DETERMINATION OF THE *TRANS*-RESVERATROL CONTENT OF CHAMPAGNE WINES BY REVERSED-PHASE HPLC

DÉTERMINATION DU CONTENU EN *TRANS*-RESVÉRATROL DES VINS DE CHAMPAGNE PAR CLHP EN PHASE INVERSE

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Abstract: Levels of *trans*-resveratrol in Champagne wines were determined by the use of reversed-phase HPLC with UV and fluorometric detection after liquid-liquid extraction with ethyl acetate. Resveratrol concentrations in Champagne wines range from 20 to 77 µg/L except for the Champagne rosé in which resveratrol reaches several hundred micrograms per litre. The resveratrol content of Champagne wines was also shown to decrease with aging on lees.

Résumé : Le contenu des vins de Champagne en *trans*-resvératrol, un composé aux propriétés biologiques remarquables (anti-oxydantes, antifongiques, anti-bactériennes, anti-tumorales, anti- inflammatoires et cardio- protectrices) a été analysé par chromatographie liquide hautes performances utilisant la détection fluorimétrique couplée à une détection UV. Le resvératrol a été extrait des vins par extraction liquide-liquide avec l'acétate d'éthyle. Les résultats montrent que le Champagne contient peu de resvératrol (entre 20 et 77 µg/L). Le mode de pressurage des raisins en Champagne (dont 3/4 sont des variétés rouges) n'extrait donc pas le resvératrol présent dans la pellicule des baies qui est un composé relativement peu soluble dans l'eau. Les concentrations en resvératrol sont beaucoup plus élevées dans les Champagnes rosés issus d'assemblages vins blancs/ vins rouges (Côteaux champenois) où les teneurs atteignent de 189 à 642 µg/L. Les teneurs en resvératrol du Champagne diminuent au cours du vieillissement en relation avec des phénomènes d'oxydation des composés phénoliques, phénomènes déjà décrits dans le cas d'autres vins effervescents (Cavas d'Espagne).

Key words: stilbenes, Champagne, resveratrol, wine

Mots clés : stilbènes, Champagne, resvératrol, vin

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INTRODUCTION

Resveratrol (*trans* 3,5,4'-trihydroxystilbene) is a phytoalexin produced by grape fruits upon fungal infection or stress (for a review see ADRIAN and JEANDET, 2006). SIEMANN and CREASY (1992) found that resveratrol could occur in grape products and particularly in wine. Interestingly, there are numerous studies which demonstrate resveratrol to have biological effects, namely as a preventive agent against vascular diseases or some cancers (for reviews, see AGGARWAL and SHISHODIA, 2006). Since the pioneering work of SIEMANN and CREASY (1992), many works have focused on the resveratrol content in wines from every important wine-producing area of the world . Surprising as it may seem, there is no similar study concerning the resveratrol content of Champagne wines. We present here data on the concentration of resveratrol in some Champagne wines following extraction with ethyl acetate and analysis by high performance liquid chromatography coupled with UV and fluorometric detection (JEANDET *et al.*, 1997).

MATERIALS AND METHODS

I - WINE SAMPLES

Wines were obtained from different Maisons or Coopératives de Champagne.

II - RESVERATROL EXTRACTION

Champagne wines were degassed in vacuo. Since resveratrol concentration in Champagne wines is relatively low, wines were not injected directly to the HPLC column as previously reported (ADRIAN *et al.*, 2000). Instead, the resveratrol was extracted from wine with an organic solvent before analysis as follows: an aliquot of 100 mL of each Champagne was extracted at room temperature with 100 mL of ethyl acetate, the organic phase being washed with water (twice) (recovery was nearly 100% for resveratrol).

The ethyl acetate was removed at 40°C in vacuo. After evaporation, the residue was completely redissolved in 10 mL of pure ethanol, then filtered through cellulose acetate filters (0.45 µm, Gelman Sciences, MI). The residue was then concentrated and redissolved in 2 x 1 mL of pure ethanol. Extracts were kept at - 80°C until HPLC analysis.

III - HPLC ANALYSIS

Analyses were performed on a Waters (Waters, Milford, MA) Spherisorb S5ODS2 C18 reversed-phase column (250 mm x 4.6 mm, 5 µm) preceded by a guard column (Waters Spherisorb S5ODS2 ; 10 mm x 4.6 mm, 5 µm). A Waters system comprising a model W 600 System controller, a model W717 sample injector, a model W490E multiwavelength detector and a model W474 fluorometer was used. For fluorometric detection, the maximum excitation wavelength was measured at 330 nm and emission at 374 nm (JEANDET *et al.*, 1997). Stilbenes were eluted from the HPLC column with a gradient comprising acetonitrile (solvent A) and water (solvent B) which was modified from that of JEANDET *et al.* (1997) and ADRIAN *et al.* (2000). Linear gradients, from 10% to 50% A, 0 - 35 min ; 50% to 85% A, 35 - 37 min ; 85% A; 37 - 45 min ; 85% to 10% A, 45 - 50 min, 10% A, 50 - 55 min (equilibration before injection of the following sample). The flow rate was 1 mL/min. The standard calibration curve was obtained from peak area vs the following amounts of *trans*-resveratrol : 2, 10, 20, 50, 100 and 200 ng ($r^2 = 0.997$) (detection limit : 1 µg /L). Replicate analyses gave coefficients of variation of 0.6 to 2.3 %. Resveratrol was identified in the chromatograms of the extracts by its retention time (25.22 to 25.46 min).

RESULTS AND DISCUSSION

The amounts of *trans*-resveratrol in the Champagne wines examined are presented in table I. As expected, the resveratrol content of Champagne wines is very low (ranging from 20 to 77 µg/L) compared to that of red wines (ADRIAN *et al.*, 2000) but consistent with that found for white wines (whose values rarely exceed 100 µg/L). Champagne is typically a white wine whose grapes are

Table I - *Trans*-resveratrol content ± [a]SD (µg/L) of some Champagne wines

Contenu en *trans*-resvératrol de quelques vins de Champagne

C1	[b]n = 1	64
C2	n = 3	56 ± 5
C3	n = 2	47 ± 3
C4	n = 5	72 ± 13
C5	n = 2	66 ± 5
C6	n = 3	59 ± 5
C7	n = 2	59 ± 8
C8	n = 4	32 ± 5
C9	n = 3	70 ± 8
C10 [c]	n = 2	20 ± 3
C11 [c]	n = 3	21 ± 3
C12 [c]	n = 1	24
C13 [c]	n = 7	22 ± 6
C14	n = 1	35
C15	n = 3	41 ± 5
C16	n = 2	77 ± 8
C17 rosé	n = 2	189 ± 5
C18 rosé	n = 1	642
C19 rosé	n = 1	362
C20 rosé	n = 1	413

aSD = standard deviation of the measurements
bn = number of bottles analysed
c = Wines vinted exclusively from the Chardonnay variety

hand-harvested and pressed very lightly in the way of limiting the extraction of phenolic compounds from the skins and stems. Although three quarters of this wine are obtained from black varieties i.e. Pinot noir and Pinot Meunier, only small amounts of resveratrol are obtained from pressing. Four Champagne wines analyzed exhibit a relatively high resveratrol content (from 189 up to 642 µg/L). These values correspond to rosé Champagne wines which are the result of the assemblage of a white wine and a red one (Côteaux Champenois) in proportions varying according to the vintage from 90/10 to 80/20 (V/V). Red wines of Champagne usually contain 1 to 3 mg/L of resveratrol (data not shown), values comparable to other red wines. Since red wines contain much more resveratrol than do white wines, it was expected that rosé Champagne wines would contain higher levels of resveratrol.

Data concerning changes in the resveratrol content of two Champagne wines as a result of aging are presented in table II. These wines were obtained from the same producer and vinted under the same conditions. The lowest resveratrol levels are found in older wines (vintages 1990 and 1995). A similar study conducted respectively on a Brut sans année (15 months of aging in contact with lees), a Millésime (three years of aging) and a Cuvée spéciale (seven years of aging) indicates that the concentration of resveratrol decreases with the age of Champagne wines. These results are somewhat in contradiction with our previous study of red Burgundy wines (JEANDET *et al*., 1995), which showed that resveratrol was relatively stable in wine since some aged red wines had more resveratrol than did young red wines. Because the polyphenolic content of Champagne wines is considerably lower than that of the red wines we analyzed, it is likely that resveratrol is less protected from oxidation than in red wines. This could explain the lower resveratrol levels in aged Champagne wines. Another explanation could be that resveratrol was oxidized during aging in contact with lees. This would be in agreement with the finding (IBERN-GOMEZ *et al.*, 2000) that Cavas (Spanish Sparkling wines) undergo colour changes as a result of the oxidation of phenolic compounds (particularly the hydroxycinnamic acids).

Table 2 - *Trans*-resveratrol content (µg/L) ± [a]SD in relation to Champagne aging

Contenu en *trans*-resvératrol et relation avec l'âge du Champagne

Vintage 1997	[b]n = 2	48 ± 5
Vintage 1995	n = 3	15 ± 3
Vintage 1990	n = 2	1
Brut sans année	n = 2	66 ± 3
Millésime	n = 3	39 ± 5
Cuvée spéciale	n = 2	21 ± 5

[a]SD = standard deviation of the measurements

[b]n = number of bottles analyzed

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