

María Castro-Puyana  
Antonio Luis Crego  
María Luisa Marina  
Carmen García-Ruiz

Departamento de Química  
Analítica,  
Facultad de Química,  
Universidad de Alcalá,  
Alcalá de Henares (Madrid), Spain

Received March 5, 2007  
Revised April 27, 2007  
Accepted April 27, 2007

## Review

# CE methods for the determination of non-protein amino acids in foods

In addition to the 20 amino acids universally distributed as protein constituents in living organisms, there are other amino acids of non-protein origin that can be found in foods. The determination of these non-protein amino acids is interesting since they can be indicative of the quality and safety of foods. This work presents for the first time an updated and comprehensive review devoted to show the possibilities of capillary electrophoresis for the determination of non-protein amino acids in food samples. The results reported have been classified according to the chemical structure of the non-protein amino acid studied. Separation conditions as well as detection systems used have been detailed since most of these amino acidic compounds do not possess chromophore groups detectable by conventional UV-Vis detection, being in this case necessary a previous derivatization step. Finally, the application of microchip electrophoresis to the determination of non-protein amino acids in foodstuffs is also included in this review.

### Keywords:

CE / Foods / Microchip electrophoresis / Non-protein amino acids

DOI 10.1002/elps.200700169

## 1 Introduction

In addition to the 20 L-amino acids universally distributed as protein constituents in living organisms, there are hundreds of other amino acids (amino and imino acids or related derivatives) of non-protein origin. Hunt [1] defined non-protein amino acids as those amino acids that are not found in protein main chain either for lack of a specific transfer RNA and codon triplet or because they do not arise from protein amino acids by post-translational modification. That is, all those amino acidic compounds which are not the coded protein amino acids (glycine, alanine, valine, leucine, isoleucine, serine, threonine, asparagine, glutamine, phenylalanine, cysteine, methionine, aspartic acid, glutamic acid, tyrosine, proline, tryptophan, histidine, lysine, and arginine) or post-translationally formed (lanthionine, cystine, cystathionine) [2], can be considered as non-protein amino acids.

Non-protein amino acids in foods mainly exist as products formed during food processing [3], as metabolic intermediates that may occur in animal tissues [4], or as additives used to increase the functional properties of foodstuffs. Their presence in foods can give interesting information on their quality and safety. Some examples in which the determination of non-protein amino acids in foods presents a high interest include those cases in which these compounds have positive effects on health (*i.e.* therapeutic effects of taurine [5]), are of indicative value for the detection of adulterations (*i.e.*  $\epsilon$ -N-2-furoylmethyl-L-lysine (furosine) in milks and milk products [3]), they cause a decrease in the nutritional value of the food through the reaction with a proteic amino acid (*i.e.* formation of cross-linking lysinoalanine [6]) or even have toxic properties [7, 8] (*i.e.* neurotoxicity of domoic acid [9]). All these reasons justify the need of development of analytical methodologies enabling the determination of non-protein amino acids in foods.

CE is a powerful separation technique increasingly used in last years in the field of food analysis due to its high efficiency, resolution, versatility and low amount of reagents and samples required [4, 10]. These interesting characteristics have promoted the publication of some reviews devoted to describe the possibilities of CE in the analysis of food components such as proteic amino acids or proteins [11, 12]. In this sense, CE has been considered a developing technology for the analysis of amino acids in foods and food proteins and as a very interesting complementary technique to HPLC

**Correspondence:** Dr. Carmen García-Ruiz, Departamento de Química Analítica, Facultad de Química, Universidad de Alcalá, Ctra. Madrid-Barcelona Km. 33.600, E-28871 Alcalá de Henares (Madrid), Spain

**E-mail:** carmen.gruiz@uah.es

**Fax:** + 34-91-885-4971

**Abbreviations:**  $\beta$ -CD,  $\beta$ -cyclodextrin; cITP, capillary isotachopheresis; DNS, dansyl chloride; DNS-Hypro, DNS-hydroxyproline; Furosine,  $\epsilon$ -N-2-furoylmethyl-L-lysine; GABA,  $\gamma$ -aminobutyric acid; ICP-MS, inductively coupled plasma MS; Levodopa, 3,4-dihydroxyphenylalanine

(the most used chromatographic technique in amino acid analysis in foods) where collaborative testing methodologies are needed. Moreover, since CE provides better resolution than HPLC, it is useful for the separation and/or rapid screening of low levels of amino acids from components of different chemistries in complex food matrixes during a single analysis [4]. However, a review devoted to the application of CE to non-protein amino acids analysis in foods has never been reported and thus has been the aim of this article. In order to describe the analytical methodologies developed by CE for the determination of non-protein amino acids in foods, these non-protein amino acids have been classified according to their structure. CE conditions employed, sample treatment, complexity of separations achieved, and samples analyzed are detailed. In addition, the application of microchip electrophoresis to the determination of non-protein amino acids in foodstuffs is also reported.

## 2 Determination of non-protein amino acids in foods by CE

Table 1 summarizes the non-protein amino acids determined in foods using CE. They have been classified in different groups according to their structure and following a criterion based on the classification established by Hunt [1]. The name and structure of the different amino acids investigated as well as CE separation conditions, compounds separated from the amino acid studied, LODs achieved, sample treatment and samples analyzed are given in this table.

Non-protein amino acids have mainly been determined by CE using two different separation modes: (i) CZE where a separation buffer without or with additives is used and the separation is based on the different electrophoretic mobilities of analytes at a certain pH, and (ii) EKC where a pseudophase (a micellar system, *i.e.* a surfactant at a concentration higher than its critical micelle concentration, usually SDS, and/or CD) is added to the electrolytic solution to perform the separation of non-protein amino acids in complex matrices (such as fishes, meats, vegetables, legumes, *etc.*) or their chiral separation based on the different interaction of amino acids with a chiral pseudophase.

As shown in Table 1, UV absorption detection has mainly been used in the analysis of non-protein amino acids by CE in spite of the absence of strong chromophore groups in many of them (*i.e.* seleno and sulfur amino acids, aliphatic amino acids, cyloalkane amino acids and hydroxyl amino acids). This has been possible due to the use of different derivatization strategies with adequate probes and in most cases selecting low UV detection wavelength. Other detection systems employed have been LIF, ESI-MS, inductively coupled plasma MS (ICP-MS), and electrochemical detection. In the case of LIF detection, a previous derivatization step was necessary to provide fluorescent groups. Derivatization was also employed when using ESI-MS in order to obtain larger molecules providing better detection

sensitivity for the amino acids studied. ICP-MS and electrochemical detection enabled to avoid previous derivatization steps.

Samples analyzed comprise beverages, seeds, vegetables and plants, food supplements, eggs, meats, fishes, and nuts. Usually, after the extraction of non-protein amino acids, a physical treatment (centrifugation, filtration) of samples has been performed. Moreover, protein hydrolysis has been employed in some cases such as (i) the determination of selenomethionine in yeast, and (ii) when the separation of non-protein amino acids from protein amino acids was performed (*i.e.*, lysinoalanine in duck eggs, aromatic or isoxazolinone amino acids in seeds or heterocycle amino acids in seeds, dried milk, fishes, meats, vegetables, and nuts).

In addition to these general aspects related with the determination of non-protein amino acids by CE, a more detailed description of the interest of their determination in food samples and of the CE methodology developed for each group of non-protein amino acids will be performed herein.

### 2.1 Seleno amino acids

The selective qualitative and quantitative determination of particular species of selenium, including selenoamino acids, is vital in order to understand selenium's metabolism and significance in biology, toxicology, clinical chemistry and nutrition [36]. At high concentrations, selenium is toxic, causing problems such as dermatitis, fatigue and hair loss. However, human nutritional studies have shown that appropriate doses of supplemental selenium enhance cellular defense against oxidative damage and may prevent certain types of cancer [13].

CE methods developed for the determination of selenoamino acids were devoted to the analysis of selenomethionine in selenized yeast, nutrition supplements, and human milk, and selenocystine in human milk.

With respect to the determination of selenomethionine, the chiral speciation of this non-protein amino acid in selenized yeast has been performed by CE with UV and ICP-MS detection under aquiral conditions through the derivatization of the two enantiomers of selenomethionine with the Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide) to form diastereomers that were separated by CZE using 30 mM ammonium phosphate buffer at pH 3.3. Selenized yeast was enzymatically hydrolyzed with proteinase K prior to its analysis by UV and ICP-MS detection. Under these conditions, the identification of selenomethionine enantiomers (LOD  $\sim 1 \times 10^{-6}$  M for UV detection at 214 nm and  $\sim 7 \times 10^{-7}$  M for ICP-MS detection) in the sample was performed using pure L-selenomethionine standard. Due to the selectivity of ICP-MS detection, where only selenium species are detected, electropherograms obtained for the selenized yeast were simpler than those observed by UV detection. It is important to remark that racemization due to sample preparation and derivatization steps was not observed although other peaks at longer migration times

**Table 1.** Classification, name, structure, CE separation conditions, food sample and sample treatment of the different food samples where non-protein amino acids have been determined using CE as separation technique<sup>a)</sup>

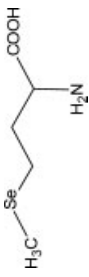


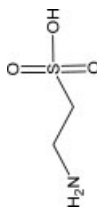
Classification	Name (Abbr.)	Structure	CE conditions	Separation from	LOD (M)	Food sample	Sample treatment	Ref.
Seleno amino acids	Seleno-methionine (Se-Met)		CZE: 30 mM phosphate buffer (pH 3.3) 75 $\mu\text{m} \times 50 \text{ cm}$ ; 23°C; -25 kV; UV-214 nm and ICP-MS	-	$\sim 1 \times 10^{-6}$ (UV) $\sim 7 \times 10^{-7}$ (ICP-MS)	Selenized yeast	Protein hydrolysis (proteinase K), filtration and derivatization (Marfey's reaction to form diastereomers) prior to CE injection	[13]
	Selenocystine (Se-Cys)		CZE: 15 mM phosphate buffer (pH 10.6) 75 $\mu\text{m} \times 64.5 \text{ cm}$ (coated); -25 kV; UV-195 nm CZE: 100 mM phosphate (pH 2.5) or acetate (pH 1.9) 10 kV; 20°C; UV-200 nm	Other arsenic and selenium species (see text)	$\sim 1 \times 10^{-5}$	Nutrition supplement  Human milk	Clean-up ( $\text{C}_{18}$ cartridge), dilution, centrifugation and filtration prior to CE injection  Defatting, protein precipitation by centrifugation, SEC fractionation, drying and water dilution prior to CE injection	[14]  [15]
Sulfur amino acids	Selenocystine (Se-Cys)		CZE: 10 mM carbonate buffer (pH 11.5); CIEF: leading electrolyte: sodium hydroxide (pH 2); terminating electrolyte: phosphoric acid (pH 10) 50 $\mu\text{m} \times 150 \text{ cm}$ ; 20°C; 18 kV; ICP-MS CZE: 100 mM phosphate (pH 2.5) or acetate (pH 1.9) 10 kV; 20°C; UV-200nm	Other selenium species (see text)	4 - 6 $\times 10^{-4}$ (CZE) 1 - 4 $\times 10^{-4}$ (CIEF)	Human milk	Defatting, protein precipitation by centrifugation, pre-concentration (drying) and dissolution prior to CE injection	[16]
	Taurine		CZE: 50 mM borate (pH 9.2) 25 $\mu\text{m} \times 70 \text{ cm}$ ; 30 kV; electro-chemical detection	-	$\sim 1 \times 10^{-7}$	Human milk  Medicinal herb, milk powder and health beverage	Defatting, protein precipitation by centrifugation, SEC fractionation, drying and water dilution prior to CE injection  (Water dissolution and sonication/filtration) Buffer dissolution prior to CE injection	[15]  [5]

Table 1. Continued


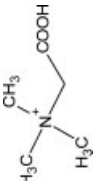
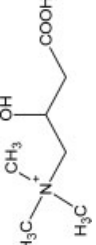
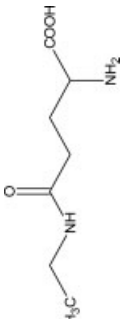

Classification	Name (Abbr.)	Structure	CE conditions	Separation from	LOD (M)	Food sample	Sample treatment	Ref.
Aliphatic monoamino-monocarboxylic amino acids	$\gamma$ -aminobutyric acid (GABA)		EKC: 100 mM borate (pH 9.7), 30 mM SDS, 20 mM $\beta$ -CD 50 $\mu$ m $\times$ 50 cm; 25°C; 20 kV; LIF: 488 nm (excitation) 520 nm (emission) EKC: 100 mM borate (pH 9.4), 30 mM SDS, 20 mM $\beta$ -CD 50 $\mu$ m $\times$ 50 cm; 25°C; 23 kV; LIF: 488 nm (excitation) 520 nm (emission) EKC: 100 mM ammonium acetate (pH 6.0), 5 mM $\beta$ -CD 50 $\mu$ m $\times$ 87 cm (coated); -15 kV; ESI-MS	Enantiomers of protein amino acids (Arg, Pro, Ala, Glu, Asp)	$\sim 2 \times 10^{-8}$	Vinegars	Filtration and derivatization (FITC) prior to CE injection	[17]
	Glycine betaine		CZE: 80 mM phosphate buffer (pH 3.0) 50 $\mu$ m $\times$ 70 cm; 15 kV; 25°C; UV-214 nm	Betaines, trigonelline, and carnitine	$\sim 1 \times 10^{-5}$	Higher plants	Solvent extraction, centrifugation, drying, water dissolution, derivatization ( <i>p</i> -bromophenacyl bromide)	
Aliphatic amino acids with nitrogen in the side chain	Carnitine		CZE: 5 mM Tris, 7 mM phosphoric acid, 0.5 mM quinine 50 $\mu$ m $\times$ 25.5 cm; 15 kV; indirect UV-254 nm	–	$\sim 2 \times 10^{-5}$	Food supplements	Dilution	[21]
	Theanine		EKC: 25 mM phosphate (pH 7.0), 100 mM SDS, 5–6% methanol 50 $\mu$ m $\times$ 50 cm; 25°C; 14 kV, UV-200 nm CZE: 20 mM borax (pH 8.0) 50 $\mu$ m $\times$ 70 cm; 23°C; 30 kV; UV-200 nm cITP: Leading electrolyte: 0.01 M HCl, 0.02 mM Tris, 0.05% HEC (pH 8.1). Terminating electrolyte: 0.01 mM DL-valine, barium hydroxide (pH 10) Conductimetric detection	Tea catechins, caffeine, gallic acid, and ascorbic acid	$\sim 2 \times 10^{-5}$	Dried fresh tea leaf, black tea, bottled black tea Green tea	Water extraction and filtration prior to CE injection Water extraction, filtration and dilution prior to CE injection	[22] [23]
Lysinoalanine			EKC: 250 mM borate (pH 9.5), 200 mM SDS, 75 mM $\beta$ -CD (methanol) 75 $\mu$ m $\times$ 40 cm, 15 kV; 25°C; UV-254 nm	Protein amino acids (Cys, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Met, Ile, Leu, Phe, Hys, Lys, and Arg)	$\sim 4 \times 10^{-4}$	Green teas and food supplements Duck eggs	Water extraction, dilution and filtration prior to CE injection Acid protein hydrolysis, water dissolution of hydrolysate and derivatization (DNS) prior to CE injection	[24] [6]

Table 1. Continued

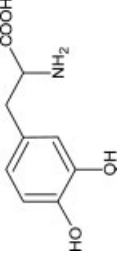


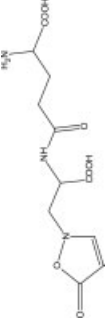
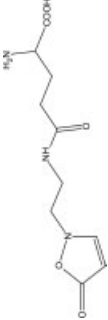
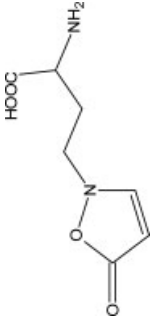
Classification	Name (Abbr.)	Structure	CE conditions	Separation from	LOD (M)	Food sample	Sample treatment	Ref.
Phenyl amino acids	3,4-Dihydroxy-phenylalanine (DOPA)		CZE: 35 mM phosphate (pH 4.55) 75 $\mu\text{m} \times 47$ cm; 17.5 kV; 30°C; UV-210nm	–	$\sim 3 \times 10^{-6}$	Broad bean and lentil	Ultrasonic extraction, centrifugation and filtration prior to CE injection	[25]
Isoxazolinone amino acids	2-Carboxymethyl-3-isoxazolin-5-one (CMI)		CZE: 25 mM phosphate buffer (pH 7.5), 8% 1-propanol 75 $\mu\text{m} \times 52.6$ cm; 22.5 kV; 30°C; UV-254 nm	$\beta$ -(isoxazolin-5-on-2-yl)-pro-pionitrile (BIP), BIA, $\gamma$ -glu-BIA, $\gamma$ -glu-AEI, and ACI	$\sim 3 \times 10^{-5}$	Seeds of lentil, seedlings, legume seedlings	Acid protein hydrolysis, amino acid extraction, centrifugation and preconcentration (vacuum) prior to CE injection	[26] [27]
	$\beta$ -(isoxazolin-5-on-2-yl)-alanine (BIA)			BIP, CMI, $\gamma$ -glu-BIA, $\gamma$ -glu-AEI, and ACI	$\sim 2 \times 10^{-5}$			
	$\gamma$ -Glutamyl- $\beta$ -(isoxazolin-5-on-2-yl)-alanine ( $\gamma$ -glu-BIA)			BIP, CMI, BIA, $\gamma$ -glu-AEI, and ACI	$\sim 1 \times 10^{-5}$			
	2-( $\gamma$ -Glutamyl-aminoethyl)isoxazolin-5-one ( $\gamma$ -glu-AEI)		CZE: 25 mM phosphate buffer (pH 7.5), 8% 1-propanol 75 $\mu\text{m} \times 52.6$ cm; 22.5 kV; 30°C; UV-254 nm	BIP, CMI, BIA, $\gamma$ -glu-BIA, and ACI	$\sim 3 \times 10^{-5}$	Legume seedlings	Amino acid extraction, centrifugation and preconcentration (vacuum) prior to CE injection	[27]
	2-(3-Amino-3-carboxypropyl)isoxazolin-5-one (ACI)			BIP, CMI, BIA, $\gamma$ -glu-BIA, and $\gamma$ -glu-AEI	$\sim 2 \times 10^{-5}$			

Table 1. Continued


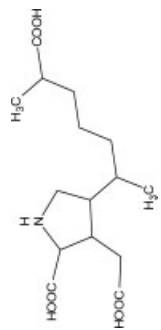
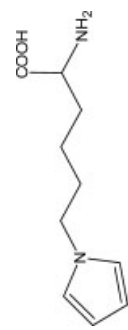
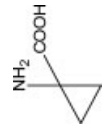
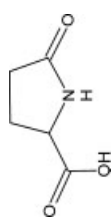
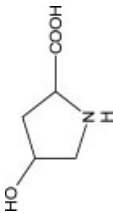
Classification	Name (Abbr.)	Structure	CE conditions	Separation from	LOD (M)	Food sample	Sample treatment	Ref.
Heterocycle amino acids	$\varepsilon$ -N-2-Furoylmethyl-L-lysine (furosine)		CZE: 20 mM phosphoric acid (pH 2.5), 60 mM TMBD 75 $\mu$ m $\times$ 30 cm, 15 kV, 25°C, UV-280 nm CZE: phosphoric acid (pH 8.0) 50 $\mu$ m $\times$ 72 cm, UV-280 nm	–	$\sim 2 \times 10^{-6}$	Dried milk	Acid protein hydrolysis, lyophilization and water dissolution prior to CE injection	[28]
	Domoic acid (DA)		CZE: 3-(N-morpholino-2-hydroxypropanesulfonic acid (pH 7.0) extended-path length capillary of 50 $\mu$ m $\times$ 57 cm, 25 kV, 20°C, UV-280 nm cITP-CZE: Leading electrolyte (preparation capillary, 90 mm $\times$ 0.8 mm id): 10 mM HCl, 20 mM $\beta$ -alanine, 0.05 % HEC (pH 3.5); BGE (analytical capillary, 150 mm $\times$ 0.3 mm id): 20 mM caproic acid, 20 mM $\beta$ -alanine, 5% methanol, 0.1% HPMC Terminating electrolyte: 5 mM caproic acid, 5% methanol UV-242 nm EKC: 100 mM borate buffer (pH 8.4), 100 mM SDS 75 $\mu$ m $\times$ 50 cm; 30 kV; 30°C; UV-280 nm	–	$\sim 5 \times 10^{-6}$	Shellfish, food supplements	Acid hydrolysis, lyophilization and buffer dissolution prior to CE injection Acid hydrolysis, solid-phase extraction and redissolved prior to CE injection Filtration and dilution prior to CE injection	[29] [30] [9]
Cycloalkane amino acids	$\varepsilon$ -N-Pyrrolyl-norleucine		CZE: 500 mM phosphoric acid (pH 6.25), 0.5 mM CTAB 50 $\mu$ m $\times$ 57 cm; – 10 kV; 25°C, UV-200 nm EKC: 50 mM boric acid (pH 9.5), 130 mM SDS 50 $\mu$ m $\times$ 57 cm; 10 kV; 25°C, UV-200 nm	Short-chain organic acids (see text) Caffeine	Standards $\sim 2.3 \times 10^{-4}$ Samples $\sim 1.2 \times 10^{-4}$ $\sim 6.9 \times 10^{-7}$	Coffee	Basic protein hydrolysis and derivatization (DEEMM) prior to CE injection Water ultrasonic extraction, centrifugation and filtration prior to CE injection Water dilution, and filtration prior to CE injection	[31] [32] [33]
	1-Aminocyclopropane-1-carboxylic acid (ACC)		EKC: 20 mM borate (pH 9.35), 40 mM SDS, 10 mM Brij 35 50 $\mu$ m $\times$ 50 cm; 25 kV; 25°C; LIF: 488 nm (excitation) 560 nm (emission)	Protein amino acids (His, Tyr, Thr, Gly, Ala, Met, Val, Phe, Leu/Ile, Asn/Gln, Trp, Cys, Glu, Asp)	$\sim 1 \times 10^{-8}$	Apples	Extraction, centrifugation and derivatization (FQ) prior to CE injection	[34]
Pyroglutamic acid								

Table 1. Continued

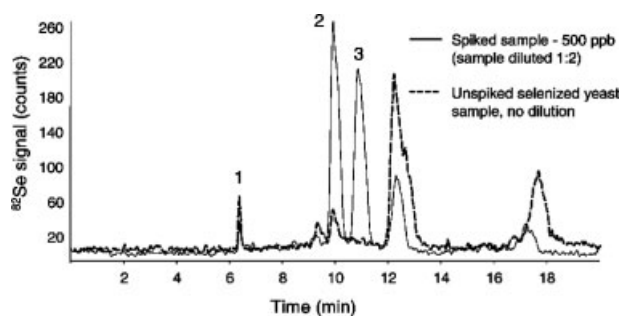
Classification	Name (Abbr.)	Structure	CE conditions	Separation from	LOD (M)	Food sample	Sample treatment	Ref.
Hydroxyl amino acids	Hydroxyproline (Hy-pro)		EKC: 20 mM borax, 125 mM SDS 50 $\mu\text{m} \times 50 \text{ cm}$ ; 10°C; 25 kV; UV-214 nm	Protein amino acids (Thr, Ser, Gln, Ala, Gly, Glu, Val, Asp, Pro, Met, Ile, Leu, Phe, Arg, Lys, Tyr)	–	Corn seed flour	Acid protein hydrolysis, drying, water dissolution, derivatization (DNS) prior to CE injection	[35]

a) Marfey's reagent, 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide; FQ, 3-(2-furoyl)quinoline-2-carboxaldehyde (producing cyanobenzoinsoindole (CBI) derivatives); TMBD, *N,N,N',N'*-tetramethyl-1,3-butanediamine; HP- $\alpha$ -CD, 2-hydroxypropyl- $\alpha$ -CD; HP- $\gamma$ -CD, 2-hydroxypropyl- $\gamma$ -CD; HEC, hydroxyethylcellulose; HPMC, hydroxypropylmethylcellulose; DEEMM, diethyl ethoxymethylenemalonate; CTAB, cetyltrimethylammonium bromide; glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), serine (Ser), threonine (Thr), asparagine (Asn), glutamine (Gln), phenylalanine (Phe), cysteine (Cys), methionine (Met), aspartic acid (Asp), glutamic acid (Glu), tyrosine (Tyr), proline (Pro), tryptophan (Trp), histidine (His), lysine (Lys), and arginine (Arg).

were observed and preliminarily attributed to selenized dipeptides and tripeptides formed during hydrolysis (see Fig. 1) [13]. On the other hand, the separation of arsenic (arsenite, arsenate, monomethylarsonic acid, dimethylarsinic acid) and selenium species (selenate, selenite, selenocystine, selenomethionine and selenocystamine) was achieved by CZE when 15 mM phosphate buffer at pH 10.6 was used. These species were separated with negative separation voltage polarity in a capillary coated with poly-(diallyldimethylammonium chloride). Using direct UV detection at very low wavelengths (195 and 200 nm) in a capillary of 75  $\mu\text{m}$  of ID and with normal stacking, LODs for standard samples were  $\sim 1 \times 10^{-5}$  M (expressed as selenium). This method was applied to the determination of selenomethionine in a selenium nutrition supplement. Due to the high concentration of inorganic ions, proteins, amino acids, food additives and sugar in this sample, cleanup (C18 cartridge) and dilution steps were necessary [14].

Different CZE methods for the separation of the two non-protein amino acids selenomethionine and selenocystine from other selenized molecules of similar relative molecular mass (glutathione and selenocystamine) and nearly equal mobility have been developed [15]. These methods have been used to identify and quantify low concentrated selenoamino acids in human milk. The comparison among migration times obtained by these methods was necessary for the identification of the seleno amino acids because migration times were different from sample to sample due to the different ionic strengths and compositions of samples. In this work, only selenocystine was clearly identified in breast milk.

An on-line hyphenation of CE, using CZE or CIEF separation modes, with ICP-MS detection was applied to selenium speciation of six selenium species (Se(IV), Se(VI), selenium carrying glutathione, selenocystamine and the two



**Figure 1.** Electropherograms of a selenized yeast sample (enzymatically hydrolyzed with proteinase K) analyzed by CE-ICP-MS. Electrophoretic conditions: fused-silica capillary with a total length of 50 cm and 75  $\mu\text{m}$  id; separation buffer, 30 mM phosphate (pH 3.3); separation temperature, 23°C; applied voltage, –25 kV; sampling depth: 4.5 mm. ICP-MS detection (MicroMist nebulizer). Peak identification: 1, selenite; 2, L-selenomethionine; 3, D-selenomethionine; other peaks are unknown and probably due to selenized dipeptides and tripeptides. Reprinted from [13], with permission.



seleno amino acids selenomethionine and selenocystine). The CZE method using an alkaline background electrolyte (10 mM sodium carbonate buffer adjusted to pH 11.5 with potassium hydroxide) separating the different species according to their different mobilities (charge to size ratio). The CIEF method used sodium hydroxide as the catholyte at the capillary inlet and phosphoric acid as the anolyte at the capillary outlet/nebulizer (pH range from 2 to 10) and the separation was produced according to the individual *pI* of the selenium species in the pH gradient generated after voltage application. Both coupling methods were very sensitive and highly resolving. However, LODs were slightly lower for CIEF-ICP-MS than for CZE-ICP-MS (see Table 1), due to higher sample intake and increased resolution of CIEF-ICP-MS, while the CZE-ICP-MS method showed advantage in the number of species analyzed, six selenium species, including two inorganic ones. The two methods were applied separately to the analysis of human milk samples, being selenocystine the only non-protein amino acid found in samples submitted to a tenfold preconcentration step [16].

## 2.2 Sulfur amino acids

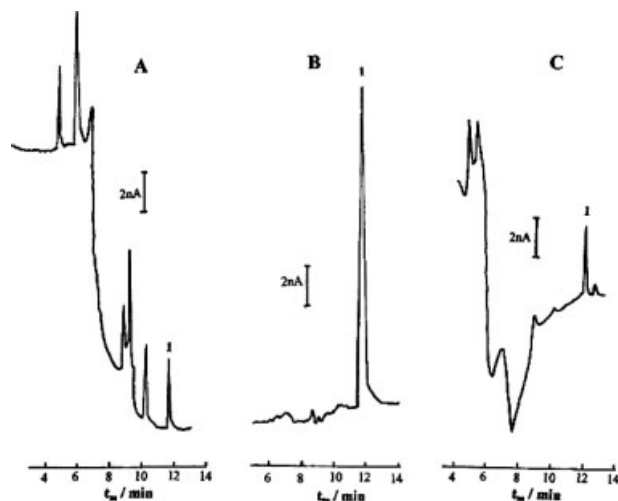
Taurine is the only sulfur amino acid analyzed in foods by CE. This compound is present in tissues in its free form and acts as physiological (*i.e.* maintenance of excitatory activity in muscle) and as a therapeutical (*i.e.* treatment of congestive heart failure) agent. However, the capacity for endogenous biosynthesis of taurine is insufficient being essential its assimilation from a dietary source [5].

A CZE method using 50 mM borate buffer at pH 9.2 was used for the determination of taurine in different foods. Due to the low sensitivity of UV or fluorescence detection of this non-protein amino acid (see its structure in Table 1), electrochemical detection with a carbon-disk as working electrode was used achieving LODs of  $\sim 10^{-7}$  M [5]. Figure 2 shows the typical electropherograms obtained for a Chinese medicinal herb (*Lycium barbarum* L. capsules), a health beverage (Lipovitan), and a milk powder using the CZE method developed. The authors concluded that, in terms of accuracy, sensitivity and reproducibility, the developed method was a useful tool to quantitate taurine in traditional Chinese medicines and beverages.

## 2.3 Aliphatic monoaminomonocarboxylic amino acids

In this section, three aliphatic amino acids possessing one amino group and one carboxylic group are included. Another important characteristic of the three amino acids included in this group is that they are not  $\alpha$ -amino acids.

$\gamma$ -Aminobutyric acid (usually abbreviated as GABA) is a non-protein amino acid that together with other 14 protein amino acids forms a group of 15 amino acids responsible for more than 90% of the amino acidic content in vinegars [17] and orange juices [18, 19]. Its separation from other protein



**Figure 2.** Electropherograms of three food samples containing taurine (peak 1) obtained by CE with electrochemical detection. (A) *Lycium barbarum* L. capsules; (B) Lipovitan, and (C) milk powder. Electrophoretic conditions: fused-silica capillary with a total length of 70 cm and 25  $\mu$ m id; separation buffer, 50 mM borate (pH 9.2); applied voltage, 30 kV; electrokinetic injection, 20 kV for 8 s. Electrochemical detection with a working electrode potential of 1.05 V. Reprinted from [5], with permission.

amino acids has been performed using an EKC system with SDS micelles and  $\beta$ -cyclodextrin ( $\beta$ -CD) (100 mM borate buffer at pH 9.5, 30 mM SDS, 20 mM  $\beta$ -CD). The use of this cyclodextrin (chiral selector) enabled the separation of the enantiomers of protein amino acids and was effective to separate them from GABA. To achieve the sensitive detection of GABA under these conditions (LODs in the nM range), derivatization with FITC prior to LIF detection was performed [17, 18]. Moreover, ESI-MS detection was also employed for a less sensitive detection (LODs in the  $\mu$ M range) of this non-protein amino acid and other protein amino acids, after derivatization with FITC, in orange juices [19] when an EKC system with a volatile buffer and a low concentration of the non-volatile  $\beta$ -CD was used. In addition, GABA (together with the protein amino acids L-arginine and L-aspartic acid) has been considered as an important parameter (its corrected peak area) for the characterization of orange juices (concentrated and pasteurized juices and nectars) [18].

Glycine betaine, named also trimethylglycine or betaine, is a quaternary ammonium non-protein amino acid with a widespread distribution in plants that has extensively been investigated since many plants accumulate it in response to environmental stress (such as salinity, high and low temperatures, *etc.*). In fact, glycine betaine is involved in osmotic adjustment, confers enzyme protection, and increases membrane stability under stress conditions. A CZE method based on the use of an 80 mM phosphate buffer at pH 3.0 was used to determine this compound in plant samples collected from high-salinity areas in North China and West



Australia (*i.e.* spinach) [20]. Prior to the CE injection, the amino acid was converted into their phenacyl ester to obtain a positive charged compound that at low pH migrates faster than other negative and neutral sample compounds which could interfere. The presence of glycine betaine in a leaf extract of a halophyte, *Suaeda glauca*, collected in a saline area in China showed that the interference caused by other amino acid esters in the determination of 1 mM glycine betaine ester was negligible.

Finally, carnitine is a quaternary ammonium non-protein amino acid that participates in bioenergetic processes aiding the transformation of fats into energy, in the control of the mitochondrial acyl-CoA/CoA ratio, peroxisomal oxidation of fatty acids, and the production of ketone bodies. A deficiency of carnitine is known to have major deleterious effects on the central nervous system. It can be synthesized in the liver and kidney of adult people (from the protein amino acids lysine and methionine) or taken up in foods (meat, milk and vegetables). This amino acid has been analyzed in several food supplements by capillary isotachopheresis (cITP) with conductimetric detection and CZE with direct and indirect UV detection. cITP was achieved on an electrophoretic analyzer, CZE with direct detection was performed on a CE instrument, and CZE with indirect detection was carried out on both instruments. Since results obtained were similar by the above-mentioned methods, the comparison of them required to take into account the difficulty of sample preparation, instrumentation and automatic injection. The disadvantage of cITP and CZE with indirect UV detection performed on the electrophoretic analyzer was the impossibility of automatic injection. On the other hand, derivatization with FMOC was necessary for CZE with direct UV detection, which is not practical when analyzing series of samples. However, derivatization has the advantage of removing all interfering proteins and peptides. Finally, CZE with indirect UV detection achieved on the CE instrument was a practical option for simplicity and automation. This method consisted of a buffer with 5 mM Tris and 7 mM phosphoric acid containing 0.5 mM quinine as absorbing probe allowing LODs of  $\sim 2 \times 10^{-5}$  M. It was applied to determine carnitine in four different food supplements [21]. The results obtained by the CE methods for several manufactory samples with different concentrations of L-carnitine were compared with those obtained when using a validated HPLC method. Statistical differences among electrophoretic methods and HPLC were not found and the authors concluded that the CE analysis was faster, consumed less solvents and total running costs were lower than in HPLC [21].

#### 2.4 Aliphatic amino acids with nitrogen in the side chain

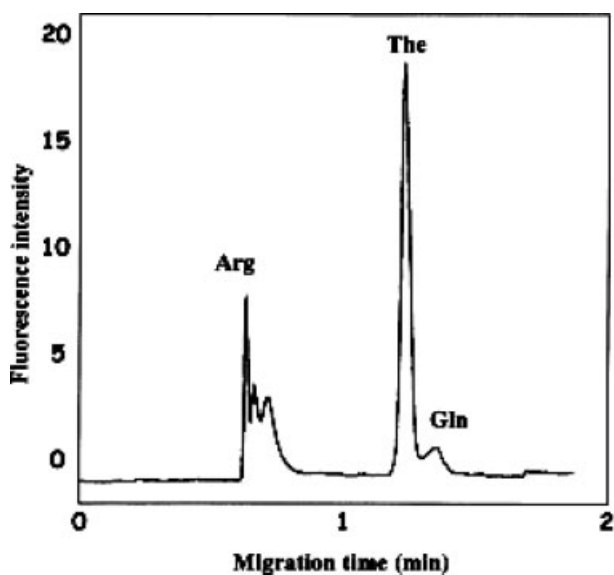
The aliphatic non-protein amino acids containing nitrogen in the side chain are grouped in this section. The two amino acids included are  $\alpha$ -amino acids with one (theanine) or two (lysinoalanine) asymmetric carbon atoms.

Theanine is a major component in tea related with its quality because it shows high correlation with the price of green tea. It has been separated from other tea catechins, caffeine, gallic acid and ascorbic acid by micellar EKC using 25 mM phosphate buffer (pH 7.0) containing 100 mM SDS and 6% methanol (for dried tea leaf and black tea samples) or 5% methanol (for bottled tea samples). This method together with direct UV detection at 200 nm enabled the detection up to  $2 \times 10^{-5}$  M of theanine [22]. In addition, a CZE method using borax at pH 8.0 has also been used for the simultaneous separation of major components (theanine, catechins, caffeine, and ascorbic acid) in green tea infusions [23]. On the other hand, a cITP method using a conductivity detector was developed for the analysis of L-theanine in tea and food supplements. The leading electrolyte consisted of 0.01 M hydrochloric acid containing 0.02 mM TRIS and 0.05% hydroxyethylcellulose (pH 8.1), and the terminating electrolyte was 0.01 mM DL-valine with barium hydroxide (pH 10). After extraction with boiling water, theanine concentrations determined by this method ranged from 0.6 to 0.9% (m/m) in green tea (four different samples) and were about 90% (m/m) in food supplements (three samples) [24].

Lysinoalanine is a cross-linking amino acid formed in the reaction of lysine with dehydroalanine residues. It is indigestible by proteases and its formation implies a loss of lysine in proteins decreasing its nutritional value [6]. This non-protein amino acid has been determined in Pidan, a popular alkali-treated duck egg in Taiwan, and the relationship between the formation of lysinoalanine and the degradation of cysteine, serine, and threonine at various alkali-treating stages was investigated. A micellar EKC method using 250 mM borate buffer (pH 9.5) with 200 mM SDS and 75 mM  $\beta$ -CD and UV detection at 254 nm was used for the separation of this non-protein amino acid from most protein amino acids (see Table 1) [6]. Results obtained in this work indicated that the formation of lysinoalanine in albumen in the first stage of the pickling process was due to the speedy increase in the pH and the abundant formation of dehydroalanine from cysteine whereas the formation of lysinoalanine in the later pickling period was related much more to the alkali treating time than to the pH factor.

In recent years, a growing interest in chemical miniaturization led to research and development of new methods and instrumentation on a microchip. Electrophoresis on microchips (usually named as chip or microchip electrophoresis) is a promising technology since it offers an easy integration of many steps including sample preparation, derivatization, and coupling of several separation procedures together [37, 38]. In addition, the use of microchips instead conventional CE systems leads to reduced analysis times. However, up to now very few applications to food samples have been performed by microchip electrophoresis. In fact, only one work where non-protein amino acids were determined in food samples by microchip electrophoresis has been reported in literature up to date. In this work, a plastic microchip with fluorescence detection was used to determine both non-pro-

tein (theanine) and protein amino acids (arginine and glutamine) in green tea [39]. The CE method used 5 mM phosphate buffer at pH 5.5 containing 0.05 mM SDS, and the derivatization of samples was carried out with 4-fluoro-7-nitro-2,1,3-benzoxadiazole. Amino acid analysis was improved by removing polyphenols (catechins) using a polyvinylpyrrolidone pretreatment. Figure 3 shows the electropherogram corresponding to the separation of the amino acids theanine, arginine and glutamine found in Japanese green tea. Although arginine was not completely separated from unknown peaks, the separation of theanine and glutamine was good within 2 min [39].



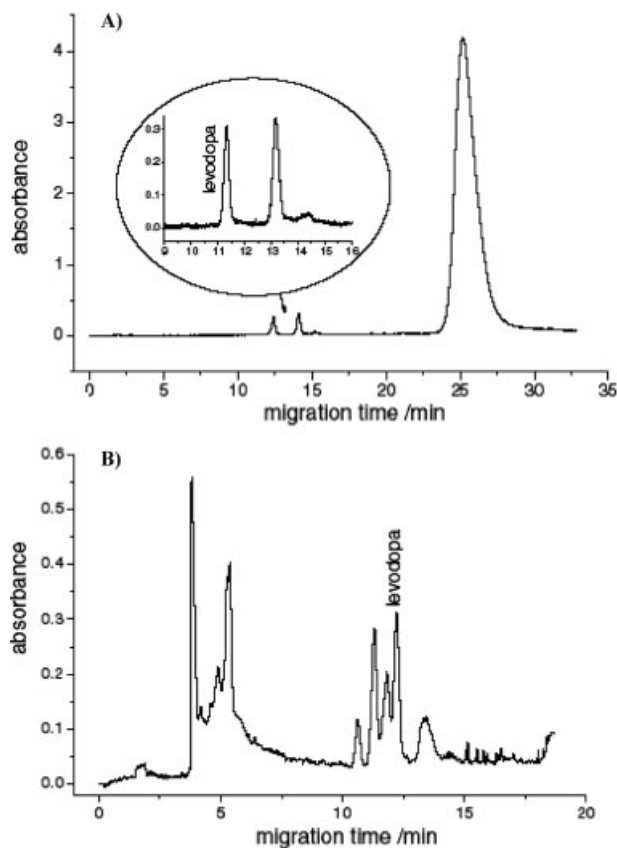
**Figure 3.** Electropherogram of Japanese green tea obtained using microchip electrophoresis with fluorescence detection. A polymethyl methacrylate (PMMA) microchip (85 mm × 50 mm) with three simple cross-channels of 100 μm width and 30 μm deep (detection point at 31 mm) was used; separation buffer, 5 mM phosphate (pH 5.5) containing 0.05 mM SDS; applied voltage, 1.7 kV; sample injection: 100 V for 60 s. Fluorescence detection: 460–490 nm (excitation wavelength) and 515 nm (emission wavelength). Reprinted from [39], with permission.

## 2.5 Phenyl amino acids

Among non-protein amino acids with a phenyl group in their structure, only the L-enantiomer of 3,4-dihydroxyphenylalanine (Levodopa or L-DOPA) has been analyzed under achiral conditions by CE in food samples. Levodopa is the precursor required by the brain to produce dopamine, a neurotransmitter (chemical messenger in the nervous system), thus it is used in the treatment of Parkinson disease [25]. However, auto-oxidation of levodopa generates toxic metabolites, such as free radicals, semi-quinones and quinones. *In vitro*, levodopa is a powerful toxin that is lethal to the culture of neurons, and a few animal studies have shown that levodopa can also be toxic [40]. Levodopa was

first isolated about one century ago from fruit of broad bean [41] and has been found in minute quantity in some beans [42].

A 35 mM phosphate buffer at pH 4.55 was used in the CZE method developed for the determination of levodopa in broad bean and lentil seeds. Under these conditions, and employing UV detection (210 nm), a LOD of  $\sim 3 \times 10^{-6}$  M was obtained [25]. Figure 4 shows the electropherograms obtained for the extracts of both samples using the described CZE method. A peak for levodopa was identified through the increase observed in the peak area after adding the standard of levodopa in the sample solution.



**Figure 4.** Electropherograms of levodopa from broad bean (A) and lentil seed (B) sample solutions employing CE with UV detection. Electrophoretic conditions: fused-silica capillary with a total length of 47 cm (effective length of 40 cm) and 75 μm ID; separation buffer, 35 mM phosphate (pH 4.55); separation temperature, 30°C; applied voltage, 17.5 kV; hydrodynamic injection (3447.4 Pa for 3 s). UV detection at 210 nm. Reprinted from [25], with permission.

## 2.6 Isoxazolinone amino acids

Isoxazolinone are N,O-heterocyclic natural compounds with high sensitivity to UV radiation and alkaline conditions. The amino acids included in this section are: 2-carboxymethyl-3-isoxazolin-5-one, β-(isoxazolin-5-on-2-yl)-alanine, γ-glutamyl-

$\beta$ -(isoxazolin-5-on-2-yl)-alanine, 2-( $\gamma$ -glutamylaminoethyl)-isoxazolin-5-one, and 2-(3-amino-3-carboxypropyl)isoxazolin-5-one. These compounds are present in legumes and seedlings, and especially in the seedlings of some *Lathyrus* species their concentration can be very high. They are biochemical precursors of a group of toxic metabolites present in some *Lathyrus* species and responsible for human diseases (*i.e.* neurotathyrism). Some of them have physiological activity on isolated neurones or cloned neuronal receptors or are of ecological importance [26, 27].

A CZE method using 25 mM phosphate buffer containing 8% 1-propanol at pH 7.5 was used to determine the five above-mentioned isoxazoline compounds in seedlings. This method allowed LOD ranging from  $1 \times 10^{-5}$  to  $3 \times 10^{-5}$  M [27]. Figure 5 shows the electropherograms of isoxazoline compounds in seedling extracts of grass pea (*Lathyrus sativus*) and of sweet pea (*Lathyrus odoratus*) using UV detection at 254 nm. Concentrations obtained by CE were in good agreement with those obtained by HPLC.

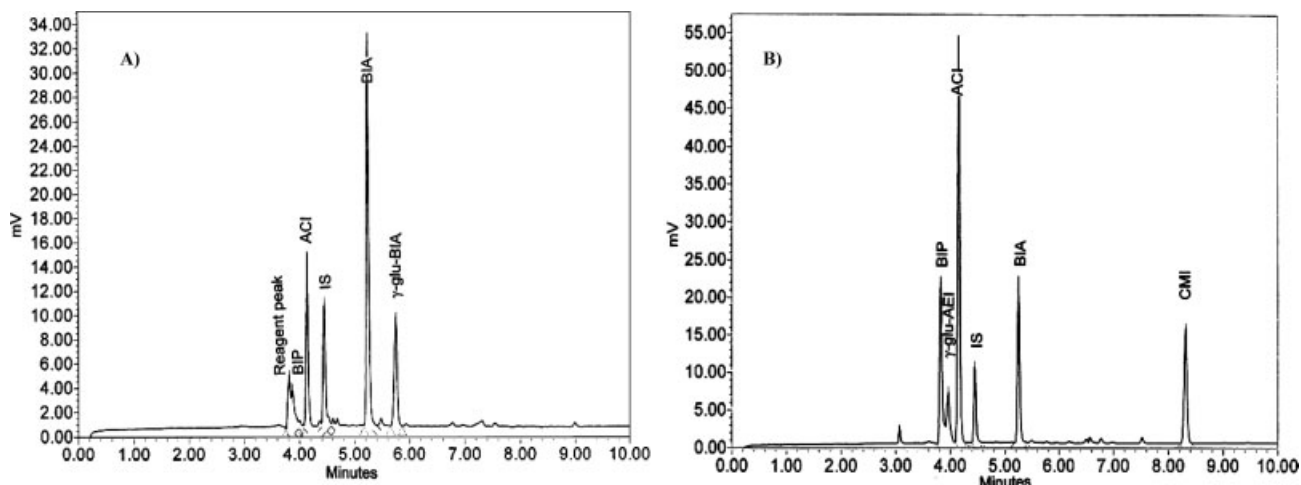
## 2.7 Heterocycle amino acids

The non-protein amino acids containing a heterocycle group in the side chain are grouped in this section. The amino acids included here contain different heterocycles such as furane in furosine, pyrrolidine in domoic acid and pyrroglutamic acid, and pyrrole in  $\epsilon$ -N-pyrrolylnorleucine (see structure in Table 1).

Furosine is a non-protein amino acid formed from lysine, which is produced in milk and dairy products by the Maillard reaction (one of the most important reactions occurring in processed food during processing, sanitizing,

packing and storage). Because furosine is formed during the acid hydrolysis of  $\epsilon$ -N-2-fructosyllysine, its determination can be used to evaluate the extent of the Maillard reaction and the level of nutritionally unavailable lysine in processed food [28]. Furosine has been analyzed in dried milk and dairy products hydrolyzed with hydrochloric acid, by CZE with UV detection at 280 nm. This method consisted of 20 mM phosphoric acid buffer containing 60 mM *N,N,N',N'*-tetramethyl-1,3-butanediamine, and allowed an LOD of  $\sim 2 \times 10^{-6}$  M. This additive was used to avoid interactions of furosine with the capillary wall and to modulate its migration behavior by controlling the EOF [28]. Moreover, a previous method for the determination of furosine [29] has been improved for greater accuracy and sensitivity using a 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid solution at pH 7.0 as running buffer together with an extended-path-length capillary enabled to obtain a CZE method whose repeatability and sensitivity fulfilled the requirements stated in the EU and Italian regulations for furosine determination in pasteurized milk and Mozzarella cheese. The data obtained when analyzing 48 different food samples including heat-treated milk, cheeses, and durum wheat products were comparable with those obtained by an HPLC method and proved to be accurate for furosine values up to at least 400 mg per 100 g protein [30].

Domoic acid is a neurotoxic tricarboxylic acid related to glutamic acid, which causes amnesic shellfish poisoning. In mammals, including human beings, it acts as a neurotoxin, causing short-term memory loss, brain damage, and death in severe cases [9]. A sensitive on-line coupled cITP-CZE method with UV detection at 242 nm has been developed to determine domoic acid in shellfish samples and food sup-



**Figure 5.** Electropherograms of isoxazoline compounds in 3-day-old seedlings of *Lathyrus sativus* (A) and *Lathyrus odoratus* (B) analyzed by CZE with UV detection. Electrophoretic conditions: coated fused-silica capillary with a total length of 60 cm (effective length of 52.6 cm) and 75  $\mu$ m id; separation buffer, 25 mM phosphate containing 8% 1-propanol (pH 7.5); separation temperature, 30°C; applied voltage, 22.5 kV; hydrostatic injection of 10 s. UV detection at 254 nm. BIP,  $\beta$ -(isoxazolin-5-on-2-yl)-propionitrile; ACI, 2-(3-amino-3-carboxypropyl)isoxazolin-5-one; BIA,  $\beta$ -(isoxazolin-5-on-2-yl)-alanine;  $\gamma$ -glu-BIA,  $\gamma$ -glutamyl- $\beta$ -(isoxazolin-5-on-2-yl)-alanine; CMI, 2-carboxymethyl-3-isoxazolin-5-one; IS, internal standard. Reprinted from [27], with permission.

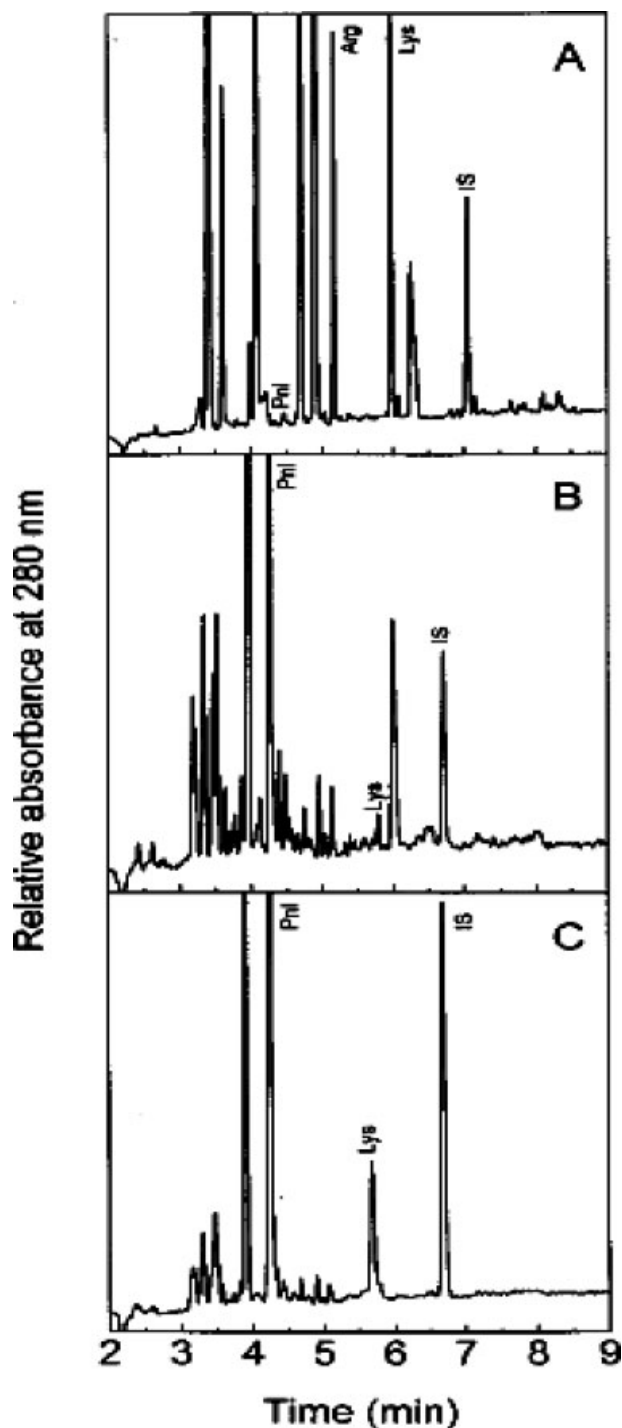
plements containing algae extract. The conditions for cITP-CZE analysis are detailed in Table 1, and enabled an LOD of  $\sim 4.8 \times 10^{-6}$  M [9].

$\epsilon$ -*N*-Pyrrolylnorleucine is a product of the reaction between the lipid peroxidation product 4,5-(*E*)-epoxy-2-(*E*)-heptanol and the  $\epsilon$ -amino group of lysine [43]. This amino acid seems to be a normal component of many fresh food products, in which it may act as a natural antioxidant. In addition, the  $\epsilon$ -*N*-pyrrolylnorleucine/lysine ratio was directly correlated with lipid and iron contents and inversely correlated with the protein content in food [31]. A micellar EKC method based on the use of 100 mM borate buffer at pH 8.4 containing 100 mM SDS was employed to determine this compound in 22 different fresh food products hydrolyzed in basic conditions (5 fishes, 3 meats, 10 vegetables, and 4 nuts). To make easy the separation from other amino acids and the UV detection (280 nm), derivatization with diethyl ethoxymethylenemalonate prior to CE injection was performed. Figure 6 shows the electropherograms obtained for three different foods: salmon, spinach, and walnut where peaks corresponding to  $\epsilon$ -*N*-pyrrolylnorleucine, arginine, and lysine are marked [31].

The pyroglutamic acid content of coffee could provide it with some central activities previously associated with caffeine, such as improved attention and enhanced cognition by modulating dopamine and adenosine central functions. In fact, pyroglutamate is known to have a number of remarkable cognitive enhancing effects [32, 33]. It has been separated from other 16 short-chain organic acids (*i.e.* oxalic, formic, fumaric, mesaconic, succinic, maleic, malic, isocitric, citric, acetic, citraconic, glycolic, propionic, lactic, furanoic, and quinic acids) by CE with direct UV detection at 200 nm in coffee samples of different origins and with different treatments [32]. The method consisted of 500 mM phosphate buffer (pH 6.25) containing 0.5 mM CTAB, and allowed an LOD of  $\sim 1.2 \times 10^{-4}$  M in these samples. The organic acid profiles obtained for the samples showed important differences depending on industrial treatment of coffee. Moreover, pyroglutamate content was considerably higher in the lyophilized coffee than in the roasted one and the authors attributed these results to some stage of the industrial process [32]. On the other hand, an MEKC method using 50 mM borate buffer (pH 9.5) with 130 mM SDS, and using UV detection at 200 nm was also developed for the simultaneous determination of caffeine and pyroglutamate in ten soluble coffees acquired in the market. This method enabled LODs of  $\sim 6.9 \times 10^{-7}$  M. The coffees with the highest content in pyroglutamate (with or without caffeine) were preliminarily tested for sedative/stimulant properties and cognition enhancing effects in mice [33].

## 2.8 Cycloalkane amino acids

The 1-aminocyclopropane-1-carboxylic acid is a disubstituted cyclic amino acid, and it is the only cycloalkane amino acid that has been analyzed by CE in food samples. This amino



**Figure 6.** Electropherograms corresponding to the separation of  $\epsilon$ -*N*-pyrrolylnorleucine (Pnl), arginine (Arg), lysine (Lys), and internal standard (IS) in (A) salmon, (B) spinach, and (C) walnut by CE with UV detection after basic hydrolysis and derivatization with diethyl ethoxymethylenemalonate. Electrophoretic conditions: uncoated fused-silica capillary with a total length of 50 cm and 75  $\mu$ m id; separation buffer, 100 mM borate (pH 8.4), 100 mM SDS; temperature, 30°C; applied voltage, 30 kV; hydrodynamic injection (2 s). UV detection at 280 nm. Reprinted from [31], with permission.

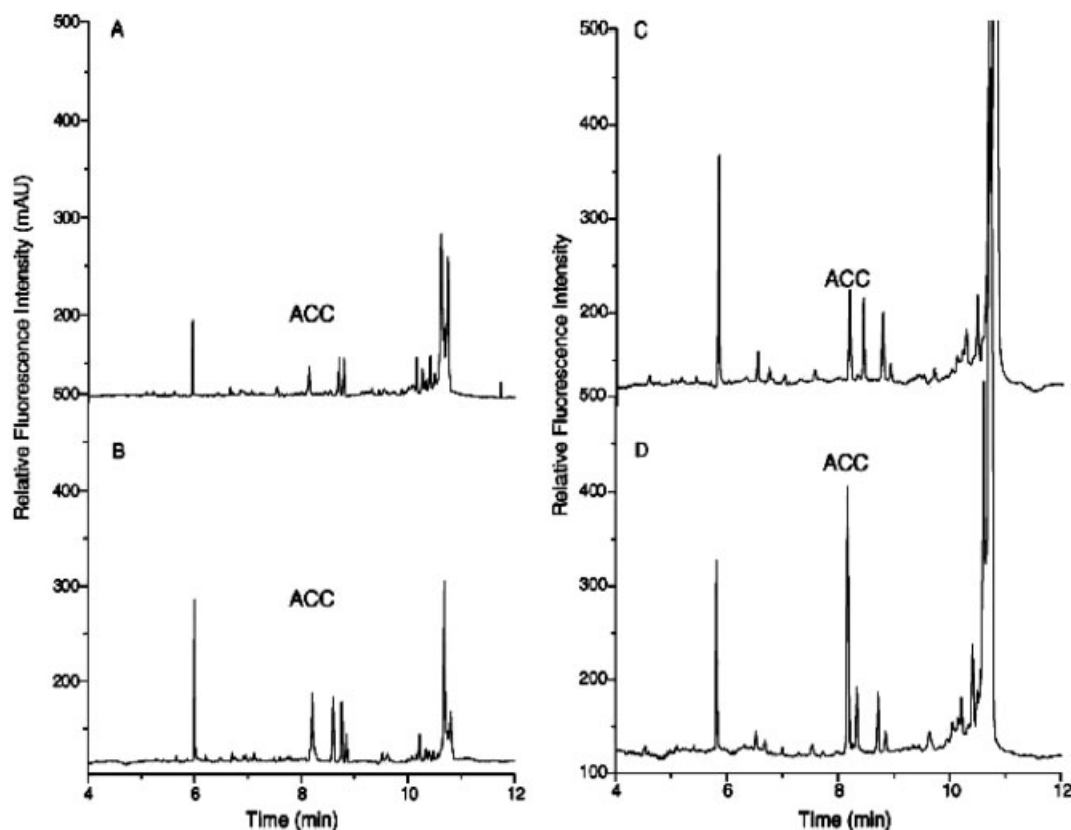
acid plays an important role in the biosynthesis of ethylene, which is associated with many physiological processes in higher plants, including fruit ripening, senescence, and abscission of plant organs. In fact, 1-aminocyclopropane-1-carboxylic acid was identified as an important immediate precursor of ethylene [34]. For this reason, the quantitative analysis of this non-protein amino acid is of interest in higher plants and their fruits.

The separation of 16 common protein amino acids (see Table 1) and 1-aminocyclopropane-1-carboxylic acid in apple extracts was performed by an EKC method with a running buffer of 20 mM borate (pH 9.35), 40 mM SDS and 10 mM Brij 35 and employed LIF detection. Since the molecular structure of this non-protein amino acid does not have a fluorophore group, a derivatization with 3-(2-furoyl)quinoline-2-carboxaldehyde prior to LIF detection was necessary [34]. Figure 7 shows the electropherograms obtained for apple extracts using different extraction solvents with or without wounding treatment (this treatment is performed by nicking the apple with blade and then keeping the wounded apple at room temperature for 24 h). As it can be seen, the

content of 1-aminocyclopropane-1-carboxylic acid in apple is higher when employing an acid extraction with wounding treatment. This figure shows that the treatment of the sample is an important parameter since the efficiency of sample extraction can be affected by different solvents and can be improved under wounding treatment of apples. The mass LOD of 1-aminocyclopropane-1-carboxylic acid was 50 amol or  $1 \times 10^{-8}$  M in terms of concentration. It is important to mention that the high sensitivity and selectivity of the method enabled the direct determination of this non-protein amino acid in crude extracts without further purification and concentration [34].

## 2.9 Hydroxyl amino acids

Hydroxyproline differs from proline in the presence of a hydroxyl group attached to the  $\gamma$ -carbon. This non-protein amino acid is a major component of the protein collagen. It helps to provide stability to the triple-helical structure of collagen by forming hydrogen bonds.



**Figure 7.** Electropherograms of 1-aminocyclopropane-1-carboxylic acid (ACC) in apple extracts by CE with LIF detection, using aqueous methanol (80%, v/v) without wounding treatment (A), using aqueous methanol (80%, v/v) with wounding treatment (B), using 5% sulfosalicylic acid without wounding treatment (C), and using 5% sulfosalicylic acid with wounding treatment (D). Electrophoretic conditions: uncoated fused-silica capillary with a total length of 65 cm (effective length of 50 cm) and 50  $\mu$ m id; separation buffer, 20 mM borate (pH 9.35), 40 mM SDS and 10 mM Brij 35; temperature, 25°C; applied voltage, 25 kV; hydrodynamic injection (50 mbar for 5 s). LIF detection: 488 nm (excitation), 560 nm (emission). Reprinted from [34], with permission.



The separation of hydroxyproline from 16 protein amino acids (indicated in Table 1) was performed using a MEKC method based on the use of 20 mM borax containing 125 mM SDS with UV detection at 214 nm. Before UV detection, a pre-concentration step of samples after derivatization with dansyl chloride (DNS) was necessary due to the low concentration of DNS-amino acids in the mixture [35]. The separation of the hydrolysis product of DNS (dansyl sulfonic acid) and DNS-hydroxyproline from corn seed flour using the MEKC method was achieved. It was observed that the concentration of SDS was an important parameter to avoid the coelution of the derivatization agent with the derivatized hydroxyproline.

### 3 Concluding remarks

This review is the first one reporting all the articles published in relation to the determination of non-protein amino acids in foods by CE. Since non-protein amino acids can be related with the quality and security of foods, their determination in these samples presents a high interest.

Determination of non-protein amino acids by CE in foods was usually performed using CZE and EKC modes. Detection systems employed include direct and indirect UV absorption, fluorescence, electrochemical, and atomic and molecular MS. The most used were optical detectors, mainly UV absorption detection, which required a previous step of derivatization due to the low UV absorptivity of most non-protein amino acids. Derivatization was also employed when using molecular MS detection, in order to increase the molecular mass of the analytes to be determined. Although electrochemical detection did not require derivatization, it was very scarcely used.

CE analytical methodologies with adequate sensitivity and selectivity were developed showing the big potential of this technique for the analysis of a great number of non-protein amino acids in a considerable variety of samples of different complexity including beverages, seeds, vegetables and plants, food supplements, yeast, eggs, meats, fishes, and nuts. In fact, some problems in the food area not previously solved by other analytical separation techniques were solved by CE. Thus, for some of the non-protein amino acids included in this review, CE methodologies proposed are the only existing methodologies. As example, as far as we know, no HPLC methods were developed for the determination of taurine, glycine betaine, 3,4-dihydroxyphenylalanine, pyroglutamic acid and all the isoxazolinone amino acids included in Table 1. On the other hand, for those amino acids for which previous HPLC methodologies were recommended, CE was successful in the development of complementary methodologies that could be useful in carrying out collaborative testing needed in the food area.

*The authors thank the Comunidad Autónoma de Madrid (Spain) (project S-0505/AGR/0312) for financial support. C. García-Ruiz also thanks the Ministry of Science and Technology*

*for the Ramón y Cajal program (RYC-2003-001). M. Castro-Puyana thanks the University of Alcalá for her predoctoral grant.*

### 4 References

- [1] Hunt, S., The Non-Protein Amino Acids, in: Barret, G. C. (Ed.), *Chemistry and biotechnology of amino acids*, 1985, 55–183.
- [2] Friedman, M., *J. Agric. Food Chem.* 1999, 47, 3457–3479.
- [3] Kvasnička, F., *J. Sep. Sci.* 2005, 28, 813–825.
- [4] Peace, R. W., Gilani, G. S., *J. AOAC Int.* 2005, 88, 877–887.
- [5] Cao, Y., Zhang, X., Chu, Q., Fang, Y., Ye, J., *Electroanalysis* 2003, 15, 898–902.
- [6] Chang, H.-M., Cheng-Fang, T., Chin-Fung, L., *J. Agric. Food Chem.* 1999, 47, 1495–1500.
- [7] Peng, H., Revell, D., McSweeney, C. S., Brooker, J. D., *Anim. Feed Sci. Technol.* 2005, 121, 139–146.
- [8] Kuo, Y.-H., Rozan, P., Lambein, F., Frias, J., Vidal-Valverde, C., *Food Chem.* 2004, 86, 537–545.
- [9] Kvasnička, F., Ševčík, R., Voldřich, M., *J. Chromatogr. A* 2006, 1113, 255–258.
- [10] Marina, M. L., Ríos, A., Valcárcel, M. (Eds.), *Analysis and Detection by Capillary Electrophoresis*, CAC, Elsevier, Amsterdam 2005.
- [11] Poinot, W., Lacroix, M., Maury, D., Chataigne, G. *et al.*, *Electrophoresis* 2006, 27, 176–194.
- [12] Cifuentes, A., *Electrophoresis* 2006, 27, 283–303.
- [13] Day, J. A., Kannamkumarath, S. S., Yanes, E. G., Montes-Bayón, M., Caruso, J. A., *J. Anal. At. Spectrom.* 2002, 17, 27–31.
- [14] Sun, B., Macka, M., Haddad, P. R., *J. Chromatogr. A* 2004, 1039, 201–208.
- [15] Michalke, B., *Fresenius' J. Anal. Chem.* 1995, 351, 670–677.
- [16] Michalke, B., Schramel, P., *J. Chromatogr. A* 1998, 807, 71–80.
- [17] Carlavilla, D., Moreno-Arribas, M. V., Fanali, S., Cifuentes, A., *Electrophoresis* 2006, 27, 2551–2557.
- [18] Simó, C., Martín-Alvarez, P. J., Barbas, C., Cifuentes, A., *Electrophoresis* 2004, 25, 2885–2891.
- [19] Simó, C., Rizzi, A., Barbas, C., Cifuentes, A., *Electrophoresis* 2005, 26, 1432–1441.
- [20] Zhang, J., Nishimura, N., Okubo, A., Yamazaki, S., *Phytochem. Anal.* 2002, 13, 189–194.
- [21] Prokorátová, V., Kvasnička, F., Ševčík, R., Voldřich, M., *J. Chromatogr. A* 2005, 1081, 60–64.
- [22] Aucamp, J. P., Hara, Y., Apostolides, Z., *J. Chromatogr. A* 2000, 876, 235–242.
- [23] Horie, H., Mukai, T., Kohata, K., *J. Chromatogr. A* 1997, 758, 332–335.
- [24] Kvasnička, F., Kratka, J., *Central Eur. J. Chem.* 2006, 4, 216–222.
- [25] Chen, X., Zhang, J., Zhai, H., Chen, X., Hu, Z., *Food Chem.* 2005, 92, 381–386.
- [26] Rozan, P., Kuo, Y.-H., Lambein, F., *Phytochemistry* 2001, 58, 281–289.



- [27] Chowdhury, B., Rozan, P., Kuo, Y.-H., Sumino, M., Lambein, F., *J. Chromatogr. A* 2001, **933**, 129–136.
- [28] Corradini, C., Cannarsa, G., Corradini, C., Nicoletti, I. *et al.*, *Electrophoresis* 1996, **17**, 120–124.
- [29] Tirelli, A., Pellegrino, L., *Italian J. Food Sci.* 1995, **7**, 379–385.
- [30] Tirelli, A., *J. Food Protection* 1998, **61**, 1400–1404.
- [31] Zamora, R., Alaiz, M., Hidalgo, F. J., *J. Agric. Food Chem.* 1999, **47**, 1942–1947.
- [32] Galli, V., Barbas, C., *J. Chromatogr. A* 2004, **1032**, 299–304.
- [33] Maeso, N., del Castillo, C., Cornejo, L., García-Acicollar *et al.*, *J. Pharm. Biomed. Anal.* 2006, **41**, 1095–1100.
- [34] Liu, X., Li, D.-F., Wang, Y., Lu, Y.-T., *J. Chromatogr. A* 2004, **1061**, 99–104.
- [35] Skočir, E., Prošek, M., *Chromatographia* 1995, **41**, 638–644.
- [36] Uden, P. C., Boakye, H. T., Kahakachchi, C., Tyson, J. F., *J. Chromatogr. A* 2004, **1050**, 85–93.
- [37] Erickson, D., Li, D. Q., *Anal. Chim. Acta* 2004, **507**, 11–26.
- [38] Bilitewski, U., Genrich, M., Kadow, S., Marsal, G., *Anal. Bioanal. Chem.* 2003, **377**, 556–569.
- [39] Kato, M., Gyoten, Y., Sakai-Kato, K., Toyō'oka, T., *J. Chromatogr. A* 2003, **1013**, 183–189.
- [40] Melamed, E., Offen, D., Shirvan, A., Ziv, I., *J. Neurol.* 2000, **247**, 135–139.
- [41] Brain, K. R., *Plant Sci. Lett.* 1976, **7**, 157–161.
- [42] Vadivel, V., Janardhanan, K., *Plant Foods Hum. Nutr.* 2002, **57**, 151–164.
- [43] Zamora, R., Navarro, J. L., Hidalgo, F. J., *Lipids* 1995, **30**, 477–483.