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Research note

Occurrence of a resveratrol ß-D-glucoside in wine: Preliminary studies

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K e y w o r d s : resveratrol, resveratrol glucoside, wine.

Introduction: Much interest had been focused upon resveratrol (*trans*-3,5,4'-trihydroxystilbene, Fig. 1), a phytoalexin present in some grape species whose synthesis is induced upon fungal infection (LANGCAKE and PRYCE 1976; HOOS and BLAICH 1988; JEANDET *et al.* 1994 a) or stress (LANGCAKE and PRYCE 1976; JEANDET *et al.* 1991). Recently, SIEMANN and CREASY (1992) have reported that resveratrol could also occur in grape products and particularly in wine where it is thought to be responsible, at least in part, for the protective effects of wine against coronary heart disease. Previous investigations have been conducted mainly in order to determine the resveratrol content in wine by using various analytical techniques: HPLC (SIEMANN and CREASY 1992; MATTIVI 1993; JEANDET *et al.* 1994 b; ROGGERO and ARCHIER 1994) and GC-MS (JEANDET *et al.* 1993). We give here good evidence of the presence of a ß-D-glucoside of resveratrol in wine by the use of glucosidases directly on the wine.

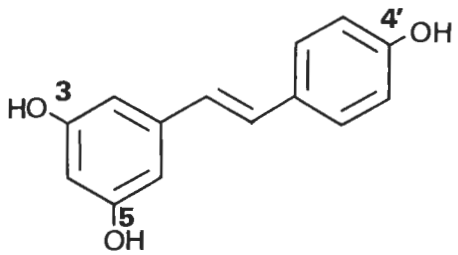

Fig. 1: Chemical structure of *trans*-resveratrol.

Materials and methods: W i n e s a m p l e s : A Bandol wine of the 1993 vintage (var. Mourvèdre) was chosen for this study since it was reported to have a high resveratrol content (ROGGERO and ARCHIER 1994).

E n z y m a t i c t r e a t m e n t o f w i n e a n d e x t r a c t i o n o f r e s v e r a t r o l : To investigate the presence of a glycosidically-bound form of resveratrol in wine, wine samples (50 ml) were treated respectively by ß-D-glucosidase (Sigma, France) or α-glucosidase (Type VI Sigma, France) at 37 °C for 48 h in the dark at pH 5.0 and pH 6.8 where the two enzymes have their maximum activity respectively. Resveratrol was protected from chemical oxidation by maintaining the samples under a stream of nitrogen. Aliquots of 3 ml were taken at various times from the incubation mixtures and extracted by 3 ml of ethyl acetate as previously described in JEANDET *et al.* (1993). For HPLC analysis, 200 µl of ethyl acetate extracts were concentrated and completely redissolved in 200 µl of pure ethanol. Samples were then applied to a lichrocart C_{18} reverse phase column (Merck, 250 x 4 mm; 5 µm) and analysed isocratically with 40 % acetonitrile/60 % water as eluant at a flow rate of 0.6 ml/min. Detection was at 307 nm and 280 nm (corresponding to the *trans*- and *cis*-resveratrol absorbance maxima respectively; JEANDET *et al.* 1994 b). Further identification of the two resveratrol isomers was carried out by GC and GC-MS according to JEANDET *et al.* (1993) or JEANDET *et al.* (1994, submitted). Resveratrol was quantified by comparison with known samples of pure resveratrol which was synthesized as described in JEANDET *et al.* (1991). All experiments were done in triplicate. Standard errors of the 3 measurements were < 5 %.

Results and discussion: As reported by ROGGERO and ARCHIER (1994), the Bandol wine contained high levels of *trans*- resveratrol (ca. 4.7 mg/l) while lower amounts of the *cis*-form (0.90 mg/l) were found in the samples analysed. Treatment of wine by α-D-glucosidase did not modify resveratrol concentration even after 48 h of incubation when compared with the non-treated wines (Fig. 2). In contrast, after the addition of ß-D-glucosidase, there is a rapid increase of the resveratrol content of wine during the first 24 h, by which time the levels of resveratrol had

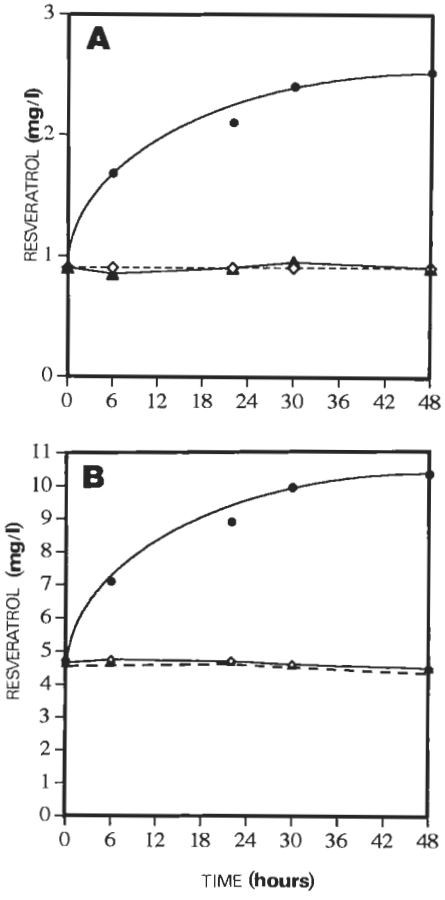

Fig. 2: Time course changes in resveratrol content of wine without (◇) or with addition of ß-D-glucosidase (▲) or α-D-glucosidase (●) at pH 5.0 and pH 6.8 (37 °C). A: Data obtained for *cis*-resveratrol; B: Data obtained for *trans*-resveratrol.

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reached 10 mg/l, *i.e.* more than two fold the values found in the controls or in the samples treated with α-D-glucosidase (Fig. 2). It is interesting to note that both the *cis*- and the *trans*-isomers of resveratrol are glycosilated (Fig. 2, A and B). In both cases, the rate of liberation of resveratrol follows apparent first-order kinetics.

Since ß-D-glucosidase is capable of liberating free resveratrol from a glycosidically-bound form present in wine, it can reasonably be assumed that this compound is glycosilated on one of its free phenol groups. The glycosidically-bound fraction of resveratrol in wine (*i.e.* corresponding to the quantity of aglucone liberated after the cleavage of the glycosidic bond) is probably underestimated in our assay since ß-glucosidase activity can partially be reduced in the presence of ethanol (AYRAN *et al.* 1987).

Glucoside derivatives of resveratrol have previously been described by several authors, *e.g.* presence of a 4'-glucoside in the inner bark of the Siberian pine (*Pinus sibirica*) (GROMOVA *et al.* 1975), in several *Rheum* species (GENICHIRO *et al.* 1977) and a 3-glucoside (piceid) as a minor bark component of Sitka spruce (*Picea sitchensis*) (ARITOMI and DONELLY 1976). To our knowledge, the presence of a resveratrol glucoside in grapevines or wine has, at present, never been reported though ROGGERO and ARCHIER (1994) have suggested the presence, together with resveratrol, of a glucoside in wine, but they have provided no direct evidence of that.

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