

SCREENING FOR POTENTIAL WINE PIGMENTS IN RED WINE USING TANDEM MASS SPECTROMETRY

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Despite a progressive decline in genuine anthocyanins during maturation, wine color is maintained. This suggests that new and more stable wine pigments are formed by the reaction of genuine anthocyanins with other wine constituents. In fact, several wine pigments have been reported in recent years and these appear to have important roles for color development.

We developed the sensitive screening method for potential wine pigments (anthocyanin derivatives) in red wine using a combination of electrospray ionization tandem mass spectrometry (ES-MS/MS) techniques. The screening of these compounds would be an important step prior to a comprehensive investigation including the synthesis of reference compounds for structural confirmation and the influence of these wine pigments on red wine color.

Potential wine pigment fractions from Shiraz (*Vitis vinifera*) wine were obtained by ion exchange chromatographic techniques. Those fractions were analyzed by ES-MS/MS in neutral loss scan mode to screen the glycosylated compounds with the three different forms (glucoside, acetyl glucoside and *p*-coumaroyl glucoside). Because wine pigments should have the same glycosylated forms as those of anthocyanins. The screened glycosylated compounds were further examined by ES-MS/MS in product ion scan mode of their aglycones to confirm their anthocyanidin origin and to elucidate their structures.

Fourteen compounds were considered to be of anthocyanidin origin on the basis of their MS/MS spectra. Their proposed structures except 4-hydroxy malvidin characterized for the first time were anthocyanidin C-4 substitutes with vinyl linkage between C-4 and the hydroxy group at C-5. The anthocyanidin derivatives screened were identified as vinyl, vinylmethyl, vinylformic acid, 4-vinylphenol, 4-vinylguaiacol and vinylcatechin adducts of malvidin as well as vinylformic acid and 4-vinylphenol adducts of peonidin and petunidin. The presence of vinylalcohol, 4-vinylcatechol and 4-vinylsyringol adducts of malvidin were also demonstrated for the first time.

ES-MS/MS was successfully applied for the screening of potential wine pigments. Fourteen compounds were screened and tentatively identified by MS/MS. Four out of those potential pigments have not previously been reported and thus represent 'new' wine pigments.

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