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

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Running title: Food analysis by CE

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Abbreviations: AD, amperometric detection**; ASP,** amnesic shellfish poisoning; **BC**, bacitracin; **BGE,** background electrolyte; **BHA**, butylated hydroxyanisole; **BHT**, butylated hydroxytoluene; **CCD**, charge-coupled device; **C^4D**, coupled contactless conductivity detection; **CD,** conductivity detection; **CytC**, cytochrome C; **DA**, domoic acid ; **DAD**, diode array detection; **DMA-EpyM**, N,N-dimethylacrylamide-ethylpyrrolidine methacrylate; **DNS,** dansyl chloride; **ED**, electrochemical detection; **FASI**, field amplified samples injection; **FITC,** fluorescein isothiocianate; **GMO,** genetically modified organism; **HEC**, hydroxyethylcelullose; **LED**, light-emitting diode; **LPA**, ligation-dependent probe amplification; **MEEKC,** microemulsion electrokinetic chromatography; **MRLs,** maximum residue limits; **OA**, ochratoxin A; **PAD**, pulsed amperometric detection; **PCR-RFLP**, PCR based restriction fragment length polymorphism; **PFSG**, programmed field strength gradients; **PG**, propyl

gallate; **PMMA**, poly(methyl methacrylate); **QIT**, quadrupole ion trap; **SERRS**, surface-enhanced resonance raman spectroscopy; **SPPM**, system for prediction of peptide migration; **TBHQ**, tert-butylhydroquinone.

Keywords: capillary electrophoresis, foods, beverages, review, MEKC, proteins, amino acids, peptides, DNA, phenols, pesticides, toxins, additives.

ABSTRACT

This review covers the application of capillary electromigration methods to analyze foods and food components, including amino acids, biogenic amines, peptides, proteins, DNAs, carbohydrates, phenols, polyphenols, pigments, toxins, pesticides, vitamins, additives, small organic and inorganic ions, chiral compounds, and other compounds in foods, as well as those applications of capillary electrophoresis (CE) for monitoring food interactions and food processing. The use of microchips as well as other foreseen trends in food analysis by CE are discussed. Papers that were published during the period June 2005-March 2007 are included following the previous review by Cifuentes (*Electrophoresis* 2006, 27, 283–303).

CONTENTS

1. Introduction.

Capillary electrophoresis (CE) is a separation technique that provides fast and efficient separations in an automated way with minimum consumption of sample and reagents. Nowadays, CE represents an interesting strategy for the determination of many compounds in foods. Thus, CE has already demonstrated its potential and versatility for solving multiple analytical problems in Food Science, including compliance with food and trade laws, adulteration and/or contamination detection, quality control investigations, study on chemical composition of foods, etc [1]. This review intends to provide to the readers with an updated overview on the innovative developments reported in food analysis using CE methods covering the literature published from June 2005 to March 2007.

Although the major applications of CE to the analysis of food and food components remained essentially those discussed in previous reviews [1], it is noteworthy the increasingly growing interest on the application of CE to the food research field that is reflected by the high number of works and reviews published on this topic during the period covered by this review. For instance, the following reviews have appeared in the literature during the mentioned period dealing with the application of CE methods to the analysis of interesting compounds in food analysis, including amino acids [2], antibiotics [3], bioactive amines [4], phenolic compounds [5], flavonoids [6], natural antioxidants [7], carboxylic acids [8], pesticides [9-11], proteins [12, 13], and pigments [14]. In addition, some other published reviews were focused on discussing the different applications of a particular CE technique, including food analysis. This is the case for instance of CE coupled to mass spectrometry (MS) [15-17], electrochromatography [18], CE coupled to chemiluminiscence detection [19], microemulsion electrokinetic chromatography (MEEKC) [20], sample treatment prior to CE-MS [21].

The following sections of this review describe the numerous CE approaches recently taken to detect compounds of significance to Food Science and Technology including the analysis of amino acids, biogenic amines, peptides, proteins, DNA, carbohydrates, phenols, polyphenols, pigments, toxins, additives, vitamins, small organic and inorganic ions, chiral compounds, pesticides and other residues in foods, as well as to investigate food interactions and food processing.

2. Amino acids, biogenic amines and nitrosamines.

Analysis of amino acids remains of great importance in Food Science since it can provide relevant information on food quality, processing, adulteration and/or food composition. Carrasco-Pancorbo *et al.* [22] developed a novel capillary electrophoresis-laser induced fluorescence (CE-LIF) method for the detection of amino acids using a copolymer made of N,N-dimethylacrylamide-ethylpyrrolidine methacrylate (DMA-EpyM) as capillary coating. DMA-EpyM provided a physically adsorbed coating by only flushing the capillary with a solution containing the copolymer between runs without adding the copolymer to the background electrolyte (BGE). The amino acids glutamic acid, aspartic acid, glycine, threonine and tryptophan were derivatized with fluorescein isothiocianate (FITC) and used during the optimization of CE-LIF method. The reproducibility study demonstrated good intraday and interday precision of the method with relative standard deviation (RSD, n=8) values of the migration times lower than 0.65% and 1.03%, respectively, indicating the good stability of the coating at both, short- and long-term use. Chen *et al.* [23] developed a capillary electrophoresis method with diode array detection (CE-DAD) for the detection of 18 amino acids in edible marine algae. Thus, amino acids were derivatized with phenylisothiocyanate that under acidic conditions produce phenylthiohydantoin derivatives. The separation of the mixture containing 18 derivatized amino acids was improved in bare fused-silica capillaries by adding 1 mM

ethylenedimine to 0.4 M tris-boric acid buffer at pH 9.5. Good precision was demonstrated for this method, providing RSD (n=7) values lower than 3.2% and 1.1% for peak area and migration time, respectively. The linearity was established over the concentration range 50 to 1000 µM for each derivatized amino acid, with correlation coefficients (r) ranging between 0.9904 and 0.9993.

Biogenic amines are a group of low-molecular mass organic bases that are present in a large variety of foods. Excess uptake of biogenic amines has been associated with different negative physiological effects in humans. In addition, biogenic amines may also be considered as carcinogens due to their ability to react with nitrites to form potentially carcinogenic nitrosamines [24]. Hence, Sanches *et al.* [25] proposed the combined use of supercritical fluid extraction and MEKC-UV for the complete analysis of volatile nitrosamines in spiked sausages. A careful optimization process of the extraction method was carried out and the results demonstrated that this approach is a good alternative for the extraction and fast analysis of volatile nitrosamines with an important reduction in the solvent waste. However, the limited sensitivity, within the mg/kg range, was an important restriction for its application to real samples that usually contain nitrosamines within the µg/kg range. This limitation could be overcome by using extraction processes that allow working with larger amount of samples.

In the last years, the increased number of novel CE methods proposed for the determination of biogenic amines in foods reflects the current interest in these compounds [4]. Recent developments for the analysis of biogenic amines in CE include novel derivatization procedures and concentration strategies prior to CE analysis. Thus, Cao *et al.* [26] studied factors affecting the derivatization reaction of eight biogenic amines with 6-oxy-(N-succinimidyl acetate)-9-(2'-methoxy-carbonyl) fluorescein and their separation by micellar electrokinetic chromatography

(MEKC-LIF). Optimal separation and detection of the derivatized amines with limits of detection (LODs) within the interval 0.25-2.5 nM were achieved using a BGE containing 30 mM boric acid at pH 9.3, 25 mM sodium dodecyl sulfate (SDS), and 20% v/v ACN. The method was successfully used to monitor biogenic amines in fish samples. Alternatively, Krausova *et al.* [27, 28] extended the application of a MEKC with UV detection to the analysis of biogenic amines in numerous meat matrices, including young bull, cow, pig and chicken livers. A new procedure based on the use of CZE with lamp-induced fluorescence detection and 4-fluor-7-nitro-2,1,3-benzoxadiazole as derivatization reagent was developed to determine histamine and polyamines including spermine, spermidine, diaminopropane, putrescine, cadaverine and diaminohexane [29]. A detection limit for the amines (10 nM) comparable to other CE analytical methods without using laser as the excitation radiation was achieved. Recently, Cortacero-Ramírez *et al.* [30] have optimized a derivatization procedure using FITC for the determination of ten biogenic amines by CZE-LIF. Parameters affecting the sensitivity and resolution of the CE separations were examined including pH, electrolyte composition, voltage, injection time and the addition of organic modifiers to the separation buffer. The reported detection limits of the amines in beer ranged from 5 to 198.3 µg/L. The linearity and precision of the method was good and allowed determining the concentration of biogenic amines in beers and samples taken at different stages of the brewing process. In-capillary derivatization with 1,2-naphthoquinone-4-sulfonate has been also proposed for the quantification of biogenic amines with UV detection [31]. This procedure is based on the zone-passing strategy with a mixed tandem injection mode. Thus, plugs of 1,2-naphthoquinone-4-sulfonate solution and BGE were injected one after the other. After the injection of the sample, the separation voltage was applied and the online reaction took place inside the capillary when the amines mixed with the reagent. Besides, field-amplified sample stacking was applied to enhance the sensitivity of the CZE-UV method for the detection of 9 biogenic amines in red wines from different Spanish

regions. The reported LODs ranged from 0.02 to 0.91 mg/L and the RSDs for the migration time and area were around 1.2 and 6.2%, respectively. Similar LOD values for the analysis of amines in fish, meat and sausage were reported by Ruiz-Jiménez and Luque de Castro [32] using on-line coupling of a pervaporation module with a CZE-UV system. The coupling was aimed at avoiding the problems arising from the complex sample matrix and the low concentration levels at which amines are present in the samples. This CZE method did not required derivatization step and the detection was based on indirect UV absorption. Similarly, using UV detection, Rossano *et al.* [33] monitored the histamime content in anchovies under different storage conditions by CZE.

3. Proteins and peptides.

Analysis of proteins and peptides represents one of the main applications of CE in Food Science since CE, besides assisting in quality control of food, provides considerable information e.g., to the identification of cultivars, to detect adulterations, etc. The most recent applications of CE to the analysis of proteins in food include the characterization of protein extracts to provide information about the protein composition with the aim of: (a) determining the origin of the ingredients [34], the technological process employed to produce a particular food [35], or the quality of a protein extract [36]; (b) detecting high-quality products adulterated with products of inferior quality [37, 38]; and (c) monitoring and optimizing technological processes [39, 40]. In addition to these applications, it is worth noting last approaches based in CE coupled to mass spectrometry (MS) and developed to predict the amino acid composition of protein hydrolysates [41, 42], as well as the novel procedure to provide simplified 2-D mapping of CE-MS data in order to simplify the discovery and detection of specific markers [43].

CE has demonstrated to be a good alternative separation technique for the analysis of milk of goat, cow and sheep [1, 13]. Genetic differences and polymorphisms in milk proteins composition are of great interest to the cheese industry because of the existing correlations to firmness, coagulation time, casein content and cheese yield. Clement *et al.* [34] analyzed the different polymorphs of proteins present in milk of different breeds of sheep (Awai, Merino, East Friesian and their crosses). To do this, milk casein and whey protein fractions were analyzed by CE-UV with an uncoated fused-silica capillary and a low pH buffer containing urea and hydroxylpropylmethylcellulose. Although ovine casein fractions were satisfactorily resolved in less than 30 min, the silent variants, due to amino acid substitutions that do not lead to a change in the net charge of the proteins, could not be detected by the proposed CE method. In a separate paper, the analysis of protein composition by CE-UV, using hydrophilically coated capillaries and an acidic buffer, allowed the characterization of some fresh cheeses (Quarg, Burgos and Mozzarella) in relation to their coagulation agent, the milk heat treatment or ultrafiltration process and the origin of the milk [35].

Food authenticity is nowadays a hot topic in food analysis. Thus, a common problem in grain legumes is the replacement of high-quality seeds by other seeds with high resemblance but that can be toxic or to present poorer quality. This is the case for lentil (*Lens culinaris* Medik.) that often is contaminated by seeds of vetch (*Vicia* spp.) that contain toxic compounds. Piergiovanni and Taranto [37] analyzed protein extracts from five lentil cultivars and two vetch species by CZE-UV using uncoated fused-silica capillaries and a BGE containing imino diacetic acid, hydroxypropylmethylcellulose and 20 % ACN. The discrimination of lentil cultivars was based mainly on differences on peak heights. These results restricted the usefulness of the procedure for differentiation of lentil cultivars, since the content of proteins may be affected by environmental and growing conditions. On the other hand, it was advantageous to discriminate

lentils and vetch seeds which showed distinctive peak profiles. In addition, the CZE-UV method allowed the detection of the vetches in contaminated lentils mixtures. A similar approach has been recently explored for detection of smoked paprika “pimenton de La Vera” adulteration [38]. Several CE parameters were optimized for the separation of methanol-soluble protein extracts from paprika samples in uncoated silica capillaries. Under optimum separation conditions, qualitative and quantitative differences were detected among the CZE-UV profiles of paprika elaborated with different varieties of pepper. With this method, it was possible to detect as low as 5% adulterant paprika in mixtures.

Capillary electrophoresis has been used to characterize rize protein isolates obtained by different extraction procedures [39]. The CE-UV method, based on a commercial kit, provided better resolution and it was able to separate the protein fractions over a wider molecular weight range than classical polyacrylamide gel electrophoresis with SDS. However, it was also demonstrated that the method employed for isolating rice protein fractions affects their purity and protein profile obtained by CE.

CE coupled to MS has been proposed as a good alternative to monitor and optimize pressurized liquid extraction of phycobiliproteins from *Spirulina platensis,* an edible microalga [40]. To do that, different extraction conditions were tested, including time, temperature, pressure of extraction, nature of pressurized liquid, and distribution of microalga inside the extraction cell. Significance of sample matrix in CE-MS analysis was also demonstrated since ultrafiltred extracts from microalga provided better CE resolution and MS signals than direct analysis of crude extracts. The combined use of pressurized liquid extraction and CE-MS under optimum conditions provided yields as high as 20% of phycobiliproteins in less than 2 h.

Apart from the aforementioned CE applications for food proteins, the detection and identification of peptides is also an important topic. Two novel procedures aimed at determining the amino acid sequence of peptides of interest for the food industry have recently been published. Thus, Simó *et al.* [41] combined the use of a theoretical model that correlates electrophoretic behavior of peptides to their sequence together with CE-MS [44, 45] for the study of the cleavage activity of a recombinant pepsin versus its corresponding natural variety using cytochrome C (CytC) as a substrate. To do this, hydrolysates were first analyzed by CE coupled to electrospray ionization mass spectrometry (ESI-MS). Using the mass values of the peptidic fragments and a sequence search program only the sequence of two peptides could unambiguously be characterized, while for the rest of fragments many possible peptidic forms came out. Thus, a simulation program called "system for prediction of peptide migration" (SPPM) was used to elucidate in a fast and simple way the most probable peptidic sequence for each fragment. Namely, SPPM was used to simulate the migration times of the probable peptides under given separation conditions, In this way, the most probable sequence of each peptide could be easily found by comparing the simulation with the experimental separation (see an example in **Figure 1**). Using this procedure a differential cleavage activity of recombinant and natural pepsine could be established in a fast and simple way.

Following a similar approach, Tessier *et al.* [42] proposed a procedure based on the combined use of two models to predict the amino acid composition of small peptides contained in protein hydrolysates. The procedure was applied to a complex rapeseed protein hydrolysate and allowed the determination of the amino acid sequences of 16 out of 30 peptides.

4. Phenols, polyphenols and pigments.

Significant attention is nowdays given to phenolic compounds because of their potential health benefits including antioxidant, antiinflammatory and antimicrobial activities, as well as the reduction of the risk of coronary heart diseases, circulatory disorders, some types of tumors and chronic diseases. In food analysis, CE has sufficiently demonstrated its potential and versatility for characterizing the phenolic fraction of different foods with the aim of determining its pharmaceutical, cosmetic, nutraceutical or technological value while ensuring quality and compliance with food and trade laws. Following this idea, diverse CE modes have been applied to the study of phenolic compounds. Thus, microemulsion electrokinetic chromatography (MEEKC) has been used in combination with the anion-selective exhaustive injection-sweeping technique for the detection of 8 phenolic compounds (syringic acid, p-cumaric acid, vanillic acid, caffeic acid, gallic acid, 3,4-dihydroxybenzoic acid, p-hydroxybenzoic acid and (+)-catechin), which are present in many plant-derived foods [46]. The method is based on the sequential injection of short plugs of an acidic solution and dionized water into the capillary filled with a microemulsion solution composed of 1.36% w/v heptane, 2.89% w/v SDS, 7.66% w/v cyclohexanol, 2% w/v ACN, and 86.1% w/v 25 mM phosphate buffer at pH 2.0. Similar to a field-amplified sample injection strategy, the acid analytes were electrokinetically injected. After that, the sample vial is replaced by a vial containing the microemulsion solution to proceed with the separation of the stacked analytes at negative polarity. This procedure afforded about 96000-fold to 238000-fold increases in detection sensitivity in terms of peak areas without any separation efficiency loss when compared to normal MEEKC separation. Hence, trace levels (about 3 ng/g) of gallic acid and catechin in foods could successfully be detected by this technique.

A new separation method, based on coelectrosmotic CE, was developed for simultaneous separation of eight phenolic compounds in cereal and beer samples [47]. Coelectrosmotic CE technique requires that the direction of the electrosmotic flow (EOF) to be the same as the electrophoretic mobility of the ions. With this technique the response was linear over three orders of magnitude with detection limits (S/N of 3) between 40 ng/mL for vanillic and syringic acids and 70 ng/mL for sinapic and p-hydroxybenzoic acids within analysis times shorter than 3.5 min.

A comparative study about the amount of elenolic acid, ligstroside aglycon, oleuropein aglycon, and (+)-pinoresinol found in the polyphenolic fraction in seven varieties of extra-virgin olive oils was carried out by CZE-UV [48]. Results indicated the importance of the extraction procedure to determine the correct amount of polyphenols in virgin olive oil. The characterization of the phenolic fraction in hops (female flowers of the species *Humulus lupulus* L.) is an interesting topic to the brewing industry. In this regard, CZE-ESI-MS has demonstrated to be an attractive technique for this type of determinations [49]. During the optimization of the CZE-ESI-MS method, the influence of several variables on the CE migration, MS sensitivity and peak shape of the analytes was investigated. Some flavonoids glycosides and chalcones were identified comparing four extraction procedures of polyphenols and phenolic acids from hop.

Recently, a fast method for the analysis of 10 characteristic compounds of the phenolic fraction (resveratrol, (-)-epicatechin, (+)-catechin, malvidin-3-glycoside, peonidin-3-glycoside, cyanidin-3-glycoside, delphinidin-3-glycoside, kaempferol, myricetin, quercetin) in grape skin has been published [50]. These compounds were extracted using superheated ethanol-water at 120 ºC and 80 bar for 30 min. After extraction, the analytes were separated and detected in 10 min by CZE

with simultaneous dual diode array absorption and fluorescence detection to improve selectivity. The method was applied to commercial extracts of grape skin demonstrating its utility for the fast screening of the phenolic fraction.

The use of synthetic phenolic antioxidants as food additives is questioned since their effect on human health is still uncertain. Consequently, most countries have imposed regulations limiting the amount that can be added to foods. MEKC has recently been applied with different detection systems to determine the content of these compounds in foods. Simultaneous determination of propyl gallate (PG), tert-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) has been carried out by MEKC with electrochemical detection (ED) [51]. Effects of the potential of the working electrode, pH of the BGE, SDS concentration, injection time, and separation voltage on MEKC-ED were investigaded. Under optimum conditions, the analytes were separated in a 20 mM boric-borate buffer at pH 7.4 and 25 mM SDS within 13 min. A 300 μm diameter carbon disk electrode exhibited a good response at +950 mV for all analytes. It was concluded that this MEKC-ED method could be used for the determination of PG, TBHQ, BHT and BHA in mushroom cream, fish soup and vegetable oil samples. The reported LODs ranged from 0.29 to 2.7 μM for PG and BHT, respectively. In another report, Guan *et al.* [52] used CZE-ED for the determination of vitamin C, chlorogenic acid, caffeic acid, quercetin, and rutin in sweet potato.

Delgado-Zamarreño *et al.* [53] have recently reported the application of MEKC-UV for BHA, BHT and PG analysis in edible oils. The BGE employed consisted of an aqueous solution with 20% acetonitrile, 20 mM tetraborate buffer at pH 9.2 and 20 mM bis-(2-ethylhexyl)sodium sulfosuccinate surfactant. Studies involving solid-phase and liquid-liquid extraction were carried out observing that the best methodology for the isolation of antioxidants was extraction with

acetonitrile from edible oil diluted with hexane. The proposed MEKC-UV method was useful to determinate BHA, BHT and DG at levels permitted in the European Union.

The separation of 13 phenolic acids in standard solutions has been also investigated by CZE-UV using a physically adsorbed polymer (DMA-EpyM) as capillary coating [22]. A BGE composed of 25 mM di-sodium hydrogen phosphate buffer and 20% v/v 2-propanol was applied to the simultaneous and reproducible detection of 4-hydroxybenzoic acid, gentisic acid, protocatechuic acid, vanillic acid, gallic acid, trans-cinnamic acid, 4-hydroxyphenylacetic acid, o-coumaric acid, p-coumaric acid, dopac, caffeic acid, ferulic acid and sinapinic acid.

Isoflavones have attracted much attention due to their recognized health benefits against many hormone-dependent diseases such as osteoporosis, cancer and cardiovascular diseases. The separation of six isoflavones (three aglycones and three glycosides) extracted from soy germ capsules was carried out by MEKC-UV with a BGE composed of 10 mM sodium tetraborate at pH 9.3, 40 mM SDS and 1% v/v methanol [54]. This method afforded the baseline separation of the analytes studied with good precision, *i.e.* RSDs (n=6) better than 0.29% and 1.5% for migration time and peak areas, respectively. Linear responses with good coefficients of correlation ($\geq$ 0.9999) were obtained using standard solutions of isoflavone mixtures in the 1.6-50 µg/mL range.

Dinelli *et al.* [55] reported the use of CZE-DAD to study compositional changes induced by UV-B radiation treatment of common bean and soybean seedling. Flavonoid content was monitored in UV-B-treated and untreated sprouts of three common beans and one soybean. The flavonoids daidzein, glycitein, genistein, and kaempferol were detected at ppm level in plant matrices within 16 min. The optimized separation buffer contained 50 mM ammonium acetate at

pH 10.5 and 20% v/v methanol. The proposed CE method allowed the identification of sprouts of two common bean genotypes (*Verdone* and *Zolfino*) as a potential dietary source of isoflavones.

Anthocyanins are a group of plant polyphenols of interest to food industry due to their anti-oxidative and antimicrobial activity. The detection of these colored compounds in wine and wine musts has been investigated by CE-MS analysis [56] using two electrolytes: an acidic one (chloroacetate-ammonium, pH 2) and a basic one with high selectivity towards derivatives containing vicinal hydroxy groups (borate-ammonium, pH 9). The optimized methods allowed the identification and quantification of several anthocyanins in red wine and must demonstrating its utility for monitoring wine-production technology.

Curcuminoids are yellow polyphenolic pigments found in the turmeric herb. The content of the curcuminoids in turmeric is directly related to its quality. The quantitative analysis of curcuminoids, such as curcumin, demethxycurcumin, and bis-demethoxycurcumin was investigated by MEEKC-DAD [57]. Optimization of the separation parameters affecting the retention factor, selectivity, efficiency and resolution was carried out. Among the parameters investigated, the type and percentage of organic cosolvent, especially 2-propanol, had a strong impact on resolution. On the other hand, an increase in the concentration of SDS surfactant resulted in an insignificant change in the separation properties. The use of high temperature and voltage provided worse resolution mainly due to a decrease in efficiency by Joule heating. The optimal composition of the microemulsion buffer was established as 50 mM phosphate buffer at pH 2.5, 1.1% v/v n-octane as oil droplets, 180 mM SDS as surfactant, 890 mM 1-butanol as cosurfactant, and 25% v/v 2-propanol as organic cosolvent.

5. Carbohydrates.

Carbohydrates are analytes of considerable significance in biochemistry, medicine, biotechnology, food science, etc. At the present, there is an important demand of analytical methods that allow the determination of these compounds in food. However, owing to the high number of isomeric forms arising from the various possible arrangements of the monosaccharides and their lack of chromophore groups, the development of analytical methods for carbohydrates is not straightforward. Within the period covered by the present review, several CE methods based on different detection strategies have been developed for carbohydrates. Andersen *et al.* [58] widen the application of an already developed CZE method that used indirect UV detection [59] to the analysis of α-galactosides (raffinose, stachyose, verbascose and ajugose) in various plant species belonging to the genera *Lupinus*, *Pissum*, *Brassica*, and *Hordeum*. The CZE method in combination with chemometrics demonstrated that the profile of α-galactosides in the seeds depended on the genera and on the plant species to a certain extend. However, authors indicated that it would be necessary to analyze a larger data set to make a prediction of plant origin when unknown α-galactosides-containing materials are analyzed.

An alternative strategy is the use of laser-induced fluorescence detection (LIF) to monitor carbohydrates by CE [60]. Prior to CE-LIF analysis, the samples containing oligosaccharides were derivatized using 9-aminopyrene-1,4,6-trisulfonic acid trisodium salt. The separations were performed in polyethylene oxide-coated capillaries using 25 mM lithium acetate solution as running buffer. This approach was applied to the analysis of structural isomers of short oligosaccharides in various plant substrates. Using this method, baseline resolution of three different galactobiose isoforms, including β-1,4, α-1,4 and α-1,3 galactose disaccharides was demonstrated.

CE analysis of carbohydrates with photometric detection using a 406 nm light-emitting diode (LED) is an interesting approach because it offers some advantages over conventional light sources due to their superior stability and low noise. Momenbeik *et al*. [61] used the LED detection system coupled to CZE to determine carbohydrates derivatized with p-nitroaniline in food samples. Several parameters affecting the separation selectivity were studied using an artificial neural network able to predict mobilities including as response factor the quality of the final separation. Under optimum conditions, provided by the model, nine derivatized sugars were baseline resolved in alkaline borate buffer in less than 20 min showing an excellent agreement between the experimental and predicted mobilities. The benefits of using a LED over a deuterium lamp were also demonstrated. A comparison between both detection systems indicated LED was 30 times more sensitive than normal deuterium lamp. The applicability of this method to the analysis of carbohydrates in complex samples was also demonstrated by the analysis of infant milk, powdered milk, rice syrup and cola drink.

6. DNAs.

Several approaches have recently been proposed based on the analysis of DNA by CE methods [62-70], these applications include e.g., the detection of food-borne pathogens, genetically modified organisms (GMOs) or the study of food authenticity. The majority of these studies were directed to increase throughput and reduce time of analysis. Among the strategies explored to speed up the detection of amplified DNA sequences in foods are the use of multiplex polymerase chain reaction (PCR) combined with CGE-LIF [62], ligation-dependent probe amplification (LPA) with CGE-LIF [63] and microchip capillary electrophoresis [64-66]. The latter strategy will be discussed later in *Microchip CE* section.

An approach to identify foodborne pathogens is the combined use of multiple locus variable-number tandem-repeat with CGE-LIF. Multiple locus variable-number tandem-repeat is based on the amplification of short nucleotide sequences that are repeated multiple times in the bacterial genome. These short sequences often vary in copy number in different strains of a species, thus providing a means of discriminating between strains. Using labeled primers with different dyes, it is possible to perform multiplex PCR followed by CGE-LIF to provide microbiological identification with less uncertainty than with classical AGE analysis. This methodology has proven to be a powerful tool to differentiate serovars of *S. enterica* and Shiga toxin-producing *E. coli* O157, and therefore, it may be a helpful tool in foodborne disease surveillance programs [67].

Following the first works published on GMOs analysis by PCR-CGE-LIF [71-75] some new applications have come out also on this topic. Thus, a PCR-CGE-LIF method for GMO detection involved the use of fluorescent labeled primers with different dyes to perform the simultaneous PCR amplification in a single tube [62]. Optimal conditions for pentaplex PCR, which included the use of five primer pairs, were established for the detection of four transgenic maize lines (Bt11, GA21, Mon810 and NK603). For each primer pair, the forward primer was fluorescently labeled with one of the three fluorophores used in the study (6-carboxyfluorescein, tetrachloro-6-carboxyfluorescein and hexacloro-6-carboxyfluorescein). This strategy allowed the generation of five fluorescent PCR products with similar size but different fluorescent features, which also provided additional discriminatory information about the amplified DNA sequence when analyzed by CGE equipped with a charge-coupled device (CCD) detector and set of appropriate filters [62]. Ligation-dependent probe amplification (LPA) is based on the amplification of products resulting from the ligation of bipartite hybridization probes. Upon incubation with DNA template, two synthetic oligonucleotides probes containing the respective

specific sequences for targeting adjacent hybridization sites on the DNA sequence of interest are ligated *in vitro*. After ligation with a thermostable DNA ligase, the ligation products are amplified by PCR and analyzed by CGE-LIF. The beneficial aspect of this technique is the enhanced multiplexing capability by using several probe pairs. Owing to the incorporation of identical primer biding sites into the 5' ends of the probes, the differences among amplification 63] demonstrated the applicability of this approach to the simultaneous detection of genetically modified maize and soy in certified reference samples with different GMO content. The potential of the mentioned method for multiplex amplification was demonstrated on mixtures containing DNA from 0.1% maize and 5% soy standards, and *vice versa*. Accuracy of quantification of GMO in processed food is an important issue yet to be attained. A study about this point was carried out and CGE-LIF technique served to determine the degree of DNA fragmentation in samples [68]. In that work, genomic DNA extracts obtained from different food products were injected directly into the capillary and separated by CGE-LIF using a commercial kit. CE signals from the DNA extract were compared with the CE signals of molecular weight DNA standards to obtain an approximate range size of DNA molecules in the sample.

Apart from the aforementioned CGE methods, based on the use of commercial polymer solutions for the separation of DNA fragments, other developments have been published aimed at achieving fast, reproducible, sensitive and cheap separations of PCR products. In this sense, Sánchez *et al.* [69] investigated a new and simple CGE-LIF method capable of providing reproducible DNA separations in bare fused-silica capillaries. Using a sieving buffer containing 20 mM Tris, 9.5 mM orthophosphoric acid, 2 mM ethylenediaminetetraacetic acid (EDTA), and 4.5% hydroxyethylcellulose (HEC) at pH 7.3 the regeneration of the capillary by flushing with 0.1 M HCl between injections was essential to obtain reproducible separations (namely, RSD

values for migration times were lower than 1.0% for the same capillary and same day, lower than 2.2% for the same capillary and four different days and lower than 2.3% for four different capillaries). The method was successfully applied to the analysis of PCR products from samples with 0.9% transgenic content, namely, Bt11 and Mon810 maizes, and Roundup Ready soy.

The analysis of DNA markers for testing the origin of raw materials and the authenticity of food products is an interesting field for CE methods. Recently, Rodríguez-Plaza *et al.* [70] proposed a novel methodology based on the use of microsatellite markers and CGE-LIF for the differentiation of Albariño and Moscatel Grano Menudo musts employed for the production of wine. Authors demonstrated reproducible and efficient separations of DNAs and, as a result, adequate size assignments could be achieved and characteristic DNA profiles were obtained by the analysis of two microsatellite markers for the two grape varieties.

7. Vitamins.

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin with potent biological activity. As a consequence, available CE methods for the analysis of vitamin C have been developed over the last years [76, 52]. A CE method with capacitively coupled contactless conductivity detection (C^4D) was used for simultaneous detection of ascorbate, among others food additives [76]. The effect of pH and buffer nature on the resolution of the analytes was studied. Under optimum conditions (10 mM histidine, 0.135 mM tartaric acid, 0.1 mM CTAB and 0.025% hydroxypropyl-β-ciclodextrin, pH 6.5) and using a very simple sample treatment procedure including dilution with water, filtration and sonication for 10 min, ascorbate was detected in less than 400 s in soft drinks with a reported LOD of 3 mg/L (S/N=3).

Electrochemical detection coupled to CE has also been applied to the detection of vitamin C in foods. Guan *et al.* [52] developed a CE-ED method for the analysis of biological active compounds in sweet potato. The electrochemical detector was based on the end-column approach in which the working electrode, a carbon-disk electrode with 300 µm diameter, was simply placed at the outlet of the separation capillary. The influence of the potential applied to the working electrode on the sensitivity and stability of this method was investigated as well as the effects of the pH and concentration of the buffer, separation voltage and injection time on the migration time of the analytes. After all studied parameters were adjusted; vitamin C could be detected in less than 15 min at levels above 71.4 µg/mL. The good linearity (r=0.9996) obtained from the regression analysis on calibration curves over the range 2-100 µg/mL and the acceptable reproducibility of the peak current (RSD=3.25%) allowed the quantitation of vitamin C in both, fresh and cooked sweet potato samples.

Folic acid and folate are forms of the water-soluble vitamin B9. Orange juice is a good source of folate, which is mainly in the form of polyglutamyl 5-methyl tetrahydrofolates. A CZE-DAD method was developed for the analysis of 5-methyl tetrahydrofolates forms in orange juice [77]. The complex sample preparation procedure included centrifugation, filtration, affinity chromatography and solid phase extraction (SPE). With this procedure, seven polyglutamyl 5-methyl tetrahydrofolates were found in most orange juices in total amounts of 1 nmol/mL, with varying distributions of individual polyglutamates. It was also demonstrated that the CZE-DAD method is a good alternative to traditional analytical procedures requiring enzymatic deconjugation and microbial assays.

8. Small organic and inorganic ions.

The determination of cations is one of the most important analyses for routine quality testing of foods and beverages. CE represents an attractive alternative for simultaneous determination of cations to the established, more complicated techniques due to its high separation efficiency, good repeatability, fast analysis, and low consumption of electrolytes and samples. Fung and Lau [78] developed a CZE method with indirect UV detection at 214 nm for the separation and determination of 14 cations (Rb^+, NH_4^+, K^+, Ca_2^+, Na^+, Mg_2^+, Mn_2^+, Co_2^+, Fe_2^+, Cd_2^+, Cr_3^+, Ni_2^+, Zn_2^+ and Cu_2^+) in beverages products. Using a bare-fused silica capillary with a three-complex buffer system (10 mM N,N-dimethylbenzylamine, 8 mM lactic acid and 2 mM 18-crown-6) adjusted to pH 4.65, the baseline separation of the analytes was achieved within 7 min observing very symmetrical and efficient peaks (see **Figure 2**). Electrokinetic injection mode provided more sensitivity on the detection of cations (12-78 ng/L *vs.* 1.4-10 ng/L, respectively), while hydrostatic injection mode demonstrated to be more precise. Alternating both injection modes, electrokinetic and hydrostatic, a wider linear dynamic range could be covered (*i.e.*, 0.005-50 µg/L). The applicability and reliability of this method was demonstrated by comparison of the results obtained from the analysis of real samples with the inductively coupled plasma combined with atomic emission spectrometry method.

9. Toxins, contaminants, pesticides, and residues.

The presence of ochratoxin A (OA) in food and feed represents a serious concern worldwide since the exposure to this ubiquitous mycotoxin, produced by different species of *Aspergillus* and *Penicillium* fungi, has been related with human nephropathies and tumors of urinary organs. Various CE methods have been developed to detect OA, including MEKC and CZE, both with LIF detection due to the good fluorescent properties of this mycotoxin. More recently, Köller *et*

al. [79] have compared the performance of CZE with enzyme linked immunosorbent assay (ELISA) for the detection of OA. CZE-LIF method demonstrated to be superior in terms of precision and reproducibility. Both methods provided limits of detection which were below the regulatory limits in foods; nevertheless, ELISA provided overestimation of the OA concentrations. A possible reason for that might be lack of adequate procedures to carry out the sample clean-up used for ELISA.

The water-soluble tricarboxylic amino acid, known as domoic acid (DA), is the recognized toxic agent of amnesic shellfish poisoning (ASP) and the only shellfish poison produced by a diatom, currently limited in its distribution to North America [80]. Although several CE-UV separations of DA have been reported in food samples, a common limitation of these CE methods was their poor sensitivity [81]. Thus, one of the main research lines in this area is the sensitivity improvement by different means, including alternative sample purification strategies, adjustments of CZE parameters and development of a CEC method [81]. Recently, a sensitive on-line coupled cITP-CZE method for the determination of DA in shellfish and algae has been published by Kvasnicka *et al.* [82]. The cITP-CZE approach provides LOD of 0.2 mg of DA/kg, which is two orders of magnitude lower than the regulatory limit (20 mg/kg); a low running cost and a minimum sample processing requirement (methanolic extraction and filtration).

Apart from the aforementioned fungal and algae toxins, the characterization of bacterial toxins is one of the utmost interesting issues in food safety. As an example, botulinum neurotoxin serotype A (BoNT/A) is a potent protein derived from *Clostridium botulinum* that induces muscle paralysis by blocking release of neurotransmitter acetylcholine. Studies focused on the discovery of novel inhibitors of BoNT/A have been carried out in the last decade. CE methods represent an attractive alternative to available HPLC methods, in terms of speed of analysis and

resolution, to evaluate the enzymatic activity of the toxin *in vitro*. Laing *et al.* [83] developed two CE methods to test combinatorial peptide libraries for potential modulating effects on BoNT/A peptidase activity. To achieve this, BoNT/A was sequentially incubated with the library compounds and the substrate labeled with 3-(4-carboxy-benzoyl)-2-quinoline-carboxaldehyde). The mixture was separated in 7 min by CZE-LIF in a bare-fused silica capillary with a BGE containing 50 mM HEPES pH 8.4, 20 mM NaCl, 0.005% Tween-20 and 1 mM EDTA. This method provided baseline separation of the reaction products. However, the presence of interferants in the analysis of some samples hampered the quantitation of the reaction products. To solve this, an alternative MEKC method was developed reformulating the buffer composition, namely exchanging 0.005% Tween-20 for 50 mM SDS [83]. This second method provided better selectivity but longer analysis times for the detection of the reaction products.

The accurate determination of pesticides in fruits, vegetables and related matrices in food analysis is of great importance. As a consequence, several CE applications for the separation of pesticides have been reported during the period covered by this review [84-89]. Juan-García *et al.* [84] reported a MEKC-DAD method for the simultaneous determination of six pesticides, including acrinathrin, bitertanol, cyproconazole, fludioxonil, flutriafol, myclobutanil, pyriproxyfen and tebuconazole in lettuce, tomato, grape, and strawberry. The suitability of two different extraction methods in combination with MEKC-DAD for pesticide determination in the mentioned matrices was tested. SPE demonstrated better recovery values and time requirements for extraction when it was compared with stir-bar sorptive extraction whereas the latter featured slightly better precision and cleaner elctropherograms. Another MEKC-UV method was recently developed for the simultaneous detection of organophosphorus pesticides in water, vegetables and grain. The LOD ranged from 7 to 150 ng/mL for five pesticides studied,

including methamidophos, acephate, dicrotophos, dichlorvos and malathion [85]. When a SPE pre-concentration step was used, an enrichment factor of 250 was easily achieved in the analysis of water samples, making it possible to determinate pesticide residues at a concentration of 0.04 ng/mL.

Most recently, Ravelo-Pérez *et al.* [86] achieved improved separations of pesticides. Authors demonstrated that the combined use of both, on-column and off-column pre-concentration techniques with MEKC-UV was beneficial to achieve sensitive and simultaneous detection of twelve pesticides, namely, carbendazinm, pirimicarb, metalaxyl, pyrimethanil, procymidone, nuarimol, azoxystrobin, tebufenozide, fenarimol, benalaxyl, penconazol, and tetradifon (**Figure 3**). Although, reversed-electrode polarity stacking mode (REPSM) allowed LOQ values ranging from 33.6 µg/L for azoxystrobin to 216 µg/L carbendazim, these values were still above the maximum residue limits required for real samples. Therefore, a SPE method to extract the studied pesticides from water samples was developed and combined with REPSM-MEKC-UV method. The LOQ values were then improved, ranging from 1.37 µg/L for azoxystrobin to 8.13 µg/L carbendazim.

Owing to its major advantages, namely, remarkable high resolution, identification capability and versatility, CE-MS coupling may be considered as a powerful tool also to analyze pesticides in complex matrices. The suitability of CE-MS was tested by applying it for the analysis of six pesticides in fruits [87]. Separations were carried out using a buffer composed of 0.3 M ammonium acetate at pH 4 with 10% methanol. LODs for the six pesticides were within the range of 0.01 and 0.05 mg/kg.

Reversed-phase CEC has been applied to the analysis of herbicides residues in food [88]. Fresh vegetable and processed food products from soy were extracted with methanol followed by SPE. The extracts were analyzed using a CEC capillary fully packed with 5 µm non-endcapped C18 particles as stationary phase, given the possibility to perform the separation using the shorter part of the capillary by reversing the electric polarity and the sample injection end. This setting was essential for obtaining fast and efficient separations under the conditions assayed. Some difficulties in the identification of the pesticides in soy products were encountered as a consequence of matrix interferences. Six synthetic pyrethroids could also be simultaneously determined in cabbage by a similar CEC approach [89]. In this case, a high-pressure syringe pump was used to provide supplementary flow to the capillary. By using SPE, the CEC method was sensitive enough to achieve LODs below the regulatory levels for the studied pyrethroids (i.e. about 5 µg/mL in extract).

Owing to the extensive use of antibiotics in animals, there is a significant risk of generating drug-resistance through the persistance of drug-residues, what is becoming a serious health problem. As a consequence, many countries have established safe maximum residue limits (MRLs) for residues of these substances in animal tissues and derived foodstuffs entering the human food chain. Therefore, sufficiently sensitive multiresidue methods that provide reliable determinations of antibiotics in foodstuff of animal origin are needed.

An important group of antibiotics are quinolones. The wide application range and intensive use of these compounds in veterinary medicine represent a potential hazard since residues of quinolones may persist in animal-derived food products. Several CE methods have been recently applied to the determination of quinolones in food. Lü and co-workers [90] studied the influence of different variables on the separation of seven quinolones (norfloxacin, fleroxacin,

ciprofloxacin, lomefloxacin, enoxacin, ofloxacin, and gatifloxacin) by CEC with supplementary flow by pressure. Among the variables studied, pH buffer, organic solvent modifier and SDS concentration affected the resolution, while the addition of trietilamine (TEA) had a positive impact on peak shape by reducing peak tailing. With this CEC method, that uses UV detection, LODs for quinolones ranged from 0.2 to 1.0 mg/L. Moreover, the applicability of the proposed CEC method was demonstrated by the detection of spiked quinolones in fish samples after SPE clean-up.

The recent application of CE-MS to the quantification of five quinolones (enrofloxacin, danofloxacin, pipemidic acid, flumequine, and ofloxacin) in fish and chicken muscles has shown the good possibilities of this technique to the analysis of residue in complex food matrices [91]. To achieve that, a pressure-assisted CE system was optimized with regards to important operating parameters such as the type of buffer, pH, and concentration. Adequate separations between quinolones are usually attained with basic buffers due to their pK values, and considering that the coupling between CE and MS requires the use of volatile CE running buffers, a BGE consisting of 60 mM ammonium carbonate at pH 9.2 was used. An excess pressure of 30 mbar during the run at 25 kV allowed the separation of the five quinolones in 20 min. The effect of the composition and flow of sheath liquid was also investigated and the best MS signal was obtained with 60 mM ammonium carbonate at 10 µL/min. Two different MS analyzers, single quadrupole and quadrupole ion trap (QIT), were compared in terms of dynamic range, precision and sensitivity in spiked fish and chicken samples. Although the method validation study showed that QIT posses a narrower dynamic range and lower precision than single quadrupole, both methods demonstrated comparable sensitivity (~ 20 ng/g), which was enough to detect these quinolones at the required MRL (100-200 ng/g) in real samples. Further improvements in the determination of a higher number of quinolones (danofloxacin,

sarafloxacin, ciprofloxacin, marbofloxacin, enrofloxacin, difloxacin, oxolinic acid, and flumequine,) by CE-MS in food samples were provided by Lara *et al.* [92]. In this case, a tandem mass spectrometry approach was adopted for simultaneous detection and confirmation of the quinolones. A meticulous study of the instrumental and chemical variables involved in the analysis was carried out using experimental design methodology. In addition, a sample preparation procedure based on two-step SPE clean-up and optimized in bovine raw milk proved to be a valuable alternative avoiding the necessity to precipitate proteins, the use of organic solvents of chlorinated acids. Using this methodology under optimized conditions, efficient ionization of analytes was achieved in order to obtain LOD values (6 ng/mL), below the established MLRs.

Bacitracin (BC) and nystatin, also known as mycostatin, are antibiotics used as animal food additives for different purposes. The MEKC-UV separation of a more stable form of bacitracin, i.e. complexed with zinc (Zn-BC complex) and nystatine was approached by Injac *et al.* [93]. The proposed micelle system consisted of 15 mM borate and 19 mM phosphate at pH 8.2, containing 20 nM SDS and 10% methanol as the BGE. Additional running pressure (15 mbar) was applied to reduce the time of analysis. The selectivity of the method was successfully tested by the analysis of different spiked feedstuff mixtures, namely pork, cattle and chicken, showing no interferences in MEKC-UV results at the wavelengths selected. The validation study of the method demonstrated good linear response over the concentration ranges of 14-52 mg/L for Zn-BC and 8-82 mg/L for nystatine. Also, the reported LOD values for Zn-BC and nystatine were 4.72 and 2.96 mg/L, respectively. The RSD values were lower than 0.61% for Zn-BC and 0.75% for nystatine.

CE methods have been successfully applied to the simultaneous determination of multiple illegal drugs in animal feeds [94, 95]. In these works, 2-thiouracil and phenylthiouracil were derivatized with 5-iodoactamidofluoresceins prior to their analysis by CZE-LIF using a 20 mM disodium hydrogen phosphate at pH 10.0 [94]. Clenbuterol, cimaterio and salbutamol were analyzed by CZE-AD using a 150 mM borax-potassium dihydrogen phosphate at pH 6.0 [95].

10. Food additives.

Food additives are compounds added to foods to maintain product consistency, to improve or maintain nutritional value, to retard product spoilage by microorganisms, to control acidity/alkalinity, and/or to enhance flavor or impart desired color. The use of these substances in food is also regulated regardless the source or process from which they are derived. In this sense, CE methods have recently been developed for the analysis of food preservatives and antioxidant [76] and food colorants [96-99].

The food preservatives benzoate and sorbate, and also the anitoxidant ascorbate were simultaneously analyzed in soft drinks by CE coupled with a C^4D detector [76]. Interestingly, a BGE consisting of MES/histidine, which has been widely used with the C^4D provided lower sensitivity than histidine/tartaric acid. CTAB was added for the suppression of the EOF and HP-β-CD was included to improve resolution. In this study, LOD values of 0.5 mg/L for benzoate and sorbate, and 3 mg/L for ascorbate were reported.

Colorants are widely used for color enhancement of foods. Although most food colorants are expected to be safe, several food colorants have demonstrated some toxicity. As a consequence, many countries have established regulations for the use of natural and synthetic colorants in

foods. Due to these regulatory restrictions, new methods are demanded for rapid detection and identification of colorants in food products.

Fifteen synthetic food colorants (Patent Blue V, Sunset Yellow, Cochineal Red, azorubine, Quinoline Yellow, Carminic acid, amaranth, erythrosine, Food Red 2G, Allura Red, indigotine, Erioglaucine, Brillant Green and Brillant Black PN) were separated and detected in 20 min by MEKC-UV using as separation buffer a mixed micellar system [96].

A similar MEKC-UV approach was applied to the determination of eleven synthetic colorants in alcoholic beverages [97]. In this case, a running buffer adjusted to pH 11 and containing SDS micelles proved to be very efficient in separating these compounds. Owing to the great number of compounds contained in wines and liquors that absorb light in the ultraviolet region, the electrophoregrams were monitored at different wavelengths within the visible region, 450 nm for yellow dyes, 525 nm for red dyes, and 625 nm for blue dyes. The sensitivity of the method was determined in wine samples with added standards and LODs ranged from 0.4 to 2.5 µg/mL. Good linearity was observed within the working range 0.5-400 µg/mL for the colorants analyzed. Besides, the average recovery was 92.6 and 104.0%, at two levels of concentration in the red wine without any sample treatment. Synthetic colorants were found in cocktail and aromatized spirits samples below the regulatory limits (30 mg/100 mL) by this procedure.

Arráez Román *et al.* [98] developed an interface for the coupling of CZE and Surface-Enhanced Resonance Raman Spectroscopy (SERRS) and its performance was tested for the separation and identification of colorants. The novelty of this approach was the use of a stainless steel needle interface as (grounded) cathode and thin layer chromatography (TLC) plates as substrates attached to a translation table. After deposition onto the moving TLC plate, the spots were read

by a Raman microscope. With this configuration, three silver colloids were tested since the type of colloid that is applied to the deposited spots for SERRS analyses, as well as the charge of the analyte were expected to have a major impact on the SERRS intensities. The CE-SERRS coupling performed well when samples contained analytes of the same charge. The SERRS spectra were recorded and differences in vibrational frequencies to distinguish different compounds were obtained.

The red food colorants carnoisine, amaranth, ponceau, erythrosine and red 2G have been recently analyzed by CZE-LIF with a diode pumped frequency-doubled Nd:YAG laser (532 nm) as excitation source [99]. Better sensitivities were achieved with this detection system when compared to results obtained with CE-UV/Vis detector. Besides, authors pointed out that the CZE-LIF method allowed the dilution of the sample and thus, minimizing the problems from matrix effect.

Sucralose is a food additive used as artificial sweetener produced by chlorinating sucrose. The use of sucralose as food aditive at certain concentrations is allowed in the EU. As a consequence, there is a demand of rapid analytical method for the determination of sucralose in foodstuffs. A CZE method with indirect UV detection was developed and optimized for the determination of sucralose in beverage and food matrices [100]. Some of the optimized parameters were the separation buffer (3mM dinitrobenzoic acid/20 mM sodium hydroxide, pH 12.1), a potential of 0.11 kV/cm, temperature of 22 ºC and detection wavelength at 238 nm by indirect UV. A validation study of this CZE method demonstrated its suitability for the analysis of sucralose in foodstuffs.

11. Food interactions and processing.

Some physical and chemical changes are usually expected during food processing and storage. In some cases, food processing is intended to improve a particular attribute (flavor, texture, storage stability, nutrients, etc.). For instance, a common practice in food processing is the incorporation of protein ingredients in the product formulation to provide food with better quality. In some cases, interactions and associations among food ingredients occur and as a consequence, functional properties of food are modified without damaging or even improving the nutritional quality. On the other hand, undesired changes on food composition can also originate from processing and storage. These unintended changes might represent a serious hazard to human health as for example, the formation of acrylamide in carbohydrate-rich cooked foods and the generation of chloropropanols in hydrolyzed vegetable proteins under certain conditions. Therefore, the study of the effects of food processing is interesting to Food Science in order to guarantee safety of food and to improve food quality for consumers and producers. In this regard, CE has shown to be a valuable tool to obtain interesting information on interactions among food ingredients and on the effect of industrial processing on foods.

Bonet *et al.* [101] studied the effect of different proteins on the functional properties of wheat dough. They also examined the effectiveness of a microbial transglutaminase as a catalyst for the formation of heteropolymers of wheat and wheat-exogenous proteins to improve the rheological properties and nutritive value of doughs. In that work, a CE method served to determine the content on glutenins, water-soluble and salt-soluble proteins from blends of wheat flour containing 20% (w/w) exogenous protein sources (soy, gelatin, albumin, lupin and beer) and with or without transglutaminase treatment.

Bovine ß-lactoglobulin is the major allergenic protein in cow's milk and exhibit proteolytic stability under conditions found in human gastrointestinal digestion. A study on the changes in peptic digestibility of this protein due to fermentation processes was carried out [102]. To achieve this, a CZE was applied to monitor the peptic hydrolysis of ß-lactoglobulin from different bovine milk products. Samples were also analyzed by ELISA to monitor the changes of antibody binding properties and then, a correlation was established with the electrophoretic data. Results indicated that fermentation of milk increased the susceptibility of ß-lactoglobulin towards peptic digestion.

Since the discovery of the formation of acrylamide in heated food, this neurotoxic compound has aroused concerns word-wide. New developments on analysis of acrylamide by CE have been reported in recent years [103, 104]. The lack of a strong UV chromophore demanded a sample derivatization step with 2-mercaptobenzoic acid prior CZE separation. The analysis by CZE-UV was carried out using uncoated fused-silica capillaries and a 40 mM phosphate buffer at pH 8.0 as BGE [103]. The method provided a LOD of 70 ng/mL and an intraday precision (RSD, n=5) of 2.2% for medium concentration level and 5.8% for low concentration level. The method was used for the analysis of acrylamide in french fries, breakfast cereals, and biscuits. Further improvements of this CE method with sample derivatization consisted of the optimization of two in-line preconcentration methods, namely, field amplified samples injection (FASI) and stacking with sample matrix removal [104]. The sensitivity enhancement in the detection of acrylamide achieved by these techniques in comparison with CZE-UV (without preconcentration) was about 70 and 10-fold, respectively.

The compound 3-chloro-1,2-propanediol is another example of potential human carcinogen that is generated in foods during cooking. A CZE-ED for determination of this compound in soy

sauces has been recently developed [105]. The detector consisted of a three-electrode cell system containing a copper working electrode, a platinum auxiliary electrode and a saturated calomel reference electrode. The separation of 3-chloro-1,2-propanediol from other compounds of similar structure was attained in 13 min with a reported LOD of 0.13 µg/mL, which is well below the maximum legal limit of this compound (1 mg/kg) in soy sauce in several countries.

12. Chiral analysis of foods compounds.

A MEKC-LIF method has been developed to separate and quantitate the derivatized L- and D-amino acids usually found in vinegars [106]. Samples were derivatized with FITC and then, diluted in water prior to MEKC-LIF analysis. During the method development process, the washing routine and the running buffer were investigated in order to achieve adequate and reproducible separations in short analysis times. Using a buffer containing 100 mM sodium tetraborate, 20 mM SDS, and 20 mM ß-cyclodextrin at pH 9.7, the separation of derivatized chiral amino acids proline, alanine, arginine, glutamic, and aspartic acid was attained in less than 20 min. The method was applied to the quantitative analysis of chiral amino acids in twelve vinegars (see some examples in **Figure 4**). The results indicated that balsamic vinegars contained the highest absolute amounts of amino acids compared to the rest of vinegars studied and also the highest relative percentage of D-Pro, which is an indicator for maturation of balsamic vinegars. A major content of D-Ala, D-Glu and D-Asp in vinegars made from sherry was indicative that these vinegars were produced by microbial fermentation. It is noteworthy the simplicity of this MEKC-LIF method compared with the established GC method for the analysis of vinegars.

13. Other applications.

Detection of aldehydes and ketones can also be carried out by using capillary electromigration methods. These compounds are interesting to food science due to their sensory relevance, and in some cases, aldehydes are indicators of quality deterioration, microbacterial fermentation and off-flavor. Generally, the determination of these compounds is accompanied of a derivatization step that prevents the analytes from evaporation and reaction with other compounds in the sample. Acetone and four aldehydes (namely, formaldehyde, acetaldehyde, hexenal and 2-trans-hexenal) have been simultaneously determined in slurries samples by CE coupled to a pervaporator by a flow injection manifold and the replenishment system of the CE instrument [107]. Using this approach the volatile analytes were removed, derivatizated and injected into the capillary meanwhile the sample matrix remained in the pervaporator. A multivariate study of the influence of ten variables on the derivatization process and separation showed that the 4-hydrazinobenzoic acid concentration, water-methanol ratio, injection pressure, injection time and capillary temperature were not statistically influential factors within the ranges under study. On the other hand, the derivatization time had a negative effect while the concentration of SDS had a significant positive effect. The optimal pervaporation time and that necessary for the individual separation/detection of the target analytes are 15 and 10 min, respectively. The LODs ranged between 0.1 and 0.6 μg/mL. The accuracy of the method and potential matrix effects were established by analyzing spiked yoghurt, juice and yoghurt-juice mixtures. A preconcentration effect between three and ten times was reported for formaldehyde an acetone, respectively. It was also demonstrated a drastic reduction of interferents in the separation, in comparison with that provided by direct injection of the yoghurt.

L-carnitine plays an essential role in the transport of long-chain fatty acids across the mitochondrial membrane. Although adults can synthesize carnitine in the liver and kidney,

exogenous supply of L-carnitine is mainly supplied by meat, milk and vegetables. Recently, there has been an increase in the production of food supplements with L-carnitine. Fast analytical techniques are needed to determine the L-carnitine concentration in these new products. The L-carnitine content in food supplements was evaluated by Prokorátová *et al.* [108] using three different CE approaches, namely, ITP-ITP, CZE with UV detection and CZE with indirect UV detection. The comparison of the three electrophoretic systems denoted that the CZE with indirect UV detection provided the best results. Isotachophoresis method demonstrated good linearity and sensitivity, however, the disadvantage of this method was the impossibility of automatic injection due to instrumental limitations. Derivatization with 9-fluorenylmethoxycarbonyl was necessary prior to CZE-UV and this is not practical for large series of samples.

A MEKC-UV method was developed to detect and quantify the methylxanthines theobromine, caffeine and theophylline in commercial brands of *yerba mate*, coffee, tea and cocoa as well as two cola drinks [109]. The separation was carried out in an uncoated fused-silica capillary using a 90 mM borate buffer at pH 8.5 containing 50 mM SDS. The analytes were separated in less than 8 min, however, peak splitting due to tautomeric species was observed.

14. Microchips and other future trends in food analysis.

Owing to the advantageous features of microchip-CE technology, including negligible consumption of reagents and samples, and the capability for fast and automatized analysis in-situ, the emergent development of microchips was already expected to see important applications in the food analysis field [1] as corroborated by the many recent applications of microchips CE in food analysis. These applications of microchips-CE include the analysis of DNA [64-66], proteins [110], amines [111], seleno-amino acids [112], antioxidants [113, 114]

and sulfite [115]. Besides, among the current developments in microchip-CE, different detection schemes have been developed to be used together with these microdevices. Some of these novel developments are next discussed.

The combined use of microchip capillary gel electrophoresis with programmed field strength gradients (PFSG) was applied to provide ultra-fast detection (11 s) of genetically modified soybean in food samples [64]. A commercial microchip CE system equipped with a diode-pumped solid-state laser was employed and the gradient in PFSG was optimized to reduce time of analysis of DNA amplified fragments without loss in resolution. Other methodologies, involving the combination of PCR-based methods with microchip CE, have been tailored for ensuring the authenticity and the origin of food products. Dooley *et al.* [65] developed a PCR based restriction fragment length polymorphism (PCR-RFLP) method, which combined with a Lab-on-a-Chip CE platform and epifluorescent detection with a 10-mW semiconductor laser, afforded the detection and identification of ten commercial fish species in food samples. Moreover, results provided by five different laboratories suggested the methodology was easy-to-perform, robust and accurate. However, owing to the increased complexity of the DNA profiles expected in samples containing more than two fish species, the suitability of the method would be limited to confirm the presence of a fish species in a fish product and at best, to recognize the presence of additional species that should not be present [65]. The same microchip CE platform was utilized for detecting contamination of Arabica coffee (*Coffea arabica*) with Robusta coffee (*Coffea robusta*) [66]. In this approach, a PCR-RFLP method was developed on the basis of single-nucleotide polymorphisms in chloroplastic DNA among *Coffea* species. Coffee mixtures containing different proportions of both coffees were analyzed by this methodology and a LOD value of 5% Robusta was reported. However, the quantitative application of the method remain uncertain since further studies to confirm that plastid copy

number is constant across a wider range of varieties, and is not influenced by environmental conditions should be carried out. Lab-on-a-Chip CE technology has been also applied to study the differences between the proteomic profiles obtained from wild and farmed fish [110].

Amperometric detection (AD) is an attractive alternative for on-chip applications due to its compatibility with these microdevices and its remarkable sensitivity. The detection of three biogenic amines, namely, tryptamine, tryptophan and tyramine in alcoholic beverages has been recently attained in less than 240 seconds by microchip CE-AD [111]. A similar strategy has been also used for the rapid detection of food-related seleno amino acids [112]. The method was based on sample derivatization with o-phthaldialdehyde in presence of 2-mercaptoethanol with the subsequent separation and detection of the selenoamino acids in a simple-cross glass microchip coupled to an end-column amperometric detector. The LODs were within the micromolar range and the RSDs of the peak current and migration time of Se-methionine were 4.7% and 1.3%, respectively. Also, microchip-CE-AD has been applied to the analysis of natural antioxidants, including phenolic and nonphenolic antioxidants, in commercial fruit juices and pulp [113]. The microchip CE plate incorporated two reservoirs for sequential sample injection and a glassy carbon electrode for the detection of analytes. Despite of the good performance of AD, the short lifetime of the electrodes is a disadvantage of this detection system. To avoid this, pulsed amperometric detection (PAD) has been proposed as a sensitive detection system suitable for the detection of a large number of analytes. Ding *et al.* [114] used this analytical strategy for the detection of antioxidants in 2 min above the low micromolar range.

Poly(methyl methacrylate) (PMMA) microchips have recently been used in combination with C^4D for the determination of anions and cations [116] and food additives [76] in beverages.

Using a PMMA device, pH variations had lower impact on the EOF allowing to modify the separation selectivity in order to achieve an adequate separation of NH_4^+, K^+, Ca_2^+, Na^+ and Mg_2^+ in beverages [116]. Besides, ten inorganic and organic anions including, Cl^-, SO_4^{2-}, oxalate, tartrate, malate, citrate, succinate, acetate, lactate, and phosphate (peaks 1-10 in **Figure 5**, respectively) were also separated by using as BGE a solution composed of 10 mM His/7 mM glutamic acid at pH 5.75. All major anionic compounds could be determined and quantified in several different samples including red wine, white wine, beer, fruit juices and milk. This microchip CE-C^4D method demonstrated excellent precision for the migration time (RSD < 0.38%) and peak area (RSD < 4.52%). Separations were completed in less than 90 seconds and the LODs ranged from 150 to 2000 µg/L for Cl^- and acetate, respectively.

ITP and CZE were coupled in a microchip configuration called column-coupling chip that was used for the determination of total sulfite in wine samples [115]. Using this strategy, wine matrix could be eliminated and the analyte of interest concentrated to a certain extent. The detection system employed for this application consisted of conductivity sensors that allowed the detection of sulfite at a concentration of 90 µg/L.

Despite the remarkable advances on food analysis by CE, the development of new CE separation and detection strategies applicable to real-life food analysis will keep growing in order to comply with the everyday more and more strict requirements on food safety and quality imposed by the consumers and regulations. It is expected that some of the limitations of current CE methods, mainly, sensitivity, will be improved by different means (more powerful detection schemes, sample preconcentration, stacking methods, etc.). In this regard, it is expected that application of these strategies will continue growing in food analysis. On the other hand, although CE-MS and microchips-CE have already been used for a significant number of

applications in food analysis, the development of new and more powerful methods based on CE-MS and microchips will also be expected in this field.

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Figure legends

Figure 1. (A) Experimental CE-MS extracted ion electrophoregram of two peptides (#14 and #16); (B) simulated electrophoregram of the three possible sequences found for peptide 16 all of them with similar m/z value (namely, #16A given by QKCAQCHTVEKGGKHKTG; #16B given by HGLFGRKTGQAPGFSYTD; and #16C given by YTDANKNKGITWGEETL). From the comparison between experimental (A) and simulation (B) it is deduced that the most probable sequence is 16C. This point is corroborated by the MS/MS spectra of peptide #16 given in (C). Redrawn from [41].

Figure 2. Electrophoregram of a standard cation mixture. Separation conditions: Separation buffer: 10 mM N,N-dimethylbenzylamine, 2 mM 18-crown-6 and 8 mM lactic acid at pH 4.65; Voltage: 20 kV; Detection: 214 nm. Cation standards: 10 µg/L each; Peaks: 1) Rb, 2) ammonium, 3) K, 4) Ca, 5)Na, 6) Mg, 7) Mn(II), 8) Co(II), 9) Fe(II), 10) Cd(II), 11) Cr(III), 12) Ni(II), 13)Zn(II), and 14) Cu(II). Redrawn from [78].

Figure 3. MEKC-UV electrophoregram of the separation of 12 pesticides. Sample dissolved in 30 mM SDS, 100 mM borate, pH 8.5. Separation buffer: 100 mM sodium tetraborate, 20 mM SDS at pH 8.5, and 6% 1-propanol; Voltage: 22 kV; Temperature: 25 ºC. Detection: 210 nm. Peaks: 1) carbendazim, 2) pirimicarb, 3) metalaxyl, 4) pyrimethanil, 5) procymidone, 6) nuarimol, 7) azoxystrobin, 8) tebufenozide, 9) fenarimol, 10) banalaxyl, 11) penconazole, and 12) tetradifon. Redrawn from [86].

Figure 4. Chiral-MEKC-LIF electrophoregram of a vinegar. Traces: A) vinegar sample 1, B) same as (A) plus 10 µg/mL of D-Asp, and C) same as (A) plus 40 µg/mL of D-Asp. Redrawn from [106].

Figure 5. Microchip CE- electrophoregram of the separation of inorganic and organic anions in wine samples. Background electrolyte: 10 mM His, 7 mM glutamic acid (pH 5.75). Run voltage: 24 kV. Injection: 21 kV for 3 s. Redrawn from [116].

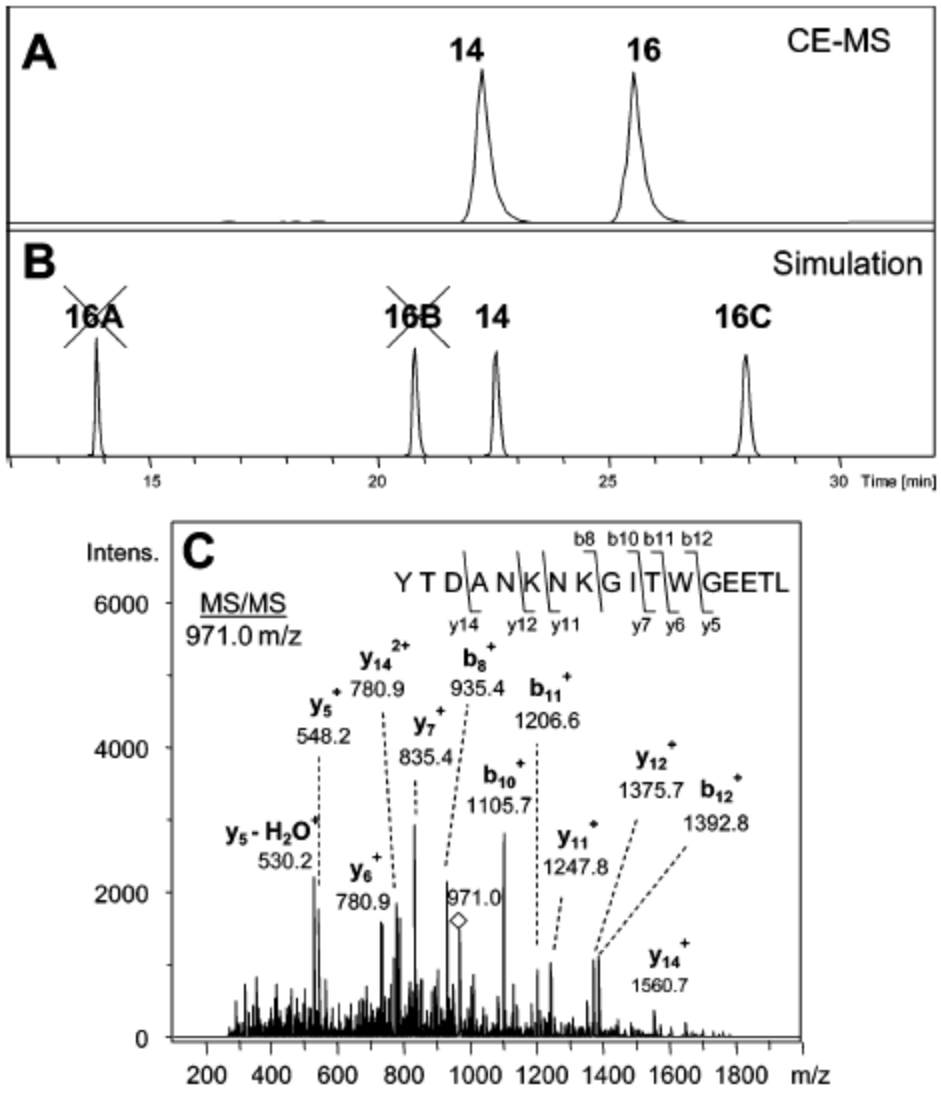
A
14
16
CE-MS
B
Simulation
16A
16B
14
16C
15
20
25
30
Time [min]
C
Intens.
6000
4000
2000
0
MS/MS
971.0 m/z
b8 b10 b11 b12
Y T D A N K N K G I T W G E E T L
y14 y12 y11 y7 y6 y5
y14 2+ 780.9
b8 + 935.4
y5 + 548.2
y7 + 835.4
b11 + 1206.6
y12 + 1375.7
b12 + 1392.8
b10 + 1105.7
y5 - H2O + 530.2
y11 + 1247.8
y6 + 780.9
971.0
y14 + 1560.7
200 400 600 800 1000 1200 1400 1600 1800 m/z

FIGURE 1

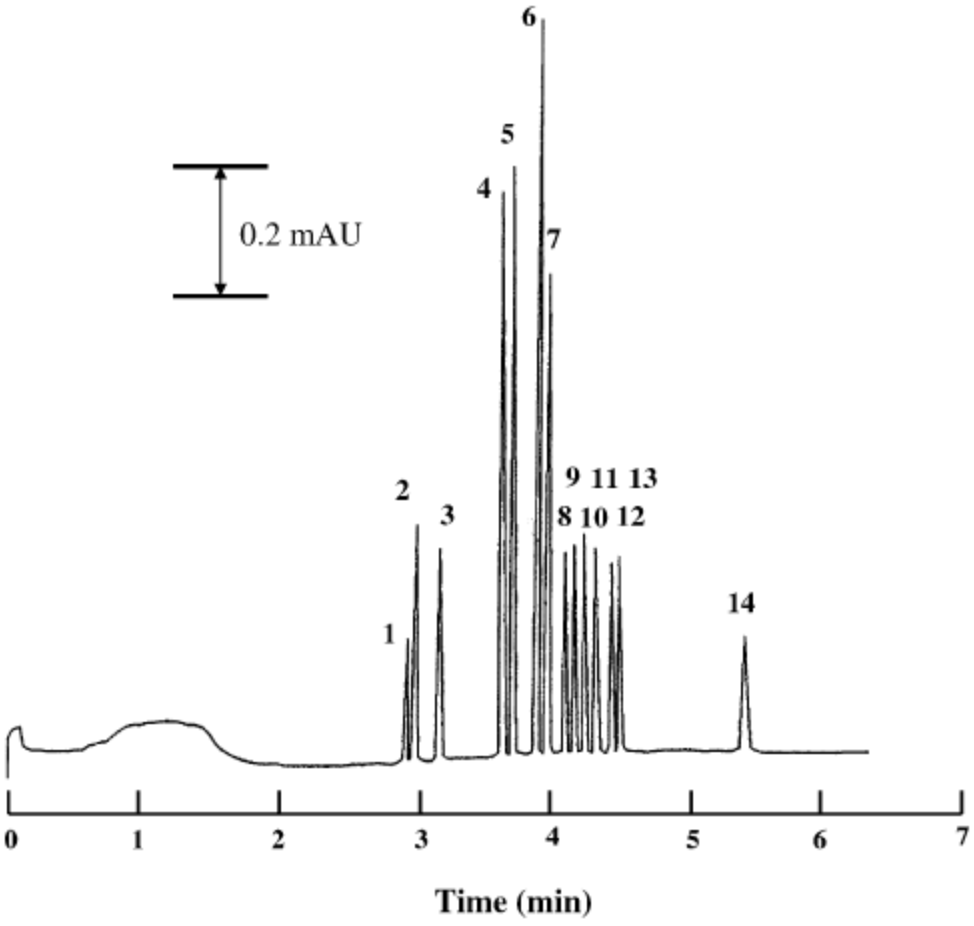

FIGURE 2

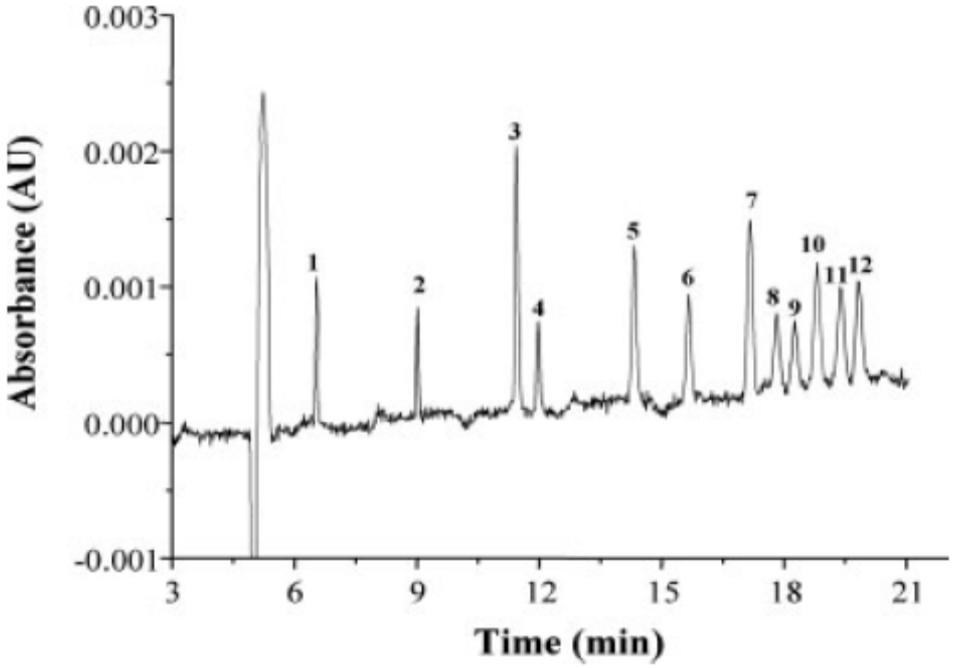
0.003
0.002
0.001
0.000
-0.001
Absorbance (AU)
3
6
9
12
15
18
21
Time (min)
1
2
3
4
5
6
7
8
9
10
11
12

FIGURE 3

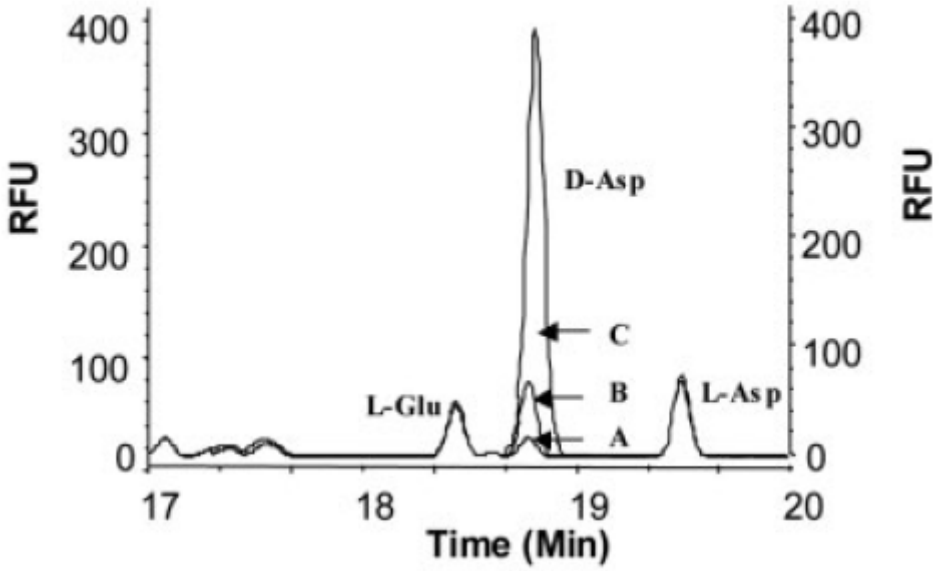

FIGURE 4

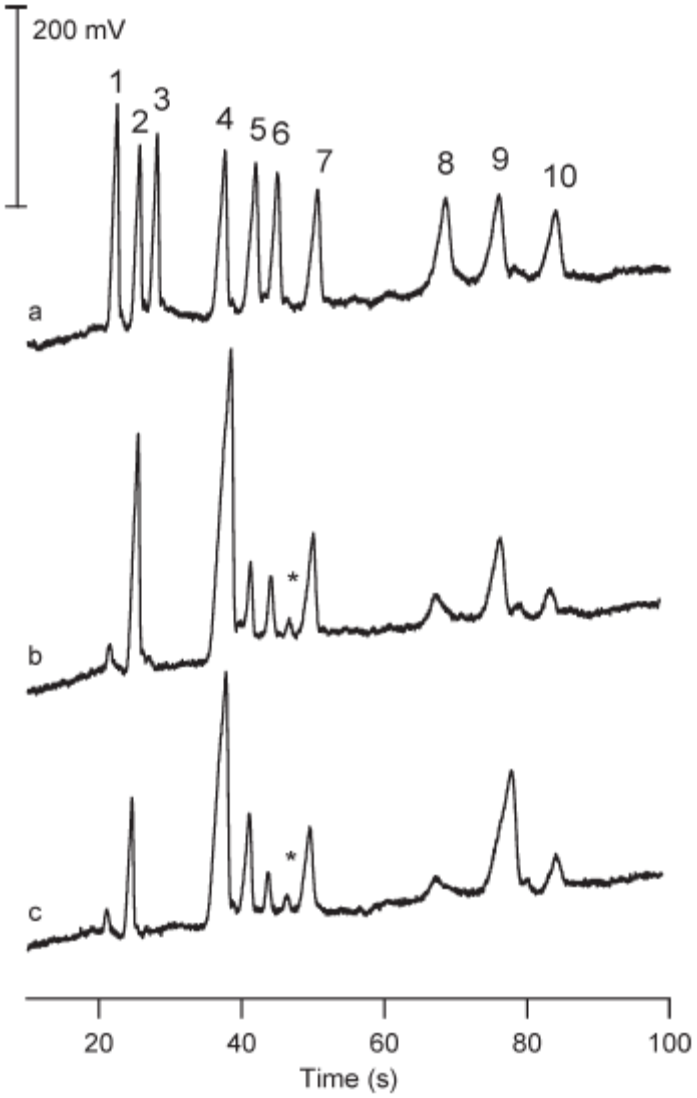

FIGURE 5