

**Ascorbate metabolism in grape berries during
development**

by

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Abstract

Ascorbate (Asc, Vitamin C) is a ubiquitous and abundant antioxidant in plants. The biological role of Asc is composed of the following three functions: as an antioxidant (radical scavenger), an enzyme cofactor and a donor/acceptor in electron transport across membranes. This present research was focussed on the additional, less comprehensively investigated function of Asc as a precursor of tartaric acid (TA) and oxalic acid (OA) in certain species. Although the *in planta* function of TA is unclear, it is well known as the dominant organic acid in grape (*Vitis vinifera*) berries. TA is therefore largely responsible for the low berry pH, whereas in other fruits, the majority of the acidity is conferred by malic, citric and ascorbic acids. Earlier research investigating the Asc-derived TA biosynthetic pathway revealed several intermediates and one characterised enzyme. Research investigating metabolism of the Asc precursor in TA and OA accumulating plant species is now required. The aim of this present research was to therefore investigate the factors which may regulate the accumulation of Asc and those which in turn regulate the fate of Asc in fruit.

Sensitive methods were developed for the extraction and analysis of Asc, TA and OA from vegetative and fruit tissues. These methods were used to demonstrate that Asc and its catabolites are widely distributed throughout the grapevine; however, the total Asc pool and the Asc to dehydroascorbic acid (DHA) ratio were shown to be significantly greater in leaves than berries at all physiological stages of maturity. This research further demonstrated that immature grape berries rapidly accumulate Asc *in situ* from D-mannose and L-

galactose (precursors of the Smirnoff-Wheeler Asc biosynthetic pathway). Developmental regulation of Asc biosynthetic, recycling (redox) and catabolic genes were demonstrated in grape berries. The gene transcription results of this study strongly suggested that Asc biosynthesis in immature berries is supported by the Smirnoff-Wheeler biosynthetic pathway whilst the alternative 'carbon-salvage' pathway of Asc biosynthesis supports post-veraison accumulation of Asc. A positive correlation between the developmental accumulation of Asc and TA was observed and the capacity for *in situ* Asc catabolism in immature berries, generating both TA and OA products, was directly demonstrated. The accumulation of Asc and TA was shown to be influenced by sunlight intensity and, putative light-responsive Asc biosynthetic and recycling genes were identified. The results also demonstrated that OA accumulates in berries independent of sunlight intensity. The outcomes of this research strongly suggested that TA biosynthesis is regulated by the availability of its precursor Asc; however, it is unlikely that the biosynthesis of OA is dependent on the availability of Asc. In conclusion, the results of this present study highlighted that both developmental and environmental factors influence transcription of Asc metabolic genes and the accumulation of Asc. Furthermore, these factors also influence the fate of Asc in grape berries. The low levels of accumulated Asc detected in the fruit at all stages of maturity are therefore likely to be the result of catabolism rather than a low biosynthetic capacity. The implications of Asc catabolism and the accumulation of TA and OA products on fruit growth and development are discussed.

Declaration

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Statement of the contributions of jointly authored papers

1. Melino, V. J., Soole, K. L. and Ford, C.M. A method for determination of fruit-derived ascorbic, tartaric, oxalic and malic acids and its application to the study of ascorbic acid catabolism in grapevines. Australian Journal of Grape and Wine Research, Jul 21 2009, 10.1111/j.1755-0238.2009.00062.x.

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Author Contributions: VJM designed and conducted all research experiments except those designed and conducted by MHA. MHA designed the artificial-shading experiment and conducted sampling of grapes for season 1 and assisted with sampling for season 2. VJM analysed the data and drafted/constructed the manuscript. CMF supervised all research. MHA, CMF and KLS contributed to the research ideas, and the editing of the manuscript.

Each of these manuscripts are displayed in this thesis in either their submitted or published forms according to the author guidelines of the specific journal.

The following authors agree that the Statement of the contributions of jointly authored papers accurately describes their contribution to research manuscripts 1., 2. and 3. and give consent to their inclusion in this thesis.

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