Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1054 (2004) 95–111
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Review

Extraction and analysis of phenolics in food

Marian Naczk[a], Fereidoon Shahidi[b,*]

[a] *Department of Human Nutrition, St. Francis Xavier University, Antigonish, NS, Canada B2G 2W5*
[b] *Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL, Canada A1B 3X9*

Abstract

Phenolics are ubiquitous compounds found in all plants as their secondary metabolites. These include simple phenols, hydroxybenzoic acid and cinnamic acid derivatives, flavonoids, coumarines and tannins, among others. The extraction of phenolics from source materials is the first step involved in their analysis. While chemical methods are used for determination of total content of phenolics, chromatographic and spectrometric analyses are employed for identification and quantification of individual compounds present. This paper provides a summary of background information and methodologies used for the analysis of phenolics in foods and nutraceuticals.

Keywords: Phenols; Flavonoids; Cinnamic acid

Contents

1. Introduction 95
2. Extraction procedures 96
   2.1. Phenolic acids 97
   2.2. Wall-bound phenolics 97
   2.3. Flavonoids 97
   2.4. Anthocynins 98
   2.5. Proanthocyanidins 98
3. Purification and fractionation procedures 98
   3.1. Liquid–solid phase procedures 98
   3.2. Liquid–liquid procedures 99
4. Quantification of phenolics 100
   4.1. Spectrophotometric assays 100
   4.2. Chromatographic techniques 107
5. Conclusions 107
References 107

1. Introduction

Phenolic compounds are considered as secondary metabolites that are synthesized by plants during normal development [1,2] and in response to stress conditions such as infection, wounding, and UV radiation, among others [3,4]. These compounds occur ubiquitously in plants [1,5] and are a very diversified group of phytochemicals derived from phenylalanine and tyrosine (Fig. 1) [6–8]. Plant phenolics include simple phenols, phenolic acids (both benzoic and cinnamic acid derivatives), coumarins, flavonoids, stilbenes, hydrolyzable and condensed tannins, lignans, and lignins. In plant, phenolics may act as phytoalexins, antifeedants, attractants for pol-

* Corresponding author. Tel.: +1 709 737 8552; fax: +1709 737 4000.
E-mail address: fshahidi@mun.ca (F. Shahidi).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.08.059

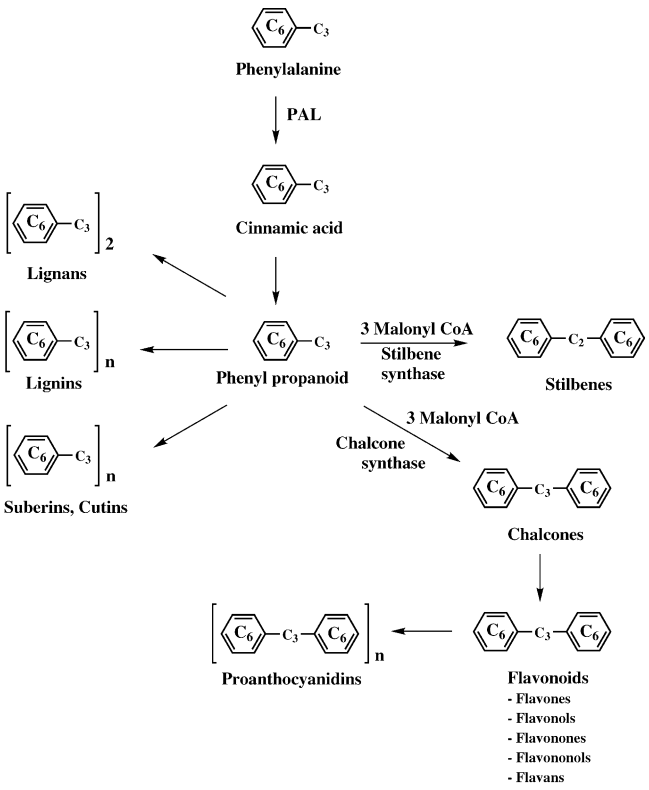


Fig. 1. Production of phenylpropanoids, stilbenes, lignans, lignins, suberins, cutins, flavonoids and tannins from phenylalanine (PAL).

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

Phenolics are not uniformly distributed in plants at the tissue, cellular and subcellular levels. Insoluble phenolics are the components of cell walls, while soluble phenolics are compartmentalized within the plant cell vacuoles [2,3,9–12]. At the tissue level, the outer layers of plants contain higher levels of phenolics than those located in their inner parts [9,13,14]. Cell wall phenolics, such as lignins (the polymer of monolignol units) and hydroxycinnamic acids are linked to various cell components [15–17]. These compounds contribute to the mechanical strength of cell walls, play a regulatory role in plant growth and morphogenesis and in the cell response to stress and pathogens [15,17–20]. Ferulic and *p*-coumaric acids, the major phenolic acids, 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]. It has been suggested that these cross-links may play a significant role in cell–cell adhesion [30], serve as a site for the formation of lignins [31–33] and contribute to the thermal stability of plant food texture [28].

A number of reviews on the analysis of polyphenolics have appeared [34–42b]. The assays used for the analysis of polyphenolics can be classified as either those which determine total phenolics content, or those quantifying an individual phenolic or a specific group or class of phenolic compounds. However, phenolic compounds must first be extracted from their source prior to any analysis.

2. Extraction procedures

Extraction 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 presence of interfering substances. The chemical nature of plant phenolics vary from simple to highly polymerized substances that include varying proportions of phenolic acids, phenylpropanoids, anthocyanins and tannins, among others. They may also exist as complexes with carbohydrates, proteins and other plant components; some high-molecular-weight phenolics and their complexes may be quite insoluble. Therefore, phenolic extracts of plant materials are always a mixture of different classes of phenolics that are soluble in the solvent system used. Additional steps may be required for the removal of unwanted phenolics and non-phenolic substances such as waxes, fats, terpenes and chlorophylls. Solid phase extraction (SPE) techniques and fractionation based on acidity are commonly used to remove unwanted phenolics and non-phenolic substances [42b].

Solubility of phenolic compounds is governed by the type of solvent (polarity) used, degree of polymerization of phenolics, as well as interaction of phenolics with other food constituents and formation of insoluble complexes. Therefore, there is no uniform or completely satisfactory procedure that is suitable for extraction of all phenolics or a specific class of phenolic substances in plant materials. Methanol, ethanol, acetone, water, ethyl acetate and, to a lesser extent, propanol, dimethylformamide, and their combinations are frequently used for the extraction of phenolics [34]. Palma and Taylor [58] used supercritical mixture of carbon dioxide and alcohol for the extraction of polyphenolics from de-oiled grapeseed. According to these authors methanol was a better carbon dioxide modifier than ethanol. Later, Murga et al. [59] demonstrated that the solvent capacity of the supercritical mixture of carbon dioxide and methanol was affected by the operation conditions. Recently, Ashraf-Khorassani and Taylor [60] developed a sequential extraction of de-oiled grapeseed to fractionate phenolics into monomeric phenolics and procyanidins. A methanol-modified carbon dioxide was first used to extract catechins and epicatechins and then pure methanol was employed for extraction of procyanidins from the seeds. Up to 80% of catechins and epicatechins, present in the seeds, were extracted using 40% methanol-modified carbon dioxide solvent system.

Extraction periods, usually varying from 1 min [72] to 24 h [73–75], have been reported. Longer extraction times

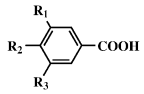
increase the chance of oxidation of phenolics unless reducing agents are added to the solvent system [76]. On the other hand, Naczk and Shahidi [64] and Naczk et al. [46] found that a two-stage extraction with 70% (v/v) acetone, 1 min each, using a Polytron homogenizer at 10,000 rpm, was sufficient for 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 [77] demonstrated that the optimum extraction time required for dry bean phenolics was 50–60 min.

The recovery of polyphenols from food products is also influenced by the ratio of sample-to-solvent (*R*). Naczk and Shahidi [46,64] found that changing *R* from 1:5 to 1:10 (w/v) increased the extraction of condensed tannins from commercial canola meals from 257.3 to 321.3 mg per 100 g of meal and total phenolics from 773.5 to 805.8 per 100 g of meal when using 70% acetone.

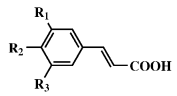
Deshpande and Cheryan [62] demonstrated that the yield of tannin recovery from dry beans was strongly influenced by variations in the sample particle size. They found that the amount of vanillin assayable tannins decreased by about 25–49% as the minimum size was reduced from 820 to 250 μm.

2.1. Phenolic acids

Krygier et al. [43] extracted free and esterified phenolic acids (Fig. 2) from oilseeds using a mixture of methanol–acetone–water (7:7:6, v/v/v) at room temperature. Following this, free phenolics were extracted with diethyl ether from the acidified aqueous suspension of phenolic extract and then the water suspension of the extract was 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 residue after exhaustive extraction with a mixture of methanol–acetone–water, was treated with 4 M NaOH under nitrogen to liberate insoluble-bound phenolic acids. Krygier et al. [43] reported alkaline hydrolysis may lead to significant losses of hydroxycinnamic acid derivatives. Recently, Nardini et al. [44] reported that the addition of ascorbic acid (1%) and ethylenediaminetetraacetic acid (EDTA; 10 mM) prevented the degradation of phenolic acids during alkaline hydrolysis. Other solvents, such as ethanol, acetone, and chloroform have also been used for the extraction of phenolics, often with different proportions of water [34,45,46a,b].



Acid		R_1	R_2	R_3
p-Hydrobenzoic	4-Hydroxybenzoic	H	OH	H
Protocatechuic	3,4-Dihydroxybenzoic	OH	OH	H
Vanillic	4-Hydroxy-3-methoxybenzoic	OCH_3	OH	H
Syringic	3, 5-Dimethoxybenzoic	OCH_3	OH	OCH_3
Gallic	3,4,5-Trihydroxybenzoic	OH	OH	OH



Acid		R_1	R_2	R_3
p-Coumaric	4-Hydroxycinnamic	H	OH	H
Caffeic	3,4-Dihydroxycinnamic	OH	OH	H
Ferulic	4-Hydroxy-3-methoxycinnamic	OCH_3	OH	H
Sinapic	4-Hydroxy-3,5-dimethoxycinnamic	OCH_3	OH	OCH_3

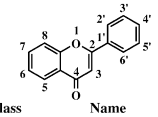
Fig. 2. Phenolic acids found in foods and nutraceuticals.

2.2. Wall-bound phenolics

A sequential alkaline hydrolysis procedure has been developed for the extraction of wall-bound phenolics [47,48]. The cell wall material was first isolated from the plant tissue [49] and then it was sequentially extracted with 0.1 M NaOH (1 h, 25 °C), 0.1 M NaOH (24 h, 25 °C), 1 M NaOH (24 h, 25 °C), and 2 M NaOH (24 h, 25 °C). Each alkaline extract was acidified with HCl to pH < 2 and then extracted three times with ethyl acetate to recover free phenolics.

2.3. Flavonoids

Flavonoids (Fig. 3) are commonly extracted from plant materials with methanol, ethanol, water or their combina-



Class	Name	Substitutions	Dietary Source
Chalcone	Butein	2, 4, 3', 4' - OH	Miscellaneous
(ring C noncyclized)	Okanin	2, 3, 4, 3', 4' - OH	Miscellaneous
Flavone	Chrysin	5, 7 - OH	Fruit skins
	Apigenin	5, 7, 4' - OH	Parsley, celery
Flavonone	Naringin	5, 4', - OH; 7 - rhamnoglucose	Citrus, grapefruit
	Naringenin	5, 7, 4' - OH	Citrus
	Taxifolin	3, 5, 7, 3', 4', - OH	Citrus
	Eriodictyol	5, 7, 3', 4' - OH	Lemons
	Hesperidin	3, 5, 3' - OH, 4' - OMe; 7 - rutinose	Oranges
	Isosakuranetin	5, 7 -OH; 4' - OMe	Citrus
Flavonol	Kaempferol	3, 5, 7, 4' - OH	Leek, broccoli, endives, grapefruit, black tea
	Quercetin	3, 5, 7, 3', 4' - OH	Onion, lettuce, broccoli, tomato, tea, berries, apples, olive oil
	Rutin	5, 7, 3', 4' - OH; 3 - rutinose	Buckwheat, citrus, red pepper, red wine, tomato skin
Flavononol	Engeletin	3, 5, 7, 4' - OH; 3 - O-rhamnose	White grapeskin
	Astilbin	3, 5, 7, 5', 4' - OH; 3 - O-rhamnose	White grapeskin
	Genistin	5, 4' - OH; 7 - glucose	Soybean
	Taxifolin	3, 5, 7, 3', 4' - OH	Fruits
Isoflavone	Genistein	5, 7, 4' - OH	Soybean
	Daidzin	4' - OH, 7 - glucose	Soybean
	Daidzein	4', 7 - OH	Soybean
Flavanol	(+)-Catechin	3, 5, 7, 3', 4' - OH	Tea
	(+)-Gallocatechin	3, 5, 7, 3', 4', 5' - OH	Tea
	(-)-Epicatechin	3, 5, 7, 5', 4' - OH	Tea
	(-)-Epigallocatechin	3, 5, 7, 3', 4' - OH	Tea
	(-)-Epicatechin gallate	3, 5, 7, 3', 4' - OH; 3 - gallate	Tea
Anthocyanidin	Epigenidin	5, 7, 4' - OH	Stored fruits
	Cyanidin	3, 5, 7, 4' - OH; 3, 5 - OMe	Cherry, raspberry, strawberry
	Delphinium	3, 5, 7, 3', 4', 5' - OH	Dark fruits
	Pelargonidin	3, 5, 7, 4' - OH	Dark Fruits

Fig. 3. Different classes of flavonoids, their substitution patterns and dietary sources.

tion, but in some cases these solvents are acidified. Following this, the extracts are often treated with HCl under N_2 in order to hydrolyze flavonoid glycosides into aglycones before high performance liquid chromatography (HPLC) analysis. Matilla et al. [50] extracted flavonoids from plant materials with 62.5% (v/v) aqueous methanol. After acidification of the extract with 6 M HCl (extract:acid, 1:4, v/v) the hydrolysis of flavonoid glycosides was carried out under nitrogen at 90 °C for 2 h. Arts et al. [51] utilized aqueous methanol (70–90%, v/v) for extraction of catechins from freeze-dried fruits, vegetables, staple foods and processed foods. Lin et al. [52] extracted tea polyphenols by steeping tea leaves in boiling water for 30 min. The infusion was then filtered and subjected to HPLC analysis. On the other hand, Moore et al. [53] have obtained tea polyphenol extracts by employing a multiple extraction of tea samples with 80% (v/v) methanol and subsequently 80% methanol containing 0.15% HCl.

2.4. Anthocynins

Anthocyanins/anthocyanidins (Fig. 4) are usually extracted from plant materials with an acidified organic solvent, most commonly methanol. This solvent system destroys the cell membranes, simultaneously dissolves the anthocyanins and stabilizes them. According to Moore et al. [54,55] the acid may change the native form of anthocyanins by breaking down their complexes with metals and co-pigments. Recently, Cacace and Mazza [56] proposed using sulfured water for the extraction of anthocyanins from black currents. The maximum amount of anthocyanins was extracted from berries at a SO_2 level of 1000–1200 ppm at 30–35 °C and a solvent to berry ratio of 19:1 (v/w). Later Ju and Howard [57] used pressurized liquid extraction (PLE) to extract anthocyanins from dried red grape skins. These authors reported that high-temperature PLE (80–100 °C), using acidified water, was as effective in extracting anthocyanins from grape skins as the acidified 60% methanol.

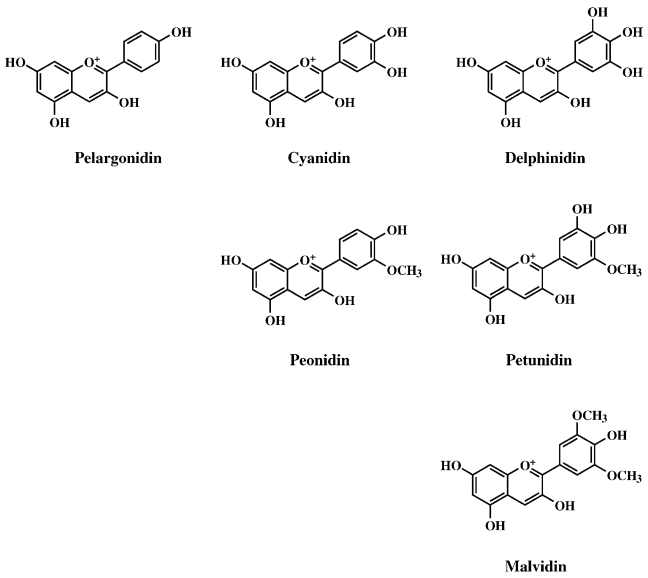


Fig. 4. Chemical structures of anthocyanidins.

2.5. Proanthocyanidins

For the extraction of proanthocyanidins, several solvent systems, namely absolute methanol, ethanol, acidified methanol, acetone, water and their combinations have been used. For example, 1% HCl in methanol was used for the extraction of proanthocyanidins from sorghum [61] and dry beans [62]. Meanwhile acetone–water (70:30, v/v) was found to serve as the best solvent system for the extraction of proanthocyanidins from rapeseed/canola [63–65], beach pea (*Lathyrus maritimus* L.) [66,67] and blueberries [68]. On the other hand, acetone–water (60:40, v/v) was used for the extraction of proanthocyanidins from cider apple [69] and grape skins [70]. Proanthocyanidins of cloves and allspice may be extracted with boiling water [71].

3. Purification and fractionation procedures

The extracts of phenolics, first concentrated under vacuum, may then be extracted with petroleum ether, ethyl acetate or diethyl ether in order to remove lipids and unwanted polyphenols [78,79]. Concentration of extracts containing anthocyanins before purification may, however, bring about losses of labile acyl and sugar residues [88]. In order to avoid this detrimental effect, several researchers have proposed to reduce the contact of the acids with pigments [89], to use neutral organic solvents or boiling water [88], or to use weak organic acids such as formic or acetic acid, for acidification of the solvents used for extraction of the pigments [34,54,55].

3.1. Liquid–solid phase procedures

The phenolic extract can be partially purified, using ion exchange resins, as described by Fuleki and Francis [80]. Amberlite particles (XAD-2) have also been utilized for isolation and purification of phenolics present in aqueous plant extracts [83,84] and acidified aqueous solutions of honey [85–87]. Amberlite particles were stirred with aqueous extracts for up to 4h and then packed into a glass column. The column was first washed with water [84] or a combination of acidified water and water [85] to remove sugar and other polar constituents. Subsequently, the phenolics were washed out from the column with methanol. Oleszek et al. [81] applied crude methanolic extract from yucca bark onto a C_{18} column (30 mm × 70 mm, 60 μm, Baker) equilibrated with water. The column was first washed out with 40% (v/v) methanol and subsequently with pure methanol. The phenolics were only detected in the 40% methanol eluate. Later, Mullen et al. [82] employed a combination of ion-exchange column (Diaion HP-20) and column containing 40 μm C_{18} silica gel support to remove sugars and other contaminants from acidified extracts of raspberry.

Phenolics extracts can also be purified and fractionated using solid phase extraction or solid phase microextraction (SPME) on C_{18} cartridges. Mateos et al. [215e] employed diol-bonded phase cartridge (Supelco C., Bellefonte, PA) to extract phenolics from olive oil. The oil was applied to the column and subsequently the column was washed with hexane and then with hexane/ethyl acetate (90:10, v/v). Following this, the phenolic fraction was eluted from the column with methanol. Molecularly imprinted polymers (MIP) are novel analytical tools for advanced solid phase extraction of targeted analytes in food [215j,k]. Analyte is used as a template during formation of the polymer. Analyte is then extracted from the polymer leaving receptors specific for a molecular recognition of targeted analyte. Recently, Molinelli et al. [215i] employed antiquercetin MIP for determination of quercetin in wine. The recovery rate of quercetin from such a column was 98.2%. Salagoity-Auguste and Bertrand [90] as well as Jaworski and Lee [91] demonstrated that a C_{18} Sep-Pak cartridge may be used to separate grape phenolics into acidic and neutral fractions. Later, Sun et al. [92] 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. 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 cartridge and catechins were first eluted with diethyl ether and then oligomeric proanthocyanidins with methanol.

Hong and Wrolstad [93] 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 [94] also isolated anthocyanins from fruit juices and wine using a SPE cartridge, but used methanol–formic acid–water (70:2:28, v/v/v) for their elution.

Column chromatography has been also employed for fractionation of phenolics extracts. Mateus et al. [95] 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 per min. The anthocyanins were subsequently eluted from the column with water–ethanol (20%, v/v). The elution of wine phenolics from Toyopearl column yielded malvidin 3-glucoside and three derived pyruvic adduct pigments. The adducts were those of malvidin 3-glucoside, malvidin 3-acetylglucoside and malvidin 3-coumarylglucoside. These glucosides accounted for 60% of the total content of monoglucosides.

Souquet et al. [101] utilized a Fractogel (Toyopearl TSK HW-50(f) gel) column (35 cm × 8 cm) to separate the non-tannin phenolics and proanthocyanidins. The non-tannin phenolics were washed out from the column with two bed volume of water followed by five bed volumes of ethanol–water–trifluoroacetic acid (TFA) (55:44.05:0.05, v/v/v). Subsequently, proanthocyanidins were washed from the column with three bed volumes of acetone–water (60:40, v/v). On the other hand, Vidal et al. [102] used a Toyopearl (TSK HW-50(f) column (50 cm × 25 cm) equilibrated with water for a semi-preparative separation of anthocyanins extracted from grape skin with ethanol–water (75:25, v/v) containing 2% acetic acid. The column was sequentially washed with water (three beds) and ethanol–water–TFA (20:80:0.05, v/v/v) to recover the main portion of anthocyanin glucosides. The remaining anthocyanins were then eluted with ethanol–water–TFA (80:20:0.05, v/v/v) solvent system.

Fractogel (Toyopearl TSK HW-40(s) gel) has also been used for separation of malt and hops to obtain proanthocyanidin dimers and trimers with high purity. The proanthocyanidin fractions, obtained after chromatography of polyphenols on Sephadex LH-20 with methanol, 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 [103].

The isolation of proanthocyanins (condensed tannins) is commonly carried out by employing Sephadex LH-20 column chromatography [96–100]. The crude extract is applied to the column which is then washed with ethanol to elute the non-tannin substances. Following this, proanthocyanidins are eluted with acetone–water or alcohol–water.

An inert glass powder (Pyrex microparticles, 200–400 μm) has been employed for fractionation of grape (seed or skin) proanthocyanidins according to their degree of polymerization [70]. 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 top of the column with chloroform. Proanthocyandins were sequentially eluted from the column by increasing proportions of methanol in a methanol–chloroform solvent system.

3.2. Liquid–liquid procedures

Countercurrent chromatography (CCC) has recently been explored as an alternative to liquid chromatographic techniques for fractionation of various classes of phenolic compounds [104,105]. Separation of compounds is based on their partitioning between two immiscible liquids [106]. One of the solvent is used as a liquid stationary phase while another is used as a mobile liquid phase. The immiscible phases usually have an auxiliary solvent, miscible in both phases, aiding in the partitioning of the analytes between two immiscible phases [107].

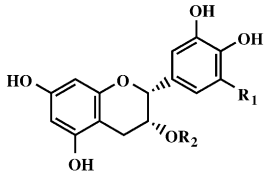
Degenhart [107] used high speed centrifugal countercurrent chromatography (HSCCC) for preparative isolation

of anthocyanins from red wines and grape skins. Anthocyanins were fractionated based on their polarities into four solvent 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, 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. [108] also employed 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 aqueous methanol (75%, v/v). Afterwards, the phenolic extract was fractionated using HSCCC 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 per min, for elution of phenolics in ascendant and descendant modes, respectively.

Degenhart et al. [109] 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 to HSCCC, are extracted from tea infusion with ethyl acetate and then cleaned up using a Sephadex LH-20 column to avoid co-elution of catechins and theaflavins. On the other hand, isolation of thearubigins required cleaning up tea infusion on an Amberlite XAD-7 column prior to HSCCC to remove all non-phenolic compounds.

Renault et al. [110] applied gradient elution combined with HSCCC for purification of anthocyanins from Champagne byproducts using ethyl acetate–*n*-butanol–water (0.2% TFA) solvent system, Vidal et al. [102] coupled a stepwise gradient elution to a multilayer coil countercurrent chromatography (MCCC) for fractionation of anthocyanins into glucosides, and the corresponding acetylated, coumaroylated and caffeoylated derivatives without using appropriate tie-lines. The most effective separation of anthocyanins was achieved when *tert*-butyl methyl ether–*n*-butanol–acetonitrile–water acidified with 0.02% (v/v) TFA (2:2:0.1:5, v/v/v/v) was used as a solvent system (lower phase of the system) and a binary stepwise gradient of solvent system consisting of *tert*-butyl methyl ether–*n*-butanol–acetonitrile–water acidified with 0.02% (v/v) TFA (2:2:0.1:5, v/v/v/v; upper phase of the system) and *tert*-butyl methyl ether–*n*-butanol–acetonitrile–water acidified with 0.02% (v/v) TFA (2:2:2.5:5, v/v/v/v; upper phase of the system).



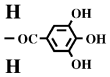
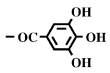
Catechin	R_1	R_2
(-)-Epicatechin (EC)	H	H
(-)-Epicatechin gallate (ECG)	H	
(-)-Epigallocatechin (EGC)	OH	H
(-)-Epigallocatechin gallate (EGCG)	OH	

Fig. 5. Chemical structures of catechins.

A simple and efficient procedure for separation of catechin gallates (Fig. 5) from spray-dried tea extract was developed by Baumann et al. [111]. Tea phenolic extract was first subjected to liquid–liquid partitioning between ethyl acetate and water. The organic layer containing catechins was then submitted to HSCCC operating in an ascending mode. Favorable partitioning was achieved using n-hexane–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.

4. Quantification of phenolics

A number of spectrophotometric methods for quantification of phenolic compounds in plant materials has been developed. These assays are based on different principles and are used to determine various structural groups present in phenolic compounds. Gas chromatographic (GC) and high performance liquid chromatographic techniques are used widely for both separation and quantitation of phenolic compounds. Structure elucidation is often achieved using combination of GC and HPLC with mass spectrometric analysis, as well as other relevant techniques.

4.1. Spectrophotometric assays

The Folin–Denis assay is the most widely used procedure for quantification of total phenolics in plant materials [71]. Reduction of phosphomolybdic–phosphotungstic acid (Folin–Denis) reagent to a blue colored complex in an alkaline solution occurs in the presence of phenolic compounds [112]. Swain and Hills [113] modified the Folin–Denis method for routine analysis of a large number of samples. The Folin–Ciocalteu assay is also used for determination of the total content of plant food phenolics [74,114–117]. Both Folin–Denis and Folin–Ciocalteu reagents are not specific and detect all phenolic groups found in extracts, including those in the extractable proteins. Another disadvantage of

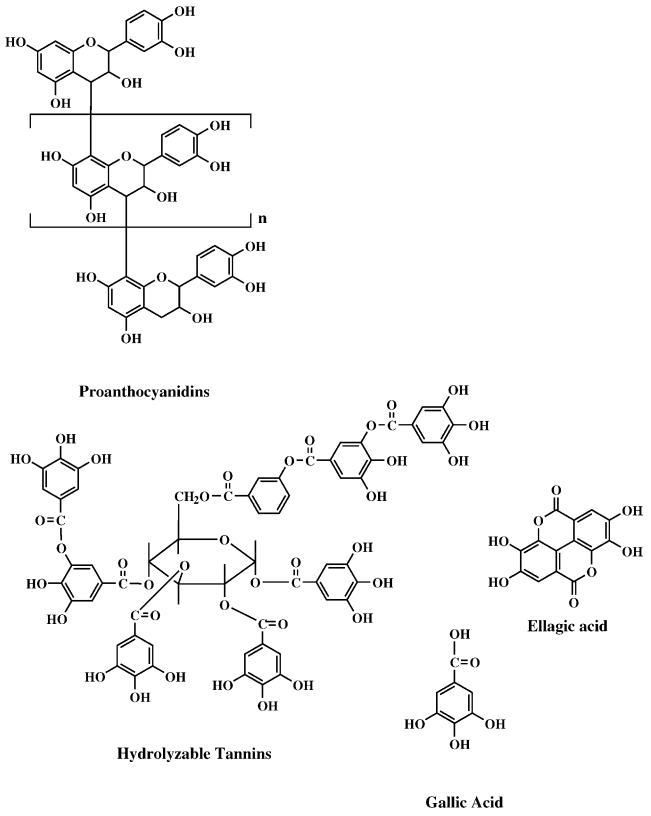


Fig. 6. Chemical structures of tannins.

this assay is the interference of reducing substances such as ascorbic acid.

The vanillin method is widely used for quantification of proanthocyanidins (condensed tannins) (Fig. 6) in plant materials [118] and, particularly, grains [73]. The vanillin test is specific for flavan-3-ols, dihydrochalcones and proanthocyanidins which have a single bond at the 2,3-position and possess free meta-hydroxy groups on the B ring [119,120]. Catechin, a monomeric flavan-3-ol, is often used as a standard in the vanillin assay. According to Price et al. [61] and Gupta et al. [119] this may lead to overestimation of proanthocyanidin contents. The vanillin assay in methanol is more sensitive towards polymeric proanthocyanidins than monomeric flavan-3-ols. This assay is generally recognized as a useful method for the detection and quantification of proanthocyanidins in plant materials due to its simplicity, sensitivity and specificity. The method can be used for quantifying proanthocyanidins in the range of 5–500 μg with precision and accuracy of greater than 1 μg when optimum concentrations of reactants and solvents are selected [121].

The 4-(dimethylamino)-cinnamaldehyde (DMCA) assay has also been proposed for estimation of proanthocyanidins [122]. The formation of a green chromophore between catechin and DMCA was first reported by Thies and Fischer [123]. However, DMCA does not react with a wide range of flavonoids, including dihydrochalcones, flavanones and flavononols as well as phenolic acids, but instead reacts with indoles and terpenes. A weak response was also detected for resorcinol, orcinol, naphtoresorcinol, and phloretin [122,124,125]. Li et al. [126] re-examined the use of the DMCA assay for determination of proanthocyanidins in plants. According to these authors, on a mole basis, the sensitivity of DMCA towards proanthocyanidins was four times greater than that of indole and 30,000 times greater than that of thymol. The DMCA reagent, in comparison to the vanillin reaction, carried out in methanol, reacts only with the terminal groups of proanthocyanidins and it is sensitive to both monomeric and polymeric units. The presence of methanol, acetone, ethyl acetate, and dimethylformamide does not have any detrimental effect on the rate and intensity of color development [122].

The proanthocyanidin assay is carried out in a solution of butanol and concentrated hydrochloric acid (95:5, v/v). In the presence of this acidic solution proanthocyanidins (condensed tannins) are converted to anthocyanidins. This occurs through autoxidation of carbocations formed by cleavage of interflavanoid bonds [127]. The yield of this reaction depends on the concentration of HCl and water, temperature and reaction time, the presence of transition metals, as well as the degree of polymerization of proanthocyanidins [40,127]. The presence of transition metals enhanced both the reproducibility and the yield of conversion of proanthocyanidins to anthocyanidins. Ferrous and ferric ions were the most effective catalysts in the formation of anthocyanidins [127]. The reproducibility of this method was significantly improved with strict controlling of the reaction temperature [40].

Various approaches have been used for screening of a large number of plant materials for their content of hydrolyzable tannins (Fig. 6). Of these, the most widely used method is based on the reaction between potassium iodate and hydrolyzable tannins. This reaction was first described by Haslam [128] and later used by Bate–Smith [129] for the development of an analytical assay for estimation of hydrolyzable tannins in plant materials. Recently, Hartzfeld et al. [130] modified this assay by including a methanolysis step followed by oxidation with potassium iodate. The assay is based on gallic acid which is a common structural component found in both gallotannins and ellagitannins. Methyl gallate, formed upon methanolysis of hydrolyzable tannins in the presence of strong acids, reacts with potassium iodate to produce a red chromophore with a maximum absorbance at 525 nm. The detection limit of the method is 1.5 μg of methyl gallate. Methanol and acetone stabilize the chromophore, while presence of water accelerates degradation of the resultant pigment. Other analytical assays proposed for quantification of hydrolyzable tannins in plant materials include the rhodanine [131] and the sodium nitrate [132] methods. The rhodanine assay may be used for estimation of gallotannins and is based on determination of gallic acid in a sample subjected to acid hydrolysis under anaerobic conditions. On the other hand, the sodium nitrate assay was developed for quantitative determination of ellagic acid in sample hydrolyzate, but this assay requires large quantities of pyridine as a solvent.

Table 1
Some HPLC procedures for determination of isoflavones in soy foods

Food	Sample preparation	Stationary phase	Mobile phase	References
Seed	Extraction with 80% MeOH; centrifugation	Aquapore C_8; (220 mm × 4.6 mm)	(A) 10% acetonitrile in H_2O with 0.1% TFA; (B) 90% acetonitrile in H_2O with 0.1% TFA; linear gradient: 100% A, 0% B, 0 min; 70% A, 30% B, 30 min; step gradient to 0% A, 100% B	Simonne et al. [193]
Soy foods	Extraction with 80% MeOH; centrifugation, evaporation of MeOH, extraction of lipids with hexane	Brownlee Aquapore C_8 reversed-phase; (300 mm × 4.5 mm)	(A) 0.1% TFA in H_2O; (B) acetonitrile; linear gradient: 100% A, 0% B, 0 min; 53.6% A, 46.4% B, 20.6 min	Coward et al. [194]
Seeds, soy foods	Extraction with 0.1% HCl:acetonitrile (1:5), filtration	YMC-pack ODS–AM-303 (250 mm × 4.6 mm, 5 μm)	(A) 0.1% acetic acid in water; (B) 0.1% acetic acid in acetonitrile; 85% A, 15% B, 0 min; 85% A, 15% B, 5 min; 71% A, 29% B, 31 min; 65% A, 35% B, 8 min; 85% A, 15% B, 3 min	Murphy et al. [195]
Seeds	Extraction with 80% MeOH	YMC-pack ODS-AQ 303 (250 mm × 4.6 mm; 5 μm)	(A) 0.1% acetic acid in H_2O; (B) 0.1% acetic acid in acetonitrile; modified gradient: 85% A, 15% B, 0 min; 69% A, 31% B, 45 min	Wang et al. [177]
Soy sauce	Direct injection	Wakosil II 5C18 HG (250 mm × 4.6 mm) fitted with a precolumn (30 mm × 4.6 mm packed with the same material	(A) 0.05% TFA in H_2O; (B) 90% acetonitrile with 0.05% TFA; 100% A, 0% B, 0 min; 100% A, 0% B, 20 min; 75% A, 25% B, 270 min, linear gradient	Kinoshita et al. [196]
Soy foods	Extraction with 80% MeOH, centrifugation	NovaPak C_{18} reversed-phase (150 mm × 3.9 mm, 4 μm) coupled to Adsorbosphere C_{18} column guard	(A) 10% acetic acid in H_2O; (B) MeOH–acetonitrile–dichloromethane (10:5:1, v/v/v). 95% A, 5% B, 0 min; 95% A, 5% B, 5 min; linear gradient: 45% A, 55% B, 20 min; 30% A, 70% B, 6 min; 95% A, 5% B, 3 min	Franke et al. [197]
Soy foods	Extraction with 96% EtOH, centrifugation, filtration	NovaPak C_{18} (150 × 3.9 mm; 4 μm)	Isocratic: acetonitrile–water (33:67, v/v)	Hutabarat et al. [198]

The complexation of phenolic with Al(III) has been used for the development of spectrophotometric methods for determination of total caffeic acid, total flavonoids and total tannins [133–135]. The total caffeic acid was measured by adding a solution of $AlCl_3$ to the methanolic extract of phenolics and adjusting the pH of this mixture to 4.8 with a solution of NH_4Cl. The absorbance of this solution was then measured at 355 nm [135]. On the other hand, the total content of flavonoids in methanolic extract of phenolics mixture was by complexing flavonoids with $AlCl_3$ at pH 3.1. The total content of flavonoids and tannins was determined by measuring the absorbance of the solution at 407 and 323 nm, respectively [135]. The modification of the $AlCl_3$ assay proposed by Zhishen et al. [136] included the reaction of phenolic extract with sodium nitrate followed by the formation of flavonoid–aluminum complex. The absorbance of the solution was then read at 510 nm.

Quantification of anthocyanins (Fig. 4) takes advantage of their characteristic behavior in acidic media. The analytical procedure for quantification of anthocyanins was first developed by Sondheimer and Kertesz [137]. This procedure was later modified by Swain and Hillis [113] who suggested to express the concentration of pigments in terms of the change in the absorbance at λ_{max} between pH 3.5 and pH $\leq$ 1.0. Fuleki and Francis [79] suggested to extend the pH differential to between 4.5 and 1.0. They proposed to determine the optical density of the two samples at 515 nm for aliquots buffered to the above pH values.

A number of approaches have been used to develop a simple and satisfactory UV spectrophotometric assay [117,138–143]. Simple phenolics have absorption maxima between 220 and 280 nm [138,139], but their absorption is affected by the nature of the 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, the development of a satisfactory UV assay is a rather cumbersome and a difficult task. In addition, suitability of the UV assay depends on the material to be analyzed. Both UV and visible spectroscopic techniques are often used for identification of isolated phenolic compounds, particularly flavonoids [144] as well to identify the presence of groups of predominant phenolic compounds [145]. Diode array detection (DAD) may also be of benefit in such analysis.

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) [146,147]. Chemometric techniques use information (such as a spectrum) and chemical indices (such as concentration of a component) and established a mathematical relation between the two. This technique assumes that the chemical index (concentration) is correct and attributes weighings of the spectral information accordingly. The setting up of the model, correlating the

Table 2
Some HPLC procedures for determination of catechins and proanthocyanidins (PA) in selected foods

Food	Sample preparation	Stationary phase	Mobile phase	References
Grapeseed	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% phosphoric acid (v/v); (B) 82% acetonitrile with 0.4% phosphoric acid; gradient: 100% A, 0% B, 0 min; 85% A, 15% B, 15 min; 84% A, 16% B, 25 min; 83% A, 17% B, 5 min; 57% A, 43% B, 3 min; 48% A, 52% B, 1 min; isocratic 52% B, 7 min; 57% A, 43% B, 1 min; 83% A, 17% B, 1 min; 100% A, 0% B, 2 min	Peng et al. [187]
Beverages	Direct injection	Spherisorb ODS2 (150 mm × 46 mm, 3 μm)	(A): H_2O; (B) MeOH; (C) 4.5% aqueous formic acid; (D) 4.5% aqueous formic acid–MeOH (90:10, v/v); gradient: 0–10 min, 100% A to 100% C; 10–20 min, 0–15% D in C; 20–30 min, 15% D in C, isocratic; 30–40 min, 15–35% D in C; 40–45 min, 35% D in C, isocratic; 45–60 min, 35–45% D in C; 60–75 min, 45–100% D in C; 75–175 min, 0–50% B in D; 175–180 min, 50–80% B in D	de Pascual-Teresa et al. [182]
Apples, grapes beans	Extraction with 90% MeOH (apples, grapes) or 70% MeOH (beans); filtration	Inertsil ODS-2 (150 mm × 4.6 mm, 5 μm) coupled with Opti-Guard PR C_{18} 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, [51]
Wine	Dealcoholized under vacuum; fractionation of polymeric fraction using Tyopearl TSK gel HW-50 (f), thiolysis	Nucleosil 120 (125 mm × 4 mm, 3 μm)	(A) 2% HCOOH in H_2O; (B) CH_3CN–H_2O–HCOOH (80:18:2, v/v/v); linear gradient: 15–75% B, 0–15 min; 75–100% B, 15–20 min	Fulcrand et al. [199]
Wine	Dealcoholized under vacuum; fractionation of procyanidins and catechins using two C_{18} Sep-Pak cartridges in series	Superspher 100 RP18 (250 mm × 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. [200]
Wine	Filtration, direct injection	Nucleosil 100 C_{18} (250 mm × 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. [173]
Cocoa	Extraction of defatted seeds with 70% acetone (v/v), followed by extraction with 70% MeOH (v/v), fractionation of phenolic from non-phenolics on column packed with Baker octadecyl for flash chromatography	Phenomenex Luna (250 mm × 4.6 mm, 5 μm)	(A) Dichloromethane; (B) MeOH; (C) acetic acid–H_2O (1:1,v/v); gradient: 0 min, 14% B in A; 0–30 min, 14–28.4% B in A; 28.4–50% B in A, 30– 60 min; 50–86% B in A, 60–65 min; isocratic 65–70 min	Hammerstone et al. [189]
Oolong tea	Extraction with acetonitrile–water (1:1, v/v)	Devolosil PhA-5 (250 mm × 46 mm)	0.1 M NaH_2 PO_4 buffer (pH 2.5)–acetonitrile (85:15, v/v) with 0.1 mM EDTA2 Na, isocratic	Sano et al., [181]
Green tea beverage	Washed with chloroform; extraction with ethyl acetate, ethyl acetate layers combined, evaporated, residue dissolved in 50% acetonitrile	μ-Bondapak C_{18} (300 mm × 3.9 mm)	Acetonitrile–ethyl acetate–0.05% phosphoric acid (12:2:86, v/v/v), isocratic	Wang et al. [178]
Green tea	Extraction with hot water, filtration, washing with chloroform, extraction of water layer with ethyl acetate, ethyl acetate layers combined and evaporated, residue dissolved in water	Hypersil ODS (250 mm × 4.6 mm, 5 μm)	0.05% H_2SO_4 aqueous–acetonitrile–ethyl acetate (86:12:2, v/v/v), isocratic	Chen et al. [201]

Table 3
Some HPLC procedures for determination of anthocyanins and anthocyanidins in selected foods

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); isocratic: 80% A, 20% B, 0–4 min; linear regression: 20–80% B in A, 4–26 min	Gennaro et al. [202]
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 preinjection 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); linear gradient: 5–20% B in A, 0–1 min; 20–25% B in A, 1–12 min; 25–32% B in A, 12–32 min; 32–55% B in A, 32–38 min; 55–100% B in A, 38–44 min; 100% B, 44–46 min; 100–5% B in A, 46–47 min	Wang and Sporns [94]
Red wine	Direct injection	Ultrasphere (C_{18}) ODS (250 mm × 4.6 mm)	(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 in A, 0–70 min; 85–100% B in A, 70–75 min; isocratic: 100% B, 75–85 min	Mateus et al. [95]
Red Wine	Centrifugation, addition of formic acid to 1.5%, filtration	Supelcosik LC-18 (250 mm × 2.1 mm), reverse-phase	(A) 5% formic acid in H_2O (v/v); (B) MeOH; gradient: 5% B in A, 0–5 min; 5–65% B in A, 5–55 min; 65–100% B, 55–58 min; 100–5% B in A, 58–60 min; 5% B in A, 60–64 min	Mazza et al. [203]
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 (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 in A, 0–2 min; 10–50% B in A, 2–32 min; 50% B in A, 32–37 min; 50–70% B in A, 37–57 min	Lee [204]

Table 4
Some HPLC procedures for determination of flavones and flavonols in selected foods

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) 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. [202]
Yellow and green French beans	Extraction with chloroform to remove chlorophyll and carotenoids, drying, extraction with 70% MeOH, evaporation of MeOH, purification of phenolics using polyamide cartridge, filtration	LiChrospher 100 RP-18 (250 × 4, 5 μm) coupled with guard (4 mm × 4 mm) packed with the same stationary phase	(A) Acetonitrile; (B) 2% acetic acid in H_2O; gradient: 10–30% A in B, 0–35 min; 30–45% A in B, 35–37 min; 45% A in B, 37–42 min; 45–10% A in B, 42–44 min	Hempel and Bohm [205]
Red beans seed coats	Extraction with MeOH, separation of tannins using Sephadex LH-20, flavonoid fraction rechromatographed on Sephadex LH-20	Shiseido Capcell Pak C_{18} preparative reverse-phase (250 mm × 10 mm, 5 μm)	Isocratic: acetonitrile–H_2O (30:70, v/v)	Beninger and Hosfield [206]
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 (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 [207]

Table 4 (*Continued*)

Food	Sample preparation	Stationary phase	Mobile phase	References
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, 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. [208]
Edible tropical plants	Extraction with 1.2 M HCl in 50% MeOH for 2 h at 90 °C, filtration	Nova Pak C_{18} (150 mm × 3.9; 4 μm)	Isocratic: MeOH–H_2O (1:1, v/v) adjusted to pH 2.5 with TFA	Miean and Mohamed [209]
Fruits and vegetables	Refluxing with 62.5% MeOH stabilized with TBHQ and containing 1.2 M HCl at 90 °C for 2 h	Nova Pak C_{18} column (150 mm × 3.9 mm, 4 μm) coupled with guard packed with Perisorb RP-18 (40 mm × 3.9 mm, 30–40 μm)	Isocratic: acetonitrile/phosphate buffer pH 2.4 (25:75; v/v)	Hertog et al. [209b–d]

information with a chemical index, is known as calibration [148].

Monedero et al. [149] developed a chemometric technique for controlling the content of phenolic aldehydes and acids during production of wine subjected to accelerated aging. Edelmann et al. [150] developed a rapid method of discrimination of Austrian red wines based on mid-infrared spectroscopy of phenolic extracts of wine. Subsequently, Brenna and Pagliarini [151] employed a multivariate analysis for establishing a correlation between the polyphenolic composition and the antioxidant power of red wines.

Briadet et al. [152] applied PCA to differentiate between Arabica and Robusta instant coffees based on their FTIR spectra. The discrimination between different species of cof-

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

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	Isocratic: H_2O–acetic acid–MeOH (80:5:15, v/v/v)	Subra Rao and Muralikrishna [210]
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 (150 mm × 4.6 mm, 5 μm)	(A) 0.01 M citrate buffer pH 5.4 adjusted with 50% acetic acid; (B) MeOH; gradient: 2–4% B in A, 0–12 min; 4–13% B in A, 12–20 min; 13% B in A, 20–26 min; 13–2% B in A, 26–30 min	Vasanthan and Temelli [211]
Citrus fruits	Coumarins	Extraction with acetone, filtration, evaporation dissolving in MeOH-acetone (1:1, v/v), filtration	Hypersil ODS (125 mm × 4 mm, 5 μm)	Isocratic: MeOH–H_2O (75:25, v/v)	Ogawa et al. [212]
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} (250 mm × 4.6 mm)	Isocratic: MeOH–acetonitrile–dichloromethane–acetic acid (50:44:3:3, v/v/v/v)	Xu and Golber [213]
Flaxseed flour, defatted	Lignans	Extraction with 1,4-dioxane–95%EtOH (1:1, v/v), centrifugation, evaporation, alkaline hydrolysis, acidification to pH 3, removal of salt using C_{18} reversed-SPE	Econosil RP C_{18} (250 mm × 4.6 mm, 5 μm)	(A) 5% Acetonitrile in 0.01M phosphate buffer, pH 2.8; (B) acetonitrile; gradient: 100–70% A in B, 0–30 min; 70–30% A in B, 30–32 min	Johnsson et al. [214]
Peanuts	Resveratrol	Extraction with 80% EtOH, centrifugation, semi-purification Al_2O_3 silica gel $60R_{18}$ (1:1)	Vydac C_{18} (150 mm × 4.5 mm) reversed-phase	(A) Acetonitrile; (B) 0.1% TFA; gradient: 0% A in B, 0–1 min; 0–15% A in B, 1–3 min; 15–27% A in B, 3–23 min; 27–100% A in B, 23–28 min	Sanders et al. [215]

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 1.2 M HCl in 50% MeOH for 2 h at 90 °C; filtration	Inertsil ODS (150 mm × 4 mm; 3 μm) 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. [50]
Nectarines, peaches, plums	Phenolic acids, catechins, flavonols, procyanidins	Extraction with 80% MeOH containing 2mM NaF; centrifugation, filtration	Nucleosil C_{18} (150 mm × 4.6 mm, 5 μm) reversed-phase 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. [176]
Red raspberry	Ellagic acids, flavones	Extraction with MeOH, filtration, addition of H_2O, evaporation, semi-purification of phenolics using Sep-Pak C_{18}, filtration	Lichrocart 100 RP-18 (250 mm × 4 mm, 5 μm) reversed-phase	(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. [179]
Propolis	Phenolic acids, flavones, flavonones, flavonols	Dilution with EtOH, alkaline hydrolysis, acidification, extraction of phenolic with ethyl acetate, evaporation, dissolving in EtOH	Lichrosorb RP18 (200 mm × 3 mm, 7 μm) coupled with C_{18} guard	(A) H_2O adjusted to pH 2.6 with H_3PO_4; (B) acetonitrile; gradient: 0–9% B in A, 0–12 min; 9–13% B in A, 12–20 min; 13–40% B in A, 20–40 min; 40–70% B in A, 40–60 min; 70% B in A, 60–85 min	Siess et al. [175]
Spinach	Flavonols, flavanones	Extraction with 70% MeOH, removal of carotenoids and chlorophyll using ODS-C_{18} packing material, centrifugation, concentration	YMC ODS-AQ (250 mm × 4.6, 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. [174]
Olive oil	Phenolic acids, secoiridoids	MeOH/H_2O (80:20, v/v) evaporation to syrup concentration; addition of acetonitrile, washing with hexane	C_{18} Erbasil column (150 mm × 4.6 mm)	(A) 2% CH_3COOH in H_2O; (B) MeOH; gradient: 95% A/5% B for 2 min; 25% B in A in 8 min; 40% B in A in 10 min; 50% B in A in 10 min; 100% B in 10 min	Montedoro et al. [215b,c]
Olive oil	Phenolic acids, secoiridoids	Same as Montedoro et al. [215b,c]	Spherisorb ODS-2 (25 cm × 4.6 mm, 5 μm)	(A) H_2O pH 3.1 adjusted with CH_3COOH; (B) MeOH; C: gradient: 90% A/10% B, for 2 min; 30% B in A in 10 min; 70% A/30% B for 20 min; 40% B in A in 10 min; 60% A/40% B for 5 min; 50% B in A in 5 min; 50% A/50% B for 5 min	Brener et al.[215]
Olive oil	Phenolic acids, secoiridoids, flavones, lignans	Solid phase–liquid extraction using diol-bonded cartridge; unwanted substances washed out with hexane and hexane/ethyl acetate (90:10, v/v), then phenolics eluted from column with MeOH	Lichrospher 100 RP-18 (250 mm × 4 mm, 5 μm) reversed-phase	(A) H_2O/CH_3COOH (97:3, v/v); (B) MeOH/acetonitrile (50:50, v/v); gradient: 5–30% B in A, 0–25 min; 35% B in A in 10 min; 40% B in A in 5 min, 70% B in A in 10 min; 100% B in 5 min	Mateos et al. [215e]
Olive oil	Phenolic acids, secoiridoids, lignans	Same as Mateos et al. [215e]	Spherisorb S3 ODS2 column (250 mm × 4.6 mm, 5 μm)	(A) H_2O/CH_3COOH (95:5, v/v); (B) MeOH; (C) acetonitrile; gradient: from 95% A/2.5% B/2.5% C to 34% A/33% B/33%C in 50 min	Gómez-Alonso et al. [215f]
Olive fruits, pomace	Secoiridoids, flavones	Extraction 80% MeOH containing 100 ppm sodium salt of diethyldithiocarbamic acid; extract purified using C_{18} cartridge.	Lichrospher 100 (250 mm × 4.6 mm, 5 μm)	(A) H_2O (pH 2.5 adjusted with 0.15 M H_3PO_4); (B) H_2O; gradient: from 10% B in A to 30% B in A in 10 min, 30% B in A for 20 min; from 40% B in A in 10 min; 40% B in A for 5 min; 60% B in A in 5 min; 70 % B in A in 5 min; 100% B in 5 min	Romero et al. [215g]

fee was based on their different contents of chlorogenic acid and caffeine. Later, Downey et al. [153] 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. [154] used a near-infrared reflectance (NIR) spectroscopic method for prediction of polyphenols in the leaves of green tea (*Camelia sinensis* (L.) O. Kuntze).

4.2. Chromatographic techniques

Various gas chromatographic techniques have been employed for separation and quantitation of phenolic acids (Fig. 2) [43,155], isoflavones [156], capsaicinoids [157], phenolic aldehydes [158] and monomers of condensed tannins [159]. 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 [43,155], acid [160] and enzymatic hydrolysis [156] or acid depolymerization of tannins in the presence of nucleophiles such as phloroglucinol [159] or benzyl mercaptan [69,70]. Prior to chromatography, phenolics are usually transformed to more volatile derivatives by methylation [161–163], trifluoroacetylation [164,165], conversion to trimethylsilyl derivatives [43,155] or derivatization with *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide [159].

HPLC techniques are now most widely used for both separation and quantitation of phenolic compounds. Various supports and mobile phases are available for the analysis of anthocyanins, procyanidins, flavonones and flavonols, flavan-3-ols, procyanidins, flavones, phenolic acids, and secoiridoids [166,215b–g]. Introduction of reversed phase columns has considerably enhanced HPLC separation of different classes of phenolic compounds [167]. Several reviews have been published on the application of HPLC methodologies for the analysis of phenolics [42b,168–172].

Tables 1–6 summarize some modern HPLC procedures employed for the analysis of various classes of food phenolics. Food phenolics are commonly detected using UV–vis, photodiode array (DAD), and UV-fluorescence detectors [48,51,53,56,95,173–180]. Other methods used for the detection of phenolics include electrochemical coulometric array detector (EC) [50,181,215h], on-line connected DAD and electroarray detector [50], chemical reaction detection technique [182], and fluorimetric detector [173,183]. A combination of HPLC technique and voltammetry has successfully been 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 phenols with those of standard solutions containing pure phenolics [184–186a]. Recently, Romani et al. [186b] compared electrochemical (differential pulse voltammetry and amperometric biosensor) HPLC/DAD procedures for analysis of phenolics in natural matrices. Of these, HPLC/DAD technique gave the most accurate, while the differential pulse voltammetry technique which employed graphite screen-printed electrodes was considered as a good and quick method for screening polyphenols in natural extracts.

High-performance liquid chromatograph coupled with mass spectrometry (HPLC-MS tandem) has commonly been used for structural characterization of phenolics. Electrospray ionization mass spectrometry (ESIMS) has been employed for structural confirmation of phenolics in plums, peaches, nectarines [176], grapeseeds [187], soyfoods [188], cocoa [189], olive oil [215g] and walnut leaves [180,190] and this has demonstrated that complexation of flavonoids with Cu^{2+} enhances the detection of flavonoids by ESIMS. Mass spectra obtained for metal–flavonoids complexes were more intense and simpler for interpretation than that of corresponding flavonoids. Identification of phenolics collected after HPLC analysis was also carried out using fast atom bombardment mass spectrometry (FABMS) [174,181,191] and electron impact mass spectrometry [174]. On the other hand, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has been employed for qualitative and quantitative analysis of anthocyanins in foods [94], while MALDI on line with a linear time-of-flight (TOF) mass spectrophotometer was used for identification of theaflavins and thearubigins from black tea [192].

5. Conclusions

Numerous studies suggest that the consumption of plant foods containing dietary phenolics may significantly contribute to human health. Hundreds of publications on the analysis of food phenolics have already appeared over the past two decades. Nonetheless, there is still no standardize procedure for sample preparation and extraction available. Therefore, there is a need for systematic investigation for sample preparation and for determination of food phenolics. Moreover, methodologies used for analysis of food phenolics have not been standardized. Furthermore, HPLC methods currently employed do not determine all health-beneficial phenolics in a sample at once. Thus, there is a need to develop more robust analytical procedures for simultaneous determination of important subclasses of food phenolics.

References

[1] J.B. Harborne, Introduction to Ecological Biochemistry, second ed., Academic Press, New York, NY, 1982.
[2] J.B. Pridham, Phenolics in Plants in Health and Disease, Pergamon Press, New York, NY, 1960.
[3] C.H. Beckman, Physiol. Mol. Plant Pathol. 57 (2000) 101.
[4] R. Nicholson, R. Hammerschmidt, Annu. Rev. Phytopathol. 30 (1992) 369.

[5] J.B. Harborne, B.L. Turner, Plant Chemosystematics, Academic Press, London, UK, 1984.
[6] F. Shahidi, Nahrung 44 (2000) 158.
[7] F. Shahidi, Interface friction and energy dissipation in soft solid processing applications in: M.J. Morello, F. Shahidi, C.-T. Ho (Eds.), ACS Symposium Series, vol. 807, American Chemical Society, Washington, DC, 2002, pp. 162–175.
[8] F. Shahidi, M. Naczk, Phenolics in Food and Nutraceuticals: Sources, Applications and Health Effects, CRC Press, Boca Raton, FL, 2004.
[9] M.L. Bengoechea, A.I. Sancho, B. Bartolomé, I. Estrella, C. Gómez-Cordovés, T. Hernández, J. Agric. Food Chem. 45 (1997) 4071.
[10] R. Ibrahim, D. Barron, in: P.M. Dey, J.B. Harborne (Eds.), Methods in Plant Biochemistry, vol. 1, Academic Press, London, UK, 1989, pp. 75–111.
[11] G.H.N. Towers, in: J.B. Harborne (Ed.), Biochemistry of Phenolic Compounds, Academic Press, London, UK, 1964, pp. 249–294.
[12] M. Wink, Adv. Bot. Res. 25 (1997) 141.
[13] F.J. Pérez-Ilzarbe, V. Martinez, T. Hernández, I. Estrella, J. Liq. Chromatogr. 15 (1991) 637.
[14] B. Fernández de Simón, J. Pérez-Ilzarbe, T. Hernández, C. Gómez-Cordovés, I. Estrella, J. Agric. Food Sci. 40 (1992) 1531.
[15] G. Wallace, S. Fry, Int. Rev. Cytol. 113 (1994) 1223.
[16] G. Musel, T. Schindler, R. Bergfeld, K. Ruel, G. Jacquet, C. Lapierre, V. Speth, P. Schopfer, Planta 201 (1997) 146.
[17] M. Baucher, B. Monties, M. Van Montagu, W. Boerjan, Crit. Rev. Plant Sci. 17 (1998) 125.
[18] S. Kamisaka, S. Takeda, K. Takahashi, K. Shibata, Physiol. Plant 78 (1990) 1.
[19] N. Lewis, E. Yamamoto, Ann. Rev. Plant Physiol. Plant Mol. Biol. 41 (1990) 455.
[20] J.A. Scalbert, Polyphenolic Phenomena, INRA, Paris, France, 1993.
[21] C.T. Brett, G. Wende, A.C. Smith, K.W. Waldron, J. Sci. Food Agric. 79 (1999) 421.
[22] K.J. Briggs, S.C. Fry, Plant Physiology, American Society of Plant Physiologists, Rockville, MD, 1987.
[23] M. Bunzel, J. Ralph, J. Marita, H. Steinhart, J. Agric. Food Chem. 48 (2000) 3166.
[24] S.C. Fry, Planta 157 (1983) 111.
[25] F. Eraso, R.D. Hartley, J. Sci. Food Agric. 51 (1990) 163.
[26] T. Ishii, Plant Sci. 127 (1997) 111.
[27] A. Renger, H. Steinhart, Eur. Food Res. Technol. 211 (2000) 422.
[28] K.W. Waldron, A. Ng, M.L. Parker, A.J. Parr, J Sci. Food Agric. 74 (1997) 221.
[29] G. Wende, K.W. Waldron, A.C. Smith, C.T. Brett, Phytochemistry 52 (1999) 819.
[30] A. Ng, A.J. Harvey, M.L. Parker, A.C. Smith, K.W. Waldron, J. Sci. Food Agric. 76 (1998) 3365.
[31] R.J. Bruce, C.A. West, Plant Physiol. 91 (1989) 889.
[32] J.H. Grabber, J. Ralph, R.D. Hatfield, J. Agric. Food Chem. 48 (2000) 6106.
[33] J. Ralph, J.H. Grabber, R.D. Hatfield, Carbohydr. Res. 275 (1995) 167.
[34] M. Antolovich, P. Prenzler, K. Robards, D. Ryan, Analyst 125 (2000) 989.
[35] S.S. Deshpande, M. Cheryan, D.K. Salunkhe, CRC Crit. Rev. Food Sci. Nutr. 24 (1986) 401.
[36] A.E. Hagerman, Y. Zhao, S. Johnson, in: F. Shahidi (Ed.), Antinutrients and Phytochemicals in Food, ACS Symposium Series, vol. 662, American Chemical Society, Washington, DC, 1997, pp. 209–222.
[37] R.L. Jackman, R.Y. Yada, M.A. Tung, J. Food Biochem. 11 (1987) 279.
[38] H.P.S. Makkar, J. Agric. Food Chem. 37 (1989) 1197.
[39] L.J. Porter, Tannins, in: J.B. Harborne (Ed.), Methods in Plant Biochemistry, vol. 1, Academic Press, San Diego, CA, 1989, pp. 389–420.
[40] A. Scalbert, B. Monties, G. Janin 37 (1989) 1324.
[41] A. Scalbert, in: R.W. Hemingway, P.S. Laks (Eds.), Plant Polyphenols: Synthesis, Properties, Significance, Plenum Press, New York, NY, 1992, pp. 259–280.
[42] (a) A.S. Tempel, J. Chem. Ecol. 8 (1982) 1289;
(b) R. Robbins, J. Agric. Food Chem. 51 (2003) 2866.
[43] K. Krygier, F. Sosulski, L. Hogge, J. Agric. Food Chem. 30 (1982) 30.
[44] M. Nardini, E. Cirillo, F. Natella, D. Mencarelli, A. Comisso, C. Scaccini, Food Chem. 79 (2002) 119.
[45] H. Kozlowska, D.A. Rotkiewicz, R. Zadernowski, F.W. Sosulski, J. Am. Oil Chem. Soc. 60 (1983) 1119.
[46] (a) M. Naczk, F. Shahidi, A. Sullivan, Food Chem. 45 (1992) 51;
(b) H.D. Smolarz, Chem. Acta (Polish) 46 (2001) 439.
[47] R.D. Hartley, W.H. Morrison, J. Sci. Food Agric. 55 (1991) 365.
[48] R.C. Rodriguez-Arcos, A.C. Smith, K.W. Waldron, J. Agric. Food Chem. 50 (2002) 3197.
[49] M.L. Parker, K.W. Waldron, J. Sci. Food Agric. 68 (1995) 337.
[50] P. Mattila, J. Astola, J. Kumpulainen, J. Agric. Food Chem. 48 (2000) 5834.
[51] I.C.W. Arts, B. van de Putte, P.C.H. Hollman, J. Agric. Food Chem. 48 (2000) 1746.
[52] Y.-S. Lin, Y.-J. Tsai, J.S. Tsay, J.-K. Lin, J. Agric. Food Chem. 51 (2003) 1864.
[53] C. Cabrera, R. Giménez, C. López, J. Agric. Food Chem. 51 (2003) 4427.
[54] A.B. Moore, F.J. Francis, F.M. Clydesdale, J. Food Protect. 45 (1982) 738.
[55] A.B. Moore, F.J. Francis, M.E. Jason, J. Food Protect. 45 (1982) 590.
[56] J.E. Cacace, G. Mazza, J. Agric. Food Chem. 50 (2002) 5939.
[57] Z.Y. Ju, L.R. Howard, J. Agric. Food Chem. 51 (2003) 5207.
[58] M. Palma, L.T. Taylor, J. Chromatogr. A 849 (1999) 117.
[59] R. Murga, R. Ruiz, S. Beltran, J.L. Cabezas, J. Agric. Food Chem. 48 (2000) 3408.
[60] M. Ashraf-Khorassani, L.T. Taylor, J. Agric. Food Chem. 52 (2004) 2440.
[61] M.L. Price, S. Van Scoyoc, L.G. Butler, J. Agric. Food Chem. 26 (1978) 1214.
[62] S.S. Deshpande, M. Cheryan, J. Food Sci. 50 (1985) 905.
[63] J. Leung, T.W. Fenton, M.M. Mueller, D.R. Clandinin, J. Food Sci. 44 (1979) 1313.
[64] M. Naczk, F. Shahidi, Presented at 8th International Rapeseed Congress, 9–11 July, Saskatoon, Canada, 1991.
[65] M. Naczk, F. Shahidi, Food Chem. 31 (1989) 159.
[66] U.D. Chavan, F. Shahidi, M. Naczk, Food Chem. 75 (2001) 509.
[67] F. Shahidi, U.D. Chavan, M. Naczk, R. Amarowicz, J. Agric. Food Chem. 49 (2001) 926.
[68] R.L. Prior, S.A. Lazarus, G. Cao, H. Muccitelli, J.F. Hammerstone, Food Chem. 49 (2001) 1270.
[69] S. Guyot, N. Marnet, J.-F. Drilleau, J. Agric. Food Chem. 49 (2001) 14.
[70] B. Labarbe, V. Cheynier, F. Brossaud, J.-M. Souquet, M. Moutounet, J. Agric. Food Chem. 47 (1999) 2719.
[71] AOAC Association of Official Analytical Chemists, Official Methods of Analysis, 12th ed., AOAC, Washington, DC, 1980.
[72] M.L. Price, L.G. Butler, J. Agric. Food Chem. 25 (1977) 1268.
[73] R.E. Burns, Agron. J. 63 (1971) 511.
[74] E.D. Maxson, L.W. Rooney, Cereal Chem. 49 (1972) 719.
[75] S.J. Cork, A.K. Krockenberger, J. Chem. Ecol. 17 (1991) 123.
[76] S.K. Khanna, P.N. Viswanatham, P.S. Krishnan, G.G. Sanwai, Phytochemistry 7 (1968) 1513.

[77] S.S. Deshpande, Investigation of dry beans (*Phaseolus vulgaris* L.): microstructure, processing and antinutrients, Ph.D. thesis, University of Illinois, Champaign, IL, 1985.
[78] T. Fuleki, F.J. Francis, J. Food Sci. 33 (1968) 72.
[79] T. Fuleki, F.J. Francis, J. Food Sci. 33 (1968) 78.
[80] T. Fuleki, F.J. Francis, J. Food Sci. 33 (1968) 266.
[81] W. Oleszek, M. Sitek, A. Stochmal, S. Piacente, C. Pizza, P. Cheeke, J. Agric. Food Chem. 49 (2001) 747.
[82] W. Mullen, J. McGinn, M.E.J. Lean, M.R. MacLean, P. Gardner, G.G. Duthie, T. Yokota, A. Crozier, J. Agric. Food Chem. 50 (2002) 5191.
[83] M.I. Gil, F. Ferreres, F.A. Tomás-Barberán, J. Agric. Food Chem. 46 (1998) 2007.
[84] R. Llorach, A. Gil-Izquierdo, F. Ferreres, F.A. Tomás-Barberán, J. Agric. Food Chem. 51 (2003) 3895.
[85] L. Yao, Y. Jiang, B. D'Arcy, R. Singanusong, N. Datta, N. Caffin, K. Raymont, J. Agric. Food Chem. 52 (2004) 210.
[86] I. Martos, M. Cossetini, F. Ferreres, F.A. Tomás-Barberán, J. Agric. Food Chem. 45 (1997) 2824.
[87] F.A. Tomás-Barberán, F. Ferreres, F. Tomás-Lorente, A. Ortiz, Z. Naturforsch. 48 (1993) 68.
[88] J.B. Adams, Changes in Polyphenols of Red Fruits During Processing: The Kinetics and Mechanism of Anthocyanin Degradation, Campden Food Pres. Res. Assoc. Tech. Bull., vol. P22, Chipping Campden, Gloucestershire, UK, 1972, pp. 1–185.
[89] C.T. Du, F.J. Francis, Anthocyanins of garlic (*Allium sativum* L.): microstructure, processing and antinutrients, Ph.D. thesis, University of Illinois, Champaign, IL, 1975.
[90] M.H. Salagoity-Auguste, A.J. Bertrand, J. Sci. Food Agric. (1984) 35.
[91] A.W. Jaworski, C.Y. Lee, J. Agric. Food Chem. 35 (1987) 257.
[92] B.S. Sun, M.C. Leandro, J.M. Ricardo-da-Silva, M.I. Spranger, J. Agric. Food Chem. 46 (1998) 1390.
[93] V. Hong, R.E. Wrolstad, J. Agric. Food Chem. 38 (1990) 698.
[94] J. Wang, P. Sporns, J. Agric. Food Chem. 47 (1999) 2009.
[95] N. Mateus, A.M.S. Silva, J. Vercauteren, V. de Freitas, J. Agric. Food Chem. 49 (2001) 4836.
[96] R.S. Thompson, D. Jacques, E. Haslam, R.J.N. Tanner, J. Chem. Soc., Perkin Trans. 1 (1972) 1387.
[97] D.H. Strumeyer, M.J. Malin, J. Agric. Food Chem. 23 (1975) 909.
[98] A.B. Davis, R.C. Hoseney, Cereal Chem. 56 (1978) 310.
[99] T.N. Asquith, C.C. Izuno, L.G. Butler, J. Agric. Food Chem. 31 (1983) 1299.
[100] G. Nonaka, S. Morimoto, I. Nishioka, J. Chem. Soc., Perkin Trans. 1 (1983) 2139.
[101] J.M. Souquet, V. Cheynier, F. Brossaud, M. Moutounet, Phytochemistry 43 (1996) 509.
[102] S. Vidal, Y. Hayasaka, E. Meudec, V. Cheynier, G. Skouroumounis, J. Agric. Food Chem. 52 (2004) 713.
[103] G. Derdelinckx, J. Jerumanis, J. Chromatogr. 285 (1984) 231.
[104] A. Degenhart, H. Knapp, P. Winterhalter, J. Agric. Food Chem. 48 (2000) 338.
[105] A. Degenhart, U.E. Engelhardt, C. Lakenbrink, P. Winterhalter, J. Agric. Food Chem. 48 (2000) 3425.
[106] Modern Countercurrent Chromatography, W.D. Conway, R.J. Petrovski (Eds.), ACS Symposium Series, vol. 593, American Chemical Society, Washington, DC, 1995.
[107] A. Degenhart, S. Hofmann, H. Knapp, P. Winterhalter, J. Agric. Food Chem. 48 (2000) 5812.
[108] X. Vitrac, C. Castagnino, P. Waffo-Teguo, J.-C. Deleunay, J. Vercauteren, J.-P. Monti, G. Deffieux, J.-M. Merillon, J. Agric. Food Chem. 49 (2001) 5934.
[109] A. Degenhart, U.E. Engelhardt, A.-S. Wendt, P. Winterhalter, J. Agric. Food Chem. 48 (2000) 5200.
[110] J.H. Renault, P. Thepenier, M. Zeche-Hanrot, L.L. Men-Olivier, A. Durand, A. Foucault, R. Margraff, J. Chromatogr. A 763 (1997) 345.
[111] D. Baumann, S. Adler, M. Hamburger, J. Nat. Prod. 64 (2001) 353.
[112] O. Folin, W. Denis, J. Biol. Chem. 12 (1912) 239.
[113] T. Swain, W.E. Hillis, J. Sci. Food Agric. 10 (1959) 63.
[114] M. Brune, L. Hallberg, A.B. Skanberg, J. Food Sci. 56 (1991), 128, 167.
[115] S.S. Deshpande, M. Cheryan, J. Food Sci. 52 (1987) 332.
[116] C.F. Earp, J.O. Akingbala, S.H. Ring, L.W. Rooney, Cereal Chem. 58 (1981) 234.
[117] J.F. Hoff, K.I. Singleton, J. Food Sci. 42 (1977) 1566.
[118] J.L. Goldstein, T. Swain, Phytochemistry 2 (1963) 371.
[119] R.K. Gupta, E. Haslam, Vegetable Tannins: Structure and Biosynthesis. Polyphenols in Cereals and Legumes, International Development Research Center, Ottawa, Canada, 1980.
[120] S.K. Sarkar, R.E. Howarth, J. Agric. Food Chem. 24 (1976) 317.
[121] R.B. Broadhurst, W.T. Jones, J. Sci. Food Agric. 29 (1978) 788.
[122] I. McMurrough, J. McDowell, Anal. Biochem. 91 (1978) 92.
[123] M. Thies, R. Fischer, Mikrochim. Acta 1 (1971) 9.
[124] J.A. Delcour, J.D. de Verebeke, J. Inst. Brew. 91 (1985) 37.
[125] D. Treutter, J. Chromatogr. 467 (1989) 185.
[126] Y.-G. Li, G. Tanner, P. Larkin, J. Sci. Food Agric. 70 (1996) 89.
[127] L.J. Porter, L.N. Hrtstich, B.G. Chan, Phytochemistry 25 (1986) 223.
[128] E. Haslam, Phytochemistry 4 (1965) 495.
[129] E.C. Bate-Smith, Phytochemistry 16 (1977) 1421.
[130] P.W. Hartzfeld, R. Forkner, M.D. Hunter, A.E. Hagerman, J. Agric. Food Sci. 50 (2002) 1785.
[131] K.H. Inoue, A.E. Hagerman, Anal. Biochem. 169 (1988) 363.
[132] T.C. Wilson, A.E. Hagerman, J. Agric. Food Chem. 38 (1990) 1678.
[133] J.L.C. Lamaison, A. Carnet, Pharm. Helv. 65 (1990) 315.
[134] J. Zhishen, T. Mengcheng, W. Jianming, Food Chem. 64 (1999) 555.
[135] O.A. Zaporozhets, O.A. Krushynska, N.A. Lipkovska, V.N. Barvinchenko, J. Agric. Food Chem. 52 (2004) 21.
[136] J. Zhishen, T. Mengcheng, W. Jianming, Food Chem. 64 (1999) 555.
[137] E. Sondheimer, Z. Kertesz, Anal. Chem. 20 (1948) 245.
[138] J.L. Owades, G. Rubin, M.W. Brenner, J. Agric. Food Chem. 6 (1958) 44.
[139] J.L. Owades, G. Rubin, M.W. Brenner, Proc. Am. Soc. Brew. Chem. 6 (1958) 66.
[140] K.R. Davis, Cereal Chem. 58 (1982) 454.
[141] R. Blair, R.D. Reichert, J. Sci. Food Agric. 35 (1984) 29.
[142] M. Naczk, P.K.J.P.D. Wanasundara, F. Shahidi, J. Agric. Food Chem. 40 (1992) 444.
[143] F. Shahidi, M. Naczk, Bull. de Liason du Groupe Polyphenols 15 (1990) 236.
[144] T.J. Mabry, K.R. Markham, M.B. Thomas, The Systematic Identification of Flavonoids, Springer-Verlag, New York, NY, 1970.
[145] J.-J. Macheix, A. Fleuriet, J. Billot, Fruit Phenolics, CRC Press, Bota Raton, FL, 1990.
[146] R. Kramer, Chemometric Techniques for Quantitative Analysis, Dekker, New York, NY, 1998.
[147] F.R. van der Voort, Food Res. Int. 25 (1992) 397.
[148] K.R. Beebe, B.R. Kowalski, Anal. Chem. (1987) 59.
[149] L. Monedero, M. Olalla, F. Martin-Lagos, H. Lopez, M.C. Lopez, J. Agric. Food Chem. 47 (1999) 1836.
[150] A. Edelmann, J. Diewok, K.C. Schuster, B. Lendl, J. Agric. Food Chem. 49 (2001) 1139.
[151] O.V. Brenna, E. Pagliarini, J. Agric. Food Chem. 49 (2001) 4841.
[152] R. Briandet, E.K. Kemsley, R.H. Wilson, J. Agric. Food Chem. 44 (1996) 170.
[153] G. Downey, R. Briandet, R.H. Wilson, K. Kemsley, J. Agric. Food Chem. 45 (1997) 4357.
[154] H. Schulz, U.H. Engelhardt, A. Wegent, H.-H. Drews, S. Lapczynski, J. Agric. Food Chem. 47 (1999) 5064.

[155] K.J. Dabrowski, F.W. Sosulski, J. Agric. Food Chem. 32 (1984) 123.
[156] J. Liggins, L.J.C. Bluck, Anal. Biochem. 264 (1998) 1.
[157] B.V. Thomas, A.A. Schreiber, C.P. Weisskopf, J. Agric. Food Chem. 46 (1998) 2655.
[158] M. Friedman, N. Kozukue, L.A. Harden, J. Agric. Food Chem. 48 (2000) 5702.
[159] P.J. Hemes, J.J. Hedges, Anal. Chem. 72 (2000) 5115.
[160] R. Zadernowski, Acta Acad. Agricult. Olst. Technol. Aliment. 21 (Suppl. F) (1987) 1.
[161] J. Jurenitsch, M. David, F. Heresch, W. Kubelka, Planta Med. 36 (1979) 61.
[162] J. Jurenitsch, R. Leinmuller, J. Chromatogr. 189 (1980) 389.
[163] S. Kosuge, M. Furuta, Agric. Biol. Chem. 34 (1970) 248.
[164] D. Chassagne, J. Crouzet, C.L. Bayonove, R.L. Baumes, J. Agric. Food Chem. 46 (1998) 4352.
[165] C.C. Sweeley, R. Bentley, M. Makita, W.W. Wells, J. Am. Chem. Soc. 85 (1963) 2497.
[166] S.D. Senter, J.A. Robertson, F.I. Meredith, J. Food Sci. 54 (1989) 1259, 1268.
[167] K. Hostettmann, M. Hostettman, in: J.B. Harborne, T.J. Mabry (Eds.), The Flavonoids: Advances in Research, Chapman & Hall, New York, NY, 1982, pp. 1–18.
[168] D.J. Daigle, E.J. Conkerton, J. Liq. Chromatogr. 6 (1983) 105.
[169] D.J. Daigle, E.J. Conkerton, J. Liq. Chromatogr. 11 (1988) 309.
[170] J.J. Karchesy, Y. Bae, L. Chalker-Scott, R.F. Helm, L.Y. Foo, Chromatography of Proanthocyanidins. Chemistry and Significance of Condensed Tannins, Plenum Press, New York, NY, 1989.
[171] K. Robards, M. Antolovitch, Analyst 122 (1997) 11R.
[172] H.M. Merken, G.R. Beecher, J. Agric. Food Chem. 48 (2000) 577.
[173] S. Carando, P.-L. Teissedre, L. Pascual-Martinez, J.-C. Cabanis, J. Agric. Food Chem. 47 (1999) 4161.
[174] R. Edenharder, G. Keller, K.L. Platt, K.K. Unger, J. Agric. Food Chem. 49 (2001) 2767.
[175] M.-H. Siess, A.M. Le Bon, M.-C. Canivenc-Lavier, M.-J. Amiot, S. Sabatier, S.Y. Aubert, M. Suschetet, J. Agric. Food Chem. 44 (1996) 2297.
[176] F.A. Tomás-Barberán, M.I. Gil, P. Cremin, A.L. Waterhouse, B. Hess-Pierce, A.L. Kader, J. Agric. Food Chem. 49 (2001) 4748.
[177] C. Wang, M. Sherrard, S. Pagala, R. Wixon, R.A. Scott, J. Am. Oil Chem. Soc. 77 (2000) 483.
[178] L.-F. Wang, D.-M. Kim, C.Y. Lee, J. Agric. Food Chem. 48 (2000) 4227.
[179] P. Zafrilla, F. Ferreres, F.A. Tomas-Barberan, J. Agric. Food Chem. 49 (2001) 3651.
[180] J.S. Amaral, R.M. Seabra, P.B. Andrade, P. Valentão, J.A. Pereira, F. Ferreres, Food Chem. 88 (2004) 373.
[181] M. Sano, M. Suzuki, T. Miyase, K. Yoshino, M. Maeda-Yamamoto, J. Agric. Food Chem. (1999) 47.
[182] S. de Pascual-Teresa, D. Treutter, J.C. Rivas-Gonzalo, C. Santos-Buelga, J. Agric. Food Chem. 46 (1998) 4209.
[183] I.C.W. Arts, P.C.H. Hollman, J. Agric. Food Chem. 46 (1998) 5156.
[184] C.E. Lunte, J.F. Wheeler, W.R. Heineman, Analyst 113 (1988) 94.
[185] S. Mahler, P.A. Edwards, M.G. Chisholm, J. Agric. Food Chem. 36 (1988) 946.
[186] (a) P.J. Woodring, P.A. Edwards, M.G. Chisholm, J. Agric. Food Chem. 38 (1990) 729;
(b) A. Romani, M. Minunni, N. Mulinacci, P. Pinelli, F.F. Vincleri, J. Agric. Food Chem. 48 (2000) 1197.
[187] Z. Peng, Y. Hayasaka, P.G. Iland, M. Sefton, P. Hoj, E.J. Waters, J. Agric. Food Chem. 49 (2001) 26.
[188] S. Barnes, L. Coward, M. Kirk, J. Sfakianos, PSEBM 217 (1998) 254.
[189] J.F. Hammerstone, S.A. Lazarus, A.E. Mitchell, R. Rucker, H.H. Schmitz, J. Agric. Food Chem. 47 (1999) 490.
[190] M. Satterfield, J.S. Brodbelt, Anal. Chem. 72 (2000) 5898.
[191] J. Bakker, P. Bridle, A. Koopman, J. Sci. Food Agric. 60 (1992) 471.
[192] M.-C. Menet, S. Sang, C.S. Yang, C.-T. Ho, R.T. Rosen, J. Agric. Food Chem. (2004) in press.
[193] A.H. Simonne, M. Smith, D.B. Weaver, T. Vail, S. Barnes, G.I. Wei, J. Agric. Food Chem. 48 (2000) 6061.
[194] L. Coward, N.C. Barnes, K.D.R. Setchell, S. Barnes, J. Agric. Food Chem. 41 (1993) 1961.
[195] P.A. Murphy, T.T. Song, G. Buseman, K. Barua, J. Agric. Food Chem. 45 (1997) 4635.
[196] E. Kinoshita, Y. Ozawa, T. Aishima, J. Agric. Food Chem. 45 (1997) 3753.
[197] A.A. Franke, J.H. Hankin, M.C. Yu, G. Maskarinec, S.-H. Low, L.J. Custer, J. Agric. Food Chem. 47 (1999) 977.
[198] L.S. Hutabarat, H. Greenfield, M. Mulholland, J. Food Compos. Anal. 14 (2001) 43.
[199] H. Fulcrand, S. Remy, J.-M. Souquet, V. Cheynier, M. Moutounet, J. Agric. Food Chem. 47 (1999) 1023.
[200] B.S. Sun, M.C. Leandro, J.M. Ricardo-da-Silva, M.I. Spranger, J. Agric. Food Chem. 46 (1998) 1390.
[201] Z.-Y. Chen, Q.Y. Zhu, Y.F. Wong, Z. Zhang, H.Y. Chung, J. Agric. Food Chem. 46 (1998) 2512.
[202] L. Gennaro, C. Leonardi, F. Esposito, M. Salucci, G. Maiani, G. Quaglia, V. Fogliano, J. Agric. Food Chem. 50 (2002) 1904.
[203] G. Mazza, L. Fukumoto, P. Delaquis, P.B. Girard, B. Ewert, J. Agric. Food Chem. 47 (1999) 4009.
[204] H.S. Lee, J. Agric. Food Chem. 50 (2002) 1243.
[205] J. Hempel, H. Bohm, J. Agric. Food Chem. 44 (1996) 2114.
[206] C.W. Beninger, G.L. Hosfield, J. Agric. Food Chem. 47 (1999) 4079.
[207] B.D. Oomah, G. Mazza, J. Agric. Food Chem. 44 (1996) 1746.
[208] A. Crozier, M.E.J. Lean, M.S. McDonald, C. Black, J. Agric. Food Chem. 45 (1997) 590.
[209] (a) K.H. Miean, S. Mohamed, J. Agric. Food Chem. 49 (2001) 3106;
(b) M.G.L. Hertog, P.C.H. Hollman, D.P. Venema, J. Agric. Food Chem. 40 (1992) 1591;
(c) M.G.L. Hertog, P.C.H. Hollman, M.B. Katan, J. Agric. Food Chem. 40 (1992) 2379;
(d) M.G.L. Hertog, P.C.H. Hollman, B. van de Putte, J. Agric. Food Chem. 41 (1993) 1242.
[210] M.V.S.S.T. Subra Rao, G. Muralikrishna, J. Agric. Food Chem. 50 (2002) 889.
[211] J.Y.T. Vasanthan, F. Temelli, J. Agric. Food Chem. 49 (2001) 4352.
[212] K. Ogawa, A. Kawasaki, T. Yoshida, H. Nesumi, M. Nakano, Y. Ikoma, M. Yano, J. Agric. Food Chem. 48 (2000) 1763.
[213] Z. Xu, J.S. Goldber, J. Agric. Food Chem. 47 (1999) 2724.
[214] P. Johnsson, A. Kamal-Eldin, L.N. Lundgren, P. Aman, J. Agric. Food Chem. 48 (2000) 5216.
[215] (a) T.H. Sanders, R.W. McMichael, K.W. Hendrix, J. Agric. Food Chem. 48 (2000) 1243;
(b) G. Montedoro, M. Servili, M. Baldioli, E. Miniati, J. Agric. Food Chem. 40 (1992) 1571;
(c) G. Montedoro, M. Servili, M. Baldioli, E. Miniati, J. Agric. Food Chem. 40 (1992) 1577;
(d) M. Brenes, A. Garcia, J.J. Rios, A. Garrido, J. Agric. Food Chem. 47 (1999) 3535;
(e) R. Mateos, J.L. Espartero, M. Trujillo, J.J. Rios, M. León-Camacho, F. Alcudia, A. Cert, J. Agric. Food Chem. 49 (2001) 2185;
(f) S. Gómez-Alfonso, M.D. Salvador, G. Fregapane, J. Agric. Food Chem. 50 (2002) 6812;
(g) C. Romero, M. Brenes, P. Garcia, A. Garrido, J. Agric. Food Chem. 50 (2002) 3835;

(h) K. Aaby, E. Hvattum, G. Skrede, J. Agric. Food Chem. 52 (2004) 4595;
(i) A. Molinelli, R. Weiss, B. Mizaikoff, J. Agric. Food Chem. 50 (2002) 1804;
(j) M.J. Whitcombe, C. Alexander, E.N. Vulfson, Trends Food Sci. Technol. 8 (1997) 140;
(k) J. Ramström, K. Skudar, J. Haines, P. Patel, O. Brüggemann, J. Agric. Food Chem. 49 (2001) 2105.