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Proceeding Paper

Within-Canopy Variation in the Ascorbic Acid Content of Tuckeroo (*Cupaniopsis anacardioides*) Fruits †

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Abstract: Although fruit canopy position is known to affect phytochemical composition in a number of commercial crops, there is limited information on its impact on the nutritional quality of native Australian fruit. This study is the first to quantify ascorbic acid in tuckeroo (*Cupaniopsis anacardioides*) fruit and investigate the impact of canopy position in this species. High levels of ascorbic acid were found in the skin (mean of 423 ± 61 mg/100 g on a dry weight basis) and arils (60.0 ± 18.8 mg/100 g), but not in the seeds (mean of 15.6 ± 4.3 mg/100 g). The tree, side and height all significantly affected fruit mass, with larger fruit located on the northern bottom side of the canopy. Skin ascorbic acid content also varied significantly with the tree (responsible for 50.8% of the total variance observed in vitamin C content) and canopy height (accounting for 0.9% of the total variance), with a marginal impact of the side (compass direction). Fruit from the top of the southern side of the tree typically had the highest ascorbic acid content. This inter-tree and within-canopy variation in the nutritional content of *C. anacardioides* fruit may have implications for sampling protocols and potential harvesters of this fruit.

Keywords: vitamin C; native Australian plants; indigenous fruit

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1. Introduction

The tuckeroo (*Cupaniopsis anacardioides* (A.Rich.) Radlk.) is an endemic native Australian tree producing smooth-skinned, yellow-to-orange fruit. It is commonly used in amenity horticulture plantings across subtropical Australia, such as in roadside parks [1,2]. When mature, the capsule-like fruits open upon drying to reveal three black seeds covered with a bright red aril. Some authors indicate that the arils of the ripe fruit were eaten by indigenous Australians [3]; however, the results of basic chemical analysis (total phenolics, flavonoids and proanthocyanidins) on the fruit have only recently been reported [4–6]. The species is a prolific fruiter, with up to 5000 fruit estimated to be produced by single mature tree [7]. Given the significant unexplored potential of native Australian plants from both a culinary [8,9] and therapeutic perspective [10], further chemical investigation of *C. anacardioides* is warranted to elucidate potential nutritional or pharmaceutical uses of this species [4]. Additionally, it is important to understand the impact of within-canopy location on the levels of nutritional/therapeutic compounds found in individual tuckeroo fruit. This could have significant implications for the choice of sampling protocols used by future researchers investigating this fruit, as well as for the potential commercial harvest of the fruit for nutritional or pharmaceutical purposes. Our aim was to add to the existing knowledge of the phytochemical composition of tuckeroo fruit through profiling its ascorbic acid (vitamin C) content in different fruit portions, with a particular focus on determining the impact of canopy position on ascorbic acid content.

2. Materials and Methods

2.1. Sample Collection and Preparation

The tuckeroo fruit samples were collected from North Rockhampton, Central Queensland (23°17′25″ S, 150°31′5″ E) during late spring (27 November 2020). Four trees from a small area (approx. 100 × 100 m) were randomly selected for inclusion in this study. From each tree, approximately 100–200 g of fruit was sampled from each of four canopy locations (the top and bottom, on both the north and south sides). Fruit mass, length and width were recorded for 10 randomly selected fruits from each position. Fruit volume was calculated by approximating the shape of each fruit as an oblate spheroid and using the corresponding volume formula:

$$V = 4/3 \times \pi \times b^2 \times c$$

where b = major axis length and c = minor axis length.

For each sample, approximately 10 fruit were separated into the skin (including the flesh), arils and seeds, with each portion diced finely. From this homogenised sample, duplicate samples were taken for ascorbic acid extractions. Moisture content was determined from the mass loss upon freeze-drying subsamples of each portion (−50 °C; 100 mT).

2.2. Reagents and Extraction Protocols

Metaphosphoric acid was obtained from Chem-Supply (Gillman, SA, Australia). All other chemicals and reagents were sourced from Sigma-Aldrich Australia (Castle Hill, NSW, Australia). All reagents were of analytical grade or higher. Ascorbic acid was extracted in duplicate as previously reported [11], using a mass of ~1 g of fresh material and 14 mL of 3% metaphosphoric acid, sonicated for 20 min. The supernatants were vacuum filtered (0.45 µm) and syringe-filtered (0.45 µm PTFE).

2.3. HPLC Analysis of Ascorbic Acid

Ascorbic acid was quantified using an Agilent 1100 HPLC system, as previously reported [11]. Ascorbic acid was identified by its retention time, ultraviolet spectral characteristics and spiking samples with an authentic ascorbic acid standard. Quantitation of ascorbic acid in the fresh samples was undertaken using an external standard calibration in the linear range of 10–120 mg L^{-1} (R^2 = 0.999). The ascorbic acid content of the samples was expressed in mg 100 g^{-1} on a dry weight (freeze-dried) basis.

2.4. Data Analysis

Statistical testing was performed in SPSS (v26); graphs were created in GraphPad Prism (v8). Where applicable, results are presented as mean ±1 standard deviation. All results are expressed in terms of dry weight unless otherwise specified.

3. Results and Discussion

3.1. Physical Characteristics

The average of mass across all tuckeroo fruit was 3.4 ± 0.9 g, with average dimensions of 19 ± 3 mm (length) × 17 ± 2 mm (width) (n = 160 for all). The average correlation between length and width (r = 0.57) was slightly higher than that reported for tuckeroo fruit from Townsville (0.3–0.39) [7]. Fruit mass was moderately correlated with length (r = 0.67) and strongly correlated with width (r = 0.87) and volume (r = 0.89) (Figure 1).

The inter-tree variation in mass was responsible for 83.6% of the total variance observed in fruit mass, which was considerably more than the intra-tree mass variation. The canopy side explained 6.4% of the total variance, while the height explained only 0.8% of the variance. Compared to fruit from the top north side (n = 10 for each position), the fruit on the top south side was consistently heavier (Figure 2). This is likely due to the different within-canopy temperatures and cumulative light availability over the growing season, resulting in differing fruit maturity stages in these canopy positions.

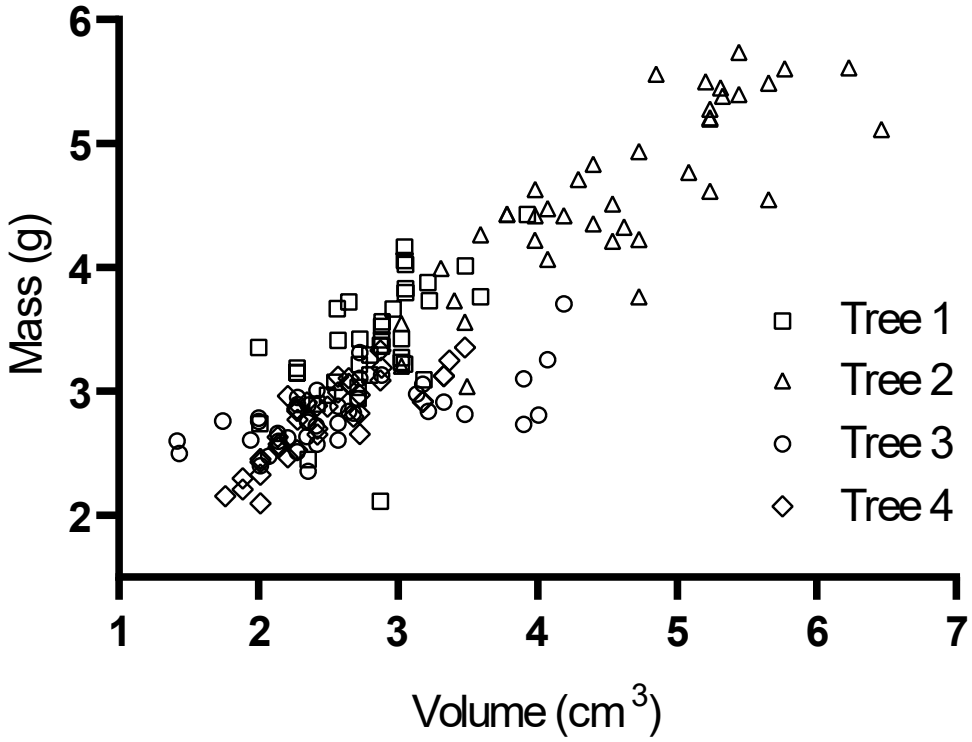

Figure 1. Scatterplot showing the relationship between volume and mass of the tuckeroo fruit.

As the masses were approximately normally distributed and logarithmic transformation did not improve the normality of the data, a factorial analysis of variance (ANOVA) was performed, with the tree, side (north or south) and height as the three factors. This demonstrated that all three variables (tree, side and height) significantly affected the fruit mass ($p < 0.01$ for all), with a significant interaction between side/height ($p < 0.001$), side/tree ($p < 0.001$) and side/tree/height ($p < 0.01$). Based on the estimated marginal means of the regression model, the fruit mass was consistently higher on the southern side compared to the northern side. The effect of height on fruit mass was negligible for fruit sourced from the southern side of the trees, but for the northern side of the trees, fruit from the bottom was heavier than fruit from the top. Similarly, the compass direction had no effect on fruit length for fruit from the top of the tree, but for fruit from the bottom of the tree, that from the southern side was longer. Fruit from the southern side was wider than fruit from both the bottom and top of the tree.

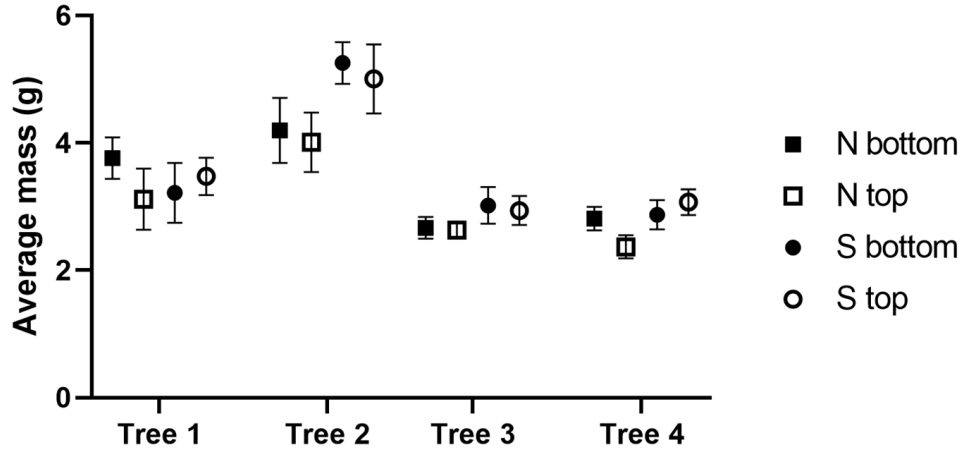

Figure 2. Barplot of fruit mass from each canopy position from each tree.

In contrast to Hawkeswood [7], who found an average of 2.07 seeds/fruit for tuckeroo fruit from Townsville, nearly all of the fruit (>95%) in the present study contained three seeds, with abortion of ovules occurring in only a handful of the ~400 fruit examined. Some level of insect damage was observed in ~10% of the fruit, primarily affecting the arils and seeds. The insect species responsible for the damage in this study was not identified; however, several lepidopteran species have previously been recorded from the fruits of *C. anacardioides*, including *Deudorix diovis* and *Cryptophlebia ombrodelta* [3].

In the fruit from the present study, the skin accounted for an average of 71.2 ± 3.3% of the total fruit mass, with seeds comprising a further 19.1 ± 4.8% (Table 1). Arils made up the remaining 9.7 ± 3.1% of the mass. These results were quite similar to those previously reported by Pham, et al. [4], although the seeds constituted slightly more weight in this study and the skin slightly less.

Table 1. Proportion of mass and ascorbic acid contents from different portions of the fruit (skin, seeds and arils). Results given as mean ±1 standard deviation on a fresh weight basis (n = 4 trees for each).

Location	Proportion of Mass (% FW)			Ascorbic Acid Content (mg/100 g DW)		
	Skin (%)	Seeds (%)	Arils (%)	Skin (%)	Seeds (%)	Arils (%)
N bottom	70.6 ± 3.8	19.8 ± 4.8	9.6 ± 2.8	426 ± 77	15.7 ± 2.9	67.8 ± 16.8
N top	70.9 ± 4.2	20.1 ± 5.5	9.1 ± 2.5	410 ± 66	18.2 ± 4.2	64.9 ± 16.7
S bottom	71.8 ± 3.9	19.0 ± 4.3	9.1 ± 2.2	418 ± 55	15.0 ± 5.1	49.7 ± 14.7
S top	71.5 ± 2.7	17.4 ± 5.9	11.1 ± 5.1	440 ± 52	13.6 ± 4.2	57.7 ± 23.9
Means	71.2 ± 3.3	19.1 ± 4.8	9.7 ± 3.1	423 ± 61	15.6 ± 4.3	60.0 ± 18.8

3.2. Ascorbic Acid Content

The ascorbic acid content of the fruit skins ranged from 92–121 mg/100 g on a fresh weight basis, or 336–521 mg/100 g (mean = 423 ± 61 mg/100 g) on a dry weight basis (Figure 3; Table 1). This indicates that in addition to its previously reported high content of phenolic compounds [4], tuckeroo fruit can be a rich source of vitamin C.

A three-way ANOVA, using the tree, side and height as fixed factors, revealed that the tree significantly impacted the ascorbic acid content of the skin ($p < 0.001$, explaining 50.8% of the total variation observed in vitamin C content), with significant interactions between the tree/side and tree/height, as well as between tree/side/height ($p < 0.05$ for all).

Examination of the estimated marginal means plot revealed that for fruit from the bottom of the tree, the side or location of the fruit had a relatively minor impact on the ascorbic acid content. In contrast, for fruit from the top of the tree, ascorbic acid content was higher on the southern side compared to the northern side (Figure 4). As the northern canopy side would be expected to receive more light and heat over the growing season, this stands in contrast to the results of Lado et al. [12], who found that the ascorbic acid content of citrus increased with light availability. However, further work is required to confirm the exact relationship between sunlight exposure and ascorbic acid content in the tuckeroo.

The ascorbic acid content of the seeds was relatively quite low, averaging between 7.4–23.0 mg/100 g (dry weight basis; mean = 15.6 ± 4.3 mg/100 g). The tree and side significantly affected seed ascorbic acid content ($p < 0.001$ and $p < 0.01$, respectively), with a significant interaction between these two factors, as well as between side/height. For fruit from both the top and bottom of the canopy, lower seed ascorbic acid content was found on the southern side compared to the northern side, with the effect being more pronounced for fruit from the top of the tree.

Finally, the ascorbic acid content of the arils was moderately higher compared to the seeds, at 31–91 mg/100 g (dry weight basis; mean = 60.0 ± 18.8 mg/100 g). As with the seeds, three-way ANOVA revealed that the tree and side significantly affected aril ascorbic acid content ($p < 0.001$ and $p < 0.01$, respectively), with a significant interaction between tree/side/height. For fruit from both the bottom and top of the tree, arils from the northern side had higher ascorbic acid levels (Figure 4). Again, further work is required

to confirm the relationship between sunlight exposure and ascorbic acid content in this species, particularly for internal fruit parts such as the seeds and arils. As these are not directly exposed to sunlight, they are less likely to show a direct correlation with irradiation levels.

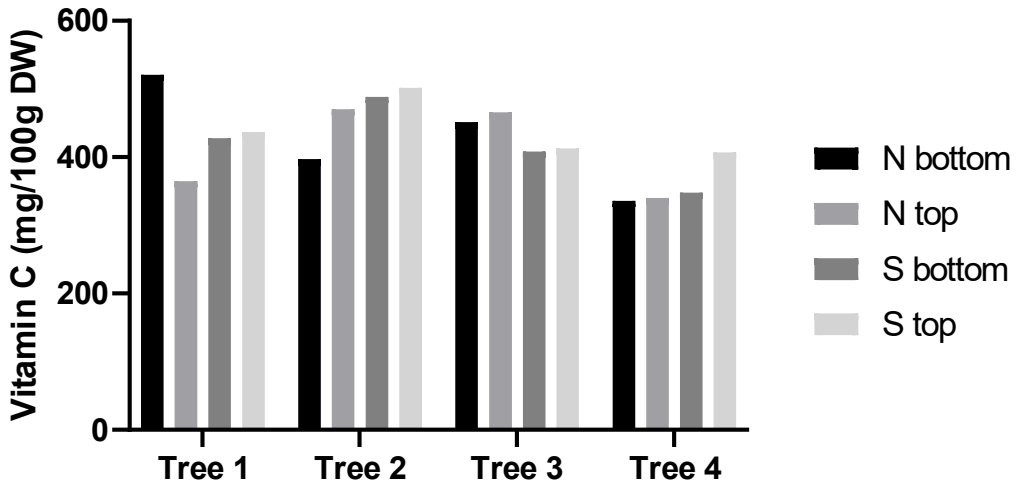

Figure 3. Mean ascorbic acid content of the fruit skins on a dry weight basis.

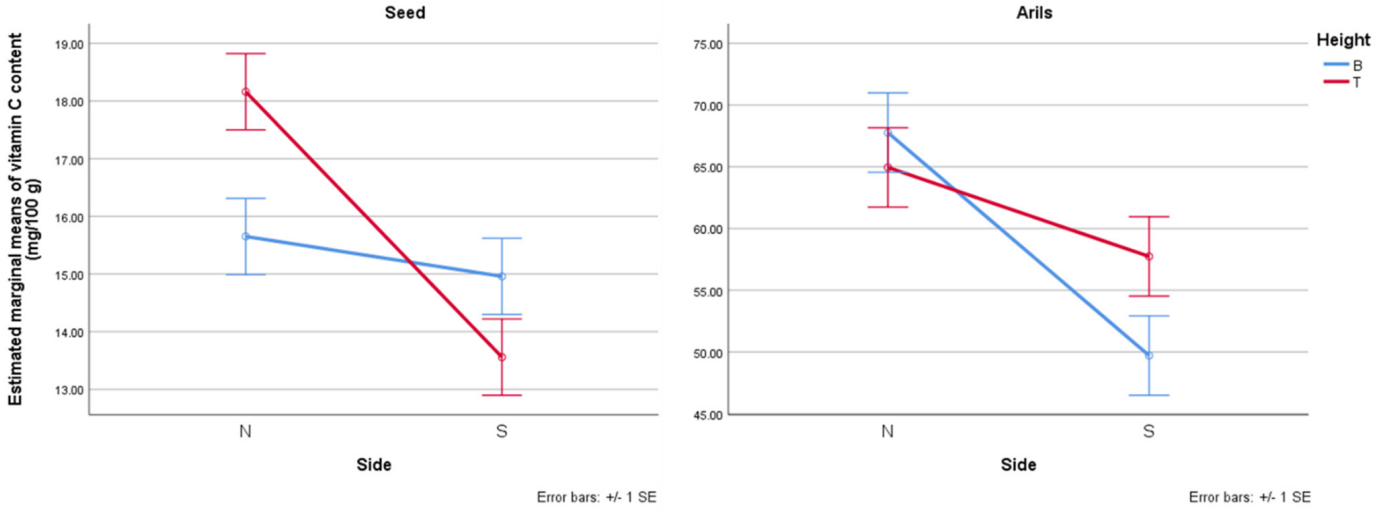

Figure 4. Estimated marginal means for the impact of side and height on ascorbic acid content of the fruit seeds and arils.

4. Conclusions

The skin/flesh of *C. anacardioides* appears to be a significant source of vitamin C (ascorbic acid), with contents over 500 mg/100 g (dry weight basis) found in this study. The arils also contain appreciable amounts of ascorbic acid, albeit at concentrations several times lower compared to the skin. The tree, side and height all significantly affected fruit mass, with fruit from the northern bottom side typically the heaviest. Similarly, the ascorbic acid content was significantly affected by height, with a marginal impact of the side (compass direction). Fruit from the top of the southern side of the tree typically had the highest ascorbic acid content. This indicates that there is some within-canopy variation in the nutritional content of *C. anacardioides* fruit, which may have implications for the choice of sampling protocols by future researchers of this species, as well as for potential harvesters of this fruit for nutritional or pharmaceutical purposes.

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References

1. Wood, E.M.; Esaian, S. The importance of street trees to urban avifauna. *Ecol. Appl.* **2020**, *30*, e02149. [CrossRef]
2. Esperon-Rodriguez, M.; Rymer, P.D.; Power, S.A.; Challis, A.; Marchin, R.M.; Tjoelker, M.G. Functional adaptations and trait plasticity of urban trees along a climatic gradient. *Urban For. Urban Green.* **2020**, *54*, 126771. [CrossRef]
3. Melzer, R.; Plumb, J. *Plants of Capricornia*; Capricorn Conservation Council: Rockhampton, Australia, 2007.
4. Pham, N.M.Q.; Chalmers, A.C.; Vuong, Q.V.; Bowyer, M.C.; Scarlett, C.J. Characterising the Physical, Phytochemical and Antioxidant Properties of the Tuckeroo (*Cupaniopsis anacardioides*) Fruit. *Technologies* **2017**, *5*, 57. [CrossRef]
5. Pham, N.M.Q.; Vuong, Q.V.; Le, A.V.; Bowyer, M.C.; Scarlett, C.J. Investigation of the Most Suitable Conditions for Dehydration of Tuckeroo (*Cupaniopsis anacardioides*) Fruits. *Processes* **2020**, *8*, 151. [CrossRef]
6. Pham, N.M.Q.; Vuong, Q.V.; Bowyer, M.C.; Scarlett, C.J. Optimization of ultrasound-assisted extraction conditions for phenolic compounds and antioxidant capacity from Tuckeroo (*Cupaniopsis anacardioides*) fruit. *Sep. Sci. Technol.* **2020**, *55*, 3151–3160. [CrossRef]
7. Hawkeswood, T. Notes on the pollination and fruit production of *Cupaniopsis anacardioides* (A. Rich.) Radlkf.(Sapindaceae) at Townsville, north Queensland. II. Fruit and seed production. *Vic. Nat.* **1983**, *100*, 121–124.
8. Richmond, R.; Bowyer, M.; Vuong, Q. Australian native fruits: Potential uses as functional food ingredients. *J. Funct. Foods* **2019**, *62*, 103547. [CrossRef]
9. Netzel, M.; Netzel, G.; Tian, Q.; Schwartz, S.; Konczak, I. Native Australian fruits—A novel source of antioxidants for food. *Innov. Food Sci. Emerg. Technol.* **2007**, *8*, 339–346. [CrossRef]
10. Mani, J.S.; Johnson, J.B.; Hosking, H.; Ashwath, N.; Walsh, K.B.; Neilsen, P.M.; Broszczak, D.A.; Naiker, M. Antioxidative and therapeutic potential of selected Australian plants: A review. *J. Ethnopharmacol.* **2020**, *268*, 113580. [CrossRef] [PubMed]
11. Johnson, J.B.; Budd, C.; Mani, J.S.; Brown, P.; Walsh, K.B.; Naiker, M. Carotenoids, ascorbic acid and total phenolic content in the root tissue from five Australian-grown sweet potato cultivars. *N. Z. J. Crop. Hortic. Sci.* **2021**. [CrossRef]
12. Lado, J.; Alós, E.; Rodrigo, M.J.; Zacarías, L. Light avoidance reduces ascorbic acid accumulation in the peel of *Citrus* fruit. *Plant Sci.* **2015**, *231*, 138–147. [CrossRef] [PubMed]