ELSEVIER

Journal of Chromatography A, 881 (2000) 403–410

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Review

Analysis of anthocyanins in foods by liquid chromatography, liquid chromatography–mass spectrometry and capillary electrophoresis

Cristina T. da Costa[a,c], Derek Horton[b], Sam A. Margolis[c,*]

[a]*Évora University, Department of Chemistry, Rua Romão Ramalho 59, 7000 Évora, Portugal*

[b]*American University, Department of Chemistry, 4400 Massachusetts Avenue, Washington, DC 20216, USA*

[c]*National Institute of Standards and Technology, Analytical Chemistry Division, Gaithersburg, MD 20899-8392, USA*

Abstract

This article reviews recent developments in the methodology for the measurement of anthocyanins that offer several advantages over classical methods of analysis. The use of UV-diode array and mass spectrometric (MS) detectors, with improved methods of liquid chromatography analysis has facilitated identification of these analytes. The use of capillary electrophoresis (CE) analysis of the anthocyanins under acid conditions has significantly increased peak resolution and improved the detection limits by several orders of magnitude. CE offers the advantage of economies of very small sample size, very small solvent consumption, and short analysis times along with the future possibility of being combined with MS detection.

Keywords: Reviews; Food analysis; Wine; Grapes; Fruits; Anthocyanins

Contents

1. Introduction

Anthocyanins are water-soluble pigments that are responsible for the red, blue, and purple colors of most flowers and fruits. Anthocyanins are glycosylated derivatives of the 3,5,7,3′-tetrahydroxyflavylium cation and are classified as flavonoids. The electron deficiency of the flavylium cation makes the free aglycones (anthocyanidins) highly reactive, and they do not occur naturally. The glycosides are more stable than the aglycones, and over 250 different anthocyanins have been isolated from plants [1]. These anthocyanins are all *O*-glycosylated with

*Corresponding author. Fax.: +1-301-977-0685.

E-mail address: sam.margolis@nist.gov (S.A. Margolis)

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00328-9

different sugar substituents. The glycosyl moiety is usually located at carbons 3, 5, 7, 3′, and 5′ and the most prevalent sugars are D-glucose, L-rhamnose, D-galactose, D-xylose and arabinose [2]. Anthocyanins are relatively unstable molecules, and their colors are dependent on pH, temperature, light, and the presence of metals [1]. Because anthocyanins are natural products they are a source of potential replacements for banned synthetic food dyes, but their use is limited because they tend to lose their color under common food-processing conditions. Acylation with cinammic acids or related acids confers additional stability to anthocyanins, and different crops have been assessed as potential sources of acylated anthocyanins for use as new food colorants [3]. The beneficial pharmacological activities and possible health benefits of the anthocyanins confer a distinct advantage for their use as food colorants. Red fruit extracts that are rich in anthocyanins are used in folk medicine and have some positive therapeutic effects as anti-inflammatory agents [4], and in the treatment of various ailments, including micro-circulation diseases [4] resulting from capillary fragility and the prevention of cholesterol-induced artherosclerosis [4]. The biological properties of anthocyanins have been mainly attributed to their antioxidant properties, and the capacity of the individual anthocyanins [4,5] as well as plant extracts containing high amounts of anthocyanins [6] to react with oxygen radicals [7]. The antioxidant mechanisms of the anthocyanins have not been established, although there are indications that hydrogen donation, metal chelation, and protein binding are involved [5].

The variability in the patterns of anthocyanin pigments as a function of plant species is used in chemotaxonomy and for quality control in the food industry (see Ref. [8] for additional references). Determination of the anthocyanin profile is widely used for verification of authenticity of food products prepared from red fruits, such as syrups, jams, and in the fruit drink industry (see Ref. [8] for additional references).

In 1992 Lee et al. [8] reviewed the chromatographic methods of analysis of anthocyanins. High-performance liquid chromatography (LC) was the method of choice for determination of anthocyanins, and it remains the most commonly used method for their measurement. Recently, anthocyanins have also been identified in plant extracts by other analytical techniques, including capillary electrophoresis (CE) and liquid chromatography–mass spectrometry (LC–MS). This report reviews the significant advances in the separation and measurement of anthocyanins published after 1992, with special emphasis on the use of LC, CE, and LC–MS analytical methods. We have cited a representative selection of the application of these methods to various plant materials and when necessary we have cited a few significant early references for the benefit of the reader.

2. Sample preparation

The anthocyanins are soluble in polar solvents, and they are commonly extracted from plant materials with methanol that contains small amounts of hydrochloric or formic acid. The acid lowers the pH of the solution and prevents the degradation of the non-acylated anthocyanin pigments. However, small amounts of acid may cause partial or total hydrolysis of the acyl moieties of acylated anthocyanins that are present in some plants. A comparison of various techniques for the extraction of anthocyanins from red grapes was recently published [9] and this report also demonstrated that solvents containing up to 0.12 mol/l hydrochloric acid cause partial hydrolysis of acylated anthocyanins (also see Ref. [9] for additional references).

Acetone has also been used to extract anthocyanins from several plant sources [10,11]. In comparison to acidified methanol, this technique for the extraction of anthocyanins from red fruits allows an efficient and more reproducible extraction, avoids problems with pectins, and permits a much lower temperature for sample concentration [11]. Solid-phase extraction (SPE) on C_{18} (SPE) cartridges or Sephadex is commonly used for the initial purification of the crude anthocyanin extracts (see review by Kraemer-Schafhalter et al. [12]). The anthocyanins are bound strongly to these adsorbents through their unsubstituted hydroxyl groups and are separated from unrelated compounds by using a series of solvents of increasing polarity.

3. LC analysis

Reversed-phase LC is the most common method used for the separation of anthocyanins. Because each laboratory has tended to develop an analytical method for a specific separation requirement, it is impossible to describe a single standard procedure. However, certain conditions are commonly followed. Detection is usually performed using UV diode-array absorbance detection (DAD), which enables the collection of on-line spectra. The UV–Vis absorbance spectrum of an anthocyanin can give information on the nature of the aglycone, glycosylation pattern, and possibility of acylation [13]. Single-wavelength detectors can selectively monitor anthocyanins between 520 and 546 nm, where no other plant phenolics show absorption. C_{18} columns are most commonly used for separations, but polystyrene columns have also been used [10,14–16]. The separation characteristics of anthocyanins on a variety of C_{18} columns produced by different manufacturers vary considerably. In our hands the C_{18} deactivated columns provide the best separations of anthocyanins and other plant phenols [17]. Some peak broadening and peak tailing was observed in non-deactivated columns, and this has been attributed to the interaction of free hydroxyl groups, present in the anthocyanins and other phenolic compounds, with silanol groups at the silica surface.

The separation of structurally similar phenolic compounds is accomplished most effectively by gradient elution, using methanol or acetonitrile as an organic modifier. The pH of the elution system is normally kept below two by the addition of a small amount of formic, acetic, or trifluoroacetic acid. Below pH 3.2, anthocyanins exist as two distinct interconvertible forms: the red flavylium cation and the blue quinoidal species [18]. According to Wulf et al. [19] 96% of an anthocyanin is in the flavylium form at pH 1.5, but only 67% is in that form at pH 2.5. Above pH 2 severe peak broadening results from the slow interconversion between species [20], leading to poor resolution and reduced detection limits.

The polarity of the aglycones (anthocyanidin) is the most important factor affecting the LC retention times. Under the usual conditions, the order of elution is delphinidin derivatives, followed by the cyanidin, petunidin, pelargonidin, peonidin, and malvidin derivatives. Thus, the retention decreases with increasing polarity (i.e. increased number of hydroxyl groups in the flavylium nucleus). The presence of sugars increases the retention of the anthocyanins, with diglucosides usually eluting before monoglycosides. Acylation also increases the retention time of the anthocyanins when compared to the similar non-acylated derivative. In 1993 Amic et al. [21] presented a theoretical calculation for the retention time of several anthocyanins, which agrees with experimental values.

The evaluation of the anthocyanin content in grapes and wine is commercially important. The color and quality of wines depends to a large extent on the anthocyanins present in grape cultivars. However, it is often difficult to make a direct correlation between the amount and composition of anthocyanins present in the grapes and in the wine. The anthocyanins in the red grapes vary greatly with the species, maturity, production area, seasonal conditions, and yield of fruit. Differences in the manufacturing process and maturation also influence the amount and type of anthocyanins in the wine. Table 1, Section A lists the references to papers concerning the separation of anthocyanin pigments from red grapes and wine published since 1992.

The anthocyanin profiles of some food products derived from red fruits are used to verify the authenticity and control the quality of these products. Despite the fact that the number of laboratories performing such analyses increases every year, there is no validated chromatographic method, and most laboratories use their own specific methods. Goiffon et al. [22] recently validated their method for the determination of the anthocyanins present in the most common red fruit juices, namely, blackcurrant, elderberry, sour cherry, strawberry, grape, blueberry, raspberry, and red currant. The references to papers published since 1992 concerning the separation of anthocyanin pigments from red fruits and other food products are listed in Table 1, Section B. Interest in replacing some food colorants with acylated anthocyanins led to the publication of several reports concerning the evaluation of different plants (some already used as food crops) as possible sources of such compounds (Table 1, Section C). Section D of

Table 1
LC analysis of anthocyanins in foods[a]

Food Product	Ref.
Section A. *Wine and red grapes*	
Wine	[14,23–25]
Red grape	[9,22,26,27,58–60]
Section B. *Red fruits*	
Blueberry	[22,28–31]
Blackberry	[31]
Blackcurrant	[22,31]
Cherries	[16,22,32]
Huckleberries	[33]
Pomegranate	[34,35]
Raspberry	[22,36]
Red Currant	[22,31,37]
Red Pears	[38]
Strawberries	[22,31,39–42]
Section C. *Crops with a high content of acylated anthocyanins*	
Red fleshed potato	[15,43,44]
Red radish	[10,45]
Elderberry	[22,46]
Basil	[16]
Section D. *Various food products*	
Black bean	[47]
Bambara groundnut	[48]
Black rice	[49]
Lychee	[50]
Olive	[51]
Onion	[52,53]

[a] This table contains a representative selection of references. Additional references can be obtained by consulting the cited references.

Table 1 lists the references to LC determination of anthocyanins from various food products.

4. LC–MS analysis

The characterization of a mixture of anthocyanins usually involves separation and collection of each compound, and subsequent analysis by nuclear magnetic resonance (NMR) and fast atom bombardment mass spectroscopy (FAB-MS) [55]. The lack of suitable standards necessitates a long and tedious procedure for the identification of known compounds. LC–MS combines the separation of LC with the selectivity and sensitivity of the MS detector permitting the identification of individual components of a mixture of compounds. The application of LC–MS methods to food analysis [54] and mass spectrometry methods to the measurement of plant phenols [55] have been recently reviewed. These reviews provide a good overview of the different available LC–MS methods, but there are very few reports on the application of LC–MS to the analysis of anthocyanins in food products or plant extracts. In 1992 Glässgen et al. [56] described the use of liquid–chromatography electrospray ionization mass spectrometry (LC–ESI-MS) for the identification of anthocyanins in plant tissue and cell cultures of Indian black carrot (*Daucus carota* L. ssp. *sativus*). The anthocyanins were separated on a narrow-bore reversed-phase LC column, with gradient elution using acetonitrile, water, and formic acid solvents, and a flow-rate of 200 μl/min. A triple quadrupole mass spectrometer was used, and collision-induced dissociation (CID) experiments were performed using argon gas. Several cyanidin derivatives were identified, including acylated and non-acylated compounds. Recently liquid chromatography–electron impact ionization mass spectrometry (LC–EI-MS) was also used to identify the anthocyanins of *Catharanthus roseus* extracts [57].

In 1995 Baldi et al. [58] used LC–MS with an atmospheric pressure-ionization ion-spray interface to analyze the anthocyanins contained in the skins of grapes (*Vitis vinifera* L.). An acidified ethanolic extract of grape skin was fractionated with organic solvents to obtain a sample containing only the anthocyanins. The anthocyanins in the extract were separated by a three-step linear gradient with water, methanol, acetonitrile, and formic acid solvents at a flow-rate of 1.5 ml/min. The flow was split 1:25 to allow a 60 μl/min flow-rate into the spray ion interface before a single quadrupole mass spectrometer. Nineteen derivatives of cyanidin, delphinidin, petunidin, malvidin and peonidin were identified by this ionization technique. The individual mass spectra showed peaks for the molecular ion, together with a fragment corresponding to the aglycone; when acylation was present, an additional fragment was detected at m/z values corresponding to loss of the acyl moiety from the molecular ion.

Tamura et al. [59] reported the use of LC–MS with continuous-flow fast atom bombardment (CF-

FAB) to separate and identify anthocyanins in the Japanese grape, Muscat Bailey A. The grape pigment extracts were separated by gradient elution on a reversed-phase column with water, acetonitrile, and 0.5 mass% trifluoroacetic acid solvents at a flow-rate of 1 ml/min. After separation, a matrix solvent of 3% (v/v) glycerol and 1 mg/g dimethyl sulfoxide in methanol was added to the carrier solution at a rate of 0.3 ml/min. The LC flow was allowed to mix with the matrix solvent before reaching a splitter that introduced 5 μl/min of solution into the mass ion source. The positive molecular ions of five malvidin derivatives were detected together with several fragments including the intact aglycone (*m/z* 331).

Atmospheric-pressure ionization (API) techniques have several advantages over other MS detection methods. In API-MS the ion source is located outside the MS, the ions are formed at atmospheric pressure, and then sampled into the mass spectrometer. These are soft ionization techniques (only the molecular ion is formed), although the application of a potential at the entrance of the mass spectrometer (fragmentor voltage) creates suitable conditions for CID, and production of fragment ions. Two API interfaces are available commercially namely, the atmospheric pressure chemical ionization interface (APCI) and the ESI interface. Recently Revilla et al. [60] analyzed the anthocyanins present in extracts of the grape skin and red wines with a LC–MS system equipped with the ES interface. The anthocyanin mixtures were applied to a reversed-phase LC column, and separated by gradient elution with methanol–water–formic acid solvents at a flow-rate of 0.8 ml/min. The identity of the analytes was established by the MS data, together with UV–Vis absorbance spectra obtained on-line with DAD.

We used an LC–MS system that was equipped with an APCI interface for analysis of anthocyanins (3-glucosides and the 3-rutinosides of cyanidin and delphinidin) from the blackcurrant fruit (*Ribes nigrum*) [17]. A sample rich in blackcurrant anthocyanins was separated on a reversed-phase LC column with a water–acetonitrile–formic acid gradient at a flow-rate of 1.5 ml/min. The LC eluent was directly introduced into the APCI interface. The molecular ion [M^+], and the mass fragments corresponding to the successive loss of the sugar residues, [M^+-146], [M^+-146–162] (Fig. 1) were detected under appropriate conditions. By increasing the fragmentor voltage the aglycone fragments produced an additional series of ions, the most important of which was a retro-Diels–Alder fragment that permitted confirmation of the identity of the aglycone.

5. CE analysis

CE is a relatively new analytical tool having excellent mass sensitivity, high resolution, low sample consumption, and minimal generation of solvent waste (1 to 2 ml/day) [61]. Very few reports have appeared in the literature on the application of CE methods to the separation of anthocyanins. In 1996 Bridle et al. [62,63] reported the separation of a mixture of standards, as well as strawberry and elderberry anthocyanins, by capillary zone electrophoresis (CZE). The authors used standard silica capillaries, and borate running buffers at pH 8. The applicability of this method is limited in part by the instability of the anthocyanins in basic media. Furthermore the predominant ionic species at basic pH does not absorb light at 580 nm. This lack of sufficient quantities of absorbing anthocyanin ions reduced the sensitivity of the detection method. At pH 8 a much more concentrated sample (87 times) was required for an equivalent CZE response at pH 8 as compared to LC at pH 1.8 [42].

In 1998, we published a CZE method to separate blackcurrant anthocyanins in a fused-silica capillary using acidic phosphate buffers (pH 1.5)–acetonitrile (2:1 v/v) [64]. The interaction of the anthocyanins with the capillary wall was evaluated by comparing the separation of anthocyanins on a fused-silica capillary column to that on a linear polyacrylamide (LPA) coated one. The pH of the running buffer was increased to 1.8 because of the instability of the coated capillary at pH 1.5. The migration times were 1 to 3 min longer with the LPA column, and the peak shape and resolution were very similar for both columns (see Fig. 2 [64]). This indicated that there was almost no interaction at pH 1.8 between the silanols on the uncoated capillary and the anthocyanins. The four anthocyanins present in the blackcurrant juice, namely cyanidin and delphinidin 3-glucosides and 3-rutinosides, were separated within

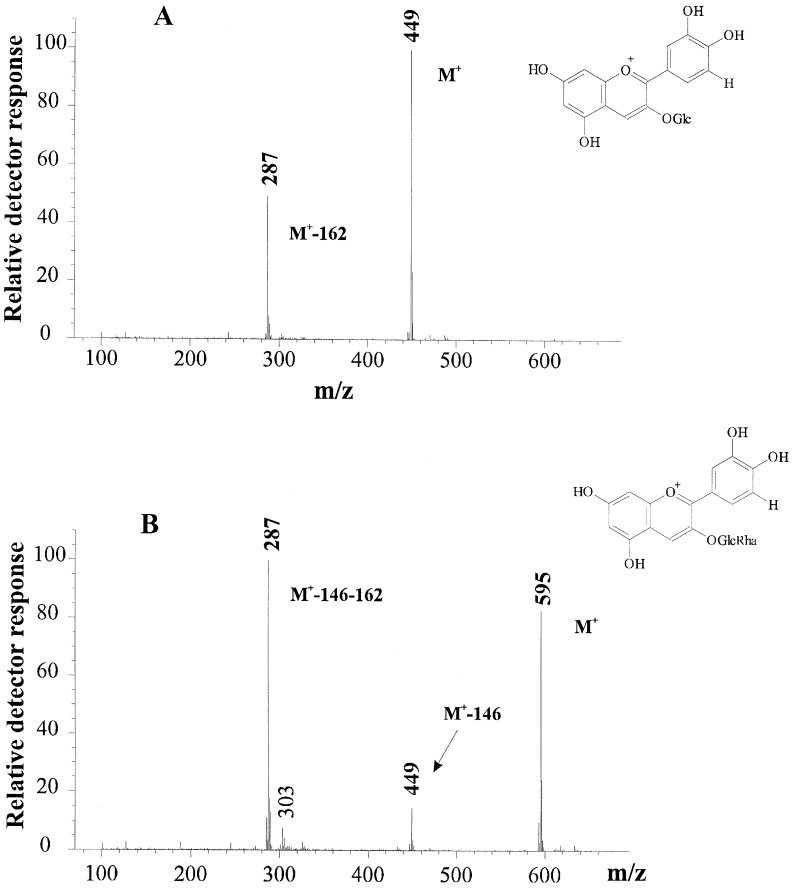

Fig. 1. APCI mass spectra of cyanidin 3-glucoside (A) and cyanidin 3-rutinoside (B) obtained on-line by LC separation of blackcurrant extracts [60].

the time range expected for an LC analysis, while consuming much less sample and solvent.

Four anthocyanin derivatives were separated by CZE at acidic pH using fused-silica capillaries, and a phosphate running buffer containing 0.25 mmol/L cetyltrimethylammonium bromide (CTAB) at pH 2.1 [65]. The concentration of the detergent was set at about one fourth of the critical micellar concentration thus avoiding the formation of micelles. The CTBA cation was essential for the migration of the anthocyanins to the detector. According to Bricard et al. [65], CTAB (a cationic surfactant) interacts with the silanol groups on the capillary wall and reduces the adsorption of the anthocyanins. We did not observe this interaction of the anthocyanins with the silanol groups [64].

Watanabe et al. [66] analyzed the elderberry pigments (*Sanbucus nigra* L.) in commercial food samples (candy, juice, and jelly) by micellar electrokinetic chromatography (MEKC). The anthocyanins, all cyanidin derivatives, were separated using 30 mmol/L sodium dodecyl sulfate (SDS) in 30 mmol/L phosphate–60 mmol/L borate buffer at pH 7.0. At acidic pH the elderberry pigments show a UV–Vis absorbance maximum at 510 nm, but this maximum shifts to 560 nm at neutral pH, which is the detection wavelength used in the MEKC analysis. The authors successfully separated the elderberry anthocyanins in less than 10 min.

6. Conclusion

Two independent methods of analysis of antho-

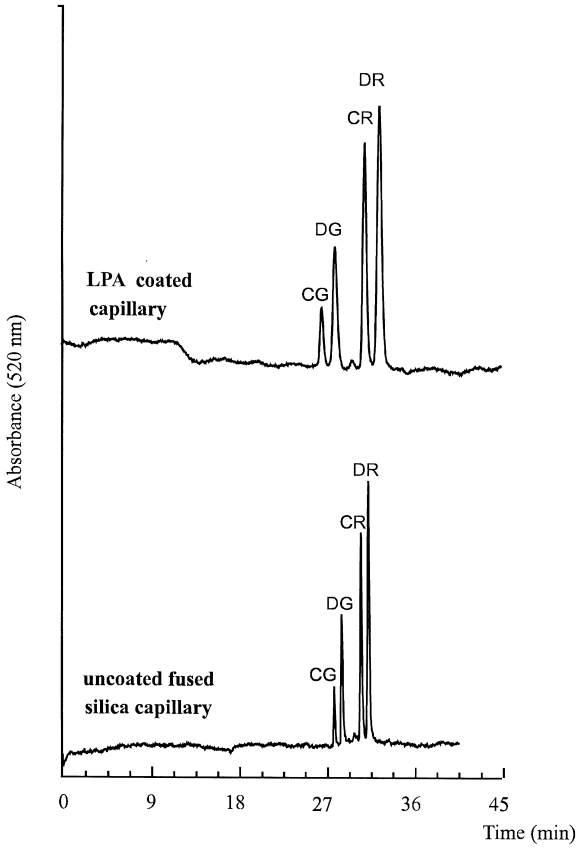

Fig. 2. Comparison of the optimized separation of blackcurrant anthocyanins on an uncoated fused-silica capillary and on a linear polyacrylamide (LPA) coated capillary. Peak identification: CG= cyanidin 3-glucoside; CR=cyanidin 3-rutinoside; DG= delphinidin 3-glucoside; DR=delphinidin 3-rutinoside [64].

cyanins have been described, liquid chromatography (LC) and capillary electrophoresis (CE). The LC method can be used either with a UV detector or a MS detector. The UV detector is useful for routine analysis and method development. The MS method has much greater selectivity and permits the partial identification of the structure of the anthocyanins. The CE method permits the use of very small samples and very small amounts of solvent, but does not offer the range of separation of complex samples that can be achieved with LC. Because the CE method uses much smaller samples it does not offer any advantage over LC with respect to sensitivity. Each of these methods has a useful role in the analysis of anthocyanins in plant materials and products.

Acknowledgements

C.T.d.C. acknowledges the financial support from Fundação Calouste Gulbenkian, Portugal.

References

[1] D. Strack, V. Wray, in: J.B. Harborne (Ed.), The Flavonoids: Advances in Research Since 1986, Chapman & Hall, London, 1993, p. 1.
[2] F.J. Francis, Food Colorants: Anthocyanins, Crit Rev. Food. Sci. Nutr. 28 (1989) 273.
[3] P. Bridle, C.F. Timberlake, Food Chem. 58 (1997) 103.
[4] H. Wang, G. Cao, R.L. Prior, J. Agric. Food Chem. 45 (1997) 304.
[5] M.T. Satue-Garcia, M. Heinonen, E.N. Frankel, J. Agric. Food Chem. 45 (1997) 3362.
[6] P.M. Abuja, M. Murkovic, W. Pfannhauser, J. Agric. Food Chem. 46 (1998) 4091.
[7] A.S. Meyer, O.S. Yi, D.A. Pearson, A.L. Waterhouse, E.N. Frankel, J. Agric. Food Chem. 45 (1997) 1638.
[8] H.S. Lee, V. Hong, J. Chromatogr. A 624 (1992) 221.
[9] E. Revilla, J.M. Ryan, G. Martin-Ortega, J. Agric. Food Chem. 46 (1998) 4592.
[10] M.M. Giusti, L.E. Rodriguz-Saona, J.R. Baggett, G.L. Reed, R.W. Durst, R.E. Wrolstad, J. Food Sci. 63 (1998) 219–224.
[11] C. Garcia-Viguera, P. Zafrilla, F.A. Tomas-Barberan, Phytochem. Anal. 9 (1998) 274.
[12] A. Kraemer-Schafhalter, H. Fuchs, W. Pfannhauser, J. Sci. Food Agric. 78 (1999) 435.
[13] V. Hong, R.E. Wrolstad, J. Agric. Food Chem. 38 (1990) 708.
[14] A.L. Waterhouse, S.F. Price, J.D. McCord, Methods Enzymol. (1999) 113.
[15] L.E. Rodriguz-Saona, M.M. Giusti, R.E. Wrolstad, J. Food Sci. 63 (1998) 458.
[16] W.B. Phippen, J.E. Simon, J. Agric. Food Chem. 46 (1998) 1734.
[17] C.T. da Costa, J.J. Dalluge, S.A. Margolis, D. Horton, Abstr. Pap. Am. Chem. Soc. Meet. 1999.
[18] D. Strack, V. Wray, in: J.B. Harborne (Ed.), Plant Phenolics, Academic Press, San Diego, CA, 1989, p. 325, Chapter 9.
[19] L.W. Wulf, C.W. Nagel, Ant. J. Enol. Agric. 29 (1978) 42.
[20] M.L. Hale, F.J. Francis, I.S. Fargerson, J. Food Sci. 51 (1986) 1511.
[21] D. Amic, D. Davidovic-Amic, J. Chromatogr. A 653 (1993) 115.
[22] J.P. Goiffon, P.P. Moulby, E.M. Gaydou, Anal. Chim. Acta 382 (1999) 39.
[23] R.M. Lamuela-Raventos, A.L. Waterhouse, Am. J. Enol. Vitic. 45 (1994) 1.
[24] T.V. Johnston, J.R. Morris, J. Food Sci. 62 (1997) 684.
[25] A. Pena, V. Garcia, M.d.l.L. Romero, S. Cappela, J. Chromatogr. Sci. 35 (1997) 161.
[26] Y. Gao, G.A. Cahoon, Am. J. Enol. Vitic. 46 (1995) 339.

[27] J.A. Fernandez-Lopez, L. Almela, J.M. Lopes-Roca, Anales Quim. 91 (1995) 380.
[28] F. Kader, B. Rovel, M. Girardin, M. Metche, Food Chem. 55 (1999) 35.
[29] L. Gao, G. Mazza, J. Liq. Chromatogr. 18 (1995) 245.
[30] L. Gao, G. Mazza, J. Food Sci. 59 (1994) 1057.
[31] C. Garcia-Viguera, P. Zafrilla, F.A. Tomas-Barberan, J. Sci. Food Agric. 73 (1997) 207.
[32] H. Wang, M.G. Nair, A.F. Iezzoni, G.M. Starsburg, A.M. Booren, J.I. Gray, J. Agric. Food Chem. 45 (1997) 2556.
[33] C.L. Price, R.E. Wrolstad, J. Food Sci. 60 (1995) 369.
[34] M.I. Gil, J. Cherif, N. Ayed, F. Artes, F.A. Tomas-Barberan, Z. Lebensm. Unters. Forsch. 201 (1995) 361.
[35] M.I. Gil, C. Garcia-Viguera, F. Artes, F.A. Tomas-Barberan, J. Sci. Food Agric. 68 (1995) 77.
[36] C. Garcia-Viguera, P. Zafrilla, F. Artes, F. Romero, P. Abellan, F.A. Tomas-Barberan, J. Sci. Food Agric. 78 (1998) 565.
[37] B. de Ancos, E. Gonzalez, M.P. Cano, Z. Lebensm. Unters. Forsch. 208 (1999) 33.
[38] M.C. Dussi, D. Sugar, R.E. Wrolstad, J. Am. Soc. Hort. Sci. 120 (1995) 785.
[39] J. Bakker, P. Bridle, S.J. Bellworthy, J. Sci. Food Agric. 64 (1994) 31.
[40] M.I. Gil, D.M. Holcroft, A.A. Kader, J. Agric. Food Chem. 45 (1997) 1662.
[41] C. Garcia-Viguera, P. Zafrilla, F. Romero, P. Abellan, F. Artes, F.A. Tomas-Barberan, J. Food Sci. 64 (1999) 243.
[42] P. Bridle, C. Garcia-Viguera, Food Chem. 59 (1997) 299.
[43] C.E. Lewis, J.R.L. Walker, J.E. Lancaster, K.H. Sutton, J. Sci. Food Agric. 77 (1998) 58.
[44] C.E. Lewis, J.R.L. Walker, J.E. Lancaster, K.H. Sutton, J. Sci. Food Agric. 77 (1998) 45.
[45] M.M. Giusti, R.E. Wrolstad, J. Food Sci. 61 (1996) 322.
[46] O. Inami, I. Tamura, H. Kikuzaki, N. Nakatani, J. Agric. Food Chem. 44 (1996) 3090.
[47] G.R. Takeoka, L.T. Dao, G.H. Full, R.Y. Wong, L.A. Harden, R.H. Edwards, J.J. Berrios, J. Agric. Food Chem. 45 (1997) 3395.
[48] E. Pale, M. Nacro, M. Vanhaelen, R. Vanhaelen-Fastre, J. Agric. Food Chem. 45 (1997) 3359.
[49] S.N. Ryu, S.Z. Park, C.T. Ho, J. Food Drug Anal. 6 (1998) 729.
[50] J. Rivera-Lopez, C. Ordorica-Falomir, P. Wesche-Ebeling, Food Chem. 65 (1999) 195.
[51] A. Romani, N. Mulinacci, P. Pinelli, F.F. Vincieri, A. Cimato, J. Agric. Food Chem. 47 (1999) 964.
[52] T. Fossen, O.M. Andersen, D.O. Ovstedal, A.T. Petersen, A. Raknes, J. Food Sci. 61 (1996) 703.
[53] H. Donner, L. Gao, G. Mazza, Food Res. Int. 30 (1998) 637.
[54] M. Careri, A. Mangia, M. Musci, J. Chromatogr. A 794 (1998) 263.
[55] D. Ryan, K. Robards, M. Antolovich, Trends Anal. Chem. 18 (1999) 362.
[56] W.E. Glassgen, H.U. Seitz, J.W. Metzger, Biol. Mass Spectrom. 21 (1992) 271.
[57] A. Piovan, R. Filippini, D. Favretto, Rapid Commun. Mass Spectrom. 12 (1998) 361.
[58] A. Baldi, A. Romani, N. Mulinacci, F.F. Vincieri, B. Casetta, J. Agric. Food Chem. 43 (1995) 2104.
[59] H. Tamura, Y. Hayashi, H. Sugisawa, T. Kondo, Phytochem. Anal. 5 (1994) 190.
[60] I. Revilla, S. Perez-Magarino, M.L. Gonzalez-SanJose, S. Beltran, J. Chromatogr. A 847 (1999) 83.
[61] K.D. Altria, Capillary Electrophoresis Guidebook, Humana Press, NJ, 1996.
[62] P. Bridle, C. García-Viguera, F.A. Tomás-Barberán, J. Liq. Chromatogr. Rel. Technol. 19 (1997) 537.
[63] P. Bridle, C. García-Viguera, Food Chem. 59 (1997) 299.
[64] C.T. da Costa, B.C. Nelson, S.A. Margolis, D. Horton, J. Chromatogr. A 799 (1998) 321.
[65] V. Bicard, A. Fougerousse, R. Brouillard, J. Liq. Chromatogr. Rel. Technol. 22 (1999) 541.
[66] T. Watanabe, A. Yamamoto, S. Nagai, S. Terabe, Anal. Sci. 14 (1998) 839.