Changes in varietal volatile composition during shelf-life of two types of aromatic red sweet Brachetto sparkling wines

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CHANGES IN VARIETAL VOLATILE COMPOSITION DURING SHELF-LIFE OF TWO TYPES OF AROMATIC RED SWEET BRACHETTO SPARKLING WINES

Fabrizio Torchio, Susana Río Segade, Vincenzo Gerbi, Enzo Cagnasso, Manuela Giordano, Simone Giacosa, Luca Rolle*

Di.Va.P.R.A. - Microbiology and Food Technology sector, University of Turin, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy.

*Corresponding author (email: luca.rolle@unito.it, Phone: 39 0116708558; Fax: 39 0116708549).

ABSTRACT

This work constitutes the first contribution to elucidate the varietal volatile composition of two types of sparkling wines manufactured with Brachetto grapes and its relative evolution during the shelf-life. After bottling, the volatile composition differed significantly among the typologies of sparkling wines evaluated, namely lightly ones (final bottle pressure < 1.7 bar) and fully ones (final bottle pressure > 3.0 bar). Free 2-phenyl ethanol was the major aromatic compound (71-78%) in Brachetto sparkling wines, but its concentration was significantly higher in fully ones. Among terpenoids, glycosylated geraniol was the predominant compound in the two types of sparkling wines, followed by nerol. These two compounds accounted for around 49% and 67% of bound aromatic precursors in lightly and fully sparkling wines, respectively. Lightly sparkling wines showed a significant lower content of four free terpenoids (*trans*-pyran linalool oxide, nerol, citronellol and 2,6-dimethyl-3,7-octadien-2,6-diol) in relation to fully ones, and a significant higher content of three free volatile compounds (geraniol, *cis*-pyran linalool oxide and *cis*-furan linalool oxide).

A central composite design (CCD) and response surface methodology (RSM) were applied to evaluate and to predict the effect of two independent variables, time and temperature, on the varietal volatile composition and the overall olfactory attributes of both lightly and fully sparkling wines during their ageing in bottle. Significant quantitative changes were observed and some of them were satisfactorily predicted by the RSM approach. Values of the determination coefficient higher than 0.800 were obtained in both lightly and fully sparkling wines for free geraniol and glycosylated linalool, as well as for olfactory preferences. Significant correlations were also found among the overall olfactory judgement and some single free terpenoids correctly predicted by the mathematical model, preferentially α-terpineol and geraniol, although only the concentration of free geraniol was higher than its odour threshold. The higher presence of varietal geraniol was positively valued by the tasters.

Keywords: Sweet sparkling wines; Volatile composition; Olfactory judgment; Response surface methodology; Central composite design; cv Brachetto.

1. Introduction

Sparkling wines are produced according to a double fermentation process. In a first phase, the base wine is elaborated. It is then left to undergo a second alcoholic fermentation, inside a sealed bottle (the *Champenoise* or traditional method) or in large containers (the *Charmat* method), by adding a suspension of yeasts and sugar.

The aroma constitutes a factor of paramount importance to produce high quality sparkling wines (Vannier, Brun, & Feinberg, 1999) and it is a result of the volatile composition. The presence of volatile compounds in the wine can be influenced by the grape cultivar (varietal aroma), the yeasts (fermentative aroma) or the ageing stage (post-fermentative aroma). Although there are many factors involved in the chemical composition of sparkling wines like the base wine characteristics (Girbau-Solà, López-Tamames, Buján, & Buxaderas, 2002; Hidalgo, Pueyo, Pozo-Bayón, Martínez-Rodríguez, Martín-Álvarez, & Polo, 2004; Torrens, Riu-Aumatell, Vichi, López-Tamames, & Buxaderas, 2010), for sparkling wines produced by the traditional method the transformations occurring in the volatile composition during the second fermentation and ageing are key factors in the aroma profile, affecting the sensory quality of sparkling wines. During the sparkling wine ageing on the yeast lees, the simultaneous degradation and synthesis of volatile compounds occurs, resulting that at any given time one of the two latter processes can predominate (Pozo-Bayón, Pueyo, Martín-Álvarez, Martínez-Rodríguez, & Polo, 2003; Usseglio-Tomasset, Bosia, Di Stefano, & Castino, 1983). The enzymes released during the yeast autolysis cause an increase in many varietal aromas (Francioli, Guerra, López-Tamames, Guadayoi, & Caixach, 1999; Riu-Aumatell, Bosch-Fusté, López-Tamames, & Buxaderas, 2006; Torrens, et al., 2010), whereas the most hydrophobic volatile compounds can be adsorbed on the yeast lees reducing their concentration in sparkling wines aged (Pozo-Bayón, Martínez-Rodríguez, Pueyo, & Moreno-Arribas, 2009).

The development of new analytical methodologies has allowed determining the volatile composition of sparkling wines, with special emphasis to terpenoids that are minor compounds but with great influence on the wine aroma. In general, varietal volatile compounds represent in terms of concentration 1-6% of the volatile composition of sparkling wines (Coelho, Coimbra, Nogueira, & Rocha, 2009). Some volatile compounds have also been proposed as age markers of Cava wines like ethyl esters, acetates (isoamyl acetate, hexyl acetate and 2-phenylethyl acetate) and norisoprenoids (vitispirane and 1,2-dihydro-1,1,6-trimethylnaphtalene (TDN)) (Francioli, et al., 1999; Francioli, Torrens, Riu-Aumatell, López-Tamames, & Buxaderas, 2003; Pozo-Bayón, at al., 2003; Riu-Aumatell, et al., 2006).

In Italy, the traditional oenological technique consists of using the second fermentation in a sealed tank (the *Charmat* method) to produce sparkling wines with high concentration of residual sugars (90-130 g/L) and low alcohol content (4.5-7.5% v/v ethanol) from white and red aromatic grapes like Muscat petit grain or Muscat white, Brachetto, Malvasia di Casorzo, Malvasia di Schierano and others (Di Stefano, Borsa, Maggiorotto, & Corino, 1995; Mateo, Gentilini, Huerta, Jiménez, & Di Stefano, 1997; Torchio, Río Segade, Gerbi, Cagnasso, & Rolle, 2011). The performance of the second fermentation in a sealed tank offers the advantage of preserving varietal aromas when aromatic grape varieties are used due to the less time in contact with the yeast lees. In this case, the wine is filtered before the bottling.

While large scientific literature is present on the volatile composition and shelf-life of sweet aromatic Moscato d'Asti DOCG sparkling wines made from aromatic white grapes of Muscat petit grain variety (Bordiga, Coisson, Travaglia, Piana, & Arlorio, 2009; Di Stefano, & Ciolfi, 1983; Gerbi, Rolle, Ghirardello, Giordano, & Zeppa, 2006), no study has been published up until now on the volatile profile of Brachetto d'Acqui DOCG sparkling wines. These wines produced from Brachetto grapes, with about 6-8 million of bottles produced for year and commercialized in all the world, are the most important aromatic red sweet sparkling wines in Italy. Therefore, the main aims of this work were to characterize the volatile composition of two types of red sweet sparkling wines made from Brachetto grapes and to model the changes occurring in volatile compounds and olfactory characteristics during their shelf-life. For this last aim, the effect of different storage conditions was evaluated using central composite design (CCD) and response surface methodology (RSM).

2. Materials and methods

2.1. Winemaking procedure

Brachetto grapes (*Vitis vinifera* L.) were harvested in 2008 from a vineyard sited in Castelboglione (Alessandria, Piedmont, North-West Italy), respecting the Brachetto d'Acqui DOCG Disciplinary of production and the optimum phenol content (Rolle, Torchio, Zeppa, & Gerbi, 2009). Briefly, after the first maceration/fermentation phase of 2 days (48 h) at 16-17 ºC by adding sulphur dioxide (2.5 g/hL), pectinolytic enzyme (3.5 g/hL, Vinozym® Vintage FCE, Novozymes, Switzerland) and *Saccharomyces cerevisiae* dried active (LSA) commercial yeast (20 g/hL) and.performing two pumping-over without aeration for each day, the grape pomace was pressed at a maximum pressure of 1.2 bar. The pectinolytic enzyme was used to facilitate a rapid extraction of anthocyanins because the berry skin of Brachetto grapes is characterized by slow extractability of these compounds (Rolle, et al., 2009; Torchio, et al., 2011). Free-run juice and press juice were mixed (base wine, ethanol < 3.5% v/v) and stored at 0 °C for 2 weeks.

Two equal portions of the juice stored were introduced in distinctive sealed tanks, where a second fermentation (*prise de mousse*) was performed by inoculating yeasts (20 g/hL, Zymaflore VL1, Laffort, France), B_1 vitamin (0.6 mg/L), ammonium phosphate (20 g/hL), and saccharose (34 g/L) only for the fully sparkling wine. Two types of wine were elaborated according to the Brachetto d'Acqui DOCG Disciplinary of Production: a sweet lightly sparkling wine (final bottle pressure < 1.7 bar, called '*Tappo raso'*) and a sweet fully sparkling wine (final bottle pressure > 3.0 bar, called '*Spumante*'). In both sweet fully and lightly sparkling wines, the alcoholic fermentation was stopped before it was completed, by a rapid decrease of temperature at 0 °C, to obtain a concentration of residual sugars comprised between 90 and 130 g/L and an alcohol content comprised between 4.5 and 7.5% v/v ethanol. Finally, the resulting wines, after adjusting their free sulphur dioxide content to 30 mg/L, were filtered and bottled.

2.2. Chemical parameters

Reducing sugars, ethyl alcohol, pH, total acidity and volatile acidity were determined according to International Organization of Vine and Wine (O.I.V.) methods (OIV, 2008).

2.3. Free and glycosylated volatile compounds extraction and determination

All wines (see section 2.5) were analyzed as described by Di Stefano (1991) and Mateo et al. (1997). A diluted (1:3) sample aliquot of 100 mL was spiked with 1-heptanol as internal standard (200 µL of 44 mg/L solution in 10% ethanol), and was loaded onto a 1 g Sep-Pak tC-18 reversed-phase solid-phase extraction (SPE) cartridge (Waters Corporation, Milford, MA, USA), previously activated with 5 mL of methanol and then rinsed with 10 mL of deionized water using a flow-rate of ca. 3 mL/min. Before elution, the cartridge was rinsed with 10 mL of deionized water to eliminate sugars, acids and other low molecular weight polar compounds. The free fraction was eluted with 12 mL of dichloromethane. The eluate was dried over anhydrous Na_2SO_4 and concentrated to about 200 µL under a stream of nitrogen. This extract containing free volatile compounds was immediately analyzed by gas chromatography/mass spectrometry (GC/MS).

The glycoconjugates were then eluted from the cartridge with 20 mL of methanol and the eluate was concentrated to dryness using a vacuum rotavapor (Buchi R-210, Switzerland) at 35 °C. The dried glycosidic extract obtained was dissolved in 3 mL of citrate-phosphate buffer (0.2 M, pH 5). The enzymatic hydrolysis was carried out using 50 mg of an AR-2000 commercial preparation with glycosidase side activities (DSM Oenology, The Netherlands) and incubating at 40 °C for 24 h. After adding 200 µL of 1-heptanol (44 mg/L solution in 10% ethanol), glycosylated precursors were then extracted following the SPE method previously described. The dichloromethane extract obtained was dried over anhydrous Na_2SO_4, concentrated to 200 µL under nitrogen and kept at -20 °C until to be analyzed.

GC/MS analysis was performed with a Shimadzu GC-2010 gas chromatograph equipped with a Shimadzu QP-2010 Plus quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan) and a DB-WAXETR capillary column (30 m × 0.25 mm, 0.25 µm, J&W Scientific Inc., Folsom, CA, USA) (Rolle, Giordano, Giacosa, Vincenzi, Río Segade, Torchio, Perrone, & Gerbi, 2011). The temperature program started at 35° C for 5 min, and increased at rate of 2 °C/min to 190 °C and 3 °C/min to 230 °C for 5 min. The carrier gas (He) flow-rate was 1 mL/min. Injections of 1 µL were performed in split mode 1:10. The injection port temperature was 250 °C, the ion source temperature was 240 °C and the interface temperature was 230 °C (solvent delay of 6.5 min). The detection was carried out by electron impact mass spectrometry in total ion current (TIC) mode, using an ionization energy of 70 eV. The mass acquisition range was *m/z* 30-330. The identification of volatile compounds was confirmed by injection of pure standards and comparison of their retention indices (a mixture of a homologous series of C5-C28 was used), MS data reported in the literature and database (http://webbook.nist.gov/chemistry/). Compounds, for which pure standards were not available, were identified on the basis of their mass spectra and their retention indices available in the literature. Semiquantitative data (µg/L) were obtained by measuring the relative peak area of each identified compound in relation to that of the added internal standard.

2.4. Sensory preference (Olfactory judgment)

All wines (see section 2.5) were evaluated by the same group of 15 trained panellists (3 females and 12 males), staff members from the University of Turin (Italy) and Consorzio di Tutela del Brachetto d'Acqui DOCG (Italy), all of them belonging to a sensory group with long experience in tasting of aromatic sparkling wines. Several previous training sessions were carried out to standardize criteria among panellists on the olfactory quality of Brachetto d'Acqui DOCG sparkling wines (ISO 3972, 2011). This was evaluated by the aroma global quality attributes using a 10 point hedonic scale (Fanzone, Peña-Neira, Gil, Jofré, Assof, & Zamora, 2012), which is most used to estimate the overall consumer acceptability because of its great relevance in the commercial value of the two types of aromatic wines.

The assessment took place in a standard sensory analysis chamber (ISO 8589, 2007) equipped with separate booths. There was an uniform source of lighting and absence of noise and distracting stimuli, ambient temperature being comprised between 19 and 21 °C across the day. Wine samples were presented for tasting at 15 °C. All samples of each session were served in completely randomized order in clear wine tasting glasses (ISO 3591, 1977) labeled with a three-digit code. In order to ascertain the judges' consistency, one sample was replicated in each session. One session of 60 min was performed for each point of the experimental design proposed by CCD.

2.5. Shelf-life experimental design and statistical analysis

One-way analysis of variance (ANOVA) was used in order to establish statistical differences in the volatile composition between sweet lightly and fully sparkling wines. Two factorial 2^2 CCD was used to optimize the ageing conditions (Granato, Favalli Branco, & de Araújo Calado, 2011). The independent variables were time (X_1) and temperature (X_2), which were studied at five different levels. The ageing time was 0, 53, 182, 312 or 365 days, and temperature was 5, 8, 15, 22 or 25 ºC. The codified values for the variables ranged between +1.414 and −1.414, taking the zero value as central point. A total of 13 experiments including five replicates of the central point were carried out (Torchio, et al., 2011). The mean values of triplicate determinations (three bottles for each CCD experiment) were adjusted to the following second-order polynomial model:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2$$

where Y is the predicted response, X_1 and X_2 correspond to the independent variables, b_0 is the value in the central point conditions, b_1 and b_2 represent the principal effects associated with each variable, b_{11} and b_{22} are the squared effects and b_{12} is the interaction effect. The equations obtained were represented as surface plots using RSM to optimize the ageing conditions. A statistical analysis was performed to predict models through regression analysis (R^2). The regression models were highly significant ($p < 0.01$) with a satisfactory value of determination coefficient ($R^2 > 0.67$), indicating that at least 67% of the variability in the response could be explained by the second-order model equations.

Pearson's correlation coefficients (R) were calculated to determine significant relationships among volatile compounds and the olfactory evaluation for the two types of Brachetto sparkling wines ($p < 0.05$). All statistical analyses were performed using the software package Statistica version 7.0 (Statsoft Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. Chemical parameters

As reported in Brachetto d'Acqui DOCG Disciplinary of production, the second alcoholic fermentation (*prise de mousse*) was stopped when ethyl alcohol was of 5.13 and 6.54 % v/v in lightly and fully sparkling wines, respectively. Moreover, the resulting wines were sweet with a residual sugar concentration of 120 and 128 g/L in lightly and fully sparkling wines, respectively. Between the two types of wines, no differences were detected in pH (3.20) and total acidity (5.05 g/L as tartaric acid). For all the wines, the values of volatile acidity (< 0.30 g/L as acetic acid) indicated good preservation of the wines.

3.2. Volatile composition of Brachetto sparkling wines at bottling

Free and glycosylated volatile compounds of both sweet lightly and fully sparkling wines after bottling are shown in Table 1.

As reported in a previous research on the aromatic profile of Brachetto grapes, terpenols, in particular geraniol, play a significant role in the varietal aroma of this cultivar (Di Stefano, & Corino, 1984). Consequently, these compounds strongly characterize the varietal aromatic profile of Brachetto d'Acqui DOCG sweet red sparkling wines.

At bottling, after second fermentation in sealed tanks, the *tappo raso* typology of sparkling wines showed a significant lower content of four free terpenoids (*trans*-pyran linalool oxide, nerol, citronellol and 2,6-dimethyl-3,7-octadien-2,6-diol or Diol 1) in relation to fully Brachetto sparkling wines, and a significant higher content of three free volatile compounds (geraniol, *cis*-pyran linalool oxide and *cis*-furan linalool oxide). In both lightly and fully sparkling wines, the concentration of citronellol, geranyl acetate, hotrienol, linalool, Diol 1 and 2,6-dimethyl-1,7-octadien-3,6-diol (Diol 2) in free form was higher than that in bound form. Geraniol, citronellol and linalool were the most abundant free monoterpenoids in lightly sparkling wines. In particular, lightly sparkling wines displayed a significantly higher content of free geraniol (two-folds) than fully ones, confirmed by a minor consumption by *Saccharomyces cerevisiae* during fermentation (Garcia Moruno, Ribaldone, Di Stefano, Conterno, & Gandini, 2002; Vaudano, Garcia Moruno, & Di Stefano, 2004) and a significantly minor content (also two-folds) of free citronellol. In fact, yeasts are responsible to trasforming most of geraniol to citronellol through enzymatic reactions, which are particularly evident in fully sparkling wines (Vaudano, et al., 2004).

The olfactory threshold of linalool (25 μg/L; Ferreira, López, & Cacho, 2000) was exceeded in both Brachetto sparkling wines, whereas α-terpineol (250 μg/L; Ferreira, et al., 2000) and citronellol (100 μg/L; Guth, 1997) were present below their odorant threshold. After bottling, geraniol reached its threshold (30 μg/L in 10% ethanol, Guth, 1997; 7.5 μg/L in water, Belitz, Grosch, & Schieberle, 2009) in Brachetto sparkling wines.

Although bound volatile compounds do not contribute directly to the wine aroma, they are odourless precursors of flavour. Glycosylated geraniol was the predominant terpenoid compound in the two types of Brachetto sparkling wines (about 600 μg/L), followed by nerol. These two compounds accounted for around 49% and 67% of bound aromatic precursors in lightly and fully sparkling wines, respectively. Glycosylated forms of *trans*-furan linalool oxide, *cis*-furan linalool oxide, α-terpineol and *trans*-pyran linalool oxide were found in significantly higher contents in lightly sparkling wines, whereas glycosylated forms of *cis*-pyran linalool oxide, citronellol and Diol 1 were more abundant in fully ones (Table 1).

The content of α-terpineol, geraniol, hotrienol and linalool in Brachetto sparkling wines was in the range reported for other sparkling wines elaborated from two Portuguese grape varieties, such as Fernão-Pires and Baga, while nerol was not detected (Coelho, et al., 2009). Another important aspect is the higher content of linalool found in Brachetto sparkling wines if compared with other sparkling wines made from different varieties (Campo, Cacho, & Ferreira. 2008; Torrens, et al., 2010).

The monoterpenoid profile of Brachetto sweet sparkling wines was deeply different to the reported for dry red wines elaborated from this winegrape variety (Bonino, Schellino, Rizzi, Aigotti, Delfini, & Baiocchi, 2003). The percentage of α-terpineol, citronellol, geranyl acetate and linalool was lower in the two types of sweet sparkling wines (2.9-9.0 times), whereas nerol and geraniol were more abundant in sweet sparkling wines than in dry wines (1.4-3.6 times), probably because in this first case there are less losses due to the lower effect of the alcoholic fermentation (partial alcoholic fermentation). On the other hand, all these Brachetto wines showed similarities regarding to hotrienol. A distinct characteristic of Brachetto sweet sparkling wines is the presence of linalool oxides that were not detected in dry wines. Since the winemaking process is a critical factor in the aroma composition of a wine, more research is necessary to confirm the power of linalool oxides to differentiate the wine typologies mentioned.

Among fermentative volatile compounds, at start point of the shelf-life study, free 2-phenyl ethanol was the major free aromatic compound in Brachetto sparkling wines, but its concentration was significantly higher in fully sparkling wines. It represents the 71% and 78% of all volatile compounds detected in lightly and fully sparkling wines, respectively. This flavour-active alcohol, one of the glycosylated compounds showing the highest content in lightly sparkling wines, was also identified in fully ones but at significantly lower concentration. Alcohols were also strongly represented in varietal free and glycosylated forms of 1-hexanol. 2-Phenyl ethanol may contribute to the aroma of Brachetto sparkling wines with rose and sweet notes, while 1-hexanol may confer herbaceous notes. However, the content of these two alcohols in Brachetto sparkling wines was below their odour-threshold, which is 14 and 8 mg/L, respectively (Ferreira, et al., 2000; Guth, 1997).

Regarding 2-phenyl ethanol, Brachetto sparkling wines presented contents intermediate to other data published for sparkling wines by Francioli et al. (2003) (3.4-5.3 mg/L) or Coelho et al. (2009) (1.800-5.870 mg/L), and by Torrens et al. (2010) or Campo et al. (2008) (16.871-21.313 mg/L). This volatile compound is related with yeast metabolism. Some works reported a progressive decrease (Francioli, et al., 2003) but also an increase (Torrens, et al., 2010) in the aromatic alcohol during ageing.

The content of 1-hexanol in Brachetto sparkling wines was lightly lower than the reported for other sparkling wines elaborated from different Spanish white winegrape varieties like Macabeu, Xarel·lo and Parellada (0.7-1.389 mg/L) (Francioli, et al., 2003; Torrens, et al., 2010) or red ones like Garnacha Tinta (2.2 mg/L) (Hidalgo, et al., 2004), but similar to the content found in sparkling wines obtained from Portuguese varieties like Fernão-Pires and Baga (245.22-743.79 µg/L) (Coelho, et al., 2009). In these works previously published, no change was reported in the 1-hexanol content during ageing.

After bottling, lightly sparkling wines showed significantly higher contents of free form of 2-phenyl ethyl acetate than fully sparkling wines. Although Francioli et al. (2003) found 2-phenyl ethyl acetate contents of 1.7 mg/L in the younger sparkling wines (< 9 months), this volatile compound displayed the lowest values in the longer aged samples and even it was not detected in other works (Coelho, et al., 2009; Torrens, et al., 2010). Compounds such as 2-phenyl ethyl acetate have been reported as the esters that exhibit the higher contribution to the sparkling wines aroma (Mamede, Cardello, & Pastore, 2005). However, the concentration found in Brachetto sparkling wines was below its sensory threshold (250 µg/L; Guth, 1997).

3.3. Evaluation of the predictive power of the mathematical model for the aroma shelf-life modelling

During storage of sparkling wines, the volatile composition could undergo considerable changes. Therefore, it is important to assess the shelf-life of both lightly and fully sparkling wines. The effect of two variables (time and temperature) was evaluated using two factorial 2^2 CCDs and, in accordance with the RSM approach, the model was significant. The predictive capability of the mathematical model was validated by calculating the coefficient of determination (R^2) between the real contents of volatile compounds and those predicted by the mathematical model. Table 2 shows the determination coefficient for each free volatile compound and for the glycosylated form of geraniol and linalool, which contribute more strongly to the odour impact and therefore to the wine aroma (Di Stefano, & Corino, 1984). As can be observed, the RSM approach could explain at least 67.7% of all variance of the results obtained for some volatile compounds like free forms of α-terpineol and geraniol, and glycosylated forms of geraniol and linalool in lightly sparkling wines, as well as free forms of 2-phenyl ethanol, Diol 2, α-terpineol, geraniol, *cis*- and *trans*-furan linalool oxides, and glycosylated forms of geraniol and linalool in fully sparkling wines. It is important to evidence that values of the determination coefficient higher than 0.800 were obtained in both lightly and fully sparkling wines for free geraniol and glycosylated linalool. Furthermore, total explained variance was about 82% for glycosidically bound geraniol in fully sparkling wines. In addition to the volatile compounds, the model also explained satisfactorily more than 80% of all variance of the results obtained for olfactory analysis of the two types of sparkling wines.

Fig. 1 and 2 show the effect of the ageing time and temperature on the content of each free terpenoid compound significantly predicted by the RSM model for lightly and fully sparkling wines, respectively. Furthermore, the mathematical equations obtained are summarized in Table 3. The tendency of α-terpineol with the increase in the storage temperature was different depending on the ageing time (Fig. $1_{[a]}$ and $2_{[a]}$). In fact, higher α-terpineol contents were favored when the ageing time increased, but this effect was more evident for the higher temperatures. As consequence, the regression coefficient was positive for time and negative for temperature (Table 3). The effect of time and temperature on the α-terpineol content was more important in fully sparkling wines than in lightly ones.

The main factor affecting the geraniol content in lightly sparkling wines was the ageing time. The maximum values of the geraniol content corresponded to intermediate times of about 182 days at any temperature. However, intermediate temperatures also revealed higher geraniol contents. In fully sparkling wines, the stronger increase in the geraniol content was undergone for the longer ageing times at low temperatures, because the higher temperatures involved the reduction of the abundance of geraniol, excepting for the initial stages of wine ageing. Fig. $1_{[b]}$ and $2_{[b]}$ show the different changes in the geraniol content depending on the sparkling wine type. In any case, the regression coefficients for the two independent variables were positive. The sparkling wines most affected by the changes in both time and temperature were lightly ones.

The behaviour of α-terpineol and geraniol during ageing of sparkling wines was different to the reported for ageing of red wines stored in oak wood barrels (Castro-Vázquez, Alañón, Calvo, Cejudo, Díaz-Maroto, & Pérez Coello, 2011). In oak barrels, the significant reduction in the two terpenoid compounds may be due to the oxidative ageing process instead of the reductive ageing process in bottle.

The content of *trans*- and *cis*-furan linalool oxides varied significantly throughout the shelf-life of fully sparkling wines (Fig. $2_{[c,d]}$). Although the maximum values of the two linalool oxides were obtained at the higher ageing times and temperatures, the regression coefficient was only positive for the temperature variable. This could be due to that the content increased with the ageing time and temperature from 182 days.

The behaviour of the Diol 2 during ageing of fully sparkling wines is shown in Fig. 2[e]. As can be observed, the increase of the ageing times caused a significant increase in the content of this compound from 172 days, whereas the negative effect of the storage temperature was much lower. Nevertheless, the regression coefficients for the two independent variables were negative possibly due to that the increase in time at the first ageing steps (up to 172 days) resulted in a smaller content of Diol 2.

Since the importance of developing quality control methods for assessing the wine authenticity, this work could also be considered as a contribution to the use of the volatile composition and its evolution across the commercial shelf-life for assessing the authenticity of Brachetto sweet sparkling wines, as already done for dry wines and other foods (Arvanitoyannis, Katsota, Psarra, Soufleros, & Kallithraka, 1999; Nasi, Ferranti, Amato, & Chianese, 2008; Tzouros, & Arvanitoyannis, 2001).

3.4. Evolution of olfactory judgment throughout wine ageing

The sensory panel found differences in the olfactory profile of the sparkling wines studied during their ageing under different environmental conditions. According to Fig. 3[a,b], the positive overall olfactory attributes decreased with the increase in time for both lightly and fully sparkling wines. However, this effect was attenuated or even inversed in the latest storage days, particularly when the lower temperatures were used. At the initial ageing phase, the higher temperatures gave better olfactory response, but the increase in temperature for a longer time caused a decrease in the intensity of the positive olfactory attributes. The negative effect of both time and temperature was more accused in fully sparkling wines than in lightly ones.

The objective measurement of the wine aroma is of great importance for winemakers because the increasing hedonic character of the consumption of this product suggests a stronger relationship between aroma and acceptability of the wine. Since the wine olfactory profile may be related to the volatile composition, a correlation study was performed among the olfactory attributes and each single free terpenoid compound correctly predicted by the mathematical model (coefficient of determination > 0.680, Table 2). Table 4 shows that α-terpineol was satisfactorily inversely correlated with the acceptability of lightly and fully sparkling wines, whereas geraniol only was significantly linked to the sensory quality of lightly sparkling wines. Furthermore, the higher presence of geraniol was positively valued by the tasters. Contrarily, the negative Pearson's correlation coefficient obtained for α-terpineol indicates worse acceptability of the product with higher content of this terpenol in agreement with the reported by Garcia Moruno et al. (2002).

4. Conclusions

This work constitutes the first aroma characterization of sweet lightly and fully sparkling wines made from Brachetto winegrape variety. Free 2-phenyl ethanol was the major aromatic compound, whereas glycosylated geraniol was the predominant terpene in the two types of Brachetto sparkling wines, followed by nerol. Significant quantitative differences were firstly found in the volatile composition of two different typologies of Brachetto sparkling wines. Lightly sparkling wines showed a significant lower content of four free terpenoids (*trans*-pyran linalool oxide, nerol, citronellol and 2,6-dimethyl-3,7-octadien-2,6-diol) in relation to fully ones, and a significant higher content of three free volatile compounds (geraniol, *cis*-pyran linalool oxide and *cis*-furan linalool oxide).

The RSM approach was successfully used to evaluate the effect of the ageing environmental conditions on the evolution of varietal volatile compounds that strongly contribute to the wine aroma during the shelf-life of red lightly and fully aromatic sparkling wines, as well as on the evolution of the overall olfactory attributes. Many changes in volatile compounds were correctly predicted by this mathematical model, particularly total explained variance was higher than 80% for free geraniol and glycosylated linalool in the two typologies of Brachetto sweet sparkling wines. Significant correlations were found among the overall olfactory quality and free forms of α-terpineol and geraniol, although only the concentration of free geraniol was higher than its odour threshold.

The modelling of the results obtained for these compounds gave mathematical equations that provide useful information for predicting the behaviour of sparkling wines under different environmental conditions. This may suppose a great advance to control the wine quality and to predict the sensory acceptance of this product by consumers. In this sense, high temperatures influence negatively the sensory quality of lightly and fully sparkling wines when long ageing times are used.

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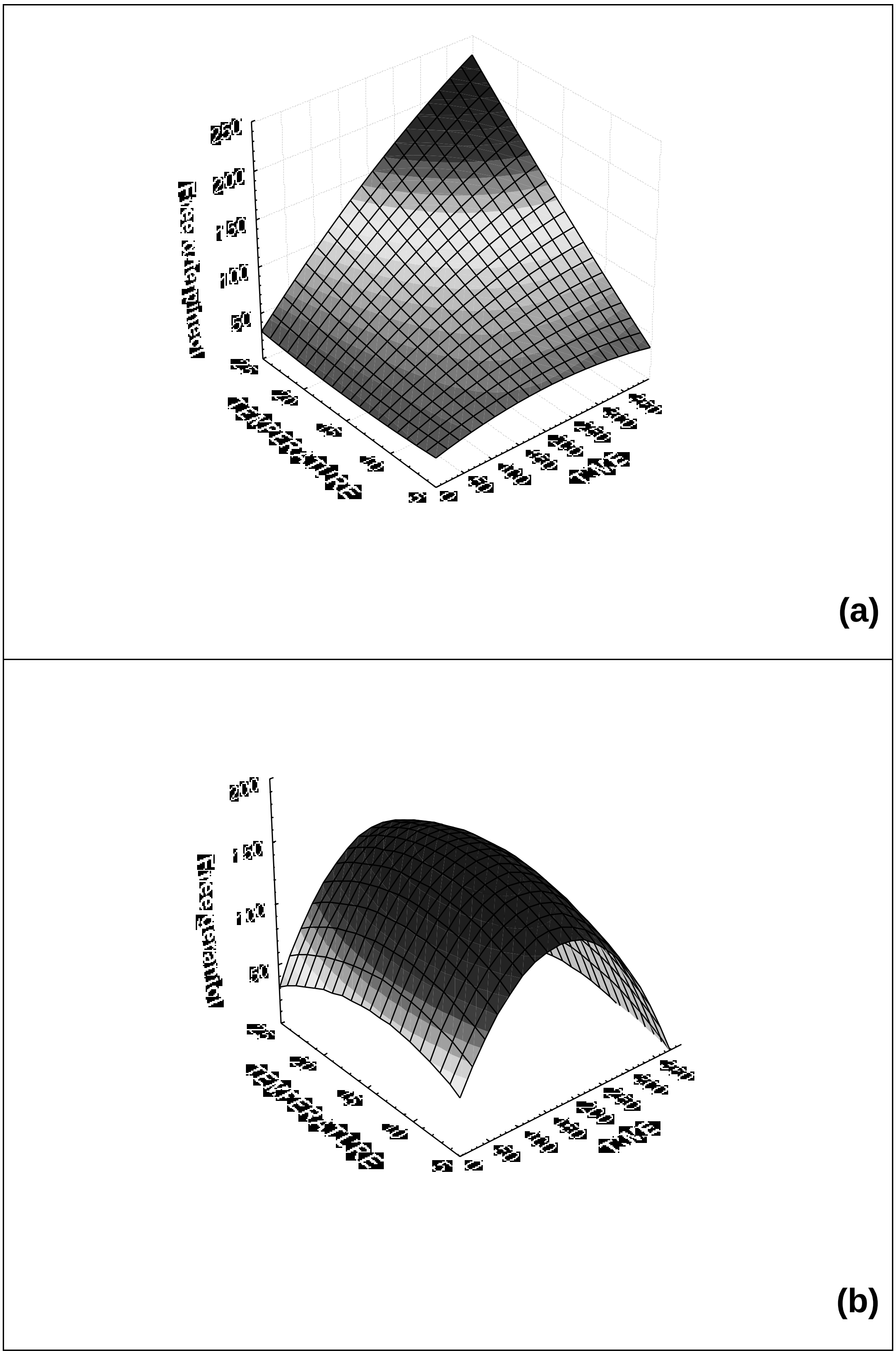

Fig. 1. Response surface plot showing the effect of time and temperature of ageing on the content of free α-terpineol (a) and free geraniol (b) in sweet lightly sparkling wines.

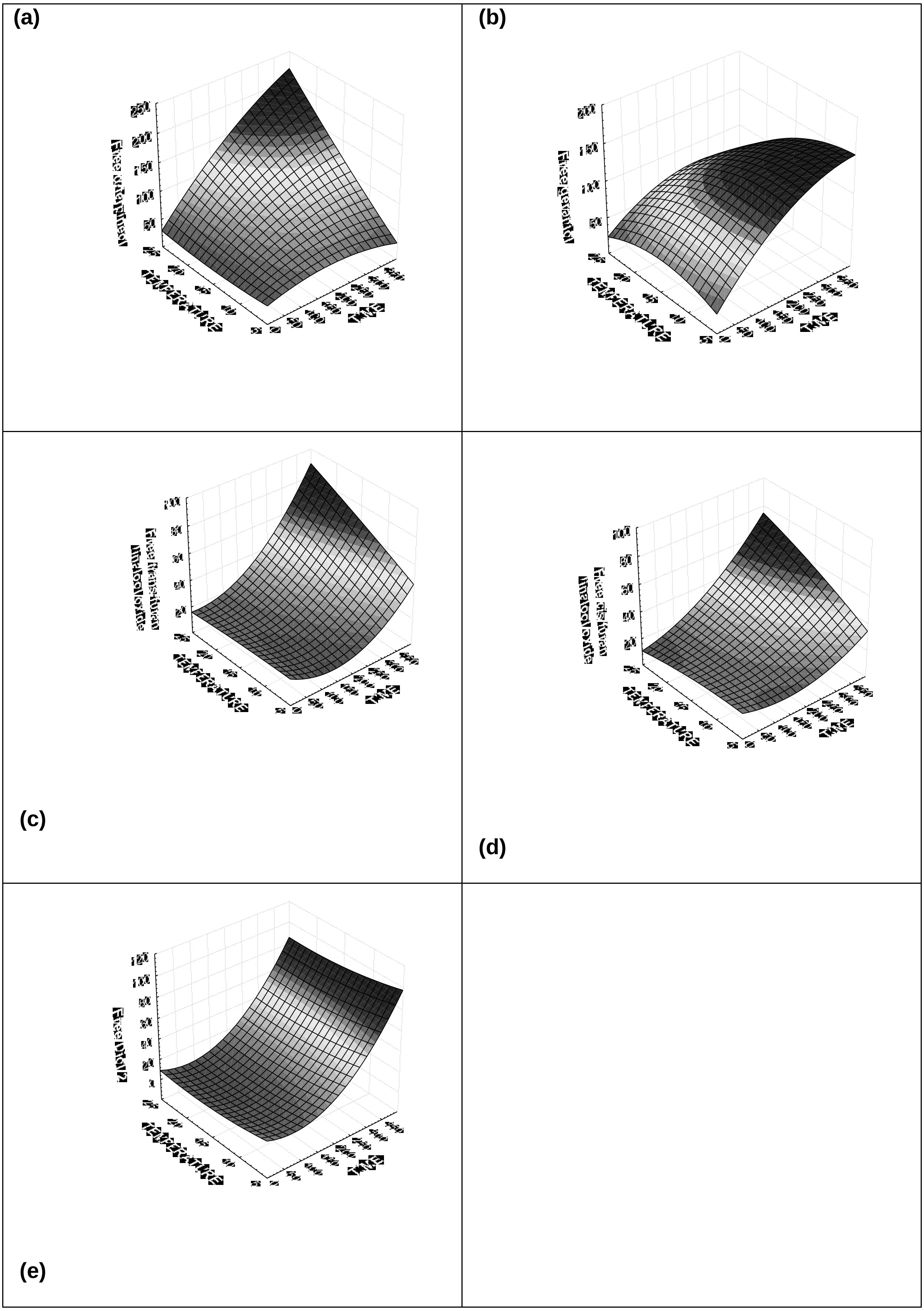
(a)
(b)
(c)
(d)
(e)

Fig. 2. Response surface plot showing the effect of time and temperature of ageing on the content of free α-terpineol (a) and free geraniol (b), free *trans*-furan linalool oxide (c), free *cis*-furan linalool oxide (d) and free Diol 2 (e) in sweet fully sparkling wines.

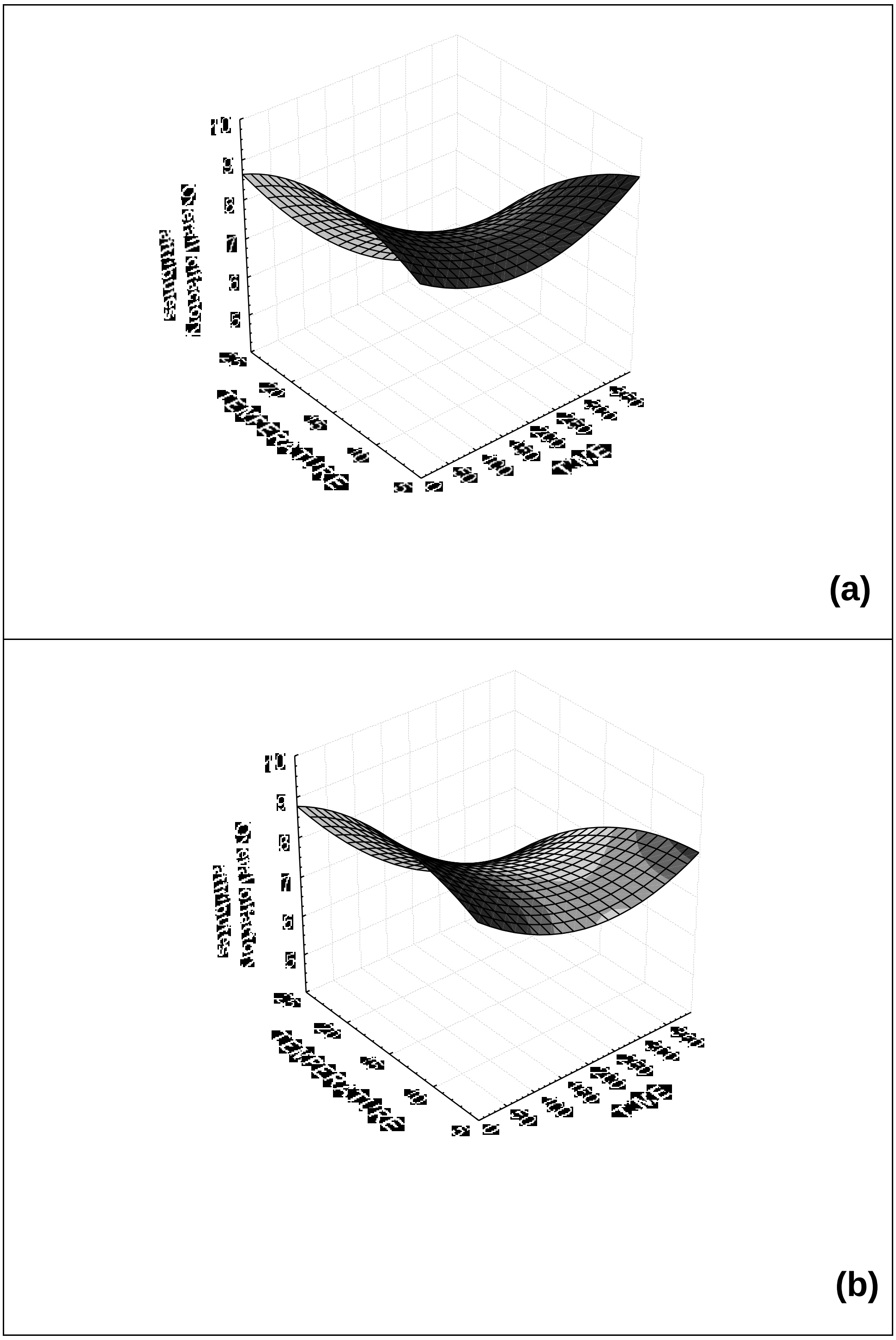

Fig. 3. Response surface plot (RSM) showing the effect of time and temperature of ageing on the overall olfactory attributes in sweet lightly (a) and fully (b) sparkling wines.

Table 1

Free and glycosylated volatile compounds (µg/L) in both sweet lightly (SL) and fully (SF) sparkling wines after bottling, start point of shelf-life study.

Volatile compounds	SL sparkling wine (bottle	SF sparkling wine (bottle pressure > 3.0 bar)	Sign[a]
Free compounds			
1-Hexanol	466 ± 3	465 ± 5	ns
trans-Furan linalool oxide	18.5 ± 2.1	18.0 ± 0.1	ns
cis-Furan linalool oxide	24.0 ± 0.2	12.5 ± 2.1	*
Linalool	41.5 ± 2.1	39.0 ± 2.8	ns
Hotrienol	16.5 ± 0.7	11.5 ± 2.1	ns
α-Terpineol	25.5 ± 2.1	26.0 ± 0.1	ns
trans-Pyran linalool oxide	30.0 ± 1.4	49.0 ± 0.3	**
cis-Pyran linalool oxide	29.0 ± 0.2	13.0 ± 1.4	**
Citronellol	43.5 ± 0.7	82.5 ± 0.7	***
2-Phenyl ethyl acetate	38.7 ± 2.8	25.5 ± 0.7	*
Nerol	29.5 ± 0.7	55.0 ±1.4	**
Geraniol	47.0 ± 1.4	25.0 ± 0.1	**
Geranyl acetate	21.0 ± 1.4	24.5 ± 0.7	ns
2-Phenyl ethanol	7780 ± 34	8706 ± 62	**
Diol 1	49.5 ± 2.1	60.5 ± 2.1	*
Diol 2	7.50 ± 0.70	9.00 ± 1.40	ns
Glycosylated compounds			
1-Hexanol	149 ± 2	149 ± 1	ns
trans-Furan linalool oxide	38.0 ± 1.2	18.0 ± 2.4	*
cis-Furan linalool oxide	88.0 ± 1.4	66.0 ± 2.8	*
Linalool	21.0 ± 1.4	18.0 ± 2.8	ns
Hotrienol	8.5 ± 0.7	8.0 ± 2.8	ns
α-Terpineol	71.0 ± 2.8	39.5 ± 2.1	**
trans-Pyran linalool oxide	47.5 ± 2.1	35.0 ± 1.4	*
cis-Pyran linalool oxide	6.5 ± 0.7	19.5 ± 0.7	**
Citronellol	9.0 ± 0.1	12.5 ± 0.7	*
2-Phenyl ethyl acetate	29.0 ± 1.4	29.0 ± 1.4	ns
Nerol	479 ± 6	465 ± 4	ns
Geraniol	599 ± 6	604 ± 4	ns
Geranyl acetate	18.0 ± 1.4	19.0 ± 1.4	ns
2-Phenyl ethanol	605 ± 4	50.5 ± 9.2	***
Diol 1	38.0 ± 1.4	56.5 ± 2.1	**
Diol 2	4.0 ± 0.2	4.5 ± 0.7	ns

All data are expressed as average value ± standard deviation (n = 3). [a]: *,**,*** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively.

Table 2

Determination coefficient (R^2) calculated for each single volatile compound and overall olfactory judgement from the contents experimentally determined and those predicted by the mathematical model.

Parameter	SL sparkling wine (bottle	SF sparkling wine (bottle pressure > 3.0 bar)
Free compounds		
1-Hexanol	0.457	0.351
trans-Furan linalool oxide	0.535	0.743
cis-Furan linalool oxide	0.009	0.704
Linalool	0.447	0.500
Hotrienol	0.313	0.561
α-Terpineol	0.682	0.745
trans-Pyran linalool oxide	0.448	0.181
cis-Pyran linalool oxide	0.610	0.136
Citronellol	0.603	0.631
2-Phenyl ethyl acetate	0.290	0.004
Nerol	0.438	0.252
Geraniol	0.848	0.880
Geranyl acetate	0.374	0.078
2-Phenyl ethanol	0.272	0.698
Diol 1	0.073	0.085
Diol 2	0.119	0.750
Glycosylated compounds		
Geraniol	0.677	0.824
Linalool	0.866	0.814
Olfactory judgements	0.896	0.812

SL: sweet lightly; SF: sweet fully.

Table 3

Second-order polynomial model by central composite design (CCD).

Free terpenoids	Wine type	Equation
trans-Furan linalool oxide	F	$Y = 19.8468 - 0.1672X_1 + 0.0398X_2 + 0.0005X_1X_1 + 0.0060X_1X_2 - 0.0079X_2X_2$
cis-Furan linalool oxide	F	$Y = 18.0734 - 0.1049X_1 + 0.2904X_2 + 0.0003X_1X_1 + 0.0061X_1X_2 - 0.0235X_2X_2$
α-Terpineol	L	$Y = 37.4943 + 0.0359X_1 - 1.4395X_2 - 0.0004X_1X_1 + 0.0246X_1X_2 + 0.0462X_2 X_2$
	F	$Y = 40.4196 + 0.0611X_1 - 1.7789X_2 - 0.0005X_1X_1 + 0.0244X_1X_2 + 0.0534X_2X_2$
Geraniol	L	$Y = 20.1166 + 0.9862X_1 + 7.0026X_2 - 0.0030X_1X_1 + 0.0042X_1X_2 - 0.2617 X_2X_2$
	F	$Y = 2.5207 + 0.6467X_1 + 5.8935X_2 - 0.0007X_1X_1 - 0.0107X_1X_2 - 0.2028 X_2X_2$
Diol 2	F	$Y = 22.9333 - 0.1227X_1 - 1.6211X_2 + 0.0008X_1X_1 - 0.0007X_1 X_2 + 0.0429X_2X_2$

L: sweet lightly sparkling wine; F: sweet fully sparkling wine.

Table 4

Significant Pearson's correlation coefficients among the olfactory judgement and each single free terpenoid compound correctly predicted by the mathematical model.

Free terpenoids	SL sparkling wine (bottle	SF sparkling wine (bottle pressure > 3.0 bar)
trans-Furan linalool oxide		-0.360
cis-Furan linalool oxide		-0.236
α-Terpineol	-0.844	-0.759
Geraniol	0.671	0.539
Diol 2		0.236