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Electrochemical Tools for Determination of Phenolic Compounds in Plants. A Review

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Electrochemical methods are a reliable tool for a fast and low cost assay of phenolic compounds (phenolics) in food samples. The methods are precise and sesnitive enough to assay low content of polyphenols. The devices can be stationary or flow through, and based on voltammetry or amperometry. The application of voltammetric methods and HPLC-ED methods for analysis of phenolics in food samples is described in this review. The advantages and disadvantages of the methods are discussed.

Keywords: Phenolic Compounds; Voltammetry; Amperometry; Coulometry; High Performance Liquid Chromatography

Abbreviations: AdSV – Adsorptive Stripping Voltammetry; CV – Cyclic Voltammetry; DAD – Diode Array Detection; DPV – Differential Pulse Voltammetry; DPPH – Diphenyl-1-picrylhydrazyl;

ED –Electrochemical Detection; FC – Folin-Ciocalteu; GCE - glassy carbon electrode; HPLC – High Performance Liquid Chromatography; MS – Mass Spectrometry; NMR – Nuclear Magnetic Resonance; LC – Liquid Chromatography, LOD – Limit of Detection; LOQ – Limit of Quantification; PbFE – Lead Film Electrode; PDA – Photodiode Array; RSD – Relative Standard Deviation; SWV – Square Wave Voltammetry; SCE – Saturated Calomel Electrode; TEAC – Trolox Equivalent Antioxidant Capacity; TP – Total Phenolics; UV – Ultra Violet; VIS – Visible

1. INTRODUCTION

The aim of the review is to summarize the basic facts about suitability of electrochemical techniques and their combination with HPLC for assay of phenolics in foods. HPLC techniques with electrochemical detection systems have an important place in the field of phenolic compounds analysis because they are sensitive, selective and reproducible in the case of quantifying phenolic compounds [1,2]. A special chapter is devoted to analysis of polyphenols in apricots using HPLC with electrochemical or spectrometric detection.

Phenolic compounds are compounds that have one or more hydroxyl groups on the aromatic skeleton. Polyphenols represent group of compounds that have more than one phenolic hydroxyl group attached to one or more benzene ring. They occur in both free and bound form as esters or glycosides in plants [3,4]. There are different approaches in their classification. They may be grouped to the class of common, widely distributed phenols, class of less common that have limited distribution in plants and class of phenolics that are present as polymers. Classification of Harborne and Simmonds is based on the number of carbons in the molecule. The simplest are phenolics (C_6) and their derivatives such as phenolic acids and related compounds (C6-C1), acetophenones and phenylacetic acids (C6-C2), derivatives of cinnamic acid (C6-C3), coumarins, isocoumarins and chromones (C6-C3) [5], chalcones, dihydrochalcones, aurones, flavans, flavones, flavanones, flavanonols, leucoanthocyanidins, anthocyanidins and anthocyanins (C15, respectively C6-C3-C6 also generally called “flavonoids”), biflavonyls (C30), benzophenones, xanthones and stilbenes (C6-C1-C6, resp. C6-C2-C6), quinones (C6, C10, C14), lignans, neolignans and tannins (dimers or oligomers, e.g. proanthocyanidinis, dimers or oligomers of flavan-3-ol) and polymeric compounds such as lignin and phlobaphenes. Due to their role in plants they commonly occur in the food of plant origin and represent inseparable part of food [6,7]. The examples of individual classes of phenolic compounds and their presence in foods are introduced in Table 1.

These compounds are important for the food industry and food processing due their ability to improve flavour and colour of food. Phenolic compounds as antioxidants are also recommended as dietary supplements to improve human health [8-11]. Many epidemiological studies suggest that regular consumption of fruits and vegetables rich in polyphenols may reduce the risk of cancer and cardiovascular diseases [12-16]. The beneficial effects of polyphenols may be based on several factors [12], however, quenching free radicals and inhibition of lipid peroxidation (antioxidant properties) are probably the most important biological activities of phenolic compounds contributing to the chemoprevention (chemoprotection) of human health [17-20], because phenolic compounds carry a major part of the antioxidant activity in a majority of plant products [21-25].

Table 1. Main classes of phenolic compounds and their presence in foods adapted from Valls et al. [26] and modified.

Class	**Basic skeleton**	**Examples**	**Presence in food**	**References**
Phenolic acids		gallic acid protocatechuic acid vanillic acid	grapes wine tea	[27-29]
Cinnamic acids		caffeic acid ferulic acid sinapic acid *p*-coumaric acid	coffee tea rice wine (sake)	[30-32]
Coumarins		coumarin aesculetin	bakery products cereals fruits spice (*Apiaceae*)	[33-36]
Chalcones		butein	bee products	[37,38]
Dihydrochalcones		phloridzin	apple juice and other apple products strawberries	[39-41]
Flavanones		naringenin eriodyctiol hesperetin hesperidin pinocembrin	citrus fruits (grapefruit) citrus-based foods honey and other bee products	[42-47]
Flavanonols		taxifolin aromadendrin	red onion herbal products	[48,49]

Leucoanthocyanidins		leucocyanidin leucodelphinidin leucomalvidin	cocoa herbal products	[50,51]
Flavones		apigenin luteolin chrysin	vegetable (parsley, celery) fruits spice	[52-54]
Flavan-3-ols		catechin epicatechin and their esters	cocoa chocolate tea fruits	[55-58]
Flavonols		kaempferol myricetin quercetin rhamnetin astragalin rutin	vegetables and fruits herbal products wines	[59-64]
Isoflavones		genistein dadzein biochanin A formononetin	*Fabaceae* products (e.g. soy foods)	[65-67]
Anthocyanidins		cyanidin delphinidin pelargonidin malvidin peonidin	fruits vegetables red wines	[68-71]
Deoxyanthocyanidins		apigeninidin luteolinidin	herbal products red wine	[72,73]
Anthocyanins	Water-soluble glycosides of anthocyanidins	cyanidin-3- glucoside delphinidin-3- glucoside	fruits vegetables red wines	[74-79]
Stilbenes		resveratrol pinosylvin viniferin piceid	wine fruits	[80-84]

2. ELECTROCHEMICAL TECHNIQUES IN FOOD ANALYSIS

Electrochemical techniques are being developed and improved for determination of phenolic compounds. These techniques are low-cost, sensitive and enable rapid analysis of sample [85]. They can be applied in analysis of samples without the necessity of modification. In addition, it is possible to determine and quantify phenolic compounds in highly complex biological matrices [86]. Analyses can be performed in a stationary system using techniques such as differential pulse voltammetry, cyclic voltammetry or biosensor applications based on enzyme catalysis. In addition to stationary systems, dynamic systems based on voltammetry or amperometry are commonly used in electrochemical analysing of phenolic compounds [26,87-96]. Electroanalytical techniques are advantageous in the analysis of phenolic compounds. Antioxidant properties of phenolic compounds are related to their ability to donate electrons. Therefore, the detection of polyphenols based on electrochemical oxidation is performed at low potentials [86,97,98].

2.1. Voltammetric techniques

Voltammetric techniques are the most widely used stationary methods used in the analysis of phenolic compounds in food [26,86,99,100]. They are briefly summarized in Table 2.

2.1.1. Cyclic voltammetry

Cyclic voltammetry (CV) on carbon electrode appears to be a suitable tool in antioxidant assays due to its simplicity, fastness, and no need to pre-treat samples [101]. Unfortunately, CV does not allow identifying antioxidants present in sample. The technique provide sum of total antioxidants in the sample and, in this way, a simpler interpretation of experimental data. Compounds that are oxidised more easily appear to be in higher amount as antioxidant capacity is more extensive [102]. Assay of charge going through CV device is another approach in measurement of antioxidants by this method. The experimental data can be related to a standard that is chemically close to major antioxidant present in the sample [103].

Kilmartin et al. [104] used CV to quantify antioxidants on carbon electrode in diluted white wine samples (10%) and red wine samples (0.25%) in a model solution containing 12 % ethanol (*v*/*v*) and 0.033 M tartaric acid adjusted to pH 3.6. In samples of red and white wines, first peak close to anode potential of 400 mV (against Ag/AgCl) was caused by phenolic compounds with *o*-diphenolic group or gallic acid, while small peak observed at 300 mV appeared in the group of red wines containing higher amount of myricetin (flavonoid containing three phenolic groups on B circle, commonly present in food, chemically 3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-4-chromenone). Peak at 470 mV was ascribed to quercetin glycosides. The main difference between red and white wines consisted in the presence of a peak at 640 mV in the voltammograms of red wine samples, which was associated with malvidin anthocyanidins and its amount was significantly lowered in samples of older red wines [104].

Table 2. Overview of voltammetric methods for the determination of phenolic compounds in foods, beverages and plants**.**

Electrochemical technique	**Type of electrode**	**Assayed analyte**	**Type of sample**	**Ref.**
Cyclic voltammetry (CV)	glassy carbon electrode	myricetin, quercetin , epicatechin, catechin, gallic acid , caffeic acid , caftaric acid and rutin	red and white wines, standards of phenolic compounds	[104]
		catechin, syringic acid, vanillic acid, gallic acid, coumaric acid, ferulic acid, rutin and quercetin	red and white wines, standards of phenolic compounds	[105]
		quercetin, gallic acid, protocatechuic acid, *o*-coumaric acid, *m*-coumaric acid, *p*-coumaric acid, caffeic acid, vanillic acid, ferulic acid	standards	[106]
		caffeic and chlorogenic acid	orange juice	[107]
		pyrogallol, catechol, epigallocatechin gallate	green tea	[108]
Differential pulse voltammetry (DPV)	glassy carbon electrode	polyphenols, phenolic acids, myricetins, malvidin anthocyanins	red wines	[109]
	glassy carbon electrode	phenolic acids and flavonoids	apple and pears peels and pulps & commercial juices	[110]
	glassy carbon electrode modified with green apple	total phenolic content	white and red wines	[111]
Square Wave Voltammetry (SWV)	carbon –polyurethane composite electrode	rutin	green tea infusion samples	[112]
	glassy-carbon electrode	(-)-epigallocatechin gallate	green tea	[113]
Adsorptive stripping voltammetry (AdSV)	boron-doped diamond electrode	chlorogenic acid	coffee products	[114]
	glassy-carbon electrode with lead film	caffeic acid	plant *Plantago lanceolata*	[115]
Linear sweep voltametry (LSV)	platinum rotating disc electrode	caffeic acid	plant *Arnica montana*	[1]

The study of Makhotkina et al. was also focused on the determination of phenolic compounds in the samples of red and white wines using CV [105]. Two main peaks were determined in samples of white wines. The first peak at 480 mV corresponded to derivatives of catechol-containing hydroxycinnamic acid, the most common phenolic compounds present in a majority of white wines. The second one was observed at the potential range from 900 to 1000 mV and probably corresponded to coumaric acid and its derivatives. On the other hand, analysis of red wines samples revealed the presence of three anodic peaks. The first peak appeared at potential of 440 mV and corresponded to oxidation of catechin-type of flavonoids. The second one appeared at 680 mV and corresponded to malvidin anthocyanidins commonly present in red wine and the third anodic at 890 mV was attributed to second oxidation of catechin-type of flavonoids [105]. The electrochemical behaviors of 10 structurally different flavonoids (quercetin, galangin, chrysin, 3-hydroxyflavone, naringenin, luteolin,

apigenin, flavone, kaempferol, and naringin) on a glassy carbon electrode were studied by cyclic voltammetry. Nitrophenyl diazonium salt was synthesized from p-nitrophenylamine. One millimolar prepared nitrophenyl diazonium salt (in 100 mM tetrabutylammonium tetrafluoroborate) in acetonitrile was used to modify the glassy carbon electrode. Nitro groups were reduced to amine groups in 100 mM HCl medium on the nitrophenyl-modified glassy carbon electrode surface. Although nitrophenyl-modified glassy carbon electrode surface was electro-inactive, it is activated by reducing the nitro group into amine group. And then, aminophenyl-modified glassy carbon electrode surface has been used for the determination of antioxidant activities of 10 flavonoid derivatives with cyclic voltammetry technique. The activity sequence of the investigated, structurally different, flavonoids follows the sequence: quercetin > galangin > chrysin > 3-hydroxyflavone > naringenin > luteolin > apigenin > flavone > kaempferol > naringin [116].

Moreover, CV of caffeine showed only one oxidation peak at 1.46 V (vs. saturated calomel electrode (SCE)) in phosphate buffer pH 7. Chemical calculations were performed using PM3 method to prove the electrochemical oxidation mechanism of caffeine studied by CV [117,118]. Phenolic acids and flavonoids were characterized by cyclic voltammetry also by Yakovleva et al. [106]. Anode peak voltages (Eap) and their pH dependences were determined for the studied phenolic acids. Correlation between the half-wave potential (E1/2) and TEAC was determined for electrochemically irreversible compounds. Mechanisms of the reaction of phenolics on the electrode involving one-and two-electron oxidation were proposed [106]. Sousa et al. [107] monitored electrochemical oxidation of caffeic, chlorogenic, sinapic, ferulic, and *p*-coumaric acids in acetate buffer (pH 5.6) on modified glassy carbon electrode. Results were compared with DPPH assay and corresponded to results obtained by this assay. Linear course was determined within the concentration range from 1×10^{-4} to 1×10^{-3} M. Method was also applied on the biological matrix as orange juice. It provided anodic peak at the potential of 0.38 V. The peak corresponded to caffeic and chlorogenic acids and increased with addition of these acids into sample. Selectivity was illustrated by the analysis of caffeic and chlorogenic acids electrodeposited on a glassy carbon electrode previously modified by electrochemical activation in the presence of ascorbic acid [107]. Roginsky et al. developed CV at an inert carbon electrode, a new method to provide a rapid measure of easily oxidizable polyphenols in beverages as different kinds of tea [108]. CV measured the antioxidant activity, which was correlated well only for the green teas, where the phenolics content is dominated by epigallocatechin gallate. Phenolic antioxidants were ranked by reducing strength and characterized for reversibility using cyclic voltammetry at a glassy carbon electrode.

Linear sweep voltammetry

Reyes-Salas et al. [1] quantified caffeic acid in tinctures of medicinal plant *Arnica montana* L. by linear sweep voltammetry with a platinum rotating disc electrode. The tinctures showed a number of electrochemical signals: two of them corresponded to caffeic acid oxidation. One of these signals was found to be controlled by a diffusion process and was used to quantify caffeic acid in the tinctures. The detection limit of caffeic acid was established to be 385 μg L^{-1}, and its concentration in tincture ranged from 161 to 265 mg/L.

2.1.2. Differential pulse voltammetry

Seruga et al. [109] analysed samples of red wines originating from three different wine regions. Amount of total phenolic compounds was measured using differential pulse voltammetry (DPV), Folin-Ciocalteu (FC), and HPLC. Application of these three principally different methods revealed suitability of DPV to determine amount of all phenolic compounds present in sample. In addition, DPV method showed high sensitivity. Differential pulse voltammograms of catechin, which was used as a standard, showed two anodic oxidation peaks. The first peak was observed at 0.440-0.475 V and moved to more positive values with the increasing amount of catechin. The second oxidation signal of catechin was observed at 0.750 V. Authors expressed the amount of total phenolic compounds in samples determined by the use of DPV in catechin equivalent. High correlation was established for amount of phenolic compounds determined using DPV, FC and HPLC methods.

Blasco et al. [119] analysed apple and pear fruits, and their juices. Authors used DPV method to determine content of flavonoids and phenolic acids. Caffeic acid and chlorogenic acid were used as cinnamics standards; (+)-catechin and (-)-epicatechin were used as flavan-3-ols, rutin and quercitrin as flavonols, arbutin as a quinone and phloridzin as a chalcone. Analyses were carried out at two different pH values (7.5 and 2.0). Two peaks were observed in apple and pear juice samples. The first peak corresponded to (+)-catechin at pH 7.5 and to (+)-catechin and cinnamics at pH 2.0. The second peak appeared at both pH values and corresponded to (+)-catechin and phloridzin in apple juice samples and to (+)-catechin and arbutin in pear juice samples. Bisetty et al. [111] investigated the changes in determination of total phenolics content in wine samples using catechin as a standard by differential pulse voltammetry (DPV). The modification of the glassy carbon electrode (GCE) was carried out using green apple as an enzymatic source of polyphenol oxidase. The method was optimized with respect to the current signal at a deposition potential of 0.2 V and within an oxidation potential of -0.2 V to 0.6 V. Good analytical responses were obtained with apple sensors for the detection of total phenolics in wine samples, with a higher concentration in red wines than in white wines.

2.1.3. Square wave voltammetry

Malagutti et al. [112] used rigid carbon-polyurethane composite electrode for determination of rutin by square wave voltammetry. Anodic oxidation peak of rutin was observed at potential about 400 mV. This electrochemical method was suggested and developed for determination of rutin in green tea. Detection limit was 7.1×10^{-9} M. Novak et al. [113] investigated electrochemical oxidation of (-)-epigallocatechin gallate, the main monomeric flavanol found in green tea, over a wide pH range at a glassy-carbon electrode using square-wave voltammetry (SWV). The best SWV responses for (-)-epigallocatechin gallate were obtained at pH 2.0, frequency of 100 Hz, step of 2 mV and amplitude of 50 mV. Under these conditions, linear responses for (-)-epigallocatechin gallate were obtained for concentrations from 1×10^{-7} M to 1×10^{-6} M, and calculated LOD and LOQ for the first oxidation peak were 6.59×10^{-8} M and 2.19×10^{-7}, respectively. The suggested electroanalytical procedure was applied for the determination of (-)-epigallocatechin gallate content in green tea. The concentrations of ellagic acid determined by this SWV method in the samples of strawberries, raspberries and blackberries were 5.52, 40.06 and 37.60 mg/100 g of fresh weight, respectively [120].

Square wave voltammetry was also used for the direct electrochemical determination of caffeine. The effect of different experimental parameters was investigated on the peak height of caffeine. The effect of ascorbic acid and paracetamol on the peak height of caffeine was studied. The suggested method has been successfully applied for the direct electrochemical determination of caffeine in different real samples such as tea, coffee, cola, and pharmaceutical formulations [117,118].

2.1.4 Adsorptive stripping voltammetry

Yardim et al. [114] established an electroanalytical methodology for determination of chlorogenic acid on a boron-doped diamond electrode using adsorptive transfer stripping voltammetry. The values obtained for chlorogenic acid were used to estimate the antioxidant properties of the coffee sample based on chlorogenic acid oxidation. By using square-wave stripping mode, the compound yielded a well-defined voltammetric response at +0.49 V with respect to Ag/AgCl in Britton-Robinson buffer at pH 3.0 (after 120 s accumulations at a fixed potential of 0.40 V). The developed protocol was successfully applied for the analysis of antioxidant capacity in the coffee products such as Turkish coffee and instant coffee. Similarly, Tyszczuk et al. [115] developed an adsorptive stripping voltammetric method for determination of caffeic acid on a lead film electrode (PbFE). The working electrode was prepared *in situ* on a glassy carbon substrate and the method was based on the accumulation of caffeic acid by adsorption on PbFE and then on its oxidation during the stripping step. In a acetate buffer based supporting medium, the oxidation signal for caffeic acid was found to be proportional to the caffeic acid concentration within the range from 1×10^{-8} to 5×10^{-7} M with the limit of detection equal to 4×10^{-9} M (with preconcentration for 30 s). The method operated in the square-wave voltammetric mode was successfully applied for determination of caffeic acid in a plant material (*Plantago lanceolata* L.).

2.2 High performance liquid chromatography

Detection (quantification) of phenolic compounds by the use of HPLC is presently one of the most frequently used approaches [86,121-129]. Advantage of HPLC application is based on the chemical properties of some phenolic compounds, such as relatively high molecular weight, hydrophobicity of aglycones and hydrophilicity of corresponding glycosides. It is possible to detect and determine very low amounts of analytes in the presence of more interfering or co-eluting components [26]. The principle of HPLC separation is based on the distribution of analytes between stationary and mobile phases. HPLC with reverse phase is the most used technique for separation of phenolic compounds [113]. In this case, stationary phase contains linked non-polar aliphatic residues (they are called "C8", "C18", etc. according to the length of the aliphatic chain) and mobile phase is made up of polar solvents (methanol, acetonitrile). Ultraviolet/visible (UV/VIS), photodiode array (PDA), and UV-fluorescent detectors are commonly used for detection of phenolic compounds [111,115,130]. All phenolic compounds contain at least one aromatic ring, so they absorb UV radiation. Therefore, UV/VIS detection is the most used detection technique. However, this method shows relatively low sensitivity. In addition, one wavelength enables to determine only limited spectrum of phenolic compounds [26]. On the other hand, PDA is the most widely used method

because it enables scanning of UV/VIS spectra of all dissolved compounds passing the detector in real time [129,131,132]. Application of fluorescent detectors in the detection of phenolic compounds is relatively unusual. They are used in the combination with UV detectors, which enable to differentiate individual phenolics on the base of their florescence [2]. Detectors based on mass spectrometry (MS) are used to elucidate the structure of phenolic compounds [133] and to characterize the type of flavonoids. Diode-array UV, tandem-MS and nuclear magnetic resonance detectors are used for their identification [26,134-138].

2.2.1 HPLC with electrochemical detection

Electrochemical detection is a highly sensitive method for the determination of compounds that are easily oxidized or reduced at low potentials [1,129,139-141]. These compounds are especially phenolic acids and flavonoids [114]. Electrochemical detection in HPLC can be divided into several classes. Potentiostatic methods operate at a constant potential of the working electrode (amperometry and potentiostatic coulometry) and amperostatic methods (amperostatic voltammetry and coulometry) operate at a constant current. Coulometric detector, which measures the electrical charge required to oxidise or reduce the total amount of the compound during its pass a cell of detector, is predominantly used for the determination of phenolic compounds [26]. This method is mainly used in the analysis of flavonoids [142] including isoflavones with limits of detection ranging from 300 µg/g to 1600 µg/g [143]. Coulometric detection can be improved in a multichannel system that is compatible with gradient elution and enables highly sensitive detection and characterization of phenolic compounds that differ in electrochemical properties [93,144]. Coulometric detection can provide complete voltammetric differentiation of analytes according to their reaction potential. Another advantage is the larger working surface of a cell, which is made of porous graphite, and the analyte is subjected to more than 90% of the oxidation-reduction reaction compared to a conventional planar working electrode [145]. Amperometric detection represents next electrochemical detection method. The principle consists in the measurement of a current induced by passing of reduced or oxidized compound via flow cell of detector. Its effectiveness is given by the flow rate of mobile phase and the working surface of electrodes. However, it provides higher limits of detection compared to above-mentioned methods, so, this arrangement is only rarely used in determination of phenolic compounds [26].

Aaby et al. [150] used HPLC coupled with diode array and coulometric array detectors to characterize and quantify phenolic compounds in achenes and flesh of ripe strawberries. Fedina et al.[146] analysed the total content of antioxidants in herb extracts, tea, coffee, wine, brandy, balsams, beer, vegetables, fruit, and berries using HPLC with amperometric detection**.** In another article, Gazdík et al. [151] determined content of neuroprotective plant phenols (rutin, quercitrin, gallic acid and 4-aminobenzoic acid) in some less common fruit species - Blue Honeysuckles (*Lonicera caerulea* v. *kamtschatica* Sevast. and *Lonicera edulis*, Turcz. ex. Freyn), Saskatoon berry (*Amelanchier alnifolia* Nutt.) and Chinese Hawthorn (*Crataegus pinnatifida* Bunge using liquid chromatography with electrochemical detection (HPLC-ED). The multifunctional HPLC-ED array method coupled with a DPPH appeared to be the optimal analytical progress, accurately reflecting the nutritive-therapeutic properties of a fruit. Similarly, Jurikova et al. [152] investigated polyphenolic profile of a number of

non-traditional fruit species and their genotypes, namely blue honeysuckle (*Lonicera* spp.), Saskatoon berry (*Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem.), black mulberry (*Morus nigr*a L.), Tomentosa cherry (*Prunus tomentosa* Thunb.) and jostaberry (*Ribes nigrum* x *Grossularia uva-crispa*). The results showed that 'Tisnovsky' and 'Smoky' together with Morus nigra 'Jugoslavska' accumulated the highest level of examined polyphenolic compounds. The analysis of various types of samples is summarized in Table 3.

Table 3. Overview of HPLC methods with electrochemical detection used for determination of polyphenolic compounds in foods, beverages and plants.

Technique	Assayed analyte	Type of sample	Ref.
HPLC-amperometric detection	total polyphenols	tea, coffee, wine, brandy, beer products vegetables, fruit, and berries	[146]
	flavonoids	red grape skin	[147]
	flavonoids	onion	[148]
	hydroxycinnamic acids	wort and beer	[149]
HPLC-coulometric array detection	phenolic compounds	strawberries	[150]
	gallic acid, 4-aminobenzoic acid, rutin, quercitrin	less common fruit species	[151]
	gallic acid, rutin, resveratrol, catalposide, quercetin, quercitrin, chlorogenic acid	less common fruit species	[152]
	methyl 4-hydroxybenzoate, methyl vanillate, methyl syringate, *trans-p*-methyl coumarate and *trans*-methyl ferulate	honey	[153,154]
	flavonoids and other phenolic compounds	beers, red and white wines, lemon juice, soya, forsythia and tobacco extracts	[92]
	protocatechuic, vanillic acid	mead	[155]
HPLC-electrochemical detection	flavonoids and phenolic acids, total polyphenols	almonds	[156]
	trans-resveratrol, cis-resveratrol and quercetin	vegetables and fruits	[157]
	protocatechuic, 4-aminobenzoic, chlorogenic and caffeic acid, vanillin, *p*-coumaric acid, rutin, ferulic acid, quercetin, resveratrol and quercitrin	apricots	[158]
	ferulic, sinapic and caffeic acid, procyanidin and catechin	pearled barley	[131]
	gallic acid, protocatechuic acid, caffeic acid, *p*-coumaric acid, rutin and quercetin	jujube (*Sizyphus jujuba*), a traditional Chinese fruit	[132]
	caffeic acid, *p*-coumaric acid, ferulic acid and hesperetin	Chinese citrus honey	[139]
	polyphenols	wine	[159]
	phenolic compounds	maple products	[160]
	gallic, protocatechuic, syringic, *p*-coumaric, caffeic, chlorogenic and ferulic acid	wine	[161]
	flavones, flavonols, flavanones and anthocyanidins	vegetable and fruits consumed in Hawai	[162]
LC-coulometric detection	caftaric, chlorogenic, and cichoric acid, cynarin, echinacoside,	dietary supplements and tea blends containing Echinacea	[163]
LC-electrochemical	*trans*-resveratrol	wines, grape juice and grape seed	[164]

detection		capsules	
	phenolic acids	honey and green tea	[165,166]
HPLC-tandem electrochemical and spectrometric detection	gallic, 4-aminobenzoic, chlorogenic , ferulic , caffeic, salicylic and *p*-coumaric acid, quercetin, quercitrin, rutin, resveratrol, vanillin, epicatechin, (–)- and (+)-catechin.	apricots	[167]
	gallic acid, procatechinic acid, *p*-aminobenzoic acid, chlorogenic acid, caffeic acid, vanillin, *p*-coumaric acid, rutin, ferrulic acid and quercetrin	239 apricot cultivars	[168]

Milbury et al. [156] determined total phenols, flavonoids, and phenolic acids in California almonds (*Prunus dulcis* (Mill.) D. A. Webb) with high-performance liquid chromatography (HPLC)/electrochemical detection and UV detection. Total phenols ranged from 127 to 241 mg of gallic acid equivalents per 100 g of fresh weight. The analyses were compiled to produce a data set of 18 flavonoids and three phenolic acids. The predominant flavonoids were isorhamnetin-3-O-rutinoside and isorhamnetin-3-O-glucoside (in combination), catechin, kaempferol-3-O-rutinoside, epicatechin, quercetin-3- O-galactoside, and isorhamnetin-3-O-galactoside in amounts of 16.81, 1.93, 1.17, 0.85, 0.83, and 0.50 mg per 100 g of fresh weight almonds, respectively. Another work of Kolouchova et al. [157] describes the occurrence and determination of *trans*-resveratrol, *cis*-resveratrol and quercetin in various vegetables and fruits using HPLC method with electrochemical detection. The concentrations of resveratrol were found to range from trace quantities up to 0.03 mg per g of dry weight of *trans*-resveratrol and 0.006 mg per g of dry weight of *cis*-resveratrol. The highest known concentration was found in red cabbage and spinach, while a surprisingly low concentration was found in garlic. The quercetin content ranged from 0.003 mg/g of dry weight to 5.9 mg/g of dry weight [157]. Yoshida et al. [131] determined the content of insoluble bound phenolic acids in pearled barley by an analytical system consisting of alkaline hydrolysis extraction, high-performance liquid chromatographic separation and electrochemical detection. Insoluble bound phenolic acids in five pearled cultivars and fifteen breeding lines of barley comprised ferulic acid (4.3-34.2 mg/100 g dry matter), sinapic acid (0.025-0.445 mg/100 g dry matter) and caffeic acid (0.002-0.016 mg/100 g dry matter). Soluble free polyphenols comprised procyanidins (12.2-80.3 mg/100 g dry matter), catechin (0.1-28.2 mg/100 g dry matter), and total pholyphenol comprised 152.4-324.0 mg of gallic acid equivalents per 100 g of dry matter. Wang et al. [132] established a simple, sensitive and accurate liquid chromatography method with electrochemical detection for simultaneous separation and determination of six phenolic compounds (gallic acid, protocatechuic acid, caffeic acid, *p*-coumaric acid, rutin and quercetin) in jujube (*Ziziphus jujuba* Mill.), a traditional Chinese fruit. Similarly, Liang et al. [139] established a sensitive and accurate method for simultaneous separation and determination of four phenolic compounds (caffeic acid, *p*-coumaric acid, ferulic acid, and hesperetin) in Chinese citrus honey by HPLC-ED. The detection and quantification limits of the four compounds with ED were 6-14 times greater than those obtained with diode-array detection (DAD). The extraction process was very simple, because of the dissolution of honey directly in water.

Novak et al. [147] used reverse-phase high-performance liquid chromatography (RP-HPLC) with electrochemical detection (ED) for determination of flavonoids present in red grape skins obtained from four varieties of Portuguese grapes. Extraction of flavonoids from red grape skins was

performed by ultrasonication, and hydrochloric acid in methanol was used as extraction solvent. The developed RP-HPLC method used combined isocratic and gradient elution with amperometric detection with a glassy carbon-working electrode. Eleven different flavonoids: cyanidin-3-O-glucoside (kuromanin), delphinidin-3-0-glucoside (myrtillin), petunidin-3-O-glucoside, peonidin-3-O-glucoside, malvidin-3-O-glucoside (oenin), (+)-catechin, rutin, fisetin, myricetin, morin and quercetin, can be separated in a single run by direct injection of sample solution. RP-HPLC-ECD was characterized by an excellent sensitivity and selectivity. Kahoun et al. [155] used HPLC with coulometric-array detection for determination of 25 phenolic compounds in different mead samples (honeywines) and, in the case of hydroxymethylfurfural, they used UV detection. Phenolic compounds concentration was determined in 50 real samples of meads and correlated with meads composition and hydroxymethylfurfural concentration. The most frequently occurred compounds were protocatechuic acid and vanillic acid (both of them were present in 98 % of all samples), the least occurred compounds were (+)-catechin (10 % of samples) and sinapic acid (12 % of samples). Vanillin and ethylvanillin, which are used as artificial additives for the taste improvement, were found in 60 % and 42 % of samples, respectively. Hydroxymethylfurfural concentration, as an indicator of honey quality, was within the range from 2.47 to 158 mg/L. The method is applicable for determination of 25 phenolic compounds in mead, honey and related natural samples.

Zielinska et al. [148] determined 4 flavonoids - quercetin (Q), quercetin-3,4'-diO-beta-glucoside (Q3,4'G), quercetin-3-O-beta-glucoside (Q3G) and quercetin4'-O-beta-glucoside (Q4'G) in onion bulbs (*Allium cepa* L.) by HPLC with amperometric detection after analysis of the hydrodynamic voltammograms of flavonoid standards within the potential range from 50 to 1000 mV. The hydrodynamic voltammetric profiles of flavonoids showed that the peak current of Q, Q3G, Q4'G and Q3,4'G increased rapidly when the applied potential exceeded +450 mV. High sensitivity and low background current were observed at the applied potential of +950 mV. The lower limits of detection (LOD) were determined at signal-to-noise ratio of 3 and showed the following values: 8.05×10^{-8} M (Q), 1.08×10^{-7} M (Q3G), 1.22×10^{-7} M (Q4'G) and 2.6×10^{-7} M (Q3,4'G). Vanbeneden et al. [149] used isocratic HPLC method with amperometric electrochemical detection for simultaneous detection and quantification of hydroxycinnamic acids and their corresponding aroma-active volatile phenols in wort and beer. The technique gave good specificity and sensitivity, and therefore could be used for routine monitoring of hydroxycinnamic acids in wort and the development of volatile phenolic flavour compounds during the beer production process and subsequent conservation.

Cao et al. [161] developed a sensitive and accurate reverse phase HPLC-ED method for simultaneous determination of seven kinds of bioactive phenolic acids (gallic acid, protocatechuic acid, syringic acid, p-coumaric acid, caffeic: acid, chlorogenic acid, ferulic acid) in five different types of wine. The separation was performed on Hypersil ODS column (250 mm × 4.0 mm, 5.0 μm) by gradient elution. The mobile phase consisted of mixture of methanol-4% acetic acid. The flow rate was 0. 8 mL/min. The working potential was 0.7 V and column temperature was 30 °C. Franke et al. [162] determined ascorbic acid, and the major dietary flavones (apigenin, luteolin), flavonols (kampferol, quercetin, myricetin), flavanones (hesperetin, naringenin and their glycosides), and anthocyanidins (pelargonidin, cyanidin, delphinidin) in fruits and vegetables commonly consumed in Hawaii by HPLC with electrochemical, diode-array and/or mass spectrometric detection. Concentrations of analysed

compounds ranging in foods as eaten from 4 to 801 mg/kg for ascorbic acid and from 172 to 905 mg/kg for citrus flavanones to as high as 259 mg/kg for flavones/flavonols and 1168 mg/kg for anthocyanidins. Long et al. [165,166] established a novel scheme of liquid chromatography with four-channel electrochemical detection for the identification and quantification of phenolic acids. The method was tested on honey and green tea samples. Luo et al.[163] determined phenolic compounds in dietary supplements and tea blends containing *Echinacea* by liquid chromatography with coulometric electrochemical detection**.** The determined phenolic compounds were caftaric acid, chlorogenic acid, cynarin, echinacoside, and cichoric acid. Samples from tablets, capsules, and bags of tea blends were extracted by sonication for less than or equal to 30 min with methanol-water (60+40). The LC method with EC detection showed better sensitivity and selectivity when compared with LC with ultraviolet detection.

Zhu et al. [164] developed a sensitive and selective liquid chromatography method with multi-channel electrochemical detection for the determination of *trans*-resveratrol in wines, grape juice, and grape seed capsules. Samples were prepared with an automated solid phase extraction workstation. A four channel detector with glassy carbon electrodes was used, which can control up to four working electrodes simultaneously with applied potentials of +800, 700, 600, 500 mV vs. Ag/AgCl, and gave a better characterization of resveratrol in the complex matrices. The calibration curve was linear over the analytical range from 5 to 1000 ng/mL. Brenna et al. [159] established a new reverse-phase HPLC method that uses a coupled revelation system based on diode-array and dual electrode electrochemical detection. This method is suitable for direct measurement of polyphenols in wines and offers distinct advantages in terms of selectivity and sensitivity when compared with alternative HPLC methods, based on UV-VIS or PDA detection, since multiple features of the analytes can be checked during sample analysis. Good peak resolution was obtained following direct injection of a 20 μL sample, and more than 20 different analytes were identified by spectral comparison with known standards and by their electrochemical behaviour at potentials of +0.4 and +0.8 V (vs Ag/AgCl). Kermasha et al. [160] developed HPLC method, using ultraviolet and electrochemical detectors for the analyses of phenolic and furfural compounds in maple products. The concentrations of compounds were calculated using external standards that conformed to linear behaviour. Most of compounds identified in saps, concentrates, and syrups were related to lignin derivatives.

Joerg et al. [153] developed a new method for the determination of phenolic acids in honey. Following ethyl acetate extraction and reversed phase column chromatography these compounds are detected electrochemically using a dual electrode detector. Oxidation in the first detector cell takes place at +0.90 V and the reaction products are reduced in the second detector cell at -0.20 V. The current resulting from these electrochemical processes was proportional to the concentration of the phenolic acids. Hydrodynamic voltammograms contribute to their identification. Concentrations range from 0.02 to 13 mg/kg in honey with detection limits from 0.1 to 1.5 ng (3 S/N). Honeys of equal and different floral types were compared and characterized. The distribution pattern of phenolic acids allows differentiating between honey dew, chestnut and blossom honey. The same authors used multichanel coulometric detection coupled with liquid chromatography for determination of phenolic esters in honey in their further work [154]. A coulometric electrode-array system with sixteen electrodes arranged in series and set at increasing potentials (300-900 mV) was used for

electrochemical detection of the compounds. Chromatographic peaks for methyl 4-hydroxybenzoate, methyl vanillate, methyl syringate, *trans-p*-methyl coumarate and *trans*-methyl ferulate were identified. The content of the esters varied between 1.3 and 5044 µg per kg of honey with detection limits of 0.1-1.0 µg per kg of honey (3 S/N). Achilli et al. [92] developed a general method for the evaluation of phenolic compounds in fermented beverages, fruit juices and plant extracts using gradient HPLC with coulometric detection. In a single injection (10 µl) it was possible to identify and determine 36 different molecules (flavonoids and simple and complex phenols), without sample extraction, purification or concentration, in several kinds of beers, red and white wines, lemon juice and soya, forsythia and tobacco extracts.

3. ANALYSIS OF PHENOLIC COMPOUNDS IN APRICOTS USING HPLC WITH ELECTROCHEMICAL OR SPECTROMETRIC DETECTION

Apricots (*Prunus armeniaca* L.) are an important source of natural polyphenols [169]. Phenolic compounds, such as catechin, epicatechin, *p*-coumaric acid, caffeic acid, ferulic acid and their esters have been identified in the fruits [170-172]. Chlorogenic acid (Figure 1) is the dominant ester in apricots [173]. Flavonols occur mostly as glycosides (mainly rutinosides) of quercetin (Figure 1), however, kaempferol and quercetin 3-rutinoside predominate [174,175].

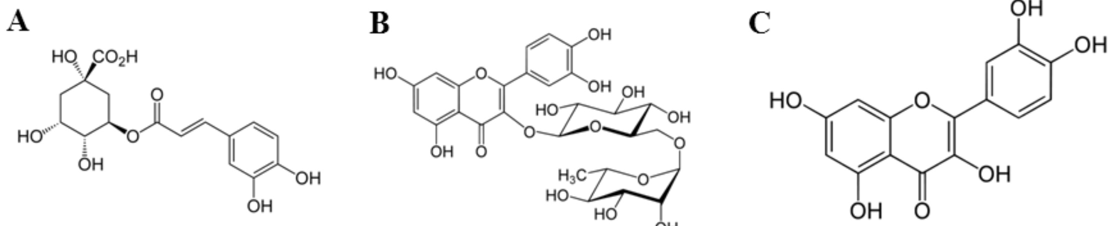

Figure 1. Structural formulas of (**A**) chlorogenic acid, (**B**) rutin, and (**C**) quercetin.

Sochor et al. [167] verified and validated HPLC technique with tandem spectrometric and electrochemical detection for determination of total antioxidant capacity (TAC) of twenty perspective genotypes of apricot (*Prunus armeniaca* L.) which were cultivated with aim to improve resistance against Plum pox potyvirus (PPV). They determined phenolic profile consisting of the following fifteen phenolic compounds: gallic acid, 4-aminobenzoic acid, chlorogenic acid, ferulic acid, caffeic acid, procatechin, salicylic acid, *p*-coumaric acid, the flavonols quercetin and quercitrin, the flavonol glycoside rutin, resveratrol, vanillin, and the isomers epicatechin, (–)- and (+)- catechin. In another study, the same authors [168] performed complex analysis of 239 apricot cultivars (*Prunus armeniaca* L.) cultivated in Lednice, South Moravia, Czech Republic. Profile of polyphenols, measured as content of ten polyphenols with significant antioxidant properties (gallic acid, procatechinic acid, *p*-aminobenzoic acid, chlorogenic acid, caffeic acid, vanillin, *p*-coumaric acid, rutin, ferrulic acid and quercetrin), was determined by high performance liquid chromatography with tandem spectrometric and electrochemical detection. Data were processed and correlated using bioinformatics techniques (cluster analysis, principal component analysis). The studied apricot cultivars were clustered according to their common biochemical properties. Zitka et al. [158] investigated a suitable method for determination of protocatechuic acid, 4-aminobenzoic acid, chlorogenic acid, caffeic acid, vanillin, *p*-

coumaric acid, rutin, ferulic acid, quercetin, resveratrol and quercitrin in apricot samples. HPLC with UV detection (Figure 2) was compared to HPLC with electrochemical detection (Figure 3). Conclusion from the acquired results was that the coulometric detection under a universal potential of 600 mV is more suitable and sensitive for polyphenols determination than UV detection at a universal wavelength of 260 nm.

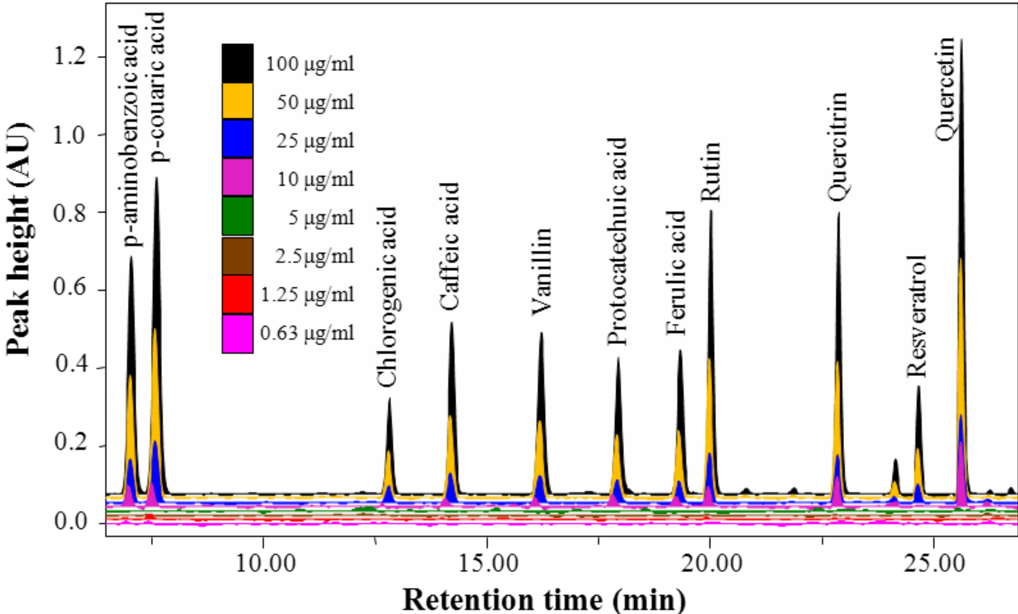

Figure 2. The HPLC-UV chromatogram for determination of phenolic compounds. Absorbance shows the quantity of particular substances in samples. Reprinted with permission from Zitka et al. [158].

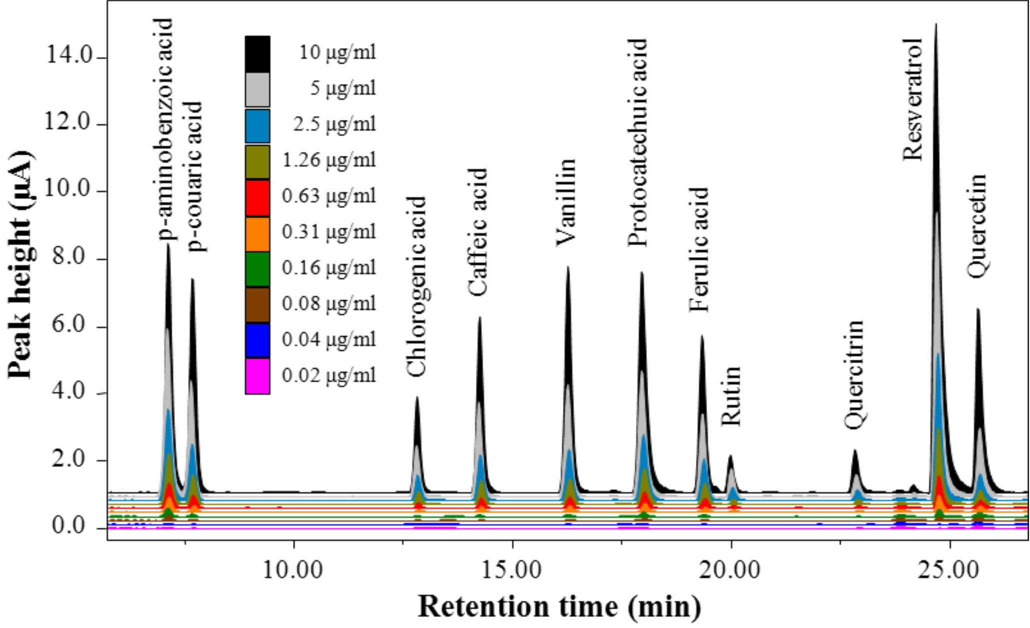

Figure 3. The HPLC-ED chromatogram for determination of phenolic compounds. Reprinted with permission from Zitka et al. [158].

Zitka et al. applied the optimized method for testing the contents of individual polyphenols in the apricot cultivars Mamaria, Mold and LE-1075. The major phenolic compounds were chlorgenic acid and rutin. Chlorgenic acid was found in amounts of 2,302 mg/100 g of fresh weight in cultivar LE-1075, 546 mg/100 g in cultivar Mamaria and 129 mg/100 g in cultivar Mold. [158]. Versari et al. [176] analysed the composition of 26 Italian commercial apricot juices obtained from organic, integrated and conventional agriculture for phenolic compounds by HPLC with diode array detection. Univariate analysis disclosed some significant differences among the composition of the apricot juices in terms of chlorogenic acid and rutin. Dragovic-Uzelac et al. [177] determined the changes of polyphenols during the ripening of three apricot cultivars ("Keckemetska ruza", "Madjarska najbolja" and "Velika rana") grown in two different geographical region of Croatia were determined by using HPLC with UV-VIS photo diode array detection. The content of individual polyphenols during ripening was quite similar, whereas their amount differed significantly. Immature fruits showed the highest level of polyphenols, which decreased at semi-mature fruits and did not change remarkably in commercial mature fruits. Among polyphenols, flavan-3-ols, chlorogenic acid and quercetin-3-rutinoside were dominant in all ripening stages of all apricot cultivars. Usenik et al. [178] investigated the role of phenols in apricot graft incompatibility. Assays of phloem with cambium from 1-year-old apricot trees of cultivars Marlen, Leskora and Betinka which were grafted on the rootstocks of different genetic origin: M-LE-1, Lesiberian, MY-KL-A, Tetra, Penta, Green Gage, Julior, MRS 2/5 and Isthara were analysed with HPLC with diode array detection. The phloroglucinol, catechin, *p*-coumaric acid and further non-identified phenols with the retention time 23-25 and 30 min were determined. The content of individual phenol compounds was related to specific cultivar/rootstock combination. The minimum number of statistical significant differences in the phenol content between tissues above and below graft union was established in homospecific combinations (*P. anneniaca* L./*P. armeniaca* L.). Cultivars Marlen, Leskora and Betinka differ in the degree of compatibility or incompatibility with rootstocks. The pattern of non-identified phenol 23 in different graft combinations is similar to catechin and *p*-coumaric acid. Verberic et al. [179] analysed chlorogenic acid, epicatechin and rutin in different cultivars of peach, apricot and sweet cherries. The analyses were performed using high performance liquid chromatography with a diode-array detector. Differences in the contents of all phenolic compounds between the cultivars were distinguished. At peach and apricot varieties we noticed that the contents of chlorogenic acid, epicatechin and rutin were higher in peel compared to pulp. Therefore it can be suggested to the consumers that, regarding health promoting properties of fruit, unpeeled fruits should be eaten or used for further processing. The highest values of chlorogenic acid, epicatechin and rutin were detected in apricot peel (on average 705.2, 86.3, 347.4 mg/kg respectively). The highest values of analysed phenolics in pulp were as follows: chlorogenic acid (125.43 mg/kg) in peach, epicatechin in apricots (43.46 mg/kg). Both apricots and peaches exhibited similar values for the content of rutin (4.84 and 4.60 mg/kg respectively). The average content of phenolics in the whole cherry fruit was similar to or higher than the content of phenolics in apricot and peach pulp (103.6 mg/kg for chlorogenic acid, 66.3 mg/kg for epicatechin and 17.42 mg/kg for rutin). The content of analysed phenolics is comparable to the content of phenolics in apple and therefore the analysed species can be considered as important source of antioxidants.

4. CONCLUSION

Electrochemical techniques represent the group of methods convenient for determination of phenolic compounds in food samples. They constitute reliable tool for rapid and low-cost tests. Their connecting with HPLC techniques enables identification as well as quantitative analyses of phenolic compounds in food matrices even in very low concentrations.

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