WILEY-VCH ELECTROPHORESIS

CHIRAL CAPILLARY ELECTROPHORESIS IN FOOD ANALYSIS

Journal:	*Electrophoresis*
Manuscript ID:	elps.200900770.R1
Wiley - Manuscript type:	Review
Date Submitted by the Author:	25-Jan-2010
Complete List of Authors:	Herrero, Miguel; Institute of Industrial Fermentations, Food Analysis Simo, Caroline; Institute of Industrial Fermentation, Food Anaysis Garcia-Cañas, Virginia; Instituto de Fermentaciones Industriales, Caracterización de Alimentos Fanali, Salvatore; Consiglio Nazionale delle Ricerche, Institute of Chemical Methodologies Cifuentes, Alejandro; CSIC, Food Analysis
Keywords:	amino acids, beverages, enantiomers, pesticides, polyphenols

CHIRAL CAPILLARY ELECTROPHORESIS IN FOOD ANALYSIS

Miguel Herrero[1,2], Carolina Simó[1], Virginia García-Cañas[1], Salvatore Fanali[3], Alejandro Cifuentes[1]*

[1]*Department of Food Analysis, Institute of Industrial Fermentations (CSIC) Juan de la Cierva 3, 28006 Madrid, Spain*

[2]*Food Science Departmental Section, Autónoma de Madrid University, Campus de Cantoblanco, 28049 Madrid, Spain*

[3]*Institute of Chemical Methodologies, Consiglio Nazionale delle Ricerche Area della Ricerca di Roma I, Via Salaria Km 29,300, 00015 Monterotondo, Rome, Italy*

Running Title: Chiral CE in food

***Corresponding author:**

Tel# 34-91-5622900 (Ext 387), Fax# 34-91-5644853, E-mail: acifuentes@ifi.csic.es

Abbreviations: β-CD (β-cyclodextrin); **γ-CD** (γ-cyclodextrin); **DM-β-CD** (2,6-di-*O*-methyl-β-cyclodextrin); **DNS** (5-(dimethylamino)naphthalene-1-sulfonyl chloride); **FITC** (fluorescein isothiocyanate); **GABA** (gamma-amino butyric acid); **HPF** (hydrolysed protein fertilizers); **HP-β-CD** (2-hydroxypropyl-β-cyclodextrin); **SBE-β-CD** (sulfobutyl β-cyclodextrin).

Keywords: amino acids, beverages, capillary electrophoresis, chiral, enantiomers, pesticides, polyphenols.

ABSTRACT

This review article addresses the different chiral capillary electrophoretic methods used to study and characterize foods and beverages through the enantiomeric separation of different food compounds such as amino acids, pesticides, polyphenols, etc. This work intends to provide an updated overview on the main applications of such enantioselective procedures together with their main advantages and drawbacks in food analysis. Some foreseeable applications and developments of these chiral CZE, CEC and MEKC methods for food characterization are also discussed. Papers that were published within the period January 2003-October 2009 are included, following the previous review on this topic by Simo et al. (*Electrophoresis* 2003, *24*, 2431–2441).

CONTENTS

1. INTRODUCTION

Nowadays, there is a clear trend towards the use of enantioselective methodologies in Food Analysis [1]. Chiral separations can be used in food and beverage studies for identifying adulterated foods and beverages, control and monitoring of fermentation processes and products, evaluation and identification of age, treatment and storage effects, evaluation of some flavor and fragrance components, fingerprinting complex mixtures, analysis of chiral metabolites from chiral and/or prochiral constituents of foods and beverages, decreasing environmental persistence of contaminants, etc [2].

Among the different separation techniques developed so far to separate chiral compounds (e.g., HPLC, GC, etc), capillary electrophoresis (CE) has emerged as a good alternative for enantiomer separations since this technique provides fast and efficient separations for this type of analysis. Moreover, the availability of many chiral selectors and the minimum consumption of such compounds during a CE run have to be considered as an additional advantage of capillary electromigration methods. These characteristics fulfill many of the requirements demanded by food chemists, regulatory agencies and quality control laboratories involved in Food Analysis [1]. Moreover, chiral CE methods are capable to provide important information about processing, quality control, compliance with food and trade laws, adulteration, contamination, product tampering and chemical composition of foods. As a consequence, there is a growing interest in the development of CE methods, including chiral procedures, applied to food analysis as can be deduced from the review works shown in Table 1 published on these topics [1,3-17].

2. SCOPE OF THE REVIEW

This review article covers the different chiral capillary electrophoretic methods (i.e., CZE, CEC and MEKC) used to study and characterize foods and food compounds including, amino acids, pesticides, polyphenols, etc. Thus, especial emphasis is given to applications in which analytes are detected in food matrices, demonstrating in this way the usefulness of chiral CE separations to solve specific problems in Food Science and Technology (see some representative examples in Table 2). Papers that were published during the period January 2003-October 2009 are included following the previous review on this topic by Simo et al [1]. Some foreseeable applications and developments of chiral CZE, CEC and MEKC methods in food analysis are also discussed.

3. AMINO ACIDS

The analysis of chiral amino acids in foods and beverages is a very useful tool to assess their quality, corroborate their authenticity or detect microbiological contaminations. Moreover, determination of specific enantiomers or enantiomer ratios of amino acids can provide valuable information about adulterations, fermentation, storage, age, etc. The huge information that can be achieved through the chiral CE analysis of amino acids together with the well known structure of amino acids and their ubiquitous character, have made of this application the most frequent as can be deduced from the high number of papers published on this topic.

The enantioseparation by CE-MS of seven chiral amino acids and one achiral amino acid was carried out to detect adulterations in orange juices [30]. A polymer coating of the inner

capillary wall was used in this case to reduce the EOF and minimize in this way the entrance of the neutral β-CD into the ion source [30]. The chiral CE-MS method provided good selectivity and the possibility to confirm the analyte identity through the molecular mass determination. However, this chiral CE-MS method provided lower sensitivity than the obtained using a chiral-MEKC-LIF method [31]. This chiral-MEKC-LIF method was developed to classify three types of commercial orange juices (i.e., nectars, orange juices reconstituted from concentrates, and pasteurized orange juices not from concentrates) based on their amino acids profile. From these results, it was demonstrated that some D-amino acids occur naturally in orange juices. Application of stepwise discriminant analysis to 26 standard samples of orange juice showed that the amino acids L-Arg, L-Asp and GABA were the most important variables to differentiate the three groups of orange juices. With these three selected amino acids a 100% correct classification of the samples was obtained either by standard or by leave-one-out cross-validation procedures. These classification functions based on the content in L-Arg, L-Asp and GABA were also applied to nine test samples and provided an adequate classification and/or interesting information on the samples [31].

Ornithine is a chiral non-protein amino acid, whose L-enantiomer favors the metabolism of corporal fatty excess, collaborates in human growth hormone synthesis, participates in ammonia detoxification in urea cycle and generates polyamines and L-proline, which are involved in cell proliferation and collagen synthesis, respectively [32]. However, D-Orn produces depletion in the urea synthesis by competition with L-Orn in the enzymatic system, giving rise to toxic consequences for the body by avoiding the elimination of ammonia [32]. Therefore, the chiral separation of ornithine is an interesting topic in food analysis, because this compound is frequently found in fermented beverages and food supplements. In a recent paper [32], ornithine was determined by CE-MS in beers submitted to different fermentation

processes. By operating the instrument in tandem mode (MS/MS), a significant increase in the signal was achieved, obtaining LOD of $2.5 \cdot 10^{-9}$ M, two orders of magnitude lower than the LOD obtained with UV detection [33,34].

Dietary supplements are more and more consumed everyday and their adequate quality control is mandatory in order to warrant their safety and composition. Thus, CE coupled with fiber-optic light-emitting diode-induced fluorescence detection was developed for the chiral separation of tyrosine using R(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as a chiral fluorescence tagged reagent for derivatization of Tyr [22]. The LOD of D- and L-Tyr derivatives were 2.9 and 2.2 µmol/L (S/N = 3), respectively. The method was successfully applied to the determination of Tyr in a commercial amino acid oral solution.

A fast in-capillary derivatization method by CE with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate was developed for the determination of amino acid enantiomers (arginine, lysine, and ornithine) providing LOD in the micro-molar range using UV detection at 260 nm and a BGE with a mixture of cyclodextrins. The optimized method was successfully applied to the determination of the enantiomers of arginine, lysine, and ornithine in dietary supplements and wines [35].

Confirmation of substantial equivalence between transgenic foods and their isogenic non-transgenic counterparts is essential in order to corroborate the safety of these new foods. In this regard, chiral-CE analysis can provide complementary information and a completely new perspective on this hot topic. Following this idea, a new chiral method using MEKC-LIF analysis was developed to identify and quantify L- and D-amino acids in three lines of

transgenic maize and their corresponding nontransgenic parental lines grown under identical conditions [18]. The procedure included amino acids extraction, derivatization with FITC and chiral-MEKC-LIF separation in a background electrolyte composed of 100 mM sodium tetraborate, 80 mM SDS, and 20 mM β-CD at pH 10.0. The analysis was completed in less than 25 min, with efficiencies up to 890 000 plates/m and LODs as low as 160 nM, allowing the detection of 1% D-Arg in the presence of 99% of its opposite enantiomer. Very similar D- and L-amino acids composition was obtained for one of the studied maize couples (natural vs transgenic) what could be presented as a new proof of their substantial equivalence. However, significant differences in their L/D ratios of amino acids were observed for the other two couples of maize studied. It is concluded that enantioselective procedures can open new perspectives in the study of transgenic organisms in order to corroborate (or not) the equivalence with their conventional counterparts, as also demonstrated through the chiral analysis of amino acids from conventional and transgenic yeasts used for sparkling wine production [36]. From this study it was concluded that the genetic modification brings a faster autolysis of the yeast, releasing a higher amount of L-amino acids to the medium in a short time. Interestingly, the pattern of release of D-amino acids was also different between the transgenic and the conventional yeast strains [36].

The use of modified cyclodextrins was investigated for the separation of five chiral amino acids in different food matrices by CE-MS, including transgenic soybeans [21]. The new synthesized 3-monodeoxy-3-monoamino-β-cyclodextrin could bring additional ionic interactions due to its positive charge, increasing in this way the selector-analyte complex formation, and allowing in this way the use of low concentration of chiral selectors for their use in the CZE-MS coupling [21]. Moreover, LOD values obtained by chiral CE-MS were in the nM range comparable or only slightly worse to those obtained by CE-LIF [21]. An

example of these applications is shown in Figure 1 in which some qualitative and quantitative differences between the wild and transgenic soybeans studied were found based on their D- and L-amino acids profile.

Chiral analysis of amino acids has also been used to identify different varieties of microalgae used as natural source of nutraceuticals and functional ingredients together with their different drying processes [37]. Thus, three microalgae species, *Spirulina platensis*, *Dunaliella salina*, and *Tetraselmis suecica* were compared in terms of their content in D- and L- amino acids analyzed by using MEKC-LIF with β-cyclodextrin in the BGE to separate and quantify the analytes previously derivatized with FITC. The method allowed the detection of amounts as low as 330 ng of D-Arg per gram of microalga. Several D-amino acids were detected in all the microalgae, observing interesting differences in their D/L-amino acids profiles, corroborating the usefulness of the chiral-MEKC-LIF approach to characterize different microalgae species as well as different microalgae drying processes [37].

The formation of D-amino acids in many fermented foods depends, among other factors, on the particular fermentation conditions, the action and autolysis of the microorganisms involved. In this sense, the analysis of chiral amino acids is an interesting analytical strategy for food scientists, since these compounds can be used as bacterial markers and can help, e.g., to detect adulterations, microbiological contaminations, etc. Following this idea, different chiral-CE methodologies have been developed to study different vinegars. Thus, the use of β-cyclodextrin together with MEKC-LIF allowed the separation of D- and L- amino acids previously derivatized using FITC in different vinegars with LOD lower than 17 nM [19]. Several D-amino acids were detected and quantified in balsamic, sherry, white wine, and cider vinegars using this MEKC-LIF procedure, observing interesting differences in their L- and D-amino acids profiles and contents allowing a straightforward characterization of the

different vinegars [19]. Recently, a chiral ligand-exchange capillary electrophoretic method has been developed for the enantioseparation and UV detection of dansyl-amino acids with Zn(II) L-arginine complex as chiral selecting system [38]. This new method was applied to the separation and quantification of some pairs of amino acids in rice vinegars showing a linear range between 0.8 and 150 μg/mL [38] as well as for the analysis of aromatic amino acid enantiomers in rice-brewed suspensions (called Laozao in Chinese), what could be used as an index to recognize the brand of Laozao [20]. An example of this application can be seen in Figure 2. Also, the use of modified cyclodextrins together with CE-MS has shown very interesting possibilities for the separation and identification of chiral amino acids in vinegars [38].

Somehow related to the food analysis area, the degree of racemization of hydrolyzed protein fertilizers (HPFs) was monitored by MEKC using a polyacrylamide coated capillary and a run buffer containing SDS and β-cyclodextrin in the BGE [39] . The quantitative separation of D- and L-Ala was carried out and the degree of racemization (RD) was calculated as D-Ala/[D-Ala+L-Ala]. The analysis of ten commercial HPFs showed that more than 60% of HPFs had an RD greater than or equal to 40%, while only one product showed a RD <5%. These results indicated that most of the HPFs on the market are obtained with strong hydrolytic processes and high contents of D-amino acids are probably less effective as plant nutrients or even potentially dangerous to plants [39].

Carnitine is a non-protein amino acid available to humans both through in vivo biogenesis involving lysine and methionine, and from a variety of dietary sources. In infants, since they are unable to synthesize it due to an immature biosynthesis pathway, this non-protein amino acid is an essential nutrient to be supplemented into the baby food products. However, such baby food products should be controlled to avoid the use of racemic carnitine. In fact, while

L-carnitine plays an important role in the metabolism of long chain fatty acids, D-carnitine has been found to have a considerable toxic influence on biochemical processes due to inhibition effects on the carnitine acetyltransferase, producing a depletion of the body's L-carnitine stock [23]. For this reason, the development of analytical methodologies enabling the determination of carnitine enantiomers is of great interest for the evaluation of the nutritional quality of baby food products. In a recent paper, the determination of D and L carnitine in different infant formulas has been described using chiral-CE-MS [23]. Succinyl-γ-cyclodextrin was used filling 50% of the capillary with this chiral selector in a 0.5 M ammonium formate BGE at pH 2.5. In order to improve sensitivity and selectivity of the CZE-MS method, MS/MS experiments with an ion trap analyzer were carried out. Thus, a 100-fold sensitivity enhancement with respect to UV detection was obtained, achieving LOD of 100 ng/g for D-carnitine. The determination of L-carnitine and its enantiomeric purity in 14 infant formulas supplemented with carnitine was successfully achieved; sample preparation only required ultrafiltration with centrifugal filter devices to retain the components with the highest molecular weights [23].

4. FUNGICIDES, HERBICIDES AND OTHER PESTICIDES

Detection of contaminants in foods is a key topic to guarantee their safety. Moreover, chiral analysis of these analytes by CE-based techniques allows monitoring the different toxicity, activity and/or degradation rate of the enantiomers involved and with that their impact on human health. Thus, chiral resolution of the fungicide imazalil was performed by CE using 2-hydroxypropyl-β-cyclodextrin as a chiral selector [34]. Eight orange samples were analyzed, and imazalil was detected in seven samples. In four of these seven oranges, the level of (–)-imazalil was the same as that of (+)-imazalil, but in the other three oranges, the level of (–)-

imazalil was found to be lower than that of (+)-imazalil, suggesting that (−)-imazalil was degraded more quickly than (+)-imazalil in oranges (see Figure 3) [24]. In a different work from the same group, vinclozolin, a fungicide with some anti-androgenic activity, was enantiomerically analyzed using MEKC together with γ-cyclodextrin [25]. The peak area ratio of (+)- and (−)-vinclozolins determined in wine was found to be 2:3, concluding that degradation rates were different between (+)- and (−)-vinclozolins. The anti-androgenic activities of (+)- and (−)-vinclozolins on dihydrotestosterone-induced transcription were also investigated. The anti-androgenic activity of (+)-vinclozolin tended to be stronger than that of (−)-vinclozolin, suggesting the possibility that vinclozolin can act as an enantioselective anti-androgen [25].

Enantiomer separation of a series of herbicides (aryloxypropionic, aryloxyphenoxypropionic, and aminopropionic acid) was carried out by CEC after binding to biotin and grafting upon streptavidin-modified porous glass beads. The results suggest that the interactions of the enantiomer during CEC are solely based on chromatographic mechanisms and that the electrophoresis plays only a minor role in this separation; in addition, it was demonstrated that the enantiomerization barrier of some herbicides can be determined using this chiral stationary phase [40].

The enantioselective transformation of pesticides will determine their persistence and impact on human health, since their transformation will define the possibility of detecting them in water, plants and/or foods. Following this idea, CE was applied to study the enantioselective transformation of five chiral pesticides in aerobic soil slurries [41]. Namely, the enantiomers of five chiral pesticides of environmental interest, metalaxyl, imazaquin, fonofos (dyfonate), ruelene (cruformate), and dichlorprop, were separated using CE with cyclodextrin chiral selectors. The work showed the influence of environmental changes on the transformation of

chiral pollutants in soils. Moreover, CE was shown to be a simple, efficient, and inexpensive way to follow the transformation of chiral pesticides in laboratory microcosms where concentrations can be made high enough (25–50 mg/L initial racemate concentration) for detection of residual parent enantiomers during most of the process [41]. In this sense, the enantiomers of four different organophosphorus pesticides (profenofos, prothiofos, sulprofos, and pyraclofos) were analyzed using different BGEs containing sodium cholate either with SDS or γ-cyclodextrin, proposing this methodology for analyzing these pesticides in soil samples [42].

5. PHENOLIC COMPOUNDS

Catechins (see chemical structures in Figure 4) can play an important role against cancer, cardiovascular diseases and other degenerative diseases [43]. Moreover, structure–activity relationships of these compounds have been investigated and the catechol functionality in the catechins has been considered to be responsible for the protective effects exerted by these compounds against a wide range of human diseases [44]. Therefore, the analysis of the content of catechins, including their different enantiomeric forms, in foods as well as in biological fluids is thus crucial for epidemiological and nutritional studies designed to examine the possible relationships between foods containing catechins and the incidence of cancer and cardiovascular diseases. Moreover, the enantiomeric profile of catechins can also be used for food characterization including food processing and storage as shown below.

Analysis of catechins in chocolate and *Theobroma cacao* was carried out by using MEKC at acidic pH and hydroxypropyl-β-cyclodextrin [45,46]. The acidic conditions were observed to improve stability of catechins. Under these conditions, separation of methylxanthines (theobromine and caffeine), procyanidin dimers B1 and B2, and catechins (epicatechin and

catechin) was obtained simultaneously to the enantioseparation of racemic catechin within 10 min. Moreover, the enantioselectivity of the method made possible to evaluate possible epimerisation at the C-2 position of epicatechin monomer (see Figure 4) potentially occurring during heat processing and storage of *T. cacao* beans. The method was applied to *T. cacao* beans from different countries; interestingly, the native enantiomer (+)-catechin was found in the beans whereas, for the first time it was reported that in chocolate, predominantly (−)-catechin is present, probably yielded by epimerisation of (−)-epicatechin occurred during the manufacture of chocolate [45,46]. Similar approach was applied to differentiate green tea samples [47]. Namely, the MEKC BGE at acidic pH with hydroxypropyl-β-cyclodextrin was applied to study the thermal epimerisation of (-)-epicatechin and epigallocatechin, to non-epi-structured (-)-catechin and (-)-gallocatechin. The latter compounds, being non-native molecules, were for the first time regarded as useful phytomarkers of tea samples subjected to thermal treatment. The proposed method was applied to the analysis of more than twenty tea samples of different geographical origins (China, Japan, Ceylon) having undergone different storage conditions and manufacturing processes, showing that it was possible to distinguish tea samples on the basis of their different contents of native and non-native catechins [47].

There is also a great interest to analyze catechins in biological fluids after ingestion of foods (rich in this kind of compounds) in order to analyze the bioavailability and biological activity of these polyphenols. Thus, a chiral CE method using β-CD in the BGE together with a high-sensitivity UV cell was developed to analyze catechin isomers in human plasma subsequent to green tea ingestion [48]. The method was successfully applied to determine catechin and epicatechin isomers in human plasma after ingestion of green tea with detection limits of 4.1 and 1.5 ng/mL, respectively. The unchanged amounts of catechin and epicatechin in plasma were about 17.4 and 1.8% of the administered dose after 2 h of starting tea ingestion [48]. Chiral CE was also applied to the separation and quantification of catechin and epicatechin

(plus ascorbic acid) in some commercial drinks and in human urine after ingestion of the mentioned drinks. Analysis involved the separation of analytes in less than 5.0 min at 240 nm using a BGE composed of 50 mM borate buffer with 3 mM β-CD at pH 8.35. Detection limits for catechin, epicatechin and ascorbic acid were 0.028, 0.011 and 0.004 μg/mL, respectively. The maximum urinary excretion of catechin and epicatechin were noted at 2.0 and 4.0 h of the administrated dose. Unchanged catechin, epicatechin and ascorbic acid amounted to about 1.500, 8.696 and 0.003% of the administered dose in the 48.0 h urine collection [49].

Different CE-based methods have been developed for chiral analysis of catechins trying to use this information also for food characterization, including food processing. Thus, the content of catechin and epicatechin enantiomers was determined in different food plants (apple juice and guaraná seeds) using CE with (2-hydroxypropyl)-γ-cyclodextrin as chiral selector [27]. (+)-Catechin and (-)-epicatechin could be verified as the most common flavan-3-ols in apple juice and guaraná seeds. However, in the case of guaraná, all four enantiomers, both (+)- and (-)-catechin and (+)- and (-)-epicatechin were identified as naturally occurring compounds. This finding was verified by further isolation and purification of the flavan-3-ols and subsequent LC-MS analysis. This method allows for the identification of the authenticity of guaraná through the analysis of the catechin and epicatechin enantiomers [27]. Also, a new cyclodextrin-modified MEKC method was applied to the enantioseparation of catechin and epicatechin using 6-O-alpha-D-glucosyl-β-cyclodextrin. Under these conditions, the resolution of racemic catechin and epicatechin were 4.15 and 1.92, respectively. With this system, catechin and epicatechin enantiomers along with other four catechins ((-)-catechin gallate, (-)-epicatechin gallate, (-)-epigallocatechin, (-)-epigallocatechin gallate) and caffeine were detected in tea samples [26].

Hop (*Humulus Luplus L.*) is a source of phenolic compounds in beer making. In hop cones, the most abundant prenylated chalcone is xanthohumol, which accounts for 80-90% of the prenylated flavonoids in hop. During the brewing process of beer, xanthohumol is converted into the corresponding isomeric prenylflavanone isoxanthohumol [28]. A hydroxypropyl-γ-cyclodextrin-modified MEKC method has been proposed for the chiral resolution of isoxanthohumol enantiomers in beer samples. Using this method, isoxanthohumol was detected in 12 beer samples and the total levels of (+)- and (−)-isoxanthohumol ranged from 0.15 to 1.4 mg/L. However, the amount of xanthohumol was below the detection limit (0.017 mg/L). Each level of (−)-isoxanthohumol was almost the same as that of (+)-isoxanthohumol, suggesting that isoxanthohumol was a racemic mixture in these beer samples. The racemization of isoxanthohumol in beer could be attributed to the production of a racemic mixture from xanthohumol during boiling and to the fact that isoxanthohumol enantiomers were easily interconverted [28]. The separation of 2R- and 2S-diastereomers of major flavanone-7-O-glycosides in five citrus was carried out by chiral-CE using BGEs containing mixtures of different cyclodextrins [29]. Under these conditions, hesperidin and narirutin were detected in sweet orange and mandarin; hesperidin and eriocitrin in lemon; naringin and narirutin in grapefruit; while naringin, neohesperidin and neoeriocitrin were detected in sour orange. Marmalade made from sour and sweet oranges was also analyzed by using the same CE method. In this matrix, characteristic flavanones of both orange varieties could be identified. The simultaneous chiral resolution of these flavanones in citrus could be achieved only by employing combined buffers with two CDs. Chiral HPLC was used as a reference method, in order to prove diastereomeric ratio ascertained by chiral CE corroborating the usefulness of this new methodology for food characterization [29].

6. FUTURE OUTLOOKS AND CONCLUDING REMARKS

Although chiral CE is becoming well established as a viable alternative to chromatography techniques, CE still lacks the sensitivity and repeatability of HPLC (or GC). However, GC and HPLC also have their own drawbacks (e.g., generally use expensive chiral-columns, the procedures for sample preparation are frequently laborious and time consuming and separations may be lengthy). Therefore, new chiral procedures able to overcome these limitations (as the ones based on CE) are very useful. In this regard, 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) are good indicators of the great potential of CE in the chiral food analysis area.

In the non-distant future, some new developments that are nowadays being worked out within the CE domain will foreseeably be applied for chiral food analysis trying to improve in this way the throughput limitations, sensitivity problems and/or lack of repeatability of CE. These developments include chip-based enantioselective separations (see an example in Figure 5) [50-52], the use of new stationary phases and/or background electrolytes for chiral CE and CEC [53-58], new chiral selectors [59-61], multi-capillary arrays and methods that combine in a single step on-line stacking and derivatization of enantiomers [64-65]. It is expected that the development of these new approaches and their applications in food analysis will be an important help to overcome some of the mentioned limitations of chiral-CE.

ACKNOWLEDGEMENTS

This work was supported by the projects: 2008IT0013 CSIC-CNR bilateral project, AGL2008-05108-C03-01 (Ministerio de Ciencia e Innovación) and CSD2007-00063 FUN-C-

FOOD (Programa CONSOLIDER, Ministerio de Educación y Ciencia). M.H. would like to thank Spanish Science and Innovation Ministry (MICINN) for a "Juan de la Cierva" contract. Authors declare no conflict of interest.

7. REFERENCES

[1] Simo C., Barbas, C., Cifuentes, A. *Electrophoresis* 2003, *24*, 2431–2441.

[2] Armstrong, D. W., Chang, C. D., Li, W. L., *J. Agric. Food Chem*. 1990, *38*, 1674-1677.

[3] Shamsi, S. A., Miller, B. E., *Electrophoresis* 2004, *25*, 3927-3961.

[4] Gubitz, G., Schmid, M. G. *Electrophoresis* 2007, *28*, 114-126.

[5] Scriba, G. K. E. *J. Sep. Sci.* 2008, *31*, 1991-2011.

[6] Juvancz, Z., Kendrovics, R. B., Ivanyi, R., Szente, L. *Electrophoresis* 2008, *29*, 1701-1712

[7] Fanali, S.*Electrophoresis* 2009, *30*, S203-S210.

[8] Fanali, S. *Methods Mol. Biol.* 2004, *243*, 265-273.

[9] Hernández-Borges, J., Rodríguez-Delgado, M. A., García-Montelongo, F. J., Cifuentes, A. *Electrophoresis* 2005, *26*, 3799-3813.

[10] Chankvetadze, B., *Electrophoresis* 2009, *30*, S211-S221.

[11] Chankvetadze, B., *J. Chromatogr. A* 2007, *1168*, 45-70.

[12] Chankvetadze, B. *Methods Mol. Biol.* 2004, *243*, 387-399.

[13] Kodama, S., Saito, Y., Chinaka, S., Yamamoto, A., Hayakawa, K. *J. Health Sci*., 2006, *52*, 489-494.

[14] Kahle, K. A., Foley, J. P. *Electrophoresis* 2007, *28*, 2503-2526.

[15] Cifuentes, A. *Electrophoresis* 2006, *27*, 283-303.

[16] Garcia-Cañas, V., Cifuentes, A. *Electrophoresis*, 2008, *29*, 294-309.

[17] Castañeda, G., Rodriguez-Flores, J., Rios, A. *J. Sep Sci.* 2005, *28*, 915-924.

[18] Herrero, M., Ibañez, E., Martin-Alvarez, P. J., Cifuentes, A. *Anal. Chem.* 2007, *79*, 5071-5077.

[19] Carlavilla, D., Moreno-Arribas, M. V., Fanali, S., Cifuentes, A. *Electrophoresis* 2006, *27*, 2551-2557.

[20] Qi, L., Liu, M. R., Guo, Z. P., Xie, M. Y., Qiu, C. G., Chen, Y. *Electrophoresis* 2007, *28*, 4150-4155.

[21] Giuffrida, A., León, C., García-Cañas, V., Cucinotta, V., Cifuentes, A. *Electrophoresis* 2009, *30*, 1734-1742.

[22] Bi, W., Lei, S., Yang, X., Xu, Z., Yuan, H., Xiao, D., Choi, M. M. F. Talanta 2009, *78*, 1167-1172.

[23] Castro-Puyana, M., Garcia-Ruiz, C., Crego, A. L., Marina, M. L. *Electrophoresis* 2009, *30*, 337-348.

[24] Kodama, S., Yamamoto, A., Ohura, T., Matsunaga, A., Kanbe, T. *J. Agric. Food Chem.* 2003, *51*, 6128–6131.

[25] Kodama, S., Yamamoto, A., Saitoh, Y., Matsunaga, A., Okamura, K., Kizu, R., Hayakawa, K. *J. Agric. Food Chem*. 2002, *50*, 1312–1317.

[26] Kodama, S., Yamamoto, A., Matsunaga, A., Yanai, H. *Electrophoresis* 2004, *25*, 2892-2898.

[27] Kofink, M., Papagiannopoulos, M., Galensa, R. *Eur. Food Res. Tech.*2007, *225*, 569-577.

[28] Kodama, S., Yamamoto, A., Sato, A., Suzuki, K., Yamashita, T., Kemmei, T., Taga, A., Hayakawa, K. *J. Agric. Food Chem*. 2007, *55*, 6547–6552.

[29] Gel-Moreto, N., Streich, R., Galensa, R. *Electrophoresis* 2003, *24*, 2716-2722.

[30] Simo, C., Rizzi, A., Barbas, C., Cifuentes, A. *Electrophoresis* 2005, *26*, 1432-1441

[31] Simo, C., Martin-Alvarez, P. J., Barbas, C., Cifuentes, A. *Electrophoresis* 2004, *25*, 2885-2891.

[32] Dominguez-Vega, E., Sanchez-Hernandez, L., Garcia-Ruiz, C., Crego, A. L., Marina, M. L. *Electrophoresis* 2009, *30*, 1724-1733.

[33] Dominguez-Vega, E., Martinez-Giron, A. B., Garcia-Ruiz, C., Crego, A. L., Marina, M. L. *Electrophoresis* 2009, *30*, 1037-1045.

[34] Martinez-Giron, A. B., Dominguez-Vega, E., Garcia-Ruiz, C., Crego, A. L., Marina, M. L. *J. Chromatogr. B*, 2008, *875*, 254-259.

[35] Martinez-Giron, A. B., Garcia-Ruiz, C., Crego, A. L., Marina, M. L. *Electrophoresis* 2009, *30*, 696-704.

[36] Giuffrida, A., Tabera, L., Gonzalez, R., Cucinotta, V., Cifuentes, A. *J. Chromatogr. B*, 2008, *875*, 243-247.

[37] Herrero, M., Ibañez, E., Fanali, S., Cifuentes, A. *Electrophoresis* 2007, *28*, 2701-2709.

[38] Qi, L., Chen, Y., Xie, M. Y., Guo, Z. P., Wang, X. Y. *Electrophoresis* 2008, *29*, 4277-4283.

[39] Cavani, L., Ciavatta, C., Gessa, C., *J. Chromatogr. A* 2003, *985*, 463-469.

[40] Andre, C., Berthelot, A., Thomassin, M., Guillaume, Y. C. *Electrophoresis* 2006, *27*, 3254-3262.

[41] Jarman, J. L., Jones, W. J., Howell, L. A., Garrison, A. W. *J. Agric. Food Chem.* 2005, *53*, 6175–6182.

[42] Huang, L., Lin, J. M., Xu, L. J., Chen, G. N., *Electrophoresis* 2007, *28*, 2758-2764.

[43] Higdon, J. V., Frei, B. *Crit. Rev. Food Sci. Nutr.* 2003, *43*, 89–143.

[44] Tejero, I., Gonzalez-Garcia, N., Gonzalez-Lafont, A., Lluch, J. M. *J. Am. Chem. Soc.* 2007, *129*, 5846–5854.

[45] Gotti, R., Fiori, J., Mancini, F., Cavrini, V. *Electrophoresis* 2004, *25*, 3282–3291.

[46] Gotti, R., Furlanetto, S., Pinzauti, S., Cavrini, V. *J. Chromatogr. A* 2006, *1112*, 345–352.

[47] Gotti, R., Furlanetto, S., Lanteri, S., Olmo, S., Ragaini, A., Cavrini, V. *Electrophoresis* 2009, *30*, 2922-2930.

[48] El-Hady, D. A., El-Maali, N. A. *Talanta* 2008, *76*, 138-145.

[49] El-Hady, D. A., Gotti, R., Ei-Maali, N. A. *J. Sep. Sci*. 2008, *31*, 2252-2259.

[50] Gong, X. Y., Hauser, P. C. *Electrophoresis* 2006, *27*, 4375-4382.

[51] Piehl, N., Ludwig, M., Belder, D. *Electrophoresis* 2004, *25*, 3848-3852.

[52] Escarpa, A., Gonzalez, M. C., Crevillen, A. G., Blasco, A. J. *Electrophoresis* 2007, *28*, 1002-1011.

[53] Yuan, L. M., Han, Y., Zhou, Y., Meng, X., Li, Z. Y., Zi, M., Chang, Y. X. *Anal. Letters* 2006, *39*, 1439-1449.

[54] Fanali, S., Catarcini, P., Presutti, C., Quaglia, M. G., Righetti, P. G. *Electrophoresis* 2003, *24*, 904-912.

[55] Li, M., Lin, X., Xie, Z. *J. Chromatogr. A* 2009, *1216*, 5320-5326.

[56] Mizrahi, S., Rizkov, D., Shames, A. I., Lev, O. *Electrophoresis* 2008, *29*, 3941-3948.

[57] Turiel, E., Martin-Esteban, A. *Anal. Bioanal. Chem*. 2004, *378*, 1876-1886.

[58] Kim, I. W., Hyun, M. H., Gwon, J., Jin, J., Park, J. H. *Electrophoresis* 2009, *30*, 1015-1021.

[59] Kwon, C., Yoo, K. M., Jung, S. *Carbohyd. Res*. 2009, *344*, 1347-1351.

[60] Park, H., Jung, S. *Electrophoresis* 2005, *26*, 3833-3838.

[61] Kodama, S., Aizawa, S., Taga, A., Yamashita, T., Yamamoto, A. *Electrophoresis* 2006, *27*, 4730-4734.

[62] Liu, K., Wang, H., Bai, J., Wang, L. *Anal. Chim. Acta* 2008, *622*, 169-174.

[63] Wang, J., Zhang, Y. Y., Wang, L., Bai, J. L. *J. Chromatogr. A* 2007, *1144*, 279-282.

[64] Ptolemy, A. S., Tran, L., Britz-McKibbin, P. *Anal. Biochem*. 2006, *354*, 192-204.

[65] Fan, L. Y., Cheng, Y. Q., Chen, H. L., Liu, L. H., Chen, X. G., Hu, Z. D. *Electrophoresis* 2004, *25*, 3163-3167.

Table 1: Reviews published in the period covered by this work (January 2003-October 2009) on chiral capillary electromigration methods and/or food analysis by CE-based approaches, following the review on this topic by Simo et al. [1].

Subject	Reference
Chiral capillary electrophoresis-mass spectrometry	[3]
Chiral separation using capillary electromigration techniques	[4]
Cyclodextrins in capillary electrophoresis enantioseparations	[5]
The role of cyclodextrins in chiral capillary electrophoresis	[6]
Chiral separations by CE employing cyclodextrins.	[7]
Enantioresolutions by capillary electrophoresis using glycopeptide antibiotics	[8]
Chiral analysis of pollutants and their metabolites by CE methods	[9]
Separation of enantiomers with charged chiral selectors in CE	[10]
The story of 20 and a few more years of enantioseparations by CE	[11]
Enantioseparation in CEC using polysaccharide-type chiral stationary phases.	[12]
Chiral capillary electrophoresis of agrochemicals	[13]
Chiral EKC and chiral microemulsion EKC	[14]
Capillary electromigration methods for food analysis	[15]
Advanced capillary electromigration methods for food analysis	[16]
Capillary electrophoresis in routine food analysis	[17]

Table 2: Food compounds analyzed by chiral capillary electromigration procedures.

Analyte	Food	Chiral selector	Reference
Several D,L-amino acids	Transgenic maize	β-CD	[18]
Several D,L-amino acids	Balsamic, sherry, white wine, and cider vinegars	β-CD	[19]
Several D,L-amino acids	Rice vinegar, Laozao	Complexation with Zn(II) L-arginine	[20]
Several D,L-amino acids	Transgenic soybean	3-monodeoxy-3-monoamino-β-CD	[21]
D,L-Tyrosine	Dietary supplements	Derivatization with R(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole	[22]
Carnitine	Infant formulas	Succinyl- γ-CD	[23]
Imazalil	Orange	2-hydroxypropyl-β-CD	[24]
Vinclozolin	Wine	γ-CD	[25]
Several catechins	Tea	6-O-alpha-D-glucosyl- β-CD	[26]
Catechin and epicatechin	Various plant food samples	Hydroxypropyl-γ-CD	[27]
Isoxanthohumol	Beer	Hydroxypropyl-γ-CD	[28]
Flavanone-7-O-glycosides	Sweet orange, mandarin, grapefruit, lemon, and sour or bitter orange	Neutral and charged CDs	[29]

FIGURE LEGENDS

Figure 1. Chiral CE-ESI-TOF-MS extracted ion electropherograms from a wild and transgenic soybean. Bare fused-silica capillary with 50 µm ID and 85 cm of total length. Running buffer: 50 mM ammonium hydrogen carbonate at pH 8.0 with 0.5 mM 3-monodeoxy-3-monoamino-β-cyclodextrin as chiral selector. All samples injected for 25 s at 0.5 psi. Separation was performed at 30 kV (38 µA) and 25ºC. Sheath liquid: water-2-propanol (50:50, v/v) delivered at 0.24 ml/h. ESI polarity in the positive mode with a 0.3 bar nebulizer and 4 l/min dry gas at 180ºC. MicrOTOF scan from 200 to 2000 m/z every 60 µs using a prepulse storage of 11.7 µs. Redrawn from [21].

Figure 2. Electropherogram measured from some mixed pairs of dansylated standard amino acid enantiomers using a running buffer of 100mM boric acid, 5mM ammonium acetate, 3mM Zn(II) and 6mM L-Arg, adjusted to pH 8.0 with solid Tris. Capillary: 50 µm id. L_t 57 cm (50 cm effective length); injection: 0.5 psi for 3 s; voltage: -20 kV; temperature: 20 ºC; UV detection: 214 nm. Peak identity: (A) 1. D-Asp, 1'. L-Asp; 2. D-Leu, 2'. L-Leu; 3. D-Ala, 3'. L-Ala; 4. D-Lys, 4'. L-Lys; (B) 5. D-Ile, 5'. L-Ile; 6. D-Tyr, 6'. L-Tyr; 7. D-Met, 7'. L-Met; 8. D-Asn, 8'. L-Asn; (C) 9. D-Glu, 9'. L-Glu; 10. D-Thr, 10'. L-Thr; 11. D-Orn, 11'. L-Orn; (D) 12. D-Phe, 12'. L-Phe; 13. D-Ser, 13'. L-Ser; 14. D-His, 14'. L-His. (E) Electropherogram measured from the supernate of a rice vinegar diluted ten times with water. Peak identity: 1. L-Asp; 2. L-Tyr; 3. L-Met; 4. D-Ser; 5. L-Ser; 6. D-His; 7. L-His. Redrawn from [38].

Figure 3. Electropherograms of (A) standard solution (20 mg/L racemic imazalil); (B) orange sample 1; (C) orange sample 2; (-) and (+) represent (-)- and (+)-imazalil, respectively. The BGE was composed of 4 mM 2HP-β-CD containing 5 mM ammonium dihydrogenphosphate-

50 mM phosphate buffer (pH 3.0). Samples were injected at a pressure of 50 mbar for 2 s. Separation was performed in a fused-silica bubble cell capillary of 64.5 cm (effective length 56 cm) x 75 µm i.d. The capillary was kept at 20 °C. The analytes were detected at 200 nm. The power supply was operated in the constant-voltage mode, at +25 kV. Redrawn from [24].

Figure 4 . Basic chemical structure of flavan-3-ols and structures of catechin and epicatechin enantiomers.

Figure 5. Chiral separation of DNS-amino acids in a microchip with 7 mm of separation length. Electrical field strength: 2012 V/cm. Electrolyte: 2% HS-γ-CD, 25 mM triethylammonium phosphate buffer, pH 2.5. Peak identification, L- and D- forms of: 1, DNS-tryptophan; 2, DNS-norleucine; 3, DNS-phenylalanine; 4, DNS-methionine; 5, DNS-aspartic acid; 6, DNSaminobutyric acid; 7, DNS-leucine; 8, DNS-norvaline; 9, DNS-glutamic acid. Redrawn from reference [51].

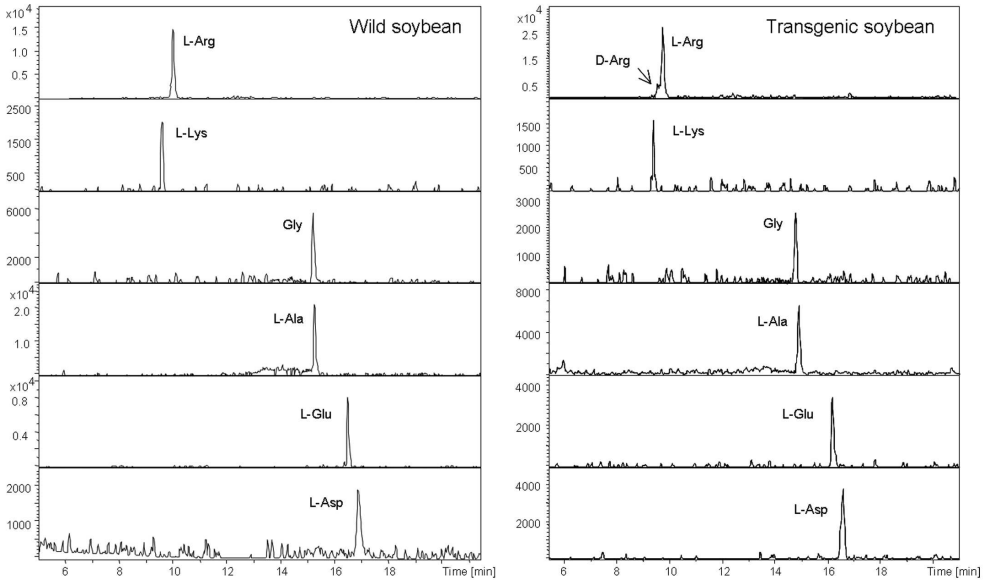

Fig 1
185x110mm (300 x 300 DPI)

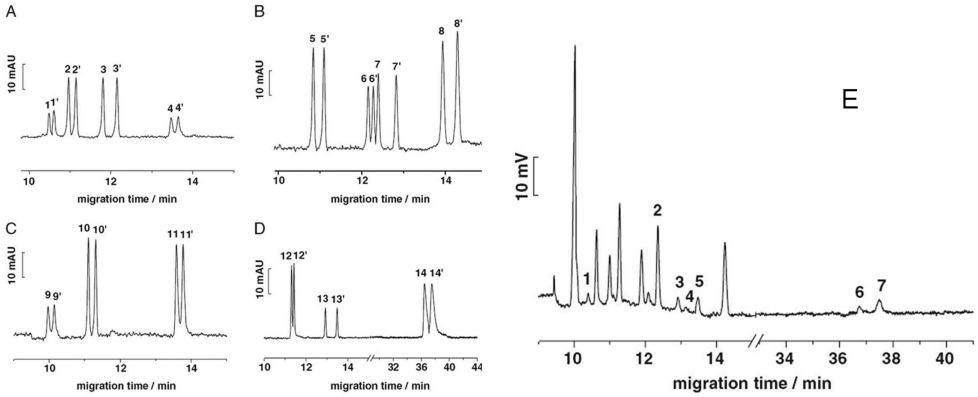

Fig 2
197x84mm (300 x 300 DPI)

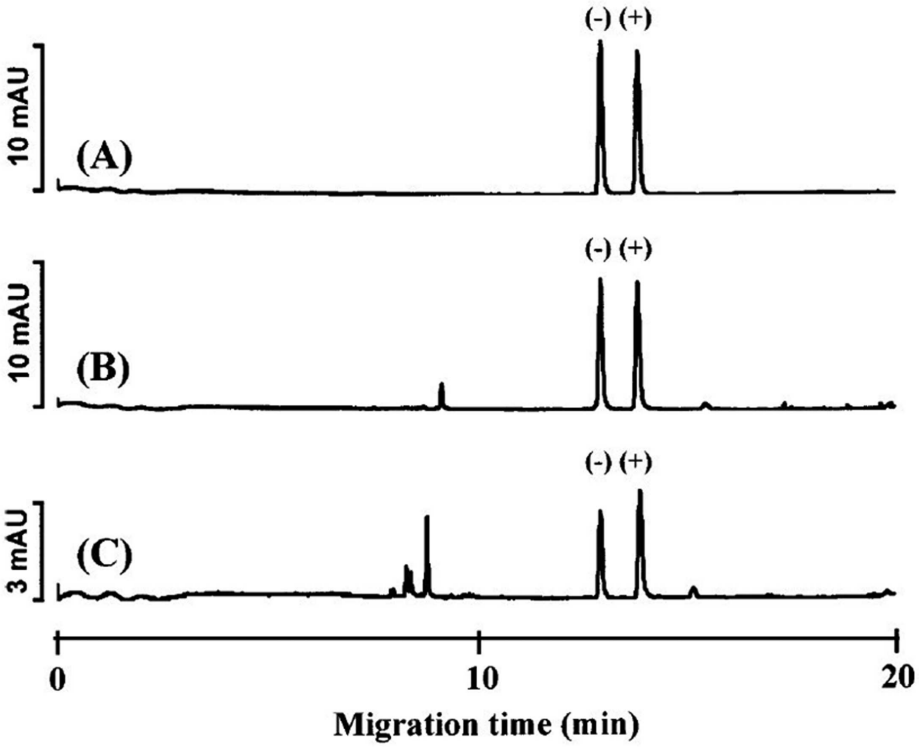

Fig 3
199x162mm (300 x 300 DPI)

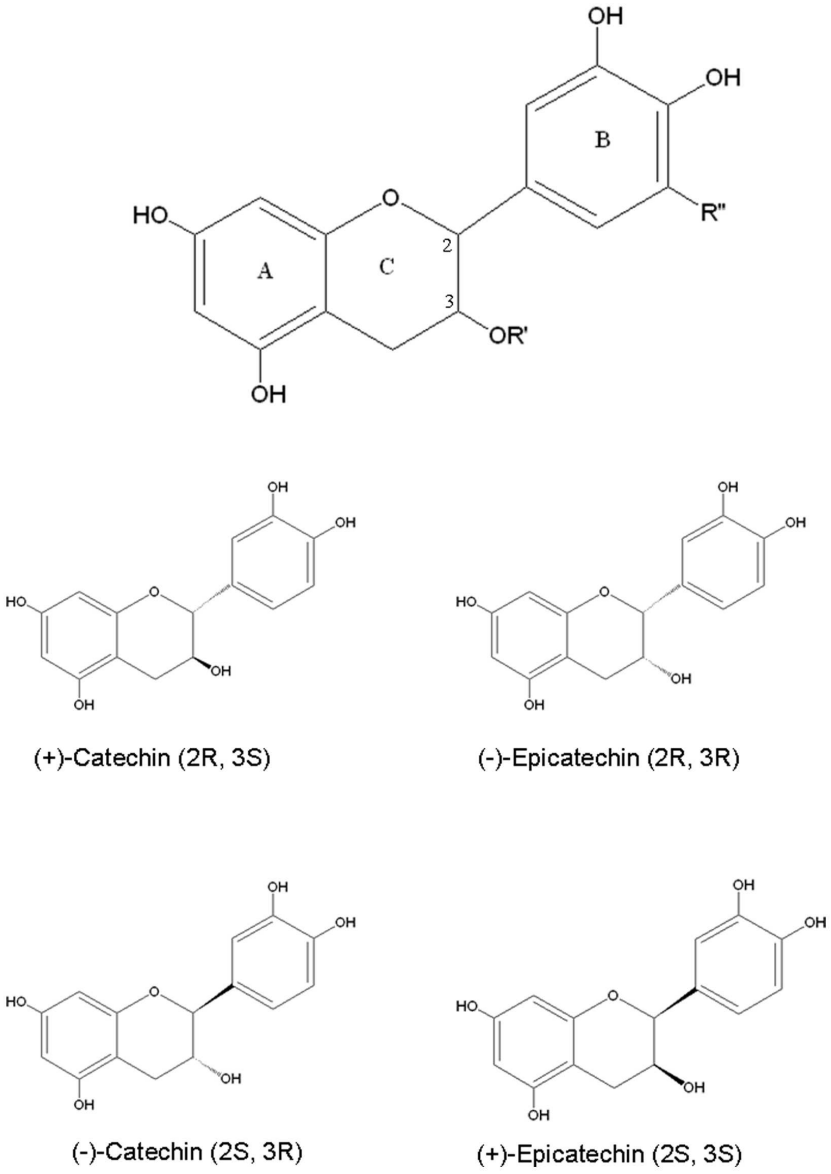

Fig 4
144x199mm (300 x 300 DPI)

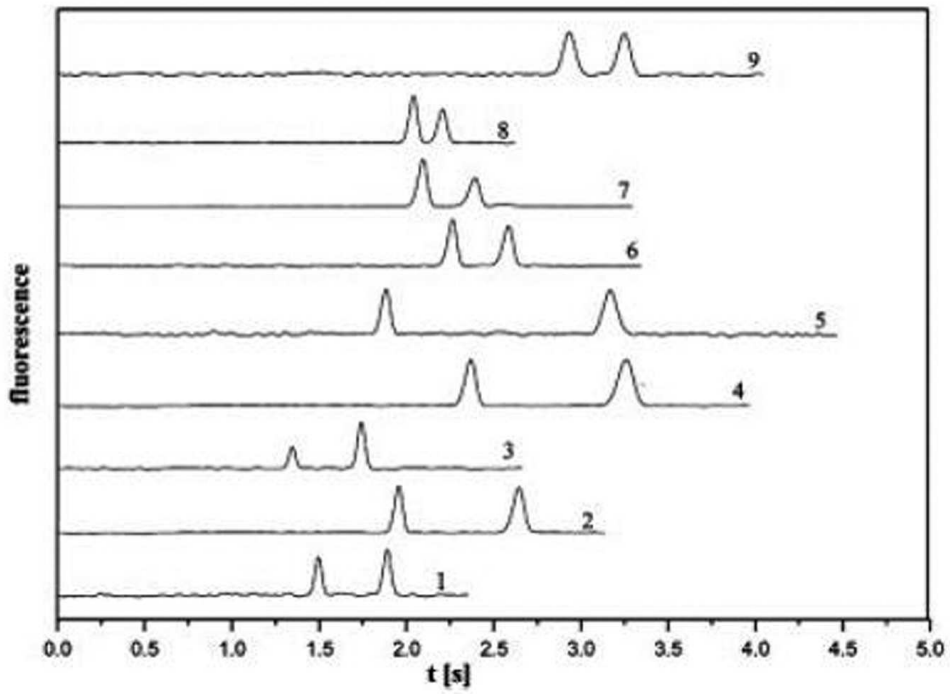

Figure 5
119x91mm (300 x 300 DPI)