NMR and FT-ICR-MS metabolomics of Australian native Green Plum fruit

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Introduction

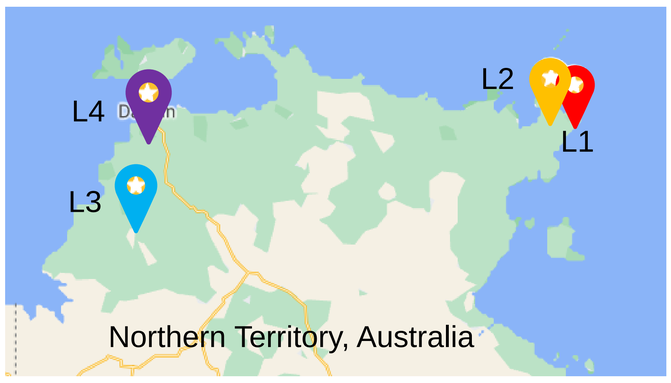
The green plum (*Buchanania obovata*) fruit grow in northern Australia and are a traditional food of the Aboriginal Australians that has been underutilised for its taste and nutrition.

The green plums are small, weighing between 1 and 2 grams, and belong to the same family, Anacardiaceae, as the mango, of which they look like miniature versions. They taste sweet, acidic and of stewed fruit and are very popular as a summer food in the Northern Territory and Western Australia where they grow.

Figure 1: Green plums were harvested by hand in the Northern Territory at a range of maturity stages

Sample Collection

Green plums were harvested from four geographical locations in the Northern Territory, Australia: L1, L2, L3 and L4 (Figure 2). They were harvested at all levels of maturity and divided into five different maturity stages based on size, fullness and colour: M1, M2, M3, M4 and M5 with M4 being ripe green plums and M5 sundried green plums.
Samples were collected from two harvest years, H1 and H2, under Northern Territory Parks and Wildlife permits and with the assistance of Wild Orchard Kakadu Plum, Gulkula Mining Company and The Arnhem Land Progress Aboriginal Corporation.



M1 M2 M3 M4 M5

	M1	M2	M3	M4	M5
L1 H1				✓	
L3 H1				✓	
L1 H2	✓	✓	✓	✓	✓
L2 H2			✓	✓	
L3 H2	✓	✓	✓		
L4 H2	✓	✓	✓		

Figure 2: The green plums were harvested from four locations and two harvest years in the Northern Territory, Australia. They were divided according to stage of maturity. The table shows the samples used for metabolomics analysis.

NMR metabolomics

Methods

Methanol extracts of green plums across the different locations, harvest years and maturity stages shown in Figure 2 were analysed with non-targeted NMR-based metabolomics at 900 MHz. NMR spectra were aligned using icoshift in Matlab and statistically analysed with OPLS in SIMCA. Results are shown as 3D score plots.

Results and Discussion

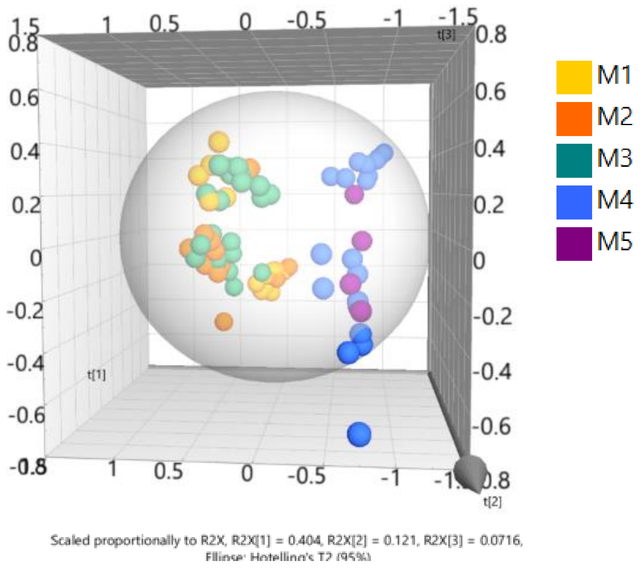


Figure 3: OPLS-DA of green plums by maturity stage by NMR, showing clear clustering of maturity stages and the major differentiation between the immature M1, M2 and M3 compared to the ripe M4 and sundried M5.

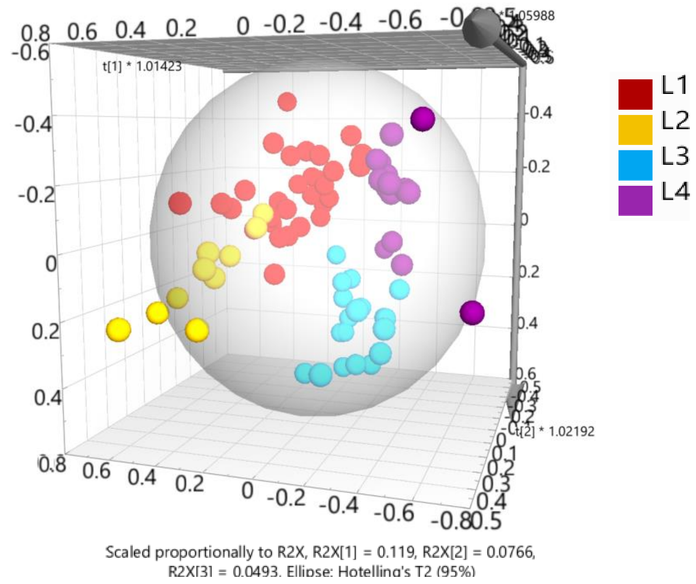


Figure 4: OPLS-DA of NMR spectra of green plums by location, showing clear clustering of each location.

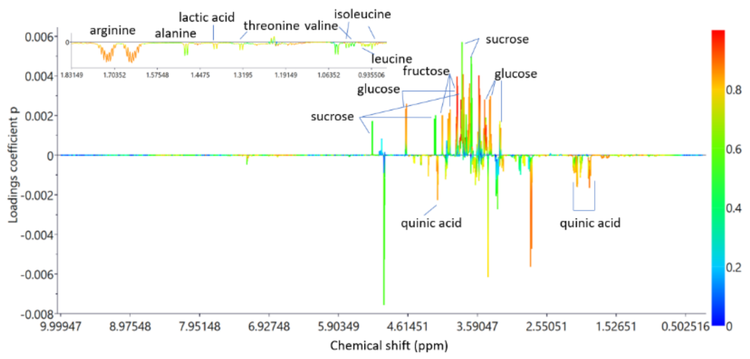


Figure 5: ^{1}H NMR spectrum S-line plot of OPLS-DA showing the metabolite differences between M3 (-p) and M4 (+p) green plums.

Figure 6: The green plum fruit grow on the tree *Buchanania obovata* in the Northern Territory and Western Australia.

FT-ICR-MS metabolomics

Methods

A second aliquot of the methanol green plum extracts was analysed by non-targeted 12 Tesla Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). Alignment and molecular compositions were performed by in-house software tools. Results from statistical analysis by OPLS-DA in SIMCA are shown as 3D score plots and loadings plots.

Results and Discussion

FT-ICR-MS analysis discovered 5012 metabolites in the green plums.

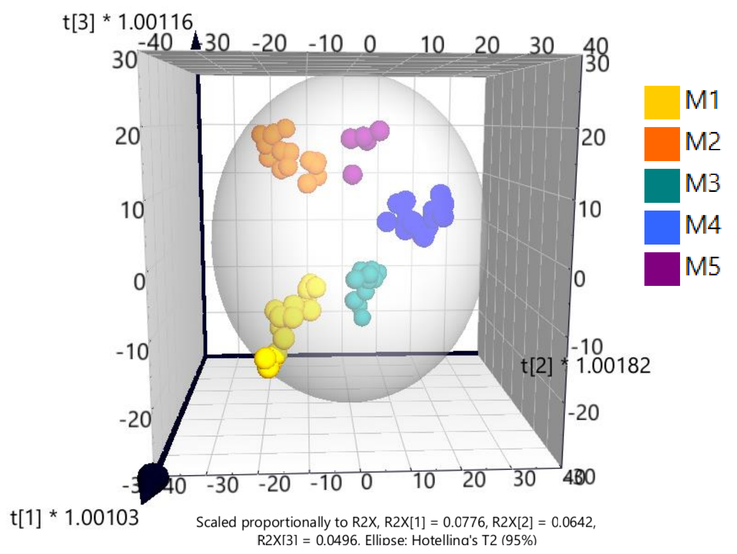


Figure 7: OPLS-DA of green plums by maturity stage by FT-ICR-MS, showing clear clustering of metabolites by maturity stage.

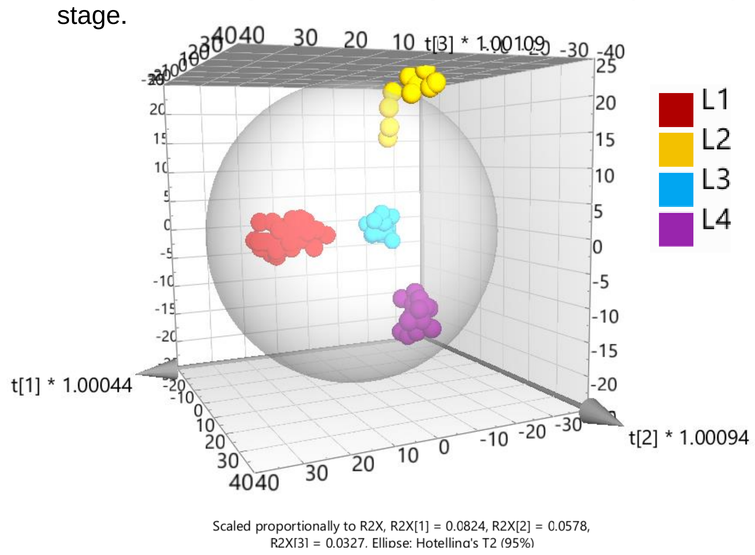


Figure 8: OPLS-DA of FT-ICR-MS of green plums by location, showing clear clustering of each location.

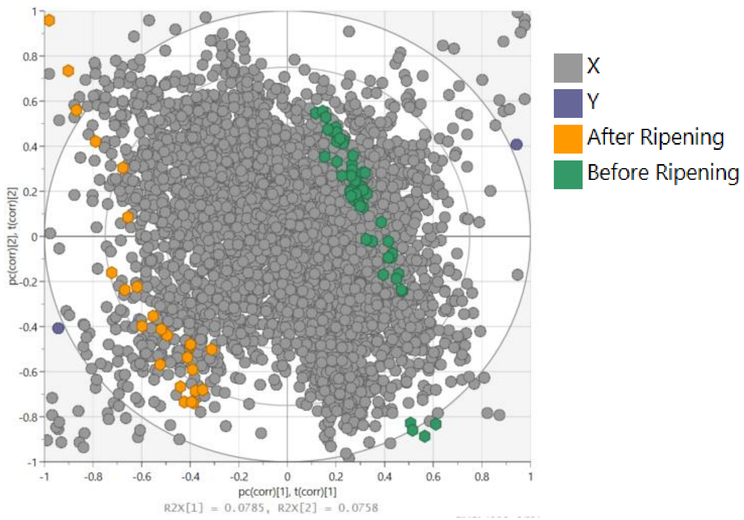


Figure 9: PLS scores and loadings biplot of FT-ICR-MS of green plums by ripening, showing before ripening (M1, M2 and M3) and after ripening (M4 and M5).

Conclusion

This work shows that green plums have differences in their metabolites as they mature, with distinct differences between the underripe and the ripe fruit. They also show differences in metabolites from each geographical location.
Further analysis, identification and quantification of metabolites will give more information about green plums properties and nutritional benefits.

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