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Sugar reduction in white and red musts with nanofiltration membranes

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ABSTRACT

In recent years the alcohol content of wine increases mainly due to climate change. Moreover, at present, consumers are increasingly demanding more aromatic and less alcoholic wines, it is due to the greater social awareness in the alcohol consumption and the regulations of the alcoholic products. The aim of this work is the reduction of sugar in the grape must to obtain wines with a slight reduction of their alcoholic degree. A reduction of sugar has been by performing two successive stages of nanofiltration. To this end, we have worked with two types of musts: one from the *Verdejo* variety of white grapes and the other from red grapes of the Tinta de Toro variety. Each must has been fermented both after treatment and, to be used as control, without any filtration in order to check the effectiveness of the process. Once fermentation is completed, wide-ranging analysis have been used to study all possible changes in the characteristics of the wine from a chemical point of view. The alcohol reduction reached by the wines obtained after nanofiltration and mixing of both white and red musts has been satisfactory.

Keywords: Membrane; Nanofiltration; Fouling; Sugar reduction; Musts; Low alcohol-content wines

1. Introduction

In the last years, the production of low alcohol wines is more and more demanded. This is mainly due to the social consciousness for the moderate consumption of alcohol and the regulations of this products. This caused, for example, increasing taxes and traffic penalties. In the last years the alcohol content of wine has been increasing due to different factors, the most important being the climate change [1]. An earlier wining should affect the final wine quality, leading to more acid and less colored wines, because the phenolic maturity should not be achieved. Thus producers struggle to achieve the same levels of phenolic ripeness and tannic characteristics without an increase in alcohol content. At the same time, consumers demand more and more reduced alcohol beverages as a result of health and social concerns. Therefore the wining should be carried out in the maturity optimum and then the necessary techniques should be used to reduce the final alcohol content.

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Wine producers have used many dealcoholisation processes or methods to produce low alcohol-content wine. The most used method in the industry is the spinning cone column (SCC). SCC are used in the food industry for the separation of volatile components from liquids and slurries [2–5]. This procedure requires several steps to remove first the wine aromas and afterwards alcohol and finally the aromas are returned to the dealcoholised wine. Because it is a long and expensive process, other techniques have been used too. Some examples are: aerobic yeasts [6], thermal and distillation processes (as evaporators, distillation columns or freeze concentration) and extraction processes [7]. Evidently all these techniques are applied to wines that are a complex and delicate system that can suffer losses in its organoleptic properties.

Many membrane filtration processes have been applied to wining for a long time. Some examples are: ultrafiltration (UF) to clarify white wine from grape must [8], sugar concentration by using nanofiltration (NF) [9] and reverse osmosis (RO) [10] in musts. Reverse osmosis is also used to reduce alcohol in wines [11], but the problem is that RO membranes are permeable to both alcohol and water, an after the filtration it is necessary to add water again to the dealcoholised wine which creates legal problems in some countries where the addition of water is forbidden. Of course, the permeated water could be separated from alcohol and added back to the retained wine. In principle, this should be allowed because this water is coming from the same wine. Other membrane processes are being used to get low alcohol wine or beer, as dialysis [12,13] or pervaporation [14] or vacuum membrane distillation [15].

In this work we obtain low-alcohol wine by sugar control in winemaking. For this purpose, nanofiltration membranes have been used to reduce sugar concentration in must before fermentation. The idea of reducing the resulting content of alcohol in wine by reducing sugar in the must by membrane processes is not new, [16–18] but we propose to use two-steps nanofiltration in order to simplify the process. This will be tested by treating musts coming from two Spanish varieties of grapes, a white one (*Verdejo*) and a red one (*Tinta de Toro*).

2. Materials and methods

2.1. Pretreatment of musts

As already mentioned, two different musts have been used:

- *Verdejo* white must: The initial must was obtained by the traditional white must production method. Once the must had been clarified, the must was filtered through plate filters with pore sizes of 0.8 μm, in order to prevent rapid membrane fouling, and to make the nanofiltration easier.
- *Tinta de Toro* red must: It was obtained by drawing off, the must was then filtered first through plate filters firstly with 0.3 μm pores and then through filters with pore size of 0.8 μm, in order to limit turbidity. In this case the solid portion (which is called crushed mass and consists in the grape skins, seeds etc.) were cold-stored in airtight stainless steel tanks for addition to musts to be fermented after nanofiltration.

2.2. Musts

30 l of must have been used in both filtrations. The *Verdejo* white must, with a sugar concentration, measured by refractometry, of 209 g/l and a turbidity of 9.5 NTU and the *Tinta de Toro* red must, with a sugar concentration, measured by refractometry, of 244 g/l and a turbidity of 3.6 NTU.

2.3. Experimental procedure

The experimental set-up used for must filtration is shown in Fig. 1. The membrane is a spiral wound module of nanofiltration HL Series Thin Film Membrane (reference HL2540 FM) made and commercialized by GE Water & Process Technologies (MWCO 150–300 dalton for uncharged organic molecules; $MgSO_4$ rejection of 98%; water flux 4.55–9.76 x 10^{-12} m/Pa·s and a membrane area of 2.5 m^2). Hussain et al. [19] explained that the Desal-HL membrane is a polysulfone membrane support coated by a proprietary polyamides made by interfacial polymerization on the support and working as the active layer [19].

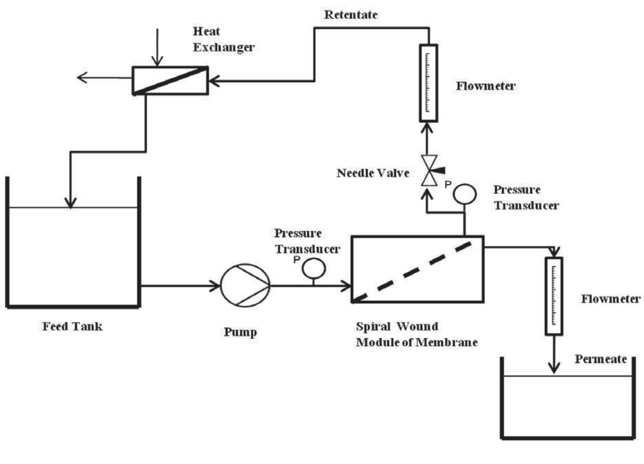

Fig. 1. Diagram of the experimental setup used for the nanofiltration of musts.

Previously to the choice of the HL membrane, a series of other membranes, in flat configuration, from GE Water & Process Technologies, were tested by using isomolecular mixtures of glucose and fructose at a total concentration of 249.58 g/l (similar to that usually found in musts) and commercial musts. Results were shown previously [20].

White must was kept at 3°C during filtration. The process was operated under 24 bar of pressure and a feed recirculation flow rate of 1 l/min. The total sugar concentrations (glucose plus fructose), both in the permeate and the retentate, have been determined by refractometry, every 30 min and the turbidity every hour. The flow in the permeate solution was measured every 30 min.

The red must was kept at 6°C. The process was operated under 24 bars of pressure and a recirculation flow rate of the feed of 1.5 l/min. The total sugar concentration, in both permeate and retentate, turbidity and flow were measured every hour. In this case, due to the high content of total solids of the red wines, along with refractometry, polarimetry has also been used to determine sugar concentration.

The differences in temperature and recirculation flow are due to the higher viscosity and thus to the more elevated friction and resistance to pumping shown by the red must.

For both musts, the way tested here as a possible method to reduce their sugar content consists of a double filtration in the steps:

1. Firstly the untreated must (T) is filtered to get a low volume of sugar rich retentate (R1) and a permeate with a medium sugar content (P1). After that, the membrane is rinsed with tap water during 2 h and with Milli-Q water during 1 h.
2. Then, the first permeate (P1) is filtered through the same membrane until the viscosity and the osmotic pressure of the retentate don't allow any ulterior reduction of the retentate volume. This process provides a retentate (R2) with a high sugar content and a second permeate (P2) with a low sugar content.

The details of the process are shown in Fig. 2.

After the filtration steps the mixing and fermentation have been produced. In the *Verdejo* must, the second permeate (P2) is mixed with the first retentate (R1) in the suitable proportions to produce the intended moderate reduction in the alcohol degree of the final wine in approximately 2 degrees. The fermentation process has been carried out in duplicate in 4 l tanks. In all cases fermentation was initiated by the inoculation of commercial yeast and at a controlled temperature. Low alcohol content wine (R1+P2) has been produced as well as the control wine (T) with the white must.

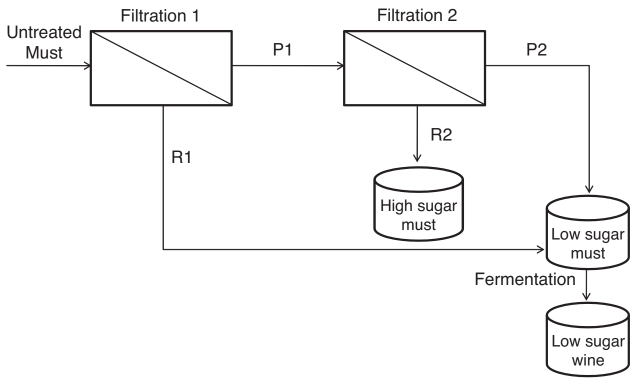

Fig. 2. Diagram of the experimental nanofiltration process.

A similar process was used for the winemaking of the *Tinta de Toro* wines but now, the fermented retentate resulting after the first filtration step (R1) and the corresponding part of the wine obtained from the fermentation of the final permeate (P2) were mixed. The R1 and P2 proportions were chosen to reduce the alcoholic content in approximately 2 degrees (as done for the white must). A 40% of crushed mass was added to the T and R1+P2 musts prior to their alcoholic fermentation. Once this fermentation was completed the crushed mass was removed and then the malolactic fermentation occurred. Each of these musts was fermented by duplicate in 35 l tanks.

Once the fermentation was completed the wines were racked and bottled for their later analysis to investigate their chemical composition and organoleptic properties. It is worth noting that several alcohol degree reductions have been obtained. To test the repeatability of these results, besides the fermentation, the analyses have been carried out in duplicate.

The performance of the process was determined by measuring the resulting permeate flux rate as a function of time, at constant and optimal conditions of pressure and recirculation flow [21]. Given that we measured the concentration of the permeate and the retentate, we can study the time evolution of the observed retention of the system, R_{obs}.

$$R_{obs}(t) = 1 - \frac{c_p}{c_o} \qquad (1)$$

where c_p is the permeate concentration and c_o is the concentration of the feed.

2.4. Cleaning

Water permeabilities were measured after and before each filtration experiment, An important reduction in permeability due to the fouling have been observed. For that reason after must filtration, the module has been cleaned in order to recover the flow. The cleaning steps consisted of: a rinsing with Milli-Q water; cleaning with a sodium dodecyl sulfate solution 0.1% w/w adjusted to pH 9 and a second rinsing with Milli-Q water. Water permeabilities were measured after and before each cleaning step too.

2.5. Must and wine analysis

A complete analysis have been made of the must and wines before and after filtrations and fermentations respectively. This analysis checks the possible changes in the properties of the must and wines obtained. The oenological parameters analyzed were determined according to the Organisation Internationale de la Vigne et du Vin (OIV) methods [22]. Specifically the glucose, fructose and the malic acid have been determinated by the enzymatic method, the tartaric acid by the colorimetric method, the potassium by atomic absorption spectroscopy and the polyphenols by UV/Vis spectrophotometry.

3. Results and discussion

3.1. Nanofiltration processes

As mentioned the total sugar concentration, in both permeate and retentate, (and consequently its retention) the turbidity and the permeate flux have been measured with time for both white and red must.

The must solution contents molecules with a wide range of sizes, in this case the small molecules penetrate into the pores of the membrane while the big molecules are deposited on the membrane surface. For this kind of solutions, the flux decrease is produced by several mechanisms [23,24]:

- The osmotic pressure increases due to the increment of the concentration of small molecules on the membrane surface (C_m) due to increased concentration in the retentate, (C_o).
- Thickening of the gel layer on the membrane surface due to the rise of the concentration of big molecules and colloids on the membrane surface (C_m).
- Increase in the viscosity of the fluid that goes through the membrane pores.
- Fouling due to the reversible or irreversible adhesion of the molecules on the membrane surface or inside the pores.

The importance of fouling mechanism is shown in Fig. 3 (a similar behavior is seen for red musts) where

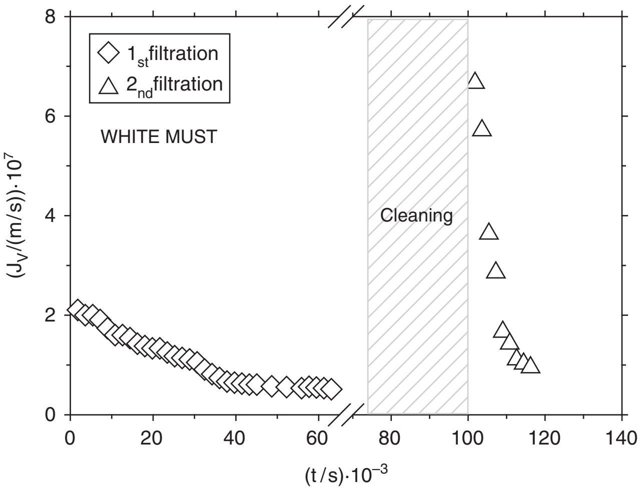

Fig. 3. Flux decay kinetic versus time for the white must.

it is seen that, because the first permeate (P1) has a smaller concentration of large molecules, the initial flow of the second filtration is higher than the initial one of the first filtration stage. In both filtrations the fouling effect is very important because must has a high range of molecular weight as mentioned previously, therefore the fouling appears into the membrane pores but also on the membrane surface. In the second stage, the effect of osmotic flow is also reduced because the concentration of sugar and other substances of low molecular weight decreased after the first filtration step. As a consequence, the time for the completion of the second nanofiltration step is shorter than for the first one, for both musts.

In order to follow the evolution of retention, the sugar concentration has been determined versus the permeation time for both white and red musts, and both filtration steps. Fig. 4 represents the observed retention, $R_0(t)$ as determined by using Eq. (1), versus the permeation time. It shows that there is a decay in retention. This should be expected due to the increase in the concentration and viscosity of the retentate that causes an increase in the concentration polarization and osmotic pressure effects. Moreover, fouling also affects the retention conditions of the membrane because it causes a decrease in the flow and it is known that lower fluxes are associated with lower retentions.

Note that retention is higher for the *Tinta de Toro* must than for the *Verdejo* one, because there are more big molecules as polyphenols on the membrane surface. In the second stage, the largest molecules have been eliminated leading to similar decays in R_0 for both the musts. This is because the concentration of small molecules increase on the membrane surface, and the effect of the osmotic pressure is the determining factor causing an additional reduction in retention.

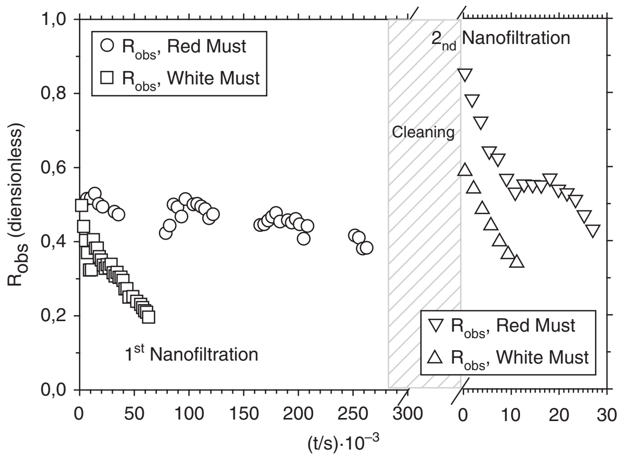

Fig. 4. Sequential representation of observed retention for the nanofiltration processes.

As mentioned, the water permeability was measured before and after every module use (filtration or cleaning process). This has allowed us to evaluate the feasibility of this process, checking the loss of permeability due to fouling during the must filtration and the permeability recovery after the cleaning process.

The final loss of permeability after the two filtration steps was a 35% for white must and almost negligible for the red must. For the red must, cleaning easily removes the substances deposited on the membrane surface. The permeability recovery after cleaning process arrives to a 80% in white must and a close to a 100% in red must.

3.2. *Analysis of the filtered musts*

The fundamental objective is the sugar reduction to obtain a moderate reduction of alcohol in wines. With this aim in mind, we mixed adequately the permeate of the second filtration process and the retentate of the first one.

The main characteristics of permeate and retentate have been analyzed for suitable known mixing proportions. Wine has a lot of substances but, for the sake of simplicity, we have classified them into three groups: sugars, other small molecular weight substances and polyphenols.

The simplest method of determining the sugar content uses the index of refraction, where only a drop of the sample is required, but unfortunately, this method is interfered by the presence of other substances in wine. The polarimetric method has less interferences, but its accuracy is not high due to the opposed rotation of the polarization plane induced by the two main sugars in wine (glucose and fructose). Finally, the enzymatic method allows a determination of the amount of glucose and fructose separately and with greater accuracy. It is also recommended by the OIV, thus it has been used for the results shown below.

Tables 1 and 2 show the results of the sugar determination for the untreated or control must and for the permeated and retained musts. The concentration factors in each filtration step and for each must have been calculated. The concentration factor was 1.9 for the first filtration and 7.0 for the second filtration, in the white must. In the red must, the concentration factor was 1.7 for the first filtration and 6 for the second step. Note that the concentration factors are similar for each filtration in both musts. The volume of the second filtration retentate (R2) is two liters for both musts, therefore the must yield is 93.3%.

In terms of sugar concentration, the application of membrane treatments merely modified the total sugar concentration of the initial must and therefore the probable alcohol contents to be reached after fermentation. The alcoholic grade of the wine that would be obtained from the musts is presented in the last column of these tables. Note that in the case of white musts, we would reduce the alcohol degree from 12 to as low as 5 degrees

Table 1
White musts (*Verdejo*). Glucose and fructose concentrations, glucose versus fructose concentrations ratio, total sugar concentration and the expected alcoholic degree estimated before fermentation

Must	Glucose (g/l)	Fructose (g/l)	G/F[a]	Sugar[b](g/l)	Probable alcoholic degree[c] (%vol)
T	109.0	94.0	1.2	203	12.0
P1	78.0	66.0	1.2	144	7.3
P2	61.0	51.0	1.2	112	5.0
R1	144.0	139.0	1.0	283	15.9
R2	141.0	128.0	1.1	269	15.5

T: control must, P1: first permeate, P2: second permeate, R1: first retentate, R2: second retentate.

[a]G/F = Glucose/Fructose ratio; [b]Addition of glucose and fructose as got by the enzimatic method; [c]Estimated from tables of the alcoholic degree to be expected from the sugar content of must [25].

Table 2
Red musts (*Tinta de Toro*). Glucose and fructose concentrations and glucose versus fructose concentrations ratio, total sugar concentration and the expected alcoholic degree estimated before fermentation

Must	Glucose (g/l)	Fructose (g/l)	G/F[a]	Sugar[b](g/l)	Probable alcoholic degree[c] (%vol)
T	135.0	128.5	1.1	263.5	14.0
P1	81.0	75.0	1.1	156	8.4
P2	59.2	53.8	1.1	113	5.6
R1	149.0	157.0	0.9	306	16.8
R2	153.5	150.5	1.0	304	16.6

T: control must, P1: first permeate, P2: second permeate, R1: first retentate, R2: second retentate.
[a]G/F = Glucose/Fructose ratio; [b]Addition of glucose and fructose as got by the enzimatic method; [c]Estimated from tables of the alcoholic degree to be expected from the sugar content of must [25].

Table 3
pH, acidity and substances of low molecular weight for the white musts (from *Verdejo* grapes)

Must	pH	A.T (g/l)	MH2 (g/l)	TH2 (g/l)	Potassium (mg/l)
T	3.37	4.79	4.0	2.8	1090
P1	3.68	4.56	4.0	2.0	1030
P2	3.23	4.16	3.6	2.0	880
R1	3.35	5.11	4.0	3.6	1010
R2	3.35	4.59	4.0	2.9	1220

A.T = Total acidity, MH2 = Malic Acid and TH2 = Tartaric Acid.

Table 4
pH, acidity and substances of low molecular weight for the red musts (from *Tinta de Toro* grapes)

Must	pH	A.T (g/l)	MH2 (g/l)	TH2 (g/l)	Potassium (mg/l)
T	3.76	3.78	3.8	2.1	1820
P1	3.75	3.45	3.7	1.6	1700
P2	3.75	3.45	3.8	1.5	1630
R1	3.74	3.80	3.8	1.9	1920
R2	3.75	3.57	3.4	2.2	1720

A.T = Total acidity, MH2 = Malic Acid and TH2 = Tartaric Acid.

after fermentation, and for the red one we should go from 14 to 5.6 degrees. However, our aim is not a drastic reduction of alcoholic degree, but a small reduction that as said would be achieved by mixing the final permeate with the initial retained must. This allows a regain of the other important substances in the winemaking process that have not passed to the final permeate.

Tables 3 and 4 show the results of the analysis of other relevant low molecular weight substances. The general trend is a slight decrease of these substance in the permeates and an increase in the retentates. But these variations are too small for most of these compounds.

The retention of these substances should be correlated with their molecular weight and with their charge, because the HL membrane has an isoelectric point of 3.3 [26] and thus it is negatively charged at the pH of the musts. This explains the ion retention as, for example, for potassium, despite of its low molecular weight (MW = 39.1 g/mol), there is a relationship between the concentrations of potassium and of free hydrogen ion (pH) and the electroneutrality condition must be accomplished [27]. In the case of the malic acid, (MW = 134.09 g/mol, pK = 3.40) with the higher pK (less ionization) and medium molecular weight, it has a lower retention (the variations are probably associated with errors of the calculation method). Things are different for tartaric acid (MW = 150 g/mol, pK = 3.03) that, with only slightly higher molecular weight and lower pK (higher ionization), is more retained than malic acid. This is due to the negative charge shown by the tartaric acid and the membrane that make them to repel each other.

The polyphenoic compounds are mainly related to the color of wine and have higher molecular weights than sugars, due to their size, they present a high retention mainly during the initial filtering phase. In *Verdejo* must, the relation between the total concentration of polyphenols in the retentate (R1) and in the permeate (P2) is approximately equal to 10. While for *Tinta de Toro* must the relation is around 30. Their high retention moves to recover these substances and to include them into the final must to be fermented, by mixing the permeate (P2) with the initial retentate (R1), in order to avoid an unbalanced final wine.

3.3. Production and analysis of wines

As mentioned, a control wine (T) and a low alcohol content wine (R1 + P2) have been produced from white and red musts.

Table 5 shows the results for the analysis of the white wines. After alcoholic fermentation, control wine (T) had an alcohol content higher in 3.3% v/v when compared with that of the reduced alcohol wine (R1+P2). Note that the reduction effectively attained has been higher

Table 5
Classical oenological parameters of the white wines after alcoholic fermentation

Wine	pH	A.T g/l	A.V g/l	MH2 g/l	TH2 g/l	Sugar g/l	Alcoholic degree %vol	Potassium mg/l
T	3.05	7.42	0.21	2.9	2.8	1.65	12.71	675
R1+P2	3.06	7.78	0.13	2.8	4.2	1.66	9.34	915

A.V = Volatile acidity.

Table 6
Classical oenological parameters for the control wine and the different products of filtration and mixtures after alcoholic and malolactic for red musts (*Tinta de Toro*)

Wine	pH	A.T g/l	A.V g/l	MH2 g/l	TH2 g/l	Sugar g/l	Alcoholic degree %vol	Potassium mg/l
T	3.89	5.05	0.65	0.10	1.17	1.30	14.28	1555
R1+P2	3.87	4.16	0.52	0.10	0.94	1.30	12.47	1380

than the 2 degrees initially anticipated. This difference is attributable to the difficulty involved in determining the exact proportions of musts to be blend.

It is worth noting that these wines with reduced alcohol content had higher levels of tartaric acid and potassium than the other wines produced, probably due to the fact that alcohol favors the reaction of tartaric acid with potassium giving potassium bitartrate that precipitates [28]. In the case of the other oenological parameters studied, no significant differences were found between the various wines elaborated.

There is a very slight decrease of total polyphenols and color index at 420 nm in the reduced degree wines as compared with the control one. This is due to the loose of polyphenols with the second retentate (R2) that is not used to make wine because it carries most of the sugars that we want to eliminate.

Table 6 shows the results for the analysis of the red wines. The results obtained for the oenological parameters for the red wine, after alcoholic and malolactic fermentation, were similar to those obtained for the white ones. The difference is that red wines with reduced alcohol have slightly lower levels of tartaric acid and potassium than the control wine as it occurs with the musts before fermentation as can be seen in Table 4. The red musts have higher retention of tartaric probably due to the extra barrier formed by the retained larger molecules on the membrane. Moreover, higher amounts of potassium are present in red wines due the process of maceration what increases the formation of bitartrates and thus its elimination by precipitation.

The final wines with a decreased alcohol content also revealed lower concentrations of phenolic compounds than those in the control wine as in white wines and due to the same reasons. This loss of polyphenols compounds during nanofiltration led to a significant reduction in color intensity. Although the color of this wine was slightly less intense than the control wine, the tone and percentages of blue, yellow and red were similar.

The sensorial analysis of the elaborated white wines with reduced alcohol content revealed no defects in terms of their color or olfactory qualities. Indeed, no differences were observed between the color of the control wine and those with a reduced alcohol content. On the nose, the control wine displayed a greater aromatic intensity when compared to those wines with reduced alcohol content. As should be expected, the greatest variations were detected during the tasting phase, as the reduction in the alcohol level modifies the taste perception of the other compounds present in the wine. The wines with a reduced content of alcohol were described by the tasters as being more acidic and lighter than the control wine.

In the sensorial analysis of red wines, no differences in color were found, although variations were observed during the olfactory phase showing slightly less fruity aromas. In the mouth, the only differences detected between the control wine and the wines with lower alcohol degrees consisted in the gustative translation of this olfactory observation.

4. Conclusions

A new method to obtain white and red wines with slightly reduced alcohol content is tested here. The method implies the manipulation of musts instead of wines, making easier to preserve the organoleptic properties of wine. The results show, when compared with those for the untreated musts, that the wine produced from R1+P2, exhibits good properties. The aromas should probably be even better if the filtration time was reduced.

Obviously, the success of the method depends on the characteristics of the wine as an answer to the demanded product; and on the production cost, which is a critical factor in the viability of the whole process. This method is inexpensive in terms of production costs and the must yield is high as mentioned before. Moreover, the economic feasibility can be even increased by using the retained R2 must for the production of sweet wines, liquors or additives for functional foods.

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