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Anthocyanin profile of mayhaw (*Cretaegus opaca*)

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Abstract

Mayhaw samples (Elite 2001, Texas Star 2000 and 1999) were harvested over a three-year period and analyzed for anthocyanin concentration, colour density, polymeric colour, and % polymeric compounds. Anthocyanins were separated by HPLC. All mayhaw samples from the three year period contained about 50% of cyanidin-3-glucoside, 10–21% cyanidin-3-galactoside, and less than 32% unidentified anthocyanins. The highest concentration of anthocyanins was detected in the Texas Star 1999 sample (about 7 mg/100 g) whereas Texas 2000 and Elite 2001 had 3.3 and 2.3 mg/100 g of anthocyanins, respectively. Colour density was lower in the Elite samples than the Texas. The polymeric anthocyanins and polymeric colour decreased in the Elite 2001 > Texas Star 2000 > Texas Star 1999 and the decrease was tentatively ascribed to sample age.

Keywords: Anthocyanins; Mayhaw; Cretaegus opaca; Phenolics; Chronic diseases

1. Introduction

Mayhaw (*Cretaegus opaca*) is cultivated in low wet areas and swamps from North Carolina to Florida and west of Arkansas to Texas in the United States. Mayhaw grows like a small tree and bears fruit that look like small crabapples. The fruit, known as "the grape of the South", is a small pome (8–19 mm diameter), yellow to bright red, fragrant, acid and juicy, resembling cranberries in appearance and crabapples in taste. The fruit is not eaten raw but made into jams, jellies, sauces, and wine of which there are several commercial manufacturers in the southeastern United States. While jellies, jams, sauces, desserts, food for wildlife, and wines have been investigated, the opportunity exists for a greatly expanded market based upon knowledge of the chemistry of mayhaw fruit chemistry.

Anthocyanins impart the red colour to grape skins. The health-enhancing properties ascribed to anthocyanins are associated with their ability to act as antioxidants and radical scavengers in biological systems (Bagchi, Sen, Bagchi, & Atalay, 2004; Kay, Mazza, Holub, & Wang, 2004; Meyers, Watkins, Pritts, & Liu, 2003; Wang & Mazza, 2002) Epidemiological and experimental evidence suggest a link between the consumption of diets rich in fruits and vegetables and a decreased risk of chronic angiogenic diseases, such as cardiovascular diseases, arthritis, chronic inflammation, and cancers (Middleton, Kandaswami, & Theoharides, 2000; Prior, 2003). Identification of anthocyanins in mayhaw is of interest because of the chemical properties, health effects, and the potential economic development they may provide in the southern regions and to consumers of mayhaw products.

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Table 1
Characteristics of Mayhaw total anthocyanins

Sample ID	Anthocyanin concentration (mg/100 g)	Colour density	Polymeric colour	% Polymeric anthocyanins
Texas Star 1999	6.87 ± 0.52	3.15 ± 0.10	0.39 ± 0.03	12.4 ± 0.24
Texas Star 2000	3.30 ± 0.22	2.34 ± 0.20	1.13 ± 0.00	48.3 ± 0.33
Elite 2001	2.29 ± 0.14	2.62 ± 0.12	1.37 ± 0.11	52.3 ± 1.22

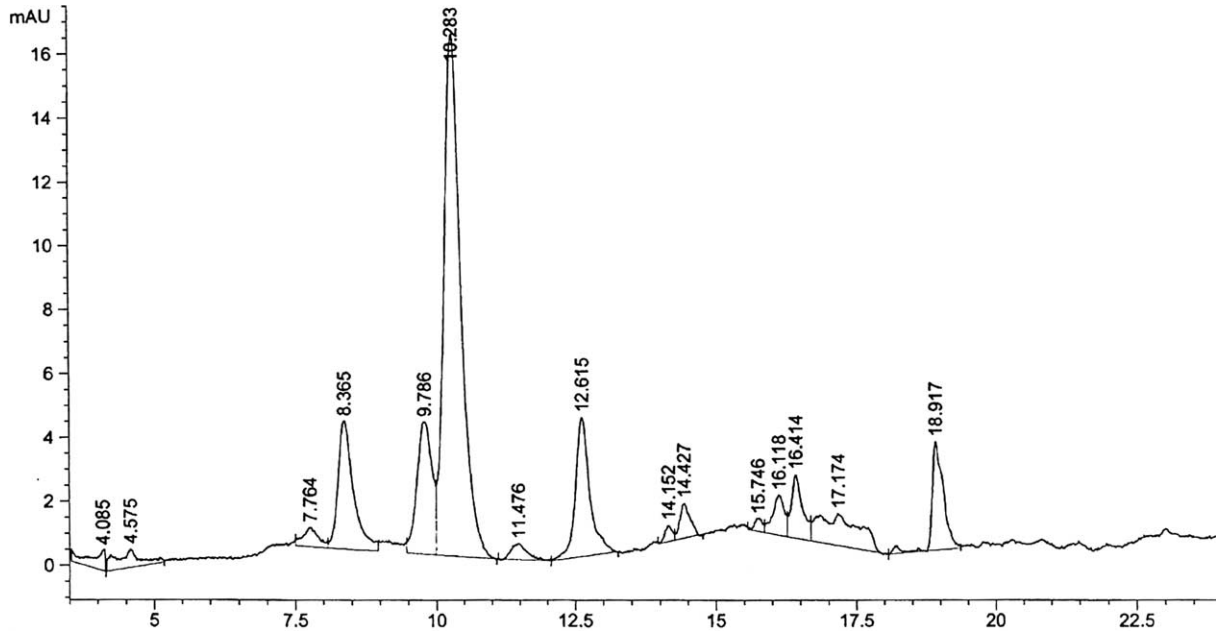

Fig. 1. HPLC profile of anthocyanins from Texas Star 1999 mayhaw. Anthocyanins were isolated as described under Section 2 and major peaks, for which standards, were available were identified as cyanidin-3-glucoside (peak at 10.283 min) and cyanidin-3-galactoside (peak at 8.365 min).

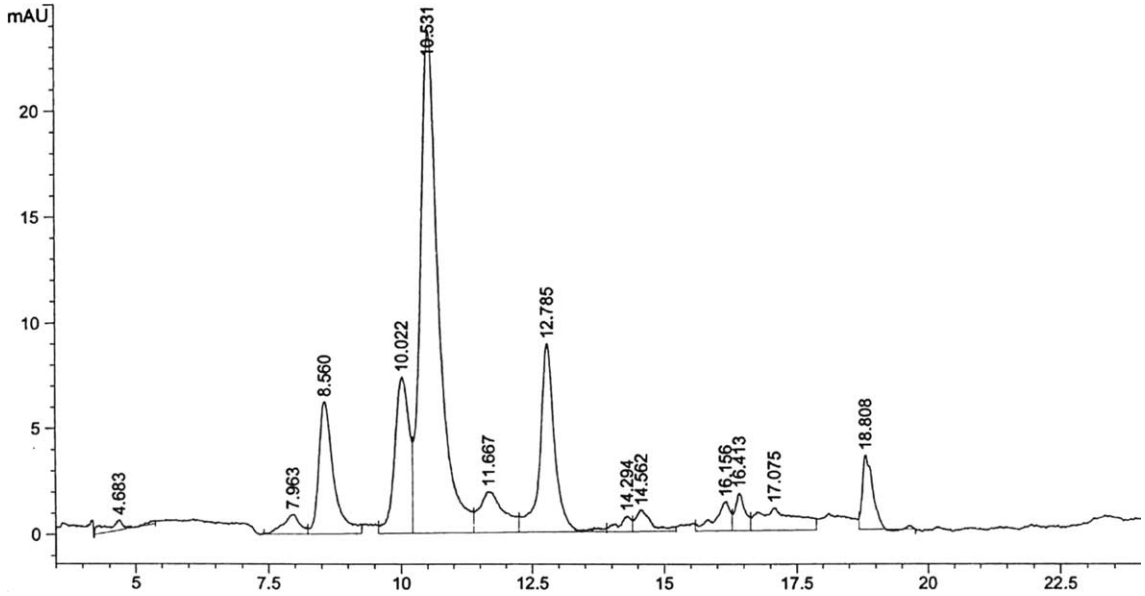

Fig. 2. HPLC profile of anthocyanins from Texas Star 2000 mayhaw. Anthocyanins were isolated as described under Section 2 and major peaks for which standards were available, were identified as cyanidin-3-glucoside (peak at 10.53 min) and cyanidin-3-galactoside (peak at 8.56 min).

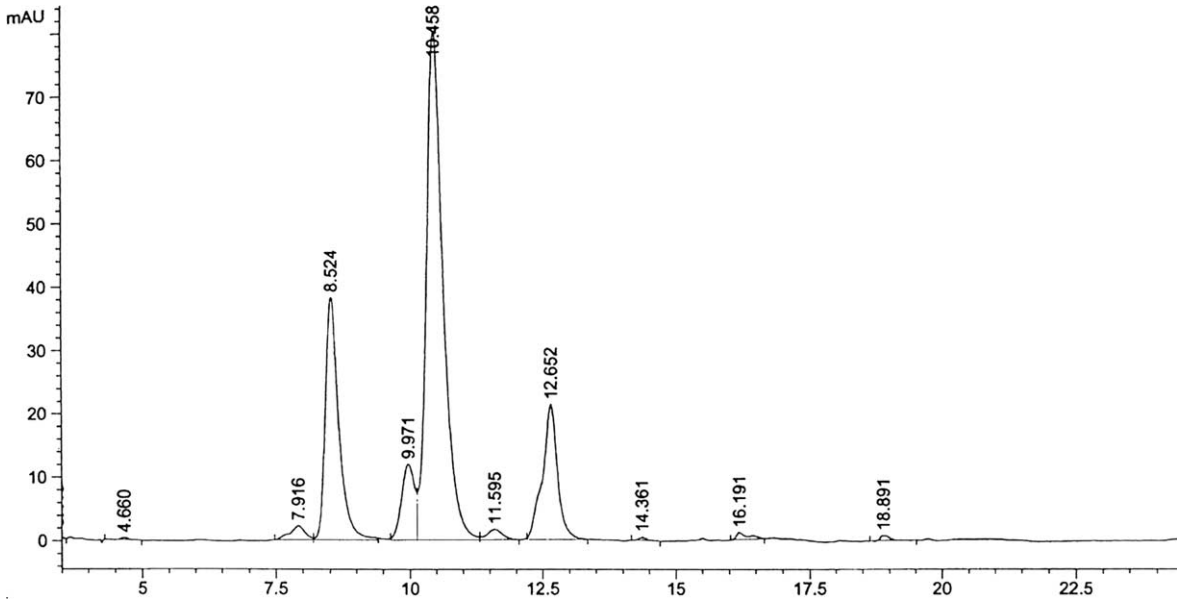

Fig. 3. HPLC profile of anthocyanins from Elite 2001 mayhaw. Anthocyanins were isolated as described under Section 2 and major peaks, for which standards were available, were identified as cyanidin-3-glucoside (peak at 10.45 min) and cyanidin-3-galactoside (peak at 8.52 min).

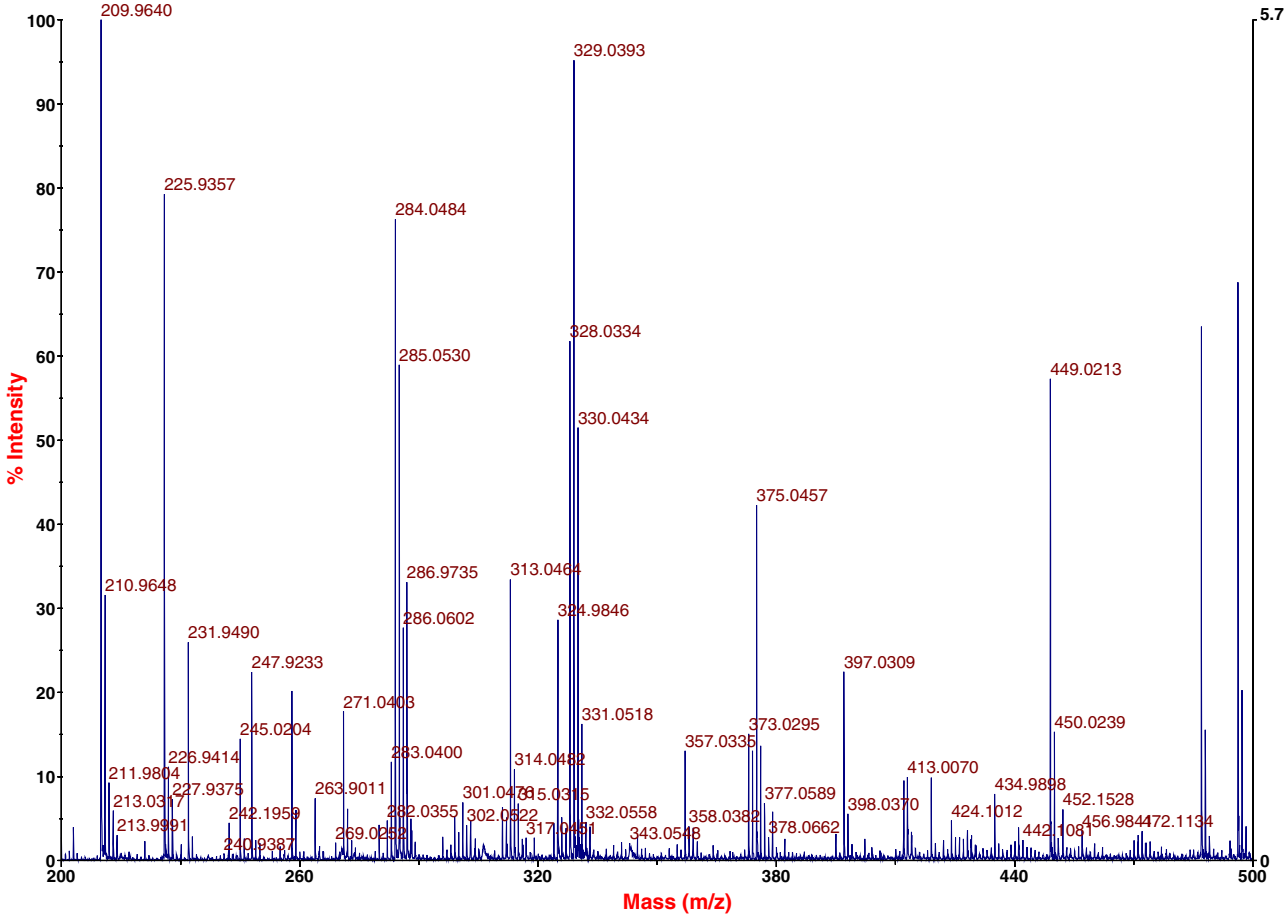

Fig. 4. MALDI-MS of mayhaw anthocyanins in the region of *m/z* 200–500, showing the peak at 449 Da associated with cyanidin-3-glucoside and cyanidin-3-galactoside.

2. Materials and methods

2.1. Materials

Mayhaw (*C. opaca*) samples (Elite 2001, Texas Star 2000 and 1999) were obtained from Vidar, TX. C_{18} Sep-Pak cartridges were from Waters (Milford, MA). All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Preparation of Mayhaw anthocyanins

Mayhaw fruits were ground in liquid nitrogen. Five grams of ground samples were extracted with 10 ml of 100% acetone, sonicated for 5 min, and separated by centrifugation at 4000*g* for 20 min. The extraction was repeated twice by using 10 ml of 70% acetone, followed by centrifugation. The acetone fractions were combined and partitioned with 75 ml of chloroform. The aqueous layer was separated by centrifugation and the residual acetone was removed by rotary evaporation. A fraction was taken up and used for subsequent analyses.

2.2.2. Characterization of Mayhaw anthocyanins

Total anthocyanin concentration was determined by the pH-differential method (Giusti & Wrolstad, 2000). All measurements were performed in triplicate and were repeated at least three times. Two buffer systems were used: 0.025 M potassium chloride buffer at pH 1.0 and 0.4 M sodium acetate buffer at pH 4.5. Two hundred microlitres of the anthocyanin sample were mixed with 1.8 ml of either potassium chloride or sodium acetate buffer and the absorbance of the solution was read at 510 and 700 nm using the following formula:

$$A = (A_{510\ \mathrm{nm}} - A_{700\ \mathrm{nm}})\mathrm{pH}_{1.0} - (A_{510\ \mathrm{nm}} - A_{700\ \mathrm{nm}})\mathrm{pH}_{4.5}.$$

Color density of the sample treated with water was determined by the relation:

$$[(A_{420\ \mathrm{nm}} - A_{700\ \mathrm{nm}}) + (A_{\lambda\ \mathrm{vis\text{-}max}} - A_{700\ \mathrm{nm}})] \times \mathrm{DF},$$

where DF is the dilution factor.

Polymeric colour of sample treated with bisulfite was calculated as:

$$[(A_{420\ \mathrm{nm}} - A_{700\ \mathrm{nm}}) + (A_{\lambda\ \mathrm{vis\text{-}max}} - A_{700\ \mathrm{nm}})] \times \mathrm{DF}.$$

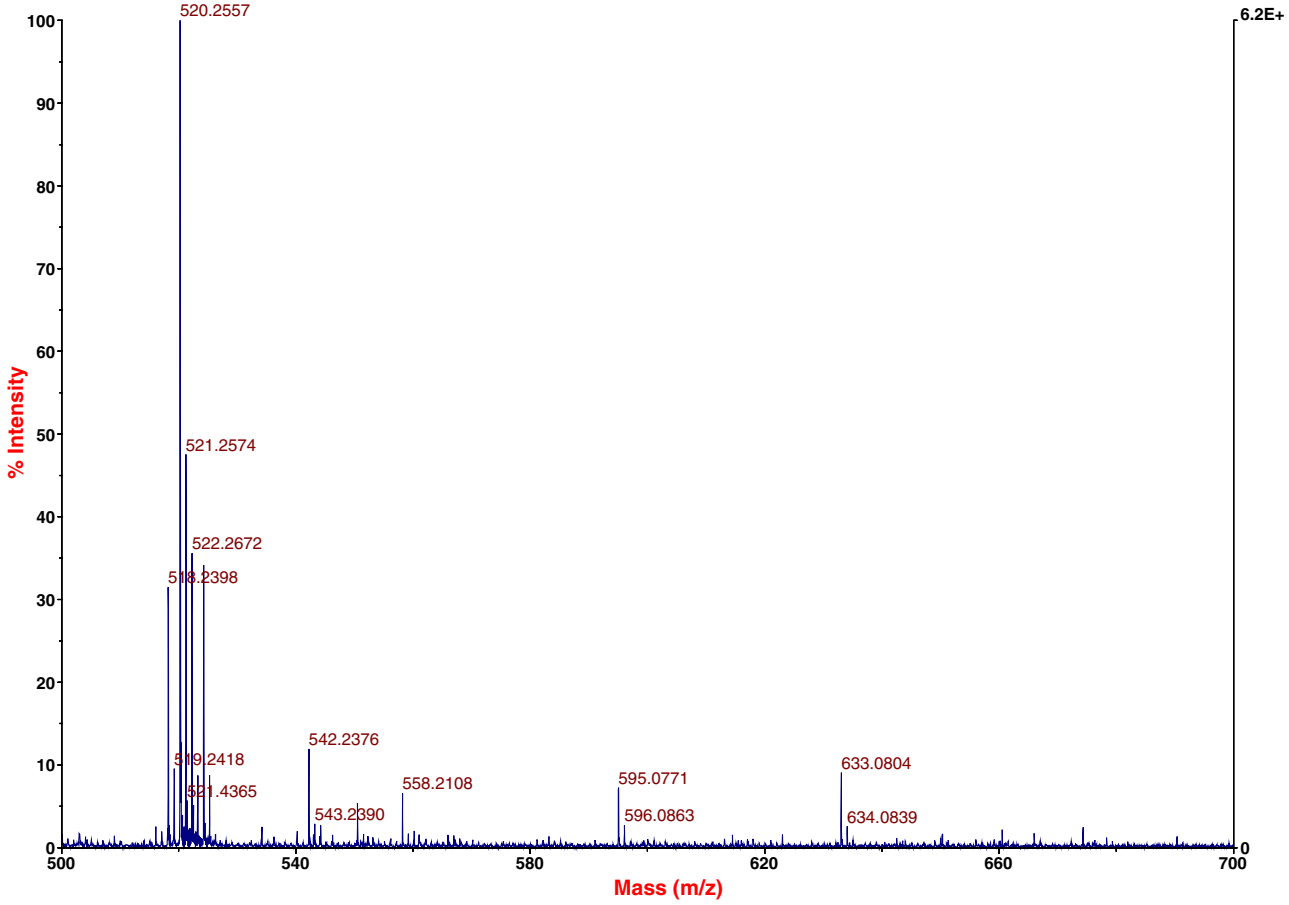

Fig. 5. MALDI-MS of mayhaw anthocyanins, in the region of *m*/*z* 500–700, showing the peaks associated with anthocyanin dimers and salt adducts.

The percent polymeric colour was determined as:

(polymeric colour/colour density) × 100.

2.2.3. HPLC analysis of Mayhaw anthocyanins

HPLC separations of mayhaw anthocyanins were performed at Oregon State University in Professor Wrolstad's laboratory. Mayhaw anthocyanins were separated by reverse-phase HPLC using an HP 1090 with autosampler and a Prodigy Prodigy C_{18} reversed-phase column (Phenomenex, Torrance, CA) containing a C_{18} ODS-3 (4 mm × 3.0 mm i.d.) guard column and running at 30 °C. Flow rate was at 1 ml/min; injection volume was 50 μl; and a diode array detector was used. Solvent A consisted of a mixture of 5% acetonitrile, 10% glacial acetic acid, and 1% phosphoric acid (made from 85% concentrate) in water (v/v/v) and solvent B was acetonitrile. The separation was as follows: 0–5 min: 100% A; 5–20 min: 100–80% A; 20–25 min: 80–60% A.

2.2.4. Identification of Mayhaw anthocyanins by MALDI

The molecular mass of mayhaw anthocyanins from C_{18}-Sep-Pak cartridge desorption were analyzed by MALDI-TOF-MS using a Voyager-DE STR. Mayhaw anthocyanins in methanol were mixed 1:1 with the matrix solution (10 mg/ml of 3-indoleacrylic acid in methanol). MALDI was performed at 2 Hz with a nitrogen laser (337 nm) with an intensity setting of about 1800. Acceleration voltage was 20 kV with a grid ratio of 0.68 and delay time of 200 ns. One hundred laser shots were averaged per spectrum. The low mass gate was turned on and set for 190 Da. External mass calibration was provided by the $[M + H]^+$ ions of ferulic acid.

3. Results and discussion

3.1. Characteristics of Mayhaw anthocyanins

Monomeric concentrations of mayhaw anthocyanins are presented in Table 1. The monomeric concentration increased with storage from 2.29 mg/100 in samples harvested in 2001 to 6.87 in samples harvested in 1999, probably as a result of anthocyanin polymerization. Anthocyanin colour density increased with storage and less colour was observed in 2001 samples (colour density = 2.62) than in 1999 samples (3.15). In contrast, less polymeric colour was observed in the Texas Star of 1999

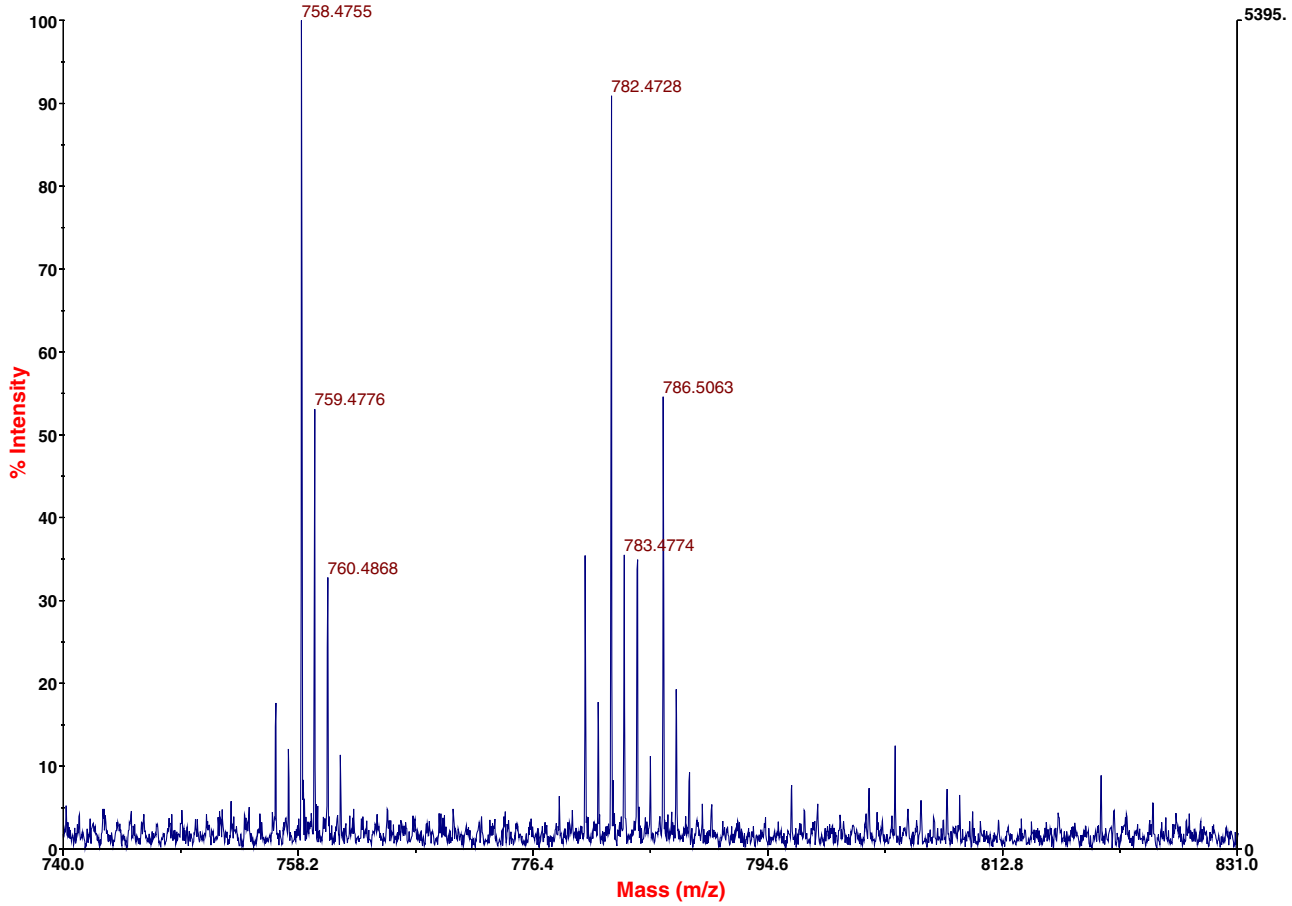

Fig. 6. MALDI-MS of mayhaw anthocyanins, in the region of *m/z* 700–831, showing the peaks associated with anthocyanin dimers and salt adducts.

than in Texas Star of 2000, than in Elite 2001. Comparison of the mayhaw anthocyanins over the 3-year period suggests that peroxidase, an enzyme present in mayhaw and many other grapes, may play an important role in anthocyanin degradation and polymeric colour and anthocyanin loss over time (Lopez-Serrano & Barcelo, 1999).

3.2. HPLC characterization of Mayhaw anthocyanins

Figs. 1–3, show the chromatograms of mayhaw samples Texas Star 1999, Texas Star 2000, and Elite 2001. A total of 15 peaks appeared in Texas Star 1999; 13 peaks appeared in Texas Star 2000, and nine peaks appeared in Elite 2001, showing that aging was associated with a higher number of compounds in the samples. In the three different samples, major peaks identified were associated with acylated anthocyanins, usually found in grapes, in terms of their retention time and UV–Vis characteristics. Cyanidin-3-glucoside and cyanidin-3-galactoside were the major anthocyanins and accounted for about 71% of the total anthocyanins. Cyanidin-3-glucoside alone accounted for about 50% of the anthocyanins and eluted after 10.45, 10.53 and 10.28 min in Elite 2001, Texas Star 2000, and Texas Star 1999, respectively. Cyanidin-3-galactoside eluted after 8.52, 8.56 and 8.36 min in Elite 2001, Texas Star 2000, and Texas Star 1999. The unidentified anthocyanins accounted for 30% of the total anthocyanins. The relative amounts of these anthocyanins decreased with time during storage from 1999 to 2001 (Figs. 1–3). The decrease in the number of HPLC peaks of mayhaw from 1999 to 2001 may be associated with the increase in anthocyanin polymerization, as indicated by the characteristics of the anthocyanins, as described in Table 1.

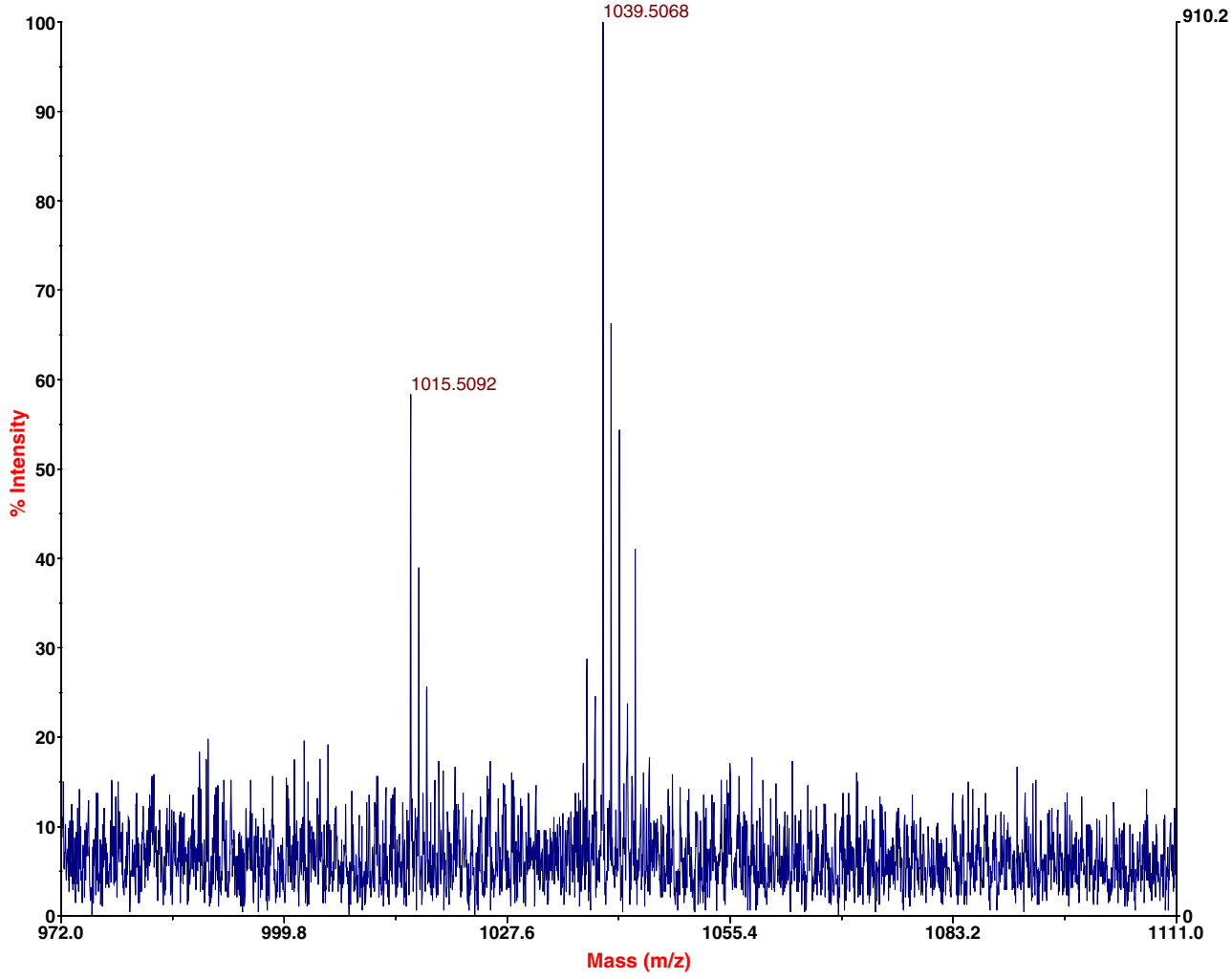

Fig. 7. MALDI-MS of mayhaw anthocyanins, in the region of *m/z* 800–1200 showing the peaks associated with anthocyanin trimers and salt adducts.

3.3. MALDI-TOF-MS of mayhaw anthocyanins

MALDI-TOF-MS profile of mayhaw anthocyanins are shown in Figs. 4–7. The monomeric unit for condensed tannins is either catechin or epigallocatechin with a molecular size of 288. Majors peaks resolved by HPLC were identified, at *m*/*z* of 449, as cyanidin-3-glucoside and cyanidin-3-galactoside. Other minor peaks were identified at *m*/*z* 595, 758 and 786 and were tentatively associated with cyanidin-3-glucoside coumarate, delphinidin 3-glucoside-coumarate-5-glucoside, and petunidin 3-glucoside-coumarate-5-glucoside, respectively (Wang & Sporns, 1999). Peaks were also observed as a combination of the trimers (*m*/*z* = 864) and salts ([M + K], [M + Na], [M + Li], or [M + K + Na + Li]) or glucosides to give the peak at *m*/*z* 1039 (Fig. 6). The MALDI profile of mayhaw anthocyanins indicated the presence of a mixture of dimers and trimers. Given the number of peaks associated with anthocyanins from HPLC separation, a variety of anthocyanin-glucoside combinations could be imagined in mayhaw (Figs. 1–3) (Wang & Sporns, 1999). Recently Mazza, Cacace, and Kay (2004) reported the advantages of techniques such as capillary electrophoresis and LC-MS for the separation of anthocyanins and the use of NMR for structural identification. These techniques will further our understanding of the properties of anthocyanins. In conclusion, fresh mayhaw juice contained less colour and more polymeric anthocyanins than stored juice. Storage was associated with an increase in anthocyanin concentration, and a decrease in polymeric colour and percent polymeric anthocyanins.

References

Bagchi, D., Sen, C. K., Bagchi, M., & Atalay, M. (2004). Anti-angiogenic, antioxidant, and anti-carcinogenic properties of a novel anthocyanin-rich berry extract formula. *Biochemistry (Mosc), 69*(1), 75–80.

Giusti, M. M., & Wrolstad, R. E. (2000). Characterization and measurement of anthocyanins by UV–Visible spectroscopy. In *Current protocols in food analytical chemistry*. New York: Wiley.

Kay, C. D., Mazza, G., Holub, B. J., & Wang, J. (2004). Anthocyanin metabolites in human urine and serum. *British Journal of Nutrition, 91*(6), 933–942.

Lopez-Serrano, M., & Ros Barcelo, A. (1999). H_2O_2-mediated pigment decay in strawberry as a model system for studying color alterations in processed plant foods. *Journal of Agricultural and Food Chemistry, 47*(3), 824–827.

Mazza, G., Cacace, J. E., & Kay, C. D. (2004). Methods of analysis for anthocyanins in plants and biological fluids. *Journal of AOAC International, 87*(1), 129–145.

Meyers, K. J., Watkins, C. B., Pritts, M. P., & Liu, R. H. (2003). Antioxidant and antiproliferative activities of strawberries. *Journal of Agricultural and Food Chemistry, 51*(23), 6887–6892.

Middleton, E., Jr., Kandaswami, C., & Theoharides, T. H. (2000). The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacological Reviews, 52*(4), 673–751.

Prior, R. L. (2003). Fruits and vegetables in the prevention of cellular oxidative damage. *American Journal of Clinical Nutrition, 78*(3 Suppl), 570S–578S.

Wang, J., & Mazza, G. (2002). Inhibitory effects of anthocyanins and other phenolic compounds on nitric oxide production in LPS/IFN-gamma-activated RAW 264.7 macrophages. *Journal of Agricultural and Food Chemistry, 50*(4), 850–857.

Wang, J., & Sporns, P. (1999). Analysis of Anthocyanins in red wine and fruit juice using MALDI-MS. *Journal of Agricultural and Food Chemistry, 47*(5), 2009–2015.