayez, N.M.; Abu-Hiamed, H.A.; Al-Sanea, E.A.; Al-Farga, A.M. Mode of action and determination of antioxidant activity in the dietary sources: An overview. Saudi J. Biol. Sci. 2021, 28, 1633–1644.
11. Apak, R. Current issues in antioxidant measurement. J. Agric. Food Chem. 2019, 67, 9187–9202.
12. Sochor, J.; Dobes, J.; Krystofova, O.; Ruttkay-Nedecky, B.; Babula, P.; Pohanka, M.; Jurikova, T.; Zitka, O.; Adam, V.; Klejdus, B. Electrochemistry as a tool for studying antioxidant properties. Int. J. Electrochem. Sci. 2013, 8, 8464–8489.
13. Ivanova, A.; Gerasimova, E.; Gazizullina, E. Study of antioxidant properties of agents from the perspective of their action mechanisms. Molecules 2020, 25, 4251.
14. David, I.G.; Popa, D.E.; Buleandră, M.; Cheregi, M.C. Electrochemical methods and (bio) sensors for rosmarinic acid investigation. Chemosensors 2020, 8, 74.
15. Adamcová, A.; Horna, A.; Šatínský, D. Determination of Phloridzin and Other Phenolic Compounds in Apple Tree Leaves, Bark, and Buds Using Liquid Chromatography with Multilayered Column Technology and Evaluation of the Total Antioxidant Activity. Pharmaceuticals 2022, 15, 244.
16. Bedlovičová, Z.; Strapáč, I.; Baláž, M.; Salayová, A. A brief overview on antioxidant activity determination of silver nanoparticles. Molecules 2020, 25, 3191.
17. Koc, T.B.; Kuyumcu Savan, E.; Karabulut, I. Determination of Antioxidant Properties and β-Carotene in Orange Fruits and Vegetables by an Oxidation Voltammetric Assay. Anal. Lett. 2022, 55, 891–903.
18. Harnisch, F.; Freguia, S. A basic tutorial on cyclic voltammetry for the investigation of electroactive microbial biofilms. Chem. Asian J. 2012, 7, 466–475.
19. Ricci, A.; Parpinello, G.P.; Teslić, N.; Kilmartin, P.A.; Versari, A. Suitability of the cyclic voltammetry measurements and DPPH• spectrophotometric assay to determine the antioxidant capacity of food-grade oenological tannins. Molecules 2019, 24, 2925.
20. Pastor, F.T.; Šegan, D.M.; Gorjanović, S.Ž.; Kalušević, A.M.; Sužnjević, D.Ž. Development of voltammetric methods for antioxidant activity determination based on Fe (III) reduction. Microchem. J. 2020, 155, 104721.
21. Jiao, Y.; Kilmartin, P.A.; Fan, M.; Quek, S.Y. Assessment of phenolic contributors to antioxidant activity of new kiwifruit cultivars using cyclic voltammetry combined with HPLC. Food Chem. 2018, 268, 77–85.
22. Wang, H.-W.; Bringans, C.; Hickey, A.J.; Windsor, J.A.; Kilmartin, P.A.; Phillips, A.R. Cyclic Voltammetry in Biological Samples: A Systematic Review of Methods and Techniques Applicable to Clinical Settings. Signals 2021, 2, 12.
23. Li, X.; Qin, Z.; Fu, H.; Li, T.; Peng, R.; Li, Z.; Rini, J.M.; Liu, X. Enhancing the performance of paper-based electrochemical impedance spectroscopy nanobiosensors: An experimental approach. Biosens. Bioelectron. 2021, 177, 112672.
24. Han, Q.; Pang, J.; Li, Y.; Sun, B.; Ibarlucea, B.; Liu, X.; Gemming, T.; Cheng, Q.; Zhang, S.; Liu, H. Graphene Biodevices for Early Disease Diagnosis Based on Biomarker Detection. ACS Sens. 2021, 6, 3841–3881.
25. Reddy, Y.M.; Kumar, S.P.; Saritha, K.V.; Gopal, P.; Reddy, T.M.; Gandara, S.J. Phytochemical profiling of methanolic fruit extract of Gardenia latifolia Ait. by LC-MS/MS analysis and evaluation of its antioxidant and antimicrobial activity. Plants 2021, 10, 545.
26. Percevault, L.; Limanton, E.; Nicolas, P.; Paquin, L.; Lagrost, C. Electrochemical Determination and Antioxidant Capacity Modulation of Polyphenols in Deep Eutectic Solvents. ACS Sustain. Chem. Eng. 2020, 9, 776–784.
27. David, M.; Serban, A.; Radulescu, C.; Danet, A.F.; Florescu, M. Bioelectrochemical evaluation of plant extracts and gold nanozyme-based sensors for total antioxidant capacity determination. Bioelectrochemistry 2019, 129, 124–134.
28. Tufan, A.N.; Baki, S.; Guclu, K.; Ozyurek, M.; Apak, R. A novel differential pulse voltammetric (DPV) method for measuring the antioxidant capacity of polyphenols-reducing cupric neocuproine complex. J. Agric. Food Chem. 2014, 62, 7111–7117.
29. Piszcz, P.; Głód, B.K. Antioxidative properties of selected polish honeys. J. Apic. Sci. 2019, 63, 81–91.
30. Della Pelle, F.; Compagnone, D. Nanomaterial-based sensing and biosensing of phenolic compounds and related antioxidant capacity in food. Sensors 2018, 18, 462.
31. Latos-Brozio, M.; Masek, A.; Chrzescijanska, E.; Podsędek, A.; Kajszczak, D. Characteristics of the Polyphenolic Profile and Antioxidant Activity of Cone Extracts from Conifers Determined Using Electrochemical and

Spectrophotometric Methods. Antioxidants 2021, 10, 1723.

32. Belfar, A.; Bensaci, C.; Belguidoum, M. Evaluation of Antioxidant Capacity by Cyclic Voltammetry of Phoenix dactylifera L.(date palm). Asian J. Res. Chem. 2022, 15, 138–144.
33. Nikolic, M.; Pavlovic, A.; Mitic, S.; Tosic, S.; Mitic, M.; Kalicanin, B.; Manojlovic, D.; Stankovic, D. Use of cyclic voltammetry to determine the antioxidant capacity of berry fruits: Correlation with spectrophotometric assays. Eur. J. Hortic. Sci. 2019, 84, 152–160.
34. Vasyliev, G.S.; Vorobyova, V.I.; Linyucheva, O.V. Evaluation of reducing ability and antioxidant activity of fruit pomace extracts by spectrophotometric and electrochemical methods. J. Anal. Methods Chem. 2020, 2020, 8869436.
35. Aslışen, B.; Koçak, Ç.C.; Koçak, S. Electrochemical determination of sesamol in foods by square wave voltammetry at a boron-doped diamond electrode. Anal. Lett. 2020, 53, 343–354.
36. Karimi, F.; Bijad, M.; Farsi, M.; Vahid, A.; Bami, A.H.; Wen, Y.; Ganjali, M.R. A new nanostructure square wave voltammetric platform for determination of tert-butylhydroxyanisole in food samples. Curr. Anal. Chem. 2019, 15, 172–176.
37. Angelis, N.P.; Mendonça, C.J.; da Rocha, R.L.; Capelari, B.T.; Prete, C.M.; Segatelli, G.M.; Borsato, D.; Tarley, R.T.C. Feasibility of a Nano-Carbon Black Paste Electrode for Simultaneous Voltammetric Determination of Antioxidants in Food Samples and Biodiesel in the Presence of Surfactant. Electroanalysis 2020, 32, 1198–1207.
38. Brainina, K.Z.; Varzakova, D.P.; Gerasimova, E.L. A chronoamperometric method for determining total antioxidant activity. J. Anal. Chem. 2012, 67, 364–369.
39. Porfírio, D.A.; Ferreira, Q.R.; Malagutti, A.R.; Valle, E.M. Electrochemical study of the increased antioxidant capacity of flavonoids through complexation with iron (II) ions. Electrochim. Acta 2014, 141, 33–38.
40. Tirawattanakoson, R.; Rattanarat, P.; Ngamrojanavanich, N.; Rodthongkum, N.; Chailapakul, O. Free radical scavenger screening of total antioxidant capacity in herb and beverage using graphene/PEDOT: PSS-modified electrochemical sensor. J. Electroanal. Chem. 2016, 767, 68–75.
