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Sensory identity of wine from the ancient and almost forgotten grape variety Timorasso

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ABSTRACT

Over the last few decades, there has been a gradual decrease in natural biodiversity, which is leading to the standardisation of wine products. Minor grape varieties possess key traits for producing wines with a specific territorial identity and they contribute to increasing biodiversity. Timorasso is an ancient Italian white grape variety native to the province of Alessandria in the Piedmont region. It fell into oblivion for a period of time due to the advent of phylloxera at the end of the 1800s and the abandonment of rural areas, which almost led to the extinction of this grapevine. This study aimed to identify the sensory properties that characterise Timorasso wine from different producers and production areas and to investigate the diversity of the sensory space. Sixteen Timorasso wines from the 2018 vintage and were evaluated for sensory description by a panel of nine semi-trained judges using the RATA method. The Timorasso wine was found to have a complex aromatic profile spanning fruity (citrus, tropical fruits, tree-fruit and dried/baked fruit), floral, vegetative, balsamic, honey and fuel/petroleum. The multiple factor analysis clearly differentiated wines with fruity notes from those with balsamic, vegetative and fuel/petroleum aromas. The hierarchical cluster analysis evidenced three main clusters. Cluster 1 comprised wines with pale-yellow colour and fruity-related aromas, low sourness, body and alcohol sensations. Cluster 2 comprised wines with dark yellow colour, low sourness, and high intensities of raisin and fuel aromas, probably due to specific oenological practices, such as grape maceration. Cluster 3 comprised wines with high sourness and vegetative odour. The results provide for the first time a sensory map of a grapevine that was almost forgotten until a few decades ago, but which is now considered to be among the most promising white wines on the Italian market.

KEYWORDS: sensory description, RATA, white wine, biodiversity, grapevine

INTRODUCTION

According to the latest International Organization of Vine and Wine report, Italy is - at the time of writing - the top wine-producing country (OIV, 2022), having a wide selection of autochthonous grapevine varieties (*Vitis vinifera L.*), many of which are quite rare and might disappear in the near future due to lack of conservation programmes (D'Amato *et al*., 2023). In fact, over time there has been a gradual decrease in natural biodiversity, which is systematically leading to product standardisation, and a decrease in richness and genetic diversity in the wine sector. In this context, minor grape varieties possess key traits for producing wines with a specific territorial identity and they contribute to increasing biodiversity (D'Amato *et al*., 2023).

Timorasso is an ancient Italian white grape variety native to the province of Alessandria in the Piedmont region. It is a variety that has been present in the area for a long time and can be produced in the Curone, Grue, Ossona, Scrivia and Borbera valleys (Mipaaf, 2014). The first mention of Timorasso wine is found in the agricultural encyclopedia written by Pier de Crescenzi in the 14th century (De Crescenzi, 1548). In the Middle Ages, this variety spread throughout the valleys around the city of Tortona (once named Derthona), becoming the most important white grape variety in Piedmont. However, due to the advent of phylloxera at the end of the 1800s, which almost led to its extinction, and then to the two world wars, the Timorasso grapevine fell into oblivion until the 1980s. The decline of the Timorasso grape variety can also be ascribed to its lower production yield, which is linked to the thick skin of the berry and its greater sensitivity to rot (having a very compact bunch). These challenges have drawn the attention of wine producers to other white varieties and, in general, to red grape varieties, which are more productive and less difficult to cultivate, and they may explain the complete lack of scientific information regarding Timorasso wine.

The phenomenon of ancient variety abandonment is common in other well-known grape-growing regions (e.g., in France and Spain) - so much so that remarkable efforts have been made to bring back grapevine varieties whose cultivation has become marginal or has almost ceased (e.g., Berués variety in the peri-urban area of Pamplona, Crespo-Martínez *et al*., 2022; Fenile, Ginestra, Pepella, and Ripoli varieties from Amalfi coast, D'Amato *et al*., 2023). This has resulted in a significant number of nearly forgotten/neglected varieties being recovered in most countries in Southern Europe (Crespo-Martínez *et al*., 2022). Along with these interventions, starting in the 1980s, a small group of wine producers rediscovered and brought back into production the Timorasso vine in order to produce Timorasso wine, which is considered as being among the most promising white wines on the Italian wine market today, with an increase in vineyard area of 212.6 % in the last 10 years (Vigasio and Montaldo, 2019).

From an agronomic point of view, the buds of the Timorasso grape consist of slightly pinkish and white cottony apexes, which grow into medium-sized, compact and elongated bunches. The berries are also medium-sized, yellow-green in colour and have a thick skin and fleshy pulp. The berries are spheroidal, but they are often deformed by the compaction of the bunch and their maturation is asynchronous in nature (Masaf, 2023; D'Agata, 2014; Raimondi *et al*., 2005) (Figure 1). This grape variety grows on light and dark clay soils with a calcareous component resulting from ancient marine deposits (Masaf, 2023).

In 2005 under the Ministerial Decree DM 27.09.2005, Timorasso wine was included in the designation of origin "Colli Tortonesi DOC" established in 1973 in order to protect its unique characteristics and to promote its high quality while guaranteeing its geographical origin (Mipaaf, 2014). In the designation, Timorasso wine appears among white wines, such as "Colli Tortonesi Timorasso" and "Colli Tortonesi Timorasso Riserva". In order to claim the designation "Colli Tortonesi DOC" followed by "Timorasso" or "Timorasso Riserva", the wine must contain at least 95 % Timorasso grapes and a maximum of 5 % of other non-aromatic white grape varieties suitable for cultivation in the Piedmont region.

FIGURE 1A-C. a) Timorasso grape buds in the "wool stage", b) Timorasso grape bud burst with green-pinky shoot tips clearly visible, and c) Timorasso grape bunch ready for harvest.

The maximum grape yield and the percentage grape/wine yield must be 8 t/ha and 65 % respectively, while the minimum alcoholic volume of the wine must be 12 %. Timorasso wine must go through an aging phase of 10 months and can claim the designation "Riserva" if it has been aged for more than 21 months (Mipaaf, 2014). Even though the Timorasso grape is grown in a limited area, 86 wineries were found to belong to the Consortium for the Protection of Colli Tortonesi Wines in April 2023 (Consortium for the protection of Colli Tortonesi, 2023). Most, if not all, wineries produce Timorasso wine, covering a wide range of growing conditions and different types of winemaking. These differences can influence the final quality of Timorasso wine, and only a detailed sensory characterisation can allow the key features of this grapevine variety to be mapped. Despite the Timorasso grape proving to be very promising in the context of the Italian wine market, no data (either sensory or chemico-physical) linked to Timorasso wine are available.

Therefore, this study aimed to identify the sensory properties that best characterise 16 Timorasso wines (2018 vintage) from different producers and valleys of production by looking at the diversity of the sensory space. To this end, the Rate-All-That-Apply (RATA) method was used involving a panel of semi-trained assessors.

MATERIALS AND METHODS

1. Wines

Sixteen Timorasso wines from the 2018 vintage from different local producers (representing approximately 26 % of the total producers at the time of the sample selection performed in the spring of 2021) were used in this study, as shown in Table 1. The producers had been selected based on their availability and in order to have a good representation of the different areas in which Timorasso can be obtained, i.e., the Curone, Grue, Ossona, Borbera, and Scrivia valleys (Figure 2). All the wines were made from 100 % Timorasso grapes. In all cases, the producers had adopted the Guyot pruning system, declared a yield of between 35 - 70 q/ha, and grown between 4000 - 7000 plants per hectare (data not shown in Table 1). The wines were stored in a dark room at 20°C until the evaluation was performed between May and July 2022. It was decided to evaluate the samples four years after wine production, because Timorasso wine expresses its particular characteristics after a few years of aging.

2. Sensory analysis

2.1. Participants

Nine assessors (6 women and 3 men) aged between 21 and 44 years (mean age 29.0 ± 7.1 years) were recruited from the students and employees of the Faculty of Agricultural and Food Science (University of Milan). The recruitment criterion was a regular consumption of wine (at least one glass a week). None of the assessors had previous or present taste or smell disorders. They all gave their written informed consent. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Ethics Committee (n. 32/12).

2.2. Procedure

The Rate-All-That-Apply (RATA) method (Reinbach *et al*., 2014; Ares *et al*., 2014) was used to identify and to quantify the sensory properties of Timorasso wine obtained from the different producers. This method can be used with both consumers (Danner *et al*., 2018; Meyners *et al*., 2016) and semi-trained assessors (Giacalone and Hedelund, 2016; Tan *et al*., 2020; Traill *et al*., 2019). In the present study, we opted for a reduced number of semi-trained assessors, which has been shown to work well when evaluating wine (Rabitti *et al*., 2022).

FIGURE 2. Map of Timorasso wine production area and location of the different producers: https://www.google.com/maps/.

TABLE 1. Description of the wine samples based on information provided by producers (Sun exposure: N = North, S = South, E = East, W = West). (part 1/2).

Label	Producer	Wine name	Growth and oenological practices	Aging	Area of production	Vol.%	Soil composition	Sun exposure	Altitude a.s.l. (m)	Vine age (years)	Harvest time
BOLUI	Boveri Luigi Michele Azienda Agricola	Derthona Colli Tortonesi Timorasso DOC	Gentle grape pressing, must settling and fermentation at controlled temperature (18-20 °C)	12 months in tank on periodically-stirred fine lees + 12 months in bottle	Costa Vescovato (Ossona valley)	14.0	Marly-calcareous	SW	250	8	End September
BOREN	Boveri Renato Vignaiolo Azienda Agricola	Derthona	Fermentation in ancient stone and cement wine vessels	10/12 months on fine lees in ancient stone and cement wine vessels + 12 months in bottle	Monleale (Curone valley)	14.0	Clay-calcareous	SE	300	13	September
CAGEN	Cascina Gentile Azienda Agricola	Derthona Colli Tortonesi Timorasso DOC	Gentle grape pressing, 1-day maceration, cold must pressing and lees separation, fermentation at controlled temperature (18°C) for 35-40 days	12 months on fine lees, batonnage every 10-15 days	Capriata d'Orba (Scrivia valley)	14.0	Loamy-clay	W-SW, E-NE	250	14	September
CAN	Canevaro Luca Azienda Agricola	"Cà degli Olmi" Derthona Colli Tortonesi Timorasso DOC	Organic grapes, manual grape harvest, gentle grape pressing, fermentation at controlled temperature	12 months in stainless steel tank on fine lees with batonnage at controlled temperature	Avolasca (Grue valley)	14.0	Clay	W	300-350	20	September
CASOC	Cantina Sociale di Tortona S.c.a.	"Vignaioli del Tortonese" Derthona Colli Tortonesi Timorasso DOC	Manual grape harvest, 6-h cryomaceration in inert atmosphere, fermentation at controlled temperature	10 months on fine lees with batonnage in stainless steel	Viguzzolo, Avolasca, Vho di Tortona (Grue and Ossona valleys)	14.0	Clay-calcareous	E-SE	250	n.a.	September
DAF	Terralba Azienda Agricola	Derthona	Gentle grape pressing, 2- or 3-day grape maceration, fermentation in stainless steel	24 months on fine lees	Berzano di Tortona (Curone valley)	13.0	Clay-calcareous	SW	270	20	Beginning September
MAN	Mandirola Enrico Azienda Agricola	Derthona Colli Tortonesi Timorasso DOC	Gentle grape pressing, fermentation at controlled temperature	12 months on fine lees	Casasco (Grue valley)	13.5	Clay-calcareous	SW	300	30	End September/ October
MAR	Mariotto Claudio Azienda Agricola	Derthona Colli Tortonesi Timorasso DOC	Gentle grape pressing, spontaneous fermentation at controlled temperature	12 months on fine lees in stainless steel with batonnage + 6 months in bottle	Vho di Tortona (Ossona valley)	14.0	Clay-calcareous	Various	220-280	8-25	End September

TABLE 1. Description of the wine samples based on information provided by producers (Sun exposure: N = North, S = South, E = East, W = West). (part 2/2).

Label	Producer	Wine name	Growth and oenological practices	Aging	Area of production	Vol.%	Soil composition	Sun exposure	Altitude a.s.l. (m)	Vine age (years)	Harvest time
MAS	Vigneti Massa	Derthona	2-3-day maceration, must pressing, fermentation at controlled temperature (18-22 °C)	12 months in stainless steel with batonnage + 6 months in bottle	Monleale (Curone valley)	13.5	Calcareous-clay-marly	S, E, W	250-310	15-26	September
MUT	Mutti Andrea Azienda Agricola	"Castagnoli" Derthona Colli Tortonesi Timorasso DOC	Fermentation at controlled temperature	12 months on fine lees	San Ruffino di Sarezzano (Grue valley)	14.5	Clay-calcareous	SW	275	32	September
POG	Poggio Paolo Giuseppe Azienda Agricola	"Ronchetto" Derthona Colli Tortonesi Timorasso DOC	Gentle grapes pressing, 2h-maceration, lees separation, alcoholic fermentation at controlled temperature with selected yeast starter, spontaneous malolactic convertion	10 months in stainless steel with batonnage	Brignano Frascata (Curone valley)	13.5	Clay-calcareous	S	320	13-28	End September
REP	Repetto Gian Paolo Azienda Agricola	"Quadro" Derthona Colli Tortonesi Timorasso DOC	Gentle grape pressing, fermentation in stainless steel at controlled temperature	9 months in stainless steel with batonnage every week + 4-12 months in bottle	Sarezzano (Ossona valley)	13.5	Clay-calcareous	SW, NW	280	16	Beginning September
RIC	Ricci Carlo Daniele Azienda Agricola	Derthona Colli Tortonesi Timorasso DOC	3-day grape maceration, spontaneous fermentation in stainless steel with, no clarification, no filtration	12 months in acacia barrel on fine lees + 12 months in bottle	Costa Vescovato (Ossona valley)	13.5	Marly	S-SW	280	22/18/16/13	End September
SAS	Sassobraglia di Fabio Cogo Azienda Agricola	Colli Tortonesi Timorasso DOC	Gentle grape pressing, lees separation, fermentation at controlled temperature	On fine lees in stainless steel	Rocchetta Ligure (Borbera valley)	13.0	Clay-calcareous	S-SE	500-750	25	End September/ October
VAUN	Valli Unite Soc. Coop. Agr	Derthona	Organic grapes, gentle grape pressing, 2-day maceration, spontaneous fermentation in stainless and cement tank, no filtration	12 months in stainless steel + 12 months in bottle	Costa Vescovato (Ossona valley)	14.5	Clay-marly	SW	330	30	End August
VEPO	La Vecchia Posta	"Il Selvaggio" Derthona Colli Tortonesi Timorasso DOC	Organic grapes, spontaneous fermentation in stainless steel, no clarification	12 months on fine lees with batonnage	Avolasca (Grue valley)	14.5	Clay-calcareous	SW	380	26	End September/ October

2.2.1. Training phase

The panel underwent a two-part training phase following international guidelines (ISO 3972, 2011; ISO 5496, 2006; OIV, 2015). First, the judges were trained to identify odours and flavours and to rank taste and tactile sensations (salty, sour and wine body) (Supplementary Table, ST1) in water solutions. Cotton buds soaked in pure aroma standards (Le Nez du Vin®) were initially used to recognise odours and flavours, followed by reference standards in table wine (Supplementary Table, ST2).

The second part of the training aimed at familiarising the panel with both the tasting procedure and the wines. Therefore, seven one-hour sessions were held, in which each judge was presented with three Timorasso wines. During this phase, all the judges tasted all the experimental wines (Table 1) at least once. The sensory terms used in this study were selected based on previous research on a large range of Italian wines, including white ones (Rabitti *et al*., 2022). This list was modified by the semi-trained judges and adapted to reflect the terms that best characterised the experimental Timorasso wines. The list of terms comprised 31 items and covered multiple sensory modalities: 1 term for appearance, 13 for odours, 13 for flavours, 2 for tastes and 2 for tactile sensations. Each term was represented by a reference standard (Supplementary Table ST2). The attributes were listed per sensory modality (i.e., appearance, odour/flavour, taste and tactile sensations), and those in the odours and flavours modality were subdivided into macro-categories (e.g., the macro-category “fruit trees” gathered the terms apple, pear and peach). This format has been reported to improve attribute processing and reduce cognitive burden in similar tasks (Ares and Jaeger, 2013). In addition, to make the task even easier, the different sensory modalities were presented in the expected order of perception: 1) appearance, 2) odours, 3) flavours, 4) taste, and 5) tactile sensations (Giacalone and Hedelund, 2016). Once the list of descriptors and reference standards had been developed, three sessions were conducted in the sensory booths to familiarise the judges with the use of the scales.

2.2.2. Evaluation phase: The Rate-All-That-Apply (RATA) method

After the training phase, the 16 wines were evaluated in duplicate over a 1-month period, with 2 tasting sessions per week, each lasting approximately 45 min. In each session, 4 wines were evaluated. The wines (30 mL of wine per glass) were checked to ensure there were no defects before every tasting session and presented at room temperature in ISO glasses (ISO 3591, 1977) covered with plastic lids to prevent volatile components from escaping and coded with a 3-digit number.

For each wine, judges were asked to select the attributes that described the samples and to rate their intensity using a 5-point scale (1 = “low”, 3 = “medium” and 5 = “high” intensity).

The sensory evaluation took place at the Sensory & Consumer Science Laboratory (SCS_Lab) of the Department of Food, Environmental and Nutritional Sciences (DeFENS) of the University of Milan, which had been designed following international guidelines ISO 8589:2014 (UNI EN ISO 8589, 2014). All the judges performed the evaluation in individual sensory booths under white light. The panelists were instructed to refrain from smoking, eating and drinking (except water) an hour before tasting.

The order in which the samples were presented systematically varied depending on the judge and replicate to balance the effects of serving order and carry-over (MacFie *et al*., 1989). The results were acquired using Fizz software version 2.31b (Biosystèmes, Couternon, France).

2.3. Data analysis

2.3.1. Panel reliability

The strategy for evaluating the reliability of the panel when selecting and rating the descriptors for the two replicates was twofold: first, a reproducibility index (RI) was calculated to assess individual performance, then a multiple factor analysis (MFA) was carried out to assess group performance.

Individual performance assessment was checked by calculating a reliability index (RI) for each assessor and for each wine, according to Giacalone and Hedelund (2016):

$$RI_j = \frac{1}{n} * \sum_{s=1}^{n} \left(\frac{des_{com}s}{des} \right)$$

where RIj is the global reliability index related to judge j, n is the number of samples (n = 16), $des_{com}s$ is the number of descriptors indicated by judge j in an identical manner in all the replicates for a given sample s (including the descriptors checked and those not checked by the judge for both replicates) and des is the total number of descriptors (n = 31) including all descriptors for appearance, odours, flavours, taste and tactile sensations. RI comprises values from 0 to 1; the higher the RI value, the higher the reliability of the judge. An RI value ≥ 0.5 was considered as the cut-off for a judge’s reliability according to Giacalone and Hedelund (2016).

A group performance assessment was performed by evaluating the proximity of the replicates to each other on the MFA wine map obtained from intensity data provided by the assessors for both replicates (Giacalone and Hedelund, 2016).

2.3.2. Sensory characterisation of the wine

To characterise Timorasso wine samples, 2-way analysis of variance (ANOVA) was carried out on individual descriptors, in which the samples and replicates and their interaction were considered as fixed effects. When the ANOVA showed a significant sample effect ($p \leq 0.05$), the least significant difference (LSD) was applied as a multiple-range test.

Data were then averaged across replicates and assessors and a second MFA was performed to visualise the Timorasso wine space. After performing the MFA, a hierarchical cluster analysis (HCA) was run on the coordinates of the wines on the total dimensional MFA space (using Euclidean distance and Ward’s agglomeration criteria) to identify wine clusters with similar sensory characteristics.

The descriptors that best characterised each cluster were identified using the test-value parameter (Lebart *et al*., 1995). The test value corresponds to a statistical criterion akin to a standardised variable (zero mean and unit variance). By ranking the terms according to their test values, it is possible to quickly characterise each of the clusters (Morineau, 1984).

RESULTS

1. Panel reliability

RIs calculated by judge and averaged across all samples are reported in Supplementary Table 3, ST3. The mean RI values ranged from 0.66 to 0.84, with an average value of 0.77. The minimum individual value registered was 0.48, all other minimum individual values were above the cut-off value of 0.5 reported by Giacalone and Hedelund (2016), reaching 0.68. These values indicate a good individual reliability in terms of selecting the same attributes for the two replicates of all wines.

To further investigate the reliability of the panel in terms of reproducibility of the entire sensory characterisation, the proximity to each other of the replicates of each wine on the three-dimensional MFA wine space (49 % of total variance) was evaluated (Supplementary Figure S1a-b). With the exception of a few wines (e.g., Mariotto, MAR1 and MAR2; Vecchia Posta, VEPO1 and VEPO2; Valli Unite, VAUN1 and VAUN2), the wine replicates were close together in the wine space, showing good panel reliability.

2. Wines sensory characterisation

2.1. Analysis of variance

The factor "*Replicates*" and the interaction "*Sample * Replicates*" were not significant ($p > 0.05$) for all descriptors, confirming the reliability of the panel. The main factor "*Wines*" was significant for 14 descriptors out of 31. The mean intensity ratings for the 14 significant sensory attributes are shown in Table 2. The sensory characteristics that discriminated the wines the most were colour, raisin odour and flavour and fuel odor ($p < 0.001$). The wines with the darkest yellow colour were Cascina Gentile (CAGEN) and Ricci (RIC), while the lightest were Boveri Luigi (BOLUI) and Sassobraglia (SAS) wines. The raisin odour and flavour distinguished Cascina Gentile (CAGEN), their intensities being significantly higher than those of the other wines. The highest intensity of fuel odour was perceived in the Daffonchio (DAF) wine, while the lowest intensities were perceived in the Cascina Gentile (CAGEN) and Poggio (POG) wines. Other descriptors that contributed to differentiating the Timorasso wines were sour taste, and balsamic and vegetative odours ($p < 0.01$). The Cantina Sociale (CASOC) wine had the highest sourness intensity, while Ricci (RIC) and Mariotto (MAR) wines had the lowest. Cantina Sociale (CASOC) wine also had the highest intensity of vegetative odour, while Boveri Luigi (BOLUI) wine had the lowest. Balsamic odour was perceived as significantly more intense in Ricci (RIC) wine compared to Boveri Luigi (BOLUI) and Vecchia Posta (VEPO). Although to a lesser extent, pineapple and ripe fruit odours, apple, pear and fuel flavours, and alcoholic sensation also contributed to discriminating the wines ($p < 0.05$). Cascina Gentile (CAGEN) showed the highest intensity of ripe fruit odour, which was absent in Cantina Sociale (CASOC) wine. A fuel flavour characterised Ricci (RIC) wine. Luigi Boveri (BOLUI) wine was characterised by a pear flavour, clearly distinguishing it from all the other wines, apart from the Poggio (POG) wine. A pineapple odour differentiated the Canevaro (CAN) from the Daffonchio (DAF) and Cantina Sociale (CASOC) wines. Sassobraglia (SAS), and Renato Boveri (BOREN) wines were mainly described as having an apple flavour, which was not detected in the Vecchia Posta (VEPO) wine. The Cascina Gentile (CAGEN) and Cantina Sociale (CASOC) wines showed the highest intensity of the alcohol sensation compared to the Sassobraglia (SAS) wine which had the lowest.

2.2. Multiple Factor Analysis

Figures 3 and 4 show the first three dimensions obtained from the normalised MFA carried out on all sensory descriptors averaged across the replicates and assessors. The total explained variance was 59.45 %, with F1 accounting for 29.62 %, F2 for 16.61 %, and F3 for 13.22 %. Wines (Figures 3a and 4a) and descriptors (Figures 3b and 4b) are shown with different dot sizes and colours respectively depending on the squared cosine. The squared cosine between the projection of a wine or a descriptor and an MFA factor is an index for assessing the quality of the representation in the range 0–1. The higher the squared cosine the better the representation in the bidimensional space. According to the squared cosine values (dot sizes) depicted in Figure 3a, the wines that are best represented on axes 1 and 2 are Sassobraglia (SAS), Ricci (RIC) and Mandirola (MAN). Meanwhile, Vecchia Posta (VEPO), Mutti (MUT), and Canevaro (CAN) are poorly represented since the cosine square value for these wines is lower than 0.2 on axes 1 and 2. In the same way, the cosine square values (different colours) depicted in Figure 3b indicate that some descriptors (such as pineapple, apple and pear odours and pineapple, pear, apple, floral, solvent and fuel flavours) are poorly represented by the first two dimensions, while colour and sour ($cos^2 > 0.8$), solvent, ripe fruit, honey and lemon odours, vegetative flavour and wine body ($cos^2 > 0.7$) best characterised the Timorasso wines, followed by vegetative odour, peach flavour and salty taste ($cos^2 > 0.6$).

In F1 (Figure 3a), the different Timorasso wines are clearly differentiated, with Sassobraglia (SAS), Mandirola (MAN) and Luigi Boveri (BOLUI) being separate from Ricci (RIC), Mariotto (MAR) and Cascina Gentile (CAGEN). F2 differentiated the wines Cantina Sociale (CASOC), Repetto (REP), and Daffonchio (DAF) from the wines Cascina Gentile (CAGEN), Massa (MAS) and Sassobraglia (SAS).

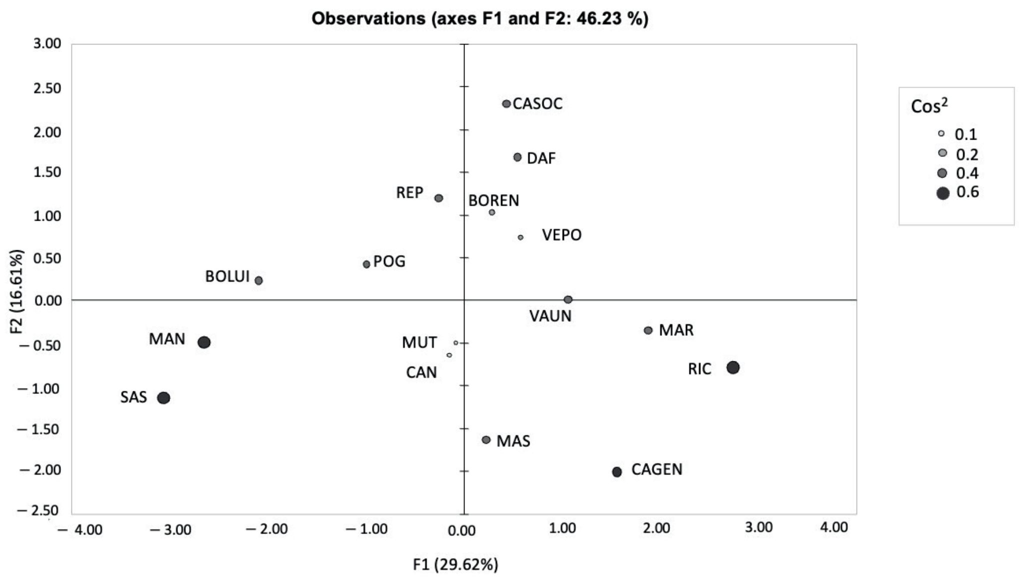
In Figure 3b, F1 clearly differentiates the vegetative, ethereal and balsamic odour macrocategories (positive side of F1) from the fruity (citrus, tropical fruits and fruit tree) and floral macrocategories (negative side of F1). Meanwhile, F2 separates the sour and salty wines (positive side of F2) from the wines with honey, ripe fruit and raisin odours/flavours.

Table 2. Mean values (range 0-5) of the 14 significant sensory descriptors. Superscripts by row indicate significantly different means according to LSD post-hoc test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Minimum and maximum mean values per row are shown in bold. O = odour; F = flavour; Veg = vegetative.

Attributes	p-value	BOLUI	BOREN	CAGEN	CAN	CASOC	DAF	MAN	MAR	MAS	MUT	POG	REP	RIC	SAS	VAUN	VEPO
Colour	***	1.4^{h}	3.5^{b}	4.9^{a}	2.7^{e}	3.1^{cd}	3.2^{bc}	1.9^{g}	3.5^{b}	3.5^{b}	2.8^{de}	3.5^{b}	2.3^{f}	4.8^{a}	1.4^{h}	3.5^{b}	3.3^{bc}
O_Pineapple	*	1.1^{ab}	0.9^{abcd}	0.9^{abc}	1.4^{a}	0.2^{d}	0.2^{d}	0.7^{bcd}	0.6^{bcd}	1.0^{ab}	0.9^{abc}	1.0^{ab}	0.8^{abcd}	0.6^{bcd}	0.5^{bcd}	0.3^{cd}	1.1^{ab}
O_Raisin	***	0.4^{bcd}	0.9^{bc}	1.7^{a}	1.0^{ab}	0.3^{bcd}	0.7^{bcd}	0.7^{bcd}	0.8^{bcd}	0.6^{bcd}	0.3^{cd}	0.5^{bcd}	0.3^{cd}	0.4^{bcd}	0.9^{bc}	0.3^{bcd}	0.1^{d}
O_Ripe Fruit	*	0.7^{de}	0.7^{de}	2.1^{a}	1.1^{bcde}	0.4^{e}	0.8^{cde}	1.2^{bcde}	1.2^{bcde}	1.2^{bcde}	1.3^{abcd}	1.0^{bcde}	1.1^{bcde}	0.9^{bcde}	1.8^{ab}	1.1^{bcde}	1.6^{abc}
O_Veg	**	0.3^{f}	0.9^{abcdef}	0.7^{cdef}	0.4^{ef}	1.7^{a}	1.4^{abc}	0.9^{bcdef}	1.0^{abcdef}	0.5^{def}	1.2^{abcd}	0.9^{abcdef}	0.9^{abcdef}	1.6^{ab}	0.7^{cdef}	1.1^{abcde}	0.7^{cdef}
O_Balsamic	**	0.4^{e}	0.8^{bcde}	0.4^{e}	0.5^{cde}	1.3^{ab}	0.5^{cde}	0.5^{cde}	0.9^{abcde}	0.6^{cde}	1.1^{abc}	1.1^{abcd}	0.8^{bcde}	1.5^{a}	0.6^{cde}	1.1^{abcd}	0.3^{e}
O_Fuel	***	1.6^{bcd}	1.8^{bcd}	1.3^{d}	1.8^{bcd}	1.6^{cd}	3.1^{a}	1.8^{bcd}	2.3^{abc}	1.7^{bcd}	2.5^{ab}	1.1^{d}	2.3^{abc}	2.4^{abc}	1.8^{bcd}	2.4^{abc}	1.7^{bcd}
F_Apple	*	0.1^{bc}	0.7^{a}	0.4^{ab}	0.2^{bc}	0.1^{bc}	0.2^{bc}	0.2^{bc}	0.2^{bc}	0.3^{abc}	0.0^{c}	0.2^{bc}	0.1^{bc}	0.6^{bc}	0.7^{a}	0.1^{bc}	0.0^{c}
F_Fuel	*	0.8^{de}	0.9^{cde}	1.1^{bcde}	1.5^{abcde}	0.9^{cde}	1.6^{abcd}	0.9^{cde}	1.0^{cde}	1.7^{abc}	1.3^{bcde}	0.7^{e}	1.1^{bcde}	2.3^{a}	1.5^{abcde}	1.9^{ab}	1.2^{bcde}
F_Pear	*	0.4^{a}	0.0^{b}	0.1^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.2^{ab}	0.2^{ab}	0.1^{b}	0.1^{b}	0.0^{b}	0.0^{b}	0.2^{ab}
F_Raisin	***	0.3^{b}	0.5^{b}	1.2^{a}	0.2^{b}	0.1^{b}	0.2^{b}	0.1^{b}	0.4^{b}	0.2^{b}	0.2^{b}	0.4^{b}	0.3^{b}	0.3^{b}	0.4^{b}	0.3^{b}	0.0^{b}
F_Veg	*	0.3^{cde}	0.9^{a}	0.5^{abcde}	0.2^{de}	0.9^{a}	0.8^{ab}	0.4^{abcde}	0.3^{cde}	0.2^{de}	0.3^{cde}	0.3^{cde}	0.8^{abc}	0.5^{abcde}	0.1^{e}	0.6^{abcd}	0.3^{cde}
Sour	**	3.3^{abcd}	3.5^{ab}	3.0^{cd}	3.0^{cd}	3.7^{a}	3.4^{abc}	3.3^{abcd}	2.8^{d}	3.0^{cd}	3.2^{bcd}	3.5^{ab}	3.4^{abc}	2.8^{d}	3.2^{bcd}	3.1^{bcd}	3.4^{abc}
Alcohol	*	3.4^{abcd}	3.2^{bcd}	3.7^{a}	3.4^{abcd}	3.7^{a}	3.2^{cd}	3.2^{bcd}	3.5^{abc}	3.6^{ab}	3.6^{abc}	3.3^{abcd}	3.5^{abc}	3.6^{abc}	3.1^{d}	3.3^{abcd}	3.6^{ab}

More specifically, when comparing Figures 3a and b, the positions of Cantina Sociale (CASOC), Daffonchio (DAF) and Boveri Renato (BOREN) wines (located in the upper right-hand quadrant) indicate that they are mainly characterised by vegetative, ethereal (Solvent, Fuel), and balsamic notes. These wines were also perceived to have a high intensity of salty taste, wine body, alcohol content and colour, along with the Valli Unite (VAUN), Mariotto (MAR), Ricci (RIC), Massa (MAS) and Cascina Gentile (CAGEN) wines located in the lower right-hand quadrant. Moreover, the latter wines are also described as having ethereal (solvent and fuel), pineapple and raisin flavours, although these descriptors are mainly driven by Cascina Gentile (CAGEN) and Massa (MAS) wines. Mutti and Canevaro (CAN) wines, despite being poorly represented, are near the floral and apple flavours on the graph, while the positions of Sassobraglia (SAS) and Mandirola (MAN) wines show that they were perceived as having high intensities of peach, floral (acacia) and honey odours/flavours. Finally, the positions of Repetto (REP), Poggio (POG) and Luigi Boveri (BOLUI) wines indicates that they were perceived as having a high intensity of sourness and are near the lemon and pear odorus/flavours on the graph.

a



b

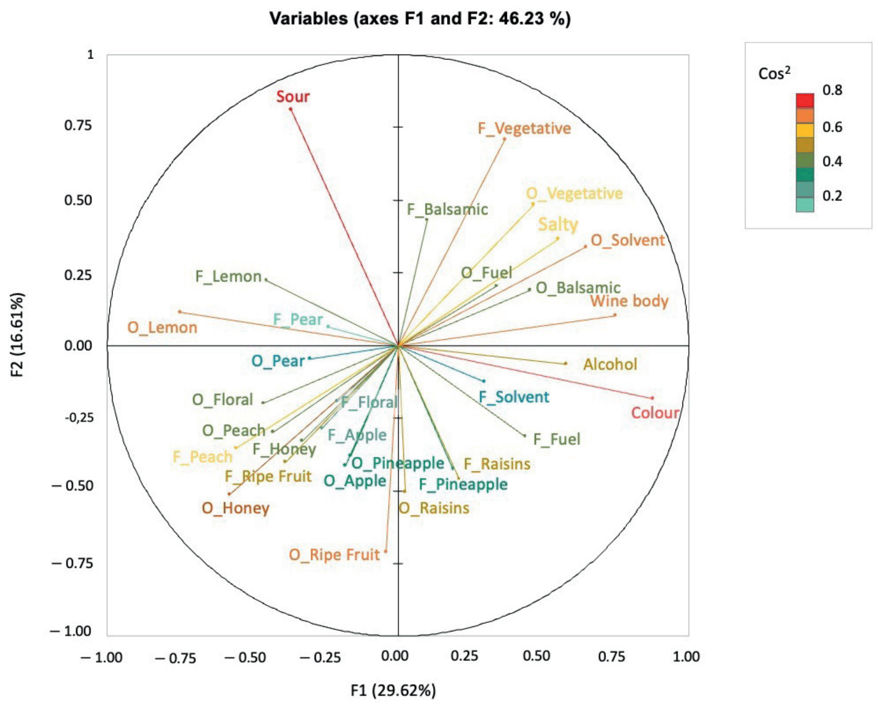


FIGURE 3. Configuration of a) Samples and b) variables from MFA performed on the 16 wines (data averaged across replicates). Dimensions 1 and 2.

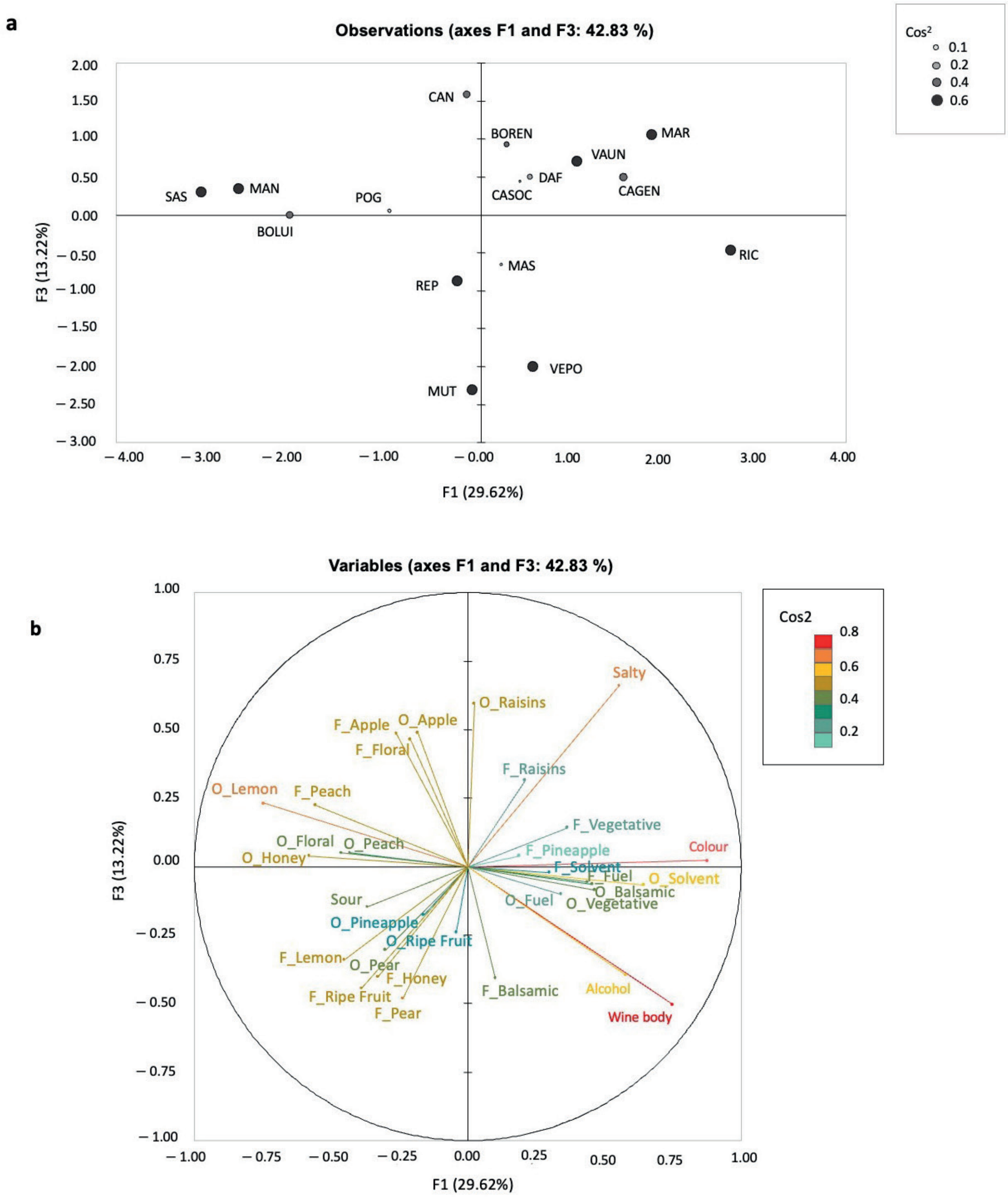


FIGURE 4. Configuration of a) Samples and b) variables from MFA performed on the 16 wines (data averaged across replicates). Dimensions 1 and 3.

Figures 4a-b (F1 *vs* F3) provide more information on some wines that are not well represented in F1 and F2, such as Vecchia Posta (VEPO), Canevaro (CAN) and Mutti (MUT). More specifically, the graphs show that Vecchia Posta (VEPO) and Mutti (MUT) wines (negative side of F3) were described as having intense wine body and alcohol content, a balsamic odour (Vecchia Posta wine) and fruity notes, such as lemon, pear, ripe fruit and honey odour (Mutti wine). These wines were on the opposite side to the Canevaro (CAN) wine (positive side of F3), which was characterised mainly by apple odour/flavour, oraisin odour and floral flavour.

3. Hierarchical Cluster Analysis

The HCA carried out on the wine coordinates of the first three MFA factors yielded three main clusters (Figure 5a-b). The first cluster (C1) contains four wines (Canevaro, Sassobraglia, Luigi Boveri and Mandirola), which contributed the most to the construction of the axes (Figures 3 and 4a-b). The second cluster (C2) contains 5 wines (Ricci, Valli Unite, Mariotto, Massa and Cascina Gentile) wines, while the third cluster (C3) contains seven (Mutti, Repetto, Vecchia Posta, Renato Boveri, Daffonchio, Poggio and Cantina Sociale). In each cluster, the most typical samples (i.e., those the furthest from the other clusters) are Sassobraglia (C1), Cantina Sociale (C2) and Cascina Gentile (C3) (Figure 5b).

To identify the terms that contributed the most to the formation of the clusters, one-way ANOVAs were performed on the attribute intensity scores, the clusters being considered as a fixed source of variance. A significant cluster effect was found on the following attributes: O. Lemon ($F = 8.6$; $p < 0.01$); F. Lemon ($F = 3.8$; $p = 0.05$); F. Peach ($F = 5.7$; $p < 0.05$); F. Floral ($F = 3.6$; $p = 0.05$); O. Vegetative ($F = 3.6$; $p = 0.05$); F. Vegetative ($F = 4.0$; $p < 0.05$); O. Solvent ($F = 3.7$; $p = 0.05$); Sour ($F = 21.5$; $p < 0.001$); Body ($F = 7.9$; $p < 0.01$); Alcohol ($F = 3.6$; $p = 0.05$); Colour ($F = 15.6$; $p < 0.001$). To describe the clusters using variables in a quantitative manner, the descriptors that best characterised the samples were identified using the test-value parameter (Lebart *et al*., 1995). The descriptors showing statistically significant values for each cluster are shown in Table 3. Cluster 1 was characterised by attributes from the fruity macrocategory, whereas wines in Cluster 2 were mainly characterised by colour intensity and the ethereal macrocategory. Finally, C3 was mainly defined by sourness and vegetative notes.

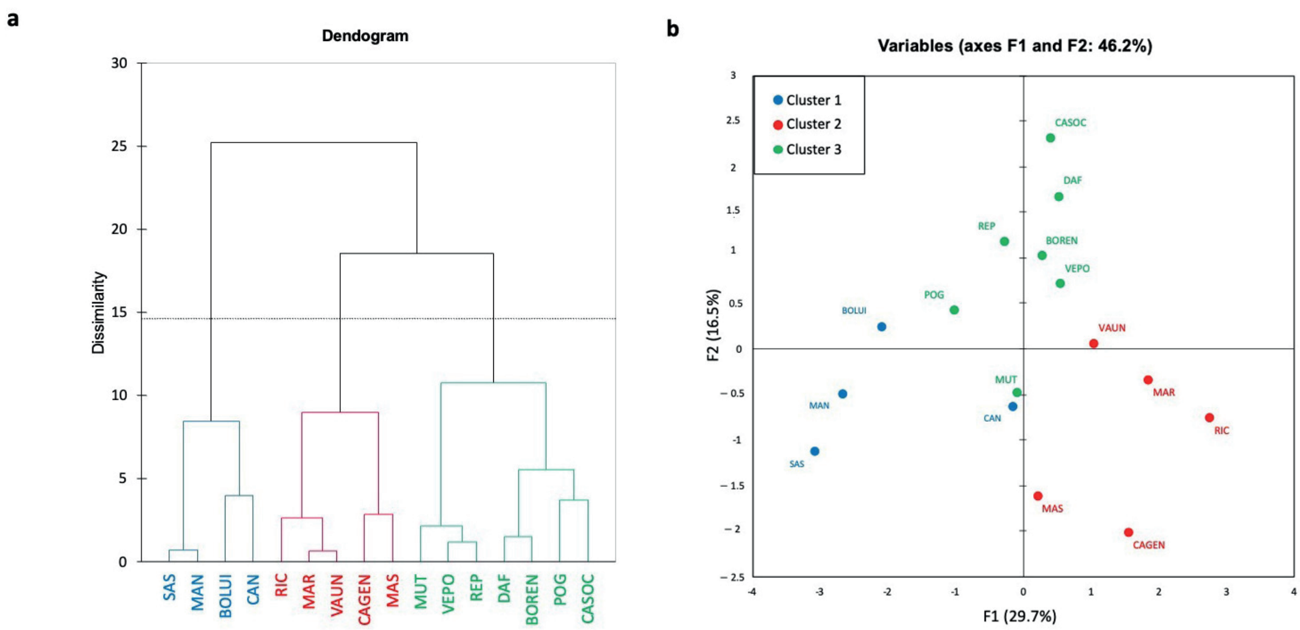


FIGURE 5A-B. Map with a) clusters and b) distance between clusters. The hierarchical classification was produced after the MFA and the total explained variance is 46.2 %.

TABLE 3. Sensory descriptors that obtained a statistically significant test value and contributed the most to the formation of each cluster. Positive means indicate a higher-than-average presence and negative means a lower-than-average presence. O = Odour; F = Flavour.

Cluster	Samples	Positive mean value	Negative mean value
1	BOLUI; CAN; MAN; SAS	O. Lemon, F. Peach	O. Vegetative, F. Vegetative, O. Solvent, Sour, Body, Alcohol, Colour
2	CAGEN; MAR; MAS; RIC; VAUN	Colour, O. Solvent	O. Lemon, F. Lemon, Sour
3	BOREN; CASOC; DAF; MUT; POG; REP; VEPO	F. Vegetative, Sour	F. Floral, Colour

DISCUSSION

The analysed Timorasso wines showed a surprisingly high sensory variability linked mainly to the aromatic component. The olfactory characteristics of Timorasso wines spanned fruity notes, such as citrus, tropical fruits, fruit tree and dried/baked fruit, and floral, vegetative, balsamic, caramelised and fuel/petroleum. This richness in the aromatic profile can be linked to different factors, such as production area and oenological practices. While Timorasso wines from different production areas were selected for the study, a clear sensory pattern related to a specific valley of production cannot be discerned, because certain valleys were not represented by a sufficiently large number of producers. For instance, the Borbera valley was represented in the study by only one of the producers (Sassobraglia, SAS) of the three in this area at the time of wine collection. Including a sufficiently large number of producers per valley in the study was not always possible for various reasons; e.g., some wineries did not produce Timorasso from the 2018 vintage due to adverse climatic conditions or some were small-scale producers with limited wine production. However, even in the case of valleys better represented by producers (e.g., Curone, Grue and Ossona), it was not still possible to identify clear sensory traits that could be attributed to a specific area. The high variability of the Timorasso wines' aromatic profiles can therefore be ascribed to differing oenological practices adopted by the producers rather than to the production area.

Nevertheless, the whole area of production of the wines (i.e., Colli Tortonesi in Piedmont) may be responsible for another key sensory characteristic: saltiness. Indeed, the Timorasso wines tested in the present study were described as salty. Examples of wines in which saltiness has been reported as a main feature are those originating from grapes grown near or within coastal regions or on Islands (Afonso *et al.*, 1998; Rabitti *et al*., 2022). However, Timorasso wine is produced in an area which is about 50 km from the coast, therefore its salty taste may be due to the clayey-marly soils which transfer mineral salts to the grapes and, consequently, to the wine giving it its unique characteristics (Mipaaf, 2014).

The MFA results showed that the Timorasso wines with fruity notes were clearly differentiated from those with balsamic, vegetative and fuel/petroleum aromas. Fuel, kerosene or petrol odours are usually found in wines like Riesling and are attributable to 1,1,6-Trimethyl-1,2-dihyronaphthalene (TDN), which belongs to the family of C13-Norisoprenoids (Sacks *et al*., 2012). Sponholz and co-workers (Sponholz and Hühn, 1997) considered this compound to be beneficial to the overall quality and identity of Riesling wines when present in concentrations of up to 4 µg/L.

In addition to petroleum notes, Timorasso wine shares other aromatic characteristics with Riesling wine. For example, German Riesling wines from the Rheingau region have been described as having the fruit-related aromas citrus, grapefruit, apple, pear and floral (Fischer *et al*., 1999). Canadian Riesling wines have also been associated with fruity-related odours, but along with petrol, honey and mineral attributes (Douglas *et al*., 2001; Marciniak *et al*., 2013). In Australian Riesling wines, tropical and citrus fruit aromas have been shown to be strong in young wines, whereas kerosene, honey and caramel were more present in older wines (Cozzolino *et al.*, 2006). All the aforementioned odours were also perceived in the Timorasso wines in the present study.

Citrus and tropical fruit aromas in wine are mainly associated with volatile thiols, which are organosulfur odor-active molecules containing a -SH group. These compounds contribute significantly to wine aroma (Carlin *et al*., 2022) due to their low odor detection threshold (Carlin *et al*., 2022; McGorrin, 2011). Although some volatile thiols present in wine are varietal thiols already present in grapes (Villano *et al*., 2017), most of them are formed during the fermentation process as a result of specific yeast strain activity (Swiegers *et al.*, 2006). Variations in citrus and tropical fruit aromas among the different Timorasso wines may be due to the different oenological practices adopted by the local producers.

The Timorasso wines differed not only in the aromatic component but also in the other sensory modalities. The HCA evidenced three main clusters which differed mainly in colour, taste and mouthfeel sensations. Some wines (Cluster 1) were pale yellow in colour and had fruity-related aromas, showing low intensities of sourness, body and alcohol sensations. By contrast, the wines in Cluster 3 mainly stood out for having the highest intensity of sour taste and vegetative odour. Finally, the other wines in Cluster 2 were very dark yellow in colour and had low sourness. According to the MFA (Figure 3a-b), these darker wines were characterised as having a raisin odour and flavour and a fuel odour; this can be attributed to aging and oxidation notes (Schüttler *et al*., 2015), as well as to specific oenological practices (e.g., maceration and aging for 12 months in acacia barrels as was performed by one producer (Ricci, RIC), whose wine was among those that received the highest rating for colour intensity). In general, all the producers of the wines in Cluster 2, except Mariotto (MAR), carried out short maceration (from 1 to 3 days), which could explain the particular characteristics of these wines.

Acidity and vegetative notes, such as those that characterised the wines in Cluster 3, are often associated with early harvest (Allamy *et al*., 2023) or adverse climatic conditions (Pons *et al*., 2017). Although the present data do not allow conclusions to be drawn in this respect, it seems unlikely that the sensory characteristics of this cluster are related to harvest period, as the producers had declared a quite broad harvest period spanning the beginning of September (two producers out of seven) to the end of September/October (two producers out of seven). In the same way, from the present data it is not possible to state that the characteristics of the wines in Cluster 3 are linked to climatic conditions. In fact, although the valleys in which Timorasso wine can be produced can have quite different climatic conditions, Cluster 3 comprises wines from three of the five valleys.

In conclusion, the present study is the first to identify the sensory characteristics that distinguish Timorasso wine, which is made from a native white grape of the Piedmont region that almost became extinct towards the end of the 1800s. Timorasso wine was found to be very complex from an aromatic point of view, with aromas spanning fruity (citrus, tropical fruits, tree-fruit, dried/baked fruit), floral, vegetative, balsamic, honey and ethereal (solvent and fuel). Although the wines were selected from the same vintage (2018) and were produced according to the regulation , high variability was found among the three identified wine clusters. Cluster 1 contained wines characterised by lemon and peach aromas, low acidity, body alcohol and colour and low intensity of vegetative and ethereal aromas. By contrast, Cluster 2 was characterised by wines with high colour and ethereal aroma intensities and low citrus aroma intensity. Cluster 3 was represented by wines with high acidity and vegetal aroma intensities, but low colour and floral aroma intensities. These results provide for the first time a sensory map of a grapevine and a wine that until a few decades ago had almost become extinct, but which is now considered as being one of the most promising white wines on the Italian market. The sensory identity and typicality of wines made from minor grape varieties can contribute to improving genetic biodiversity, improving the reputation of less well-known Italian producers and increasing their competitiveness on the market.

Future research should aim to further developing the characterisation of the Timorasso grape and its wine through the use of combined instrumental and sensory approaches, as well as exploring the evolution of chemico-physical and sensory properties of the wine during aging. Moreover, since the number of Timorasso wine producers is systematically increasing, future studies should involve more producers within a given valley to be able to verify the potential influence of geographical location on Timorasso wine quality.

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