

Phenolic and Volatile Composition of Wines Made from *Vitis vinifera* Cv. Muscat Lefko Grapes from the Island of Samos

Stelios Karagiannis,* Anastasios Economou, and Panagiotis Lanaridis

The Athens Wine Institute, National Agricultural Research Foundation, 1 Sofokli Venizelou Street, Athens 141 23, Greece

A study of the phenolic and volatile composition of wines produced from the white cultivar Muscat lefko from the island of Samos was conducted. Dry, fortified, naturally sweet wines and mistelles (aged and nonaged) have been studied. The phenolic components (flavan-3-ols, hydroxycinnamates, and flavonols) were measured by high-performance liquid chromatography after solid phase extraction (SPE). The terpenes (free and glycosidically linked) were determined by the use of gas chromatography–mass spectrometry (GC-MS) after SPE. The fermentation aroma components were analyzed by GC-MS after liquid–liquid extraction. It was found that the dry wines contained lower amounts of most of the phenolics and higher quantities of terpenes and fermentation aroma compounds than the sweet wines. The aged mistelle wines contained lower levels of coumaric and caftaric acids, higher concentrations of the free acids, and markedly fewer free and bound terpenes and fermentation aroma components compared to the other sweet wines. The naturally sweet wine contained relatively increased amounts of phenolics, 2,3-butanediol, and glycosidically linked terpenes.

Keywords: Wine volatiles; fermentation aroma; terpenes; phenolics; Muscat lefko

INTRODUCTION

Phenolic compounds are important constituents of wines because they greatly contribute to the sensory properties and other attributes of wines. In particular, phenolics are associated with bitterness, astringency, and color stability and also possess potent antioxidant, anticancer, and anti-inflammatory properties (Kunhau, 1976; Singleton and Noble, 1976; Noble, 1990; Singleton, 1992; Teissedre et al., 1996; Soleas et al., 1997). Until recently, these beneficial effects were associated with red wines that, generally, contain total phenols in concentrations 10–20-fold higher than those in white wines. To date, this is why the analysis of phenolics in wine has been mainly concentrated on red varieties. However, the claim that the consumption of red wines results in more pronounced physiological effects than the consumption of white wines is nowadays seriously questioned (Goldberg et al., 1999). Therefore, it becomes increasingly important to study and screen quantitatively different varieties of white wines with respect to their phenolic content. In white wines, the hydroxycinnamic acid derivatives (i.e., the esters of caffeic and coumaric acid with tartrate, caftaric acid, and coumaric acid) constitute the vast majority of total phenols. In addition, small quantities of flavanols and flavonols may also exist.

It is, also, widely recognized that the characteristic taste and aroma of grapes and wines of some white cultivars such as Muscat, Riesling, and Gewürztraminer are associated with the presence of various volatile monoterpene compounds (Williams et al., 1981; Marais, 1983; Bayonove, 1992). Parameters such as soil, climate, viticultural practices, and wine-making processes strongly

influence the free (volatile) and glycosidically bound (nonvolatile) monoterpene concentrations in grapes and wines (Marais, 1983; Marais and van Wyk, 1986; Macaulay and Morris, 1993; Reynolds and Wardle, 1996; Salaha et al., 2000). Furthermore, it is known that several volatile compounds are responsible for the fermentation aroma in wines: ethyl esters of C6, C8, and C10 fatty acids and acetates of higher alcohols enhance the fruity and floral character of white wines, whereas large amounts of higher alcohols and volatile acids may degrade wine aroma (Bertrand et al., 1994; Navarre, 1998). Therefore, it is obvious that quantitative determination of all the above-mentioned substances is necessary to elucidate the aromatic intensity of white wines.

Muscat lefko is a famous white grape cultivar highly esteemed in Greece for its potential to produce high-quality sweet and dry wines. As no previous report exists in the pertinent literature on wines produced from this cultivar, the objective of this study was to examine the phenolic and volatile composition of several Muscat lefko wines (dry and sweet wines as well as aged and nonaged mistelles) produced by the same winery on the island of Samos.

MATERIALS AND METHODS

Chemicals. All chemicals used in this work were of analytical grade. Catechin, caffeic acid, geraniol, and vanillin were purchased from Fluka (Steinheim, Germany) and epicatechin, *p*-coumaric acid, and nerol from Sigma (Steinheim, Germany). HPLC grade solvents (acetonitrile and methanol) were purchased from LabScan Analytical Sciences (Dublin, Ireland). Sodium hydroxide and phosphoric acid were purchased from Riedel-de Häen (Seelze, Germany), whereas dichloromethane and analytical grade methanol were provided by Merck. Caffeoyltartaric acid ester was kindly donated by A. Waterhouse (University of California, Davis). Myricetin and

* Corresponding author [e-mail wineinst@otenet.gr; telephone (+301) 2828111; fax (+301) 2844954].

Table 1. Solvent Gradient for the Analysis of Phenolics (Solvent A, Phosphate Buffer, pH 2.6; Solvent B, 20% Solvent A and 80% Acetonitrile)

flavanols (280 nm), flavonols (365 nm)			hydroxycinnamates (313 nm)		
time (min)	% A	% B	time (min)	% A	% B
0–5	100	0	0–5	100	0
5.01–23	82	18	5.01–23	82	18
23.01–35	70	30	23.01–35	70	30
35.01–40	60	40	35.01–45	60	40
40.01–45	0	100	45.01–50	0	100
45.1–50	100	0	50.01–0	100	0

quercetin were donated by G. Volikakis (University of Athens, Greece). Volatile ethyl esters, fatty acids, higher alcohols, acetates, 2,3-butanediol, and butyric and 2-methylpropionic acids were purchased from PolyScience (Niles, IL). Gallic acid, linalool, citronellol, and α -terpineol were provided by Merck (Darmstadt, Germany). Sep-Pak SPE C-18 cartridges (500 mg of sorbent) were purchased from Waters (Milford, MA).

Experimental Procedure. The sample pretreatment for the analysis of phenolics was similar to previously reported methods (Jaworski and Lee, 1987; Oszmianski et al., 1988). The SPE cartridge was conditioned sequentially with 4 mL of methanol and 4 mL of Britton–Robinson (B–R) buffer solution at pH 7. Five milliliters of the wine was subjected to rotary evaporation at 30 °C to remove ethanol, the pH was adjusted to 7 using NaOH, and the solution was made up to original volume (5 mL) with Millipore water. Four milliliters of this solution was passed through the preconditioned Sep-Pak C-18 SPE cartridge, and the cartridge was further washed with 4 mL of B–R buffer, pH 7; the neutral phenolics (such as the flavanols, flavonols, and vanillin) were retained on the packing material, whereas the acidic phenolics (acids and esters) were eluted. The neutrals were recovered by washing the cartridge with methanol and keeping the first 4 mL for the analysis. The eluate was buffered to pH 2 with HCl and applied to a second SPE cartridge previously activated with 4 mL of methanol and 4 mL of water at pH 2. The acidic fraction was retained on the cartridge. The cartridge was washed with 4 mL of water at pH 2, and the acidic compounds were recovered with 4 mL of methanol. Calibration curves for catechin, epicatechin, and vanillin were constructed using standard solutions and measured at 280 nm by HPLC. The hydroxycinnamates were quantified using the response factor for caffeic acid at 313 nm, whereas myricetin and quercetin were quantified using the response factor for quercetin at 365 nm. A calibration curve for gallic acid was constructed using standard solutions with detection at 313 nm. All of the standards were made up in methanol. A Hewlett-Packard model 1050 chromatographic workstation (Waldbronn, Germany) was used interfaced to a quaternary gradient pump, a UV detector, a degassing unit, a Rheodyne valve (equipped with a 5 μ L loop), and an analytical RP C-18 Kromasil column (5 μ m, 250 mm \times 4.5 mm i.d., purchased from MZ, Mainz, Germany) operating at 30 °C. A two-solvent gradient was used for the analysis as indicated in Table 1. The solvents were filtered through a 0.45 μ m filter prior to use and stored at 4 °C. The flow rate of the mobile phase was 0.6 mL/min.

Analysis for free and glycosidically linked terpenes was carried out according to the method of Di Stefano (1991). Twenty-five milliliters of centrifuged wine and 0.1 mL of 1-octanol [19.4 mg/L (internal standard)] were added to 25 mL of deionized water. The mixture was then passed under suction through an SPE column containing 1 g of C-18 (IST Ltd., U.K.) already activated with 10 mL of CH₃OH and 20 mL of H₂O. The hydrophilic compounds were eliminated by addition of 20 mL of H₂O. Extraction of free and mono- and dihydroxylated terpenes was done with 35 mL of CH₂Cl₂. The extract was dried over Na₂SO₄, and the solvent was removed up to 1 mL by distillation through a Vigreux column. Two microliters of the sample was injected into the GC-MS for analysis. Extraction of trihydroxylated and glycosidically linked terpenes was

achieved with 30 mL of CH₃OH. The solvent was removed in a rotary evaporator (25–30 °C), and then 3 mL of phosphate–citrate buffer, pH 5, was added as well as 70 mg of the β -glucosidase enzyme Novoferm 12 G (Novo Nordisk Ferment Ltd, Dittingen, Switzerland). The enzyme was allowed to react for 24 h at 37 °C. After addition of 0.1 mL of the internal standard, the free terpenes produced were extracted with 30 mL of CH₂Cl₂. The solvent was then removed as described above to a final volume of 1 mL. Two microliters of the sample was injected into the GC-MS for analysis. The GC-MS unit consisted of a Hewlett-Packard 6890 gas chromatograph coupled to an HP 5972 mass selective detector (Avondale, PA). The GC was equipped with a Hewlett-Packard 25 m \times 0.2 mm \times 0.2 μ m Innowax [cross-linked poly(ethylene glycol)] capillary column. The chromatographic conditions were as follows: initial temperature, 60 °C for 5 min and then ramped at a rate of 1.5 °C/min to 140 °C and at 3 °C/min to 205 °C using helium as the carrier gas (column head pressure = 18 psi, flow rate = 1 mL/min). The injector and transfer line temperatures were held at 200 and 280 °C, respectively. Identification of compounds was accomplished by comparing retention times and mass spectra (SCAN) made with those of reference standards. Quantitative analysis was carried out by the use of the selective ion monitoring (SIM) mode. Selected ions were *m/z* 71 and 93 for linalool, *m/z* 121 and 136 for α -terpineol, *m/z* 69 and 82 for citronellol, and *m/z* 69 and 93 for nerol and geraniol.

Ethyl esters (hexanoate, octanoate, and decanoate), fatty acids (hexanoic, octanoic, and decanoic), higher alcohols (isobutanol, amyl alcohols, 1-hexanol, and 2-phenylethanol), acetates of higher alcohols (isoamyl acetate and 2-phenylethyl acetate), diethyl succinate, 2,3-butanediol, and volatile acids (butyric and 2-methylpropionic) were also measured by GC-MS. The extraction procedure is thoroughly described in detail (Bertrand, 1992). Chromatographic conditions were as follows: initial temperature, 50 °C for 5 min and then ramped at 3 °C/min to 200 °C using helium as carrier gas (column head pressure = 17 psi, flow rate = 1 mL/min). The injector and transfer line temperatures were held at 250 and 280 °C, respectively. Identification of compounds was accomplished by comparing retention times and mass spectra with those of reference standards. Quantitation was carried out by the use of the SCAN mode because these substances are found in relatively large amounts (>0.1 mg/L) in wine and no overlapping of peaks occur.

All analyses were carried out in duplicate. Concentrations given in the tables are mean values.

Samples. The vineyard of the island of Samos covers a surface of 1400 ha yielding ~5–10 t/ha of Muscat lefko grapes. Grape juice contains sugars at a minimum of 195 g/L and 5.5 g/L minimum total acids. Pneumatic presses are used for the pressing of the grapes, and free-run juice is added to the first pressings. High pressings are kept separately. Vinification takes place in containers of 40–60 t without yeast inoculation. All of the wines analyzed in the present study are commercial and produced from the only winery existing on the island of Samos. Because the Muscat lefko cultivar is exclusively grown on the island of Samos, the wines selected for this study were considered as typical of this cultivar. The following types of wine were examined: dry wines (samples 1–3) produced by conventional white vinification, with sample 1 having been vinified from selected grapes; mistelles (samples 4–6) in which no fermentation took place due to addition of alcohol to the must. Samples 4 and 5 were matured in barrels for 5 years. The fermentation of fortified sweet wines (samples 7 and 8) was interrupted upon addition of alcohol. The grapes for a naturally sweet wine (sample 9) were exposed to sunlight for 5 days before pressing. The fermentation of this sample was stopped without any alcohol addition. No maceration was carried out in any of these wines. All of the wines were vinified and bottled in 1997, except for samples 4 and 5 (aged mistelles) that were vinified in 1992 and bottled in 1997. The results of the standard analyses are given in Table 2.

Table 2. Standard Analyses of Dry, Sweet Wines and Mistelles from the Muscat Lefko Cultivar from the Island of Samos

sample	type of wine	volatile acidity (g/L)	total acidity (g/L)	pH	sugars (g/L)	alcohol content (% vol)
1	dry Muscat	0.34	5.6	3.30	1.9	12.48
2	dry Muscat	0.32	5.6	3.26	1.4	12.30
3	dry Muscat	0.29	5.6	3.23	1.5	12.35
4	aged mistelle	0.52	5.4	3.45	196	15.03
5	aged mistelle	0.41	5.0	3.51	194	15.00
6	nonaged mistelle	0.26	4.1	3.55	192	15.15
7	fortified sweet	0.29	4.6	3.35	116	15.08
8	fortified sweet	0.28	4.8	3.35	123	15.00
9	naturally sweet	0.33	5.3	3.41	140	14.09

Table 3. Analysis of Some Phenolics (in Milligrams per Liter) in Dry, Sweet Wines and Mistelles from the Muscat Lefko Cultivar from the Island of Samos

sample	catechin	epicatechin	caftaric acid	coutaric acid	caffeic acid	coumaric acid
1	0.3	0.6	4.4	5.5	3.1	2.5
2	0.4	0.5	3.8	4.8	3.1	2.6
3	0.3	0.1	2.5	4	3.3	3.3
4	4.4	0.8	14.6	7.6	8.4	13.7
5	4.3	0.9	14.6	5.9	6.3	11.5
6	4.9	0.8	43.7	24.4	1.6	3.0
7	2.4	0.7	19	9.1	1.3	1.1
8	4.9	1.3	27.2	13.8	2.0	2.1
9	4.9	0.7	38.5	17.6	4.0	3.3

RESULTS AND DISCUSSION

In all Muscat wines (samples 1–9) the contents of both catechin and epicatechin were found to be quite low (Table 3). Indeed, the values determined are much lower than those of typical red wines but in accordance with the values expected for white wines with no maceration (Goldberg et al., 1999). A characteristic feature of the dry wines is their low contents of caftaric acid and coutaric acid compared to the sweet wines (samples 4–9). On the other hand, the concentrations of the free acids (caffeic and coumaric) are only slightly higher in the dry wines than in the nonaged sweet wines except the naturally sweet wine (sample 9). It is, therefore, clear that the exhaustive fermentation from which the dry wines are produced leads to a drop in the levels of the esters without a considerable rise of the concentrations of the free acids. This observation agrees with earlier studies reporting that the caftaric and coutaric acids decrease in the course of the fermentation process but is not accompanied by a proportional accumulation of the free acids (Cheyner et al., 1989; Ramos et al., 1993). This result leads to the conclusion that the hydrolysis products are rapidly further metabolized (Ramos et al., 1993). It can be assumed that the predominant reason for the drop in the levels of the esters is enzymatic oxidation rather than the activity of hydrolysis esterases (Nagel et al., 1979). In samples 4 and 5 (mistelles aged in barrels), the distribution of hydroxycinnamic derivatives is rather interesting; the ratios of caffeic acid/total caffeoates and coumaric acid/total coumarates were much higher compared to the rest of the sweet wines (samples 6–9). In contrast to the dry wines, hydrolysis of the esters was accompanied by a marked increase in the levels of the free acids, suggesting that hydrolysis of the relevant esters is the predominant path for the formation of the free acids (Ritchey and Waterhouse, 1999). It is worth pointing out that the HPLC chromatograms of these samples were complicated and revealed the presence of compounds not existing in the rest of the wines, probably

Table 4. Analysis of Fermentation Aroma (in Milligrams per Liter) in Dry, Sweet Wines and Mistelles from the Muscat Lefko Cultivar from the Island of Samos

sample	total ethyl esters	total fatty acids	total higher alcohols	total acetates	diethyl succinate	2,3-butane-diol	total volatile acids
1	1.7	16.2	268	1.6	1.2	486	3.4
2	1.5	12.4	299	1.1	0.8	742	3.0
3	1.5	12.1	220	1.2	1.2	167	4.0
4	0.4	4.3	82	0.1	10.4	222	7.5
5	0.6	5.6	61	0.1	7.9	130	4.7
6	0	0.3	6	0.1	0	201	0.9
7	1.0	10.5	241	0.3	2.0	811	2.5
8	1.4	11.2	193	0.3	4.6	924	3.8
9	0.6	5.3	375	0.9	6.7	2986	4.1

due to the extraction of these compounds from the wood of the barrel (Gimenez Martinez et al., 1996). For instance, vanillin was detected by HPLC in samples 4 and 5 (0.7 and 0.4 mg/L, respectively), whereas mere traces were found in the rest of the Muscat wines. The same was true for gallic acid, which was measured as 4.0 mg/L in sample 4 and 2.7 mg/L in sample 5 but was found only in traces in the other samples. In addition, various oxidation products are believed to contribute to the more varied HPLC profile of these wines. The nonaged mistelle contained the highest concentrations of caftaric and coutaric acids probably because it was not subjected to fermentation, which is responsible for breaking down the esters, as mentioned earlier. It is also possible that the phenyl oxidase, responsible for the oxidation of these esters, is partly inactivated owing to the addition of alcohol to prevent fermentation (Singleton et al., 1985). Characteristic HPLC profiles of the nonaged and aged mistelles (samples 4 and 6, respectively) are illustrated in Figure 1. The content of caffeic and coumaric acids was similar to that of the other sweet wines. In the case of the naturally sweet wine (sample 9), the concentrations of the caftaric and coutaric esters were very high and only slightly lower than the corresponding concentrations in the nonaged mistelle (sample 6). This can be attributed to the fact that the grapes were enriched with these compounds during the drying stage before pressing. So, although some fermentation did take place with the associated decrease in the esters, the initial concentration of the latter was so elevated that a considerable amount remained after the fermentation process stopped. This enrichment induced by drying might also be the cause for the slightly increased levels of caffeic and coumaric acid, compared to the "fortified" sweet wines (samples 7 and 8). In the case of the fortified sweet wines (samples 7 and 8), the concentrations of caftaric and coumaric esters were intermediate between the dry wines (samples 1 and 3) and the nonaged sweet wines (samples 6 and 9), reflecting the limited fermentation these samples were subjected to. The content of free acids was similar to that of the nonaged mistelle (sample 6). It is interesting that only traces of myricetin, quercetin, and their glucosides were detected in all of the white wines under investigation.

In terms of fermentation aroma, the dry Muscat wines (samples 1–3) were found to have the highest content in C6, C8, and C10 fatty acids, their ethyl esters, and acetates of higher alcohols compared to the sweet wines (Table 4). Their concentrations in higher alcohols did not exceed the level of 300 mg/L, over which wines can present herbaceous flavors due to those compounds (Navarre, 1998). Also, the amounts of the other substances (diethyl succinate, 2,3-butanediol, and volatile

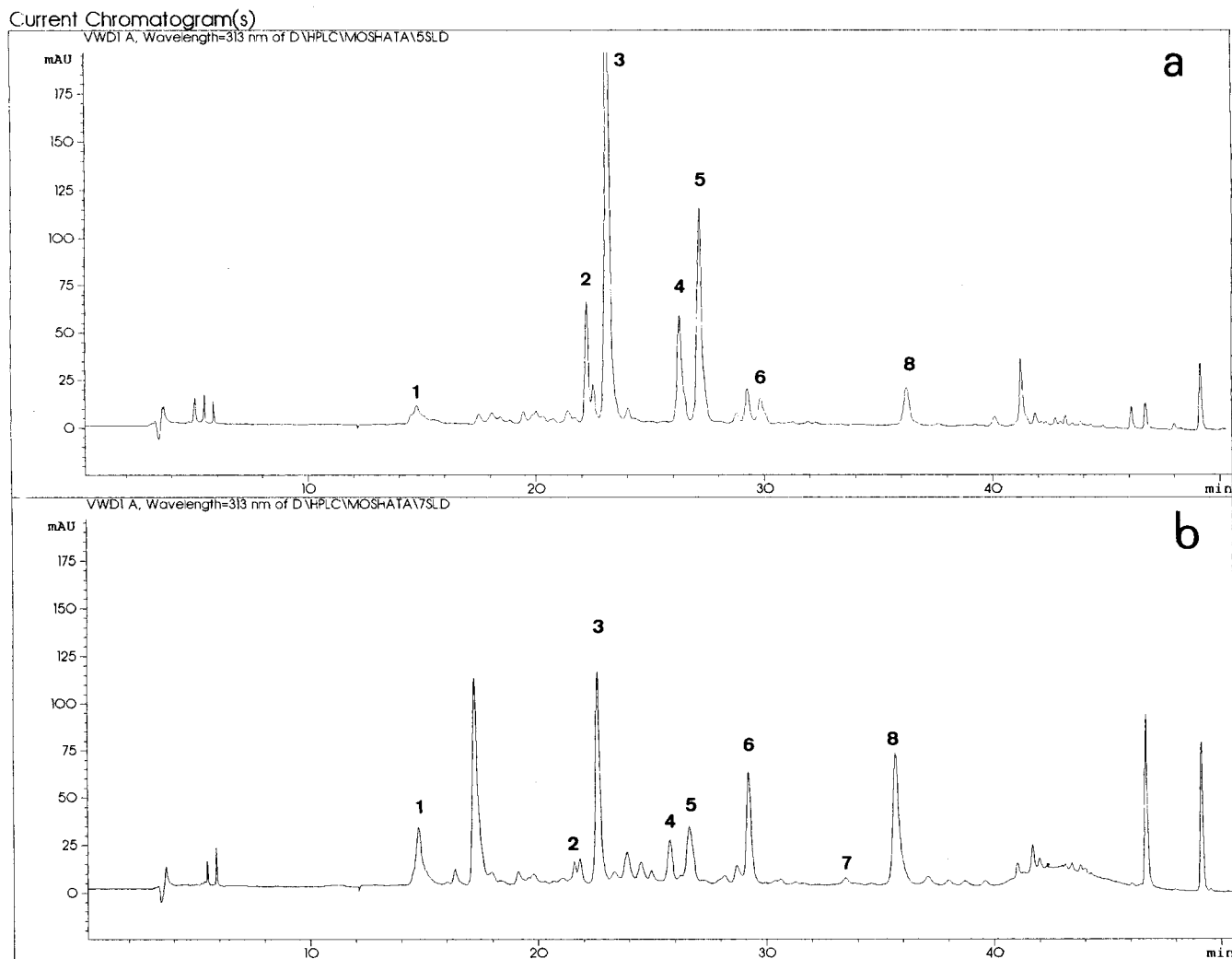


Figure 1. Representative HPLC profiles after direct injection of the sample and detection at 313 nm for the determination of the phenolic composition of (a) sample 6 (nonaged mistelle) and (b) sample 4 (aged mistelle), produced from the Muscat lefko cultivar from the island of Samos. Peaks: (1) gallic acid; (2) *cis*-caftaric acid; (3) *trans*-caftaric acid; (4) *cis*-coutaric acid; (5) *trans*-coutaric acid; (6) caffeic acid; (7) vanillin; (8) coumaric acid.

Table 5. Analysis of Free Terpenes (in Micrograms per Liter) in Dry, Sweet Wines and Mistelles from the Muscat Lefko Cultivar from the Island of Samos

sample	nerol oxide	linalool	α -terpineol	citronellol	nerol	geraniol
1	25	359	256	39	94	166
2	23	315	175	40	86	145
3	26	277	218	31	62	128
4	69	29	251	7	11	14
5	60	49	325	4	12	16
6	21	366	206	12	55	128
7	50	238	445	28	40	98
8	45	228	374	8	32	76
9	41	275	182	18	33	54

acids), generally contributing to unpleasant odors (Bertrand et al., 1994), were not high (Table 4). The aged mistelles (samples 4 and 5) contained very low concentrations of ethyl esters, fatty acids, acetates, and higher alcohols, although we would expect much lower concentrations for unfermented wines. It appears that these samples were allowed to ferment for a short period prior to alcohol addition. Also, it is obvious that these wines lack any positive fermentation aroma. On the contrary, the diethyl succinate concentration was rather high, indicating that formation of this volatile compound is

Table 6. Analysis of Glycosidically Linked Terpenes (in Micrograms per Liter) in Dry, Sweet Wines and Mistelles from the Muscat Lefko Cultivar from the Island of Samos

sample	nerol oxide	linalool	α -terpineol	citronellol	nerol	geraniol
1	19	43	10	13	307	203
2	18	32	tr ^a	17	415	288
3	22	27	7	14	463	370
4	6	7	2	7	142	55
5	7	16	4	2	316	152
6	50	125	18	59	1320	860
7	47	47	22	25	521	426
8	26	26	9	22	606	457
9	57	99	26	43	950	719

^a Traces.

avored during prolonged conservation of wines in barrels. Diethyl succinate is reported as having an odor reminiscent of camphor (Bertrand et al., 1994). Also, the levels of the volatile acids were relatively increased in samples 4 and 5 compared to the other wines. As expected, the nonaged mistelle (sample 6) did not contain any fermentation aroma (Table 4). The only volatile compound of this group found at significant level was 2,3-butanediol. It appears that synthesis of 2,3-

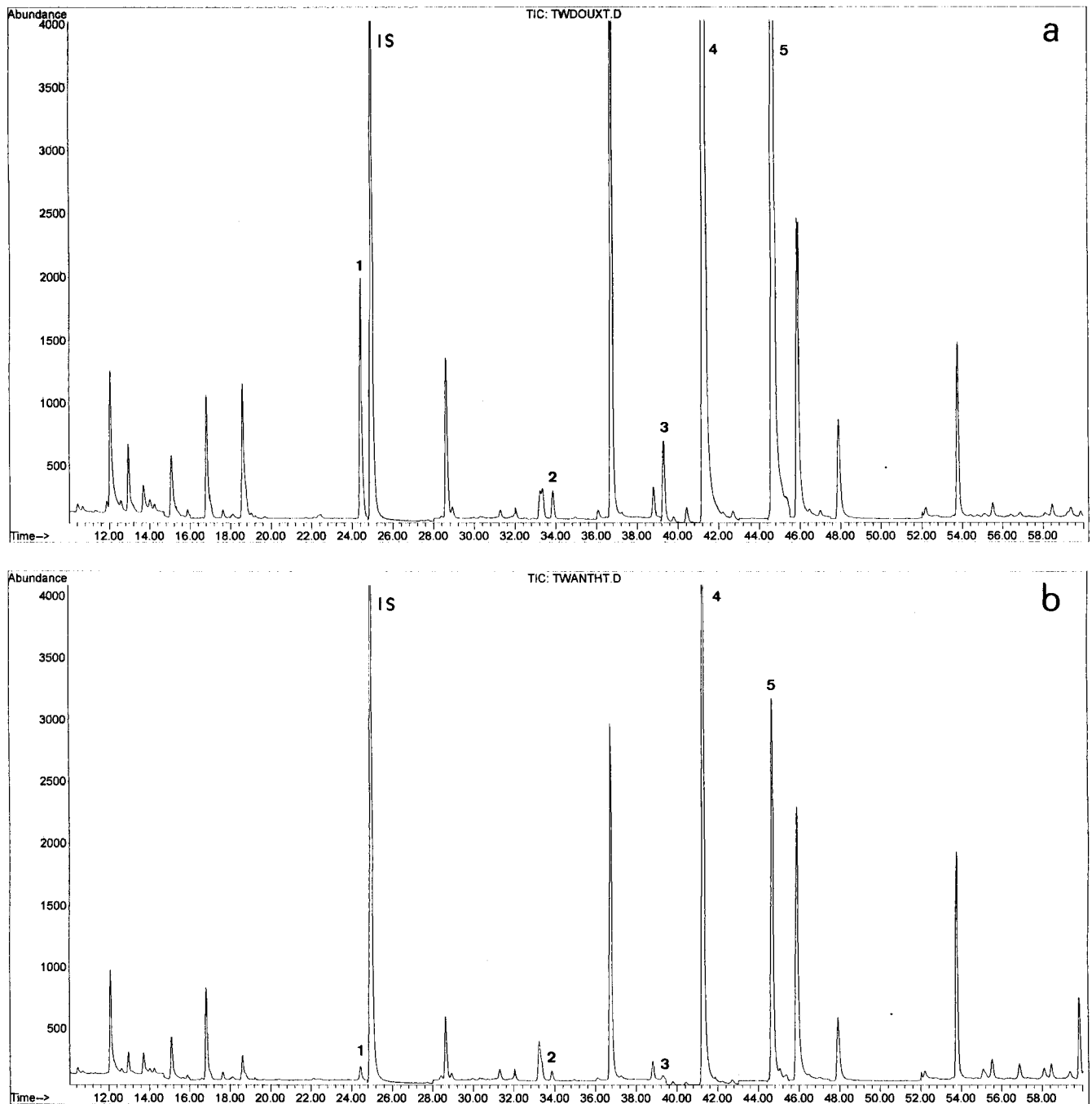


Figure 2. Representative GC-MS profiles for the determination of the glycosidically bound terpenes in (a) sample 6 (nonaged mistelle) and (b) sample 5 (aged mistelle), produced from the Muscat lefko cultivar from the island of Samos. Peaks: (1) linalool; (2) α -terpineol; (3) citronellol; (4) nerol; (5) geraniol. IS is the internal standard.

butanediol begins during grape maturation or must processing, which is in accordance with previous results (Usseglio-Tomasset, 1978). Furthermore, it is remarkable that C6, C8, and C10 fatty acids are present in mistelle, although in very low concentrations. This finding is in agreement with previous work (Santamaria et al., 1995) demonstrating that hexanoic, octanoic, and decanoic acids are found in negligible quantities in grape juices. In sample 9 (naturally sweet wine), a markedly increased concentration of 2,3-butanediol was observed in comparison to the fortified sweet wines (Table 4). This fact could be associated with reactions taking place during exposure of the grapes to the sun. Also, a relatively high concentration of higher alcohols was

determined in the same sample, confirming the appearance of herbaceous odors.

In the case of terpenes the levels of free linalool, citronellol, nerol, and geraniol in the dry wines (samples 1–3) were the highest compared to the sweet Muscat wines with the exception of the nonaged mistelle (sample 6), which contained the highest concentration of linalool (Table 5). On the contrary, the levels of free nerol oxide and α -terpineol of the dry wines were the lowest of all wines examined. On the basis of the fact that linalool and geraniol are the most important compounds for the terpene-like character of Muscat wines (Marais, 1983), we may assume that the dry wines (samples 1 and 3) have more intense varietal

aroma than the rest of the Muscat wines except sample 6. Furthermore, as mentioned before, the dry wines possess the richest fermentation aroma. From the analytical data, we would expect that sample 1 is the most aromatic one among the three dry wines examined (Tables 4 and 5). The concentrations of free linalool, citronellol, nerol, and geraniol of the aged mistelles (samples 4 and 5) were the lowest of all Muscat wines examined (Table 5). The surprisingly low levels of linalool, nerol, and geraniol indicate that oxidation of these free terpenes takes place during prolonged aging in barrels and eliminates any terpene-like character in the resultant wines. On the other hand, large amounts of nerol oxide and α -terpineol were measured in the aforementioned samples. However, these two terpenes do not have such a significant impact on the varietal aroma of Muscat wines as linalool and geraniol. These results are consistent with a previous study (Marais, 1983) reporting that some of the most intense aromatic terpene compounds with low aroma thresholds (linalool, nerol, and geraniol) may be transformed into α -terpineol and some terpenes with high aroma thresholds such as linalool oxides and that these rearrangements may result in the loss of Muscat aroma during storage of grapes and wines. It is reported that aroma threshold values of linalool, nerol, and geraniol are 100, 400–500, and 130 $\mu\text{g/L}$, respectively, whereas those of linalool oxides are $>3000 \mu\text{g/L}$ (Ribéreau-Gayon et al., 1975).

The concentrations of nonvolatile glycosidically bound terpenes in the aged mistelles were the lowest of all Muscat wines examined (Table 6), indicating that hydrolysis of these compounds is favored during wine aging. Chromatograms depicting the difference in the levels of glycosidically bound terpenes between nonaged and aged mistelle wines are shown in Figure 2. The concentrations of free linalool and geraniol of sample 6 were very high (Table 5), indicating a rich Muscat aroma. On the contrary, the nerol oxide level of the same sample was the lowest of all Muscat wines examined. A previous study (Wilson et al., 1984) reported that nerol oxide is not present naturally in Muscat grapes and is possibly formed during juice storage or processing. The concentrations of glycosidically linked terpenes in sample 6 were almost the highest among all of the samples (Table 6). This observation was expected because a fraction of these nonvolatile substances are hydrolyzed during the alcoholic fermentation (Marais, 1983). Although sample 9 (naturally sweet wine) is not so rich in free terpene content (Table 5), it contains high concentrations of glycosidically bound terpenes, especially those of nerol and geraniol (Table 6). Hydrolysis of these terpene precursors during its aging can induce more flavor in this type of wine. The fortified sweet wines (samples 7 and 8) were unexceptional, exhibiting characteristics between the dry and the sweet wines in terms of the volatile compound content.

The concentrations of free linalool, nerol, and geraniol in Muscat lefko wine, as determined in the present study, are in the normal range of values expected for white wines (Bertrand et al., 1994). In the case of dry wines, the levels of citronellol are slightly higher than those mentioned in Bertrand et al. (1994). On the other hand, the levels of α -terpineol are markedly higher than those reported in the literature (0–80 $\mu\text{g/L}$; Bertrand et al., 1994).

CONCLUSIONS

The analysis of the phenolic content and the primary and fermentation aroma in several Muscat lefko wines vinified on the island of Samos revealed many differences among them that could be accounted for by the different vinification and conservation treatments.

Future research could focus on the measurement of additional compounds contributing to organoleptic properties (e.g., tertiary aroma for the aged wine) and to health impact (e.g., resveratrol and urethane) as well as on the comparison of Muscat lefko with other Muscat cultivars with emphasis on their organoleptic compositions.

LITERATURE CITED

- Bayonove, C. Les composés terpeniques. In *Les Acquisitions Récentes en Chromatographie du Vin*; Lavoisier Tec & Doc: Paris, France, 1992.
- Bertrand, A. Les principales substances chimiques actuellement dosées par chromatographe. In *Les Acquisitions Récentes en Chromatographie du Vin*; Lavoisier Tec & Doc: Paris, France, 1992.
- Bertrand, A.; Guedes de Pinho, P.; Anocibar-Beloqui, A. Les constituants organiques majoritaires du vin, les constituants volatils: teneurs trouvées, exemples d'analyses. *Feuill. O.I.V.* **1994**, No. 971.
- Cheynier, V.; Souquet, J. M.; Moutounet, M. Glutathione Content and Glutathione to Hydroxycinnamic Acid Ratio in *Vitis vinifera* Grapes and Musts. *Am. J. Enol. Vitic.* **1989**, *40*, 320–324.
- Di Stefano, R. Proposition d'une méthode de préparation de l'échantillon pour la détermination des terpènes libres et glycosides des raisins et des vins. *Bull. O.I.V.* **1991**, *64*, 219–223.
- Gimenez Martinez, R.; Lopez Garcia de la Serana, H.; Villalon Mir, M.; Quesada Granados, J.; Lopez Martinez, M. C. Influence of Wood Heat Treatment Temperature and Maceration Time on Vanillin, Syringaldehyde and Gallic Acid Contents in Oak Wood and Wine Spirit Mixtures. *Am. J. Enol. Vitic.* **1996**, *47*, 441–446.
- Goldberg, D. M.; Karumanchiri, A.; Soleas, G. J.; Tsang, E. Concentrations of Selected Polyphenols in White Commercial Wines. *Am. J. Enol. Vitic.* **1999**, *50*, 185–193.
- Jaworski, A.; Lee, C. Y. Fractionation and HPLC Determination of Grape Phenolics. *J. Agric. Food Chem.* **1987**, *35*, 257–259.
- Kunhau, J. The Flavonoids. A class of semi-essential food components: Their role in human nutrition. *World Rev. Nutr. Diet.* **1976**, *24*, 117–191.
- Macaulay, L. E.; Morris, J. R. Influence of cluster exposure and winemaking processes on monoterpenes and wine olfactory evaluation on Golden Muscat. *Am. J. Enol. Vitic.* **1993**, *44* (2), 198–204.
- Marais, J. Terpenes in the aroma of grapes and wines: A review. *S. Afr. J. Enol. Vitic.* **1983**, *4* (2), 49–60.
- Marais, J.; van Wyk, C. J. Effect of grape maturity and juice treatments on terpene concentrations and wine quality of *Vitis vinifera* L. cv. Weisser Riesling and Bukettraube. *S. Afr. J. Enol. Vitic.* **1986**, *7* (1), 26–35.
- Nagel, C. W.; Baranowski, J. D.; Wulf, L. W.; Powers, J. R. The Hydroxycinnamic Acid Tartaric Acid Ester Content of Musts and Grape Varieties Grown in the Pacific Northwest. *Am. J. Enol. Vitic.* **1979**, *30*, 198–201.
- Navarre, C. *L'œnologie*; Lavoisier Tec & Doc: Paris, France, 1998.
- Noble, A. C. Bitterness and astringency in wine. In *Bitterness in Foods and Beverages*; Rouseff, R. L., Ed.; Elsevier: New York, 1990; pp 25–145.
- Oszmianski, J.; Ramos, T.; Bourzeix, M. Fractionation of Phenolic compounds in red wine. *Am. J. Enol. Vitic.* **1988**, *39*, 259–262.

- Ramos, T.; Fleuriet, A.; Rascalou, M.; Macheix, J. J. The Effect of Anaerobic Metabolism of Grape Berry Skins on Phenolic Compounds. *Am. J. Enol. Vitic.* **1993**, *44*, 13–16.
- Reynolds, A. G.; Wardle, D. A. Impact of viticultural practices on grape monoterpenes and their relationship to wine sensory response. In *Proceedings of the Fourth International Symposium on Cool Climate Viticulture and Enology*; Henick-Kling, T., Wolf, T. E., Harkness, E. M., Eds.; Comm. Services: New York, 1996.
- Ribéreau-Gayon P.; Boidron, J. N.; Terrier, A. Aroma of Muscat grape varieties. *J. Agric. Food Chem.* **1975**, *23*, 1042–1047.
- Ritchev, J. G.; Waterhouse, A. A Standard Red Wine: Monomeric Phenolic Analysis of Commercial Cabernet Sauvignon Wines. *Am. J. Enol. Vitic.* **1999**, *50*, 91–99.
- Salaha, M. J.; Lanaridis, P.; Tsoutsouras, E.; Tzourou, I.; Karagiannis, S. Arôme variétal de raisins et de vins issus des cépages Muscat blanc de l'île de Samos et Muscat d'Alexandrie de l'île de Lemnos. Effet de terroir et incidence de la macération pelliculaire. In *Proceedings of the 6th Symposium International d'Enologie*; Lavoisier Tec & Doc: Paris, France, 2000.
- Santamaria, M. P.; Lopez, R.; Gutierrez, A. R.; Diez de Bethencourt, C. A.; Garcia-Escudero, E. Incidence de la température de fermentation sur l'évolution des acides gras totaux. *Vitis* **1995**, *29* (2), 101–104.
- Singleton, V. L. Tannins and the quality of wines. In *Plant Polyphenols*; Hemingway, R. W., Lacks, P. E., Eds.; Plenum Press: New York, 1992; pp 859–880.
- Singleton, V. L.; Noble, A. C. Wine flavor and phenolic substances. In *Phenolic, Sulfur and Nitrogen Compounds in Food Flavors*; Charalambous, G., Katz, I., Eds.; ACS Symposium Series 26; American Chemical Society: Washington, DC, 1976; pp 47–70.
- Singleton, V. L.; Salgues, M.; Zaya, J.; Trousdale, E. Caftaric Acid Disappearance and Conversion to Products of Enzymic Oxidation in Grape Must and Wine. *Am. J. Enol. Vitic.* **1985**, *36*, 50–56.
- Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. Wine as a biological fluid: History, production and role in disease prevention. *J. Clin. Lab. Anal.* **1997**, *11*, 287–317.
- Teissedre, P. L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H.; German, J. B. Inhibition of *In Vitro* Human LDL Oxidation by Phenolic Antioxidants from Grapes and Wines. *J. Sci. Food Agric.* **1996**, *70*, 55–61.
- Usseglio-Tomasset, L. Confronto tra la composizione del mosto e del vino. In *Chimica Enologica*; Edizioni AEB: S. Polo-Brescia, Italy, 1978; pp 170–173.
- Williams, P. J.; Strauss, C. R.; Wilson, B. Classification of the monoterpenoid composition of Muscat grapes. *Am. J. Enol. Vitic.* **1981**, *32* (3), 230–235.
- Wilson, B.; Strauss, C. R.; Williams, P. J. Changes in free and glycosidically bound monoterpenes in developing Muscat grapes. *J. Agric. Food Chem.* **1984**, *32*, 919–924.

Received for review April 11, 2000. Revised manuscript received August 1, 2000. Accepted August 3, 2000.

JF000459C