Agricultural and Food Electroanalysis

Edited by

ALBERTO ESCARPA
MARÍA CRISTINA GONZÁLEZ
MIGUEL ÁNGEL LÓPEZ

Analytical Chemistry,
Physical Chemistry and Chemical Engineering Department,
University of Alcalá,
Spain

WILEY
At the time we were editing this book, Professor Mascini passed away. Probably, one of the latest excellent contributions done in his vast successful career is found in this book. In memoriam, editors would like to dedicate him these words as proof of his valuable contribution to the field of electrochemical biosensors in food analysis.
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List of Contributors

Lúcio Angnes, Instituto de Química, Universidade de São Paulo, Brazil

Alberto Sánchez Arribas, Departamento de Química Analítica y Análisis Instrumental, Universidad Autónoma de Madrid, Spain

Craig E. Banks, School of Chemistry and the Environment, Division of Chemistry and Environmental Science, Manchester Metropolitan University, UK

Mónica Moreno Barambio, Departamento de Química Analítica y Análisis Instrumental, Universidad Autónoma de Madrid, Spain

Simona Benedetti, Dipartimento di Scienze per gli Alimenti, la nutrizione e l’ambiente Università degli Studi di Milano, Italy

Erika Bustos, Centro de Investigación y Desarrollo Tecnológico en Electroquímica, Parque Tecnológico Querétaro Sanfandila, México

Gang Chen, School of Pharmacy, Fudan University, China

Wendell K. T. Coltro, Institute of Chemistry, Federal University of Goias, Brazil

Stella M. Cosio, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Italy

Agustín Costa-García, Departamento de Química Física y Analítica, Facultad de Química, Universidad de Oviedo, Spain

Alberto Escarpa, Departamento de Química Analítica, Química Física e Ingeniería Química, Universidad de Alcalá, Spain

Fabiana Silva Felix, Instituto de Química, Universidade de São Paulo, Brazil

M. Teresa Fernández-Abedul, Departamento de Química Física y Analítica, Facultad de Química, Universidad de Oviedo, Spain

Carlos D. Garcia, Department of Chemistry, The University of Texas at San Antonio, USA
Luis A. Godínez, Centro de Investigación y Desarrollo Tecnológico en Electroquímica, Parque Tecnológico Querétaro Sanfandila, México

María Cristina González, Departamento de Química Analítica, Química Física e Ingeniería Química, Universidad de Alcalá, Spain

Araceli González-Cortés, Departamento de Química Analítica, Facultad de Ciencias Química, Universidad Complutense de Madrid, Spain

M. Begoña González-García, Departamento de Química Física y Analítica, Facultad de Química, Universidad de Oviedo, Spain

Miguel A. López, Departamento de Química Analítica, Química Física e Ingeniería Química, Universidad de Alcalá, Spain

Saverio Mannino, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Italy

Juan Manríquez, Centro de Investigación y Desarrollo Tecnológico en Electroquímica, Parque Tecnológico Querétaro Sanfandila, México

Marco Mascini, Dipartimento di Chimica Ugo Schiff, Università degli Studi di Firenze, Italy

Sandra Mendoza, Departamento de Investigación y Posgrado en Alimentos, Universidad Autónoma de Querétaro, México

Solomon Lemma Mengistu, Free University of Bolzano, Italy

Arben Merkoçi, Institució Catalana de Recerca i Estudis Avançats (ICREA), Spain; Nanobioelectronics and Biosensors Group, Universitat Autònoma de Barcelona, Spain

Jonathan P. Metters, School of Chemistry and the Environment, Division of Chemistry and Environmental Science, Manchester Metropolitan University, UK

Maria F. Mora, Jet Propulsion Laboratory, California Institute of Technology, USA

Geza Nagy, General and Physical Chemistry, Faculty of Science, University of Pécs, Hungary

Lívia Nagy, Szentagothai Research Centre, University of Pécs, Hungary

Ilaria Palchetti, Dipartimento di Chimica Ugo Schiff, Università degli Studi di Firenze, Italy

Briza Pérez-López, Nanobioelectronics and Biosensors Group, Universitat Autònoma de Barcelona, Spain; LEITAT Technological Center, Spain
José M. Pingarrón, Department of Analytical Chemistry, Faculty of Chemistry, University Complutense of Madrid, Spain

Manuel Chicharro Santamaría, Departamento de Química Analítica y Análisis Instrumental, Universidad Autónoma de Madrid, Spain

Matteo Scampicchio, Free University of Bolzano, Italy

Joseph Wang, Departments of Nanoengineering, University of California, USA

Paloma Yáñez-Sedeño, Department of Analytical Chemistry, Faculty of Chemistry, University Complutense of Madrid, Spain
Preface

This pioneer book *Agricultural and Food Electroanalysis* provides a description and rationale use of modern electroanalytical techniques, strategies, and approaches in the exciting field of agricultural and food analysis. Electrochemical techniques offer very valuable features such as very good sensitivity, tunable selectivity, low cost, simple use, inherent miniaturization, high compatibility with modern technologies required from microfabrication techniques to build “lab-on-a-chip” devices, high compatibility with surface modification employing biological reagents as well as exciting nanomaterials such as nanoparticles, nanotubes, and nanowires. Without any question, with the incursion of advanced approaches such as screen printed technology, biosensors, microchips, and nanotechnology, among others, electroanalysis is living a truly *Renaissance* and new frontiers have been clearly opened in the last years.

This book is divided in three parts and contains 16 chapters written by truly well-recognized experts in the field.

The first chapter, *Electroanalysis and Food Analysis*, has the important role to introduce the readers in the whole book where the adequacy of electroanalysis to agricultural and food analysis is exposed.

Following this initial introductory chapter, the book is structured in three parts. The first part discusses different *Electroanalytical Techniques in Batch and Continuous Systems* as highly remarkable tools in the agricultural and food field. In this sense, Chapter 2 deeply explores the sweep potential electroanalytical techniques, while Chapter 3 allows the readers to obtain fundamental information on voltammetric techniques coupled to flow systems which could proportionate faster analysis, reproducible results, high sensitivity, with additional advantages such as the requirement of less sample, and the use of simpler instrumentation.

Separation techniques coupled to electrochemical detectors are also studied in this section. Chapter 4 deals with the design and integration of electrochemical detectors within the HPLC separation system and their compatibility and compromise with the chromatographic conditions necessary to achieve the optimum resolution of the analytes in agricultural and food field. Chapter 5 introduces the key strategies in capillary electrophoresis using electrochemical detection for separating and detecting a variety of constituents in foods and agricultural products. That includes the commonly used separation modes of capillary electrophoresis, its coupling with electrochemical detection, and its application in agricultural and food analysis.

The largest part of the book, organized in the following nine chapters, is dedicated to the *Electrochemical Sensing in Food Analysis*. Chapter 6 introduces microelectrodes and
microelectrode arrays which can be used for both fundamental and applied electrochemistry. Different approaches to fabricate such transducers are critically overviewed with special emphasis on the requirement of sensors that can be used at the site of sampling, being cost-effective and reproducible. Besides, the importance of potentiometric sensors and electrochemical biosensing approaches is deeply studied in Chapters 7–11.

Electrochemical transducers combined with an enzyme as a biochemical component constitute the largest category of biosensors, thus becoming an important tool for the detection of highly concern analytes in agricultural and food monitoring. This matter is considered in Chapter 8. The design, chemical construction, and application in agricultural and food electroanalysis of the further most important biosensing approaches such as immunosensors and genosensors are studied in Chapter 9 and Chapter 10, respectively. Additionally, Chapter 11 discusses the recent trends that have led to powerful nanomaterial-based electrochemical biosensing devices and examines the related prospects and challenges suggesting considerable promise for diverse applications in the food and agricultural field.

The next two chapters of this book section address the novel micro- and nanotechnologies impact in the field. Electroanalysis on board of microfluidics and lab-on-a-chip platforms is studied in Chapter 12 and selected nanoelectrochemistry applications for food analysis are covered in Chapter 13. To conclude this part, Chapter 14 deals with the principles and food applications using electrochemical impedance spectroscopy.

The book finishes with two chapters configuring the third part regarding Industrial Implications: Electroanalysis in Food Process Control (Chapter 15) and Instrumental Aspects of Food Analysis by Electrochemical Methods (Chapter 16). Unlike traditional chemical analysis, performed in well-equipped laboratories with the aim to identify and quantify small amounts of analytes, the goal of process analytical chemistry is to supply quantitative and qualitative information about a chemical process that can be used not only to monitor and control the process, but also to optimize its efficient use of energy, time, and raw materials. In addition, it is possible to simultaneously minimize plant effluent release and to improve product quality. These important concepts adapted to food and agricultural electroanalysis are focused in Chapter 15. In contrast, despite the clear advantages associated with electrochemical detectors, the training of personnel and somehow the limited availability of commercial instruments has traditionally limited the development of electrochemical methods applied to agricultural and food-related samples. In the light of such considerations, Chapter 16 aims to provide a brief overview of the key instrumental aspects linked to agricultural and food electroanalysis.

In sum, and in editor’s opinion, this valuable text offers a comprehensive vision about different electrochemical techniques following their basic principles, instrumentation, and main applications in the field of food and agricultural analysis. Also, editors hope that the critical and attractive vision of the book will help readers to get introduced in the exciting area of agricultural and food electroanalysis.

Finally, the editors would like to thank to all the authors for their excellent contributions to this pioneer book in the agricultural and food analysis field.

Alberto Escarpa, María Cristina González, and Miguel Ángel López
Editors
1

Electroanalysis and Food Analysis

Paloma Yáñez-Sedeño and José M. Pingarrón
Department of Analytical Chemistry, Faculty of Chemistry, University Complutense of Madrid, 28040, Madrid, Spain

1.1 Introduction and Adequacy of Electroanalysis for Food Analysis

Electroanalysis is a powerful analytical tool for food analysis. Since the early times of polarography and potentiometry until the current developments of chemical sensors, biosensors and lab-on-a-chip (LOC) devices involving electrochemical detection principles and many other electroanalytical methodologies have demonstrated their usefulness to accomplish the requirements imposed by the food industry for the analytical monitoring and control of raw materials and foodstuffs. This is particularly true in the last decades where impressive advances exhibited in automation, miniaturization, and easy handling of electroanalytical devices including both the corresponding instrumentation and the electrodes employed as electrochemical transducers, have led to user-friendly methods of analysis that are competitive against other well-established analytical techniques such as chromatography and spectroscopy. This, together with the inherent affordable costs of electroanalytical approaches and the superior sensitivity that can be achieved using modern voltammetric techniques or coupling with amplification response methodologies involving nanomaterials, makes modern electroanalysis a more than suitable strategy to face up to the increasingly demanding requirements of food industry to ensure food quality, control, and safety [1].

The development and innovation in food industry rely basically on the concepts of food safety and food quality. However, lately, products as foodstuffs supplemented with compounds such as omega-3 acids, vitamins, fiber, and so on, which confer them particular properties sought by specific strata of society, have burst into the modern food industry providing products with a high added value. Obviously, as the food chain is increasingly
complex, there is a high demand for the development of efficient traceability systems which are able to guarantee the firmness of the whole chain. These systems should possess high sensitivity, ability to be implemented rapidly, and permit automatic screening.

Food quality can be understood as a set of factors which are able to differentiate food products according to their organoleptic characteristics, composition, and functional properties. An increased regulatory action together with an increased consumer demand for information have led to the extensive labeling of major and minor constituents of the foodstuffs. The scientific evaluation of the food freshness is another important task concerning food quality assessment. A list of the most current compounds to be analyzed for food quality assessment can be found in [2]. Moreover, continuous monitoring of food industrial processes allow real-time detection of possible errors in the chain production as well as taking decisions to rectify such errors in an immediate manner. The assessment of food safety is the other key axe for the modern food industry. In general, one can speak about food contamination when dealing with harmful substances or microorganisms that are not intentionally added to the food. Contaminants may enter the food chain during growth, cultivation, or preparation, accumulate in food during storage, form in the food through the interaction of chemical components or may be concentrated from the natural components of the food [3]. However, chemicals are also added during food processing in the form of additives. At present, pathogen microorganisms, pesticides, animal-drug residues, and antimicrobial drug resistance are the main concerns for food safety. Food regulatory agencies have established control programs, such as the HACCP (Hazard Analysis Critical Control Point) program, to avoid the entering of these substances into the food chain [4].

Electroanalysis has played a relevant role in food quality and food safety assessment and, in the last few years, it is increasingly significant due to the combination of sensors and biosensors technology, even in a disposable manner, with efficient electrochemical transduction techniques allowing the implementation of rapid and reliable detection methods. To provide an overview of the state of art in the use of electrochemical techniques in the field of agricultural and food analysis, we discuss in this chapter some examples on the latest advances in this field, pointing out on relevant methodologies related to the measurement techniques, including the development of electrochemical sensors and biosensors for food components, and the use of nanostructured electrodes.

1.2 Methodologies Related to Measurement Techniques

1.2.1 Continuous Detection Methods

In general, the application of electrochemical techniques for the detection of analytes in a continuous mode has demonstrated to be able to improve the sensitivity and selectivity of well-established analytical methods. Electrochemical detection has shown to be appropriate to be combined with high-performance liquid chromatography (HPLC), flow injection analysis, capillary electrophoresis, or microfluidics-based methodologies. There are numerous examples regarding food analysis where the improvements achieved using electrochemical detection techniques can be illustrated. With respect to liquid chromatography, methods are still being developed for detecting healthy food components. Representative
examples are the simultaneous determination of hydroxy polymethoxy-flavones in citrus products and orange juice [5], and a very recent method for the determination of phenolics in olive oil [6]. In both cases, HPLC with coulometric detection at multichannel CoulArray detector was used which enabled a high sensitivity to be obtained. Moreover, methodologies developed for the detection of drugs and pesticide residues using electrochemical detection can be found in the recent literature. As examples, the efficient separation and sensitive determination of sulfonamides in shrimps using a monolithic column and amperometric detection at a boron-doped diamond electrode [7], and the detection of carbamate pesticides in fruits and vegetables using an acetylcholinesterase biosensor where the enzyme was immobilized on a polyaniline–carbon nanotubes composite electrode [8], can be cited.

Flow-injection methods with electrochemical detection continue to attract great attention in the field of food analysis due to the inherent simplicity of these approaches and the good analytical performance provided. A recent and interesting application involves a single-line flow injection system combined with multiple pulse amperometric detection with a boron-doped diamond electrode for the simultaneous determination of two pairs of food colorants: tartrazine (TT) and sunset yellow (SY) (TT–SY) or brilliant blue (BB) and SY (BB–SY) in sports drink beverages, gelatin, and powdered juice. A dual-potential waveform was applied to the electrode for both colorants in each pair to be determined with detection limits ranging between 0.80 and 3.5 μM [9]. Batch injection analysis (BIA) combined with electrochemical detection has also been applied in this field taking advantage of the versatility, reproducibility, high analytical frequency, sensitivity, portability, and sample size provided by this combination [10]. Using these systems, precise sample plugs are directly injected onto the working electrode surface which is immersed in a large-volume blank solution, and the electrochemical responses are recorded directly. For example, amperometric detection at a Prussian Blue-modified graphite-composite electrode was recently described for determining H2O2 in high- and low-fat milk samples [11]. In this method, an electronic micropipette injected 100-μl aliquots of 10-fold diluted samples directly onto the modified electrode immersed in the BIA cell (Figure 1.1). The detection limit was low (10 μM), and good recovery values were achieved for spiked samples.

Within this family of continuous methodologies, it is also important to mention the sequential injection lab-on-valve (SI-LOV) technique, which allows increasing sampling capacity and the automation of the analytical methods [12]. In a recent article, an SI-LOV system was used for the sensitive determination of hypoxanthine [13]. As one of the purine bases, hypoxanthine is produced during the degradation process of fresh meat and fish, so that the content of this compound can be envisaged as a valuable indicator of food freshness [14]. In the cited work, a Fe3O4/multiwalled carbon nanotubes (MWCNTs)/β-cyclodextrin (β-CD) (Fe3O4/MWCNTs/β-CD) modified electrode was employed to measure the electrochemical oxidation of hypoxanthine. A diagram of the SI-LOV system used is depicted in Figure 1.2. After aspiration of 500 μl phosphate buffer solution (PBS) into the holding coil, various microvolumes of carrier, air, sample solution, and PBS, were aspirated and transferred into electrochemical flow cell (EFC) for the analyte accumulation on the modified electrode at 0.1 V. Then, the stripping voltammogram of hypoxanthine was recorded. Under the optimized conditions, a linear dependence between log Ip vs. log [hypoxanthine] was found in the $5.0 \times 10^{-8} - 1.0 \times 10^{-5}$ range, and the method was applied in determining hypoxanthine in meat samples.
Figure 1.1  (a) Schematic diagram of the batch injection cell containing the three-electrode system. (b) BIA amperometric responses of PB-modified graphite composite electrode for 100–600 μmol/l H₂O₂. Reproduced from Ref. [11] with permission from Elsevier

Figure 1.2  (a) Schematic diagram of SI-LOV manifold for hypoxanthine analysis: C, carrier (H₂O); SP, syringe pump; HC, holding coil; W, waste; A, air; S, sample; PBS, phosphate buffer solution, EFC, electrochemical flow cell (internal volume 200 μl). (b) Stripping voltammograms for (a–i) 0.05–10 mmol/l hypoxanthine. Inset: log Ip vs. log hypoxanthine concentration calibration plot. Reproduced from Ref. [13] with permission from Elsevier
During the past 20 years, a great progress has been made in the development of miniaturized systems for chemical analysis. In this context, microfluidics has attracted special interest because of offering remarkable sensitivity, inherent miniaturization, low cost, portability, compatibility with mass fabrication, and on-site analysis. The manipulation of small amounts of fluids through microchannels can be combined with miniaturization technologies for developing “LOC” devices, which intend the integration of different steps involved in the analytical process. When a separation step is required, capillary electrophoresis (CE) has demonstrated to be very appropriate for miniaturized technology. A representative example of application of these systems to agricultural and food analysis is the determination of phenolic compounds (tyrosol, hydroxytyrosol, and oleuropein glucoside) in olive oil using glass microchip electrophoresis with end-channel amperometric detection at a 100-μm gold wire working electrode [15]. Other recent application makes use of a microfluidic device for the simultaneous detection of five sulfonamides in meat involving preconcentration of the analytes in the microfluidic device (Figure 1.3) followed by their electrokinetic separation and amperometric detection at Al₂O₃–gold nanoparticles (AuNPs)-modified carbon paste electrodes. A linear range between 0.01 and 2,025 pM, and detection limits between 0.91 and 2.21 fM were obtained [16].

Capillary electrophoresis with amperometric detection was also used for the determination of four electroactive preservatives (methylparaben, ethylparaben, propylparaben, and butylparaben) and, indirectly, two nonelectroactive preservatives (potassium sorbate and sodium lactate) in various types of foodstuffs [17]. Moreover, high-performance micellar electrokinetic capillary chromatography with amperometric detection (MECC–AD) has been also employed for the fast determination of melamine (2,4,6-triamino-s-triazine), which was occasionally used to increase the apparent protein content of milk products [18].

### 1.2.2 Stripping Analysis

Mercury film electrode has been widely used in stripping voltammetry for a long time owing to its excellent electrochemical behavior. However, the toxicity of mercury has led to the development and use of the environmentally friendly bismuth film electrode (BiFE). This electrode is characterized by the simple preparation process, large enough accessible potential window, high sensitivity, well-defined, and undistorted stripping signal, as well as good resolution of neighboring peaks. In addition, BiFE is less sensitive to the presence of
dissolved oxygen than MFE [19]. An interesting example of recent applications of stripping methodologies with BiFE in food analysis is the determination of azo-compounds used as dyes for food, beverages, and textile industry coloring, which represent a human hazard because their degradation products, including amines, are carcinogenic. A bismuth/poly(p-aminobenzene sulfonic acid) (p-ABSA) composite film-coated glassy carbon electrode (GCE) (Bi/poly(p-ABSA)/GCE), prepared by depositing bismuth on the poly(p-ABSA) modified electrode at \(-0.9\) V, was used in this method. Azo-compounds such as 1-(2-pyridylazo)-2-naphthol (PAN), 4-(2-pyridylazo)-resorcinol (PAR), and azobenzene were determined by differential pulse voltammetry in orange and lemon beverages [20]. Other metallic films have also been employed for the preparation of modified electrodes to be applied in stripping methodologies for food analysis. For example, adsorptive stripping voltammetry (AdSV) with an \textit{in situ} plated lead film GCE was used for the determination of vitamin B1 (thiamine) in juices. Thiamine was preconcentrated at \(-1.25\) V and then electrochemically reduced by scanning the potential from \(-1.25\) to \(-1.55\) V using square wave voltammetry (SWV). A range of linearity between 0.0133 and 0.265 mg/l thiamine was reported with this methodology [21].

Despite the attempts to substitute mercury electrodes by other greener film electrodes, some recent applications involving Hg electrodes still appear in the literature. As an example, a method for the determination of the antibiotic ceftiofur (CF) in milk has been reported implying the adsorptive accumulation of the drug on a hanging mercury-drop electrode (HMDE) and reductive SWV. CF is a widely used broad-spectrum third-generation cephalosporin, which is approved for the treatment of infections in cattle, swine, sheep, goats, turkeys, and chickens. By application of the AdSV methodology, a linear calibration plot between 52.4 and 524 ng/ml, which allowed testing of the established tolerance level of 100 ng/ml for CF residues in bovine milk, was achieved. The method was applied to determine CF in spiked milk samples [22]. HMDE and SWV were also used for trace determination of azoxystrobins [methyl (E)-2-[6-(2-cyanophenoxy)-pyrimidin-4-yloxy]phenyl]-3-methoxyacrylate] and dimoxy-strobins [(E)-o-(2,5-dimethylenoxymethyl)-2-methoxyimino-N-methyl phenylacetamide] in potatoes, grapes, and grape juice. These compounds are synthetic pesticides, the strobilurins, which are derived from the natural occurring \(\beta\)-methoxyacrylates. In this method, limits of quantification as low as 119 \(\mu\)g/l in grape juice and 45 \(\mu\)g/kg in potatoes and grapes were found using deposition potential and deposition time values of \(-300\) mV and 30 s, respectively [23]. Differential pulse stripping voltammetry at an HMDE was also used for the simultaneous determination of tetracycline antibiotics in spiked animal feed and fresh fish muscle dosed with the drugs. The voltammograms from the drug mixture produced complex, overlapping profiles, and chemometrics methods were applied for calibration modeling. The analytical linear ranges were within 0.02–0.18 \(\mu\)g/ml and the corresponding limit of detections (LODs) were within 3–5 \(\mu\)g/l [24].

However, metal traces in foods were also determined by stripping analysis. For example, in a recent method, the sequential voltammetric determination of Hg(II) and Cu(II) at a gold electrode, and of Cu(II), Pb(II), Cd(II), Zn(II) at an HMDE by SW anodic stripping voltammetry in matrices involved in food chain as whole meal, wheat, and maize meal, was proposed. The supporting electrolyte was 0.01 mol/l EDTA-Na\(_2\) + 0.06 mol/l NaCl + 2.0 mol/l HClO\(_4\), and the analytical procedure was validated by the analysis of standard reference materials [25].
1.2.3 Potentiometry and Chronopotentiometry

Potentiometric detection using ion selective electrodes (ISEs) has had wide application in the field of food analysis since long time ago due to the fair selectivity, wide linear dynamic range, low cost, and automation ability of the derived methods [26]. Advances ISEs, developed in the last years, have also found application in this area. For example, solid-contact ion-selective platforms based on GCEs coated with electropolymerized polyaniline (PANI) and tetrasubstituted thiacalix[4]arene ionophores were reported for the discrimination of the brands of apple juices and herbal liqueurs. The samples were diluted and spiked with Fe^{3+}, and the variation of the signal from this ion, which is related to its reactivity with the organic ligands, was monitored. The method was also applied to the determination of antioxidants (ascorbic, malic, oxalic acids, hydroquinone, and quercetin) in the range from $5.0 \times 10^{-6}$ to $1.0 \times 10^{-2}$ M [27]. A potentiometric fumarate (FUM) ion selective electrode (Pt/Hg/Hg^{2+} FUM/graphite) has been recently developed for the determination of the acidulant additive fumaric acid in powdered foods such as gelatin, instant pudding, and ice cream. The achieved sensitivity was $(-29.2 \pm 0.6)$ mV/decade over a concentration range between $7.5 \times 10^{-7}$ and $1.0 \times 10^{-2}$ M [26]. A graphite carbon electrode coupled with a flow system was also used for the potentiometric determination of citrate in fruit juices and an isotonic drink. The fundamentals of this procedure involve the ion-exchange adsorption process of citrate on the electrode surface and the subsequent potential change. The electrode exhibits a linear response, with a slope of $-29.0 \pm 1.0$ mV/decade, in a $0.07-7.0$ mM concentration range with an LOD of $3.0 \mu$M [28].

Chronopotentiometric analytical methods have also found application in the field of food analysis. In this technique, the oxidation or reduction of species at a constant current is carried out, and the transition time is measured as the quantitative characteristic [29]. In this context, recent chronopotentiometric methods have been reported for histamine determination in foodstuffs. One of these methods was based on the oxidation of the amine at a planar gold disc electrode in the presence of electrogenerated chlorine which facilitates charge transfer between the analyte and the electrode surface. Well-defined signals were observed at $+1.15$ V in hydrochloric acid medium, giving rise to a linear calibration plot in the $2-100$ mg/l concentration range with an LOD of $0.27$ mg/l histamine. The method was applied to the determination of histamine in fermented sausages [30]. The same authors used a mercury film electrode to develop a chronopotentiometric method for histamine in cheese [29].

1.2.4 Electronic Tongues

Electronic tongues are multisensor systems with marked mix-response, capable of giving a wide and complete response toward the analyzed species [31]. Advanced mathematical procedures for signal processing based on pattern recognition and/or multivariate analysis, able to extract meaningful data from the complex readings, are usually needed in their applications [32]. Electronic tongues involving arrays of electrodes suitable for voltammetric experiments have been applied in food quality studies, namely in wines [33], milk [34], and fruit juices [35]. An illustrative recent example is the development of an electronic tongue that combined non-noble metals (Ni, Co, and Cu) and noble metals (Au, Pt, Rh, Ir, and Ag) for the determination of chloride, nitrate, and nitrite in minced meat. A rational
design of the waveform used by the electronic tongue and multivariate analysis including cross validation and partial least square (PLS) to build suitable management and prediction models for the analysis were reported [36]. Another electronic tongue has been developed for the simultaneous determination of the ethanol acetaldehyde, diacetyl, lactic acid, acetic acid, and citric acid content in probiotic fermented milk. The sensor array comprised of seven nonspecific, cross-sensitive sensors coupled with a reference Ag/AgCl electrode. Samples of plain, strawberry, apple-pear, and forest-fruit flavored probiotic fermented milk were analyzed and the results were used for the development of neural network models for rapid estimation of the aroma compounds content in probiotic fermented milk [37].

Monitoring of biotechnological processes, including fermentation, by determination of physicochemical parameters allows the suitable control of the process ongoing including the detection of possible relevant perturbations. A sensor array composed of potentiometric and voltammetric sensors (Figure 1.4a) was proposed as an efficient tool to control the production process of beer. The sensor array consisted of 10 miniaturized ion-selective electrodes and silicon based three-electrode voltammetric transducers. The obtained results were processed using PLSs and PLS-DA (partial least squares-discriminant analysis). The samples originated from batch of homemade beer fermentation and from two stages of the process: fermentation reaction and maturation of beer [38]. Also, a bioelectronic tongue has been constructed for the estimation of the polyphenol content in wine. The approach involved an array of four voltammetric enzyme biosensors (Figure 1.4b) using epoxy–graphite composites and a chemometric processing tool, which is able to interpret the chemical signals and extract meaningful data from the complex readings. One blank