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CHANGES IN CHROMATIC CHARACTERISTICS AND PHENOLIC COMPOSITION DURING WINEMAKING AND SHELF-LIFE OF TWO TYPES OF RED SWEET SPARKLING WINES

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ABSTRACT

The Brachetto variety is the most important aromatic red grape used for the production of sweet sparkling wines in Italy. The phenolic composition and chromatic characteristics were studied in base and red sweet sparkling wines made from this variety. The present work represents the first study on sparkling wines manufactured with Brachetto grapes. The amount of pigments extracted in the base wine was low as a consequence of the necessity for short maceration time (48 h) and low alcohol content (< 3.5 % v/v). The second fermentation (*prise de mousse*) caused a pigment content decrease, accompanied by both a color intensity decrease and a tonality increase. In terms of phenolic compounds and chromatic characteristics, lightly sparkling wines (final bottle pressure < 1.7 bar) agreed with fully sparkling wines (final bottle pressure > 3.0 bar) at the end of the second fermentation and, therefore, the chromatic quality was independent on the winemaking methodology used, excepting for lightness and color intensity.

Response surface methodology was applied to predict the effect of two independent variables, namely time and temperature of storing, on the phenolic composition and chromatic properties in both lightly and fully sparkling wines. So, it is possible to evaluate the development of

two types of sweet sparkling wines during their ageing in bottle and their commercial shelf-life. A central composite design (CCD) and response surface methodology (RSM) were used for this purpose. Quantitative changes were observed in the color parameters. Although the time variable influenced strongly them, the two sparkling wines were stable enough only at low temperatures after 12 months of ageing. During the wine ageing in bottle, an increase in the formation of polymeric red pigments can be suggested taking into account the losses observed in monomeric anthocyanins. The results obtained showed that the color of fully sparkling wines was more stable than that of lightly ones and, therefore, the shelf-life of the former ones could be longer.

Keywords: Sweet sparkling wines; color characteristics; response surface methodology; central composite design; phenolic compounds; cv Brachetto.

1. Introduction

Phenolic compounds constitute a decisive factor in the quality of a red wine because they are responsible for color, bitterness and astringency which are key sensory attributes for the acceptability of a wine by the consumer (García-Puente Rivas, Alcalde-Eon, Santos-Buelga, Rivas-Gonzalo & Escribano-Bailón, 2006; Monagas, Martín-Álvarez, Bartolomé & Gómez-Cordovés, 2006; Parpinello, Versari, Chinnici & Galassi, 2009). Among these compounds, anthocyanins are the main ones that contribute to red-bluish color in young wines. They are located in berry skins and are extracted into the must/wine during the winemaking process (Mateus, Machado & De Freitas, 2002; Revilla, García-Beneytez & Cabello, 2009). Moreover, it is well-known that molecular interactions among the wine components play a determinant role in the color stability (Boulton, 2001). Anthocyanins can react with other phenolic compounds to produce more stable pigments resulting in a color change towards reddish-brown in aged wines (Boulton, 2001; Cheynier, Duenas-Paton, Salas, Maury, Souquet, Sarni-Manchado & Fulcrand, 2006; Fulcrand, Duenas, Salas & Cheynier, 2006; Cavalcanti, Santos & Meireles, 2010). On the other hand, seed proanthocyanidins strongly influence wine astringency (Vidal, Francis, Noble, Kwiatkowski, Cheynier & Waters, 2004) but their association with anthocyanins during the ageing stage causes the decrease of this sensory property (Cheynier, Duenas-Paton, Salas, Maury, Souquet, Sarni-Manchado & Fulcrand, 2006; Fulcrand, Duenas, Salas & Cheynier, 2006).

Sparkling wines are usually produced according to the traditional method that consists of a double fermentation process. The base wine elaborated in a first phase is then left to undergo a second alcoholic fermentation, inside a sealed bottle by adding a suspension of yeasts and sugar, followed by ageing on yeast lees under anaerobic conditions for several months. However, the performance of the second fermentation in autoclaves offers the advantage of preserving variety aromas when aromatic grape varieties are used due to the less time in contact with yeast lees. In this case, the wine is filtered before bottling.

Although there are many factors involved in the chemical composition of the base wine (Anderson, Smith, Williams & Wolpert, 2008; Coelho, Coimbra, Nogueira & Rocha, 2009; Girbau-Solà, López-Tamames, Buján & Buxaderas, 2002; Pozo-Bayón, Polo, Martín-Álvarez & Pueyo, 2004; Riu-Aumatell, López-Barajas, López-Tamames & Buxaderas, 2002), it undoubtedly seems that the second fermentation and the storage conditions are also important factors. The last ones affect the sparkling wine quality since both parameters are involved in the distinctive characteristics between sparkling and base wines (Girbau-Solà, López-Barajas, López-Tamames & Buxaderas, 2002; Hidalgo, Pueyo,

Pozo-Bayón, Martínez-Rodríguez, Martín-Álvarez & Polo, 2004; Pozo-Bayón, Hernández, Martín-Álvarez & Polo, 2003; Pozo-Bayón, Monagas, Polo & Gómez-Cordovés, 2004; Pozo-Bayón, Pueyo, Martín-Álvarez, Martínez-Rodríguez & Polo, 2003; Riu-Aumatell, Bosch-Fusté, López-Tamames & Buxaderas, 2006; Torrens, Riu-Aumatell, Vichi, López-Tamames & Buxaderas, 2010). Therefore, the varietal phenolic compounds, together with those that give rise from the chemical reactions occurring during the winemaking and ageing processes, influence on color, taste and structure of the final product.

In spite of the obvious implications of phenolic compounds in the sensory quality of wines, there are very few studies developed to establish the changes of these compounds during the manufacture of sparkling wines. Taking into account that sparkling wines are usually produced with white grape varieties, the most of studies were performed on the quality and characteristics of these distinctive wines made from white varieties or even from a mixture of red and white varieties (Bosch-Fusté, Sartini, Flores-Rubio, Caixach, López-Tamames & Buxaderas, 2009; Jeandet, Chaudruc, Robillard, Peters, Tusseau, Conreux et al., 2006; Mazauric & Salmon, 2005, 2006; Pozo-Bayón, Hernández, Martín-Álvarez & Polo, 2003). There are very few studies on the sparkling wines manufactured only with red varieties for producing *rosé* (partially fermented with skins) and *blanc de noir* (fermented without skins) ones. So, two studies aimed at determining the phenolic composition of *rosé* sparkling wines (Pozo-Bayón, Hernández, Martín-Álvarez & Polo, 2003; Pozo-Bayón, Monagas, Polo & Gómez-Cordovés, 2004). In the first one, the authors observed that the most influential factors on the phenolic composition of sparkling wines are the grape variety used and the winemaking technique (Pozo-Bayón, Hernández, Martín-Álvarez & Polo, 2003). On the other hand, the second work showed quantitative changes in terms of anthocyanin and pyranoanthocyanin pigments and, therefore, chromatic characteristics are important parameters for evaluating the quality of the sparkling wines produced with red grape varieties (Pozo-Bayón, Monagas, Polo & Gómez-Cordovés, 2004).

The growing interest in the production of sparkling wines from red grape varieties has promoted studies for assessing the color stability during the ageing stage and for elucidating the pigments involved. Red wines may have a complex phenolic composition that dramatically changes during their shelf-life (Revilla & Ryan, 2000). On the other hand, dessert and sparkling wines are produced in limited geographical areas according to traditional winemaking methods, which can include the use of grapes with special features as raw material. Furthermore, the wine distinctive character may be supported by the use of autochthonous red grape varieties.

Despite the relatively scarce literature published with regards to the phenolic composition of red sweet sparkling wines, no study deals with the evolution of phenol indices and chromatic characteristics during the shelf-life of such products. Wineries need to optimize the storage conditions as changes in sensory properties occur, color being one of the first characteristics that consumers observe in a wine. Therefore, the main aim of this work was to monitor the changes occurring in the phenolic composition and chromatic characteristics during the winemaking and ageing processes of two types of red sweet sparkling wines produced from aromatic red grapes cv Brachetto (Di Stefano, Borsa, Maggiorotto & Corino, 1995). With 6.5 million of bottles produced for year and commercialized in all the world, the Brachetto d'Acqui DOCG wine is the most important red sweet sparkling wine in Italy. The preservation of the optimal chromatic characteristics during the shelf-life is the main problem of this type of dessert wine, more than the maintenance of aromatic compounds. Therefore, the effect of different storage conditions was also evaluated using central composite design (CCD) and response surface methodology (RSM) was then applied to optimize these conditions.

2. Materials and methods

2.1. Winemaking procedure

Red grapes of Brachetto cultivar (*Vitis vinifera* L.) were harvested in 2008 year from a vineyard sited in Castelbogione (AL) (Piedmont, North-West Italy), when technological maturity parameters of the grape respected the Brachetto d'Acqui DOCG Disciplinary of production and the phenols content reached was optimal for the variety (Rolle, Torchio, Zeppa & Gerbi, 2009). Grape berries were destemmed and crushed. The harvested mass (8000 kg) was distributed in a tank with a total volume of 10,000 L for the first fermentation phase. Total sulphur dioxide (2.5 g/hL) and pectinolytic enzyme (3.5 g/hL, Vinoxym[®] Vintage FCE, Novozymes, Switzerland) were added to the grape must, which was then inoculated with *Saccharomyces cerevisiae* dried active yeast (LSA) commercial yeast (20 g/hL). The maceration time was 2 days (48 h) and temperature was maintained at 16-17 °C. Two pumping-over without aeration for each day were performed using rotating irrigator. A volume of juice equal to one-third of total must volume was pumped over for each cycle. After this maceration/fermentation stage, the grape pomace was pressed by an industrial pneumatic

press (X-Pert 450, Bucher Vaslin, Italy) with a maximum pressure of 1.2 bar. Free-run juice and press juice were mixed (base wine, ethanol < 3.5 % v/v) and stored at 0 °C for 2 weeks.

The juice stored was divided in two equal portions which were introduced in distinctive autoclaves for a second fermentation (*prise de mousse*). Each juice portion was inoculated with yeasts (20 g/hL, Zymaflore VL1, Lafforte, France), B₁ vitamin (0.6 mg/L), ammonium phosphate (20 g/hL), and saccharose (34 g/L) only for the fully sparkling wine. As reported in Brachetto d'Acqui DOCG Disciplinary of production, two typologies of wine were elaborated: 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). 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. The resulting wines, after adjusting their free sulphur dioxide content to 30 mg/L, were filtered and isobaric filling was used during bottling. For the shelf-life study, the bottles were stored at temperatures comprised between 5 and 25 °C for one year in agreement with the CCD experimental matrix. For each CCD experiment, three bottles were analyzed.

2.2. Standard parameters

Reducing sugars, ethyl alcohol, pH, total acidity, volatile acidity and sulphur dioxide were determined according to International Organization of Vine and Wine (O.I.V.) methods (OIV, 2008). Organic acids (malic acid and tartaric acid) were quantified by HPLC (Thermo Electron Corporation, Waltham, MA, USA) using an UV detector (UV100) at 210 nm. The analyses were performed isocratically at 0.8 mL/min and 65 °C with a 300 x 7.8 mm i.d. cation exchange column (Aminex HPX-87H) and a Cation H⁺ Microguard cartridge (Bio-Rad Laboratories, Hercules, CA, USA). The mobile phase was 0.0065 mol/L sulphuric acid (Schneider, Gerbi & Redoglia, 1987).

2.3. Spectrophotometric parameters

Phenolic compounds in Brachetto wines were determined by spectrophotometric methods (Di Stefano, Cravero & Gentilini, 1989; Rigo, Vianello, Clementi, Rossetto, Scarpa, Vrhovsek & Mattivi, 2000) using an UV-1601PC spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD,

USA). Total anthocyanins (TA) and monomeric anthocyanins (MA) were expressed as malvidin-3-glucoside chloride while flavanols reactive to vanillin (flavanols vanillin assay, FVA) and total flavonoids (TF) were expressed as (+)-catechin. Proanthocyanidins (PRO) were determined after acid hydrolysis with warming (Bate-Smith reaction) using a ferrous salt (FeSO_4) as catalyst. They were expressed as cyanidin chloride. The relative standard deviations of phenolic compound determinations, based on repeated analyses ($n = 20$) of ten samples, were 1.14, 3.90, 2.80, 0.93 and 1.74 % for TA, MA, FVA, TF and PRO, respectively (Torchio, Cagnasso, Gerbi & Rolle, 2010).

The wine color was assessed by the Glories chromatic parameters, such as color intensity and tonality (Glories, 1984), and by the CIELab space (OIV, 2006). The spectrophotometric measurements were carried out using a 2 mm path length. Although the CIELab parameters are less used by oenologists, it is well known that they permit to define better the wine color and a greater differentiation (Pérez Magariño & González San José, 2006). The parameters that define the CIELab space are red/green color component (a^*), yellow/blue color component (b^*) and clarity (L^*), from which the parameters correlated with the color perception are obtained, like chroma (C^*) and hue angle (H^*). The CIELab color difference parameter (ΔE^*) was calculated as: $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{0.5}$ (OIV, 2006).

2.4. Shelf-life experimental design

Two factorial 2^2 CCDs were developed to optimize the ageing conditions (Granato, Favalli Branco & de Araújo Calado, 2010). Time (X_1 , days) and temperature (X_2 , °C) were chosen as the independent variables. The ageing time was evaluated up to one year, in general the maximum time employed by the winery in the turnover of the bottled wine of each vintage. The temperature selected varied between 5 °C (winery cold store) and 25 °C (maximum temperature in sale ambients).

The variables were codified such that their values ranged between +1.414 and -1.414, taking the zero value as central point. The variables were coded according to the following equation:

$$x_i = (X_i - \text{mean } X_i) / \Delta X_i$$

where x_i is the coded value of an independent variable, X_i is the real value of an independent variable, mean X_i is the real value of an independent variable at the central point and ΔX_i is the step change of X_i corresponding to a variation unit of the dimensionless value. Thus:

$$x_1 = (X_1 - 182)/129$$

$$x_2 = (X_2 - 15)/7$$

Table 1 shows the factorial design matrix, with variables in both coded and non-coded form, for a total of 13 experiments including five replicates of the central point. Mean values of triplicate determinations were analyzed to fit 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 second-degree polynomial equations were calculated with Statistica, data program version 7.0 (Statsoft Inc., Tulsa, OK, USA), and expressed as surface plots using RSM in order to visualize the relationship between the response and experimental values of each factor and to find the optimum conditions. A statistical analysis from Statistica 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 ($R^2 > 0.90$), indicating that at least 90 % of the variability in the response could be explained by the second-order model equations.

3. Results and discussion

The physico-chemical characteristics of the base wine are shown in Table 2. The standard parameters are those typically found in a base wine destined to the production of sweet sparkling wines. In fact, the manufacture of these wines requires a very short fermentation/maceration stage (24-48 h) because the base wine obtained after the first fermentation does not must exceed 3.5 % ethanol. As a consequence of the short maceration time and low alcohol content, the extraction of phenolic compounds from grape berries into the wine is usually reduced. Moreover, Brachetto grapes were characterized by an anthocyanin content lower than 600 mg/kg with a prevalence of cyanidin and peonidin derivative forms (Rolle, Torchio, Zeppa & Gerbi, 2009). The extraction kinetic of anthocyanins is particularly important for grape varieties rich in 3'-hydroxylated anthocyanins because these pigments are preferentially extracted during the initial phase of the maceration (Rolle, Torchio, Zeppa & Gerbi, 2009). They may be more easily oxidized by the polyphenols oxidase present in the juice with respect to those cultivars containing an anthocyanin profile made up mainly of

molecules tri-substituted in the B-ring and, therefore, more protected against oxidation (González-Neves, Gil & Barreiro, 2008). Some researchers have also demonstrated that the grape ripeness and berry skin hardness influence anthocyanin content in the final product (Rolle, Torchio, Zeppa & Gerbi, 2009). In fact, the anthocyanin extractability varied between 62.0 and 72.4 % in berry skins of Brachetto grapes which were macerated for 48 h in a model hydroalcoholic solution containing 3 % ethanol and classified in four groups according to two ripeness stages (184 and 250 g/L sugar) and two ranges for berry skin hardness (0.22-0.40 and 0.45-0.70 N) (Rolle, Torchio, Zeppa & Gerbi, 2009). This low extractability involves often unsatisfactory chromatic characteristics of the base wine (Rolle, Zeppa & Gerbi, 2006). Therefore, new winemaking techniques have been proposed in order to improve the anthocyanin extraction by employing a continuous pre-fermentative maceration system (Extract System ES30[®]) and using dry ice for low temperature maceration (Rolle, Zeppa & Gerbi, 2006).

As can be seen in Table 2, the amount of pigments extracted was low when the values of total anthocyanins and color intensity were compared with others previously published for red wines after alcoholic fermentation. Although the phenolic composition of red wines has been extensively studied after malolactic fermentation for several grape varieties, very few works have been performed on the composition of these ones after alcoholic fermentation. So, total anthocyanins varied between 701 and 772 mg/L and color intensity ranged 8.804 and 12.830 at the end of alcoholic fermentation, depending on the winemaking technique used for producing Mencía wines (Soto Vázquez, Río Segade & Orriols Fernández, 2010). Despite the higher value reported of total anthocyanins for monovarietal red wines elaborated from Monastrell grapes at the end of alcoholic fermentation (485 mg/L), the percentage of monomeric anthocyanins (70.6 %) was slightly lower than that obtained for Brachetto wines (Lorenzo, Pardo, Zalacain, Alonso & Salinas, 2005). In this case, color intensity was 13.88. Nevertheless, total anthocyanins varied between 22.4 and 31.5 mg/L in *rosé* wines made from Monastrell variety which corresponded to color intensity ranged 0.63 and 0.80 (Salinas, Garijo, Pardo, Zalacain & Alonso, 2003). On the other hand, lower values of color intensity in the base wine were obtained for Garnacha variety (0.07) (Pozo-Bayón, Monagas, Polo & Gómez-Cordovés, 2004) in relation with Brachetto grapes.

The second alcoholic fermentation (*prise de mousse*) was stopped at the relative ethyl alcohol content planned, as reported in Brachetto d'Acqui DOCG Disciplinary of production, and the wines were filtered and bottled. Standard and spectrophotometric parameters were determined in the two types of products obtained just after bottling (Table 2). The resulting wines were sweet with a residual sugar concentration of 120 and 128 g/L, and an ethanol content of 5.13 and 6.54 % v/v in lightly and fully sparkling wines, respectively. Volatile acidity concentrations were less than 0.3 g/L

showing a good preservation state. Similar free sulphur dioxide concentrations allowed establishing comparisons between the color parameters corresponding to both lightly and fully sparkling wines. The second fermentation methodology seems to affect the phenolic composition and chromatic characteristics of sweet sparkling wines. Total anthocyanins, monomeric anthocyanins and total flavonoids already present in the base wine underwent quantitative changes, which resulted in a pigment content decrease, accompanied by both a color intensity decrease and a tonality increase. The strongest changes corresponded to anthocyanins and color intensity. So, the content of total anthocyanins and monomeric anthocyanins decreased 36 and 53 %, respectively, after the second fermentation. It involved a marked decrease in color intensity (18.4-23.8 %) but a slight increase in tonality (10.5-9.3 %) when the second fermentation took place. Nevertheless, phenolic compounds and chromatic characteristics of lightly sparkling wines did not differ significantly from those ones of fully sparkling wines and, therefore, the chromatic quality at the end of the second fermentation is independent on the winemaking methodology used to produce sweet sparkling wines from Brachetto variety. Two exceptions were only found, color intensity being higher for fully sparkling wines and, therefore, lightness being significantly lower for the ones.

Color intensity values fall within the range reported by Ribéreau-Gayon et al. (Ribéreau-Gayon, Glories, Maujean & Dubourdiou, 2003), which varies from 3 to 18 depending on the wine type and the variety. Regarding tonality, Ribéreau-Gayon et al. (Ribéreau-Gayon, Glories, Maujean & Dubourdiou, 2003) published that the normal values for a young red wine are comprised between 0.5 and 0.7 but they increase during the wine ageing up to 1.3. Therefore, all the values determined of color intensity and tonality are in the normal range.

A high proanthocyanidin concentration was obtained for Brachetto wines in relation with other results previously published of 107 mg/L for *rosé* sparkling wines manufactured from Garnacha variety (Pozo-Bayón, Monagas, Polo & Gómez-Cordovés, 2004). Taking into account that skin tannins are easily extracted during fermentation (Monagas, Gómez-Cordovés, Bartolomé, Laureano & Ricardo da Silva, 2003; Sun, Pinto, Leandro, Ricardo da Silva & Spranger, 1999), it is expected that proanthocyanidins are mostly of skin origin as consequence of the short maceration stage. In fact, Brachetto skins are rich in these compounds (Di Stefano, Borsa, Maggiorotto & Corino, 1995). This can be supported by FVA/PRO ratios less than 0.8 because they are in accordance with the few terminal units of skin tannins (high polymerization degree) (Vidal, Damien, Souquet, Fulcrand & Cheynier, 2002).

On the other hand, significant changes in the phenolic composition and chromatic characteristics were found through the wine ageing. Therefore, it is important to assess the shelf-life

of both lightly and fully sparkling wines. In fact, the effect of two variables (time and temperature) was evaluated on anthocyanins, flavonoids, color intensity, tonality, CIELab and ΔE^* parameters. Table 3 shows the second-order polynomial models obtained for the different parameters studied.

Time and temperature of the ageing stage had only linear effects on total anthocyanin concentration and they did not show any interaction effect. Fig. 1_[1.1-1.2] represents the effect of the independent variables on total anthocyanins. Their content decreased mainly with the increase in time, this effect being attenuated or even inversed in the latest storing days particularly when the lower temperatures were used. Only for the longer times, the increase in temperature caused also a decrease in the predicted response. The effect of the changes in both time and temperature was more accused in fully sparkling wines than in lightly sparkling wines.

Despite many differences found, the tendency observed for monomeric anthocyanins (Fig. 1_[1.3-1.4]) and total flavonoids (Fig. 1_[1.5-1.6]) was very similar than that one corresponding to total anthocyanins. Furthermore, the effect of the independent variables on the response was more accused for total flavonoids, particularly in fully sparkling wines.

The increase observed in flavonoids and anthocyanins when Brachetto wines were stored during the longer times, particularly at lower temperatures, could be justified by a partial depolymerization of anthocyanin-tannin and/or other anthocyanin-condensed products, liberating anthocyanins (Pozo-Bayón, Monagas, Polo & Gómez-Cordovés, 2004). During the wine ageing in bottle, an increase in the formation of polymeric red pigments can be suggested taking into account the already observed losses of monomeric anthocyanins.

Fig. 2_[2.1-2.2] shows different changes in color intensity depending on the wine type. This color parameter had a similar tendency than that one associated with anthocyanins and flavonoids for lightly sparkling wines in relation with the ageing time and temperature. In fully sparkling wines, the effect of the storing temperature was practically negligible whereas a clear decreasing tendency was observed when the ageing time was increased in any case (Fig. 2_[2.2]). On the other hand, Fig. 2_[2.3-2.4] represents the relationship between tonality, time and temperature which is illustrated by a three-dimensional representation of the response surface. The higher values of tonality corresponded to the longer times in lightly and fully sparkling wines, excepting for the lower temperatures for which the higher values of tonality were associated with the shorter times. Moreover, it can be observed that greater tonality was obtained for the higher temperatures but the inverse tendency was seen for the shorter times. This behavior of tonality in the first periods of sparkling wine storage is probably explained by the presence of free sulphur dioxide. In fact, it is well-known that anthocyanins are readily bleached by this additive with reversible formation of colorless addition

products (Di Stefano & Cravero, 1989; Picinelli, Bakker & Bridle, 1994). The independent variables had only linear effects on color intensity and tonality and they did not show any interaction effect. In addition, these linear effects were stronger in lightly sparkling wines than in fully sparkling wines.

Regarding CIELab parameters, many differences were found between both lightly and fully sparkling wines. Fig. 3_[3.1-3.2] shows that the wine clarity (L^*) increased particularly with the ageing time but from 175 and 250 days onward non-significant differences were observed for lightly and fully sparkling wines, respectively. Moreover, the effect of temperature was not significant for the first ones whereas an increase in the wine darkness was observed when the ageing temperature increased in fully sparkling wines for the longer times. A similar tendency was reported for the yellow/blue color component (b^*). In this case, the effect of temperature was practically negligible for the two wine types. In spite of the yellow/blue color component increased with the ageing time, non-significant differences were observed between 125 and 250 days for both lightly and fully sparkling wines (Fig. 3_[3.5-3.6]). However, this color component decreased with the increase in the ageing time from 250 days onward, particularly in lightly sparkling wines.

The response model for red/green color component (a^*) (Fig. 3_[3.3-3.4]) is very related with that one corresponding to chroma (C^*) (Fig. 3_[3.7-3.8]). As occurred for other CIELab parameters, the most important effect on the predicted response resulted to be the ageing time. Moreover, the increase in the two CIELab parameters with the ageing time was stronger at low temperatures. The behavior of red/green color component and chroma depended also on the ageing temperature. So, at the longer times this color component and chroma decreased when the temperature increased. Nevertheless, it was found an inverse tendency at the shorter times which was again inverted for red/green color component at the higher temperatures.

As can be seen in Fig. 3_[3.9-3.10], the maximum values of hue (H^*) were obtained at the longer times and higher temperatures for lightly sparkling wines. Nevertheless, these were obtained at intermediate times for fully sparkling wines and, in these conditions, the greatest hue was also associated with the higher temperatures.

Time and temperature of the ageing stage had only linear effects on the CIELab parameters and the independent variables did not show any interaction effect. When both lightly and fully sparkling wines were submitted to the same storing conditions, it was found that fully sparkling wines showed higher color intensity and, therefore, higher darkness. Furthermore, higher contribution of red and yellow color components, brightness and hue was usually observed. The higher anthocyanin content in the last wines may be responsible for the higher contribution of the red color component. The higher hue values indicate the contribution of other pigments different

from anthocyanins which usually involve the possible pyranoanthocyanin formation (Álvarez, Aleixandre, García & Lizama, 2006). It results in red-orange hues typical of *rosé* wines.

Regarding ΔE^* parameter (Fig. 4_[4.1-4.2]), in the scientific literature it was reported that the perceptibility threshold of CIELab colorimetric differences is approximately $\Delta E^* = 0.8-1$ while values less than 10 were designated for 'small' differences (Gonnet, 2001). Although the results of extensive visual matching experiences have revealed that the perceptibility of colorimetric differences by the human visual system continuously changes with the direction (lightness, chroma or hue) and the amplitude of those differences (Gonnet, 2001), on the basis of the knowledge acquired it is possible to assure a significant change in color for both lightly and fully sparkling wines from 6 months of storage. The behavior of this index was more related to the time variable than the temperature one.

Finally, Table 4 reports the results obtained for both lightly and fully sparkling wines after storing 365 days at 15 °C and it can be observed that the highest concentration of anthocyanins and flavonoids was found in fully sparkling wines. Regarding chromatic characteristics, they were higher in fully sparkling wines, excepting clarity and tonality. The decrease experimented in color intensity after ageing (22.6-26.3 %) resulted to be similar than that reported for sparkling wines elaborated from Garnacha (28.6 %) (Pozo-Bayón, Monagas, Polo & Gómez-Cordovés, 2004), Trepát (27.0-33.3 %) (Girbau-Solà, López-Barajas, López-Tamames & Buxaderas, 2002) or Monastrell (0-41.8 %) (Girbau-Solà, López-Barajas, López-Tamames & Buxaderas, 2002) grapes, in the same period but in contact with yeast lees.

4. Conclusions

The present research permitted to characterize for the first time sweet lightly and fully sparkling wines elaborated from Brachetto grapes in basis of the phenolic composition and chromatic characteristics. Despite the importance and diffusion of Brachetto wines, literature on their phenolic content and chromatic properties is rather defective. Moreover, a better understanding of the evolution of key parameters in the color stability during the winemaking and ageing would help to control the wine quality and, therefore, may have an impact on the wine technological processes. In this sense, the phenolic composition and chromatic characteristics of lightly and fully sparkling wines were stable enough during the storing time of one year only at low temperatures. The time variable influenced strongly total

anthocyanins, monomeric anthocyanins, total flavonoids, color intensity, tonality and CIELab parameters. Among both wine types, the results obtained showed that fully sparkling wines were more stable than lightly ones and, therefore, the shelf-life of the former ones could be longer. Moreover, the knowledge of the relationship between phenol indices and chromatic characteristics of a base wine and of the resulting sparkling red wines is of interest to winemakers as it provides useful information for improving the final product. However, for better understanding the shelf-life of these two typologies of Brachetto sparkling wines, further studies are necessary to know the evolution of aromatic compounds and to correlate all these changes with consumer judgments.

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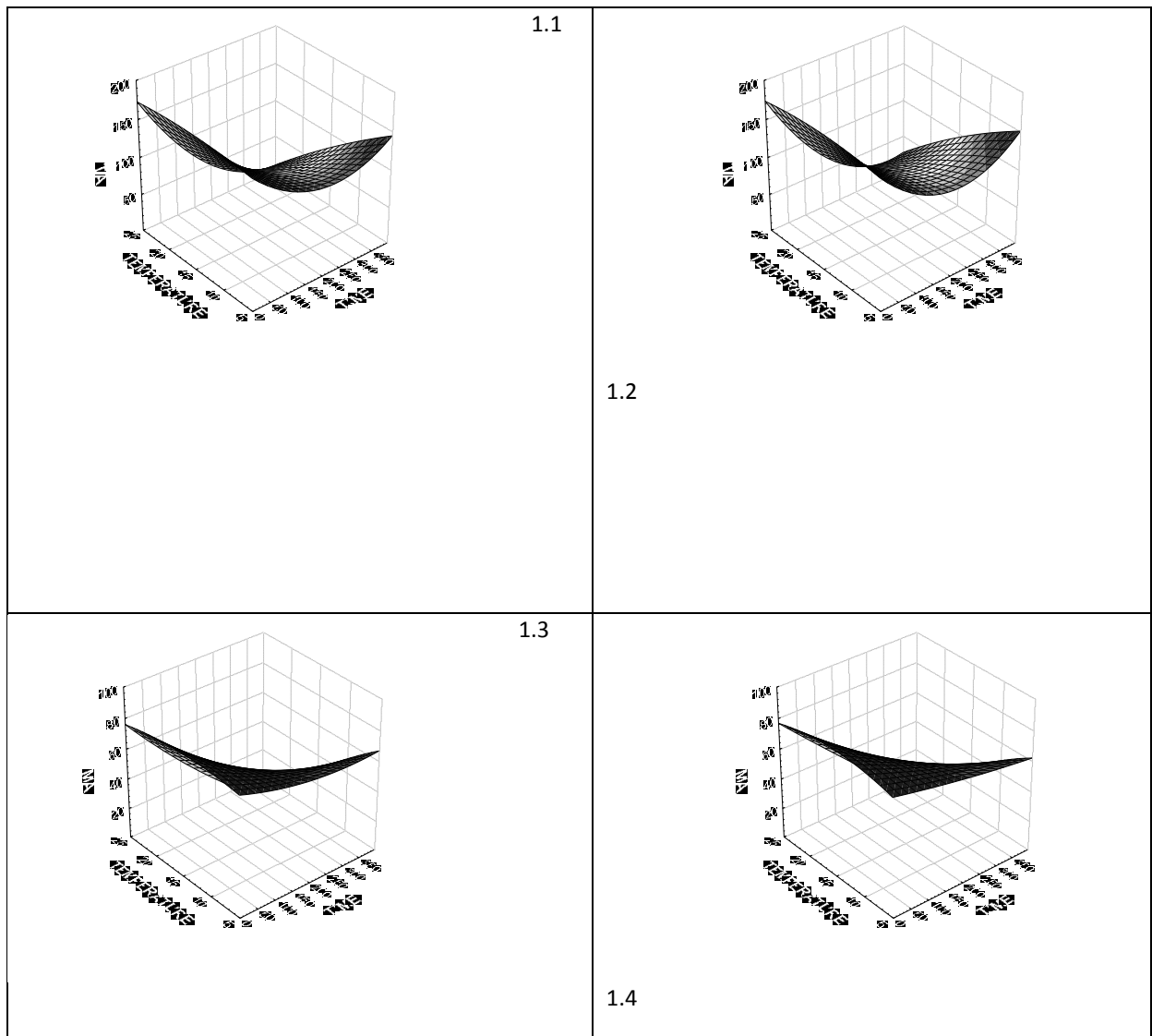
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Fig. 1. Response surface plot showing the effect of time and temperature of ageing on total anthocyanins ($TA_{[1.1-1.2]}$, mg/L malvidin-3-glucoside chloride), monomeric anthocyanins ($MA_{[1.3-1.4]}$, mg/L malvidin-3-glucoside chloride) and total flavonoids ($TF_{[1.5-1.6]}$, mg/L (+)-catechin) in sweet lightly (left) and fully (right) sparkling wines.



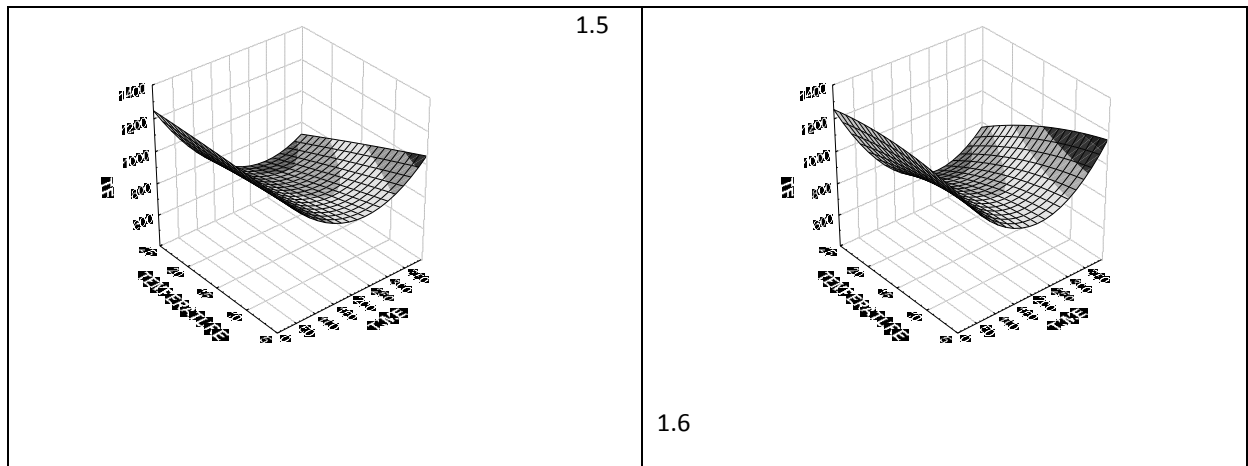


Fig. 2. Response surface plot showing the effect of time and temperature of ageing on color intensity ($CI_{[2.1-2.2]}$) and tonality ($T_{[2.3-2.4]}$) in sweet lightly (left) and fully (right) sparkling wines.

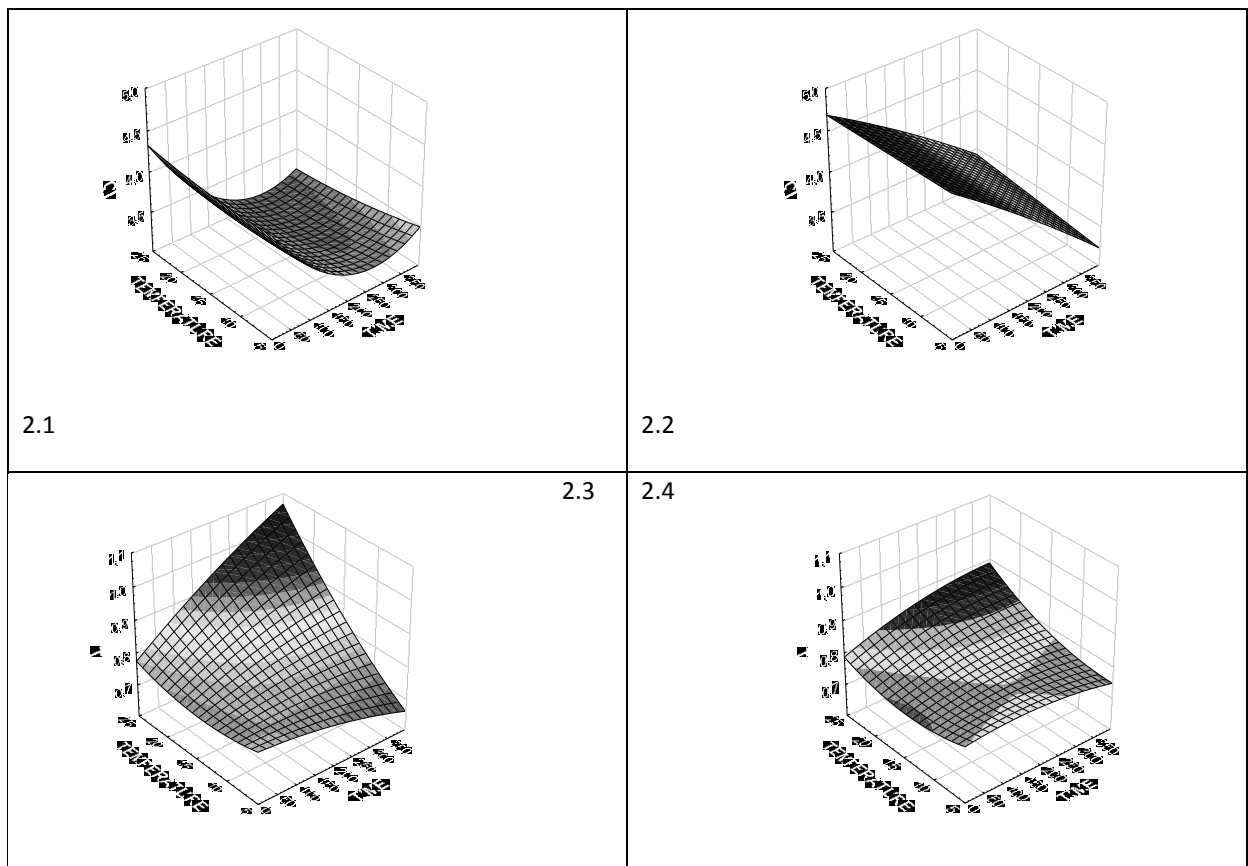
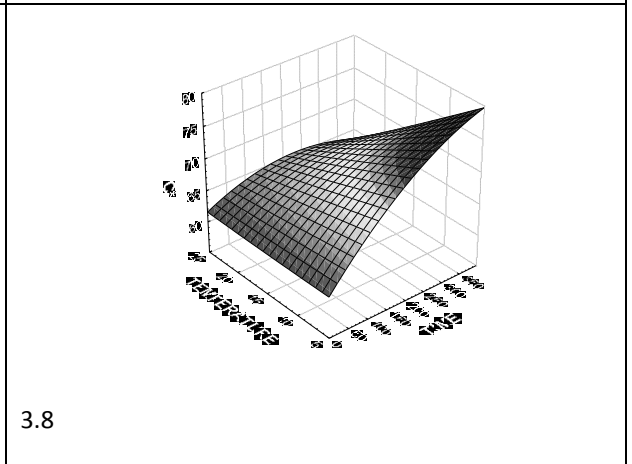
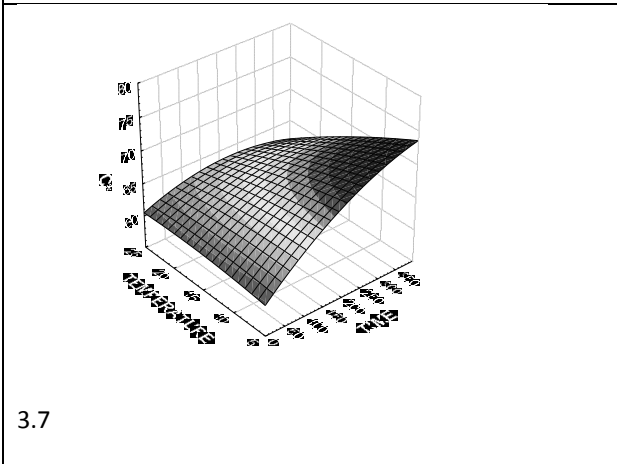
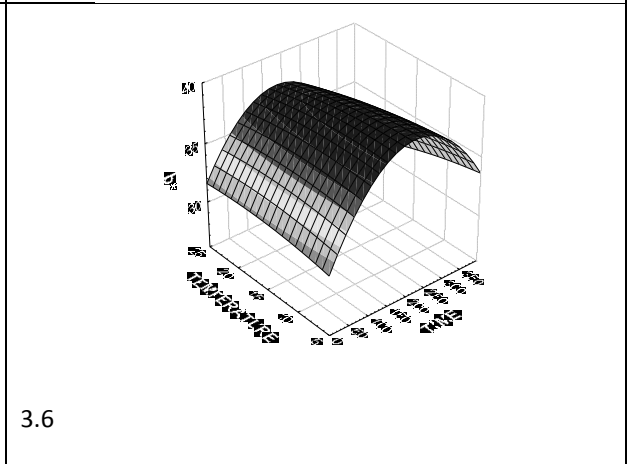
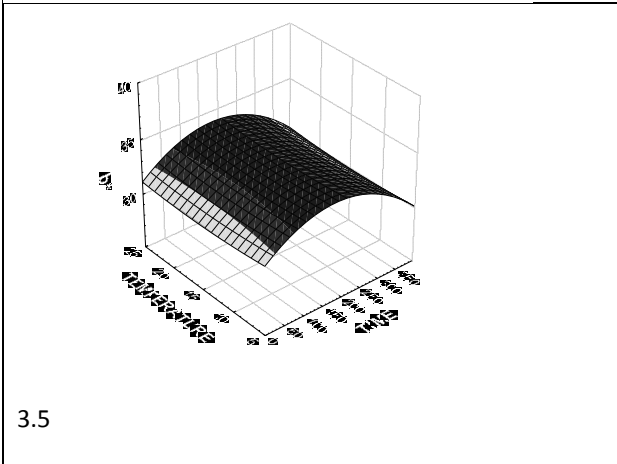
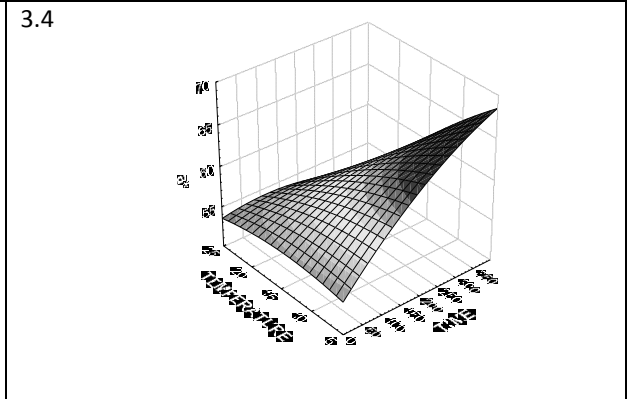
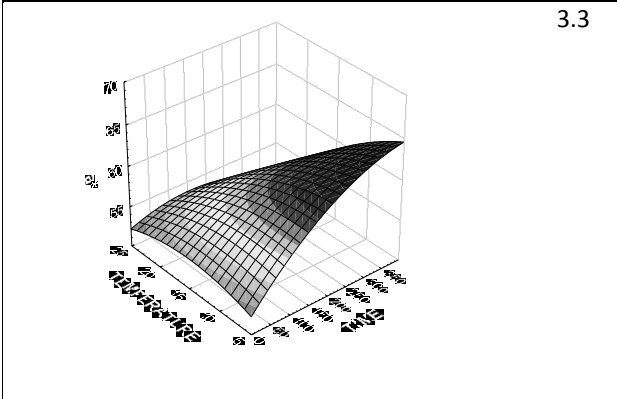
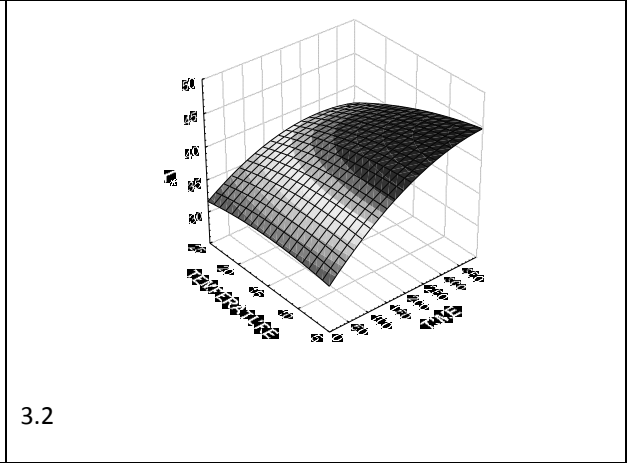
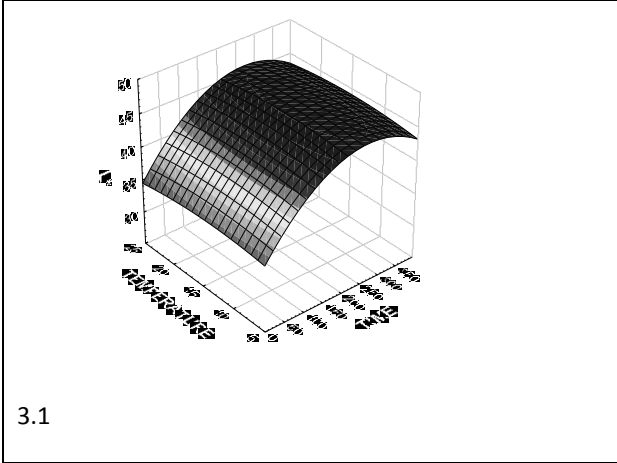


Fig. 3. Response surface plot showing the effect of time and temperature of ageing on clarity ($L^*_{[3.1-3.2]}$), red/green color component ($a^*_{[3.3-3.4]}$), yellow/blue color component ($b^*_{[3.5-3.6]}$), chroma ($C^*_{[3.7-3.8]}$) and hue angle ($H^*_{[3.9-3.10]}$) in sweet lightly (left) and fully (right) sparkling wines.



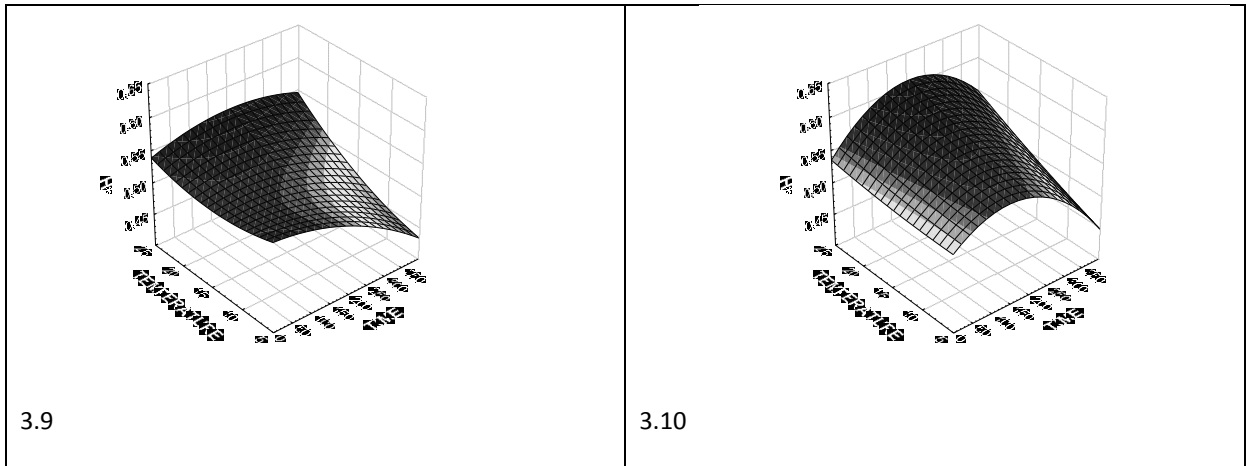


Fig. 4. Response surface plot showing the effect of time and temperature of ageing on ΔE^* parameter calculated with respect to the wine color after bottling (start point of shelf-life study) in sweet lightly (left) and fully (right) sparkling wines.

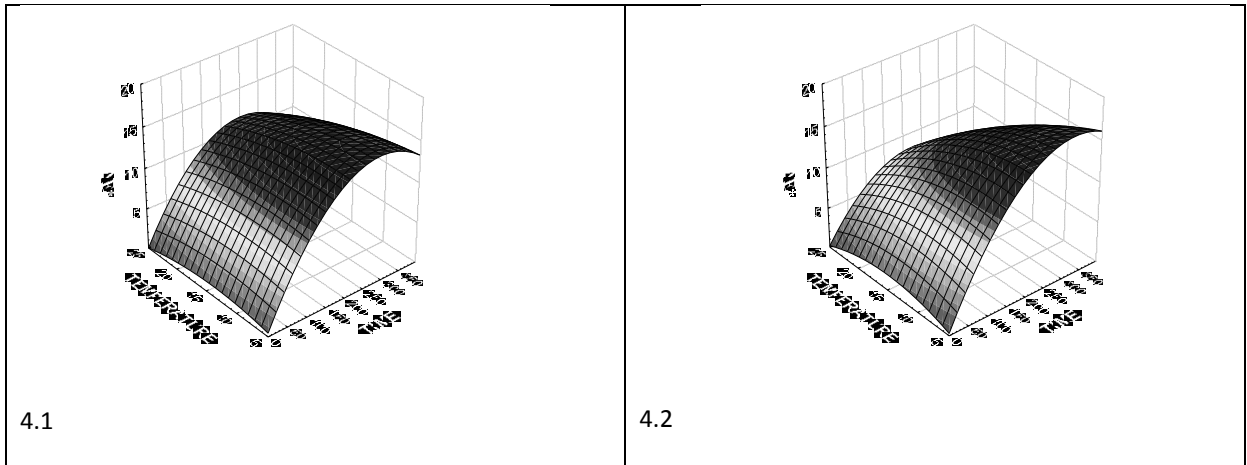


Table 1. Experimental matrix of CCD.

Experiment	Ageing time (coded value) (x_1)	Ageing temperature (coded value) (x_2)	Ageing time (real value) (X_1 , days)	Ageing temperature (real value) (X_2 , °C)
1	-1.4141	0	0	15
2	-1	-1	53	8
3	-1	+1	53	22
4	0	-1.4141	182	5
5	0	+1.4141	182	25
6	0	0	182	15
7	0	0	182	15
8	0	0	182	15
9	0	0	182	15
10	0	0	182	15
11	+1	-1	312	8
12	+1	+1	312	22
13	+1.4141	0	365	15

Table 2. Physico-chemical characteristics of the base wine and both sweet lightly and fully sparkling wines after bottling, start point of shelf-life study.

Parameter	Base wine	Sweet lightly sparkling wine (bottle pressure <1.7 bar)	Sweet fully sparkling wine (bottle pressure >3.0 bar)
Residual sugars (g/L)	145	120±7	128±6
Alcoholic degree (% v/v ethanol)	3.40	5.13±0.06	6.54±0.04
Total acidity (g/L tartaric acid)	6.67	5.06±0.8	5.03±0.9
Volatile acidity (g/L acetic acid)	0.05	0.13±0.02	0.27±0.04
pH	3.40	3.19±0.02	3.20±0.01
Tartaric acid (g/L)	3.5	1.7±0.1	1.7±0.1
Malic acid (g/L)	2.6	2.6±0.2	2.5±0.1
Free sulphur dioxide (mg/L)	nr	31±5	28±5
Total sulphur dioxide (mg/L)	24	158±8	143±10
TA (mg/L malvidin-3-glucoside chloride)	286	183±3	182±4
MA (mg/L malvidin-3-glucoside chloride)	221	104±4	103±2
FVA (mg/L (+)-catechin)	nd	644±34	652±15
TF (mg/L (+)-catechin)	1619	1355±41	1340±24
PRO (mg/L cyanidin chloride)	nd	829±56	866±31
Color intensity (PO 10 mm)	5.64	4.30±0.13	4.60±0.17
Tonality	0.68	0.76±0.02	0.75±0.02
L*	nd	34.2±0.7	31.3±0.8
a*	nd	52.66±0.57	53.24±0.83
b*	nd	31.35±0.34	31.05±0.74
C*	nd	61.28±0.66	61.63±1.09
H*	nd	0.54±0.01	0.53±0.01

L*: clarity; a*: red/green color component; b*: yellow/blue color component; C*: chroma; H*: hue angle; nr: not relievable, nd : not determinate.

Table 3. Second-order polynomial model by central composite design (CCD).

Parameter	Wine type	Equation
Total anthocyanins	L	$Y = 165.6011 - 0.3893X_1 + 1.5676X_2 + 0.0010X_1^2 - 0.0172X_1X_2 - 0.0515X_2^2$
	F	$Y = 163.6117 - 0.4540X_1 + 2.1255X_2 + 0.0012X_1^2 - 0.0157X_1X_2 - 0.0692X_2^2$
Monomeric anthocyanins	L	$Y = 75.6341 - 0.0373X_1 + 1.0373X_2 + 0.0001X_1^2 - 0.0097X_1X_2 - 0.0409X_2^2$
	F	$Y = 71.2994 + 1.7393X_2 - 0.0090X_1X_2 - 0.0607X_2^2$
Total flavonoids	L	$Y = 1220.1238 - 2.9880X_1 + 2.2766X_2 + 0.0069X_1^2 - 0.0442X_1X_2$
	F	$Y = 1194.7988 - 3.6052X_1 + 12.9461X_2 + 0.0090X_1^2 - 0.0492X_1X_2 - 0.4214X_2^2$
Color intensity	L	$Y = 4.2966 - 0.0071X_1 + 1.3548E-5X_1^2$
	F	$Y = 4.7535 - 0.0035X_1$
Tonality	L	$Y = 0.7954 - 0.0003X_1 - 0.0086X_2 + 4.9356E-5X_1X_2 + 0.0003X_2^2$
	F	$Y = 0.8162 + 7.3120E-5X_1 - 0.0103X_2 + 1.6084E-5X_1X_2 + 0.0004X_2^2$
Clarity (L*)	L	$Y = 34.2133 + 0.0809X_1 + 0.2023X_2 - 0.0002X_1^2 - 0.0077X_2^2$
	F	$Y = 30.9367 + 0.0655X_1 + 0.2624X_2 - 7.3118E-5X_1^2 - 0.0009X_1X_2 - 0.0094X_2^2$
Red/green (a*)	L	$Y = 50.2920 + 0.0643X_1 + 0.4647X_2 - 6.1386E-5X_1^2 - 0.0018X_1X_2 - 0.0156X_2^2$
	F	$Y = 52.9750 + 0.0645X_1 + 0.2539X_2 - 4.3912E-5X_1^2 - 0.0022X_1X_2 - 0.0093X_2^2$
Yellow/blue (b*)	L	$Y = 31.7410 + 0.0289X_1 - 0.1004X_2 - 8.0444E-5X_1^2$
	F	$Y = 30.4499 + 0.0743X_1 + 0.1466X_2 - 0.0002X_1^2$
Chroma (C*)	L	$Y = 58.9718 + 0.0643X_1 + 0.1711X_2 - 5.5315E-5X_1^2 - 0.0015X_1X_2$
	F	$Y = 61.5898 + 0.0836X_1 - 6.9311E-5X_1^2 - 0.0021X_1X_2$
Hue (H*)	L	$Y = 0.5588 - 0.0001X_1 - 0.0050X_2 - 5.4997E-7X_1^2 + 1.3887E-5X_1X_2 + 0.0002X_2^2$
	F	$Y = 0.5214 - 0.0004X_2 - 1.6665E-6X_1^2 + 1.0643E-5X_1X_2$
Color difference (ΔE^*)	L	$Y = -0.6669 + 0.1056X_1 + 0.2775X_2 - 0.0002X_1^2 - 0.0006X_1X_2 - 0.0100X_2^2$
	F	$Y = -1.8469 + 0.1094X_1 + 0.5149X_2 - 0.0002X_1^2 - 0.0016X_1X_2 - 0.0173X_2^2$

L: sweet lightly sparkling wine; F: sweet fully sparkling wine; X_1 : time (days); X_2 : temperature ($^{\circ}\text{C}$).

Table 4. Physico-chemical characteristics of both sweet lightly and fully sparkling wines after storing 365 days at 15 °C.

Parameter	Sweet lightly sparkling wine	Sweet fully sparkling wine
	(bottle pressure <1.7 bar)	(bottle pressure >3.0 bar)
TA (mg/L malvidin-3-glucoside chloride)	74.3±0.8	81.9±6.8
MA (mg/L malvidin-3-glucoside chloride)	39.6±1.1	42.1±8.5
TF (mg/L (+)-catechin)	780±17	843±15
Color intensity (PO 10 mm)	3.17±0.02	3.56±0.39
Tonality	0.81±0.01	0.78±0.04
L*	45.6±0.20	42.7±3.37
a*	59.19±0.10	60.68±1.78
b*	30.81±0.14	35.00±2.05
C*	66.73±0.15	70.05±2.57
H*	0.48±0.01	0.52±0.01

L*: clarity; a*: red/green color component; b*: yellow/blue color component; C*: chroma; H*: hue angle.