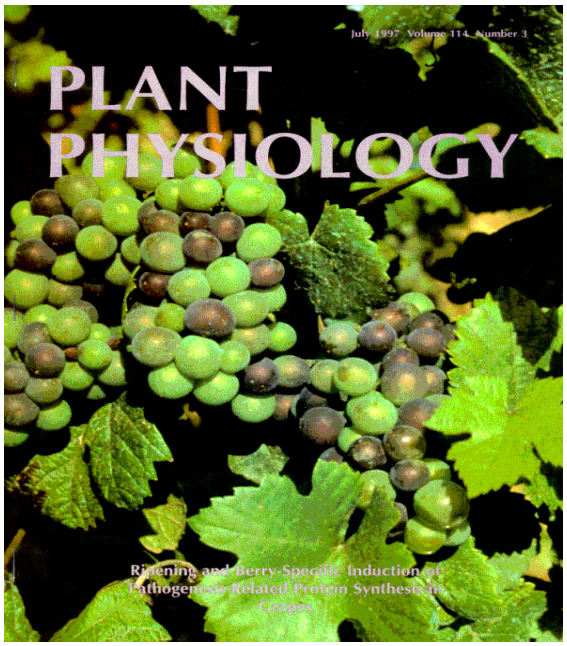
Assay and characterisation of grape berry proteins of importance to winemaking and crop protection



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **UA 96/1**

Principal Investigator: **Peter Hoj**

Research Organisation: **Adelaide University**

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Final report for Project UA 96/1-

Title: Assay and characterisation of grape berry proteins of importance to winemaking and crop protection.

Research organisation: Adelaide University

Principal Researcher: Professor Peter B. Høj

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Executive summary:

The present project set out to characterise the properties of haze-forming proteins not only in relation to their measurement and ease of removal but also in terms of their biology. The outcomes have been many and the knowledge acquired has on several occasions been used to troubleshoot problems in export wines that have been submitted to Industry Services of The Australian Wine Research Institute with precipitate problems. Without the knowledge and expertise generated through this project and through AWR8, this quality advice could not have been given. Significant portions of the work reported in the resulting publications have been achieved in collaboration with staff at The Australian Wine Research Institute through project AWR8. Indeed, some of the leads uncovered in this project have now been pursued to a stage where the reduction of bentonite requirements for fining of white wine can in principle be achieved (see the annual progress report for AWR8, 2000/2001).

Publication of results from this project has happened in a timely manner and the reader is referred to those for detailed overviews and outcomes, viz

- Tattersall, D.B., van Heeswijck, R and Høj, P.B (1997) Identification and Characterisation of a Fruit-Specific Thaumatin-like Protein Which Accumulates at Very High Levels in Conjunction With the Onset of Sugar Accumulation and Berry Softening in *Vitis vinifera.* Plant Physiol **104**, 759-770 (This Article was selected as the cover-story for the July issue of 1997).

- D.B. Tattersall, K.F. Pocook, Y. Hayasaka, K. Adams, R. van Heeswijck, E.J.Waters, P.B. Høj (2001) Pathogenesis related proteins - their accumulation in grapes during berry growth and their involvement in white wine heat instability. In "Molecular Biology and Biotechnology of the Grapevine" K.A. Roubelakis-Angelakis. Kluwer Academic Publishers, Dordrecht (ISBN 0-7923-6949-1), pp 183-202

- Hayasaka, Y, Adam K.S., Pocock, K.F, Baldock, G.A., Waters E.J. and Høj, P.B. (2001) Use of electrospray mass spectrometry for mass determination of grape (*Vitis vinifera*) juice pathogenesis-related proteins: A potential tool for varietal differentiation. J Agric Food Chem. **49,** 1830-1839

- P.B. Høj, D.B. Tattersall, K. Adams, K.F. Pocook, Y. Hayasaka, R. van Heeswijck, E.J.Waters (2001) The 'Haze Proteins' of Wine – A Summary of Properties, Factors Affecting Their

[*] Through AWR 8 (led by Dr E. Waters).

Accumulation in Grapes, and the Amount of Bentonite Required for Their Removal From Wine. Proceedings of the American Society for Enology and Viticulture's 50th Meeting, Seattle Washington, June 19-23, 2000 pp 149-154. (2001).

Main findings:

- The proteins predominantly responsible for haze formation in wine are derived from the grape berry and have been identified as homologues of plant pathogenesis related proteins (PR-proteins). These PR-like proteins very likely help protect the ripening berry against fungal pathogens at a time where sugar accumulation and berry softening makes the berry vulnerable to infection. Tests on agar plates show that the purified haze-proteins have an antifungal effect when tested artificially against *Botrytis cineria* and some other fungi. For this reason, attempts to downregulate the synthesis of these haze proteins by anti-sense gene technology is no longer favoured.

- Synthesis of the haze-forming proteins is initiated at berry veraison and, everything else being equal, the riper the grapes the higher the amount of haze-forming PR protein and the higher the bentonite requirement to stabilise the resultant wine. These conclusions were established using both cDNAs cloned in our laboratory and using antibodies we raised against isolated haze proteins. The findings were subsequently extended to the study of other fruits and represented a significant step forward in our understanding not only of grape berry ripening physiology but of fruit ripening physiology in general.

- Mechanical harvesting coupled with transport results in enhanced PR-like protein levels through the extraction of protein from skins. The bentonite requirement for protein fining can be doubled if extensive extraction of PR-like proteins occurs from skins following mechanical harvesting and prolonged juice/skin contact. (an outcome through AWR 8). This insight immediately suggests the benefits of on-site crushing or nearby crushing of fruit which has been mechanically harvested with a large degree of juicing.

- The observation that less bentonite is required in sparkling wine making than in full bodied white winemaking, can be explained by a combination of having less ripe berries, performing hand harvesting and prolonged storage on lees, all factors correlating with low bentonite requirement.

- Under current winemaking conditions protease treatment of juice and wine cannot be used effectively for stabilisation of white wine. Introduction of procedures for the denaturation of haze proteins during winemaking may lead to protease susceptibility, and significantly reduced bentonite requirements. Our experiments have identified proteases and commercial enzyme preparations which, when added to wine heated for short periods of time, will degrade a good portion of the haze proteins and also reduce the bentonite requirements for fining to stability. Preliminary sensory assessment of the wines indicate that the heating is not likely to introduce detrimental sensory effects, but this assertion needs to be tested in greater detail. If and when disposal of bentonite becomes a significant environmental issue, this finding may have practical application. Furthermore, the amount of wine which has to be recovered from the bentonite-lees would be reduced through the application of such technology.

- We attempted to develop rapid antibody based assays for haze proteins with the view to better predict fining requirements. Very sensitive antibody based ELISA assays were developed and these were capable of detecting a few nanograms of haze forming protein but could not readily be adopted for routine assays in a winery.

- Comparison of predicted molecular masses for hazeforming proteins from different varieties indicated that small differences might be found amongst their otherwise identical set of hazeforming proteins. It was

therefore hypothesised that varieties could be discriminated on the basis of the masses of their haze-forming proteins. Electrospray mass spectrometry of grape proteins revealed this to be so. The advantage of this approach is that it may extended to wine where sufficient amounts of protein might exist for the same determinations to be made. This offers an alternative to DNA-based diagnostics, which currently does not work in wine, presumably due to degradation of grape DNA during the fermentation process. The mere existence and further development of our massspectrometry based technology help cement label integrity in world-wine.