Further Insights into the Floral Character of Touriga Nacional Wines

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ABSTRACT: Higher-quality Touriga Nacional (TN) wines are characterized by a fruity-citric aroma described as sweet and fresh citrus evoking the bergamot fruit (*Citrus bergamia*). In fact, "bergamot-like" descriptor is currently employed to rate higher quality TN wines. The aim of this work was to identify among volatile compounds present in bergamot fruit extracts (mainly terpenes) which of them contributes the most to the bergamot overall perception, and relate these data with the volatile composition of TN wines. The identification of the most important descriptors was done by sensory analysis. Among 18 descriptors 3 were selected: bergamot-like aroma, orange like, and violet. A GCO of a typical TN wine extract allows the identification of 3 related odorant zones ZO1, ZO2, and ZO3 related with bergamot-like aroma. Using AEDA, ZO2 was confirmed to be one of the most important odorant zones. Using AEDA the presence of linalool and linalyl acetate was confirmed. A similarity test was performed with a non-TN wine added with linalool and linalyl acetate alone or in combinations. The highest similarity value was observed when linalool (SV = 5.9) was added. In fact, results obtained from the analysis of several red wines from different varieties show that terpenols are present in higher amounts in wines coming from TN variety, which proves that these compounds can be the clue to the varietal aroma of TN wines.

Keywords: AEDA, bergamot-like descriptor, GCO, sensory analysis, Touriga Nacional wines

Introduction

Touriga Nacional (TN) is a *Vitis vinifera* variety, planted in Portugal, which is considered one of the best red varieties to produce quality wines. Higher-quality TN wines present a "typical aroma" which is recognized by experts (oenologists, wine opinion makers, journalists) as a characteristic of the TN variety. The wines that present such aroma profile have higher prices in the market. This fruity-citric aroma is often characterized as sweet and fresh citrus evoking the bergamot fruit (*Citrus bergamia*). Bergamot essential oil is mainly used in perfumery and aromatherapy, but is also employed in the pharmaceutical, food, and confectionery industries. Their chemical composition depends on the fruit origin and on the extraction method (Mondello and others 1998; Minh Tu and others 2003; Verzera and others 2003; Tranchida and others 2006). The typical volatile compounds are linalool, linalyl acetate, α-pinene, β-pinene, myrcene, β-bisabolene, nerol, neryl acetate, geraniol, geranyl acetate, and α-terpineol, and the principal nonvolatile constituents are bergamotene, bergaptene, and critroptene (Verzera and others 2003; Tranchida and others 2006). Some other volatiles from other *Citrus* species were also identified (Choi and others 2001; Minh Tu and others 2003). "Bergamot-like" descriptor is currently employed to rate higher-quality TN wines to describe the floral-like aroma present in some wines. Previous works have shown that TN wines have higher levels of terpenol compounds compared with other wines made from other red wine varieties (Barbosa and others 2003; Oliveira and others 2006). There is a consensus that monoterpenes have an important role on the wine aroma. As suggested by several studies, terpenoid compounds are closely associated with sensory expression of the wine bouquet, being used for variety characterization. The terpenols are interesting for their flowery odors (Wirth and others 2001). The determination of terpene profile in some wine varieties was carried out by several authors (Williams and others 1980; Gunata and others 1990; Sabon and others 2002; Barbosa and others 2003). In a recent work the terpene composition of 158 wines from 14 Portuguese wine varieties was determined (Barbosa and others 2003). The relationship between grape flavor content and wine quality is dependent on terpene content for most of the varietal aroma (Gunata and others 1990). The literature refers to some levels of terpenols for white wines; its presence when above the olfactif perception limit corresponds to wine with floral characteristics as it is the case of Muscat wines (Cordonnier and Bayonove 1974; Delcroix and others 1994). This fact justifies the wine industry's interest in knowing the terpene content of their wines. The monoterpenes such as linalool, α-terpineol, citronellol, nerol, geraniol, and so on can exist in 2 forms: free and glycosidically conjugated forms. The free aroma compounds are volatile substances that have an olfactive impact and are for the most part related to wine quality. The free form is present in several wine varieties in different amounts (Gunata and others 1990). In addition to the free form of monoterpenes, there are the glycosidically bound monoterpenes that are quantitatively the most important forms, although they do not have a direct contribution on wine aroma. Despite the apparent importance of terpenes in the global aroma, no recent works relating to the levels of terpens in wines and their sensory impact exist. The perception thresholds existing in the literature date from the 1990s (Etievant 1991).

The levels of terpenes for red wines are less studied as they are not considered to contribute to the red wine flavor. Descriptors usually related with red wine are red and black fruits, black, violet, butter, spicy, balsamic, and woody like (Noble and others 1987).

TN cultivar is one of the most important red wine varieties. It is one of the most important varieties used to produce Port wine at Douro Region in Portugal. Because of its quality this variety is

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nowadays planted in several wine production regions around the world. Wines produced with this variety have higher commercial prices.

Precedent works have shown that the aroma of TN wines is mainly related to the terpene composition (Barbosa and others 2003). This knowledge can be important information for enologists as they can better control the levels of these compounds in grapes by correct viticulture practices and consequently increase the floral perception of TN wines. They can also increase terpene contents by changing some technological parameters during wine production. For example, recent works performed in red wines show differences among free and glycosidically bound volatile compounds in red wine measured after malolactic fermentation (MLF). According to these results, MLF resulted in a significant decrease in the concentration of total glycosides. Ugliano and Moio (2006) show that glycoside-related aroma compounds such as linalool, farnesol, and β-damascenone were increased after MLF. Fernandez and others (2003) and Hernandez and others (2003) used selected yeasts and demonstrated that they can also increase the contents of terpenes in wines.

The aim of this work was to determine which among volatile compounds present in bergamot fruit (one of the TN descriptors, determined by an industry panel) contributes the most to the bergamot overall perception, relating these data with the volatiles of TN wines. In order to evaluate the relationship between "bergamot-like" descriptor and TN wine quality, several monovarietal wines coming from different wine regions were analyzed.

Materials and Methods

TN wines selected by industry as higher quality

Seven TN wines coming from different Portuguese wine regions were selected by an industry expert panel a corresponding to TN higher-quality wines with the typical aroma, called by this panel a "bergamot-like" aroma.

Other monovarietal red wines

Other monovarietal red wines used in this study were a total of 75 different wines from Tinta Roriz (TR), Tinto Cão (TC), Tinta Franca (TF), Tinta Barroca (TB), and TN.

Bergamot essential oil

Two drops of this oil (from Segredo da Planta, Produtos Naturais e Biológicos Lda, Seixal, Portugal) were diluted in 100 mL of a 20% ethanol. This solution was used for the sensory and chemical analyses. For some sensory tests, earl grey tea (infusion) was also used as its composition is bergamot.

Sensory studies: sensory panels

Two different sensory panels were constituted: first an expert panel, and then a trained panel. The expert panel comprised winemaking producers who selected among several monovarietals TN wines those corresponding to the highest quality TN wines. The panel was constituted by 8 persons selected from a group of 15 on the basis of their availability to perform wine evaluation. The trained panel comprising 12 graduate students, the majority with previous wine tasting experience, were selected from a group of 19 persons on the basis of their sensory performances. The selection process was similar to Issanchou and others (1995).

The typical high-quality TN wines were selected by the industry panel and were used to conduct 4 free choice profiling sessions; this procedure was used to select the most important descriptors that describe the global aroma of TN wines (descriptor selection).

In 2 sessions, the judges from the trained panel generated descriptive terms concerning the TN wines selected by the expert panel. These preliminary terms were then discussed and modified and, then, by consensus, the most appropriate terms defining the typical character of TN wines were selected (Table 1).

Four training sessions were held. In each session, 1 commercial high-quality TN wine was presented with 18 reference aroma standards to help the panelists identify and remember the sensory attributes found in the evaluated wine samples presented in transparent 170-mL (6.5 oz) tulip-shaped wine glasses. Individual aroma standards containing 20 mL of red wine were presented in black 170-mL (6.5 oz) tulip-shaped wine glasses, covered with plastic Petri dish lids. All evaluations were conducted in an open space room at the temperature (22 ± 1 °C) under white lightning.

In each session, 2 different TN typical wines were analyzed. The 18 preliminary terms were then discussed and modified and, then, by consensus, the most appropriate terms defining differences among these wines were selected (Table 1) for use in the formal descriptive analysis. Thirteen of them were selected for the formal descriptive analysis. Aromas like "banana" (1), "chocolaty" (4), "apple" (5) were discarded; "eucalyptus" (8) and "minty" (14) were put together (menthol and eucalyptus essences) as "balsamic" note (Table 1). In 2 latter sessions, a TN wine was again presented to the panel. The panelists were asked to note the presence or absence of the 13 attributes selected.

The "similarity value" (SV) of each sample with the higher quality TN was determined by a comparison test using a discontinuous scale from 0 to 10.

Similarity tests

The identification of the most important descriptors related to bergamot-floral characteristics of TN high-quality wines was performed according to AFNOR NFV-09-021 (1991) by the trained panel (Falqué-Lopez and others 2004).

Table 1 — Attributes used for the descriptive analysis by the 12 panelists and composition of reference standards (standards were made out of several commercial brands available at Continente Grocery Store, Matosinhos, Porto, Portugal)

	Aroma[a]	Composition
1	Banana	Banana fruit skinless
2	Woody	Oak chips with some drops of oil
3	Caramel	Liquid caramel
4	Chocolaty	Chocolate powder
5	Apple	Apple preserves
6	Clove	Clove-spice grains
7	Bergamot	Earl grey tea
8	Eucalyptus	Eucalyptus essence
9	Dried Fruit	Mixed dried fruit (raisins + prunes + figs)
10	Wild Fruit	Wild fruit preserves (cassis + cherry + strawberry)
11	Nutty	Mixed nuts (pecan + walnuts + almonds)
12	Passion Fruit	Passion fruit pulp
13	Honey	Home made honey
14	Minty	Menthol essence
15	Black pepper	Black pepper spice grains
16	Grassy	Fresh cut grass
17	Orange like	Scraped mandarin skin
18	Violet	Violet essence

[a]Only available during training sessions.
All the standards were labeled and presented in a 20 mL of red wine basis in black 170-ml (6.5 oz) tulip-shaped wine glasses covered with plastic Petri dish lids.

Identification of key sensory descriptors

First, triangular tests for bergamot aroma—3 sessions with 4 sets—were performed. No-typical wine was spiked with earl grey tea (or bergamot tea, a direct infusion prepared at room temperature for 5 s). The panel detected the different sample with an RSD of 19% which corresponds to 79.5% of correct answers. Following this, 6 sessions were held. In each session, 3 wines were presented with 13 reference standards to help panelists identify and remember sensory attributes found (Rainey 1986) in the evaluated wine samples, Silva Ferreira and others (2003). The composition is listed in Table 1.

Organic extract selection

Different organic solvents—hexane, ether, ethyl acetate, and dichloromethane—were used to obtain extracts from the bergamot oil dilution and from the TN wine. Similarity tests were performed between the aromas of the obtained extracts and the diluted bergamot oil and the wine, respectively, following the same procedure as done by Silva Ferreira and others (2002). Two milliliters of each organic extract was concentrated under nitrogen stream until 0.5 mL. A drop was then put on "perfume sampling papers," and the aroma was compared with the diluted bergamot oil and the original wine.

Gas chromatography/olfactometry

Two microliters of the extract of the diluted bergamot oil and the TN wine was injected into the GC equipped with an olfactometric detector. Chromatographic conditions were the following: Hewlett Packard HP 5890 gas chromatograph; column BP-21 (50 m × 0.25 mm, 0.25 μm) fused silica (SGE, Portugal); hydrogen (5.0, Gasin, Portugal); flow (1.2 mL/min); injector temperature, 220 °C; oven temperature, 40 °C for 1 min programmed at a rate of 2 °C/min to 220 °C, maintained for 30 min; splitless time, 0.5 min; split flow, 30 mL/min.

The make-up gas employed on the olfactometric device (SGE, Portugal) is air (80% N_2; 20% O_2) (Gasin, Portugal). Two streams were used—one was bubbled in water, nose moister; the other was applied at the exit of the GC column in order to lower the temperature of the effluent.

The descriptors' collection on GCO was performed by 5 trained persons (lab students) and was repeated several times until a constant number of descriptors and a quality evaluation were obtained. The descriptors retained were those that obtained the higher number of citations, considering each member of the panel.

GC-olfactometry and GC-MS analysis

The GCO analysis was employed on dichloromethane (DCM) extracts of TN wines in order to determine odorant zones related to bergamot aroma. The extracts of bergamot oil were also analyzed to determine the dilution factors of the most important odorant zones. Each extract was analyzed twice by 3 trained panelists.

Aroma extract dilution analysis

The relative importance of each different odor zones in bergamot oil and in TN wine extracts was evaluated by "aroma extract dilution analysis," AEDA, as done by Ullrich and Grosch (1987). Two milliliters of the extract was concentrated to 1:10 under nitrogen stream. Then, 2 μL of the concentrated dichloromethane extract were separated on a capillary column. The odor-active regions and the odor qualities were assigned by 5 assessors (GCO). The extract was stepwise diluted with dichloromethane (1 + 1 by volume), and the odor zones were reevaluated. The process stopped when no aromas were detected by the assessor. Flavor dilution (FD) factors of the odor-active compounds were determined. The FD factors of the odor-active compounds were determined in the following dilution series: the original extract was diluted stepwise with dichloromethane (1:1 by volume). This factor was calculated as 2^{n-1}, with n being the number of dilutions (factor 2) required for no odor to be perceived. The AEDA was performed according to the Etievant and others (1995) method. The identification of odorants was made by comparing retention index (RI), mass spectra, and odor properties of unknowns with those of authentic standard compounds analyzed by identical conditions. To calculate the RI values, we added a series of n-alkanes (from 8 to 32 carbon atoms). In case standards were not available, compounds were positively identified based on Wiley 7N mass spectral database (Hewlett Packard) or tentatively identified according to the bibliography.

Gas chromatography/mass spectrometry

Dichoromethane extracts were analyzed using a Varian CP-3800 gas chromatograph (U.S.A.) equipped with a Varian Saturn 2000 mass selective detector (U.S.A.) and a Saturn GC/MS workstation software version 5.51. The column used was STABILWAX-DA (60 m × 0.25 mm, 0.25 μm) fused silica (Restek, U.S.A.). The injector port was heated to 220 °C. The split vent was opened after 30 s. The carrier gas was Helium C-60 (Gasin, Portugal) at 1 mL/min, constant flow. The oven temperature was 40 °C (for 1 min), then increased at 2 °C/min to 220 °C and held for 30 min. All mass spectra were acquired in the electron impact (EI) mode. The ion trap detector was set as follows: the transfer line, manifold, and trap temperatures were, respectively, 230, 45, and 170 °C. The mass range was 33 to 350 m/z, with a scan rate of 6 scans/s. The emission current was 50 μA, and the electron multiplier was set in relative mode to autotune procedure. The maximum ionization time was 25000 μs, with an ionization storage level of 35 m/z. The injection volume was 1 μL and the analysis was performed in full scan mode.

Identification was achieved from comparisons of mass spectra obtained from the sample with those from pure standards injected in the same conditions, by comparing the Kovats indices and the mass spectra present in the NIST 98 MS Library Database or in the literature.

Alpha-pinene, linalool, terpineol, geraniol, nerol, citronelol, linalylacetate, and limonen were quantified by GC-MS as earlier described by Silva Ferreira and others (2003).

Statistical analysis

Analysis of variance (ANOVA) using Excel^{TM} software from Windows 98 v 7.0 (Microsoft Corp., Redmond, Wash., U.S.A.) was applied to the experimental data, and the results were considered significant if the associated P-value was < 0.05.

Results and Discussion

Terpenols in red wines

Among the 75 red wines analyzed, levels of terpenols (linalool, terpineol, geraniol, nerol, and citronellol) were much higher in TN wines than in the other red wines tested (Figure 1). In fact, wines from TN are considered to have floral characteristics, which could be associated with the presence of terpenol compounds.

Identification of key sensory descriptors

According to the procedure described in Materials and Methods, it was found that bergamot is the aroma that describes best the typical aroma of TN wine. The comparison of the TN wine (selected by

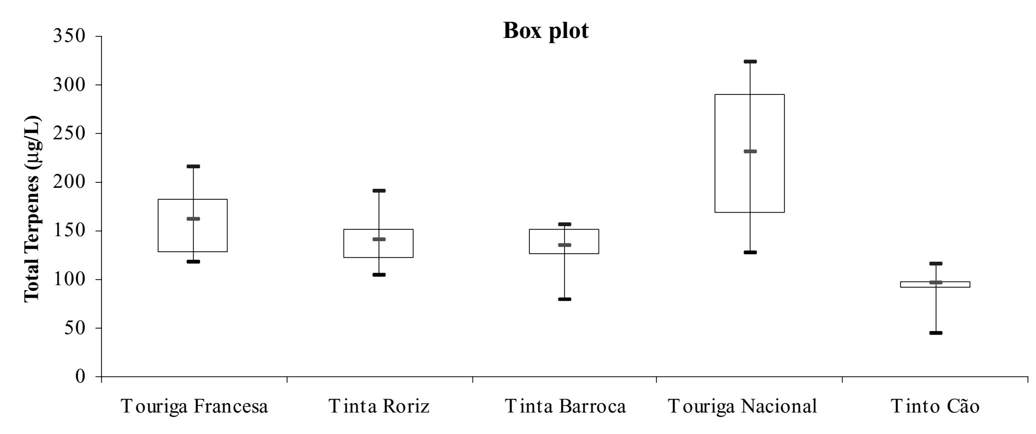

Figure 1 — Distribution of terpenol compounds (linalool, terpineol, geraniol, nerol, and citronellol)

industry panel) with a no-typical wine spiked with reference materials for 3 combine attributes showed that the wine spiked with earl grey tea, scraped mandarin skin, and violet was the most similar to TN wine.

Determination of compounds related to sensory descriptors

GCO analysis of bergamot oil extract. The 2nd step of this work was to determine which compound among the global aroma of bergamot has the major sensory contribution. To our knowledge, no study has been published in order to determine the relative participation of each volatile compound in the global aroma of bergamot essential oil. The objective of this study was to perform an aroma extract dilution analysis (AEDA) to characterize the odorants that are responsible for the aroma of the bergamot essential oil. The selected organic solvent that represents best the global aroma of bergamot is the dichloromethane. The AEDA analysis was applied on this extract.

The GCO analysis of the bergamot essential oil extract revealed 99 odor-active compounds. However, among all these compounds, only 38 compounds exceeded an FD factor of 8, and only 18 substances presented a FD higher than 64 (Table 2). Some previous studies by Minh Tu and others (2003) and Choi and others (2001) with other species of *Citrus* have applied this methodology. For *Citrus hyuganatsu,* Choi and others (2001) using the AEDA have identified 10 odors. Among them linalool and octanol were regarded as the key compounds of this *Citrus* variety. For *Citrus inflate*, Minh Tu and others (2003) have identified by AEDA–GCO the major contributor which was the *E*-ocimene.

On the basis of the AEDA results, several groups of aroma-active compounds contributed to some desirable and undesirable flavors in bergamot oil; nevertheless 13 provided the typical citric, lemon, and lime aroma characteristics of this essential oil (Table 3). The comparison of the Kovats retention indices on polar stationary comparison phase and the mass spectra to the authentic compounds confirmed the presence of 5 volatile monoterpenes (α-pinene, linalool, linalyl acetate, α-terpineol, and citronellol). The results showed that the major contributors were α-pinene (FD = 1024), linalool (FD = 512), and linalylacetate, γ-terpinene(E)-β-ocimene (all with FD = 256) (Table 3). The 1st one, which exhibited a pine-like aroma, was found to be the strongest aroma contributor to this essential oil, since it had the highest FD factor. The presence of linalool and linalyl acetate was confirmed, and their quantification by GC-MS showed that these 2 volatile compounds were present in the highest concentration; this suggested their important role in the flavor of bergamot oil. These results were confirmed by recent works by Tranchida and others (2006). Gamma-terpinene, (E)-β-ocimene, and β-phellandrene, tentatively identified according to the Kovats retention index cited in the bibliography, were also important for the overall aroma property of bergamot essential oil (Lawrence 1987; Tranchida and others 2006).

GCO analysis of the TN wine extract. The GCO analysis was simultaneously performed with the same TN wine used in the AFNOR analysis. Figure 2 shows the GC-MS chromatogram with the 3 most important odorant zones related to bergamot aroma selected.

Three odorant zones (OZ) were identified with aroma related with bergamot-like aroma (Figure 2): a 1st odorant zone, OZ1 (RI = 1023, FD = 4), with an aroma described as pineapple/pine/fruity, a 2nd one, OZ2 (RI = 1560, FD = 4), with a floral, earl grey aroma descriptor;

Table 2 — Total compounds detected by GCO-AEDA in the bergamot oil extract

Flavor dilution (FD)	Compounds
1024	2
512	3
256	7
128	6
64	6
32	6
16	8
8	15
4	19
2	14
1	13

Table 3 — Volatile compounds of bergamot essential oil with citric odor notes

Compound	Kovats retention index (polar column)	Reliability of identification	Flavor dilution (FD)
α-pinene	1018	b	1024
Unknown	1042	c	128
Unknown	1045	c	128
Limonene	1207	a	8
β-phellandrene	1216	b	4
(E)-β-ocimene	1238	b	256
γ-terpinene	1252	b	256
Linalool	1555	a	512
Linalyl acetate	1566	a	256
α-terpineol	1696	a	4
Citronellol	1773	a	1
Unknown	1929	c	2
Unknown	2357	c	1

[a]GC-MS, odor description, and Kovats Retention Index of pure standard compounds.
[b]GC-MS, odor description, and/or Kovats Retention Index in agreement with the literature.
[c]Nonidentified compounds.

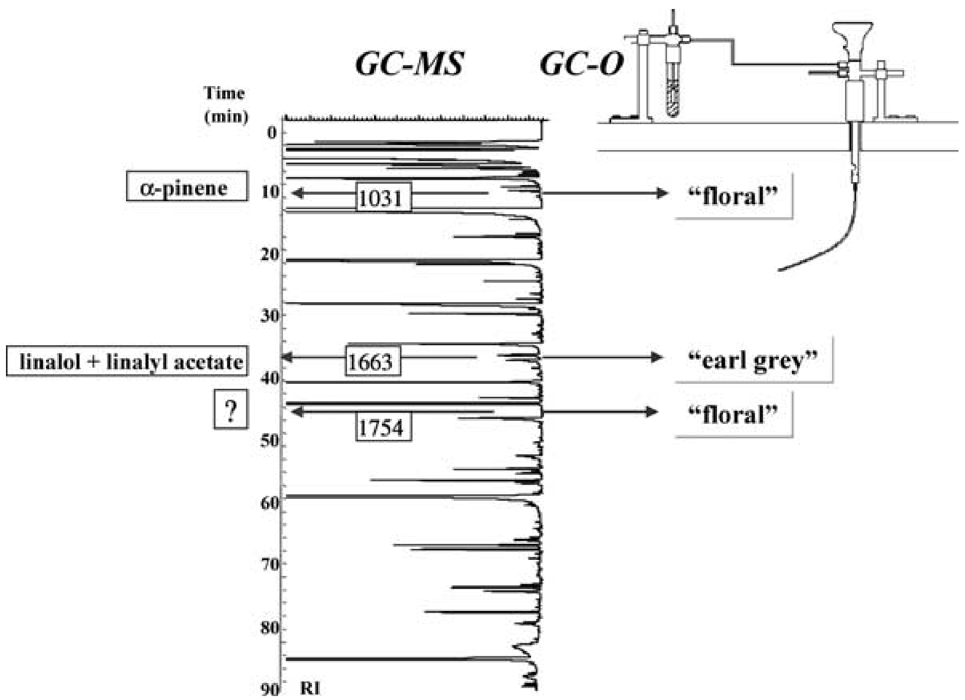

Figure 2—GCO and GC-MS analysis of a TN wine extract used in the AFNOR analysis. Identification of odorant zones on TN wine.

and finally a 3rd odorant zone, OZ3 (RI = 1940, FD = 32), described as floral.

A GCO of a DCM extract of a non-TN wine was also performed. The aromatic intensities of these zones were much less. Among the 3 chromatographic odorant zones related to the floral aroma the most similar with bergamot-like aroma was OZ2. This OZ corresponded to the presence of linalool and linalyl acetate identified by GC/MS. In order to investigate further contribution of these molecules into the "bergamot-like" aroma of TN wines, these 2 compounds were added either separately or together to a TR wine in concentrations that are naturally found in TN wines. This TR wine was selected as it has very low concentration in terpenols (Figure 1). The similarity values obtained for each pair of comparison test between TR wine and the spiked samples are given in Table 4.

The highest similarity value was observed when linalool was added to the TR wine (SV = 5.9); the addition of linalyl acetate has a small impact (SV = 3.3). The ANOVA calculations for the data showed differences between the samples ($P = 7.65$ e-5) at 95% level and no significant differences between assessors. All pair additions contributed in a high degree to TN aroma perception; SV ranged from 5.5 to 5.9.

Linalool, linalyl acetate, and maybe other terpenol compounds are important key odorants on the perceived aroma of TN typical wines as characteristic descriptors related to bergamot-like aroma. More results are needed to validate this information statistically.

Table 4—Results obtained from sensory analysis (12 persons)

Added compounds to TR	SV	SD
TN	9.7	1.1
TR + linalol	5.9	1.9
TR + linalyl acetate	3.3	2.1
TR + linalol + linalyl acetate	5.5	2.6
TR	2.8	2.4

SV = similarity value; SD = standard deviation.

Conclusions

In this work, attempts were made to elucidate the varietal aroma of TN wines. Seventy-five red wines coming from this variety were analyzed and it was observed that the levels of free terpenol were higher than those from other monovarietal red wines. On the other hand, sensorial panel experts from wine industry attribute to TN high-quality wines the "bergamot-like" aroma descriptor. The study of bergamot oil essence allows the determination of the volatiles with higher FD (factor dilution), which were related to the presence of α-pinene, linalool, and linalyl acetate. Simultaneously, using a trained panel and results from GCO, 3 descriptors, "bergamot-like," violet, and orange like, have been selected. By GCO analysis it was found that one of the 3 most important odorant zone related to the former descriptors corresponds to the presence of linalool and linalyl acetate. Sensory tests were performed in order to evaluate the importance of these 2 molecules on the global aroma of TN wines. For this purpose, a similarity test was performed with a non-TN wine that was added with linalool and linalyl acetate alone or in combinations. The highest similarity value (SV = 5.9) was observed when linalool was added. These results and the fact that higher concentrations of linalool are found in TN wines prove that these compounds are one of the important clues of the varietal aroma of TN wines.

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