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Review

Advanced separation methods of food anthocyanins, isoflavones and flavanols

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ABSTRACT

In recent years, increasing knowledge of the positive health effects of food polyphenols has prompted the need to develop new separation techniques for their extraction, fractionation and analysis. This article provides an updated and exhaustive review of the application of counter-current chromatography, high performance liquid chromatography, capillary electrophoresis, and their hyphenation with mass spectrometry to the study of food polyphenols. Flavonoids constitute the largest class of polyphenols, widely spread in the plant kingdom and common in human diet which has been the most widely studied with respect to their antioxidant and biological activities. The main subgroups are anthocyanins, catechins, isoflavones, flavonols and flavones. They are reported to exhibit antioxidant, anti-carcinogenic, anti-inflammatory, anti-atherogenic, anti-thrombotic, and immune modulating functions, among others. Since red fruit anthocyanins, soy isoflavones and flavanols from grapes and teas are currently the most used phenolic compounds for producing new nutraceuticals and functional foods, this review is focused on these three flavonoid groups.

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Abbreviations: APCI, atmospheric-pressure chemical ionization; C, catechin; CCC, counter-current chromatography; CD, cyclodextrin; CE, capillary electrophoresis; CEC, capillary electrochromatography; CGE, capillary gel electrophoresis; CIEF, capillary isoelectric focusing; CITP, capillary isotachophoresis; CMC, critical micellar concentration; CPC, centrifugal partition chromatography; Cy, cyanidin; CZE, capillary zone electrophoresis; DAD, diode array detector; DCCC, droplet counter-current chromatography; DP, degree of polymerization; Dp, delphinidin; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; ELISA, enzyme-linked immunoabsorbent assay; EOF, electroosmotic flow; ESI, electrospray ionization; HILIC, hydrophilic interaction chromatography; HPLC, high pressure liquid chromatography; HSCCC, high-speed counter-current chromatography; ICP, induced coupled plasma; IL, ionic liquids; ITMS, ion-trap mass spectrometers; ITP, isotachophoresis; LOD, limit of detection; LOQ, limit of quantification; MALDI, matrix-assisted laser desorption ionization; MEKC, micellar electrokinetic chromatography; MLCCC, multilayer coil counter-current chromatography; MRM, multiple reaction ion monitoring; MS, mass spectrometry; Mv, malvidin; NMR, nuclear magnetic resonance; PA, proanthocyanidin; Pg, pelargonidin; Pt, petunidin; QqQ, triple-quadrupole mass spectrometer; RP, reversed phase; SDS, sodium dodecyl sulphate; SIM, selected ion monitoring; SRM, selected reaction monitoring; TLC, thin layer chromatography; TOF, time-of-flight mass spectrometer; UPLC, ultra-high pressure liquid chromatograph; UV, ultraviolet.

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1. Introduction

Polyphenols are found ubiquitously in a variety of fruits, vegetables, nuts, seeds, flowers, bark, beverages and manufactured foods, as a component of the natural ingredients used. Cocoa, apples, tea, berries, coffee, wine, jams, chocolates, or onion are common sources for polyphenols in human diets [1].

Although traditionally their interest has mainly been related to their organoleptic properties, such as colour [2] (anthocyanins or curcumin, for example), astringency [3] (tannins), bitterness (flavanols) or taste [4], in recent decades they are increasingly being recognised for their nutritional value, since they may help reduce the risk of chronic disease and, in general, have a positive effect on health [5,6]. They have been reported to have anti-carcinogenic [7], anti-atherogenic [8], anti-ulcer [9], anti-thrombotic [10], anti-inflammatory [11], anti-allergenic, immune modulating, anti-aggregative [12], anti-microbial [13], vasodilatory [14], and estrogenic [15] effects. They can accomplish these roles as antioxidants, chelators of divalent cations, or as modulators or inhibitors of the activity of such enzymes as topoisomerases, protein kinases, or cyclo-oxygenase [9,16,17].

These recently discovered properties of phenolic compounds have been exploited for cosmetics, medicines, pharmaceuticals, nutritional supplements or functional foods. The food industry has launched numerous new functional products, the health functionality of which is closely connected with their polyphenols content, which is usually higher than the content of the traditional products. Milks enriched with soy isoflavones, chocolates enriched with procyanidins, beverages with higher amounts of anthocyanins, functional drinks enriched with tea extracts, and many others are all part of the functional foods revolution [18]. On the other hand, the use of synthetic antioxidants in the food industry is severely restricted as to both application and level. This is the reason why more attention is now being paid to natural antioxidants extracts from plants.

All these healthy properties are strongly dependent on the polyphenols chemical structure [6]. Because the number of phytochemicals already identified is only a small part of those that exist in nature, there is a considerable interest in new methods of separation, isolation and characterization of polyphenol structures from foods.

For the purpose of this review, we aim to give a detailed description of three advanced separation techniques that are currently applied for food analysis and new food polyphenols identification.

(a) Counter-current chromatography is a technique that allows the fractionation and isolation of pure compounds, to yield the large amounts required for identification by MS and NMR methodologies, or for a further utilization as standards in analytical methods or as bioactive compounds for biological studies.
(b) High pressure liquid chromatography is the classic separation technique for analyzing polyphenols. Its hyphenation with mass detectors is being crucial for detecting and identifying minor and unknown polyphenols in complex food samples.
(c) Capillary electrophoresis is an interesting alternative to HPLC, its main advantage being that it takes less time and uses smaller quantities of solvent. Recent advances in hyphenation with mass detectors make this technique a promising field of application.

2. Polyphenol structures

The identification of polyphenols has blossomed during the last decades [19–21]. The development of new isolation, separation and identification techniques has made it possible to constantly increase the database of phenolic compounds with new structures, and to provide a better understanding of the mechanisms that govern their effects. The more recent advances have been related with the identification and quantifications of polyphenols in food complex matrices and in biological fluids and tissues [22–25].

However, several difficulties arise, because the term "polyphenols" includes a lot of different families with widely differing structures and properties, and every year a large number of new polyphenol structures are identified. This means that no universal method can be used with all the phenolic compounds: different approaches must be used depending on the specific foods and polyphenols of interest.

Table 1 shows the most important families of polyphenols in foods, classified by their skeleton structure [26]. Flavones, flavonols, flavanones, flavanols, isoflavonoids and anthocyanins are known as flavonoid compounds and all of them share the same basic structure. Fig. 1 shows this basic structure and the numbering system of

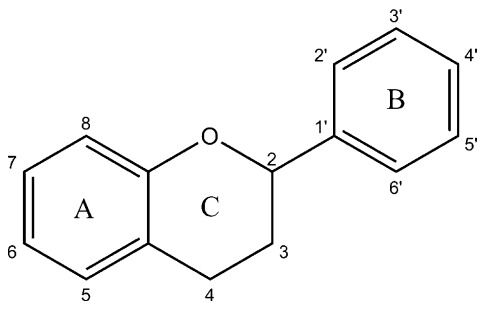

Fig. 1. Basic structure and numbering system of flavonoids.