CAPILLARY ELECTROPHORESIS IN FOOD ANALYSIS

Editors:
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Current and Future Developments in Food Science

(*Volume 2*)

Maria Luisa Marina (Ed.)

Capillary Electrophoresis in Food Analysis

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FOREWORD

The present book on Capillary Electrophoresis in Food Analysis edited by Dr. Maria Castro-Puyana, Dr. Miguel Herrero and Prof. Maria Luisa Marina represents very comprehensive and updated source of information on the topic.

The book opens with the chapter on the basic principles of Capillary Electrophoresis written by Kalaycıoğlu and Erim. This chapter is a good reading for every researcher interested in this technique and not just limited to food scientists. The aspects such as separation mechanism, basic instrument, separation parameters, modes of Capillary Electrophoresis, on-line sensitivity enhancement, detection methods and separation strategies for some groups of analytes are discussed in a very concise and clear way.

The second chapter by Li and co-authors deals with sample preparation techniques used in food analysis. In particular, the updated overview on the techniques based on phase separation, field-assisted extraction, membrane separation, chemical conversion and online sample preparation is provided.

Following five chapters deal with the analysis of specific groups of compounds such as lipids, carbohydrates and proteins (Chapter 3 by Oliveira and coauthors), peptides (Chapter 4 by Stepanova and Kasicka), amino acids (Chapter 5 by Castro-Puyana and Marina), vitamins (Chapter 6 by Van Schepdael, Wang and co-authors) and polyphenols (Chapter 7 by Marina and co-authors). Together, these chapters provide the most recent and important advances in the analysis of such relevant analytes as powerful tools for food characterization.

The book also comprises of interesting chapters devoted to very important aspects in the field of food analysis such as the application of Capillary Electrophoresis to the quantification of food additives (Chapter 8 by Rios and co-authors), food authenticity (Chapter 11 by Kvasnicka), controlling of chemical food safety (Chapter 12 by Hernández-Mesa and co-authors) or quality and safety of dietary supplements (Chapter 13 by Donati and Aturki).

Three chapters dealing with advanced methodological aspects in Capillary Electrophoresis complete the book, including Chapter 9 by Marina and co-authors on the application of chiral Capillary Electrophoresis to food analysis, Chapter 10 by Crevillén, Escarpa and co-authors on the application of Microchip Electrophoresis to food analysis and the concluding Chapter 14 by Recber and Celebier on the potential of Capillary Electrophoresis-Mass Spectrometry as analytical methodology to perform metabolomic approaches to food analysis.

I would like to congratulate the editors, all the contributing authors, as well as the readers of this book for publishing this timely and well-balanced book on application of Capillary Electrophoresis in food analysis. The strength of this book is based on the long time professional activity of all three editors in the field covered by the book. This allowed them to select a very strong team of authors for covering almost all the most important aspects of food analysis based on Capillary Electrophoresis.

This book will definitely become a reference source for everyone using Capillary Electrophoresis for food analysis and will promote further use of this technique, as well as its advanced modalities such as Capillary Electrophoresis-Mass Spectrometry, Microchip Electrophoresis, multichannel- and multidimensional Capillary Electrophoresis for sophisticated problem solving in food analysis.

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PREFACE

Modern food analysis comprises different important fields within Food Science, including food quality, food safety and traceability assessment, detection of frauds, authentication of origin, determination of the nutritional value of food products, or even the assessment of the potential bioactivities that food ingredients may exert. Moreover, food analysis has a significant social impact due to the consumers' concern about everything related to food. Among these fields, the researches aimed to observe the effects that bioactive compounds present in food may confer are probably those that have increased their importance the most. This is not only related to an increase of interest in this particular subfield but also in the improvement and evolution of modern analytical tools that have allowed to tackle analytical challenges not reachable some decades ago. In this regard, the development of faster, more sensitive, accurate, and powerful analytical methods capable to providing information about all these aspects is crucial.

In this context, capillary electrophoresis (CE) is positioned among other more extended separative tools, mainly those based on gas and liquid chromatography. However, despite not being so broadly used, capillary electromigration methods, with CE leading the way, possess exceptional properties related to their high analysis speed and separation efficiencies combined with the small samples and reagent requirements and a great variety of potential applications.

The present volume is aimed at providing an updated overview of the current state-of-the-art related to the use of CE in the field of food analysis. The book is structured, including some general chapters dealing with the basic principles of capillary electrophoresis, providing an overview of the most relevant and important characteristics and potential of this analytical tool. The theoretical background of capillary electromigration is included as well as the description of the basic instrumentation needed and the different separation modes that make CE such a versatile tool. Moreover, sample preparation aspects, specifically directed towards the subsequent application of CE are also presented, which are relevant considering the great influence of sample preparation steps and procedures on the analytical results that are attainable. A second group of chapters is focused on the required approaches for the analysis of important groups of food components, such as lipids, carbohydrates, proteins, peptides, amino acids, vitamins, polyphenols, and food additives. Each chapter includes information showing the advantages of the use of CE as well as its different separation modes for those applications compared to other separation and analytical techniques. Two additional chapters deal with more specific developments within the general use of CE, namely, the development of approaches for chiral separations and the use of miniaturized devices. Indeed, CE has demonstrated very good capabilities for the efficient separation of chiral compounds. The different approaches and compounds that can be used as chiral selectors are presented and discussed. Besides, CE has been demonstrated as one of the most suitable analytical tools to be miniaturized and to construct lab-on-a-chip devices based on electromigration even allowing to perform multiple simultaneous analyses. Instrumentation, detection, and microchip design aspects are included in the chapter devoted to microchip CE. Finally, several chapters are focused on specific fields of the study showing the latest developments and applications presented in each topic. Those chapters include information related to the use of CE for food authentication, food safety, the analysis of dietary supplements as well as the use of CE coupled to mass spectrometry for its use in food metabolomics-related researches.

Together, the chapters included in this volume, written by renowned experts in their respective fields, provide a wide perspective of the use of capillary electrophoresis-based

approaches in the field of food analysis. Readers with a background in Food Science, from Ph.D. students to experts and researchers working in food and analytical chemistry, as well as chemists working in food control laboratories, might find this information interesting.

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CHAPTER 1

Capillary Electrophoresis: Basic Principles

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Abstract: Capillary Electrophoresis (CE) is a powerful separation and analysis technique that has been rapidly progressing since it was first introduced. The application range of CE is so diverse that it ranges from small analytes to large and complex macromolecules. This chapter aims to provide a deep understanding of the basic principles of CE. The first part of the chapter involves the theoretical basis, instrumentation, and separation mechanism of CE. The second part focuses on capillary electrophoretic separation modes and the third part describes the detection methods in CE. The fourth and final part covers capillary electrophoretic strategies for specific analyte groups.

Keywords: Capillary electrophoresis, CEC, CGE, Chiral separation, Contactless conductivity detector, CZE, Indirect detection, LIF, MEKC, Proteins, Sample stacking, Small anions, Small organic acids.

INTRODUCTION

Capillary Electrophoresis (CE) is a separation method that takes the basis of separation principle from classical electrophoresis and has the device design of modern chromatographic techniques. CE was first described as a new separation technique between 1980-1990, then a relatively new separation technique by comparison with High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC). Today, CE has been widely applied to real samples and can be defined as a modern separation technique.

Classical electrophoresis was first introduced by the Swedish chemist Arne Tiselius in 1930 [1]. This invention earned Tiselius a Nobel Prize in 1948. Electrophoresis, in its simplest definition, means the migration of charged particles under the electric field.

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Classical electrophoresis experiments began in U-shaped tubes with electrodes attached to both ends, containing an electrolyte inside. Later, the separation media preferred were gels, as the reproducibility of migration velocities in open solution was low. This method, is known today as slab-gel electrophoresis

and it is an essential tool of biochemistry laboratories, especially in protein separations. The technology has developed over the years and made it possible to produce silica columns with a diameter of micrometers. Thus, reproducible migration velocities have been obtained in open solutions in capillary columns. J.

W. Jorgensen and K. D. Lukacs introduced the CE technique in 1981 with an article published in Analytical Chemistry [2]. The method first evolved with hand-made devices in research labs, and later, modern CE devices were introduced to the market.

The present chapter describes the basic principles and mechanisms of capillary electrophoretic techniques. It covers instrumentation, different separation modes, and detection methods in CE, and separation strategies for specific analyte groups, such as small inorganic anions and organic acids, proteins, metal ions, and chiral molecules.

SEPARATION MECHANISM

In this section, general and some unique principles, strengths, and weaknesses of the CE technique will be given. Although the CE separation principle is based on classical electrophoresis, there are essential differences between the two methods. The high electrical resistance of the capillary column makes it possible to apply a very high voltage (HV) between the two electrodes. In commercial CE devices, up to 30 kV voltage can be achieved. The high voltage applied gives the CE technique a tremendous separation speed. This high speed also increases the separation efficiency of the method. An advantage of CE over other chromatographic techniques is that separation can be done in an aqueous medium. If necessary, hydrophilic organic solvents can also be added to the aqueous separation medium. Another advantage of CE is that different types of analytes can be separated in the same silica column using the same instrument design. One designation that has been used since the early years of CE is that the species can be separated in a broad spectrum from Li-ion to DNA by capillary electrophoretic methods. Indeed, many species in inorganic, organic, and macromolecular structures have been shown to be separated by CE. The different applications of CE can be seen in the most recent reviews [3 - 5]. Another advantage of CE compared to liquid chromatography methods is that silica capillary columns are less costly. Since the injection volume in CE is in nL size, it is an economical method in terms of capillary columns and the small amounts of sample and

chemical consumption. The capillary column can be regenerated by washing with separation electrolytes and suitable solvents

between each injection. Due to this advantage, many sample solutions can be injected directly into the capillary column without the need for pre-purification.

The weakness of CE is the detection limit. In CE, the separation column is also the detection cell. Since the light path of the detection cell is in micrometers, if the molar absorptivity of the analyte is not high enough in UV detection, detection limits will be high. There are unique methods to overcome this obstacle in CE. These methods will also be mentioned in this section. The schematic device design of CE is shown in Fig. (**1**).

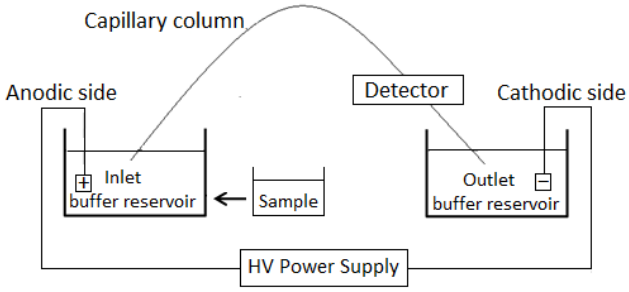

Fig. (1). Basic components of capillary electrophoresis instrumentation.

Fig. (**1**) shows two small buffer vials and a sample vial. The ends of a fused-silica column are immersed in buffer vials during electrophoresis. For electrophoretic separation, the separation medium must be an electrolyte solution with electrical conductivity. In CE separations, the use of a buffer solution for the background electrolyte medium is preferred due to the formation of H^+ and OH^- ions in the electrode regions as a result of electrolysis. H^+ and OH^- concentrations due to electrolysis are at the micromolar levels. However, the migration mobilities of pH- sensitive analytes and the surface charge of silica capillary column are affected by the pH change of the medium. Therefore, it is common practice to use a buffer as the separation medium in CE applications, with some exceptions.

The two electrodes are connected to a high voltage (HV) source. Voltage up to 30 kV can be provided in CE devices. Capillary columns are generally fused-silica columns. The outer layer of a fused-silica capillary is coated with a thin layer of polymer to prevent breakage and provide flexibility while using it. The detection

CHAPTER 2

Sample Preparation in Capillary Electrophoresis for Food Analysis

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Abstract: This chapter introduces sample preparation techniques in Capillary Electrophoresis (CE) for food analysis. Food sample preparation prior to CE analysis aims to transfer target analytes from random statuses in the original food matrix to highly ordered pre-detection statuses, which is an entropy reduction procedure and cannot happen spontaneously. Generally, this is a time-consuming, labor-intensive, and error-prone step in complex sample analysis, especially in food analysis. Nevertheless, to match the fast analysis nature of CE, food samples have to be prepared efficiently in a relatively short time. Therefore, many highly efficient and fast sample preparation techniques were applied in CE for food analysis, including phase separation, field-assisted extraction, membrane separation, chemical conversion, and online coupling of sample preparation/analysis techniques. The principles and operation of each of the above-listed sample preparation techniques and some application examples are shown in different sections.

Keywords: Chemical conversion, Field- assisted extraction, Food analysis, Membrane separation, Sample preparation, capillary electrophoresis, phase partition.

INTRODUCTION

Sample preparation is a critical step in capillary electrophoresis for food analysis, which affects sensitivity, selectivity, accuracy, and speed of analytical results. From an analytical science viewpoint, foodstuff is a type of sample with wide varieties and high complexity, which contain a large number of target analytes and in a very complex matrix [1]. Moreover, these target analytes may be extremely similar to each other but present a trace amount in the food sample matrix [2 - 4]. Before injecting foodstuff into capillary, the preparation process can isolate and/or preconcentrate target analytes from food matrix based on their different physical, chemical, and biological properties, making them suitable for

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electrophoretic analysis [5]. Sample preparation is a time-consuming process, since transferring analytes from random status in the original sample matrix to highly ordered pre- detection statuses is an entropy reduction procedure which cannot occur spontaneously [6, 7]. Nevertheless, to match the fast analysis nature of capillary electrophoresis (CE), food samples have to be efficiently prepared in a relatively short time [8]. Therefore, many high efficiency and fast sample preparation methods and techniques were applied in CE for food analysis as shown in Fig. (**1**), including phase separation, field-assisted extraction, membrane separation, chemical conversion, and online coupling techniques.

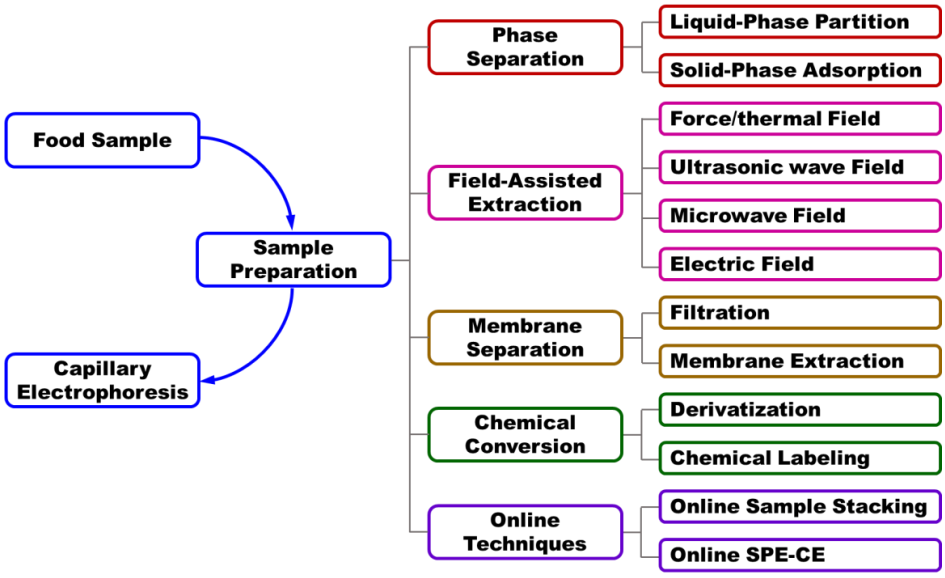

Fig. (1). Sample preparation in capillary electrophoresis for food analysis.

According to the morphology of sample, techniques to pretreat food are divided into solid and liquid sample preparation techniques. Solid sample preparation techniques involve Soxhlet extraction, Supercritical Fluid Extraction (SFE), Accelerated Solvent Extraction (ASE), Ultrasonic-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), and so on. Their attributes are shown in Table **1**. Soxhlet extraction is a classic solid sample preparation technique. Due to its shortcomings of time-consuming, large solution consumption, low efficiency and reproducibility, this technique is being replaced by SFE, ASE, UAE, and MAE in food analysis.

Table 1. Sample preparation techniques for solid food samples.

Method	Principle	Advantage	Disadvantage
Soxhlet extraction	Continuous extraction using solvent reflux and siphon	Easy operation, low instrumental cost, deal with a large amount of sample	Time-consuming, large solvent consumption, may cause components decompose
Supercritical Fluid Extraction (SFE)	The supercritical fluid was used as solvent in extraction	Fast, low solvent consumption, high throughput, high selectivity, large concentration multiple, relatively low extraction temperature, suitable for thermo-sensitive analytes	High instrumental cost, complicated operation, may have the risk of sample lost
Accelerated Solvent Extraction (ASE)	Solid-liquid extraction in high-temperature and high-pressure field	Fast, low solvent consumption, easy operation, large concentration multiple	High instrumental cost, low extraction efficiency, not suit for thermally unstable substance
Ultrasonic-Assisted Extraction (UAE)	Ultrasonic field effects are used to accelerate extraction, such as cavitation and thermal effects	Fast, low cost, easy operation, deal with a large amount of sample	Manual operation, require filtration, extraction efficiency is affected by cavitation effect strength, the size and density of solid samples and solvent property
Microwave-Assisted Extraction (MAE)	Microwave field is applied to enhance extraction performance	Fast, low solvent consumption; homogeneous heating; easy operation	Require polar extraction solvent, require filtration and other auxiliary steps

Liquid sample preparation techniques involve Liquid-Liquid Extraction (LLE), Liquid-Phase Microextraction (LPME), Solid-Phase Extraction (SPE), and Solid-Phase Microextraction (SPME). Their attributes are shown in Table **2**. LLE and SPE are traditional liquid sample preparation techniques; they are widely used in food sample analysis. By device miniaturization, their mini versions of LPME and SPME prove to provide better sample preparation performances [9].

PHASE SEPARATION SAMPLE PREPARATION TECHNIQUES

Isolating target analytes from sample matrix through the phase separation process, including liquid-phase partition and solid-phase adsorption, is involved in almost all complicated sample pretreatment before instrumental analysis. Beyond eliminating matrix interference, phase separation sample preparation techniques also enable analytes preconcentration and background solution conversion to enhance the feasibility of electrophoretic analysis. LLE, LPME, SPE, and SPME are phase separation techniques frequently used for food sample preparation.

CHAPTER 3

Recent Trends in the Analysis of Lipids, Carbohydrates, and Proteins in Food by Capillary Electrophoresis

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Abstract: Highly selective and sensitive analytical methods are necessary for food analysis because diverse components can be found in this complex sample matrix, sometimes occurring at only trace levels. Besides, simple and cost-effective methods are needed to meet the requirements of governmental food standards organizations and industries. Capillary Electrophoresis (CE) is a technique that meets these requirements offering high-resolution separations and high-throughput. It only demands small amounts of samples and chemicals for experiments and its versatility due to the different separation modes possible and the combination with different detection systems, has favored its application to determine diverse compounds in food analysis. This chapter summarizes significant issues and challenges involved in the determination of lipids, carbohydrates, and proteins, as well as recent advances in the analysis of these food components by several CE modes and detection systems.

Keywords: Analytical methods, carbohydrates, Electromigration methods, Food quality control, Lipids, Proteins, Sample preparation protocols.

INTRODUCTION

It is essential to study food components in order to ensure the health and safety of consumers. Besides, the determination of certain food compounds is mandatory for compliance with legal standards, quality assurance, nutritional value purpose, and fraud evaluation.

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Food nutrition labels generally include information on the provided energy, lipids composition (mainly cholesterol, saturated and *trans* fats), and carbohydrate, sugars, protein and salt contents. Additionally, it is essential to inform in food labels the potentially allergenic nutrients, *e.g.*, gluten and lactose, for celiacs and lactose intolerant people, respectively.

When considering macronutrients which is the focus of this chapter, different approaches can be used to determine a variety of compounds within these classes. Capillary Electrophoresis (CE) is a technique that operates in liquid media employing capillary columns to perform separation and is widely used for macronutrient determination in food. Solvated ions, as well as ionized and neutral species, can be separated using the various separation CE modes, which make CE a very versatile technique.

This chapter describes the application of CE methods for lipids, carbohydrates, and proteins determination in foods over the past five years. The fundamentals of the CE modes and detection systems employed in methods will be discussed, and the main analytical methods conditions will be summarized in tables throughout the chapter. Furthermore, several references will be provided for readers who wish to go deeper into the topic addressed

LIPIDS

Lipids comprise naturally occurring organic compounds that are generally insoluble in water and soluble in organic substances, which present several physicochemical and biological properties [1]. They are essential for energetic, metabolic, and structural activities and play essential roles in nutrition and health [2]. Just like carbohydrates and proteins, lipids contents need to be accurately measured and informed in food packages [3].

Some lipids are considered essential for the organism, such as omega-3 and omega-6 fatty acids. They need to be ingested from the diet because they are not synthesized by the *de novo* synthesis route in the body. These fatty acids are required to maintain cell membranes structure, brain function, the transmission of nerve impulses, and metabolic processes [4]. However, there is considerable awareness that abnormal levels of specific lipids, such as cholesterol, saturated fatty acids, and *trans*-fatty acids, may cause cardiovascular diseases, among other diseases such as obesity and type 2 diabetes [5, 6].

The determination of lipids in food is very challenging due to several varieties of matrices, diverse composition of fatty acids, and a wide range of total fat contents.

However, CE presents some advantages to lipids analysis when compared to chromatographic techniques, *e.g.*, derivatization reactions and specific columns are generally not required, and faster analysis times can be achieved. Besides, the technique follows the green chemistry principles, as small amounts of samples, reagents, and solvents are needed to perform analysis, and less waste is generated [7].

According to Fahy *et al.* [8], lipids can be classified into eight main categories: fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides, sterols, and prenols. In this section, several methods reported in the literature for the study of the different classes of lipids employing CE will be revised, approaching different CE modes and detection systems, in order to show recent advances in lipids analysis employing the technique. Besides, challenges involving CE analysis for lipids determination in food will be summarized.

Fatty acids are a diverse group of molecules synthesized by chain elongation of an acetyl-CoA primer with malonyl-CoA (or methylmalonyl-CoA) groups [8]. They consist of carbon, hydrogen, and oxygen, arranged as a linear carbon chain skeleton of diverse lengths with a carboxyl group at one end. Fatty acids can be saturated (no double bond), monounsaturated (one double bond), or polyunsaturated (two or more double bonds). In food, they mostly occur as triesters, linked to glycerol forming triacylglycerols [9]. In minor amounts, naturally occurring free fatty acids, such as Hydroxy, Acetylenic, Allenic, and Cumulenic, can be found [10].

Extensive uses of fatty acids have been reported due to their different structures, mainly in food and oleochemicals industries. Nine major commodity oils are tracked worldwide, including coconut, soybean, sunflower, palm, palm kernel, cottonseed, canola, peanut, and olive, that accounts for approximately 97% of total oil production. Additionally, fatty acids are being used to manufacture soaps, detergents, lubricants, coatings, cosmetics, and other products [3]. (Table **1**) shows the methods for fatty acids determination reported to different food matrices, disclosed in chronological order, considering the last five years. Analytes, sample preparation procedure, background electrolyte (BGE), and instrumental conditions are summarized, as well as the CE mode and detection system used to fatty acids separation and identification.

CHAPTER 4

Analysis of Peptides by Capillary Electromigration Methods

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Abstract: These peptides themselves and especially as products of enzymatic or chemical cleavage of parental proteins, belong to the important components of foodstuffs. They significantly influence their nutritional, biological, technological, and functional properties. Some of these peptides were found to have effects on human health and nutrition, *e.g.*, by affecting human digestive, endocrine, cardiovascular, immune, and nervous systems. Hence, qualitative and quantitative analysis of peptides in foods is of great importance. For the separation and quantification of peptides in foods, capillary electromigration methods represent one of the most suitable analytical methods. This chapter presents a comprehensive overview of the developments and applications of high performance capillary and microchip electromigration methods (zone electrophoresis, isotachophoresis, isoelectric focusing, affinity electrophoresis, electrokinetic chromatography and electrochromatography) for separation and analysis of peptides in foods and food products in the time period since 2010 up to the middle of 2020. Various aspects of the application of capillary electromigration methods for peptide analysis in foods, such as sample preparation, peptide preseparation, preconcentration, derivatization, adsorption suppression, and detection, are described and discussed. Several particular applications of capillary electromigration methods for separation and analysis of peptides in various food samples of animal, plant, and microbial origin are demonstrated.

Keywords: Bioactive peptides, Capillary electrophoresis, Electromigration methods, Food analysis, Peptides in foods.

INTRODUCTION

Peptides themselves and especially as products of enzymatic (proteolytic) and chemical hydrolysis of parental proteins belong to the important components of foodstuffs. They possess significant effect on their nutritional, biological, functional and technological properties. Food proteins are a major source of biologically active peptides.

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Food-derived biopeptides are mostly released from animal, plant or microbial proteins by enzymatic hydrolysis under *in-vivo* or *in-vitro* conditions using variable proteases. The peptides remain biologically inactive within the parent proteins until they are released during gastrointestinal digestion, during the processing of food or by using proteolytic enzymes or microbial fermentation [1 - 3]. Bioactive peptides exhibit many vitally important activities in all living organisms. They act as hormones, neurotransmitters, enzyme substrates and inhibitors, co-enzymes, ionophores, antibiotics, and drugs. Peptides in foods possess many biological effects. Based on their mode of action, they can be classified as antioxidant, antimicrobial, antifungal, antithrombotic, anticoagulant, antihypertensive, antidiabetic, opioid, hypo-cholesterolemic, immunomodulatory, mineral binding, and other [3 - 5]. Antihypertensive peptide inhibitors of angiotensin converting enzyme have been derived from milk, corn and fish proteins. Opioid peptides were found to be released by cleavage of wheat gluten or milk casein by pepsin. Immunomodulatory peptides were derived from hydrolysates of rice and soybean proteins by trypsin. Antioxidant properties were observed for peptides originating from milk proteins. Both hydrophilic and lipophilic antioxidant activity were found at caseinophosphopeptides obtained by a tryptic digestion of casein. Because of the possible health-influencing effects of peptides by affecting the human digestive, endocrine, cardiovascular, immune, and nervous systems, the separation and qualitative and quantitative analysis of bioactive peptides in foods is a subject of great interest and importance and a challenge for analytical methods.

Besides the most spread separation methods, different modes of (ultra)high-performance liquid chromatography ((U)HPLC), capillary electromigration (CE) techniques represent alternative and/or complementary separation and analytical tools for food analysis and foodomics, including analysis of peptides in foods and food products [6 - 8].

CE methods enable the separation of a wide set of compounds ranging from small molecules, such as metal ions, inorganic and organic acids and bases, and amino acids, through the medium sized peptides and oligonucleotides up to the large biopolymers, such as proteins, polysaccharides and nucleic acids (see the recent comprehensive review [9] and book [10] on the latest developments and applications of CE methods for the analysis of various types of (bio)molecules and (bio)particles). Based on the separation principles, CE methods can be divided into two major groups. The first one includes purely electrophoretic modes, such as zone electrophoresis in a free solution (CZE) or in gel and sieving media (CGE and CSE), Isotachophoresis (CITP), and Isoelectric Focusing (CIEF), Affinity Electrophoresis (ACE), which separate the soluble charged compounds according to their electrophoretic mobilities governed by the

charge/size (charge/mass) ratio of their molecules. The second group of CE methods contains the combined electro- chromatographic methods, particularly, the Capillary Electrokinetic Chromatography (EKC), especially its most spread micellar mode, Micellar EKC (MEKC), and Capillary Electrochromatography (CEC). These methods can separate both charged and non-charged compounds and their separation is based mainly on their different interactions with stationary (CEC) or pseudostationary (MEKC) phases.

The advantages of all the above CE methods include high separation efficiency, short analysis time, relatively simple method development, low sample and solvent consumption, and low running costs. Their main drawback is their relatively low concentration sensitivity due to the short optical path of the most frequently used UV-Vis absorption detector.

Peptide separations and analyses by CE methods in general have been regularly reviewed within a two-year period in the last 20 years (see the last review of this series [11] and the references cited therein). CE methods combined with variable detection modes, UV-absorption, fluorescence, contactless conductivity, and especially mass spectrometry (MS), are frequently employed in the fields of peptidomics and proteomics [12 - 14].

This chapter presents a comprehensive overview of the developments and applications of CE methods for the separation and analysis of peptides in foods and food products from 2010 until the middle of 2020. Various aspects of application of CE methods for peptide analysis in foods, such as sample preparation, peptide preseparation, preconcentration, derivatization, adsorption suppression, and detection, are described and discussed. Moreover, several particular applications of CE methods for separation and analysis of peptides in various food samples of animal, plant and microbial origin are demonstrated.

METHODOLOGY

Sample Preparation

One major problem in the analysis of peptides in foods in general is that the food matrix is usually very complex. Various food matrix components, additives, and food treatment during processing and storage can interfere with peptide determinations in foodstuffs. Additionally, the sensitivity of the employed detection methods or the separation power of the CE technique might be not sufficient for the separation of complex peptide mixtures and for direct analysis of peptides present at low concentrations. For these reasons, the sample preparation, comprising peptide preseparation and/or preconcentration, is often an important and necessary step in the CE analysis of peptides in foods.

CHAPTER 5

Amino Acid Analysis by Capillary Electromigration Methods

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Abstract: The relevance of amino acids analysis is widely recognized in different fields. In Food Science, the determination of amino acids is of special interest since it can provide valuable information related to the nutritional, quality, and safety properties of food samples. For this reason, the development of robust, efficient, sensitive and cost-effective analytical methodologies is essential. Among the different analytical techniques, capillary electrophoresis has shown great potential in the last decades as a powerful tool to carry out the analysis of amino acids in food samples. This chapter aims at providing a comprehensive overview of the most recent applications of capillary electrophoresis for the analysis of protein and nonprotein amino acids in foodstuffs. The main experimental conditions concerning the separation and detection of amino acids are discussed and given in tables.

Keywords: Capillary electrophoresis, Food, Nonprotein amino acids, Protein amino acids.

INTRODUCTION

Amino acids are organic compounds which contain in their structure an amino group, a carboxylic group, and a side chain (R group) linked to a carbon atom. The R group is specific for each amino acid so that it determines not only the amino acid identity but also its properties. The wide variety of side chains that may be part of the amino acid structure makes possible the existence of hundreds of differen amino acids. Among them, just twenty have specific codons in the genetic code and are the protein building blocks in living organisms.

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Protein amino acids have a relevant role since they act as key compounds in the regulation of metabolic pathways to improve the health, survival, growth, development, and reproduction of organisms [1, 2]. These amino acids have been traditionally classified as "*essential*" or "*nonessential*" based on the need (or not) to be provided by the diet for nitrogen balance or growth [3]. The human body is able to produce nonessential amino acids in quantities needed for healthy functioning [4]. In contrast, those amino acids that cannot be produced by the human body must be ingested from the diet. These are the group of essential amino acids where phenylalanine (Phe), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), threonine (Thr), tryptophan (Trp) and valine (Val), are included. In some cases, dietary supplementation with one or more amino acids can be used to treat some health problems. Considering that elevated levels of amino acids and their products are pathogenic factors for neurological disorders, oxidative stress, and cardiovascular disease, it is crucial to maintain an optimum balance among amino acids in the diet and circulation to guarantee body homeostasis [3]. Along with the twenty protein amino acids, there are hundreds of other amino acids that are not found in the main protein 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 [5]. This kind of nonprotein amino acids mainly exist in foods as products formed during food processing, as metabolic intermediates, or as additives to increase the nutritional and functional properties of foodstuffs [6].

In the food analysis framework, amino acids are responsible for nutritional and organoleptic properties. Technological processes (fermentation, aging, etc.) may affect their concentration or even generate new amino acids. In this way, amino acids and their relative concentration can be considered as an important marker of authenticity, quality, and safety of food [7]. For this reason, the determination of protein and nonprotein amino acids has been exploited for years in the food field. Consequently, the relevance of high-throughput and sensitive analytical techniques to determine these components in foodstuffs is unquestionable.

Since its introduction over thirty years ago, the popularity and attractiveness of Capillary Electrophoresis (CE) have considerably increased owing to its intrinsic advantages (short analysis time, high sensitivity, and low consumption of solvents). Nowadays, CE is considered an excellent and reliable analytical tool. The high interest in the development and application of CE approaches to perform amino acid analysis in different matrices including foodstuffs can be deduced from the review articles published on this topic during the last ten years and collected in Table **1**.

Although not included in this table, other interesting reviews have also been published on the use of capillary electromigration methods for the analysis of a large variety of food-related molecules with different chemical properties including amino acids [17 - 22].

Table 1. Representative reviews published in the last ten years on amino acid analysis in food by capillary electromigration methodologies.

Year	Title	Ref.
2010	MEKC: A powerful tool for the determination of amino acids in a variety of biomatrices.	8
2012	Recent novel MEKC applications to analyze free amino acids in different biomatrices: 2009-2010.	9
2012	Recent advances in amino acid analysis by capillary electrophoresis.	10
2013	Recent trends in the analysis of amino acids in fruits and derived foodstuffs.	11
2014	Recent advances in amino acid analysis by capillary electromigration methods, 2011–2013.	12
2016	Recent advances in amino acid analysis by capillary electromigration methods, 2013-2015.	13
2016	Capillary electrophoresis determination of non-protein amino acids as quality markers in foods	6
2016	Analysis of amino acids, proteins, carbohydrates and lipids in food by capillary electromigration methods: a review.	14
2018	Recent advances in amino acid analysis by capillary electromigration methods: June 2015–May 2017.	15
2019	Advances in the determination of nonprotein amino acids in foods and biological samples by capillary electrophoresis.	16

Among the different CE modes, Capillary Zone Electrophoresis (CZE) and Micellar Electrokinetic Chromatography (MEKC) are the preferred options to carry out the amino acid analysis in food samples. Other modes, Capillary Electrochromatography (CEC) and Capillary Isotachophoresis (ITP) have been used in a lesser extent. Also, Microchip Capillary Electrophoresis (MCE) can result in a very attractive tool for rapid amino acid analysis. Regarding detection systems, UV, fluorescence, conductivity and mass spectrometry (MS) have been employed in combination with the different CE modes. Among them, UV and laser-induced fluorescence (LIF) are the most popular for the determination of amino acids in food samples. However, the absence of chromophore or fluorophore groups in most of the amino acid structures makes that a derivatization step be often essential in order to enhance their detectability using optical detection. Derivatization procedures also provide merits for electrochemical detection or MS. In the former,

CHAPTER 6

Vitamins Analysis by Capillary Electrophoresis

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Abstract: Vitamins are a series of trace compounds obtained from food that play important roles in human health. Vitamin analysis is essential for nutritional assessment and food production. A comprehensive overview of capillary electrophoresis for vitamins analysis is given. This chapter includes papers published since 1996 and can be seen as a guidance note of vitamins analysis using capillary electrophoresis. The analyses are discussed for water-soluble vitamins and fat-soluble vitamins according to different separation modes. In addition, various sample pretreatment methods avoiding matrix interferences with the analysis of vitamins are also described. Articles pertaining to different vitamins from a variety of food and beverages, dietary supplements, and pharmaceutical samples are included. This chapter highlights the unique performance of capillary electrophoresis for the qualitative analysis of vitamins in food.

Keywords: Capillary electrophoresis, Vitamins, Food.

INTRODUCTION

As one of the seven major nutrients, vitamins are important for human nutrition. They can be subdivided into water-soluble and fat-soluble vitamins [1, 2]. Vitamins cannot be produced within the body with the exception of vitamin D. They can be obtained either from food products or *via* pharmaceutical formulations. The dietary sources of these vitamins are represented in Table **1** [3, 4].

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Table 1. Food sources of vitamins.

Nutrient	Food Sources
Thiamin (Vitamin B_1)	Nuts and seeds, legumes, wholegrain/enriched cereals and breads, pork
Riboflavin (Vitamin B_2)	Organic meats, milk, bread products, fortified cereals
Niacin (Vitamin B_3)	Meat, fish, poultry, enriched and whole-grain breads and bread products, fortified cereals
Pantothenic Acid (Vitamin B_5)	Chicken, beef, potatoes, oats, cereals, tomato products, liver, kidney, yeast, egg yolk, broccoli, whole grains
Biotin (Vitamin B_7)	Liver and smaller amounts in fruits and meats
Folic Acid (Vitamin B_9)	Enriched cereal grains, dark leafy vegetables, liver, orange juice, seeds, legumes, leafy green vegetables
Cobalamin (Vitamin B_{12})	Milk, milk products, eggs, seafood, fish, poultry, meat. It is not present in plant foods.
Ascorbic Acid (Vitamin C)	Found in vegetables and fruits, especially citrus fruits, tomatoes, tomato juice, potatoes, brussels sprouts, cauliflower, broccoli, strawberries, cabbage, spinach
Vitamin A	Liver, dairy products, fish, darkly colored fruits and leafy vegetables
Vitamin D	Fatty fish, fortified milk products, fortified cereals
Vitamin E	Vegetable oils, unprocessed cereal grains, nuts, fruits, vegetables, meats
Vitamin K	Green vegetables, brussels sprouts, cabbage, plant oils, margarine

Some vitamins can also be used as biomarkers for certain diseases [5]. Therefore, is necessary to have fast methods for vitamins analysis in different sample matrices.

High Performance Liquid Chromatography (HPLC) is one of the most common techniques for the analysis of vitamins [6 - 8]. However, it usually requires a longer analysis time and larger sample volume with much more solvent consumption. As an alternative tool to HPLC, Capillary Electrophoresis (CE) offers unique advantages due to its fast speed, less sample consumption and low reagent cost [9].

The separation of CE in different modes is based on the differential migration in an electric field [10]. Capillary Zone Electrophoresis (CZE) is based on differences in the charge-to-mass ratio enabling to analyze charged analytes [11]. Water-soluble vitamins can be easily charged and can be separated using CZE [12]. Micellar Electrokinetic Chromatography (MEKC) is another mode of CE whereby the separation of neutral compounds was achieved [13]. MEKC was

mostly used for the simultaneous analysis of water-soluble vitamins [14, 15]. In addition, other separation modes were also used to analyze vitamins in foods, such as Microchip Electrophoresis (MCE), Nonaqueous Capillary Electrophoresis (NACE),

Microemulsion Electrokinetic Chromatography (MEEKC) and Capillary Electrochromatography (CEC).

Currently, a variety of detection techniques have been used for CE in different food analyses, including UV, laser-induced fluorescence (LIF), chemilum-inescence (CL), electrochemical (EC) and MS detection [16]. Most CE-UV methods are mainly used for high concentration analyte analysis or simultaneous determination of several vitamins [17]. More sensitive detectors and/or on-line preconcentration techniques are developed to improve CE sensitivity [18]. LIF could provide 1000 times more sensitivity than the UV detector [19]. However, it is only suitable for analytes containing a fluorophore. EC detection coupled with CE is attractive due to its good selectivity towards electroactive analytes. MS detectors have a higher sensitivity but the instrumentation required is expensive [20]. A stable CE current and high ionization efficiency were achieved by the developed interfaces [21].

This chapter provides an overview of CE method developments in vitamins analysis from 1996 to 2019. The different types of samples together with separation mode, buffer, detection type and obtained sensitivity are listed in **Tables 2-6**.

Table 2. Summary of vitamin B analysis by CE.

Analyte	Sample	Separation Mode	Detection	Buffer	LOD	Ref
Analyte	**Sample**	**Separation Mode**	**Detection**	**Buffer**	**LOD**	**Ref**
Thiamine(B_1)	Tablets	CZE	UV	65 mM boric acid,	2×10^{-4} mM,	[38]
TH, THP,			200 nm	8 mM sodium tetraborate,	1×10^{-4} mM,	
THPP				pH 8.24	1×10^{-4} mM	

CHAPTER 7

Application of Capillary Electrophoresis to the Determination of Polyphenols in Food Samples

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Abstract: Polyphenols are naturally occurring compounds found in fruits, vegetables, cereals and beverages. Nowadays, there is a high interest in these compounds because of their potential health benefits associated with the protection against the development and progression of many degenerative diseases due to their antioxidant capacity. However, their composition changes both qualitatively and quantitatively depending on the natural source. Thus, the determination of these compounds is not straightforward. Among the different techniques employed for their analysis, capillary electrophoresis is a very interesting alternative due to its high separation efficiency, high resolution power, short analysis time and low consumption of samples and reagents. This chapter presents an overview of the recent developments and applications of capillary electrophoresis for the analysis of phenolic compounds from food samples, including articles published since 2010 to date. In addition, the characteristics of the most relevant developed methodologies using different separation modes are broadly discussed.

Keywords: Capillary electrophoresis, Food samples, Phenolic compounds, Polyphenols.

INTRODUCTION

Polyphenols are a broad family of secondary natural metabolites which are products of the pentose phosphate, shikimate and phenylpropanoid pathways in plants [1]. Structurally, they contain aromatic rings, with one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymeric compounds (see Fig. **1**) [2].

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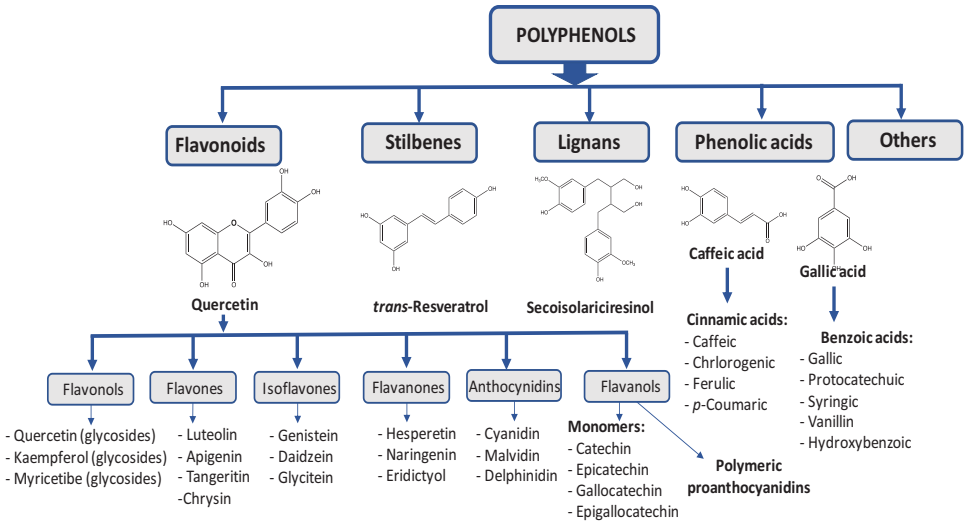

Fig. (1). Classes of polyphenols based on the number of phenol rings and their structural elements.

The majority of polyphenols in nature are found as conjugates with mono- and polysaccharides or as functional derivatives like esters and methyl esters [3]. They can be classified into different classes according to the chemical structures of the aglycones such as flavonoids (flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols), stilbenes, phenolic acids, lignans, and others, as shown in Fig. (**1**) which represents a classification based on the work of D'Archivio *et al.* [4]. Plant polyphenols provide protection against pathogens and predators, playing an important role in growth and reproduction. They are also responsible for the colour and the sensory characteristics of fruits and vegetables [5]. Moreover, polyphenols present several health beneficial properties such as anti- inflammatory, anticarcinogenic, antimicrobial, antithrombotic, cardioprotective, vasodilatory, and anti-neurodegenerative effects [6]. Many of these properties are due to their antioxidant capacity [7, 8]. Their antioxidant properties are mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers [9].

Polyphenols are common constituents of plants and foods of plant origin. The polyphenol composition from one to another natural source changes both qualitatively and quantitatively. For instance, some phenolic compounds are extensively spread while others are limited to specific natural sources (*i.e.* isoflavones in legumes). That is why the determination of polyphenols is a challenging analytical assignment. Generally, two different approaches are used in order to achieve their determination. One is the quantification of plant

polyphenols through spectrophotometric methods. For example, Folin-Ciocalteau and Folin- Denis methods have been widely employed as spectrophotometric methods to measure total phenolic compounds in plants for many years. The main disadvantage of these methods is that they only allow for an estimation of the phenolic content because they are not specific for phenolic compounds. Furthermore, these methods are not selective and robust being highly affected by matrix effects [10]. Moreover, spectrophotometric methods do not provide quantitative measurement of individual compounds because they do not allow an analytical separation of the compounds. Therefore, the second approach comprises the separation of polyphenols before their detection. With this purpose, chromatographic techniques have mainly been employed although Capillary Electrophoresis (CE) has also demonstrated its potential in the separation of phenolic compounds from plant extracts. Regarding chromatographic techniques, High-Performance Liquid Chromatography (HPLC) is by far the preferred technique for the separation and quantification of polyphenols from natural sources. Gas Chromatography (GC) has also been employed with this aim although to a lesser extent due to the need for a derivatization step of compounds [11]. In the last years, supercritical fluid chromatography (SFC) has also been used in the separation and identification of phenolic compounds. SFC is more versatile than HPLC, more cost-effective and environmentally friendly, with higher output, better resolution and faster analysis times than general liquid chromatographic methods [12]. On the other hand, CE has already been exhibited as an attractive alternative to HPLC or GC for the analysis of phenolic compounds. CE is a versatile analytical tool for the routine determination of a wide variety of phenolic compounds in different types of samples due to its high separation efficiency, high resolution power, short analysis times and low consumption of samples and reagents [13, 14]. CE methods developed for the analysis of phenolic compounds were generally based on the use of UV-Vis detection while detection by mass spectrometry (MS) is less frequent [15 - 17].

This chapter presents an overview of the recent developments and applications of CE to the determination of phenolic compounds in different food matrices covering the last ten years (from 2010 until the present). The characteristics of the developed methodologies using different CE separation modes are detailed in Tables and the most recent and relevant applications of CE in this field are discussed.

ANALYSIS OF PHENOLIC COMPOUNDS

Different separation modes were employed in the analysis of phenolic compounds by CE. The most employed CE mode was Capillary Zone Electrophoresis (CZE) although Electrokinetic Chromatography (EKC) using cyclodextrins (CDs) in the

CHAPTER 8

Analysis of Food Additives by Capillary Electrophoresis

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Abstract: Electrophoretic approaches are increasingly used for the determination of food additives in real samples based on the easy operation modes and enhanced separation efficiency of Capillary Electrophoresis (CE). This chapter presents a summary of recent breakthroughs related to the development of different analytical strategies focused on enhancing the study of food samples and reviews the determination of food additives by CE, including some promising approaches. The effectiveness of these strategies to solve alimentary issues is also discussed.

Keywords: Capillary electrophoresis, Detection, Food additives, Microchips, Nanomaterials, Preconcentration, Screening.

INTRODUCTION

Food additives have become more and more noticeable for regulatory and consumer agencies. These kinds of chemicals are deliberately added to food products for different reasons [1]. However, potential health risks to humans have increased particularly when these chemicals are ingested in excess. This fact is leading governments and controlling authorities to regulate the permitted levels of food additives [2]. To avoid food fraud and protect consumers there is need for developing rapid analytical methods suitable for the simultaneous determination of many component additives mixtures with high performance, appropriate reproducibility, and good sensitivity.

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Electrophoretic analytical separation techniques have been used to determine food additives in food. A comparison of LC and GC with the CE technique reveals some interesting facts (see Table **1**).

Table 1. Comparison of capillary electrophoresis (CE), liquid chromatography (LC) and gas chromatography (GC) techniques for the determination of food additives in food.

	HPLC	GC	CE
INVESTMENT	High	Medium	Low
SOLVENT CONSUMPTION	High	High	Low
SKILLS NEEDED	Medium	High	Medium
SAMPLE PRETREATMENT	Medium	High	Low
SENSITIVITY	High	Medium	Low

CE is an interesting alternative because of its low investment costs and the possibility of performing multiple fast separations and detection with little solvent and sample consumption; however, it has a low concentration sensitivity with optical detection. CE separation is achieved by the application of an electric field through a narrow capillary [3]. This technique has become an important approach and a good alternative separation technique for the determination of a large number of food additives allowing the simultaneous determination of diverse chemicals and with the ability to analyze charged and neutral species [4]. The main advantages of CE for daily analysis are small injection volume and low waste, reduced reagent and sample consumption, shorter analysis times than those commonly obtained by LC, and the fact that it enables the separation of analytes exhibiting a poor behavior in GC [5]. In addition, complex samples can be injected into the CE system after an easy and simplified sample pre-treatment step without a detriment in the separation which makes CE more attractive for the analysis of diverse food matrices [6]. CE also provides the possibility to achieve analyses in miniaturized format which has demonstrated to be an extraordinary alternative for microchip technology because volumes in the region of nanoliters can be easily handled, it requires fixed parts and provides quick separations with a high resolution. Microchip-based CE has some other additional advantages namely fast analysis time, small size and low cost. These features are the main objectives of many analytical methods [7].

CE is generally used with a low cost and easy UV-Vis detector. However, one of the limitations of this detection device is the low concentration sensitivity because of the short length of the detection path and the low sample volumes commonly injected. To improve sensitivity many approaches have been developed using various types of detectors (electrochemical (ECD), laser-induced fluorescence

(LIF), or mass spectrometry (MS)), chemical reactions (chemiluminescence, derivatization and nanomaterials) and sample treatment units for preconcentration and clean-up (Fig. **1**). These sample treatment units can be used off-line in batch mode or incorporated on-line by coupling or integrating them with the CE system.

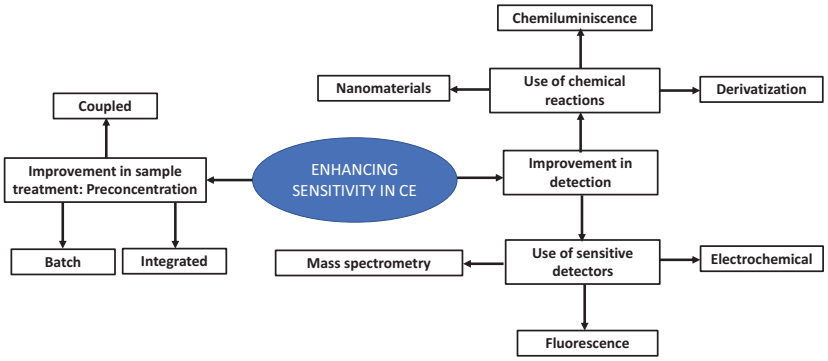

Fig. (1). Analytical strategies applied to enhance sensitivity in CE.

CE has become a promising separation technique based on its versatility given that it permits to separate very important molecules by using new and different coatings; it may be miniaturized into a microfluid-based device suited for several techniques such as Mass Spectrometry, and it has also proved its on-line coupling with sample pretreatments approaches for on-line analysis and applicability for the analysis of complex samples [8].

The aim of this chapter is to introduce food additives, their importance and uses, and discuss the common analytical strategies developed for the determination of food additives by CE including preconcentration, separation, screening, detection and the use of microchips. Some recent applications for monitoring additives in food samples by electrophoretic methods are included.

FOOD ADDITIVES: IMPORTANCE AND USES

Food additives are chemicals incorporated during food production for several purposes, for example, to increase the safety of food by preventing the undesirable effects of microbial growth and also to enhance food color or make food odor, flavor, and texture more attractive [9] but above all, these additives are employed to facilitate food preparation.

The use of food additives is not novel. The need and importance of preserving food already existed in ancient times, when plain salt was used to preserve fish or to make meat last longer. Similarly, the resins added to Greek or Roman wine

CHAPTER 9

Chiral Capillary Electrophoresis in Food Analysis

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Abstract: Chiral analysis is a powerful tool in Food Science for quality and safety assessment since the enantiomeric composition of food samples can reveal adulterations, the effects of processing and storage or give valuable information on the bioactivity, traceability or even toxicity of foods. This chapter describes the potential of Capillary Electrophoresis in the chiral analysis of food and beverages. The separation modes used in CE for the chiral analysis of food samples are described, including different strategies for sample preparation and sensitivity enhancement. The most relevant applications developed in the period from 2010 to the present are depicted and the main conclusions and future prospects are outlined.

Keywords: Amino acids, Capillary electrochromatography, Catechins, Chiral capillary electrophoresis, Chiral separation, Enantiomers, Electrokinetic chromatography, Food samples, Organic acids, Sweeteners.

INTRODUCTION

Chiral analysis is nowadays an important area in different fields due to the different biological activities that enantiomers may have. In fact, the enantiomeric determination of chiral compounds has become paramount in a big variety of samples such as pharmaceutical, biomedical, agrochemical, environmental or food matrices, among others.

In food science, chiral analysis is a powerful tool for food quality and safety assessment.

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This is due to the fact that numerous food components are chiral (amino acids, organic acids, vitamins, bioactive compounds or sweeteners, among others) but also different toxic compounds potentially present in food are chiral. As a consequence, the enantiomeric composition of food samples can reveal adulterations, alterations due to the effects of processing or storage (including microbial racemization of the enantiomers naturally present in food or beverages), can explain changes in organoleptic properties or can give valuable information on the bioactivity or traceability of food samples.

Aimed to individually determine the enantiomers of chiral compounds, different analytical techniques have been employed. Separation techniques such as High Performance Liquid Chromatography (HPLC) or capillary LC, Gas Chromatography (GC), Supercritical Fluid Chromatography (SFC) and electromigration techniques such as Capillary Electrophoresis (CE) or Capillary Electrochromatography (CEC) have been the most used and their potential to achieve chiral analysis in food science has been reviewed in several relevant articles [1 - 3]. However, the use of other chiral techniques such as countercurrent chromatography, sensors, biosensors, and direct mass spectrometry (MS) in food science has also been recently reviewed [3]. Among chromatographic and electromigration techniques, HPLC has been the most employed for enantiomer determinations due to its applicability for preparative and analytical purposes, easy operation mode, wide range of chiral stationary phases commercially available, and the possibility of working in two dimensional chromatography, although some drawbacks can also be mentioned such as the use of organic solvents and expensive chiral columns [3]. In this context, CE has demonstrated in the last years very interesting characteristics and even some advantages for chiral analysis. Among these advantages, the high efficiency that can be obtained and the easy change of the chiral selector for a given enantiomeric separation (by simply adding the selector to the separation medium) can be highlighted [4, 5]. Moreover, the fact that the use of a chiral chromatographic column is not necessary (although it can be employed in CEC if desired) together with the low consumption of reagents required to work in CE, confers this technique a special attraction from economic and environmental points of view. In addition to these interesting features, the coupling of CE with MS has increased the potential of this technique for chiral analysis [6 - 8], although the use of some strategies was necessary in order to avoid the entrance of non-volatile chiral selectors into the ionization source of the mass spectrometer. Some of these strategies were partial filling techniques, counter migration of chiral selector or employing the indirect separation mode for chiral analysis consisting of using pure chiral reagents to originate diastereomers having different electrophoretic mobilities in CE with achiral separation media.

Although several articles have reviewed the main advances in the application of capillary electromigration methods for food analysis and foodomics in the last decade [9 - 13], and some others were focused on the analysis of relevant food components such as non-protein amino acids [14, 15], just one review article has been published in the last decade specifically devoted to chiral capillary electrophoresis in food analysis [16].

This chapter presents the potential of CE in the chiral analysis of food samples. With this aim, the CE separation modes enabling the separation of enantiomers are described, including specific features for sample preparation and sensitivity enhancement, and the most recent and relevant applications of chiral CE for food analysis are discussed. The characteristics of the methodologies developed are detailed and presented in Tables and the main conclusions and future prospects are outlined.

CHIRAL SEPARATION MODES IN CE

Chiral separations are definitely one of the most challenging tasks in the Separation Sciences. The separation of two species that have the same physicochemical properties and that are mirror images of one another can only be achieved under a chiral environment, *i.e*., when interacting with an appropriate chiral selector. CE is a microscale analytical separation technique valuable in the chiral analysis of numerous application fields. There are two possibilities that enable chiral separations in CE based on how a given pair of enantiomers interact with this chiral environment. Firstly, these can be classified into indirect and direct modes. The indirect mode relies on the covalent reaction of the enantiomers with an enantiomerically pure derivatizing agent. This turns the separation of a pair of enantiomers into a separation of a pair of diastereoisomers which now possess different physicochemical properties and, thus, can be separated under achiral conditions. As will be further detailed in the *Applications* section of this Chapter, indirect procedures have been used in previous years but this approach is not as popular as the direct strategies. This is because of the additional experimental time and costs derived from having enantiomerically pure chemicals that enable the formation of the purest diastereoisomers. On the contrary, the direct mode is based on the direct interaction of a chiral selector and the given enantiomers to form temporary diastereoisomers that ideally will have different electrophoretic mobilities and consequent chiral separation. The direct mode is the gold standard approach in CE that can be further divided into two modes depending on whether the chiral selector is solubilized in the background electrolyte (Electrokinetic Chromatography, EKC) or forms part of the capillary column (CEC).

CHAPTER 10

Food Analysis by Microchip Electrophoresis

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Abstract: The most significant advances in food analysis using microchip electrophoresis (ME) technology will be discussed in this book chapter, covering the works published from 2009 to 2019, which will be organized in three sections based on the detection mode employed: electrochemical and fluorescence detection, and bioanalytical-based approaches. The most innovative methodologies, relevant applications, and latest advances in instrumentation to achieve a truly portable lab-o--a-chip will be discussed. Commercial instruments will also be briefly mentioned as a demonstration of the maturity of ME technology.

Keywords: Biosensors, Bioassays, Electrochemical detection, Fluorescence detection, Food analysis, Lab-on-a-chip, Microfluidic, Microchip electrophoresis.

INTRODUCTION

In recent years, there is an increasing concern for health and the environment, including food quality and safety [1-3]. In fact, food is currently considered not only a source of nutrients but also an affordable way to prevent future illnesses [4].

Food analysis encompases a large variety of food-related molecules with different chemical properties, including amino acids, phenols, carbohydrates, DNAs, toxins, pesticides, residues, food additives, and small organic and inorganic compounds. In many cases, food contaminants and residues are at very low concentrations in very complex matrices.

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This is why the development, validation and application of new or alternative analytical methods are highly relevant to characterize the identity of genuine foods, determine analytes of utmost interest such as allergens, toxins and foodborne pathogens, and detect frauds and adulterations [5, 6].

Microchip Electrophoresis (ME) is not only the miniaturized format of capillary electrophoresis but also a technology that provides additional functionalities such as parallelization (higher throughput), integration of multiple analytical steps and portability, and also several advantages such as shorter analysis times, lower sample and reagent consumption, and lower waste generation (green chemistry). In consequence, ME has become a good alternative to more conventional methods, which are time-consuming and expensive [7, 8]. Moreover, ME is a mature technology with a wide range of possibilities for lab-on-a-chip (LOC) applications [9]. In this sense, LOC technology aims to perform all functions carried out in laboratories, but on a much smaller scale (chip) and with new characteristics and capabilities [10]. One of the pillars of LOC systems is microfluidics, which is defined as the science and technology that process or manipulate small amounts of fluid (from 10^{-9} to 10^{-18} L) using channels measuring from tens to hundreds of micrometers [11]. Microfluidics offers new capabilities in controlling the concentration of molecules in space and time due to the omnipresence of laminar flow. The use of this technology in food analysis gave rise to a new concept, *Food Microfluidics* [9].

From the early times of the appearance of the ME technology, our research group has published several reviews dealing with the use of ME in food analysis [12-14]. In these reviews, the evolution in this field can be followed up; from the pioneers' works, which were focused on the exploration of fast separations of relevant compounds in simple food samples using home-made microchip systems (proof of concept) [12], to the use of the first commercial instruments based on microchip technology for food analysis [14].

In this chapter, we intend to review the most significant advances in food analysis by ME over the last decade (2009-2019). Innovative methodologies, relevant applications, and advances in instrumentation to achieve a truly portable lab-on-a-chip will be highlighted. In addition, commercial instruments and components will be cited throughout the manuscript for helping beginners to know companies involved in this field. As Professor George Whitesides stated about LOC technology, "*The science is working its way into technology, and preparing to become products*" [10] thus it is fundamental to point out commercial ME products achieved to date.

This chapter is structured in three sections. First, the main detection principles used in ME, electrochemical and fluorescence detection, will be discussed. Second, bioanalytical-based approaches will be described and third the main conclusions and ME future prospects for food analysis will be highlighted.

DETECTION MODES

In the context of food analysis by ME, both electrochemical (amperometry and conductometry) and laser induced fluorescence (LIF), are the most used detection modes. The use of mass spectrometry is residual in food ME. As it will be discussed in the next sections, LIF detection provides the lowest limits of detection, but it is often not miniaturized [15, 16]. In this sense, electrochemical detection is ideal in ME because it can be easily miniaturized without affecting the analytical performance [17, 18].

Electrochemical Detection

Electrochemical detection is an excellent approach for ME due to its easy miniaturization without losing analytical performance (unlike optical methods), good sensitivity and moderate selectivity. Also, the derivatization step is not widely needed [17-19].

In this section, amperometric and conductivity detection will be critically discussed, showing their advantages and limitations in some selected food analysis applications. Within each section, we will discuss the literature from two perspectives: works reporting methodological improvements (such as the use of nanomaterials) and/or relevant applications, and other research works proposing advanced technical innovations, such as fully integrated equipment.

Amperometric Detection

Among all electrochemical techniques, amperometric detection (AD) is the most used mode in ME. The basis of this technique is that a stable voltage is applied to a working electrode relative to a reference electrode, and the current generated from the oxidation or reduction reaction of electroactive species is measured on the working electrode surface. To increase this surface and improve the analytical performance, many of the works discussed in this section use mainly carbon and metallic-based nanomaterials [1, 20].

Marques Petroni *et al.* proposed a simple methodology to fabricate screen-printed carbon electrodes by manual deposition of home-made conductive carbon ink over patterned acrylic substrate [21]. The electrode was aligned at the end of the separation channel of a PDMS microchip with a cross-shaped pattern. Polymer

CHAPTER 11

Application of Capillary Electrophoresis to Food Authentication

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Abstract: This chapter deals with the issue of food authenticity and applications of capillary electrophoresis in this field. An overview of food adulteration, including the frequency of adulteration of selected commodities and a list of methods used to prove authenticity or detect food adulteration, is presented. An overview of applications of capillary electrophoresis for food authentication is supplemented by specific cases described in more detail.

Keywords: Adulteration, Capillary electrophoresis, Falsification, Food authentication.

INTRODUCTION

Cases of food adulteration can be encountered from the time when they began to be manufactured for sale. Mentions on counterfeiting and penalties for dishonest producers and traders are found in a number of historical texts. In Biblical times, meat inspection rules and fitness criteria were specified. They can be found in the Book of Leviticus. Galen, an ancient physician who developed the principle of humours, linking body type with health and personality, warned against the adulteration of herbs and spices [1]. The Industrial Revolution brought the great age of food adulteration to its height. During this period, untainted, safe and healthy food was a difficult commodity to buy. The major problem was that adulterated food was not easy to detect. Science was based in universities and the practice was never applied to the food industry. Friedrich Engels (German-born political theorist) in his study "The Condition of the Working Class in England" stated: "…*workers were being exploited by the middle classes who were mixing sugar with ground rive, chicory with coffee, and earth with cocoa, then selling the adulterated products as pure and at full price…" [2].*

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Methods have also been developed for years for counterfeit detection. In fact, already in 1820, the German chemist Frederick Accum published a treatise on food adulteration and culinary poisoning where he exposed fraudulent practice and toxic contamination in food and drink, providing methods for their detection. The treatise, popularly known as "There is Death in the Pot" aroused great attention and has spread throughout Europe [3].

In principle, counterfeiting procedures do not fundamentally change; the examples described in historical sources are similar. The main categories of counterfeiting are listed in Table **1**, while it is clear that the individual categories are not fundamentally defined and overlap [4 - 8].

Table 1. Examples of food adulteration issues.

Way of Adulteration	Examples
Abuse of a well-known brand	- fake sale of the product under a more expensive brand; - use of packaging, labels, names resembling a well-known brand, *etc*.;
Setting food cheaper component	- non-compliance with the requirements for the content of the so-called glaze in frozen meats; - the use of blood proteins instead of muscle in meat products; - additions of fibre to meat products; - undeclared additions of soya, cereal, pea and other vegetable proteins to meat products; - additions of cow's milk to buffalo in the production of true mozzarella; - dilution of milk with water; - partial or complete replacement of Basmati rice by cheaper varieties; - additions of common wheat flour (*Triticum aestivum*) to pasta presented as pasta made from semolina (made from wheat flour *Triticum durum*); - dilution of olive oil with other vegetable oils; - additions of starch hydrolysates or sugar syrups to honey; - additions of water, sugar, acids and dyes to fruit juices, nectars, beverages; - replacing more expensive fruits with cheaper ones (apple puree instead of strawberries, apple juice instead of more expensive juices, *etc*.); - reducing the cocoa butter content of chocolate by adding other oils; - addition of roasted skins to ground coffee or cocoa; - dilution of wine with water; - additions of water, sugar, acids and dyes to fruit juices, nectars, beverages

(Table 1) cont.....

Way of Adulteration	Examples
Presence of undeclared components	- undeclared use of other types of meat in meat products; - unauthorized or undeclared use of mechanically separated meat in meat products; - undeclared use of offal in meat products; - undeclared addition of substitutes such as maltodextrins in coffee; - addition of meat species in processed vegetarian foods; - undeclared use of genetically modified food
Food exchange for another cheaper	- issuing other vegetable oils as olive; - issuing sea trout for salmon; - issuing cheaper varieties as more expensive
Failure to comply with the declared technological procedure	- dispensing thawed meat and fish fresh; - declaring toasted bread as fresh - undeclared use of gamma radiation in production - dispensing the reconstituted juice from the concentrate with freshly pressed juice; - use of synthetic alcohol for the production of spirits; - issuing ordinary oil for cold-pressed oil (virgin olive oil); - incorrect indication of the age of the distillate
Incorrect indication of geographical origin or method of production	- farming of fish produced on farms; - labelling of usual production as bio (organic); - issuing imported wines for domestic - incorrect declaration of floral or geographical origin of honey
Declared content of the component higher than the actual content	- placing a higher number of eggs in pasta - declaration of the proportion of muscle proteins in meat products distorted by the addition of plant or blood proteins - the use of misleading labels and markings (images, names, graphic symbols inadequate to food composition)

Trends in commodity counterfeiting are being evaluated according to the frequency of publications in databases; they do not change in the long time span they develop according to current cases. Counterfeiting is motivated by economic profit, so expensive and luxurious foods (spirits, wine, spices) are most often counterfeited or, conversely, foods that are sold in large volumes (meat and dairy products, fats and oils, fruit juice). This also corresponds to the trends in the interest of the individual commodities expressed as the number of citations of articles with a given focus in various databases. Table **2** summarizes the trends of interest in individual commodities.

CHAPTER 12

Chemical Food Safety Applications of Capillary Electrophoresis Methodologies

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Abstract: Chemical hazards may be present in food due to their intended use during food production (*i.e.*, residues), they may be intentionally added to food products to confer specific attributes (*i.e.*, food additives), or they may simply occur at any of the stages of the food supply chain (*i.e.*, contaminants). Since these chemical hazards represent a health risk to consumers, legislation has been developed to establish the maximum concentration levels of these substances in food, and to define control measures to monitor their presence in food products. In general, liquid chromatography (LC) and gas chromatography (GC) are used as analytical techniques in laboratories that are responsible for carrying out routine food safety analyses. During the last decades, capillary electrophoresis (CE) has been extensively investigated as an alternative (or complementary) separation tool to chromatographic techniques, and today, it is already a consolidated technique that can be implemented in routine food safety laboratories. This chapter presents the state of the art of CE in the field of chemical food safety and gives an overview of relevant applications in this area.

Keywords: Capillary electrophoresis, Chemical hazards, Contaminants, Food additives, Food safety, Residues.

INTRODUCTION

The intensification of agricultural and livestock activities related to an increasing population rate and a higher demand for food, as well as the current globalization of the food chain, poses numerous challenges for food safety. In this context, bacteria, viruses, parasites and chemical hazards can occur at any point in the food

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chain, from primary production to delivery to the final consumer, endangering public health. It is the mission of policy-makers to promote health and ensure food safety by establishing food systems and infrastructures to respond to and manage food risks, such as the Rapid Alert System for Food and Feed (RASFF) implemented by the European Union (EU) to avoid food safety risks [1]. Consequently, food safety authorities can take actions and prevent similar risks. As part of food safety management, competent authorities have also implemented control plans to assess the prevalence of hazards in feed, food and animals [2]. In this sense, selective and analytical methods are required to successfully execute these control plans.

With respect to chemical hazards, and according to the Codex Alimentarius Commission, they can be classified into residues (*i.e*. substances intentionally used during food production for agricultural or livestock benefits), contaminants (*i.e*. substances that are not intentionally added to food or feed for food-producing animals, but are however present in such food or feed as a consequence of food-related activities such as processing, transport, storage, *etc*. or environmental contamination), and additives (*i.e*. substances intentionally added to confer food some specific organoleptic properties) [3]. This general classification may be even simpler, and chemical hazards can be differentiated according to whether their presence in food is due to intentional use (*i.e*. residues and additives) or not (*i.e*. contaminants). In order to standardize at an international level, and without prejudice to national regulations, the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Meeting on Pesticide Residues (JMPR) has established allowable daily intakes (ADIs, chronic values) and acute reference doses (ARfDs) of pesticide residues in food. In addition, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has set tolerable daily intakes (TDIs), ADIs and other guidance values for food additives, contaminants, such as naturally occurring toxins, and residues of veterinary drugs in food [4].

Nowadays, there is a wide range of regulations and guidelines aimed to control chemical hazards in food to ensure that they do not exceed concentrations levels considered ‘safe’ or levels related to a significant health risk. From the perspective of European regulations and guidelines on food safety, and as examples, the performance of analytical methods intended for the analysis of veterinary drugs, pesticides, and mycotoxins in food must meet the criteria established in Commission Decision 2002/657/EC [5], SANTE/12682/2019 [6] and SANTE/12089/2016 [7], respectively. These regulations and guidelines establish that mass spectrometry (MS) generally coupled with Liquid Chromatography (LC) or Gas Chromatography (GC) is the analytical technique of choice to control chemical hazards in food. However, GC-MS or LC-MS analysis

of some chemical hazards may be limited by their volatility and polarity properties.

Capillary Electrophoresis (CE) is an alternative (or complementary) separation technique to LC and GC which offers several advantages, such as the requirement of low sample volumes and low solvent consumption in accordance with the principles of Green Chemistry. CE has been applied primarily to the separation of charged substances (*i.e*. Capillary Zone Electrophoresis (CZE) mode), although the separation of neutral compounds can also be achieved by generally applying the Micellar Electrokinetic Chromatography (MEKC) mode [8]. CE is recommended for the separation of polar substances that are difficult to be separated by chromatographic techniques, especially considering that Hydrophilic Interaction Chromatography (HILIC)-based methods are less robust than traditional Reverse Phase Liquid Chromatography (RPLC) methods implemented in food safety laboratories.

CE is routinely used in pharmaceutical applications [9] and in forensic laboratories, namely for the analysis of deoxyribonucleic acid (DNA) [10]. However, although the applicability of CE for food analysis has been extensively demonstrated [11 - 14], this technique has not yet been implemented in food safety laboratories for routine applications and still remains a research tool. In general, the implementation of CE has been limited by its lower concentration sensitivity and drawbacks associated to CE-MS hyphenation, especially considering that both are key aspects in the analysis of chemical hazards in food. Food safety applications involve the detection of substances at low concentration levels (within the ng kg^{-1} and μg kg^{-1} ranges), and as mentioned above, MS detection is necessary for the unambiguous determination of residues, contaminants and additives in food since they involve legal consequences. However, CE technology has rapidly evolved in recent years and numerous preconcentration strategies (on-line, in-line and off-line approaches) can be applied to achieve low limits of detection (LODs) [15, 16]. Efficient and robust interfaces for the hyphenation of CE with MS are now commercially available, contributing to the further development and implementation of CE-MS applications [17, 18].

In this context, this chapter shows the most outstanding current applications of CE in the field of chemical food safety, mainly focused on the analysis of residues (*i.e*. veterinary drugs and pesticides), contaminants (*i.e*. toxic metals, biogenic amines, natural toxins, food processing contaminants and contaminants related to food packaging) and food additives (*e.g*. preservatives, colorants and sweeteners). In addition, a particular section is dedicated to the CE analysis of nanoparticles in food, as this is an emerging concern in food safety and these materials can be

CHAPTER 13

The Role of Capillary Electrophoresis to Guarantee the Quality and Safety of Dietary Supplements

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Abstract: At present, dietary supplements are commercially available products, globally consumed as an addition to the usual diet. Considering that dietary supplements are a source of nutrients, they are widely utilized to improve human health and prevent various diseases; therefore they are expected to be safe. There is still no common definition regarding the role of supplements which cannot be considered functional foods nor drugs. Dietary ingredients in supplements are exempt from food additives or drugs regulations. For this reason, these supplements are marketed without any data on identity, including ingredient information, effectiveness, toxicology and safety. Therefore, efficacy and safety are necessary claims required to preserve consumer health. To face this imperative challenge, sensitive and selective analytical techniques capable of providing a full characterization of the supplements in terms of their components are needed. In the last decades, Capillary Electrophoresis (CE) has shown to be a powerful tool that offers solutions to almost any analytical issue arising in several application fields. Due to its simplicity of operation and versatility, it has become a complementary separation tool to other separation techniques such as gas and liquid chromatography in the analysis of dietary supplements. This chapter aims to give a comprehensive overview of the most important applications of CE for the analysis of dietary supplements in terms of their main key components.

Keywords: Amino acid, Antioxidant, Capillary electrophoresis, Contaminant, Dietary supplement, Vitamin.

INTRODUCTION

Dietary supplements are preparations taken in addition to the normal diet in order to assure a balanced nutrient intake for optimal health. Supplements are available on the market as tablets, capsules, powders, gels or liquids and include at least one of the following substances: vitamins, minerals, amino acids, fatty acids, proteins (enzymes), hormones or botanicals [1 - 3].

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In recent years, consumption of dietary supplements has greatly increased since their use has become a common habit to compensate for nutrient deficiency. Despite this, inadequate information relating to their safety, quality and benefits is provided [4].

The usage scenario of dietary supplements varies significantly over the world. Whereas in some countries supplements are considered as medicines for illnesses in others they are taken to enhance physical well-being. As a consequence, dietary supplements regulations vary considerably throughout the world [5, 6]. For instance, in the United States, dietary supplements are labelled as foods and include only the preparations orally taken under the Dietary Supplements Health Education Act of 1994 (DSHEA). As pre-market requirement, the U.S. Food and Drug Administration (FDA) usually demands data proving the harmlessness of these products to people. Therefore, food additives and ingredients in the supplements are classified as GRAS (generally recognized as safe) under the terms of use described on their labelling. However, for many ingredients there is no information about their safety, even though FDA requires from manufacturers to report side effects related to dietary supplements [3, 5, 7]. On the other hand, the European Union (EU) makes provision for two possible forms of dietary supplements regulation, depending both on products' nature and use. Namely, supplements are classified as “Food supplements” and follow the foodstuffs rules (Directive 2002/46/EC of the European Parliament) when they are sold to provide health benefits or to reduce the risks of chronic diseases (*e.g*., cardiovascular diseases, osteoporosis, hypertension, overweight or obesity). In that case, centralized pre-market authorization is not needed. In the EU, the harmonized legislation regulates only vitamins and minerals ((EU), 2006; (EU), 2015b); for all other ingredients, the European Commission draws up a list of substances the use of which must be controlled because they are known or suspected to have an adverse effect on health (Annex III of Regulation (EC) No 1925/2006) ((EC), 2015a). All the non-harmonized ingredients are under national legislation and manufacturers must follow the rules established by the regulatory agencies for each country. On the contrary, botanical dietary supplements are classified as “Medicines” and are regulated under the EU medicinal law Directive 2004/24/EC (European Commission 2004) if therapeutic claims are made. Safety, effectiveness and premarket authorization of these products are regulated by the European Medicines Agency (EMA) [2, 6, 8].

The absence worldwide of a common legislation on dietary supplements causes lack of adequate information on the adverse effects of these products. Moreover, several reports on adulteration and contamination with unsafe substitutes, toxic metals, pharmaceuticals, pesticides, and pathogenic organisms are known. Lastly, supplements are often taken at the same time as other drugs and oftentimes there

is no information on the safety of such combinations. Consequently, several people have experienced serious adverse reactions associated with some supplements or due to the interaction of supplements with prescription and over-the-counter drugs [4, 9].

In view of the foregoing, it is difficult to solve the challenge of the borderline between medicinal products and food supplements, owing to the lack of specific legislation so far. For these reasons, an effective collaboration between regulators is necessary since national decisions may have global consequences. Therefore, it is of utmost importance to evaluate efficient and sensitive analytical methods to guarantee quality, efficacy and safety of dietary supplements, taking into account legal requirements to maintain the associated claims [7, 8, 10, 11]. To realize these requirements, conventional chromatographic techniques including High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) are the analytical methods commonly used. Nowadays, Capillary Electrophoresis (CE) is largely promoted as a valid and suitable alternative to conventional chromatographic techniques for the determination of compounds belonging to several application fields [12 - 15].

CE has emerged as a highly efficient, versatile and selective separation technique allowing fast and cost-effective analyses (simple fused-silica capillaries *vs*. LC columns), requiring only small amounts of sample and reagents [16, 17]. One of the main peculiarities of CE is the easy instrumentation needed. In addition, this analytical tool allows operating in a number of separation modes including among others, Capillary Zone Electrophoresis (CZE), Micellar Electrokinetic Capillary Chromatography (MEKC), Capillary Isotachophoresis (ITP) and Capillary Electrochromatography (CEC). This chapter aims at showing the capabilities of such methodologies in determining the dietary supplements' key components in order to assure their quality and safety.

CZE is the most widely used CE mode for the determination of the key ingredients of dietary supplements. The discrimination of the charged analytes is based on their different migration mobilities within fused-silica capillaries of narrow diameter (20 to 200 μm), filled with a background electrolyte (BGE), under the influence of a high electric field strength generated by applying a high voltage between the ends of the capillary. High separation efficiency and great resolution, rapid analyses and minimal consumption of reagents have made CZE a powerful tool and a valid alternative to liquid chromatography for the analysis of dietary supplements.

MEKC, on the other hand, expands the applicability of CZE to neutral molecules by combining the principles of electrophoresis and chromatography. MEKC

CHAPTER 14

An Overview of Food Metabolomics: CE-MS Based Targeted and Non-targeted Analysis

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Abstract: The safety and quality of food products, which is one of the most significant issues today, is a cause of increasing concern for consumers. For this reason, food policies are tightly determined by the governments with new conditions and regulations. Systematic monitoring of chemical pollutants such as pesticides, toxins, environmental and industrial contaminants, and residues in food products is critical in protecting public health. Capillary Electrophoresis with mass spectrometry (CE-MS) is frequently used in food analysis to ensure food safety and food quality. In this review, an updated overview of the targeted analysis of residues, contaminants, exogenous toxic ingredients, endogenous toxic ingredients, bioactive components, carbohydrates, amino acids, peptides and proteins in different food matrices through CE-MS is presented. In addition, the advantages of CE-MS based non-targeted analysis and its effectiveness in the field of food safety and quality are discussed in the light of recent studies. From a future perspective, the role of CE-MS based food metabolomics in food science is discussed together with recent developments on metabolomics applications.

Keywords: CE-MS, Food, Food safety, Food quality, Food metabolomics, Foodomics, Targeted analysis, Non-targeted analysis.

INTRODUCTION

Food safety and the need to control foods have become more important today than ever before. As a result of the incredible developments in the food industry and food trade today, new techniques are emerging every day in the production of foodstuffs, new product formulas are being designed and the product range is growing rapidly. Therefore, the importance of food safety services is obviously increasing.

Food products offered to consumers must be of high quality and produced in a safe manner with no harm to human health.

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This is why new legal arrangements are made in this regard, new audit mechanisms are established and proper standards are published. However, the implementation of these measures largely depends on the effectiveness of laboratory studies [1, 2].

In laboratories providing food analysis services, the points to be considered in sampling, preparation of samples for analysis and sampling for each food product to be analyzed are applied with great care. In addition, data obtained by analytical methods are evaluated and reported based on relevant standards. These laboratories are accredited through national and international accreditation organizations and they operate in accordance with the generally accepted test methods and test criteria valid globally. The purpose of all these analysis studies is ultimately to ensure food safety and quality assurance. Therefore, laboratories must keep up with developing technology, have experienced and educated staff and have up-to-date knowledge. Food analysis is the whole of detailed studies for the healthy consumption of food products. The content of this definition is very wide [3, 4]. Besides food safety, consumers have become more conscious about healthy food consumption especially since the 90's [5]. One of the biggest factors leading people to consume healthy foods may be the increasing number in cancer cases [6].

Cancer is one of the deadliest diseases at present, threatening today's people both socially and economically. As in the last century, it is one of the most important diseases that humanity will seek solution in the next century [7]. Cancer research continues to include studies to identify various molecular targets that are thought to be related to cancer prevention and treatment. However, the inability to reach the desired point with mono-therapeutic approaches with the use of a single drug leads researchers to apply combined therapy methods or to develop approaches that affect different metabolic pathways at the same time [8]. Another approach to fight cancer is to prevent it through small but effective lifestyle changes including a healthy diet and the use of food supplements [6].

Studies to establish a link between diet and the risk of developing cancer first started in the 80’s and increased rapidly in the following years [9 - 12]. Cancer-related epidemiological data have been collected for the past 50 years, and the fact that some types of cancer are more common or less common in some cultures is evidenced [13 - 19]. For example, while lung, colon, prostate and breast cancer are common in western societies, they are less common in eastern societies [20 - 22]. Similarly, head and neck cancer are most common in India, while stomach cancer is most common in Japan [23]. Although it is possible to explain the data revealed by epidemiological research with genetic factors, when the post-migration cancer statistics of populations from the same ethnic group were

examined, it was observed that in most cases similar results were obtained to those of the statistics of the geographic location they migrated from. Therefore, environmental factors and diet significantly increase the risk of developing cancer. For example, Ziegler *et al.* evaluated the risk of breast cancer for women migrating from China, Japan and Philippines to the United States [24]. The data obtained as a result of the study shows that; 4 to 7 times more cases of breast cancer detected in American women were almost identical to those of the Asian women settled in America as a result of migration. McMichael *et al.* evaluated the statistics of catching stomach, pancreas, colon and rectum cancer due to the change in the feeding styles of the communities that migrated from Europe to Australia. While the risk of continental European populations getting colon cancer is half of the Australian population, it was observed that after living in Australia for some time, this risk was equivalent to that of the Australian population [25]. It is possible to replicate similar examples [26 - 28]. Vecchia *et al.* show that some compounds in vegetables and fruits in the Mediterranean-style diet that are called 'micronutrients' reduce the risk of cancer [29]. In such a situation, a global evaluation must be concerned with evaluating the anticancer or cancer prevention properties of foods. Another issue discussed at this point includes the effect of consuming organic foods on increasing life quality and protecting against diseases [30]. Therefore, the content of foods caused by organic farming must be evaluated at a metabolome level to reveal the mechanisms of action on improving health.

In the light of this brief discussion above, the importance of food analysis could be discussed now in a better perspective than ever before since analysis of foods is a critical issue on both food safety and food quality which could be identified by determining the harmful and/or 'beneficial to health-life hacking' components in foods and food products.

Capillary Electrophoresis (CE) is an analytical technique, which theory was already known for years, but the fully-automated device was first introduced by Beckman Coulter (then Beckman Instruments) in 1989. Two different forces would change the migration of an analyte in CE. These are electroosmotic flow (EOF) and electrophoretic mobility. CE separates ions based on their electrophoretic mobility with the use of an applied voltage. The electrophoretic mobility depends on the charge of the molecule, the viscosity, and the atom's radius. Since neutral species do not have any charge, they have no electrophoretic mobilities and only ions move with the electric field. Ions with greater charge will move faster than the others. The other cause of ions separation in CE is their size. The smaller particle has less friction and an overall faster migration rate [31, 32]. This situation makes CE a powerful technique in comparison to High-Performance Liquid Chromatography (HPLC) where the polar-ionic compounds are hard to be separated and analyzed in reverse-phase mode and their separation

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