Alcohol reduction in red and white wines by Nanofiltration of musts before fermentation

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Abstract

One of the consequences of global warming is the early ripening of grapes which promotes a sugar content increase. Fermentation of their must leads to wines with an alcoholic degree higher than desired. The scope of this study is to select a nanofiltration (NF) technique to reduce the alcohol content of wines approximately 2 degrees by controlling the sugar content of grape must before its fermentation.

For this purpose the performance of single-stage and two-stage NF processes using a spiral wound membrane unit were compared for white must (Spanish *Verdejo)* while for red must (Spanish *Garnacha*) a two-stage procedure was tested. During the single-stage NF intermittent backflush due to the osmotic pressure effect was tested. Results showed that backflushing had an undesirable effect because it increased the flux decay by disturbing the cake stabilization on the membrane. The corresponding wines obtained by adequate mixing of permeated and retained or control musts showed a 1 to 2 degrees alcohol reduction. Sensory evaluation and principal component analysis (PCA) revealed that there were no significant differences between the control and the filtered wines. Among the processes studied, the best NF technique was the two-stage process without backflush.

Keywords:

Winemaking, Alcohol reduction, Membrane Technology, Nanofiltration, Must

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1. Introduction

Over the last years, due to global warming, observations from various world winemaking regions have provided evidence of modified vine development and fruit maturation patterns. Among the most important climate change-related effects there is an increased grape sugar concentration that leads to high wine alcohol levels, lower acidities and modification of varietal aroma compounds (Mira de Orduña, 2010). Premature grape harvest and winemaking should affect the final wine quality, because the acidic and phenolic maturity should not be fully achieved (Garcia-Martin et al., 2011) leading to more acid and less colored wines. A commendable oenological practice establishes that the quality of wines depends essentially on the maturity of phenolic components contained in the grape berries. Since phenolic maturity is directly linked to a high sugar concentration, grapes are being picked having high potential alcohol content, up to 17%, with low acidity (Massot et al., 2008).

But in some countries, as USA, wine producers have to struggle with a supplementary tax added to beverages with alcohol content over 14.5%. Moreover, this over maturity leads to difficulties in wine making as some difficulties appear in alcoholic fermentation and in microbiological stabilization. It also causes a gustatory disequilibrium since the strengthening of warm sensation in the mouth could mask the fruity aromas and taste of wine. Meanwhile, consumers show preference and demand wines with less alcohol content (between 9 and 13%), tendency reinforced by the new social trends of limiting alcohol consumption (Labanda et al., 2009; Masson et al., 2008; Massot et al., 2008).

Therefore, in order to produce a full flavored wine, the harvest should be carried out in the optimum ripeness of the fruits and then innovative techniques to control sugars in musts should be applied.

In order to use a mild and highly specific technology, membranes are a good election. Recently, the OIV introduced in the “International Code of Oenological practices” the application of membrane techniques for the treatment of musts and wine

in order to enable the selective holding back or passing of some compounds. (OIV, 2012).

If the molecular weight of sugars in must is taken into account, nanofiltration (NF) seems to be the most appropriate technique to control their concentration (García-Martín et al., 2009). In our previous work (Salgado et al., 2012), several experiments were performed aiming to select the most appropriate NF membrane for sugar control in grape must. Here, the performance on must NF of 3 flat sheet membranes was compared: NF270 (Dow Filmtec), HL (GE) and SR3 (Koch Membrane System). The results obtained showed that the HL and SR3 membranes were the most appropriate to reduce the content of sugar of red must. Specifically SR3 showed the best passage of sugar and less fouling. As a continuation of the mentioned study, the SR3 membrane was successfully used for sugar control in grape must at a higher scale using a spiral wound module (SWM) (Salgado et al., 2014).

The scope of the present study is to select the most appropriate NF technique to reduce the alcohol content of wines approximately 2 degrees by controlling the sugar content of the grape must before its fermentation. For that purpose the performance of single- stage and two-stage NF processes using a SWM unit were compared. This was tested by treating musts coming from two Spanish varieties of grapes, a white one (*Verdejo*) and a red one (*Garnacha*).

2. Theory

When the overall filtration process is taken into account, the permeate flux per unit of membrane area can be written in terms of the applied transmembrane pressure, Δp, the osmotic pressure gradient, $\Delta \pi$, the viscosity of the solution, η, and the system resistance. This is the sum of the membrane resistance, R_m, plus a series of terms that depend on the fouling caused by the solute and the membrane itself, R_f (Goldsmith,

1971; Jonsson, 1984; Kozinski and Lightfoot, 1971; Wijmans et al., 1984). Thus the permeate flux can be written as

$$Jv = \frac{\Delta p - \Delta \pi}{\eta\left(R_m + R_f\right)} \tag{1}$$

The efficiency of a membrane is determined by its true retention, R, which is defined as

$$R_i = 1 - \frac{C_{\mathrm{p},i}}{C_{\mathrm{m},i}} \quad (i = 1,2...N) \tag{2}$$

for the i-th component present as solute in the feed. Here $C_{\mathrm{m},i}$ is the concentration of the i-th component on the membrane active layer and $C_{\mathrm{p},i}$ the permeate concentration of the i-th component. One of the methods to calculate the experimentally inaccessible concentration $C_{\mathrm{m},i}$. is the use of the Film Theory of concentration polarization. This model is based on the use of the mass transfer coefficient, $K_{\mathrm{m},i}$, in order to describe the solute transport in the membrane active layer (Kuhn et al., 2010; Prádanos et al., 1994) as

$$C_{\mathrm{m},i} = C_{\mathrm{p},i} + \left(C_{0,i} - C_{\mathrm{p},i}\right) e^{\left(Jv/K_{m,i}\right)} \tag{3}$$

Here, J_v is the flux through the membrane; $C_{0,i}$ and $K_{m,i}$ are the feed concentration and the mass transfer coefficient of the i-th component respectively.

The hydrodynamics and mass transport in a spiral wound module are critically influenced by the presence of the spacer material in the feed channel. The appropriate equations for the spiral wound unit and used in the present study have been explained in detail in our previous work (Salgado et al., 2014) and according to it $K_{m,I}$ can be evaluated as (Koutsou et al., 2009; Schock and Miquel, 1987; Schwinge et al., 2004)

$$K_{m,i} = 0.14 x D_i^{0.58} x d_h^{-0.36} x \upsilon_{eff}^{0.64} x \rho_f^{0.22} x \eta_f^{-0.22} \tag{4}$$

where D_i is the diffusion coefficient of the *i*-th component, d_h and υ_{eff} are the hydraulic diameter and the effective velocity characteristic of the feed channel respectively, and η_f and ρ_f stand for the viscosity and density of the feed respectively.

Taking into account that the membrane is semipermeable, the $K_{m,i}$ calculated using Eq. 4, that should be valid for an impenetrable wall, need to be corrected to $K^s_{m,i}$ according to Geraldes & Afonso (Geraldes and Afonso, 2007):

$$K^s_{m,i} = k_{m,i}\left[\left(\frac{Jv}{K_{m,i}}\right)+\left(\frac{Jv/K_{m,i}}{\exp\left\{Jv/K_{m,i}-1\right\}}\right)\right] \quad \text{for} \quad Jv/K_{m,i} \le 1 \tag{5}$$

3. Materials and Methods

3.1. Membrane and experimental set-up

Grape must filtrations were performed in a pilot plant scale unit with a NF SWM. The experimental set-up used is shown in Fig. 1**.** It consists in a feed vessel, with a cryogenic unit to assure that the feed´s temperature is kept at 16ºC. The feed is extracted from the thermostated reservoir by means of a regulatable piston pump Hydra – Cell G03. Two pressure transducers are placed before and after the SWM to measure the inlet and outlet pressure. In order to adjust manually the pressure inside the module a needle valve is placed at the exit of the unit. Cross flow is adjusted through this valve and the speed control of the pump. The retentate flow rate is measured with a flowmeter ranging from 0 to 10 L/min. In order to decrease the retentate temperature a heat exchanger was placed before its return to the feed vessel. The permeate flux was monitored using a three-tube flow system with flow capacity from 0 to 10 L/min.

Figure 1

The membrane used for NF was a KMS SR3 (reference 3839 SR3- NYV), made and commercialized by Koch Membrane Systems. As mentioned, the selection of the SR3 membrane was based on previous experiments testing different nanofiltration membranes in flat sheet configuration using commercial musts (Salgado et al., 2012).The main characteristics of the membrane and SWM have already been exposed in our previous study (Salgado et al., 2014), they are summarized in the supplementary material (see Table S1).

3.2. Grape musts

Two different grape must varieties were used, one white and one red, *Verdejo* and *Garnacha* respectively. Both varieties were cultivated in the experimental vineyard of the Agriculture Technology Institute of Castilla y León (experimental field of Zamadueñas, Valladolid, Spain) from 2012 vintage. Both, grapes, white and red, were transported in plastic boxes of 15 kg to the experimental wine cellar of the Agricultural Engineering School (University of Valladolid, Palencia, Spain), where the musts were elaborated.

3.2.1. Garnacha red must

After the reception, about 100 kg of Garnacha grapes were destemmed and crushed and potassium metabisulphite was added (80 mg/L of SO_2) in order to prevent oxidation or spoilage caused by bacteria. The must was obtained by drawing off, without press. In this case, the solid parts (crushed mass which consist of the grape skins, seeds, remaining must and so forth), were cold-stored at 4ºC in airtight plastic boxes for ulterior addition to musts for the fermentation after nanofiltration. The must was filtered first through 3 µm and then through 0.8 µm cellulose filter plates in order to prevent fast membrane clogging and to make the nanofiltration easier.

3.2.2. Verdejo white must

In this case, nearly 200 kg of Verdejo grapes were destemmed, crushed, sulphited and pressed to obtain the respective must. Potassium metabisulphite was added (80 mg/L of SO_2) with the same purpose as for red must. Pectolytic enzymes (10 mg/L of Enozym Altair, Agrovin) were added to enhance first clarification.

The cleared must was filtered through 0.8 µm cellulose filter plates in order prevent ulterior membrane fouling and thereby facilitating the nanofiltering process.

The main oenological parameters of the pre-filtered red and white must before the nanofiltration process are given in the first column (as control must) of the Tables 2 and 3, respectively.

The portions of the musts that were going to be nanofiltered were transported in 35 L stainless steel vessels to the Laboratory of Membrane Processes of the Faculty of Science (University of Valladolid, Valladolid, Spain). The remaining volumes of musts were cold-stored at 4ºC in airtight vessels and kept as control musts in the cold chamber of the Agricultural Engineering College (University of Valladolid, Palencia, Spain).

3.3. Procedure

In order to select the most appropriate nanofiltration process for sugar reduction in musts, different techniques were studied. In the case of the white must, two nanofiltrations were carried out: a single-stage method and a two- stage one. For the red must a two-stage nanofiltration was analyzed.

Before using the SR3 module, it had to be conditioned following successive cleaning steps to enhance its performance (refer to Fig. S1 of the supplementary material for more detail).

All must filtrations were carried out in a batch concentration mode. Permeate was sent to the thermostated permeate vessel in order to collect it and the retentate was recirculated to the thermostated feed vessel.

Between filtrations, membrane setting was carried out and water permeability was determined.

After all filtration processes, a cleaning procedure, according to manufacturer's instructions (refer to Fig. S2 of the supplementary material), was carried out and the final water permeability of the membrane was determined.

3.3.1. *Two-stage Nanofiltrations*

25 L of must (red and white) were treated in a double nanofiltration in the following steps:

- Nanofiltration (first stage) of untreated must (C) providing a permeate with a medium sugar content (P1) and a sugar rich retentate (R1). The later also contains the main portion of the high molecular weight compounds such as polyphenols, polysaccharides and proteins.
- Nanofiltration (second stage) of the first permeate (P1) providing a retentate (R2) and a second permeate (P2) with a lower sugar content.
- For both musts, red and white, the second permeate (P2) was mixed with the first retentate (R1) in appropriate proportions to produce the intended moderate reduction in the alcohol degree of the final wine. This mixture preserves the specific grape features linked to the high molecular weight components retained in R1.

A scheme that describes briefly each two-stage nanofiltration procedure and operating conditions is depicted in Fig. 2 for the Garnacha red must and in Fig. 3a for the Verdejo white must.

Figure 2

3.3.2. Single-stage Nanofiltration

25 L of white must were treated using one nanofiltration stage. During this procedure 2-minutes-stops were performed every 30 minutes by manually opening the

needle valve of the retentate loop, at the exit of the SWM (see Figure 1). Thereby, the applied pressure inside the module is zero. In this way, only the osmotic pressure acts as driving force creating a backflush of permeate to the retentate. This overturn of the flow promotes shear, which may affect the deposition and detachment behavior of the fouling species on the membrane surface.

This process provides a sugar rich retentate (R1) and a permeate (P1) with a low sugar content.

After this, permeate (P1) was blended with untreated white must (VC) in adequate proportions to create a mixture with a similar sugar content as in the two-stage process.

Fig. 3b provides a scheme of the single-stage nanofiltration process carried out.

Figure 3

3.4. Winemaking process

The elaboration of wines was carried out at the experimental winery of the Agricultural Engineering School (University of Valladolid, Palencia, Spain). Both wine varieties, red and white, were manufactured following the corresponding traditional procedure (detailed in Fig. S3 of the supplementary material).

Three different Garnacha red wines were elaborated: A control made from the control must (GC) and two low alcohol content wines obtained from the mixture of musts (P2+R1): G2NF1 and G2NF2. These low alcohol samples represent the duplicate of the fermentation of the same blend of musts.

Also, three different Verdejo white wines were manufactured: A control obtained from the control must (VC), and two low alcohol content wines: one made from the mixture proceeding from the single-stage nanofiltration (P1+C): V1NF and one produced from the mixture (P2+R1) obtained from the two-stage nanofiltration process: V2NF.

3.5. Analytical methods

Musts were analyzed before and after the filtration process according to the principles and methods summarized in Table 1.

Table 1

The chromatographic system used consisted in an HPLC apparatus from Waters with a Refractive Index detector Waters 2414, an isocratic pump Waters 1515, the Waters 1707 Autosampler, and a thermostated column compartment together with the software Breeze 2. A Supelco Supelcogel Pb guard column and column were used for the sugars (glucose and fructose) separation and a Shodex DE-413 guard column and column for malic and tartaric acid detection.

Total and Free SO_2 were determined by idometry according to the Ripper method (García - Barceló, 1990). This technique was automated by means of an SO2-Matic 23 apparatus from Crison.

Alcohol Degree of wines was measured by ebulliometry (Amerine and Ough, 1976) using a Barus apparatus from GAB System.

UV/Vis spectrophotometric methods were performed using the UV/Vis spectrophotometer (Lan Optics 2000 UV, Labolan, Spain).

3.6. Consumer sensory test

Sensory evaluation of the wines was conducted with 48 consumer volunteers from18 to 65 years old of various socioeconomic backgrounds. A total of 68.75% of the consumers were male and 85.42 % were between 18 and 34 years of age.

Consumer tests were carried out in the Sensory Science Laboratory of the Agricultural Engineering College at the University of Valladolid, Palencia (Spain), in individual booths.

The sensory analysis session for each panelist consisted in doing first the acceptability test of the white wine samples and then of the red wine samples. Consumers tasted the samples served sequential monodically. Samples were presented in glasses coded with 3-digit random numbers and served in a randomized order. Water and crackers were available for rinsing.

Here, the wines were evaluated on the basis of the acceptance of the sensory descriptors colour, odour, flavour, persistence and overall liking on a 9-point hedonic scale. The scale of values ranged from ‘‘like extremely’’ to ‘‘dislike extremely’’ corresponding to the highest and lowest scores of 9 and 1, respectively.

Principal component analysis (PCA) was used to know the effect of the different filtrations on the sensorial and physicochemical characteristics of the wines. Consumer sensory data collected from the acceptability test were subjected to PCA in order to see which the favorite wines were. PCA was performed with the correlation matrix (derived from the data matrix). SPSS for Windows (version 20.0) was used for data processing (Rodriguez-Nogales et al., 2012).

4. Results and discussion

4.1. Nanofiltration processes

As mentioned, water permeability (L_p) and resistance (R_m) of the SWM were determined before each filtration process to control its performance. The values obtained are given in Table S2 of the supplementary material.

Fig. 4 depicts the kinetics of the permeate flux of the three filtration processes.

Figure 4

Fig. 5 compares the filtration processes of the red and white musts during the first and second stages (Fig. 5a and b respectively), in terms of the initial permeate flux ($J_v/J_{v,0}$). Thus the influence of the initial membrane fouling is avoided.

Figure 5

According to Eq. 1, the factors that would mainly promote the flux decline during the first- stage of nanolfiltration (due to the presence of high molecular weight compounds in the feed) are:

1) Formation and thickening of the cake layer on the membrane surface (R_f).
2) Increase of the viscosity (η) of the fluid that goes through the membrane pores.
3) Reversible or irreversible fouling of the membrane during the process (R_m).

As expected, the contribution of these factors is more significant in the case of Garnacha red must (see Fig. 5a) because of its higher concentration of molecules with a molecular weight higher than 300 Da such as polyphenols (see Total polyphenols index in Table 2) and proteins (as shown in table S1, the molecular weight cut-off of the membrane is 200 Da). Moreover, the importance of fouling and cake formation is shown in Fig. 4a and 4c. Here, the initial flow of the first- stage is considerably lower than the initial one of the second-stage, where the feed is mainly composed of low molecular weight molecules.

The permeate flow is also influenced by the osmotic pressure ($\Delta\pi$) increase (see Eq. 1) due to the increment of the concentration of small molecules in the retentate, (C_o) and therefore on the membrane active layer (C_m). This contribution should be similar for both musts, since the concentration of small solutes (such as glucose, fructose malic and tartaric acids) is similar in both as shown in Tables 2 and 3. This fact can be mainly appreciated in Fig. 5b, where the flow kinetics in the second filtration stage is illustrated. Thus the influence of the high molecular weight compounds is avoided.

The effect of the 2-minutes-stops carried out during the single stage NF process of Verdejo must can be assessed in Fig. 5a. Here, lower permeate flow values than for the two-stage NF process are reached. Apparently, the permeate backflush affects in an unexpected way the deposition of foulants and their attachment on the membrane surface, this is, on the growing cake surface. This agrees with the results obtained by (Sioutopoulos et al., 2010). In their research they studied the influence of shear on cake formation and fouling of reverse osmosis and ultrafiltration membranes. They observed that when applying higher stirring rotation speed (i.e. higher shear) in the dead end filtration cell, lower permeate fluxes were obtained. They attributed this to the formation of a thinner cake. Results suggest that the re-suspension of the deposited molecules promoted by shear leads to a thinner cake or the formation of smaller aggregates. This may have higher resistance to the permeate flux and therefore be more effective in reducing the permeate flow.

The concentration of glucose and fructose was measured for the retentate and permeate for both musts along all filtration stages and processes. R_i was calculated according to Eqs. 2 to 5.

Fig. 6 shows a comparison of the sugars rejection during the first (Fig. 6a) and the second stages (Fig. 6b) of filtration of both musts.

It may be observed a slight reduction in the time evolution of the retention for the three processes and also for the 2 stages. This decrease was expected due to the rise of the concentration of sugars in the retentate that finally cross the membrane.

During the first filtration, the retention is higher for the Garnacha must. This is due to the presence of higher amounts of high molecular weight compounds (higher than 300 Da), which contribute to the membrane fouling and cake formation. As observed in previous works (Salgado et al., 2013), this cake layer formed on the membrane surface acts as a pseudo-membrane that changes both: permeability and selectivity of the overall membrane. In the absence of larger molecules (Fig. 6b) the phenomena related with cake formation are mitigated. Thus, the sugar retention is

similar for the musts obtained from Garnacha and Verdejo varieties, during the second stage..

Fig. 6a shows that the process in which the stops were carried out (Verdejo 1NF) sugars rejection is higher than in the process without them. This suggests that the thinner cake formed, probably composed of smaller aggregates, as mentioned, may be less porous and therefore less permeable to sugars too.

Figure 6

4.2. Analysis of the filtered musts

Concentrations of the resultant permeates and retentates were analyzed, for each stage and NF process. Results for the Garnacha red must and for the Verdejo white must are shown in Tables 2and 3 respectively. Note that some parameters have not been determined for some samples because they were considered irrelevant.

Table 2

Table 3

Results show that the general trend is a high reduction of total sugars in the permeates and an increase in the retentates.

Regarding the effect of NF on the concentration of low molecular weight compounds such as malic and tartaric acid, Tables 2 and 3 show that the variations are not so significant for these compounds. Furthermore, since the purpose is to produce low alcohol wines, the permeate has to be mixed with untreated must (P1+C) or with the retentate (P2+R1) in adequate proportions before its fermentation. In this way, the reconstructed must will be chemically very similar to the original one but with a lower sugar content and the variation of the other compounds will be reduced. In accordance to the total sugar content of the mixtures, the probable alcoholic degree of the resulting wine can be estimated from tables (García - Barceló, 1990). In this way, Table 2 shows that the blend (P2+R1) of red musts predicts a 1.67º reduction of the alcohol content. In

the case of white musts (Table 3), the mixtures (P1+C) and (P2+R1) predict a decrease by 2.17º and 1.49º respectively. In all cases this predictive parameter shows that the alcohol reduction would be around 2º as intended. Note that the reduction is not exactly the same in all cases due to the difficulty involved in determining the exact proportions of musts to blend.

The influence of the NF procedures on the phenolic compounds was measured in terms of the total polyphenols index (TPI) and the color index (CI), since they are related to the color of must (see Tables 2 and 3). In the case of the main red musts samples (i.e. GC, R1 and (R1+P2)), the content of anthocyanins was also measured. Results show that nanofiltration did not allow the passage of polyphenolic compounds due to their higher molecular weight. Therefore their concentration increased in the retentates and was lower in the permeate samples. Moreover, it can be seen that in the case of some permeate samples CI could not be detected (N/D). The blending of the permeate with untreated must (single-stage NF) or with the retentate (two-stage NF) reduces the final loss of these compounds. Furthermore, as shown in Table 3, the mixture with the first retentate (P2+R1) promotes a higher recovery of these substances than with untreated must. In this way, if the chemical and sensory characteristics of the wine obtained from the blend (P2+R1) are similar or better than those of the blend (P1+C) it can be said that the best technique is the two-stage NF. Besides, this process minimizes volume losses, as it will be discussed later.

4.3. Production and analysis of wines

As mentioned in section 3.4, 6 different wines were elaborated. Three Garnacha red wines: GC, G2NF1, G2NF2 and three Verdejo white wines: VC, V1NF and V2NF. Table 4 shows the results of the chemical analysis of the six wine samples.

Table 4

In the case of the Garnacha wines, after the malolactic fermentation, wines G2NF1 and G2NF2 had an alcohol degree lower by 1.2º and 1.4º %vol respectively in comparison to the control C wine. In both cases the alcohol reduction achieved was lower than the 2º expected. This could be due to the additional input of untreated must (i.e. sugar content) remaining in the crushed grape mass that was blended with the mixture (R1+P2) prior to the alcoholic fermentation. Regarding the parameters of Total Acidity (T.A.) and pH, no significant differences were determined between the 2 nanofiltered samples and also in comparison with the control wine. Volatile acidity (V.A.) is similar for the G2NF1 and G2NF2 but slightly higher when compared to the control wine. This could be understood as a minor deterioration of must during the NF process, since the V.A. values correspond to the fatty acids including those related with the acetic series (i.e. acetic, acetate, formic, propionic, butyric). As also observed for the resulting musts, nanofiltration affected the concentration of polyphenols and the parameters related (CI and anthocyanins). Table 4 shows that wines G2NF1 and G2NF2 presented a 14% and 16.5% TPI loss respectively when compared with the control wine.

Regarding the Verdejo samples, after alcoholic fermentation, V1NF wine had a 1.93º %vol lower alcohol content when compared to the control, but no alcohol degree reduction was achieved in the V2NF wine. Note that the sugar content of the must mixture (P2+R1) would have led to a lower alcohol degree. It is probable that some microbiological contamination could have promoted this. Also, the fermentation of this blend took longer than the one of the other musts causing the high VA and IC values measured for this sample. The degradation of the wine related to these parameters respectively is for example the formation of acetic acid and the oxidation of compounds related with the color of it.

Also a 15% TPI loss was determined for the V1NF sample.

All in all, it can be said that among the processes studied, the best NF technique is the two- stage process without backflush. This technique allows not only an

appropriate sugar content reduction, but the mixture (P2+R1) promotes a higher recovery of polyphenolic compounds (i.e. color). Besides it minimizes volume losses since the retentate of the first stage and the permeate of the second stage are used for the appropriate blend of must and. Only the permeate of the second stage and the volume retained in the pump and in the module are of no use. At a larger scale (industrial scale) these dead volumes are negligible and it is has been estimated that in the 2NF technique proposed the volume losses would be around 18%.

4.4. Chemical and sensory characteristics of the resulting wines

Sensory evaluation of the wines was carried out only with 5 samples: 3 with lower alcohol content (G2NF1, G2NF2 and V1NF) and the respective control samples (GC and VC). The V2NF wine was not included in this analysis because fermentation was not correct. However, the process of sugar reduction was satisfactory, so the results of this experience have been kept.

Results of the chemical analysis (presented in Table 4) and acceptability test were put into a matrix form. This data matrix consisted of 5 wine samples (rows) by 12 variables (columns): 7 physicochemical and 5 sensorial.

The data matrix of variables analyzed was subjected to PCA in order to decrease the number of results associated with the data set while still explaining the maximum amount of variability present in the data (Shin et al., 2010). In this way, a new set of orthogonal variables (PCs) was generated.

The first 2 PCs explain the 90.32% of the total variance in the data set. Fig.7 shows the plot of the 5 wine samples, the 5 sensorial and 7 physicochemical variables in the first 2 PCs. Furthermore, the first principal component, PC1, accounts for 69.92% of the variability data and PC2 explains the 20.41% of the data variance.

Specific patterns of correlations between the variables can be appreciated from the plot between the PCs, where the position of the variables respect to one other and their corresponding correlations can be visualized. In order to analyze this, the Pearson

correlation between the sensorial and physicochemical variables tested was performed. Results showed that the chemical variables related with color, namely TPI, CI and Anthocyanins are strong and positively correlated with the sensorial variable colour ($r=0.945$; $r=0.972$ and $r=0.947$ respectively with a significance level $p<0.05$). Moreover, the sensorial variable flavour is positively correlated with pH ($r=0.959$, $p<0.05$) and negatively correlated with total acidity ($r=-0.994$, $p<0.05$). Therefore it can be said, that these sensorial variables (evaluated by the consumers) are appropriately correlated with the chemical variables that describe them.

From the sensorial point of view it may be appreciated that none of the samples was particularly preferred by the consumers. Moreover, since there is no significant difference between the control or the filtered samples, this general trend can be attributed to an absence of substantial moddifications apart from the alcohol reduction.

It can be noticed that red wines, especially the control (GC) and G2NF1, were preferred by their odour and colour. The sample G2NF2 presented a high volatile acidity and lower TPI and therefore it is located further from the other 2 red wines and was less preferred. Since both, G2NF1 and G2NF2 were obtained from the same nanofiltrated must, it can be said that the differences of G2NF2 were caused by the fermentation and they are not related with the nanofiltration process. Results show that the filtration did not affect significantly the odour and colour acceptance of the resulting red wines, since the G2NF1 had the highest colour and odour acceptance. Regarding white wines, they showed the highest acceptance in flavour and overall liking, especially the control one. But they were not located in the space defined by the colour and odour descriptors. Moreover, the sample V1NF presented lower persistence in mouth, flavour and overall liking. These features could be related, from the sensorial point of view, to a wine with a lower alcohol degree, even though the alcohol degree is not strongly correlated with any other descriptor. Besides, Verdejo is a variety characterized by its aroma components (volatile compounds). That is why nanofiltration could be more effective in the loss of these compounds in this variety.

Figure 7

5. Conclusion

After the assessment of the different NF techniques studied, the main following conclusions can be raised:

- The use of backflush during the single-stage NF of Verdejo must is not appropriate since itcaused lower permeate flow values . This means that it does not improve the productivity of the process.
- .
- The mixture of the second must permeate with the first retentate (P2+R1) promoted a higher recovery of polyphenolic compounds than with untreated must (P1+C).

Regarding the wines produced, the following conclusions can be made:

- .
- The techniques studied here for sugar control in grape juice allow the partial reduction of alcohol in the resulting wine. Results show that the two-stage NF process promotes a higher IPT recovery and less volume losses.
- Sensory evaluation and PCA analysis showed that none of the wine samples was particularly preferred by the consumers, showing that there were no significant differences between the control and the filtered wines.
- NF did not affect significantly the odour and colour of the resulting red wines, since the G2NF1 had the highest colour and odour acceptance.

Moreover, the depletion of aroma components observed during Verdejo filtration may be analyzed in future studies by the recovery of these compounds using pervaporation before NF.

All in all, it can be said that this study reveals the feasibility of single-and-two-stage NF processes for sugar reduction in grape must without a significant alteration of important compounds such as polyphenols, malic and tartaric acids. This allows the production of wines with sensorial and chemical characteristics similar as wines obtained of the fermentation of untreated musts. Therefore, this technique could be applied at a larger scale for the production of low alcohol content wines.

Nomenclature

Roman

$C_{0,i}$	feed concentration of the i-th component (kg m^{-3})
$C_{m,i}$	concentration of the i-th component on the membrane active layer (kg m^{-3})
$C_{p,i}$	permeate concentration of the i-th component (kg m^{-3})
D_i	diffusion coefficient of the i-th component (m^2 s^{-1})
J_v	permeate flux per unit of area through the membrane (m^3 m^{-2} s^{-1})
$J_{v,0}$	permeate flux per unit of area through the membrane at time t =0 (m^3 m^{-2} s^{-1})
$K_{m,i}$	mass transfer coefficient (m s^{-1}) of the i-th component at impermeable membranes (m s^{-1})
$K^s_{m,i}$	mass transfer coefficient of the i-th component at semipermeable membranes (m s^{-1})
R_f	resistance due to fouling (m^{-1})
R_i	membranes true retention for the i-th component
R_m	membrane resistance (m^{-1})

Greek

$\Delta\pi$	osmotic pressure gradient (Pa)

η	solution viscosity (Pa s)
η_f	feed viscosity (Pa s)
υ_{eff}	effective velocity (m s^{-1})
ρ_f	feed density (kg m^{-3})

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Appendix A. Supplementary data

The following are the supplementary data to this article:

Supplementary data

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Tables

Table 1

Methods used for the determination of some oenological parameters of musts.

Parameter	Principle	Method
Glucose and Fructose	Ion exchange Chromatography	HPLC
Tartaric and Malic Acid	Chromatography	HPLC
pH	Potentiometry	pH- meter
Volatile acidity	Acid- base titration	García-Tena [a]
Total acidity	Potentiometric titration	OIV [b]
SO_2 T and SO_2 F	Iodometry	Ripper automated
Alcoholic degree	Ebullometry	Barus apparatus [c]
Total Polyphenols	UV absorbance	UV/Vis spectrophotometry
Anthocyanins	Vis absorbance	UV/Vis spectrophotometry [d]
Color	Vis absorbance	UV/Vis spectrophotometry [b]

[a] (García - Barceló, 1990)
[b] (OIV, 2011)
[c] (Amerine and Ough, 1976)
[d] (Ribereau-Gayon P. and Stonestreet E., 1965), (Lasanta et al., 2014)

Table 2

Oenological parameters of Garnacha red musts after the two-stage NF process and of the control must (C).

	Control (C)	Garnacha two-stage NF				
		P1	R1	P2	R2	P2+R1
Glucose (g/L)	105 ± 2	27.6± 0.6	135 ± 3	5.73 ± 0.13	92 ± 2	90 ± 2
Fructose (g/L)	106 ± 4	27.0 ± 1.0	138 ± 5	7.5 ± 0.3	92 ± 3	93 ± 3
Probable alcoholic Degree[a] %vol	12.5 ± 0.3					10.9 ± 0.3
TH_2 (g/L)	4.4 ± 0.5	3.0 ± 0.4	4.3 ± 0.5	1.51 ± 0.19	4.8 ± 0.6	3.9 ± 0.5
MH_2 (g/L)	0.59 ± 0.01	0.53 ± 0.01	0.47 ± 0.01	0.43 ± 0.01	0.58 ± 0.01	0.49 ± 0.01
pH	3.02 ± 0.01	----	3.12 ± 0.01	----	----	3.19 ± 0.01
T.A. (gTH_2 /L)	3.21 ± 0.01	----	3.06 ± 0.1	----	----	2.70 ± 0.14
TPI	8.8 ± 0.3	1.18 ± 0.01	12.9 ± 0.5	0.29 ± 0.01	----	7.93 ± 0.18
CI	0.34 ± 0.04	0.02 ± 0.01	0.28 ± 0.04	N/D	N/D	0.20 ± 0.06
Anthocyanins (mg/L)[b]	63 ± 3	----	91 ± 7	----	----	56.9 ± 1.2

C: Control must, P1: permeate of the first stage, R1: retentate of the first stage, P2: permeate of the second stage, R2: retentate of the second stage.

T.A: Total acidity expressed as g TH_2 per liter, TH_2: Tartaric acid, MH_2: Malic acid, TPI: Total Polyphenol Index, CI: Color Index

N/D: not detectable

[a] Estimated from tables of the alcoholic degree to be expected on the basis of 16.83 g sugars from must per 1% alcohol (García - Barceló, 1990)

[b] expressed as mg malvidin-3 glycoside per liter

Table 3

Oenological parameters of Verdejo white musts after the single-stage and two-stage NF processes and of the control must (C).

	Control (C)	Verdejo single- stage NF			Verdejo two-stage NF				
		P1	R1	P1+C	P1	R1	P2	R2	P2+R1
Glucose (g/L)	117.0 ± 1.6	48.4 ± 0.7	157 ± 2	97.9 ± 1.3	48.9± 0.7	149 ± 2	12.75 ± 0.17	127.9 ± 1.8	105.2 ± 1.4
Fructose (g/L)	120.4 ± 1.6	46.6 ± 0.6	164 ± 2	102.1 ± 1.4	46.8 ± 0.6	156 ± 2	13.36 ± 0.18	126.7 ± 1.7	106.8 ± 1.4
Probable alcoholic Degree[a] %vol	14.05 ± 0.05	---	----	11.88 ± 0.04	----	----	----	----	12.56 ± 0.04
TH_2 (g/L)	3.8 ± 0.5	2.4 ± 0.3	3.5 ± 0.4	4.1 ± 0.5	2.4 ± 0.3	3.5 ± 0.4	1.7 ± 0.2	2.6 ± 0.3	3.3 ± 0.4
MH_2 (g/L)	1.47 ± 0.01	1.42 ± 0.01	1.20 ± 0.01	1.31 ± 0.01	1.51 ± 0.01	1.23 ± 0.01	1.40 ± 0.01	1.42 ± 0.01	1.66 ± 0.02
pH	3.40 ± 0.01	----	3.37 ± 0.01	3.35 ± 0.01	----	3.36 ± 0.01	----	----	3.37 ± 0.01
T.A. (gTH_2 /L)	2.66 ± 0.03	----	3.06 ± 0.16	3.27 ± 0.03	----	3.04 ± 0.08	----	----	2.91 ± 0.11
TPI	5.47 ± 0.12	0.06 ± 0.01	8.37 ± 0.01	4.11 ± 0.03	----	8.30 ± 0.16	0.01 ± 0.01	----	5.97 ± 0.08
CI	0.07 ± 0.01	0.01 ± 0.01	0.12 ± 0.01	0.06 ± 0.01	----	0.13 ± 0.01	N/D	----	0.09 ± 0.01

C: Control must, P1: permeate of the first stage, R1: retentate of the first stage, P2: permeate of the second stage, R2: retentate of the second stage.

T.A: Total acidity expressed as g TH_2 per liter, TH_2= Tartaric acid, MH_2: Malic acid, TPI: Total Polyphenol Index, CI: Color Index.

N/D: not detectable

[a]Estimated from tables of the alcoholic degree to be expected on the basis of 16.83 g sugars from must per 1% alcohol (García - Barceló, 1990)

Table 4

Chemical analysis for the main oenological parameters of the red and white wines

Chemical parameter	Garnacha			Verdejo		
	C	2NF1	2NF2	C	1NF	2NF
pH	3.21 ± 0.01	3.29 ± 0.01	3.31± 0.01	3.37 ± 0.01	3.31 ± 0.01	3.42 ± 0.01
T.A. (gTH_2 /L)	4.60 ± 0.03	4.26 ± 0.03	4.05 ± 0.05	3.75 ± 0.05	3.92 ± 0.03	3.75 ± 0.05
V.A. (g /L)	0.23 ± 0.01	0.31 ± 0.01	0.34 ± 0.01	0.37 ± 0.01	0.18 ± 0.01	0.63 ± 0.05
TPI	42.9 ± 0.4	36.93 ± 0.04	35.85 ± 0.07	6.31 ± 0.08	5.37 ± 0.01	7.75 ± 0.05
Anthocyanins (mg/L)[a]	371 ± 14	355 ± 2	288 ± 3	4.4 ± 1.9	2.63 ± 0.01	2.2 ± 1.9
CI	11.1 ± 0.2	8.19 ± 0.07	6.94 ± 0.02	0.09 ± 0.01	0.07 ± 0.01	0.10 ± 0.01
Alcoholic Degree (%vol)	12.40 ± 0.18	11.20 ± 0.01	11.00 ± 0.01	13.88 ± 0.01	11.95 ± 0.01	14.00± 0.01

Garnacha C: Garnacha Control wine, Garnacha 2NF1and 2NF2: Red wines obtained from fermentation of the mixture (R1+P2), Verdejo C: White control wine, Verdejo 1NF: white wine obtained after the fermentation of the mixture (C+P1), Verdejo 2NF: white wine obtained after the fermentation of the mixture (R1+P2)

T.A: Total acidity expressed as g TH_2 per liter, TH_2= Tartaric acid, MH_2: Malic acid, TPI: Total Polyphenol Index, CI: Color Index

[a] expressed as mg malvidin-3 glycoside per liter

Figure Captions

Figure 1. Scheme of the experimental device used in the Nanofiltration processes.

Figure 2. Scheme of the procedure carried out for the Garnacha red must two-stage Nanofiltration and ulterior fermentations.

Figure 3. Scheme of the Nanofiltration procedures carried out for the Verdejo white must and ulterior fermentation. a) two-stage and b) single-stage nanofiltration.

Figure 4. Kinetics of the permeate flux of the three filtration processes. (a) and (c) for the two-stage filtration processes, of red and white must respectively. (b) shows the kinetics of the white permeate must during the single-stage NF process. Dashed lines represent the 2-minutes-stops with osmotic backflushing.

Figure 5. Comparison of the filtration processes of the red and white musts during the first (a) and second stage (b), in terms of the initial permeate flux ($J_v/J_{v,0}$).

Figure 6. Comparison of the sugars rejection during the first (a) and the second stages (b) of filtration of both musts.

Figure 7. Principal Component Analysis of the wine samples and the physicochemical and sensorial characteristics. Symbols. Physicochemical variables: Anthocyanins (A); Total Acidity (TA); Volatil Acidity (VA); Total Polyphenol Index (TPI); Color Index (CI); Alcoholic Degree (AD). Sensory descriptors: Color (C); Odor (O); Flavor (F); Persistence (P); Overall liking (OL). Wine samples: Verdejo: Control (VC); single-stage (V1NF). Garnacha: Control (GC); two-stage (G2NF1) and fermentation duplicate (G2NF2).

Alcohol reduction in red and white wines by Nanofiltration of musts before fermentation

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Abstract

One of the consequences of global warming is the early ripening of grapes which promotes a sugar content increase. Fermentation of their must leads to wines with an alcoholic degree higher than desired. The scope of this study is to select a nanofiltration (NF) technique to reduce the alcohol content of wines approximately 2 degrees by controlling the sugar content of grape must before its fermentation.

For this purpose the performance of single-stage and two-stage NF processes using a spiral wound membrane unit were compared for white must (Spanish *Verdejo*) while for red must (Spanish *Garnacha*) a two-stage procedure was tested. During the single-stage NF intermittent backflush due to the osmotic pressure effect was tested. Results showed that backflushing had an undesirable effect because it increased the flux decay by disturbing the cake stabilization on the membrane. The corresponding wines obtained by adequate mixing of permeated and retained or control musts showed a 1 to 2 degrees alcohol reduction. Sensory evaluation and principal component analysis (PCA) revealed that there were no significant differences between the control and the filtered wines. Among the processes studied, the best NF technique was the two-stage process without backflush.

Keywords:

Winemaking, Alcohol reduction, Membrane Technology, Nanofiltration, Must

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1. Introduction

Over the last years, due to global warming, observations from various world winemaking regions have provided evidence of modified vine development and fruit maturation patterns. Among the most important climate change-related effects there is an increased grape sugar concentration that leads to high wine alcohol levels, lower acidities and modification of varietal aroma compounds (Mira de Orduña, 2010). Premature grape harvest and winemaking should affect the final wine quality, because the acidic and phenolic maturity should not be fully achieved (Garcia-Martin et al., 2011) leading to more acid and less colored wines. A commendable oenological practice establishes that the quality of wines depends essentially on the maturity of phenolic components contained in the grape berries. Since phenolic maturity is directly linked to a high sugar concentration, grapes are being picked having high potential alcohol content, up to 17%, with low acidity (Massot et al., 2008).

But in some countries, as USA, wine producers have to struggle with a supplementary tax added to beverages with alcohol content over 14.5%. Moreover, this over maturity leads to difficulties in wine making as some difficulties appear in alcoholic fermentation and in microbiological stabilization. It also causes a gustatory disequilibrium since the strengthening of warm sensation in the mouth could mask the fruity aromas and taste of wine. Meanwhile, consumers show preference and demand wines with less alcohol content (between 9 and 13%), tendency reinforced by the new social trends of limiting alcohol consumption (Labanda et al., 2009; Masson et al., 2008; Massot et al., 2008).

Therefore, in order to produce a full flavored wine, the harvest should be carried out in the optimum ripeness of the fruits and then innovative techniques to control sugars in musts should be applied.

In order to use a mild and highly specific technology, membranes are a good election. ~~In fact, membrane filtration has been applied in winemaking for a long time for different tasks such as: cross-flow microfiltration (MF) and ultrafiltration (UF) for~~

clarification (Cassano et al., 2008), sugar concentration using nanofiltration (NF) (Versari et al., 2003) and reverse osmosis (RO) (Rektor, 2007) in musts. Recently, the OIV introduced in the "International Code of Oenological practices" the application of membrane techniques for the treatment of musts and wine in order to enable the selective holding back or passing of some compounds. (OIV, 2012).

If the molecular weight of sugars in must is taken into account, nanofiltration (NF) seems to be the most appropriate technique to control their concentration (García-Martín et al., 2009). In our previous work (Salgado et al., 2012), several experiments were performed aiming to select the most appropriate NF membrane for sugar control in grape must. Here, the performance on must NF of 3 flat sheet membranes was compared: NF270 (Dow Filmtec), HL (GE) and SR3 (Koch Membrane System). The results obtained showed that the HL and SR3 membranes were the most appropriate to reduce the content of sugar of red must. Specifically SR3 showed the best passage of sugar and less fouling. As a continuation of the mentioned study, the SR3 membrane was successfully used for sugar control in grape must at a higher scale using a spiral wound module (SWM) (Salgado et al., 2014).

The scope of the present study is to select the most appropriate NF technique to reduce the alcohol content of wines approximately 2 degrees by controlling the sugar content of the grape must before its fermentation. For that purpose the performance of single- stage and two-stage NF processes using a SWM unit were compared. This was tested by treating musts coming from two Spanish varieties of grapes, a white one (*Verdejo*) and a red one (*Garnacha*).

2. Theory

When the overall filtration process is taken into account, the permeate flux per unit of membrane area can be written in terms of the applied transmembrane pressure, Δp, the osmotic pressure gradient, $\Delta \pi$, the viscosity of the solution, η, and the system

resistance. This is the sum of the membrane resistance, R_m, plus a series of terms that depend on the fouling caused by the solute and the membrane itself, R_f (Goldsmith, 1971; Jonsson, 1984; Kozinski and Lightfoot, 1971; Wijmans et al., 1984). Thus the permeate flux can be written as

$$Jv = \frac{\Delta p - \Delta \pi}{\eta\left(R_m + R_f\right)} \tag{1}$$

The efficiency of a membrane is determined by its true retention, R, which is defined as

$$R_i = 1 - \frac{C_{\mathrm{p},i}}{C_{\mathrm{m},i}} \quad (i = 1,2...N) \tag{2}$$

for the i-th component present as solute in the feed. Here $C_{\mathrm{m},i}$ is the concentration of the i-th component on the membrane active layer and $C_{\mathrm{p},i}$ the permeate concentration of the i-th component. One of the methods to calculate the experimentally inaccessible concentration $C_{\mathrm{m},i}$. is the use of the Film Theory of concentration polarization. This model is based on the use of the mass transfer coefficient, $K_{\mathrm{m},i}$, in order to describe the solute transport in the membrane active layer (Kuhn et al., 2010; Prádanos et al., 1994) as

$$C_{\mathrm{m},i} = C_{\mathrm{p},i} + \left(C_{0,i} - C_{\mathrm{p},i}\right) e^{\left(Jv/K_{m,i}\right)} \tag{3}$$

Here, J_v is the flux through the membrane; $C_{0,i}$ and $K_{m,i}$ are the feed concentration and the mass transfer coefficient of the i-th component respectively.

The hydrodynamics and mass transport in a spiral wound module are critically influenced by the presence of the spacer material in the feed channel. The appropriate equations for the spiral wound unit and used in the present study have been explained in detail in our previous work (Salgado et al., 2014) and according to it $K_{m,I}$ can be evaluated as (Koutsou et al., 2009; Schock and Miquel, 1987; Schwinge et al., 2004)

$$K_{m,i} = 0.14 x D_i^{0.58} x d_h^{-0.36} x \upsilon_{eff}^{0.64} x \rho_f^{0.22} x \eta_f^{-0.22} \quad (4)$$

where D_i is the diffusion coefficient of the *i*-th component, d_h and υ_{eff} are the hydraulic diameter and the effective velocity characteristic of the feed channel respectively, and η_f and ρ_f stand for the viscosity and density of the feed respectively.

Taking into account that the membrane is semipermeable, the $K_{m,i}$ calculated using Eq. 4, that should be valid for an impenetrable wall, need to be corrected to $K^s_{m,i}$ according to Geraldes & Afonso (Geraldes and Afonso, 2007):

$$K^s_{m,i} = k_{m,i}\left[\left(\frac{Jv}{K_{m,i}}\right) + \left(\frac{Jv/K_{m,i}}{\exp\{Jv/K_{m,i} - 1\}}\right)\right] \quad \text{for} \quad Jv/K_{m,i} \le 1 \qquad (5)$$

3. Materials and Methods

3.1. Membrane and experimental set-up

Grape must filtrations were performed in a pilot plant scale unit with a NF SWM. The experimental set-up used is shown in Fig. 1**.** It consists in a feed vessel, with a cryogenic unit to assure that the feed´s temperature is kept at 16ºC. The feed is extracted from the thermostated reservoir by means of a regulatable piston pump Hydra – Cell G03. Two pressure transducers are placed before and after the SWM to measure the inlet and outlet pressure. In order to adjust manually the pressure inside the module a needle valve is placed at the exit of the unit. Cross flow is adjusted through this valve and the speed control of the pump. The retentate flow rate is measured with a flowmeter ranging from 0 to 10 L/min. In order to decrease the retentate temperature a heat exchanger was placed before its return to the feed vessel. The permeate flux was monitored using a three-tube flow system with flow capacity from 0 to 10 L/min.

Figure 1

The membrane used for NF was a KMS SR3 (reference 3839 SR3- NYV), made and commercialized by Koch Membrane Systems. As mentioned, the selection of the SR3 membrane was based on previous experiments testing different nanofiltration membranes in flat sheet configuration using commercial musts (Salgado et al., 2012).The main characteristics of the membrane and SWM have already been exposed in our previous study (Salgado et al., 2014), they are summarized in the supplementary material (see Table S1).

3.2. Grape musts

Two different grape must varieties were used, one white and one red, called *Verdejo* and *Garnacha* respectively. Both varieties were cultivated in the experimental vineyard of the Agriculture Technology Institute of Castilla y León (experimental field of Zamadueñas, Valladolid, Spain) from 2012 vintage. Both, grapes, white and red, were transported in plastic boxes of 15 kg to the experimental wine cellar of the Agricultural Engineering School (University of Valladolid, Palencia, Spain), where the musts were elaborated.

3.2.1. Garnacha red must

After the reception, about 100 kg of Garnacha grapes were destemmed and crushed and potassium metabisulphite was added (80 mg/L of SO_2) in order to prevent oxidation or spoilage caused by bacteria. The must was obtained by drawing off, without press. In this case, the solid parts (crushed mass which consist of the grape skins, seeds, remaining must and so forth), were cold-stored at 4ºC in airtight plastic boxes for ulterior addition to musts for the fermentation after nanofiltration. The must

was filtered first through 3 μm and then through 0.8 μm cellulose filter plates in order to prevent fast membrane clogging and to make the nanofiltration easier.

3.2.2. Verdejo white must

In this case, nearly 200 kg of Verdejo grapes were destemmed, crushed, sulphited and pressed to obtain the respective must. Potassium metabisulphite was added (80 mg/L of SO_2) with the same purpose as for red must. Pectolytic enzymes (10 mg/L of Enozym Altair, Agrovin) were added to enhance first clarification.

The cleared must was filtered through 0.8 μm cellulose filter plates in order prevent ulterior membrane fouling and thereby facilitating the nanofiltering process.

The main oenological parameters of the pre-filtered red and white must before the nanofiltration process are given in the first column (as control must) of the Tables 2 and 3, respectively.

The portions of the musts that were going to be nanofiltered were transported in 35 L stainless steel vessels to the Laboratory of Membrane Processes of the Faculty of Science (University of Valladolid, Valladolid, Spain). The remaining volumes of musts were cold-stored at 4ºC in airtight vessels and kept as control musts in the cold chamber of the Agricultural Engineering College (University of Valladolid, Palencia, Spain).

3.3. Procedure

In order to select the most appropriate nanofiltration process for sugar reduction in musts, different techniques were studied. In the case of the white must, two nanofiltrations were carried out: a single-stage method and a two- stage one. For the red must a two-stage nanofiltration was analyzed.

Before using the SR3 module, it had to be conditioned following successive cleaning steps to enhance its performance (refer to Fig. S1 of the supplementary material for more detail).

All must filtrations were carried out in a batch concentration mode. Permeate was sent to the thermostated permeate vessel in order to collect it and the retentate was recirculated to the thermostated feed vessel.

Between filtrations, membrane setting was carried out and water permeability was determined.

After all filtration processes, a cleaning procedure, according to manufacturer's instructions (refer to Fig. S2 of the supplementary material), was carried out and the final water permeability of the membrane was determined.

3.3.1. *Two-stage Nanofiltrations*

25 L of must (red and white) were treated in a double nanofiltration in the following steps:

- Nanofiltration (first stage) of untreated must (C) providing a permeate with a medium sugar content (P1) and a sugar rich retentate (R1). The later also contains the main portion of the high molecular weight compounds such as polyphenols, polysaccharides and proteins.
- ~~Membrane rinse~~
- Nanofiltration (second stage) of the first permeate (P1) providing a retentate (R2) and a second permeate (P2) with a lower sugar content.
- For both musts, red and white, the second permeate (P2) was mixed with the first retentate (R1) in appropriate proportions to produce the intended moderate reduction in the alcohol degree of the final wine. This mixture preserves the specific grape features linked to the high molecular weight components retained in R1.
- ~~The mixture (P2+R1) was fermented according to the corresponding traditional winemaking procedure.~~

A scheme that describes briefly each two-stage nanofiltration procedure and operating conditions is depicted in Fig. 2 for the Garnacha red must and in Fig. 3a for the Verdejo white must.

Figure 2

3.3.2. Single-stage Nanofiltration

25 L of white must were treated using one nanofiltration stage. During this procedure 2-minutes-stops were performed every 30 minutes by manually opening the needle valve of the retentate loop, at the exit of the SWM (see Figure 1). Thereby, the applied pressure inside the module is zero. In this way, only the osmotic pressure acts as driving force creating a backflush of permeate to the retentate. This overturn of the flow promotes shear, which may affect the deposition and detachment behavior of the fouling species on the membrane surface.

This process provides a sugar rich retentate (R1) and a permeate (P1) with a low sugar content.

After this, permeate (P1) was blended with untreated white must (VC) in adequate proportions to create a mixture with a similar sugar content as in the two-stage process.

Fig. 3b provides a scheme of the single-stage nanofiltration process carried out.

Figure 3

3.4. Winemaking process

The elaboration of wines was carried out at the experimental winery of the Agricultural Engineering School (University of Valladolid, Palencia, Spain). Both wine varieties, red and white, were manufactured following the corresponding traditional procedure (detailed in Fig. S3 of the supplementary material).

Three different Garnacha red wines were elaborated: A control made from the control must (GC) and two low alcohol content wines obtained from the mixture of

musts (P2+R1): G2NF1 and G2NF2. These low alcohol samples represent the duplicate of the fermentation of the same blend of musts.

Also, three different Verdejo white wines were manufactured: A control obtained from the control must (VC), and two low alcohol content wines: one made from the mixture proceeding from the single-stage nanofiltration (P1+C): V1NF and one produced from the mixture (P2+R1) obtained from the two-stage nanofiltration process: V2NF.

3.5. Analytical methods

Musts were analyzed before and after the filtration process according to the principles and methods summarized in Table 1.

Table 1

The chromatographic system used consisted in an HPLC apparatus from Waters with a Refractive Index detector Waters 2414, an isocratic pump Waters 1515, the Waters 1707 Autosampler, and a thermostated column compartment together with the software Breeze 2. A Supelco Supelcogel Pb guard column and column were used for the sugars (glucose and fructose) separation and a Shodex DE-413 guard column and column for malic and tartaric acid detection.

Total and Free SO_2 were determined by idometry according to the Ripper method (García - Barceló, 1990). This technique was automated by means of an SO2-Matic 23 apparatus from Crison.

Alcohol Degree of wines was measured by ebulliometry (Amerine and Ough, 1976) using a Barus apparatus from GAB System.

UV/Vis spectrophotometric methods were performed using the UV/Vis spectrophotometer (Lan Optics 2000 UV, Labolan, Spain).

3.6. Consumer sensory test

Sensory evaluation of the wines was conducted with 48 consumer volunteers from18 to 65 years old of various socioeconomic backgrounds. A total of 68.75% of the consumers were male and 85.42 % were between 18 and 34 years of age.

Consumer tests were carried out in the Sensory Science Laboratory of the Agricultural Engineering College at the University of Valladolid, Palencia (Spain), in individual booths.

The sensory analysis session for each panelist consisted in doing first the acceptability test of the white wine samples and then of the red wine samples. Consumers tasted the samples served sequential monodically. Samples were presented in glasses coded with 3-digit random numbers and served in a randomized order. Water and crackers were available for rinsing.

Here, the wines were evaluated on the basis of the acceptance of the sensory descriptors colour, odour, flavour, persistence and overall liking on a 9-point hedonic scale. The scale of values ranged from ‘‘like extremely’’ to ‘‘dislike extremely’’ corresponding to the highest and lowest scores of 9 and 1, respectively.

Principal component analysis (PCA) was used to know the effect of the different filtrations on the sensorial and physicochemical characteristics of the wines. Consumer sensory data collected from the acceptability test were subjected to PCA in order to see which the favorite wines were. PCA was performed with the correlation matrix (derived from the data matrix). SPSS for Windows (version 20.0) was used for data processing (Rodriguez-Nogales et al., 2012).

4. Results and discussion

4.1. Nanofiltration processes

As mentioned, water permeability (L_p) and resistance (R_m) of the SWM were determined before each filtration process to control its performance. The values obtained are given in Table S2 of the supplementary material.

Fig. 4 depicts the kinetics of the permeate flux of the three filtration processes.

Figure 4

Fig. 5 compares the filtration processes of the red and white musts during the first and second stages (Fig. 5a and b respectively), in terms of the initial permeate flux ($J_v/J_{v,0}$). Thus the influence of the initial membrane fouling is avoided.

Figure 5

According to Eq. 1, the factors that would mainly promote the flux decline during the first- stage of nanolfiltration (due to the presence of high molecular weight compounds in the feed) are:

1) Formation and thickening of the cake layer on the membrane surface (R_f).
2) Increase of the viscosity (η) of the fluid that goes through the membrane pores.
3) Reversible or irreversible fouling of the membrane during the process (R_m).

As expected, the contribution of these factors is more significant in the case of Garnacha red must (see Fig. 5a) because of its higher concentration of molecules with a molecular weight higher than 300 Da such as polyphenols (see Total polyphenols index in Table 2) and proteins (as shown in table S1, the molecular weight cut-off of the membrane is 200 Da). Moreover, the importance of fouling and cake formation is shown in Fig. 4a and 4c. Here, the initial flow of the first- stage is considerably lower than the initial one of the second-stage, where the feed is mainly composed of low molecular weight molecules.

The permeate flow is also influenced by the osmotic pressure ($\Delta\pi$) increase (see Eq. 1) due to the increment of the concentration of small molecules in the retentate, (C_o) and therefore on the membrane active layer (C_m). This contribution

should be similar for both musts, since the concentration of small solutes (such as glucose, fructose malic and tartaric acids) is similar in both as shown in Tables 2 and 3. This fact can be mainly appreciated in Fig. 5b, where the flow kinetics in the second filtration stage is illustrated. Thus the influence of the high molecular weight compounds is avoided.

The effect of the 2-minutes-stops carried out during the single stage NF process of Verdejo must can be assessed in Fig. 5a. Here, lower permeate flow values than for the two-stage NF process are reached. Apparently, the permeate backflush affects in an unexpected way the deposition of foulants and their attachment on the membrane surface, this is, on the growing cake surface. This agrees with the results obtained by (Sioutopoulos et al., 2010). In their research they studied the influence of shear on cake formation and fouling of reverse osmosis and ultrafiltration membranes. They observed that when applying higher stirring rotation speed (i.e. higher shear) in the dead end filtration cell, lower permeate fluxes were obtained. They attributed this to the formation of a thinner cake. Results suggest that the re-suspension of the deposited molecules promoted by shear leads to a thinner cake or the formation of smaller aggregates. This may have higher resistance to the permeate flux and therefore be more effective in reducing the permeate flow.

The concentration of glucose and fructose was measured for the retentate and permeate for both musts along all filtration stages and processes. R_i was calculated according to Eqs. 2 to 5.

Fig. 6 shows a comparison of the sugars rejection during the first (Fig. 6a) and the second stages (Fig. 6b) of filtration of both musts.

It may be observed a slight reduction in the time evolution of the retention for the three processes and also for the 2 stages. This decrease was expected due to the rise of the concentration of sugars in the retentate that finally cross the membrane.

During the first filtration, the retention is higher for the Garnacha must. This is due to the presence of higher amounts of high molecular weight compounds (higher

than 300 Da), which contribute to the membrane fouling and cake formation. As observed in previous works (Salgado et al., 2013), this cake layer formed on the membrane surface acts as a pseudo-membrane that changes both: permeability and selectivity of the overall membrane. In the absence of larger molecules (Fig. 6b) the phenomena related with cake formation are mitigated. Thus, the sugar retention is similar for the musts obtained from Garnacha and Verdejo varieties, during the second stage.

Fig. 6a shows that the process in which the stops were carried out (Verdejo 1NF) sugars rejection is higher than in the process without them. This suggests that the thinner cake formed, probably composed of smaller aggregates, as mentioned, may be less porous and therefore less permeable to sugars too.

Figure 6

4.2. Analysis of the filtered musts

Concentrations of the resultant permeates and retentates were analyzed, for each stage and NF process. Results for the Garnacha red must and for the Verdejo white must are shown in Tables 2and 3 respectively. Note that some parameters have not been determined for some samples because they were considered irrelevant.

Table 2

Table 3

Results show that the general trend is a high reduction of total sugars in the permeates and an increase in the retentates.

Regarding the effect of NF on the concentration of low molecular weight compounds such as malic and tartaric acid, Tables 2 and 3 show that the variations are not so significant for these compounds. Furthermore, since the purpose is to produce low alcohol wines, the permeate has to be mixed with untreated must (P1+C) or with the retentate (P2+R1) in adequate proportions before its fermentation. In this way, the

reconstructed must will be chemically very similar to the original one but with a lower sugar content and the variation of the other compounds will be reduced. In accordance to the total sugar content of the mixtures, the probable alcoholic degree of the resulting wine can be estimated from tables (García - Barceló, 1990). In this way, Table 2 shows that the blend (P2+R1) of red musts predicts a 1.67º reduction of the alcohol content. In the case of white musts (Table 3), the mixtures (P1+C) and (P2+R1) predict a decrease by 2.17º and 1.49º respectively. In all cases this predictive parameter shows that the alcohol reduction would be around 2º as intended. Note that the reduction is not exactly the same in all cases due to the difficulty involved in determining the exact proportions of musts to blend.

The influence of the NF procedures on the phenolic compounds was measured in terms of the total polyphenols index (TPI) and the color index (CI), since they are related to the color of must (see Tables 2 and 3). In the case of the main red musts samples (i.e. GC, R1 and (R1+P2)), the content of anthocyanins was also measured. Results show that nanofiltration did not allow the passage of polyphenolic compounds due to their higher molecular weight. Therefore their concentration increased in the retentates and was lower in the permeate samples. Moreover, it can be seen that in the case of some permeate samples CI could not be detected (N/D). The blending of the permeate with untreated must (single-stage NF) or with the retentate (two-stage NF) reduces the final loss of these compounds. Furthermore, as shown in Table 3, the mixture with the first retentate (P2+R1) promotes a higher recovery of these substances than with untreated must. In this way, if the chemical and sensory characteristics of the wine obtained from the blend (P2+R1) are similar or better than those of the blend (P1+C) it can be said that the best technique is the two-stage NF. Besides, this process minimizes volume losses, as it will be discussed later.

4.3. Production and analysis of wines

As mentioned in section 3.4, 6 different wines were elaborated. Three Garnacha red wines: GC, G2NF1, G2NF2 and three Verdejo white wines: VC, V1NF and V2NF-. Table 4 shows the results of the chemical analysis of the six wine samples.

Table 4

In the case of the Garnacha wines, after the malolactic fermentation, wines G2NF1 and G2NF2 had an alcohol degree lower by 1.2º and 1.4º %vol respectively in comparison to the control C wine. In both cases the alcohol reduction achieved was lower than the 2º expected. This could be due to the additional input of untreated must (i.e. sugar content) remaining in the crushed grape mass that was blended with the mixture (R1+P2) prior to the alcoholic fermentation. Regarding the parameters of Total Acidity (T.A.) and pH, no significant differences were determined between the 2 nanofiltered samples and also in comparison with the control wine. Volatile acidity (V.A.) is similar for the G2NF1 and G2NF2 but slightly higher when compared to the control wine. This could be understood as a minor deterioration of must during the NF process, since the V.A. values correspond to the fatty acids including those related with the acetic series (i.e. acetic, acetate, formic, propionic, butyric). As also observed for the resulting musts, nanofiltration affected the concentration of polyphenols and the parameters related (CI and anthocyanins). Table 4 shows that wines G2NF1 and G2NF2 presented a 14% and 16.5% TPI loss respectively when compared with the control wine.

Regarding the Verdejo samples, after alcoholic fermentation, V1NF wine had a 1.93º %vol lower alcohol content when compared to the control, but no alcohol degree reduction was achieved in the V2NF wine. Note that the sugar content of the must mixture (P2+R1) would have led to a lower alcohol degree. It is probable that some microbiological contamination could have promoted this. Also, the fermentation of this blend took longer than the one of the other musts causing ~~But~~ the high VA and IC values measured for this sample~~. suggest that some problems during the alcoholic~~

fermentation could have occurred. The degradation of the wine related to these parameters respectively is for example the formation of acetic acid and the oxidation of compounds related with the color of it.

Also here, a 15% TPI loss was determined for the V1NF sample.

All in all, it can be said that among the processes studied, the best NF technique is the two- stage process without backflush. This technique allows not only an appropriate sugar content reduction, but the mixture (P2+R1) promotes a higher recovery of polyphenolic compounds (i.e. color). Besides it minimizes volume losses since the retentate of the first stage and the permeate of the second stage are used for the appropriate blend of must and. Only the permeate of the second stage and the volume retained in the pump and in the module are of no use. At a larger scale (industrial scale) these dead volumes are negligible and it is has been estimated that in the 2NF technique proposed the volume losses would be around 18%.

4.4. Chemical and sensory characteristics of the resulting wines

Sensory evaluation of the wines was carried out only with 5 samples: 3 with lower alcohol content (G2NF1, G2NF2 and V1NF) and the respective control samples (GC and VC). The V2NF wine was not included in this analysis because fermentation was not correct. However, the process of sugar reduction was satisfactory, so the results of this experience have been kept.

Results of the chemical analysis (presented in Table 4) and acceptability test were put into a matrix form. This data matrix consisted of 5 wine samples (rows) by 12 variables (columns): 7 physicochemical and 5 sensorial.

The data matrix of variables analyzed was subjected to PCA in order to decrease the number of results associated with the data set while still explaining the maximum amount of variability present in the data (Shin et al., 2010). In this way, a new set of orthogonal variables (PCs) was generated.

The first 2 PCs explain the 90.32% of the total variance in the data set. Fig.7 shows the plot of the 5 wine samples, the 5 sensorial and 7 physicochemical variables in the first 2 PCs. Furthermore, the first principal component, PC1, accounts for 69.92% of the variability data and PC2 explains the 20.41% of the data variance.

Specific patterns of correlations between the variables can be appreciated from the plot between the PCs, where the position of the variables respect to one other and their corresponding correlations can be visualized. In order to analyze this, the Pearson correlation between the sensorial and physicochemical variables tested was performed. Results showed that the chemical variables related with color, namely TPI, CI and Anthocyanins are strong and positively correlated with the sensorial variable colour ($r=0.945$; $r=0.972$ and $r=0.947$ respectively with a significance level $p<0.05$). Moreover, the sensorial variable flavour is positively correlated with pH ($r=0.959$, $p<0.05$) and negatively correlated with total acidity ($r=-0.994$, $p<0.05$). Therefore it can be said, that these sensorial variables (evaluated by the consumers) are appropriately correlated with the chemical variables that describe them.

From the sensorial point of view it may be appreciated that none of the samples was particularly preferred by the consumers. Moreover, since there is no significant difference between the control or the filtered samples, this general trend can be attributed to an absence of substantial moddifications apart from the alcohol reduction.

It can be noticed that red wines, especially the control (GC) and G2NF1, were preferred by their odour and colour. The sample G2NF2 presented a high volatile acidity and lower TPI and therefore it is located further from the other 2 red wines and was less preferred. Since both, G2NF1 and G2NF2 were obtained from the same nanofiltrated must, it can be said that the differences of G2NF2 were caused by the fermentation and they are not related with the nanofiltration process. Results show that the filtration did not affect significantly the odour and colour acceptance of the resulting red wines, since the G2NF1 had the highest colour and odour acceptance. Regarding white wines, they showed the highest acceptance in flavour and overall liking,

especially the control one. But they were not located in the space defined by the colour and odour descriptors. Moreover, the sample V1NF presented lower persistence in mouth, flavour and overall liking. These features could be related, from the sensorial point of view, to a wine with a lower alcohol degree, even though the alcohol degree is not strongly correlated with any other descriptor. Besides, Verdejo is a variety characterized by its aroma components (volatile compounds). That is why nanofiltration could be more effective in the loss of these compounds in this variety.

Figure 7

5. Conclusion

After the assessment of the different NF techniques studied, the main following conclusions can be raised:

- The ~~effect~~ use of ~~the~~ backflush ~~studied~~ during the single-stage NF of Verdejo must is not appropriate since it caused lower permeate flow values ~~than for the two-stage NF process~~. This means that it does not improve the productivity of the process.
- ~~Apparently, the permeate backflush affects the foulants deposition and attachment behavior on the membrane surface suggesting that a thinner cake or the formation of smaller aggregates may have higher resistance to the permeate flux. Moreover, it may be less porous and therefore less permeable to sugars too~~.
- The mixture of the second must permeate with the first retentate (P2+R1) promoted a higher recovery of polyphenolic compounds than with untreated must (P1+C).

Regarding the wines produced, the following conclusions can be made:

- ~~Alcohol degree reduction was achieved for the red wines produced of the two-stage NF process (i.e. G2NF1 and G2NF2). In the case of white wines only the~~

~~wine proceeding of the single- stage NF (V2NF) process had the appropriate alcohol degree content~~.

- The techniques studied here for sugar control in grape juice allow the partial reduction of alcohol in the resulting wine. Results show that the two-stage NF process promotes a higher IPT recovery and less volume losses.
- Sensory evaluation and PCA analysis showed that none of the wine samples was particularly preferred by the consumers, showing that there were no significant differences between the control and the filtered wines.
- NF did not affect significantly the odour and colour of the resulting red wines, since the G2NF1 had the highest colour and odour acceptance.

Moreover, the depletion of aroma components observed during Verdejo filtration may be analyzed in future studies by the recovery of these compounds using pervaporation before NF.

~~All in all, it can be said that among the processes studied, the best NF technique is the two- stage process without backflush. This technique allows not only an appropriate sugar content reduction, but the mixture (P2+R1) promotes a higher recovery of polyphenolic compounds (i.e. color). Besides it minimizes volume losses since the retentate of the first stage is used for the appropriate blend of must.~~

All in all, it can be said that this study reveals the feasibility of single-and-two-stage NF processes for sugar reduction in grape must without a significant alteration of important compounds such as polyphenols, malic and tartaric acids. This allows the production of wines with sensorial and chemical characteristics similar as wines obtained of the fermentation of untreated musts. Therefore, this technique could be applied at a larger scale for the production of low alcohol content wines.

Nomenclature

Roman

$C_{0,i}$	feed concentration of the i-th component (kg m^{-3})
$C_{m,i}$	concentration of the i-th component on the membrane active layer (kg m^{-3})
$C_{p,i}$	permeate concentration of the i-th component (kg m^{-3})
D_i	diffusion coefficient of the i-th component (m^2 s^{-1})
J_v	permeate flux per unit of area through the membrane (m^3 m^{-2} s^{-1})
$J_{v,0}$	permeate flux per unit of area through the membrane at time t =0 (m^3 m^{-2} s^{-1})
$K_{m,i}$	mass transfer coefficient (m s^{-1}) of the i-th component at impermeable membranes (m s^{-1})
$K^s_{m,i}$	mass transfer coefficient of the i-th component at semipermeable membranes (m s^{-1})
R_f	resistance due to fouling (m^{-1})
R_i	membranes true retention for the i-th component
R_m	membrane resistance (m^{-1})

Greek

$\Delta\pi$	osmotic pressure gradient (Pa)
η	solution viscosity (Pa s)
η_f	feed viscosity (Pa s)

υ_{eff} effective velocity (m s^{-1})

ρ_f feed density (kg m^{-3})

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Appendix A. Supplementary data

The following are the supplementary data to this article:

Supplementary data

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Tables

Table 1

Methods used for the determination of some oenological parameters of musts.

Parameter	Principle	Method
Glucose and Fructose	Ion exchange Chromatography	HPLC
Tartaric and Malic Acid	Chromatography	HPLC
pH	Potentiometry	pH- meter
Volatile acidity	Acid- base titration	García-Tena [a]
Total acidity	Potentiometric titration	OIV [b]
SO_2 T and SO_2 F	Iodometry	Ripper automated
Alcoholic degree	Ebullometry	Barus apparatus [c]
Total Polyphenols	UV absorbance	UV/Vis spectrophotometry
Anthocyanins	Vis absorbance	UV/Vis spectrophotometry [d]
Color	Vis absorbance	UV/Vis spectrophotometry [b]

[a] (García - Barceló, 1990)
[b] (OIV, 2011)
[c] (Amerine and Ough, 1976)
[d] (Ribereau-Gayon P. and Stonestreet E., 1965), (Lasanta et al., 2014)

Table 2

Oenological parameters of Garnacha red musts after the two-stage NF process and of the control must (C).

	Control (C)	Garnacha two-stage NF				
		P1	R1	P2	R2	P2+R1
Glucose (g/L)	105 ± 2	27.6± 0.6	135 ± 3	5.73 ± 0.13	92 ± 2	90 ± 2
Fructose (g/L)	106 ± 4	27.0 ± 1.0	138 ± 5	7.5 ± 0.3	92 ± 3	93 ± 3
Probable alcoholic Degree[a] %vol	12.5 ± 0.3					10.9 ± 0.3
TH_2 (g/L)	4.4 ± 0.5	3.0 ± 0.4	4.3 ± 0.5	1.51 ± 0.19	4.8 ± 0.6	3.9 ± 0.5
MH_2 (g/L)	0.59 ± 0.01	0.53 ± 0.01	0.47 ± 0.01	0.43 ± 0.01	0.58 ± 0.01	0.49 ± 0.01
pH	3.02 ± 0.01	----	3.12 ± 0.01	----	----	3.19 ± 0.01
T.A. (g TH_2 /L)	3.21 ± 0.01	----	3.06 ± 0.1	----	----	2.70 ± 0.14
TPI	8.8 ± 0.3	1.18 ± 0.01	12.9 ± 0.5	0.29 ± 0.01	----	7.93 ± 0.18
CI	0.34 ± 0.04	0.02 ± 0.01	0.28 ± 0.04	N/D	N/D	0.20 ± 0.06
Anthocyanins (mg/L)[b]	63 ± 3	----	91 ± 7	----	----	56.9 ± 1.2

C: Control must, P1: permeate of the first stage, R1: retentate of the first stage, P2: permeate of the second stage, R2: retentate of the second stage.

T.A: Total acidity expressed as g TH_2 per liter, TH_2: Tartaric acid, MH_2: Malic acid, TPI: Total Polyphenol Index, CI: Color Index

N/D: not detectable

[a] Estimated from tables of the alcoholic degree to be expected on the basis of 16.83 g sugars from must per 1% alcohol (García - Barceló, 1990)

[b] expressed as mg malvidin-3 glycoside per liter

Table 3

Oenological parameters of Verdejo white musts after the single-stage and two-stage NF processes and of the control must (C).

	Control (C)	Verdejo single- stage NF			Verdejo two-stage NF				
		P1	R1	P1+C	P1	R1	P2	R2	P2+R1
Glucose (g/L)	117.0 ± 1.6	48.4 ± 0.7	157 ± 2	97.9 ± 1.3	48.9± 0.7	149 ± 2	12.75 ± 0.17	127.9 ± 1.8	105.2 ± 1.4
Fructose (g/L)	120.4 ± 1.6	46.6 ± 0.6	164 ± 2	102.1 ± 1.4	46.8 ± 0.6	156 ± 2	13.36 ± 0.18	126.7 ± 1.7	106.8 ± 1.4
Probable alcoholic Degree[a] %vol	14.05 ± 0.05	---	----	11.88 ± 0.04	----	----	----	----	12.56 ± 0.04
TH_2 (g/L)	3.8 ± 0.5	2.4 ± 0.3	3.5 ± 0.4	4.1 ± 0.5	2.4 ± 0.3	3.5 ± 0.4	1.7 ± 0.2	2.6 ± 0.3	3.3 ± 0.4
MH_2 (g/L)	1.47 ± 0.01	1.42 ± 0.01	1.20 ± 0.01	1.31 ± 0.01	1.51 ± 0.01	1.23 ± 0.01	1.40 ± 0.01	1.42 ± 0.01	1.66 ± 0.02
pH	3.40 ± 0.01	----	3.37 ± 0.01	3.35 ± 0.01	----	3.36 ± 0.01	----	----	3.37 ± 0.01
T.A. (gTH_2 /L)	2.66 ± 0.03	----	3.06 ± 0.16	3.27 ± 0.03	----	3.04 ± 0.08	----	----	2.91 ± 0.11
TPI	5.47 ± 0.12	0.06 ± 0.01	8.37 ± 0.01	4.11 ± 0.03	----	8.30 ± 0.16	0.01 ± 0.01	----	5.97 ± 0.08
CI	0.07 ± 0.01	0.01 ± 0.01	0.12 ± 0.01	0.06 ± 0.01	----	0.13 ± 0.01	N/D	----	0.09 ± 0.01

C: Control must, P1: permeate of the first stage, R1: retentate of the first stage, P2: permeate of the second stage, R2: retentate of the second stage.

T.A: Total acidity expressed as g TH_2 per liter, TH_2= Tartaric acid, MH_2: Malic acid, TPI: Total Polyphenol Index, CI: Color Index.

N/D: not detectable

[a]Estimated from tables of the alcoholic degree to be expected on the basis of 16.83 g sugars from must per 1% alcohol (García - Barceló, 1990)

Table 4

Chemical analysis for the main oenological parameters of the red and white wines

Chemical parameter	Garnacha			Verdejo		
	C	2NF1	2NF2	C	1NF	2NF
pH	3.21 ± 0.01	3.29 ± 0.01	3.31± 0.01	3.37 ± 0.01	3.31 ± 0.01	3.42 ± 0.01
T.A. (gTH_2 /L)	4.60 ± 0.03	4.26 ± 0.03	4.05 ± 0.05	3.75 ± 0.05	3.92 ± 0.03	3.75 ± 0.05
V.A. (g /L)	0.23 ± 0.01	0.31 ± 0.01	0.34 ± 0.01	0.37 ± 0.01	0.18 ± 0.01	0.63 ± 0.05
TPI	42.9 ± 0.4	36.93 ± 0.04	35.85 ± 0.07	6.31 ± 0.08	5.37 ± 0.01	7.75 ± 0.05
Anthocyanins (mg/L)[a]	371 ± 14	355 ± 2	288 ± 3	4.4 ± 1.9	2.63 ± 0.01	2.2 ± 1.9
CI	11.1 ± 0.2	8.19 ± 0.07	6.94 ± 0.02	0.09 ± 0.01	0.07 ± 0.01	0.10 ± 0.01
Alcoholic Degree (%vol)	12.40 ± 0.18	11.20 ± 0.01	11.00 ± 0.01	13.88 ± 0.01	11.95 ± 0.01	14.00± 0.01

Garnacha C: Garnacha Control wine, Garnacha 2NF1and 2NF2: Red wines obtained from fermentation of the mixture (R1+P2), Verdejo C: White control wine, Verdejo 1NF: white wine obtained after the fermentation of the mixture (C+P1), Verdejo 2NF: white wine obtained after the fermentation of the mixture (R1+P2)

T.A: Total acidity expressed as g TH_2 per liter, TH_2= Tartaric acid, MH_2: Malic acid, TPI: Total Polyphenol Index, CI: Color Index

[a] expressed as mg malvidin-3 glycoside per liter

Figure Captions

Figure 1. Scheme of the experimental device used in the Nanofiltration processes.

Figure 2. Scheme of the procedure carried out for the Garnacha red must two-stage Nanofiltration and ulterior fermentations.

Figure 3. Scheme of the Nanofiltration procedures carried out for the Verdejo white must and ulterior fermentation. a) two-stage and b) single-stage nanofiltration.

Figure 4. Kinetics of the permeate flux of the three filtration processes. (a) and (c) for the two-stage filtration processes, of red and white must respectively. (b) shows the kinetics of the white permeate must during the single-stage NF process. Dashed lines represent the 2-minutes-stops with osmotic backflushing.

Figure 5. Comparison of the filtration processes of the red and white musts during the first (a) and second stage (b), in terms of the initial permeate flux ($J_v/J_{v,0}$).

Figure 6. Comparison of the sugars rejection during the first (a) and the second stages (b) of filtration of both musts.

Figure 7. Principal Component Analysis of the wine samples and the physicochemical and sensorial characteristics. Symbols. Physicochemical variables: Anthocyanins (A); Total Acidity (TA); Volatil Acidity (VA); Total Polyphenol Index (TPI); Color Index (CI); Alcoholic Degree (AD). Sensory descriptors: Color (C); Odor (O); Flavor (F); Persistence (P); Overall liking (OL). Wine samples: Verdejo: Control (VC); single-stage (V1NF). Garnacha: Control (GC); two-stage (G2NF1) and fermentation duplicate (G2NF2).

Figure 1

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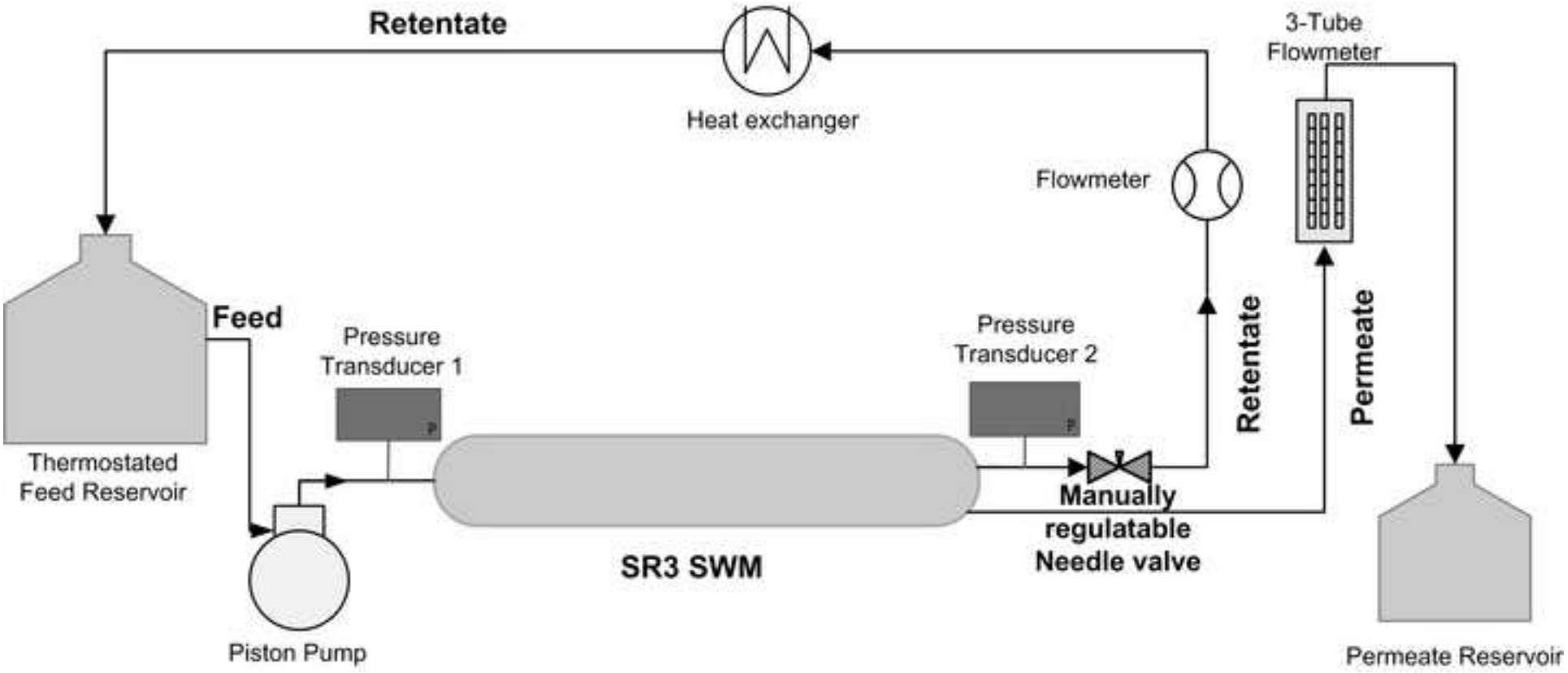

Figure 2

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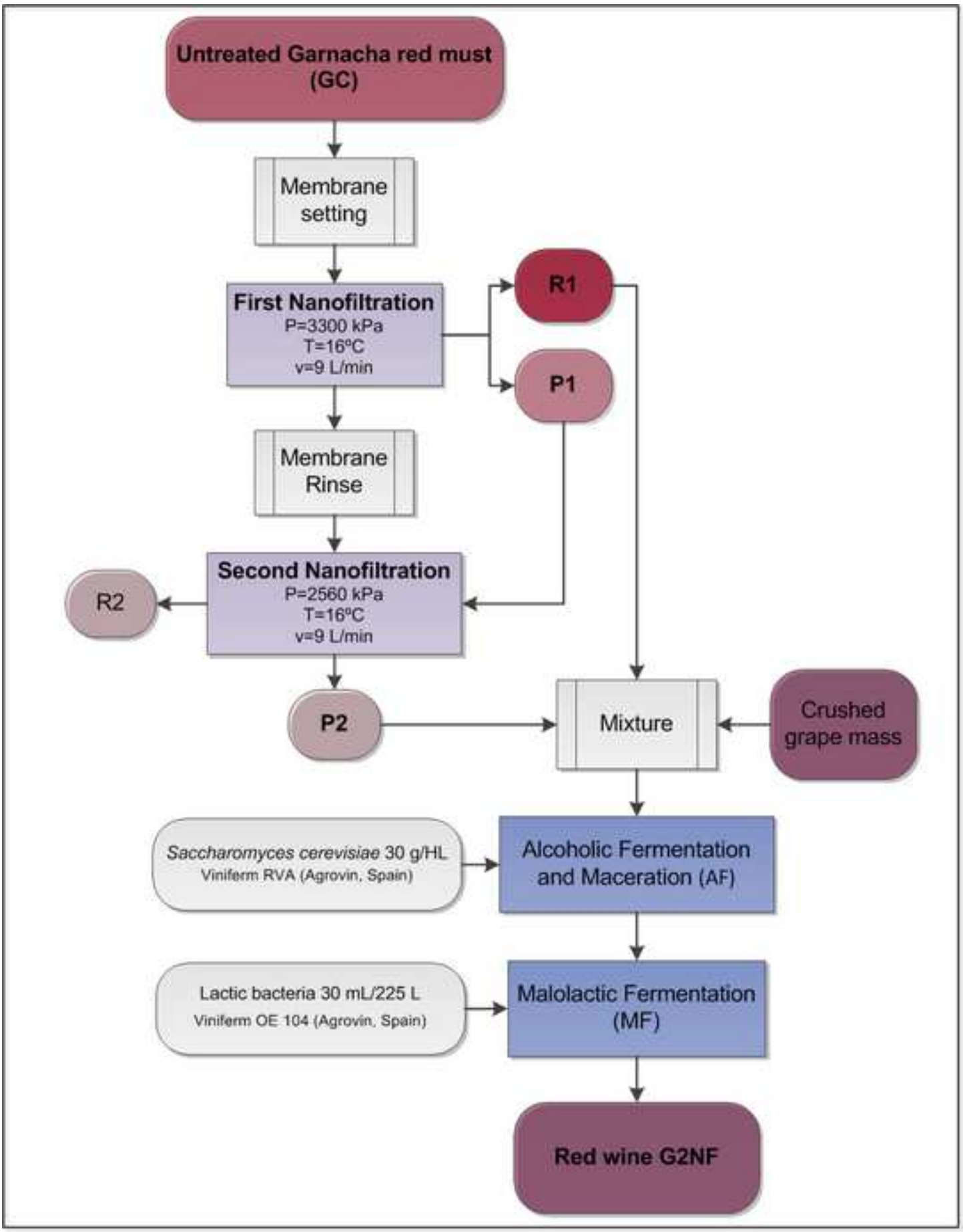

Figure 3

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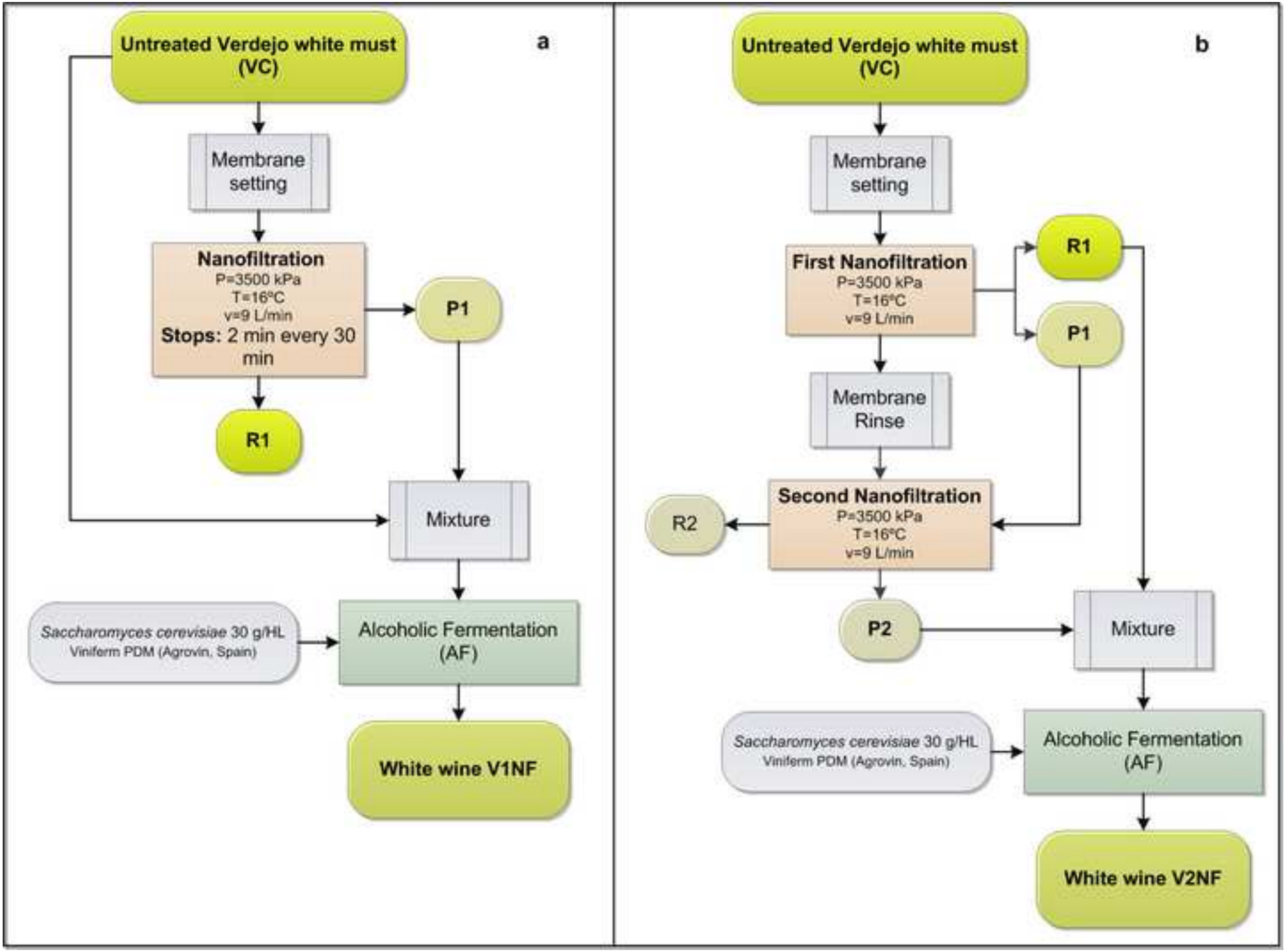

Figure 4

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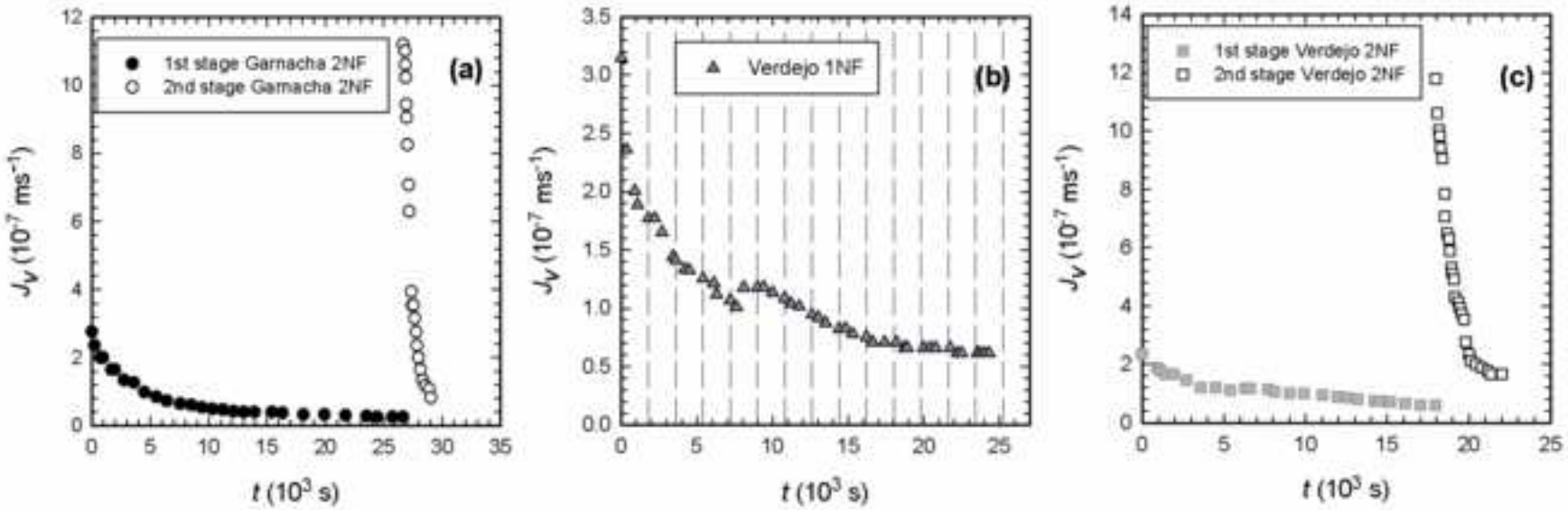

Figure 5

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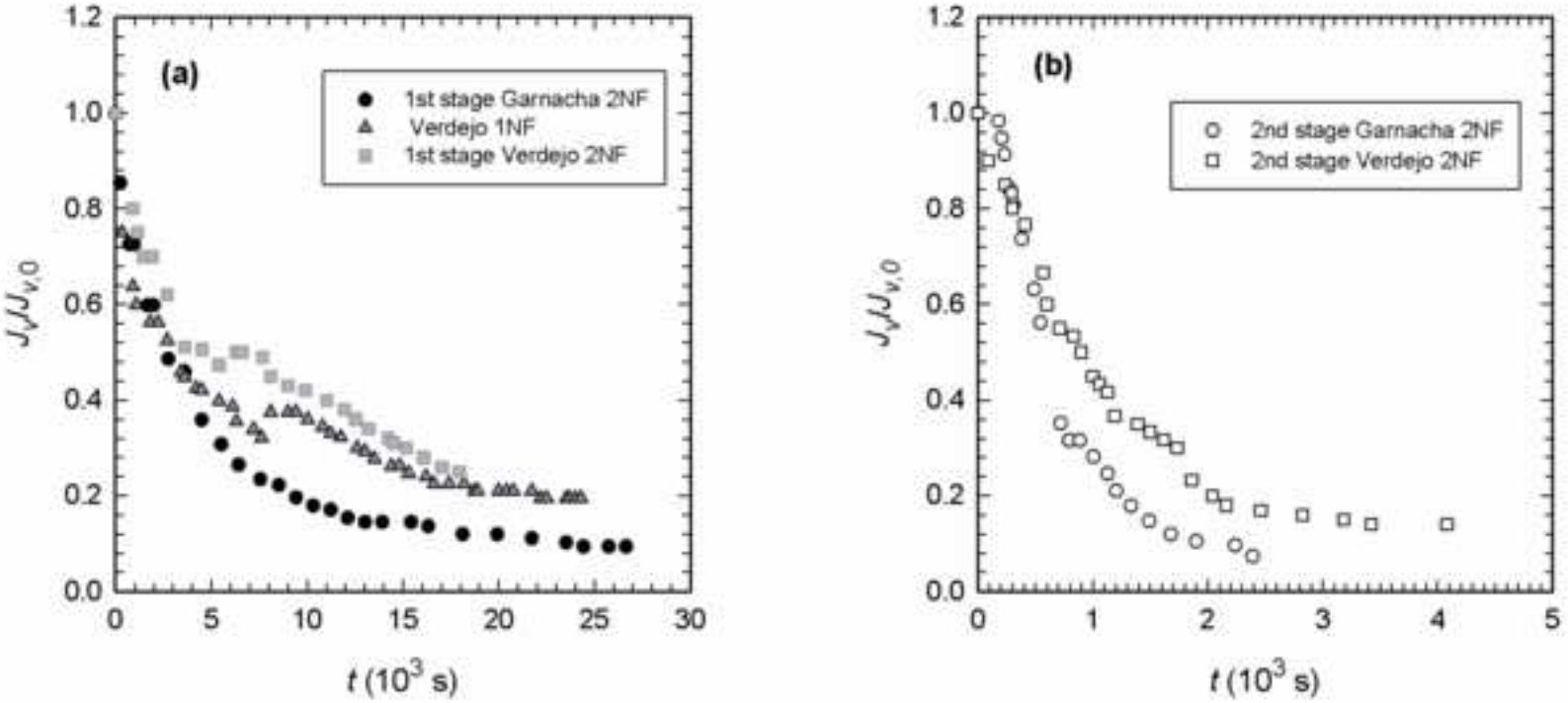

Figure 6

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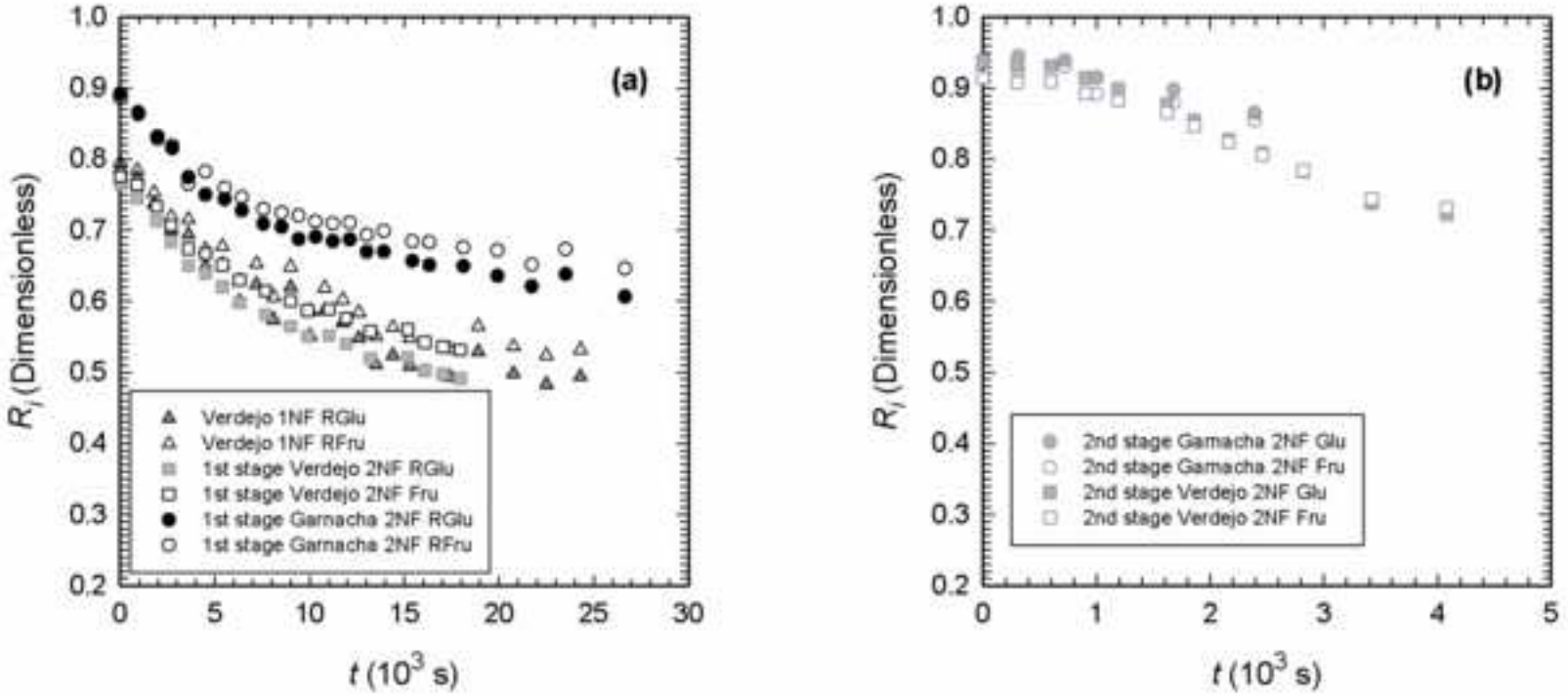

Figure 7

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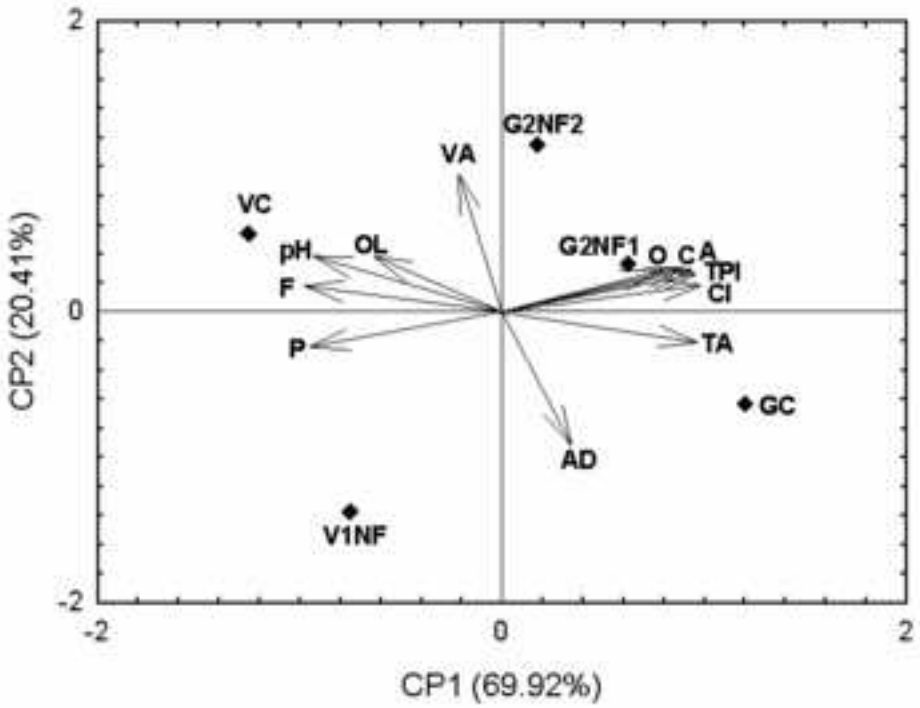

Supplementary Material

Table S1
Main characteristics of the 3839 SR3-NYV spiral wound module

MWCO (Da)[a,b]	Lactose Rejection (%)[a,b]	pH range	Max. Pressure (10^5 Pa)[b]	Max. Temperature (ºC)[b]	Active membrane Area A_m (m^2)[b]	Feed spacer porosity[c], ε	Feed spacer height, H (10^{-3}m)[b]	Leaf width W (m)[d]
200	99.900	3- 10	41.400	50.000	7.061	0.850	0.787	3.608

[a] 5% Lactose at 1380 kPa
[b] Provided by the manufacturer
[c] (Vrouwenvelder et al., 2010)
[d] Own determination

Table S2
Hydraulic permeability, L_p, and membrane resistance, R_m, both initially and after filtration and cleaning procedure.

Process	Water Permeability L_p (10^{-12}m/Pa·s)	Membrane Resistance R_m (10^{14} m^{-1})
Initial: Before filtrations	7.88	1.27
After red must two- stage NF	6.15	1.62
After white must single- stage NF	6.04	1.65
After white must two- stage NF	5.78	1.72
After manufacturers cleaning procedure	7.82	1.27

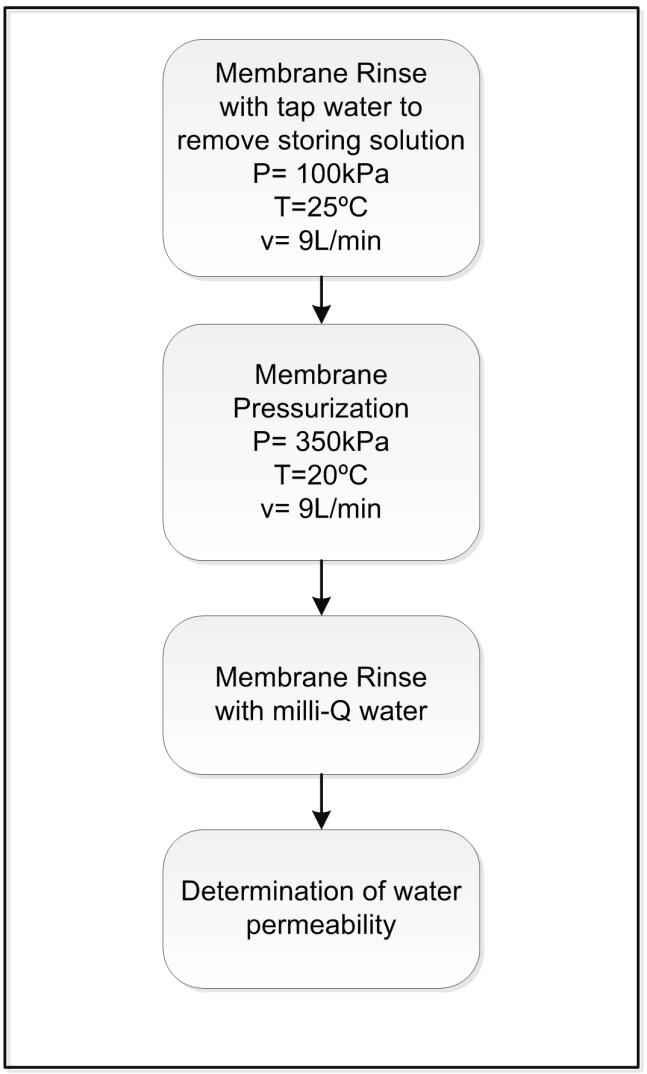

Figure S1. Procedure for Membrane conditioning

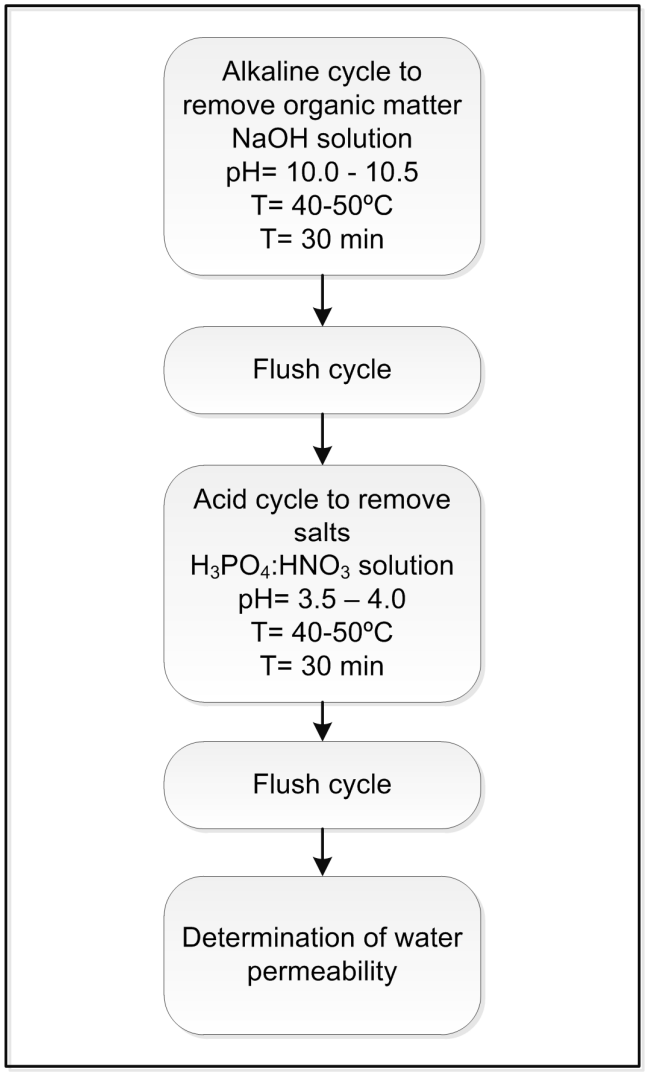

Figure S2. Cleaning Procedure for the SR3 Spiral Wound Module according to manufacture´s instructions

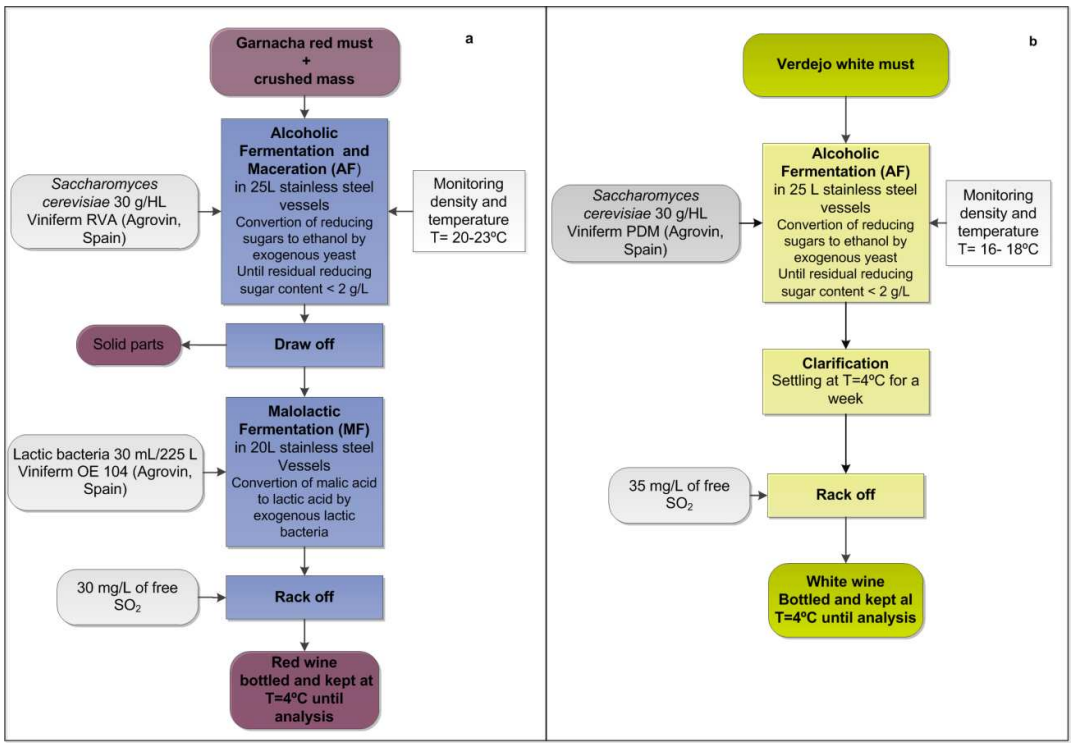

Figure S3. Traditional winemaking procedures for (a) red and (b) white wines

References

Vrouwenvelder, J.S., Picioreanu, C., Kruithof, J.C., van Loosdrecht, M.C.M., (2010). Biofouling in spiral wound membrane systems: Three-dimensional CFD model based evaluation of experimental data. Journal of Membrane Science 346(1), 71-85