Recent advances in the application of capillary electromigration methods for food analysis and Foodomics

Virginia García-Cañas, Carolina Simó, María Castro-Puyana,
Alejandro Cifuentes*

Laboratory of Foodomics, Institute of Food Science Research (CIAL), CSIC,
Nicolas Cabrera 9, Campus de Cantoblanco, 28049 Madrid, Spain.

Abbreviations: ABA, abscisic acid; AD, amperometric detection; BPs, bromophenols; BZDs, benzimidazole compounds; CEIA, CE-based immunoassay; CIP, ciprofloxacin; CSEI, cation-selective exhaustive injection; EDCs, disrupting compounds; ENR, enrofloxacin; FASS, field-amplified sample stacking; GMO, genetically modified organism, HVF, hydrogenated vegetable fat; LLE, liquid-liquid extraction; MRLs, maximum residue limits; MSPD, matrix solid-phase dispersion; MSPE, magnetic solid phase extraction; mtDNA, mitochondrial DNA; NSM, normal stacking mode; PCA, principal component analysis; PDMS, polydimethylsiloxane, PHU, phenylurea herbicides; PSP, paralytic shellfish poisoning; qc-PCR, quantitative competitive PCR; QuEChERS, quick, easy, cheap, effective, rugged, and safe; TBP, tubulin-based polymorphism; TCs, tetracyclins; TFA, trans fatty acids; W/O, water-in-oil.


Keywords: capillary electrophoresis, CE, food analysis, Foodomics.

Total number of words including figure and table legends: 18130

*Corresponding author: a.cifuentes@csic.es
Abstract

In this work, the analysis of foods and food components using capillary electromigration methods is reviewed. The present work presents and discusses the main CE applications performed in Food Science and Technology including the new field of Foodomics, reviewing recent results on food quality and safety, nutritional value, storage, bioactivity, as well as applications of CE for monitoring food interactions and food processing. The CE analysis of a large variety of food related molecules with different chemical properties, including amino acids, peptides, proteins, phenolic compounds, carbohydrates, DNA fragments, vitamins, toxins, pesticides, additives and other minor compounds is described. The use of microchips, CE-MS and chiral-CE in food analysis is also discussed as well as other current and foreseen trends in this area of research. Following the previous review by Castro-Puyana et al. (Electrophoresis, 2012, 33, 147–167), the current review covers the papers that were published from February 2011 to February 2013.
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1. Introduction

For years, the production of safe foods with the desired quality has been a major goal for the food industry. This implies that robust, efficient, sensitive and cost-effective analytical methodologies must be available to verify compliance with food regulations in order to guarantee the safety, quality and traceability of foods. Moreover, the new field of Foodomics is rapidly emerging [1-2], this new discipline entails a shift from more conventional methods towards the application of advanced omics techniques to investigate topics that were considered unapproachable in food science and nutrition few years ago. Among the analytical separation techniques commonly used for food analysis and Foodomics, CE can provide versatility as well as fast, efficient and automated separations with small sample volumes and low consumption of solvents. The present review focuses on recent advances and applications of CE to the analysis of compounds of relevance to food science and Foodomics, covering the literature published from February 2011 to February 2013, following the previous review by Castro-Puyana et al. [3]. It is noteworthy to mention the interest in the application of CE to the food analysis and Foodomics research fields, reflected by the high number of review articles published on these topics during the period covered by this review. Table 1 summarizes these review papers recently published on the state of the art of capillary electromigration techniques with regard to new developments and applications in food analysis and Foodomics. As can be seen, different CE separation modes [7,21], detection schemes [17, 23], concentration procedures [22], and miniaturized CE systems [12-14] have been recently reviewed. The application of CE to the analysis of contaminants [16, 20], chiral compounds [19], flavonoids [4], amino acids [21] in different food products has been also reviewed. Also, in the mentioned period several reviews have been published focusing on the application of CE to the analysis of transgenic foods [9], nutraceuticals [8], wine [15], natural products [18], as well as other food issues including authentication and traceability of foods from animal origin [10]. Besides, new results from the application of CE in the field of Foodomics have been also recently reviewed [3,11-13].
The following sections of this review describe the different CE approaches used to detect compounds of relevance to food science and technology including the analysis of amino acids; biogenic, heterocyclic and other hazardous amines; peptides and proteins; phenols, polyphenols and pigments; carbohydrates, DNA; vitamins; small organic and inorganic ions; toxins, contaminants, pesticides and residues; chiral compounds; and also important compounds to investigate food interactions and food processing. An overview of the last developments and applications of microchip CE to food analysis is provided, as well as the recent results obtained by CE-MS in Foodomics applications.

2. Amino acids, biogenic amines and other hazardous amines

CE is a very attractive analytical technique for the separation of amino-compounds in complex matrices as foods due to its high separation efficiency. Latest developments concerning CE methods for the analysis of amino acids (from 2008 to 2011) have been described by Poinsot et al., in 2012 [27]. Since most amino acids and biogenic amines do not have chromophores their UV or fluorescence detection is not possible. A labeling step is then usually needed when UV or fluorescence detection wants to be selected. The majority of the published works are based on off-line derivatization prior to separation. By using LIF detection an important sensitivity enhancement can be achieved at the expenses of time-consuming procedures for sample preparation. As shown below, several CE-LIF approaches have been developed for the analysis of amino acids and biogenic amines in food matrices. Thus, a new CE-LIF method was developed for the analysis of a group of nine amino acids (alanine, arginine, aspartic acid, cysteine, glutamic acid, lysine, methionine, proline and tryptophan) plus serotinine [28]. For a fast derivatization, a microwave-assisted protocol using 5-(4,6-dichlorotriazinyl)aminofluorescein as fluorescent dye was developed. LODs were in the 7-50 ng/mL range, and the method was applied to the analysis of Italian wines for the detection of wine adulterations and commercial frauds. CE-LIF was also employed for the analysis of a group of three amino acids (arginine, valine and tryptophan) together with other three vitamins, namely, riboflavin, folic acid, and niacinamide using FITC as derivatization
agent (with the exception of riboflavin, which was analyzed underivatized) [29]. The applicability of the proposed CE-LIF method was shown by the detection of these health promoting compounds in beverages. When coupled to MS, CE offers further advantages in terms of selectivity and sensitivity increase for amino acid analysis in complex samples. Some developed methodologies have been used to determine non-protein amino acids as indicators of food quality and safety. CE-MS enabled the simultaneous determination of six nonprotein amino acids (ornithine, β-alanine, gamma-aminobutyric acid, alloisoleucine, citrulline and pyroglutamic acid) previously derivatized with butanol, with LODs between 0.04 and 0.19 ng/g. The method was successfully applied to the detection of olive oil adulterations with soybean oil [30].

Many contaminants can occur as a result of a transformation process of the original contaminant entered in the food. This is sometimes the case of biogenic amines. As a result of raw materials contamination and inappropriate processing and storage conditions, toxicological risks associated to the formation of biogenic amines may occur. Intake of foods containing high concentrations of biogenic amines may represent a health hazard as a result of the direct toxic effects of these compounds. As with amino acid analysis, the detection of biogenic amines is rather difficult due to their lack of chromophore groups. For this reason a derivatization procedure is usually carried out to obtain good sensitivity. Derivatization approaches for the chromatographic and electrophoretic analysis of biogenic amines in wines has been recently revised [31]. Moreover, the analysis of these compounds in foods as food quality markers by capillary electromigration techniques is gaining great interest. A MEKC-LIF method using the non-ionic surfactant Brij 35 was developed for the analysis of seven FITC-derivatized biogenic amines (phenylethylamine, tyramine, cadaverine, histamine, tryptamine, spermidine and putrescine) in less than 9 min [32]. Very good detection limits were obtained (0.42-1.26 nM), and the method was applied to red wine samples and fruit molasses analysis. It was observed that biogenic amine type and amount were dependent on the type of grapes and hygienic conditions during the wine making process. Recently, a
capillary ITP-CZE-UV method was proposed for routine control of three underivatized biogenic amines (namely, histamine, 2-phenylethylamine and tyramine) in red wine samples as quality marker [33]. The evaluated LODs of the method were 0.33-0.37 mg/L, and after analysis of several commercial Czech red wines, determined concentration of histamine and tyramine were 2.0-7.0 mg/L and 1.5-2.5 mg/L, respectively. 2-Phenylethylamine was not found in the selected red wine samples. Meat freshness can also be evaluated by considering biogenic amine content. For this purpose, ITP with conductivity detection was the selected analytical platform for the determination of underivatized putrescine, cadaverine, histamine, tyramine, spermidine and spermine in meat samples (pork, beef and poultry) subjected to a different storage times. LODs of the proposed method were between 0.26 and 2.3 mg/L [34]. Among analyzed biogenic amines, putrescine and cadaverine were found to be good indicators of freshness because their levels rapidly increased during the process of meat aging. Better LODs (10-42 µg/L) were obtained in the analysis of seven unlabeled biogenic amines (spermine, spermidine, histamine, cadaverine, β-phenylethylamine, tyramine and tryptamine) by using CZE with AD [35]. Several additives were tested to improve biogenic amine separation. Among all tested additives, 18-crown-6-ether provided the best results in terms of separation. The method was applied to the analysis of biogenic amines in tap water, as well as in other environmental samples (lake water, pond water and sewage water).

Since 2008, when melamine was illicitly added to milk powder as a forgery of high protein content in order to mask milk dilution with water, a variety of analytical methodologies have been developed for the analysis of this compound in food matrices. Melamine may be found in food from illegal direct addition or from the animal feed carried over into the food. It has been described that cypromazine, a triazine derivative pesticide, can metabolize via dealkylation reactions and undergo environmental degradation to form melamine. It has been suggested that the occurrence of melamine in certain foods have not yet been distinguished between the intentional adulteration of food or feed and residues from the legitimate use of cypromazine. For this reason, Li at al. [36] developed a MEKC method for the simultaneous
analysis of trace levels of cyromazine and melamine in dairy products. The method was based on cation-selective exhaustive injection (CSEI) in combination with sweeping-MEKC-UV. More than 6000-fold enhancement in detection sensitivity for the two compounds was demonstrated when using CSEI-sweeping-MEKC instead of MEKC, with LODs equal to 43.7 and 23.4 pg/mL for cyromazine and melamine, respectively. Using a similar approach, analysis of melamine spiked in milk, gluten, chicken feed, and cookies, was analyzed by sweeping-MEKC [37]. In a recent study, melamine was analyzed by CE-UV [38]. The method development was mainly focused on the extraction of melamine from milk samples. The most widely used extraction methods, SPE and liquid-liquid extraction (LLE) were compared with ultrasonic-assisted extraction in terms of simplicity, cost and effectiveness of extraction. The method permitted the detection of melamine at levels as low as 0.163 mg/kg. Although melamine was not detected in the selected commercial products, the usefulness of the methodology was demonstrated in the analysis of milk and yogurt products with spiked melamine at three different levels (0.5, 1 and 2 mg/kg). Recently, a new CEC-MS method was proposed by Huang et al. [39] as an alternative to LC-MS, employing poly(divinyl benzene-alkene-vinylbenzyl trimethylammonium chloride) monoliths as stationary phase for the analysis of melamine and its three by-products (ammelene, ammelide, and cyanuric acid). With the use of mass spectrometer detection the LODs of the four compounds were reduced by three orders of magnitude regarding UV detection, down to 2.2-19.4 µg/L. The method was applied to the analysis of milk powder spiked with melamine and its related by-products. In another application, a CE-LIF method was developed to analyse six aliphatic amines (methylamine, ethylamine, n-propylamine, n-butylamine, n-pentylamine and n-hexylamine) labeled with MeCy5-OSu within 11 min [40]. The method was successfully applied to the analysis of environmental (lake water and sewage water) and food (wine) samples. Detection limits were between 0.6 and 2.0 nM.

3. Peptides and proteins
CE shows interesting features and advantages over other traditional separation techniques, although it has not been fully exploited in massive proteomic analyses yet. Several recent reviews have highlighted the last methodological development in the analysis of proteins and peptides by CE-based approaches [41-43]. Proteins and peptides play a key role in nutritional, functional and technological properties of foods. As an example, proteins have a decisive role in the conversion of animal muscle into meat. Protein degradation and protein oxidation have been identified as processes that modify proteins as well as the tenderness of meat [44]. Alterations in the sarcoplasmic protein fractions of beef strip subjected to treatments that influence meat tenderness (aging and hydrodynamic pressure processing) by different mechanisms was studied by both CE-UV and HPLC-UV [45]. It was demonstrated that postmortem changes in the soluble protein fraction of beef may be useful as potential indicators of meat tenderness. Although minimal effects were observed when meat was subjected to hydrodynamic pressure processing treatments, significant differences in sarcoplasmic proteins were observed after postmortem aging [45].

Several proteins in a variety of foods including peanut, milk, and egg, are interesting targets in food analysis, not only for food quality control but also for highlighting the presence of allergens. Detailed method description for the analysis of β-lactoglobulin in infant foods by CE-LIF using on-capillary derivatization, was recently described by Garrido-Medina et al. [46]. Using immunoaffinity CE, β-lactoglobulin and α-lactalbumin were immunocaptured inside the capillary (coated with hydroxypropylcellulose) through the use of magnetic beads functionalized with antibodies [47]. After elution from the beads, analyte focusing and separation were performed by transient ITP. Using an automated fraction collector interface, separated proteins were collected in a MALDI plate and subsequently analyzed by TOF MS. In a different work, quantitative analysis of lysozyme, conalbumin and ovalbumin in hen egg white, and of β-lactoglobulin A, β-lactoglobulin B and α-lactalbumin in defatted milk powder were carried out by CE-UV [48]. In this case, in order to avoid protein adsorption onto the
capillary wall during separation, a new polydopamine-\textit{graft}-PEG copolymer coating proved to be very effective. Recently, phosphoproteomic analysis of food milk was carried out combining the capabilities of CE and MS in an on-line configuration \cite{49}. To achieve in-line preconcentration, transient ITP mode was used and ultra-low flow (below 10 nL/min) ESI for MS detection was also investigated. The developed method was used for the identification of phosphopeptides in a skimmed milk digest sample and results were compared to those obtained with conventional nano-LC-MS method. Although highly phosphorylated peptides (with ultra-low pI and negatively charged even at very low pH) migrate toward the inlet of the capillary rather than toward the MS with subsequent lack of information, overall, CE-MS method was superior to nano-LC-MS, which was only able to identify monophosphorylated peptides.

In order to prevent microbial contamination of foods, several strategies have been developed to eliminate foodborne pathogens, among them, γ-irradiation is one of the most effective processes. CE-UV demonstrated to be a very useful methodology to separate proteins from two foodborne pathogens, \textit{Listeria monocytogenes} and \textit{Staphylococcus aureus}, previously treated at different irradiation doses \cite{50}. Several proteins were found to be related to the resistance or sensitivity of food pathogens to γ-irradiation. While in \textit{Listeria monocytogenes}, two proteins (70.2 and 85.4 kDa) were significantly changed (P≤0.05) at different doses of irradiation, in \textit{Staphylococcus aureus}, one ribosomal protein (16.3 kDa) was significantly decreased at a low dose of irradiation and a transcriptional regulator CtsR protein (17.7 kDa) was increased significantly at all doses of irradiation treatment compared to control (non-irradiated).

In another application, Baxter et al. \cite{51} studied the effect of salinity on the pasting and textural properties of rice flour as well as on the protein content and composition of rice endosperm. CE-UV was used for the analysis of glutelin protein fraction (the major storage protein present in rice endosperm) in the different cultivars. It was observed that rice grown
under saline conditions had significantly lower yields but substantially higher protein content, mainly attributed to increases in the amount of glutelin fraction. Higher salinity also altered some technological properties of rice flours, and thus, higher pasting temperatures and lower peak and breakdown viscosities were observed under these conditions. Protein content of the rice flour had significant effect on these properties, however, the changes on physicochemical properties of rice was rather complex and may involve constituents other than proteins.

In many cases peptides are released during the industrial processing. During cheese ripening, for example, the selected coagulant is of great importance to the development of flavor and texture of the final product. Moreover, storage conditions may also affect to the proteolysis of cheese. In this sense, Masotti et al. [52] studied the proteolytic pattern by CE-UV of fresh goat milk cheese both at market (under different times, temperatures, light irradiation) and at the cheese factory (in cold rooms under darkness) storage conditions. For identification purposes, migration times of peptides from cheese were compared with those present in the patterns of in vitro enzymatic hydrolysates of the thermized goat milk. It was observed that the formation and the progressive increase in peptides originated from \( \alpha_{s1} \)-casein proteolysis could be used to monitor the proteolytic phenomena occurring during aging.

Some food-derived proteins and peptides can confer additional health benefits beyond nutrition [53]. Thus, the interest of food products with potential health benefits is rapidly increasing. Although a variety of food products are currently under study, main efforts have been focused on milk, cheese and other dairy products as sources of bioactive proteins and peptides. In this sense, CE-TOF MS was recently used for separation and identification of bioactive peptides in hypoallergenic infant milk formulas [54]. An example showing the typical peptide profiles from three different infant formulas is given in Figure 1 in which CE-MS allowed the identification of up to 38 bioactive peptides. A variety of biological activities were confirmed. Among them, a significant number of these peptides were reported as
inhibitors of angiotensin converting enzyme, and thus, with potential anti-hypertensive effects.

Profiling analysis of proteins has been also reviewed for the analysis of genetically modified crops [55]. CE-UV was employed for protein profiling in extracts from transgenic and non-transgenic variants of maize and soybeans [56]. Some protein changes were attributed to the genetic modification; however, it was indicated that larger number of samples might be needed to obtain statistically significant results. Following an alternative approach, CE-UV was applied to the analysis of enzymatic (tryptic) hydrolysates of water-soluble proteins from *Bacillus thuringiensis* (Bt)-transgenic and two native non-transgenic maize varieties [57]. Some significant qualitative and quantitative differences between the transgenic and the two native non-transgenic varieties were found. For further application of this methodology to the differentiation of maize species, the analysis of a larger number of plants grown under different conditions was mentioned to be necessary. Recently, Montealegre et al. [58] have demonstrated the good potential of using anionic carbosilane dendrimers as nanoadditives in EKC for profiling of soybean and olive seeds proteins. In a separate report, Montealegre et al have approached the analysis of olive proteins using CGE-UV [59]. Some of their results highlighted the usefulness of the method in combination with discriminant analysis to classify the samples according to their geographical origin.

4. Phenols, polyphenols, pigments and lipids.

The many health-promoting properties (e.g., antioxidant, antibacterial, anti-inflammatory, antiallergic, antithrombotic, etc) related with the presence of polyphenolic compounds in foods, make the analysis of these compounds an interesting topic in food research and nutrition. Concerning the use of CE, different separation modes, preconcentration procedures, and detectors have been used to analyze polyphenols in a wide variety of samples in the period of time covered by this review [60-71].
A simple and rapid NACE method coupled to both UV and fluorescence detection has been proposed by Godoy-Caballero et al. [60] to characterize a group of phenolic acids representative of a variety of compounds encountered in extra virgin olive oil. Variables involved in the injection, separation and detection were optimized by response surface analysis. The hydrophobicity of the selected BGE (25 mM boric acid and 18 mM KOH in a mixture of 74:26 (v/v) 1-propanol:methanol) allowed its miscibility with the olive oil sample so that the characterization and determination of phenolics could be performed without any sample pretreatment. The quantification of the major compounds present in virgin extra olive oil (hydroxytyrosol, tyrosol, and vanillic acid) was carried out by the direct injection of the sample dissolved in 1-propanol, simplifying the analysis. Authors also developed a simple LLE method based on a relationship 5:1 (w/v) olive oil/ethanol to achieve the necessary preconcentration for the determination of phenolics compounds present at lower concentrations [61]. Response surface analysis along with central composite design and the Derringer-Suich multi-criteria method were also used in a different paper to optimize the simultaneous separation by CE-UV of thirteen phenolic compounds from extra virgin olive oil [62]. Phenolic compounds were extracted from olive oil using a LLE procedure previous to CE analysis. An aqueous BGE containing 50 mM boric acid at pH 10.2 and a voltage of 30 kV were pointed out by these multivariate statistical methodologies as the optimal experimental parameters to carry out the separation. Under these conditions, and using a preconcentration by sample stacking, which resulted in a 6-fold increase in peak areas, the polyphenols were determined in 12 min with LODs between 0.05 to 0.22 mg/L.

The determination of polyphenols for quality control and assessment of wines is considered a priority because of their effects on health and taste. CE-UV has been employed for the simultaneous determination of twenty polyphenols in forty-nine Spanish wines [63]. Principal component analysis (PCA) of the polyphenols profile and the peaks areas of the most abundant compounds enabled to determine the significant features contributing to wine discrimination according to the geographical areas of production. Polyphenols such as tyrosol,
gallic, protocatechuic, ρ-coumaric, and caffeic acids were identified as the most representative discriminant compounds. On the other hand, CE-AD has been also used to analyze polyphenols in white wines [64]. In this work, AD was carried out using carbon nanotube-modified electrodes which provided not only electrocatalytic properties but also enhanced signal stability and increased resistance to passivation. LODs between 2.3 and 3.2 μM for caffeic, chlorogenic, ferulic and gallic acid and (+)-catechin were attained without any preconcentration step.

Different phenolic acids and flavonoids from Brassica vegetables have been also studied by CE. Lee et al. [65] developed a simple solid phase extraction and a rapid CZE-UV methodology for the isolation and separation of four phenolics acids, namely sinapic, ferulic, ρ-coumaric and caffeic acid. The four analytes were simultaneously separated in less than 7 min with LODs ranging from 1.1 to 2.3 mg/kg of vegetable. The developed CZE-UV method showed to be a viable alternative to HPLC-UV since the results obtained in the quantification of these phenolics acids by both techniques in broccoli, broccolini, Brussels sprouts, cabbage and cauliflower were in agreement. Further investigation of the same group has also shown the potential of the developed CZE-UV method for the separation and quantification of two key flavonoids (kaempferol and quercetin) found in broccoli [66]. Prior to separation by CE, it was necessary an on-line preconcentration by large volume sample stacking due to the low concentration of flavonoids in the sample. This stacking procedure enabled sensitive detection, obtaining LODs of 0.9 and 0.6 mg/kg of broccoli for kaempferol and quercetin, respectively.

CE coupled to MS was employed by Verardo et al. [67] to investigate the phenolic fraction of buckwheat. Nineteen different compounds including phenolic acids, procyanidins and galloylated propelargonidins were identified. The presence of swertiamacroside and 2-hydroxy-3-O-β-D-glucopyranosil-benzoic acid in buckwheat was confirmed while identified
for the first time, whereas 5,7,4’-trimethoxyflavan and dihydroxy-trimethoxyisoflavan were tentatively identified for the first time. Recently, Koyama et al. [68] have also investigated changes in the phenols content of buckwheat sprouts with the aim to determine the optimal growth period for accumulating the most abundant functional phenols. By CE-UV analysis it was found that germinated buckwheat soaked for 20 h contained 1.5 times more rutin than the amount measured before germination. Moreover, the use of HPLC and LC-MS revealed that in addition to rutin, the buckwheat sprouts contained phenols (isoorientin, orientin, isovitexin, vitexin) that were produced in the germination process.

Seven isoflavones, including aglycones and glucosides, have been separated and quantified for the first time by CZE-MS in soy drink samples [69]. The isoflavones were separated in CE as anions and MS detection was carried out in ESI positive ion mode. A programmed nebulizing gas pressure was applied along the analysis to provide high resolution to prevent drops in the current. Using this methodology, LODs for the analytes under study ranged from 0.52 to 3.2 μg/L. In a separate report, the potential of an “electronic tongue” multisensor system in identification of various tea samples and in quantitative analysis of separate tea polyphenols was demonstrated by Papieva et al. [70]. In this later work, the authors previously applied MEKC-UV to obtain the simultaneous separation of these compounds and next, data obtained by MEKC were used as a reference for calibration of the multisensor.

The coloring ability of saffron is due to the presence of crocins. They are different glycosides (monoglycosyl or diglycosylesters) of a C20 carotenoid aglycon crocetin. Besides crocins, the other two main bioactive metabolites are safranal, which is the main essential oil constituent, and picrocrocin, which is the main bitter constituent. A MEKC-UV method capable of quantifying the three types of constituents has been developed by Gonda et al. [71]. Prior to MEKC analysis, a simple extraction protocol based on the use of BGE (20 mM disodium phosphate, 5 mM sodium tetraborate, 100 mM SDS, pH 9.5) as extracting solvent was applied. The developed method enabled the quantification of picrocrocin, safranal, crocetin-
Di-(β-D-gentiobiosyl) ester and crocetin (β-D-glycosyl)-(β-D-gentiobiosyl) ester in less than 18 min with LODs ranging from 0.006 to 0.04 mg/ml. This methodology may be considered for routine screening and quality management of saffron samples.

Recently, glycerophospholipids content in vegetable oils have attracted much attention owing to their influence on the antioxidant capacity and their oxidative stability of oils. However, these compounds have scarcely been studied in vegetable foods by capillary electromigration methods. To this regard, a novel NACE-ESI-MS method has been recently developed for the analysis of olive fruit and oil samples [72]. The method enabled the identification of the main molecular species of each glycerophospholipid found in the olive samples analyzed.

5. Carbohydrates

Carbohydrates are one of the most important components of food and can be classified as sugars, oligosaccharides, and polysaccharides depending on their molecular weight. They are the main source of energy for almost all physiological functions and are involved in many biological processes. Naturally occurring carbohydrates are consumed as a part of a healthy diet, although sugars can be also added to foods during manufacturing. In the period of time covered by this review, several CE methodologies have been developed to overcome the main drawbacks for the determination of carbohydrates by CE, namely, the lack of both easily ionizable groups (due to their high pKa values) and chromophore groups which makes difficult their CE separation and UV detection.

An option to solve the lack of chromophores is the use of indirect UV detection. For instance, Rizelio et al. [73] developed a CE methodology for the determination of fructose, glucose and sucrose in seven multifloral honey samples. A satisfactory and rapid separation (less than 2 min) was achieved using a BGE comprised of 20 mM sorbic acid, 0.2 mM CTAB and 40 mM sodium hydroxide (pH 12.2). Under these conditions, the reported LODs for the three sugars were in the range from 22 and 29 mg/L. The determination of these carbohydrates enabled
describing the quality of honey since not only the fructose/glucose ratio may have an impact on the honey flavor but also the determination of sucrose content is useful to detect the adulteration of honey by the addition of syrups. Indirect UV detection was also employed to the rapid determination of the key components in the taste intensity of fruit and vegetable crops of great economic value such as tomato, pepper, muskmelon, winter squash, and orange [74]. Sugars such as fructose, glucose and sucrose along with organic acid (oxalate, malate, and citrate) and the amino acid glutamate were determined under optimal conditions which enable a large number of injections (200) without disturbances in the same capillary. The methodology developed in this work is recommended for routine analysis of production quality systems or plant breeding programs [74]. On the other hand, in a work by Meinhart et al. [75], MEKC with anionic surfactant and indirect UV detection was applied to the separation of thirteen carbohydrates in six different food matrixes. The combination of central composite design and empirical models for prediction allowed the optimization of six procedures which were successfully applied to the separation of carbohydrates in condensed milk, orange juice, rice brand, red wine, coffee and breakfast cereals.

Analysis of carbohydrates can be also performed using CE with direct UV detection. In these approaches, a chromophore group is introduced in the carbohydrate through derivatization process enabling its direct UV determination. One of the popular labels is the reagent 1-phenyl-3-methyl-5-pyrazolone which can react with reducing carbohydrates under mild conditions without the requirement of acids catalyst or causing desialylation and isomerization, and shows strong UV absorbance at 245 nm. Using this reagent, it was possible to develop a CE-direct UV methodology to the simultaneous analysis of compositional monosaccharides released from the polysaccharides from herbal Lycipus lucidus Turecz. and Jujube as well as the analysis of monosaccharides and disaccharides in beer and milk [76]. This strategy has been also applied by Taga et al. [77] to analyze reducing carbohydrates and fructosyl saccharides in maple syrup and maple sugar.
In order to avoid time-consuming and expensive derivatization steps for carbohydrate labeling or the indirect UV detection mode which is poor in sensitivity, Sarazin et al. [78] proposed an alternative strategy to analyze neutral carbohydrates (fructose, glucose, lactose and sucrose) in beverages (red wine and apple juice), forensic and pharmaceutical samples. The strategy is based on the use of a BGE composed of 98 mM sodium hydroxide and 120 mM sodium chloride and direct UV detection via the formation of an absorbing intermediate (malonaldehyde or related compounds) by photo-oxidation in the detection window. To achieve this, the authors previously carried out the optimization of the CE separation using a design of experiments based on resolutions between adjacent peaks and analysis time of the four carbohydrates of interest and five potentially interfering carbohydrates (ribose, xylose, maltose, mannose, and galactose) [79], and investigated in-depth the photo-oxidation reaction of carbohydrates in the detection window under high alkaline conditions [80].

C4D has been also used as detection mode for the analysis of carbohydrates by CE [81,82]. For instance, the quantitative and qualitative analysis of saccharides in drinks and foodstuffs is important for checking the foodstuff energy values, the control of their production procedures, the time of their storage, and possibly also for disclosing illegal additions. Thus, Tuma et al. [81], developed a CE-C4D method for monitoring low-molecular saccharides (glucose, fructose, galactose, mannose, ribose, sucrose and lactose) in fruit juices, cola drinks, milk, red and white wines, yoghurts, honey and a foodstuff additive. Under the optimal experimental conditions (75 mM sodium hydroxide at pH 12.8) the separation of the studied carbohydrates was obtained in less than 3 min with LODs lower than 1 μM. In a work by Vochyanova et al. [82], an in-house designed apparatus based on capillary with an effective length of 4 cm in combination with C4D detection was successfully applied for the rapid (less than 1 min) determination of sucrose, glucose and fructose in high-energy drinks. These saccharides were separated in the anionic form, in solutions of alkali hydroxides, and their determined contents were identical with the declared values within the reliability interval in most cases (RSD less than 2%).
6. DNAs

The breakthrough of emerging analytical approaches such as next-generation sequencing technologies has opened a new era in the analysis of DNA. Over the last years, several generations of instruments have been developed with improved capabilities for DNA sequencing at costs that were unconceivable few years ago. However, while these technologies keep continuously evolving, numerous DNA analytical methods, including genotyping, detection of amplification products, sequencing PCR fragments, and oligonucleotide analysis, among other applications, are still performed by CGE. Although with lower throughput capability than the aforementioned new technologies, CGE is also well suited for the simultaneous analysis of multiple DNA sequences due to its high resolving power, short analysis time, and simplicity. Last developments in CGE include novel sieving matrices [83], novel capillary coatings [84] and additives [85,86] to provide high resolution and miniaturization into microchips [87-89]. A recent example of these innovative solutions in DNA separations is represented by the employ of the nonhazardous DNA staining reagent GelGreen in CGE-LIF [90]. Using this dye, detection of DNA fragments exhibited twofold improved sensitivity at lower costs in comparison with the conventionally used YOPRO-1 dye.

Detection of genetically modified organisms (GMOs), food-borne pathogens, and authenticity testing are the prevalent CGE applications for DNA analysis in foods. In the case of GMOs, the enforcement of new regulations implies that analytical tools must be available to verify their compliance in terms of control for non-authorized GMOs; and also, for labeling and traceability of approved GMOs. In this regard, there is a need for suitable strategies that enable rapid detection, identification and accurate quantification of the steadily increasing number of approved and unapproved GMOs that can be present in a food sample. In response to this demand, several CE-based approaches have been recently developed aimed at either increase as many as possible the number of GMOs that can be simultaneously detected in a
single analysis or improve the detection sensitivity. For instance, Holck and Padersen [91] have exploited the capability of the commercial sequencer ABI Prism® for the simultaneous detection of different fluorescent dyes in order to detect and quantitate five GM maize lines in samples. In that work, the multiplex quantitative competitive (qc)-PCR strategy combined with CGE-LIF was designed to amplify and detect target DNA sequences. As it has been shown in a previous report [92], CGE-LIF offered a great capability for the accurate and sensitive estimation of transgenic DNA fragments and its respective competitor DNA fragments in qc-PCR reactions. A common problem of GMO analysis in complex food samples is the presence of contaminants from the food matrix or from the chemicals used for DNA isolation can inhibit the PCR reactions. Taking into consideration the differences in type, composition, and degree of processing of foods, DNA extraction protocols must be developed and applied on a case-by-case basis. Also, the availability of sensitive techniques such as CGE-LIF for the analysis of amplified DNA has proven to be essential for the detection of recombinant DNA in samples with inhibitory substances and low quantity of DNA and/or degraded DNA. An illustrative example of this is the multiplex PCR-CGE-LIF based procedure developed by León et al. [93] for the simultaneous detection of recombinant yeasts in wine samples. In that study, a novel methodology, based on the use of polyvinylpolypyrrolidone, was developed for the extraction of PCR-quality yeast DNA from wine samples. CGE-LIF analysis of the amplified DNA extracts from wine demonstrated high sensitivity, good analysis speed and impressive resolution of DNA fragments, making this technique very convenient to optimize multiplex PCR parameters and to analyze the amplified DNA fragments. Recently, Jiang et al. [94] used CGE with UV detection to investigate novel chitosan functionalized magnetic particles as a rapid and simple alternative to conventional methods for the genomic DNA extraction from soybean seed samples. The extracted DNA was directly used as the template for PCR, followed by CGE-UV analysis using a phosphate-based buffer containing 1% (w/v) hydroxypropylmethylcellulose as sieving matrix. The electrophoretic analysis of amplified transgenic DNA fragments demonstrated the suitability of the procedure for providing DNA extracts ready for further amplification.
The CGE approaches developed for the detection of DNA markers in food authenticity testing applications have been recently reviewed in a report [10]. In the case of dairy products, certain mitochondrial DNA (mtDNA) regions have been used for discrimination among closely related milk-producing species. Gonçalves et al. [95] have developed a method for the simultaneous detection of cow, sheep, goat, and buffalo based on single multiplex PCR targeting short species-specific mtDNA sequences, followed by CGE analysis. Electrophoretic analyses were performed using two commercial sequencers to analyze PCR products generated with and without fluorescent primers, respectively. The method showed a sensitivity of at least 1% (v/v) milk mixtures in both electrophoretic methods for fragment size detection. In another report, Hernández-Chávez et al. [96] adapted the CGE-LIF method developed by García-Cañas et al. [97] with the aim to detect amplified mitochondrial DNA markers for the detection of chicken, turkey and pork in heat treated meat mixtures. The good applicability of the method was demonstrated by the detection of 1% turkey meat in meat samples. Authenticity testing of plant species is also relevant topic in food analysis. In last years, the tubulin-based polymorphism (TBP) has gained attention due to its simplicity, versatility and low cost. The conventional TBP method is based on the amplification of selective plant β-tubulin introns by PCR and their subsequent analysis by PAGE and silver staining. Recently, Gavazzi et al. [98] have improved the sensitivity, resolution, and reproducibility of TBP analysis by replacing PAGE by CGE-LIF for the detection of wheat, maize, barley, soybean, rape, sunflower and alfalfa in feeds.

Foodborne disease, commonly referred to as food poisoning, occurs when food becomes contaminated with harmful species. Although chemical species such as pesticides, among others, can originate important health problems (see below); however, the vast majority of food poisonings are the direct result of microbiological hazards induced by bacteria, toxigenic molds, viruses, parasites, or microalgae. To this regard, there is much interest on the development of new methods allowing the rapid, sensitive and simultaneous detection of
food-borne pathogenic species. With that aim, Oh et al. [99] have developed a CE-SSCP method for the detection of ten food-borne pathogenic bacteria. In their study, authors investigated the suitability of three different regions of 16S rRNA for discriminant analysis of bacteria. DNA amplifications were performed using primer pairs where either the forward or reverse primer was fluorescently labeled. On the other hand, separations were performed in non-coated capillaries at 35 ºC and using a polymer matrix solution containing Pluronic F108 PEO-PPO-PEO triblock copolymer. Differences in the conformation of single-stranded DNA molecules provided signals with different migration times for each species under study, enabling the simultaneous detection of the ten species in the same sample. Using the same triblock copolymer system, Chung et al., [100] have also approached the simultaneous detection of ten food-borne pathogens by CE-SSCP technique. In this case, the multiplex ligation-dependent probe amplification technique was used for target DNA amplification. This analytical strategy was tested on inoculated milk samples, demonstrating high specificity in the detection of six food-borne pathogens (Figure 2).

The development of new typing methods for food spoiling microorganisms and food-borne pathogens is still a relevant research area in food microbiology. In this context, the analysis of amplification products by CGE-LIF is usually preferred due to the great advantages provided by this technique in terms of resolution and sensitivity, which can be translated in a high discriminatory power. The report by Vigentini et al. [101], focused on the genotyping of the wine spoilage yeast Dekkera/Brettanomyces bruxellensis, is a good example of the investigation on new markers to discriminate food spoilage strains by CGE-LIF. In that case, authors studied the discriminating power of introns 5’ splice site sequences using a commercial kit with the ABI Prism® sequencer. Based on a similar idea, the polymorphic tandem repeat regions of Listeria monocytogenes have been recently studied using the same CE instrument [102].

7. Vitamins
Analysis of foods as source of vitamins is of great relevance since, with exception of vitamin C, the human body cannot synthesize them. Lack of vitamins can cause different diseases even thought small concentrations are indispensable to maintain good health. During the last two years, new CE methodologies have been developed mainly to the simultaneous determination of water soluble (e.g., vitamins B) or insoluble (e.g., tocopherols) vitamins in different food matrices [104-107]. Besides, the determination of vitamin C by CZE in tomato samples has demonstrated to be a very useful way to teach students of undergraduate labs the usefulness of CE as analytical technique [108].

The simultaneous and quantitative analysis of the five B-group vitamins (thiamine hydrochloride (B1), riboflavin (B2), nicotinic acid (B3), pyridoxine hydrochloride (B6), and cyanocobalamin (B12)) and vitamin C (ascorbic acid) in corn flour and fortified corn flakes samples has been performed by a partially validated MEKC methodology [103]. The results obtained in that work, which were comparable to those obtained by HPLC-MS, demonstrated the broad range in vitamins composition within different varieties of corn flakes while they were in accordance with the stated values on the package.

The application of CE with LIF detection to analyze B vitamins has been rarely reported; however, its potential to the separation and determination of three essential amino acids (arginine, valine, and tryptophan) and three B vitamins (folic acid, niacinamide, and riboflavin) with simultaneous derivatization has been recently demonstrated [104]. FITC was used as the reagent for fluorescence labelling of the three amino acid, folic acid and niacinamide, whereas riboflavin was detected without derivatization. An optimum separation of underivatized riboflavin and the other five derivatized compounds was attained with 25 mM sodium borate buffer at pH 9.85 providing LODs ranging from 0.5 to 1.5 nM for the FITC derivatives and 2.5 \times 10^{-3} \text{nM} for riboflavin. The applicability of the developed method was shown by the trace analysis of the amino acids and vitamins in health drink samples.
On-line preconcentration techniques have been also used for the simultaneous determination of B vitamins. Dziomba et al. combined, for the first time, field amplified sample stacking (FASS) with sweeping to determine thiamine, nicotinamide, pyridoxine, riboflavin and folic acid by MEKC [105]. Compared with CZE, the methodology developed in this work, enabled to obtain an enhancement factor of about 40-fold in sensitivity. Under optimized conditions, the developed methodology was applied to the determination of these vitamins in bacterial growth media and *Ilex paraguariensis* leaves thus demonstrating its potential to monitor vitamins in industrial bioreactor and to estimate their content in plant.

Recently, Mu et al. [106] employed approaches such as experimental design and artificial neural networks to optimize CE separation of nicotinic acid and its amide derivative nicotinamide in food (russula alutacea, instant dry yeast, vitamins water and vitamin functional drinks). Using the statistical models, experimental variables, such as buffer concentration, voltage, and SDS concentration, were optimized to obtain good resolution and minimum analysis time. This CE method could be an environmental friendly alternative to HPLC for routine analysis of these compounds since is simple, rapid (less than 5 min) and less expensive and contaminant.

Tocopherols are water insoluble vitamins that are present in vegetable oils. These compounds are relevant to food science and nutrition due to their nutritional functions and ability to prevent the oxidation of vegetable oils. A novel NACE method for the determination of tocopherols in vegetable oil samples (maize germ, olive, and sunflower) has been recently reported [107]. On-line UV and fluorescence detection were employed, demonstrating the latter an improved sensitivity and selectivity. Besides, fluorescence detection allowed a simplified sample treatment. A comparison of the UV and fluorescence signal obtained for a sunflower oil sample (before and after been fortified) can be observed in Figure 3. Tocopherol signals were not detected in the UV electropherograms due to the low sensitivity
and to the overlapping with other signals from the matrix. However, by fluorescence the signals were free of interferences and enabled to achieve LODs lower than $\mu$g/mL [107].

8. Small organic and inorganic compounds

Analysis of small ions by CE is one of the most important applications of this technique also in food science. CE is a good alternative to other more established techniques for individual or multiple determination of small ions since it provides high efficiency, good repeatability, fast analysis, and low consumption of electrolytes and samples. Low molecular ions (organic and inorganic) are present extensively and naturally in many foods and play important roles in several physiological activities. CE has demonstrated its potential for the analysis of this type of compounds through the determination of sodium in milk and milk products [109]: using a background electrolyte consisting of 10 mM imidazole adjusted to pH 3.75 by the addition of oxalic acid allowed baseline separation of Na from other milk cations and from Li ion, which was adopted as an internal standard [109]. The potential of carboxylic group functionalized magnetic nanoparticles was investigated by Carpio et al. [110] to preconcentrate metals from juices before CE analysis. Under the optimized conditions, detection limits for Co, Zn, Cu, Ni, and Cd were 0.004, 0.003, 0.004, 0.008, and 0.009 mg/L, respectively [110]. CE has also given impressive results for the analysis of organic and inorganic anions [111]. A procedure for the CE determination of lactic, malic, tartaric, and citric acids in fruits, juices, nectars, wines and beer was developed using 3-nitrobenzoic acid as a light-absorbing component of the running buffer and indirect photometric detection [112]. CE was also used to determine the concentrations of nitrate, nitrite and oxalate in two vegetables which are popular at Black Sea coast, namely kale (*B. oleracea* var. acephala) and sultana pea (*Pisum sativum* var. saccharatum) [113]. The results were compared with the concentrations in spinach and chard. The average amount of nitrate in fresh kale leaves (2016 +/- 519 mg/kg) was greater than that in spinach and lower than that in chard. The nitrite ion concentrations were much more in the leaves of kale (111 +/- 4 mg/kg) than in stalks on the contrary of chard. Oxalate concentration was lower in kale (2970 +/- 672 mg/kg) than in spinach and chard [114]. An environmentally
friendly, simple and sensitive isotachophoretic method was developed to identify and quantify orthophosphates, pyrophosphates, tripolyphosphates, nitrites and nitrates in various food products [114]. The mentioned ions were analyzed in meat (different canned products, smoked, cooked and long-matured pork ham, headcheese) and seafood (raw and cocktail prawns, squids and different mixes of seafood) products [114].

CE has been also applied to analyse small ions in nutritional supplements and energy drinks. Thus, a simple CE method was developed to determine glucosamine in nutritional supplements using in-capillary derivatisation with o-phthalaldehyde [115]. No significant bias was observed ($r^2 = 0.989, p < 0.01$), between results obtained by the proposed CE method and the official colorimetric method to determine glucosamine [115]. Caffeine has been determined in energy drinks by microemulsion electrokinetic chromatography (MEEKC) with diode array detection [116] and quinine in beverages by on-line coupling ITP to capillary zone electrophoresis with UV spectrophotometric detection [117].

CE-MS has also found interesting applications to analyze small compounds in food science. A CE-MS/MS methodology enabling the simultaneous determination of betaines (glycine betaine, trigonelline, proline betaine and total content of carnitines) was also developed and applied to determine these compounds in vegetable oils [118]. The method, with LODs and LOQs at 0.1 ppb level, was applied for the determination of the selected betaines in seed oils and extra virgin olive oils. In extra virgin olive oils, carnitines were not detected, proposing them as a feasible novel marker for the detection of adulterations of olive oils.

CE methods have been also developed for the analysis of flavor compounds in foods. As an example, bromophenols have been identified as key off-flavor compounds found in seafood. A new CE method was established for simultaneous assay of five bromophenols (BPs), 4-BP, 2,4,6-triBP, 2,4-diBP, 2-BP and 2,6-diBP, in seafood (Trachypenaeus curvirostris and Lepidotrigla microptera). The contents of 4-BP, 2,4,6-TriBP, 2,4-DiBP and 2,6-DiBP in $T$. 

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were 3.9, 2.5, 7.3 and 0.6 ng/g, respectively. The feasibility of this method for the
determination of bromophenols in freshwater fish and crustaceans (*Macrobrachium*
*nipponense* and *Carassius auratus* var. Pengzesi) was also tested. The results indicated that
the contents of these flavor compounds in freshwater fish and crustaceans were lower than the
detection limits mentioned above [119]. A strongly aromatic compound, called sotolon, was
determined by CE without specific sample pre-treatment. The limit of quantitation and limit
of detection values were 3.13 ppm (S/N = 9) and 0.781 ppm (S/N = 3), respectively. Using
this system, sotolon was clearly detected from a maple-flavored food additive [120].

CE and miniaturized CE-AD methods have been employed to the determination of aldehydes,
including formaldehyde, acetaldehyde, propanal, butanal, pentanal, hexanal, glutaradehyde,
2,3-butanedione and methylgloxal in different food samples such as wines, oils, and water-
soaked products [121,122]. After being derivatized with an electroactive compound, 2-
thiobarbituric acid, these nine non-electroactive aldehydes were converted to electroactive
adducts, and therefore detectable by CE-AD approach. CE with on-line preconcentration and
UV detection has been also used for the determination of aldehydes (namely, sinapaldehyde,
syringaldehyde, coniferaldehyde, and vanillin) for the authenticity control of whiskey samples
[123]. The main objective was to obtain a screening method to differentiate authentic samples
from seized samples suspected of being false using the phenolic aldehydes as chemical
markers. The CE reliability in the aldehyde analysis in the real sample was compared
statistically with LC-MS/MS methodology, and no significant differences were found, with a
95% confidence interval between the methodologies [123].

9. Toxins, contaminants, pesticides, and residues

Due to the hazardous effects of microbial toxins to human health, the occurrence of these
compounds in food represents a serious concern worldwide. As a safety measure, many
countries have established maximum acceptable levels of certain toxins in foods.
Furthermore, as new toxins are being identified as well as their potential risk to public health,
the imposition of regulatory limits for toxins in foods is likely to continue. Some of the main drawbacks of the existing in vivo methods for toxin analysis are that they cannot provide qualitative information concerning the nature of the toxin components in a complex mixture, tend to be less sensitive and precise when compared to instrumental analytical procedures, and are increasingly unacceptable as methods of regulatory evaluation owing to ethical considerations associated with the use of live animals. In vitro biological methods often feature excellent sensitivity but may be characterized by false-positive reactions and are only recently becoming available in commercial test kits appropriate to routine use. Accordingly, there is a demand for reliable methods that can provide appropriate analysis to enforce legislative limits at acceptable cost and time. In last years, a myriad of methods have been developed to detect a single compound or a group of related toxins. Among the instrumental methods, HPLC has been the most widely used to study toxins in complex mixtures. However, HPLC analysis of certain toxins can represent a significant challenge as in the case of paralytic shellfish poisoning (PSP) toxins, which have highly polar nature and lack of useful UV chromophores. Therefore, most of the available HPLC methods for the analysis of PSP toxins involve laborious sample preparation and require highly trained personnel for the interpretation of results. On the other hand, their ionizable nature makes PSP toxins perfect candidates for CE analysis as it has been demonstrated by different research groups. Recently, Zhang and Zhang [124] have developed a novel CE-based immunoassay (CEIA) method using electrochemical detection for the simultaneous detection of two PSP toxins, saxitoxin and decarbamoylsaxitoxin, in shellfish samples. Authors used a competitive model in which a labeled antigen was first mixed with different concentrations of non-labeled antigen (standard solution or sample extract) that competed for binding with the limited and fixed amount of antibody to form immunological complexes that are subsequently separated by CE. During method development stage, the influence of incubation time on immunocomplex formation, as well as the effect of BGE composition, pH, and electric field on the separation was studied. Under optimized conditions, separations were completed in 6 min and the method provided quantitative results comparable to those obtained with ELISA and the mouse bioassay. Based
on the same idea, the application of CEIA method has been recently expanded to the analysis of brevetoxin B, a neurotoxic shellfish poisoning toxin [125]. In this case, the developed CEIA method was also compared with a commercial ELISA kit, demonstrating that the former allowed sensitive and fast detection for the determination of trace (LOD 0.1 ng/mL) brevetoxin B in shellfish samples.

Mycotoxins have been also object of investigation by CE in recent years. For instance, the electrophoretic analysis of citrinin, a nephrotoxic and hepatotoxic mycotoxin produced by filamentous fungi of the genera Penicillium, Aspergillus and Monascus, has been attempted by Zhu et al. [126]. The CE method was used to evaluate the performance of an in-house prepared immunoaffinity column containing silica gel immobilised with anti-citrinin antibodies for selective extraction and enrichment of citrinin in contaminated food samples. In a separate report, a MEKC-UV method has been used for monitoring the production of ochratoxin A in different strains [127]. The detection of this ubiquitous mycotoxin by CE was the basis for validating the suitability of a novel molecular protocol, based on specific DNA amplification by polymerase chain reaction, for the detection of ochratoxigenic moulds in foods.

The extensive use of antibiotics in animals is attracting much attention since the persistence of drug-residues in food is increasing the risk of introducing resistant bacteria into the food chain, which potentially represents a serious health problem. In response to this concern, European Union has established rigid regulations that impose strict maximum residue limits (MRLs) for these substances in animal tissues and derived foodstuffs entering the human food chain. The enforcement of these regulations implies that suitable analytical tools must be available to verify their compliance. In recent years, much effort has been directed to the development of sensitive multiresidue methods that provide reliable determinations of several antibiotics in foodstuff of animal origin. In particular, sulfonamides constitute an important group of antibiotics with a wide range of therapeutic and prophylactic purposes, including the
treatment of microbial pathogens and for growth-promoting purposes in farm animals. Several CE methods have been recently applied to the determination of sulfonamides in food [128]. The recent application of CEC-MS to the analysis of 9 sulfonamide compounds (sulfadiazine, sulfamerazine, sulfapyridine, sulfamethazine, sulfisoxazole, sulfadimethoxine, sulfaquinoxaline, sulfamonomethoxine and sulfathiazole) in meat samples has shown the good possibilities of this technique for the analysis of residues in complex food matrices [129]. In that work, different poly(divinylbenzene-alkylmethacrylate) monolithic stationary phases, column-end roughness, and mobile phases were tested in order to achieve optimal sulfonamide separations and sensitivity. Particularly, the roughness of the monolithic column end cross-section was found to have a strong influence on the mass signal stability. Also, in order to counteract the diluting effect caused by the sheath liquid-assisted CE-ESI interface, authors investigated an on-line sample concentration procedure to enhance the sulfonamide sensitivities. That point was approached by using ammonium formate of pH 4 as sample matrix in order to stack anionic forms of sulfonamides on the border of the monolithic inlet end. With the proposed CEC-MS method, the LODs for sulfonamides ranged from 0.01 to 0.14 μg/L. The MEKC-UV separation of seven sulfonamides (sulfamethazine, sulfamerazine, sulfathiazole, sulfachloropyridazine, sulfamethoxazole, sulfacarbamide, and sulfaguanidine) and three amphenicol-type antibiotics (chloramphenicol, thiamphenicol, and florfenicol) in poultry tissue has been approached by Kowalski et al. [130]. SPE extraction with C18 cartridges was implemented as sample pretreatment procedure. The proposed micelle system consisted of methanol and a mixture of 15 mM sodium tetraborate decahydrate at pH 9.3 and 25 mM sodium dodecylsulfate (20:80, v/v) as the BGE. The selectivity of the method was successfully tested by the analysis of spiked turkey muscle, showing no interferences in MEKC-UV results at 200 nm (Figure 4). The validation study of the method demonstrated good linear response over the concentration ranges of 0.025 μg/g for sulfonamides and 0.005–1 μg/g for chloramphenicol. Also, the reported LOD values for sulfonamides and amphenicol-type antibiotics were lower than 7.8 and 5.7 ng/g, respectively.
Quinolones are a relevant group of antibiotics widely used in humans and in food-producing animals for treatment of several bacterial infections. The intensive use of these antimicrobials in animals represents a potential hazard since residues of quinolones may persist in animal-derived food products. The CE-UV analysis of the quinolone enrofloxacin (ENR) and its primary metabolite ciprofloxacin (CIP) in milk has been approached by Piñero et al. [131]. In their study, authors carried out the optimization of the separation conditions as well as the investigation of several strategies to extract quinolones and/or clean-up milk samples. The selected sample treatment involved clean-up/extraction procedure based on protein precipitation with hydrochloride acid followed by a defatting step with centrifugation and SPE using a hydrophilic-lipophilic balance cartridge. CE separations were performed at 25 °C and 25 kV using a BGE containing 50 mM phosphoric acid with a pH value of 8.4, providing the separation of both analytes in 6 min. LODs obtained for both quinolones were below the established MRLs and the precision of the method was acceptable, providing RSD values below 9.9% and 0.9% for peak area and migration time, respectively. An exhaustive validation of the analytical procedure indicated good suitability of the method for its application in routine identification and quantification of CIP and ENR. In a different report, Hermo et al. [132] performed a comparative study of different analytical techniques including CE-UV, LC-UV, LC-MS and LC-MS/MS for the multiresidue determination of a series of quinolones regulated by the European Union (marbofloxacin, ciprofloxacin, danofloxacin, enrofloxacin, sarafloxacin, difloxacin, oxolinic acid and flumequine) in bovine and porcine plasma. SPE was selected for clean-up and preconcentration of the analytes before their injection into each separation system. The effect of several parameters affecting the separation was systematically studied for each separation technique. In the case of CE-UV analysis, parameters such as capillary length and time of injection were optimized using pig plasma samples fortified at 10 mg/L of each quinolone. Also, the potential use of two different internal standards, namely piromidic acid and tosofloxacin, was evaluated for quantification by CE-UV. Owing to matrix effect, different slopes between calibration curves were obtained when cow or pig plasma was used. Although CE-UV demonstrated lower
sensitivity than LC-UV, the electrophoretic technique was satisfactorily applied to identify and quantify enrofloxacin in plasma samples from orally medicated animals, obtaining similar results to using LC-UV.

Tetracyclins (TCs) are broad-spectrum antibiotics that are commonly applied in veterinary practice. Although TCs are licensed for use in a variety of food-producing animals, including cattle, pig, poultry, and fish, the presence of TC antibiotic residues in foodstuffs has harmful effects on consumers’ health. Consequently, many countries have established MRLs for this type of antibiotics. Mu et al. [133] have optimized a novel methodology based on the combined use of matrix solid-phase dispersion (MSPD) and CZE-UV for the simultaneous determination of three TCs (tetracycline, oxytetracycline and doxycycline) in milk samples. MSPD procedure involved blending sorbent with the milk sample; defatting the mixture with hexane; and eluting TCs with 0.1 M citric acid aqueous solution/methanol (1:9). Among the different sorbents tested during the method development stage, better results in terms of recovery and clean-up performance were obtained using C18 than with fluorisil and silica gel sorbents. Under optimal CZE-UV conditions, TCs were detected in 5 min with good intra-day and inter day precision for the migration time (RSD < 0.13% and 0.98%, respectively). The method demonstrated good linearity in the range of 1-200 µg/mL, as well as good sensitivity, providing LOD values for TCs that were below 0.1 µg/mL. The applicability of the method was demonstrated by the analysis of commercial and spiked milk samples.

The analysis of penicillin residues in food samples by different chromatographic methods has received much attention in last years. A novel analytical procedure that combines on-line concentration method with water-in-oil (W/O) MEEKC has been developed for the simultaneous analysis of 6 penicillin antibiotics [134]. The on-line concentration method was based on normal stacking mode (NSM) using a basic running buffer. On the other hand, the optimal W/O microemulsion solution was composed of 5% SDS, 80% 1-butanol, and 15 % sodium acetate solution at pH 8. For NSM, the capillary was filled with the microemulsion
solution, and then a plug of 1-propanol was introduced into the capillary. The sample, dissolved in deionized water was injected hydrodynamically into the capillary, and subsequently, the separation voltage was applied with the microemulsion solution in the inlet vial. Using this procedure, the analytes stacked in the organic solvent zone because of the difference in solution viscosity, whereas the negatively charged water droplets also entered the stacked sample zone sweeping the analytes previous W/O MEEKC separation. A systematic study of the effect of some parameters, including pH of the solution plug, composition and length of the organic plug, and time of injection, on the sensitivity and resolution allowed the selection of the optimal separation conditions for the detection of the six antibiotics and an internal standard. A comparison of the NSM-W/O MEEKC method with other CE and LC methods reported in the literature suggested that the sensitivity of the developed was better than LC-UV and CE-UV, but poorer than the sensitivity obtained with LC-MS. The application of the NSM W/O MEEKC method to the analysis of spiked porcine liver and kidney samples indicated the suitability of the proposed method for detecting trace-levels of penicillins in complex food samples.

MRLs have been also established for antihelmintics drugs, more specifically for benzimidaloze compounds (BZDs). These synthetic antiparasitic agents, when improperly used, may be present in animal-derived food products, which represent a risk to develop several toxic effects in humans. LC-MS is the preferred technique for benzimidazole drugs detection; however, CE has demonstrated to be a rapid, inexpensive and green alternative. On the other side, the inferior sensitivity obtained by CE compared to LC has focused the attention to develop various electrophoresis-based preconcentration procedures to enhance the sensitivity of CE technique. Recently, magnetic solid phase extraction (MSPE) has been combined with FASS-CZE-DAD for the determination of ten BZDs in swine tissue samples [135]. Functionalized Fe₃O₄-SiO₂ magnetic particles with methacrylic acid-co-ethyleneglycol diamethacrylate were investigated for BZDs. Several parameters such as amount of adsorbent, pH, extraction time, desorption time, and desorption solvent were
optimized to obtain maximum extraction efficiency. In addition, FASS was used as on-line preconcentration technique. Specifically, sample enrichment was performed by electrokinetically injecting a sample with lower conductivity compared with the BGE. In this case, a sample solution of acetonitrile-trifluoroacetic acid (100:0.5, v/v) provided the best results. The analysis under optimized extraction and separation conditions of swine muscle and liver samples spiked with BZDs showed no interferences from the matrix. The sensitivity of the MSPE-FASS-CZE-DAD method provided LODs lower than 13 ng/g for BZDs spiked in swine samples, and intraday (n=4) and interday (n=9) precision for the whole procedure lower than 14 %RSDs. Domínguez-Álvarez et al. [136] have investigated the potential of CE-MS for the multiresidue determination of 10 BZDs in eggs. In that study, optimal separation and MS detection conditions were water:isopropanol (70:30, v/v) containing 6 M formic acid as BGE, a temperature of 25 ºC, a separation voltage of 22 kV, isopropylalcohol/water (50:50, v/v) as sheath liquid, and a cone voltage of 120 V. Once the optimal CE-MS conditions were selected, a comparative study of different sample preparation methods, including solvent extraction-based methods followed by a clean-up step using SPE with a novel polymeric sorbent, and a procedure based on QuEChERS (quick, easy, cheap, effective, rugged, and safe) method, for ten BZDs spiked in egg yolks, was carried out. Some steps in the latter method were varied in order to improve its performance. The whole optimized method (QuEChERS with preconcentration prior to CE-MS) was validated, demonstrating reliability and robustness to detect residues of these drugs in egg samples with LODs between 3 and 51 μg/L and recoveries in the 74-112% range.

CE presents a broad potential to separate a wide spectrum of small molecules, which is being exploited for the accurate determination of pesticides in complex matrices in food analysis. However, owing to its limited sensitivity and as in the analysis of other food contaminants, novel methods are focused on the development of preconcentration strategies. A recent example of this is represented in the work by Santalad et al. [137] that focused on the determination of 6 carbamate insecticides (methomyl, propoxur, carbofuran, carbaryl,
isoprocarb, and promecarb) in fruit samples with reversed electrode polarity stacking sample preconcentration combined with MEKC. The preconcentration procedure involved the injection of the sample into the capillary hydrodynamically and then, a change of -15 kV in the polarity was performed for 0.5 min that was subsequently reversed to +15 kV throughout the separation. The critical parameters were optimized in order to achieve the conditions for separation and preconcentration of the target insecticides. In the case of on-line preconcentration, sample solvent, sample injection volume, switching voltage, and reversal time were investigated. The preconcentration efficiency of the proposed procedure demonstrated stacking enhancement factors from four to 13-folds compared to the values obtained in normal MEKC. The method showed to be simple, rapid (8 min) and sensitive for the detection of the six target insecticides, providing LODs in the range of 0.01-0.1 mg/L which is acceptable with respect to their MRLs for the insecticides in raw fruit samples. In addition to the efficient preconcentration of the sample, development of innovative and sensitive separation methods is of great importance for the detection and quantification of pesticides. In this sense, competitive CE immunoassay with LIF detection (CEIA-LIF) was proposed by Liu et al. [138] as a promising technique to detect and quantify metolcarb in food samples like rice and cucumber. The application of MSPD extraction with CE has been also investigated to the analysis of green vegetable and rice samples for the detection of three phenylurea herbicides (PHUs), namely isoproturon, linuron and iduron [139]. In that work, separations were performed using 20 mM phosphate buffer containing 12 mg/ML poly-β-CD at pH 7.5 as BGE with a CE instrument coupled with an electrochemiluminescence detector. Initially, the optimum Pt electrode potential for the detection of the three herbicides was established in the detection system. Then, several variables affecting the separation were investigated using a standard mixture of the three PHUs. The results obtained from optimization experiments showed that the use of poly-β-CD was key to separate the three relatively apolar neutral analytes. In order to validate the feasibility of the procedure to analyze the three herbicides, the performance of the method was evaluated with spiked green
vegetable and rice samples. CE analysis provided electrophoregrams without interfering signals and LODs lower than 0.2 μg/L.

CE methods have been successfully applied to the determination of colchicine, an illegal drug in food-producing animals [140]. In that work, the detection of this alkaloid in milk samples was approached using three different capillary electromigration techniques, namely, aqueous CZE, NACE and MEKC. Also, on-column preconcentration using sweeping-MEKC enabled improving the sensitivity of the method up to 3 ng/mL. On the other hand, the buffer composition used for non-aqueous capillary electrophoretic method (1 mM HClO₄ in a mixture of methanol:acetonitrile (1:2, v/v) containing 60 mM ammonium formiate) was highly compatible with MS detection, opening new ways for the investigation of analytical solutions to colchicine determination in food samples.

There are other food contaminants whose determination has been also approached by CE. This is the case for the determination of some endocrine disrupting compounds (EDCs) in honey. The contamination of foodstuffs with these compounds could occur during food production process and also via the plastic packaging material. Owing to the anionic nature of these phenolic compounds, their detection by CE-ESI-MS in positive separation mode is challenging. Domínguez-Álvarez et al., [141] have developed a new CE-ESI-MS method using positive separation mode and a programmed nebulizing-gas pressure along the analysis. This approach provided high resolution of EDCs (2,4-dichlorophenol, 2,4,5-trichlorophenol, pentachlorophenol, bisphenol-A, 4 tert-butyl-phenol, and 4-tert-butyl benzoic acid) and stability of the MS signal throughout the analysis. The method was tested in extracts obtained from spiked honey samples by LLE. Following this procedure, the limits of detection were in the 1-4 ng/g range and RSD values in the 2-9% range demonstrating the good possibilities of the method for the analysis of EDCs in honey.

10. Food additives
Different compounds can be added to food products to enhance or modify their color, aroma, flavor, and/or taste, as well as their stability and to control their antibacterial and antioxidant activities. CE methods have extensively been applied to analyze these additives. Although most food colorants are expected to be safe, several food colorants have demonstrated some toxicity. As a consequence, many countries have established regulations for the use of natural and synthetic colorants in foods. Due to these regulatory restrictions, new methods are still demanded for rapid detection and identification of colorants in food products [142]. Following this trend, a new method based on partial filling MEKC for the quantitative determination of Sudan dyes (I, II, III, and IV) in chilli sauces was developed [143]. Under optimized conditions, the azo dyes are baseline separated in less than 8 min with limits of detection ranging from 0.57 to 0.71 μg/mL (S/N > 3). Using an internal standard, the repeatability of the quantitative determination is improved almost four times. The applicability of the method for rapid screening and determination of Sudan dyes was corroborated by analyzing spiked chilli sauce samples with recoveries from 85 to 99%. In a second work by the same group [144] the reported partial filling MEKC conditions were demonstrated to be compatible with mass spectrometry detection. The use of MEKC was essential to achieve the separation of the neutral Sudan dyes (I, II, III, and IV), while the partial filling technique was necessary to avoid the contamination of the ion source with non-volatile micelles. MEKC separation and MS detection conditions were optimized in order to achieve a fast, efficient, and sensitive separation of the four dyes. Filling 25% of the capillary with an MEKC solution containing 40 mM ammonium bicarbonate, 25 mM SDS, and 32.5% (v/v) acetonitrile, a baseline separation of the four azo-dyes was obtained in 10 min. Tandem MS improved the sensitivity and selectivity of the analysis. LODs were 5, 8, 15, and 29 times better were obtained for Sudan III, I, II, and IV, respectively, using partial filling MEKC-MS/MS instead of partial filling MEKC-MS. Under optimized conditions, LOD from 0.05 to 0.2 μg/mL were obtained. The suitability of the developed method was demonstrated through the fast and sensitive determination of Sudan I, II, III, and IV in chilli powder samples. This
determination could not be achieved by MEKC-UV due to the existence of several interfering compounds from the matrix [144].

Food preservatives are added to foods to prevent alteration and degradation by microorganisms during storage. However, excessive addition of some preservatives may induce health problems (allergy, dermatitis, convulsion, hives, etc.). The possibilities of CE to analyze food additives have been further demonstrated in the reviewed period [145] including methods for the determination of benzoic acid and sorbic acid in beverages, vinegar and fruit jam samples [146]. A CE method was developed and compared with HPLC for the determination of benzoyl peroxide, as benzoic acid, in wheat flour, concluding that the performance of the CE method was comparable, and the quantitative results were in good agreement with those using HPLC. Meanwhile the proposed CE method has the advantages of better resolution, shorter analysis time and lower cost, and would be a good alternative to HPLC for routine monitoring of benzoyl peroxide amount in wheat flour [147]. Another type of preservatives as parabens were determined by CEC using a commercial C18 silica (3 μm, 40 cm x 100 μm i. d.). An optimal separation of the parabens was obtained within 18.5 minutes with a pH 8.0 mobile phase composed of 50:50 (v/v) tris(hydroxymethyl)aminomethane buffer and acetonitrile. The method was successfully applied to the quantitative analysis of paraben preservatives in sweetener samples with direct injection of the sample [148]. Tertiary butylhydroquinone is a highly effective antioxidant and in foods it is used as a preservative for unsaturated vegetable oils and many edible animal fats since it does not change flavor or odor of the material to which it is added. A CE-AD method was developed for the determination of tertiary butylhydroquinone in food samples without derivatization or purification [149].

The addition of collagen or its hydrolysates as a protein source or water binding agent is a common practice in the production of meat products. However, its level of addition is restricted by international regulations. Glycine, proline, and hydroxyproline are the most
abundant amino acids in collagen, which is the main constituent of connective tissue. Thus, a CE method was developed for the determination of hydroxyproline content in meat products using UV detection at 214 nm. From the samples tested (36), commercial ham showed the lowest average collagen content (< 7.16 g per 100 g protein) by the CE method. On the other hand, frankfurter and Mexican "chorizo" sausages showed average collagen contents of 12.88 and 11.17 g per 100 g protein, respectively. According to the authors, the CE method developed could be used by regulatory agencies to ensure compliance with maximum levels of collagen addition in processed meats [150]. A MEKC approach with in-capillary derivatization was also developed for the determination of hydroxyproline in milk powder, liquid milk, milk drink and soymilk powder samples. Under the optimal conditions, derivatization and separation procedure could be completed within 7 min and the detection limit for hydroxyproline was 1.6 ng/mL [151].

11. Food interactions and processing

Chemical composition of food can be altered by industrial process (heating, roasting, storage, etc.). These changes may give rise to the formation of new compounds which might represent a risk to human health so that analytical methodologies able to monitor these changes or to determine hazardous compounds are necessary. For instance, furosine has been demonstrated to be an indicator of the thermal treatment occurring in food processing and its presence and concentration in food products is related to the initial step in the Maillard reaction. The determination of furosine in flour samples of different origin (wheat, chestnut, lupin, einkorn, chickpeas), and food products such as pasta, milk, and tigelle bread by CE-MS/MS has been reported by Bignardi et al. [152]. By using this methodology it was possible to investigate the effect of process such as drying, milling, heat treatment, and storage in the furosine content.

5-Hydroxymethylfurfural is other compound which is formed as an intermediate product during the Maillard reaction and from hexoses degradation and caramelization. Although it is nearly absent in fresh and untreated foods, its concentration tends to rise during heating, so
that its analytical control has been used to evaluate both the quality of the heat processing and the organoleptic characteristics of the final product. For instance, a MEKC method was developed for the simultaneous determination of furanic compounds in honey and vegetable oils [153]. The MEKC method allowed the simultaneous determination of 2-furfural, 3-furfural, 5-methylfurfural, 5-hydroxymethylfurfural, 2-furoic acid and 3-furoic acid in honey and vegetable oils (palm, walnut, grape seed and rapeseed) [150]. Other different MEKC methods have been developed and applied to the determination of 5-hydroxymethylfurfural in honey samples [154], breakfast cereals, toasts, honey, orange juice, apple juice, jam, coffee, chocolate and biscuits [155].

Acrylamide, which has been classified as “probably carcinogenic in humans”, is other example of product that appears in many foods after high-temperature cooking (frying and roasting). A MEKC method with quantum dot-mediated LIF detection was proposed with the aim to detect sub-ppm levels of acrilamide in potato crisps detection [156]. The proposed method demonstrated sufficient sensitivity (LODs of 0.1 mg/kg) to meet the maximum level of acrylamide specified by World Health Organization requirement for potato crisps.

Spreadable processed cheese, (widely consumed in Brazil) uses hydrogenated vegetable fat (HVF) as a substitute for dairy ingredients to improve the consistency, taste and texture of the cheese, and to reduce manufacturing costs. However, the addition of HVF during spreadable cheese manufacture is associated with health implications due to the presence of trans fatty acids (TFA). Despite this, HVF is still available on the Brazilian market and is widely used in the manufacture of several dairy products. Therefore, monitoring the amount of TFAs in spreadable cheeses for quality control purposes is highly relevant. De Castro Barra et al. [157] optimized a CZE method with indirect UV detection for monitoring TFAs during industrial processing of spreadable cheeses, without the need for derivatisation steps. Compared with the official GC method of the American Oil Chemists’ Society, the developed CE method could be considered an alternative for routine analysis of TFAs in spreadable cheeses.
CZE with UV detection has been also used to evaluate the effects of combined treatments in fields, including water stress and exogenous abscisic acid (ABA) on phenolic accumulation in berries and wine [158]. The results obtained show as ABA supply increased catechin and malvidin synthesis while resveratrol was enhanced only for water-stress. Moreover, additions of ABA to grape berry skins subjected to water stress could have induced anthocyanin biosynthesis in a synergistic manner. The ability to determine each individual phenol may provide a way to optimize the operational conditions taking into account that many viticulture and enological factors influence the type and concentration of flavor components.

CE has also shown to be a valuable technique to obtain information on interactions among food ingredients. For instance, the bioactivities and bioavailability of plant polyphenols may be affected by covalent reaction between polyphenol and proteins. Both processing conditions and gastrointestinal conditions may promote formation of covalent complexes for polyphenol-rich foods and beverages such as wine [159]. Thus, different CE methods were developed by pairing capillaries with different diameters with appropriate alkaline borate buffers to distinguish free protein from covalently bound protein-polyphenol complexes and to monitor polyphenol oxidation products. To do that, epigallocatechin gallate was used as model compound and BSA as a model protein.

12. Chiral analysis of food compounds

Today, there is no doubt about the potential of enantiomeric separations using capillary electromigration methodologies, especially due to its high separation efficiency and the need of small quantities of chiral selectors [20]. Mechanistic aspects regarding CE enantioseparations have been recently reviewed [160]. The use of cyclodextrins and their derivatives as chiral selectors is involved in the majority of chiral CE separations. It has been recently highlighted the crucial role of the cyclodextrin cavity size on enantioseparation as well as and the significant effects on the resolution obtained by small differences in the
structural characteristics of chiral molecules [161,162]. On the other hand, the use of chiral metal complexes for enantioseparation following the chiral ligand exchange principle is gaining importance since it provides high separation selectivity of chiral molecules [163].

A number of recent papers addressed the enantiomeric separation by CE of different food components. Through a variety of chiral applications, the usefulness of CE methods has been demonstrated to determine quality control, bioactivity and safety of foodstuffs [164,165]. Besides, over the last years stereoselective analysis of food compounds has increasingly demonstrated its usefulness to assess food adulteration [166]. The use of chiral metal complexes for enantioseparation following the chiral ligand exchange principle provides high selectivity of chiral molecules, and following this idea, D-isocitric, L-isocitric acid and citric acids were separated using a ligand exchange CE method with Ni(II)-D-quinic acid system. Following a similar approach, but with different ligand exchange (namely, Sc(III)/Cu(II)-D-quinic acid system), enantiomers of D/L-malic, D/L-tartaric and D/L-isocitric acids, as well as and citric acid, were analyzed [167]. The content of these α-hydroxy acids enantiomers was also used to distinguish authentic and adulterated fruit juices.

Natural products have also been the subject of research in this field. A variety of natural product compounds that exhibit chirality have been studied using CE techniques [168]. Chirality is also of major importance in the biological activity of compounds, and thus, Kodama et al. [169] studied the content of the naturally occurring antioxidant (R)-lipoic acid in food supplements. The use of a sulfonated capillary and trimethyl-β-cyclodextrin as chiral selector were the key features of the developed chiral CE-UV method. The presence of the racemic lipoic acid evidenced the presence of lipoic acid of synthetic origin in some commercial dietary supplements which claimed to use only naturally occurring lipoic acid.
Dominguez-Vega et al. [170] presented a novel polysaccharide-based chiral stationary phase (cellulose tris(3-chloro-4-methylphenylcarbamate)) for the enantioseparation of FMOC-derivatized amino acids by CEC-UV. In this case, amino acid derivatization may give rise to additional interactions with the chiral selectors commonly used, improving the separation performance. When compared to nano-LC, better efficiencies and resolution were observed with CEC. Chiral discrimination of 20 out of 23 amino acids could be achieved. The CEC-UV method was applied to the analysis of the non-protein amino acids in commercial food supplements. The method allowed detecting down to 0.15% of the enantiomeric impurity (D-citrulline) in the commercial samples manufactured based on L-citrulline.

13. CE microchip technology in food analysis

In the last years, miniaturized technology has kept growing in terms of technical development and emerging applications. The application of CE microchips in food analysis is generating a large interest due to its advantageous features, including negligible consumption of reagents and samples, and the capability for fast and automatized analysis in situ. Commercial equipments based on microchip CE technology have received wide acceptance in food analysis laboratories. More precisely, the instruments based on gel sieving separations and LIF detection have been helpful for highthroughput analysis of protein samples obtained from cereals for quality testing [171,172]. On the other hand, technical advances in microchip CE include new detection systems. In this sense, microchip CE in combination with electrochemical systems is gaining relevance in food applications. This increasingly adopted detection strategy is sensitive, easy to miniaturize without loss of performance and compatible with microfabrication techniques. Among the available electrochemical methods, amperometric detection has been the most employed in combination with microchips CE for food applications in last years. For instance, Dossi et al. [173] studied the performance of a mixed-valent ruthenium oxide/hexacyanoruthenate polymeric film electrochemically deposited onto glassy carbon electrodes for the microchip CE-AD separation of two biogenic amines and their amino acid precursor in beer. In a different approach, Ding et al. [174]
developed a PDMS microchip CE device coupled with an electrode fabricated by electrodeposition of an alloy of Cu-Sn-Cr and tested on spiked milk samples containing five aminoglycoside antibiotics (spectinomycin, streptomycin, amikacin, paromomycin, and neomycin). In that study, acidic conditions were chosen for separation, whereas AD was performed under alkaline conditions. This was possible by the use of two auxiliary channels that allowed the use of different conditions for each analytical operation. The microchip CE under optimum separation and detection conditions provided LODs below 4.6 μM. Also, the application of microchip CE-AD to the analysis of phenolic compounds in olive oil has been recently investigated [175]. In this case, a glass microchip with a three-electrode configuration was employed in combination with an amperometric detector situated in the detection reservoir. A gold wire electrode, chosen as working electrode with an end-channel configuration, was aligned at the outlet of the separation channel. The application of this developed microdevice enabled the detection of tyrosol, hydroxytyrosol and oleuropein glucoside in olive oil and fruit. In a different report, Fernández-la-Villa et al. [176] upgraded a previous microchip CE based on potentiometric detection. The new development allowed better portability for in situ applications. Another interesting feature of this device was the multichannel architecture that allowed different models of integrated electrodes including interdigitated arrays and microelectrodes. The performance of the method was evaluated by the separation of several types of analytes, including vitamins, polyphenolic acids, and flavones.

14. Foodomics and other future trends of CE in food analysis

Regarding the new discipline of Foodomics (see a scheme in Figure 5), it is interesting to mention that the first book on this topic has already been published in 2013 [177]. The book presents the fundamentals of Foodomics, exploring the use of advanced mass spectrometry techniques in food science and nutrition including the use of CE-MS in many of these applications. It includes an overview of Foodomics principles and applications and covers modern instruments and methods of proteomics, including the study and characterization of
food quality, antioxidant food supplements, and food allergens. It also discusses advanced mass spectrometry-based methods to study transgenic foods and the microbial metabolome, principles and practices of lipidomics, green Foodomics, mass spectrometry-based metabolomics in nutrition and health research and the Foodomics' impact on our current understanding of micronutrients (phenolic compounds and folates), optimal nutrition, and personalized nutrition and diet related diseases. The book also describes the use of chemometrics in mass spectrometry and Foodomics and explores the potential of systems biology approaches in food and nutrition research. In this regard, it is interesting to mention some recent applications of Foodomics that involve the use of CE-based methodologies. For instance, our research group has demonstrated the great potential of CE-TOF MS to the metabolite profiling of cultured human HT-29 colon cancer cells [178,179]. Thus, Simó et al. [178] developed a CE-TOF MS method that provided the simultaneous and reproducible analysis of more than 80 metabolites in less than 20 min per sample with minimum consumpiton of sample and reagents. The importance of selecting an appropriate purification strategy in metabolomics was highlighted in that work. Also, Ibañez et al. [180] exploited the benefits of analytical multiplatforms to improve the description of the metabolome status in cultured HT-29 cells. In that study, CE-TOF MS analyses were complemented with UPLC-TOF MS using different chromatographic modes to carry out a broad metabolomic study on the anti-proliferative effect of dietary polyphenols on HT-29 cells. Using this strategy, the intracellular levels of 22 highly related metabolites, that are known to be essential for the maintenance of the cellular functions, were altered in HT-29 cells by the treatment with dietary polyphenols. After functional enrichment and pathway analysis using Ingenuity Pathway Analysis (IPA) software, changes in glutathione metabolism as well as in the levels of polyamines induced by the treatment with polyphenols were suggested that may have important implications in the proliferation of HT-29 cells. In further reports, the Foodomics approach has been applied in a cross-omic platform study including data integration from Transcriptomics, Proteomics and Metabolomics for the comprehensive study of the health benefits of rosemary polyphenols on human colon cancer cell models [181,182]. Thus, a
global Foodomics strategy was adopted following a hypothesis-free approach in which the biological information from each expression level considered individually is compared with the biological information obtained after combining the three expression levels [181]. In that report, authors remarked the impressive analytical power of the global Foodomics approach, but also, the limitations and challenges, mainly related with the lack of bioinformatic tools able to handle and integrate complex multidimensional data generated by the different omics platforms. In another global Foodomics study on the antiproliferative effect of dietary polyphenols on human leukemia lines, Valdés et al. [183] combined whole-transcriptome microarray data with MS-based metabolomics data (via CE-TOF MS and UPLC-TOF MS analysis). Functional enrichment and pathway analyses using IPA software, as a previous step for a reliable interpretation of transcriptomic and metabolomic profiles, were performed based on the results from each single platform. These preliminary analyses were followed by an integrative approach that involved overlaying transcriptomic datasets on canonical pathways obtained from metabolomics analysis and vice versa. Using this Foodomics approach stronger evidences on the chemopreventive and antioxidant response of leukemia cells to dietary polyphenols were provided. Also, Foodomics has been recently applied to assess the substantial equivalence between GMOs and the unmodified counterparts [184]. In their study, two GM tomato varieties over-expressing miraculin glycoprotein and a panel of traditional tomato cultivars were selected to prove a methodology based on three different metabolomics platforms. More specifically, data from GC-TOF MS, LC-QTOF MS and CE-TOF MS were summarized in single consensus datasets for further multivariate analysis. The combination of the three platforms allowed the statistical analysis of datasets containing over 175 unique tentatively identified metabolites and more than 1,400 peaks with no or imprecise metabolite annotation. This analytical setup provided metabolite coverage of 85% of the chemical diversity found in the LycoCyc database. Results showed that >92% of the tested peaks in the transgenic lines deviated less from the control line than the accepted limit estimated using the reference panel of traditional cultivars. As it can be deduced from these works, CE-MS offers great potential to provide relevant contributions in Foodomic studies.
The studies shown in this review demonstrate the relevant role of capillary electromigration methods in food analysis and Foodomics over the last two years. It is expected that the development of novel CE methods applicable to food analysis will keep growing to serve authorities on the verification of the compliance of the everyday more strict regulations. In this regard, a number of on-line preconcentration strategies aimed to overcome the concentration sensitivity limitation of the technique have been reported during the period covered in this review in the food analysis field. In the near future, it is expected that novel materials, including also nanomaterials, will provide new improvements in terms of sensitivity and selectivity in capillary electromigration techniques that will be applied in food analysis. This point can be also extrapolated to microchip-CE development that it is also expected to keep technologically growing as well as its applications in food analysis. Despite the predominant use of electrochemical detection with microchip CE, novel applications of microchip CE devices and fluorescence detection can be also foreseen in the non-distant future in food analysis [185]. The use of MS is becoming a more frequent alternative detection in CE and it is expected to be also more generally used in food science. It is also expected that chiral CE separations keep increasing in food analysis, while chiral CE-MS applications are expected to grow [186,187], once the important limitations induced by the incompatibility between chiral selectors and ESI-MS can be overcome.

Acknowledgements

This work was supported by AGL2011-29857-C03-01 (Ministerio de Economía y Competitividad, Spain), and CSD2007-00063 FUN-C-FOOD (Programa CONSOLIDER, Ministerio de Educacion y Ciencia, Spain) projects. M.C-P. thanks the Ministerio de Ciencia e Innovación for her Juan de la Cierva contract (JCI-2009-05297).
15. References


[35] Li, W-L., Ge, J-Y., Pan, Y-L., Chu, Q-C., Ye, J-N. *Microchim. Acta* 2012, 177, 75-80.


[181] Ibáñez, C., Valdés, A., García-Cañas, V., Simó, C., Celebier, M., Rocamora, L.,
Gómez, A., Herrero, M., Castro, M., Segura-Carretero, A., Ibáñez, E., Ferragut, J.A.,

[182] Valdés, A., García-Cañas, V., Rocamora-Reverte, L., Gómez-Martínez, A., Ferragut,


Watanabe, S., Yano, M., Hiwasa-Tanase, K., Ezura, H., Saito, K., PLoS ONE 2011, 6,
e16989.


[186] Giuffrida, A., Contino, A., Maccarrone, G., Messina, M., Cucinotta, V. *Electrophoresis*
2011, 32, 1176-1181.

Figure captions

Figure 1. Base peak electropherograms of i) Infant Formula 1 (partially hydrolyzed whey), ii) Infant Formula 2 (highly hydrolyzed whey) and iii) Infant Formula 3 (casein and highly hydrolyzed casein bovine proteins) using a) C18 and b) STX cartridges (for peptide purification). Redrawn from [54] with permission from Wiley-VCH.

Figure 2. Multiplex detection of foodborne pathogens. The electropherograms are demonstration of ten-plex pathogen detection using genomic DNA mixture (A) and detection six strains incubated in milk (B). Redrawn from [100] with permission from Wiley-VCH.

Figure 3. Fluorescence and UV electropherograms corresponding to a sunflower oil sample before (left) and after (right) been fortified with 392 μg/g of α-tocopherol, 192 μg/g of (β + γ)-tocopherol, and 151 μg/g of δ-tocopherol. Samples were injected in background electrolyte:ethanol:hexane (60:20:20), being the final added concentrations 26.1 μg/mL of α-tocopherol, 12.8 μg/mL of (β + γ)-tocopherol, and 10.1 μg/mL of δ-tocopherol. Redrawn from [107] with permission from Elsevier.

Figure 4. Typical electropherogram of (A) drug-free turkey muscle and (B) blank turkey muscle spiked with sulfaguanidine (1), florfenicol (2), thiamphenicol (3), cloramphenicol (4), sulfamethazine (5), sulfamerazine (6), sulfachloropyridazine (7), sulfamethoxazole (8), sulfathiazole (9), sulfacarbamide (10) and flumequine (I.S.). Conditions: UV detection at 200 nm, unmodified silica capillary (57cm×50 μm I.D.), temp. 22 °C, running buffer composed of (20:80, v/v) methanol and mixture of 15mM Na₂B₄O₇ and 25 mM SDS. Redrawn from [130] with permission from Elsevier.

Figure 5. Scheme of the new discipline of Foodomics including the main tools employed and the main fields of application.