Journal of Chromatography A, 1428 (2016) 97–114

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review

Capillary electrophoresis determination of non-protein amino acids as quality markers in foods

Raquel Pérez-Míguez, María Luisa Marina, María Castro-Puyana*

Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, Faculty of Biology, Environmental Sciences and Chemistry, University of Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain

ARTICLE INFO

Article history:
Received 23 April 2015
Received in revised form 19 June 2015
Accepted 20 July 2015
Available online 22 July 2015

Keywords:
Capillary electrophoresis
Non-protein amino acids
Foods
Quality markers
Microchip Electrophoresis

ABSTRACT

Non-protein amino acids mainly exist in food as products formed during food processing, as metabolic intermediates or as additives to increase nutritional and functional properties of food. This fact makes their analysis and determination an attractive field in food science since they can give interesting information on the quality and safety of foods. This article presents a comprehensive review devoted to describe the latest advances in the development of (achiral and chiral) analytical methodologies by capillary electrophoresis and microchip capillary electrophoresis for the analysis of non-protein amino acids in a variety of food samples. Most relevant information related to sample treatment, experimental separation and detection conditions, preconcentration strategies and limits of detection will be provided.

Contents

* Corresponding author. Tel.: +34 918856430; fax: +34 918854971.
E-mail address: maria.castrop@uah.es (M. Castro-Puyana).

http://dx.doi.org/10.1016/j.chroma.2015.07.078
0021-9673/

1. Introduction

The determination of protein amino acids has been exploited for years in the food field because they can provide relevant information on the quality and safety of food samples [1–3]. In addition to these compounds, it is also possible to find other amino acids of non-protein origin in foods which exist as metabolic intermediates, as products formed during food processing or as additives in food to increase some nutritional and functional properties [4,5]. Non-protein amino acids have been defined as those amino acids that are not found in protein main chain either for lack of a specific transfer RNA and codon triplet or because they do not arise from protein amino acids by post-translational modification [6]. Even though non-protein amino acids have been studied to a lesser extent, they have also shown to play an important role in the quality and safety of foods. Thus, different research works have demonstrated the relevance of determining non-protein amino acids to detect food adulterations, to investigate fermentation, storage and thermal treatments, to evaluate the nutritional quality of foods or to determine their toxic effects [7–12].

Non-protein amino acids can be chiral having one or more chiral centers providing, therefore, at least one pair of enantiomers. Obviously, each enantiomer can originate different effects when interacting with chiral environments as enzymes, proteins and receptors [3]. Although the L-enantiomers are the natural form of amino acids, their exposure to certain processing conditions may originate their racemization to D-enantiomers. Processing-induced amino acid racemization includes from heat treatments, fermentation or storage to microbiological processes [13,14]. Moreover, D-enantiomers can be found in foods as a consequence of the fraudulent addition of racemic mixtures of non-protein amino acids in supplemented foodstuffs where regulations establish the use of pure L-enantiomers. The use of racemic mixtures originates economical benefits due to the minor cost that these mixtures have with respect to the use of pure enantiomers. In general terms, enantioselective separations may be relevant in different food areas in order to propose quality markers to assess food authenticity and to detect adulterations, to evaluate the effects of processing, fermentation, microbiological activity and storage, to analyze chiral metabolites and to investigate health-promoting compounds or evaluate flavor and fragrance aromas [2,3,15,16]. In the specific case of chiral non-protein amino acids, the analysis of their enantiomers in foods is a very useful tool not only to assess food quality and authenticity [17], but also to evaluate processing, manufacturing of food supplements and detect microbiological contaminations [12,18].

Undoubtedly, these research activities are of great interest in the area of Food Analysis due to the increasing concern of consumers about the quality of food. Therefore, taking into account that assuring product food quality, authenticity, and safety is the main demand in the food field, there is an increasing need of analytical methodologies enabling the determination of non-protein amino acids in foodstuffs. Among the advanced analytical techniques that can be used to solve some of the challenges that Food Science has to face, Capillary Electrophoresis (CE) has already demonstrated its high potential for the (achiral and chiral) determination of many compounds in foods, including amino acids, to ensure compliance with food and trade laws [1,16,19,20]. Among the CE modes used to determine non-protein amino acids in food samples, CZE (based on the free mobility of analytes in the aqueous solution under an applied electric field) and MEKC (based on the combination of electrokinetic migration and the partitioning mechanism between the bulk solution and micelles) are the most employed, whereas EKC and CEC (based on the interaction of each enantiomer with a chiral selector present in the mobile phase or with a chiral stationary phase, respectively) are the most employed CE modes to achieve an enantiomeric separation.

The present article reviews the most recent advances in the analysis of non-protein amino acids in foods using capillary electrophoretic methodologies (CZE, MEKC, EKC, and CEC) and microchip electrophoresis under achiral as well as chiral conditions covering the research articles published during the period February 2007 to February 2015, following the previous article published by our research group on this topic [1]. To make easier the discussion of the literature data and demonstrate the usefulness of CE to face different challenges related to food quality, this review has been divided in two different sections focused on the achiral and chiral determination of non-protein amino acids in foods. These sections gather the non-protein amino acids according to their structure and describe the different CE approaches (including experimental conditions, preconcentration strategies and sample treatments) developed to analyze these compounds in a great variety of food samples.

2. Determination of non-protein amino acids in foods by CE under achiral conditions

With the aim of providing an updated view on the achiral CE methodologies developed for the analysis of non-protein amino acids in the period of time reviewed in this article, Table 1 summarizes the main characteristics of the developed methodologies. It can be observed that, as expected, most of the research articles published employed CZE as separation mode, although the use of other modes such as electrokinetic chromatography using micelles as pseudostationary phase (MEKC mode), CEC and microchip capillary electrophoresis (MCE) has also been reported. With respect to the detection systems, UV and fluorescence are the most popular despite the need to include an additional analytical step (derivatization) to add a chromophore or fluorophore group into the molecule. *o*-phthaldialdehyde (OPA), fluorescein isothiocyanate (FITC), 4-chloro-7-nitrobenzofurazan (NDB-Cl) or 9-fluoroenylmethylchloroformate (FMOC), among others, have mainly been used as reagents for the derivatization of non-protein amino acids. To a lesser extent, mass spectrometry (MS), inductively coupled plasma-MS (ICP-MS), electrochemical and capacitively coupled contactless conductivity (C^4D) have also been reported as detection systems coupled to CE for determining non-protein amino acids. It should be highlighted that a broad range of food samples, from beverages (such as tea, soy based beverages, cow milk, energy drinks, etc.), to flour products, shellfish, vegetables (tomato, *Brassica* or *Allium* species, etc.), rice, meat products, or vegetable and olive oils have been analyzed by the developed CE approaches as shown in Table 1. The achiral determination of non-protein amino acids in these food matrices has been carried out mainly with quality control purposes. Thus, research works focused on the detection of adulterations, the study of the effects of fermentation, storage or thermal treatments, or the evaluation of the nutritional quality of different foods samples have enabled to point out these compounds as potential markers of food quality.

A more detailed description of the different achiral CE methodologies developed for the determination of non-protein amino acids and their applications in the area of Food Analysis will be provided next.

2.1. Aliphatic monoamino-monocarboxyl amino acids

γ-Aminobutyric acid (GABA) is a non-protein amino acid whose structure contains one carboxylic group and one primary amino group attached to the gamma carbon atom. It is distributed throughout the nervous system and it is the main inhibitory