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Analysis of 4-vinylphenol and 4-vinylguaiacol in wines from the Württemberg region (Germany)

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1,312 wines from the Württemberg region (Germany) were analyzed by means of HPLC-UV/Vis-FLD for their content of 4-vinylphenol and 4-vinylguaiacol. Vinylphenols were mainly detected in white wines in concentrations between non-detectable and 1,207µg/l. Only 2.13 % of all wines show vinylphenol contents above the sensory threshold. Statistical analysis revealed that there is no correlation between the content of vinylphenols and the results of the sensory evaluation of 'Qualitätswein'. Thus, we suppose that vinylphenols do not have a significant impact on the aroma profile of German wines from the Württemberg region.
Keywords: wine, HPLC, 4-vinylphenol, 4-vinylguaiacol, Württemberg

Analyse von 4-Vinylphenol und 4-Vinylguajacol in Weinen aus dem deutschen Weinbaugebiet Württemberg. *1.312 Weine aus dem deutschen Anbaugebiet Württemberg wurden mittels HPLC-UV/Vis-FLD auf ihren Gehalt an 4-Vinylphenol und 4-Vinylguajakol hin untersucht. Vinylphenole konnten hauptsächlich in Weißweinen nachgewiesen werden mit Konzentrationen zwischen nicht nachweisbar und 1.207µg/l. Nur 2,13 % der Weine zeigen Vinylphenolkonzentrationen oberhalb des sensorischen Schwellenwerts. In der statistischen Auswertung zeigten sich keine Korrelationen zwischen dem Gehalt an Vinylphenolen und der in der sensorischen Prüfung für 'Qualitätswein' vergebenen Punktzahl. Wir vermuten daher, dass Vinylphenole in deutschen Weinen aus dem Anbaugebiet Württemberg keinen signifikanten Einfluss auf das Aromaprofil der Weine haben.*
Schlagwörter: Wein, 4-Vinylphenol, 4-Vinylguajakol, HPLC, Württemberg

L'analyse du 4-vinyl-phénol et du 4-vinyl-guaïacol dans les vins de la région viticole allemande du Württemberg. *1.312 vins de la région viticole allemande Württemberg ont été examinés par voie de HPLC-UV/Vis-FLD en vue de déterminer leur teneur en 4-vinyl-phénol et en 4-vinyl-gaýacol. Pour la plupart, les vinyl-phénols ont pu être détectés dans les vins blancs, les concentrations se situant entre non détectable et 1.207µg/l. 2,13 % des vins seulement présentaient des concentrations de vinyl-phénols au-dessus de la valeur limite sensorielle. L'analyse statistique n'a pas montré de corrélations entre la teneur en vinyl-phénols et le nombre de points attribués lors de l'évaluation sensorielle. Nous supposons donc que les vinyl-phénols dans les vins allemands de la région viticole du Württemberg n'ont aucune influence significative sur le profil aromatique des vins.*
Mots clés: vin, 4-vinyl-phénol, 4-vinyl-gaýacol, HPLC, Württemberg

According to literature vinylphenols are one of the most significant problems in winemaking, since they can alter a wine's flavor significantly. On the one hand they are known to contribute to 'band-aid, spiciness, and gouache'-like off-flavors (van Wyck and Rogers, 2000). But on the other hand they are also known to positively contribute to the broom-like floral aromas and spicy flavors of 'Chardonnay' and 'Gewürztraminer', respectively (Versini, 1985; Versini et al., 1992; Rapp and Versini, 1996). One study showed that nearly one third of the wines from France analyzed shows vinylphenol concentrations above the sensory threshold of 770µg/l. Thus, they play a major role in the flavor profile of these wines (Chatonnet et al., 1995). Other studies have shown that vinylphenols are usually present in concentrations below 1mg/l. If their concentration exceeds this 1mg/l margin, the wines normally inevitably show a deteriorated flavor (Chatonnet et al.,

1992; CHATONNET et al., 1993). Therefore, a very narrow range between sensory threshold and concentrations having a detrimental effect on flavor seems to exist for these wine flavor compounds.

The formation of 4-vinylphenol and 4-vinylguaiacol mainly occurs during alcoholic fermentation from their precursors p-coumaric and ferulic acid, respectively, by certain yeasts like *Saccharomyces cerevisiae* and non-*Saccharomyces cerevisiae* strains, such as *Candida* sp., and bacteria like *Pediococcus* sp., or *Cryptococcus* sp. These strains possess a stereospecific decarboxylase for the E (trans)-forms of the aforementioned hydroxycinnamic acids (DUGELAY et al., 1993; SHINOHARA et al., 2000; COUTO et al., 2006). In literature they are often referred to as POF+, i.e. 'phenolic off-flavor positive' (GRANDO et al.,1993). Oligomeric procyanidins are known to inhibit this enzyme, which explains the generally low vinylphenol concentrations found in red wine (CHATONNET et al., 1990). Some commercially available enzymes have been blamed for giving rise to vinylphenol concentrations especially in white wines due to decarboxylase side-activities in non-purified preparations (KOHLER et al., 1999).

Analytically, vinylphenols have been measured using GC and MS techniques, mostly coupled with various sample extraction methods (DOMINGUEZ et al., 2002; BOUTOU and CHATONNET, 2007; PIZARRO et al., 2007). These methods are quite laborious and costly due to the complicated sample preparation and the specialized laboratory equipment. More recently, also HPLC methods have been used for the simultaneous measurement of vinyl- and ethylphenols with the aid of coulometric or fluorimetric detectors, some of them without the need of sample preparation prior to analysis (SIEGRIST et al., 1993; LARCHER et al., 2007; NICOLINI et al., 2007).

While there is already some data published on vinylphenol concentrations in wines from several regions of the world, there is still data lacking from Germany. Furthermore, to-date it is not clear if these compounds play an important role in the flavor profile of German wines. Thus, our main goal was to apply a modified simple HPLC method for the measurement of vinylphenols in wine and to analyze German wines from the Württemberg region. We also correlated the vinylphenol concentrations with the results from the sensory panel of the approval board for 'Qualitätswein' in order to assess the impact of these compounds on wine quality and their sensory relevance.

Materials and methods

Wine samples

1,312 varietal wines or cuvees from 58 grape varieties were collected from the 'Qualitätsweinprüfstelle' (approval board for 'Qualitätswein') in Weinsberg (Germany) directly before each sensory evaluation. The samples were transferred from the freshly opened wine bottle into HPLC vials, immediately transferred to the air-conditioned HPLC laboratory (21°C room temperature), and injected into the HPLC. To avoid discrimination of the wine's polyphenolic constituents, we did not filter the samples prior to analysis, since some authors have experienced problems with several filter types (LAMUELA-RAVENTÓS et al., 1995).

Sensory evaluation

The sensory panel consisted of 3 or 4 wine professionals and a maximum of 60 samples was presented in ISO tasting glasses at each series in the sensory evaluation room of the 'Qualitätsweinprüfstelle' in Weinsberg (Germany) at ambient room temperature. Panelists were asked to assess the wines using the 5-point system of the DLG (German Agricultural Society, Frankfurt/M., Germany), where 5 is the best, 0 the worst rating. Wines have to receive at least 1.5 points to comply with the German regulations for 'Qualitätswein'.

Chemicals

KH_2PO_4, and H_3PO_4, acetonitrile, water (HPLC grade) and methanol (gradient grade) were from VWR (Darmstadt, Germany). 4-Vinylphenol and 4-vinylguaiacol were from AlfaAesar (Karlsruhe, Germany) and used without any further purification.

HPLC analysis

Analysis of vinylphenols was performed using the method published by NICOLINI et al. (2007) in a slightly modified form. Briefly, all samples were directly injected without pre-filtration into a Dionex UltiMate 3,000 HPLC (Dionex, Idstein, Germany) equipped

Table 1: Vinylphenol concentration (µg/l) of wines from the Württemberg region (n.d.: not detected)

Variable	n	Min.	Max.	Mean	Standard deviation
4-Vinylphenol	1312	n.d.	1207	76.6	154.4
4-Vinylguaiacol	1312	n.d.	835	27.5	63.1

Table 2: Vinylphenol concentration (µg/l) of wines from the Württemberg region according to wine type (n.d.: not detected) different characters at mean values indicate significant statistical difference at $p < 0.05$ level (n = 1,312)

Compound	Wine type	n	Min.	Max.	Median	Mean	Standard deviation
4-Vinylphenol	White wine	548	n.d.	1207	117	176 a	197.4
	Weißherbst	129	n.d.	256	n.d.	22 b	49.3
	Blanc de noirs	12	n.d.	86	n.d.	19 b	27.3
	Rosé	34	n.d.	134	n.d.	16 b	35.6
	Red wine	589	n.d.	524	n.d.	1 b	22.0
4-Vinylguaiacol	White wine	548	n.d.	836	40	64 a	84.6
	Blanc de noirs	12	n.d.	28	n.d.	9 b	11.1
	Rosé	34	n.d.	62	n.d.	7 b	16.8
	Weißherbst	129	n.d.	119	n.d.	6 b	16.2
	Red wine	589	n.d.	18	n.d.	n.d. b	0.7

with a photodiode-array and a fluorescence detector. The isocratic eluent consisted of 50mM KH_2PO_4 (adjusted to pH 3.4 with H_3PO_4), acetonitrile, and methanol (65:30:5; v/v/v) and the flux was set to 1.5ml/min. A LiChroCART 250-4 LiChrospher 100 RP-18 (5µm) column (Merck, Darmstadt, Germany) was used for the separation of the compounds and was maintained at 40°C. Injection volume was 10µl. Detection and quantification of vinylphenols were achieved using the fluorescence detector with external standardization. The respective wavelengths were set at 225nm (excitation) and 320nm (emission). A linear calibration curve was obtained with standard concentrations between 20 and 2,000µg/l. Detection limit was 11µg/l for both substances. The photodiode-array detector was set to 280, 310, 320, 360, and 520nm to check peak purity and to detect other possible interfering phenolic substances.

Statistical analysis

All statistical analysis was performed using Excel (Microsoft, Redmond, USA), XLStat (Addinsoft, Paris, France), and SPSS (SPSS, Chicago, USA). ANOVA was conducted on variety, wine type, region, winery, vintage, quality level, taste, storage vessel type, and DLG points using a confidence interval of 95 %. Tukey's $HSD_{0.05}$ was used as the means separation test.

Results and discussion

The concentrations of the vinylphenols are given in Table 1. 4-Vinylphenol concentrations are higher than those of 4-vinylguaiacol. This is mainly due to the fact that grapes and wines generally show higher contents of p-coumaric than of ferulic acid (Pour Nikfardjam et al., 2006; Pour Nikfardjam et al., 2007). Both phenols act as precursors for the formation of vinylphenols and thus more 4-vinylphenol than 4-vinylguaiacol can be formed. In accordance with literature vinylphenol concentrations were significantly higher in white wines than in 'Weißherbst' (rosé made from a single grape variety), rosé, red or blanc de noirs wines (Table 2). This is obviously due to the higher proanthocyanidin concentrations in red grape varieties, which suppress the enzymatic decarboxylation of p-coumaric and ferulic acid, respectively (Chatonnet et al., 1990; Chatonnet et al., 1993).

The German quality wine system is divided into several categories: 'Qualitätswein' (quality wine) and 'Prädikatswein' (quality wine with predicate). The latter is further divided into the following subcategories with increasing quality: 'Kabinett', 'Spätlese', 'Auslese', 'Beerenauslese', 'Trockenbeerenauslese', and 'Eiswein'. According to our results there is no significant influence of the wine category on vinylphenol concentrations, yet there is a certain trend towards 'Beerenauslese' and 'Spätlese'. The higher contents of vinylphenols might be due to the much more advanced ripeness stage of the grapes. At harvest, grapes for 'Spätlese' wine have to exhibit at least 85°Oe (= 20°Bx), for 'Beerenauslese' the value of 125°Oe (= 29.41°Bx) is even much higher. At more progressed ripeness stages grapes generally show higher concentrations of polyphenols (Ong and Nagel, 1978; Singleton et al., 1986; Pérez-Magariño and González-San José, 2006), which might then act as a pool for the decarboxylase of the fermenting yeasts leading to vinylphenols.

The residual sugar content of the wines (dry, half-dry, mild, sweet) showed no significant influence on vinylphenol concentrations, yet a strong influence was found for the vintage year. 2007 was the year with the highest concentrations of vinylphenols followed by vintages of older age. Presumably, vinylphenols are decomposed

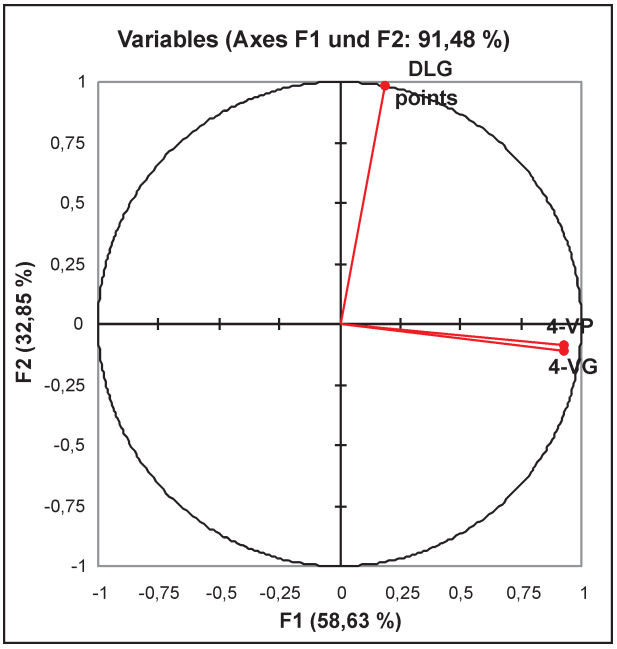

Fig. 1: Principal component analysis of wines from the German Württemberg region based on 4-vinylphenol, 4-vinylguaiacol content and DLG points (n = 1,312)

during wine aging. Especially in red wines they are known to react with anthocyanins leading to pyranoanthocyanins and related structures (Oliviera et al., 2006). In white wines they might react with procyanidins, flavor compounds, proteins and other wine constituents.

A highly significant correlation was found between vinylphenol concentration and origin of the wines. Especially high concentrations were found in wines from the 'Remstal-Stuttgart' area, which is located around the city of Stuttgart and along the river Rems in the south-west of Germany. The main varieties from this region are 'Riesling', 'Trollinger', and 'Kerner'. Especially 'Riesling' showed high contents of vinylphenols (means: 4-vinlyphenol 220µg/l; 4-vinylguaiacol 103µg/l). We could not find any explanation for this significant difference between the regions.

In Germany the storage vessel type (wooden barrel, Barrique) can be added as an indication of quality on the label

- if at least 75 % of the wine has been stored in wooden barrels for at least 6 months (in case of red wine) or 4 months (if other than red wine); or
- if a respective part of the wine has been stored in barrels made of oak wood with a volume of less than 350 l.

A significant correlation was found between vinylphenol concentration and storage vessel type. The highest amounts were found in wines, which had no vessel type indication. Presumably, these wines were made in vessels other than wood, such as stainless steel or glass-fiber reinforced plastic. It is well known that storage in wooden vessels bears the peril of a possible infection with *Brettanomyces* yeast. *Brettanomyces* possesses a vinylphenol reductase, which converts the vinylphenols into the respective highly odorous ethylphenols. The latter are responsible for the *Brettanomyces* off-flavor (Chatonnet et al., 1992; Chatonnet et al., 1993). In fact we could detect significantly higher ethylphenol concentrations in the wines from wooden casks (Pour Nikfardjam & May 2008). Apart from a possible *Brettanomyces* infection, the much more oxidative milieu in wooden casks would also support the decomposition of vinylphenols. This shows that in these cases a decomposition of the vinylphenols is very likely and, thus, the higher concentrations in wines from non-wood vessels is understandable.

The ratio between 4-vinylphenol and 4-vinylguaiacol was dependent on variety and highest in 'Rivaner', 'Chardonnay', 'Pinot gris', 'Müller-Thurgau', and 'Silvaner' with values of 5.786, 5.039, 3.928, 3.898, and 3.748, respectively. Especially these varieties are known for their discreet fruitiness and broom-like flavors, in which vinylphenols are thought to play a major role (Versini et al., 1992; Chatonnet et al., 1995). Yet as

Table 3: Statistical results (independent two sample t-test) of the sensory results of the wines with vinylphenol concentrations above and below threshold level (n = 1,312)

Variable	n	Min.	Max.	Mean	Standard deviation	t-value (observed)	t-value (critical)	p-value (two sample)
DLG points (above threshold)	29	1.0	3.9	2.328	0.602	1.809	1.962	0.0707
DLG points (below threshold)	1284	0.0	4.5	2.101	0.667			

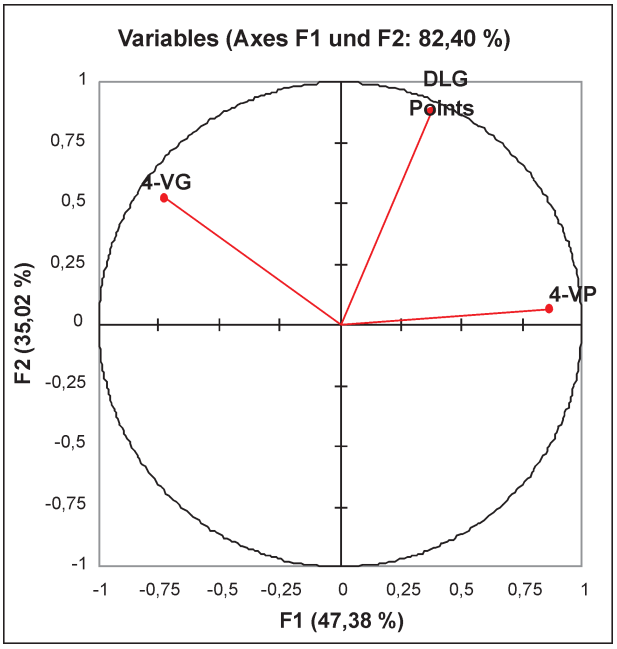

Fig. 2: Principal Component Analysis of wines from the German Württemberg region based on 4-vinylphenol, 4-vinylguaiacol content and DLG points, and showing vinylphenol concentrations above the sensory threshold of 770µg/l (n = 28)

shown in Figure 1 no correlation exists between the sensory results (DLG points) and the concentrations of vinylphenols in wine.

Generally, only 28 wines out of 1,312 (=2.13 %) show vinylphenol contents above the sensory threshold of 770µg/l. These wines received between 1.0 and 3.9 DLG points with a mean of 2.3, and the first and third quartile at 2.0 and 2.6, respectively. Thus, they were slightly higher rated than the wines with vinylphenol concentrations below sensory threshold level, which received points between 0 and 4.5 points with a mean of 2.1, and the first and third quartile at 1.8 and 2.5, respectively. Yet there was no statistically significant difference between both groups ($p < 0.07$) (Table 3).

If principal component analysis is conducted only for those 28 wines with vinylphenol concentrations above sensory threshold level, the correlation between all factors is even worse (Fig. 2). No correlation can be observed between DLG points and 4-vinylguaiacol. A negative correlation exists between both vinylphenols and only a slight positive correlation exists between DLG points and 4-vinylphenol. Thus, it can be concluded that the contents are generally too low to have a direct impact on the sensory quality of the wine. When the concentration exceeds sensory threshold levels, the impact of vinylphenols on quality rating tends to be slightly better than for wine below threshold. Yet this trend is not statistically significant ($p < 0.07$). Thus, despite this slight trend and due to the small number of wines reaching vinylphenol concentrations above sensory threshold we speculate that the impact of vinylphenols as such for the flavor profile of German wine from the Württemberg region is rather negligible.

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References

Boutou, S. and Chatonnet, P. 2007: Rapid headspace solid-phase microextraction/gas chromatographic/mass spectrometric assay for the quantitative determination of some of the main odorants causing off-flavours in wine. J. Chromatogr. A 1141: 1-9

Chatonnet, P., Boidron, J.N. and Pons, M. 1990: Elevage des vins rouges en fûts de chêne : Evolution de certains composés volatils et de leur impact aromatique. Sci. Alim. 10: 565-587

Chatonnet, P., Dubourdieu, D., Boidron, J.N. and Pons, M. 1992: The origin of ethylphenols in wines. J. Sci. Food Agric. 60: 165-178

Chatonnet, P. Dubourdieu, D., Boidron, J.N. and Lavigne, V. 1993: Synthesis of volatile phenols by *Saccharomyces cerevisiae* in wines. J. Sci. Food Agric. 62: 191-202

Chatonnet, P., Dubourdieu, D. and Boidron, J.N. 1995: The influence of *Brettanomyces/Dekkera* sp. yeast and lactic acid bacteria on the ethylphenol content of red wines. Am. J. Enol. Vitic. 46: 463-468

Couto, J.A., Campos, F.M., Figueiredo, A.R. and Hogg, T.A. 2006: Ability of lactic acid bacteria to produce volatile phenols. Am. J. Enol. Vitic. 57: 166-171

Dominguez, C. Guillén, D.A. and Barroso, C.G. 2002: Determination of volatile phenols in fino sherry wines. Anal. Chim. Acta 458: 95-102

Dugelay, I., Gunata, Z., Sapis, J.C., Baumes, R. and Bayonove, C. 1993: Role of cinnamoyl esterase activities from enzyme preparations on the formation of volatile phenols during winemaking. J. Agric. Food Chem. 41: 2092-2096

Grando, M.S., Versini, G., Nicolini, G. and Mattivi, F. 1993: Selective use of wine yeast strains having different volatile phenols production. Vitis 32: 43-50

Köhler, H.J., Curschmann, K., Geßner, M. and Schmitt, A. 1999: Enzymeinsatz: Leichtere Verarbeitung - bessere Qualität. Dt. Weinmagazin (22): 13-17

Lamuela-Raventos, R.M., Romero-Perez, A.I., Waterhouse, A.L. and de la Torre-Boronat, M.C. 1995: Direct HPLC analysis of cis- and trans-resveratrol and piceid

isomers in Spanish red *Vitis vinifera* wines. J. Agric. Food Chem. 43: 281-283

Larcher, R., Nicolini, G., Puecher, C., Bertoldi, D., Moser, S. and Favaro, G. 2007: Determination of volatile phenols in wine using high-performance liquid chromatography with a coulometric array detector. Anal. Chim. Acta 582: 55-60

Nicolini, G., Larcher, R., Bertoldi, D., Puecher, C. and Magno, F. 2007: Rapid quantification of 4-ethylphenol in wine using high-performance liquid chromatography with a fluorimetric detector. Vitis 46: 202-206

Oliviera, J., Santos-Buelga, C., Silva, A.M.S., de Freitas, V. and Mateus, N. 2006: Chromatic and structural features of blue anthocyanin-derived pigments present in Port wine. Anal. Chim. Acta 563: 2-9

Ong, B.Y. and Nagel, C.W. 1978: Hydroxycinnamic acid-tartaric acid ester content in mature grapes and during the maturation of White Riesling grapes. Am. J. Enol. Vitic. 29: 277-281

Pérez-Magariño, S. and González-San José, M.L. 2006: Polyphenols and colour variability of red wines made from grapes harvested at different ripeness grade. Food Chem. 96: 197-208

Pizarro, C., Pérez-del-Notario, N. and González-Sáiz, J.M. 2007: Determination of Brett character responsible compounds in wines by using multiple headspace solid-phase microextraction. J. Chromatogr. A 1143: 176-181

Pour Nikfardjam, M.S., Márk, L., Avar, P., Figler, M. and Ohmacht, R. 2006: Polyphenols, anthocyanins, and trans-resveratrol in red wines from the Hungarian Villány region. Food Chem. 98: 453-462

Pour Nikfardjam, M.S., Köhler, H.J., Schmitt, A., Patz, C.D. and Dietrich, H. 2007: Polyphenolic composition of German white wines and its use for the identification of cultivar. Mitt. Klosterneuburg 57: 146-152

Pour Nikfardjam, M. and May, B. 2008: Brettanomyces - Status quo in Württemberg. Dt. Weinbau (21): 16-18

Rapp, A. and Versini, G. 1996: Volatile phenolic compounds in wine. Dt. Lebensm.-Rundsch. 92: 42-48

Shinohara, T., Kubodera, S. and Yanagida, F. 2000: Distribution of phenolic yeasts and production of phenolic off-flavors in wine fermentation. J. Biosci. Bioeng. 90: 90-97

Siegrist, J., Salles, C. and Etievant, P. 1993: HPLC determination of volatile phenols in wines. Chromatographia 35: 50-54

Singleton, V.L., Zaya, J. and Trousdale, E. 1986: Compositional changes in ripening grapes: Caftaric and coutaric acids. Vitis 25: 107-117

van Wyck, C.J. and Rogers, I.M. 2000: A "phenolic" off-odour in white table wines: Causes and methods to diminish its occurrence. S. Afr. J. Enol. Vitic. 21: 52-57

Versini, G. 1985: Sull'aroma del vino Traminer Aromatico o Gewürztraminer. Vignevini 12: 57-65

Versini, G., Dalla Serra, A., Falcetti, M. and Sferlazzo, G. 1992: Rôle du clone, du millésime et de l'époque de la récolte sur le potentiel aromatique du raisin de Chardonnay. Rev. Oenol. 18: 19-23

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