Full title: A kinetic approach to describe the time evolution of red wineas a function of packaging conditions adopted: influence of closure and storage position.

Short title: Wine shelf-life: influence of closure and storage position.

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Abstract

During bottle storage the barrier against the external atmosphere is provided by the closure.

In order to evaluate the influence of capsule and storage position in preserving the quality of the wine, this research project aimed to study the chemical and sensorial evolution of a red wine stored over a period of 12 months in glass bottles closed with natural cork stoppers with or without capsule and maintained in different storage position (horizontal vs vertical).

The different storage conditions adopted deeply affected wine quality suggesting that their rational optimization could allow the maintenance of red wine quality during storage.

In particular, wine degradation rate was higher when the wine was stored in glass bottles closed with a natural cork without the capsule, regardless of the position of storage. On the contrary wine decay was significantly delayed when it was stored in the horizontal position, independently from the closure system used.

Key words

Red wine shelf-life; storage position; capsule; chemical characterization; sensorial characterization.

1. Introduction

Bottle aging is a necessary stage in the wine-making process because it helps to modify various organoleptic properties to obtain high quality wines (Gao *et al.*, 2015). During red wine storage, spontaneous clearing, colour stabilization and reactions that lead to the formation of more complex compounds have been observed (Del Alamo-Sanza & Nevares Dominguez, 2006; Marquez, Serratosa & Merida, 2014). As the storage time in bottle increases, reactions of copigmentation and polymerization of anthocyanins take place (Eiro & Henionen, 2002), causing the formation of more stable compounds responsible for the change from the bluish-red hues of young wines to the orange-red onescharacteristic of aged wines (Atanasova, Fulcrand, Cheyner & Moutonet, 2002.). As oxygen is one of the main factors affecting wine evolution as well as its deterioration (Moutonet & Vidal, 2006; Silva & Lambri, 2006; Kwiatkowski, Skouroumounis, Lattey & Waters, 2007;Ghidossi, Poupot, Thibon & Mietton-Peuchot, 2012; Dombre, Rigou, Wirth & Chalier,2015), changes occurring after fermentation are partly driven by chemical oxidations deriving from wine-making and storage (Wirth *et al*., 2012).

Nowadays, glass containers are still preferred for bottling wine (Ghidossi *et al*., 2012) being them readily recyclable and characterized by a high impermeability to gases and vapours, stability over time and transparency (Mentana, Pati, La Notte & Del Nobile, 2009). During storage the only barrier against the external atmosphere is represented by the closure system, and the evolution of phenolic compounds on the development of wine colour and mouthfeel mainly depends on the transfer of oxygen through the bottle stopper (Silva, Lambri & De Faveri, 2003; Gao *et al.*, 2015). In this condition, oxygen diffusion into the bottled wine appears strongly dependent on the effective sealing of the closure (Skouroumounis *et al*., 2005; Venturi *et al*., 2016a). Indeed, oxygen permeability may greatly change from cork to cork, and this heterogeneity is one of the main factors affecting variation among bottles (Wirth *et al*., 2010).

Although some authors examined the changes occurring during red wine stabilization in bottle (Perez-Magarino & Gonzales-San Jose, 2004; Del Alamo-Sanza & Nevares Dominguez, 2006; Vincenzi, Marangon, Tolin & Curioni, 2011; Sàenz-Navajas, Avizcuri, Ferreira & Fernandez-Zurbano, 2014; Marquez, Serratosa & Merida, 2014), most of the studies carried out so far analyzed only the effect of different types of stoppers on the chemical composition, colour and flavour of bottled wines (Gao *et al*., 2015 and ref. within). Little is known about the influence of the capsule, combined with a natural cork stopper, on the evolution of the red wine during bottle aging. Moreover, at the best of our knowledge, no study has been focused till now on the effect of storage position (horizontal vs vertical) on the evolution of red wines maintained in glass bottles closed with natural cork stoppers.

In this research project both chemical and sensorial evolution of a not structured red wine, stored in glass bottles closed with natural cork stoppers with or without capsule, were evaluated over a period of 12 months with the aim to determine the influence of the capsule and the storage position (horizontal vs vertical) in preserving the quality of wine.

2. Materials and methods

2.1.Experimental setup

The red wine was analyzed before the beginning of the experiment and its chemical parameters are reported in Table 1.

The red wine, collected from one single vat, was stored in glass bottles (0.750 L) at the same time in a commercial winery bottling line using a fully automated bottling/filling station. After the washing process, the bottles passed automatically to the filling machine and then to the corking. In order to eliminate the presence of oxygen in the bottles, before capping the air in the headspace was replaced with an inert gas. In particular we used N_2, the chemical inertia of which makes it particularly suitable in fields where the high reactivity of oxygen causes unwanted actions.

All the bottles were closed with the same natural cork stopper. This 100% natural product was extracted from a single cork strip and perfected using the cutting-edge technology. As reported in Figure 1, the bottles were divided equally in two groups: one group was closed with a natural cork (one-piece cylindrical cork stoppers of natural origin deriving from the bark of the cork-oak tree (*Quercus suber*) by punching) and aluminium capsule, while the other was closed with cork but without the capsule.

Bottles were transported by an air-conditioned truck (T=20±1°C) from the bottling/filling facility located at Ruvo di Puglia (BA) to the Food Technology Laboratory of the Department of Agriculture, Food and Environment (DAFE) of the University of Pisa after one day from bottling/packaging. Sampling and analyses were performed at the DAFE. Sampling of wine was carried out after 3 days and 3, 6, 9 and 12 months of storage. Throughout the observation period samples were stored under controlled temperature (T = 20±1°C). Each group was further divided (Figure 1) in order to verify the influence of the storage position (vertical vs horizontal) on the time evolution of wine.

2.2.Chemical evolution

All chemical determinations for the characterization of the starting wine were performed at the laboratory of Food Technology of DAFE (University of Pisa) according to the official methods proposed by the International Organization of Vine and Wine (OIV), and described in the Compendium of International Methods of Wine and Must Analysis (OIV, 2014).

2.2.1 Total SO_2 (TSO_2). Time evolution of total SO_2 concentration (TSO_2) was determined by the Ripper titrimetric method (Zoecklein, Fugelsang, Gump & Nuri, 1999).

This method is based on the redox reaction in which sulfur dioxide, in the form of the bisulphite ion, reacts with iodine as follows:

$$HSO_3^- + I_3^- + H_2O \rightarrow SO_4^{2-} + 3H^+ + 3I^- \quad (1)$$

Unreacted iodine forms a blue complex with starch indicator to signify the endpoint. The addition of sodium bicarbonate prior to starting the titration creates an inert blanket of carbon dioxide gas which prevents interference caused by oxygen in air. Red wines may require decolorizing with activated carbon prior to performing the titration to ensure that the endpoint color change is observable. When determining total sulfur dioxide, the sample is pretreated with sodium hydroxide solution in order to adjust the pH. This causes chemically bound forms of sulfur dioxide to be released in solution as free sulfur dioxide. It is essential to titrate at the endpoint when the blue color persists for 30 seconds to ensure that all forms of sulfur dioxide in solution have reacted.

2.2.2. Total Anthocyanins (TAnt). As described in a previous paper (Zinnai, Venturi & Andrich, 2011) time evolution of total anthocyanins concentration (TAnt) was determined by measuring the absorbance at 540 nm after dilution of the sample with a solution composed by ethanol/water/HCl (70/30/1 by vol.). The concentration of anthocyanins was calculated (as gL^{-1} of malvin equivalent) using the equation:

$$[\text{TAnt}] = \frac{\boldsymbol{Abs(540nm)}}{\boldsymbol{\varepsilon}} \cdot \boldsymbol{dil.} \cdot \mathbf{332} \qquad (2)$$

where $Abs_{(540nm)}$ = Absorbance at 540nm; ε = molar attenuation coefficient, according to Beer's law;dil. = dilution of wine sample, generally ranging from 1:20 to 1:50 (v/v); 332 = molecular weight of malvidin.

2.3 Mathematical model

As reported in previous papers (Zinnai, Venturi, Quartacci & Andrich, 2011; Venturi *et al*., 2015), the identification of the best values to be assigned to the model parameters was carried out by the specific statistical program BURENL (Buzzi Ferraris & Manca, 1996).

This program identifies, in a space of j-dimensions (where j represents the number of equation parameters), the minimum value of F function which is obtained from the sum of the squares of differences occurring between calculated $\left(\boldsymbol{R^*_{calc.,i}}\right)$ and experimental $\left(\boldsymbol{R^*_{exper.,i}}\right)$ values:

$$\text{F} = \sum_{\text{i}=1}^{\boldsymbol{N}} \left(\boldsymbol{R^*_{calc.,i}} - \boldsymbol{R^*_{exper.,i}}\right)^{\mathbf{2}} \qquad (3)$$

where i = i-calculated/experimental value, and N = total number of experimental values.

2.4 Sensorial evaluation

The sensorial profiles of wines as a function of the storage conditions were evaluated by a trained panel included in the "expert panel" of the DAFE; all assessors had previous experience in sensory descriptive analysis, mainly in wine evaluation. According to the DAFE internal procedure for assessor selection and training (Venturi, Zinnai, Fantoni, Gabelloni & Razionale, 2013; Venturi *et al*., 2014; Venturi *et al*., 2016b) based on a normalized technical procedure reported in the literature (Pérez Elortondo *et al*., 2007), expert assessors are requested to repeat and pass re-qualification tests at least once a year in order to verifythat they are still able of evaluating the samples.

The assessors were provided with a specifically developed sensorial sheet consisting of a not structured, parametric, descriptive wine scoring chart. The panellists ranked the wine stored in each closing method on a scale from 0 to 10, made comments on the quality, and evaluated the intensity of each parameter, including visual, aroma, and taste attributes, as well as an hedonic parameter such as the Overall Appreciation (Martin & Rasmussen, 2011). Tasting was carried out in the morning, in a well-ventilated quiet room and in a relaxed atmosphere. The wines were then presented to assessors at the same time; a randomized serving order was proposed. All assessments were repeated in duplicate in two different days by the same group of panellists.

2.5 Statistical analysis

In order to evaluate the statistical significance of the data regarding the chemical characterization, determinations were carried out on samples coming from two different bottles for each group and, for the same bottle, they repeated twice.

The reliability of the data sets (four treatments for two replicates) was evaluated by One Way Completely Randomized ANOVA (CoStat, Cohort 6 software). Comparisons among means were performed by the Bartlett's X2 corrected test ($P< 0.05$). Tukey's HSD multiple mean comparison test ($P< 0.05$) was used to state the differences among variables.

As regards the sensorial analysis for each tasting session the reliability of the assessments was evaluated by Two Way Completely Randomized ANOVA (software R, version 3.3.1) and by the Bartlett's test (P<0.05), with Product (packaging and storage conditions adopted) and Panellist as main factors. Tukey's HSD multiple mean comparison test (P<0.05) was used to state the differences among variables.

Pearson's correlation coefficient test was also carried out to measure the strength of the correlation among TSO_2 degradation kinetic constant (k_{TSO2}) and the main wine attributes that are generally associated with oxidation. Generally, a coefficient of about ±0.7 or more is regarded as indicating fairly strong correlation, and in the region of ±0.9, it indicates very strong correlation. In the region of ±0.5 the correlation is moderate, and in the range -0.3 to +0.3 it is weak (Leighton, Schönfeldt & Kruger, 2010).

3. Results and discussion

3.1.Chemical evolution of wine

To avoid differences at bottling time in the concentration of O_2, the red wine collected from one single vat was bottled at the same time. Consequently, the different evolution of the oxidative processes detected during time can be attributed to the different oxygen intake during storage.

Kinetics of $TSO_2$and TAnt degradation. As sulfur dioxide (SO_2) plays an important protective role against oxidation in wine, the chemical degradation of this compound during storage may represent a good index of oxidative degradation of the product as a function of the storage conditions (Venturi *et al*., 2016a).

Generally, in wine SO_2 can exist in a variety of "free" (FSO_2) and "bound" (BSO_2) forms. Most of free ionic sulfur dioxide occurs as bisulfate ions (HSO_3^-), while the remaining free SO_2 exists as undissociated sulfurous acid (H_2SO_3). Because in wine SO_2 rapidly binds with several constituents (i.e. acetaldehyde, anthocyanins, tannins, piruvic acid, sugars, etc.), it often occurs as hydroxysulfonates with a consequent reduction of its active (free) concentration.

In this context, as SO_2 exists in many interconvertible forms, the active level of SO_2 is influenced by many factors (i.e. pH, concentration of binding compounds, oxygen availability, etc.) also during wine storage. In this study the concentration of TSO_2 (FSO_2 + BSO_2) has been assumed as a measure of oxidative degradation induced by the storage conditions since in wine only the free fraction represents a chemical intermediate among all the above mentioned forms of SO_2 and its concentration can be affected by several chemical reactions other than oxidation.

As reported in a previous paper (Venturi *et al.*, 2016a), the time evolution of TSO_2 concentration could be described by a first order kinetic equation:

$$-d[TSO_2]_{t=t}/dt = k_{TSO2} \cdot [TSO_2]_{t=t} \quad (4)$$

where k_{TSO2} is the kinetic constant related to TSO_2 degradation,and $[TSO_2]_{t=t}$ is the concentration of total SO_2 at the generic reaction time t=t.

After integration, the following equation can be obtained:

$$[TSO_2]_{t=t} = [TSO_2]_{t=0} \cdot e^{-kTSO2 \cdot t} \quad (5)$$

The two functional parameters k and $[TSO_2]_{t=0}$ were assumed to be a valid measure of the effect induced by oxidation during wine storage as a function of storage conditions (presence of capsule, storage position). To identify the best values to assign to the functional parameters (k and $[TSO_2]_{t=0}$) involved in equation (5) the statistical program Burenl was used (Buzzi Ferraris & Manca, 1996). The high values assumed by the squares of the correlation coefficients calculated for the linearized form of the kinetic equation in the Taylor series (Table 2) give an indication of the suitability of the theoretical approach followed and the reliability of the kinetic equations proposed.

Color is one of the most important organoleptic characteristics of red wine affecting the quality evaluation of the product (Bimpilas, Panagopoulou, Tsimogiannis & Oreopoulou, 2016.). As anthocyanins are the main compounds involved in the color of young red wines, and the change of color during aging from red–purple to brick-red hues is greatly influenced by the level of oxygen dissolved in the wine during storage (Avizcuri *et al.*, 2016), the time evolution of the concentration of anthocyanins (TAnt) may represent a further index of oxidative degradation of wine during storage.

The same experimental approach proposed above for describing TSO_2 time evolution was also followed to describe TAnt.

As reported in Table 2, the influence of the closure system (with or without capsule) as well as of the storage position (vertical or horizontal) on the oxidation of red wine is evident and the differences in the degradation rate of SO_2 after one year of storage are statistically significant. In particular, the reduction of the concentration of TSO_2 was faster when the red wine was stored in glass bottles closed with natural corks without the application of a capsule, regardless of the position during storage. Furthermore, independently from the closure system, the time evolution of the red wine during storage, in terms of TSO_2 degradation, was delayed when bottles were stored in the horizontal position. On the contrary, after 12 months of storage, TAnt was almost the same in all the storage conditions adopted.

3.2.Sensorial evolution of stored wine

The results of the ANOVA applied to the main attributes used to describe appearance, smell and taste are reported in Figure 2. Among all the descriptors ranked by the panel components, only the main parameters generally related to the wine evolution, together with the parameters that showed statistically significant differences in one or more storage conditions, are reported and discussed.

After 12 months of storage (Figure 2), in agreement with what observed for the chemical evolution, the wine maintained in the horizontal position showed the best sensorial profile, regardless of the closure system, whereas the fastest deterioration was observed when it was stored in glass bottles closed with a natural cork without capsule and maintained in vertical position.

According to the literature (Del Alamo-Sanza & Nevares Domiguez, 2006; Marquez *et al*., 2014; Gao *et al*., 2015), a period of 12 months of bottle-aging allowed to improve the "Overall pleasantness" of the young red wine. Among all the storage conditions, only the wine stored in glass bottles closed with natural cork without capsule and maintained in vertical position showed a significant degradation of its sensorial starting quality.

3.3.Matrix of correlation

In order to evidence whether the rate of TSO_2 degradation could represent a chemical index of the evolution of red wine during storage, the kinetic constant k_{TSO2} (Table 2) was correlated for all storage conditions with the sensory attributes generally related to wine oxidation. Among them, only the parameters that showed some significant differences during storage time (see Figure 2) were selected. The Pearson's correlation coefficients are reported in Table 3.

Thus, it is possible to evidence that TSO_2 degradation kinetic constant (k_{TSO2}) was strictly inversely correlated with positive sensorial attributes such as "Balance", "Frankness" and "Overall Pleasantness" whereas the negative attribute "Oxidation" was strongly correlated with k_{TSO2}.

4. Conclusions

A new "integrated approach" deriving from the merging of chemical, kinetic and sensorial data can be applied in order to identify the best storage conditions to preserve the quality of red wines.

As reported in previous papers (Venturi et al., 2016a; Venturi et al., 2017b, c), k_{TSO2} was effectively used as an index to describe the influence of packaging and storage conditions on the oxidative evolution of different wines, regardless of the wine style (i.e. white, red, full-bodied red). Furthermore, also the time evolution of both full-bodied red (Venturi et al., 2013; Venturi et al., 2014; Venturi et al., 2017a) and rosé (Venturi et al., 2016b; Venturi et al., 2017a) wines as a function of the shape of the glass utilised during tasting was well correlated with the TSO_2 degradation rate.

Thus, the TSO_2 decay rate constant (k_{TSO2}) could be considered a useful index also to describe the chemical evolution of red wine under investigation, in combination with the main sensorial attributes generally associated with its oxidative evolution, as a function of closure and storage position.

On the contrary, in the present experimental conditions, k_{TAnt} did not result a good index of the chemical evolution of the red wine. This experimental evidence is in agreement with the sensorial evaluation of the "Color intensity" (Fig. 2), suggesting that k_{TAnt} could be considered a useful oxidation index over a period of bottle aging longer than 12 months.

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Figure 1: Experimental protocol adopted.

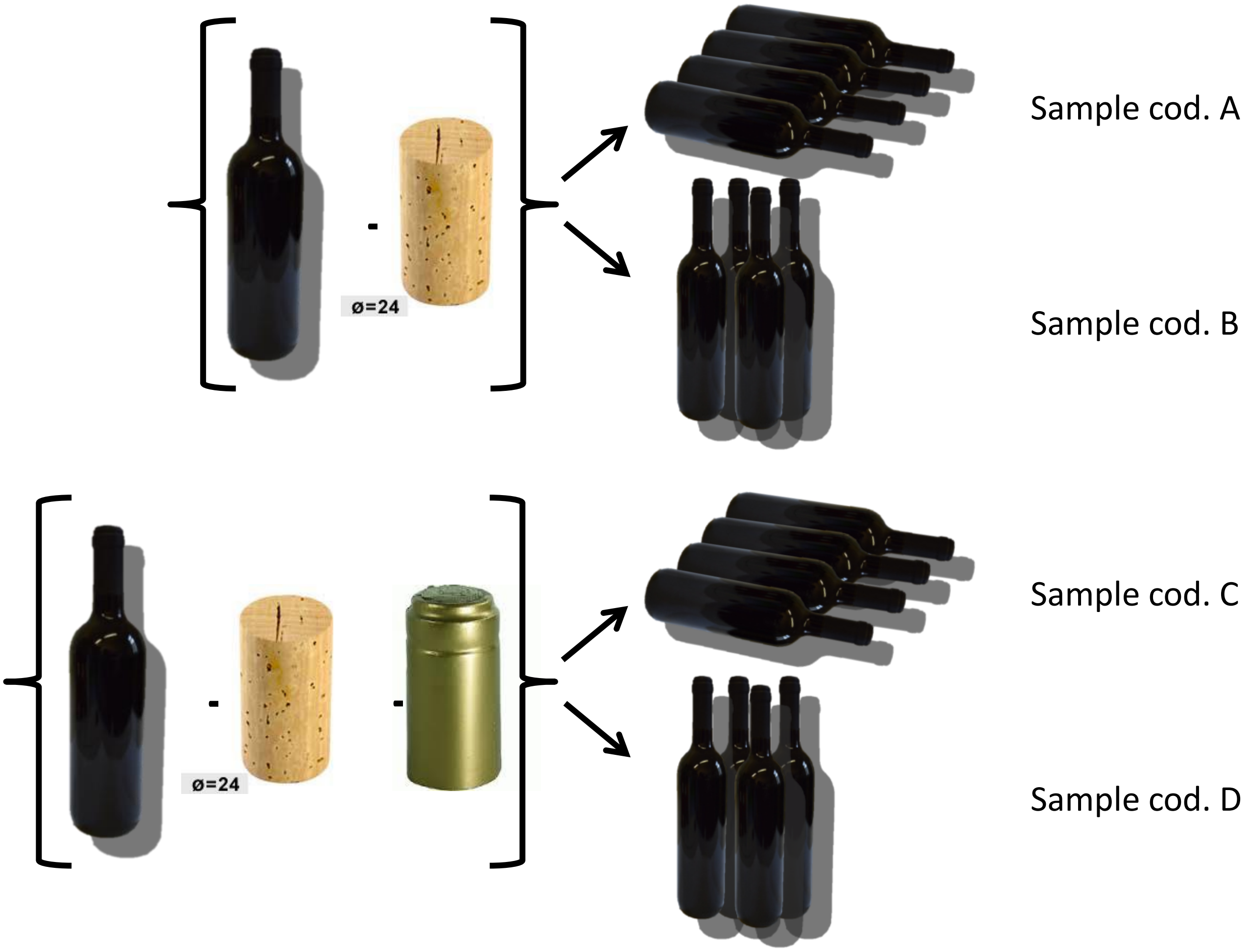

Figure 2: Main sensorial descriptors ranked by panel components as a function of closure and storage conditions adopted (A, B, C, D = samples after 12 months of storage; T = sample at starting time (3 days after bottling). Parameters not sharing the same letter have a significantly different mean value (P<0.05).

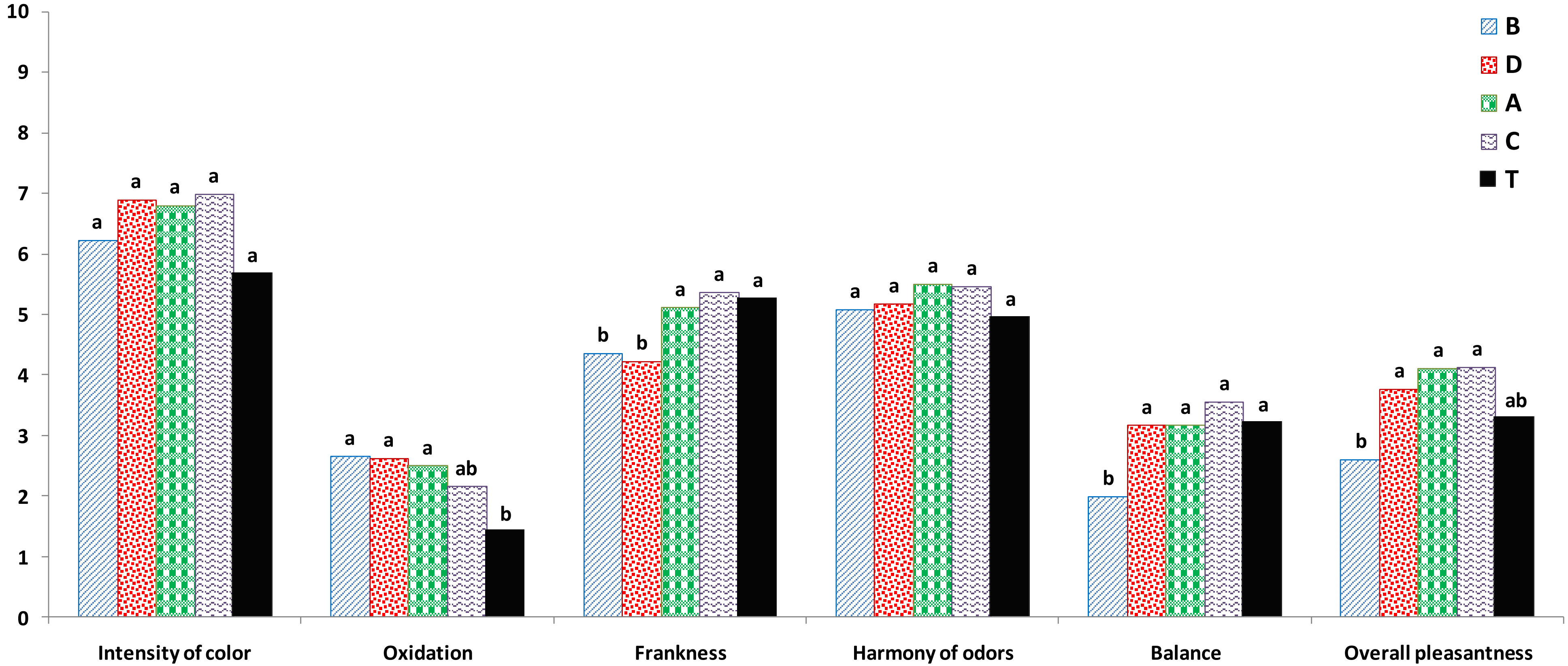

Table 1: Initial chemical composition of the red wine

Parameter	Mean value ± c. i.*
Alcohol (%v/v)	11.46± 0.06
pH	3.41 ± 0.01
Titratable Acidity (g/L as tartaric acid)	5.32 ± 0.01
Net Volatile Acidity (g/L as acetic acid)	0.40 ± 0.01
Total Phenols (g/L as gallic acid)	3.39 ± 0.05
Proanthocyanidins (g/L as catechins)	1.34 ± 0.03
Total Anthocyanins (g/L as malvin)	0.310±0.002

*c.i. = confidence interval, P<0,05.

Table 2: Kinetic parameters describing the time evolution of TSO_2 and TAnt concentrations as a function of closure and storage conditions.

Sample*	$kTSO_2$±c.i.** ($months^{-1}$)·10^4	$[TSO_2]_{t=0}$± c.i. (mg L^{-1})	r^2	kTAnt ($months^{-1}$)	$[TAnt]_{t=0}$± c.i. (mg L^{-1})	r^2
A	8.61±0.01	93.59±0.03	0.86	0.026±0.001	282.20±0.01	0.90
B	18.65±0.67	94.26±0.03	0.81	0.031±0.001	282.20±0.01	0.95
C	4.89±0.01	90.45±0.04	0.65	0.030±0.001	285.50±0.01	0.95
D	12.89±0.79	92.85±0.04	0.93	0.027±0.001	285.50±0.01	0.87

*A = Glass + natural cork without capsule, horizontal storage position
B = Glass + natural cork without capsule, vertical storage position
C = Glass + natural cork + capsule, horizontal storage position
D = Glass + natural cork + capsule, vertical storage position
**c.i.=confidence interval, $P<0,05$.

Table 3– Correlation matrix relating the kinetic constant describing TSO_2 degradation to wine sensory attributes (storage time = 12 months).

Parameter	$kTSO_2$
Balance	-0.93
Frankness	-0.87
Oxidation	0.87
Overall pleasantness	-0.92