Statistical study of the best oenological treatments for protein and tartaric stabilization in white wines

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Author: Miguel Ángel López González

Director: Ricard Boqué Martí

e-mail: miguelangel.lopezg@estudiants.urv.cat, ricard.boque@urv.cat

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Abstract

The influence of protein and tartaric stabillity treatments over the final wine quality of wines from Sauvignon blanc, Verdejo and Xarel·lo grape varieties was evaluated in the present study. Microfermentations from the three varieties mentioned above were carried out and then subjected to different combinations of protein stability treatments (bentonite, isinglass, PVPP and vegetal protein) and tartaric stability (cooling stabilization, mannoprotein, potassic bitartarate and carboximethyl celulose). Variables analyzed were chemical variables (total and volatile acidity, pH and alcohol by volume), colour variables (a^* , b^* and L^* components from CIELAB colour space, colour intensity, tonality and Folín index), phenolic content varables (Folín index and total phenolic acids), and fermentative aromas content variables (aromas and off-flavours content). In order to evaluate treatments different data analysis techniques were used: univariate (two factor ANOVA) and multivariate (PCA and two factor MANOVA) techniques. A quality index was calculated for each treatment combination in order to determinate which of the combination was the more suitable for each grape variety studied. The results provided by univariate and multivariate data analysis evidenced the significant influence of tartaric stability treatments over the variables studied and the null influence of protein ones. Quality index calculation determined in a general way that the best combination of treatments were which that uses mannoprotein as a tartaric stabilizer, followed by carboximethyl celulose ones and leaving potassium bitartarate and cooling stabilization in the last place.

Resum

Al present estudi es va avaluar la influència dels tractaments d'estabilització tartàrica i proteica sobre la qualitat final dels vins de les varietats Sauvignon blanc, Verdejo i Xarel·lo. Es van dur a terme micro fermentacions de les tres varietats anomenades i es van sotmetre a diferents combinacions de tractaments d'estabilització proteica (bentonita, cua de peix, PVPP, proteïna vegetal) i tartàrica (estabilització per fred, manoproteines, bitartrat de potassi i carboximetil cel·lulosa). Es van analitzar variables químiques (acidesa total tartàrica i volàtil, pH i contingut alcohòlic), de color (components a*, b* i L* del espai de color CIELAB, intensitat colorant, tonalitat i índex de Folín), de contingut en compostos fenòlics (índex de Folín i àcids fenòlics totals) i de contingut en aromes fermentatius (compostos aromàtics i off-flavours). Per avaluar la influència dels tractaments es van emprar tècniques d'anàlisi de dades univariant (ANOVA de dos factors) i multivariant (PCA i MANOVA de dos factors). De les combinacions de tractaments aplicades es van realitzar el càlcul d'un índex de qualitat per determinar quina d'aquestes era la més adient per a cadascuna de les varietats de raïm estudiades. Els resultats de l'anàlisi de dades univariant i multivariant van evidenciar la influència significativa dels tractaments d'estabilitat tartàrica i la influència pràcticament nul·la dels tractaments d'estabilitat proteica. El càlcul de l'índex de qualitat va determinar de manera general que les millors combinacions de tractaments a aplicar són aquelles les quals empren el tractament d'estabilització tartàrica de manoproteines, seguides pel tractament amb carboximetil cel·lulosa i deixant els tractaments amb addició de bitartrat de potassi i estabilització per fred a l'últim lloc.

Resumen

En el presente estudio de evaluó la influencia de los tratamientos de estabilización tartárica y proteica sobre la calidad final de vinos de las variedad Sauvignon Blanc, Verdejo y Xarel·lo. Se realizaron micro fermentaciones de las tres variedades mencionadas y se sometieron a diferentes combinaciones de tratamientos de estabilización proteica (bentonita, cola de pescado, PVPP y proteína vegetal) y tartárica (estabilización por frío, manoproteínas, bitartarato de potasio y carboximetil celulosa). Se analizaron variables químicas (acidez total tartárica y volátil, pH y contenido alcohólico), de color (componentes a*, b* y L* del espacio de color CIELAB, intensidad colorante, tonalidad e indíce de Folín), de contenido en compuestos fenólicos (índice de Folín y Acidos fenólicos totales) y de contenido en aromas fermentativos (compuestos aromáticos y off-flavours). Para evaluar la influencia de los tratamientos se utilizaron técnicas de análisis de datos univariante (ANOVA de dos factores) y multivalente (PCA y MANOVA de dos factores) De las combinaciones de tratamientos aplicadas se realizó el cálculo de un índice de calidad para determinar cuál de estas era la más adecuada para cada variedad de uva. Los resultados del análisis de datos univariante y multivariante evidenciaron la influencia significativa de los tratamientos de estabilidad tartárica y la influencia prácticamente nula de los tratamientos de estabilidad proteica. El cálculo del índice de calidad determinó de manera general que las mejores combinaciones de tratamientos a aplicar son aquellas las cuales se utiliza el tratamiento de estabilización tartárica de manoproteínas, seguidos por el tratamiento de carboximetil celulosa y dejando los tratamientos de adición de bitartarato potásico y estabilización por frío en el último lugar.

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Introduction

The present study is part of a more general project developed by VITEC (Parc Tecnològic del Vi, Falset), whose main objective is to increase the shelf life period of white wines from the principal production regions (those with certificate of origin Penedès, Rueda and Cava) and to enhance their main varietal markers from Xarel·lo, Verdejo and Sauvignon Blanc varieties.

There are several treatments to improve shelf life of wines. Some of these are based on assuring protein and tartaric acid stability, which could bring problems of solid precipitation, turbidity as well as undesired colours and tonality.

Sometimes, protein and tartaric stability treatments can interact with phenolic acids, reducing in that way the phenolic content of wines. Phenolic compounds are the main responsible of colour in wines, so that, this kind of treatments could have an influence on different colour parameters as well.

The aim of the present study is to know how the different combination of protein and tartaric acid stabilization treatments can affect to phenolic composition and colour and, therefore, can influence the final quality of the product. Once the relationship between the different treatments and the phenolic content and colour is known, the best combination of tartaric and protein treatments will be determined for each variety, by the calculation of a global quality index.

Materials and methods

Samples

Micro fermentations of grape-juice obtained from grapes of the three main varieties studied (Xarel-lo, Verdejo and Sauvignon blanc) were subjected to different combinations of protein and tartaric stability treatments, shown in Table 1 with their coding. The name of a sample is formed first by the code of the protein treatment and then, after a dot, by the code of the tartaric treatment.

Protein		Tartaric acid						
Treatment	Code		Treatment	Code				
Bentonite		b	Cooling stabilization	f				
			Cooling+					
Bentonite + Isinglass		bc	mannoprotein	m				
			addition					
Pure Polivinilnirrolidone		nv	Cooling + Potassium	V				
r dre r olivillipit olidone		μv	bitartrate	v				
Vegetal protein		n	Cellulose	C				
		Р	carboxymethyl	ť				

Table 1. Stabilization treatments applied and their coding.

For each combination of treatments, three experimental replicates were performed. In total, each grape variety had a number of 48 samples (16 treatments x 3 replicas). The definition of each treatment applied is explained below:

Protein stabilization treatments

Bentonite

Bentonite is a kind of clay composed by aluminium-silicate and formed from volcanic ash. It has the property of being negatively charged so it is a good agent for wine clarification. Negative charges from bentonite react with the positive side of proteins, making them precipitate. Bentonite is typically used on white wine due to its high power to reduce colour. Bentonite works better at lower pH because the

positive charge in the proteins is stronger when the pH is acid.[The Australian Wine Research Institute n.d.]

Bentonite + Isinglass

Issinglass is a protein collagen typically used in white wine clarification. It reacts with polyphenolic compounds, removing turbidity and enhancing fruity characters. It has the advantage of not changing the phenolic profile in a big way and not reducing so much wine astringency. On the other hand, an excess of isinglass can provide fishy odours and increase the protein haze forming.[The Australian Wine Research Institute n.d.]

Pure Polyvinylpolypyrrolidone (PVPP)

PVPP is commonly used to reduce phenolic compound content and also browning and astringency effects in white wines. PVPP is a synthetic polymer, as a difference from the other fining agents frequently used. It has the advantage of being insoluble in wine and easy to remove from it. It is also gentle with aromas, preserving most of them while reducing off-flavours and bitterness.[The Australian Wine Research Institute n.d.]

Vegetal protein

Vegetal protein is a natural fining agent obtained from vegetables, mainly potatoes and peas, usually from by-products of their processing. The proteins are usually added in a powder form and what they do in wine is to cause a reciprocal flocculation with colloids and the precipitation of floccules. Vegetal protein has the advantage of being a non-allergenic fining agent in comparison with other proteins used for the same purpose (egg albumen, isinglass,...). It is also popular for being used to elaborate vegan wines.[López Casado 2014]

Tartaric acid stabilization treatments

Cooling stabilization

Tartaric stabilization by cooling is the most used treatment in oenology to avoid tartaric precipitation, as it is the easiest and cheapest way. The fundamental of this technique is based on the solubility of tartrate salts. Wine temperature is reduced by storing it at very low temperature, close to the freezing point. This reduces the solubility constant and causes a controlled precipitation of tartrate salts. Wine is usually stored from 3 days to 3 weeks, but usually remains one week at this temperature. The cooling treatment is also combined with other techniques, to enhance its effect. Two examples are explained below.[Lasanta and Gómez 2012]

Cooling + mannoprotein addition

Mannoproteins are obtained from the hydrolysis of *Saccharomyces cerevisiae* cell walls, which are formed by glycoproteins. The aim of using mannoproteins is to inhibit the crystallization of tartrate salts. Mannoproteins lower the tartrate salts crystallization by biding crystal nucleation points and avoiding crystal expansion. Mannoproteins have the advantage of being formed during the fermenttion process, from liberation by yeasts and lees after fermentation by autolysis. Mannoproteins are also useful to assure protein, phenolic and sensory stability.[Lasanta and Gómez 2012]

Cooling + potassium bitartrate

Salt formation is the way of precipitation of tartaric acid. The most common salt of how the tartaric acid precipitates is potassium bitartrate. Even seeming a contradiction, the aim of adding potassium bitartrate to avoid precipitation of itself in wine is to use it as a crystal seed. In a supersaturated solution of potassium bitartrate, two stages take place. On the first stage, there is a formation of a crystal nucleus that is called seed crystal. Then, in a second stage, there is a migration of potassium bitartrate is used to supersaturate the wine and to introduce on it a crystal seed to accelerate crystal nucleation and formation and, therefore, its precipitation.[Dharmadhikari n.d.]

Carboxymethyl cellulose

Carboxymethyl cellulose, or CMC, is used as an emulsifier in the food industry, but also as a good tartaric stabilizer. CMC is a derivative from cellulose. It has the particularity of being negatively charged, so when used as a tartaric stabilization agent it interacts with the positive charge of tartrate salt crystals to prevent them to keep growing. Compared cooling or mannoprotein addition, CMC has a longer stabilization effect. It has also the advantage of interacting with polyphenols, so it is useful to assure phenolic stability at the same time.[Guise et al. 2014]

Analytical methods

pH was measured following the OIV-MA-BS-13 method. Total Acidity (*TA*) was measured by potentiometric titration following OIV-MA-BS-12 method.

Alcohol by volume (ABV) was determined by following the OIV-MA-BS-01 method.

Volatile Acidity (VA) was measured by following Garcia-Tena's method, based on wine fractional distillation and separated titration of the collected fractions.[GAB sistemática analítica n.d.]The analysis was carried out using the electric voltammeter from GAB Sistemática Analítica.

Folin-Ciocalteu Index (FI) was measured using the MA-F-AS2-10-INDFOL method from OIV.

Colour Intensity (CI) was measured by following the OIV-MA-BS-26 method.

Tonality (*Ton.*) and the LAB colour space components L^* , a^* and b^* were measured following the OIV-MA-BS-27 method. To calculate L^* , a^* and b^* components, *MSCV* software from Universidad de la Rioja-Universidad de Zaragoza. Lightness (L^*) is expressed in a 0 (black) to 100 (colourless) interval. Component a^* expresses colour spectra from green (negative values) to red (positive values). Component b^* expresses colour spectra from blue (negative values) to yellow (positive values).

Total phenolic acid contents (*Phen.*) is the sum of all phenolic acids present in wine and was determined by gas chromatography. The phenolic acids analysed were trans-GRP, trans-castaric, cis-coutaric, caffeic, cis-fertaric, trans-fertaric, coumaric and ferulic acids.

Fermentative aroma variables, aromatic compounds content (*Ar.*) and off flavours (*Off*), where determined by gas chromatography. The aromatic compounds content is the result of the sum of all desired fermentative aromatic compounds found in white wine. The aromatic compounds determined were ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl isovalriate, diethyl succinate, ethyl acetate, isoamyl acetatehexyl acetate, 2-phneylethyl acetate, isoamyl alcohol, isobutanol, benzil alcohol and 2-phenyethyl alcohol, which bring nice aromas such as fruity and floral aromas. The variable "off flavours" is the result of the sum of all fermentative off-flavours found in white wine. The off-flavours determined where hexanoic acid, octanoic acid and decanoic acid, which provide disgusting aromas such as rancid, cheese and vomit aromas.[Francis n.d.]

Statistical methods

Univariate methods

Two Factor ANOVA

The two factor analysis of variance (ANOVA) is an statistical method whose main objective is to analyze if there exist differences between groups on the value of a dependent variable and conditioned by two independent variables (factors).[Lærd Statistics 2017a] ANOVA compares group means to determine if there is one that differs from the rest and if this difference is significant. It is also used to find out if there is interaction between factors.[Boqué and Maroto 2004]

Two factor ANOVA was performed using the data analysis tool from *Microsoft Excel 2007* software. It was carried out for each variable (oenological parameter), where protein and tartaric treatment were placed as factors. To evaluate significance a one-sided *F* test was carried out, where a significance level $\alpha = 0.05$ was selected.

Multivariate methods

Principal Component Analysis

Principal Component Analysis (PCA) is a multivariate data analysis technique [N Miller and C Miller 2005] that transforms a set of variables into a reduced set of non-correlated variables, by carrying out a data compressing. [Johnson n.d.] Data compressing is carried out by creating new orthogonal variables called principal components (PCs). The criteria to determine this new axis set is that PCs go in the space direction where the data have the maximum variance. PCA calculates PCs in a hierarchical way, that is, PC_1 explains the maximum of variance, PC_2 explains the maximum of variance not explained by PC_1 , and so on. PCs are orthogonal between them and, therefore, uncorrelated. This means information (variance) explained by PC_1 is not explained by PC_2 , and so on. The number of possible PCs is equals the minimum number of samples or original variables; however, the aim is not representing all these PCs, but reducing dimensions and just focusing on the PCs that are significant and preserve most of the original structure of the data. Each sample is represented by the coordinates on this new axis set. This coordinates are called scores. The correlation between samples and variables is defined by its correlations coefficients, called loadings. Scores and loadings are displayed in plots to show the results from PCA. [Ferré and Boqué Martí 2004] PCA is used then as an exploratory method to look for similarities between samples or between variables, to find groups or trends in the data, to determine what variables are correlated and what variables are important in sample differentiation[Johnson n.d.], and also to detect abnormal (outlier) samples in the dataset.

PCA models were built using *The Unscrambler X (v10.3)* software. The variables (oenological parameters) in the study had different magnitudes, so weighting of data was carried out by dividing each individual value by the standard deviation of the variable. Data were also mean centred, i.e. the mean value of each variable for the set of samples was subtracted from each individual value.

Two Factor MANOVA

Two factor multivariate analysis of variance (MANOVA) is an extension of two factor ANOVA explained in the previous section. The purpose of two factor MANOVA is to determine whether there exist differences between groups on the values of two or more dependent variables conditioned by two independent variables (factors).[Lærd Statistics 2017b]

Two factor MANOVA was performed using the *IBM SPSS Statistics* multivariate general linear model tool. To evaluate significance of differences of multiple variables at the same time, multivariate analysis was carried out using the *Wilks Lambda* one-sided *F* test. An inter-subject test was also applied in order to determine the effect of each kind of treatment applied and to check if the interaction of both of them had a significant influence on the value of each variable studied. Once significant differences were determined, a *post hoc* analysis *Tukey* test was carried out in order to determine which of the treatments differs from the rest. An α value of 0.05 was selected to carry out the test.

Global quality index determination

In order to determine what combination of tartaric and protein stability treatments is the best to assure wine quality, each sample was evaluated by scoring it from 0 to 10, in a so called global quality index (*QI*). *QI* was calculated using an equation were the value of *pH*, *ATT*, *L**, *a**, *b**, *Ar*. and *Off*. was considered. Variable normalizing was necessary to work with the same magnitude. Normalizing was carried out by transforming the values into a scale from 0-10 by applying equations 1[Cubero and Berzal 2015] and 2. It is important to comment that to transform values into a scale from 0 to 10, first of all it was necessary to determine how the variable values will affect the *QI* value. For example, if high values for a given variable are expected to affect positively the quality of wine, then the higher the value is, the more scoring the variable gets. On the other hand, if low values from a given variable are expected to affect negatively the quality of wine, then the lower the values are, the higher may score on *QI*. To solve this problem two different equations were proposed. First equation (Eq.1) gives more scoring to high values. For giving high scoring to low variable values, Eq. 1 was transformed into Eq.2.

There was another problem to solve, as not all variables are in a close scale. One example is a^* and b^* , whose values could get values from minus infinite to plus infinite. To solve this, the lowest value from all samples of the variable studied was taken as the lower limit (original minimum on eq. 1 and 2) while the highest value was taken as the upper limit (original maximum on eq. 1 and 2). Something similar occurs when the variable has a lower limit but there is not an upper limit; in this case, the higher values of the variable from all samples studied will act as an upper limit.

$$y = \min + \frac{x - original \ minimum}{original \ maximum} (max - min)$$
(1)

$$y = min + \frac{x - original \ maximum}{original \ minimum \ - original \ maximum} (max - min)$$
(2)

Some assumptions were considered for the variables selected:

- *pH* values are expected to be inside the range of 3.2-3.5. These are the most common values that are expected in white wines[Comfort 2010]. In the particular case of *pH* the two formulas proposed to transform the variables into a 0-10 range were used. The objective is to score higher the values between the 3.2-3.5 *pH* range, so the closest to 3.35 the *pH* is the higher the scoring is expected to be. To solve the problem for *pH* values higher than 3.35 Eq. 1 was used to score higher values close to 3.35, while for values lower than 3.35 Eq. 2 was used with the same purpose. When using Eq. 1 the original maximum will be 14 while the original minimum will be 3.35. When using Eq.2 the original maximum will be 3.35 while the original minimum will get the value of 0.
- *TA* values are expected to be as low as possible. That means that tartaric treatments applied are effective avoiding precipitation of tartrate salts. In this case Eq. 2 was used. The original minimum will have the value of 0 while the original maximum will be the highest value of all samples studied.
- L* values are expected to be high, what means having a lighter wine. In this case, Eq.1 is used, where the original maximum will have the value of 100 and the original minimum will have the value of 0.
- a* and b* values are expected to be negative. The more negative a* values are the greener the wine is, while the more positive the b* values are the less yellow the wine is, what is expected in white wines. In both cases, Eq.2 was used, in which the original minimum got the same value as the lowest value of the samples studied, while the original maximum got the same value as the highest value of the samples studied.
- Ar. values are expected to be as high as possible. White wines are characterized for being aromatic wines with presence of fruity and floral aromas. Eq.1 is used in this case by taking the value of 0 for the original minimum and the highest value of the variable from all the samples studied as the original maximum.
- *Off.* values are expected to be as low as possible in order to score lower those wines having more disgusting aromas. Eq.2 was used in this case, by taking the value of 0 for the original minimum and the highest value of the variable from all the samples studied as the original maximum.

Variable weighting was necessary to reflect the importance of each variable on the *QI* value. *QI* equation (Eq. 3) was set up by determining how the value of the different variables was beneficial to wine quality.

$$QI = pH \cdot w_{pH} + TA \cdot w_{TA} + L^{x} \cdot w_{L} + a^{*} \cdot w_{a} + b^{*} \cdot w_{b} + Ar \cdot w_{Ar} + Off \cdot w_{Off}$$
(3)

It was intended to give more weight to aromatic compounds and colour variables, followed by the total acidity content and, finally, by the *pH* value. The weightings proposed were the following ones:

 $W_{pH} = 0,1, W_{-TA} = 0,1, W_{L} = 1,33, W_{a^*} = 1,33, W_{b^*} = 1,33, W_{Ar.} = 0,2 \text{ and } W_{Off} = 0,2.$

Results and discussion

To look out whether the treatments have an influence on the different physical and chemical properties, the different tests were performed with different sets of variables described below:

- All parameters
- Chemical parameters, which include pH, ABV, volatile acidity and total acidity
- Colour parameters, including colour intensity, tonality, Folin index, L*, a* and b* CIELAB variables
- Phenolic content, including Folin index and total phenolic acid content
- Fermentative aromas, including aromatic compounds content, which are wanted in white wines and off-flavour compounds content, which are not desired in wine.

In PCA plots and *QI* determination, colouring was applied in order to distinguish the different tartaric treatments. Green = Carboxymethyl cellulose, Blue = Potassic bitartrate, Orange = cooling and Red = Mannoprotein. In ANOVA and MANOVA tables, significant results were highlighted in yellow.

Principal component analysis

All variables

In the PCA scores plot for Sauvignon Blanc samples (Fig. 1a), three different groups are seen, one composed by potassium bitartrate treatment samples, a second one composed by carboxymethyl cellulose samples and, finally, another one composed by cooling and mannoprotein samples. In this last group it is possible to distinguish two subgroups for F and MA samples, respectively; however, these are close enough to consider there are no significant differences between them. By looking at the loadings plot (Fig. 1b), it can be seen that potassium bitartrate samples are characterized for having the highest values on colour intensity, *b** CIELAB component, *FI* and total phenolic acid content variables. Carboxymethyl cellulose samples are likely to have higher values on total acidity, tonality, *a** and *L** CIELAB components. Finally, cooling and mannoprotein samples have the highest values of *ABV*, *pH* and fermentative aromas variables. It is worth commenting that carboxymethyl cellulose samples form a highly dispersed group, which might mean that the protein treatment interacts with CMC.



Figure 1a. Scores plot for Sauvignon Blanc samples (all variables). Figure 1b. Loading plot for Sauvignon Blanc samples (all variables). (PC₁ explains a 40% of total data variance while PC₂ explains a 20%).

This tendency is repeated in the other grape varieties. For Verdejo samples (Fig. 2a), it is clearly seen that samples treated with potassium bitartrate are widely differentiated from the rest of treatments. Carboxymethyl cellulose samples also form a group, but very close to the third group composed by cooling and mannoprotein treatment samples. That could mean that carboxymethyl cellulose samples

do not have the same effect on Verdejo wines as on Sauvignon Blanc wines. Looking at the loadings plot for Verdejo (Fig. 3b), it can be seen that potassium bitartrate samples show high values for colour intensity, Folin index and b^* variables. Carboxymethyl cellulose samples are characterized for having high values of tonality, a^* , L^* and total acidity variables. As in the Sauvignon Blanc plot, cooling and mannoprotein samples have the highest values of *ABV* and *pH* and fermentative aroma variables.



Figure 2a. Scores plot for Verdejo samples (all variables). Figure 2b. Loading plot for Verdejo samples (all variables). (PC₁ explains a 36% of total data variance while PC₂ explains a 20%)

Xarel·lo samples showed the same tendency described above. In the scores plot (Fig. 3a) three groups can be seen, one composed by potassium bitartrate samples, another one composed by carboxymethyl cellulose samples and a third group composed by cooling and mannoprotein samples. In this case, a wide differentiation between groups is observed. In the loadings plot (Fig. 3b) it can be seen that potassium bitartrate samples show the highest values of Folin index, b^* and colour intensity variables, whereas carboxymethyl cellulose samples are characterized for having the highest values of total phenolic acid content, *pH*, *ABV* and tonality variables. Cooling and mannoprotein samples are characterized for having mid values of volatile acidity, total acidity, L^* and a^* variables and for having the highest values of fermentative aroma variables.



Figure 3a. Scores plot for Xarel·lo samples (all variables). Figure 3b. Loading plot for Xarel·lo samples (all variables). (PC₁ explains a 41% of total data variance while PC₂ explains a 28%)

The information shown in the PCA plots of the three grape varieties evidences that there exist three different groups depending on the tartaric treatment applied.

First, a group formed by the samples treated with potassium bitartrate is seen, for which is possible to affirm that it is differentiated from the rest for having the highest values on colour variables (b^* , CI) and phenolic content variables (*Phen* and *FI*). It is possible to observe that there exists a correlation between phenolic content and colour variables, as the colour intensity and the blue and green tonalities rise when the polyphenol content is higher.

A group with samples subjected to the carboxymethyl cellulose treatment can also be seen. This is different from the mentioned above, and is characterised for having high values on total acidity and volatile acidity. This fact makes thinking that carboxymethyl cellulose treatments could be less effective to avoid tartrate precipitations in wine, as more quantity of tartaric acid is left in the wine.

Finally, a third group can be seen formed by samples subjected to mannoprotein treatments and cooling stabilization. These last two treatments do not show significant differences between them and among the rest of treatments and seems to have moderated values in most of the variables studied.

For a more accurate analysis, a PCA was carried out for the variable sets mentioned before.

Chemical variables

In the scores plot for the Sauvignon Blanc varieties (Fig. 4a), a big difference for carboxymethyl cellulose samples is seen if the PCA is carried out just picking chemical variables. The remaining samples are very close each other, forming a group very differentiated from the carboxymethyl cellulose one. It is important to say that carboxymethyl cellulose samples show the same dispersion described in the score plots for all variables. This fact may mean that there could be some interaction between protein and tartaric treatments just on these samples, which makes it to get different values. By looking at the loadings plot (Fig. 4b) what is seen is that carboxymethyl cellulose samples have the highest values for total acidity and volatile acidity content.



Figure 4a. Chemical variables scores plot for Sauvignon Blanc samples. Figure 4b. Chemical variables loadings plot for Sauvignon Blanc samples. (PC₁ explains a 67% of total data variance while PC₂ explains a 18%)

For Verdejo samples, looking at the results shown in the scores plot (Fig. 5a) it can be considered that there exist differences between carboxymethyl cellulose from the other treatments, which are very similar among them. The loadings plot (Fig. 5b) shows that carboxymethyl cellulose samples are



differentiated by the values of total acidity and volatile acidity variables, which are higher than the remaining samples.

Figure 5a. Chemical variables scores plot for Verdejo samples. Figure 5b. Chemical variables loadings plot for Verdejo samples. (PC₁ explains a 55% of total data variance while PC₂ explains a 38%)

Finally, for the Xarel-lo samples the same tendency described in Sauvignon Blanc and Verdejo plots is observed in the scores plot (Fig. 6a). Carboxymethyl cellulose samples are displayed far from the remaining samples, which makes to think that a big difference from the chemical values on carboximetil cellulose exist compared with the remaining samples, that are very near each other. The loadings plot (Fig. 6b) show that carboxymethyl cellulose samples have the highest values for *ABV* and *pH* variables, something different if it is compared with Sauvignon Blanc and Verdejo samples, where carboxymethyl cellulose is distinguished for having high values on total acidity and volatile acidity.



Figure 6a. Chemical variables scores plot for Xarel·lo samples. Figure 6b. Chemical variables loadings plot for Xarel·lo samples. (PC₁ explains a 81% of total data variance while PC₂ explains a 11%)

As a general observation, TA and VA variables are located in the opposite side of pH in the loadings plots. This means that they are inversely correlated variables. High values of TA and VA would give more acidity to wine, lowering the pH value in this way. This confirms the results mentioned before. For Sauvignon Blanc and Verdejo varieties, carboxymethyl cellulose treatments preserve tartaric acid

content, which involves a risk of suffering tartrate salt precipitations in the future. On the other hand, this provides some acidity to wine, which improves its organoleptic profile. It is possible to affirm that the remaining treatments are more aggressive in removing the tartaric acid, so that these treatments would be better to avoid tartrate salts precipitation.

Colour variables

The scores plot for Sauvignon blanc samples (Fig. 7a) shows two differentiated groups. One is formed by potassium bitartrate treatments, which are characterized for having the highest values on *FI*, b^* and *CI* variables if we look at loadings plot (Fig. 7b). A second group is found formed by the remaining treatments. The remaining treatments are too close between them and no significant differences between them can be considered. By looking at the loadings plot (Fig. 7b) this group is characterized for having the highest values on tonality a^* and L^* .



Figure 7a. Colour variables scores plot for Sauvignon Blanc samples. Figure 7b. Colour variables loadings plot for Sauvignon Blanc samples. (PC₁ explains a 78% of total data variance while PC₂ explains a 11%)

The results provided by PCA for Verdejo samples are similar to the ones described for the Sauvignon Blanc ones. By looking at the scores plot (Fig. 8a) two groups are differentiated, one composed by potassium bitartrate samples and the other one composed by the remaining treatments. Many of the samples of the potassium bitartrate group are close to the remaining group of samples, even being a bit different. There are also some samples from potassium bitartrate that are included in the remaining samples group. This evidences that even there are significant differences these are not big. For the potassium bitartrate differentiated group, it can be seen in the loading plot (Fig. 8b) that this has the highest values on *FI*, *b** and *CI* variables, while the remaining samples have the highest values on *L**, *Ton* and *a** variables.



Figure 8a. Colour variables scores plot for Verdejo samples. Figure 8b. Colour variables loadings plot for Verdejo samples. (PC₁ explains a 69% of total data variance while PC₂ explains a 17%)

The results for Xarel-lo samples show the same tendency described above. The scores plot (Fig. 9a) and the loadings plot (Fig. 9b) show that the potassium bitartrate treatment forms a separated group characterised for having the highest values on *FI*, b^* , and *CI*. There is a second group formed by mannoprotein and cooling treatments, which has the highest values for L^* and a^* variables. Finally, a group formed by carmboxymethyl cellulose samples can also be observed, although a bit dispersed, having the highest values on L^* and Ton. However, this group is close enough to the mannoprotein and cooling samples to consider there are significant differences.



Figure 9a. Colour variables scores plot for Xarel·lo samples. Figure 9b. Colour variables loadings plot for Xarel·lo samples. (PC₁ explains a 75% of total data variance while PC₂ explains a 16%)

As a general observation of the results provided by PCA from the three grape varieties studied, it is possible to affirm that potassium bitartrate treatments preserve better the green tonalities while at the same time preserve polyphenol content and colour intensity. The problem is that, at the same time, they show high b^* and low L^* values, meaning more yellow tonalities and less brightness than the other samples, a fact that that is not desired in white wines. It is then possible to say that treatments displayed in the centre of the scores plot are equilibrated regarding a^* and b^* variables and so they are the best ones to preserve desired colours in wine. This could be due to that potassium bitartrate treatments work on wine by accelerating tartrate crystals nucleation, without using polyphenol binding to induce salt precipitation, as carboxymethyl cellulose and mannoprotein treatments do. In this way the phenolic content is preserved, which brings to wine green tonalities and higher colour intensity. This fact is observed in the loadings plot, where the Folin index is directly correlated to yellow coloration and colour intensity and inversely correlated to L^* , green colours and tonality. Finally, there is a negative aspect regarding potassium bitartrate treatments, as they are not useful to bring luminosity to wine because there is not enough removal of polyphenols to achieve it.

Phenolic compounds content variables

As a general observation, the PCAs built with the phenolic compound variables do not show a big difference between the different samples studied (Figs. 10a, 11a and 12a). All the samples are displayed close enough to consider that they are not much different. Even if there are not big differences between samples, some grouping can be seen, which would help to determine what samples have higher values of the variables studied.

For Sauvignon Blanc samples and looking at the loadings plot (Fig. 10b), PC1 explains most of the variance on the total phenolic acid content and Folin index. The more on the right side of PC1 the samples are on the scores plot (Fig. 10a), the higher is the value on these variables, so that potassium bitartrate samples are the ones having a higher content of phenolic compounds.



Figure 10a. Phenolic compound content variables scores plot for Sauvignon Blanc samples. Figure 10b. Phenolic compound content variables loadings plot for Sauvignon Blanc samples. (PC₁ explains a 67% of total data variance while PC₂ explains a 33%)

In the case of Verdejo samples, PC2 in the loadings plot (Fig. 11b) shows the variance of total phenolic acid content and Folin index variables. When observing the scores plot (Fig. 11a) it can be seen that potassium bitartrate samples have the highest values of these variables, and so are the wines with the highest content of phenolic compounds.



Figure 11a. Phenolic compound content variables scores plot for Verdejo samples. Figure 11b. Phenolic compound content variables loadings plot for Verdejo samples. (PC₁ explains a 56% of total data variance while PC₂ explains a 46%)

Finally, looking at the Xarel·lo loadings plot (Fig. 12b) it can be seen that PC1 explains the variance of total phenolic acid and Folin index variables. In this case, the scores plots (Fig. 12a) shows that carboxymethyl cellulose and potassium bitartrate samples have the highest values for these variables.



Figure 12a. Phenolic compound content variables scores plot for Xarel·lo samples. Figure 12b. Phenolic compound content variables loadings plot for Xarel·lo samples. (PC₁ explains a 73% of total data variance while PC₂ explains a 27%)

In general, it is not possible to get much relevant information from the plots shown. One of the things seen is that, in all of them, the phenolic content is always higher in the potassium bitartrate samples, a fact that was also observed when analyzing just colour variables.

Aromatic compounds Variables

For Sauvignon blanc samples, the analysis carried out shows that there exist three different groups. In the scores plot (Fig 13a), at first glance two clearly differentiated groups can be observed, the first one formed by mannoprotein samples and the other one formed by potassium bitartrate and cooling stability treatment samples. The third group is composed by samples from the 4 different treatments

studied, but this does not follow any logical grouping, so that these samples were treated as outliers. Carboxymethyl cellulose treatment samples are so dispersed that they not show any grouping. Looking at the loadings plot (Fig. 13b) it is possible to see that the two first groups mentioned are characterized for having higher contents on aromatic compounds. PC1 differentiates samples by its content on aromatic compounds, so mannoprotein and potassium bitartrate treatments are better on preserving these flavours.



Figure 13a. Scores plot of the aromatic compounds content variables for Sauvignon Blanc samples. Figure 13b. Loadings plot of the aromatic compounds content variables for Sauvignon Blanc samples. (PC_1 explains a 61% of total data variance while PC_2 explains a 39%)

For the Verdejo variety, two differentiated groups are observed in the scores plot (Fig. 14a). There is one group formed by cooling treatment samples and another group formed by the remaining variables. In this last group, a differentiation is seen depending on the tartaric treatment applied, but the samples are close enough to consider there is no such significant difference between them. With the information provided by the loadings plot (Fig. 14b) it is possible to affirm that the cooling stabilization treatment, which has the lowest values on aromatic compounds and the highest values on off-flavours, is aggressive regarding the aromatic profile of wine, as it removes the desired flavours and preserves the off-flavours. On the other hand, the group formed by the remaining variables shows quite the opposite, they tend to preserve positive aromas and remove off-flavours.



Figure 14a. Scores plot of aromatic compounds content variables for Verdejo samples. Figure 14b. Loadings plot of aromatic compounds content variables for Verdejo samples. (PC₁ explains a 58% of total data variance while PC₂ explains a 42%)

In the case of the Xarel·lo variety, from the scores plot (Fig. 15a) built it is difficult to get conclusive information. The scores plot shows a group containing all treatment samples, which means that for this variety the treatments applied have barely an effect on the aromatic profile.



Figure 15a. Scores plot of aromatic compounds content variables for Xarel·lo samples. Figure 15b. Loadings plot of aromatic compounds content variables for Xarel·lo samples. (PC₁ explains a 97% of total data variance while PC₂ explains a 3%)

As a general observation from the results of the three varieties studied, it is possible to affirm that the mannoprotein treatment is the best treatment in order to preserve aromas in general, but even more on those that are positive to wine. Then, carboxymethyl cellulose and potassium bitartrate treatments have medium content values on aromatic compounds and off-flavours, a fact that makes to think that they have no effect on the aromatic profile. Finally, the cooling treatment could be considered as the worst treatment because it cannot eliminate off-flavours.

Two-factor ANOVA

By looking at the two-factor ANOVA test (Tab. 3), the same tendency described in the PCA plots is observed. In general, it can be seen that tartaric treatments tend to have a significant influence on most of the variable values, while for protein treatments there are just a few variables that show significant differences. Concerning interaction, it can be seen that, in some cases, differences are significant; however, it is not a general trend as it is seen for tartaric treatments. This could be due to that replicates of each sample are experimental and not analytical, so it is possible that the replicate values differ between them.

Table 3. Two factor ANOVA results for all varieties studied. Lines marked in yellow mean that there are significant differences at α =0,05 level.

		Sau	vignon B	lanc		Verdejo			Xarel·lo	
	aviable	F	Р	F	F	Р	F	E	Р	F
v	ariable	value	value	critical	value	value	critical	F value	value	critical
	Tartaric	6,209	0,002	2,901	11,278	0,000	2,901	77,869	0,000	2,901
рН	Protein	0,201	0,895	2,901	1,946	0,142	2,901	1,662	0,195	2,901
	Interaction	0,633	0,760	2,189	1,088	0,399	2,189	2,344	0,037	2,189
	Tartaric	9,206	0,000	2,901	24,572	0,000	2,901	31,260	0,000	2,901
TA	Protein	2,797	0,056	2,901	1,665	0,194	2,901	2,075	0,123	2,901
	Interaction	2,585	0,023	2,189	2,375	0,035	2,189	2,248	0,045	2,189
	Tartaric	15,690	0,000	2,901	8,900	0,000	2,901	43,324	0,000	2,901
ABV	Protein	1,624	0,203	2,901	3 <i>,</i> 356	0,031	2,901	6,097	0,002	2,901
	Interaction	0,903	0,534	2,189	3,070	0,009	2,189	4,932	0,000	2,189
	Tartaric	2,714	0,061	2,901	2,530	0,075	2,901	20,317	0,000	2,901
VA	Protein	0,822	0,491	2,901	2,641	0,066	2,901	2,486	0,078	2,901
	Interaction	2,142	0,055	2,189	2,379	0,034	2,189	1,154	0,356	2,189
	Tartaric	10,134	0,000	2,901	10,326	0,000	2,901	26,405	0,000	2,901
FI	Protein	1,693	0,188	2,901	0,686	0,567	2,901	4,598	0,009	2,901
	Interaction	0,636	0,758	2,189	0,558	0,820	2,189	1,472	0,200	2,189
	Tartaric	45,215	0,000	2,901	31,307	0,000	2,901	29,794	0,000	2,901
CI	Protein	0,975	0,417	2,901	0,896	0,454	2,901	1,815	0,164	2,901
	Interaction	0,939	0,506	2,189	0,372	0,940	2,189	2,492	0,028	2,189
	Tartaric	14,312	0,000	2,901	17,849	0,000	2,901	30,410	0,000	2,901
Ton.	Protein	0,595	0,623	2,901	1,521	0,228	2,901	7,623	0,001	2,901
	Interaction	1,052	0,423	2,189	0,346	0,952	2,189	4,045	0,002	2,189
	Tartaric	57,634	0,000	2,901	1,505	0,232	2,901	31,675	0,000	2,901
L*	Protein	0,699	0,560	2,901	1,246	0,309	2,901	1,438	0,250	2,901
	Interaction	0,992	0,466	2,189	0,922	0,519	2,189	2,108	0,059	2,189
	Tartaric	58,563	0,000	2,901	23,749	0,000	2,901	32,596	0,000	2,901
a*	Protein	0,324	0,808	2,901	0,537	0,661	2,901	0,697	0,561	2,901
	Interaction	0,957	0,492	2,189	0,270	0,978	2,189	1,175	0,343	2,189
	Tartaric	19,067	0,000	2,901	27,860	0,000	2,901	23,451	0,000	2,901
b*	Protein	0,467	0,707	2,901	1,444	0,248	2,901	4,019	0,016	2,901
	Interaction	0,563	0,817	2,189	0,566	0,814	2,189	1,305	0,273	2,189
	Tartaric	6,705	0,001	2,901	0,435	0,730	2,901	109,339	0,000	2,901
Phen.	Protein	1,473	0,240	2,901	2,768	0,058	2,901	35,683	0,000	2,901
	Interaction	1,141	0,364	2,189	1,024	0,442	2,189	4,971	0,000	2,189
	Tartaric	19,748	2E-07	2,901	7,747	5E-04	2,901	1,339	0,279	2,901
Ar.	Protein	0,491	0,691	2,901	0,704	0,557	2,901	0,896	0,454	2,901
AI.	Interaction	0,699	0,705	2,189	0,401	0,925	2,189	0,820	0,602	2,189
	Tartaric	0,923	0,441	2,901	10,716	5E-05	2,901	0,718	0,548	2,901
Off.	Protein	0,591	0,626	2,901	0,277	0,842	2,901	1,331	0,281	2,901
о <u>л</u> .	Interaction	0,433	0,907	2,189	0,587	0,798	2,189	0,906	0,532	2,189

For Sauvignon Blanc samples, it is clearly seen that there is more predominance of the tartaric treatments than the protein treatments. By observing the chemical variables group, it can be seen that there exist significant differences between tartaric treatments, with the exception of the volatile acidity variables. Regarding colour variables, in all of them significant differences on the tartaric treatments factor as well as phenolic compounds content variables are seen. Regarding fermentative aroma variables, just aromatic compounds show significant differences, while for the off-flavours content there is not a tendency, fact that was also observed in the PCA analysis.

For the Verdejo variety, results are a bit different compared to Sauvignon Blanc. Chemical variables all have significant differences for the tartaric treatment factor, with the exception of volatile acidity. The colour variables group seems to have variability on the value of its variables, with the exception of brightness. The phenolic acid content is not significant for none of the treatments applied, while on the fermentative aroma variables case there are significant difference on tartaric treatments for aromas an off-flavours.

Lastly, for the Xarel-lo variety, the same tendency described above is observed. All variables have significant differences for tartaric treatments, with the exception of aromatic compounds that do not show any significant differences, a fact that was also observed in the PCA plots for fermentative aroma variables.

On the three varieties studied, significant differences are also observed when analyzing protein treatments and interaction between the two types of treatments applied. They do not follow a clear tendency as the one seen on the tartaric treatment factor. However, this can be due to that the replicates carried out are experimental and not analytical, and deviations between replicates of each sample exist. This could explain the dispersion observed on some PCA plots for some sample groups subjected to a same tartaric treatment.

MANOVA

In general, MANOVA results confirm the results observed with PCA and ANOVA tests.

Multivariate analysis

By looking at the results of *Wilk's Lambda* multivariate test with all variables and for Sauvignon blanc samples (Tab. 4), it can be seen that the treatments applied have a significant influence on data variation and also show that there exists an interaction between treatments. For more accuracy, the *Wilk's Lambda* test was also performed for each variable set described above.

The same tendency is observed in the chemical variables set, which shows that there are significant differences when applying protein and tartaric treatments and a significant presence of interaction between them. On the other hand, colour variables and phenolic content variables sets explain that just tartaric treatments have influence on the variance of the data, with no interaction observed.

	Effect	F calc.	F tab.	Degrees of freedom	Significance
	Prot.	0,031	3,419	39	0
All variables	Tart.	0	22,171	39	0
	Int.	0,003	1,667	117	0,001
Chamies I	Prot.	0,374	2,888	12	0,002
Chemical	Tart.	0,103	8,725	12	0
variables	Int.	0,118	2,353	36	0
Colour	Prot.	0,593	0,866	18	0,619
Colour	Tart.	0,023	12,031	18	0
variables	Int.	0,249	0,826	54	0,788
Phenolic	Prot.	0,743	1,653	6	0,148
content	Tart.	0,347	7,208	6	0
variables	Int.	0,653	0,817	18	0,674
Fermentative	Prot.	0,872	0,733	6	0,625
aromas	Tart.	0,263	9 <i>,</i> 798	6	0
content variables	Int.	0,727	0,596	18	0,889

Table 4. *Wilk's Lambda* test results for Sauvignon Blanc samples and for the different sets: all variables, chemical variables, colour variables, phenolic content and fermentative aromas.

Regarding Verdejo samples, the results from the *Wilk's Lambda* test (tab. 5) show that, in general, there is a significant influence of the effects on the data. Looking at the variables sets separately, for the all variables set there is a significant influence of protein and tartaric treatments, but there is no interaction observed. Looking just at the chemical variables, it is observed that protein and tartaric treatments have significant influence on the data values, existing also an interaction between them. For colour and phenolic content variables sets there is a significant influence for tartaric treatments but not for protein treatments nor interaction.

Table 5. *Wilk's Lambda* test results Verdejo samples and for the different sets: all variables, chemical variables, colour variables, phenolic content and fermentative aroma content.

	Effect	F calc.	F tab.	Degrees of freedom	Significance
	Prot.	0,136	1,478	39	0,085
All variables	Tart.	0,001	14,876	39	0
	Int.	0,015	1,051	117	0,383
Chamical	Prot.	0,472	2,109	12	0,026
variables	Tart.	0,048	13,863	12	0
variables	Int.	0,166	1,888	36	0,006
Colour	Prot.	0,549	1,008	18	0,461
variables	Tart.	0,055	7,604	18	0
variables	Int.	0,249	0,826	54	0,788
Phenolic	Prot.	0,75	1,601	6	0,162
content	Tart.	0,496	4,337	6	0,001
variables	Int.	0,681	0,73	18	0,767
Fermentative	Prot.	0,898	0,573	6	0,750
aromas	Tart.	0,224	11,476	6	0
content variables	Int.	0,715	0,630	18	0,862

Xarel·lo samples showed similar results as the described above. The results of the multivariate test (tab. 6) show a total influence of both kind of treatments applied and interaction between them, with the exception of fermentative aroma variables, which are only significant when analysing the tartaric effect.

	Effect	F calc.	F tab.	Degrees of freedom	Significance
	Prot.	0,014	4,962	39	0
All variables	Tart.	0	22,385	39	0
	Int.	0,001	2,061	117	0
Chamical	Prot.	0,33	3,343	12	0,001
variables	Tart.	0,01	30,342	12	0
variables	Int.	0,123	2,295	36	0,001
Colour	Prot.	0,144	4,211	18	0
variables	Tart.	0,017	13,771	18	0
variables	Int.	0,039	2,34	54	0
Phenolic	Prot.	0,185	13,717	6	0
content	Tart.	0,029	49,966	6	0
variables	Int.	0,282	3,043	18	0,001
Fermentative	Prot.	0,778	1,385	6	0,235
aromas	Tart.	0,392	6,171	6	0
content variables	Int.	0,629	0,900	18	0,581

Table 6. *Wilk's Lambda* test results for Xarel·lo samples and for the different sets: all variables, chemical variables, colour variables, phenolic content and fermentative aroma content.

As a general observation, what has been described so far has been seen repeatedly in the tests performed. The fact that tartaric treatments have more influence on the data variance than protein ones is noticed by obtaining positive results on all variable sets studied.

The fact that for protein treatments and interaction effects significant results are obtained in given situations could be explained again as an error associated to the differences between replicates of the same sample. Particularly, for Xarel-lo samples these positive results for the protein treatments and interaction effects seem not to be false positive results, as they are obtained with enough frequency.

Inter-subject test

An inter-subject test (tab. 7) was performed in order to analyse the variables separately and determine whether the effect of protein and tartaric treatments and the interaction between them were significant. The results showed, in a general way, that protein treatments had null effect on data. By looking at the results of the tartaric treatments, it can be seen that the samples have significant differences on the values of all variables studied, with the exception of volatile acidity that appears as non-significant. There was no interaction observed for most of the variables studied, except for tartaric acidity. In general, the result of the inter-subject test is the same as the obtained in the ANOVA test for the Sauvignon blanc samples.

		Protein			Tartaric		Interaction			
Dependent variable	DF	F	Sig.	DF	F	Sig.	DF	F	Sig.	
рН	3	0,201	0,895	3	6,209	0,002	9	0,633	0,760	
ТА	3	2,797	0,056	3	9,206	0	9	2,585	0,023	
ABV	3	1,624	0,203	3	15,690	0	9	0,903	0,534	
VA	3	0,822	0,491	3	2,714	0,061	9	2,142	0,055	
FI	3	1,720	0,183	3	10,139	0	9	0,638	0,756	
CI	3	0,975	0,417	3	45,215	0	9	0,939	0,506	
Ton.	3	0,615	0,611	3	14,381	0	9	1,028	0,439	
L*	3	0,699	0,560	3	57,634	0	9	0,992	0,466	
a*	3	0,324	0,808	3	58,563	0	9	0,957	0,492	
b*	3	0,467	0,707	3	19,067	0	9	0,563	0,817	
Phen.	3	1,450	0,247	3	6,668	0,001	9	1,143	0,363	
Ar.	3	0,491	0,691	3	6,668	0,001	3	0,699	0,705	
Off	3	0,590	0,626	3	19,748	0	3	0,433	0,907	

Table 7. Inter-subject test results for Sauvignon Blanc samples.

The inter-inter subject test for Verdejo samples (tab. 8) did not show very relevant information in comparison with the results obtained for the Sauvignon Blanc samples. For the protein treatment results, just alcohol content variable appears as significant and having a significant interaction effect. For tartaric samples, the phenolic content and the fermentative aroma variables appear to be significant. Total acidity as well as volatile acidity variables are influenced by the interaction of the two effects studied. The result are very similar to the ones obtained with PCA, where for Verdejo samples the scores plots did not show widely differentiated groups, as it was seen for Sauvignon blanc and Xarel·lo samples. However, different groups were observed when analysing the fermentative aroma content variables.

		Protein			Tartaric		Interaction			
Dependent variable	DF	F	Sig.	DF	F	Sig.	DF	F	Sig.	
рН	3	1,946	0,142	3	11,278	0,514	9	1,088	0,399	
ΤΑ	3	1,665	0,194	3	24,572	0,697	9	2,375	0,035	
ABV	3	3,356	0,031	3	8,900	0,455	9	3,070	0,009	
VA	3	2,641	0,066	3	2,530	0,192	9	2,379	0,034	
FI	3	0,696	0,562	3	10,229	0,490	9	0,553	0,824	
CI	3	0,896	0,454	3	31,307	0,746	9	0,372	0,940	
Ton.	3	1,510	0,231	3	17,958	0,627	9	0,343	0,953	
L*	3	1,246	0,309	3	1,505	0,124	9	0,922	0,519	
a*	3	0,537	0,661	3	23,749	0,690	9	0,270	0,978	
b*	3	1,444	0,248	3	27,860	0,723	9	0,566	0,814	
Phen.	3	2,768	0,058	3	,433	0,039	9	1,027	0,440	
Ar.	Ar. 3		0,557	3	7,747	0,001	9	0,401	0,925	
Off	3	0,277	0,842	3	10,713	0	9	0,587	0,798	

Table 8. Inter-subject test results for Verdejo samples.

The inter-subject test for Xarel·lo (tab. 10) showed similar results as the obtained with ANOVA and PCA. Protein treatments have significant influence on the value of ABV, Folín index, tonality, phenolic content and b* variables. All the variables were influenced significantly by the effect of tartaric treatments, with the exception of the fermentative aroma variables, which were not significant. The interaction effect was observed too for pH, total acidity, alcohol content, colour intensity, tonality and phenolic content variables.

		Protein			Tartaric			Interaction	on
Dependent variable	DF	F	Sig.	DF	F	Sig.	DF	F	Sig.
pН	3	1,662	0,195	3	77,869	0	9	2,344	0,037
ΤΑ	3	2,075	0,123	3	31,260	0	9	2,248	0,045
ABV	3	6,097	0,002	3	43,324	0	9	4,932	0
VA	3	2,486	0,078	3	20,317	0	9	1,154	0,356
FI	3	4,576	0,009	3	26,285	0	9	1,474	0,200
CI	3	1,815	0,164	3	29,794	0	9	2,492	0,028
Ton.	3	7,563	0,001	3	30,338	0	9	4,085	0,001
L*	3	1,438	0,250	3	31,675	0	9	2,108	0,059
a*	3	0,697	0,561	3	32,596	0	9	1,175	0,343
b*	3	4,019	0,016	3	23,451	0	9	1,305	0,273
Phen.	3	37,087	0	3	112,122	0	9	5,207	0
Ar.	3	0,896	0,454	3	1,339	0,279	9	0,820	0,602
Off	3	1,331	0,281	3	0,718	0,548	9	0,906	0,532

Table 10. Inter-subject test results for Xarel·lo samples.

Finally, as a general observation, it can be seen again the influence of the tartaric treatments on the data variations, a fact that is confirmed by the similarity between the results obtained with PCA, ANOVA and *Wilk's lambda* tests. The fact that in the Xarel·lo case positive results for protein treatments and interaction effect are obtained, makes us thinking that, for this particular grape variety, there are significant differences caused by the interaction of both treatments, which are not false positive as was mentioned in other cases. This could be explained due to the Xarel·lo chemical composition, which allows protein stability treatments show its effect.

Post hoc analysis: Multiple comparisons test

A post hoc analysis was carried out using a *HSD Tukey* test (tab. 11 and 12) to look for significant differences in a factor. This test compares data from all variables with one of the treatments. The results of *HSD Tukey* test for protein treatment samples and for the Sauvignon blanc (tab. 11) variety confirmed that these treatments have a barely effect on variable values, without any positive result observed.

On the contrary, the *HSD Tukey* test for tartaric samples of the Sauvignon blanc (tab. 11) variety showed the opposite result. When comparing the samples submitted to tartaric treatments some significant differences can be observed when compared to the treatment with potassium bitartrate, which brings positive results for all colour variables. That is, when all treatments are compared with potassium bitartrate is when significant differences are observed, and these shown up in the colour variables (*FI*, *CI*, *Ton.*, *L**, *a** and *b**). Also, when comparing with carboxymethyl cellulose samples positive results are seen, especially in the chemical variables. Some other significant differences are seen when comparing cooling samples with mannoprotein samples for the aromatic compounds content.

		pН	ΤΑ	ABV	VA	FI	CI	Ton.	L*	a*	b*	Phen.	Ar.	Off
	bc	0,928	0,347	0,874	0,876	0,425	0,727	0,872	0,509	0,965	0,998	0,391	0,820	0,869
b	р	0,986	0,090	0,193	0,448	0,980	0,950	0,903	0,869	0,957	0,692	0,986	0,997	0,986
	pv	0,893	0,067	0,413	0,982	0,908	0,937	0,979	0,974	0,759	0,969	0,368	0,991	0,893
	b	0,928	0,347	0,874	0,876	0,425	0,727	0,872	0,509	0,965	0,998	0,391	0,820	0,869
bc	р	0,993	0,875	0,575	0,876	0,660	0,957	1,000	0,920	1,000	0,791	0,592	0,912	0,687
	pv	1,000	0,811	0,849	0,982	0,142	0,380	0,661	0,764	0,955	0,992	1,000	0,652	1,000
	b	0,986	0,090	0,193	0,448	0,980	0,950	0,903	0,869	0,957	0,692	0,986	0,997	0,986
р	bc	0,993	0,875	0,575	0,876	0,660	0,957	1,000	0,920	1,000	0,791	0,592	0,912	0,687
	pv	0,983	0,999	0,963	0,676	0,716	0,682	0,707	0,986	0,963	0,916	0,566	0,957	0,721
	b	0,893	0,067	0,413	0,982	0,908	0,937	0,979	0,974	0,759	0,969	0,368	0,991	0,893
pv	bc	1,000	0,811	0,849	0,982	0,142	0,380	0,661	0,764	0,955	0,992	1,000	0,652	1,000
	р	0,983	0,999	0,963	0,676	0,716	0,682	0,707	0,986	0,963	0,916	0,566	0,957	0,721

Table 11. Sauvignon Blanc multiple comparisons HSD Tukey test results for protein samples.

Table 12. Sauvignon Blanc multiple comparisons HSD Tukey test results for tartaric samples.

		pН	ΤΑ	ABV	VA	FI	CI	Ton.	L*	a*	b*	Phen.	Ar.	Off
	С	0,019	0,001	0,000	0,300	0,034	0,000	0,000	0,000	0,000	0,000	0,894	0,941	0,625
b	f	0,966	0,999	0,947	0,732	0,135	0,000	0,000	0,000	0,000	0,000	0,003	0,692	0,981
	m	0,928	0,815	0,888	0,982	0,000	0,000	0,014	0,000	0,000	0,000	0,028	0,000	0,995
	b	0,019	0,001	0,000	0,300	0,034	0,000	0,000	0,000	0,000	0,000	0,894	0,941	0,625
С	f	0,006	0,000	0,000	0,039	0,920	0,273	0,994	1,000	0,797	0,746	0,017	0,358	0,396
	m	0,004	0,006	0,000	0,504	0,064	0,971	0,074	0,217	0,304	0,994	0,131	0,000	0,769
	b	0,966	0,999	0,947	0,732	0,135	0,000	0,000	0,000	0,000	0,000	0,003	0,692	0,981
f	С	0,006	0,000	0,000	0,039	0,920	0,273	0,994	1,000	0,797	0,746	0,017	0,358	0,396
	m	0,999	0,749	0,590	0,504	0,014	0,506	0,125	0,227	0,052	0,874	0,802	0,000	0,923
	b	0,928	0,815	0,888	0,982	0,000	0,000	0,014	0,000	0,000	0,000	0,028	0,000	0,995
m	С	0,004	0,006	0,000	0,504	0,064	0,971	0,074	0,217	0,304	0,994	0,131	0,000	0,769
	f	0,999	0,749	0,590	0,504	0,014	0,506	0,125	0,227	0,052	0,874	0,802	0,000	0,923

The *post hoc* analysis results for Verdejo samples (tab. 13 and 14) showed determinant information for the study. First, the results obtained for protein treatment effect (tab. 13) showed that there are significant differences between vegetal protein treatment and bentonite + isinglass for the *ABV* variable. The remaining comparisons of treatments were no significant. This fact confirms that there are not big differences between protein treatments, which evidences their lack of effect on the data.

The results observed in the test carried out for the tartaric effect (tab. 14) showed a different effect as the observed before on protein samples. The comparison among the different tartaric samples showed several significant differences between them. Carboxymethyl cellulose and potassium bitartrate treatments seem to be the more different treatments, as the comparison between them tends to be significant on almost all variables. For potassium bitartrate samples, there exist significant differences when comparing colour variables with the other tartaric treatments studied. Carboxymethyl cellulose is significantly different from the remaining tartaric treatments when comparing them for the chemical variables. Finally, mannoprotein and cooling treatments are significantly different when focusing just on fermentative aromas. Mannoprotein seems to be different from the other treatments for aromatic compounds content, while cooling treatment seem to be different just for the off-flavours content.

		рН	ΤΑ	ABV	VA	FI	CI	Ton.	L*	a*	b*	Phen.	Ar.	Off
	bc	0,256	0,206	0,953	1,000	1,000	0,664	0,430	0,432	0,668	0,686	0,997	0,551	0,992
b	р	0,983	0,294	0,093	0,120	1,000	0,584	0,385	0,387	0,764	0,481	0,674	0,747	0,944
	pv	0,983	0,419	0,992	1,000	0,624	0,451	0,228	0,399	0,777	0,202	0,066	0,674	0,989
	b	0,256	0,206	0,953	1,000	1,000	0,664	0,430	0,432	0,668	0,686	0,997	0,551	0,992
bc	р	0,133	0,996	0,029	0,128	1,000	0,999	1,000	1,000	0,998	0,987	0,795	0,988	0,992
	pv	0,441	0,968	0,849	1,000	0,641	0,985	0,975	1,000	0,998	0,800	0,103	0,997	0,934
	b	0,983	0,294	0,093	0,120	1,000	0,584	0,385	0,387	0,764	0,481	0,674	0,747	0,944
р	bc	0,133	0,996	0,029	0,128	1,000	0,999	1,000	1,000	0,998	0,987	0,795	0,988	0,992
	pv	0,884	0,995	0,163	0,112	0,669	0,996	0,987	1,000	1,000	0,939	0,475	0,999	0,822
	b	0,983	0,419	0,992	1,000	0,624	0,451	0,228	0,399	0,777	0,202	0,066	0,674	0,989
pv	bc	0,441	0,968	0,849	1,000	0,641	0,985	0,975	1,000	0,998	0,800	0,103	0,997	0,934
	р	0,884	0,995	0,163	0,112	0,669	0,996	,987	1,000	1,000	0,939	0,475	0,999	0,822

Table 13. Verdejo multiple comparasions HSD Tukey test results for protein samples.

Table 14. Verdejo multiple comparasions HSD Tukey test results for tartaric samples.

		рН	ΤΑ	ABV	VA	FI	CI	Ton.	L*	a*	b*	Phen.	Ar.	Off
	С	0,001	0,001	0,002	0,412	0,156	0,000	0,000	0,414	0,000	0,000	0,818	0,458	0,256
b	f	0,256	0,256	0,908	0,673	0,000	0,000	0,000	0,507	0,000	0,000	0,947	0,224	0,012
	m	0,662	0,662	0,992	0,993	0,001	0,000	0,001	1,000	0,000	0,000	0,714	0,000	0,573
С	b	0,001	0,001	0,002	0,412	0,156	0,000	0,000	0,414	0,000	0,000	0,818	0,458	0,256
	f	0,097	0,097	0,000	0,051	0,036	0,374	0,193	0,999	1,000	0,017	0,988	0,964	0,000
	m	0,000	0,000	0,004	0,278	0,170	0,901	0,053	0,396	0,315	0,387	0,998	0,015	0,935
	b	0,256	0,256	0,908	0,673	0,000	0,000	0,000	0,507	0,000	0,000	0,947	0,224	0,012
f	С	0,097	0,097	0,000	0,051	0,036	0,374	0,193	0,999	1,000	0,017	0,988	0,964	0,000
	m	0,024	0,024	0,776	0,822	0,880	0,780	0,919	0,488	0,291	0,422	0,955	0,045	0,000
	b	0,662	0,662	0,992	0,993	0,001	0,000	0,001	1,000	0,000	0,000	0,714	0,000	0,573
m	С	0,000	0,000	0,004	0,278	0,170	0,901	0,053	0,396	0,315	0,387	0,998	0,015	0,935
	f	0,024	0,024	0,776	0,822	0,880	0,780	0,919	0,488	0,291	0,422	0,955	0,045	0,000

The *post hoc* analysis test for Xarel·lo samples (tab. 15 and 16) showed some significant results on the test carried out for the protein effect (tab. 15), mostly on the values of *ABV*, *FI* tonality b^* and phenol content. Contrary to Verdejo and Sauvingnon Blanc samples, Xarel·lo got more significant results in the *HSD Tukey* test for the protein effect. These results agree with the tests carried out until this point of the investigation. The protein effect seems to have a significant effect on data variation just for the Xarel·lo samples.

The results obtained in the *HSD Tukey* test for the tartaric effect (tab. 16) showed that there exist big differences among these treatments. Many significant results were obtained, and most of them came from the comparison with potassium bitartrate and carboximethyl cellulose treatments, which gave positive results for the colour and chemical variables sets, respectively. Regarding cooling and mannoprotein treatments, they seem not having a significant influence. This evidences their difference with potassium bitartrate and carboxymethyl cellulose. At the same time carboxymethyl cellulose and potassium bitartrate are far away from each other as when compared between them they show significant differences in almost all variables studied.

		рН	ΤΑ	ABV	VA	FI	CI	Ton.	L*	a*	b*	Phen.	Ar.	Off
b	bc	0,438	0,372	0,885	0,621	0,837	0,599	0,992	0,738	0,958	0,588	0,998	0,683	0,380
	р	0,155	0,096	0,002	0,862	0,994	0,880	0,610	0,922	0,597	0,878	0,884	0,982	0,998
	pv	0,713	0,742	0,118	0,366	0,012	0,127	0,014	0,201	0,656	0,084	0,000	0,997	0,993
	b	0,438	0,372	0,885	0,621	0,837	0,599	0,992	0,738	0,958	0,588	0,998	0,683	0,380
bc	р	0,916	0,868	0,016	0,210	0,935	0,956	0,774	0,979	0,875	0,204	0,797	0,457	0,294
	pv	0,968	0,923	0,405	0,972	0,085	0,741	0,007	0,751	0,913	0,631	0,000	0,565	0,537
	b	0,155	0,096	0,002	0,862	0,994	0,880	0,610	0,922	0,597	0,878	0,884	0,982	0,998
р	bc	0,916	0,868	0,016	0,210	0,935	0,956	0,774	0,979	0,875	0,204	0,797	0,457	0,294
	pv	0,691	0,512	0,383	0,091	0,022	0,433	0,001	0,512	1,000	0,015	0,000	0,998	0,971
	b	0,713	0,742	0,118	0,366	0,012	0,127	0,014	0,201	0,656	0,084	0,000	0,997	0,993
pv	bc	0,968	0,923	0,405	0,972	0,085	0,741	0,007	0,751	0,913	0,631	0,000	0,565	0,537
	р	0,691	0,512	0,383	0,091	0,022	0,433	0,001	0,512	1,000	0,015	0,000	0,998	0,971

Table 15. Xarel·lo multiple comparisons HSD Tukey test results for protein samples.

Table 16. Xarel·lo multiple comparisons HSD Tukey test results for tartaric samples.

		рН	ΤΑ	ABV	VA	FI	CI	Ton.	L*	a*	b*	Phen.	Ar.	Off
b	С	0,000	0,000	0,000	0,000	0,714	0,000	0,000	0,000	0,000	0,000	0,000	1,000	1,000
	f	0,298	0,298	0,161	0,008	0,000	0,000	0,279	0,000	0,000	0,000	0,000	0,999	0,717
	m	0,960	0,960	0,405	0,972	0,000	0,000	0,026	0,000	0,000	0,000	0,044	0,340	0,759
с	b	0,000	0,000	0,000	0,000	0,714	0,000	0,000	0,000	0,000	0,000	0,000	1,000	1,000
	f	0,000	0,000	0,000	0,035	0,000	1,000	0,000	0,512	0,082	0,162	0,000	1,000	0,699
	m	0,000	0,000	0,000	0,000	0,000	0,959	0,000	0,554	0,952	0,969	0,000	0,374	0,742
	b	0,298	0,298	0,161	0,008	0,000	0,000	0,279	0,000	0,000	0,000	0,000	0,999	0,717
f	С	0,000	0,000	0,000	0,035	0,000	1,000	0,000	0,512	0,082	0,162	0,000	1,000	0,699
	m	0,124	0,124	0,004	0,002	0,987	0,932	0,659	1,000	0,025	0,346	0,000	0,413	1,000
	b	0,960	0,960	0,405	0,972	0,000	0,000	0,026	0,000	0,000	0,000	0,044	0,340	0,759
m	С	0,000	0,000	0,000	0,000	0,000	0,959	0,000	0,554	0,952	0,969	0,000	0,374	0,742
	f	0,124	0,124	0,004	0,002	0,987	0,932	0,659	1,000	0,025	0,346	0,000	0,413	1,000

In general, the results observed in the multiple comparison tests completely agree with the ones obtained in the PCA analysis. First of all, it is possible to say that protein treatments have a null effect on data variation, as the exception of Xarel-Io variety that has some significant effect on the value of some variables, although not as much as the tartaric treatments. Secondly, by comparing the tartaric treatment effects we can observe are seen differences that coincide with the groups formed in the PCA scores plots. At this point of the study, it is possible to confirm that chemical variables are more influenced by carboxymethyl cellulose treatments, colour variables are more influenced by potassium bitartrate treatments and, for Sauvignon blanc and Verdejo varieties, cooling stabilization has influence on off-flavour compounds content and mannoprotein has influence on aromatic compounds content.

Quality index determination

The results obtained in the determination of *QI* for the Sauvignon Blanc samples (tab. 17) show that the best treatments to apply are the mannoprotein ones, especially those combined with PVPP, bentonite + isinglass and bentonite, which show the higher values, about 7 points out of 10. These treatments are followed by cooling stabilization and potassium bitartrate ones, with very similar scores, between 6 and

5 out of 10. Finally, carboxymethyl cellulose treatments are the worst treatments to assure protein and tartaric stability in Sauvignon Blanc wines, getting the lowest scoring, especially those combined with vegetal protein, bentonite + isinglass and bentonite, which provide values below 5 points out of 10.

	pН	ΤΑ	L*	a*	b*	Ar.	Off	QI
pv.m	9,77	1,63	9,87	2,85	8,65	9,04	6,19	7,03
bc.m	9,82	1,48	9,83	2,85	9,13	8,9	5,99	7,02
b.m	9,76	1,68	9,85	2,62	7,33	10	6,09	7
b.f	9,76	2,01	9,88	2,29	9,02	1,08	8,26	5,87
pv.b	9,78	1,92	9,53	8,91	0,79	2,65	7,89	5,84
bc.f	9,79	1,81	9,91	0	8,86	0	10	5,66
p.m	9,77	1,65	9,83	3,29	7,6	7,07	0,7	5,46
bc.b	9,78	1,84	9,46	10	0	1,63	6,65	5,41
pv.c	9,95	0	9,93	2,27	6,85	3,49	5,66	5,37
p.b	9,78	1,76	9,59	9,06	1,36	1,09	6,27	5,3
pv.f	9,8	1,78	9,92	1,92	10	0,88	3,94	5 <i>,</i> 03
p.f	9,78	1,67	9,92	0,33	8,33	0,5	4,12	4,55
b.b	9,84	1,65	9 <i>,</i> 63	7,54	2,97	2,17	1,02	4,47
p.c	9,91	0,36	9,86	2,01	6	5,06	0,25	4,47
bc.c	9,88	0,94	9,91	1,76	9,6	0,15	1,71	4,29
b.c	9,84	2,15	9,93	0,91	9,09	1,45	0	4,15

Table 17. Quality index calculation for Sauvignon blanc samples. The results are sorted from the highest to the lowest value obtained.

For Verdejo samples (Tab. 18) similar results were obtained. All samples treated with mannoproteins are in the top of the ranking, with the highest values higher than 7, so they are supposed to be the more suitable treatments for Verdejo wines. Contrary to Sauvignon Blanc samples, carboxymethyl cellulose has the second position in the raking, with scores from 5.98 to 6.48. The last position corresponds to potassium bitartrate samples and those treated by cooling stabilization, showing very similar *QI* results.

	pН	ΤΑ	L*	a*	b*	Ar.	Off	QI
p.m	9,93	1,80	9,89	2,90	8,24	10,00	8,28	7,63
bc.m	9,88	1,82	9,87	3,55	7,74	8 <i>,</i> 85	8,60	7,48
b.m	9,92	1,69	8,85	4,02	8,53	9,15	8,01	7,45
pv.m	9,92	1,75	9,90	2,74	8,69	8,34	8,13	7,31
b.c	9,90	0,00	9,91	1,73	6,26	7,34	8,19	6,48
pv.c	9,97	0,97	9,93	1,57	7,48	3,74	9,05	6,18
p.c	9,92	1,21	9,93	0,76	7,18	2,41	10,00	5,98
b.b	9,92	1,87	9,58	10,00	0,00	1,50	8,71	5,83
pv.b	9,94	1,81	9,65	8,54	4,16	1,35	5,94	5,61
bc.c	9,94	1,12	9,91	2,20	7,13	0,58	9,06	5,60
bc.f	9,95	1,81	9,89	0,00	9,29	5,26	4,07	5,60
p.b	9,93	1,62	9,66	8,56	3,33	0,97	6,42	5,51
p.f	9,96	1,80	9,92	2,09	9,75	3,56	3,31	5,45
b.f	9,96	1,88	9,87	2,43	9,10	5,88	0,00	5,21
bc.b	9,93	1,79	9,66	7,91	3,27	0,00	4,74	4,90
pv.f	9,96	1,77	9,89	1,55	10,00	2,65	0,06	4,57

Table 18. Quality index calculation for Verdejo samples. The results are sorted from the highest to the lowest value obtained.

Finally, the Xarel·lo samples (Tab. 19) show results similar to the ones described above, but closer to those of Verdejo samples. The best treatment for Xarel·lo is PVPP in combination with caboxymethyl

cellulose. Mannoprotein and carboxymethyl cellulose tretments gave similar results, and this is why they appear mixed in the classification, unlike what happens with the Sauvignon blanc and Verdejo samples, where clear groupings were seen depending on the treatment applied. So, we may consider both as the best treatments for this variety, with *QI* values from 5.68 to 6.06. In the third place, we find the potassium bitartrate samples, with values from 5.36 to 5.57, and lastly we find the cooling stabilization treatments, showing the lowest values, from 4.97 to 5.4.

	pН	ΤΑ	L*	a*	b*	Ar.	Off	QI
pv.c	9,87	1,35	9,96	3,69	8,97	0,91	8,69	6,06
b.m	9,58	0,16	9,91	3,33	7,44	2,04	9,42	6,02
pv.m	9,51	0,21	9,93	2,88	8,73	0,73	9,42	5,87
b.c	9,82	1,82	9,97	2,44	7,91	0,90	8,96	5,84
p.m	9,59	0,22	9,91	2,87	8,01	0,90	9,51	5,84
bc.m	9,54	0,05	9,89	3,11	8,05	10,00	0,00	5,77
bc.c	9,92	1,32	9,95	3,16	8,62	0,62	7,67	5,68
b.b	9,53	0,19	9,48	10,00	0,05	0,59	9,38	5,57
pv.b	9,49	0,01	9,81	5,73	6,61	0,38	7,92	5,56
bc.f	9,40	0,19	9,91	1,50	10,00	0,79	7,35	5,44
bc.b	9,49	0,00	9,69	7,30	2,94	0,56	8,45	5,41
p.b	9,58	0,19	9,59	8,62	0,00	0,44	9,36	5,36
pv.f	9,40	0,33	9,88	0,78	9,70	0,96	6,73	5,23
p.c	9,95	0,49	9,95	1,31	5,08	0,00	10,00	5,22
b.f	9,41	0,28	9,92	0,82	9,56	0,77	6,85	5,20
p.f	9,41	0,25	9,92	0,00	9,15	0,41	6,92	4,97

Table 19. Quality index calculation for Xarel·lo samples. The results are sorted from the highest to the lowest value obtained.

As a general observation, we can consider that the best treatment for all varieties are those that make use of mannoprotein. On the tests carried out, wines treated with mannoproteins stood out for their high aromatic compound content and their low content in off flavour compounds. The rest of variables had moderate values, a fact that could be translated into equilibrated wines. It's difficult to determine which of the treatments carried out would be the second best, but carboxymethyl cellulose has been demonstrated to be suitable for Xare-Io and Verdejo samples but not for Sauvignon Blanc ones. Finally the worst treatments are cooling and potassium bitartarate, probably as they are the most aggressive, as they avoid undesired flavours and colours but eliminating the desired ones as well.

Conclusions

The results obtained in this study bring the following conclusions:

Tartaric treatments are the ones having an effect on the variable values for the three wine varieties studied. PCA showed grouping by tartaric treatments when analyzing different variables sets, a fact repeated in ANOVA and MANOVA tests.

Carboxymethyl cellulose treatments are the less suitable treatments in order to reduce tartaric acid content. PCA plots for all variables and chemical variables showed that carboxymethyl cellulose samples are the ones that have the highest values on these variables and the ones giving more acidity to wine, an organoleptic factor desired in white wines but probably producing more tartarte salts precipitation.

It is possible to consider potassium bitartrate treatments as the ones that better preserve colour properties. It is the treatment giving the greener tonalities and preserving colour intensity without affecting the phenolic content. On the contrary, it produces yellow tonalities and does not bring brightness.

Mannoprotein treatments are the best ones to preserve good fermentative aromas and to remove offflavours, as they show the highest content of aromatic compounds and the lowest contents of offflavours. The cooling treatment is the less recommended treatment to keep fermentative aromas, as it reduces both off-flavours but also desired flavours.

The mannoprotein treatment could be considered as the better one for all varieties studied as it provides the best results in the *QI* determination. The second best treatment depends on the grape variety. For Sauvignon blanc samples, cooling and potassium bitartrate treated samples provide moderate results and carboxymethyl cellulose treatments provide the lowest scores. For Verdejo an Xarel·lo samples, carboxymethyl cellulose is the second more suitable treatment to be applied to wine in order to preserve protein and tartaric stability and to keeps good aromas and a good colour profile. Potassium bitartrate and cooling treatment could be considered as the worst ones for Verdejo and Xarel·lo samples.

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References

- Boqué, Ricard, and Alicia Maroto. 2004. 'El Análisis De La Varianza (ANOVA)'. Universitat Rovira i Virgili.: 1–6.
- Comfort, Shea. 2010. 'Creating a Balanced Must'. https://winemakermag.com/1013-creating-abalanced-must (August 27, 2017).
- Cubero, Juan Carlos, and Fernando Berzal. 2015. 'Estadística Descriptiva Y Preprocesamiento. Introducción a SPSS'. Universidad de Granda. Departamento de Ciencias de la Computación: 1–34. http://elvex.ugr.es/decsai/intelligent/workbook/D0 SPSS.pdf.
- Dharmadhikari, Murli. 'Methods for Tartrate Sabilization of Wine'. https://www.extension.iastate.edu/wine/methods-tartrate-stabilization-wine (August 15, 2017).
- Ferré, Joan, and Ricard Boqué Martí. 2004. 'Análisis de Componentes Principales Aplicado a La Representación de Datos Multidimensionales'. *Técnicas de laboratorio* (290): 214–19. http://dialnet.unirioja.es/servlet/articulo?codigo=866765.
- Francis, Leigh. 'Fermentation-Derived Aroma Compounds and Grape-Derived Monoterpenes'. In 15th Australian Wine Industry Technical Conference 2013,.

GAB sistemática analítica. 'Técnica Operativa de La Acidez Volatil (Método García-Tena)'.

- Guise, R. et al. 2014. 'Comparison between Different Types of Carboxylmethylcellulose and Other Oenological Additives Used for White Wine Tartaric Stabilization'. *Food Chemistry* 156: 250–57. http://dx.doi.org/10.1016/j.foodchem.2014.01.081.
- Johnson, Dallas E. *Métodos Multivariados Aplicados Al Análisis de Datos*. Internationa Thomson Publishing Company.
- Lærd Statistics. 2017a. 'Two-Way ANOVA in SPSS Statistics'. https://statistics.laerd.com/spsstutorials/two-way-anova-using-spss-statistics.php (May 3, 2017).
- Lærd Statistics. 2017b. 'Two-Way MANOVA in SPSS Statistics'. https://statistics.laerd.com/spsstutorials/two-way-manova-using-spss-statistics.php (May 3, 2017).
- Lasanta, C., and J. Gómez. 2012. 'Tartrate Stabilization of Wines'. *Trends in Food Science and Technology* 28(1): 52–59.
- López Casado, Itziar. 2014. Uso de Clarificantes de Proteína Vegetal En Vino Tinto Ecológico. https://biblioteca.unirioja.es/tfe_e/TFE000663.pdf.
- N Miller, James, and Jane C Miller. 2005. *Statistics and Chemometrics for Analytical Chemestry*. 5th ed. Essex: Pearson Education Limited.

The Australian Wine Research Institute. 'Fining Agents'. https://www.awri.com.au/industry_support/winemaking_resources/frequently_asked_questions/ fining_agents/#isinglass (August 15, 2017).