# Analysis of Anthocyanins in Red Wine and Fruit Juice Using MALDI-MS

Jian Wang and Peter Sporns*

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is a powerful new technique that will have a great impact on food analysis. This study is the first to demonstrate the applicability of MALDI-MS to perform both qualitative and quantitative analyses of anthocyanins in food. 2,4,6-Trihydroxyacetophenone (THAP) was found to be a good matrix for analysis of anthocyanins, with the best spot-to-spot repeatability. After a simple sample preparation, the presence of anthocyanins as cations with molecular masses was found in ratios expected from their fruit sources. Quantification of anthocyanins should be possible by choosing appropriate internal standards. MALDI-MS responses were linear, groups of chemically similar anthocyanins had similar responses, and addition of an internal standard had no effect on relative responses of the other anthocyanins.

## INTRODUCTION

Anthocyanins are an important family of flavonoid compounds and have been thoroughly studied. They are widely distributed in nature, occurring in most higher plants, and are responsible for most of the red or purple colors in red wine and fruit juice. The anthocyanin profile for any given plant is distinctive, and the amount and composition of anthocyanins are important to red wine and fruit juice color (Gao et al., 1997; Hong and Wrolstad, 1990a). Anthocyanin analysis has been very useful in studying the changes in anthocyanins in red wine or fruit juice and in distinguishing among different species.

Anthocyanins from many plants, including fruits, have been separated and analyzed by paper chromatography (Dekazos, 1970; Camire and Clydesdale, 1979; Francis, 1985; Mazza and Velioglu, 1992; Gao and Cahoon, 1995), thin-layer chromatography (Wrolstad and Struthers, 1971; Barritt and Torre, 1973; Pouget et al., 1990; Dussi et al., 1995), and high-performance liquid chromatography (HPLC) (Wulf and Nagel, 1978; Camire and Clydesdale, 1979; Goiffon et al., 1991; Mazza and Velioglu, 1992; Gao and Mazza, 1994; Gao et al., 1997). HPLC coupled with photodiode array detection has often been used to qualitatively and quantitatively analyze anthocyanins and their glycosylated or acylated groups utilizing relative retention times and UV–vis spectra (Williams et al., 1978; Hong and Wrolstad, 1990a,b; Goiffon et al., 1991; Dallas et al., 1996). Fast atom bombardment mass spectrometry (FAB-MS) and nuclear magnetic resonance (NMR) have proven to be powerful supporting techniques for detailed structural determination of anthocyanins (Terahara and Yamaguchi, 1986; Baubils and Berber Jiménez, 1995;

Farina et al., 1995; Escribano et al., 1996; Bakker et al., 1997a,b; Takeoka et al., 1997). HPLC coupled with a mass spectrometer (HPLC/MS) using an atmospheric pressure ionization (API) interface has also been successfully applied to identify anthocyanins of *Vitis vinifera* L. (Baldi et al., 1995). Recently, electrospray ionization (ESI) mass spectrometry and ion trap multiple mass spectrometry (MS/MS) have also been used as another tool for detection and identification of anthocyanins (Takeoka et al., 1997; Piovan et al., 1998).

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was first introduced in 1987 and originally developed for large biomolecules (Karas et al., 1987). MALDI-MS has advantages over other methodologies including the ease of use, speed of analysis, high sensitivity, wide applicability combined with a good tolerance toward contaminants, and the ability to analyze complex mixtures (Karas, 1996). However, simple MALDI-MS instruments cannot tell the difference between isomers, which have the same mass. The potential application of MALDI-MS in food systems allows for the analysis of most molecules. Presently MALDI-MS food applications are limited and reviewed by Sporns and Wang (1998). While this manuscript was under review, Sugui et al. (1998) reported using MALDI to analyze 3-deoxyanthocyanidins and anthocyanins in sorghum plant tissue. This study focused on qualitative aspects and detection limits for some anthocyanins. However, our study represents the first application of a rapid MALDI-MS procedure for both qualitative and quantitative analyses of anthocyanins in several important foods.

## MATERIALS AND METHODS

**Materials and Reagents.** Merlot '96, Pinot Noir '96, and Cabernet Sauvignon '97 were purchased from local markets in Edmonton, Alberta, Canada. Zinfandel '98 was homemade wine from a kit. Concord grape juice, cranberry juice, and

* Author to whom correspondence should be addressed [phone (780) 492−0375; fax (780) 492-4265; e-mail psporns@ afns.ualberta.ca].

raspberry syrup were also from local markets. Malvidin 3,5-glucoside was purchased from Indofine Chemical Co. (St. Louis, MO). Pelargonidin 3-glucoside, cyanidin 3-glucoside, cyanidin 3-rutinoside, peonidin 3-glucoside, and malvidin 3-glucoside were obtained from Extrasynthese S.A. (Genay Cedex, France). 1-Kestose and nystose were a gift from Dr. A. Ohta, Nutritional Science Center, Meiji Seika Kaisha, Japan. 2,4,6-Trihydroxyacetophenone monohydrate (THAP) was obtained from Aldrich Chemical Co. (Milwaukee, WI).

**Extraction of Anthocyanins from Red Wine, Fruit Juice, and Syrup.** A 200 mL sample of red wine was first prepared by removal of ethanol (about $^1/_4$ volume), using a rotavopor in vacuo with a bath temperature maintained at 35 °C, and then made up to 200 mL with doubly deionized water in a volumetric flask. Fruit syrup was diluted with an equal volume of water because of its high viscosity. Either 10 mL of ethanol-free red wine or fruit juice or 20 mL of diluted syrup sample was loaded onto a Sep-pak C18 cartridge (Waters Associates, Milford, MA) with a flow rate of 1 mL/min, washed with 10 mL of doubly deionized water three times, and eluted with 2 mL of methanol/formic acid/water in a ratio of 70:2:28. The reddish purple anthocyanin extracts were kept in a freezer at −20 °C until used.

**MALDI-MS.** MALDI-MS was performed using the Proflex III linear mode, Bruker Analytical Systems Inc. (Billerica, MA). Anthocyanins cocrystallized with matrices on the probe were ionized by a nitrogen laser pulse (337 nm) and accelerated under 20 kV with time-delayed extraction before entering the time-of-flight mass spectrometer. 2,4,6-Trihydroxyacetophenone (THAP) in acetone (0.5 μL) was applied on the probe first, air-dried, and then 1 μL of extracted anthocyanin solution or 2 μL of preparative HPLC fraction spotted directly over the THAP crystals and further air-dried. MALDI-MS was performed at an attenuation of 31 (the lower the attenuation, the higher the laser strength) to obtain the best signal-to-noise ratios and the best possible isotopic resolution with two-point external calibration using [kestose + K]$^+$ (exact isotopic mass = 543.1327) and [nystose + K]$^+$ (exact isotopic mass = 705.1856) as calibrants, resulting in a mass accuracy of ≤400 ppm. Each spectrum represents the sum of 30 laser pulses.

The responses of individual anthocyanins in MALDI-MS were recorded using cyanidin 3-rutinoside as the internal standard. The response of malvidin 3-glucoside in a food sample using MALDI-MS was obtained by adding malvidin 3-glucoside to Concord grape juice extracts, diluted with an equal volume of appropriate solution from the original anthocyanin extracts. The preparation of matrix and samples was as described above. In this study, the peak with a mass of 449 (cyanidin 3-glucoside) was used as a reference peak and therefore was arbitrarily set to a value of 1.0 to study the response of added malvidin 3-glucoside in the juice extract. Each data point represented the mean of spectra collected from three sample positions. Therefore, each spectrum was collected from three randomly chosen spots per sample position for a total of 3 × 30 laser pulses, or 90 laser pulses in total. The major isotopic C$^{12}$ peak height was used for quantification. Statistics were carried out using Microsoft Excel 97.

**Preparative HPLC.** The preparative HPLC system consisted of a Varian VISTA 5500 high-performance liquid chromatograph, a Varian 9090 autosampler, and a Spectro Monitor III UV detector. The chromatography system was equipped with a 75 mm × 4.5 mm preinjection C18 saturator column containing silica-based packing (12 μm) and a 50 mm × 4.6 mm guard column containing Supelco LC-18 reverse-phase packing (20−40 μm). Anthocyanins were separated on a SPLC-18-DB 250 mm × 10 mm (5 μm) preparative reverse-phase column (SUPELCOSIL, SUPELCO, Bellofonte, PA). The solvents used were 5% (v/v) aqueous formic acid (solvent A) and formic acid/water/methanol in a ratio of 5:5:90 (v/v/v) (solvent B). The flow rate was maintained at 4.5 mL/min, with a linear gradient profile consisting of solvent A with the following proportions (v/v) of solvent B: 0 min, 5% B; 0−1 min, 5−20% B; 1−12 min, 20−25% B; 12−32 min, 25−32% B; 32−38 min, 32−55% B; 38−44 min, 100% B; 44−46 min, 100% B; 46−47 min, 100−5% B. Detection was at 525 nm. The total run time
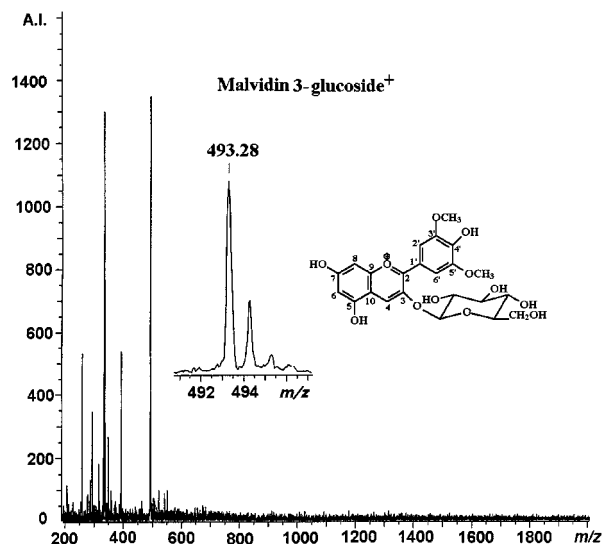


**Figure 1.** MALDI-MS spectrum of standard malvidin 3-glucoside (3.90 × 10$^{-5}$ M). Note that matrix peaks are all at masses less than 400.

was 55 min. Sample extracts (500 μL) were injected into the HPLC for preparative collection. A Shimadzu CLASS-VP chromatography data system was used to monitor the eluted peaks, and the anthocyanin fractions of interest were collected for direct UV−vis spectrometry and MALDI-MS analysis.

The chromatography of individual standard anthocyanins was achieved using the same conditions as above except employing a linear gradient profile as follows: 0 min, 5% B; 0−1 min, 5% B; 1−2 min, 5−17% B; 2−7 min, 17−19% B; 7−9 min, 19−28% B; 9−17 min, 28−34% B; 17−21 min, 34−70% B; 21−22 min, 70−100% B; 22−23 min, 100% B; 23−24 min, 5% B. Total run time was 26 min. The flow rate was set at 5.5 mL/min. For these analyses, an injection volume of 140 μL was used.

## RESULTS AND DISCUSSION

Analytes are usually desorbed and ionized in the MALDI-MS source, forming protoned or alkali adduct ions. However, because anthocyanins under acidic condition are predominately in the aromatic oxonium ion form, they easily ionize in MALDI-MS to form molecular weight cations M$^+$ in the positive ion mode (Figure 1).

**Anthocyanins in Red Wine.** Figure 2 is the MALDI-MS anthocyanin spectra from red wine extracts. Anthocyanins listed in HPLC elution order from important wine varieties are shown in Table 1. Malvidin 3-glucoside, as expected, was the major anthocyanin (Wulf and Nagel, 1978; Nagel and Wulf, 1979; Gao et al., 1997). Table 1 also shows some of the anthocyanin masses obtained using MALDI-MS, which are in excellent agreement with their theoretical values. The peak with a mass of 493 (malvidin 3-glucoside) is the major peak. Three major anthocyanins were successfully isolated as preparative HPLC fractions from Zinfandel wine extract and then were applied to MALDI-MS giving the correct masses for peonidin 3-glucoside, petunidin 3-glucoside, and malvidin 3-glucoside (Figure 3). The UV−vis spectra patterns and $\lambda_{max}$ absorbances of these three preparative HPLC anthocyanin fractions are almost the same as reported before (Wulf and Nagel, 1978). On the basis of the C18 cartridge selectivity, the HPLC relative retention time, the general UV−vis spectra pattern and $\lambda_{max}$ absorbances, and the MALDI-MS determined masses, the reddish purple pigments adsorbed and

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**Table 1. Anthocyanins in *Vitis vinifera* L. Grapes**

| anthocyanins and their derivatives | exact mass | MALDI-MS mass[a] | Cabernet Sauvignon (Wulf and Nagel, 1978) | Merlot (Nagel and Wulf, 1979) | Pinot Noir (Gao et al., 1997) |
|---|---|---|---|---|---|
| delphinidin 3-glucoside | 465.10 | 465.17 | * | * | * |
| cyanidin 3-glucoside | 449.11 | | * | * | * |
| petunidin 3-glucoside | 479.12 | 479.23 | * | * | * |
| peonidin 3-glucoside | 463.12 | 463.13 | * | * | * |
| malvidin 3-glucoside | 493.13 | 493.23 | * | * | * |
| delphinidin 3-glucoside-acetate | 507.11 | | * | * | |
| cyanidin 3-glucoside-acetate | 491.12 | | * | * | |
| petunidin 3-glucoside-acetate | 521.13 | | * | * | |
| malvidin | 331.08 | | * | | |
| peonidin 3-glucoside-acetate | 505.13 | | * | * | |
| malvidin 3-glucoside-acetate | 535.15 | 535.33 | * | * | |
| delphinidin 3-glucoside-coumarate | 611.14 | | * | | |
| cyanidin 3-glucoside-coumarate | 595.15 | | * | | |
| malvidin 3-glucoside-caffeoate | 655.17 | 655.45 | * | * | |
| petunidin 3-glucoside-*p*-coumarate | 625.15 | 625.39 | * | * | |
| peonidin 3-glucoside-*p*-coumarate | 609.16 | 609.38 | * | * | |
| malvidin 3-glucoside-*p*-coumarate | 639.17 | 639.42 | * | * | |

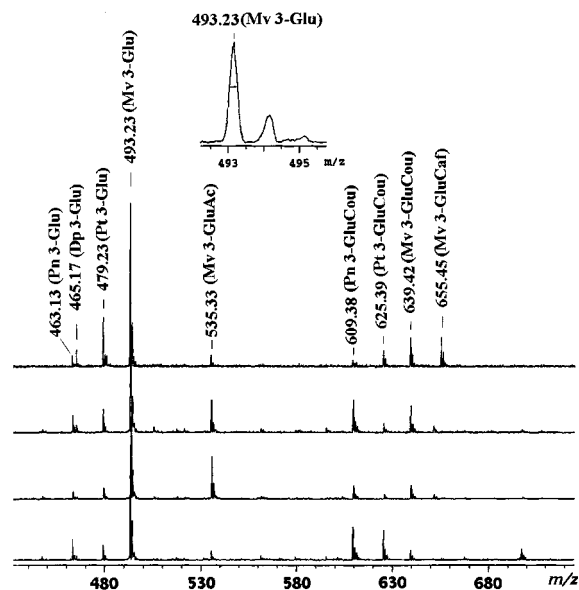[a] Masses from Zinfandel wine extracts.



**Figure 2.** MALDI-MS natural cation spectra of anthocyanins from red wine extracts. The spectra from top to bottom are from Zinfandel '98, Merlot '96, Cabernet Sauvignon '97, and Pinot Noir '96 red wine extracts. Pn 3-Glu = peonidin 3-glucoside; Dp 3-Glu = delphinidin 3-glucoside; Pt 3-Glu = petunidin 3-glucoside; Mv 3-Glu = malvidin 3-glucoside; Mv 3-GluAc = malvidin 3-glucoside-acetate; Pn 3-GluCou = peonidin 3-glucoside-coumarate; Pt 3-GluCou = petunidin 3-glucoside-coumarate; Mv 3-GluCou = malvidin 3-glucoside-coumarate; Mv 3-GluCaf = malvidin 3-glucoside-caffeoate.



**Figure 3.** MALDI-MS natural cation spectra of anthocyanins from preparative HPLC fractions. The spectra from top to bottom are from Zinfandel '98 red wine extracts, HPLC the third fraction (malvidin 3-glucoside), the first fraction (petunidin 3-glucoside), and the second fraction (peonidin 3-glucoside). Pn 3-Glu = peonidin 3-glucoside; Dp 3-Glu = delphinidin 3-glucoside; Pt 3-Glu = petunidin 3-glucoside; Mv 3-Glu = malvidin 3-glucoside; Mv 3-GluAc = malvidin 3-glucoside-acetate; Pn 3-GluCou = peonidin 3-glucoside-coumarate; Pt 3-GluCou = petunidin 3-glucoside-coumarate; Mv 3-GluCou = malvidin 3-glucoside-coumarate; Mv 3-GluCaf = malvidin 3-glucoside-caffeoate.

eluted from the Sap-pak C18 cartridge were confirmed to be the anthocyanins assigned in Figure 2.

**Anthocyanins in Concord Grape Juice, Cranberry Juice, and Raspberry Syrup.** Concord grape juice contains the 3-glucosides, 3-glucoside-*p*-coumarate, 3,5-diglucosides, and 3-glucoside-*p*-coumarate-5-glucosides of cyanidin, peonidin, delphinidin, petunidin, and malvidin with cyanidin 3-glucoside and delphinidin 3-glucoside as the major pigment constituents (Hrazdina, 1975; Williams and Hrazdina, 1978). MALDI-MS results confirmed the presence and relative amounts of these anthocyanins and their derivative profiles, in good agreement with the literature. The peaks with masses 449 (cyanindin 3-glucoside) and 465 (delphinidin 3-glucoside) are the major peaks (Figure 4; Table 2). Because
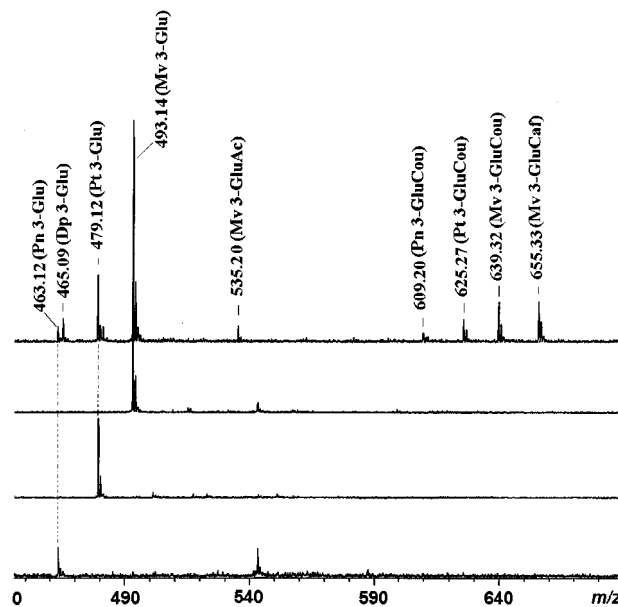
delphinidin 3-glucoside-*p*-coumarate and cyanidin 3,5-diglucosides have almost the same mass of 611, these anthocyanins came out at the same *m/z* of 611 in MALDI-MS spectra, as did peonidin 3,5-diglucosides and petunidin 3-glucoside-*p*-coumarate at *m/z* 625.

Previous research has confirmed the presence of the 3-galactoside and 3-arabinoside of cyanidin and peonidin as the four major anthocyanins in cranberries with the additional small amount of cyanidin 3-glucoside or peonidin 3-glucoside (Camire and Clydesdale, 1979; Hong and Wrolstad, 1990a). Figure 5 shows the anthocyanin spectra from cranberry juice, closely matching the exact isotopic masses of cyanidin 3-arabinoside
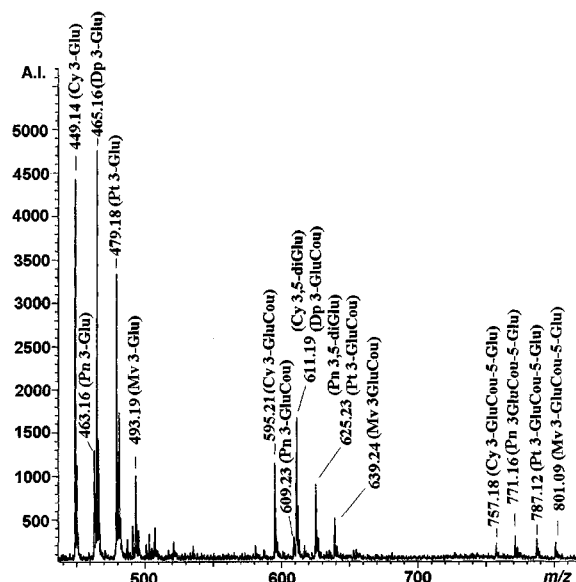
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**Figure 4.** MALDI-MS natural cation spectrum of anthocyanins from Concord grape juice extracts. Cy 3-Glu = cyanidin 3-glucoside; Pn 3-Glu = peonidin 3-glucoside; Dp 3-Glu = delphinidin 3-glucoside; Pt 3-Glu = petunidin 3-glucoside; Mv 3-Glu = malvidin 3-glucoside; Cy 3-GluCou = cyanidin 3-glucoside-coumarate; Pn 3-GluCou = peonidin 3-glucoside-coumarate; Cy 3,5-diGlu = cyanidin 3,5-diglucoside; Dp 3-GluCou = delphinidin 3-glucoside-coumarate; Pn 3,5-diGlu = peonidin 3,5-diglucoside; Pt 3-GluCou = petunidin 3-glucoside-coumarate; Mv 3-GluCou = malvidin 3-glucoside-coumarate; Cy 3-GluCou-5-Glu = cyanidin 3-glucoside-coumarate-5-glucoside; Pn 3-GluCou-5-Glu = peonidin 3-glucoside-coumarate-5-glucoside; Pt 3-GluCou-5-Glu = petunidin 3-glucoside-coumarate-5-glucoside; Mv 3-GluCou-5-Glu = malvidin 3-glucoside-coumarate-5-glucoside.

**Table 2. Anthocyanins from Concord Grape Juice**

| anthocyanin | exact mass | MALDI-MS mass |
|---|---|---|
| delphinidin 3,5-diglucoside | 627.16 | |
| cyanidin 3,5-diglucoside | 611.16 | 611.19 |
| petunidin 3,5-diglucoside | 641.17 | |
| peonidin 3,5-diglucoside | 625.18 | 625.23 |
| malvidin 3,5-diglucoside | 655.19 | |
| delphinidin 3-glucoside | 465.10 | 465.16 |
| cyanidin 3-glucoside | 449.11 | 449.14 |
| petunidin 3-glucoside | 479.12 | 479.18 |
| peonidin 3-glucoside | 463.12 | 463.16 |
| malvidin 3-glucoside | 493.13 | 493.17 |
| delphinidin 3-glucoside-coumarate | 611.14 | 611.19 |
| cyanidin 3-glucoside-coumarate | 595.15 | 595.21 |
| petunidin 3-glucoside-coumarate | 625.15 | 625.23 |
| peonidin 3-glucoside-coumarate | 609.16 | 609.23 |
| malvidin 3-glucoside-coumarate | 639.17 | 639.24 |
| delphinidin 3-glucoside-coumarate-5-glucoside | 773.19 | |
| cyanidin 3-glucoside-coumarate-5-glucoside | 757.19 | 757.18 |
| petunidin 3-glucoside-coumarate-5-glucoside | 787.21 | 787.12 |
| peonidin 3-glucoside-coumarate-5-glucoside | 771.21 | 771.16 |
| malvidin 3-glucoside-coumarate-5-glucoside | 801.22 | 801.09 |

(theoretical value, 419.10), cyanidin 3-galactoside (449.11), peonidin 3- arabinoside (433.11), and peonidin 3-galactoside (463.12).

Anthocyanins expected in raspberries included cyanidin 3-sophoroside (mass, 611; the major anthocyanin), cyanidin 3-rutinoside, cyanidin 3-(2$^G$-glucosylrutinose), cyanidin 3-glucoside, pelargonidin 3-sophoroside, and
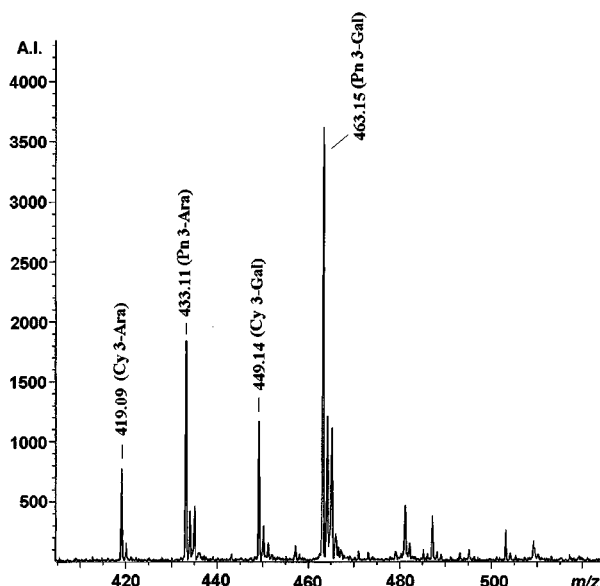


**Figure 5.** MALDI-MS natural cation spectrum of anthocyanins from cranberry juice extracts. Cy 3-Ara = cyanidin 3-arabinoside; Pn 3-Ara = peonidin 3-arabinoside; Cy 3-Gal = cyanidin 3-galactoside; Pn 3-Gal = peonidin 3-galactoside.
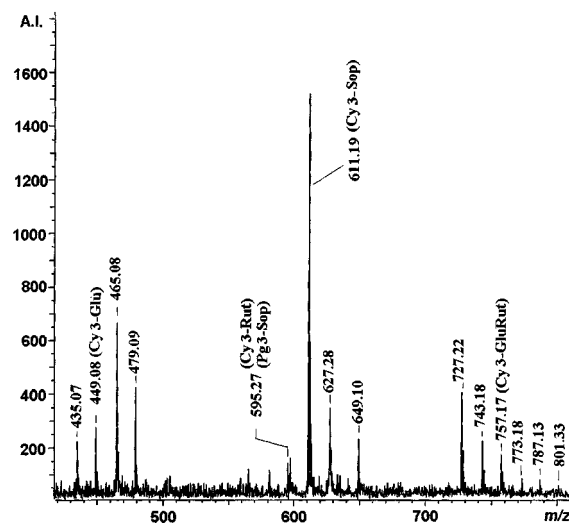


**Figure 6.** MALDI-MS natural cation spectrum of anthocyanins from raspberry syrup extracts. Cy 3-Glu = cyanidin 3-glucoside; Cy 3-Glu = cyanidin 3-rutinoside; Pg 3-Sop = pelargonidin 3-sophoroside; Cy 3-Rut = cyanidin 3-sophoroside; Cy 3-GluRut = cyanidin 3-(2$^G$-glucosylrutinoside).

pelargonidin 3-(2$^G$-glucosylrutinose) (Goiffon et al., 1991; Matthew and Wrolstad, 1993). The anthocyanin MALDI-MS spectrum from raspberry syrup extracts shows the molecular cations M$^+$ at *m/z* 449, 595, 611, and 757 corresponding to the exact isotopic masses of cyanidin 3-glucoside (449.11), cyanidin 3-rutinoside (595.17) or pelargonidin 3-sophoroside (595.17), cyanidin 3-sophoroside (611.16), and cyanidin 3-(2$^G$-glucosylrutinose) (757.22) (Figure 6).

**Responses of Anthocyanin 3-Glucosides.** For quantification of anthocyanins using MALDI-MS, the relative responses of the analytes are a key factor. Ideally, the intensity or response of an analyte should be linearly correlated to its relative molar ratios in a MALDI-MS sample. The relative responses of known amounts of individual anthocyanins in MALDI-MS are shown in Figure 7. For monoglucoside anthocyanins, the responses were only slightly different, but for a diglu-
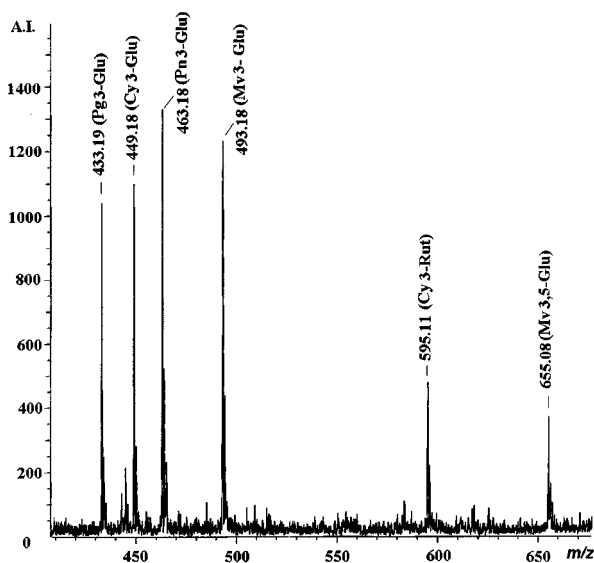
**Figure 7.** MALDI-MS natural cation spectrum of standard individual anthocyanins. Pg 3-Glu = pelargonidin 3-glucoside ($1.60 \times 10^{-5}$ M, peak height 1060.8); Cy 3-Glu = cyanidin 3-glucoside ($1.58 \times 10^{-5}$ M; peak height 1122.0); Pn 3-Glu = peonidin 3-glucoside ($1.42 \times 10^{-5}$ M; peak height 1357.6); Mv 3-Glu = malvidin 3-glucoside ($1.47 \times 10^{-5}$ M; peak height 1256.6); Cy 3-Glu = cyanidin 3-rutinoside ($2.21 \times 10^{-5}$ M; peak height 489.6); Mv 3,5-Glu = malvidin 3,5-diglucosides ($2.03 \times 10^{-5}$ M; peak height 381.5).
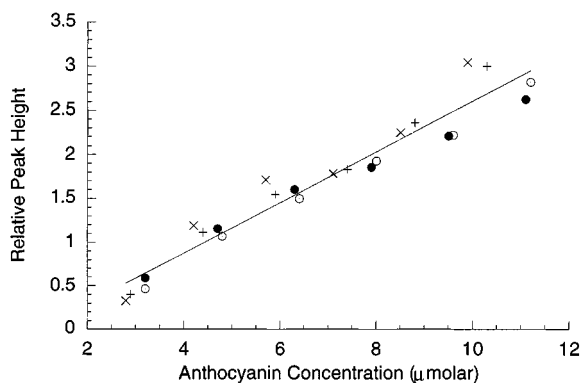


**Figure 8.** Linear responses of individual anthocyanins in MALDI-MS. ○, pelargonidin 3-glucoside; ●, cyanidin 3-glucoside; ×, peonidin 3-glucoside; +, malvidin 3-glucoside. For the total data points, $Y = 0.29X - 0.29$ ($R^2 = 0.94$). The concentration of individual fructooligosaccharides ranged from $3.19 \times 10^{-6}$ M to $1.12 \times 10^{-5}$ M for pelargonidin 3-glucoside, $3.16 \times 10^{-6}$ M to $1.11 \times 10^{-5}$ M for cyanidin 3-glucoside, $2.83 \times 10^{-6}$ M to $1.00 \times 10^{-5}$ M for peonidin 3-glucoside, and $2.94 \times 10^{-6}$ M to $1.03 \times 10^{-5}$ M for malvidin 3-glucoside, and internal standard cyanidin 3-rutinoside ($4.42 \times 10^{-5}$ M) in methanol/ formic acid/water = 70:2:28.

coside anthocyanin, e.g., malvidin 3,5-diglucoside, or an anthocyanin with a second carbohydrate moiety to a 3-glucoside, e.g., cyanidin 3-rutinoside, the relative molar response was only about one-fourth of that noted for monoglucoside anthocyanins. Figure 8 indicates the exact responses of different concentrations of monoglucoside anthocyanins for MALDI-MS using cyanidin 3-rutinoside as the internal standard. The linear models were fit to the responses of all monoglucoside anthocyanins ($R^2 = 0.94$). Other evidence that the responses of anthocyanins fit a linear model was the response of malvidin 3-glucoside added to Concord grape juice extracts. The addition of this anthocyanin to food extracts also showed a linear response with $R^2 = 0.99$ (Figure 9). Also, the relative responses of anthocyanins,
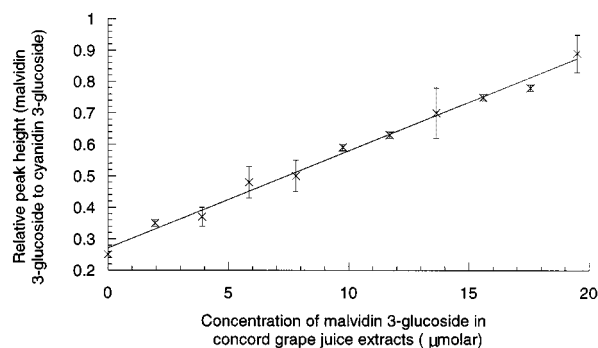


**Figure 9.** Linear MALDI-MS response of malvidin 3-glucoside ($Y = 0.031X + 0.27$, $R^2 = 0.99$) in Concord grape juice extracts. The concentration of malvidin 3-glucoside ranged from $1.95 \times 10^{-6}$ to $1.95 \times 10^{-5}$ M.
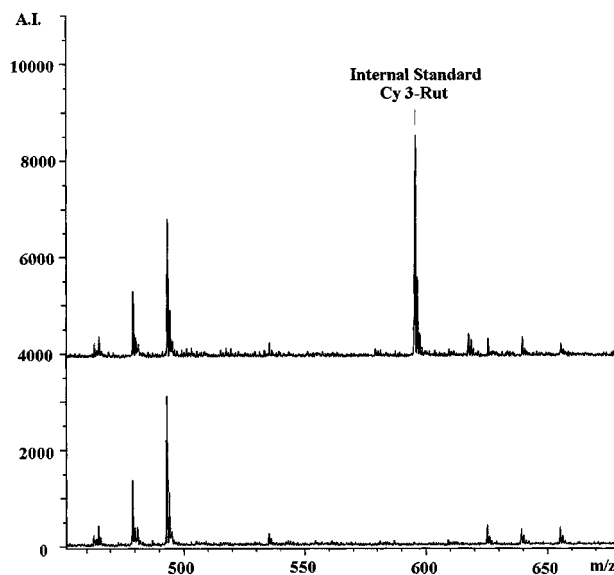


**Figure 10.** MALDI-MS natural cation spectra of anthocyanins with and without internal standard. The spectra are from Zinfandel '98 red wine extracts, which were diluted 20 times from original extracts. Top: with internal standard cyanidin 3-rutinoside ($4.42 \times 10^{-5}$ M). Bottom: without internal standard. Note that the addition of the internal standard has no effect on the relative responses of the other anthocyanins.

for example, anthocyanins in red wine extracts, were not affected in MALDI-MS by an addition of internal standard cyanidin 3-rutinoside as shown in Figure 10. All these factors indicate that the responses of individual anthocyanins in MALDI-MS are predictable and unaffected by each other. When the individual anthocyanin standard curves are plotted using an appropriate internal standard or using the standard addition method (Abell and Sporns, 1996; Wang et al., 1999), all the anthocyanins in food can be also quantitatively analyzed.

For comparison, Figure 11 indicates the relative responses of standard individual anthocyanins in the HPLC system with detection at 525 nm. The peak areas of individual anthocyanins with almost the same molar amount of anthocyanins were quite different. This was because the maximum absorbance of anthocyanins was slightly different and the various isomeric forms that can be formed were very dependent on pH and the solvent variation. For quantification using HPLC, delphinidin 3-glucoside or cyanidin 3-glucoside are often used as external standards and the amount of individual anthocyanins in samples expressed in terms of these
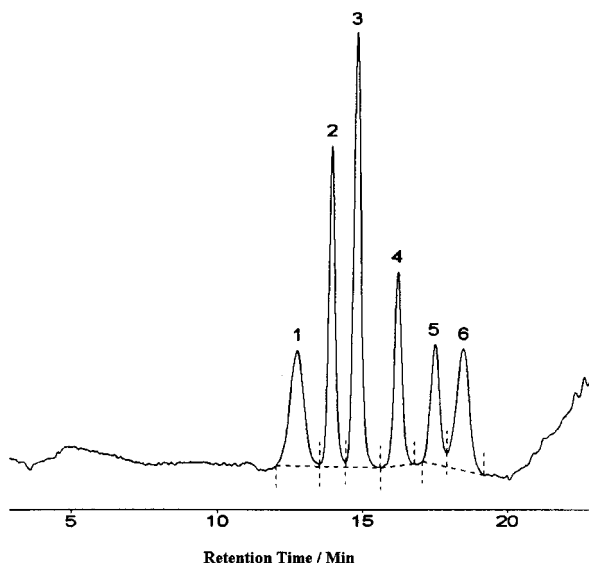
**Figure 11.** HPLC chromatography of standard anthocyanins in Figure 7. Integration values are (1) malvidin 3,5-diglucoside (peak area 227 729), (2) cyanidin 3-glucoside (peak area 295 507); (3) cyanidin 3-rutinoside (peak area 439 029); (4) pelargonidin 3-glucoside (peak area 206 928); (5) peonidin 3-glucoside (peak area 157 198); (6) malvidin 3-glucoside (peak area 225 614).

standard anthocyanins (Takeoka et al., 1997; Gao et al., 1997). Because responses vary in different solvent systems, the absolute amount of anthocyanins in samples may be misinterpreted by comparing to a single external standard. Compared to HPLC, MALDI-MS shows a more consistent response for a group of anthocyanins. Certainly for at least monoglucoside anthocyanins, it seems that any individual anthocyanin can be used as a standard for others.

In conclusion, MALDI-MS is a very valuable technique for the rapid analysis of anthocyanins. MALDI-MS can be used to obtain the exact masses of various anthocyanins in food and can provide the anthocyanin profile in a food sample within a few minutes. Also, the use of MALDI-MS to quantify anthocyanins has been demonstrated with the use of appropriate internal standards. Finally, we feel that MALDI-MS can make a significant contribution to the use of anthocyanin "fingerprints" to determine the authenticity of samples and to explore varietal and growth differences.

### LITERATURE CITED

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