Data Article

A dataset for anthocyanin analysis in purple-pericarp sweetcorn kernels by LC-DAD-MS

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ARTICLE INFO

ABSTRACT

This dataset refers to the accompanying article "Optimization of extraction procedure and development of LC-DAD-MS methodology for anthocyanin analysis in anthocyanin-pigmented corn kernels", published in Food Chemistry [1]. Here, we present concentrations, profiles, MS^2 spectra of individual anthocyanins (including isomers of cyanidin-3-(6"-malonylglucoside)) of purple-pericarp sweetcorn (PPS) kernels. Furthermore, an additional mass spectrum of an artefact-anthocyanin produced in acidified extraction solutions were reported. This data is further discussed in the accompanying research article [1]. Delphinidin-3-glucoside was used as an internal standard to compensate for individual anthocyanin losses during extraction with acidified solutions. The generated data could be used for anthocyanin identification and quantification in different anthocyanin-containing plant matrices.

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Specifications table

Subject	Analytical Chemistry
Specific subject area	Optimization methodology for identification and quantification of anthocyanins from PPS kernels
Type of data	Table
	Figures
How data were acquired	Calibration curves of concentration ratios of external standards/internal standard were established using a Shimadzu UHPLC-DAD system.
	Anthocyanins were identified by the Shimadzu-8050 electrospray ionisation mass spectrometry (ESI-MS).
Data format	Raw MS chromatograms
	Calibration curves
	Analyzed
Parameters for data collection	PPS and white (anthocyanin-free) sweetcorn cobs at 26 days after pollination (DAP) were collected from the Gatton Research Facility, Gatton, QLD, Australia.
	All data collected from the current experiments were analyzed as technical replicates (n=3).
	Selected ion monitoring (SIM) and product ion monitoring were operated at a collision energy of -20V.
Description of data collection	Full MS scans together with product ion monitoring in positive mode were operated for anthocyanin identification.
	The current data were collected by integrating the peak areas of anthocyanin signals at 520 nm.
Data source location	Gatton Research Facility, Department of Agriculture and Fisheries, Queensland Government, QLD, Australia.
	Health and Food Sciences Precinct, The University of Queensland, Coopers Plains, QLD, Australia.
Data accessibility	With the article
Related research article	Hong, H.T., Netzel, M.E. and O'Hare, T.J. (2020), Optimization of extraction procedure and development of LC-DAD-MS methodology for anthocyanin analysis in anthocyanin-pigmented corn kernels, Food Chemistry

Value of the data

- The MS data provide additional information regarding structure identification of anthocyanins in PPS kernels.
- An additional mass spectrum of an artefact-anthocyanin was provided to further support the identification of artefact-anthocyanins generated in acidified extracts of PPS kernels.
- The presented data about genuine and artefact-anthocyanins in PPS kernels are crucial for correct anthocyanin profiling (anthocyanin "fingerprint") and calculation of anthocyanin concentrations
- The calibration curves of concentration ratios of external standards/internal standard provide additional information about the method of anthocyanin quantification. Artefact-anthocyanins produced in acidified extraction solutions were reported for the first time.
- This potential data source could be used for anthocyanin identification and quantification in different anthocyanin-containing plant matrices.

1. Data

The dataset in this article (Table 1) describes raw data collected from UHPLC-DAD at 520 nm with varying concentrations from 0.05 to 50 mg/L of the external standards (cyanidin-3-glucoside (Cy3G), pelargonidin-3-glucoside (Pg3G) and peonidin-3-glucoside (Pn3G)) and internal standard (delphinidin-3-glucoside (Del3G)) at 1 mg/L. Figs. 2–4 show the calibration curves of the concentration ratios of (Cy3G, Pg3G and Pn3G)/(Del3G). Figs. 5–10 show

Table 1

Data collected from UHPLC-DAD at 520 nm for the standard calibration curves for the concentration ratio of Cy3G, Pg3G and Pn3G/Del3G.

Concentration (mg/L)		Response				Ratio		
Cy3G, Pg3G, Pn3G	Del3G	Cy3G	Pg3G	Pn3G	Del3G	Cy3G/Del3G	Pg3G/Del3G	Pn3G/Del3G
50	1	798.3	758.3	824.3	22	36.3	34.47	37.47
20	1	331	303.5	325.4	21.9	15.1	13.9	14.9
10	1	166.6	140.1	148.7	21.5	7.8	6.5	6.9
5	1	83.4	74.2	80.6	21.3	3.9	3.5	3.8
1	1	17.4	15.1	17.1	21.9	0.8	0.7	0. 8
0.2	1	3.6	3.1	2.9	22.5	0.2	0.1	0.1
0.05	1	0.8	0.7	0.7	21.5	0.0	0.03	0.03

product ion mass spectra of selected precursor ions at m/z 605 for pelargonidin-3-(3",6"-dimalonylglucoside), m/z 549 for peonidin-3-(6"-malonylglucoside), m/z 635 for (peonidin-3-(3",6"-dimalonylglucoside), m/z 535 for (cyanidin-3-(6"-malonylglucoside) and m/z 621 for (cyanidin-3-(3",6"-dimalonylglucoside), m/z 519 for pelargonidin-3-(6"-malonylglucoside), respectively in PPS kernels. The UHPLC-MS single ion monitoring (SIM) spectra of target anthocyanins in PPS is shown in Fig. 11 describing four anthocyanin isomers of cyanidin-3-(6"-malonylglucoside) in PPS kernels. Fig. 12 shows product ion mass spectrum of selected precursor ions at m/z 649 (artefact peonidin-3-(methylmalonatemalonylglucoside)) produced in acidified PPS extracts after storage at room temperature for 5 hours.

2. Experimental design, materials, and methods
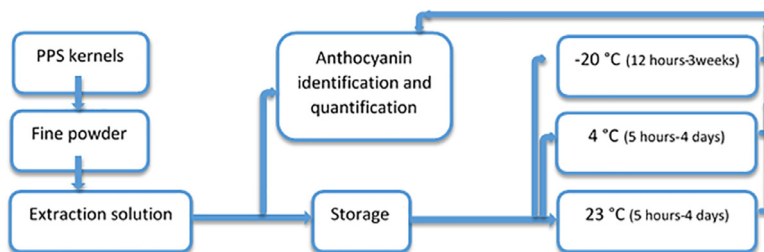
2.1. Experimental Design

Fig. 1.

Fig. 1. Experimental design for anthocyanin identification and quantification in PPS kernels.

2.2. Materials

Individual PPS was self-pollinated by hand to exclude foreign pollen. Five cobs of PPS and a white (anthocyanin-free) sweetcorn cob were harvested randomly at 26 DAP in autumn 2018 at the Gatton Research Facility, Gatton, QLD, Australia. Cobs were dehusked and immediately transported (1 h transit) to the Health and Food Sciences Precinct at Coopers Plains, The University of Queensland, QLD, Australia, where they were stored at -20°C prior to analysis.
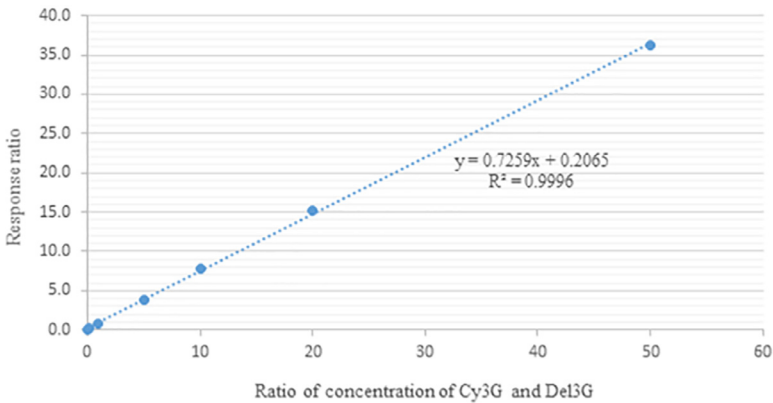
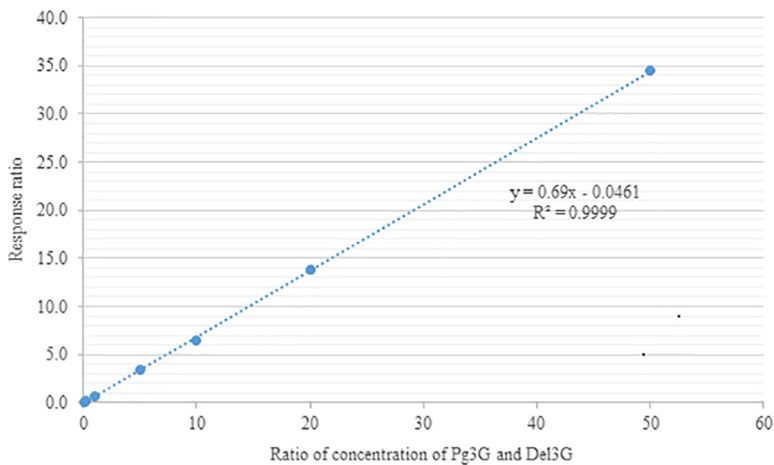**Fig. 2.** Standard calibration curve of concentration ratios of Cy3G/Del3G measured by a DAD at 520 nm.

Fig. 3. Standard calibration curve of concentration ratios of Pg3G/Del3G measured by a DAD at 520 nm.

2.3. Methods

2.3.1. Solutions

An extraction solvent of 80% v/v aqueous methanol acidified with 1% formic acid was used to dissolve 2 mg each of Cy3G, Pg3G and Pn3G in 10 mL to prepare master stock solutions of 200 mg/L. Del3G (1 mg) was dissolved as an internal standard into the extraction solution to have a final concentration of 100 mg/L. Solutions for nine calibration standard concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20, and 50 mg/L of each external standard, together with 1 mg/L of Del3G were prepared from the master stock solutions by diluting with a white (anthocyanin-free) sweetcorn matrix solution.

Anthocyanins of PPS were extracted using an extraction solvent of 80 v/v % aqueous methanol acidified with 0.1 M hydrochloric acid (HCl). The liquid chromatography mobile phase A consisted of acetonitrile, deionized water and formic acid (92:7:1, v/v/v), and mobile phase B of 1% formic acid in acetonitrile.
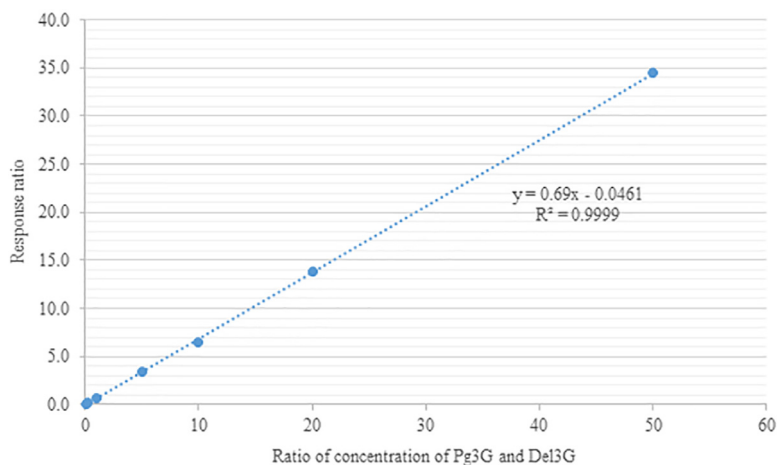
Fig. 4. Standard calibration curve of concentration ratios of Pn3G/Del3G measured by a DAD at 520 nm.
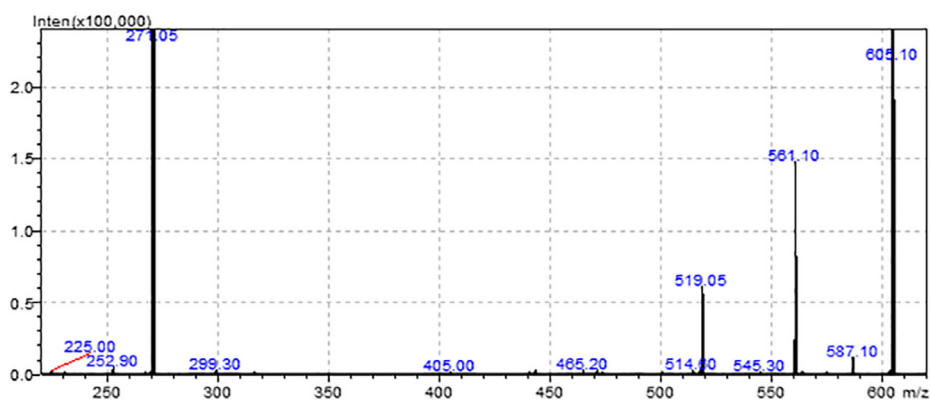

Fig. 5. MS^2 spectrum of pelargonidin-3-(3'',6''-dimalonylglucoside) from PPS kernels.

2.3.2. Sample preparation and extraction

Three rows of kernels from each cob (n=6) were snap frozen by liquid nitrogen and then cryo-milled using a ball mill (MM400 Retsch Mixer Mill, Haan, Germany) operated at 30 Hz for 60 s. The powdered sample (about 0.5 g) was transferred to a 15 mL Falcon tube with three technical replicates, 120 μL of master stock solution of Del3G (internal standard) and 3.88 mL of cold extraction solution (4°C). The mixture was sonicated for 10 min at 4°C and then shaken on a horizontal reciprocating shaker (RP 1812; Victor Harbor, SA, Australia) at 250 rpm/min for 10 min under dim light and cool temperature (4°C) before being centrifuged at 4000 rpm for 10 min at 4°C. The supernatant was removed and the pellet residue re-extracted twice with 4 mL of extraction solution using the same procedure. The combined supernatants (12 mL) were filtered through a 0.22 μm hydrophilic PTFE syringe filter into a UHPLC vial for analysis.

2.3.3. Anthocyanin identification

Anthocyanins were detected by a DAD detector at 520 nm and the molecular masses determined according to Hong, Netzel & O'Hare [2]. The remaining compounds were determined by their unique absorption maximum (DAD) and their mass-to-charge ratio (*m/z*) and fragment pat-
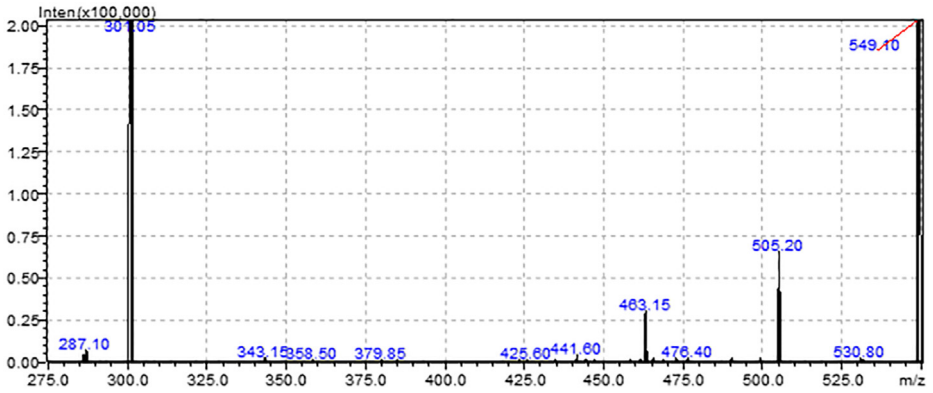
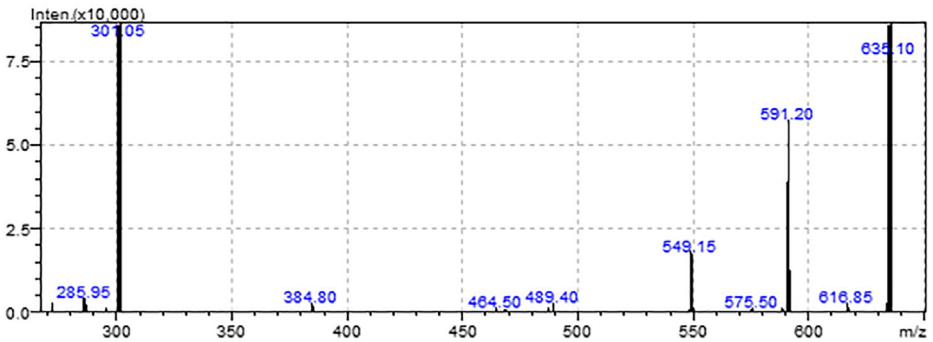Fig. 6. MS^2 spectrum of peonidin-3-(6"-malonylglucoside) from PPS kernels.

Fig. 7. MS^2 spectrum of peonidin-3-(3",6"-dimalonylglucoside) from PPS kernels.
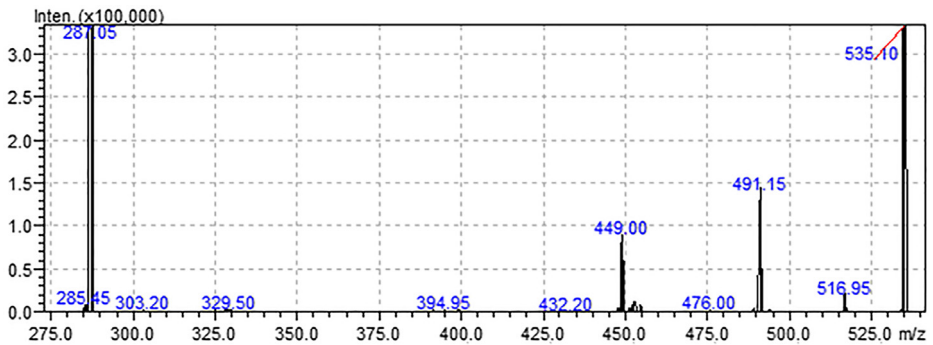

Fig. 8. MS^2 spectrum of cyanidin-3-(6"-malonylglucoside) from PPS kernels.

tern via mass spectrometry in positive ion mode (Figs. 4–9). Comparison of compound elution order to previously published studies [3–7] was further used to confirm anthocyanin identity.

Anthocyanin quantification. The concentration of individual anthocyanins was calculated by comparison of the intensity of each peak from the DAD spectra at 520 nm to the standard calibration curve of concentration ratios of external standards/internal standard. Equivalent concentrations were used for all anthocyanin malonyl-glucosides based upon their flavylium cations. Total an-
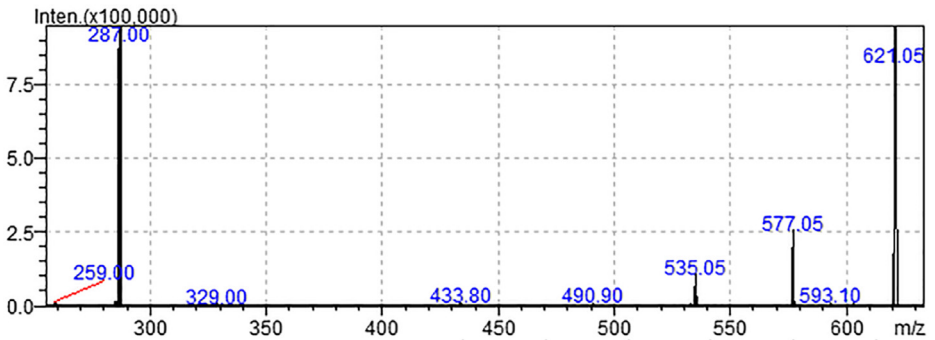
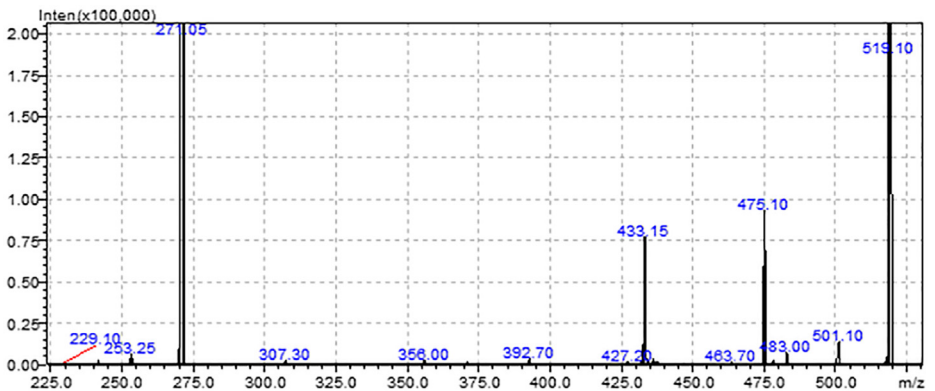**Fig. 9.** MS^2 spectrum of cyanidin-3-(3",6"-dimalonylglucoside) from PPS kernels.

Fig. 10. MS^2 spectrum of pelargonidin-3-(6"-malonylglucoside) from PPS kernels.
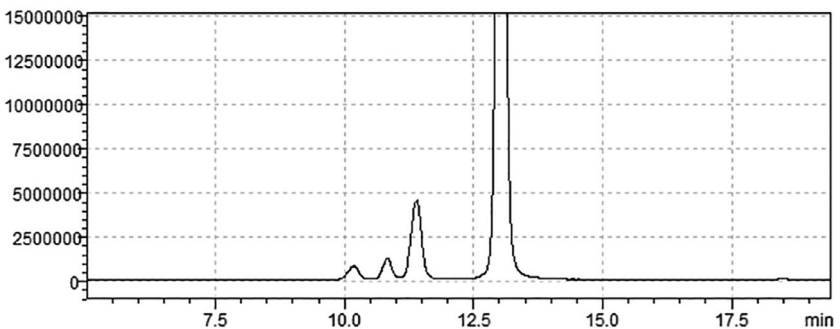

Fig. 11. Four cyanidin-3-(malonylglucoside) isomers of PPS were detected by UHPLC-ESI-MS.

thocyanin content was calculated as the sum of individual anthocyanin concentrations. Selected Ion Monitoring (SIM) in positive mode was scanned by mass spectrometry and the relative contribution of the co-eluted compounds within each peak was calculated (Fig. 10).

2.3.4. Stability studies

The acidified extraction solution of the PPS sample was stored at -20°C, 4°C and room temperature (23°C) for 5 hours, 12 hours, 24 hours, 4 days and three weeks. The stability of the six major anthocyanins in PPS was investigated and artefact anthocyanins were identified.
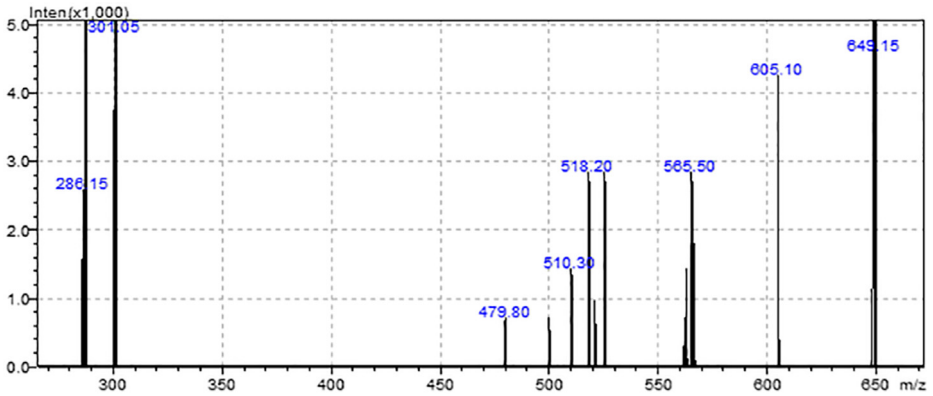
Fig. 12. MS^2 spectrum of artefact peonidin-3-(methylmalonatemalonylglucoside), one of nine artefact-anthocyanins produced in acidified PPS extracts after storage at room temperature for 5 hours.

Acknowledgments

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] H.T. Hong, M.E. Netzel, T.J. O'Hare, Optimization of extraction procedure and development of LC-DAD-MS methodology for anthocyanin analysis in anthocyanin-pigmented corn kernels, Food Chem (2020).
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