

Hydrolysable tannins in aged wine spirits: a fresh perspective using alternative ageing technology and high-resolution mass spectrometry

Tiago A. Fernandes,^{1,2} Alexandra M.M. Antunes,¹ Sheila C. Oliveira Alves,³ Ilda caldeira,^{3,4} Ofélia Anjos,^{5,6} Sofia Catarino,^{7,8} Sara Canas³

¹Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Engenharia Química, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal; tiago.a.fernandes@tecnico.ulisboa.pt

²Departamento de Ciências e Tecnologia, Universidade Aberta, Rua da Escola Politécnica, 141-147, 1269-001 Lisboa, Portugal.

³Instituto Nacional de Investigação Agrária e Veterinária, Quinta de Almoimha, Polo de Dois Portos, 2565-191 Dois Portos, Portugal.

⁴MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

⁵Instituto Politécnico de Castelo Branco, Quinta da Senhora de Mércules, 6001-909 Castelo Branco, Portugal.

⁶Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

⁷LEAF—Linking Landscape, Environment, Agriculture and Food Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

⁸CEFEMA – Center of Physics and Engineering of Advanced Materials, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1, 1049-001 Lisboa, Portugal.

Wine spirits (WSs) are usually aged in wooden barrels, but using wood pieces instead of barrels, with or without micro-oxygenation, is a technological alternative that has been investigated by our team. The current research was focused on identifying ellagitannins, their derived species, and the evolution of degradation pathways. For this propose, in this study, the behavior of hydrolysable tannins in a wine spirit aged in 50 L demijohns with chestnut wood staves and three levels of micro-oxygenation or nitrogen, was examined.^{1,2} Gallotannins and ellagitannins were identified by LC-ESI-HRMS/MS using a Q-TOF in samples collected at 8, 21, 60, 180, 270, and 365 days of ageing, and their relative abundances compared according to the ageing technology. The studied compounds derive from the wood and have a significant sensory impact in the aged wine spirits due to their association with astringency, which is closely related to the quality of these beverages. For the first time, the importance of oxygen in gallotannins and ellagitannins formation/degradation pathways in WS was established, and results aided to explain the steady increase in gallic and ellagic acid contents on WS during ageing. The results also highlighted the presence of penta-O-galloyl-β-D-glucose, tetra-O-galloyl-β-D-glucose, tri-O-galloyl-β-D-glucose, di-O-galloyl-β-D-glucose, and mono-O-galloyl-β-D-glucose, 2,3-(S)-hexahydroxydiphenoyl-β-D-glucose, pedunculagin, isomers vescalagin/castalagin, as well as two products stemming from ethanol-promoted oxidation of castalagin/vescalagin and vescalalin/castalin, in the WS aged with chestnut wood (**Figure 1**).

This study provided a better understanding of ellagitannin derivatives and determined their presence on WSs, which are associated with an increase in gallic and ellagic acid concentrations during ageing.²

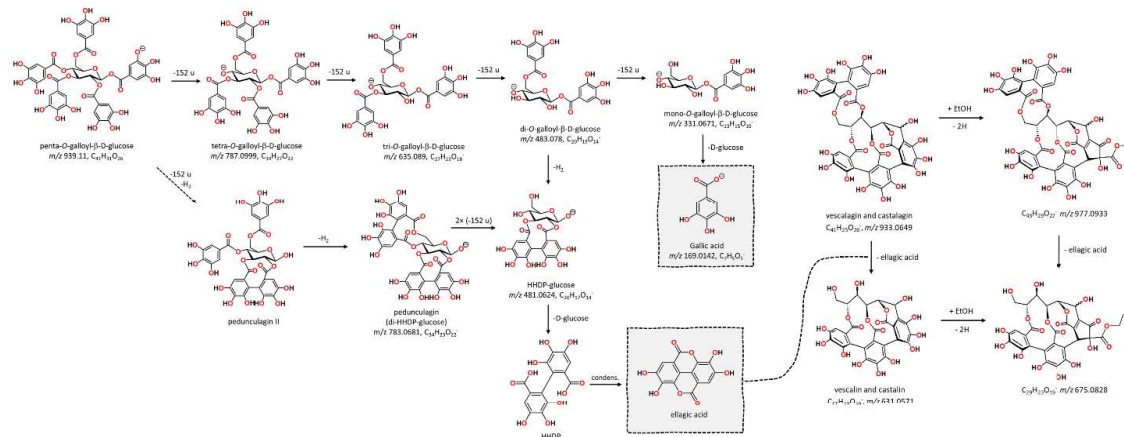


Figure 1. Proposed fragmentation patterns and degradation pathways of gallotannins and ellagitannins. Structures of vescalagin/castalagin isomers and their degradation pathway for the two derivatives that were tentatively assigned to the ethanol-promoted oxidation products of vescalagin/ castalagin and vescalalin/castalin.

Acknowledgements: The authors are very grateful to Victor de Freitas as the Scientific Consultant of the Project POCI-01-0145-FEDER-027819, and to João Pedro Catela, Nádía Santos, Manuela Gomes, Eugénia Gomes and Inês Antunes from Adega Cooperativa da Lourinhã, José Abílio Gonçalves and Sérgio Gonçalves from Tanoaria J. M. Gonçalves, Ana Partidário from INIAV—Oeiras, Pedro Rodrigues and Diogo

Rodrigues from AZ3Oeno Portugal, Sílvia Lourenço, João Amaral, Deolinda Mota from INIAV—Polo de Dois Portos, and A. Pedro Belchior for their technical support.

Funding: This research was funded by National Funds through FCT - Foundation for Science and Technology under the Project OXYREBRAND - POCI-01-0145-FEDER-027819 (PTDC/OCE-ETA/27819/2017). The authors thank the research units and Fundação para a Ciência e a Tecnologia, I.P.: CEF (UIDB/00239/2020); CQE (UIDB/00100/2020; UIDP/00100/2020); LEAF (UIDP/04129/2020; UIDB/04129/2020); MED (UIDB/05183/2020) and contracts CEECIND/02725/2018, CEECIND/02001/2017 and DL 57/2016/CP1382/CT0025.

References:

1 S. Canas, F. Danalache, O. Anjos, T.A. Fernandes, I. Caldeira, N. Santos, L. Fargeton, B. Boissier, S. Catarino, *Molecules*. 25(22), 5266 (2020).

2 T.A. Fernandes, A.M.M. Antunes, I. Caldeira, O. Anjos, V.de Freitas, L. Fargeton, B. Boissier, S. Catarino, S. Canas, *Food Chemistry*. 382, 132322 (2022).