JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2007,** *55,* 5205–5212 **5205**

Sweet-like Off-flavor in Aglianico del Vulture Wine: Ethyl Phenylacetate as the Mainly Involved Compound

LARA TAT,* PIERGIORGIO COMUZZO, FRANCO BATTISTUTTA, AND ROBERTO ZIRONI

Dipartimento di Scienze degli Alimenti, Università degli Studi di Udine, Via Marangoni 97, 33100 Udine, Italia

Interest in high-quality and peculiar products is a recent trend in the enological field; for this reason, production of wines from autochthonous vine varieties is requested by consumers. Aglianico wine from the Italian region "Basilicata" is an example of a promising product strictly connected to the territory; nevertheless, it is affected by a frequent sweet-like off-flavor. In this study the compositional cause of this off-flavor was investigated by SPME-GC−olfactometry, SPME-GC-MS, and sensory tests. Ethyl phenylacetate (EPhA) was found to be the compound mainly responsible, and its sensory threshold was determined near 73 μg/L; products with the odorant concentration near and up to these values were always recognized as significantly different from the other wines and were often far from wine technical pleasantness; besides EPhA gave to the wines a strong honey-like character. Some preliminary hypotheses about its mechanism of formation (shikimate pathway) are presented in this study: these hypotheses could explain the correlation between EPhA and volatile phenols that was found by both sensory tests and GC quantitative analysis of wines affected by different levels of defect.

KEYWORDS: **Sensory analysis; sensory threshold; SPME-GC-O; SPME-GC-MS; compositional cause**

INTRODUCTION

Aglianico del Vulture is a Denominazione di Origine Controllata (DOC) wine production of Basilicata, an Italian region. The respective grape variety is a red one, and it is widespread in southern Italy; it has ancient origins (reliable historic references go back to the 14th century) (*1*). The better conditions for its cultivation are hilly (300−600 m above the sea), volcanic, and sandstone grounds. It is a late-ripening variety being harvested in October−November, due to its thick skin that gives resistance against bad climatic conditions and cryptogamic diseases.

Aglianico del Vulture wine is very often affected by an untypical olfactory character, which is described by the producers as a "sweet-like" smell and which is considered as a defect by the local enologists. When the problem occurs is not clear, and up to now, its description was mainly based on some empirical observations: some enologists affirm that this peculiar odor could be mainly related to wines produced from grapes harvested in particular vintages or in specific zones of the Vulture region; other technicians say that it could be connected with some cryptogamic diseases. The only quite sure opinion is that it is not present in Aglianico wines produced without skin contact. The main question related to this problem is the identification of the responsible volatile compounds, which at present still unknown. For this reason, the purpose of this work was to clarify this last point and to find the relationships between this "sweet-like" olfactory deviation and wine sensory characters.

MATERIALS AND METHODS

Reagents. Ethyl phenylacetate (EPhA) and all other chemicals (4-ethylphenol, 4-ethylguaiacol, phenylacetaldehyde, phenylacetic acid, 2-phenylethanol, 2-phenylethyl acetate) were supplied by Sigma-Aldrich (St. Louis, MO).

Wine Sampling. Wine samples with different origins have been used for different purposes during the experimental trials; for a better comprehension, some details are reported in **Table 1**.

Aglianico wines used in step I were supplied by different wineries located in the Vulture region. At sampling they were all stored in 100−300 hL steel tanks, being ready to be bottled and sold by the winery; wines were sampled by filling 1 L bottles, which were immediately sealed with crown cap closures.

In step II a commercially available red wine made with Refosco dal Peduncolo Rosso grapes (DOC Isonzo del Friuli, Friuli Venezia Giulia, Italy) was supplied by a local producer (Brotto Marcello Winery, Gorizia, Italy).

Commercial wines used in steps III−V were all purchased from local Vulture wineries. All wine samples were stored at 15−16 °C for a few days at most, until analyses.

Solid-Phase Microextraction−Gas Chromatography−Olfactometric Detection (SPME-GC-O). Six clearly defective wines were utilized, in the first step of this work, for the individuation of the sweet-

* Corresponding author (telephone +39 0432 590738; fax +39 0432 590719; e-mail lara.tat@uniud.it).

10.1021/jf0637067 CCC: $37.00
Published on Web 05/27/2007

Table 1. Description of the Wines and Analytical Methods Used in the Study as Well as Purposes of Each Choice

operating step	wines	analysis	purpose
I	6 clearly defective Aglianico del Vulture wines, ready to be bottled but not in commerce yet	GC-O GC-MS	individuation and identification of the responsible compound
II	a not defective red wine, spiked with increasing amounts of the compound identified in step I	sensory analysis (detection threshold) GC-MS	evaluation of the detection threshold of the responsible compound in wine construction of a calibration curve for the responsible compound and for its possible precursors
III	18 commercial[a] Aglianico del Vulture wines	GC-MS	quantification of the responsible compound and some possible precursors in Aglianico commercial wines
IV	9 samples[a] selected from the group of 18 wines reported in step III	sensory analysis (multi-sample difference test)	study of the relationship between the amount of the compound in commercial Aglianico del Vulture wines and some sensory attributes
V	5 samples[a] selected from the group of 9 wines reported in step IV	sensory analysis (free choice profile)	sensory description of different commercial Aglianico del Vulture wines

[a] With different levels of defect.

like odor by SPME-GC-O. The samples came from the Basilicata region and were selected by some local enologists; they were stabilized steel-aged wines.

A 2 cm 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane fiber (Supelco, Bellefonte, PA) was used for headspace analysis of wines; 50 mL glass vials were filled with 25 mL of each sample, and 6 g of sodium chloride was added. A nitrogen flow was used to minimize pollution from environmental odors (*2*): nitrogen was blown inside the vial, before filling, and a laminar flow was kept at its neck during filling. Vials were then closed, and SPME was run at 37 °C, for 15 min. For this purpose, vials were kept in the water bath for 15 min before SPME to reach thermal equilibration (*2*). SPME was immediately followed by GC injection.

Instrumentation, column, and chromatographic conditions for both GC-O and GC-MS were the same as reported by Comuzzo et al. (*3*), except for the carrier gas flow of 2 mL/min and the fiber remaining in the injector for 5 min after injection.

GC-O analyses were run following the general criteria of the OSME method, as reported by Mistry et al. (*4*), with the main aim to qualitatively identify the odor active zones in the different samples.

Briefly, this qualitative evaluation was performed by enologists who well knew both the olfactory deviation and Aglianico sensory characters; they were simply asked to indicate if and where in the chromatographic profile they perceived exactly the same "sweet-like smell" that they previously described as "the defect" in the analyzed wines.

The judges had to screen the whole chromatographic profile, taking turns to avoid tiredness (7 min per judge); turns and replicates were planned in such a way that each judge sniffed every 7 min chromatographic zone.

Solid-Phase Microextraction−Gas Chromatography−Mass Spectrometric Detection (SPME-GC-MS). The six wines analyzed by GC-O were utilized for identification of the odorous peak by SPME-GC-MS.

Identification was performed by comparison of the mass spectrum of the odorous peak with those reported in the Wiley 5 mass spectra library, and by comparison of Kovats retention index, calculated from the retention times of *n*-alkanes, with those available in the literature (*5*); a standard of ethyl phenylacetate (CAS Registry No. 101-97-3) was utilized to obtain confirmation of mass spectrometric identification.

In a further step of the study, the identified odorant (EPhA) and other substances, which could be originated by the same biosynthetic pathways (4-ethylphenol, 4-ethylguaiacol, phenylacetaldehyde, phenylacetic acid, 2-phenylethanol, 2-phenylethyl acetate), were quantified in different wines by SPME-GC-MS. For this purpose, a calibration curve for each compound was constructed by adding different amounts of these substances in a basic red wine. A standard solution in ethanol was prepared (ethyl phenylacetate, 2.000 g/L; 4-ethylphenol, 5.000 g/L; 4-ethylguaiacol, 0.4000 g/L; phenylacetaldehyde, 0.5000 g/L; phenylacetic acid, 0.1500 g/L; 2-phenylethanol, 90.0000 g/L; 2-phenylethyl acetate, 3.000 g/L; isobutyric acid, 2.5000 g/L; isovaleric acid, 0.0500 g/L); then this solution was diluted 1:1000 in wine (sol. M) and analyzed in GC-MS. Subsequent dilutions in wine of sol. M (1:2, 1:5, 1:10, 1:20, 1:50, 1:200) were analyzed, too. A commercial red wine (Refosco dal Peduncolo Rosso, 2004 vintage), not aged in wood, from DOC region "Isonzo del Friuli", was used for the construction of the calibration curves. This local wine is similar to Aglianico with regard to some compositional and sensory characters, such as extract, color, and body; moreover, they are both mainly characterized by fermentative aroma, rather than varietal ones.

Eighteen commercial Aglianico del Vulture wines, from different wineries of the Basilicata region (2000−2004 vintages), were then used for the quantification of the responsible substance and the possible related compounds.

All GC-MS analyses were carried out on a Varian (Palo Alto, CA) 3400 gas chromatograph coupled to a Varian Saturn ITDMS ion trap mass spectrometer. The SPME sampling, column, and chromatographic conditions were the same as those reported for GC-O analysis. For the MS system, the temperatures of the manifold and transfer line were 170 and 250 °C, respectively; analysis was performed by injecting in TIC mode, and electron impact mass spectra were recorded using an ionization voltage of 70 eV and an ionization current of 10 μA.

Quantitative determinations were carried out from absolute areas of characteristic ion peaks: m/e 91 for ethyl phenylacetate, phenylacetaldehyde, and phenylacetic acid; m/e 104 for 2-phenylethyl acetate; m/e 105 for 2-phenylethanol; m/e 107 for 4-ethylphenol; and m/e 137 for 4-ethylguaiacol.

Samplings for SPME-GC-MS analyses of both the commercial wines and the different solutions related to the calibration curve were carried out in triplicate.

Sensory Tests. Sensory analyses were performed as a further step of this research. The panel was constituted by young enologists (20−25 years old) with experience in sensory analysis and at least 2 years of work experience. Wines (30 mL at 20 °C) were presented in one session, in three coded, tulip-shaped wine glasses.

The rapid method (E 679-79) of the American Society for Testing and Materials (ASTM; *6*) was used to evaluate the threshold of the impact odorant in wine. The threshold was evaluated ortonasally in a basic red wine prepared by adding different amounts of a standard solution of ethyl phenylacetate. The wine was the same used for calibration curves. The addition of the standard solution was not such as to cause a modification of the alcohol degree of the wine. The panelists (39 members) each received five 3-Alternative Forced Choice (3-AFC) tests with ascending concentrations spaced by a factor of 3, starting from 3 to 250 μg/L in wine. In each 3-AFC presentation, three samples are presented: two are controls, and one contains the substance under test. The judges have to examine each sample from left to right and select the odd product. Randomization of the position of the different sample, within each 3-AFC presentation, was carried out for the different panelists; the option of going back to repeat the evaluation of each sample was possible within the single 3-AFC presentation but not possible among the different 3-AFC groups of products. Biases were limited by retesting those panelists who were correct at the lowest (starting from a concentration of 13 ng/L) or failed at some intermediate or at the highest level (going to a concentration of 185 mg/L). The threshold for each subject was evaluated as the geometric mean of the highest concentration missed and the next higher concentration. The group threshold is the geometric mean of the individual ones.

The relationship between the concentration of the odorous compound and its sensory perception in wine was evaluated with an attribute difference test (*7*) and a descriptive sensory test. For the first one (a multisample test with rating approach), subjects rated the intensity of the selected attributes ("honey", "tobacco", and "technical pleasantness") on a numerical scale (nine points). Nine samples were evaluated; they were selected from the group of 18 commercial Aglianico del Vulture wines analyzed in GC-MS on the basis of their amount of ethyl phenylacetate. The selection of the attributes (tobacco and honey) was based on bibliographic search: the odorant is used as a flavoring agent in the tobacco industry (www.philipmorrisusa.com), and it is described as having a honey smell (www.oxfordchemicals.com). "Technical pleasantness" was selected to correlate the amount of the considered compound in wines and the technical hedonic judgment. The samples were evaluated ortonasally. The subjects received wines sequentially in a balanced randomized order.

With regard to the descriptive sensory test, a free choice profile method (*8*) was performed on the commercial Aglianico del Vulture wines. Eight panelists were selected on the basis of olfactory threshold for the studied compound. They had to evaluate appearance, odor, taste, and aftertaste of the products. The test was performed on five of the nine samples analyzed with the attributes difference method: the samples were selected for their different levels of EPhA. Each panelist extracted a personal vocabulary, developed his or her own score sheet, and evaluated samples with a numerical scale (five points). The subjects received samples simultaneously in a balanced randomized order.

Statistical Analysis. Data from GC quantitative determinations were statistically elaborated with regression analysis and principal component analysis (PCA) to evaluate the relationship between the amount of ethyl phenylacetate and some possible related compounds in wines.

A two-factor (samples and panelists) analysis of variance (ANOVA) and least significant difference (LSD) were carried out on data from the attribute difference test to investigate the effect of the amount of EPhA on sensorial attributes. Before data from the attribute difference test were analyzed, the score of each panelist was compared with the group mean by correlation analysis (attribute by attribute) and by PCA; data from people who did not show a good agreement with the panel were not considered in the subsequent statistical elaborations.

Linear correlation analysis was used to evaluate the relationship between the different levels of the defective component in different wines and the mean values of the ratings collected for the attributes from the attribute difference test.

Table 2. Amounts (Micrograms per Liter) of Ethyl Phenylacetate and Some Possible Related Substances[a] in the Biosynthetic Pathways As Quantified in Commercial Aglianico del Vulture Wines

wine	PA	EPhA	2-PhEA	4-EG	4-EP	PhAA	2-PhE
1	56.46	52.59	126.60	68.85	488.13	322.95	34816
2	57.51	16.19	120.23	50.59	411.61	86.94	42854
3	59.80	89.90	126.64	148.01	866.22	565.57	30230
4	62.66	112.70	135.01	55.15	461.28	893.61	32386
5	55.32	9.44	170.33	47.06	399.45	7.04	41553
6	56.98	76.63	123.15	157.40	886.82	516.23	27429
7	58.88	40.39	131.70	74.48	531.78	250.39	65381
8	56.42	17.17	128.51	50.97	420.04	ND[b]	43734
9	62.80	42.00	134.73	69.49	447.58	71.14	39362
10	53.77	97.80	116.64	70.51	513.58	354.86	39909
11	79.07	32.76	154.17	65.58	465.93	6993.27	53585
12	65.37	12.10	166.04	47.44	403.64	4165.45	48062
13	63.10	13.59	139.79	46.56	415.17	2105.46	49572
14	54.00	63.09	99.74	46.88	414.63	573.03	20131
15	47.07	150.25	116.94	169.28	2231.55	1435.78	25645
16	58.25	64.68	249.74	85.70	581.79	400.02	37717
17	48.38	18.30	128.94	52.71	415.61	ND	41712
18	67.32	35.67	117.51	57.05	431.77	ND	45369

[a] PA, phenylacetaldehyde; EPhA, ethyl phenylacetate; 2-PhEA, 2-phenylethyl acetate; 4-EG, 4-ethylguaiacol; 4-EP, 4-ethylphenol; PhAA, phenylacetic acid; 2-PhE, 2-phenylethanol. [b] Not detected.

Data from the free choice profile method were analyzed by Generalized Procustes Analysis (GPA).

The statistical packet Statistica for Windows (StatSoft, Tulsa, OK), version 6.0, was used to apply descriptive methodologies such as PCA. The Fixed Nonlinear Regression module from Statistica was used to evaluate the goodness of linear correlations. The packet Senstools for Windows (OP & P Product Research BV, Utrecht, SG, The Netherlands), version 2.3, was used for panelists' statistics (correlations and PCA), ANOVA, and GPA.

RESULTS AND DISCUSSION

The SPME-GC-O analysis clearly indicated that the "sweet-like odor" (the same described as "the defect" in wines) is related to a single chromatographic peak that was recognized by all of the judges and in all of the defective wines at 28.42 min (in the operating conditions). SPME-GC-MS analysis demonstrated that the detected compound was the ethyl ester of phenylacetic acid (EPhA; CAS Registry No. 101-97-3; retention index, 1771) in

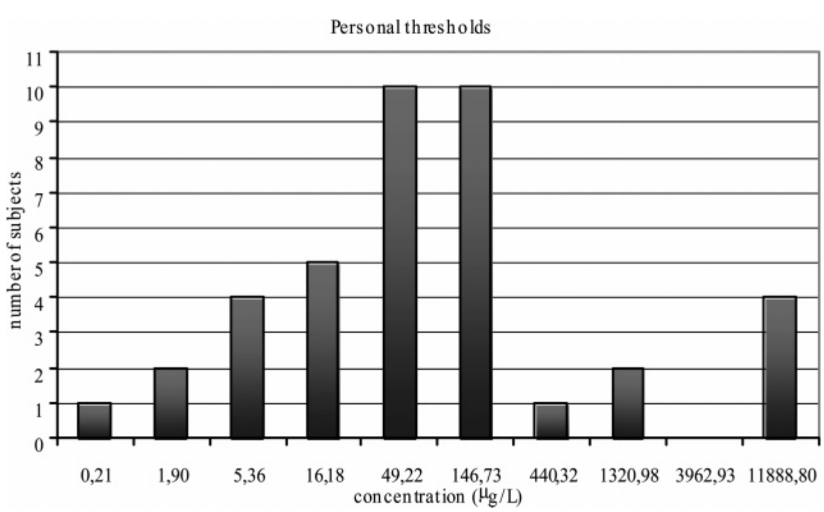

Figure 1. Distribution of individual thresholds as a function of EPhA concentration in wine for a group of 39 subjects. The samples were evaluated ortonasally (ASTM E-679 rapid method).

Table 3. Results of Two-Factor (Samples and Panelists) Analysis of Variance (ANOVA) and Least Significant Difference (LSD) Carried out on Data from an Attribute Difference Test[a]

ANOVA Table for Attribute "Honey"

source of variation	degrees of freedom	sum of squares	mean square	*F*	*p*
between subjects	24	270.22	11.26	2.48	3.41E–04
samples	8	256.24	32.03	7.06	3.67E–08
residue (error)	192	871.54	4.54		
total	224	1398			

LSD Table for Attribute "Honey"

		LSD at different levels of probability		
wine	mean ± SD ($n = 25$)	$p = 0.05$ (1.19)[b]	$p = 0.01$ (1.57)[b]	$p = 0.001$ (2.01)[b]
6	3.36 ± 1.87	a	a	a
1	3.44 ± 2.12	ab	ab	ab
2	4.6 ± 2.22	bc	abc	ab
3	4.72 ± 2.48	c	abc	ab
5	4.92 ± 2.36	c	abc	ab
9	4.96 ± 2.68	c	bc	ab
8	5.2 ± 2.42	c	c	abc
7	5.4 ± 2.6	c	c	bc
4	7.2 ± 1.78	d	d	c

[a] Different letters within a column represent means that are significantly different at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$. [b] Critical LSD values.

Table 4. Results of Two-Factor (Samples and Panelists) Analysis of Variance (ANOVA) and Least Significant Difference (LSD) Carried out on Data from an Attribute Difference Test[a]

ANOVA Table for Attribute "Tobacco"

source of variation	degrees of freedom	sum of squares	mean square	*F*	*p*
between sets	26	452.52	17.4	4.19	2.30E–09
object	8	212.74	26.59	6.40	1.99E–07
residue (error)	208	864.59	4.16		
total	242	1529.85			

LSD Table for Attribute "Tobacco"

		LSD at different levels of probability		
wine	mean ± SD ($n = 27$)	$p = 0.05$ (1.09)[b]	$p = 0.01$ (1.44)[b]	$p = 0.001$ (1.85)[b]
9	3.48 ± 2.26	a	a	a
2	3.85 ± 2.46	ab	a	ab
5	4.07 ± 2.64	ab	a	ab
4	4.26 ± 2.65	ab	a	ab
7	4.41 ± 2.19	ab	ab	ab
8	4.59 ± 2.37	b	ab	ab
1	4.7 ± 2.58	bc	ab	ab
3	5.7 ± 2.43	cd	bc	bc
6	6.7 ± 1.56	d	c	c

[a] Different letters within a column represent means that are significantly different at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$. [b] Critical LSD values.

Table 5. Results of Two-Factor (Samples and Panelists) Analysis of Variance (ANOVA) and Least Significant Difference (LSD) Carried out on Data from an Attribute Difference Test[a]

ANOVA Table for Attribute "Technical Pleasantness"

source of variation	degrees of freedom	sum of squares	mean square	*F*	*p*
between sets	24	305.72	12.74	5.42	4.71E–12
object	8	109.12	13.64	5.80	1.24E–06
residue (error)	192	451.32	2.35		
total	224	866.16			

LSD Table for Attribute "Technical Pleasantness"

		LSD at different levels of probability		
wine	mean ± SD ($n = 25$)	$p = 0.05$ (0.86)[b]	$p = 0.01$ (1.13)[b]	$p = 0.001$ (1.45)[b]
6	3.4 ± 1.61	a	a	a
4	3.6 ± 2.04	ab	ab	a
9	4.24 ± 1.94	abc	ab	ab
1	4.36 ± 1.78	bc	abc	ab
2	4.6 ± 1.73	c	bcd	ab
3	4.68 ± 2.14	cd	bcd	ab
7	4.72 ± 1.77	cd	bcd	ab
5	5.48 ± 1.87	de	cd	b
8	5.6 ± 1.91	e	d	b

[a] Different letters within a column represent means that are significantly different at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$. [b] Critical LSD values.

agreement with Baek and Cadwallader (*5*). This compound is normally present in wines (*9*−*11*) at a concentration of a few micrograms per liter, but it seems not as high as in defective Aglianico products (**Table 2**), in which it can reach values near 100−150 μg/L. Obviously this first quantitative approach has to be confirmed by constructing databases (for both Aglianico and other varieties) with time.

The biosynthetic pathways that lead to the formation of this ester are quite common in both plants and microorganisms; ethyl phenylacetate and phenylacetic acid are pheromones for different species of Hymenoptera (*Atta bisphaerica*, *Atta levigata*, *Atta sexdens rubropilosa*; www.pherobase.net). With regard to wine, the most probable precursors of ethyl phenylacetate can be related to the shikimic acid pathway (biocyc.org; *12*), such as phenylalanine and tyrosine, and also cinnamic acids. EPhA could be produced during alcoholic fermentation by esterification of phenylacetic acid by yeasts; this could also explain why "the odor" appears only after fermentation, whereas it is usually not detectable in musts.

Table 2 results (range of EPhA concentration in Aglianico wines) were used to project a threshold sensory test: results are reported in **Figure 1**. The distribution of individual thresholds as a function of EPhA concentration shows a Gaussian profile; the curve is not symmetrical, with the major slope at high levels of concentration. The sensory panel threshold of EPhA in wine, as detected by enologists, was 73 μg/L (geometrical mean of the individual thresholds); this value is much lower than that reported by Moyo et al. (*13*) (250 μg/L). The reasons might be the utilization of different sensory methods, panels, and matrices (the volatile composition and the colloidal profile of wine can cover or influence the perception of a single compound). It is interesting to note the presence of a not negligible percentage of panelists with a very high sensory threshold (11800 μg/L). Subjects with individual threshold >2 times the standard deviation of the threshold distribution (in this case it was 305.2) are called anosmics (*14*): they lack specific olfactory receptors, and they use nonspecific ones. For some odorants, a bimodal curve can be observed, with population showing two different thresholds; the bigger the difference between the anosmics' threshold and the population threshold, the smaller is the adaptability of the odorant to nonspecific receptors (*15*). Specific receptors are associated with very important compounds related to fundamental functions in the animal kingdom (nutrition, sociality, reproduction). In this case, approximately 10% of panelists were anosmics for ethyl phenylacetate, showing a threshold 163 times higher than the rest of the judges; this means that the perception of EPhA seems to depend at 99.4% on a specific receptor. Obviously, these data must be broadened over an increasing number of subjects.

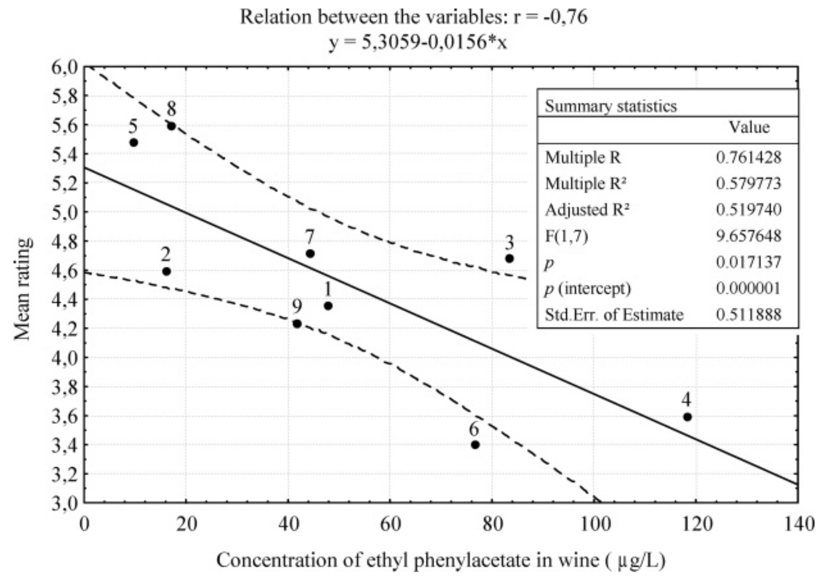

Figure 2. Attribute "technical pleasantness" from attribute difference test, as a function of the concentration of ethyl phenylacetate in wine. Wines are labeled with codes, and a summary statistics table, from a regression analysis, is reported.

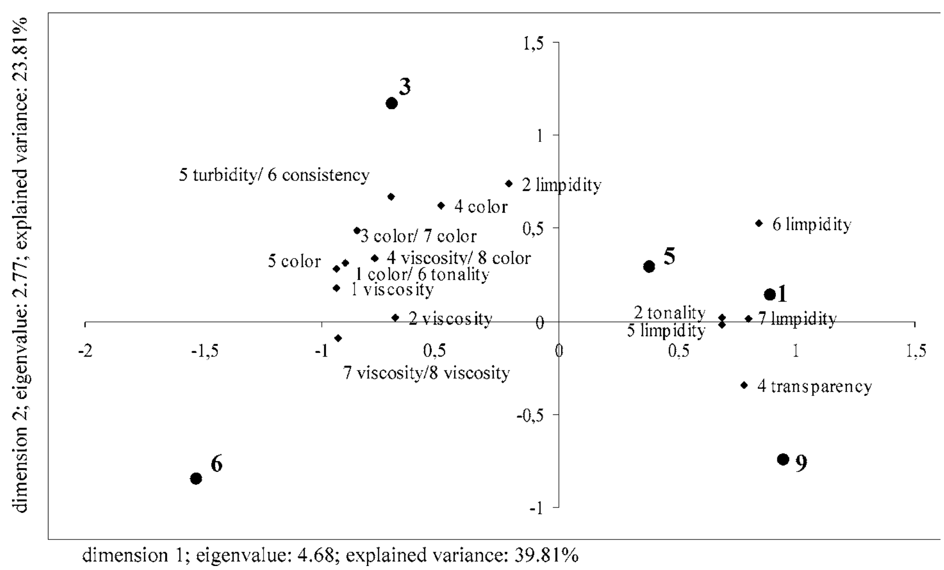

Figure 3. GPA analysis of visual, olfactive, gustative, and retronasal sensory data from a free choice profile. Visual attributes compare only. Wines are labeled with codes, and the numbers indicated in front of the attributes represent the panelists that used that term.

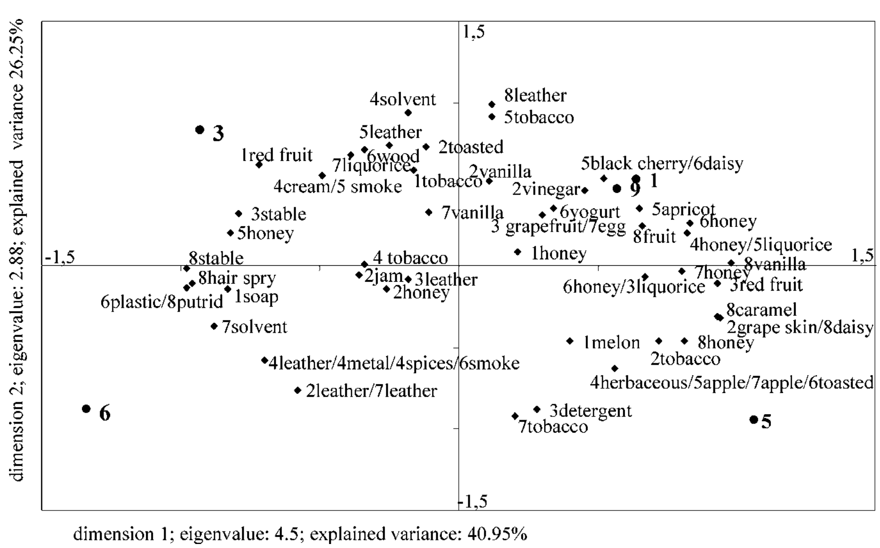

Figure 4. GPA analysis of olfactive sensory data from a free choice profile. Wines are labeled with codes, and the numbers indicated in front of the attributes represent the panelists that used that term.

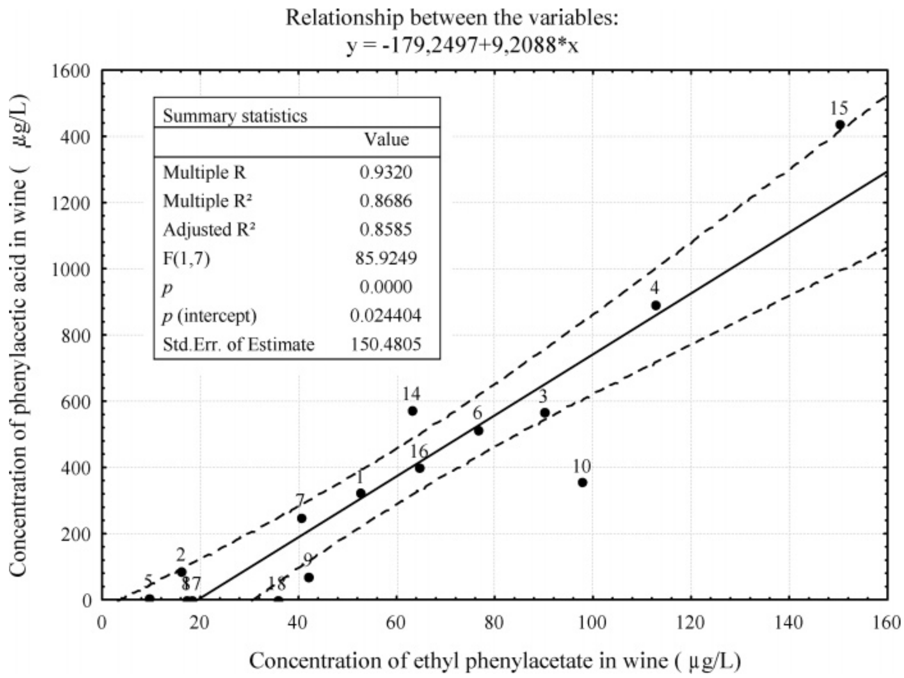

Figure 5. Relationship between the concentrations of phenylacetic acid and ethyl phenylacetate in commercial wines. Wines are labeled with codes, and a summary statistics table, from a regression analysis, is reported.

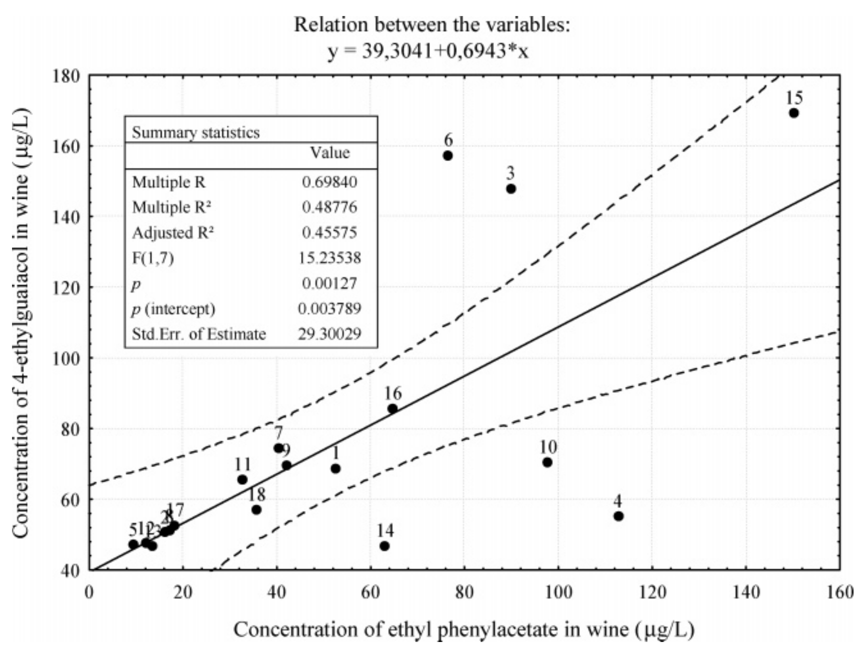

Figure 6. Relationship between the concentrations of 4-ethylguaiacol and ethyl phenylacetate in commercial wines. Wines are labeled with codes, and a summary statistics table, from a regression analysis, is reported.

The attribute difference test confirmed these results (**Tables 3** and **4**) because products with EPhA concentration near or higher than the detected sensory threshold were marked as significantly different from the others. In sample 4 (**Table 3**), the panel recognized the most intense smell of honey; it was statistically different ($p < 0.001$) from nearly all other samples. This fact seems not to be casual because sample 4 is the most concentrated in EPhA (see **Table 2**) among the wines selected for sensory analysis. Probably the level of the ester in this sample is similar to the recognition threshold or higher. Apart from this wine, EPhA is not recognized, and less concentrated samples have been indistinctly classified; in fact, wines 1 and 6 are the least intense in honey smell despite their different levels of EPhA concentration.

Table 4 reports the results related to the attribute "tobacco"; wines 3 and 6 were marked as significantly different ($p < 0.01$ and $p < 0.001$) from the major part of the others. Their EPhA concentrations were 89.90 and 76.43 μg/L, respectively; this fact seems not to be casual because both of these concentrations are near the detection threshold, confirming the values previously reported. Wines with lower amounts of EPhA showed a low tobacco character and were indistinctly classified by the judges. Nevertheless, also sample 4, which had the highest EPhA concentration (113 μg/L), showed very few tobacco characters. A possible explanation is the following: if the EPhA amount in wines was close to the detection sensory threshold, panelists recognized wines as different, and they described the perceived sensory character as tobacco, because tobacco was the only

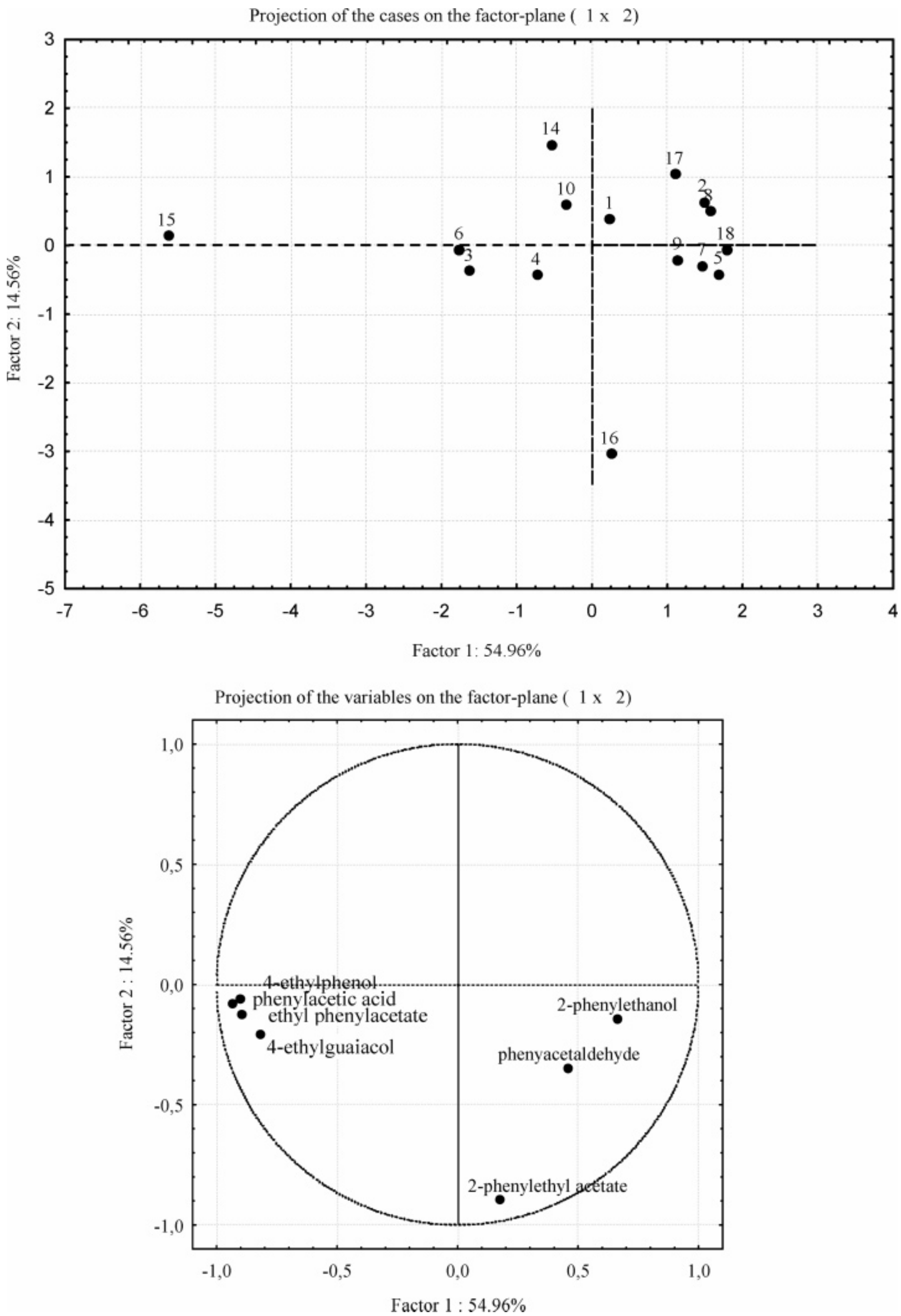

Figure 7. PCA based on amounts of ethyl phenylacetate and some possible related compounds in the metabolism. Concentrations of volatile compounds were analyzed in commercial Aglianico del Vulture wines and are reported in **Table 1**; wines are labeled with codes.

attribute they had. If the EPhA concentration increased, the sensory note became clearly "honey-like"; "tobacco" is probably not a good attribute to describe defective wines.

To evaluate, with a formal sensory method, whether there is a relationship between the level of EPhA in wines and hedonic technical judge, the panel was asked to rate "technical pleasantness"; this attribute (**Table 5**) characterizes two of the three less concentrated wines (samples 5 and 8). Significant differences with regard especially to sample 8, wines 4 and 6, with high levels of EPhA, were considered to be less pleasant by enologists. Regression analysis (**Figure 2**) between concentration of EPhA and technical pleasantness shows significant results at $p < 0.05$. Two samples are out of this trend: wines 2 (low EPhA but intermediate technical pleasantness) and 3 (high EPhA but intermediate technical pleasantness); this depends on the fact that technical pleasantness is related to a complex of characteristics. Obviously these data must be verified on a more numerous set of samples.

Enologists with the lowest sensory thresholds were requested to effect a flavor profile test. The most significant results are relative to visual and olfactory descriptors. **Figure 3** shows the distribution of samples in the two-dimensional space by GPA, starting from the totality of descriptors; wines with low and medium levels of EPhA are on the right side of the graph, contrarily of samples with high amounts. The distribution of visual descriptors on this two-dimensional space shows that these last wines are characterized by intense color and viscosity, so more defective wines seem to be rich in phenolic fraction (color) and extract (viscosity, consistency). This fact confirms the empiric observation that the studied odor develops in wines obtained by maceration. GPA on olfactory descriptors only leads to a distribution of samples that seems well related to the concentration of EPhA (**Figure 4**): wines with medium level of ester (samples 1 and 9) are separated from the one with low amount (sample 5) on the right side of the graph, whereas the most defective samples, 3 and 6, are on the left. A lot of negative descriptors characterize wines 3 and 6, especially leather and stable. This fact confirms once again the empiric observation that the studied odor develops together with volatile phenols and suggests cinnamic acids as a possible starting point for the formation of the off-flavor. In fact, cinnamic acids are common precursors of the ester and of the volatile phenols (4-ethylphenol, 4-ethylguaiacol), as said before.

This fact was verified by GC quantitative analysis: the content of ethyl phenylacetate and some possibly related volatile compounds was analyzed in commercial wines. **Table 1** reports the amounts of 4-ethylphenol, 4-ethylguaiacol, phenylacetaldehyde, phenylacetic acid, 2-phenylethanol, and 2-phenylethyl acetate in different samples. Data showed correlation between EPhA and phenylacetic acid (**Figure 5**); in fact, the acid is the most probable direct precursor because it could be normally esterified by the yeast to the relative ester. The regression summary for phenylacetic acid concentration, as dependent variable, is reported in the figure; the *p* values are low, and this confirms that a linear model fits well. Data relative to wines 11−13 were eliminated from the elaboration because areas of chromatographic peaks were invalidated by coelution by an undetected compound.

A relationship exists also among EPhA and the volatile phenols, both with 4-ethylguaiacol and with 4-ethylphenol; **Figure 6**, with the results of regression analysis, shows that the correlation between EPhA and 4-ethylguaiacol is linear, even if the R^2 is weaker than that of phenylacetic acid. Anyway, the projection of variables on the factor plane from PCA in **Figure 7** shows that EPhA, 4-ethylguaiacol, 4-ethylphenol, and phenylacetic acid are close by; these relationships suggest once again common precursors (e.g., cinnamic acids) as possible starting points for the formation of the studied off-flavor (*12*).

For these reasons, the research shall be developed by the investigation of the conditions that lead to EPhA formation in Aglianico wines; the identification of the responsible factors (plant growing conditions, seasonal/climatic factors, molds and cryptogamic diseases, yeast strain and growing conditions, *Brettanomyces*) will suggest suitable technological approaches to control the problem in wineries.

ACKNOWLEDGMENT

We thank Patrizio Bianchi for diligent work in this research.

LITERATURE CITED

(1) Manzo, M. L'Aglianico nella storia. In *Colori, Odori ed Enologia dell'Aglianico*; Moio, L., Ed.; Poligrafica F.lli Ariello Editori sas: Napoli, Italy, 2004; pp 17−21.

(2) Tat, L.; Comuzzo, P.; Stolfo, I.; Battistutta, F. Optimization of wine headspace analysis by solid-phase microextraction capillary gas chromatography with mass spectrometric and flame ionization detection. *Food Chem.* **2005**, *93*, 361−369.

(3) Comuzzo, P.; Tat, L.; Tonizzo, A.; Battistutta, F. Yeast derivatives (extracts and autolysates) in winemaking: release of volatile compounds and effects on wine aroma volatility. *Food Chem.* **2006**, *99*, 217−230.

(4) Mistry, B. S.; Reineccius, T.; Olson, L. K. Gas chromatography−olfactometry for the determination of key odorants in foods. In *Techniques for Analyzing Food Aroma*; Marsili, R., Ed.; Dekker: New York, 1997; pp 265−292.

(5) Baek, H. H.; Cadwallader, K. R. Contribution of free and glycosidically bound volatile compounds to the aroma of Muscadine grape juice. *J. Food Sci.* **1999**, *64*, 441−444.

(6) American Society for Testing and Materials (ASTM). Standard practice for determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits. In *Annual Book of ASTM Standards*; ASTM: Philadelphia, PA, 1991; pp 34−39.

(7) Meilgaard, M.; Civille, G. V.; Carr, B. T. *Sensory Evaluation Techniques*, 2nd ed.; CRC Press: New York, 1991.

(8) Williams, A. A.; Langron, S. P. The use of free-choice profiling for the evaluation of commercial ports. *J. Sci. Food Agric.* **1984**, *35*, 558−568.

(9) Baumes, R.; Cordonnier, R.; Nitz, S.; Drawert, F. Identification and determination of volatile constituents in wines from different vine cultivars. *J. Sci. Food Agric.* **1986**, *37*, 927−943.

(10) Ferreira, V.; Purificacion, F.; Gracia, J. P.; Cacho, J. F. Identification of volatile constituents in wines from *Vitis vinifera* var Vidadillo and Sensory contribution of the diferent wine flavour fractions. *J. Sci. Food Agric.* **1995**, *69*, 299−310.

(11) Perestrelo, R.; Fernandes, A.; Albuquerque, F. F.; Marques, J. C.; Camara, J. S. Analytical characterization of the aroma of Tinta Negra Mole red wine: identification of the main odorants compounds. *Anal. Chim. Acta* **2006**, *563*, 154−164.

(12) Dewick, P. M. *Chimica, Biosintesi e Bioattività delle Sostanze Naturali*; Piccin: Padova, Italy, 2000.

(13) Moio, L.; Piombino, P.; Genovese, A.; Ugliano, M.; Pessina, R. L'aroma del vino Aglianico. In *Colori, Odori ed Enologia dell'Aglianico*; Moio, L., Ed.; Poligrafica F.lli Ariello Editori sas: Napoli, Italy, 2004; pp 109−138.

(14) Amoore, J. R. Specific Anosmia. In *Smell and Taste in Health and Disease*; Getchell T. V., Doty, R. L., Bartoshuk L. M., Snow J. B., Eds.; Raven Press: New York, 1991; pp 655−664.

(15) Pelosi, P. Classificazione e misura degli odori. In *Caratteristiche Olfattive e Gustative degli Alimenti*; Montedoro, G., Ed.; Chiriotti Editori: Pinerolo (TO), Italy, 1985; pp 35−82.

Received for review December 21, 2006. Revised manuscript received April 5, 2007. Accepted April 11, 2007. The project was supported by Basilicata Region in cooperation with Agenzia Lucana di Sviluppo e di Innovazione in Agricoltura (ALSIA) and with the collaboration of Private and Co-operative Wineries of the Vulture Region and of the Camera di Commercio of Potenza.

JF0637067