Chemical Analysis of Synthetic Antioxidants in Foodstuffs

Subjects: Engineering, Chemical | Chemistry, Analytical

Contributor: Danielle Gonçalves-Filho, Djenaine De Souza

The information obtained by the systematic search in ScienceDirect® databases, indicated the predominance of the use of separation chromatography, followed by detection techniques in the development of analytical methods for the detection of phenolic antioxidants in foodstuffs. This is because these techniques allow the simultaneous determination of different types of antioxidants, through the separation of these compounds at different stages of a column, thus obtaining different retention times, which are related to the physicochemical characteristics of the antioxidants and their interaction between the stationary and mobile phase. After the separation, the antioxidants are identified and quantified using specific chromatographic detectors, such as ultraviolet–visible, diode array, thermal conductivity, and mass spectroscopy, resulting in a suitable sensitivity and selectivity. However, separation and detection chromatographic, despite being very accurate in the detection of antioxidants, have as their main disadvantage the use of large amounts of organic solvents or inert gases, with elevated purity and, consequently, high cost. Additionally, the use of these techniques requires rigorous steps of extraction and cleanup to prepare the foodstuff samples for analysis, remove interference compounds, and/or preconcentrate the antioxidants to obtain reliable information. Extraction steps can increase the time and costs in the analysis, promote a reduction in the analytical frequency, and generate a great quantity of residues, which goes against a very important principle, taken very seriously today, green chemistry, which orients the reduction or elimination of toxic residues in chemical products and processes, including all cycles of a chemical, in its design, manufacture, use, and final disposal.

Keywords: food additives ; synthetic antioxidants ; butylated hydroxytoluene

1. Introduction

Voltammetric techniques, based on electrical potential measurements, are divided according to the mode of potential applications and, for the analysis of antioxidants, have been reported for the employ of differential pulse voltammetry (DPV), linear scan voltammetry (LSV), and square wave voltammetry (SWV). However, a potential constant application technique, such as multiple pulse amperometry (MPA), also has been reported. The sensitivity reported using DPV and SWV is similar to that obtained using chromatographic detection but presents as the main advantage the simplicity in the detection of phenolic antioxidants in foodstuffs due to the fact that complex and expensive extraction steps are not necessary, often requiring only one step of liquid–liquid extraction, possibly the synthetic antioxidant detection in the extract obtained from the initial sample, without the need for complex cleanup steps [1][2][3][4].

The analytical signals from the voltammetric determination of synthetic antioxidants are related to the redox reaction that occurs in the interface electrode/solution, and the reactants and/or products from the reaction can be adsorbed in the working electrode surface, resulting in a reduction in the intensity of analytical signals and difficulties in their reproducibility. Therefore, the adequate choice of material for the preparation of the working electrode is the limiting factor in the success of antioxidant detection. For this, the use of various types of working electrodes has been reported, including chemically modified surfaces by nanoparticles, polymers, and carbon forms. The following will report some characteristics of the chromatographic detection and voltammetric techniques employed in the determination of synthetic antioxidants in foodstuffs, indicating the applicability of extraction steps and adequate working electrodes.

2. Chromatographic Analysis of Synthetic Phenolic Antioxidants

The official methodology adopted by regulatory agencies around the world to identify and quantify the synthetic antioxidants in foodstuffs involves the use of previous gas chromatography (GC) and high-performance liquid chromatography (HPLC) for separations, followed by detection using different detector types [5][6][7][8].

HPLC can be applied in the separation of any compound that is soluble in a liquid phase, which can be modified according to the polarity of the blend antioxidant. The separation depends on the interaction of antioxidants with the mobile and stationary phase, which results in the retention times being similar, requiring specific extraction steps for improvement in the selectivity of the analysis. The detectors are generally nondestructive, and therefore, synthetic phenolic antioxidants can be collected for further analysis, which is very important in the traceability and adulteration evaluation; besides, the detection can be carried out at room temperature, avoiding the destruction of thermally sensitive antioxidants. However, the most common detector, the ultraviolet detector (HPLC/UV–VIS), presents low selectivity in synthetic phenolic antioxidant detection, and for this, in the last decades, the selectivity and sensitivity in the HPLC detection have been improved with the detection of mass spectrometry, which is used to determine the mass-to-charge ratio of ions from the ionization of molecules of interest. However, the HPLC analysis requires the use of a large number of organic solvents, and the analysis is usually very time-consuming, resulting in band broadening and, therefore, lower resolution in comparison with GC [9].

Bibai Du et al. [10] used high-performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) in the determination of 20 synthetic antioxidants, including BHT. The separation used a gradient elution with a mobile phase constituted by ammonium acetate solution and methanol, and the stationary phase was the combination of two C_{18} columns, where one was used for chromatographic separation and the other was placed between the injector and the eluent mixer to eliminate possible background contamination of the chromatographic system. The detection was performed by mass spectrometry with ionization electrospray (ESI) and atmospheric pressure chemical ionization (APCI) for multiple reaction monitoring. Among the 20 synthetic antioxidants evaluated, 10 were detected in vegetable oil samples, 13 in powdered milk samples, and 9 in infant fruit puree samples. Considering BHT, the detection limit observed was 0.09 ng/mL, and recovery in vegetable oils, powdered milk, and baby fruit puree presented values between 70% and 80%, indicating that more effective extraction procedures are needed in these antioxidants analyses.

GC results in high sensitivity, excellent resolution, and good separation capacity. However, its use is only possible in the detection of volatile and thermally stable compounds. The mobile phase is an inert gas with a constant flow, and the stationary phase is a column thermostat, which can have variation in the temperature, resulting in suitable resolution and lower retention time. Detectors in GC are destructive, making their use unviable in the adulteration analysis; however, they can be useful for various foodstuff quality control laboratories.

Farajzadeh et al. [11] used GC coupled to a flame ionization detector (GC-FID) in the quantification of BHT and BHA in honey samples. For this, helium gas and a capillary column of poly dimethyl siloxane were used as mobile and stationary phases, respectively. With constant elution, the partition of antioxidants resulted in detection limits of 1.7 ng/mL for BHT and 4.1 ng/mL for BHA. These results were only achieved by the employ of a previous dispersive liquid–liquid extraction, allowing enrichment factors and extraction recoveries in the range of 144–186% and 72–93%, respectively.

Despite the high cost, GC coupled to a mass spectrometer (GC-MS) is a robust separation and detection technique that offers a superior signal-to-noise ratio, is easily automatized, and features fast data analysis, which provides comparatively more accurate and reproducible results. Therefore, Gupta et al. [12] determined phenolic antioxidants in packed fruit juices using gas chromatography–tandem mass spectrometry (GC–MS/MS), resulting in a sensitive, selective, accurate, and precise analytical procedure. However, these analyses were only possible after the use of the QuEChERS (quick, easy, cheap, effective, rugged, and safe) procedure of the extraction, which extracted simultaneously various phenolic antioxidants, removed matrix interferences, and enriched the antioxidants from the matrix in a single step.

The adequate choice of pretreatment, extraction, cleanup, and preconcentration steps will directly influence the instrumental performance, with either GC or HPLC detection. The extraction step employs specific solvents and adopts standard procedures, which can, sometimes, result in a high consumption of time, elevated cost, high level of contamination, and low extraction efficiency. Furthermore, these factors are intensely affected by the type and concentration of the extraction solvent, extraction temperature, extraction time, and extraction pH, as will be explained below [9][13][14].

3. Extraction Methods for Synthetic Phenolic Antioxidants

The quantification of synthetic antioxidants in foodstuffs is a problem in food quality control laboratories and regulatory, environmental, and health agencies due to the complexity associated with these matrices, especially those rich in lipids since they contain a mixture of synthetic phenolic antioxidants. The presence of vitamins, minerals, moisture, chlorophyll, other antioxidants, phenolic compounds, fats, and proteins, considered interferences, can directly influence the repeatability, reproducibility, sensitivity, and selectivity of the chemical analysis. Thus, phenolic antioxidant detection

requires previous preparation to eliminate the possible interferences, extraction to isolate the antioxidant in a suitable medium, and cleanup steps before chromatographic determination. In some samples, are necessary the concentration of antioxidants due to their presence at low concentrations, low levels of detection required by regulatory agencies, and the complex nature of the matrices that are present.

However, the extraction step, besides isolating the synthetic antioxidant of interest, can also remove other components of the sample, following damage in the chemical analysis. Therefore, in recent years, great advances have been noted in the development of suitable extraction methods that provide high recovery and reproducibility, in addition to being faster, cheaper, more ecological, and easier to automate [13].

All reports indicated that minimizing the preparation steps mainly reduces sources of errors and time and cost in the chemical analysis. Additionally, it was observed that the major trends in sample preparation focused on miniaturization, automation, high-throughput performance, online coupling with analytical instruments, and low-cost operations with extremely low or no solvent consumption [15][16]. The following are some of the extraction methods more frequently employed in the analysis of the synthetic antioxidant in foodstuffs.

3.1. Liquid–Liquid Extraction (LLE)

Liquid–liquid extraction (LLE) is an extraction method widely used in the past in complex sample extraction, separation, and purification processes, mainly due to its simplicity, efficiency, and ease of application in standard analytical methods. It is a technique that separates the analyte of interest from a complex matrix, based on the difference in solubility of the analyte, in two solvents of different polarities [17][18][19][20].

Kim et al. [21] evaluated a method for the determination and validation of an uncertainty measurement for the simultaneous determination of the synthetic phenolic antioxidants BHA, BHT, OG, PG, and TBHQ in edible oils commonly consumed in Korea. Antioxidants were extracted from the samples using 20 portions of hexane-saturated acetonitrile, in which the acetonitrile phase was collected and evaporated, and the extracts obtained were analyzed using LC–MS/MS under previously optimized conditions. Antioxidant recoveries ranged from 91.4% to 115.9% with relative standard deviations between 0.3% and 11.4% and uncertainties from 0.15% to 5.91%, indicating that the method is suitable for verifying the safety of edible oil products containing residues of these antioxidants.

However, as shown in the research above and in other published works, the efficiency of LLE is directly related to the use of large amounts of toxic and flammable organic solvents. Furthermore, due to the low concentration of the antioxidants in the obtained extract, the preconcentration step is necessary in the extract obtained. The use of LLE has been much questioned in recent years due to the need for large amounts of solvents, and the extraction is laborious, poses a risk to user safety, presents difficulty in automatization, and in some cases, occurs the formation of emulsions, damaging the separation of the organic from the aqueous fractions and, consequently, making difficult the extraction and detection of the antioxidant.

3.2. Solid-Phase Extraction (SPE)

Solid-phase extraction (SPE) is an extraction method that emerged in the 1970s, used to isolate and concentrate the analyte of interest from a sample by retaining it in a solid phase, which will later be recovered by elution from a liquid or fluid or by thermal desorption in the gas phase. The main advantages of SPE over LLE are the simplification of the trace enrichment matrix (sample cleaning) and medium change (transfer of the sample matrix to a different solvent or gas phase) and use of a smaller volume of solvent, in addition to being easy to set up.

SPE is widely used in chemical analysis, including environmental, pharmaceutical, clinical, food, and industrial applications. Over time, various sorbent formats were developed to facilitate the extraction of different sample types. Furthermore, it is a technique that allows its automation, so a full system integration for autonomous extraction, separation, and detection is possible, although flexible and cost-effective manual sample processing remains the most common practice in food analysis [22][23].

Many types of sorbents are commercially available and used in food analysis, such as alumina, magnesium silicate, and graphite carbon; however, silica is the most used because its surface is more reactive, allowing modifications by chemical reactions to improve the extraction specificity in addition to being stable. However, when used in solid matrices, sample treatment steps, such as homogenization, filtration, sonication, centrifugation, and liquid–liquid pre-extraction, are sometimes used, which makes the technique extremely time-consuming and expensive. Another disadvantage of this

technique is that the cartridge must be uniformly coated to avoid reproducibility difficulties in the analytical determination step [9][23].

In the last years, graphene has also been employed as a sorbent for SPE in foodstuff samples, as performed by Mateos et al. [24], who evaluated the usefulness of graphene as a sorbent for the isolation of BHA and PG from samples of precooked spaghetti and hard bouillon cubes. Therefore, SPE was influenced by the type and volume of the eluent, the amount and volume of graphene, and the concentration of antioxidants in the samples. HPLC analysis indicated that PG and BHA levels were below the established legal limits. Hard bouillon cubes, free from both antioxidants were fortified with different amounts of PG and BHA, and recoveries very close to 100% were achieved, demonstrating that graphene is a suitable sorbent in the SPE extraction of antioxidants in foodstuffs.

3.3. Ultrasound Extraction

SPE and LLE have several associated disadvantages, such as high capital investment and energy consumption and the use of toxic organic substances used for extraction. For this, ultrasound-assisted extraction (UAE) is suitable in terms of being environmentally friendly and having clean extraction and low-investment-required technique. Additionally, UAE is easy to use, multidirectional, and flexible when compared with other extraction techniques. High-intensity sonication is performed for extraction and process applications, while low-intensity sonication is used as a nondestructive analytical technique for quality assurance and process control. Ultrasound application enlarges the solvent selection range of generally recognized safe, instead of toxic, organic solvents [9][25].

Cacho et al. [26] used UAE in the extraction of BHA, BHT, and TBHQ in edible vegetable oil, resulting in a recovery range of 86% to 115% in the extracts. Žniderščič, Mlakar, and Prosen [27] used UAE in the degasification of beer, before BHT, BHA, and TBHQ extraction, which was followed by GC–MS/MS analysis, resulting in limit quantification between 0.08 and 0.10 ng/g, depending on the compound.

3.4. Solid-Phase Microextraction

In recent years, many innovations in analytical processes have also been applied in the field of sample preparation, which has resulted in the replacement of classic methods with faster, cheaper, less toxic procedures with less waste generation and with equal or better performance than classical methods, called the green extraction methods. Current trends in sample preparation have focused on low-cost operations, moving towards miniaturization, automation, high-efficiency performance, online analytical instruments, and extremely low or solvent-free consumption. Reducing the sample preparation steps can be effective in the errors, time, and cost decrease, and has some advantages for measuring trace and ultratrace analytes in complex matrices. For this, microextraction methods, such as solid-phase microextraction (SPME), stir bar boundary extraction (SBSE), and liquid-phase microextraction (LPME) have become important for sample preparation compared with conventional techniques. Microextraction means that all modes of these techniques require the use of small volumes of extraction medium during extraction conditions [9][28].

SPME is a sample preparation method that uses fused silica-coated externally with an appropriate stationary phase, which, during the elution of the sample, is retained in the coating and can later be extracted. The column used in this technique is infinitely smaller than that used in conventional SPE, in addition to allowing you to compress all the steps of sample preparation into one. This considerably reduces preparation time and solvent use and, in addition to cost, generates less waste for disposal. It can be easily coupled with GC and HPLC chromatography analysis and improve detection limits [19][29].

SPME has been successfully applied to a wide variety of compounds in gaseous, liquid, and solid samples, especially for the extraction of volatile organics and semivolatile compounds in environmental, biological, and foodstuffs samples, followed by GC and GC/MS analysis. Furthermore, SPME has also been introduced for direct coupling with HPLC and LC/MS to analyze weakly volatile or thermally unstable compounds and various polar compounds. These SPME methods are based on the adsorption of compounds in the liquid phase coated on the fiber surface [9][19][29].

Žniderščič et al. [27] employed SPME in the extraction of BHT, BHA, and TBHQ in beverages previously degassed. Antioxidants were adsorbed in an SPME fiber, the volatile compounds were removed under reduced pressure, and the extract desorbed was analyzed by GC–MS/MS, resulting in limits of detection of 0.005, 0.025, and 0.005 µg/L for BHT, BHA, and TBHQ, respectively, and recovery between 98% and 109% in beverages.

3.5. Stir Bar Sorptive Extraction

The stir bar sorptive extraction (SBSE) is an extraction technique developed in the late 1990s based on SPME. The larger coating on the stir bar, about 50 to 250 times larger than the fiber used in SPME, allows for greater adsorption and recovery capacity, ensuring greater efficiency and better reproducibility in the extraction of the compounds of interest from complex samples. In addition, it significantly reduces the use of solvents and, due to its greater absorption power, reduces the amount of sample preparation, which is often time-consuming and laborious and generates experimental errors. Normally, SBSE can be applied in the extraction of various organic compounds in the aqueous matrix of foodstuffs. Therefore, the extraction bar can be added to the sample and by the stirring process and carry out the extraction process. After the extraction time, the compounds adsorbed on the bar can be desorbed and determined by GC or HPLC. The biggest disadvantage of this technique is that it is not possible to automate it [9][19].

Nurerk et al. [30] optimized an extraction methodology using SBSE to extract BHA, BHT, and TBHQ from juice, milk, infant formula, and coffee cream samples. For this, it employed a stir bar adsorbent trapped in poly(3,4-ethylenedioxythiophene) interconnected porous cryogen, and the extraction efficiency was optimized by evaluating the effect of adsorbent compositions, extraction time, agitation speed, sample pH, desorption conditions, sample volume, and ionic strength. The analysis of the extracted synthetic phenolic antioxidants was performed by HPLC, and the detection limits for BHA, BHT, and TBHQ ranged between 0.05 and 0.15 µg/kg, and recoveries ranged from 87% to 101%. The developed composite stir bar adsorbent was convenient to use with good physical and chemical stability, allowing efficient extraction for 12 cycles of extractions.

3.6. Liquid–Liquid Microextraction

Liquid-phase microextraction (LPME) is an extraction technique that promotes the preconcentration and separation of the analyte from its matrix widely used in recent years, mainly due to its simple operation, low cost, and high efficiency. LPME is an easy, fast, efficient, and cost-effective sample preparation technique. Like SPME, sample extraction, concentration, and entry can be integrated into a single step. LPME extraction typically consists of a small amount of a water-immiscible solvent and an aqueous phase containing the target analyte. The acceptor phase is not only immersed for direct extraction but also suspended in the sample for headspace extraction, and the received phase volume varies in microliters or less; besides, greater enrichment factors must be obtained due to the relationship between the high volume of the sample and the acceptor phase [9][19][31].

LPME can be classified into single-phase droplet microextraction (SDME), hollow fiber liquid-phase microextraction (HF-LPME), and dispersive liquid microextraction (DLLME), according to the operation mode. Therefore, different LPME approaches have been developed to analyze various compounds in foodstuffs, with each group having a variety of modifications. The advantages of LPME can be summarized as a simple and highly selective extraction method, environmentally friendly due to lower solvent usage, where the µL of the solvent is used to extract an analyte from multiple samples and mainly can be combined with HPLC, GC, and capillary electrophoresis (CE) [9][19][31].

Liu et al. [32] used LPME allied to ultrasound-assisted and deep eutectic solvents for the extraction of TBHQ in edible oils, in which the extracts were directly analyzed HPLC with an ultraviolet detector after the separation by a reverse-phase column. In this methodology, they obtained a detection limit of 0.02 µg/mL and a recovery range of 98.5%–112%. This methodology was applied to determine TBHQ in 13 edible oil samples, and the result was close to that of the conventional LLE, but with the use of toxic solvents, which proves to be an environment-friendly method.

DLLME, first described in 2006, has been successfully applied to extract pesticides from water samples. Like LLME, DLLME relies on three-component solvent systems (aqueous sample, dispersive solvent, and extractive solvent). A suitable mixture of extraction solvent (organic) and dispersive solvent (water-miscible organic solvent) can be injected into the aqueous sample, and therefore, turbid solvent must be formed. Subsequently, using a centrifuge, the analytes are separated from the organic phase. DLLME's main advantages over conventional techniques are simplicity, fast operation, low cost, easy handling, low use of organic solvents, high recovery, high factor enrichment, and adaptability to HPLC and GC techniques [9][33][34].

DLLME was used by Biparva et al. [35] in the extraction of BHA and BHT in fruit juice samples for HPLC analysis. The detection limits obtained were 2.5 and 0.9 mg/L for BHA and BHT, respectively, with recovery percentages between 95% and 100% after the preconcentration of BHT and BHA. Already, Fang et al. [36] used DLLME as a cleanup step in the edible oil sample analysis, and HPLC detection resulted in detection limits from 0.002 to 0.04 mg/kg and a recovery percentage of around 74% in the oil samples. This method resulted in less time and less consumption of reagents that could be used on several occasions to detect synthetic antioxidants.

3.7. Cloud-Point Extraction

Cloud-point extraction (CPE) is the more ecological sample pretreatment, which consists of three steps: (i) solubilization of the analytes in micellar aggregates, (ii) cloudiness, and (iii) phase separation for the analysis. Nonionic surfactants may be able to form a micelle in aqueous solutions and become cloudy at a specific temperature, which is described as cloud-point temperature. At this point, the micellar solution is divided into two phases: a small-volume phase that enriches in terms of surfactant and dilutes the aqueous phase. When metal ions react with an appropriate binder, they can form an aqueous complex of low solubility, and therefore, these ions must be extracted from the aqueous solution in the small-volume phase enriched in terms of surfactant [37][38][39].

CPE is a simple, sensitive, and fast method of concentration and separation of essential elements because of employing water and avoiding the use of expensive, toxic, and flammable organic solvents in large volumes. In addition, CPE is expected to have several significant advantages, such as faster operation, easier handling, shorter time, lower cost, higher recovery and enrichment factor, and less stringent requirements for separation. Diluted surfactant solvents can be used as an extracting medium in CPE, resulting in less laboratory waste and cost-effectiveness, and are likely to be cost-effective reagents. Furthermore, surfactants are less flammable than organic solvents, reducing the risk to the analyst [37][38][39].

CPE was employed by Chen et al. [35] to extract and preconcentrate PG, TBHQ, BHA, and BHT from edible oils. Using a nonionic surfactant and assisted ultrasound, these antioxidants were extracted and analyzed by HPLC after the separation in a reverse-phase column, following limits of detection in the range of 1.6 to 9.0 ng/mL and a recovery range of 90% to 98%. The comparison with the CPE method using neutral surfactant and LLE using methanol proved that the proposed method allows a preconcentration factor of 25 times, improving the analytical sensitivity.

3.8. QuEChERS

A fast, easy, cheap, effective, robust, and secure (QuEChERS) approach can extract multiclass analytes simultaneously, remove matrix interferences, and enrich matrix analytes in a single step. This approach has advantages; for example, (i) it reduces analysis time and cost, (ii) it requires a small number of steps, and (iii) it minimizes the consumption of chemicals. Initially, the QuEChERS method was used in different matrices for analysis in food, biological, and waste industries. QuEChERS extraction is divided into two stages, an initial single-phase extraction with a solvent, followed by salting-out extraction/partitioning with salts, and finally, a dispersive solid-phase extraction to clean the extract (due to possible interferences present as a consequence of complex matrices) [12][40].

Gupta et al. [12] employed QuEChERS as an extraction procedure of synthetic antioxidants in fruit juice samples for GC–MS/MS analysis. With a detection limit of 8.14 to 25.45 ng/L, the proposed method was successfully applied to six different packaged fruit juice samples, resulting in a recovery in the range of 73.2% to 119.9%, thus being considered effective and cost-effective for routine antioxidant detection in foodstuff samples.

In another research, Guldberg et al. [41] employed QuEChERS for the extraction of BHA and PG in fish silage and fish oil used to produce animal feed. This extraction was performed to develop and validate a new method to determine these antioxidants by LC–MS/MS and allowed for the quantification of antioxidants in all matrices with low detection limits of 0.012 to 0.015 mg/kg and a recovery range of 97% to 101%.

4. Working Electrodes Used in Synthetic Phenolic Antioxidant Analysis

Like other organic compound detections, for the electrochemical determination of synthetic phenolic antioxidants, the main step that will determine the success of the methodology is the surface on which the redox reaction will occur; already in the use of chromatographic techniques, the determining step of the methodology is the extraction. For electrochemical analysis for all the articles found in the last few years, just a simple liquid–liquid extraction was enough, without additional cleanup steps to determine the antioxidants in food.

In practice, for electrochemical analysis, the sample containing the compounds of interest is added in the electrochemical cell, which is composed of electrical conductors (working, reference, and auxiliary electrodes) and the ionic conductor (supporting electrolyte). The potential difference is applied, resulting in an interfacial reaction that occurs between the working electrode surface and a solution.

Therefore, the choices of working electrodes are based on the chemical structure of antioxidants, which will result in a specific potential interval where the antioxidant oxidizes and/or reduces, following the signal proportional to the antioxidant

concentration. Additionally, the working electrodes must present low cost, high surface area, and good electric conductivity. Besides, the signal-to-noise features and reproducibility in the signals and still results in voltammetric methodologies with suitable robustness, efficiency, sensitivity, inexpensiveness, and aim at meeting green chemistry principles should also be considered [42].

The preparation of working electrodes requires a specific procedure according to the material employed, allowing for specific protocols that combine the surface cleaned by specific solvents or surfactants, alteration of the exposed microstructure by mechanical polishing, manipulation of the surface chemistry by an electrochemical process that can improve the intensity and reproducibility of the analytical signal [43]. Additionally, working electrodes with different types of materials and geometries are commercially available, and many can be employed for surface modification and specific uses.

Carbon is the most common material used as a working electrode due to its broad anodic potential range, low cost, chemical inertness, and mechanical stability. There are different forms of carbon, among them the most used are graphite, glassy carbon, carbon fiber, nanotubes, graphene, and diamond.

Medeiros et al. [44] developed a methodology for the simultaneous determination of BHA and BHT in foods using SWV combined with a boron-doped diamond (BDD) electrode. Through the evaluation of the supporting electrolyte and suitable voltammetric parameters, it was possible to observe that SWV together with a cathodically pretreated DDBE electrode can be used with some benefits for the quantitative determination of BHA and BHT, alone or mixed as commonly found in food products. Very low limits of detection were obtained in the simultaneous determination of BHA (0.14 µmol/L) and BHT (0.25 µmol/L). Furthermore, addition and recovery analysis allowed to conclude that the matrix effect did not present significant interference. The concentration values obtained for BHA and BHT are like those obtained by the HPLC method. Thus, the SWV method reported here is effective for the simultaneous determination of BHA and BHT in food products.

References

1. Gan, T.; Zhao, A.X.; Wang, S.H.; Lv, Z.; Sun, J.Y. Hierarchical Triple-Shelled Porous Hollow Zinc Oxide Spheres Wrapped in Graphene Oxide as Efficient Sensor Material for Simultaneous Electrochemical Determination of Synthetic Antioxidants in Vegetable Oil. Sens. Actuators B Chem. 2016, 235, 707–716.
2. Richard Prabakar, S.J.; Sriman Narayanan, S. Flow Injection Analysis of BHA by NiHCF Modified Electrode. Food Chem. 2010, 118, 449–455.
3. Noel Robledo, S.; Alicia Zón, M.; Daniel Ceballos, C.; Fernández, H. Qualitative and Quantitative Electroanalysis of Synthetic Phenolic Antioxidant Mixtures in Edible Oils Based on Their Acid-Base Properties. Food Chem. 2011, 127, 1361–1369.
4. Manoranjitham, J.J.; Narayanan, S.S. Electrochemical Sensor for Determination of Butylated Hydroxyanisole (BHA) in Food Products Using Poly O-Cresolphthalein Complexone Coated Multiwalled Carbon Nanotubes Electrode. Food Chem. 2021, 342, 128246.
5. Codex STAN 192-1995; Codex General Standard for Food Additives (GSFA) Online Database. FAO/WHO: Geneva, Switzerland, 2019; p. 490.
6. Tracy, M. Improved Determination of Antioxidants in Edible Oils Using Solid Core LC Columns; Thermo Fisher Scientific: Sunnyvale, CA, USA, 2014; pp. 6–9.
7. European Union Reference Laboratory. Evaluation Report on the Analytical Methods Submitted in Connection with the Application for the Authorisation of Feed Additives According to Regulation (EC) No 1831/2003; European Union Reference Laboratory for Feed Additives (EURL-FA) JRC: Geel, Belgium, 2012; pp. 2–8.
8. International Union of Pure and Applied Chemistry. Commission on Oils, Fats and Derivatives. In Standard Methods for the Analysis of Oils, Fats and Derivatives; 1st Supplement to the 7th Edition; International Union of Pure and Applied Chemistry: Research Triangle Park, NC, USA, 1992; p. 151.
9. Demirhan, B.; Kara, H.E.Ş.; Demirhan, B.E. Overview of Green Sample Preparation Techniques in Food Analysis. In Ideas and Applications Toward Sample Preparation for Food and Beverage Analysis; IntechOpen: London, UK, 2017.
10. Du, B.; Shen, M.; Pan, Z.; Zhu, C.; Luo, D.; Zeng, L. Trace Analysis of Multiple Synthetic Phenolic Antioxidants in Foods by Liquid Chromatography–Tandem Mass Spectrometry with Complementary Use of Electrospray Ionization and Atmospheric Pressure Chemical Ionization. Food Chem. 2021, 375, 131663.

11. Farajzadeh, M.A.; Abbaspour, M.; Mogaddam, M.R.A.; Ghorbanpour, H. Determination of Some Synthetic Phenolic Antioxidants and Bisphenol A in Honey Using Dispersive Liquid–Liquid Microextraction Followed by Gas Chromatography-Flame Ionization Detection. Food Anal. Methods 2015, 8, 2035–2043.
12. Gupta, M.K.; Anand, A.; Asati, A.; Thati, R.; Katragunta, K.; Agarwal, R.; Mudiam, M.K.R. Quantitative Determination of Phenolic Antioxidants in Fruit Juices by GC-MS/MS Using Automated Injector Port Silylation after QuEChERS Extraction. Microchem. J. 2021, 160, 105705.
13. Sandra, P.; David, F.; Vanhoenacker, G. Chapter 5 Advanced Sample Preparation Techniques for the Analysis of Food Contaminants and Residues. Compr. Anal. Chem. 2008, 51, 131–174.
14. André, C.; Castanheira, I.; Cruz, J.M.; Paseiro, P.; Sanches-Silva, A. Analytical Strategies to Evaluate Antioxidants in Food: A Review. Trends Food Sci. Technol. 2010, 21, 229–246.
15. Carocho, M.; Morales, P.; Ferreira, I.C.F.R. Natural Food Additives: Quo Vadis? Trends Food Sci. Technol. 2015, 45, 284–295.
16. Carocho, M.; Morales, P.; Ferreira, I.C.F.R. Antioxidants: Reviewing the Chemistry, Food Applications, Legislation and Role as Preservatives. Trends Food Sci. Technol. 2018, 71, 107–120.
17. Zhang, J.; Hu, B. Liquid-Liquid Extraction (LLE). Sep. Purif. Technol. Biorefineries 2013, 61–78.
18. Silva, L.L.B.; Mageste, A.B.; de Valverde, B.P.; Caires, L.L.R.; do Hespanhol, M.C.; Ferreira, G.M.D. Liquid-Liquid Equilibrium of Aqueous Two-Phase Systems Formed by Non-Ionic Surfactant (Triton X-165 or Triton X-305) and Ionic Liquid Based on the Choline Cation (Choline Bitartrate or Choline Dihydrogen Citrate). J. Mol. Liq. 2020, 318, 114030.
19. Nasiri, M.; Ahmadzadeh, H.; Amiri, A. Sample Preparation and Extraction Methods for Pesticides in Aquatic Environments: A Review. TrAC—Trends Anal. Chem. 2020, 123, 115772.
20. de Pinho, G.P.; Neves, A.A.; de Queiroz, M.E.L.R.; Silvério, F.O. Optimization of the Liquid-Liquid Extraction Method and Low Temperature Purification (LLE-LTP) for Pesticide Residue Analysis in Honey Samples by Gas Chromatography. Food Control 2010, 21, 1307–1311.
21. Kim, J.M.; Choi, S.H.; Shin, G.H.; Lee, J.H.; Kang, S.R.; Lee, K.Y.; Lim, H.S.; Kang, T.S.; Lee, O.H. Method Validation and Measurement Uncertainty for the Simultaneous Determination of Synthetic Phenolic Antioxidants in Edible Oils Commonly Consumed in Korea. Food Chem. 2016, 213, 19–25.
22. Poole, C.F.; Poole, S.K. Principles and Practice of Solid-Phase Extraction. Compr. Sampl. Sample Prep. 2012, 2, 273–297.
23. Beyer, A.; Biziuk, M. Applications of Sample Preparation Techniques in the Analysis of Pesticides and PCBs in Food. Food Chem. 2008, 108, 669–680.
24. Mateos, R.; Vera, S.; Díez-Pascual, A.M.; San Andrés, M.P. Graphene Solid Phase Extraction (SPE) of Synthetic Antioxidants in Complex Food Matrices. J. Food Compos. Anal. 2017, 62, 223–230.
25. Tiwari, B.K. Ultrasound: A Clean, Green Extraction Technology. TrAC—Trends Anal. Chem. 2015, 71, 100–109.
26. Cacho, J.I.; Campillo, N.; Viñas, P.; Hernández-Córdoba, M. Determination of Synthetic Phenolic Antioxidants in Soft Drinks by Stir-Bar Sorptive Extraction Coupled to Gas Chromatography-Mass Spectrometry. Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess. 2015, 32, 665–673.
27. Žnideršič, L.; Mlakar, A.; Prosen, H. Development of a SPME-GC-MS/MS Method for the Determination of Some Contaminants from Food Contact Material in Beverages. Food Chem. Toxicol. 2019, 134, 110829.
28. Biparva, P.; Abbas, A. Microextraction Techniques as a Sample Preparation Step for Metal Analysis. At. Absorpt. Spectrosc. 2012, 30, 61–88.
29. Kataoka, H. New Trends in Sample Preparation for Clinical and Pharmaceutical Analysis. TrAC—Trends Anal. Chem. 2003, 22, 232–244.
30. Nurerk, P.; Bunkoed, O.; Jullakan, S.; Khongkla, S.; Llompart, M.; Poorahong, S. A Dumbbell-Shaped Stir Bar Made from Poly(3,4-Ethylenedioxythiophene)-Coated Porous Cryogel Incorporating Metal Organic Frameworks for the Extraction of Synthetic Phenolic Antioxidants in Foodstuffs. J. Chromatogr. A 2021, 1655, 462497.
31. Martins, F.C.O.L.; Batista, A.D.; Melchert, W.R. Current Overview and Perspectives in Environmentally Friendly Microextractions of Carbamates and Dithiocarbamates. Compr. Rev. Food Sci. Food Saf. 2021, 20, 6116–6145.
32. Liu, W.; Zhang, K.; Yu, J.; Bi, Y. A Green Ultrasonic-Assisted Liquid-Liquid Microextraction Based on Deep Eutectic Solvent for the HPLC-UV Determination of TBHQ in Edible Oils. Food Anal. Methods 2017, 10, 3209–3215.
33. Berijani, S.; Assadi, Y.; Anbia, M.; Milani Hosseini, M.R.; Aghaee, E. Dispersive Liquid-Liquid Microextraction Combined with Gas Chromatography-Flame Photometric Detection. Very Simple, Rapid and Sensitive Method for the Determination of Organophosphorus Pesticides in Water. J. Chromatogr. A 2006, 1123, 1–9.

34. Rezaee, M.; Assadi, Y.; Milani Hosseini, M.R.; Aghaee, E.; Ahmadi, F.; Berijani, S. Determination of Organic Compounds in Water Using Dispersive Liquid-Liquid Microextraction. J. Chromatogr. A 2006, 1116, 1–9.
35. Biparva, P.; Ehsani, M.; Hadjmohammadi, M.R. Dispersive Liquid-Liquid Microextraction Using Extraction Solvents Lighter than Water Combined with High Performance Liquid Chromatography for Determination of Synthetic Antioxidants in Fruit Juice Samples. J. Food Compos. Anal. 2012, 27, 87–94.
36. Fang, Y.; Tian, W.; Pei, F.; Li, P.; Shao, X.L.; Fan, Y.; Hu, Q. Simultaneous Determination of Pesticide Residues and Antioxidants in Blended Oil Using a Liquid-Liquid Extraction Combined with Dispersive Solid Phase Extraction Method. Food Chem. 2017, 229, 347–353.
37. Arpa Şahin, Ç.; Efeçinar, M.; Şatiroğlu, N. Combination of Cloud Point Extraction and Flame Atomic Absorption Spectrometry for Preconcentration and Determination of Nickel and Manganese Ions in Water and Food Samples. J. Hazard. Mater. 2010, 176, 672–677.
38. Citak, D.; Tuzen, M. A Novel Preconcentration Procedure Using Cloud Point Extraction for Determination of Lead, Cobalt and Copper in Water and Food Samples Using Flame Atomic Absorption Spectrometry. Food Chem. Toxicol. 2010, 48, 1399–1404.
39. Wang, S.; Meng, S.; Guo, Y. Cloud Point Extraction for the Determination of Trace Amounts of Cobalt in Water and Food Samples by Flame Atomic Absorption Spectrometry. J. Spectrosc. 2013, 2013, 735702.
40. Sarraf, M.; Beig-babaei, A.; Naji-Tabasi, S. Application of QuEChERS Method for Extraction of Functional Compounds. SN Appl. Sci. 2020, 2, 1858.
41. Guldberg, T.S.; Sandrød, M.; Øiaas, J.B.; Holten, T.; Zahlsen, K.; Kvitvang, H.F. Analysis of Synthetic Antioxidants in Salmon Silage Using QuEChERS Extraction Method Followed by Determination by LC-MS/MS; a Single-Laboratory Validation Study. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 2021, 1174, 122715.
42. Martins, F.C.O.L.; Sentanin, M.A.; De Souza, D. Categories of Food Additives and Analytical Techniques for Their Determination. In Innovative Food Analysis; Elsevier: Amsterdam, The Netherlands, 2021; pp. 123–156.
43. De Souza, D.; Gonçalves-Filho, D.; Franco, D.L. Pesticide Residues Analysis by Electroanalytical Techniques. In Sustainable Agriculture Reviews 48; Inamuddin, Ahamed, M.I., Lichtfouse, E., Eds.; Springer: Cham: Switzerland, 2021; pp. 1–75. ISBN 978-3-030-54719-6.
44. Medeiros, R.A.; Rocha-Filho, R.C.; Fatibello-Filho, O. Simultaneous Voltammetric Determination of Phenolic Antioxidants in Food Using a Boron-Doped Diamond Electrode. Food Chem. 2010, 123, 886–891.
