Research Note

The aroma glycosides composition of Burgundy Pinot noir must

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Introduction: The sensory significance of hydrolysis products of glycosidically bound compounds to varietal aroma has been well established (Williams and Francis 1996). Varieties such as Muscat (Voirin *et al.* 1990 and 1992), Syrah (Razungles *et al.* 1993) and Riesling (Skouroumounis and Winterhalter 1994) have been the most studied cultivars to establish databases of aroma glycosides. For Pinot noir, only volatile compounds released after enzymatic hydrolysis of glycosidic extracts from clones were reported (Pigella *et al*. 1998). Tt has previously been found that enzymatic hydrolysis takes place sequentially (Gunata *et al*. 1988). According to this finding and to the glycosidase specificity, the structure of aroma glycosides needs to be known to control the enzymatic hydrolysis process for the release of volatile compounds. This study aimed at the identification of aroma glycosides from a must with the analysis of glycosidic extract by GC-MS after   trifluoroacetylation.

Material and Methods: Must was obtained from grapes (cv. Pinot noir) collected in 1997 at the vineyard of the experimental station of the University of Burgundy. Tn order to remove polyphenolic compounds, grape juice was stirred for 30 min at 4 oC in the presence of PVPP (1:10, w/v). The glycosidic fraction was obtained according to the procedure described by Williams *et al.* (1982). Clear juice (2.0 l) obtained after centrifugation at 10,000 *g* for 15 min at 10 oC was passed through a column (200 x 30 mm i.d.) filled with C18 reversed-phase adsorbent (30 g). Water-soluble compounds were removed with 1.0 l of distilled water and the retained material was eluted with 300 ml of methanol at rates up to 2 ml·min^{-1}. Enzymatic hydrolysis was performed on a glycosidic extract corresponding to 200 ml of clear juice. The sample concentrated to dryness was dissolved by 0.5 ml of 0.2 M citrate-phosphate buffer at pH 5 and extracted 5 times using pentane-dichloromethane (2:1) and 0.5 ml of an enzymatic extract (20 mg·ml^{-1} of enzymatic preparation AR2000 (Gist Brocades) in buffer 0.2 M citrate-phosphate, pH 5). After incubation at 40 oC for 16 h, 10 1-g of 5-nonanol were added as internal standard and the mixture was extracted twice with 250 1-l of pentane/dichloromethane (2:1). This aglycon extract was directly analysed by GC-MS. Sugars of glycosides were converted into alditol acetates after

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acid hydrolysis (2 M trifluoroacetic acid solution at 120 oC for 1.5 h) and analysed by GC (Harris *et al.* 1984). Synthetic glycosides and natural glycosides obtained from 40 ml must were trifluoroacetylated according to the method described by Voirin *et al.* (1992).

GC was performed with Chrompack CP 9001 gas chromatograph equipped with a HP-5 capillary column (30 m x 0.25 mm i.d.; 0.25 1-m bonded phase) for trifluoroacetylated glycosides and a Rtx®-2330 capillary column (15 m x 0.32 mm i.d.; 0.2 1-m bonded phase) for alditol acetates. The operating conditions used for GC of trifluoroacetylated glycosides were those reported by Voirin *et al.* (1992). The operating conditions for alditol acetates: injector and detector temperature 265 oC; nitrogen carrier gas flow rate 1.4 ml·min^{-1}. Column temperature was raised from 175 to 240 oC at 8 oC·min^{-1}, hold for 1 min at 240 oC and then increased to 265 oC at 8 oC·min^{-1}**.**

ET mass spectra were recorded by coupling an HP 5890 gas chromatograph equipped with DB™Wax capillary column (30 m x 0.32 mm i.d.; 0.5 1-m) for aglycons and a HP-5 capillary column (30 m x 0.32 mm i.d.; 0.25 1-m) for trifluoroacetylated derivatives and a splitless injector to a HP 5970 mass spectrometer. For trifluoroacetylated derivatives, column temperature programs were performed according to Voirin *et al*. (1992) and for GC of aglycons, column temperature was increased from 40 to 250 oC at 3 oC·min^{-1}; the carrier gas was N$_2$ at 2 ml·min^{-1}. The injector temperature was maintained at 250 oC, the detector temperature at 250 oC. Helium was used as carrier gas at 35 cm·s^{-1} at 200 oC. The mass range was scanned between 70 and 600 amu for trifluoroacetylated derivatives and between 28 and 300 amu for aglycons, ionisation voltage 70 eV.

Results and Discussion: GC analysis of alditol acetate from glycosidic moiety released by acidic hydrolysis of the glycosidic extract Pinot noir must showed the presence of glucose (77 %), apiose (12 %), arabinose (9 %) and rhamnose (2 %). These sugars are commonly reported as components of the sugar moiety of flavour glycosides in *Vitis vinifera* (Voirin *et al.* 1990, 1992). The high molar ratio of glucose can be explained by the presence of anthocyanins in the glycosidic extract obtained after elution of must on C18 column. From these results and with regard to glycosides already identified in other *V. vinifera* cvs (Voirin *et al.* 1992), the presence of glucoside, arabinosyl glucoside, rhamnosyl glucoside and apiosyl glucoside can be postulated.

Glycosides have been characterized by means of GC and GC/ET-MS of their trifluoroacetylated derivatives (Voirin *et al.* 1990, 1992). Several constituents of the C18 fraction presented a mass spectrum containing ions typically related to the electron impact mass fragmentation of trifluoroacetylated derivatives of glycosides (ions at m/z 193, 205, 207, 319). A comparison of the GC data reported by Voirin *et al.* (1992) for synthetic glycosides with the detected compounds of our trials revealed a dominance of disaccharides in the fraction analysed. The chromatographic and MS data for synthetic TFA glycosides and certain compounds detected in natural glycosidic extract were coincident (Table). Thus, benzyl and 2-phenylethyl glucopyranosides and 6-0-(a-L-rhamnopyranosyl)-ß-D-glucopyrano-

Table

Trifluoroacetylated derivatives of glycosides identified or tentatively identified in Pinot noir must from Burgundy

Peak no. in Figure	Compound	RRT unknown	ref
1	benzyl Glc*p*	1.115	1.119
2	2-phenylethyl Glc*p*	1.259	1.266
3	hexoside	1.380	
4	hexoside	1.452	
5	benzyl Rha*p*-Glc*p*	1.727	1.714
6	benzyl Ara*f*-Glc*p*	1.801	1.807
7	benzyl Apio*f*-Glc*p*	1.810	
8	2-phenylethyl Rha*p*-Glc*p*	1.825	1.836
9	benzyl pentosyl hexosides	1.988	
10	hexoside	2.017	
11	benzyl pentosyl hexoside	2.095	
12	pentosyl hexoside	2.222	

RRT: Retention time relative to phenyl ß-D-glucopyranoside

sides and benzyl 6-0-(a-L-arabinofuranoside)-ß-D-glucopyranoside were identified unequivocal in Pinot noir must. The presence of benzyl ß-D-apiofuranosyl ß-D-glucopyranoside was highly probable with regard to the MS and GC data obtained in our study and those reported by Voirin *et al.* 1990. This confirms our results concerning the sugar moiety in the composition of glycosides. All identified compounds were also found in must of the other cultivars studied for their bound aroma compounds composition (Voirin *et al.* 1990, 1992, Lopez-Tamames *et al.* 1997). Many unknown peaks in the chromatogram of TFA must glycosides (cv. Pinot noir) showed fragment ions characteristics of glycosides with osidic parts of hexoside and pentosyl hexoside (Table). Except for two peaks at RRT 1.988 and 2.095 for which ET-MS data correspond to glycosides with benzyl alcohol aglycone, characterization of their aglycone structures was not possible due to the lack of published reference mass spectra.

The glycosidic fraction obtained from Pinot noir must was hydrolysed by the enzymatic preparation AR2000. The trifluoroacteylated glycosides present in the initial extract and after enzymatic action were analysed by GC and the results are given in the Figure. The chromatographic profile of the glycosidic extract was modified by action of enzymatic preparation AR2000. Tn the range of high retention times, most of the detected peaks corresponding to mono- and diglycosides disappeared after action of enzymatic preparation. Total hydrolysis of detected glycosides was then performed. Aliphatic and aromatic alcohols, phenols and C13 norisoprenoid released after enzymatic hydrolysis of the glycosidic extract were identified by GC-MS and all have already been reported as constituents of glycosides in several Pinot noir clones by Pigella *et al.* (1998). Benzyl and 2-phenylethanol were the major components, which is in accordance with previous data given above for the composition of glycosides.
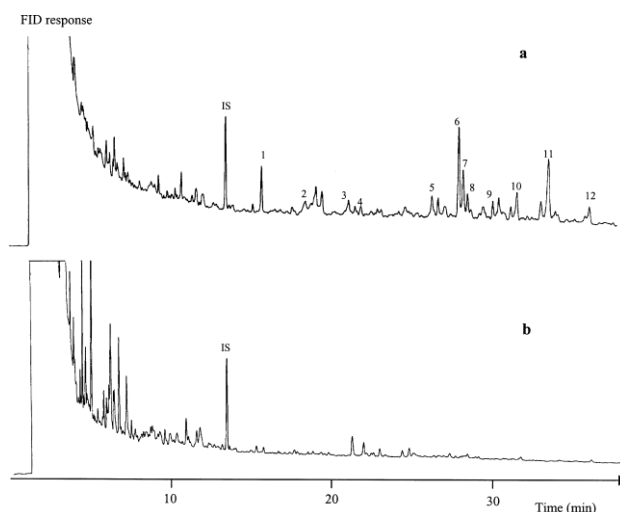
D-glucopyranoside; 5: benzyl a-L-rhamnopyranosyl ß-D-glucopyranoside; 6: benzyl a-L-arabinofuranosyl ß-D-glucopyranoside; 7: benzyl ß-D-apiofuranosyl ß-D-glucopyranoside; 8: 2-phenylethyl a-L-rhamnopyranosyl ß-D-glucopyranoside; 3,4,9,10,11,12: Unknown glycosides.

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