FIGHT FOOD WASTE
Cooperative Research Centre
REDUCE - TRANSFORM - ENGAGE

SME Solutions Centre
Final Report

Anthocyanin retention in Queen Garnet plums during processing and bottling

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INDUSTRY PARTNER FORWARD

What is the value of having engaged in industry-led research?

It was extremely valuable to have regular meetings with the team at USQ and to feel involved in every step of the project. Typically, Nutrafruit would donate product to researchers and read the final scientific paper, with very limited involvement. In contrast, the FFW CRC project gave Nutrafruit the ability to highlight any production obstacles or limitations and work directly with the researchers to come to a resolution. Over the course of the project, Nutrafruit's product range and future production plans changed and the project transformed accordingly. This allowed the milestones to shift in ways that were more relevant to the needs of the business over time, instead of committing to experiments that no longer applied to company goals.

Has your organisation gained from being involved in this project?

USQ has provided Nutrafruit with insightful recommendations to maximise anthocyanin content in the fresh fruit. This will greatly impact the growers, allowing them to be more self-sufficient with on-farm tools and requiring less handholding and follow-up from Nutrafruit. As a result of these tools, the fruit in stores will be able to reach quality specifications. In previous seasons, fruit that was underripe would go to waste due to the suboptimal aesthetic and eating quality. With improved characteristics, the fruit will move through the stores quicker, increasing demand and therefore increasing the moving supply to reduce waste at the orchards. Outside of the fresh fruit season, the processing fruit used to produce value-added products will also be of superior quality with the help of tools to increase anthocyanin levels. As a wellness brand, anthocyanin is the key component in supplying health benefits to consumers.

Involvement in next steps to drive project outcomes to commercial outcomes and impact?

Nutrafruit will be hosting a workshop with its growers to review findings from the FFW CRC project. This will be a platform for them to ask any questions and will serve as a basic training session for next year's fresh fruit season. The tools and strategies recommended by USQ will be included in the workshop. Growers will be given individual feedback based on their crop this year and Nutrafruit will assist them in improving quality. Nutrafruit will also use recommendations set by USQ to improve the current and future value-added product range.

What possible next research projects are on the agenda now?

No projects have been scheduled. Nutrafruit is currently focused on market research to inform the future product ranges for the business. In addition, another project has started at USQ using the newly produced QG extract as an ingredient in a novel hydrogel for chronic wound treatment. Normal wound healing occurs around an acidic pH and chronic wounds have an alkaline pH. The anthocyanins in the QG extract respond to changes in wound pH by changing colour, and therefore this could be used as a tool to rapidly assess whether wound healing is occurring or if further treatment should be sought.

EXECUTIVE SUMMARY

Queen Garnet (QG) plums have considerably higher anthocyanin content (antioxidant) than other common plum varieties, and consequently have great potential to be marketed as value-added health products. 340,000 QG plum trees have been planted across Australia with fruit volume forecasts that currently exceed domestic demand. Of this, 30% of fruit in 2020 was composite, lower-grade fruit sent for processing, however 15% still ended up in landfill. Nutrafruit's business plan is to expand its reach by spreading awareness of the benefits of the QG plum and grow the product market so this surplus fruit can be used. This can be achieved if the value-added products retain the key anthocyanin that gives the plum its renowned benefits. The objective of this research was to first identify any potential new product opportunities, quantify any potential issues regarding anthocyanin content loss throughout the supply chain, propose potential strategies to overcome this issue if it is significant, and enable some analytical capability at Nutrafruit to inform QA/QC and product claims.

For Nutrafruit to be able to produce a high-value product containing large amounts of anthocyanin, it was necessary to track how anthocyanin content changes in the plum during ripening, storage and processing. In this project, it was observed that anthocyanin content of QG plums varies considerably between different growers (68 - 158 mg/100g fresh weight). This difference in plum quality is potentially detrimental to the Nutrafruit brand and could impact product consistency and quality. Plums of lower visual appeal and anthocyanin content are more likely to be unsaleable and therefore end up as food waste. An outcome of this project is the potential for an affordable colourimeter to be distributed to growers and producers to allow for rapid prediction of anthocyanin content from simple colour measures. This will assist growers in being able to conduct quality control and will potentially help in preventing early harvest. Nutrafruit have pivoted to a new range of products (QG essence and extract) using alternative processes to their initial nectar pasteurisation and freeze dry processes and have already commenced commercialisation with a contract manufacturer. Further analyses were conducted to examine not just the anthocyanin content, but also the total carotenoid, phenolic and flavonoid content of the plums. The analyses conducted in this research can help inform market opportunities and potential uses/formulations of QG plum products.

Project Impacts:

1. Nutrafruit have begun commercialisation of their QG essence and extract, which are a higher-value product with a longer shelf-life than QG plum nectar. This longer shelf-life will help to protect Nutrafruit against future supply chain disruptions such as occurred during COVID-19. Production of the QG essence and extract does not require heat and therefore has greater retention of anthocyanin.
2. Investigation into the differences in QG plum quality and anthocyanin content between different growers has highlighted that improvements need to be made to increase consistency and average plum quality. Improving this will reduce the harvest of QG plums with suboptimal aesthetic and eating quality, which end up being wasted due to being unsaleable. Tools and training will be provided to Nutrafruit by USQ to assist with Nutrafruit's planned workshop for growers.
3. An affordable colourimeter has been successfully used to predict anthocyanin content of QG plums as 94% of the variance in anthocyanin content can be explained by the QG plums becoming bluer in colour (b* value becoming more negative). This has implications for use as a rapid quality control tool to track anthocyanin content across the fruit supply chain from fruit onset to harvest through to processing. Training documents and an interactive spreadsheet containing the calculations required will be provided to Nutrafruit for this purpose.

4. It is estimated that 5% of plums previously lost as waste can now be retained and or upcycled using the colourimetric QC method and commercialisation of the higher-value, longer shelf-life QG extract and essence - this equates to an estimated 300 Mt per annum saved from food loss

Objectives

The initial objectives of this project were to first identify any potential new product opportunities, quantify any potential issues regarding anthocyanin content loss throughout supply chain, propose potential strategies to overcome this issue if it is significant, and enable some analytical capability at Nutrafruit to inform QA/QC and product claims. During the project some product focus pivots occurred which informed project activities as the work progressed and additional work in alternative process and storage trials were also carried out. A stretch objective of in-line and in-situ monitoring of anthocyanin content was achieved through development of a rapid colourimetric analysis and calibration tool that could be easily used by farmers and factories.

Results

This appears to be the first time that the ripening and associated increase in anthocyanin content of QG plums was tracked from fruit set to harvest, and this was done at Warroo Orchard for both the 2020-21 and 2021-22 seasons (~October-February). The rise in anthocyanin content was highly comparable in both seasons, however ripening and harvest occurred earlier in the 2021-22 season. Anthocyanin content increases only marginally in the first ~50 days, which is then followed by a rapid quadrupling in anthocyanin by the next test date 15 days later. This is the start of the exponential increase in anthocyanin content, measured in mg/100g of Fresh Weight (FW), that occurs from day 50 – 75. The total anthocyanin content at harvest was 158 mg/100g FW in 2022, which was similar to the 2021 season where the harvest plums contained 154 mg/100g FW. However, anthocyanin content of QG plums varies considerably between different growers (68 - 158 mg/100g fresh weight) as well as varying moisture content (80-86%). This difference in plum quality is potentially detrimental to the Nutrafruit brand and could impact product consistency and quality. A new rapid analysis approach was developed to address this problem using an affordable colourimeter suitable for distribution to growers and manufacturers. Using the colourimeter, it was found that anthocyanin content is negatively correlated to the skin and flesh b* value (correlation -0.90, $P < 0.001$). This was followed by linear regression modelling, that showed that 94% of the variance in anthocyanin content is explained by the plum skin and flesh becoming bluer (b* value becoming more negative). This will assist growers in being able to conduct quality control and will potentially help in preventing early harvest, as anthocyanin content is also correlated to moisture content. Preliminary work investigating the effects of blanching for 2 minutes or placing the plums at room temperature in brown paper bags for 2 days was promising, as this resulted in a 17.4% and 22.6% increase in anthocyanin content respectively. Freeze-dried plum powder was sent to Southern Cross University for HPLC analysis, and these results were highly comparable to the UV-Vis spectrophotometer results for anthocyanin content and also highlighted that QG plums contain ~4mg carotenoids/100g FW.

Next steps

Tools and training for use of the colourimeter for anthocyanin prediction will be provided by USQ to Nutrafruit. Based on the findings of this research, Nutrafruit is planning on hosting a workshop with its growers to review findings from the FFW CRC project. This will be a platform for them to ask any questions and will serve as a basic training session for next year's fresh fruit season. USQ will continue to support the release/launch of high value products by providing analyses and consultation at cost if needed. The potential for use of Nutrafruit's QG extract in a separate project at USQ focused on chronic wound healing is currently being explored, and Nutrafruit will be advised of the outcome of this project.

Timing

- Tools and training provided to Nutrafruit by 31/12/2022
- Analyses and consultation at cost on offer up until 30/06/2023
- Results of the chronic wound healing project up until 30/06/2024

Project milestones

Milestone 1: ☑ Commencement date: 1 September 2020 Completion date: 31 December 2020 *Activities:* • Resourcing of project ☑ • Spectroscopic ☑ and Chromatographic method development ☑ • Alternate Process Scoping in lab using other readily available fruit ☑ *See Milestone 1 report for details*	**Milestone 2:** ☑ Commencement date: 1 January 2021 Completion date: 31 March 2021 *Activities:* • Baseline kinetic study ☑ • Chromatography studies ☑ *See Milestone 2 report for details. Nutrient tracking from fruit set to end product was also done.*
Milestone 3: ☑ Commencement date: 1 April 2021 Completion date: 31 March 2022 *Activities:* • Alternative Process study ☑ • Training for Nutrafruit and Manufacturing Partners ☑ *Some alternative supply chain studies were carried out in 2021 which were reported in the Milestone 2 report, and also using microwave pasteurisation (see extra QG Plum HPLC report) and modified storage trials.*	**Milestone 4:** Commencement date: 1 July 2021 Completion date: 11 July 2022 *Activities:* • In-line and in-situ monitor scoping ☑ • Final project report ☑ • One-page case study (for public release) ☑

Project impacts

Food Waste Reduction, plans for increased profitability and industry training:

- The project has provided insight into the opportunities for high-value anthocyanin containing products for Nutrafruit which have knock on effects for increased product potential and reduced waste
- Investigation into the differences in QG plum quality and anthocyanin content between different growers has highlighted that improvements need to be made to increase consistency and average plum quality. Improving this will reduce the harvest of QG plums with suboptimal aesthetic and eating quality, which end up being wasted due to being unsaleable.
- An affordable colourimeter has been successfully used to predict anthocyanin content of QG plums as 94% of the variance in anthocyanin content is explained by QG plums becoming bluer in colour (b* value becoming more negative). This has implications for use as a rapid quality control tool to track anthocyanin content across the fruit supply chain from fruit onset to harvest through to processing.

Approved by - Francesca Goodman-Smith, TRANSFORM Program Leader

1. INTRODUCTION

Queen Garnet (QG) plum is a variety of the Japanese plum (*Prunus salicina* Lindl.) that has a high anthocyanin content of up to 272 mg/100 g fresh fruit (1), which is considerably higher than other common plum varieties that average an anthocyanin content of 54 mg/100g fresh fruit (2). Anthocyanins have previously been shown to exert antioxidant and anti-inflammatory effects that have applications in cancer prevention(3), lowering of blood pressure and blood glucose (4) and reduction of obesity (5). Consequently, QG plums have great potential to be marketed as value-added health products, however some hurdles remain that need to be overcome before this can be realised. 340,000 QG plum trees have been planted across Australia with fruit volume forecasts that currently exceed domestic demand. Of this, 30% of fruit in 2020 was composite, lower-grade fruit sent for processing, however 15% still ended up in landfill. Plums of lower anthocyanin content and reduced visual appeal are more likely to be unsaleable and therefore end up as food waste. A stretch goal of this project is to investigate the potential for an affordable colourimeter to be distributed to growers and manufacturers to allow for rapid prediction of anthocyanin content from simple colour measures.

Nutrafruit's business plan is to expand its reach by spreading awareness of the benefits of the QG plum and grow the product market so this surplus fruit can be used. This can be achieved if the value-added products retain the key anthocyanin that gives the plum its renowned benefits. The objective of this project was to first identify and quantify the issues regarding anthocyanin content loss during manufacturing, propose potential strategies to overcome this issue if it is significant, and enable some analytical capability at Nutrafruit to inform QA/QC and product claims. This may involve recommendations to pivot from producing pasteurised nectar to an alternative product, as it is likely that the heating during the double pasteurisation may be reducing anthocyanin content. Value added products were scoped at the start of the project and Nutrafruit explored their own options to land on essence and extract products for future market reach. There is a future planned reduction in production of the lower value nectar product and increased production of higher value, longer shelf-life freeze dried powders, essences and extracts.

2. METHODOLOGY

The QG plums were collected at multiple timepoints from Warroo Orchard (Queensland) between fruit set to commercial harvest for both the 2020-21 and 2021-22 seasons. At each time point within 24 hours of collection, approximately 1 kg of plums were washed, pureed using a Nutribullet and then stored at -80°C in preparation for freeze-drying. Approximately 4 kg of the plums collected at harvest were stored at 4°C to track the changes in anthocyanin content over time during cold storage and an additional 4 kg were stored at -20°C for comparison. In the 2021-22 season, QG plums were collected from four other growers across Queensland and Victoria to compare to Warroo Orchard.

2.1 Sample preparation

All samples were freeze dried in a CHRIST ALPHA 2-4 LDplus freeze dryer for approximately 72 hours. The weight of the plum puree was compared to the weight of the freeze-dried plum powder to determine the moisture content of the plums. A portion of the plum powder was then sealed in airtight falcon tubes and stored in the dark for between 48 – 72 hours prior to testing. The remainder of the plum powder was immediately placed at -80°C for long term storage.

2.2 Total anthocyanin content – UV Vis Spectrophotometry

The pH differential method developed by Lee et al. (2005) was conducted according to the published method with the following modifications (6). A solvent was prepared with 100% ethanol and 0.1 M HCl at a ratio of 85:15% *v:v*. The solvent extraction was repeated for each plum sample in triplicate, whereby 15 ml of this solvent was combined with 1g of the freeze-dried plum powder and placed on a shaker bath at 25°C for 20 minutes. The solution was then centrifuged using a Heraeus Megafuge 8 Centrifuge (Thermo

Scientific) at a speed of 3500 g for 5 minutes to collect the supernatant. The solvent extraction and centrifugation processes were repeated on the same sample an additional three times to ensure that all anthocyanins were extracted from the plum powder. The supernatants from the repeated extractions were then pooled together and mixed, and then a pipette was used to measure out 1 ml from this solution into two 15 ml tubes.

The pH 1.0 buffer and pH 4.5 buffer were prepared as described by Lee et al. (2005). For each sample, two falcon tubes were prepared with each containing 1 ml of the extracted supernatant and one containing 4 ml of the pH 1.0 buffer and the other containing 4 ml of the pH 4.5 buffer. The tubes were then incubated in the dark at 20 - 25°C for 20 minutes. A UV spectrophotometer was used to measure the absorbance at 520 and 700 nm (to correct for haze) for both the pH 1.0 buffer sample and pH 4.5 buffer sample. A blank of distilled water was used to zero the UV spectrophotometer. The predominant anthocyanin in QG plum is cyanidin-3-glucoside (7), so the total anthocyanin content was calculated as cyanidin-3-glucoside equivalents (mg/L) using the following equation (6)

$$\frac{\text{Absorbance} \times \text{Molecular weight} \times \text{Dilution factor} \times 10^3}{\text{Molar extinction coefficient} \times 1\ (\text{pathlength in cm})}$$

The molar extinction coefficient for cyanidin-3-glucoside is 26 900 L x mol^{-1} x cm^{-1} and the molecular weight is 449.2 g mol^{-1}. The absorbance is $(A_{520nm} - A_{700nm})_{pH\ 1.0} - (A_{520nm} - A_{700nm})_{pH\ 4.5}$. The total mg of anthocyanin present in the sample was determined by converting the concentration of anthocyanin in the extract from mg/L to mg/mL, and then multiplying this concentration by the volume of ethanol: HCl solvent used during extraction. Then the final value reported in the results was obtained by dividing this mg of anthocyanin amount present in the extract by the mass of freeze-dried plum powder. This was converted into fresh weight concentration by multiplying by the solid content % of the plum determined during freeze-drying.

2.3 Alternate processes to study impact on anthocyanin content of plums

Explorative study to determine the changes to anthocyanin content following changes to temperature or other variables.

2.3.1 Plum blanching

Five whole plums were submerged in a water bath heated to 85°C for either 2 or 5 minutes followed by submerging them in an ice bath to cool rapidly back to room temperature. These plums were then prepared for total anthocyanin content analysis by UV Vis Spectrophotometry as above. This experiment was later repeated with the additional modification of submerging falcon tubes containing plum puree or thinly sliced plums for 2 minutes. This was to compare the effects of blanching whole plums to plum puree and sliced plums.

2.3.2 Effects of short-term changes to storage temperature

Sixteen plums were removed from cold storage at 4°C and placed at room temperature for 0 – 7 days either placed individually in brown paper bags or loosely placed covered from light, as modified from Wang et al. (8). At the 0-, 2-, 5- and 7-day timepoints, four plums in paper bags and four plums loosely placed covered from light were prepared for total anthocyanin content analysis by UV Vis Spectrophotometry as above.

2.3.3 Microwave pasteurisation of plums

The process used at Nutrafruit to pasteurise the plums involved a pasteurisation of 104 – 107°C for 30 seconds followed by a second pasteurisation of a minimum 94°C for 19 seconds. The plums that were collected at harvest were stored for 4 months at 4°C, and then nine plums were pureed, and a quantity of 120 g was weighed out into two plastic containers. A microwave oven was used to heat the plum puree in these containers, using periods of 30 seconds of heating followed by stirring the contents and checking the

temperature using a thermometer. The first container was heated to a maximum temperature of 91.4°C after 4 minutes and the second container was heated to a maximum temperature of 87.3°C after 4 minutes. In both cases the heating was repeated twice (two 30 second periods) after reaching this maximum temperature to ensure that it was the maximum. This was used as the first pasteurisation and the first container was then prepared for total anthocyanin content analysis by UV Vis Spectrophotometry as above.

The second pasteurisation was conducted on the second container that was heated to 87.3°C after the first pasteurisation. The same process as before was repeated, and after 2 minutes and 30 seconds the plum puree had reached a maximum temperature of 85°C. This second container was then prepared for total anthocyanin content analysis by UV Vis Spectrophotometry as above.

2.4. Southern Cross University HPLC analysis

The freeze-dried plum powder that was placed at -80°C for long term storage immediately following freeze-drying was sent to the Analytical Research Laboratory at Southern Cross University for HPLC analysis. The samples sent for HPLC analysis are shown in Table 1.

Table 1. Samples sent for HPLC analysis at Southern Cross University.

	Test Type		
Sample + Collection Time	**HPLC profile**	**Anthocyanin/anthocyanidins**	**Carotenoids**
Fruit set – 29/10/21	✓		
Ripening plum – 12/01/21	✓		
Harvest – 01/02/21	✓	✓	✓
Blanch control (no heat) – 19/02/21	✓		
Blanch 2 minute – 19/02/21	✓		
4-month storage – 25/05/21	✓		
Pasteurised once (89°C after 4 min) – 25/05/21	✓	✓	✓
Pasteurised twice (85°C after 2 min 30 sec) – 25/05/21	✓	✓	✓

Anthocyanin content was measured by chromatography with a Phenomenex Luna C18 HPLC column (250 × 4.6 mm) using a gradient method as described in the British Pharmacopoeia 2016 (BP2016) monograph for analysis of anthocyanin content. Samples were prepared in acidic methanol (10 mL), sonicated for 15 minutes then centrifuged. An aliquot (2 mL) of the supernatant was then diluted with 2 M phosphoric acid up to 10 mL, equilibrated for 15 minutes then an aliquot taken into a HPLC vial for analysis. The mobile phases were solvent A (8.5% formic acid, Milli-Q water) and solvent B (8.5% formic acid, 22.5% acetonitrile (Scharlau, Chem-Supply, Port Adelaide, SA, Australia), 22.5% methanol, 41.5% water). The gradient started at 7% solvent B, increased to 25% over 35 minutes, then to 65% solvent B over 10 minutes, at a flow rate of 1 mL/minute and an injection volume of 10 µL. Specific detection and calibration was performed at 535 nm. Solvents for reference and sample preparation were 2% hydrochloric acid in methanol and 2M phosphoric acid. Quantification was calculated as described in the British Pharmacopoeia based on reference standard, peak area at 535 nm and sample dilution. Total anthocyanins were calculated as cyanidin 3-glucoside chloride.

HPLC profiling was performed on 1200 series Agilent HPLC-MS system with single quad MS scanning ions from 100 to 1500 amu and diode array detector (DAD) UV-Vis peak spectra from 200 to 600 nm. Separation was performed with a Phenomenex Kinetex C18 HPLC column (100 × 4.6 mm) using a gradient method of water and acetonitrile with 0.005% trifluoroacetic acid over 30 min. The solvent gradient for separation started at 10% acetonitrile which was increased as a gradient to 30% acetonitrile over 10 minutes, then to 95% acetonitrile over 8 minutes, at a flow rate of 0.75 mL/minute. Sample were prepared in 50:50 ethanol water solvent for analysis to identify the range of hydrophilic to lipophilic constituents.

The carotenoids were determined as per the 2020 USP Lutein method by UV-Vis spectroscopy using an Agilent Cary 4854UV-Vis spectrophotometer. The test samples were prepared by extraction in 70% ethanol and their absorption measured at 446nm in a 10 mm quartz glass cuvette. Using the absorption coefficient of extinction for lutein in ethanol at 446 nm of 2550 as per the USP monograph. The quantity of carotenoids, calculated as lutein was given by the formula Concentration = A / (F x C), where A equals absorption of sample at 446 nm, F equals coefficient of extinction and C equals concentration of sample as g/mL.

2.5. Correlation of total anthocyanin content to colourimeter measurement

An affordable (~$500) colourimeter called Nix Pro 2 (https://www.nixsensor.com/nix-pro/) was used to identify whether there is a relationship between the colour of the plum skin or flesh and total anthocyanin content. The companion app for the Nix Pro 2 called 'Nix Pro Color Sensor App' is needed to operate the handheld colourimeter, and it was downloaded for free from https://www.nixsensor.com/apps/. The app settings used in this study were the illuminant set to D65, the Observer to 2° and the average number of scans to scan a colour to 3. A brief explanation of the CIELAB colour space and a screenshot of the Nix Pro Colour app is shown in Figure 1.

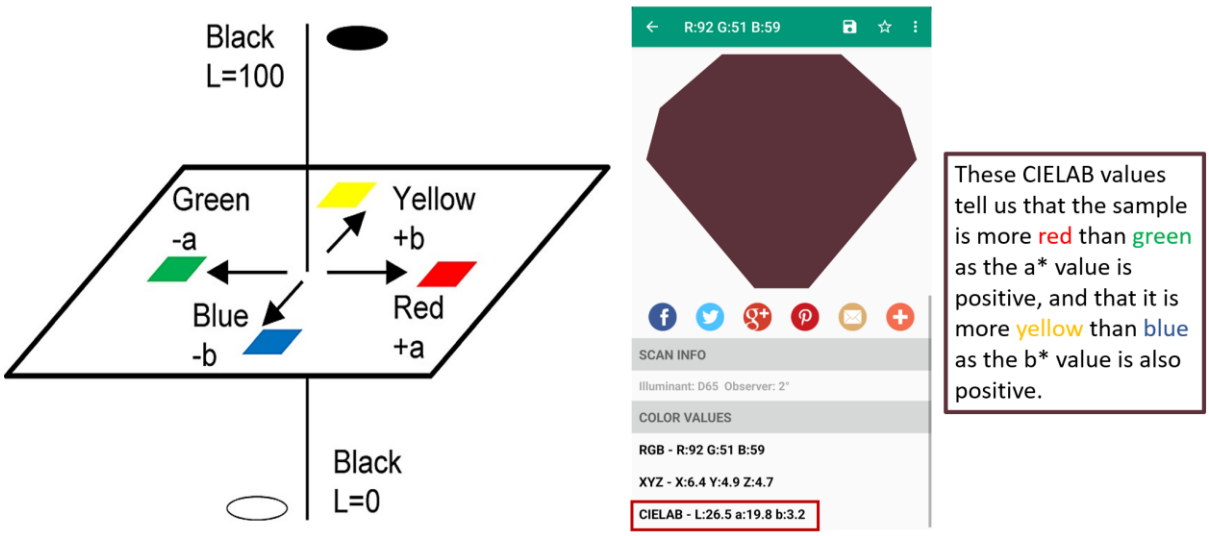

Figure 1. Diagram explaining the CIELAB colour space and a screenshot from the Nix Pro 2 app.

Plums were washed and then patted dry using a paper towel. Using the app, the Nix Pro 2 was then used to scan the surface of the plum (skin) making sure to vary the location of each scan to try and capture the natural variation in colour that occurs in a plum (Figure 2). The app scans 3 times (prompted each time by the app) to calculate the CIELAB values, and then the reported scan can be directly saved to a folder within the app for later export to Excel (Figure 3). The plums were then sliced in half, patted dry and then the Nix Pro 2 was used to scan the plum flesh as explained above.

Figure 2. Example of CIELAB values for Queen Garnet plums grown in the same location

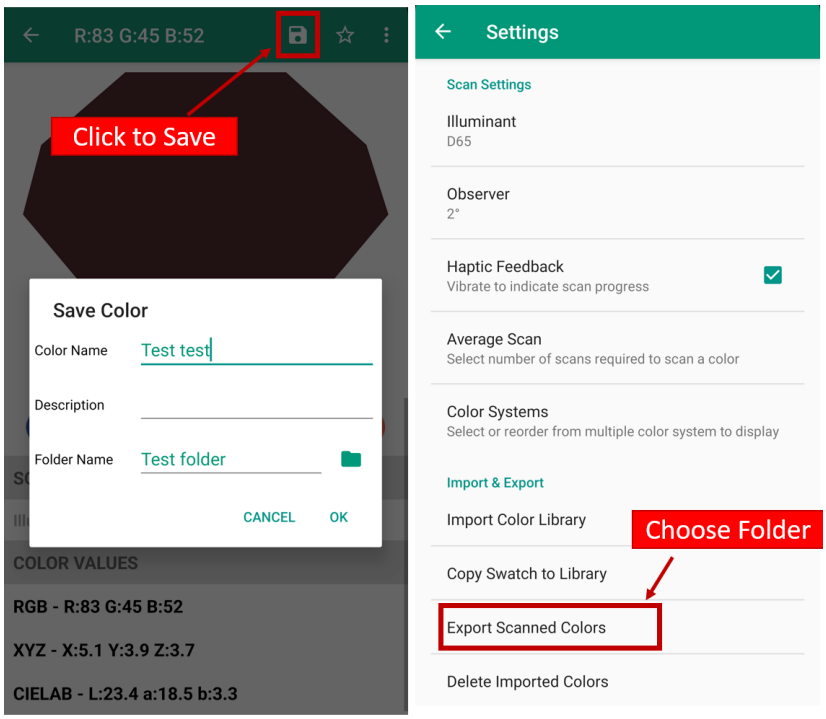

Figure 3. Diagram to show how to save scanned colours and then later export them into an excel file in the settings. All colours saved to the folder will be exported in the same excel file.

Anthocyanin data was correlated to the recorded L*, a*, and b* values from the Nix Pro 2 colourimeter to determine which variable should be used for further calculations to potentially predict anthocyanin content in plums. This correlation was performed using Pearson correlation (R package Corrplot) and a P value of <0.05 was considered as statistically significant. This correlation was then explored further using regression modelling, using the R^2 value as an indicator of the goodness of fit. To help to account for the variation in skin and flesh colour of the plums, the method for scanning the plums was later refined by repeating the scan 3 times per plum/sample (total of 9 scans) to calculate an average. The impact of this refined method on accuracy of predicting total anthocyanin content compared to the original total of 3 scans per plum is presented in the results section.

3. RESULTS

3.1. Comparison of 2021 to 2022 Harvest

The changes in anthocyanin content during ripening for both the 2020-21 and 2021-22 season are displayed in Figure 4. The increase in anthocyanin content was highly comparable in both seasons initially, however in the 2021-22 season the anthocyanin content of the plums started increasing earlier than the previous year. For example, there was a difference of 30 mg/100g Fresh Weight (FW) between the two seasons at the same timepoint (75 days after fruit set). This more rapid increase in anthocyanin content was in line with the earlier ripening of the plums and subsequent earlier harvest that occurred in 2022. The plums were larger in size in 2022 and had a higher moisture content of 80% compared to 77% in 2021. The total anthocyanin content at harvest was 158 mg/100g FW in 2022, which was similar to the 2021 season where the harvest plums contained 154 mg/100g FW.

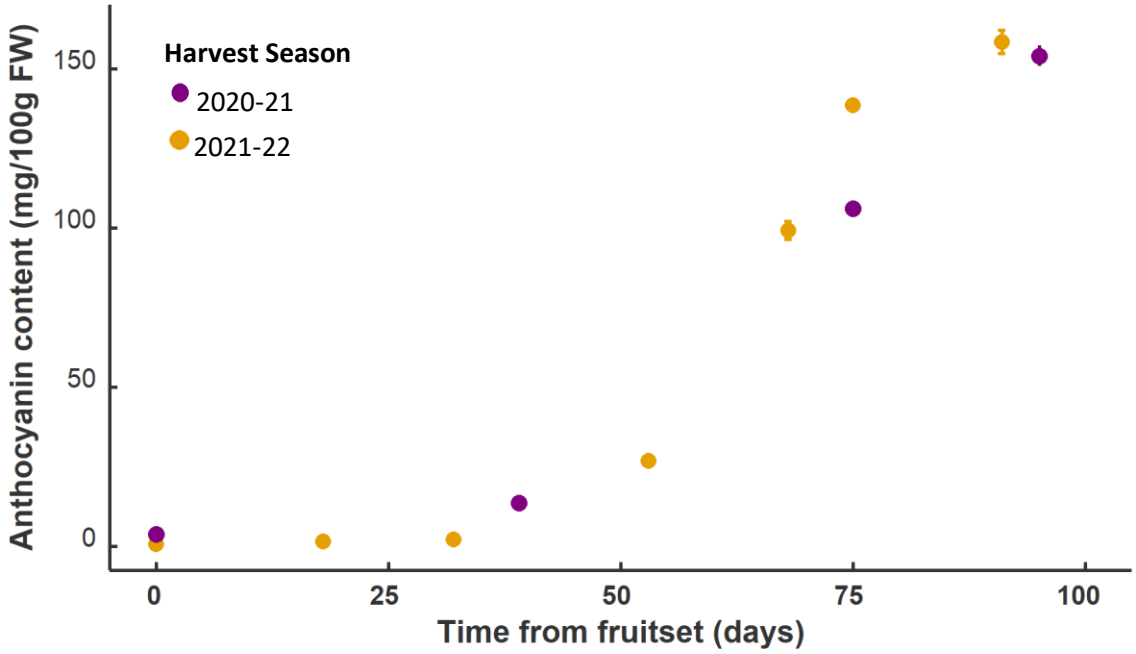

Figure 4. Queen Garnet plum anthocyanin content (mg/100 g fresh weight) tracked from fruit set to harvest. Each timepoint was tested in triplicate from a pureed plum batch produced from approximately 10 plums.

3.2. Comparison of Queen Garnet plums grown at different orchards

The two harvest collections from Warroo Orchard were compared to the four other QG plum growers (Figure 5-7). It was observed that there was considerable variance in anthocyanin content between the growers (68 - 158 mg/100g FW).

Figure 5. Comparison of the Queen Garnet plums at harvest from different growers.

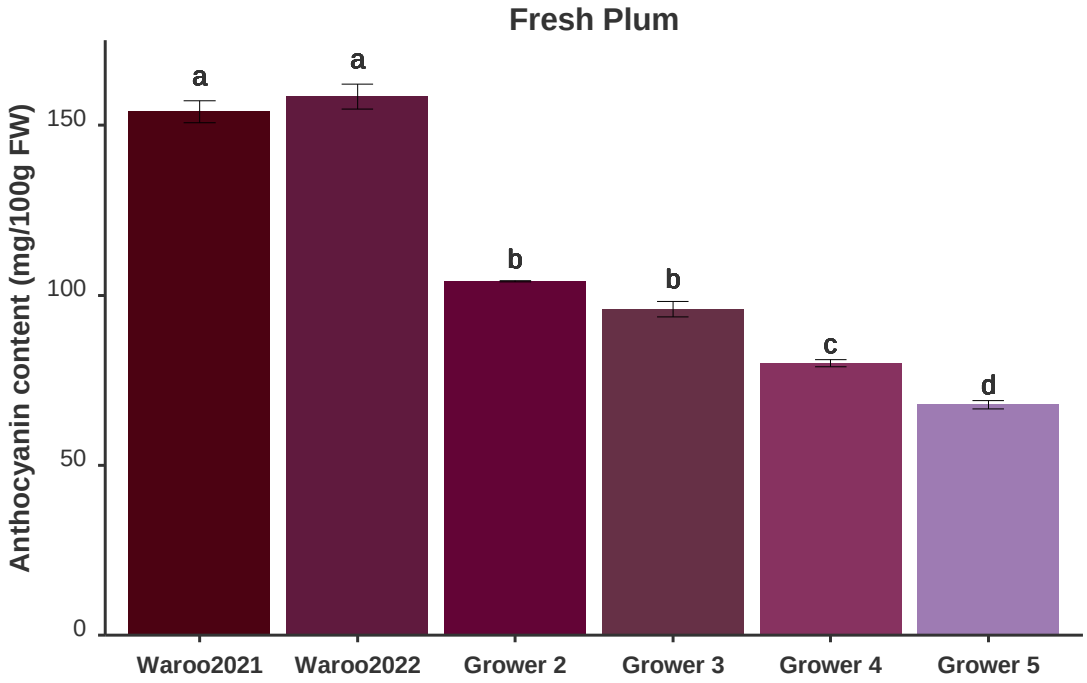

Figure 6. Total anthocyanin content of plums at harvest (fresh weight) from different growers. Values are presented as mean ± STD, n = 10-15. Means without a common letter differ, P < 0.05.

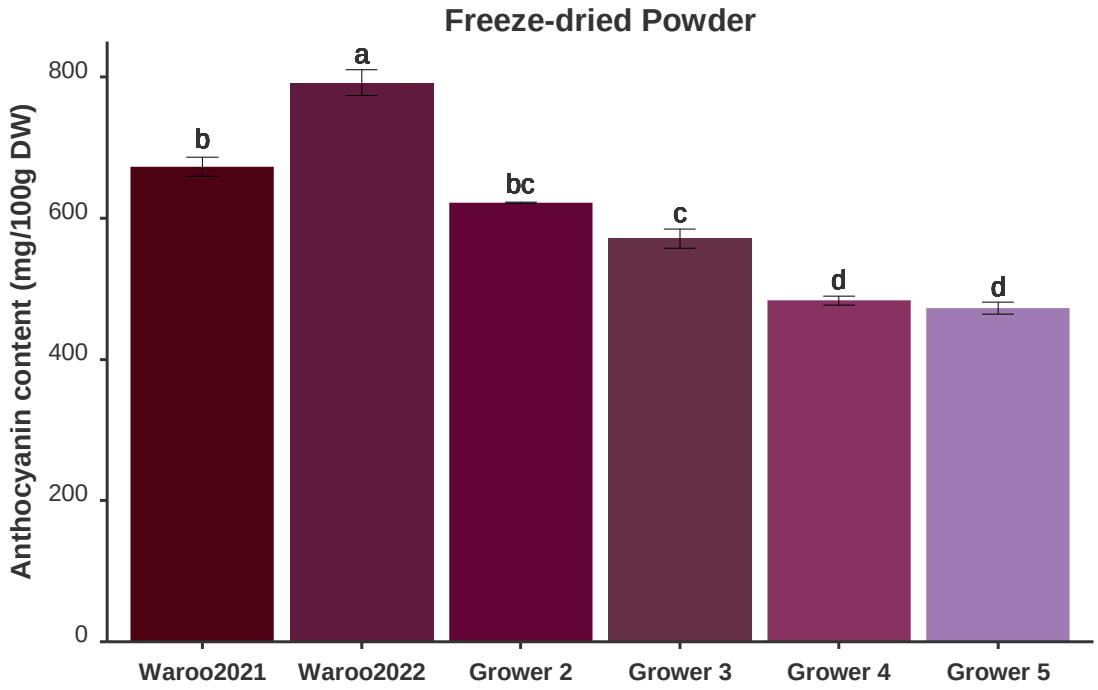

Figure 7. Total anthocyanin content of Queen Garnet plums at harvest after freeze-drying to remove all moisture from the samples. Values are presented as mean ± STD, n = 10-15. Means without a common letter differ, P < 0.05.

Another considerable difference between the QG plum growers was the moisture content of the harvest plum (Figure 8). As can be seen in Figure 9, there is a relatively strong relationship between decreasing moisture content and increasing anthocyanin content. At 53 days after fruit set there appears to be a sharp decrease in moisture content that precedes the increase in anthocyanin content (Figure 9).

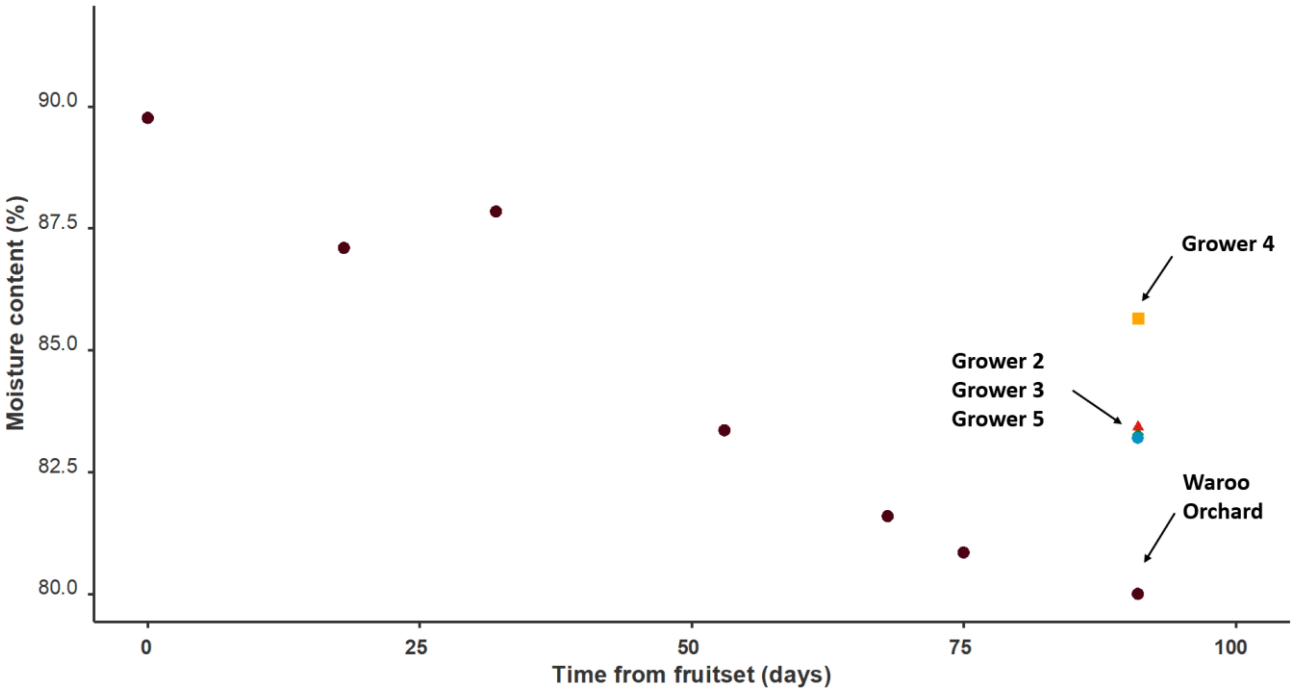

Figure 8. Moisture content of the harvest Queen Garnet plums from different growers plotted against the changing moisture content during ripening of plums at Warroo Orchard.

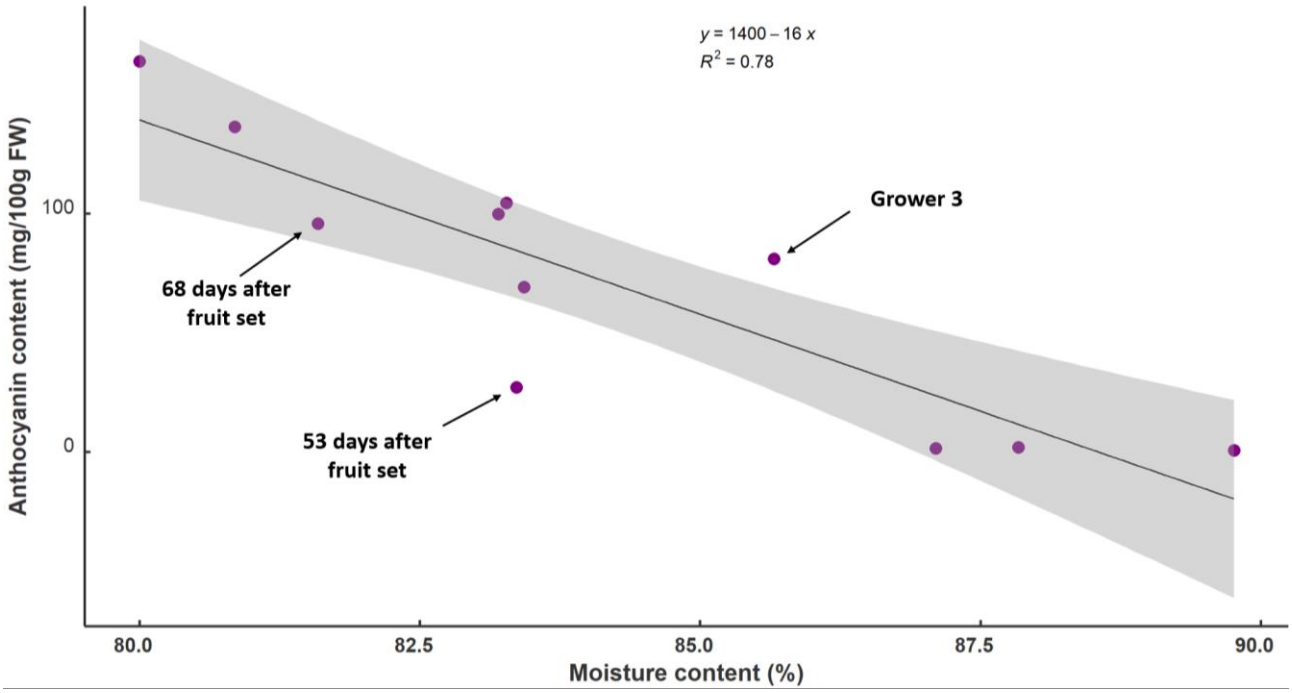

Figure 9. Regression analysis of the relationship between anthocyanin content and moisture content. The trendline is a linear regression line and the grey area in the graph represents the range in which the true regression line lies at a certain level of confidence (95% in the plot).

3.3. Comparison of Queen Garnet plums to other plum varieties

The anthocyanin content of QG plums was considerably higher than other plum varieties supplied by Favco and local fresh fruit and vegetable grocers (Figure 10 and Figure 11).

Figure 10: Other plum varieties supplied by Favco and local Toowoomba fresh fruit and vegetable grocers.

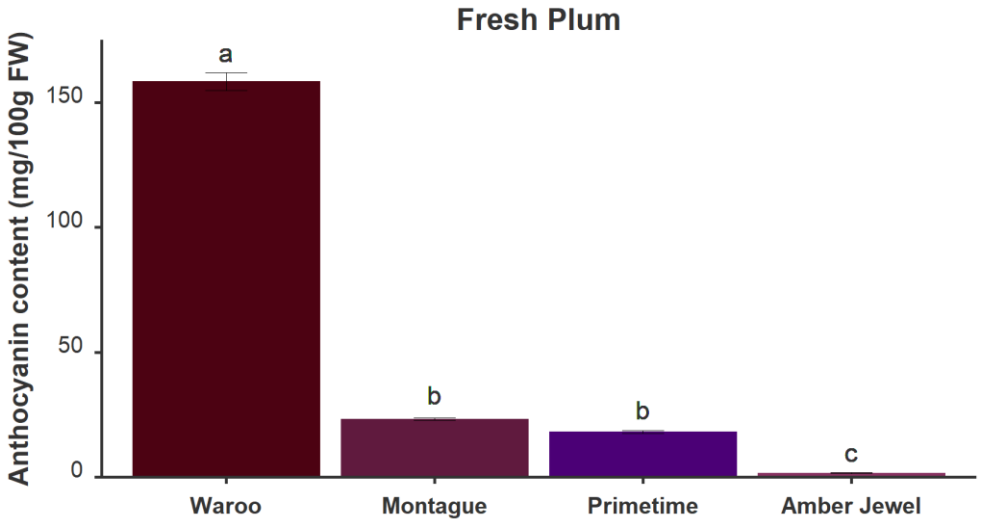

Figure 11: Anthocyanin content of Queen Garnet plum grown in Warroo Orchard 2021-22 compared to other plum varieties.

3.4. Storage trials

Soon after harvest, QG plums were either placed in cold storage (4°C) or pitted and frozen (-20°C) in preparation for tracking changes in anthocyanin content each month for the remaining project time (Figure 12). It can be observed in Figure 10 that in the 2021-22 season there was a sharp drop in anthocyanin content in the first 50 days after harvest, and this was then followed by an increase in anthocyanin content for freezer samples and a stabilisation of the plums in the fridge. The anthocyanin content had to be calculated as dry weight rather than fresh weight due to the effects of freezing on moisture content. By the end of the 140-day storage trial, the plums stored in the freezer retained close to harvest levels of anthocyanin content whereas the plums stored in the fridge had decreased by 124 mg anthocyanin/100g DW. The changes in anthocyanin content in the 2020-21 season were considerably different and showed a sharper decrease over time.

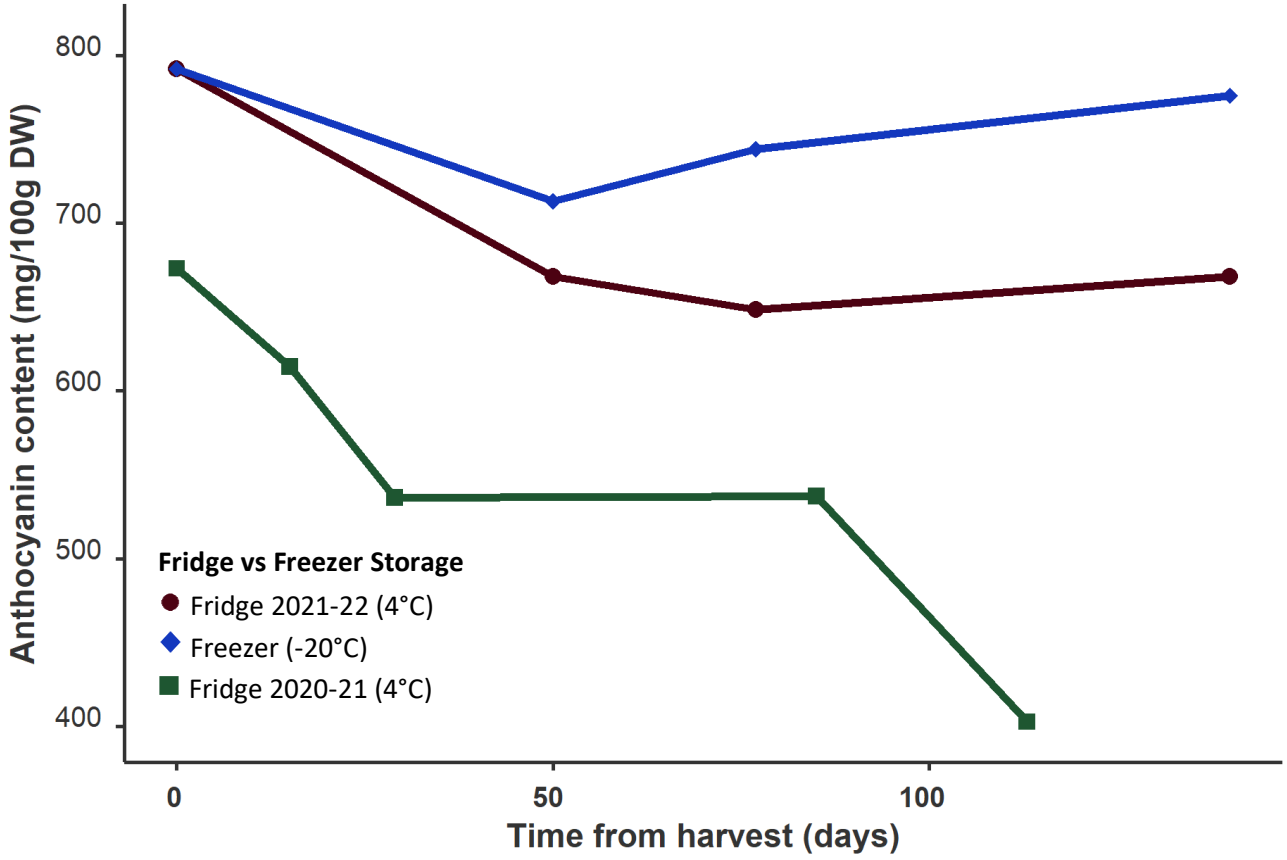

Figure 12. Changes in total anthocyanin content (dry weight) of plums stored in either the fridge (4°C) or freezer (-20°C) after harvest. This was for plums in the 2021-22 season from Warroo Orchard.

3.5. Alternative process changes to anthocyanin content

3.5.1 Plum blanching

Blanching the plums for 2 minutes increased anthocyanin content by 101 mg/100g DW (p = 0.005) (Figure 13). This experiment was repeated to compare the effects of blanching plum puree or sliced plum to the whole plum, and only blanching of the whole plum increased anthocyanin content (Figure 14).

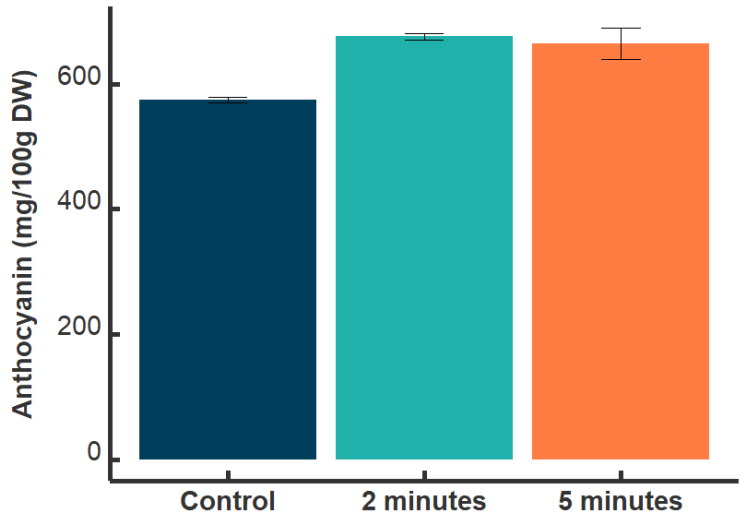

Figure 13. The effect of submerging Queen Garnet plums in a water bath heated to 85°C on anthocyanin content.

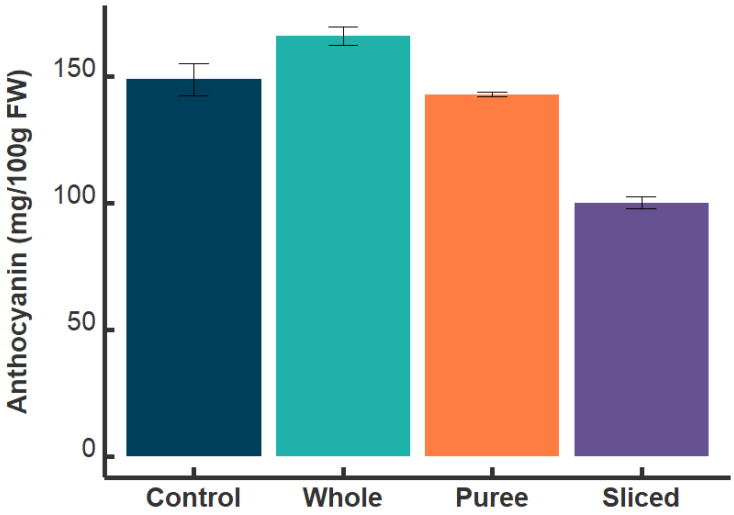

Figure 14. The effect of submerging Queen Garnet plums in a water bath heated to 85°C on anthocyanin content. Plums were either whole, pureed or thinly sliced prior to blanching.

3.5.2 Short-term storage condition changes

Plums were either placed individually in paper bags or loosely placed covered from light, and at the 0-, 2-, 5- and 7-day timepoints four plums were tested per variable (Figure 15). After two days of storage at room temperature enclosed within a paper bag, the anthocyanin content had increased by 128 mg/100g DW (P = 0.007). This increase remained significant after 5 days of storage at room temperature, and by the 7th day had decreased to be back in line with the control. There was no significant difference in anthocyanin content for the plums that were stored loose covered from light.

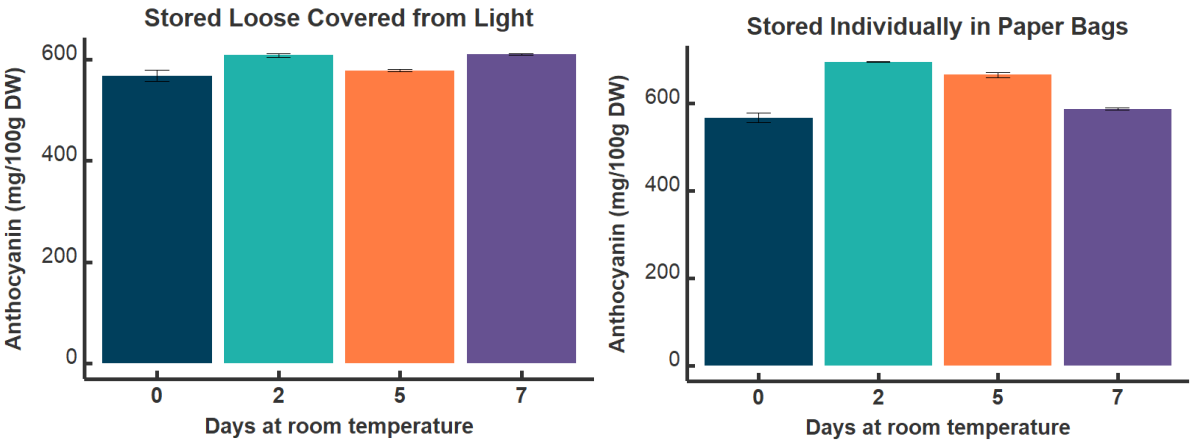

*Figure 15. The effect of moving plums from cold storage to room temperature on total anthocyanin content. The * denotes a significant difference compared to control ($p < 0.05$).*

3.5.3 Microwave pasteurisation of plums

After the first pasteurisation of plums using a microwave oven the anthocyanin content sharply decreased, and there was only a small decrease following the second pasteurisation on the same sample (Figure 16). It should be noted that the control used is the four-month storage sample, as this pasteurisation occurred at that timepoint.

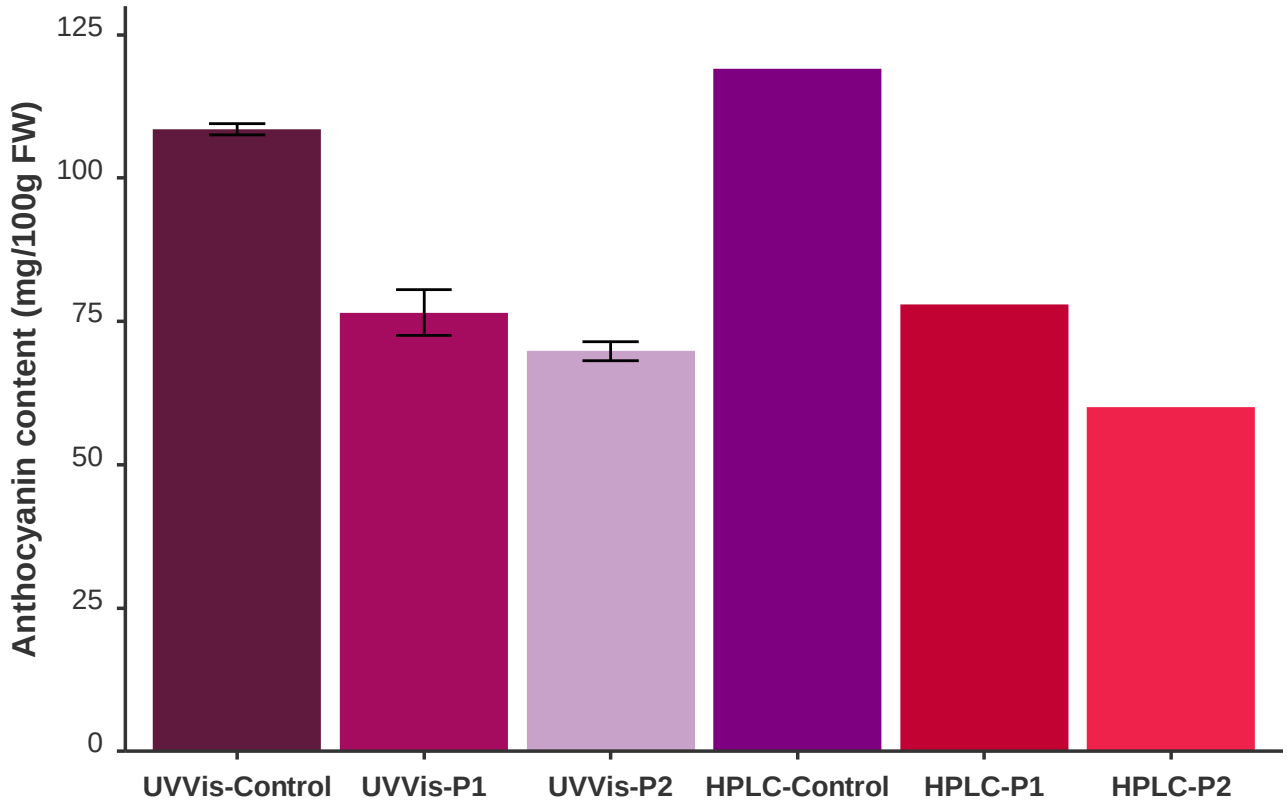

Figure 16. Comparison of pasteurised plums to control using the UV-Vis spectrophotometry and HPLC profile methods. P1; pasteurised once, P2; pasteurised twice. The same samples were tested for both methods.

3.6. HPLC analysis

The total anthocyanin content of the plums at harvest was 164 mg/100g fresh weight (FW), and this was similar to the result of 154 ± 5.6 mg/100g FW from the UV-Vis spectrometry testing done at USQ (Figure 17).

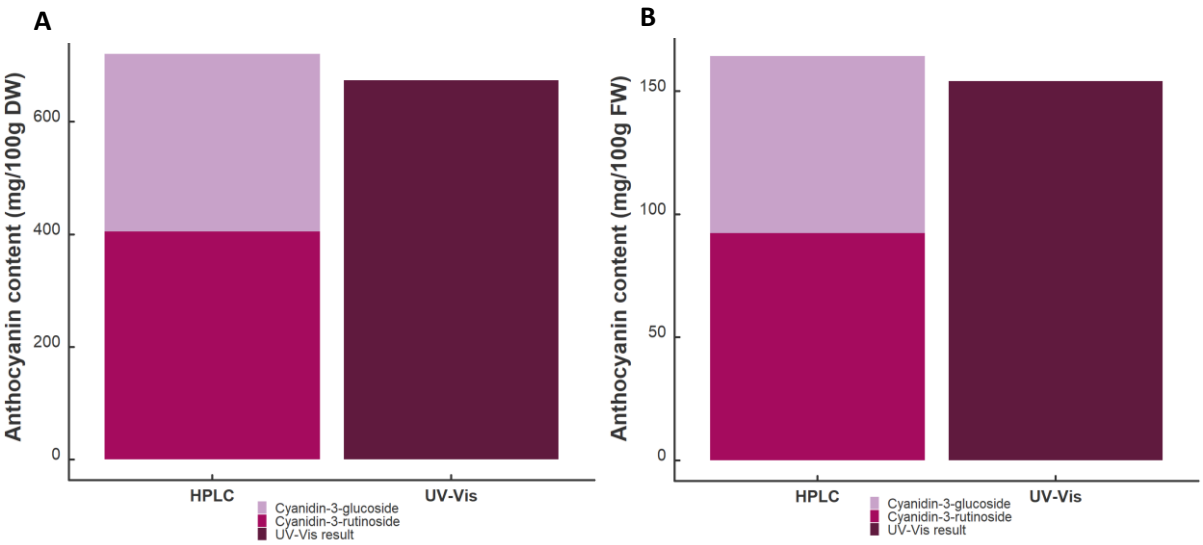

*Figure 17. Quantified anthocyanin/anthocyanidin in Queen Garnet plums at harvest. **A**. Quantity in freeze-dried powder (dry weight). **B**. Quantity calculated back to fresh weight of plum. UV-Vis is the technique using a UV-Vis spectrophotometry.*

Cyanidin-3-glucoside accounted for 71.8 mg/100g FW and cyanidin-3-rutinoside the other 92.3 mg/100g FW. The change in anthocyanin content during ripening and storage is shown in Figure 18.

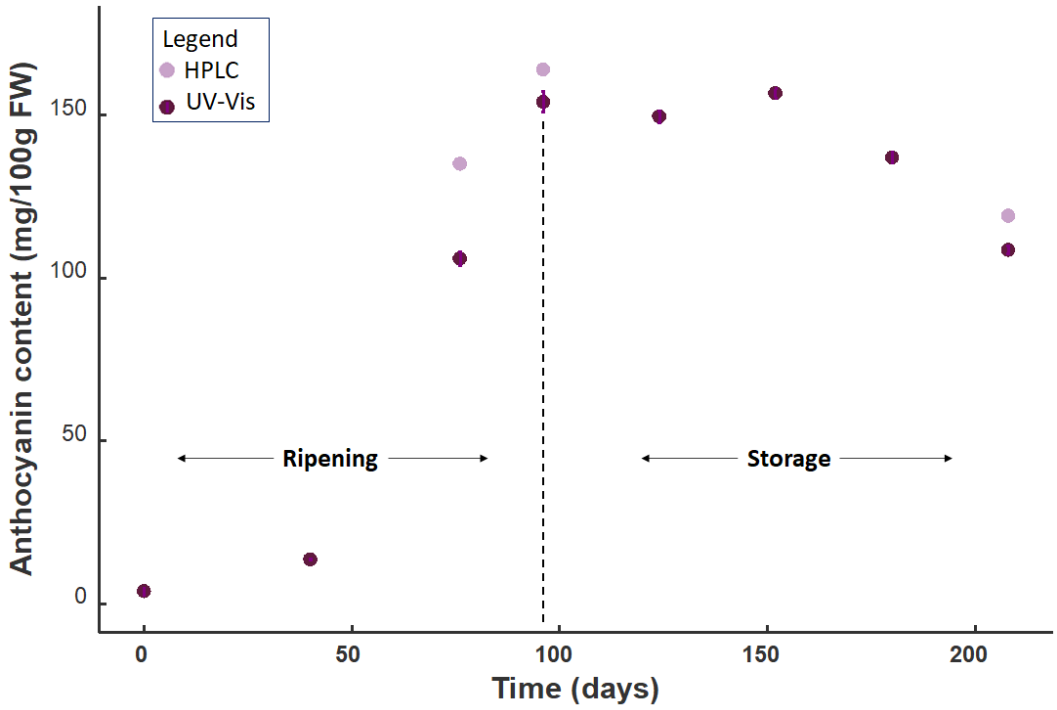

Figure 18. Change in anthocyanin content during ripening and storage (fresh weight) of Queen Garnet plums grown in Warroo Orchard 2020-21 season.

All the samples were prepared at the same concentration in the HPLC profiles, so the peak areas can be compared to indicate the relative abundance of the anthocyanins, phenolic acids, and flavonoids in the samples. After 4 months of storage and pasteurisation of the plum puree, the anthocyanin and flavonoid content was reduced compared to harvest (Figure 19 and 20). The anthocyanin content was too low to measure in the fruit set sample, and instead it was rich in procyanidin and epicatechin (proanthocyanidins). The fruit set sample had considerably higher phenolic content than the other samples, and this level reduced during ripening (Figure 21).

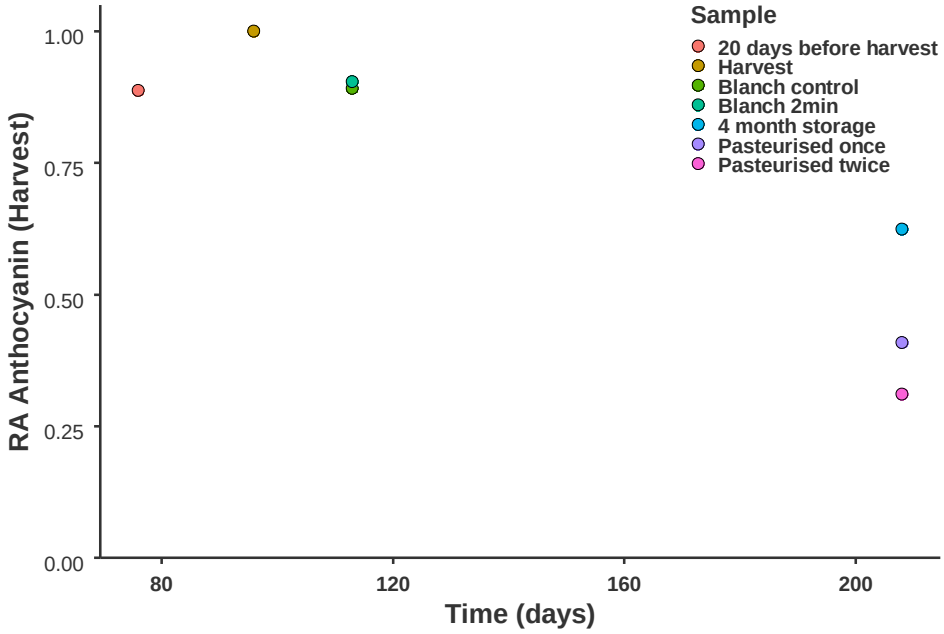

Figure 19. Relative abundance of total anthocyanin content calculated from peak area of HPLC profile analysis. Harvest was used as the maximum as it had the largest peak area.

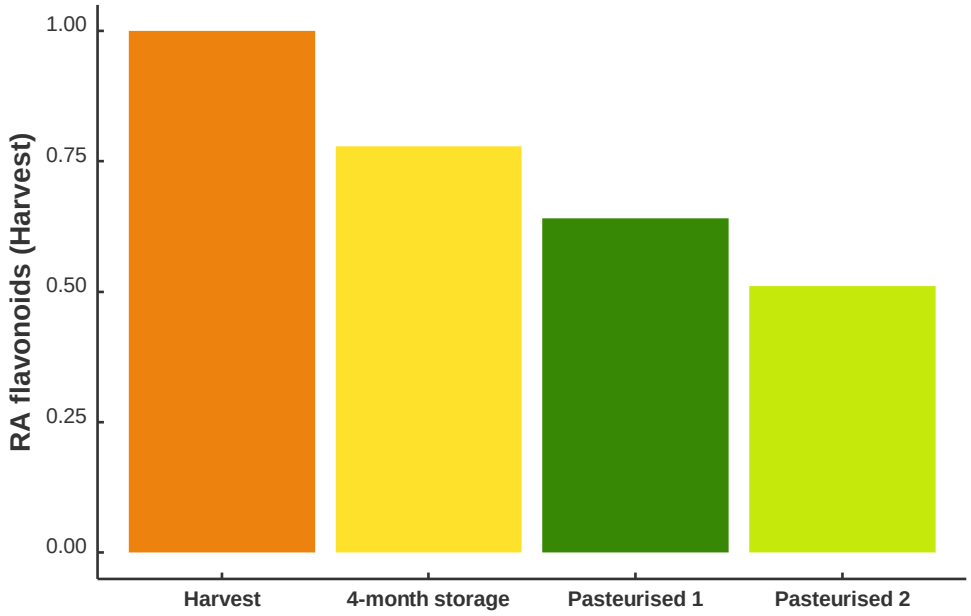

Figure 20. Relative abundance of total flavonoids calculated from total peak area of HPLC profile analysis. The harvest plum was used as the maximum.

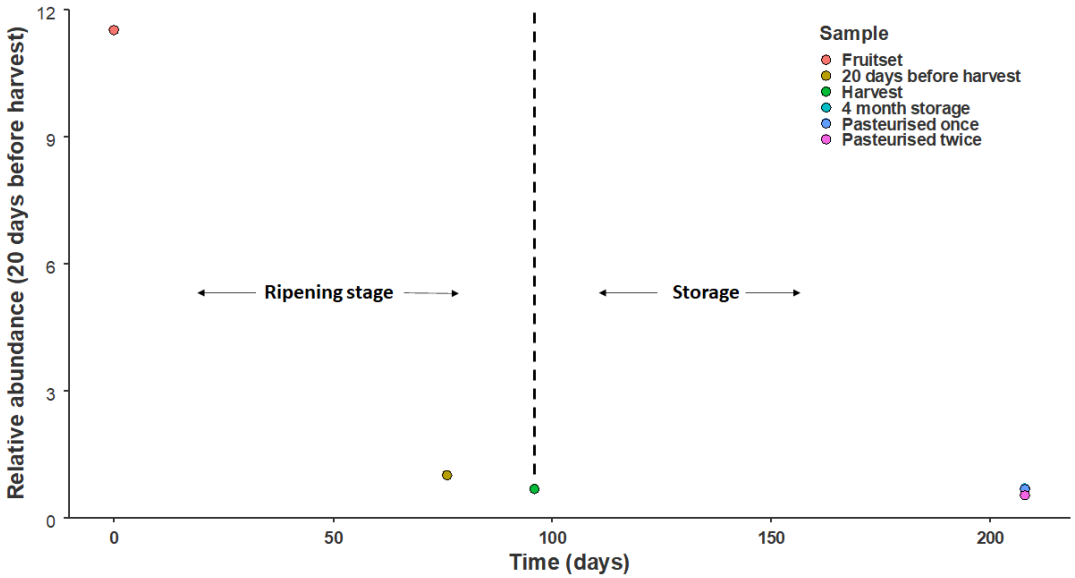

Figure 21. Relative abundance of total phenolics calculated from total peak area of HPLC profile analysis. The ripening plum at 20 days before harvest (12/01/21) was used as the maximum.

Carotenoids were quantified in the harvest plum samples as well as after 4 months of storage and pasteurisation, and it can be observed in Figure 22 that there was no difference between samples and total carotenoid content remained around 4 mg/100g FW even after long-term storage and pasteurisation.

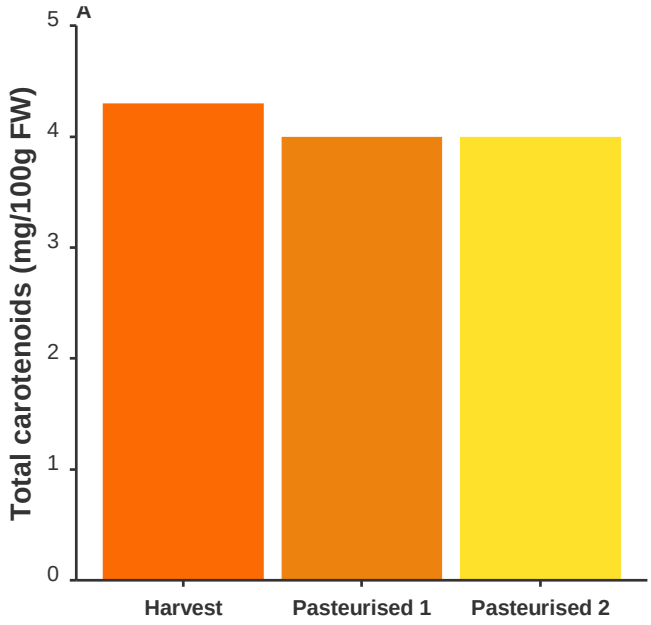

Figure 22. Quantified total carotenoids in QG plums at harvest and after 4 months of storage and pasteurisation.

3.7. Colourimeter correlation to anthocyanin content

Anthocyanin data was correlated to the recorded L*, a*, and b* values from the Nix Pro 2 colourimeter to determine which variable should be used for further calculations to potentially predict anthocyanin content in plums. Adding together the colour scans from the skin and flesh of a plum had the strongest correlation with anthocyanin content (Figure 23). The strongest correlation was -0.96, and this was between anthocyanin content and skin + flesh b*. This correlation was then explored further using regression modelling as shown in Figure 24. The R^2 value is used as an indicator of the goodness of fit, and in this graph, it means that 77% of the time the anthocyanin content can be correctly calculated from the skin + flesh b* value.

To help to account for the variation in skin and flesh colour of the plums, the method for scanning the plums was refined by repeating the scan 3 times per plum/sample (total of 9 scans) to calculate an average. A further improvement was to change from using the anthocyanin content per dry weight values to anthocyanin content per fresh weight, as this takes into account the differences in moisture content and is more accurate as fresh plums were scanned using the colourimeter. When only these data points were included in the analysis, the R^2 value is elevated to 0.94, and this means that 94% of the time the anthocyanin content can be correctly predicted, and the error is within the grey band region (Figure 24).

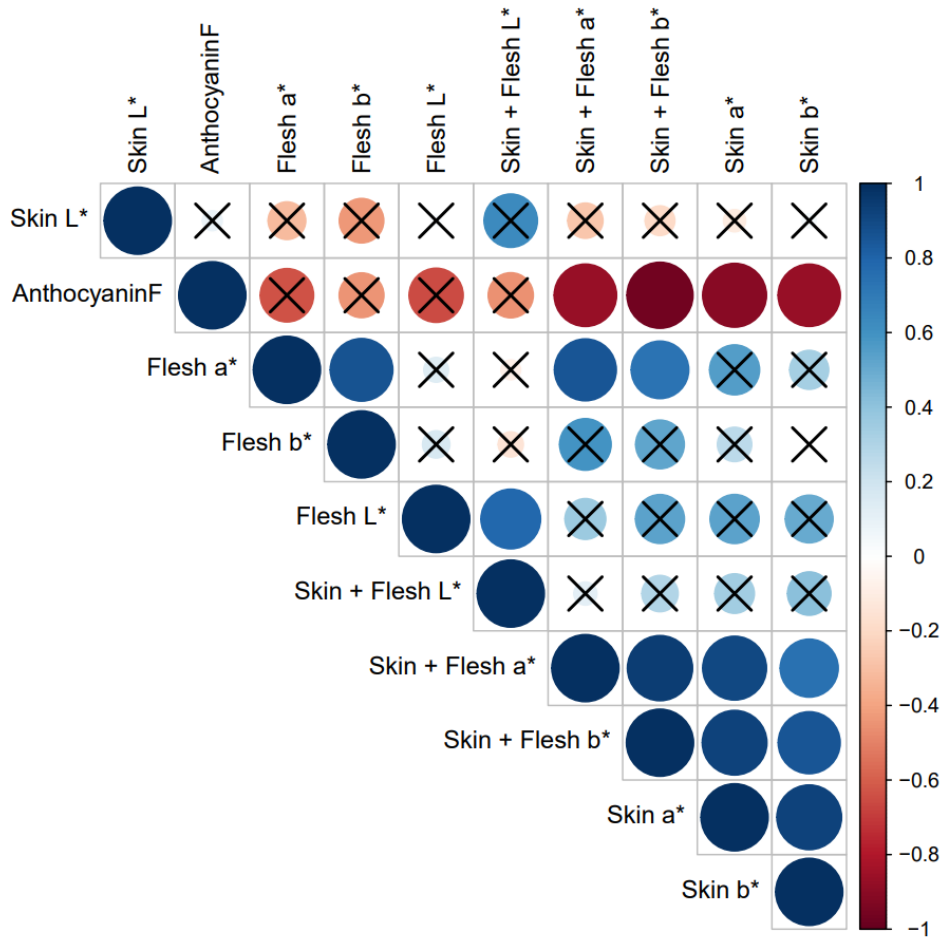

Figure 23. Correlation matrix to show the relationship between variables. Blue shows a positive correlation and red a negative correlation. A larger size dot and higher intensity of colour indicates a stronger correlation between the variables. Skin and Flesh L, a* and b* values are those recorded as explained in the methods section.*

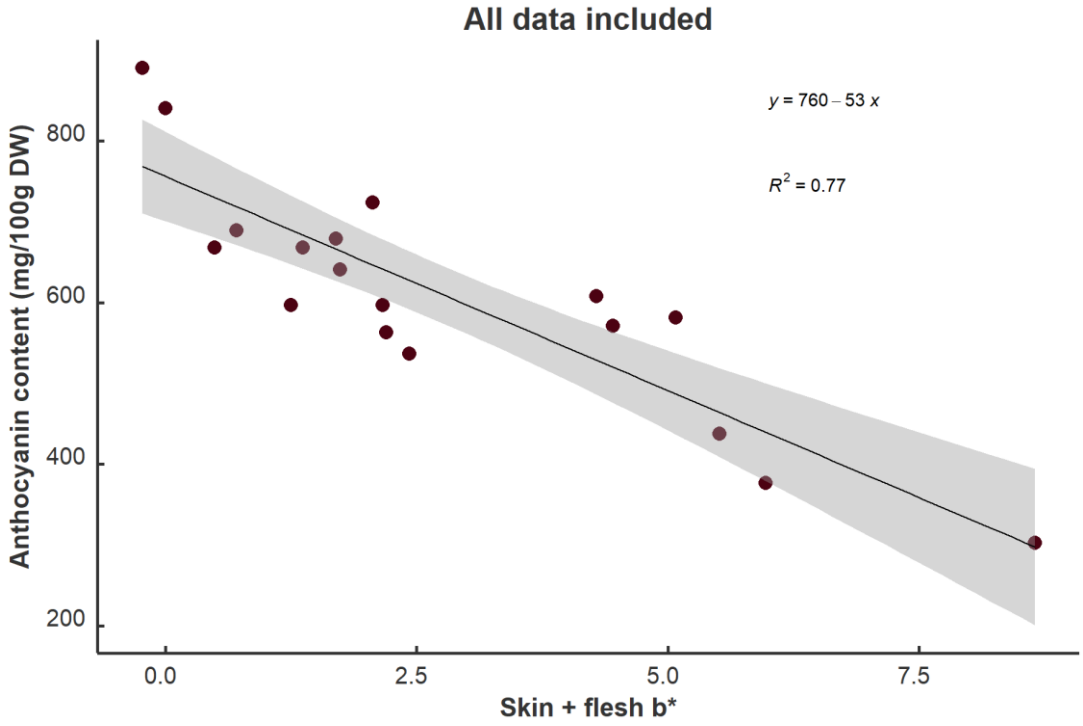

Figure 24. Regression analysis of the relationship between anthocyanin content and skin + flesh b value. Anthocyanin content is the dependent variable, and skin + flesh b* value is the independent variable. The*

trendline is a linear regression line and the grey area in the graph represents the range in which the true regression line lies at a certain level of confidence (95% in the plot).

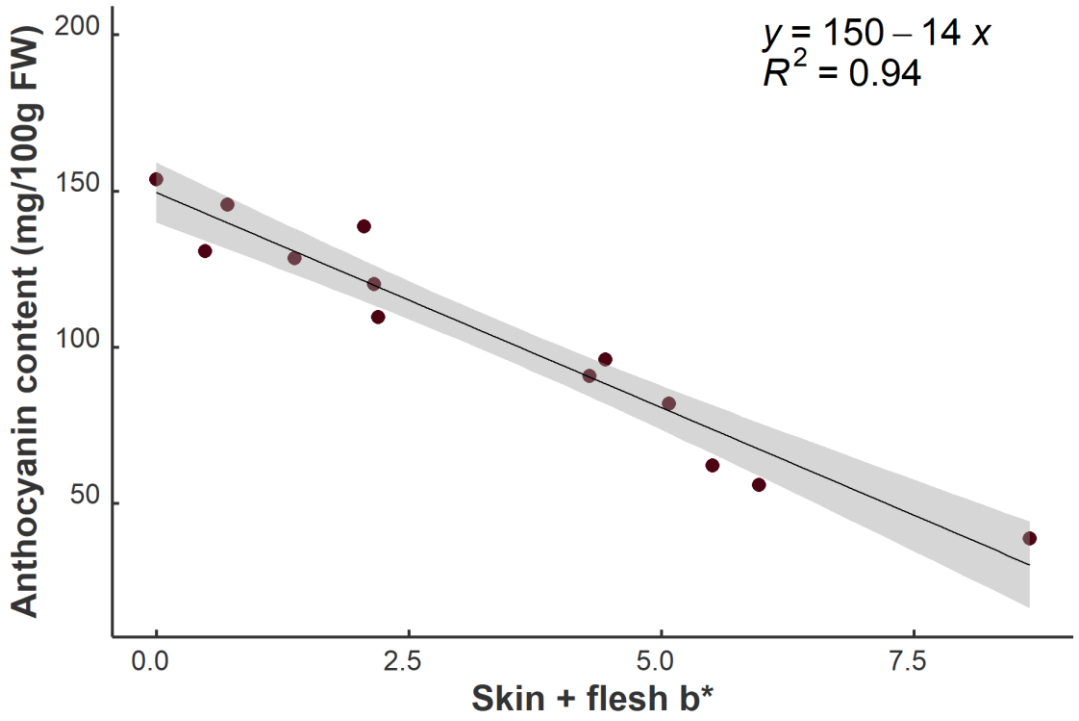

Figure 25. Regression analysis of the relationship between anthocyanin content and skin + flesh b value using only colour scans where the process was repeated 3 times to calculate an average for each plum. Anthocyanin content has been changed to a per fresh weight amount, rather than dry weight.*

4. DISCUSSION

For Nutrafruit to be able to produce a high-value product containing large amounts of anthocyanin, it was necessary to track how anthocyanin content changes in the plum during ripening, storage, and processing. This information is needed to be able to document anthocyanin content in consumer products and to potentially boost anthocyanin content through optimising harvest time, product processing, formulation, and storage practices. Several sampling points in the 2020-21 and 2021-22 seasons at Warroo Orchard were obtained to track the development and retention of anthocyanins. The anthocyanin content of the plums at harvest across the two seasons were highly similar (154 mg/100g FW in 2021 and 158 mg/100g FW in 2022), despite the earlier harvest timepoint and significantly increased rainfall that occurred in 2021-22. This finding is similar to a past study by Netzel et al. (2012), who reported the anthocyanin content of QG plum to be slightly above 150 mg/100g FW (1). This similarity is highly encouraging, as it suggests that anthocyanin content of the plums is consistently high and there is unlikely to be major fluctuations in average plum anthocyanin content. However, there remains considerable variation in individual plums grown in the same location at the same time, as can be noted in Figure 5 where testing of plums darker in colour reported an anthocyanin content of 194mg/100g FW.

The QG plums grown at Warroo Orchard over the two seasons were consistent, however they varied considerably in colour and anthocyanin content (68 - 158 mg/100g FW) to other QG plums grown at four other locations across Australia. This difference in plum quality is potentially detrimental to the Nutrafruit brand and could impact product consistency and quality. Plums of lower visual appeal and anthocyanin content are more likely to be unsaleable and therefore end up as food waste. A comparison of the growing practices and harvest timing between the different growers is needed so that the success of Warroo Orchard can be translated to the other growers to improve their anthocyanin content. Nutrafruit is

planning to host a workshop with its growers to review findings from the 2021-22 season and apply these teachings to the 2022-23 season.

The next stage in quality control of the QG plums, is retaining or increasing the anthocyanin content as well as phenolic, carotenoid and flavonoid content during storage before the plums can be either sold or processed. It can be observed in Figure 12 that there was a considerable difference in the changes to anthocyanin content in the 2020-21 and 2021-22 season when plums were stored at 4°C (in the same fridge both times). There was no obvious explanation for this variance, however, it was noted that there were some plums in the 2021-21 season that developed surface mould during the storage period. Whilst these plums were not tested, they were stored together with all samples so they may have had an effect on the results for that season. In the 2021-22 season, it can be observed that there was a sharp decrease in anthocyanin content in the first 50 days of storage, and this was followed by a stabilisation for samples stored in the fridge and a gradual increase for plums stored in the freezer (-20°C). There was limited explanation for this increase in the literature, although it has been previously observed in blackberry long-term storage trials (9). The authors of that study theorised that it may have been explained by the improved extraction of anthocyanins from the blackberries due to ice crystal damage of cell structures and thus easier release of anthocyanin. Regardless, it is recommended that QG plums be stored at -20°C rather than 4°C as this eliminated the risk of moulding occurring and also resulted in improved retention of anthocyanins. Furthermore, total flavonoids were also decreased by long-term storage of QG plums at 4°C and this may be able to be minimised by storage at -20°C.

Another avenue that has been explored as an extension to the project, is whether anthocyanin content can be increased or retained in plums by altering temperature or other variables. This could be an alternative solution to the loss of anthocyanin during storage if processing is unable to start immediately following harvest. This was first attempted by blanching the plums by submerging them in a water bath heated to 85°C for 2 or 5 minutes followed by submerging them in ice bath to cool rapidly. It was found that blanching the plums for 2 minutes increased anthocyanin content by 101 mg/100g DW ($p = 0.005$). This finding is supported by a previous study by Jiang et al. (2020), who found that steam blanching of whole blueberries for two minutes at 100°C increased anthocyanin content by deactivating the polyphenol oxidase enzyme (10). Blanching only induced an anthocyanin increase in whole plums and had no effect on pureed plum or thinly sliced plum. It has been shown that blanching blueberries before processing into juice increased the recovery of anthocyanin pigments and greatly increased radical-scavenging activity of the juice (11).

The effects of moving plums from cold storage to room temperature for 2 – 7 days was also explored based on the findings from a previous study testing Friar plums (8). After two days of storage at room temperature enclosed within a paper bag, the anthocyanin content had increased by 128 mg/100g DW. This increase remained significant after 5 days of storage at room temperature, and by the 7th day had decreased to be back in line with the control. In the previous study testing Friar plums, ethylene production peaked at the 5th day after removal from cold storage to room temperature storage and it has been previously reported that ethylene release increases anthocyanin synthesis (12). However, the result in the current study is only a preliminary finding, as temperature was controlled through air-conditioning and this needs to be repeated in an environmentally controlled chamber to confirm that temperature and humidity are being maintained over the full duration.

One of the main products Nutrafruit produced and sold from QG plum prior to this project was the QG plum nectar. A major goal of this project was to determine whether anthocyanin content was being retained during processing to produce this nectar, and also to investigate alternate processes that could lead to a higher-value product or a longer shelf-life to reduce product wastage. Early in project meetings,

the USQ team suggested ingredients and alternate products to diversify the Nutrafruit product portfolio, however at the time Nutrafruit were focused on enhancing their existing products. Unfortunately, due to the effects of COVID-19, Nutrafruit did not process the QG plums in 2021 and therefore the effects of thermal processing of the plums had to be replicated at USQ using an alternative microwave pasteurisation. It was found that this pasteurisation drastically reduced anthocyanin content, as is supported by previous studies showing the effect of pasteurisation on blueberries (13) and pomegranate (14). This information was reported to Nutrafruit, who had also been going through an evolutionary step change in their future business model thinking during 2021, probably necessitated by supply chain stressors of COVID 19. In response they have pivoted to a new range of products (QG essence and extract) using alternative processes to their initial nectar pasteurisation and freeze dry processes and have already commenced commercialisation with a contract manufacturer. The potential for use of this QG extract in a chronic wound healing study at USQ is currently being explored, and Nutrafruit will be advised of the outcome of this project.

An interesting finding of this project was that the total carotenoids in the QG plums at harvest was 4.3 mg/100g FW. This was higher than previously reported for QG plum (7), where peel carotenoid content was approximately 2.5mg/100g FW and flesh content was 0.5 mg/100g FW. For reference, a carrot contains roughly 8 mg/100g FW, a sweet potato contains 7.4 mg/100g FW, and a butternut pumpkin contains 3.1 mg/100g FW of total β-carotene equivalents (including β-carotene) according to the taken from Australian Food Nutrient Database. Another paper reported that Cherry plum contained 1.96 mg/100 g, Kirks plum 1.95 mg/100 g, Italian plum 1.90 mg/100 g and Graf Althans plum 1.24 mg/100 g of total carotenoids (15). This highlights that QG plum contains not only higher levels of anthocyanin compared to other plums, but also contains greater carotenoid content, and this should be explored further with the possibility of marketing this as another benefit of consuming QG plum.

To assist growers and producers with inline and orchard measurement of anthocyanin content, an affordable colourimeter was trialled to track changes in the colour of plum skin and flesh. The purpose of this scoping work was to identify whether there is a relationship between the colour of the plum skin or flesh and total anthocyanin content. The colourimeter trialled in this project was durable and affordable ($500), easy to use as it is linked to a phone app and is thus suitable for wider distribution. The colourimeter used was called Nix Pro 2 (https://www.nixsensor.com/nix-pro/). Anthocyanin data was correlated to the recorded L*, a*, and b* values from the Nix Pro 2 colourimeter to determine which variable should be used for further calculations to potentially predict anthocyanin content in plums. It was found that the strongest correlation was -0.90, and this was between anthocyanin content and colour measures of the skin and flesh added together for the b* value (more information in Figure 1). This correlation was negative, which means that as the b* value decreased (plums became bluer), the anthocyanin content increased. This has implications for use as a rapid quality control tool to track anthocyanin content across the fruit supply chain from fruit onset to harvest through to processing. It is planned that these colour calibration results will be published and potentially presented at the International Union of Food Science & Technology conference.

5. IMPACT AND ONGOING MONITORING

1. Nutrafruit have begun commercialisation of their QG essence and extract, which are a higher-value product with a longer shelf-life than QG plum nectar. This longer shelf-life will help to protect Nutrafruit against future supply chain disruptions such as occurred during COVID-19. The QG essence and extract do not require pasteurisation or heat to produce and therefore have greater retention of anthocyanin content.

2. Investigation into the differences in QG plum quality and anthocyanin content between different growers has highlighted that improvements need to be made to increase consistency and average plum quality. Improving this will reduce the harvest of QG plums with suboptimal aesthetic and eating quality, which end up being wasted due to being unsaleable. Tools and training will be provided to Nutrafruit by USQ to assist with Nutrafruit's planned workshop for growers to improve in this area.
3. An affordable colourimeter has been successfully used to predict anthocyanin content of QG plums as 94% of the variance in anthocyanin content is explained by the QG plums becoming bluer in colour (b* value becoming more negative). This has implications for use as a rapid quality control tool to track anthocyanin content across the fruit supply chain from fruit onset to harvest through to processing.
4. UniSQ researchers estimate that 5% of plums previously lost as waste can now be retained and or upcycled using the colourimetric QC method and commercialisation of the higher-value, longer shelf-life QG extract and essence - this equates to an estimated 300 Mt per annum saved from food loss

6. CONCLUSIONS AND RECOMMENDATIONS

Conclusions

QG plum anthocyanin content has been shown to be consistently high across multiple growing seasons, however variations in harvesting time and grower practices can result in this anthocyanin content being more than halved in the final harvest plum. Both long-term storage at 4°C and pasteurisation reduce anthocyanin and total flavonoid content, and this affects quality of product. Nutrafruit have pivoted to a new range of products (QG essence and extract).

Recommendations

Use of the findings from this research to investigate the relationship between anthocyanin content and the grower practices used in each farm to improve the quality and consistency of QG plums. Tools such as the affordable colourimeter tested in this project will assist growers in being able to conduct quality control and will potentially help in preventing early harvest and consequently unsaleable product. Total carotenoid content was found to be 2-fold higher in QG plum compared to other plum varieties, and this should be explored further with the possibility of marketing this as another benefit of consuming QG plum.

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