DETERMINATION OF BENZOTHIAZOLE IN GRAPES AND WINES

DÉTERMINATION DE BENZOTHIAZOLE DANS LES RAISINS ET LES VINS

S. KARAGIANNIS*, P. LANARIDIS
and Marie-Jeanne SALAHA

Athens Wine Institute (N.AG.RE.F.), 1 Sofokli Venizelou Str,
14123 Lykovrissi, Athens, Greece.

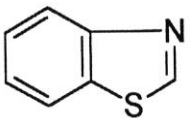
Résumé : Le benzothiazole a été dosé dans les raisins des cépages Muscat Lefko de l'île de Samos et Muscat d'Alexandrie de l'île de Lemnos, issus de parcelles différentes quant à l'altitude, la composition du sol et l'exposition au soleil, et dans les vins correspondants. Ce composé a également été déterminé dans les raisins de dix autres cépages blancs cultivés en Grèce et dans les vins correspondants. Les résultats montrent que cette substance est présente dans tous les raisins examinés ; elle pourrait être formée au cours de la maturation du raisin. Les teneurs en benzothiazole des vins blancs grecs varient de 0.8 à 6.1 µg/l ; elles sont toujours inférieures au seuil de perception.

Key words : Benzothiazole, sulfur compounds, Greek wines

Mots clés : Benzothiazole, composés soufrés, vins grecs

INTRODUCTION

Benzothiazole was the first 'heavy sulfur compound' found in wine, by DUBOIS and BRULE (1970). The term 'heavy sulfur compounds' comprises all volatile sulfur substances of wine with a boiling point higher than 90°C (ANOCIBAR-BELOQUI *et al.* 1995) which are often responsible for undesirable off-flavors. Apart from sulfur, benzothiazole also contains nitrogen in its molecule:



Benzothiazole production in beverages and foodstuffs can possibly take place via different precursors and reactions. It can be formed from the degradation of thiamine (BELITZ and GROSCH 1985) or the reaction of cysteine or cystine with a sugar or an acid (KATO *et al.* 1973). It is likely that formation of benzothiazole is favoured by heat because, in the case of milk, its concentration shows a drastic increase in heated milk (pasteurized or UHT) in comparison with raw milk (MOIO *et al.* 1994).

In low concentrations, benzothiazole does not particularly influence the organoleptic characteristics of wines. However, high concentrations of this compound give rise to an offensive character described as 'mouse odor' (KECK 1989). According to the same author, the odor threshold of benzothiazole is 24 µg/L in model solution and 70 µg/L in wine. LAVIGNE-CRUÈGE (1996) also measured its odor threshold and found it 50 µg/L in model solution, 350 µg/L in white wine and 200 µg/L in red wine. The odor of benzothiazole has been described in the past by terms such as caoutchouc (BERTRAND *et al.* 1994; LAVIGNE-CRUÈGE 1996), burning and rubbery smell (MOIO *et al.* 1994) and the range of its concentrations in wine is determined by various researchers to be 0.4 - 7 µg/L (KECK 1989), 1.5-13 µg/L (LAVIGNE-CRUÈGE 1996), 0-7 µg/L (white wine), 0-15 µg/L (red wine) (BERTRAND *et al.* 1994) and 0.3-13.8 µg/L (RAUHUT *et al.* 1999).

The majority of volatile sulfur compounds is not present in grapes and is formed during fermentation. Some exceptions have been reported such as dimethylsulfoxide, found in both free-run juice and grape skins (LEE 1991), carbonyl sulfide and carbon disulfide formed in freshly crushed, unclarified grape juice (ESCHENBRUCH *et al.* 1986). To our knowledge, benzothiazole has not been measured in grapes or musts until today, with the exception of a research (LAVIGNE-CRUÈGE 1996) demonstrating that its concentration

*Correspondence : wineinst@otenet.gr

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at the beginning and the middle of fermentation of a Semillon blanc must was equal to zero. In the same work it is showed that benzothiazole is found only at the end of the fermentation process. However, in the present investigation, the presence of benzothiazole in grapes is reported and its concentration is measured in grapes and correspondent wines of several Greek white cultivars.

MATERIALS AND METHODS

I - GRAPES

Three series of benzothiazole measurements were carried out in grape juices. Two of them aimed at comparing the concentration of benzothiazole in grapes of the same varieties from different vineyards. For this purpose berries from the varieties Muscat lefko and Muscat of Alexandria, cultivated respectively in Samos and Lemnos islands of the Aegean sea, were used. Another series of measurements took place in order to record possible variations of benzothiazole levels in grapes of some white varieties such as Assyrtiko, Athiri, Savatiano, Rhoditis, Batiki etc., cultivated in various areas of Greece. Clusters with appreciable mold or other damage were discarded during berry sampling. Berries were stored in deep freeze (-15°C) prior to analysis.

II - WINES

All cultivars examined gave white wines under the same vinification procedure: Grapes yielded 65 - 68 p. cent of must after pressings of a screw press. All musts were sulfited to 80 mg/L and were clarified up to 100 NTU. Turbidity measurements were carried out with a HACH 2100P turbidimeter. The samples were allowed to ferment with their endogenous yeasts at controlled temperature (20°C). The resultant wines were racked and received a bisulfite addition of 50 mg/L before bottling. All vinifications were carried out in 1998 at the experimental winery of the Athens Wine Institute.

III - DETERMINATION OF BENZOTHIAZOLE

180 g of berries were crushed for 30 sec at 0°C in a blender. The grape juice was then filtered through a gauze and centrifuged at 15000 g for 15 min at 0°C. If necessary, the supernatant juice was centrifuged at 22000 g for another 15 min. Wines were also centrifuged in case they presented notable turbidity.

25 mL of limpid grape juice or wine and 0.1 mL 1-octanol 20.8 mg/L (internal standard) were added to 25 mL deionized water. The solution was then passed through a column containing 1 g C_{18} (ISOLUTE) initially activated with 15 mL methanol and 15 mL H_2O. C_{18} phase is able to retain lipophilic compounds, such as benzothiazole, from aqueous solutions. Then, 15 mL H_2O were added in the column, in order to remove the hydrophilic compounds. Benzothiazole extraction was achieved with 30 mL CH_2Cl_2. The organic extract was then dried over Na_2SO_4 and CH_2Cl_2 was evaporated up to 0.8 mL by distillation through a Vigreux column. 2 µL of the sample was injected to the GC / MS for analysis.

The unit consisted of a HP 6890 gas chromatograph coupled with a HP 5972 mass selective detector. The GC was equipped with a 25 m x 0.2 mm x 0.2 µm Innowax (crosslinked polyethylene glycol) capillary column which was held at 60 °C for 0.50 min and then ramped at 20°C/min to 115°C and at 1.5°C/min to 140°C using helium gas (back pressure: 18 psi, flow rate: 1 mL/min). The injector temperature was held to 220°C and splitless mode was used. Identification of benzothiazole was accomplished by comparing retention time (R.T. 17.50 min) (figure 1) and mass spectrum (SCAN mode) (figure 2), and with those of the reference compound (Fluka). Selected Ion Monitoring (SIM) mode was used for the quantitative analysis. Target ions were m/z : 84 for 1-octanol and m/z : 108, 135 for benzothiazole (figure 3).

Calibration curves were determined with the use of standard solutions. In the case of grape juices, standard solutions contained glucose 200 g/L, tartaric acid 5 g/L, NaOH added to adjust pH to 3.5 and known amounts of benzothiazole 4.1 – 16.4 µg/L. The equation of the calibration curve was $y = 45.8 x + 0.02$ with $r^2 = 0.992$. In the case of wines, standard solutions contained ethanol 12% vol. instead of glucose. The equation of the calibration curve was $y = 54.9 x + 0.01$ with $r^2 = 0.998$.

The repeatability of the method was tested from 8 analyses of the same grape juice and of the same white wine, as well as the recovery of known amounts of benzothiazole added to a grape juice and to a white wine. Coefficient of variation and recovery were in the case of grape juices 3.5 p. cent and 89 p. cent, and in the case of wines 2.7 p. cent and 91 p. cent respectively.

RESULTS

Table I lists the amounts of benzothiazole in the grapes (given in µg/kg of berries) and the correspondant wines of the cultivars Muscat lefko of Samos island and Muscat of Alexandria of Lemnos island. It is noted that the grapes derived from several vineyards differing in altitude, soil composition, and sun exposure. Grapes had potential alcohol content 14.0 – 14.5 p. cent vol. during harvest. Benzothiazole concentration in grapes of Muscat lefko varies from 0.6 µg/kg up to 3.3 µg/kg. The amounts in the correspondant wines

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TABLE I
Benzothiazole concentration in grapes and wines of Muscat lefko and Muscat of Alexandria varieties (year : 1998)

Tableau I - Concentration de benzothiazole dans les raisins et les vins issus des cépages de Muscat Lefko et de Muscat d'Alexandrie (année 1998)

Variety/Vineyard	Benzothiazole	
	Grapes (µg/kg)	Wines (µg/L)
Muscat lefko		
T3	3.3	1.0
T4	0.6	1.1
T5	1.4	1.6
T7	1.1	0.8
Muscat of Alexandria		
L1	1.2	1.3
L2	1.5	2.0
L3	0.8	1.0
L4	0.6	1.0

range between 0.8 µg/L and 1.6 µg/L. In the case of Muscat of Alexandria, benzothiazole concentration in grapes varies from 0.6 µg/kg – 1.5 µg/kg whereas in wines it ranges from 1.0 µg/L – 2.0 µg/L. These findings indicate that the levels of benzothiazole in the grapes of the same variety exhibit some variation due to each vineyard position (geoclimatic conditions of grapegrowing). The levels of benzothiazole in grapes of Muscat lefko are relatively higher than those of Muscat of Alexandria.

Table II shows the amounts of benzothiazole in grapes and wines of several white varieties cultivated in Greece. Grapes had potential alcohol content 12.0 - 12.5 p. cent vol. during harvest.

Combining the data of tables I and II, it follows that benzothiazole ranges in grapes from 0.5 µg/kg – 6.8 µg/kg. We can assume that the synthesis of this sulfur-containing substance is related to numerous parameters (variety, degree of industrial ripeness, soil composition, climatic conditions, etc.) during berry maturation. Furthermore, the fact that benzothiazole was found in grapes of all vine varieties examined, indicates that it is formed in berries during grapegrowing and not during alcoholic fermentation as it hap-

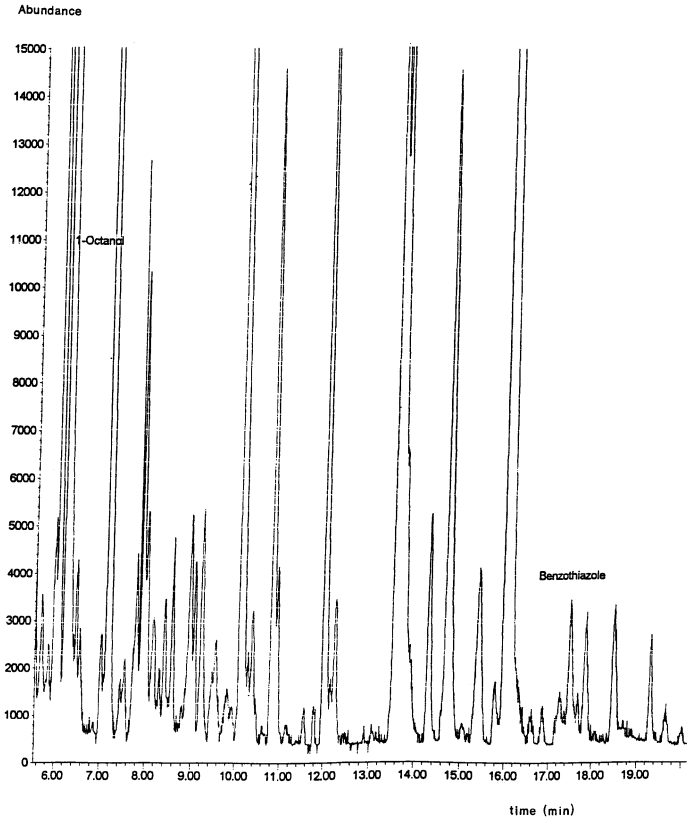


Fig. 1 - Typical chromatogram (SCAN mode) for benzothiazole identification (R.T. 17.50 min).

Fig. 1 - Chromatogramme type (mode scan) pour l'identification du benzothiazole (R.T. 17,50 min).

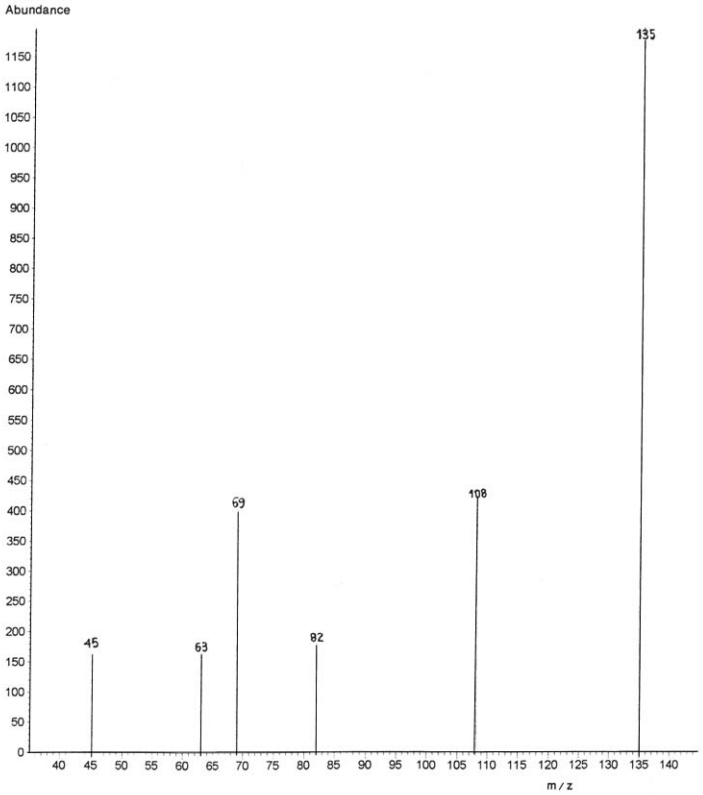


Fig. 2 - Mass spectrum (SCAN mode) of peak with R.T. 17.50 min for benzothiazole identification.

Fig. 2 - Spectre de masse (mode SCAN) des pics pour l'identification du benzothiazole (R.T. 17,50 min).

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TABLE II
Benzothiazole concentration in grapes and wines of several white varieties cultivated in Greece (year : 1998)

Tableau II - Concentration de benzothiazole dans les raisins et les vins issus de plusieurs cépages blancs cultivés en Grèce (année 1998)

Cultivar	Area	Benzothiazole	
		Grapes (µg/kg)	Wines (µg/L)
Athiri	Island of Rhodes	3.5	5.7
Assyrtiko	Island of Santorini	3.9	4.5
Dafni	Island of Crete	3.9	1.5
Batiki	Tyrnavos	4.0	6.1
Plyto	Island of Crete	1.7	2.5
Riesling	Attika	0.9	1.2
Rhoditis	Thiva	1.4	2.3
Rokaniaris	Argolida	0.8	1.4
Savatiano	Thiva	6.8	4.0
Sklavos	Argolida	0.5	1.0

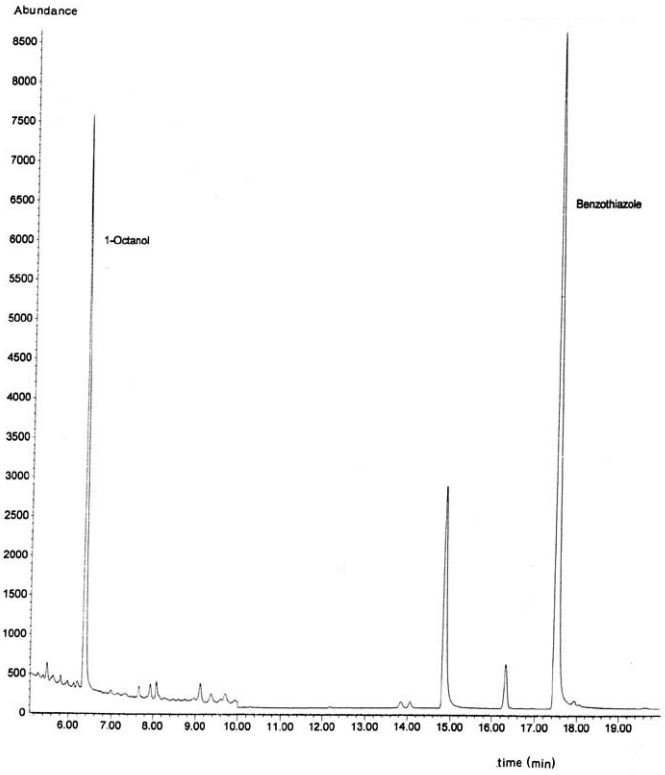


Fig. 3 - Typical chromatogram (SIM mode) for benzothiazole measurement.

Fig. 3 - Typical chromatogramme (mode SIM) pour mesurer le benzothiazole.

pens with almost all the other volatile sulfur compounds. Benzothiazole levels in Greek white wines examined, vary between 0.8 µg/L – 6.1 µg/L and they are in accordance with the range of concentrations given in the literature (BERTRAND *et al.* 1994; KECK 1989; LAVIGNE-CRUÈGE 1996). Also, no wine contained benzothiazole in amounts higher than the odor thresholds, as these have been reported before (KECK 1989; LAVIGNE-CRUÈGE 1996) and no wine presented 'mousy' or 'rubbery' odors.

As mentioned before, the formation of benzothiazole in grapes is possibly due to the reaction of sulfur-containing amino acids with a monosaccharide or an organic acid (KATO *et al.* 1973) or to reactions involving thiamine (BELITZ and GROSCH 1985). The different quantities of those substances in grapes derived from different varieties, and geoclimatic conditions are probably responsible for the variation of benzothiazole levels in mature grapes. However, we shouldn't exclude a possible origin from degradation of phytosanitary products although, to our knowledge, no such correlations have been mentioned in literature until today.

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