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Review

Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis

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Abstract

Consumption of plant foods, particularly fruits, vegetables and cereal grains is encouraged because they render beneficial health effects. Phenolics and polyphenolics are among the most desirable food bioactives because of their antioxidant activity, brought about by a number of pathways, or due to other mechanisms. The analysis of phenolics and polyphenolics requires their extraction possible purification and structure elucidation. This overview provides a cursory account of the source, extraction and analysis of phenolics in fruits, vegetables and cereals.
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Keywords: Phenolics; Polyphenols; Sources; Extraction; Analysis

Contents

1. Introduction 1523
2. Phenolics in cereals 1524
3. Phenolics in fruits 1525
4. Phenolics in vegetables 1526
5. Extraction of phenolics 1527
6. Analysis and quantification of phenolic compounds 1528
7. Spectrophotometric techniques 1528
8. Chromatographic techniques 1529
   8.1. Liquid chromatography 1529
   8.2. High-performance liquid chromatography 1530
   8.3. High-speed countercurrent chromatography 1531
   8.4. Other chromatographic techniques 1535
9. Application of capillary electrophoresis 1535
10. Conclusions 1536
References 1537

1. Introduction

Phenolics are secondary metabolites synthesized by plants, both during normal development [1,2] and in response to stress conditions such as infection, wounding and UV radiation, among others [3,4]. These compounds are a very diversified group of phytochemicals derived from phenylalanine and tyrosine [1,5–8]. Plants may contain simple phenolics, phenolic acids, coumarins, flavonoids, stilbenes, hydrolyzable and condensed tannins, lignans and lignins (Table 1). In plants, phenolics may act as phytoalexins, antifeedants, attractants for pollinators, contributors to plant pigmentation, antioxidants and protective

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Table 1
Dietary sources of plant phenolics

Phenolic compounds	Dietary source
Phenolic acids	
Hydroxycinnamic acids	Apricots, blueberries, carrots, cereals, pears, cherries, citrus fruits, oilseeds, peaches, plums, spinach, tomatoes, eggplants
Hydroxybenzoic acids	Blueberries, cereals, cranberries, oilseeds
Flavonoids	
Anthocyanins	Bilberries, black and red currants, blueberries, cherries, chokecherries, grapes, strawberries
Chalcones	Apples
Flavanols	Apples, blueberries, grapes, onions, lettuce
Flavanonols	Grapes
Flavanones	Citrus fruits
Flavonols	Apples, beans, blueberries, buckwheat, cranberries, endive, leeks, lettuce, onions, olive, pepper, tomatoes
Flavones	Citrus fruits, celery, parsley, spinach, rutin
Isoflavones	Soybeans
Xanthones	Mango, mangosteen
Tannins	
Condensed	Apples, grapes, peaches, plums, mangosteens, pears
Hydrolyzable	Pomegranate, raspberries
Other phenolics	
Alk(en)ylresorcinols	Cereals
Arbutin	Pears
Avenanthramides	Oats
Capsaisinoids	Pepper
Coumarins	Carrots, celery, citrus fruits, parsley, parsnips
Lignans	Buckwheat, flaxseed, sesame seed, rye, wheat
Secoiridoids	Olives
Stilbenes	Grapes

agents against UV light, among others [8]. In food, phenolics may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability of food. In addition, health-protecting capacity of some and antinutritional properties of other plant phenolics are of great importance to both consumers and producers [8].

Distribution of phenolics in plants at the tissue, cellular and subcellular levels is not uniform. Insoluble phenolics are found in cell walls, while soluble phenolics are present within the plant cell vacuoles [2,3,9–12]. The outer layers of plants contain higher levels of phenolics than those located in their inner parts [9,13,14]. Cell wall phenolics, linked to various cell components [15–17], contribute to the mechanical strength of cell walls as well as playing a regulatory role in plant growth and morphogenesis and in the cell in response to stress and pathogens [15,17–20]. Ferulic and *p*-coumaric acids are the major phenolic acids of cell walls. These compounds may be esterified to pectins and arabinoxylans or cross-linked to cell wall polysaccharides in the form of dimers such as dehydroferulates and truxillic acid [21–29]. These cross-links may play a significant role in cell–cell adhesion [30], serve as a site for lignin formation [31–33] and contribute to thermal stability of plant food texture [34].

The content of some phenolics may increase under stress conditions such as UV radiation, infection by pathogens and parasites, wounding, air polution and exposure to extreme temperatures [35]. In grapes, synthesis of stilbenes (*trans*- and *cis*-resveratrols (3,5,4′-trihydroxystilbene), *trans*- and *cis*-piceids (3-*O*-β-D-glucosides of resveratrol), *trans*- and *cis*-astringins (3-*O*-β-D-glucosides of 3′-hydroxyresveratrol), *trans*- and *cis*-resveratrolosides (4′-*O*-β-D-glucosides of resveratrol) and pterostilbene (a dimethylated derivative of stilbene)) is induced by fungal infection (*Botris cinerea*), injury, UV radiation and wilting as well as such factors as grape cultivar, developmental stage of the berry and soil cultivation practices [36–43]. In carrots, the synthesis of 6-methoxymellein (isocoumarin) is stimulated by exposure to ethylene [44–47] and to UV radiation [48], by microbial infection [49,50], by wounding [47] and storage at elevated temperatures [44].

The level of phenolics in plant sources also depends on such factors as cultivation techniques, cultivar, growing conditions, ripening process, as well as processing and storage conditions, among others. For example, peeling, chopping, boiling, microwaving and frying onions may reduce the total content of quercetin conjugates from 1% in case of chopping to up to 75% in case of boiling onions in water [51–54]. On the other hand, storage of whole parsnips at +4 °C for 7 days was found to increase the level of furanocoumarins from 1 to 33 mg/kg fresh weight, while storage of parsnips at −18 °C up to 50 days had little effect on the content of furanocoumarins [55].

2. Phenolics in cereals

Phenolic acids and flavonoids are present in cereals in the free and conjugated forms. The highest concentration of phenolic acids and flavonoids is in the aleurone layer of cereal grains, but these compounds are also found in embryos and seed coat of grains [56]. Phenolic acids are found abundantly in cell wall and are linked to hemicelluloses in different forms such as 2-*O*-(5′-*O*-(E)-feruoyl-β-D-xylopyranosyl)-(1 → 4)-D-xylopyranose [57–60]. According to Izydorczyk et al. [61] water binding capacity of pentosans crosslinked via ferulic acid is greater than those purified. Phenolic acids are known to contribute to the antioxidative potential of cereal grains [62–66] and may also be used for the prediction of the end-use of cereal products [67]. Cereal grains with elevated levels of phenolic acids in caryopsis exhibit greater resistance to both disease and insect [68,69], but exhibit reduced extractability of endosperm [67,70,71]. Moreover, cross-linking of arabinoxylans with phenolic acids lowers the arabinoxylan solubility and swelling in water as well as reduces their microbial degradation in the human colon [72]. Significant amounts of alk(en)ylresorcinols containing nonisoprenoid side chain (15–25 carbons in length) attached to the hydroxybenzene ring have also been detected in some cereals (barley, rye, wheat) [73,74]. These compounds are also located in the outer layers of grains [73] and displayed antioxidant as well as membrane modulating effects [73,75,76].

Barley phenolics include tyrosine, tyramine and its derivatives, phenolic acids, their esters and glycosides, anthocyanins, proanthocyanidins, lignans and substances related to lignin [77–79]. Phenolic acids are mainly located in the outer layers (husk, pericarp and aleurone) of the grain [80,81]. Ferulic acid

is the predominant free phenolic acid in barley seeds [81] and barley brans [27,82,83]. On the other hand, *p*-hydroxybenzoic acid is the major bound phenolic acid detected in barley extracts obtained by sequential treatment of grain with acid, α-amylase and cellulase [79]. The proanthocyanidins of barley are implicated in the formation of haze in beer [84,85]. These compounds are located in the testa of the grain [86] and are mixture of oligomeric prodelphinidins and procyanidins [87].

Bran–aleurone fraction of buckwheat contains bound syringic, *p*-hydroxybenzoic, vanillic and *p*-coumaric acids [88]. On the other hand, several free phenolic acids, namely ferulic, *p*-hydroxybenzoic, caffeic and chlorogenic acids were detected in buckwheat grits [74]. Rutin, quercetin, orientin, vitexin, isovitexin and isoorientin were also detected in buckwheat [89] while four catechins, namely (−)-epicatechin, (+)-catechin 7-*O*-β-D-glucopyranoside, (−)-epicatechin 3-*O*-*p*-hydroxybenzoate and (−)-epicatechin 3-*O*-(3,4-di-*O*-methyl)gallate were identified in the ethanolic extracts of buckwheat groats [90].

In corn, insoluble bound phenolic acids constitute the predominant fraction of phenolic acids present [32,91]. The embryo and aleurone layer of corn contain phenolic acids linked covalently to amine functionalities, such as feruoylputrescine, *p*-coumarylputrescine, diferuloylputrescine, di-*p*-coumarylputrescine, *p*-coumarylspermidine, diferuloylspermidine and diferuloylspermine [92]. Feruloylated disaccharides detected in acid hydrolyzate of corn hulls include *O*-(2′-*O*-*trans*-feruoyl-α-L-arabinofuranosyl)-(1 → 3)-β-D-xylanopyronose, *O*-(2′-*O*-methoxyl-5′-*O*-*trans*-feruoyl)-α-L-arabinofuranosyl-(1 → 3)-β-D-xylanopyronose and *O*-(2′-*O*-methoxyl-5′-*O*-*cis*-feruoyl)-α-L-arabinofuranosyl-(1 → 3)-β-D-xylanopyronose [93]. Furthermore, 16 steryl cinnamic acid derivatives, mainly located in the interior portion of the inner pericarp layer and germ [94–96], have also been identified.

The phenolics of oats contain a mixture of benzoic and cinnamic acid derivatives as well as quinones, flavones, flavonols, chalcones, flavanones, anthocyanins and amino phenolics. Bound-phenolic acids may be linked to sugars, polysaccharides, lignins, amines, long-chain alcohols, glycerol, as well as long chain omega-hydroxy fatty acids [74,97–100]. At least 25 and 20 avenanthramides, conjugates of cinnamic acid with anthranilic acids and *N*-acylanthranilate alkaloids, respectively, were identified in groat and hull extracts [101,102].

Wheat kernels contain a number of phenolic compounds, namely ferulic, vanillic, gentisic, caffeic, salicylic, syringic, *p*-coumaric and sinapic acids as well as vanillin and syringaldehyde, none of which contribute to antioxidant activity of products [91,103–107]. Of these, ferulic acid was the primary phenolic acid in the grain, accounting for up to 90% of total phenolic acids [60,62,70,91,103,108]. Ferulic acid is found, predominantly, in the aleurone cell walls of kernel [67,109,110] and it is esterified to arabinose [111,112], stanols and sterols [95] and glucose [113]. Campestanyl and sitostanyl ferulates were the main steryl ferulates present in wheat grain [114]. Wheat bran also contained ferulic acid dehydrodimers (DiFA) [27,115,116], which strengthen the aleurone walls during the maturation of wheat grain by formation of bridges between two arabinoxylan chains [117,118]. These dehydrodimers are products of oxidative coupling of ferulic acid catalyzed by peroxidase [119]. The cross-linking of cell walls with phenolic acids provides a physical barrier against insects and microorganisms [120]. Several *n*-alkylphenols containing 17, 19, 21, 23 and 25 carbon atoms coupled to a resorcinol ring at the 5 position were also identified in wheat grain [121,122]. Tricin (5,7,4′-trihydroxy 3′,5′-dimethoxy flavone) was found to be the dominant flavone pigment in wheat [123,124]. In addition, two C-glycosylflavones, namely 6-C-pentosyl-8-C-hexosylapigenin and 6-C-hexosyl-8-C-pentosylapigenin were isolated from wheat bran [124,125].

3. Phenolics in fruits

The phenolics in apple varieties are hydroxycinnamic acid (HCA) derivatives, flavan-3-ols (monomeric and oligomeric), flavonols and their conjugates, dihydrochalcones and procyanidins [13,126–141]. Chlorogenic acid was the major HCA identified in the apple fruit accounting for up to 87% of the total amount [130]. Anthocyanins are found in the vacuoles of epidermal and subepidermal cells of the skin of red apple varieties [142–146]. Phlorizidin (phloretin 2′-β-D-glucoside) and phloretin 2′-β-D-xylosyl-(1 → 6)-β-D-glucoside are the major dihydrochalcones found mainly in apple fruits [130,145,147–150]. Several flavonol glycosides have been identified in apple fruits, namely rutin, hyperin (quercetin-3-β-D-galactoside), isoquercitrin (quercetin-3-β-D-glucoside), reynoutrin (quercetin-3-β-D-xyloside), avicularin (quercetin-3-α-L-arabinofuranoside) and quercitrin (quercetin-3-α-L-rhamnoside) [130,145,148,150,151]. Apple procyanidins are a mixture of oligomers and polymers made of (−)-epicatechin and (+)-catechin as monomeric units [134].

Blueberries are a rich source of phenolic acids, catechins, flavonols, anthocyanins and proanthocyanidins, all of which are antioxidative in nature [152–159]. Gallic, caffeic, *p*-coumaric, ferulic and ellagic acids were identified in blueberry fruits [158]. A number of anthocyanins were also isolated and identified in blueberries, namely 3-galactosides and 3-arabinosides of cyanidin, delphinidin, peonidin, petunidin and malvidin and 3-glucosides of cyanidin, delphinidin, peonidin, petunidin and malvidin [160–162]. In addition, catechin, myricetin, quercetin and kaempferol were detected in blueberries [158]. Oligomeric B-type procyanidins from dimers to octamers have been identified in blueberry [162]. Blueberries also contain a mixture of polymeric procyanidins with a degree of polymerization ranging from 14.4 to 114.1 [163]. Blueberry leaves were found to serve as a good source of phenolics that possess high antioxidant activity. The antioxidant activity of crude phenolic extracts of blueberry leaves in β-carotene-linoleate model system was comparable to that displayed by the synthetic antioxidant butylated hydroxyanisole (BHA). The radical scavenging effect of crude phenolic extracts, at 100 μg/assay, was over 90% [164].

Cranberries serve as a good source of anthocyanins [161,162,165,166], flavonol glycosides [167], proanthocyanidins [162–167] and phenolic acids [168]. Sinapic, caffeic and *p*-coumaric acids are the major bound phenolic acids while *p*-coumaric, 2,4-dihydroxybenzoic and vanillic acids are the pre-

dominant free phenolic acids [168]. The most abundant anthocyanins in American cranberries are 3-*O*-galactosides and 3-*O*-arabinosides of cyanidin and peonidin, while 3-*O*-glucosides of cyanidin and peonidin are dominant in European cranberries [161,169]. Furthermore, polymeric proanthocyanidins comprise 63% of total proanthocyanidins in cranberries [163].

Grape berries and their skins contain phenolic acids such as caftaric acid (*trans*-caffeoyltartaric acid), coutaric acid (*p*-coumaryltartaric acid) and *trans*-fertaric acid [170–172], flavonols such as quercetin 3-glucuronide, quercetin 3-glucoside, myricetin 3-glucuronide and myricetin 3-glucuronide [171,173] and flavanonols, such as astilbin (dihydroquercetin 3-rhamnoside) and engeletin (dihydrokaempferol 3-rhamnoside) [171,174]. A number of stilbenes, namely *trans*- and *cis*-resveratrols (3,5,4′-trihydroxystilbene), *trans*- and *cis*-piceids (3-*O*-β-D-glucosides of resveratrol), *trans*- and *cis*-astringins (3-*O*-β-D-glucosides of 3′-hydroxyresveratrol), *trans*- and *cis*-resveratrolosides (4′-*O*-β-D-glucosides of resveratrol) and pterostilbene (a dimethylated derivative of stilbene) have been detected in both grape leaves and berries [41,43,175,176]. *cis*-Piceid was found to be the predominant stilbene in berry skins during fruit ripening while resveratrol was the main stilbene in wilting berries [43]. Grape seeds and skins are also an excellent source of proanthocyanidins, flavonols and flavan-3-ols [171,177–179]. Procyanidins are the predominant proanthocyanidins in grape seeds, while procyanidins and prodelphinidins are dominant in grape skins and stems [171,178,180–183].

Pomegranates are a rich source of hydrolyzable tannins and anthocyanins. Several anthocyanins such as cyanidin 3-glucoside, delphinidin 3-glucoside, cyanidin 3,5-diglucoside, delphinidin 3,5-diglucoside and pelargonidin 3-glucoside have been detected in pomegranate juice [184]. The presence gallotannins, ellagic acid tannins and gallagyl tannins such as punicalagin and punicalin has also been reported [184].

In citrus fruits, cinnamic acid derivatives, coumarins and flavonoids (flavonones, flavones and flavonols) are the major groups of phenolic compounds [185,186]. Phenolic acids are found in the flavedo of citrus fruits [186,187] in the form of esters, amides and glycosides [187–190]. Naringin, naringenin 7-neohesperidoside and narirutin, naringenin 7-rutinoside are the major flavanone glycosides found in grapefruit, while narirutin and hesperedin, hesperetin 7-rutinoside in sweet oranges and naringin, neohesperidin and hesperetin 7-neohesperidoside in sour oranges [191]. On the other hand, hesperedin, narirutin and didymin (isosakuranetin 7-rutinoside) are the predominant flavanone glycosides in navel [192] and blood oranges [189]. Polymethoxylated flavones (PMF) are unique phenolic compounds in citrus species [193], and their profile is a fingerprint of each species [194,195]. Nobiletin (5,6,7,8,3′,4′-hexamethoxyflavone) and sinensetin (5,6,7,3′,4′-pentamethoxyflavone) have been identified in orange, while tangeretin, 3,5,6,7,8,3′,4′-heptamethoxyflavone, 5,7,8,4′-tetramethoxyflavone, and 5,7,8,3′,4′-pentamethoxyflavone in grapefruits [196,197]. Diosmin (4′methoxy-5,7,3′-trihydroxyflavone-7-rutinoside) and neodiosmin (4′ methoxy-5,7,3′-trihydroxyflavone-7-neohepseridoside) are the predominant glycosylated flavones identified in citrus fruits [198]. Immature fruits contain higher levels of these flavones was detected in immature than mature fruits [198].

4. Phenolics in vegetables

Phenolic acids and isocoumarins were the predominant phenolics in carrots [199]. Major phenolic acids included *p*-hydroxybenzoic acid, syringic acid and 3′-caffeoylquinic acid (neochlorogenic acid), 5′-caffeoylquinic acid (chlorogenic acid), 3′-, 4′- and 5′-feruoylquinic acids, 3′- and 5′-*p*-coumaroylquinic acids, 3′,4′- and 3′,5′-dicaffeoylquinic acids and 3′,4′- and 3′,5′-diferuoylquinic acids [199,200]. The presence of coumarins, namely 6-methoxymellein and 6-hydroxymellein, was also reported in carrot tissues [201,202]. These compounds were predominantly accumulated in the periderm tissue of carrot root [47].

Red, iceberg and romaine lettuce contain caffeoyltartaric, chlorogenic, dicaffeoyltartaric and 3′,5′-dicaffeoylquinic acids [203–207]. Lettuce is also a good source of flavonoids. Several quercetin conjugates, namely quercetin 3-(6-malonylglucoside), quercetin 3-glucoside, quercetin 3-glucuronide, quercetin 3-rhamnoside, quercetin 3-galactoside, and quercetin 3-(6-malonylglucoside)-7-glucoside were detected in both red pigmented and green leaf lettuce [203,208,209]. Red lettuce varieties contained higher levels of flavonoids than did green lettuce varieties [51,208].

Spinach harvested in the spring contained higher levels of total phenolics than that grown in the fall [210]. Patuletin (quercetagetin 6-methyl ether), jaceidin and spinacetin (quercetagetin 6,3′-dimethyl ether) conjugates [211,212] are the major flavonoids in spinach leaves [210]. In addition a number of antimutagenic flavonoids, including flavonol glucuronides and disaccharides, methylenedioxyflavonol glucuronides, flavanones [213] and methoxyflavone glucuronides [210,214] were also identified.

Onions are rich sources of flavonoids [51,215–217] such as quercetin, isorhamnetin, myricetin, kaempferol and their conjugate [218,219]. Of these, quercetin and its conjugates are the predominant flavonols present [51,195,220]. These flavonols are mostly concentrated in the skin. In the scales, abaxial epidermis of scales contained a higher level of flavonols than did the mesophyll and approximately 50% of flavonols were detected in the top quarter part of the scales. Anthocyanins, namely peonidin 3-glucoside, cyanidin 3-glucoside and cyanidin 3-arabinoside and their malonylated derivatives, cyanidin 3-laminariobioside and delphinidin and petunidin derivatives [221], are located in the red onion skin and the outer fleshy layer [52].

Flavonoids and capsaicinoids are the predominant phenolics found in pepper fruits (*Capsicum* species). Conjugates of quercetin and luteolin are the major flavonoids found in pepper [222–225]. Capsaicinoids, acid amides of vanillylamine and C_8 and C_{13} branched fatty acids [226,227], are accumulated predominantly in the epidermal tissue of the placenta [228–229]. These compounds are responsible for the development of pungency in pepper [230,231]. The apical pepper fruits contain higher levels of caspaicinoids compared to those harvested from the middle and basal segments of the plant [232]. Over 15 cap-

saicinoids have been isolated and identified [227,233]. Of these, capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) and dihydrocapsaicin contribute about 90% to the total pungency [229,234].

Flavonols, the predominant phenolics, are located mostly in the tomato skin [235]. Cherry tomatoes contained a much higher level of flavonols than larger size tomato cultivars [51]. These compounds are a mixture of rutin, quercetin 3-rhamnosyldiglucoside, kaempferol 3-rhamnosylglucoside and kaempferol 3-rhamnosyldiglucoside [236].

5. Extraction of phenolics

Solubility of phenolics is governed by their chemical nature in the plant that may vary from simple to very highly polymerized substances. Plant materials may contain varying quantities of phenolic acids, phenylpropanoids, anthocyanins and tannins, among others. There is a possibility of interaction of phenolics with other plant components such as carbohydrates and proteins. These interactions may lead to the formation of complexes that may be quite insoluble. Solubility of phenolics is also affected by the polarity of solvent(s) used. Therefore, it is very difficult to develop an extraction procedure suitable for extraction of all plant phenolics. The phenolic extracts from plant materials are always a diversified mixture of plant phenolics soluble in the solvent system used. Additional steps may be required to remove the unwanted phenolics and non-phenolic substances such as waxes, terpenes, fats and chlorophylls.

Solvents, such as methanol, ethanol, propanol, acetone, ethyl acetate, dimethylformamide and their combinations have also been used for the extraction of phenolics, often with different proportions of water [237–242]. For example, rutin and chlorogenic acid were efficiently extracted from buckwheat herbs by agitated maceration in 30% ethanol at 60 °C for 2 h [243]. Sequential alkaline hydrolysis [244,245] as well as a number of enzymatic procedures for efficient release of bound phenolic have been described. Alpha-amylase or combination of α-amylase and cellulase have been utilized to release phenolic acids in barley [79,246] and use of combinations of commercial enzyme such as Viscozyme L., Ultraflo L., Termamyl and Lallzyme in barley spent grain [247], and cell degrading enzyme in rye grain [248] have been reported.

The recovery of polyphenols from plant materials is also influenced by the extraction time and related factors. Reported extraction periods vary from 1 min [249] to 24 h [250,251]. Longer extraction times increase the chance of oxidation of phenolics unless reducing agents are added to the solvent system [252]. On the other hand, Naczk and Shahidi [253] and Naczk et al. [240] found that a two-stage extraction with 70% (v/v) acetone, 1 min each at 10,000 rpm using a Polytron homogenizer, was sufficient for the extraction of tannins from commercial canola meals. Further extraction (up to six stages) only marginally enhanced the yield of extraction of other phenolic compounds. However, Deshpande and Cheryan [254] has demonstrated that the optimum extraction time required for dry bean phenolics is 50–60 min.

The extraction of polyphenols from plant material may also be influenced by the ratio of solvent-to-sample (*R*). Naczk and Shahidi [253] and Naczk et al. [240] found that changing *R* from 1:5 to 1:10 increased the extraction of condensed tannins from commercial canola meals from 257.3 to 321.3 mg/100 g of meal and total phenolics from 773.5 to 805.8 g/100 g of meal when using 70% acetone.

Deshpande and Cheryan [254] demonstrated that the yield of tannin recovery from dry beans was strongly influenced by variations in the sample particle size. They found that the amount of vanillin assayable tannins decreased by about 25–49% as the minimum size was reduced from 820 to 250 μm. Recently, Luthria and Mukhopadhyay [239] showed the extraction of phenolic from eggplant was influenced by sample preparation parameters.

Krygier et al. [255] extracted free and esterified phenolic acids from oilseeds using a mixture of methanol/acetone/water (7:7:6, v/v/v) at room temperature. Following this, the free phenolics were extracted with diethyl ether from the extract and the extract was then treated with 4 M NaOH under nitrogen to liberate esterified phenolic acids. The hydrolyzate was acidified and the liberated phenolic acids were extracted with diethyl ether. The left over sample, after exhaustive extraction with a mixture of methanol/acetone/water was treated with 4 M NaOH under nitrogen to liberate insoluble bound phenolic acids. According to Krygier et al. [255] alkaline hydrolysis may lead to some degradation of hydroxycinnamic acid derivatives, but this can be prevented by the addition of 1% ascorbic acid and 10 mM ethylenediaminetetraacetic acid (EDTA) [256]. High yield of phenolic extraction and a low level of the phytotoxic fagopyrin was achieved after maceration of herb in 30% ethanol at 60 °C for 2 h.

Anthocyanins are usually extracted from plant material with an acidified organic solvent, most commonly methanol. This solvent system destroys the cell membranes, simultaneously dissolves the anthocyanins, and stabilizes them. However, according to Moore et al. [257,258] the acid may bring about changes in the native form of anthocyanins by breaking down their complexes with metals and co-pigments. The acidic extracts of anthocyanins are first concentrated under vacuum and then may be extracted with petroleum ether, ethyl acetate or diethyl ether in order to remove lipids and unwanted polyphenols [259,260]. The extract can also be partially purified, using ion exchange resins, as described by Fuleki and Francis [261]. Concentration of acidic extracts of anthocyanins before purification may, however, bring about losses of labile acyl and sugar residues [262]. In order to avoid this detrimental effect, several researchers have proposed to reduce the acid contact with the pigments [263], to use neutral organic solvents or boiling water [262], or to use weak organic acids such as formic or acetic acid, for acidification of the solvents used for the extraction of pigments [237,257,258]. Anthocyanins may also be recovered from the extract using solid phase extraction (SPE) or solid phase microextraction (SPME) on C_{18} cartridges. Hong and Wrolstad [264] fractionated anthocyanins by elution of the extract through a SPE cartridge with an alkaline borate solution. Only anthocyanins with *o*-dihydroxy groups (cyanidin, delphinidin and petunidin) were preferentially eluted from the SPE cartridge with borate solution due to the formation of hydrophilic borate–anthocyanin complex.

Wang and Sporns [265] also isolated anthocyanins from fruit juices and wine using a SPE cartridge, but used methanol–formic acid–water (70:2:28, v/v/v) solvent system for their elution.

Several solvent systems have been used, including absolute methanol, ethanol, acidified methanol, acetone, water and their combination for the extraction of condensed tannins. For example, 1% HCl in methanol was used for the extraction of tannins from sorghum [266] and dry beans [254]. Meanwhile, acetone/water (70:30, v/v) solution was the best solvent system for the extraction of tannins from rapeseed/canola [267,268], beach pea (*Lathyrus maritimus* L.) [269,270] and blueberries [162]. On the other hand, acetone/water (60:40, v/v) solution was used for extraction of condensed tannins from cider apple [131] and grape skins [183]. Tannins of cloves and allspice may be extracted with boiling water over a 2 h period [271]. Recently, Ashraf-Khorassani and Taylor [272] applied a sequential extraction procedure for the extraction of monomeric phenolics and proanthocyanidins from grapeseed. Epicatechins and catechins were first extracted from the seed with a methanol-modified carbon dioxide and then pure methanol was used for the extraction of procyanidins.

Application of subcritical water extraction (SWE) for isolation of bioactive phytochemicals from plant material has recently been reported [273,274]. The SWE is carried out using hot water, above its boiling point, that is sufficiently pressurized to 15 bar at 200 °C and 85 bar at 300 °C, to maintain a liquid state [275,276]. The efficiency of SWE is affected by temperature, extraction time as well as the presence of small quantities of organic solvents and surfactants [273]. The polarity of water under pressure varies with temperature. At lower temperatures the water is a very polar system but at higher temperatures ($\geq$250 °C) the polarity of pressurized water is similar to that displayed by polar organic solvents [274–276]. Thus, the solubility of analytes in superheated water can be manipulated by changing the extraction temperature. Under subcritical conditions the polar analytes are selectively extracted at lower temperatures while less polar analytes are extracted at higher temperatures. SWE has been applied for the extraction of antioxidants from rosemary leaves. Rosmarinic acid was preferentially extracted at a lower temperature (100 °C) while carnosic acid was effectively extracted at a higher temperature (200 °C) [277–279]. Recently, Herrero et al. [278,279] used ethanol, hexane, petroleum ether and water, under pressure, for the extraction of antioxidants from *Spirulina paltensis* microalga. The extraction temperature markedly affected both the extraction yield and antioxidant activity of extracts and the highest experimental values were obtained at 170 °C. Hexane and petroleum ether extracts exhibited somewhat higher antioxidant activities than ethanol and water extracts. However, ethanol was proposed as the extracting medium for its GRAS status and highest extraction yields.

6. Analysis and quantification of phenolic compounds

A number of reviews on the analysis of polyphenolics have also been published [237,280–290]. According to Robards [241] selection of proper analytical strategy for studying bioactive phenolics in plant materials depends on the purpose of the study as well as the nature of the sample and the analyte. The assays used for the analysis of phenolics can be classified as either those which determine total phenolics content (TPC), or those quantifying a specific group or class of phenolic compounds.

Quantification of phenolic compounds in plant materials is influenced by their chemical nature, the extraction method employed, sample particle size, storage time and conditions, as well as assay method, selection of standards and presence of interfering substances such as waxes, fats, terpenes and chlorophylls.

7. Spectrophotometric techniques

A number of spectrophotometric methods have been developed for quantification of plant phenolics. These assays are based on different principles and are used to determine different structural groups present in phenolic compounds. The Folin–Denis assay [271,291] is widely used for determination of total phenolics, while the vanillin [266] and proanthocyanidin [292] assays have been utilized for the estimation of total proanthocyanidins. Spectrophometric assays for quantification of total anthocyanins are based on their characteristic behavior under acidic conditions [259–261]. On the other hand, complexation of the phenolic with Al(III) is the principle of spectrophotometric assays used for quantification of total caffeic acid and total flavonoids [293–295]. Furthermore, simple phenolics have absorption maxima between 220 and 280 nm [296,297], but their absorption is affected by the nature of solvent employed and the pH of the solution. Moreover, the possibility of interference by UV-absorbing substances such as proteins, nucleic acids and amino acids should be considered. Therefore, development of a satisfactory UV assay is a rather cumbersome and difficult task. Moreover, it is difficult to find a specific and suitable standard for quantification of phenolics. This is due to the complexity of plant phenolics as well as existing differences in the reactivity of phenols towards reagents used for their quantification.

Traditional spectroscopic assays may lead to overestimation of polyphenol contents of crude extracts from plant materials due to the overlapping of spectral responses. These problems can be overcome by using a chemometric technique to analyze the spectra such as partial least squares (PLS) or principal component analysis (PCA) [298,299]. Chemometric techniques use information (such as a spectrum) and chemical indices (such as concentration of a component) and establish a mathematical relation between the two. They assume that the chemical index (concentration) is correct and attribute weightings of the spectral information accordingly. The setting up of the model, correlating the information with a chemical index, is known as calibration [300].

Monedero et al. [301] developed a chemometric technique for controlling the content of phenolic aldehydes and acids during production of wine subjected to accelerated aging. The wine was aged by the addition of oak wood extracts obtained by maceration of charred oak shavings. Charring time and/or the interactions between charring temperature and time were essential factors to control the content of 10 of the 11 phenolic compounds studied. Edelmann et al. [302] developed a rapid method

of discrimination of Austrian red wines based on mid-infrared spectroscopy of phenolic extracts of wine. The samples of wine were cleaned up by solid-phase extraction (SPE) before collection of spectra using FTIR spectrophotometry. These authors also reported that the use of UV–vis spectroscopy was limited to the authentication of the Burgundy species Pinot Noir. Subsequently, Brenna and Pagliarini [303] employed a multivariate analysis for establishing a correlation between the polyphenolic composition and the antioxidant power of red wines.

Briandet et al. [304] applied PCA to differentiate between Arabica and Robusta in instant coffees based on their FTIR spectra. Spectra used in this study were obtained by using the diffuse reflection infrared Fourier transform and attenuated total reflection sampling techniques. According to these authors, the discrimination between species of coffees was based on the different chlorogenic acid and caffeine contents. Later, Downey et al. [305] successfully applied factorial discriminant analysis and PLS to develop a mathematical model for varietal authentication of lyophilized samples of coffee based on near- and mid infrared spectra.

Schulz et al. [306] used a near-infrared reflectance (NIR) spectroscopic method for prediction of polyphenols in the leaves of green tea (*Camelia sinensis* (L.) O. Kuntze). The contents of gallic acid and catechins were determined using a reversed-phase HPLC methodology. The PLS method was used to calibrate NIR spectra with the contents of gallic acid and catechins in tea. The models predicted the contents of catechins and gallic acid with good accuracy. On the other hand, Mangas et al. [307] used linear discrimination analysis for differentiation of bitter and non-bitter cider apple varieties. The most discriminant variables were chlorogenic acid, phloretin 2-xyloglucoside and an unidentified phenolic acid derivative with a maximum absorption at 316.7 nm. Using this model, 91.3 and 85.7% correct classification was obtained for internal and external evaluation of the model, respectively. Subsequently, Silva et al. [308] reported that principal component analysis of phenolic compounds can be used for discrimination of samples of quince peel and pulp.

Various nuclear magnetic resonance (NMR) spectroscopic techniques have been employed for structural elucidation of complex phenolics isolated from foods without previous separation into individual components (241, 285). These include ^{1}H NMR and ^{13}C NMR, two-dimensional homonuclear (2D ^{1}H–^{1}H) correlated NMR spectroscopy (COSY), heteronuclear chemical shift correlation NMR (C–H HECTOR), totally correlated NMR spectroscopy (TOCSY), nuclear Overhauser effect in the laboratory frame (NOESY) and rotating frame of reference (ROESY) [309–317]. Combinations of high-resolution spectroscopic techniques with novel mathematical data treatment techniques now provide a greater insight into structural elucidation of mixtures of phenolic compounds without their prior separation into individual components [314,318,319].

8. Chromatographic techniques

Various chromatographic techniques have been employed for separation, preparative isolation, purification and identification of phenolic compounds [241,282,289,320–322]. Chromatographic procedures have also been used to study the interaction of phenolics with other food components [323].

8.1. Liquid chromatography

Many liquid chromatographic methodologies have been described in the literature for fractionation of tannins (proanthocyanidins) using Sephadex G-25 [324–326], Sephadex LH-20 [327–332]; Sepharose CL-4B [333], Fractogel (Toyopearl TSK-HW 40(s) gel) [334–336], Fractogel (Toyopearl) TSK 50(f) [183,337], inert glass microparticles [183] as well as C_{18} Sep-Pak cartridge [131,338–340].

Hoff and Singleton [333] developed a chromatographic procedure for separation of tannins from non-tannin materials using bovine serum albumin immobilized on Sepharose CL-4B as a column packing material. The non-tannin polyphenolics were first separated by washing the column with an acetate buffer. The protein–tannin complexes were then dissociated by elution of the column with organic solvents such as methanol or dimethylformamide to release and recover tannins.

The preparative isolation of proanthocyanins is, however, most commonly achieved by employing Sephadex LH-20 column chromatography [327,329,331,332]. The crude extract is applied to the column which is then washed with ethanol to elute the non-tannin substances. Following this, proanthocyanins are eluted with acetone/water or alcohol/water. For purification of proanthocyanins, the crude extract of phenolic substances is applied to a Sephadex LH-20 column which is then eluted with water containing increasing proportions of methanol [341].

Kennedy and Taylor [342] developed a high-performance gel chromatographic technique for the analysis of proanthocyanidins using two polysteryne–divinylbenzene columns (300 mm × 7.5 mm, 5 μm, 500 Å × 100 Å) coupled in series along with a guard column containing the same material (50 mm × 7.5 mm, 5 μm). *N*,*N*-Dimethylformamide containing 1% (v/v) glacial acetic acid, 5% (v/v) water and 0.15 M lithium chloride was used a mobile phase. It was stated that addition acetic acid to the mobile phase reduced the potential for oxidation while the addition of lithium chloride eliminated self-aggregation of proanthocyanidins. The estimated average molecular mass of fractionated proanthocyanidins varied from 1193 to 7023 for hop extracts and from 1111 to 11524 for grape skin extracts. Furthermore, a statistically significant correlation existed between the retention times and the average molecular mass of proanthocyanidins.

Derdelinckx and Jerumanis [334] employed Fractogel (Toyopearl TSK HW-40(s) gel) for separation of malt and hop proanthocyanidin dimers and trimers after chromatography of polyphenols on Sephadex LH-20 with methanol. The proanthocyanidin fractions were applied to the Fractogel column which was then eluted with methanol. The four major peaks of hops polyphenolics corresponded to the B3 and B4 procyanidin dimers and two unidentified procyanidin oligomers. On the other hand, malt polyphenolics were separated into a mixture of four proanthocyanidin trimers, two procyanidin trimers and an unknown procyanidin oligomer. According to Derdelinckx and Jerumanis [334], Fractogel (Toyopearl TSK HW-40(s) gel)

allows one to obtain the proanthocyanidins in an advanced state of purity.

Recently, Labarbe et al. [183] applied an inert glass powder (Pyrex microparticles, 200–400 μm) for fractionation of grapeseed or skin proanthocyanidins according to their degree of polymerization. Proanthocyanidins were extracted from samples with acetone/water (60:40, v/v). Following this, the non-tannin phenolics and proanthocyanidins were fractionated on a Fractogel (Toyoperl TSK HW-50(f) gel) column (35 cm × 8 cm), as described by Souquet et al. [178]. The non-tannin phenolics were washed out from the column with 2 bed volume of water followed by 5 bed volumes of ethanol/water/trifluoroacetic acid (55:44.05:0.05, v/v/v). Subsequently, proanthocyanidins were washed from the column with 3 bed volumes of acetone/water (60:40, v/v). Purified proanthocyanidins were dissolved in methanol and then applied onto the column filled with glass microparticles and equilibrated with methanol/chloroform (25:75, v/v) and massively precipitated on the top of column with chloroform. Proanthocyandins were sequentially eluted from the column using increasing proportions of methanol in a methanol/chloroform solvent system.

Oh et al. [323,343] employed a gel chromatographic technique to study the tannin–protein interaction. Tannins were immobilized on Sepharose-4B via epoxy activation. Protein was then applied to the column at pH 4 and the elution of protein from the column was achieved using a pH gradient or by anionic and nonionic detergents.

Salogoity-Auguste and Bertrand [339] as well as Jaworski and Lee [338] demonstrated that a C_{18} Sep-Pak cartridge can be used to separate grape phenolics into acidic and neutral fractions. Later, Sun et al. [340] successfully used a C_{18} Sep-Pak cartridge for fractionation of grape proanthocyanidins according to their degree of polymerization. The procedure involved passing the extract of grape phenolics through two preconditioned neutral C_{18} Sep-Pak cartridges connected in series. The phenolic acids were then washed out with water; catechins and oligomeric proanthocyanidins were subsequently eluted with ethyl acetate and anthocyanidins and polymeric proanthocyanidins with methanol. The ethyl acetate fraction was redeposited on the same C_{18} Sep-Pak cartridges and catechins were first eluted with diethyl ether and then oligomeric proanthocyanidins were eluted with methanol.

Fulcrand et al. [344] fractionated wine phenolics into simple (phenolic acids, anthocyanins, flavonols and flavanols) and polymeric components using a Fractogel (Toyopearl) HW-50(f) column (bed 12 mm × 120 mm). Simple phenolic were eluted from the column with ethanol/water/trifluoroacetic acid (55:45:0.005, v/v/v) while polymeric phenolics were then recovered with 60% (v/v) acetone. Later, Mateus et al. [335] employed a Fractogel (Toyopearl) HW-40(s) column for fractionation of anthocyanin-derived pigments in red wines. Two liters of wine were directly applied onto the Toyopearl gel column (200 mm × 16 mm i.d.) at a flow rate of 0.8 mL/min. Anthocyanins were subsequently eluted from the column with water/ethanol (20:80, v/v). The elution of wine phenolics from Toyopearl column yielded malvidin 3-glucoside and three derived pigments, namely malvidin 3-glucoside pyruvic adduct, malvidin 3-acetylglucoside pyruvic adduct and malvidin 3-coumarylglucoside pyruvic adduct. These glucosides accounted for 60% of the total monoglucosides content.

8.2. High-performance liquid chromatography

High performance liquid chromatographic (HPLC) techniques are now most widely used for both separation and quantitation of phenolic compounds. Tables 2–6 summarize some modern HPLC procedures applied for the analysis of various classes of plant phenolics. HPLC methodologies have also been employed for separation of proanthocyanidins, but their ability to provide complete mass distribution is still thwarted [345–347]. According to Yanagida et al. [348] the elution order does not follow the degree of polymerization and the peaks of highly polymerized oligomers tend to overlap on chromatograms. Shoji et al. [349] applied a combination of normal-phase chromatography and HPLC for separation and identification of apple procyanidins up to decamers. Various supports and mobile phases are available for the analysis of anthocyanins, procyanidins, flavonones, flavonols, flavan-3-ols, procyanidins, flavones and phenolic acids [321,350]. Introduction of reversed phase columns has considerably enhanced the HPLC separation of different classes of phenolic compounds [351]. Several reviews have been published on the application of HPLC methodology for the analysis of phenolics [320,321,352–354].

Plant phenolics are commonly detected using UV–vis and photodiode array (DAD) detectors [176,213,355–359]. Other methods used for the detection of phenolics include electrochemical coulometric array (EC) detector [360–361], chemical reaction detection technique [362] and fluorimetric detector [355,363]. A combination of HPLC technique and voltammetry has been successfully employed for detection, identification and quantification of flavonoid and non-flavonoid phenolics in wine. Positive identification may be obtained by comparing the capacity factor (k') and electrochemical behavior of wine phenolics with those of standard solutions containing pure compounds [364–366].

Mass spectrometric (MS) detectors coupled to high-performance liquid chromatograph (HPLC–MS tandem) have been commonly employed for structural characterization of phenolics. Electrospray ionization mass spectrometry (ESI-MS) has been employed for structural confirmation of phenolics in plums, peaches, nectarines [357], grapeseeds [358], soyfoods [367] and cocoa [345]. Satterfield and Brodbelt [368] demonstrated that complexation of flavonoids with Cu^{2+} enhanced the detection of flavonoids by ESI-MS. Mass spectra obtained for metal–flavonoids complexes were more intense and simpler for interpretation than those of corresponding flavonoids. Identification of phenolics collected after HPLC analysis was also carried out using fast atom bombardment mass spectrometry (FAB-MS) [213,361,369] and electron impact mass spectrometry [213]. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI–MS) has also been employed for qualitative and quantitative analysis of anthocyanins in foods [265].

Table 2
Some HPLC procedures for determination of catechins and proanthocyanidins (PA) in some plant sources

Food	Sample preparation	Stationary phase	Mobile phase	References
Grape seed	Extraction with EtOH; fractionation of PA using Sephadex LH-20	Exsil 100 ODS C_{18}, reversed-phase (250 mm × 4.6 mm, 5 μm) coupled to C_{18} column guard	A: 0.2% H_3PO_4 (v/v); B: 82% acetonitrile with 0.4% H_3PO_4; gradient: 100%A, 0%B–85%A, 15%B, 0–15 min; 85%A, 15%B–84%A, 16%B, 15–40 min; 84%A, 16%B–83%A, 17%B, 40–45 min; 83%A, 17%B–57%A, 43%B, 45–48 min; 57%A, 43%B–48%A, 52%B, 48–49 min; isocratic 48%A, 52%B, 49–56 min; 48%A, 52%B-57%A, 43%B, 56–57 min; 57%A, 43%B–83%A, 17%B, 57–58 min; 83%A, 17%B–100%A, 0%B, 58–60 min	Peng et al. [358]
Wine	Filtration; direct injection	Nucleosil 100 C_{18}, (250 × 4 mm, 5 μm) coupled to C_{18} column guard	A: 2 mM $NH_4H_2PO_4$, adjusted to pH 2.6 with H_3PO_4; B: 20%A with acetonitrile; C: 0.2 M H_3PO_4 adjusted to pH 1.5 with ammonia; gradient: 100%A, 0–5 min; 0–4% B, 5–15 min; 4–8%B, 15–25 min; 8%B, 92%C, 25.1 min; 8–20%B, 25.1–45 min; 20–30%B, 45–50 min; 30–40%, 50–55 min; 40–80%B, 55–60 min	Carando et al. [359]
Apples, grapes beans	Extraction with 90% MeOH (apples, grapes) or 70% MeOH (beans); filtration	Inertsil ODS –2 (150 × 4.6 mm, 5 μm) coupled with Opti-Guard PR C18 Violet A guard.	A: 5% acetonitrile in 0.025 M phosphate buffer pH 2.4; B: 25% acetonitrile in 0.025 M phosphate buffer pH 2.4; isocratic, 0–5 min, 10%B; 5–20 min, linear gradient: 5–20 min, 10–80%B; 20–22 min, 80–90%B; isocratic 22–25 min, 90%B; linear gradient, 25–28 min, 10%B; isocratic, 28–37 min, 10%B	Arts and Hollman [363]
Foods, beverages	Extraction with H_2O or 70% acetone; purification using Supercosil Envil-18 20mL SPE column conditioned with MeOH and then with H_2O; phenols eluted with acetone/H_2O/CH_3COOH (70:29.5:0.5)	Phenomenex Luna silica column (250 × 4.6 mm, 5 μm)	A: dichloromethane; B: MeOH; C: 50% CH_3COOH; gradient: 14%B and 4%C–28.4%B and 4%C, 0–30 min; 28.4%B and 4%C–50%B and 4%C, 30–60 min; 50%B and 4%C–86%B and 4%C, 60–65 min; 86%B and 4%C, 65–70 min	Lazarus et al. [363b]
Wine	Dealcoholized under vacuum; fractionation of procyanidins and catechins using two C_{18} Sep Pak cartridges in series	Superspher 100 RP18 (250 × 4 mm; 4 μm)	A: H_2O; B: H_2O–acetic acid (90:10, v/v); *catechins*: 10–80%B, 0–5 min; 80–100% B, 5–29 min; 100%B, 29–45 min; procyaidins: 10–70%B, 0–40 min; 70–85%B, 40–55 min; 85–100%B, 55–74 min	Sun et al. [340]

8.3. High-speed countercurrent chromatography

High-speed countercurrent chromatography (HSCCC; centrifugal partitioning chromatography) is an all-liquid chromatographic technique very suitable for preparative isolation of pure compounds [370,371]. Separation of compounds is based on their partitioning between two immiscible liquids [372].

Degenhart et al. [373] used HSCCC for preparative isolation of anthocyanins from red wines and grape skins. Anthocyanins were fractionated based on their polarities into four solvents systems. Solvent I, consisting of *tert*-butyl methyl ether/*n*-butanol/acetonitrile/water (2:2:1:5, v/v/v/v) containing 0.1% trifluoroacetic acid (TFA), was used as a medium for fractionation of monoglucosides and acylated diglucosides. Solvent II, consisting of ethyl acetate/*n*-butanol/water (2:3:5, v/v/v) and 0.1% TFA, was used as a medium for separation of visitins and diglucosides. Solvent III, consisting of ethyl acetate/water (1:1, v/v) and 0.1% TFA, was used as a medium for extraction of coumaryl and caffeoyl monoglucosides. Solvent IV, consisting of ethyl acetate/*n*-butanol/water (4:1:5, v/v/v) was employed as a medium for fractionation of acetylated anthocyanins.

Vitrac et al. [374] also applied HSCCC for fractionation of red wine phenolics. Phenolics were extracted first from red wine into ethyl acetate. Subsequently, the phenolic extract was chromatographed using a 1.5 cm × 60 cm cation exchange Dowex (Sigma) column. Non-phenolic constituents were washed out from the column with water and then phenolics were eluted with methanol/water (75:25, v/v). Afterwards, the phenolic extract was fractionated using centrifugal partition chromatography in both ascendant and descendant modes. The solvent systems water/ethanol/hexane/ethyl acetate in the ratios of 3:3:4:5 (v/v/v/v) and 7:2:1:8 (v/v/v/v) were used, at a flow rate of 3 mL/min, for elution of phenolics in ascendant and descendant modes, respectively.

Degenhart et al. [375] demonstrated that HSCCC can be used for isolation of theaflavins, epitheaflavic acids and thearubigins from black tea using hexane/ethyl acetate/methanol/water (2:5:2:5 and 1.5:5:1.5:5, v/v/v/v). Theaflavins prior HSCCC were extracted from tea infusion with ethyl acetate and then cleaned up using a Sephadex LH-20 column to avoid coelution of catechins and theaflavins. On the other hand, isolation of thearubigins required cleaning up tea infusion on an Amber-

Table 3
Some HPLC procedures for determination of anthocyanins and anthocyanidins in some plant sources

Food	Sample preparation	Stationary phase	Mobile phase	References
Red onions	Extraction with MeOH containing 0.1% HCl, filtration	Prodigy ODS2 (250 mm × 4.6 mm, 5 μm)	A: 10% formic acid in H_2O (v/v); B: MeOH–H_2O–formic acid (50:40:10, v/v/v); gradient: 20%B, 0–4 min; 20–80%B, 4–26 min	Gennaro et al. [52]
Red wine fruit juices	Dealcoholization of wine under vacuum; dilution of fruit juice with water; separation of anthocyanins using C_{18} Sep-Pak	SPLC-18-DB (250 mm × 10 mm, 5 μm) preparative reverse-phase coupled with preinje-ction C_{18} saturator with silica-based packing (75 mm × 4.5 mm, 12 μm) and guard with Supelco LC-18 reverse-phase packing (50 mm × 4.6 mm, 20–40 μm)	A: 5% formic acid in H_2O; B: formic acid–H_2O–MeOH (5:5:90, v/v/v); gradient: 5–20%B, 0–1 min; 20–25%B, 1–12 min; 25–32%B, 12–32 min; 32–55%B, 32–38 min; 55–100%B, 38–44 min; 100%B, 44–46 min; 100–5%B, 46–47 min	Wang and Sporns [265]
Red wine	Direct injection	Ultrasphere (C18) ODS (250 mm × 4.6 mm; 5 μm)	A: H_2O–formic acid (9:1, v/v); B: CH_3CN–H_2O–formic acid (3:6:1, v/v/v); gradient: 20–85% B, 0–70 min; 85–100%B, 70–75 min; 100%B, 75–85 min	Mateus et al. [335a]
Bilberry	Extraction with 50%MeOH containing 2%HCl for 20min, filtration	Ultrasphere ODS column (250 mm × 4.6 mm, 5 μm)	A: 0.4%TFA in H_2O; B: 0.4% TFA in acetonitrile; gradient: 15% B, 0–6 min; 15–22%B, 6–20 min; 22–30%B, 20–35 min;	Zhang et al. [335b]
Red blood orange juice	Homogenization with (acetone–EtOH–hexane, 25:25:50, v/v/v), centrifugation, concentration of acetone–EtOH layer, separation of anthocyanins using C_{18} Sep-Pak	Prodigy ODS3 column (150 mm × 4.6 mm, 5 μm)	A: 0.1% phosphoric acid in H_2O; B: 0.1% phosphoric acid in acetonitrile; gradient: 10%B, 0–2 min; 10–50% B, 2–32 min; 50%B, 32–37 min; 50–70%B, 37–57 min	Lee [335c]

Table 4
Some HPLC procedures for determination of flavones and flavonols in some plant sources

Food	Sample preparation	Stationary phase	Mobile phase	References
Red onions	Extraction with MeOH stabilized with BHT; dilution with MeOH	Supelcosil LC-18 (250 mm × 4.6 mm, 5 μm) column coupled with a Spherisorb Supelguard LC-18	A: 0.01 M sodium phosphate adjusted to pH 2.5 with H_3PO_4; B: MeOH; linear gradient: 87–60%A in B, 0–13.5 min; 60–10%A in B, 13.5–39 min; 10–0%A in B, 39–42 min; 0–87%A in B, 42–46 min	Gennaro et al. [52]
Orange peel oils	Filtration, chilling to precipitate waxes, molecular distillation, residue taken to analysis	OmniSpher C18 column (125 mm × 2 mm; 3 μm).	A: 35% aqueous acetonitrile containing 0.01% formic acid; B: acetonitrile containing 0.01% formic acid. Gradient: A–B, 0–55 min	Weber et al. [335d]
Spinach	Extraction of freeze dried sample with 40% MeOH for 20 h at 4 °C; centrifugation	Phenomenex Luna phenyl-hexyl column (250 mm × 4.6 mm, 5 μm) coupled with security guard column Phenomenex C_{18} ODS (4 mm × 3 mm)	A: H_2O/MeOH/formic acid (69:30:1); B: MeOH; gradient: 15–45%B, 0–18 min; 45–100%B, 18–23 min; 100%B, 23–27 min	Bergquist et al. [214]
Buckwheat	Extraction with 80% MeOH, filtration, evaporation, dissolving in MeOH–H_2O–oxalic acid (13:36:1, v/v/v) filtration	Capcell Pak C_{18}-SG 120, column, (100 mm × 4.6 mm, 3 μm)	A: MeOH–H_2O–acetic acid (13:36:1, v/v/v); B: MeOH–H_2O–acetic acid (73:25:2, v/v/v); gradient: 10–50%B in A, 0–20 min; 50%B in A, 20–25 min; 50–10%B in A, 25–30 min	Oomah and Mazza [335e]
Tomatoes onions lettuce celery	Extraction with 1.2 M HCl in 50% MeOH for 2 h at 90 °C; extract adjusted to pH 2.5 with TFA, filtration	C_{18} symmetry (150 mm × 3.9 mm, 5 μm) reversed-phase column, coupled with C_{18} symmetry guard	A: acetonitrile; B: H_2O adjusted to pH 2.5 with TFA; gradient: 15–35% A in B, 0–20 min	Crozier et al. [51]
Edible tropical plants	Extraction with 1.2 M HCl in 50% MeOH for 2 h at 90 °C, filtration	Nova Pak C_{18} column (150 mm × 3.9 mm; 4 μm)	Isocratic: MeOH–H_2O (1:1, v/v) adjusted to pH 2.5 with TFA	Miean and Mohamed [335f]

Table 5
Some HPLC procedures for determination of other classes of phenolics in selected plant sources

Food	Phenolics	Sample preparation	Stationary phase	Mobile phase	References
Finger millet	Free phenolic acids	Extraction with 70% EtOH, centrifugation, concentration, adjusting pH to 2–3, extraction with ethyl acetate, evaporation, dilution in MeOH	Shimpak C_{18} (250 mm × 4.6 mm) reversed-phase column	Isocratic: H_2O–acetic acid–MeOH (80:5:15, v/v/v)	Subba Rao and Muralikrishna [335g]
Barley	Phenolic acids	Extractions: hot H_2O; acid hydrolysis; acid and α-amylase hydrolysis; acid and α-amylase and cellulase hydrolysis; centrifugation	Supelcosil LC-18 column (150 mm × 4.6 mm, 5 μm)	A: 0.01M citrate buffer pH 5.4 adjusted with 50% acetic acid; B: MeOH; gradient: 2–4%B, 0–12 min; 4–13%B, 12–20 min; 13%B, 20–26 min; 13–2%B, 26–30 min	Yu et al. [79]
Citrus fruits	Coumarins	Extraction with acetone, filtration, evaporation dissolving in MeOH-acetone (1:1, v/v), filtration	Hypersil ODS column (125 mm × 4 mm, 5 μm)	Isocratic: MeOH–H_2O (75:25, v/v)	Ogawa et al. [335h]
Rice bran oil	γ-Oryzanol	Solubilization of oil in hexane–ethyl acetone (9:1, v/v), removal of lipids using silica column (250 mm × 25 mm)	Microsorb-MV C_{18} column (250 mm × 4.6 mm)	Isocratic: MeOH–acetonitrile–dichloromethane–acetic acid (50:44:3:3, v/v/v/v)	Xu and Golber [335i]
Rye	Phenolic acids, ferulic acid dehydrodimers	Enzymatic removal of starch; saponification: 2 M NaOH, 1 h, 25 °C; adjustment pH < 2; extraction of phenolics with ethyl acetate	LiCroCART 100 Merck RP C_{18} column	Solvent A: 0.02 M phosphate buffer (pH 2.15; solvent B: MeOH + A (40:60); 0–50 min isocratic (75%A + 25%B); linear over 50 min to 100%B; isocratic over 20 min 100%B	Andreasan et al. [335j]
Herbal medicines	Phenolic acids	Sonication with methanol:water:TFA (50:50:1) for 30 min; centrifugation, filtration through 0.45 μm nylon filter	Agela XBP C_{18} column (150 mm × 4.6 mm, 5 μm)	A: 0.02% TFA in water; B: 0.02% TFA in MeOH; gradient: 0–5 min 25%B; 5–10 min 25–30%B; 10–16 min 30–45%B; 16–18 min 45%B; 18–25 min 45–80%B; 25–30 min 80%B; 30–40 min 80–25% B	Wen et al. [335k]
Wheat	Phenolic acids	Grain homogenized with 4M NaOH and then shaken for 2 h in the dark, acidification pH < 2; centrifugation, extraction of phenolic with ethyl acetate	C18 ODS reversed phase column (250 mm × 4.6 mm, 5 μm) coupled with precolumn (30 mm × 4.6 mm)	A: MeOH; B: 10mM phosphate buffer pH 3.5; gradient: 15%A, 0–10 min; 15–20% A, 10–20 min; 20–30%A, 20–30 min; 30–40%A, 30–35 min; 40–55%A, 35–40 min; 55–80%A, 40–45 min; 80–100%A, 45–47 min; 100%A, 47–55 min; 100–15%A, 55–57 min; 15%A, 57–60 min	McKeehen et al. [103]
Oregano leaves	Phenolic acids	Sequential extraction of leaves with hexane, ethyl acetate, dichloromethane, MeOH (soxhlet, 6 h each solvent); methanolic extract was concentrated, flushed with nitrogen	RP C_{18} column (250 mm × 4.6, 10 μm) column	A: H_2O/2%CH_3COOH; B: MeOH/2%CH_3COOH; gradient 6–37%B, 0–60 min; 37–100%B, 60–70 min; 100%B, 70–90 min; 100–6%B, 90–105 min; 6%B, 105–130 min	Gerothanassis et al. [335l]
Pineapple juice	Sinapyl derivatives	Centrifugation; filtration; filtrate passed through MeOH-activated C_{18} cartridge; cartridge washed with 0.01% HCl and then MeOH; MeOH filtrate stored over night at 0 °C; flitration; evaporation	Supelco LC-18 column (250 mm × 4.6 mm, 5 μm)	A: MeOH; B: acetonitrile; C: phosphate buffer pH 2.4; gradient: 10%A/90%C–22%A/78%C, 0–10 min; 22%A/78%C–22%A/25%B/53%C, 10–35 min	Wen et al. [335m]
Wine	Phenolic acids	Extraction with ethyl acetate	Phenomenex Luna C_{18} column (150 mm × 4.6 mm, 5 μm) coupled with Phenomenex C_{18} ODS guard column (4 mm × 3 mm)	A: 0.1% aqueous formic acid; B: MeOH; gradient: 5–30%B, 0–50 min; 30%B, 50–65 min; 30–5%B; 5% B, 65–75 min	Robbins and Beans [335n]
Eggplant	Phenolic acids	Pressurized liquid extraction with MeOH, EtOH, acetone and MeOH/H_2O	Phenomenex Luna C_{18} column (150 mm × 4.6 mm, 5 μm) coupled with Phenomenex C_{18} ODS guard column (4 mm × 3 mm)	A: 0.1% aqueous formic acid; B: MeOH; gradient: 5–30%B, 0–50 min; 30%B, 50–65 min; 30–5%B; 100%B, 65–75 min	Luthria and Mukhopadhyay [239], see also Robbins and Beans [335n]

Table 6
Some HPLC procedures for determination of multiple classes of phenolics in selected foods

Food	Phenolics	Sample preparation	Stationary phase	Mobile phase	References
Lingoberry, cranberry, onions, broccoli	Catechins, flavanones, flavones, flavonols	Extraction with 1.2M HCl in 50% MeOH for 2 h at 90 °C; filtration	Inertsil ODS (150 mm × 4 mm; 3 μm) column coupled with C-18 guard	A: 50 mM H_3PO_4 pH 2.5; B: acetonitrile; catechins: 86%A in B, isocratic; other flavonoids—gradient: 95%A in B, 0–5 min; 95–50%A in B, 5–55 min; 50%A in B, 55–65 min; 50–95%A in B, 65–67 min	Mattila et al. [360]
Nectarines, peaches, plums	Phenolic acids, catechins, flavonols, procyanidins	Extraction with 80% MeOH containing 2 mM NaF; centrifugation, filtration	Nucleosil C_{18} (150 mm × 4.6 mm, 5 μm) reversed-phase column coupled with guard containing the same stationary phase	A: 5%MeOH in H_2O; B: 12%MeOH in H_2O; C: 80%MeOH in H_2O; D: MeOH; gradient: 100%A, 0–5 min; 0–100%B in A, 5–10 min; 100%B, 10–13 min; 100–75%B in C, 13–35 min; 75–50%B in C, 35–50 min; 50–0%B in C, 50–52 min; 100%C, 52–57 min; 100%D 57–60 min	Tomas-Barberan et al. [357]
Red raspberry	Ellagic acids, flavones	Extraction with MeOH, filtration, addition of H_2O, evaporation, semi-purification of phenolics using Sep-Pak C18, filtration	Lichrocart 100 RP-18 (250 mm × 4 mm, 5 μm) reversed-phase column	A: 5% formic acid in H_2O; B: MeOH; gradient: 10–15%B in A, 0–5 min; 15–30%B in A, 5–20 min; 30–50%B in A, 20–35 min; 50–90% B in A, 35–38 min	Zafrilla et al. [367]
Buckwheat herbs	Rutin, chlorogenic acids	Maceration with 30% MeOH, 60 °C for 2 h	Eurospher-100 RP8 column (250 mm × 4 mm, 5 μm)	A: H_2O/MeOH/CH_3COOH (90:10:0.5); B: MeOH/CH_3COOH (100:0.5); gradient: 10–40%B, 0–6 min; 40% B, 6–9 min; 40–35% B, 9–10 min; 35%B, 10–18 min; 35–100%B, 18–19 min; 100%B, 19–32 min; 100–10%B, 32–33 min; 10%B, 33–40 min	Hinneburg and Neubert [243]
Spinach	Flavonols, flavanones	Extraction with 70% MeOH, removal of carotenoids and chlorophyll using ODS-C_{18} packing material, centrifugation, concentration	YMC ODS-AQ column (250 mm × 4.6 mm, 5 μm)	A: H_2O containing 0.01% TFA; B: acetonitrile containing 0.01% TFA; gradient: 100%A, 0–10 min; 100–50%A in B, 10–40 min; 50–0%A in B, 40–50 min	Edenharder et al. [213]
Artichoke	Phenolic acids, flavonoids	Extraction with 60%aqueous MeOH, 1 h, evaporation, dissolving in H_2O; purification and fractionation using MeOH-activated C_{18} reversed phase cartridge. Phenolic acids were eluted with 10%aqueous MeOH, and then flavonoids with MeOH	Phenomenex C18 Hydro-Synergi column (150 mm × 3.0 mm, 4 μm) coupled with Phenomenex C_{18} ODS guard column (4 mm × 3 mm)	A: 2% CH_3COOH; B: 0.5% CH_3COOH/acetonitrile (50:50); gradient: 10–18%B, 0–20 min; 18–24%, 20–30 min; 24–30%B, 30–45 min, 30%B, 45–65 min; 30–55%B, 65–70 min; 55–100%B, 70–75 min; 100%B 75–83 min, 100–10%B, 83–85 min	Schütz et al. [335o]
Commercial grains	Phenolic acids, alkyl- and alkenyl-resorcinols	Phenolic acids extracted with MeOH/10% CH_3COOH (85:15); bound phenolic acids were liberated by acid and alkaline hydrolysis. Other phenolics were extracted 80% MeOH	Inertsil ODS-3 column (150 mm × 4 mm, 3 μm) with a C18 guard column was used for phenolic acids. Nova Pak C18 column was employed for other phenolics	Phenolic acids: A: phosphate buffer pH 2.5; B: acetonitrile. Gradient: 95%A, 0–5 min; 95–85%A, 5–17 min; 85–80%A, 17–40 min; 80–50%, 40–60 min; 50%A, 60–65 min; 50–95%A, 65–67 min Other phenolics: A: phosphate buffer pH 2.4; B: MeOH; gradient: 5–60%B, 0–50 min; 60–90%B, 50–56 min; 90%B, 56–68 min; 90–100%B, 68–100 min	Mattila et al. [74]
Mango peels	Flavonols xanthones	Extraction with 80% acetone for 3 h, residue extracted again with same solvent for 10 min, evaporation, aqueous extract passed through column filled with polyamide CC6 (0.05–0.16 mm), phenolics recovered with MeOH	Phenomenex C18 Hydro-Synergi column (150 mm × 3.0 mm, 4 μm) coupled with Phenomenex C_{18} ODS guard column (4 mm × 2 mm)	A: 2% CH_3COOH B: 0.5% CH_3COOH/acetonitrile (50:50); gradient: 0–25%B, 0–15 min; 25–30%B, 15–50 min; 30–80%B, 50–60 min; 80–100%B, 60–65 min; 100–0%B, 65–65.5 min	Schieber et al. [335p]
Apple ciders	Phenolic acids, dihydrochalcones, flavonols, procyanidins	Degassing, filtration	Nucleosil 120 C_{18} reversed phase column (250 mm × 4.6 mm, 3 μm)	A: 2% CH_3COOH; B: MeOH; gradient: 0–45%B, 0–55 min; 45%B, 55–75 min	Madrera et al. [126]

lite XAD-7 column prior to HSCCC to remove all non-phenolic compounds.

A simple and efficient method for separation of catechin gallates from spray-dried tea extract was developed by Baumann et al. [376]. Tea phenolic extract was first subjected to liquid–liquid partitioning between ethyl acetate and water. The organic layer containing catechins was then submitted to high-speed centrifugal countercurrent chromatography operating in an ascending mode. Favorable partitioning was achieved using *n*-hexanes/ethyl acetate/water (1:5:5, v/v/v) or ethyl acetate/methanol/water (5:1:5 and 5:2:5, v/v/v). Sephadex LH-20 column with methanol as a mobile phase was used for a final purification of catechin gallates.

8.4. Other chromatographic techniques

Other chromatographic techniques have also been employed for purification and separation of food phenolics. Of these, paper chromatographic (PC) and thin-layer chromatographic (TLC) techniques are still widely used for purification and isolation of anthocyanins, flavonols, condensed tannins and phenolic acids using different solvent systems [196,267,282,377–384].

PC on Whatman No. 3 has been employed for separation of anthocyanins using butanol/acetic acid/water, chloroform/acetic acid/water, or butanol/formic acid/water as possible mobile phases [282]. On the other hand, two-dimensional PC has been used for the analysis of procyanidin oligomers. Chromatograms were developed using 6% acetic acid as a mobile phase in the first direction and 2-butanol/acetic acid/water as mobile phase in the second direction [334,385].

Azar et al. [386] have identified phenolics of bilberry juice *Vaccinium myrtillus* using a two dimensional TLC. Phenolic acids were chromatographed on a 0.1 mm cellulose layer with solvent I: acetic acid/water (2:98, v/v) and solvent II: benzene/acetic acid/water (60:22:1.2, v/v/v). However, TLC analysis of flavonols was carried out on silica gel plates using ethyl acetate/methyl/ethyl/ketone/formic acid/water (5:3:1:1, v/v/v/v) or on cellulose plates using solvent I: *t*-butanol/acetic acid/water (3:1:1, v/v/v) and solvent II: acetic acid/water (15:85, v/v). The phenolic acids were detected by first spraying the chromatograms with deoxidized *p*-nitroaniline and then with a 15% solution of sodium carbonate in water, while flavonols were detected by spraying with a 5% aluminum chloride solution in methanol. Two-dimensional cellulose TLC plates have also been employed for separation of procyanidins. *t*-Butanol/acetic acid/water (3:1:1, v/v/v) was used for the development in the first direction while 6% acetic acid was used for the development in the second direction. Detection of polyphenols on TLC was carried out using ferric chloride, potassium ferricyanide or vanillin–HCl solutions [320]. TLC on silica using ethyl acetate/formic acid/water (90:5:5, v/v/v) or toluene/acetone/formic acid (3:3:1, v/v/v) has been used for monitoring the isolation of procyanidins by column chromatography [387] and by HPLC [355], respectively. On the other hand, phenolic acids have been separated on silica TLC plates using *n*-butanol/acetic acid/water (40:7:32, v/v/v) as a mobile phase [388]. Furthermore, TLC on microcrystalline cellulose using 1-propanol/water/acetic acid (20:80:1, v/v/v) has been successfully employed for separation of five major green tea catchins (EC, GC, EGC, ECG, EGCg) [389].

Various gas chromatographic (GC) methodologies have been employed for separation and quantitation of phenolic acids [255,356], isoflavones [390], capsaicinoids [391], phenolic aldehydes [392] and monomers of condensed tannins [393]. Novel high-temperature gas chromatographic columns, electronic pressure controllers and detectors have significantly improved the resolution and have also led to an increase in the upper range of molecular weights of substances that could be analyzed by GC. Preparation of samples for GC may include the removal of lipids from the extract, liberation of phenolics from ester and glycosidic bonds by alkali [255,356], acid [394] and enzymatic hydrolysis [390] or acid depolymerization of tannins in the presence of nucleophiles such as phloroglucinol [393] or benzyl mercaptan [131,183]. Prior to chromatography, phenolics are usually transformed to more volatile derivatives by methylation [234,395,396], trifluoroacetylation [397,398], conversion to trimethylsilyl derivatives [255,356] or derivatization with *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoro-acetamide [393].

9. Application of capillary electrophoresis

Capillary electrophoresis is a novel and versatile analytical tool for separation of many classes of compounds [399,400] based on the electrophoretic migration of charged analytes. Small internal diameter capillary columns minimize the ohmic heating problems that may have an adverse effect on bandwidths. In addition small sample sizes can be used and separations require little or no organic solvents [399]. Various separation techniques, based on the principle of analyte migration in the electric field, have been developed. These include capillary zone electrophoresis, micellar electrokinetic chromatography, capillary electrochromatography, capillary isoelectric focusing and capillary isotachophoresis [401]. Capillary electrophoresis techniques have many advantages over HPLC methodology. These include less stringent purification of sample, minimum consumption of chemicals, better resolution, higher efficiency and simultaneous separation and identification of complex multicomponent mixture of structurally different chemical species at the same time involving highly polar compounds [400–404]. According to Herrero et al. [402] capillary techniques have a great potential for a broader application in separation of natural multicomponent mixtures after solving such issues as reproducibility and sensitivity. During the last 5 years more than 20 reviews on advances in the application of electromigration methods for analysis of natural antioxidants, foods and food components have been published [405]. Phenolics present in grapes, wines, olives, spices, medicinal herbs, tea, fruits and oilseeds have been studied using electromigration methods [402,403,405].

Hall et al. [406] used capillary electrophoresis for separation of food antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Later, Andrade et al. [407] utilized capillary zone electrophoresis to evaluate the effect of grape variety and wine ageing on the composition

of non-colored phenolics in port wine. Non-colored phenolics were extracted from wine into diethyl ether, then concentrated to dryness and redissolved in methanol. Subsequently, phenolics were separated on a fused-silica capillary column with a 0.1 M sodium borate (pH 9.5) at 30 °C and the voltage of 20 kV producing a current of 90 μA. Subsequently, Peng et al. [408] utilized capillary electropheresis with electrochemical detection for simultaneous determination of catechin, epicatechin and *trans*-resveratrol in red wine. The analytes, under optimum conditions, were completely separated in 20 min with the detection limits between 2×10^{-7} and 5×10^{-7} g/mL.

Moane et al. [409] utilized capillary electrophoresis for direct detection of phenolic acids in beer. Separation of phenolic acids was performed with fused silica capillary in 25 mM phosphate buffer, pH 7.2 at 25 kV. The sample was injected to capillaries using a reversed-polarity injection technique to remove non-phenolic cationic and neutral compounds. These substances interfere with electrochemical detection of phenolic acids by passivation of the electrode surface. Recently, Pan et al. [410] developed a method for determination of protocatechuic aldehyde and protocatechuic acid by capillary electrophoresis with amperometric detection. Under optimum conditions these two analytes were completely separated in 8 min with a detection limit of 0.10 g/mL for protocatechuic aldehyde and 0.25 μg/mL for protocatechuic acid.

Chu et al. [411] separated pure forms of *cis*- and *trans*-resveratrol isomers from wine using capillary electrophoresis in micellar mode. Direct separation of resveratrols in wine samples was performed with fused silica capillaries in 25 mM sodium borate, 25 mM sodium phosphate and 75 mM SDS (pH 9.3) at 16 kV and 20 °C using a UV detector set at 310 nm. The detection limit of the method was 1.25 mM. On the other hand, Kreft et al. [412] utilized capillary electrophoresis with a UV detector for determination of rutin content in different fractions of buckwheat flour and bran. The extraction of rutin from buckwheat fractions was carried out using 60% ethanol containing 5% ammonia in water. Identification of both resveratrol and rutin was confirmed by spiking the samples with standards.

Capillary electrophoresis has been used for separation of limonoid glucosides in citrus seeds [413] as well as limonoid glucosides and phlorin in citrus juices [414]. Recently, Braddock and Bryan [415] applied capillary electrophoresis for quantification of limonin glucoside and phlorin in extracts from citrus byproducts. The separation of phenolics was performed using a fused silic capillary in 75 mM borax (pH 9.4) at 15 kV. The phenolics were detected using UV detector set up at 214 nm. The limits of detection for limonin glucoside and phlorin were 2.0 and 0.2 mg/L, respectively.

Crego et al. [416] optimized conditions for separation of complex mixture of rosemary phenolics by capillary electrophoresis. The separation was performed using fused-silica capillary, 53 cm in length, and the best resolution was achieved with 50 mM tetraborate buffer at pH 9.5 and 10% acetonitrile. Reproducible results (%relative standard deviation (R.S.D.) of 2.91–5.14% for peak area and R.S.D. of 0.57–0.79% for analysis time) were obtained in less than 16 min. Later Herrero et al. [401] quantitatively characterized rosemary phenolics using capillary electrophoresis coupled with orthogonal electrospray to mass spectrometry. The separation was carried out using bare fused-silica capillary with 50 μm i.d. The reproducibility of this procedure expressed as R.S.D. was 1% for analysis time and 5.9% for peak area. Six phenolics, namely isoquercitrin, carnosic acid, rosmarinic acid, homoplantaginin and gallocatechin were detected using this methodology.

Horie et al. [417] reported a separation of five catechins together with ascorbic acid, caffeine and theanine in green tea infusions by capillary zone electrophoresis techniques. Later, Larger et al. [418] utilized micellar electrochromatography with UV detection for separation detection of flavonoids in green and black tea infusions. (−)-Epicatechin gallate and (+)-catechin were only detected in green tea, but (−)-epicatechin, (−)-epigallocatechin gallate, and (−)-epicatechin were found in both teas. Subsequently, Bonoli et al. [419] successfully applied micellar electrochromatography for detection of catechins in green tea, namely (+)-catechin, (−)-epigallocatechin, (−)-gallocatechin, (−)-gallocatechingallate, (−)-epigallocatechin-3-gallate, (−)-epicatechingallate, and (−)-epigallocatechin gallate. Futhermore, Cifuentes et al. [420] demonstrated that the separation of complex mixtures of procyanidin B3, procyanidin B2, procyanidin B1, (+)-catechin, (−)-epicatechin, *cis*- and *trans*-*p*-coumaric acids can be achieved in less than 5 min with the application of micellar electrochromatography technique. Herrero-Martinez et al. [421] reported that micellar electrochromatography technique could be used for separation of major products of acid depolymerization of proanthocyanidins in the presence of cysteine. Identification of the depolymerization products provides valuable information about composition and size of proanthocyanidins present in the extraction mixture.

10. Conclusions

Consumption of fruits, vegetables and cereals rich in dietary phenolics may contribute to disease risk reduction and human health promotion. This paper provides only an overview of plant phenolics found in selected cereals, fruits and vegetables. A number of excellent books and reviews on various aspects of the chemistry and biological properties of plant phenolics have recently been published. Biological role of phenolics in plants is well described in books edited by Hemingway and Laks [422], Macheix et al. [423a], Scalbert [20] and Tomás-Barberán and Robins [423b]. More comprehensive reviews of the chemistry of plant phenolics are provided in books published by Haslam (1989), Macheix et al. [423a], Mazza [424], Mazza and Oomah [425], Scalbert [20] and Shahidi and Naczk [8]. Plant phenolics are structurally diversified class of phytochemicals. This makes the development of standardized procedures for simultaneous extraction of all phenolics a very difficult task. On the other hand many existing extraction procedures exhibit both low selectivity and low extraction yields. Therefore, there is still a need for a systematic research to improve both selectivity and extraction yields. Advances in subcritical fluid extraction are promising and these extraction procedures may be, in the near future, widely used for isolation of bioactive phytochemicals from plant materials. Moreover, there is a need to develop more

robust HPLC and capillary electrophoretic methodologies for simultaneous determination of important classes of phenolics in a sample at once.

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