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

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Abbreviations: **AAs**, amino acids; **AFILMC**, analyte focusing ionic liquid micelle collapse capillary Electrophoresis ; **BAs**, biogenic amines; **C⁴D**, contactless conductivity detection; **CA**, carnosic acid; **CEIA**, CE-based immunoassay; **CIP**, ciprofloxacin; **COC**, cyclic olefin copolymer; **CS**, carnosol; **CSEI**, cation-selective exhaustive injection; **DML**, dimethyl labeling; **ECL**, electrochemiluminescence; **ELISA**, enzyme-linked immunosorbent assay; **ENRO**, enrofloxacin; **EOF**, electroosmotic flow; **FA**, fatty acids; **FASI**, field-amplified sample injection; **FASS**, field-amplified sample stacking; **FESI-RMM**, field-enhanced sample injection with reverse migrating micelles; **FLD**, fluorescence detector; **FMOC**, 9-fluorenylmethoxy-carbonylchloride; **GMO**, genetically modified organism; **HCTA-OH**, hexadecyltrimethylammonium hydroxide; **HF-LPME**, hollow-fiber liquid-phase microextraction; **HS-ITME**, headspace in-tube microextraction; **ILUAE**, ionic liquid-based ultrasonic assisted extraction; **LECE**, ligand exchange capillary Electrophoresis; **LED-IF**, light-emitting diode induced fluorescence detection; **LIF**, laser-induced fluorescence detection; **LVSS**, large-volume sample stacking; **MCE**, microchip Electrophoresis; **MMMIPs**, montmorillonite magnetic molecularly imprinted polymers; **MRLs**, maximum residue limits; **MSPD**, matrix solid-phase dispersion; **MSPE**, magnetic solid phase extraction; **MSS**, combining micelle to solvent stacking; **mt-MIP**, multi-template molecularly

imprinted polymer; **MWNTs**, multiwalled carbon nanotubes; **NFA**, nitrofurantoin antibiotics; **PAA**, polyacrylic acid; **PAHs**, polycyclic aromatic hydrocarbons; **PCA**, principal component analysis; **PLS**, partial least squares; **PSSNa**, sodium polystyrene sulfonate; **QuEChERS**, quick, easy, cheap, effective, rugged, and safe; **RP-HPLC**, reverse phase HPLC; **RT-PCR**, reverse transcription polymerase chain reaction; **SAs**, sulphonamides; **SFME**, surfactant-free microemulsion; **SMZ**, sulfamethoxazole; **TFA**, trans FASI; **TMP**, trimethoprim.

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Abstract

This review work presents and discusses the main applications of capillary electromigration methods in food analysis and Foodomics. Papers that were published during the period February 2015-February 2017 are included following the previous review by Acunha et al. (*Electrophoresis* 2016, 37, 111-141). The paper shows the large variety of food related molecules that have been analyzed by CE including amino acids, biogenic amines, carbohydrates, chiral compounds, contaminants, DNAs, food additives, heterocyclic amines, lipids, peptides, pesticides, phenols, pigments, polyphenols, proteins, residues, toxins, vitamins, small organic and inorganic compounds, as well as other minor compounds. This work describes the last results on food quality and safety, nutritional value, storage, bioactivity, as well as uses of CE for monitoring food interactions and food processing including recent microchips developments and new applications of CE in Foodomics.

1. Introduction

The major concern for food industries, consumers and regulatory laboratories related to food intake is food safety. As a result, the development of new analytical methods and tools is crucial to this first step in food analysis. Moreover, food quality validation as well as the evaluation of other biological properties of foods imply the use of robust, efficient, sensitive, and cost-effective analytical methodologies. CE has been applied to analyze a huge number of food matrices related to either food analysis or Foodomics [1-6], as can be also confirmed by the information given in **Table 1** [7-30]. Thus, due to its high separation efficiency and speed, together with the extremely small sample and reagents requirements, CE has demonstrated to be a very useful analytical tool in food science. **Table 1** summarizes the main review papers published within the period covered by this work on capillary electrodriven techniques in food analysis and Foodomics [7-30]. As can be seen, recent applications of CE-MS in metabolomics [7] or recent developments in CE-MS [25], as well as detection schemes for CE [15, 24], chiral methods [20, 22], concentration procedures [21], and miniaturized CE systems [17, 30] have been recently reviewed.

The application of CE to the analysis of contaminants [19, 23, 30], amino acids [13, 18], non-protein amino acids as food quality markers [10], the simultaneous CE separation of cations and anions [16] and the analysis of proteins, carbohydrates and lipids in food by CE [18] has also been recently reviewed. Regarding the use of specific modes of CE in food analysis, some reviews have described the last developments and applications of capillary electrochromatography [11], open tubular-capillary electrochromatography [12] and microchip [17, 30] in different applications related to food analysis.

It is also noteworthy the high number of review papers dealing with the development and applications of Foodomics in the period covered by this work. Thus, the use of different analytical techniques in Foodomics [8, 14, 29] has been reviewed including the use of Foodomics imaging by MS and NMR [8] and the application of direct high-resolution mass

spectrometry in Foodomics [29]. Some other works have reviewed the application of Foodomics to differentiate organic and conventional foods [9], for exploring safety, quality and bioactivity of foods [26], in microbiological investigations [27] and for investigations of food toxins [28].

The present work describes in the following sections the different CE approaches used to detect compounds of relevance to food analysis including amino acids, biogenic amines, carbohydrates, chiral compounds, contaminants, DNAs, food additives, heterocyclic amines, lipids, peptides, pesticides, phenols, pigments, polyphenols, proteins, residues, toxins, vitamins, small organic and inorganic compounds, as well as other minor compounds. **Table 2** shows a summary of some selected applications of CE including type of compound analyzed, sample, CE mode/separation BGE, detection and the suitable reference.

2. Amino acids, biogenic amines, heterocyclic amines and other hazardous amines

As basic components of proteins, or in their free form, amino acids (AAs) can provide relevant information on the quality and safety of food samples. CE is considered as a powerful separation technique for AAs analysis and a reliable alternative to reverse-phase high-performance liquid chromatography (RP-HPLC), improving the separation capacity for polar AAs. Latest advances in AAs analysis by CE (from 2013 to 2015) have been recently reviewed by Poinot et al. [13]. A wider review covering the evolution of AAs analysis and other biomolecules in food samples by electromigration techniques over the last 20 years was presented by De Oliveira et al. in 2016 [18]. In these works, UV-vis detector is the universal detection approach most commonly used in combination with commercial CE, although a derivatization step is often required in order to enhance AAs detectability [31]. CE-UV has been recently applied for determination of six amino acids in Sudanese food, demonstrating that asparagine concentration in food raw material can be correlated with concentration of acrylamide in food final product [31]. NBD-Cl (4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole) was used as fluorescent labeling agent for the determination of primary and secondary amino

acids by pre-column derivatization. The low cost, and the generation of low number of by-products are the main advantages of this derivatization reagent. Another CE-UV method based on micellar electrokinetic chromatography (MEKC) was developed to quantify 22 free AAs in passion fruit juices [32]. This time, a selective derivatization reaction with 9-fluorenylmethoxycarbonylchloride (FMOC) was used. This separation method was capable to characterize different types of juices using principal component analysis (PCA) to detect adulteration on industrial juices. Phenylalanine content in cereal products was also analyzed by a rapid MEKC-UV, using a running buffer composed by phosphoric acid, sodium dodecyl sulfate and methanol at pH 1.9, showing limits of detection (LOD) at 0,2 mg g⁻¹ level [33]. An alternative detection method based on laser-induced fluorescence (LIF) was used by Nehmé et. al for the sensitive analysis of AAs from microalgae extracts at nanomolar level (3–30 nM) [34]. The authors obtained satisfactory electrophoretic resolution using sodium tetraborate buffer (100 mM; pH 9.4) and β -cyclodextrin (10 mM) as background electrolyte (BGE). Microwave-assisted derivatization (680 W, 80°C) was applied to enhance AAs labeling with fluorescein isothiocyanate.

Some AAs of non-protein origin can also be found in food as additives or as by-products formed during food processing. Pérez-Míguez et al. reviewed a wide variety of new CE-based methodologies for the analysis of non-protein AAs, which were shown to have high potential as biomarkers to detect food adulterations and to evaluate food quality [10]. The chiral behavior of non-protein AAs is a relevant factor to be considered in their analysis. Although L-forms are generally present in nature, racemization into the D-form may occur by different processing conditions used by the food industry. An electrokinetic chromatography method was also developed by Pérez-Míguez et al. for the enantiomeric separation of a group of non-protein AAs of interest in the pharmaceutical and food analysis fields, using sulfated- α -cyclodextrin or sulfated- β -cyclodextrin as chiral selectors [35]. As shown in Figure 1, the use of anionic cyclodextrins, 100 mM formate buffer at pH 2.0 as running buffer and pre-

capillary derivatization with Fmoc enabled the enantioseparation of the eight target Fmoc-non-protein AAs with high chiral resolution.

The presence of biogenic amines (BAs) with aliphatic, aromatic or heterocyclic structures are considered as indicator of microbial foods spoilage. They are primarily generated as a consequence of microbial amino acid decarboxylation, and the intake of abnormal levels of these substances may cause severe toxicological effect. Therefore, the detection of BAs plays a crucial role in food quality control and the development of new methods that improve separation and detectability of BAs is still a focused area. Thirteen different BAs were simultaneously detected by UV detection in a CE-UV method, without any derivatization process. This was possible because the amines without UV absorption could be indirectly detected by the difference in absorptivity coefficient with respect to a UV absorbing probe. Using imidazole as the UV probe and α -cyclodextrin as additive at pH 4.5, the target BAs were separated in 9 min, with limits of detection at the low μmol level [36].

Among other hazardous amines, melamine has been found as adulterant in milk products and animal feed in order to increase the apparent protein content. Although methods based on GC-MS or HPLC are common used for melamine detection, a new approach was proposed by Zhang et al. using polydopamine-assisted partial hydrolyzed poly(2-methyl-2-oxazolinze) as coating for determination of melamine in milk by CE [37]. Using this coating, no solvent extraction is required, reaching low concentration of 4 mg kg^{-1} melamine in non-fat milk powder. For the determination of underised imidazole derivatives (2-methylimidazole, 4-methylimidazole, and 2-acetyl-4-tetrahydroxybutyl imidazole) generated during caramelization processed in food and beverages, a cation-selective exhaustive injection and sweeping (CSEI-sweeping)-MEKC method was developed to improve detection limit of the target analytes. In this work, an activated carbon-polymer monolithic column was employed as solid-phase microextraction sorbent to improve detectability and reduce matrix effect, providing LODs in the range of $33.4\text{--}60.4 \text{ g L}^{-1}$ [38]. Acrylamide is also a toxic by-product

formed during the Maillard reaction of glucose (GL) with asparagine (AS) in cooked food above 120 °C. Abd El-Hady et al. proposed an analyte focusing ionic liquid micelle collapse capillary Electrophoresis (AFILMC-CE) approach to simultaneously determine AAs, and its precursors (AS and GL) during bread baking process. Ionic liquid-based ultrasonic assisted extraction (ILUAE) was applied and the samples were prepared in 1-butyl-3-methylimidazolium bromide micellar matrix with higher conductivity than the running phosphate buffer (pH 8.5), which allowed the separation and sensitive determination of the target analytes [39].

3. Peptides and proteins

Functional and biological properties of foods may be affected by the content of peptides and proteins which demonstrate the interest on the development of analytical methodologies to perform their analysis in food samples. In fact, within the period of time covered by this work, different reviews have pointed out the last developments in the analysis of peptides and proteins by capillary electromigration methods [40, 41].

Within the last two years, proteins analysis has been accomplished by simple CZE, CGE and CEC methodologies. CZE methods with UV detection have been used to investigate the protein profile of wheat [42], camel milk [43] liquid whole eggs [44], and cheese samples [45]. The analysis of wheat proteins fractions may provide relevant information for correct allergy diagnosis. In this sense, a preliminary CZE methodology with UV detection was developed with the purpose of measure quantitatively the wheat grain proteins soluble in chloroform-methanol mixture, some of which are relevant for wheat food allergy [42]. Using 0.1 M phosphoric acid/ β -alanine buffer (pH 2.5) containing urea, hydroxyl-propyl-methyl-cellulose and acetonitrile was possible to separate the proteins of this fraction (in their native form) in only 6 min with good selectivity and efficiency. The therapeutic properties (anti-cancer, anti-diabetic, hypo-allergenic) related to camel milk have led to not only an increase in its production on large scale but also a great interest in the characterization of their

proteins. The simultaneous separation and quantification of major whey and casein proteins in camel milk was carried out using an extended light path capillary and 100 mM phosphate buffer at pH 2.5 [43]. Higher amounts of β -casein, α -lactalbumin and lactoferrin as well as lower amounts of α -casein and k -casein were found in camel milk compared to bovine milk. In addition camel milk was devoid of β -lactoglobulin (the main whey protein in bovine milk). Compared with traditional SDS-PAGE, the developed CZE method provided comparable results with higher resolution and simplicity. To carry out the protein analysis in cheese samples, Znaleziona et al. compared the potential of a new positively charged surfactant (N-dodecyl-N,N-dimethyl-(1,2-propandiol ammonium chloride) with commonly used coating agents for the dynamic coating of the capillary wall [45]. The use of this new surfactant, at a concentration of 10 mM, in 100 mM acetate buffer (pH 5.5) reversed the EOF and gave rise to the efficient separation of model basic and neutral proteins (lysozyme, cytochrome c, ribonuclease A, and myoglobin). Moreover, the developed method enabled the lysozyme determination in cheese in 7 min reaching a LOD of 0.9 mg L⁻¹. The lysozyme concentration obtained was similar to those reported by HPLC methods. A CZE-UV methodology was also used to study the applicability of whey proteins and carbohydrates (lactose or glucose) as indicators of each stage of the Maillard reaction under adverse storage conditions. The utility of the CZE method was limited to the first stage where it enabled to evaluate the extent of glycated proteins by Maillard reaction providing information when the loss of lysine is not significant [46].

Two recent articles have shown the potential of CGE as alternative to CZE for protein analysis. On the one hand, CGE has been employed to predict different cultivars of citrus fruits [47]. Prior to CGE analysis, the protein extraction was performed by different extraction protocols. Satisfactory protein yields from citrus peel and pulp were reached using enzyme-assisted extraction. The protein profiles obtained by CGE were used to develop linear discriminant analysis able to differentiate citrus fruit samples according to their cultivar. On the other hand, sodium dodecyl sulfate (SDS)-CGE has been also used to carry out the

quantification of whey proteins in infant formulas and milk powders [48]. The whey content measured by the SDS-CGE methodology was in close agreement with the declared values (95 % of the analyzed samples were within 10 % of the declared values).

CEC methodologies devoted to protein analysis have been also reported within the time covered by this review. Mainly these works have been focused on the development of effective procedures to prepare open tubular columns with stable coating for the separation of proteins. In this research line, Xiao et al., prepared a polydopamine-coated open tubular column in which ammonium persulfate was employed as oxygen source to induce the self-polymerization of dopamine to polydopamine [49]. The separation of proteins in chicken egg white and milk was successfully carried out by CEC which demonstrated the validity of the polydopamine-coated column to perform protein analysis in real samples. Further work by the same research group was focused on the development of titanium oxide nanoparticles coated open tubular columns [50]. In this approach, the self-polymerization of dopamine under alkaline conditions was employed to form a film in the inner surface of a fused-silica capillary. Then, by using a simple liquid phase deposition process, titanium oxide nanoparticles were deposited onto the modified surface capillary. Figure 2A shows the scheme of the preparation of titanium oxide nanoparticles modified column. After investigating the performance of the proposed open tubular column for the separation of proteins, its feasibility was verified by the successful separation of acidic proteins in egg samples (see Figure 2B).

The combination of the data obtained by MALDI-MS and CZE-UV was employed by Baptista et al., to accomplish the characterization of the peptide profile in prato cheese samples [51]. The samples were fractionated according to their solubility at pH 4.6, so that while CZE was applied to evaluate the pH 4.6 insoluble fraction, MALDI-MS was used to analyze the fraction soluble at pH 4.6 and 70 % ethanol. The combination of the data obtained from both techniques showed that the analyzed samples had similar casein hydrolysis profiles

in spite of the differences in raw material, ripening period and process conditions. The discriminant analysis of the data by PLS revealed that only nine peptides were responsible for the discrimination of the cheese samples. In another application, Marcolini et al., investigated the bioaccessibility of carnosine during in vitro digestion of beef meat [52]. Carnosine is a bioactive peptide which can act as antioxidant, antiglycating or as wound-healing promoter. Its bioaccessibility (i.e. the compound fraction that is released from the food matrix in the gastrointestinal tract during digestion) is a relevant factor to determine its available amount for intestinal absorption. Modification in the carnosine bioaccessibility may be caused by interactions with food matrix. For this reason, the research was focused on the evaluation of its bioaccessibility by means of the study of the effect of pH on the interaction of carnosine with digested meat matrix. To determine the carnosine concentration in two bresaola samples (a typical Italian cured meat based product) CZE and NMR were employed in parallel. The analysis was performed on both the raw material and after in vitro digestion of samples, and a good agreement between the results obtained by both techniques was achieved.

4. Phenols, polyphenols and lipids

Phenolic compounds are secondary metabolites present in plants, algae and some beverages. Their main function is the protection and adaptation of the natural organisms against environmental factors. Besides, their role as potential health promoting ingredients is attracting great interest since several functional activities have been related to different phenolic compounds, such as antioxidant, antiallergenic, antiarterogenic, antiinflammatory, antiproliferative, antimicrobial, antithrombotic, cardioprotective and vasodilatory effects [53]. Therefore, the study of this metabolites is becoming increasingly important in food analysis. The use of electrophoretic methods for the analysis of phenolic compounds has been widely described [54], being a versatile and robust alternative to the most common separation technique for this kind of compounds, i.e., HPLC, when separations with high efficiency and resolution are required.

To obtain a good separation an exhaustive optimization, the different parameters that affect the separation such as buffer type, concentration and pH, type and dimension of the capillary, temperature, voltage and injection mode, have to be carefully studied. In this regard, Gatea et al. [55] developed a CZE method for the separation of 20 phenolic compounds in propolis and plant extracts. The main phenolic compounds present in these samples are mainly flavonoids and phenolic acids. In this work, both the extraction and the separation conditions were optimized. In order to maximize the extraction of the compounds of interest, their solubility was studied evaluating the effects of temperature, nature of the extraction solvents and pH. On the other hand, several separation parameters were optimized. Tetraborate with SDS as an anionic surfactant at alkaline pH was selected as buffer. The increment on the buffer concentration enhanced the resolution of the peaks, however, the migration time also increased. The best results were achieved with a concentration of 45mM. The SDS concentration and pH were evaluated. The best separation conditions were obtained with 0.9 mM of SDS at pH 9.35. The optimized method allowed the separation of 20 phenolic compounds in 27 min achieving a good accuracy. In the same way, Sanli et al. [56] optimized a green CE method for the separation of 8 phenolic compounds in red wine (catechin, syringic acid, apigenin, myricetin, luteolin, quercetin, caffeic acid, and gallic acid). The method was developed without using toxic organic modifiers. Borate buffer was selected at pH range of 8.7-9.1. All the analytes increased their migration time with the increase of pH except myricetin, that at the higher pH tested could not be separate from apigenin. The optimum pH was 8.9 since good resolution, selectivity and peak shapes of all the analytes were achieved in relatively short analysis times (15 min). Borate buffer concentration had a significant importance in the separation, therefore a good equilibrium between resolution and migration time was established using a concentration of 40 mM. The higher temperature tested (25 °C) and the higher applied potential (26 kV) provided the better peak shapes at the less migration time. The optimization of this method gave rise to a sensitive, simple, rapid and environmentally friendly method.

The use of CE for the study of the phenolic compound profiles may be very useful for the classification of different natural samples. Some examples of this interesting application are described below.

The high resolution power of a CE-UV method using a fused silica capillary, 25 mM borate buffer with 10% methanol at pH 9.3 as BGE, 20°C and 25 kV allowed the fingerprint study of the extracted compounds of 10 *Mentha* herbal samples and 20 peppermint teas. The different phenolic profiles of each sample, together with spectrophotometric methods for the determination of the total phenolic compounds and the antioxidant capacity of each extract allowed distinguishing the *Mentha* and peppermint tea samples by a PCA model according to their potential protective antioxidant effect [57].

A CE method for the classification and the study of the impact of the aging length, aging process and mashbill of different Irish whiskies on their phenolic profile has been reported [58]. In this case, a field amplified sample stacking (FASS) preconcentration method was employed to increase the sensitivity of the analytes of interest. This method is very useful for samples of low conductivity such as the whiskey matrices. The separation was carried out with 25 mM sodium phosphate buffer at pH 6.35 as buffer and 25 kV under reversed-polarity conditions. Results showed that the length of aging in Irish whiskies positively affect the concentration of phenolic acids; whiskey aging in sherry casks produced a final product with a greater number of phenolic compounds types; and finally the phenolic profile of single pot still whiskies resulted in a rich concentration of phenolic aldehydes and a diversity of phenolic acids.

Coated capillaries have been developed for the analysis of phenolic compounds. In particular a dynamically coated capillary with chitosan and multiwall carbon microtubes for the determination of 12 phenolic compounds of chrysanthemum [59]. In this work, the combination of chitosan and multiwalled carbon nanotubes (MWNTs) was employed as a dynamic coating material for the first time. The parameters of the separation were optimized

accordingly to the different chemical properties of the analytes of interest, including the chitosan concentration, surfactant-coated MWNTs (SC-MWNTs), buffer and SDS concentrations and buffer pH. For the procedure of capillary coating, chitosan and SC-MWNTs were introduced in the inner wall of the capillary; firstly, the new capillary surface was activated by dissociation of the silanol groups, then the static modification of the capillary by chitosan was carried out, and finally, the double-coated capillary was obtained through the dynamical modification with SC-MWNTs of the chitosan coated capillary. In Figure 3 A the structure modification of the MWNT before (Figure 3A-1) and after (Figure 3.A-2) the functionalization with surfactants is shown. The efficiency of the separation of the 12 phenolic compounds with the optimized separation conditions greatly improved with the use of the chitosan-SC-MWNTs strategy in comparison with the separation carried out on a conventional fused silica capillary as can be observed in Figure 3B.

When minor compounds such as phenolic compounds are the subject of study, sample preparation methods play an important part of the analysis, since these compounds are present in low concentrations. Therefore, the elimination of the matrix effect, as well as the enrichment of the target analytes becomes essential. In this regard, different sample preparation strategies coupled to CE have been developed for the analysis of phenolic compounds. For instance, a headspace in-tube microextraction (HS-ITME) for the extraction of traces of three bromophenols in tap water and *Trachypenaeus curvirostri*, followed by their determination by CE was developed by Yue et al. [60]. Dispersive liquid/liquid microextraction combined with CE was employed for the detection and quantification of vanillin in milk powder [61]. Large-volume sample stacking (LVSS) technique has been employed to preconcentrate 7 phenolic compounds of different commercial rice of Pakistan and their analysis by non-aqueous capillary Electrophoresis (NACE) arriving at a 15-55 fold improvement in sensitivity thanks to the preconcentration step [62]. The selective extraction and separation of 6 trace phenolic compounds in water samples were possible by the development of a novel multi-template molecularly imprinted polymer (mt-MIP) that was

used as adsorbent of a solid phase extraction (SPE) and coupled to CE [63]. In this work, high sensitivity with low limits of detection and quantification (0.17 to $0.31 \mu\text{g L}^{-1}$ and 0.57 to $1.03 \mu\text{g L}^{-1}$, respectively) were attained, showing non-matrices interferences due to the high selectivity of the cleanup step.

Another application of a sample pretreatment method followed by CE analysis was developed by Xu et al. [64], they used trace amounts of poly- β -cyclodextrin wrapped carbon nanotubes (poly- β -CD-MWCNT) for the extraction of flavonoids in honey samples. After this extraction, the analytes were separated and determined by CE with light-emitting diode induced fluorescence detection (LED-IF). This extraction method is a miniaturization model of SPE, reducing the consumption of sorbent amount and organic solvents. For the separation, a buffer composed by 10 mM sodium borate, 8% methanol and 2% 1-propanol was employed at 35°C and using a separation voltage of 30 kV . This method provided low LODs (0.07 - 17.99 ng mL^{-1}), good precision and high enrichment factor, reducing the matrix effect.

Lipids are one of the main macronutrients in food and they constitute a source of energy but also they are responsible of important functional activities. Fatty acids (FA) are a group of lipids with a huge importance in food. Recently, a CZE-UV method has been employed for the detection of milk adulteration [65]. One of the main dairy products adulterations consists on the addition of whey on milk. Consequently, in this work, the six most important FA present in milk and whey were selected by GC; this six FA could be the most significant to detect the adulteration. The CE separation of these FA was achieved in a fused silica capillary with a fluoropolymer external coating operated at 25°C and 19 kV within an analysis time of 15 min . This method allowed the detection and quantification of whey addition in a range of 4 - 20% of adulteration, showing to be a good method for controlling the quality of milk.

Trans fatty acids (TFA) are present in process food and they are associated with serious cardiovascular problems in humans due to these FA increase the level of low-density lipoprotein cholesterol and decreased high-density lipoprotein cholesterol. Government

agencies are controlling the levels of TFA in foods, therefore their content in food has to be determined. Usually, TFA are analyzed by GC-FID, however, the separation and determination of FA by GC present some limitations such as the need for derivatization steps due to the low volatility of the FA, as well as their low thermal stability. On the other hand, CE for the separation of FA offers some advantages comparing to GC, for example, the shorter analysis time, absence of derivatization and low temperature separations. For this reason, a new CZE-UV method for the analysis of TFA of different process foods (butter toffee, cake mix, stuffed wafers, chocolate, a mix for Brazilian cheese bread, wafer stick, coconut donut and guava paste biscuit) has been reported [66, 67]. For the separation of the TFA, the composition of the BGE buffer has a critical role since it has to maintain the FA in anionic form, at the same time that it has to act as a chemical selector of cis or cis-trans homologous and organic solvents must be present to avoid micelle formations. With all these requirements in mind, the BGE composition in these works was composed by 12.0 mmol L⁻¹ of tetraborate buffer, 12.0 mmol L⁻¹ of Brij 35, 33% methanol and 17% acetonitrile. The separation was carried out on a fused silica capillary at 27 °C under normal polarity and a constant voltage of 27 kV. No significant differences were found when comparing the results obtained with the new CE method against the results obtained in the GC analysis of TFA. Therefore, these works presented a good application of CZE-UV for the analysis of TFA in process foods.

5. Carbohydrates

Carbohydrates are the main source of energy for almost all physiological functions and are involved in many biological processes. The analysis of carbohydrates by CE pose a great challenge, namely, due to the the lack of both easily ionizable groups (due to their high-pK_a values) and chromophore groups for detection. Dominguez et al. [68] proposed a CE method for the simultaneous determination of three carbohydrates (fructose, glucose and sucrose) and the amino acid proline in honey samples from Argentina and Sweden. The separation was

carried out in 5 minutes in a fused-silica capillary using -25 kV at 25°C and a solution composed of 10 mM sodium benzoate and 1.5 mM cetyltrimethylammonium bromide at pH 12.4 as BGE. The results showed that the method meet the requirements of both Codex Alimentarius and Código Alimentario Argentino.

CZE with direct UV detection was used for the first time for the determination of mono- and disaccharides, sugar alcohols and ethanol in fermentation broths. The composition of the BGE needed to be adjusted given the complexity of the carbohydrate mixture to improve separation and/or throughput. Although 130 mM KOH provided the best selectivity, the low viscosity of the BGE and the resulting size-to-charge ratio of the carbohydrates did not lead to the highest resolution of the mixture. Thus, 130 mM NaOH resulted in the best resolution among single-salt BGEs, whereas a mixture of 65 mM LiOH and 65 mM NaOH not only increased the resolution but also increased the analysis time. The interest of this method lies in its ability to monitor ethanol and carbohydrates on the same instrument to provide a complete picture of the fermentation sample [69]. A similar method like the one previously described was used for the quantification of carbohydrates in breakfast cereals (BCs) in order to monitor sugar levels because their influence on the high prevalence rates of obesity and other diet-related diseases. The method described by Toutounji et al. [70] analysed 13 breakfast cereals products and detected sucrose in all of them, while lactose, maltose, glucose and fructose were detected in some. Due to the complexity of the matrix it was not possible to detect other components such as proteins or lipids. The method studied, FSCE with direct UV detection was shown to be advantageous for measuring sugar content in BCs compared to traditional reducing sugar and glucose-specific methods.

In order to identify potential animal sources of sialylated oligosaccharides that can be used as health promoting ingredients in functional foods, Monti et al [71] developed a CE method, using an uncoated capillary, 30 kV as running voltage at 25°C and UV detection at 200 nm. The oligosaccharides analysed were 3-sialyllactose (3-SL), 6-sialyllactose (6-SL) and

disialyl-lacto-N-tetraose (DSLNT). These components were majority in human milk, whereas in animal samples DSLNT was missing. In bovine (goat and cow) milk was only detected 3-SL and mare milk has resulted to be as a promising font of oligosaccharides. The procedure applied in this study confirmed its suitability for the detection, the identification and the quantification of sialylated oligosaccharides in these samples with satisfactory results.

A CE method to separate and determine carbohydrates in food samples was developed using a dynamically coated capillary with indirect UV detection. Under optimal conditions, 32 carbohydrates including mono-, di-, oligosaccharides, and sugar alcohols were separated in less than 12 minutes. The running voltage was –15 kV at 25°C and the UV detection was set at 350 nm. The optimal BGE was composed of 20 mM sorbic acid, 0.005 % (w/v) HDB and 40 mM NaOH at pH 12.2. To evaluate the applicability of the CE method, it was used to determine the content of the carbohydrates in food samples such as milk, juice, honey, candied jujube, beer and chitosan oligosaccharide capsule [72].

6. DNAs

The next-generation sequencing (NGS) technologies has transformed genomic science, due to their improved capability for rapidly and inexpensively sequence billions of nucleic acid bases. Despite their high-throughput capabilities and the significant reduction in operational costs, NGS is still unaffordable for many laboratories. However, CE is a high-throughput separation method still playing an essential role in DNA analysis. For instance, sanger sequencing via CGE is still commonly used to correct for errors in assembling the sequence data, in long repeats of DNA polymer. Thus, CGE is reported as an analytical technique to assist and improve quality control in next-generation sequencing [73]. In the field of food science, the recently published papers about CE methods applied to DNA analysis are mainly aimed at detecting food fraud, food-born pathogens or allergenic ingredients.

Exposure to food allergens pose significant health risks to allergic consumers. Therefore, the fast and effective detection of potential allergens is essential for food manufacturers to ensure accurate labeling of their products. In this regard, Cheng et al. developed a decaplex PCR assay combined with CE analysis for the simultaneous detection of 10 common food allergens from hazelnut, pistachio, oat, sesame, peanut, cashew, barley, wheat, soybean and pecan [74]. This method showed LOD between 2 and 20 copies of haploid genome, and the relative LOD was as low as 0.005% (w/w), demonstrating that multiplex-PCR (MPCR) assay is a suitable procedure for routine simultaneous detection of multiple food allergens. Another multiplex alternative was proposed by López-Calleja et al., who developed a method based on multiplex ligation-dependent probe amplification (MLPA) for simultaneous detection of five food allergens including sunflower, poppy, flaxseed, sesame and soy in processed food [75]. Ligated MLPA-halb probes were amplified by PCR and the resulting amplicons were detected by CE. MLPA is a high throughput technique, which allows the detection of multiple DNA sequences with more flexibility. The easy validation of MLPA is another advantage compared to MPCR.

The detection of DNA markers for food authentication using CE methods has also been recently reported in literature. For instance, Di Renzo et al. were able to detect adulterations by the addition of table grapes during the wine making process, developing an analytical procedure for table grape DNA tracing in industrial musts. The combination of high resolution melting (HRM) and CE allowed the screening to discover adulterations and accurate genotyping, being VrZAG62 and VrZAG79 the most informative markers [76]. In other work, Bazakos et al. studied the potential of a small number of polymorphic (SNPs) to evaluate the authentication and traceability of extra virgin olive oil from three different Mediterranean countries. Specific SNPs were used as PCR analytical targets and a PCR-restriction fragment length polymorphism (RFLP) capillary Electrophoresis approach was used to discriminate several olive oils of Mediterranean origin from three different countries,

Greece, Tunisia, and Lebanon [77]. CE in combination with the DNA extraction protocol resulted in lower LODs compared to previous works.

As part of the authenticity and traceability testing process of food products, the detection and identification of genetically modified organisms (GMOs) in food samples represents an important issue, considering the legal restriction in terms of approved and unapproved GMOs that can be present in foodstuffs. In this regard, Patwardhan et al. developed a sensitive post-PCR detection method by using PCR-chip capillary Electrophoresis (PCR-CCE), to detect and identify specific GMOs in mixtures [78]. Using this approach, different GMOs such as MON531 cotton, EH 92-527-1 potato, Bt176 maize, GT73 canola, or GA21 maize were simultaneously detected by targeting event-specific nucleotide sequences. Another multiplex PCR method combined with CE was also developed by Zhang et al. to specifically detect four GM rice events. This time, fluorescence multiplex PCR was performed using fluorescence-labeled primers for endogenous, exogenous and event-specific genes, reaching sensitivity levels as low as a 0.1% [79].

As an alternative to more traditional microbiological detection methods based on plate count, new developments based on DNA analysis combined with CE were recently reported in literature for the rapid, sensitive, and simultaneous detection of food-borne pathogenic species. A multiplex detection method of nine food-borne pathogens was reported by Villamizar-Rodríguez et al., using mPCR and CE, which allows an important reduction in the required time for detection of the target pathogens [80]. The method was validated in five different food matrices (meat, dairy products, prepared food, canned fish and pastry products), showing comparable results with the traditional approaches (coincidence percentage in the range 78-92%). On the other hand, So-Young et al. proposed capillary Electrophoresis - single strand conformation polymorphism (CE-SSCP) coupled with stuffer-free multiplex ligation-dependent probe amplification (MLPA) to simultaneously detect 13 species of foodborne pathogens in a single electrogram [81]. After method optimization, 50-

500 pg genomic DNA were detected per microbe. This methodology could be useful for early detection of foodborne diseases associated with animal-derived foods in the food industry. For foodborne enteroviruses detection, Ruan et al. developed a novel and ultrasensitive method based on reverse transcription polymerase chain reaction (RT-PCR) for virus RNA amplification. Separation and detection was carried out with a CE-LIF system using glycine buffer (pH 9.5), and polyethylene glycol (PEG 6000). The amplification products were labeled with highly sensitive SYBR Gold reaching detection limits as low as 1.33×10^2 copies mL⁻¹ for norovirus, 1.86×10^2 copies mL⁻¹ for rotavirus, and 1.40×10^2 copies mL⁻¹ for astrovirus [82].

7. Vitamins

Vitamins are a group of indispensable compounds for the development and normal growth of the human body, which can be classified into two main groups: water-soluble and fat-soluble. Water-soluble vitamins include B group vitamins and ascorbic acid (vitamin C). These vitamins play specific and vital functions in metabolism, and are included in energy and sport drinks for their essentiality in the normal biological functions such as coenzymes. Navarro-Pascual-Ahuir et al. [83] developed a method for the determination of these vitamins in ten commercially available samples, 4 energy drinks, 4 sport drinks and 2 fruit nectars, by MEKC. The CE system was equipped with a DAD and uncoated fused-silica capillaries. The running voltage was 20 kV at 25°C and the UV detection was performed at 214 nm for all vitamins except for vitamin C that was 265 nm. The results showed (see Figure 4) that the found levels of B vitamins were similar as the values on the product labels. In all the energy drinks analysed, the levels of B12 vitamin were below the LOQ. In fruit nectars, the contents of C vitamin were below from that declared in the label whereas in sport drinks only ascorbic acid was found as the main vitamin component.

In order to characterize Anatolian monofloral and honeydew honeys according to their mineral, vitamin B2, total phenolic contents and antioxidant activities, Kaygusuz et al. [84]

used a CE method coupled with LIF detector to determine vitamin B2 contents. The separation was carried out in a fused-silica capillary using 30 kV at 25°C and UV detection at 488 nm and UV emission at 520 nm. The results showed that riboflavin concentrations of heather honey samples are considerable higher than vitamin B2 contents of other honeys. A similar method like the one previously described was used for the determination of the vitamin B2 level in saffron developed by Hashemi et al. [85]. The CE system was equipped with a LIF detector and a fused silica capillary. The analytical conditions were 25 kV at 25°C and a solution composed of 20 mM borate buffer at pH 9.5 as BGE. Five commercial samples of saffron, three from Iran and two from Spain, were analysed. Comparing the riboflavin contents of these samples with the reported riboflavin contents of other food sources in the literature, it could be established that saffron is one of the most riboflavin-rich foods.

8. Small organic and inorganic compounds.

Organic and inorganic compounds play an important role in the structure, properties and nutritional food value, but also their detection and analysis are crucial since some of them can be considered impurities, contaminants or even toxic compounds. Therefore, the analysis of organic and inorganic small molecules in food analysis can be involved in both, nutritional and food safety and quality evaluations since there are a huge variety of compounds.

In this regard, the wide range of applications of CE and its versatility become CE in a very useful analytical tool for the analysis of these small compounds, providing high efficiency, sensitivity, fast analysis, and low consumption of solvents and samples.

The utility of CE for the analysis of organic acids in food has been widely demonstrated. Organic acids are responsible of the food flavor as well as they have nutritional functions. Besides, organic acids can be used as indicators of some food processes such as the bacterial activity in food fermentation. For example, a simple and fast CE method has been developed for the separation and quantification of 9 aliphatic and 3 aromatic organic acids in probiotic

products of lactic acid bacteria with LOD values from 0.001-1.43 $\mu\text{g mL}^{-1}$ and LOQ values from 0.004 to 4.72 $\mu\text{g mL}^{-1}$ in a simple, rapid and reliable analysis [86]. 8 representative organic acids from rice wine and beer, including oxalic, tartaric, formic, citric, malic, lactic, succinic and acetic acids, were analyzed and quantified using a direct injection of the sample with a simple dilution in a CE instrument with indirect UV detection. Authors developed a BGE solution including a buffer and a chromophore for the detection of the closely related organic acids, in particular, 2,4-Dihydroxybenzoic acid (DHBA) was selected as chromophore based on its close mobility to the analytes as well as the short analysis time, symmetry peak shape and the sensitive signal that this compound provided. This method allowed the quantification of the rice wine and beer organic acid content (5454.15 and 527.3 mg L^{-1} , respectively) [87]. Li et al. [88] studied the organic acid composition in blueberry juices through a CE-MS method. They optimize the CE-MS parameters including the composition and pH of the buffer, the sheath liquid composition, gas flow rate or ESI voltage to enhance the ionization efficiency and to make the ESI stable. The optimal sheath liquid composition consisted on 70:30 isopropanol/water (v/v) containing 7.5 mmol L^{-1} acetic acid at a flow rate of 6 $\mu\text{L min}^{-1}$ [88]. Besides, CE-UV has been employed for the determination of the organic acids composition and quantification of 31 wine samples [89] and for the classification of 38 samples of commercial wine from four wine-producing provinces of Argentina according to the grape variety applying the Tucker3 algorithm [90] as well as for the measure of the changes in organic acids levels in coffee seeds as function of different fermentation treatments [91]. Moreover, CE has shown an interesting application as a tool for the downstream process monitoring by the detection of organic impurities in the process of lactic acid purification from 15 second-generation renewable feedstock [92].

Other bioactive compounds present in plants have been successfully analyzed by different CE methods. Wang et al. [93] employed an accelerated solvent extraction method for the extraction of six bioactive anthraquinones from slimming tea, and they developed a rapid and effective CZE method for the analysis of these interesting compounds that could be employed

for the study of the quality of tea. A NACE-UV method was employed for the analysis of bioactive curcuminoids and some degradation products [94]. Besides, six representative alkaloids (nicotine, theophylline, theobromine, caffeine and harmaline, piperine) in onion nectar, a scarce sample, were analyzed employing a SPE method for the extraction of the target compounds and MEKC-UV for their separation. With this method, the amount of sample was reduced up to 2000 times reaching limits of detection of $0.0153 \mu\text{g L}^{-1}$ [95].

Silicon in plants was determined by CE with indirect UV detection using cinnamic acid as absorbing reagent. The method consisted on a simple sample preparation and a separation on a fused silica capillary with an external protective polyimide coating using a BGE composition of 0.4 mM tetradecyltrimethylammonium bromide and 2 mM cinnamic acid (pH 10.5) and an applied voltage of -20 kV and 20°C of temperature, achieving low detection limit values [96].

A novel approach for the separation and quantification of inorganic cations (sodium, potassium, calcium and magnesium) from zucchini, mushroom and apple samples has been reported. The method was carried out by direct injection placing a small piece of plant tissue into a CE vial, and applying a voltage for electrokinetic injection. Poor repeatability due to the expulsion of plant fluids as a result of the capillary wall squashing of the sample that resulted in hydrodynamic injection, was solved by controlling the viscosity of the BGE with 2% hydroxypropylmethyl cellulose [97].

Iodine species play an important role in life processes such as growth and development of body structures, cell development and differentiation processes and metabolic balance. SC-MWNTs were used as pseudo-stationary phase in CE for the separation of iodine species. Several parameters, including the effect of surfactant on the dispersion of the MWNTs and the MWNTs concentration, were optimized considering the different chemical behavior of the target compounds. The method was applied for the analysis of iodate, tetraiodothyronine, triiodothyronine, diiodothyronine, and diiodotyrosine in seaweed samples [98].

A dispersive micro solid-phase extraction with sulfonated nanocellulose as sorbent material followed by CE was employed for the extraction and analysis of silver nanoparticles (AgNPs) in orange juice and mussels. The new sorbent material provided negatively charged sulfate groups enhancing the extraction and preconcentration of AgNPs from complex matrices. The AgNPs were desorbed into an aqueous solution and injected and analyzed by CE [99].

9. Toxins, contaminants, pesticides and residues

Toxins represent a serious threat to public health and their control in food poses a great challenge to scientists and policy makers, as new toxins are being identified and new regulatory limits need to be established. The paralytic shellfish toxins (PSTs) are naturally occurring neurotoxins produced by the marine dinoflagellates and freshwater cyanobacteria [100]. Bivalve molluscan shellfish such as clams, oysters or mussels obtain food by pumping water through their system and filtering out small organisms. In this process, if large amount of toxic microorganisms are present in the water, shellfish can accumulate high levels of the PSTs, introducing these toxic substances in the food chain, which can result in human intoxication and even death [101]. Saxitoxin (STX) is one of PSTs whose determination is crucial in order to ensure the safety of seafood consumption. This toxin was determined by a CE-ICP-MS method using with Eu^{3+} and diethylenetriamine- N,N,N',N'',N'' -pentaacetic acid for chelate labeling, which allowed the ultrasensitive detection of STX at 0.38 fmol in seafood samples [102]. For the sensitive analysis of five PSTs simultaneously, Li et al. developed a CE method with amperometric detection [103]. Using field-amplified sample injection (FASI), higher enrichment efficiency was achieved, considering the effectiveness of this preconcentration technique applied to CE. This methodology allowed the effective separation of five PSTs including saxitoxin (STX) and its analogues (dcSTX, neoSTX) or gonyautoxin (GTX) epimers, using 40 mM Britton–Robinson buffer (pH 9.5) as BGE. Compared to other methods based on HPLC-FD or MEKC-LIF, which offer lower resolution

for the target toxins, the proposed strategy allowed the complete separation of the five PSTs, reaching LOQs below the ppb level in drinking water.

CE still plays a special role in the determination of pesticide residues in food products, as evidenced by the recent publication of two reviews that cover the last developments for pesticides residues analysis in general [19], and for phenoxy acid herbicide residues in particular [23]. In fact, most of published papers about this topic are mainly focused on herbicides analysis, which continues to be a high priority in the food safety area. Thus, Wuethrich et al. applied simultaneous electrophoretic concentration and separation (SECS), as environmentally friendly sample preparation strategy, for organophosphonates and quaternary ammonium herbicides determination in beer samples [104]. After sample preparation, negatively charged organophosphonate herbicides glyphosate, glufosinate, and aminomethylphosphonic acid were analyzed by LC–MS, whereas cationic analytes, such as paraquat and diquat were detected by two-step stacking in CE–UV. Obtained detection limits were as low as 3 and 15 ng mL⁻¹ for diluted and non-diluted beer samples, respectively. Halosulfuron-methyl is an effective herbicide frequently used in much lower concentration than other pesticides. For the sensitive determination of this sulfonylureas derivative in sugarcane juice and tomato, capillary Electrophoresis –tandem mass spectrometry (CE–MS/MS) was used, after QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction [105]. The electrophoretic separation was performed in NH₄HCO₃ electrolyte (adjusted to pH 8.5) reaching LODs of 2ppbs for both studied matrices. The analysis of widespread used phenylurea herbicides such as monuron, monolinuron and diuron has been accomplished by electrochemiluminescence (ECL) detection after CE separation [106]. As extraction method, a matrix solid-phase dispersion (MSPD) procedure was proposed, showing quantitative recoveries (90.0–99.2%), with very low variability (RSD < 3.2%). The resulting method can provide effective technical support for the monitoring of these potentially carcinogenic substances in agricultural products. For low water-soluble compounds, NACE is normally the considered option. Thus, Xu et al. proposed a NACE-based method for the

determination of imazalil, prochloraz and thiabendazole fungicides in fruits and juice samples [107]. Low low-viscosity solvents such as Methanol–acetonitrile mixture (35:65, v/v), containing containing 30 mmol L⁻¹ ammonium chloride and 0.5% phosphoric acid, were introduced to favor rapid separation speed. Applying DLLME, recoveries values from 72% to 102% were achieved, with low detection limits (0.47 to 0.72 µg kg⁻¹).

In terms of sample preparation improvements, ‘tailor-made’ molecular imprinted polymers were also shown as powerful alternatives for the selective extraction of pesticide residues. The common organophosphorus pesticide trichlorfon was selectively determined using synthesized molecularly imprinted polymers material as as biomimetic antibody [108]. The competitive reactions between horseradish peroxidase (HRP) labeled trichlorfon hapten and free trichlorfon with the biomimetic antibody was evaluated. This method allows the detection of trace levels of trichlorfon, achieving LOD values of 0.16 mg L⁻¹, which improved the sensitivity of other methods reported in literature. On the other hand, a dual-layer solid-phase extraction based on molecular imprinting technology was proposed in order to enhance the selectivity fot the analysis of pesticide residues in olive oil [109]. This time, organophosphorus and triazine type pesticides were simultaneously determined in a single procedure. The recoveries obtained for dimethoate (95%) and terbuthylazine (94%) demonstrate the reliability of this dual pesticide residue methodology for the trace analysis of pesticide residues based on molecular imprinting technology.

Among the most widespread environmental contaminants, polycyclic aromatic hydrocarbons (PAHs) have been approached by capillary and microchip Electrophoresis analysis, as reported in a recent review by Ferey et al. [30]. Different suitable strategies based on electrokinetic chromatography (EKC) and micellar electrokinetic chromatography (MEKC) to separate neutral analytes such as PAHs were discussed in this paper. The addition of organic solvents, cyclodextrins (CDs), or urea to the BGE improves selectivity in MEKC analysis. Both CD-EKC and monolith-based CEC offer the additional advantage of an easier transfer to

chip format, showing great potential for PAH determination. Other contaminants such as the endocrine disruptor bisphenol A, or the ubiquitous environmental carcinogens α -Naphthol and β -Naphthol were successfully determined applying field-amplified sample injection (FASI), as a powerful strategy to substantially improve the sensitivity of CE-UV [110]. Using this preconcentration technique, the authors were able to increase the sensitivity by more than 10 times for the target contaminants in drinks and lake water. With the same purpose, Jiang et al. implemented field-enhanced sample injection with reverse migrating micelles (FESI-RMM) in capillary Electrophoresis for the trace analysis of phthalate [111]. Surfactant-free microemulsion (SFME) with toluene, isopropanol, and water was used to reinforce hollow-fiber liquid-phase microextraction (HF-LPME). The optimized method was applied to the determination of three phthalate plastizisers in beverage and urine samples at low detection levels (0.18, 0.24, and 0.14 ng mL⁻¹, respectively).

Antimicrobial resistance has become a global public health threat due to the extensive use of antibiotics to treat microbial infections in humans and animals. For this reason, worldwide regulatory authorities have established maximum residues limits (MRLs) that set out the level of residue that could safely remain in the tissue or food product derived from a food-producing animal that has been treated with an antibiotic. To comply with those regulations, several analytical methods based on CE have been developed in the last years for the reliable determination of antibiotics in foodstuffs of animal origin. An important group of antibiotics are beta-lactams, which represent about one third of the total yield of antibiotics. Six beta-lactam residues including amoxicillin, cephalexin, oxacillin, penicillin G, cefazolin, and cefoperazone were analysed in milk and egg matrices by MEKC [112]. In this work, an improved online preconcentration strategy based on LVSS with polarity switching was applied, reducing peak width, with no loss of separation efficiency. Using a running buffer of 10 mM phosphate and 22 mM SDS at pH 6.7, a sample size of 1.47 kPa $\times$ 690s, and 20 kV reverse voltage, the obtained LODs were below 0.26 ng g⁻¹. An alternative on-line enrichment method was applied by Liu et al. [113], combining micelle to solvent stacking (MSS) with

FASS for the analysis of trimethoprim (TMP) and sulfamethoxazole (SMZ) by CZE. Sample matrix, trapping solution, running buffer, sample solution volume and detection conditions were evaluated. Under the optimized conditions the obtained LODs for TMP and SMZ were 7.7 and 8.5 ng mL⁻¹, which were much lower (301 and 329 times better) compared to a typical injection.

Sulphonamides (SAs) are another important group of antibiotics residues frequently found in animal-derived food. The high efficiency and relatively low cost of SAs have stimulated their ubiquitous utilization in veterinary practices for prophylactic and therapeutic purposes. The use of magnetic nanoparticles as a rapid alternative to conventional methods was recently proposed for SAs residues analysis by Li et al. [114]. Graphene-Fe₃O₄ nanoparticles were synthesized and employed as sorbent for magnetic solid-phase extraction of SAs in milk. The resulting graphene-based magnetic solid phase extraction-CE (MSPE-CE) method allowed the rapid and sensitive determination of the target sulfonamides: sulfadoxine, sulfisoxazole, sulfamerazine, and sulfamethoxazole. However, the wide recovery range (62.7–104.8%) and the narrow linear range (5–200 and 10–200 µg L⁻¹) are important limitations for this method. In another work, three different SAs (sulfadimidine, sulfadiazine, and sulfathiazole) were determined by CE using chemiluminescence detection based on the inhibiting effect of SAs on Ag(III)–luminol reaction [115]. SPE was applied as clean-up and enrichment procedure during sample treatment. The validation study of the method demonstrated good linear response, and the reported LODs for the target antibiotics were in the range 0.65–2.75 µg mL, which allowed the simultaneous analysis of the three SAs in pork meat, chicken meat, and milk.

Another MSPE strategy based on montmorillonite magnetic molecularly imprinted polymers (MMMIPs) was proposed by Wang et al. [116] for the analysis of fluoroquinolones (FQs) in bovine milk samples. In this study, MMMIPs were prepared using montmorillonite as carrier, fleroxacin (FLE) as template molecule, and Fe₃O₄ magnetite as magnetic component. The

developed MSPE-CE method exhibit lower LODs (42.8–61.9 $\mu\text{g L}^{-1}$), compared to on-line column-switching HPLC coupled with an FLD detector, which demonstrate the high selective enrichment capabilities for analyzing fleroxacin, gatifloxacin, lomefloxacin, and norfloxacin in complex samples. For the single determination of norfloxacin, Liu et al. combined the selectivity of capillary electrophoresis immunoassay (CEIA) with the sensitivity of laser-induced fluorescence (LIF) detection [117]. After evaluation of the influence of incubation time, buffer pH and concentration, and separation voltage on the separation and determination of norfloxacin, accurate quantitation was performed within 3 min using $\text{Na}_2\text{B}_4\text{O}_7/\text{NaH}_2\text{PO}_4$ buffer (30 mmol L^{-1} , pH 8.0) for background electrolyte and 25 kV for the separation voltage. The LODs obtained with this method were 3.2 times lower than alternative ELISA-based methods, and much lower than that obtained by HPLC-MS/MS, as demonstrated in several analyzed samples such as milk, chicken, pork, and fish. An improved on-line concentration method was introduced by Xu et al. consisting of FASS combined with sweeping for the determination of the fluoroquinolones, ENRO and CIP in multiple animal-origin foodstuffs [118]. Gamma-cyclodextran (γ -CD) was introduced to the sample matrix to increase the affinity between the analytes and the pseudostationary phase of background solution, improving the sensitivity of the sweeping technique by 376 and 406 times for ENRO and CIP, respectively, compared to conventional (CE) method.

Simultaneous CE determination of different classes of antibiotics was also accomplished in two different works. Zhai et al. reported a simple MEKC method for the analysis of chloramphenicol, CIP, nitrofurantoin antibiotics (NFA) and their metabolites in fishery products. As NFA are metabolized within a few hours, they can hardly be detected in animal tissues. Instead, highly mutagenic and teratogenic nitrofurantoin metabolites are generated. Employing an optimal buffer at pH 9.0, containing 20 mmol L^{-1} sodium dihydrogen phosphate, 20 mmol L^{-1} disodium hydrogen phosphate, 80 mmol L^{-1} sodium deoxycholate, and 10 % methanol (v/v), a total of 11 antibiotics and metabolites were completely separated and determined [119]. As nitrofurantoin metabolites cannot be detected by UV due to the lack of chromophore groups, 2-

nitrobenzaldehyde was used as derivatization reagent. The resulting detection limits for each compound were in the range 0.3–1.5 $\mu\text{g kg}^{-1}$ in fishery products. In another report, Long et al. optimized a CE-ECL method to separate and analyze ampicillin and sarafloxacin in milk samples [120]. Separation of target antibiotics was carried out in a 5 mmol L^{-1} phosphate buffer solution at pH 5.5, and detected at 0.0013–0.018 $\mu\text{g mL}^{-1}$ level in a ECL detector cell containing 5 mmol L^{-1} $\text{Ru}(\text{bpy})_3^{2+}$.

10. Food additives

A broad range of CE methodologies have been developed to carry out the determination of colorants, sweeteners, and, preservatives demonstrating the potential of CE to the analysis of food additives.

The reason to add colorants to food samples is that they provide attractive colors to food ingredients that generate a great influence on the food perception of consumers. Even though most of colorants are safe, they are some that have potential hazards to human health and therefore their use is not allowed. A simple approach combining SPE (to clean up the samples) with CZE was proposed to perform the routine quantitative analysis of nine illegal azo dyes in beverages, grape wines, and foodstuffs [121]. All the analyzed dyes were separated in 16 min and the LODs achieved were between 25.1 to 75.1 $\mu\text{g L}^{-1}$. None of these colorants were detected in the different samples analyzed. Banned food dyes have been also analyzed in foods to demonstrate the potential of a novel compact fluorescence detection system designed for CE analysis [122]. Basically, this system was based on the use of two laser pointers as excitation sources, a Y-style optical fiber to transport the excitation light, a four-branch optical fiber to collect the emission light, a polydimethylsiloxane cell that combine capillary and fibers. The potential of the proposed instrumental setup in food safety analysis was demonstrated by the determination of different illegal colorants (rhodamine 6G, safranin T, rose bengal, and phloxine B, fluorescent brightener VBL, and fluorescent brightener FBA 351) in chili, red dates and wheat flour samples. LODs for the six dyes were

in the range of 3.7-64.4 $\mu\text{g kg}^{-1}$ which were below the limits established by the European Community.

Artificial sweeteners are highly used in food samples as low calorie alternative to natural sugar. These non-caloric compounds are mainly recommended for people that need (or desire) reduce sugar intakes for health reasons. However, due to their potential toxicity (for instance saccharin may have possible carcinogenic effect) their permitted amounts in foods are established by the legislation to guarantee consumer safety. This fact makes necessary the development of analytical methodologies capable to carry out a sensitive analysis of artificial sweeteners in food samples. In this sense, a CZE method with capacitively coupled to C^4D were developed for the simultaneous determination of three sulfanilamide sweeteners (acesulfame-K, saccharin and cyclamate) in beverages [123]. Separation was carried out in 10 min using 20 mM acetic acid as separation buffer, and field-amplified sample injection as on-line preconcentration technique to improve the sensitivity. In this way, the baseline resolution was achieved in 10 min with LODs between 4.4 and 8.8 $\mu\text{g L}^{-1}$ which implies a sensitivity enhancement of 560 times. Sugar alcohols (also called alditols or polyols) are also high intensity sweeteners whose used for the replacement of sucrose have gained popularity. The analysis of this kind of compounds is important to obtain nutritional information and to perform the quality control of alditols-containing products. However, their determination is quite problematic due to their high pKa values (in the range of 12-14), their high hydrophilicity and the lack of a chromophore group in their structure. Nevertheless, two different CE methodologies developed within the period covered by this work have been applied with success to the determination of different alditols in sugar-free chocolate [124] or alditols-containing products (such as xylitol mother liquor, orange juice, peanut milk and chewing gum) [125]. On the one hand, erythritol, maltitol, xylitol and sorbitol were simultaneously analyzed in sugar-free chocolate samples by using a CZE- C^4D method [124]. To do that, polyols were extracted from real sample by a simple procedure based on the use of water and ultrasonic energy. The use of 25 mM borate (pH 8.5) as BGE gave rise to the formation of

negatively charged borate esters (generated by the interaction between the polyols and the borate ions) which were separated in less than 6 min. The validation of the method shown LODs of 2.7-4.8 $\mu\text{g g}^{-1}$ and recoveries values from 70 to 116 % (with RSD from 0.2 to 19 %). The application of the developed method to the real samples demonstrated that most of them contained maltitol as the major sweetener. On the other hand, Xiao et al, optimized a CZE method with indirect LIF detection for determining four different sugar alcohol in several alditols-containing products [125]. For that purpose, xylitol, sorbitol, arabinitol and mannitol were separated using a BGE composed of 40 mM borate (pH 9.6), 5 % methanol (v/v) and 10^{-5} M fluorescein as background fluorescence. In this case, the LODs were in the range of 19-24.4 $\mu\text{g mL}^{-1}$ while the recoveries were between 99.6 and 105.5 %.

Preservatives such as benzoate and sorbate are often added to processed food in combination with monosodium glutamate. The simultaneous identification and quantitation of these three compounds in canned and processed food employing CZE with UV detection was reported by Aunga and Pyell [126]. The approach was based on the use of 25 mM borax, 5 mM o-phthalaldehyde (OPA) and 6 mM 3-mercaptopropionic acid (3-MPA) as BGE and the in-capillary derivatization of monosodium glutamate with OPA (in presence of 3-MPA). Once performed the optimization of parameter to minimize the reaction band broadening, it was possible to accomplish the simultaneous detection of derivatized glutamate and non-derivatized benzoate and sorbate.

Different CE methods have been also developed to carry out the simultaneous separation of a high number of food additives. In this line, Ding et al., developed two different MEKC-UV methodologies. On the one hand, the use of a buffer solution consisting of 15 mM tetraborate, 60 mM boric acid, and 100 mM SDS gave rise to the separation of ten preservatives (dehydroacetic acid, sorbic acid, benzoic acid, methyl paraben, ethyl paraben, isopropyl paraben, propyl paraben, isobutyl paraben, butyl paraben, and isopentyl paraben) in 20 min reaching LODs from 0.4 to 0.5 mg L^{-1} [127]. This method was applied to the determination of

the studied preservatives in 413 food samples collected from supermarkets and farmer's market. On the other hand, the same authors employed a system based on the use of 20 mM tetraborate, 42 mM boric acid, and 100 mM deoxycholate to achieve the simultaneous separation of caffeine, quinine (flavoring agent), six artificial sweetener (alitame, aspartame, neotame, stevioside, saccharin, and acesulfame-K) and three preservatives (sorbic acid, benzoic acid, and dehydroacetic acid) commonly used in beverage, yogurt and candied fruit samples [128]. In this case, the method was successfully applied to analyze the eleven additives in nine different samples including apple vinegar, cola, fanta, grape juice, haw beverage, yogurt, jelly, pineapple compote and ficus carica. None of food additives analyzed were found to exceed the permitted level. Lately, Le et al., have developed a simple and inexpensive method based on the use of CE with C⁴D detection for the screening of several food additives in a great variety of food samples [129]. For instance, the developed methodology was applied to the determination of carboxylated-based acidulants and preservatives in instant noodles, beer, wine, coffee, vinegar and tea samples, and artificial sweeteners (aspartame, cyclamate, saccharine and acesulfame K) in jelly, beverage and black bean sweet soup samples. Good agreement between the results obtained with the proposed method and those obtained using a confirmation method (HPLC-UV) was achieved which verify the reliability of the data provided by CE-C⁴D.

11. Food processing

The effect of the heat treatments in foods has been investigated in the period of time covered in this review. Thus, Mateos-Vivas et al. [130] analysed the effect of pasteurization and high-pressure processing on the contents of free nucleotide monophosphates in human milk. They used low-temperature long-time pasteurization, known as Holder pasteurization (HoP), in which milk was heated to 62.5°C in a water bath for 30 min and high-pressure processing (HPP) in which milk was pressurized at 400, 500 and 600 MPa for 5 min. The influence of the time elapsed since the arrival of the samples and the analysis was also evaluated: samples

analysed within 5 days after their arrival and samples analysed after storage at -18°C for 6 months. CE-ESI-MS was used for the separation of the samples. The results showed that freezing period no decrease the content of nucleotides in any of the samples. In these samples, there were no significance differences between HoP and HPP treatments. Similarly, Deglaire et al. [131] studied the impact of Holder pasteurization in human milk in order to evaluate whether the kinetics of peptide release during gastrointestinal digestion of term human milk were impacted by HoP. This study demonstrated that pasteurization impacted the human milk peptidome prior to digestion and induced different kinetics of peptide release during gastric and intestinal digestion, mainly depending on their protein origin and their potential behaviour toward heat denaturation. Another heat treatment developed by Uysal et al. [44] studied the effect of the temperature on liquid whole egg (LWE) proteins by using ultraviolet-visible (UV-VIS) spectroscopy and CE. Homogenized LWE was heat-treated at 60°C , 64°C and 68°C for five minutes. Separations were carried out in an uncoated fused-silica capillary using 10 kV at 25°C and the UV detection was performed at 200 nm. The optimal BGE was composed of 300 mM borate buffer at pH 9.2 containing 25 mM SDS. Electropherograms of samples demonstrated that conalbumin and lysozyme were influenced by the treatment, while ovalbumin and ovomucoid were no affected.

On the other hand, CE proved to be a sensitive method to estimate the extent of glycated proteins by Maillard reaction, providing valuable information when the loss of lysine is not significant. However, its utility is limited to the beginning of the first stage of the reaction. Leiva et al. [46] examined different indicators of each stage of the reaction under adverse storage conditions in two model systems containing whey proteins and glucose or lactose. The running voltage was 20 kV at 25°C and the UV detection was performed at 214 nm. The differences observed between glucose and lactose reactivity were significant and varied greatly with each stage of the reaction.

12. Chiral analysis of food compounds

A great variety of food components are chiral molecules that can exist, at least, as one pair of enantiomers which may originate different effects such as taste or aroma, among others. That enantiomers can be present naturally in food, can be generated in food processing or be originated from microbiological sources. This implies that chiral analysis of food components may provide relevant information related to food quality and safety, food processing, authenticity and adulterations, etc. In the field of chiral separation, CE has clearly demonstrated its high potential being nowadays one of the separation techniques most employed to carry out enantiomeric analysis due to its high separation efficiency, versatility and feasibility to use different chiral selectors.

During the period of time covered by this review, two different works have demonstrated the possibilities of ligand exchange capillary Electrophoresis (LECE) for the chiral determination of short chain organic acids. On the one hand, the ligand exchange principle was employed using L-histidine as chiral ligand and copper (II) as central ion in an open tubular CEC methodology for the enantiomeric determination of malic acid in apple juice [132]. On the other hand, the combination of a Cu (II)/D-quinic acid system with hexadecyltrimethylammonium as EOF reversal agent was used to perform the simultaneous separation of tartaric and malic acid in wines [133]. The developed LECE method enabled the enantiomeric quantification of both acids in less than eight min achieving LODs of 1.5 and 3 mg L⁻¹ for tartaric and malic acid respectively. D-Malic acid was detected in different wines in a broad range of concentrations (100-1700 mg mL⁻¹) whereas D-tartaric acid was detected in two of the wines analyzed at the level of 90 and 200 mg L⁻¹. The results obtained using this methodology are relevant for the quality control of wine since while the addition of DL-malic acid for wine acidification is legal, the use of DL-tartaric acid is not allowed by the International Organization of Vine and Wine [133].

Analysis of hydroxyeicosatetraenoic acids (endogenous eicosanoids generated in mammalian system that have important physiological and pathological functions) often requires

separating both regioisomers and enantiomers. For this reason, Kodama et al. developed a cyclodextrin-MEKC method to demonstrate the potential of CE for the chiral analysis of lipids [134]. The use of a solution consisting of 30 mM phosphate/15 mM borate (pH 9.0), 30 mM hydroxypropyl- γ -CD and 75 mM SDS as BGE gave rise to the simultaneous separation of 8-, 11-, 12-, and 15- hydroxyeicosatetraenoic acids into their enantiomers within 35 min. The feasibility of this methodology was demonstrated through the chiral analysis of 8-, and 12-hydroxyeicosatetraenoic acids in two species of red algae (*Gracilaria vermiculophylla* and *Gracilaria arcuata*).

Non-protein amino acids are relevant markers of food quality and safety. They can be present in foods as metabolic intermediates, as products formed during processing or as additives. Many of them are chiral molecules in which L enantiomer is the natural form while the presence of the D-enantiomer is because of the racemization from L-form produced by different formation metabolic pathways, processing conditions, or due to the fraudulent addition of racemic mixtures [10]. Pérez-Míguez et al., presented different analytical methodologies based on EKC-UV with anionic cyclodextrins for the enantioseparation of eight FMOC-derivatized non protein amino acids [35]. Moreover, after optimizing different experimental parameters the EKC method was applied to the enantiomeric analysis of citrulline in food supplements. LODs were 1.8×10^{-7} M and 2.1×10^{-7} M for L and D-citrulline respectively and after analysis of different supplements it was possible to show that the percentage of L-citrulline with respect to the labeled content decreased with the storage time. However, since the D-enantiomer was not detected in any of the samples analyzed it was not possible to attribute the decrease of L-citrulline to a racemization process.

All the above-mentioned methodologies are based on direct strategies in which the enantiomeric discrimination takes place in a chiral environment by the interaction between the enantiomeric mixture and a chiral selector. In spite of these direct methodologies are the most commonly used in chiral separations, during the period of time covered in this work, an

indirect approach has been also described to carry out the enantiomeric separation of D/L aldoses [135]. In this work, the analytes were labeled with (S)-(+)-4-(N,N-dimethylaminosulfonyl)-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole to form pairs of diastereomers which were separated using as BGE phenylboronate buffer containing SDS. The developed MEKC-LIF method was applied to the enantiomeric separation of D/L galactose in the hydrolysates of red seaweed, demonstrating its usefulness in the quality control of edible algae.

13. CE microchip technology in food analysis.

Microchip Electrophoresis (MCE) can result in a very attractive tool for rapid analysis of different hazardous substances in food, helping to ensure a food safety control. For instance, MEC has been employed for the detection of biogenic amines [136], polycyclic aromatic hydrocarbons [30] or two dyes, rhodamine 6G and rhodamine B [137] in food.

MCE shares advantages with CE like the high efficiency or the low reagent and sample consumption. Besides these benefits, MCE presents other advantages such as its great potential for miniaturization and the possibility of carrying out fast and real-time analysis.

However, despite these important analytical advances, its use is still limited, mainly due to the high cost of glass or silica microchips, but also because the difficulties that the thinner microchannels normally used in MCE add to the analysis. For this reason, Guo et al. [136] proposed the use of plastic materials as an alternative to reduce costs and simplified the manufacturing process. They achieved a stable cathodic EOF on a cyclic olefin copolymer (COC) microchannels achieving high efficiency separations of 4 biogenic amines in fish meat by employing sodium polystyrene sulfonate (PSSNa) as BGE. PSSNa was selected because it can act as a perfect anionic buffer component of the BGE, as well as it significantly increases the viscosity of the solution allowing a beneficial electrophoretic separation in the microchip.

In addition, PSSNa can produce a pseudo-stationary phase on COC microchips, improving the separation selectivity.

In the same vein, Wei et al. [137] established a MCE method using COC microchips for the analysis of rhodamine dyes in chili powder. The main problem of the separation of these compounds in COC microchips is the strong adsorption of the dyes on the COC surface. Authors solved this limitation using a highly organic solvent BEG with an ionic polymer, in particular, they used methanol as organic solvent and polyacrylic acid (PAA) as a multifunctional additive. The BGE composition was 30 mM phosphate at pH5, 80% methanol and 0.36% PAA. PAA worked as a viscosity regulator, selectivity modifier and as reducing agent of the analyte adsorption on the microchannel surface. With the optimal conditions, a successful separation of the two rhodamine dyes was achieved.

Another example of the use of MCE to ensure the food safety and quality was reported by Rezende et al. [138]. Authors developed a MCE method coupled to C⁴D, a powerful analytical platform for the analysis of ionic species in beverages, for the study of the authenticity of seized whiskey samples, that probably could be adulterated by the addition of tap water. For the study of this possible fraud, the presence of anionic species in the seized samples was monitored and compared with the original samples.

Besides food safety, MCE has been also employed for establishing food authentication. In particular, the wine differentiation and authentication, attending to compounds that may provide information about the botanical origin, provenance, vintage and quality such as phenols, organic acids, aldehydes, sugars, alcohols or neuroactive amines analyzed by MCE has been reviewed [17].

14. Foodomics applications

Foodomics studies the food and nutrition domains through the application and integration of omics technologies to improve consumers' well-being, health and knowledge [6]. Foodomics

applies mainly, transcriptomics, proteomics and metabolomics to boost investigations on food safety, quality and traceability and, to understand at molecular level how food and food ingredients act in our body. These studies present a high complexity because they have to solve important issues derived from food complexity, the huge natural variability, the large number of different nutrients and bioactive food compounds, their very different concentrations, their bioavailability and transformation in the human tract, the numerous targets with different affinities and specificities that might exist in the human body, etc., Due to the high complexity of this kind of studies, researchers in food science are moving to more advanced strategies and, in this context, CE- and CE-MS based methods can play an important role in Foodomics [139]. The works published on this topic have shown the huge potential of CE-MS in Foodomics through several interesting applications in Metabolomics. Thus, the optimization of an effective protocol for cell metabolomics was presented with special emphasis in the sample preparation and subsequent analysis of intracellular metabolites from adherent mammalian cells by CE-MS. As case study, colon cancer HT-29 cells, a human cell model to investigate colon cancer, were employed. The feasibility of the whole method for cell metabolomics was demonstrated via a fast and sensitive profiling of the intracellular metabolites from HT-29 cells [140]. The potential of CE-MS for metabolites profiling was also corroborated through the combined use of CE-MS with a novel non-covalent coating that allows the fast and efficient analysis of anionic metabolites [141]. A CE-MS metabolomic approach allowed the discovery of bioactive and flavor components in fermented food. Namely, CE-MS was applied to compare the metabolites found in milk fermented with GABA-producing *Lactococcus lactis* 01-7, compared with those found in control milk fermented without GABA production [142]. A metabolomics approach utilizing CE-TOF-MS, gas chromatography (GC)-TOF-MS and liquid chromatography (LC)-quadrupole (q)-TOFMS resulted in measurement of a total of 732 annotated peaks in a group of soybean samples that included six conventional and three genetically modified (GM) glyphosate-tolerant lines. The results did not show clear metabolic differences between the

conventional and GM lines [143], contrarily with what has already been observed by other authors who investigated transgenic maize and soybean [144-146].

Several of the Foodomics works recently published have involved the use of other different strategies [147] and have allowed the study of the effects of extracellular production of hydrogen peroxide by rosemary polyphenols on the anti-proliferative activity of rosemary polyphenols against HT-29 colon cancer cells [148] or how these compounds, i.e., rosemary polyphenols, induce unfolded protein response and changes in cholesterol metabolism in HT29 colon cancer cells [149]. These different analytical strategies have also involved the use of other liquid techniques (e.g., UHPLC, nano-LC) hyphenated to high resolution mass spectrometry and they have allowed the investigation of the faecal metabolomic fingerprint after moderate consumption of red wine [150], the peptidomic study of the in vitro gastrointestinal digestion of bovine haemoglobin [151].

Interestingly, several of the analytical steps necessary to carry out a metabolomic analysis are common among these hphenated techniques, including the data treatment. In this regard, the development of new data analysis strategies is also mandatory [152-154].

In a Proteomics work from our group, the proteome variations were investigated in order to understand (and to explain at molecular level) the effect of carnosic acid (CA) and carnosol (CS), two major compounds present in rosemary, against colon cancer HT-29 cells proliferation [155]. Namely, the shotgun proteomics study based on stable isotope dimethyl labeling (DML) and nano-liquid chromatography-tandem mass spectrometry (nano-LC-MS/MS) revealed that CA and CS induce different Nrf2-mediated response. Furthermore, it was also observed that each diterpene affects protein homeostasis by different mechanisms. CA treatment induces the expression of proteins involved in the unfolded protein response in a concentration dependent manner reflecting endoplasmic reticulum stress, whereas CS directly inhibits chymotrypsin-like activity of the 20S proteasome. The unbiased proteomics-wide method demonstrated to be a powerful tool to reveal differences on the mechanisms of

action of two related bioactive compounds in the same biological model [155]. The great activity of Rosemary polyphenols to reduce colon cancer progression was corroborated through an *in vivo* study combined with proteomics [156]. Namley, the effects of a polyphenol-enriched rosemary extract on xenograft tumor growth was studied *in vivo* using athymic nude mice together with a shotgun proteomic analysis based on nano-LC–MS/MS together with stable isotope DML (see Figure 5). The results showed that the daily administration of a polyphenol-enriched rosemary extract reduces the progression of colorectal cancer *in vivo* with the subsequent deregulation of 74 proteins [156].

15. Conclusions

The works presented in this review demonstrate the relevant role of CE in food analysis and Foodomics over the last two years. However, the existing limitations of this technique, mainly, its limited sensitivity and low reproducibility, still remain as important challenges for the scientific community. Some additional problems already mentioned in our previous review paper need still to be addressed in Foodomics, including, the lack of information in the databases (e.g. on the identity of many metabolites), the limitations generated by the current bioinformatic tools, our poor knowledge on many molecular processes taking place in cells, or the difficulty to combine the huge data generated by transcriptomics, proteomics and metabolomics approaches (e.g., via systems biology). These limitations are not specific of Foodomics, but general to any holistic approach that encompass the integration of big data from several levels of expression. In spite of these important limitations, the global picture that Foodomics can provide is a good response to solve many of the complex questions that still remain in current and future food science.

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Table 1. Some representative review papers on food analysis and Foodomics by CE published in the period February 2015-February 2017.

Subject	Publication year	Reference
CE-MS for metabolomics	2017	[7]
Foodomics imaging by MS and NMR	2016	[8]
Foodomics to differentiate organic and conventional foods	2016	[9]
CE of non-protein amino acids as food quality markers	2016	[10]
Capillary electrochromatography in food analysis	2016	[11]
Open tubular-capillary electrochromatography	2016	[12]
Amino acid analysis by CE methods	2016	[13]
Analytical chemistry methods in Foodomics	2016	[14]
CE with chemiluminescence detection in food analysis	2016	[15]
Simultaneous CE separation of cations and anions	2016	[16]
Microchip Electrophoresis for wine analysis	2016	[17]
CE of amino acids, proteins, carbohydrates and lipids in food	2016	[18]
CE of pesticides in environmental and food samples	2016	[19]
Cyclodextrins as chiral selectors in CEC	2016	[20]
Liquid microextraction and separation	2016	[21]
Chiral separations using miniaturized techniques	2016	[22]
Analysis of phenoxy acid herbicide residues	2016	[23]
Coupled solid-phase extraction-capillary Electrophoresis	2016	[24]
Capillary Electrophoresis with mass spectrometry	2015	[25]
Foodomics: exploring safety, quality and bioactivity of foods	2015	[26]
Foodomics in microbiological investigations	2015	[27]
Foodomics for investigations of food toxins	2015	[28]
Direct high-resolution mass spectrometry in foodomics	2015	[29]
Capillary and microchip analysis of PAHs	2015	[30]

Table 2. Some selected CE methods for food analysis and Foodomics published in the period February 2015-February 2017.

Compound	Analyzed Sample	CE mode/BGE	Detection technique	Ref.
<i>Amino acids, biogenic amines, heterocyclic amines and other hazardous amines</i>				
Phenylalanine	Cereals	MEKC BGE: 30 mmol L ⁻¹ phosphoric acid, 100 mmol L ⁻¹ SDS, and 25% methanol (v/v) at pH 1.9	UV	[33]
Amino acids	Microalgae	CE BGE: sodium tetraborate buffer (100 mM; pH 9.4) and β -cyclodextrin (10 mM)	LIF	[34]
Aliphatic, aromatic and heterocyclic biogenic amines	Beer	CE BGE: 12.0 mmol L ⁻¹ Imidazole as the UV probe and 10.0 mmol L ⁻¹ α -cyclodextrin as the additive (at pH 4.50 with acetic acid).	UV	[36]
2-methylimidazole, 4-methylimidazole, 2-acetyl-4-tetrahydroxy-butylimidazole	Caramel colors	CSEI-sweeping-MEKC BGE: pH 2 phosphoric acid (20 mM) and 140 mM SDS	UV	[38]
Acrylamide, asparagine and glucose	Bread samples	AFILMC-CE BGE: 12.5 mmol L ⁻¹ phosphate buffer (pH 8.5)	UV	[39]
Biogenic amines	Fish meat	MCE BGE: 0.3% PSSNa (pH 11.5)	LIF	[136]
<i>Peptides and proteins</i>				
Proteins	Milk	CZE BGE: 100 mM phosphate (pH 2.5)	UV	[43]
Peptides	Human milk	CE	MS	[131]
Proteins	Liquid eggs	CE BGE: 300 mM borate buffer with 25 mM SDS (pH 9.2)	UV-VIS	[44]
Peptides	Cheese	CZE BGE: 10 mM sodium phosphate, 6 M urea, 0.05 % hydroxypropyl methyl-cellulose (pH 3.0)	UV	[51]
Glycated proteins	Model systems	CE	UV	[46]
<i>Phenols, polyphenols</i>				
Different phenolic compounds	Rice	NACE BGE: 20 mM borate buffer in	UV	[62]

methanol (pH 9.0)				
Flavonoids	Honey	CE BGE: 10 mM sodium borate, 8% methanol, 2% 1-propanol	LED-IF	[64]
<i>Lipids</i>				
Fatty acids	Milk	CE BGE: 100mM NaH ₂ PO ₄ and Na ₂ HPO ₄	UV	[65]
Transfatty acids	Processed foods	CE BGE: Na ₂ B ₄ O ₇ , Brij 35, methanol, acetonitrile and water	UV	[66, 67]
<i>Carbohydrates</i>				
Sugars	Honey	CE BGE: 10 mM sodium benzoate, 1,5 mM ceteryl- trimethylammonium bromide (pH 12.4)	UV	[68]
Mono- and disaccharides	Broths	CZE	UV	[69]
Carbohydrates	Breakfast cereals	FSCE	UV	[70]
Oligosaccharides	Animal Milk	CE	UV	[71]
Carbohydrates	Food samples	CE BGE: 20 mM sorbic acid, 0.0056% (w/v) HDB and 40 mM NaOH (pH 12.2)	UV	[72]
<i>DNAs</i>				
Free nucleotide monophosphates	Human milk	CE	MS	[130]
Allergenic ingredients: pistachio, oat, sesame, peanut, cashew, barley, wheat, soybean, maize.	Commercial food products: cakes, cookies, crackers, waffle sticks, cocktail nuts, porridge oats, juice, noodles, pistachios, chocolate, mixed nuts, milk, candies, soy sauce paste, and powdered beef soup	Multiplex-PCR-CE	UV	[74]
SNPs located within lupeol synthase genomic fragment and within cycloartenol synthase genomic fragment	Virgin olive oil	SNP-based PCR-RFLP- CE	UV	[75]
Foodborne pathogen genes (GroEL, glyA, MMS, tuf, inv, ipaH, nuc, vvh, and 16S rRNA)	Animal-derived food samples (specifically, milk and sliced ham)	CE-SSCP-MLPA	UV	[81]

<i>Vitamins</i>				
Water-soluble vitamins	Energy drinks, sport drinks and fruit nectars	CE	DAD	[83]
Vitamin B2	Anatolian monofloral and honeydew honeys	CE	LIF	[84]
Vitamin B2	Saffron samples	CE BGE: 20 mM borate buffer (pH 9.5)	LIF	[85]
<i>Small organic and inorganic compounds</i>				
Organic acids	Blueberry juice	CE BGE: 40 mM ammonium acetate (pH 6.0)	MS	[88]
Alkaloids	Onion nectar	MEKC BGE: 20 mM sodium tetraborate, 100 mM SDS (pH 9.30)	UV	[95]
<i>Toxins, contaminants, pesticides and residues</i>				
Saxitoxin	Shellfish sample (mussel)	CE BGE: 20 mmol L ⁻¹ NaH ₂ PO ₄ , 5.0 mmol L ⁻¹ Na ₂ B ₄ O ₇ buffer solution (pH 6.0)	ICP-MS	[102]
Saxitoxin and its analogues dcSTX, neoSTX, and gonyautoxin epimers	Drinking water	FASI-CE BGE: 40 mM Britton–Robinson buffer (pH 9.5)	UV	[103]
Organophosphonates and quaternary ammonium herbicides	Beer	SECS BGE: 150 mmol L ⁻¹ sodium phosphate at pH 2	UV	[104]
Imazalil, prochloraz and thiabendazole fungicides	Apples, cherry tomatoes and grape juice	NACE BGE: methanol-acetonitrile (35:65, v/v), 30 mmol L ⁻¹ ammonium chloride and 0.5% phosphoric acid.	UV	[107]
Bisphenol A, α -Naphthol and β -Naphthol	Drinks and lake water	FASI-CE BGE: 0.5 M Na ₂ B ₄ O ₇ solution (pH 9.5) in 30 % (v/v) methanol	UV	[110]
Amoxicillin, cephalixin, oxacillin, penicillin G, cefazolin, and cefoperazone	milk and egg matrices	LVSS-MEKC BGE: 10 mM phosphate and 22 mM SDS at pH 6.7	UV	[112]
Sulfadoxine, sulfisoxazole, sulfamerazine, and sulfamethoxazole	Milk	Graphene-based MSPE- CE BGE: 60 mmol L ⁻¹ Na ₂ HPO ₄ , 2 mmol L ⁻¹ ethylenediamine-tetraacetic acid disodium salt and 24% (v/v) methanol	UV	[114]
Norfloxacin	Chicken, pork, fish, milk	CEIA BGE: Na ₂ B ₄ O ₇ /NaH ₂ PO ₄ buffer (30 mmol L ⁻¹ , pH 8.0)	LIF	[117]

Chloramphenicol, CIP, nitrofurantoin antibiotics, and their metabolites	Fishery products	CE BGE: 20 mmol L ⁻¹ NaH ₂ PO ₄ , 20 mmol L ⁻¹ Na ₂ HPO ₄ , 80 mmol L ⁻¹ sodium deoxycholate, and 10% methanol (v/v) at pH 9.0		[119]
<i>Food additives</i>				
Sugar alcohols	Sugar-free chocolates	CZE BGE: 25 mM borate (pH 8.5)	C ⁴ D	[124]
Preservatives	Food samples (including sauce, pickle, ham, beverage, candied fruit, cheese, pastries, moon cake, jelly)	MEKC BGE: 15 mM tetraborate, 60 mM boric acid, 100 mM SDS	UV	[127]
<i>Chiral compounds</i>				
Tartaric and malic acids	Wines	LECE BGE: 100 mM D-quinic acid, 10 mM Cu(II), 0.5 mM Al(III), 0.5 mM HCTA-OH (pH 5.0)	UV	[133]
Citrulline	Food supplements	EKC BGE: 10 mM sulfated- α -cyclodextrin in 100 mM formate (pH 3.0)	UV	[35]

Figure captions

Figure 1. Electropherograms illustrating the chiral separation of different FMOC-non-protein amino acids (0.2 mM) obtained under the best separation conditions. Pyro; using sulfated α -CD (S- α -CD) at 15°C, Norval; using sulfated γ -CD (S- γ -CD) at 25°C, Norleu; using sulfated α -CD (S- α -CD) at 15°C, DOPA; using sulfated γ -CD (S- γ -CD) at 25°C, Amino adipic; using sulfated γ -CD (S- γ -CD) at 15°C, SeMet; using sulfated γ -CD (S- γ -CD) at 15°C, Cit; using sulfated γ -CD (S- γ -CD) at 15°C, and Pipe; using sulfated γ -CD (S- γ -CD) at 25°C. *Indicates the derivatizing reagent (FMOC). Redrawn from [35] with permission from Elsevier.

Figure 2. (A) Illustration of the formation of polydopamines and titanium oxide nanoparticles deposited on the polydopamine coated open tubular column. (B) CZE-UV electrochromatograms corresponding to the separation of standard proteins (a) and diluted egg white (b). Experimental conditions: capillary, 50 μm x 39.2 cm (29.2 cm effective length); BGE, 40 mM phosphate (pH 9.0); voltage, 15 kV; temperature, 25 $^{\circ}\text{C}$; detection wavelength, 214 nm; injection, 3.45×10^3 Pa for 5 s. Peak identification: (1) conalbumin, (2-9) glycoisoforms of ovalbumin. Redrawn from [50] with permission from Elsevier.

Figure 3. (A) SEM images of MWNTs after (pristine MWNTs) (1) and before the functionalization with surfactant (SC-MWNTs) (2); B: Electropherograms of the 12 phenolic compound of chrysanthemum samples without chitosan/SC-MWNTs (1) and with chitosan/SC-MWNTs (2). Redrawn from [59] with permission from Springer.

Figure 4. Electropherograms of a fruit nectar with added vitamins (A), a sport drink (B) and an energy drink (C) obtained using a BGE containing 40 mM borate at pH 8.5, 40 mM of SDS and 5% MeOH. Peak identification: 1, caffeine; 2, sorbic acid and 3, benzoic acid. Redrawn from [83] with permission from Elsevier.

Figure 5. Simplified representation of proteomics data. (A) Total ion chromatogram of a nanoLC–MS/MS run. (B) Mass spectrum at minute 87.50 from panel A. The doubledcharged MTDQEAIQDLWQWR peptide ($M_r = 1818.8359$ Da) was found as light, medium and heavy labeled in all the samples. (C) Extracted ion chromatogram of the m/z ranges 924.43–924.45 (light), 926.44–926.46 (medium) and 928.45–928.47 (heavy). The intensity of the heavy labeled form (from rosemary pre-treated mice) was lower than the light (from pooled sample) and the medium (from vehicle-control) forms. Redrawn from [156] with permission from Elsevier.