Review

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Chiral electromigration methods in food analysis

This review article addresses the different chiral capillary electrophoretic methods that are being used for the study and characterization of foods and food compounds (*e.g.*, amino acids, organic acids, sugars, pesticides). An updated overview, including works published till December 2002, on the principal applications of enantioselective procedures together with their main advantages and drawbacks in food analysis is provided. Some anticipated applications of chiral electromigration methods in food characterization are also discussed.

Keywords: Amino acid / Capillary electrophoresis / Chiral separation / Enantiomer / Organic acid / Peptide / Pesticide / Review / Sugar DOI 10.1002/elps.200305442

Contents

1 Introduction

The increased consumers concern about what is in their food and the safety of the food they eat has brought about the use and development of new analytical techniques in food science [1]. These new analytical techniques must address an important number of problems providing information about processing, quality control, ensuring compliance with food and trade laws, adulteration, contamination, product tampering, and the chemical composition of foods. Therefore, faster, more powerful, cleaner, and cheaper analytical procedures are being required by food chemists, regulatory agencies and quality control laboratories to meet these demands. As a consequence, there is a growing interest in the development of new analytical procedures meeting all the requirements aforementioned for use in food analysis.

As indicated by Armstrong *et al.* [2], enantioselective separations can be used in food and beverage studies for: (i) identifying adulterated foods and beverages, (ii) more exact control and monitoring of fermentation processes and products, (iii) evaluation and identification of age, treatment and storage effects, (iv) more exact evaluation of some flavor and fragance components, (v) fingerprinting complex mixtures, (vi) analysis of chiral metabolites of many chiral and prochiral constituents of foods and beverages, (vii) 50% less material (*e.g.*, flavors, preservatives, additives, *etc.*) can be used in some cases, (viii) decreasing environmental persistence of some compounds.

Among the different analytical techniques that can be employed to separate chiral compounds (*e.g.*, HPLC, GC, *etc.*), the use of capillary electrophoresis (CE) has emerged as a good alternative for enantiomer separations since this technique provides fast and efficient separations in this type of analysis. Moreover, the availability of many chiral selectors and the minimum consumption of such compounds during a CE run has to be considered as an additional advantage of chiral electromigration methods.

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Abbreviations: **ANDSA**, 7-aminonaphthalene-1,3-disulfonic acid; **HP-β-CD**, 2-hydroxypropyl-β-cyclodextrin; **OPA**, *o*-phthaldialdehyde; **S-PEA**, *S*-(−)-1-phenylethylamine; **TATG**, 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranose; **TM-β-CD**, heptakis-(2,3,6-tri-O-methyl)-β-CD

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2 Scope of the review

The remarkable possibilities of CE for the separation of chiral compounds were first demonstrated by Gasman *et al.* in 1985 [3] and since then a huge number of chiral compounds have been analyzed by CE. However, its main application still remains within the separation of chiral drugs as can be deduced from the different reviews (see, *e.g.*, [4–9]) and the book [10] devoted to this topic. The use of CE in food analysis has been also reviewed in several publications [11–13]. However, to our knowledge, no revision work on the use of chiral CE in food analysis has been accomplished until now. The goal of this revision work is to carry out such revision. To do this, an updated overview (including works published till December 2002) on the principal applications of chiral electromigration procedures together with their main advantages and drawbacks in food analysis is shown. A critical revision of the principal food compounds that have been analyzed using chiral electromigration procedures (see Table 1) is also included and the interest of their analysis from a food laboratory point of view are presented. Moreover, some foreseeable applications of these chiral electromigration methods in food characterization are also discussed.

3 Organic acids

Organic acids are a heterogeneous class of compounds that contain at least one carboxylic acid group. Several hundred compounds may be included, depending on how expansive a definition is used. Short-chain organic acids are mainly studied in this section because their properties are mostly due to the carboxylic function. As it will be shown below, the presence of organic acid racemates in food products can indicate their use as additives, which is not always permitted and needs to be controlled. On the other hand, different isomers of the same acid can present different flavor or taste and their analysis can be of interest for quality control.

Lactic acid in sake, as well as in wine, is a major organic acid and it is thought to have a great influence on the taste. Whereas naturally occurring lactic acid bacteria are used in the traditional brewing of sake, the use of lactic acid addition has recently been predominant in order to simplify sake brewing [14]. Authors say that, although sensory studies are needed, D-lactic acid in water has a different sour taste from L-lactic. L-Tartaric acid is distributed in plants, especially grapes, while D-tartaric acid is not common in nature, it has only been found in some

Table 1. Food compounds analyzed by chiral electromigration procedures

Analyte	Food	Chiral selector	Ref.
DL-Tartaric acid	Grape juice, red wine, sake, tablet candy	Copper(II)-D-quinic acid	[15]
DL-Malic acid	Apple juice	Copper(II)-L-tartrate	[16]
DL-Lactic acid	Yoghurt, sake	HP-β-CD	[34]
DL-Valine, DL-glutamic acid, DL-aspartic acid, DL-leucine, DL-phenylalanine	Alkaline-treated duck eggs	β-CD	[46]
DL-Arginine, DL-proline, DL-asparagine, DL-serine, DL-alanine, DL-glutamic acid, DL-aspartic acid	Orange juice	β-CD	[48]
DL-Valine, DL-isoleucine, DL-leucine	Sport nutritional supplements	β-CD	[50]
DL-Glutamic acid, DL-aspartic acid	Beer, yoghurt	Derivatization with OPA/TATG	[51]
28 Aldose derivatives	Apple juice, wine	Derivatization with S-PEA	[53]
Propiconazole, bioallethrin, fenpropathrin, phenothrin, bitertanol, triadimenol, dimethromorph	Fortified water samples	Different commercially available surfactants and CDs	[71]
Vinclozolin	Wine	γ-CD	[72]
Eriocitrin, hesperidin	Lemon juice	γ-CD	[79]
Medicarpin, vesitone	Transgenic alfalfa	HP-β-CD/HP-γ-CD	[80]
Pantothenic acid	Soft drinks	HP-β-CD	[81]

plants with ethnomedical applications. Racemic tartaric acid can be used in food products provided that it is declared on the label. Therefore, the enantiomeric determination of tartaric acid as a food additive is very important for the safety and quality control of food products [15]. As in the other cases, malic acid exits in nature in only one enantiomeric form, L-malic acid. In some countries, the use of synthetic racemic mixture of malic acid is legally allowed in food products with a declaration of the addition in the label. Therefore, the enantiomeric determination of malic acid as a food additive is important in safety and quality control of food products [16].

Chiral analysis of short-chain organic acids is complicated because their short chain makes difficult the three-point interaction generally accepted as necessary for chiral recognition. Moreover, they lack a powerful UV-absorbent chromophore. That is why many methods for chiral short-chain organic acids analysis have been developed with derivatization to diastereomers. Relatively recent works have shown the possibility of their direct chiral separation in CE by different mechanism: ligand-exchange CE, macrocyclic antibiotics and cyclodextrins (CDs).

3.1 Indirect separation by derivatization to diastereomers

The diastereomeric derivatizing method is described as one of the most effective procedures for small molecules, and for aliphatic and nonchromophore compounds [17]. Nevertheless, derivatization of the carboxylic group is complicated, as it frequently requires several steps and racemization can take place during the derivatization process. Although improved detectability is an important reason to perform a derivatization procedure, there can be other reasons as well in chiral separation. These goals include (i) transformation in diastereomers to obtain the separation without chiral selectors, (ii) more points for steric interactions with the chiral selector. That is the reason why almost all precolumn derivatization is employed in this case. Only labeling reactions, *i.e.*, with a covalent bond formed between the analyte and the labeling reagent, will be considered in this point.

Fluorescence labeling of carboxylic acids with alkyl halides usually requires the use of organic solvents and phase transfer, because the reactivity in aqueous solution is very low [18]. Alkyl bromides, such as phenacylbromide and 4-bromomethyl-7-methoxy-coumarin, are commonly applied. A disadvantage of these reagents is their poor selectivity [19]. In indirect derivatization procedures, the carboxylic acid function is first activated by a carbodiimide, *e.g.*, dicyclohexyl-carbodiimide, or the water-soluble *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride, or 2-bromo-1-methylpyridinium iodide and is subsequently reacted with a fluorescent alcohol or amine [20]. This reaction principle has been used for bigger compounds than short-chain organic acids, *e.g.*, phenoxy acid herbicides and acidic monosaccharides with 7-aminonaphthale-1,3-disulfonic acid (ANDSA) [21], but these compounds will be discussed below [22–24]. The labeling of a dicarboxylic acid with 1-pyrenyldiazomethane (PDAM) can be performed without the addition of carbodiimide [25]. Due to steric hindrance only the 1-pyrenylmethyl monoester can be formed.

3.2 Direct separation

It is necessary to point out that, although much more elegant, this methodology suffers the problem of limited sensitivity in UV detection, because short-chain organic acids do not contain a chromophore group, and the problem gets increased with the UV absorption of the added selector.

3.2.1 Ligand-exchange capillary electrophoresis

The separation mechanism of ligand-exchange CE is based on the formation of diastereomeric ternary-mixed metal complexes between the chiral selector ligand and the analytes. Resolution is due to differences in complex stability constants of the two mixed complexes with the analyte enantiomers [26, 27]. The method, firstly applied in HPLC, was shown to be also applicable to the chiral separation of aromatic hydroxy acids using copper(II) complexes of L-hydroxyproline or aspartame as chiral selectors [28]. Authors have studies in progress to increase sensitivity by using an on-line combination of two capillaries filled with either chiral selective or achiral background electrolytes. Yet again, the analytes were aromatic compounds. More recently, ligand-exchange CE was also applied to 11 aromatic α-hydroxy acid standards and different resolution degrees were obtained. Aliphatic α-hydroxy acids did not show resolution, following the authors. Only at high selector concentration some aliphatic α-hydroxy acids were partially resolved, but serious detection problems occurred [26].

Later on, malic acid has been enantioseparated by ligand-exchange CE using copper(II)-L-tartrate as a chiral selector. The running conditions for optimum separation of malic acid were found to be 1 mM copper(II) sulfate-1 mM L-tartrate (pH 5.1) with an effective voltage of −20 kV at 30°C, using direct detection at 280 nm. With this system, D- and L-malic acid in apple juice were ana-

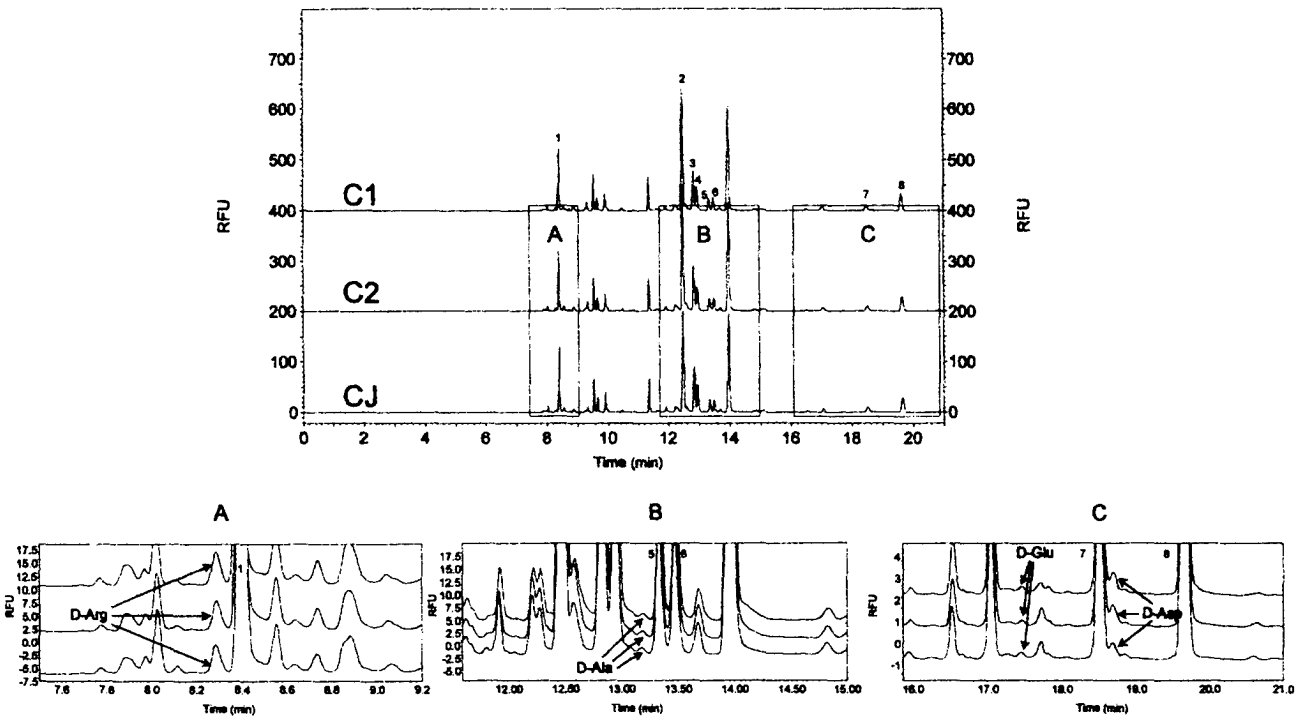

Figure 2. Chiral FITC-amino acids in orange juices. (A) Orange concentrate from Brazil; (B) orange concentrate from Florida; (C) Commercial orange juice from concentrate. Peaks: 1, L-Arg; 2, L-Pro; 3, GABA; 4, L-Asn; 5, L-Ser; 6, L-Ala; 7, L-Glu; 8, L-Asp. Running buffer: 100 mM sodium tetraborate, 30 mM SDS at pH 9.4 with 20 mM β-CD. Uncoated fused-silica capillary: l_t = 45 cm, l_d = 40 cm, 50 μm ID · Capillary temperature: 15°C. Run voltage: 20 kV. Injection at 0.5 psi for 5 s. LIF detection at 488 nm (excitation wavelength) and 520 nm (emission wavelength). Reprinted from [48], with permission.

athletes to improve performance. It is necessary to control the enantiomeric purity of these nutritional supplements, since the different biological or physiological properties of amino acids could lead to nutritionally poorer and less safe products by generating nonmetabolizable and biologically nonutilizable forms of amino acids, The method developed by Boniglia *et al.* [50] uses a previous derivatization with 9-fluorenylmethyl-chloroformate (FMOC) for UV detection at 254 nm. The method was optimized varying β-CD and SDS concentration in the presence of 2-propanol. The running buffer used for the chiral separation of these amino acids was 50 mM phosphate at pH 7.5, 15 mM SDS, 12 mM β-CD, and 15% 2-propanol.

Tivesten *et al.* [51], based on a previous work [52], developed an optimized CE method for chiral separation of DL-Asp and DL-Glu in beer and yoghurt samples. They used *o*-phthaldialdehyde (OPA) combined with a chiral thiol such as 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranose (TATG) for chiral labeling of DL-Asp and DL-Glu. The reaction of each enantiomer, prior to CE analysis, with the chiral reagent produces diastereomers which can be separated in a nonchiral environment (indirect chiral CE separation). Moreover, OPA/TATG reagents improve amino acids sensitivity providing UV detection at 340 nm and LIF detection at 350/415 nm. The method development involved the optimization of running buffer used (nature, pH, use of different surfactants, nonaqueous media). The optimum running buffer containing 100 mM octyl-β-D-glucopyranoside (a neutral surfactant) at a pH 6.5 with 5% acetonitrile, allowed the separation of complex food and biological samples containing protein and other amino acids.

5 Sugars

Though most naturally occurring sugars are D-sugars, their mirror images, the L-sugars, are also of interest. Whereas D-sugars are calorific and cariogenic, L-sugars should not give calories, and should not cause dental caries. This makes them of potential interest to the food industry as a substitute for sucrose. Since both D- and L-sugars taste sweet, this poses problems for any theories of a chiral sweetness receptor, as it would not be

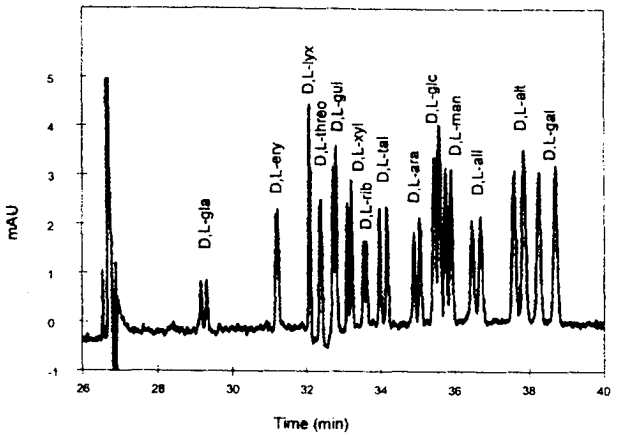

Figure 3. Electropherogram of 28 aldoses derivatized with S-PEA. Running buffer, 50 mM borate buffer plus 30% acetonitrile at pH 10.3. Uncoated fused-silica capillary, l_t = 77 cm, l_d = 70 cm, 50 μm ID. Capillary temperature, 20°C. Run voltage, 28 kV. UV detection at 200 nm. Reprinted from [53], with permission.

expected that both a right and a left hand could fit a right-hand glove. On the other hand, sugars may serve as markers of food authenticity, processing and adulteration.

In spite of the different properties of D- and L-sugars mentioned above, few applications of CE methods for carbohydrates in food have been described and most of them do not determine the enantiomeric composition of the sugar mixture. Noe *et al.* [53] developed a CE method that separated 28 aldose derivatives in about 45 min (Fig. 3). Separation was based on the well-known formation of negatively charged complexes of the sugar with hydroxy groups with tetrahydroxyborate after derivatization with *S*-(−)-1-phenylethylamine (S-PEA) as chiral UV tag. Based on this method, the sugar composition of wine and apple juice was established. The total aldose, uronic acid and deoxyaldose enantiomer composition of these beverages was accessible in a single run and usually there was no need for a previous sample clean-up process.

6 Herbicides and pesticides

About 25% of all agrochemicals used in the world are chiral compounds [54] and, currently, they are introduced in the environment as racemates despite the fact that their activity is usually the result of the preferential reactivity of only one enantiomer. Moreover, organisms produce or degrade chiral compounds by stereospecific enzymatic processes, and therefore, the application of chiral mixtures may result in undesirable environmental loading with organic pollutants. Furthermore, the environmental persistence of the unneeded enantiomers can be relatively longer than that of the useful antipode. This can cause problems with unwanted residues in various food products. In general, hydrophobic persistent organic compounds will accumulate from a lower trophic level to a higher trophic level. In a food web, the concentration increases to the highest trophic level [55].

Analytical separation methods that are highly efficient and stereoselective are needed not only to monitor the enantiopurity of commercial pesticide formulations but also to understand the enantiomeric discrimination in environmental compartments and their accumulation in the food chain. CE has proven suitable for this purpose, nevertheless, efforts have been mainly directed to the study of the chiral separation mechanism and most of the applications have been developed for standards or production samples.

Field studies on the fate of highly polar, chiral compounds, like sulfophenylcarboxylates, have been developed with CE and α-CD as chiral selector [56]. El Rassi [57] in a comprehensive review of CE of pesticides described several chiral selectors and novel chiral selectivity mechanisms, but no application to food or other complex samples was included. Mechref and El Rassi [21] evaluated the labeling of phenoxy acid herbicides with ANDSA and the chiral separation of the labeled compounds with octylmaltopyranoside, a neutral chiral surfactant. Limits of detection as low as 2.2 ppt could be obtained with LIF detection and the concept of field-amplified sample stacking (FASS). Authors propose the method for evaluating contamination at the low-ppb levels, but real samples were not included. Desiderio *et al.* [58] demonstrated the efficacy of sulfobutyl-β-cyclodextrin (SBE-β-CD) for the effective stereoselective resolution of chlorbufam, napropamide, bromacil, and ethofumesate, but only commercial preparations were tested. Ingelse *et al.* [59] separated the optical isomers of the herbicides fluazifop, halossifop, fenoxaprop, and partially flamprop with CE and 1-allylterguride as chiral selector.

Other studies on this subject have also been conducted only with standards. Thus, hepta-tyr, a glycopeptide antibiotic of the teicoplanin family, showed a good resolution with phenoxy acid herbicides. Due to the intense UV absorption, the use of the partial-filling method was employed [60]. Vancomycin was also employed with the same technique [61]. Otsuka *et al.* [62] developed a stereoselective separation of phenoxy acid herbicide enantiomers by CE with heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD) as chiral selector and electrospray ionization mass spectrometry as detector. Enantiomeric separation of phenoxy acid herbicides have been studied with diamino-β-CD and a recognition model has been

proposed [63]. Electrokinetic chromatography has been applied to the separation of organochlorine pesticides [64], and phenoxy acid herbicides with HP-β-CD as the most suitable chiral pseudophase [65, 66].

Pyrethroids are broadly used synthetic insecticides. Commercially available pyrethroids are esters. The analysis of the acidic part is excellent for environment-originated samples. The acidic parts usually have two chiral centers in their cyclopropane ring and they present large differences in their biological activity [67]. Enantiomers and diastereoisomers of chrysanthemic, permethrinic and deltamethrinic pyrethroic acids were separated from each other, using positively ionizable permethylmonoamino-β-CD (PMMA-β-CD). The optimum conditions of separation were found to be 16 mM PMMA-β-CD and pH 6.5, where analytes and selector were in oppositely ionized states.

However, pesticides are often difficult to extract, isolate and quantify from complex food matrices. Several studies have investigated CE determination of pesticides in specific food matrices, such as soybeans, onions or potatoes [68–70], but they do not deal with chiral analysis. Therefore, there is a large field of work joining together this knowledge. Practical applications of chiral CE analysis in food matrices have only been developed by Shea *et al.* [71] and Kodama *et al.* [72]. Shea *et al.* developed the enantiomeric and isomeric analysis of seven commonly used pesticides (propiconazole, bioallethrin, fenpropathrin, phenothrin, bitertanol, triadimenol, and dimethromorph) separately in fortified water samples. Recoveries ranged from 45 to 89% and limits of detection ranged from 0.18 to 2.1 ppb. Commercially available surfactants used alone or in combination with CDs were employed. Kodama and co-workers analyzed vinclozolin enantiomers in wine. This carbidiimide fungicide was separated using γ-CD added to a buffer with SDS working in MEKC conditions. Sample treatment was 25 times extraction of 20 mL of wine sample on a solid-phase cartridge for concentration and then a new solid-phase extraction on a different cartridge to purify the extract. The anti-androgenic activities of both isomers were studied on dihydrotestosterone-induced transcription.

7 Other compounds

Flavonoids are one of the largest natural occurring phenolic compounds widespread in foods and beverages. These compounds are commonly synthesized by plants (vegetables, fruits, spices, medicinal plants) and can occur in the free state (aglycone) or as glycosides. Flavonoids consist mainly of flanoves, isoflavones, flavonols, flavanones, catechines, anthocyanidins, and chalcones. Many of them are associated to flavor and color of foods. Owing to their polyphenolic nature, these compounds are often associated to antioxidant (also associated to prevention of coronary diseases), antimicrobial and/or antiviral activities [73]. Flavonoids also seem to exhibit anticancer activity, although the exact target of such inhibition has not been definitely established. Other reported activities include the inhibition of various enzymes involved in mediating inflammatory responses. In short, flavonoids play an important nutritional and pharmaceutical role and, therefore, their use is increasing as an important component of the human diet [74].

Due to their large biodiversity and specific appearance in different species of plants, they could be good indicators of food quality and security. It is well known that numerous physiological reactions are associated to stereoselective ligand-receptor interactions. In numerous cases, these compounds present at least one asymmetric carbon and, therefore, they could present optical activity. A lot of natural flavonoids come from a stereospecifically enzymatical pathway giving rise to only one particular enantiomer. Therefore, chiral separation of these kind of compounds is a powerful tool for characterization of quality and safety of foods coming from plants.

Analysis of flavonoids by CE is a relatively recent application. Even though HPLC has shown a large usefulness for flavonoids analysis [75, 76], the higher resolving power of CE can be an interesting alternative for separating the numerous compounds that are typically found in the complex mixtures of flavonoids. Moreover, CE coupled to UV diode-array and/or MS detection provides "on-line" valuable structural information of these similar compounds. Although flavonoids have been mainly separated by CE under nonchiral conditions [77, 78], some works have demonstrated the interesting possibilities of CE for enantiomeric separations of this type of food compounds. In this way, Gel-Moreto *et al.* [79] described a method for chiral analysis of six diastereomeric flavanone-7-*O*-glycosides (naringin, prunin, narirutin, and neohesperidin) by CE. These flavanone-7-*O*-glycosides exist as a pair of diastereomers because of the presence of a chiral center in the aglicone and the optically active sugar residue. In this work, these flavonoids were separated in their 2*S*- and 2*R*-diastereoisomers. Although no chiral separation in one run was obtained for the six flavanone-7-*O*-glycosides mentioned above, the chiral separation was achieved using different CDs, namely, native (β-CD, γ-CD), neutral derivatives (2,6-di-*O*-methyl-β-CD (DM-β-CD, HP-β-CD) and charge derivatives (carboxymethyl-β-CD, (CM-β-CD), 2-carboxy-ethyl-β-CD (CE-β-CD)). Stereospecifity depends on the size of the cavity of the CD and the interactions between hydroxyl groups and

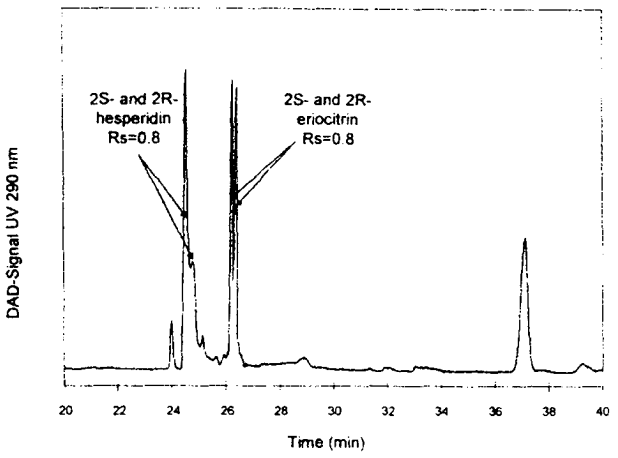

Figure 4. Chiral separation of 2*S*- and 2*R*-diastereomers of eryocitrin and hesperidin in lemon juice. Running buffer, 0.2 M borate buffer, 5 mM γ-CD at pH 10.0. Uncoated fused-silica capillary, l_t = 67 cm, l_d = 60 cm, 75 μm ID. Capillary temperature, 25°C. Run voltage, 15 kV. Injection at 0.5 psi for 2 s. UV detection at 290 nm. Reprinted from [79], with permission.

derivatized groups from CDs. In this study, differences on chiral resolution of the 2*S*- and 2*R*-diastereoisomers according to CD concentration and runnig buffer pH were also observed. This group [79] presented the analysis of the major flavanone-7-*O*-glycosides (eryotricin and hesperidin) in lemon juice, using a 0.2 M borate buffer at pH 10.0 and 5 mM γ-CD as can be seen in Fig. 4.

Another application of flavonoids enantiomer separation by CE for food characterization is presented by Allen *et al.* [80]. The pterocarpan medicarpin (coming from pterocarpan vesitone) is a flavonoid identified as a major natural fungitoxic present in alfalfa. This chiral compound normally accumulates as (−)-medicarpin enantiomer. Also it is known that (+)-medicarpin is 2–3-fold as effective as the (−)-medicarpin. To increase production of fungitoxic isomer in alfalfa, and made disease resistance stronger, it has begun to clone and introduce (+)-pterocarpan biosynthetic genes into alfalfa. Allen *et al.* [80] developed a chiral CE method for a rapid screening of the enantiomeric purity of (±)-medicarpin and (±)-vesitone flavonoids biosynthesized in these transgenic legumes. The method development involved the optimization of different chiral selectors (α-CD, β-CD, γ-CD, HP-α-CD, HP-β-CD, HP-γ-CD, and DM-β-CD) as well as running buffer pH. The optimized method used as running buffer a CD mixture (2 mM HP-β-CD and 20 mM HP-γ-CD) in a 25 mM borate at pH 10.0 with 10% v/v methanol. Under these conditions, a separation resolution of 1.47 and 1.80 for the medicarpin and vesitone enantiomers was obtained.

Pantothenic acid belongs to the B-complex vitamins. Its structure presents one asymmetric carbon atom ($HOCH_2$-$C(CH_3)_2$-C*H(OH)-CO-NH-CH_2-CH_2-COOH). D-(+)-Pantothenic acid is a precursor of coenzyme A, while L-(−)-pantothenic acid is inactive. Kodama *et al.* [81] presented a work for pantothenic acid enantiomers separation in a soft drink by CE. Even though this compound has no aromatic rings, analysis was achieved without a previous derivatization step. Under these conditions, they could detect L-enantiomer acid up to a 3% of the total pantothenic acid. Several CDs were tested for direct chiral CE separation (α-CD, β-CD, DM-β-CD TM-β-CD, and HP-β-CD). The lack of aromatic rings affects the inclusion complexation mechanism; in this way, only HP-β-CD was found to be effective for the separation of D- and L-pantothenic acid. Other parameters were modified: CD concentration, phosphate buffer concentration, pH, capillary temperature, and methanol addition. The optimum running buffer was composed of 60 mM phosphate buffer and 60 mM HP-β-CD at pH 7.0.

8 Future outlooks

Although CE is becoming well established as a viable alternative to both chromatography and gel electrophoresis in food analysis laboratories [11, 12], CE nowadays lacks the sensitivity of HPLC (or GC) and the throughput capability of traditional slab-gel electrophoresis. From a chiral point of view, GC and HPLC have been, up to now, the techniques mainly used to carry out this type of enantiomeric separations, providing in some cases unequivocal results. However, these techniques generally use expensive chiral columns, the procedures for sample preparation are frequently laborious and time-consuming and separations may be lengthy. Considering the drawbacks of these chromatographic techniques when applied to chiral separations, new analytical procedures able to overcome these limitations (as the ones based on CE) would be very useful.

In order to improve both sensitivity and mainly selectivity, chiral CE can be interfaced with other techniques such as electrospray-MS to bring about a very powerful hyphenated technique. On-line coupling of chiral CE with electrospray-MS may solve the identification problems associated with unknown chiral compounds in food analysis. A review about this interesting topic (*i.e.*, chiral CE-MS) has been recently written by Shamsi [82], highlighting the different approaches for coupling CE-MS for analysis of chiral compounds. In this review, a discussion is shown about the different strategies (*e.g.*, partial-filling technique, voltage switching, *etc.*) that have been developed to overcome the lack of electrospray efficiency and ion-

source contamination due to relatively large concentrations of involatile chiral additives that have to be added into the separation buffer. One of the few applications of chiral CE-MS to food-related compounds is the one mentioned above by Otsuka *et al.* [62] who, using CE with TM-β-CD and electrospray ionization-MS, developed a stereoselective separation of three chiral phenoxy acid herbicides (*i.e.*, fenoprop, dichloroprop, mecropop). However, in this work some suppression of analyte electrospray-MS signal was noted due to introduction of CDs into the running buffer. From all the above it can be concluded that the application of chiral CE-MS to the analysis of food compounds is an important and unexplored working field.

Some other new and interesting developments that are nowadays being worked out within the CE domain will foreseeably be applied for chiral food analysis in the nondistant future. These developments include chip-based enantioselective separations [83, 84], CE interfaced with biosensors [85, 86] and multicapillary arrays [87, 88]. The development of these systems will be an important help to overcome throughput limitations and sensitivity problems of CE.

9 Concluding remarks

Compared with GC or HPLC, CE can be considered as a novice in food analysis. However, the relative novelty of the use of chiral analysis in foods can make of chiral CE the technique of choice to carry out such analysis. The high resolving power, rapid method development, easy sample preparation, and low operation expense (allowing the use of sophisticated and/or very expensive chiral selectors) of CE are good indicators of the great potential of this technique in the domain of chiral food analysis.

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