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Impact of Must Sugar Reduction by Membrane Applications on Volatile Composition of Verdejo Wines

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ABSTRACT: Climate changes are inducing increased sugar levels of must, which produces negative effects on wine quality, as unbalanced wines with high degrees of alcohol. So, effective strategies to control the increase of sugar levels in must have been studied. One of them is the use of a membrane process, and this is applied in this work. The sugar level of white must from Verdejo (*Vitis vinifera* variety) was reduced using diverse membrane processes, and the effect of this fact on the volatile composition of the corresponding wines is studied. The study was carried out during three consecutive vintages. An important impact of the reduction of sugar levels of must on the volatile composition of the obtained wines was detected, which was due to some retention phenomena of aromatic and precursor compounds. To minimize the volatile composition modifications, an appropriate selection of the nanofiltration membrane must be done.

KEYWORDS: *must, sugars, membrane, nanofiltration, white wines, volatile compounds, alcohol degree, amino acids, Verdejo*

INTRODUCTION

Nowadays, the wine sector is highly affected by climate changes, mainly due to the increase of maximum temperatures.[1−3] This fact has an important repercussion on the synthesis of sugars[4,5] and then on the final alcohol content of wine.[6]

It is well-known that, during grape ripening, sugars are accumulated in grapes, while acid levels decrease. *Industrial maturity* has been defined as the moment in which grapes reach the maximum values of probable alcohol degree, and *technological maturity* as the moment in which grapes show the most appropriate composition to make a determinate type of wine. Then, *technological maturity* is mainly associated with the balance among levels of sugars, acids, and aroma precursors, in the case of white grapes, and among levels of sugars, acids, phenolic compounds, and aroma precursors in the case of red ones.[7] The ripening changes of sugar and acid levels occur faster in hot regions and countries.[6] So, in these zones, grapes quickly reach *industrial maturity* whereas they rarely reach *technological maturity*.[7] Consequently, wines made from these grapes will probably be unbalanced and unpleasant, and will probably show high levels of alcohol, low acidity, and poor aromatic notes.

A slight increase of the average temperatures has been detected during the later years in different Spanish regions. As a result, some imbalanced white wines, with high alcohol content, low acidity, and low aromatic notes, have been produced in these zones. Similar results have been also described in other countries.[8] In general, wines with alcohol content higher than 15% (v/v) are often rejected by consumers because they are heavy and burning in the mouth.[3,9,10] Furthermore, in the case of white wines, alcohol degrees over 13% have been usually described as unpleasant.[11] Besides this, although the presence of alcohol had a positive effect on the perception of aroma notes,[12] this effect depends on the level of ethanol in the medium as well as on the type of volatile compounds and aroma notes;[13] and a masking effect of aroma perception by high levels of ethanol has been also described.[14]

In spite of the final quality and acceptance of wines, musts with high contents of sugars usually show additional enological problems, such as difficulty in carrying out alcoholic fermentation, with sluggish fermentation, and even fermentation stops.[15] This fact gives origin to new problems, due to the microbiological instability of wines with high levels of residual sugars.

To mitigate the cited problems, some strategies useful to control and reduce sugar levels of must have been developed during the years. Some of them are based on the dilution of must, a fact usually carried out using must from unripe grapes, which have low levels of sugar and high values of acid. Similarly, wines could be also made directly from unripe grapes. However, both strategies make difficult the elaboration of good quality wines, due to unripe grapes usually giving herbaceous notes. Other strategies are based on transforming fermentable sugar into nonfermentable substrates. Some authors studied this possibility applying glucose oxidase to transform glucose into gluconic acid.[16−18]

The most recently applied technologies are based on the use of membrane processes which retain the sugars of musts.[19−22]

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The effect of membrane strategies on the quality of wines made from modified must has been poorly studied. A preliminary work of our group showed that the volatile composition of wine was significantly modified when sugar levels of must were reduced by using membrane technologies.[23] This fact was attributed to membrane retention phenomena in which different aroma compounds and precursors could be involved. Some similar results were pointed out after the membrane filtration of red wines.[24] Based on these results, the work shown in this paper was focused on the use of diverse membranes to reduce the sugar levels of white musts from Verdejo (*Vitis vinifera* variety). The desired aim was to establish the best conditions to obtain less alcoholic white wines without modifying their aromatic profile. A maximum reduction of two probable alcohol degrees of musts was looked for. This is the legal limit allowed by European legislation.[25] To achieve this aim, different assays were carried out during three consecutive vintages.

This paper is mainly focused on the volatile composition of the obtained wines. The repercussion of must modifications on different volatile compounds was evaluated and discussed. Other interesting parameters, such as titratable and volatile acidity and levels of amino acids, were also considered. These parameters were useful for the discussion of the volatile profile changes detected.

MATERIALS AND METHODS

Reagents and Standards. All standards of amino acids, the derivative agent DEEMM (diethyl ethoxy methylene malonate), dichloromethane of HPLC quality, and some of the volatile standards (2,3-butanediol, 1-octanol, benzyl alcohol, 2-phenylethyl alcohol, *trans*-3-hexen-1-ol, methionol, ethyl butanoate, ethyl lactate, ethyl octanoate, ethyl decanoate, α-terpineol, β-citronellol, linalool, geraniol, nerol, acetoin, 1-pentanol, isoamyl alcohol, *cis*-3-hexen-1-ol, benzaldehyde, and γ-butyrolactone) were purchased from Sigma-Aldrich (Quimica S.A., Madrid, Spain). 2-Octanol, ethyl isovalerate, isobutyl alcohol, isoamyl acetate, ethyl hexanoate, hexyl acetate, 3-methyl-1-pentanol, 1-hexanol, 1-heptanol, ethyl 3-hydroxybutanoate, β-phenylethyl acetate, diethyl succinate, hexanoic acid, octanoic acid, decanoic acid, and dodecanoic acid were purchased from Fluka (Quimica S.A., Madrid, Spain). 1-butanol, HPLC grade acetonitrile and methanol were purchased from Labscan (Dublin, Ireland) and 4-vinyl guaiacol was purchased from Panreac (Quimíca, S.A., Barcelona, Spain). Hydrogen, nitrogen, helium, and air gases were provided by Carburos Metálicos (Barcelona, Spain). Ultrapure water generated by the Milli-Q system Millipore (Bedford, MA, USA) was used.

Products and Processes. All the assays were carried out with Verdejo grapes, autochthonous white variety from the Denomination of Origin Rueda cited in the Autonomous Community of Castilla y León (North-West of Spain).

Due to the seasonality of grape production, the different assays described in this paper were carried out with grapes from three consecutive vintages. The initial musts were always obtained in the same way, and traditional white winemaking processes were applied. Grapes were transported to the experimental winery of the Oenological Station of Castilla y Leon (Rueda) in plastic boxes of 15 kg. After the reception, grapes were destemmed, crushed, sulfited (80 mg/L of SO_2), and pressed to obtain the respective must. Pectinolytic enzymes (10 mg/L of Novoclear Speed, Lamothe Abiet, France) were added to enhance first clarification. Once the must was cleared, one part of the must was filtered through 0.8 μm membrane plates in order to ensure optimum must clarity and to prevent ulterior membrane fouling. Thereby, clarified aliquots of must were used in the diverse membrane filtration options assayed, which were mainly nanofiltration process, although ultrafiltration membranes were also used.

Alcoholic fermentation was always carried out under controlled and similar conditions. Musts were inoculated with 20 g/HL of IOC *Saccharomyces cerevisiae* yeasts (Institut Oenologique de Champagne, France), and the fermentation temperature was controlled and maintained at 18 °C. Once the alcoholic fermentation was completed, the white wines were racked out, bottled, and maintained under controlled conditions until their analysis.

The first assays were carried out considering previous published results.[22] An aliquot of perfectly clarified must was treated by double filtration in the following steps: First, the clarified must was filtered to get a low volume of sugar rich retentate (R1) and a permeate with a medium sugar content (P1). After that, the first permeate (P1) was filtered through the same membrane until the viscosity and the osmotic pressure of the retentate did not allow any ulterior reduction of the retentate volume. This process provides a second retentate (R2) with high sugar content, and a second permeate (P2) with a low sugar content. A spiral wound module of nanofiltration HL series thin film membrane (HL2540FM, GE Water & Process Technologies, Barcelona, Spain) was used. According to the commercial company, this membrane is characterized by 98% cutoff retentation for magnesium sulfate and 2.67 water permeability.

To reduce the probable alcohol degree of the initial must (T), this must was mixed, in adequate proportions, with permeate P2. Maximum reduction of two probable degrees was looked for. The must obtained after mixing was labeled as (T+P2). Furthermore, the permeate P2 was mixed with adequate proportions of retentate R1 to obtain a new "must" with similar sugar content to the (T+P2) one, which was labeled as (R1+P2).

Initial (T), (T+P2), and (R1+P2) musts were fermented to obtain their respective wines, which were labeled similarly to the original musts. The (T+P2) wine and the (R1+P2) one were wines with reduced alcohol degree (RAD) with respect to the control one (T). In addition, in order to obtain complementary information about the possible retention of compounds with important repercussions on volatile profile of wines, retentate R1 was also fermented, and its corresponding alcoholic product was obtained; this was labeled as R1 wine.

Thirty liters of perfectly clarified must was treated by nanofiltration processes, while microvinifications were carried out with 4 L of each type of must. All the cited products were made in duplicate.

Considering the results of the first assays, new experiences were designed to be carried out from grapes of the second vintage. Single nanofiltration treatments to the initial must were applied. Three different types of membranes, HL (HL2540FM), DL (DL1812C-28D), and DK (DK1812C-28D), all of them provided by GE Water & Process Technologies (Barcelona, Spain), were used. According to the commercial company, DL is characterized by 96% cutoff retentation for magnesium sulfate and 2.27 water permeability, and DK by cutoff retention 98% for magnesium sulfate and 1.98 water permeability.

Perfectly clarified aliquots of the initial must were treated by nanofiltration using each one of the cited membranes. So, three different types of permeates were obtained. These permeates (P) were labeled with the code of the membrane used to obtain each one (HL, DL, and DK, respectively).

Similarly, to the first assays, permeates were used to reduce the sugar levels of the initial must. Adequate quantities of each type of permeate were mixed with aliquots of initial must (T), obtaining three different diluted musts, which were labeled with the same code that the permeates used to obtain them. Initial and diluted musts were fermented in similar conditions to obtain their corresponding wines, which were labeled in the same way as their respective musts. This time, microvinifications were carried out using 16 L of each type of must. The cited types of wines were made in duplicate.

Considering the results of the second assays, and to corroborate them, new assays were planned to be carried out with grapes from the third vintage. The membrane which previously gave the best results was chosen to carry out these experiments. To check the possibility of improving the obtained results, the combination of ultrafiltration (UF) and nanofiltration (NF) was studied. The ultrafiltration membrane GH (GH1812C-28D, GE Water & Process Technologies, Barcelona,

Table 1. Mean Values of the Levels of Each Studied Volatile Compound in All Obtained Wines during the Three Developed Assays[a]

wine	1st assays				2nd assays				3rd assays		
	T[b]	T+P2	R1+P2	R 1	T	HL	DL	DK	T	NF	UF+NF
Fusel Alcohols											
isobutyl alcohol	49.7 ± 7.6 c[c]	22.0 ± 1.0 a	15.8 ± 1.0 a	35.8 ± 3.8 b	20.8 ± 0.5 b	17.1 ± 2.1 a	17.7 ± 1.0 a	15.9 ± 0.8 a	14.4 ± 0.1 b	12.4 ± 0.3 a	12.2 ± 0.2 a
isoamyl alcohol	324 ± 61 c	219 ± 21 b	153 ± 9 a	286 ± 5 bc	198 ± 12 c	171 ± 20 b	166 ± 14 ab	139 ± 2 a	169 ± 2 b	168 ± 3 b	163 ± 1 a
benzyl alcohol	0.464 ± 0.066 c	0.320 ± 0.021b	0.139 ± 0.006 a	0.195 ± 0.022 a	0.083 ± 0.009 b	0.070 ± 0.011 a	0.065 ± 0.010 a	0.083 ± 0.010 b	0.110 ± 0.003 b	0.091 ± 0.003 a	0.101 ± 0.005 b
2-phenylethyl alcohol	39.5 ± 3.4 a	38.4 ± 2.0 a	37.2 ± 2.3 a	36.5 ± 3.2 a	15.7 ± 1.2 b	13.7 ± 1.2 a	13.6 ± 0.7 a	16.5 ± 0.3 b	19.2 ± 0.6 b	16.0 ± 0.1 a	15.4 ± 0.6 a
∑fusel alcohols	414 ± 72 c	280 ± 23 b	206 ± 11 a	358 ± 2 bc	235 ± 13 b	202 ± 23 a	197 ± 15 a	172 ± 3 a	203 ± 3 c	196 ± 3 b	190 ± 1 a
Other Alcohols											
1-butanol	0.751 ± 0.062 b	0.389 ± 0.034 a	0.327 ± 0.024 a	2.22 ± 0.22 c	1.81 ± 0.10 c	1.55 ± 0.17 b	1.43 ± 0.09 b	0.780 ± 0.033 a	1.37 ± 0.03 b	1.39 ± 0.02 b	1.13 ± 0.05 a
2,3-butanediol	0.116 ± 0.025 b	0.123 ± 0.002 b	0.096 ± 0.007 ab	0.068 ± 0.008 a	0.072 ± 0.004 a	0.073 ± 0.005 a	0.080 ± 0.002 b	0.121 ± 0.005 c	0.087 ± 0.003 a	0.112 ± 0.005 b	0.109 ± 0.007 b
1-pentanol	0.075 ± 0.003 c	0.043 ± 0.001 b	0.030 ± 0.005 a	0.070 ± 0.008 c	0.054 ± 0.003 b	0.048 ± 0.004 a	0.045 ± 0.005 a	0.044 ± 0.001 a	0.150 ± 0.003 b	0.140 ± 0.002 a	0.180 ± 0.003 c
3-methyl-1-pentanol	0.100 ± 0.026 ab	0.082 ± 0.005 a	0.080 ± 0.022 a	0.128 ± 0.024 b	0.094 ± 0.003 a	0.082 ± 0.011 a	0.089 ± 0.009 a	0.086 ± 0.003 a	0.086 ± 0.001 b	0.083 ± 0.003 ab	0.079 ± 0.006 a
heptanol*	18.2 ± 0.4 c	15.8 ± 0.9 b	11.1 ± 0.5 a	14.5 ± 0.4 b	8.87 ± 0.94 b	7.16 ± 0.69 a	7.52 ± 1.10 ab	7.59 ± 0.18 ab	12.1 ± 0.2 b	11.7 ± 0.9 b	9.94 ± 1.09 a
∑other alcohols	1.06 ± 0.21 b	0.653 ± 0.037 a	0.545 ± 0.052 a	2.50 ± 0.27 c	2.04 ± 0.10 c	1.76 ± 0.19 b	1.65 ± 0.01 b	1.04 ± 0.02 a	1.81 ± 0.04 b	1.81 ± 0.02 b	1.81 ± 0.06 a
C6 Alcohols											
1-hexanol	0.473 ± 0.049 b	0.432 ± 0.024 b	0.296 ± 0.014 a	0.272 ± 0.010 a	0.611 ± 0.028 b	0.550 ± 0.046 b	0.532 ± 0.042 b	0.615 ± 0.010 b	0.720 ± 0.010 c	0.640 ± 0.009 a	0.670 ± 0.017 b
trans-3-hexen-1-ol*	14.0 ± 1.1 b	12.7 ± 0.5 b	7.22 ± 1.10 a	8.39 ± 0.10 a	117 ± 6 a	110 ± 10 a	110 ± 60 a	104 ± 5 a	110 ± 3 b	100 ± 2 a	97.8 ± 7.7 a
cis-3-hexen-1-ol*	41.6 ± 2.5 c	36.8 ± 2.0 b	21.0 ± 2.1 a	22.6 ± 0.1 a	105 ± 3 a	106 ± 8 a	120 ± 11 b	94.2 ± 4.2 a	62.7 ± 3.3 c	37.8 ± 1.6 a	42.5 ± 3.0 b
∑C6 alcohols	0.530 ± 0.052 b	0.481 ± 0.026 b	0.324 ± 0.017 a	0.303 ± 0.010 a	0.833 ± 0.037 a	0.767 ± 0.064 a	0.761 ± 0.059 a	0.813 ± 0.014 a	0.900 ± 0.010 b	0.780 ± 0.011 a	0.810 ± 0.022 a
Acetates											
hexyl acetate	0.039 ± 0.002 c	0.024 ± 0.002 b	0.016 ± 0.001 a	0.024 ± 0.001 b	0.333 ± 0.033 b	0.333 ± 0.012 b	0.240 ± 0.044 a	0.222 ± 0.007 a	0.710 ± 0.010 b	0.620 ± 0.012 a	0.630 ± 0.011 a
isoamyl acetate	3.79 ± 0.49 b	1.54 ± 0.12 ab	1.15 ± 0.05 a	5.22 ± 0.01 c	13.8 ± 2.2 b	13.8 ± 1.0 b	8.89 ± 1.85 a	7.15 ± 0.18 a	32.4 ± 2.8 b	28.4 ± 0.7 a	29.1 ± 0.4 a
β-phenylethyl acetate	1.98 ± 0.18 b	1.03 ± 0.11 a	0.962 ± 0.044 a	3.46 ± 0.39 c	1.75 ± 0.28 bc	1.93 ± 0.16 c	1.54 ± 0.08 b	1.14 ± 0.04 a	3.88 ± 0.14 b	3.21 ± 0.12 a	3.22 ± 0.15 a
∑acetates	5.81 ± 0.67 b	2.59 ± 0.22 a	2.12 ± 0.10 a	8.70 ± 0.40 c	15.9 ± 2.5 b	16.1 ± 0.9 b	10.7 ± 1.9 a	8.50 ± 0.22 a	36.9 ± 2.9 b	32.2 ± 0.8 ab	32.9 ± 0.4 a
Ethyl Esters											
ethyl butyrate	0.476 ± 0.083 b	0.246 ± 0.065 a	0.250 ± 0.013 a	0.609 ± 0.031 c	1.06 ± 0.09 b	1.01 ± 0.09 ab	0.868 ± 0.199 ab	0.786 ± 0.021 a	1.22 ± 0.05 b	1.11 ± 0.05 a	1.12 ± 0.01 a
ethyl 3OH butanoate*	4.36 ± 0.85 c	3.73 ± 0.55 bc	2.56 ± 0.37 ab	2.73 ± 0.30 a	1.98 ± 0.19 a	1.71 ± 0.39 a	2.10 ± 0.24 a	1.93 ± 0.26 a	1.28 ± 0.11 a	1.70 ± 0.41 a	1.55 ± 0.38 a
ethyl isovalerate*	19.2 ± 2.6 b	12.9 ± 1.6 a	11.7 ± 0.7 a	12.1 ± 2.6 a	5.95 ± 0.53 bc	5.55 ± 0.18 ab	5.24 ± 0.35 a	6.28 ± 0.26 c	3.89 ± 0.26 a	3.65 ± 0.16 a	4.05 ± 0.51 a
ethyl hexanoate	0.850 ± 0.100 c	0.497 ± 0.003 a	0.461 ± 0.012 a	0.662 ± 0.021 b	2.61 ± 0.46 a	2.89 ± 0.08 a	2.37 ± 0.53 a	2.25 ± 0.08 a	3.82 ± 0.12 c	3.26 ± 0.12 a	3.55 ± 0.09 b
ethyl lactate	8.72 ± 0.95 bc	5.48 ± 0.38 a	6.52 ± 0.45 ab	10.2 ± 0.3 c	5.90 ± 0.25 c	5.11 ± 0.47 a	5.43 ± 0.13 ab	5.69 ± 0.08 bc	1.37 ± 0.03 a	1.56 ± 0.04 b	1.53 ± 0.02 b
ethyl octanoate	1.20 ± 0.13 b	0.856 ± 0.149 a	0.788 ± 0.020 a	1.20 ± 0.06 b	2.53 ± 0.40 ab	2.87 ± 0.11 b	2.49 ± 0.32 ab	2.25 ± 0.01 a	3.17 ± 0.18 a	2.80 ± 0.41 a	2.97 ± 0.21 a
ethyl decanoate	0.395 ± 0.028 b	0.378 ± 0.032 b	0.242 ± 0.004 a	0.541 ± 0.002 c	0.905 ± 0.116 a	1.01 ± 0.09 a	0.869 ± 0.052 a	0.854 ± 0.053 a	0.824 ± 0.061 a	0.963 ± 0.021 b	0.971 ± 0.037 b
diethyl succinate	0.148 ± 0.008 b	0.081 ± 0.005 a	0.076 ± 0.003 a	0.235 ± 0.005 c	0.050 ± 0.006 a	0.046 ± 0.002 a	0.052 ± 0.004 a	0.104 ± 0.002 b	0.017 ± 0.001 b	0.015 ± 0.001 a	0.016 ± 0.001 b
∑ethyl esters	11.8 ± 1.7 b	7.55 ± 0.51 a	8.36 ± 0.48 a	13.5 ± 0.2 b	13.1 ± 1.0 a	12.9 ± 0.5 a	12.1 ± 1.0 a	11.9 ± 0.1 a	10.4 ± 0.3 b	9.71 ± 0.48 a	10.2 ± 0.2 ab
Fatty Acids											
hexanoic acid	2.09 ± 0.26 b	1.51 ± 0.12 a	1.24 ± 0.05 a	2.28 ± 0.06 b	5.02 ± 0.24 b	5.20 ± 0.30 b	5.18 ± 0.16 b	3.82 ± 0.14 a	4.56 ± 0.07 b	4.40 ± 0.06 a	4.40 ± 0.10 a
octanoic acid	4.82 ± 0.35 b	4.42 ± 0.32 ab	4.15 ± 0.16 a	4.24 ± 0.02 ab	12.2 ± 0.8 b	13.2 ± 0.5 c	13.1 ± 0.6 bc	10.4 ± 0.2 a	14.4 ± 0.1 b	13.2 ± 0.2 a	13.3 ± 0.2 a

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Table 1. continued

wine	1st assays				2nd assays				3rd assays		
	T[b]	T+P2	R1+P2	R 1	T	HL	DL	DK	T	NF	UF+NF
					Fatty Acids						
decanoic acid	1.30 ± 0.09 b	1.25 ± 0.06 b	1.24 ± 0.11 b	1.01 ± 0.06 a	3.22 ± 0.23 ab	3.84 ± 0.32 c	3.36 ± 0.21 b	2.77 ± 0.07 a	4.19 ± 0.12 b	3.85 ± 0.16 a	4.11 ± 0.15 b
dodecanoic acid	0.058 ± 0.008 c	0.041 ± 0.005 b	0.029 ± 0.002 a	0.050 ± 0.007 bc	0.197 ± 0.042 a	0.297 ± 0.027 b	0.276 ± 0.022 b	0.187 ± 0.003 a	0.290 ± 0.023 a	0.281 ± 0.049 a	0.301 ± 0.029 a
∑fatty acids	13.0 ± 1.5 c	11.1 ± 0.7 b	9.28 ± 0.43 a	10.7 ± 1.0 ab	21.9 ± 1.1 b	24.1 ± 0.5 c	23.0 ± 0.9 bc	18.3 ± 0.4 a	23.4 ± 0.2 b	21.8 ± 0.5 a	22.1 ± 0.4 a
					Terpenes						
α-terpineol*	4.76 ± 1.24 a	4.60 ± 1.12 a	3.43 ± 0.44 a	4.34 ± 0.30 a	nd[d]	nd	nd	nd	nd	nd	nd
β-citronellol*	2.90 ± 0.78 b	3.46 ± 0.57 b	1.86 ± 0.13 a	1.44 ± 0.25 a	2.31 ± 1.21 a	2.09 ± 0.09 a	2.43 ± 0.15 a	2.56 ± 0.03 a	1.63 ± 0.14 b	1.08 ± 0.05 a	1.28 ± 0.09 a
geraniol*	7.49 ± 0.93 b	8.94 ± 0.95 b	4.09 ± 1.08 a	9.80 ± 0.83 b	1.14 ± 0.14 a	0.940 ± 0.230 a	0.925 ± 0.241 a	0.828 ± 192 a	1.42 ± 0.05 b	1.06 ± 0.05 a	1.09 ± 0.02 a
linalool*	31.2 ± 3.1 b	4.03 ± 1.05 a	3.70 ± 1.31 a	105 ± 8 c	21.9 ± 0.8 d	17.0 ± 1.0 c	14.3 ± 2.6 b	10.1 ± 0.1 a	3.42 ± 0.33 b	1.17 ± 0.08 a	0.780 ± 0.035 a
nerol*	2.28 ± 0.43 a	1.63 ± 0.53 a	2.08 ± 0.14 a	1.36 ± 0.65 a	nd	nd	nd	nd	nd	nd	nd
∑terpenes*	46.3 ± 10.2 b	21.0 ± 5.9 a	13.1 ± 2.0 a	121 ± 8 c	25.4 ± 1.5 c	20.0 ± 1.1 b	17.6 ± 2.8 b	13.5 ± 0.1 a	6.46 ± 0.46 b	3.31 ± 0.31 a	3.15 ± 0.06 a
					Others						
γ-butyrolactone	6.82 ± 0.92 b	3.85 ± 0.26 a	2.90 ± 0.16 a	10.2 ± 0.6 c	2.25 ± 0.20 ab	2.07 ± 0.20 a	2.37 ± 0.14 b	2.14 ± 0.01 ab	0.570 ± 0.025 b	0.501 ± 0.011 a	0.480 ± 0.028 ab
acetoin*	8.95 ± 0.69 c	2.86 ± 0.48 b	1.96 ± 0.12 a	14.6 ± 3.0 d	13.3 ± 0.9 c	5.61 ± 1.13 b	3.92 ± 0.29 a	27.0 ± 0.2 d	5.22 ± 0.49 a	8.14 ± 0.59 b	8.47 ± 0.51 b
benzaldehyde*	20.4 ± 2.8 c	14.5 ± 1.5 b	7.17 ± 0.46 a	31.8 ± 3.4 d	9.04 ± 1.09 c	7.72 ± 0.78 bc	6.48 ± 0.60 ab	5.27 ± 0.81 a	7.58 ± 0.59 b	5.59 ± 0.37 a	4.08 ± 0.36 a
methionol	2.24 ± 0.23 c	1.65 ± 0.13 b	1.45 ± 0.05 b	0.888 ± 0.164 a	0.775 ± 0.018 ab	0.789 ± 0.080 ab	0.844 ± 0.108 b	0.648 ± 0.069 ab	0.650 ± 0.011 a	0.610 ± 0.010 a	0.610 ± 0.044 b
4-vinylguaiacol	0.734 ± 0.008 b	0.460 ± 0.020 a	0.383 ± 0.025 a	0.914 ± 0.009 c	0.969 ± 0.028 b	0.866 ± 0.037 a	0.802 ± 0.078 a	0.768 ± 0.024 a	3.95 ± 0.06 c	2.99 ± 0.04 a	3.41 ± 0.03 b
ΣAll compounds	455 ± 48 c	307 ± 23 b	231 ± 12 a	405 ± 3 bc	292 ± 10 c	262 ± 24 b	249 ± 19 ab	216 ± 4 a	282 ± 5 b	267 ± 4 a	263 ± 2 a

[a]Data are expressed in mg/L with the exception of values marked with *, which are expressed in μg/L. [b]T, control wines; T+P2, wine obtained from modified must results of mixing initial must (T) and the two step nanofiltration permeate (P2); R1+P2, wine obtained from modified must results of mixing the first step nanofiltration retentate (R1) with P2; R1, alcoholic product obtained by fermentation of R1 retentate; HL, DL, and DK, obtained wines from modified must result of mixing the initial must with one step nanofiltration permeates obtained with HL, DL, and DK membranes; NF and UF+NF, modified wines from the must results of mixing the initial must with permeate from one step nanofiltration (NF) or with permeate from ultrafiltration + nanofiltration processes (UF+ NF). [c]Mean values ± standard deviation with different letters (by row and vintage) are significantly different by LSD test with $\alpha = 0.05$. $n = 4$ (2 replicate wines × 2 analytical determination). [d]nd = not detected.

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Spain) was used. This membrane was characterized by cutoff retention 1 kDA and water permeability of 0.98.

Furthermore, a comparative study of the effect of nanofiltration and ultrafiltration was carried out. Some aliquots of perfectly clarified initial must were filtered through nanofiltration membrane, and NF permeates were obtained. Other parts of initial clarified must were filtered through an ultrafiltration membrane. After that, UF permeates were filtered through a nanofiltration membrane and UF+NF permeates were obtained. After that, similarly to previous assays, musts with lower levels of sugars than the initial one were obtained by mixing adequate quantities of NF and UF+NF permeates with initial must. The diluted musts were labeled with the same codes of the permeates. As in the other assays, the initial and diluted musts were fermented under similar conditions to obtain the respective control (T) and reduced alcohol degree wines, which were labeled respectively as NF and UF+NF wines. All the cited types of wines were made in duplicate.

Analytical Parameters and Methodologies. The enological parameters of must and wines, namely, pH, titratable and volatile acidity, reducing sugars, and alcohol degree, were analyzed according to OIV methods.[26] °Brix was measured in must by refractometry.

Studied volatile compounds were analyzed by the methodology described in a previous published work[27] with slight modifications. The compounds were isolated from wine by liquid−liquid extraction using 5 mL of dichloromethane in 250 mL of wine. The extraction was done by continuous slight stirring during 3 h. An ice bath was employed to maintain the temperature under 4 °C. Air was removed by saturation with N_2 to avoid oxidative degradation. The organic phase was separated by centrifugation at 10000*g*/10 min at 4 °C. 1 μL of dichloromethane extract was injected in splitless mode into a 7890A GC system (Agilent technologies S.L., Madrid, Spain), with a FID detector (temperature 250 °C, H_2 = 40 mL/min, air = 400 mL/min, and N_2 = 45 mL/min). A Carbowax 20 M column (60 m × 0.32 mm, 0.25 μm film thickness) from Quadrex Corporation (Symta, Madrid, Spain), was used for separation. Helium flow was 0.8 mL/min. Oven temperature was initially 40 °C for 8 min, then raised to 85 °C (10 °C/min), then maintained at this temperature for 1 min, and then raised to 230 °C (2 °C/min), and this temperature was maintained for 35 min. Some major and minor volatile compounds, which included alcohols, ethyl esters, acetates, volatile fatty acids, lactones, terpenes, volatile phenols, and some other compounds like acetoin, benzaldehyde, and methionol, were analyzed and quantified. Quantifications were carried out considering the relative response areas for each of the volatile compounds with respect to 2-octanol, which was used as the internal standard. The corresponding calibration curves of each quantified compound were calculated.

Compound identification was carried out comparing the retention time of each peak with the retention time of the respective standards, and using their mass spectral data. A Hewlett-Packard 5973 mass detector fitted with a Hewlett-Packard 6890 GC was used. The ionization of the samples was achieved at 70 eV under the SCAN mode. The mass range studied was from 30 to 250 *m*/*z*. A ChemStation equipped with the library NIST 08 was used for interpretation of MS spectra.

Amino acids were analyzed and quantified by HPLC according to a published paper.[28] Amino−enone derivatives were obtained by reaction of 1.75 mL of borate buffer 1 M (pH = 9), 750 μL of methanol, 1 mL of the sample, 20 μL of internal standard (2-aminoheptanoic acid, 1 g/L), and 30 μL of DEEMM. Reaction was carried out in screw cap tubes, which were maintained 30 min in an ultrasonic bath. After that, the mixtures were heated and maintained at 70 °C for 2 h to allow complete degradation of excess DEEMM and reagent products. HPLC analysis was performed in an Agilent Technologies LC series 1100, with a diode array detection system. Before the injection the samples were filtered through PVDF filters with a pore size of 0.45 μm. The chromatographic separation was carried out on a reverse-phase ACE C 18 column (250 mm × 4.6 mm i.d. × 5 μm particle size), thermostated at 16 °C. The solvents were (A) 25 mM acetate buffer pH = 5.8 with 0.5% tetrahydrofuran and (B) acetonitrile/methanol (80:20). The gradient used was as follows: from 8% to 10% solvent B in 27 min, then 3.5 min to rise to 17% B; maintain this level for 3 min; then rise to 72% B in 30.5 min; and finally rise to 100% B in 7 min. The flow rate was 0.9 mL/min. For detection the diode array detector monitored at 260 and 280 nm.

The target compounds were identified by comparing their retention time and UV−vis spectra with their respective standards. The quantification was carried out using the internal standard method and the respective calibration curve of each quantified amino acid.

Statistical Analysis. Univariant analysis of variance (ANOVA) was applied to detect the effect of the processes applied to the initial must. An LSD (least significant difference) test was used to determine significant differences among levels of the studied parameters.

A factorial analysis, by principal components, was applied to detect natural associations of wines, and to analyze variable correlations. The normal distribution of the data was tested by Pearson parameter. Varimax was used as rotation criteria. The number of final considered factors was determined under the criterion of eigenvalue higher than unity.

All the tests were realized using StatGraphic Centurión XVI software (StatPoints Technologies, Inc.).

RESULTS AND DISCUSSION

The observation of the whole obtained results showed significant differences among the volatile composition of the obtained wines. In general, wines obtained from must with lower sugar contents showed lower levels of volatile compounds than their respective control wines. In spite of these general comments, the obtained results are shown and commented assay by assay, which were carried out with grape from three consecutive vintages.

Results of the First Assays (Carried out with Grape from the First Vintage). The applied double nanofiltration processes gave a permeate (P2) with significantly lower levels of sugars than the initial must (T). The reduction of the sugar levels was around 45% (Table 2). Consequently, the wines made from the diluted or modified T+P2 musts showed a considerable reduction of their alcohol degree (Table 3). Higher reductions than two alcoholic degrees were observed, probably due to the usual deviation occurring between the probable alcohol degree of must and the % v/v ethanol (°Alc) actually produced during fermentation.[29]

R1+P2 wines also showed a significantly lower content of alcohol than the control ones, but also lower than T+P2 wines. Residual sugar levels of the obtained wines (Table 3) were very similar among all wines, and then the observed differences of the alcohol degree of the wines could not be associated with this parameter. Furthermore, all analytical data were in correspondence with a controlled alcoholic fermentation development, and then it is possible to assert that undesirable secondary fermentation deviations did not occur. The three wines showed similar pH, but T+P2 and R1+P2 wines showed slightly higher values of titratable acidity and lower values of volatile acidity. The last fact could be directly associated with the reduction of sugar levels of musts. The metabolism of yeasts is the main source of volatile acidity of wines made from healthy grapes and controlled alcoholic fermentation.[30] It is convenient to remember that acetic fermentation is a secondary metabolic route associated with the alcoholic fermentation. This reason agrees with the higher values of the volatile acidity of R1 wines, which reached an alcoholic content close to 17°Alc.

The volatile composition of the three types of studied wines, control (T) and both reduced alcohol degree (RAD) wines, was very different. In general, levels of volatile compounds were higher in T wines than in RAD ones (Table 1). Important

Table 2. Mean Values of the Enological Parameters Evaluated in the Musts and Permeates Obtained in Each Assay Carried Out during Three Consecutive Vintages

	1st assays			2nd assays				3rd assays			
	T[a]	P2	R1	T	P(HL)	P(DL)	P(DK)	T	P(NF)	P(UF)	P(UF+NF)
°Brix	21.5 ± 0.1 b[b]	10.4 ± 0.1 a	27.4 ± 0.1 c	20.6 ± 0.1 d	13.9 ± 0.1 b	18.3 ± 0.1 c	12.2 ± 0.1 a	21.3 ± 0.1 c	10.2 ± 0.1 a	17.6 ± 0.1 b	10.3 ± 0.1 a
sugar (g/L)	203 ± 10 b	112 ± 7 a	283 ± 13 c	199 ± 10 d	124 ± 7 b	173 ± 9 c	106 ± 7 a	209 ± 10 c	88.0 ± 6.0 a	175 ± 9 b	88.0 ± 6.0 a
pH	3.37 ± 0.04 b	3.23 ± 0.04 a	3.35 ± 0.04 b	3.08 ± 0.04 b	3.12 ± 0.04 b	2.99 ± 0.04 a	3.00 ± 0.04 a	3.78 ± 0.04 a	3.81 ± 0.04 a	4.39 ± 0.04 b	4.68 ± 0.04 c
total acidity (g/L)	4.79 ± 0.20 b	4.16 ± 0.20 a	5.11 ± 0.20 b	6.76 ± 0.20 b	7.22 ± 0.20 c	6.33 ± 0.20 a	6.05 ± 0.20 a	3.57 ± 0.20 d	2.91 ± 0.20 c	2.43 ± 0.20 b	2.16 ± 0.20 a
probable alcohol degree %	12.0 ± 0.1 b	5.00 ± 0.14 a	15.9 ± 0.1 c	11.8 ± 0.1 d	7.40 ± 0.14 b	9.90 ± 0.14 c	6.00 ± 0.14 a	12.3 ± 0.1 c	5.00 ± 0.14 a	9.80 ± 0.14 b	5.10 ± 0.14 a

[a]T, initial must; P2, two step nanofiltration permeate; R1, first nanofiltration retentate; P(HL), P(DL), and P(DK), one step nanofiltration permeates obtained with the respective membranes HL, DL, and DK; P(NF) and P(UF+NF), permeate from one step nanofiltration and from two step ultrafiltration + nanofiltration, respectively. [b]Mean values ± uncertainty (calculated according to ISO 17025 Norm) with different letters (by row and vintage) are significantly different by LSD test with $\alpha = 0.05$.

Table 3. Mean Values of the Enological Parameters Evaluated in the Wines Obtained in Each Assay Carried Out during Three Consecutive Vintages

	1st assays				2nd assays				3rd assays		
wine	T[a]	T+P2	R1+P2	R1	T	HL	DL	DK	T	NF	UF+NF
pH	3.05 ± 0.04 a[b]	3.08 ± 0.04 ab	3.06 ± 0.04 a	3.14 ± 0.04 b	3.01 ± 0.04 a	2.98 ± 0.04 a	2.96 ± 0.04 a	2.98 ± 0.04 a	3.39 ± 0.04 b	3.29 ± 0.04 a	3.34 ± 0.04 ab
titratable acidity (g/L)	7.42 ± 0.20 a	7.78 ± 0.20 a	7.78 ± 0.20 a	7.43 ± 0.20 a	7.51 ± 0.20 a	7.47 ± 0.20 a	7.47 ± 0.20 a	7.49 ± 0.20 a	4.69 ± 0.20 a	4.74 ± 0.20 a	4.55 ± 0.20 a
volatile acidity (g/L)	0.201 ± 0.060 a	0.130 ± 0.060 a	0.130 ± 0.06 a	0.350 ± 0.060 b	0.190 ± 0.060 a	0.170 ± 0.060 a	0.150 ± 0.060 a	0.170 ± 0.060 a	0.230 ± 0.060 a	0.120 ± 0.060 a	0.230 ± 0.060 a
sugar (g/L)	1.65 ± 0.12 a	1.64 ± 0.12 a	1.66 ± 0.12 a	2.12 ± 0.13 b	2.44 ± 0.14 b	2.39 ± 0.14 ab	2.16 ± 0.12 a	3.16 ± 0.16 c	0.970 ± 0.100 a	0.900 ± 0.090 a	0.900 ± 0.090 a
alcoholic degree % v/v	12.7 ± 0.1 b	10.1 ± 0.1 a	9.34 ± 0.14 a	16.8 ± 0.1 c	13.0 ± 0.1 c	12.1 ± 0.1 b	11.6 ± 0.1 a	12.0 ± 0.1 b	12.9 ± 0.1 b	11.6 ± 0.1 a	11.7 ± 0.1 a

[a]T, control wines; T+P2, wine obtained from modified must results of mixing initial must (T) and the two step nanofiltration permeate (P2); R1+P2, wine obtained from modified must results of mixing the first step nanofiltration retentate (R1) with P2; HL, DL, and DK, obtained wines from modified must result of mixing the initial must with one step nanofiltration permeates obtained with HL, DL, and DK membranes; NF and UF+NF, modified wines from the must results of mixing the initial must with permeate from one step nanofiltration (NF) or with permeate from ultrafiltration + nanofiltration processes (UF+NF). [b]Mean values ± standard deviation with different letters (by row and vintage) are significantly different by LSD test with $\alpha = 0.05$. $n = 4$ (2 replicate wines × 2 analytical determination).

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Table 4. Factor Loadings after Varimax Rotation of the Factors with Eigenvalues Higher than Unity Obtained with Data from the First Assays

	F1	F2	F3	F4
1-butanol	**0.953**[a]	0.256		
isobutyl alcohol	0.669	**0.724**		
2,3-butanediol	0.407	**0.684**	0.540	
1-pentanol	**0.786**	0.571		
isoamyl alcohol	**0.700**	0.654	0.260	
3-methyl-1-pentanol	**0.772**		0.407	
heptanol	0.373	**0.852**		0.332
benzyl alcohol		**0.949**		
2-phenylethyl alcohol		0.523	**0.675**	
1-hexanol		**0.935**	0.250	
trans-3-hexen-1-ol		**0.900**		0.251
cis-3-hexen-1-ol		**0.939**		0.290
hexyl acetate	0.458	**0.884**		
isoamyl acetate	**0.966**			
phenylethyl acetate	**0.989**			
ethyl butanoate	**0.957**			
ethyl 3-hydroxybutanoate		**0.863**	0.392	
ethyl isovalerate	0.297	**0.886**		
ethyl hexanoate	0.640	**0.732**		
ethyl lactate	**0.861**		0.414	0.250
ethyl octanoate	**0.812**	0.478		
ethyl decanoate	**0.755**			0.579
diethyl succinate	**0.994**			
hexanoic acid	**0.886**	0.403		
octanoic acid		**0.789**	0.297	
decanoic acid	0.410	**0.608**	0.333	
dodecanoic acid	0.662	**0.685**	0.250	
γ-butyrolactone	**0.981**			
acetoin	**0.984**			
benzaldehyde	**0.938**			0.296
methionol		**0.926**		
α-terpineol	0.298	0.466	**0.620**	0.349
β-citronellol	0.378	**0.618**	0.381	0.519
geraniol	0.457			**0.764**
linalool	**0.974**			
nerol		0.503	**0.840**	
4-vinylguaiacol	**0.932**	0.252		
eigenvalue	21.1	10.5	2.36	1.58
cumulative variance %	45.5	83.6	90.1	94.5

[a]Loading values of each variable in each factor. Values less than 0.250 were not considered. Bold values are the highest ones for each analytical variable.

differences were observed in some cases, especially in the levels of alcohols. The maximum differences were detected between the levels of isobutyl and isoamyl alcohols of the control and R1+P2 wines. The last ones showed lower content, with reductions around 68% and 53% of the cited alcohols. These decreases could be explained by possible membrane retention of the principal precursors of fusel alcohols, amino acids as leucine, isoleucine, and phenylalanine.[31] This hypothesis is also supported by the obtained data of the R1 wine, which showed similar global quantities of fusel alcohols as control wines (Table 1). Levels of other alcohols showed similar effects to those observed for fusel ones. Significantly lower levels of 1-butanol and heptanol were detected (56% and 40% respectively). Furthermore, R1 wine showed higher global content of other alcohols than control ones, being especially

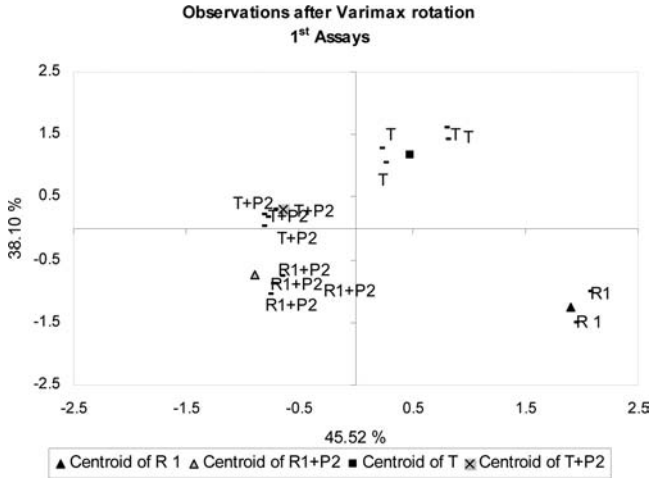

Figure 1. Distribution of samples of the first assays on the plane defined by the factors 1 and 2. T = control wines; T+P2 = wine obtained after mixing control must (T) with P2 permeate; R1+ P2 = wine obtained after mixing P2 permeate and R1 rententate; R1 = alcoholic product obtained after fermenting of R1 retentate.

rich in 1-butanol. These results could be also explained considering the retention of precursors by the membrane. It is important to have in mind that the synthesis pathway of alcohols is similar for the majority of them. However, some exception as the synthesis pathway of 2,3-butanediol should be considered. Pyruvic acid has been described as the precursor of this alcohol.[32] This fact could explain the different behavior of this alcohol, the levels of which were similar in control and RAD wines.

The hypothesis of amino acid retention by nanofiltration membrane was tested during second assays, and it will be commented on later.

Levels of C6 alcohols of T+P2 and T wines were similar. This fact was positive, since Verdejo wines are characterized by herbaceous notes,[33] which are associated with the presence in wines of different C6 alcohols.[30] R1+P2 wines showed lower levels of C6 alcohols than control wine, probably due to the low levels of precursors of these alcohols in R1 retentate, a fact corroborated by the low levels of C6 alcohols in R1 wine. This data suggests that precursors of C6, as linoleic and linolenic fatty acid, were not retained by the membrane.

The levels of acetates were significantly lower in RAD wines (around 50% less) than in control wines. These results seem to be associated with previously commented facts, as the lower levels of alcohols detected on RAD wines, and the lower volatile acidity of these wines (Table 3). These facts together could explain a reduction of acetate synthesis. Similar reasons could explain the high levels of acetates found in R1 wine, which showed higher values of alcohols and also of volatile acidity.

The reduced levels of acetates detected in RAD wines are a potentially negative effect to the quality of the wines. Acetates, especially isoamyl acetate and hexyl acetate, have an important contribution to the fruity characteristics of the white wines.[34] Other potentially negative effects detected were the significant reductions (around 40%) of the levels of ethyl esters in RAD wines with respect to control ones. These compounds contribute favorably to the fruity notes of young wines.[35,36] The data of ethyl esters could be a direct consequence of lower levels of ethanol in the medium. This reason agrees with the

Table 5. Mean Values (mg/L) of Levels of Total Amino Acids and Studied Individual Amino Acids in Must and Permeates Obtained during the Assays Carried Out with Grape from the Second and Third Vintage

	2nd assays				3rd assays		
	T[a]	P(HL)	P(DL)	P(DK)	T	P(NF)	P(UF+NF)
α-alanine	70.9 ± 0.7 c[b]	68.5 ± 1.1 ab	69.7 ± 1.1 bc	67.5 ± 0.1 a	95.9 ± 0.6 b	93.7 ± 1.7 b	76.0 ± 0.8 a
γ-aminobutyric acid (GABA)	56.9 ± 0.9 b	54.7 ± 0.8 a	58.4 ± 1.1 c	55.0 ± 0.1 a	82.3 ± 3.3 a	92.5 ± 1.0 b	85.9 ± 2.2 ab
arginine	474 ± 6 c	397 ± 6 ab	388 ± 8 a	402 ± 2 b	851 ± 4 c	816 ± 7 b	761 ± 2 a
asparagine	5.25 ± 0.08 c	4.63 ± 0.16 b	4.32 ± 0.19 a	4.53 ± 0.01 ab	6.50 ± 3.35 c	4.20 ± 0.00 b	3.30 ± 0.10 a
aspartic acid	35.3 ± 0.4 c	34.2 ± 0.4 b	37.6 ± 0.6 d	31.7 ± 0.1 a	53.2 ± 0.5 c	38.3 ± 0.3 b	22.9 ± 0.3 a
glutamic acid	94.8 ± 1.0 d	87.9 ± 1.0 b	91.0 ± 0.9 c	83.7 ± 0.4 a	119 ± 3 c	94.2 ± 0.2 b	65.2 ± 0.3 a
glutamine	102 ± 1 c	91.1 ± 1.3 b	92.4 ± 1.9 b	87.9 ± 0.9 a	43.9 ± 0.4 c	32.1 ± 0.3 b	21.3 ± 0.6 a
glycine	4.98 ± 0.04 c	4.64 ± 0.08 b	4.37 ± 0.06 a	4.55 ± 0.01 b	3.67 ± 0.01 a	6.45 ± 0.02 b	7.83 ± 0.07 c
histidine	10.2 ± 0.5 b	7.73 ± 0.83 a	9.93 ± 1.74 b	8.76 ± 0.77 ab	nd[c]	nd	nd
isoleucine	11.5 ± 0.1 c	10.3 ± 0.1 b	12.5 ± 0.1 d	9.76 ± 0.03 a	29.5 ± 0.5 c	18.1 ± 0.3 b	2.22 ± 0.01 a
leucine	9.56 ± 0.12 c	9.16 ± 0.12 b	13.1 ± 0.2 d	7.97 ± 0.02 a	34.5 ± 0.2 c	14.4 ± 0.1 b	1.12 ± 0.03 a
lysine	1.96 ± 0.02 c	1.67 ± 0.03 b	2.03 ± 0.06 d	1.29 ± 0.02 a	8.79 ± 0.06 c	0.47 ± 0.01 a	1.17 ± 0.01 b
methionine + cysteine	1.65 ± 0.04 a	1.69 ± 0.03 a	3.55 ± 0.39 b	1.54 ± 0.07 a	3.71 ± 0.06 c	1.48 ± 0.03 b	0.820 ± 0.008 a
phenylalanine	17.1 ± 0.2 c	16.1 ± 0.2 b	18.6 ± 0.3 d	15.2 ± 0.1 a	43.0 ± 0.1 c	23.7 ± 0.4 b	4.59 ± 0.11 a
proline	32.1 ± 4.6 a	31.5 ± 1.4 a	37.9 ± 1.9 b	37.6 ± 1.4 b	62.1 ± 2.7 b	87.1 ± 2.0 c	53.9 ± 1.1 a
serine	45.3 ± 0.6 b	45.7 ± 0.5 b	52.2 ± 0.9 c	40.6 ± 0.1 a	71.9 ± 2.0 c	57.9 ± 1.1 b	27.6 ± 0.6 a
threonine	66.9 ± 1.0 c	64.0 ± 1.1 b	74.6 ± 1.5 d	58.8 ± 1.0 a	92.7 ± 0.4 c	71.4 ± 1.3 b	26.9 ± 0.1 a
tryptophanic acid	4.22 ± 0.20 b	4.18 ± 0.07 b	6.65 ± 0.20 c	3.82 ± 0.02 a	32.3 ± 0.1 c	25.7 ± 0.6 b	18.4 ± 0.3 a
tyrosine	8.64 ± 0.14 b	8.24 ± 0.22 a	8.75 ± 0.23 d	7.92 ± 0.08 b	15.9 ± 0.1 b	15.5 ± 0.2 b	8.80 ± 0.09 a
valine	20.7 ± 0.2 b	18.7 ± 0.2 a	21.0 ± 0.3 b	18.3 ± 0.2 a	44.0 ± 0.4 c	33.5 ± 0.4 b	10.9 ± 0.1 a
total nitrogen compounds	1135 ± 12 c	1026 ± 15 a	1077 ± 16 b	1011 ± 12 a	1793 ± 14 c	1625 ± 11 b	1253 ± 3 a

[a]T, initial must; P (HL), (DL), and (DK), one step nanofiltration permeates obtained with the respective membranes HL, DL, and DK; P(NF) and (UF+NF), permeate from one step nanofiltration and from two step ultrafiltration + nanofiltration, respectively. [b]Mean values ± standard deviation with different letters (by row and vintage) are significantly different by LSD test with $\alpha = 0.05$. $n = 4$ (2 replicate wines × 2 analytical determination). [c]nd = not detected.

high levels of ethyl esters detected in R1 wine. In addition, the effect on the levels of fatty acids should also be considered. Fatty acid levels of RAD wines were lower than those of control wines. These data suggested, once more, a possible retention of amino acids as threonine and glutamic acid, which are the main precursors of some some medium-chain fatty acids.[37−39] Having lower levels of fatty acids and ethanol, the possibility of ethyl ester formation was notably reduced in RAD wines.

Levels of terpenes showed also significant differences among wines, but not all the studied terpenes showed similar trends. So, levels of α-terpineol were statistically equal, while levels of linalool were drastically lower in RAD wines than in control wines. The differences raised values close to 90%. These results suggested an important retention of free terpenes or of their precursors by the membrane. This supposition agrees with the high global levels of terpenes found in R1 wine. This fact could be potentially negative to the quality of wines, due to the contribution of terpenes to the wine varietal notes,[30] so as to the floral ones.[40]

Similar trends to those previously commented were observed in the rest of the studied compounds, and similar reasons could explain the observed effects.

Previous comments are derived of univariate analysis of the data, but it was considered interesting to evaluate the cited modifications all together. So, a multivariate statistical analysis was used. Factorial analysis was selected, due to its capacity to point out natural groups of samples, and the association among the variables.

Four factors showed an eigenvalue higher than unity and all together explained the 94.5% of the global variance (Table 4). The majority of the analyzed volatile compounds were mainly associated with factors 1 and 2, which together explained more than 80% of the total variability. The distribution of the score values of each sample in the plane defined by these factors (Figure 1) allowed an easy visualization of differences and similarities among wines. Control wines showed positive scored values, while RAD wines showed negative scored, at least for one of the cited factors. This fact was mainly due to the generally lower levels of the volatile compounds observed in RAD wines. In spite of this, some similarities between T+P2 wines and control wines were pointed out, due to their proximity in the plane. Furthermore, factorial analysis showed important global differences among R1 wine and the rest of the studied wines. The differences were associated with both factors, 1 and 2, and then with the majority of the volatile compounds studied.

Summarizing, significant differences among volatile profiles of the studied wines were detected. The differences seem to be a direct consequence of the process of mixing the initial white must with the nanofiltration permeates, and it seems to be associated with membrane retention phenomena, in which different aroma compounds and their precursors could be involved. However, it is also convenient to have under consideration the possible influence of the dilution factor, which could be better controlled.

Considering all the previous comments, new assays were planned. To reduce the retention of precursors during filtration processes, nanofiltration treatment was reduced to a single step process. Furthermore, the reduction of the sugar levels in modified must will be carried out carefully, in order to raise the desired levels.

Results of the Second Assays (Carried out with Grape from the Second Vintage). These assays were carried out using three different nanofiltration membranes (DK, DL, and

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Table 6. Factor Loadings after Varimax Rotation of the Factors with Eigenvalues Higher than Unity Obtained with Data from the Second Assays

	F1	F2	F3	F4
1-butanol	**0.855**[a]	−0.451		
isobutyl alcohol	**0.900**			0.363
2,3-butanediol	−0.615	**0.728**		
1-pentanol	**0.834**		0	
isoamyl alcohol	**0.963**			
3-methyl-1-pentanol	0.608			**0.711**
heptanol	**0.557**		−0.262	0.365
benzyl alcohol	0.401	**0.808**		
2-phenylethyl alcohol	0.401	**0.769**	−0.401	
1-hexanol	0.560	**0.715**		
trans-3-hexen-1-ol	**0.838**			0.379
cis-3-hexen-1-ol		−0.547		**0.626**
hexyl acetate	**0.706**		0.633	
isoamyl acetate	**0.704**		0.623	
phenylethyl acetate	0.364	−0.460	**0.652**	−0.346
ethyl butanoate	**0.761**		0.564	
ethyl 3-hydroxybutanoate				**0.898**
ethyl isovalerate		**0.831**	0.437	
ethyl hexanoate	0.316		**0.910**	
ethyl lactate	**0.526**	0.440	−0.471	0.473
ethyl octanoate			**0.904**	
ethyl decanoate			**0.837**	−0.420
diethyl succinate	−0.545	**0.797**		
hexanoic acid	0.495	**-0.768**	0.287	
octanoic acid		**-0.740**	0.615	
decanoic acid		−0.606	**0.630**	−0.395
dodecanoic acid	−0.358	**-0.749**	0.449	
γ-butyrolactone		−0.258	−0.563	**0.654**
acetoin		**0.951**	−0.255	
benzaldehyde	**0.914**			
methionol		**-0.777**		
β-citronellol	−0.268	0.257	**0.623**	0.436
geraniol	**0.502**			0.274
linalool	**0.944**			
4-vinylguaiacol	**0.815**		0.299	
eigenvalue	13.0	10.2	4.82	2.34
cumulative variance %	34.1	58.8	74.3	86.5

[a]Loading values of each variable in each factor. Values less than 0.250 were not considered. Bold values are the highest ones for each analytical variable.

HL), one of them (HL) being the same membrane used in the first assays. This fact allowed comparing the results from double step (obtained in the first assays) with those obtained in this case working in single step nanofiltration treatment of must. The obtained results showed expected results; one step (HL) was less effective than the double step process. Reductions of levels of sugar around 38% and 45% were respectively obtained. Furthermore, data allowed observing that effectiveness of nanofiltration processes depends strongly on the type of membrane (Table 2). So, DK was the most effective membrane, giving permeates with levels of sugars around 53% of those of the initial must. Then, this membrane gave permeates with similar reduction as the double process carried out with HL membrane. The worst effectiveness to reduce the levels of sugars was obtained with DL membrane: reductions around 13% were observed. Furthermore, some differences in the retention of acid by each membrane were also observed.

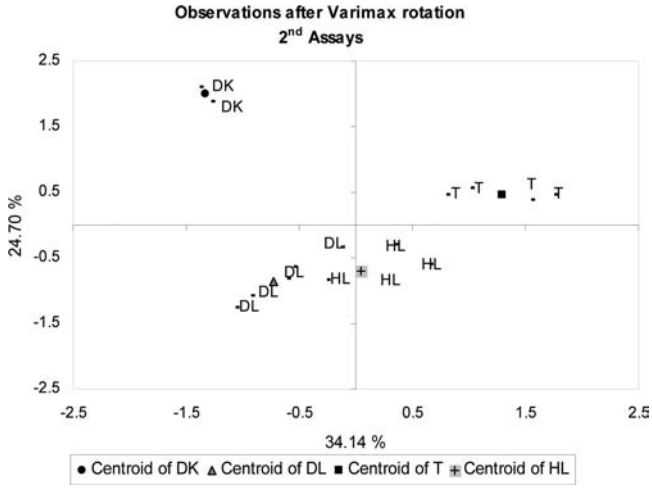

Figure 2. Distribution of samples of the second assays on the plane defined by the factors 1 and 2. T = control wines; HL, DL, and DK = RAD (reduced alcohol degree) wines obtained from T must mixed with HL, DL, and DK permeates, respectively.

Permeates of DL and DK showed lower levels of titratable acidity than the initial must, but losses were quantitatively different for each membrane.

Data from wines showed that the desired reduction of two alcohol degrees was not achieved (Table 3). In this case, maximum reductions of 1.5°Alc were obtained. Similar reasons to those commented previously can be considered to explain this fact. In spite of the undesired deviation, the obtained reductions could be considered satisfactory. White wines with 12 or less alcohol degrees (°Alc) are usually better considered than those with 13°Alc, which could be defined even as unpleasant.

The four types of wines showed similar pH, and values of titratable and volatile acidity (Table 3). However, they showed very different volatile composition (Table 1). The levels of the volatile compounds analyzed in the four types of wines showed statistically significant differences among wines. In general, volatile composition of the RAD wines was lower than that of the control ones. These general results were similar to those found in the first assay; however, in this case, the observed differences were quantitatively smaller than those found in the first assays. Probably, this fact could be related to the minor reductions of the alcohol degrees achieved in these assays, which is associated with less intense modifications of the initial must. In spite of this, quantitative and significant differences among the levels of volatile compounds of each type of RAD wines were detected. DK wines showed the most different volatile composition. This fact should be correlated with the commented high capacity of DK membrane to retain sugars and acids, so as other compounds with special importance in the synthesis of wine volatiles as amino acids. So, although in general, the levels of fusel and other alcohols were lower in the RAD wines than in the control ones, the DK wines showed the lowest values of some of these alcohols as isoamyl alcohols and butanol. These results agree with those obtained in the first assays, and they agree also with the data from levels of amino acids detected in the initial must and in the permeates of the second assays (Table 5). DK permeates presented the lowest levels of total amino acids and significant low levels of many of the studied amino acids. These data corroborated the previous hypothesis relative to membrane retention phenomena, which

Table 7. Factor Loadings after Varimax Rotation of the Factors with Eigenvalues Higher than Unity Obtained with Data from the Third Assays

	F1	F2	F3	F4	F5
1-butanol		**0.936**[a]			
isobutyl alcohol	**0.866**	0.447			
2,3-butanediol	**0.932**				0.265
1-pentanol		**0.912**	0.262		
isoamyl alcohol	0.286	**0.835**			0.272
3-methyl-1-pentanol	0.291	**0.710**	0.388		0.359
heptanol	0.258	**0.841**	0.289	0.251	0.262
benzyl alcohol	**0.829**		0.335		
2-phenylethyl alcohol	**0.805**	0.502	0.273		
1-hexanol	**0.946**				
trans-3-hexen-1-ol	**0.583**	0.532	0.324	0.268	0.255
cis-3-hexen-1-ol	**0.965**				
hexyl acetate	**0.893**	0.330			
isoamyl acetate	**0.596**	0.296	0.259	0.549	0.256
phenylethyl acetate	**0.794**	0.381	0.449		
ethyl butanoate	**0.701**	0.427		0.377	
ethyl 3-hydroxybutanoate	0.576		0.331	0.339	**0.628**
ethyl isovalerate		0.371		**0.822**	
ethyl hexanoate	**0.841**		0.298		
ethyl lactate	**0.955**				
ethyl octanoate	0.297		**0.919**		
ethyl decanoate	**0.842**	0.304		0.214	
diethyl succinate	**0.685**	0.293		0.515	
hexanoic acid	**0.606**	0.381		0.256	
octanoic acid	**0.830**	0.328	0.426		
decanoic acid	0.531	0.264	**0.702**		
dodecanoic acid			**0.969**		
γ-butyrolactone	**0.727**	0.469	0.415		
acetoin	**0.894**	0.340			
benzaldehyde	**0.752**	0.586			
methionol		0.647	0.558		
β-citronellol	**0.858**				0.350
geraniol	**0.898**				
linalool	**0.872**	0.376			
4-vinylguaiacol	**0.913**			0.304	
eigenvalue	20.6	5.72	3.36	1.39	1.04
cumulative variance %	49.5	69.4	81.7	88.0	91.8

[a]Loading values of each variable in each factor. Values less than 0.250 were not considered. Bold values are the highest ones for each analytical variable.

justify the low levels of amino acids in the permeate and then in the modified musts.

Levels of 2,3-butanediol and the C6 alcohols showed similar results as in the first assays; their levels were generally similar in all wines.

Levels of acetates were lower in DL and DK wines than in control wines, and, again, DK wines showed the quantitatively highest differences, showing levels around 50% lower than the other wines. These results could not be associated with the levels of volatile acidity, which was similar in all the wines. However, they were well correlated with the lowest levels of isoamyl alcohols detected in DK wines.

Levels of ethyl esters were similar in the four types of studied wines (Table 1). The higher levels of diethyl succinate detected in DK wines were an exception, and this fact could be explained considering the high levels of proline observed in DK permeate. Proline was positively associated with the synthesis of diethyl

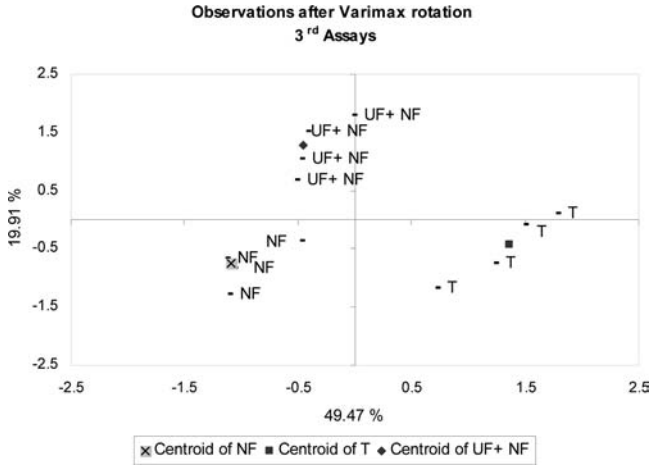

Figure 3. Distribution of samples of the third assays on the plane defined by the factors 1 and 2. T = control wines; NF and UF+NF = RAD (reduced alcohol degree) wines obtained from T must mixed with NF and UF+NF permeates, respectively.

succinate.[38] Generally, slightly lower levels of these compounds were quantified in RAD. These results agreed with those of the first assays, although the differences among wines were quantitatively lower in the second assays. In general, levels of ethyl esters were well correlated with those of fatty acids.

Results of terpenes showed also similar results to those found and commented previously. The RAD wines showed lower global levels of these compounds than control wine. The quantitative differences were variable among each individual analyzed terpene and also among wines. Significant differences among all the wines were detected only in the case of the major analyzed terpene, the linalool. Once more, the lowest levels were detected in DK wines, which showed values over 50% lower than control wines. This data also seems to point out the different retention capacity of the DK membrane.

In general, data of the other studied compounds also showed similar results to those obtained in the first assays. Levels of benzaldehyde of DK wines were the lowest ones. This fact agreed with the low levels of aspartic acid and glutamine detected on DK permeates (Table 5). Both amino acids have been correlated with the synthesis of benzyl alcohol,[38] and this alcohol has been described as the main precursor of the benzaldehyde.[41]

Some peculiar results were detected. DK wines showed high values of acetoin. This fact was unexpected, and it was not well correlated with the rest of the obtained data. Acetoin is a metabolite of a secondary reaction coupled with alcoholic fermentation, and it is especially associated with acetic deviation. However, volatile acidity values did not indicate any acetic deviation, and no reason was found to explain these data. Acetoin contents over its perception threshold 150 mg/L[31] have negative effects on wine quality,[30] however levels measured in DK wines were not superior to the cited values.

Summarizing the previous results, DK wines showed important differences whereas DL and HL wines were more similar between them, and HL wines seemed to be the more similar to control wine. To corroborate these comments data was analyzed by factorial analysis. Results of factorial analysis were similar to those of the first assays. Four factors showed an eigenvalue higher than unity, and all together explained the 86.5% of the total variance (Table 6). The graphical representation of the distribution of samples (scored values)

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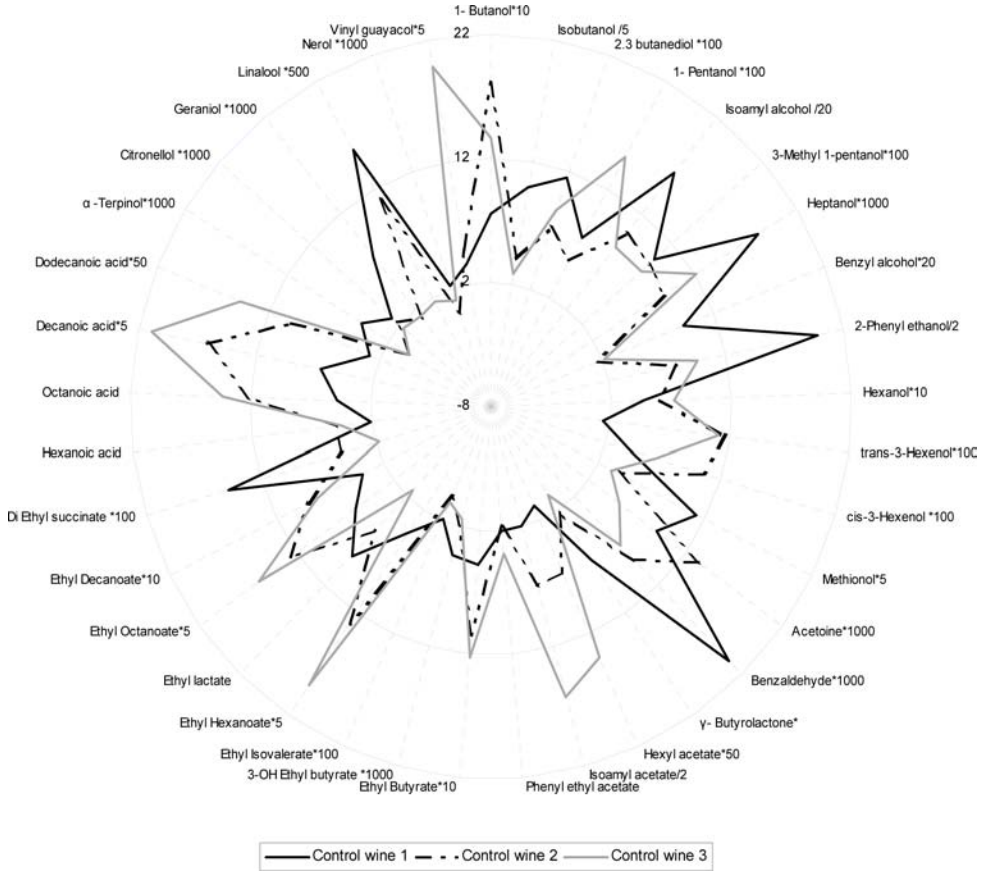

Figure 4. Means values of volatile compounds of Verdejo control wines obtained in the three assays, each one carried out from grapes of three consecutive vintages. * and / symbols accompanying legends of each analyzed compound indicate the factor applied to make possible the use of similar range values for all of them.

on the plane defined by the first two factors pointed out easily the differences and similarities of the studied wines (Figure 2). All the RAD wines were placed on a different zone of the plane than control wines; this fact evidenced their differences. Besides, comparing the distances between the centroids of each RAD wine to the centroid of control wines, it was possible to corroborate that HL wines were the RAD wines with the volatile profile more similar to control wines. Furthermore, it was corroborated that the volatile composition of DK wines was very different from the rest of the studied wines.

All the previous comments showed the important role of the membrane on achieving good results. Then, to minimize secondary unexpected and undesired effects an adequate membrane should be selected. According to the characteristics of the used membranes, those with high water permeability seem to be more adequate for the aim of this work.

Furthermore, comparing results of the second and first assays is possible to assert that the one step nanofiltration process seems to be a better option than the two step nanofiltration process. This conclusion is especially derived from the fact that a single process is more economic and allowed the making of wines with volatile composition relatively similar to control wines.

Results of the Third Assays (Carried Out with Grapes from the Third Vintage). Considering the results obtained with the second assays, HL was selected to carry out the experiences of the third assays. The analytical values of the permeates obtained in this case (Table 2) showed that the effectiveness of nanofiltration (NF) could be increased optimizing the operation conditions: So, a strict control of temperature, time of processing, and membrane cleanness allowed obtaining, in a single step process, retention ratios of sugar similar to those obtained applying a double step process (first assay). Furthermore, results showed that double step, ultrafiltration (UF) followed by nanofiltration (NF), was not more effective than one step NF treatment. Similar reductions of sugar levels were obtained in both cases, and slightly higher losses of acidity were detected in permeates of double step treatment. UF membranes showed low effectiveness to retain sugars, but notable losses of acid, more than 30%, were detected in UF permeates. These data indicated that UF permeates were not appropriated to modify sugar levels of the initial must.

The RAD wines obtained in these assays showed maximum differences of 1.3 degree of alcohol with control wines, and values of studied enological parameters were very similar among all the wines (Table 3).

Data of the volatile composition of the studied wines showed similar results to those observed in previous assays, with generally lower levels of volatile compounds in RAD than in control wines. No data pointed out any different effects of membrane applications to those found in previous assays. However, differences between volatile composition of control and RAD wines were quantitatively lower than those found in the first and second assays. This fact could be associated with the minor reduction of the alcohol degrees observed in the wines elaborated in the third assays. However, wines from third and second assays differed only by 0.2°Alc, and this fact seems

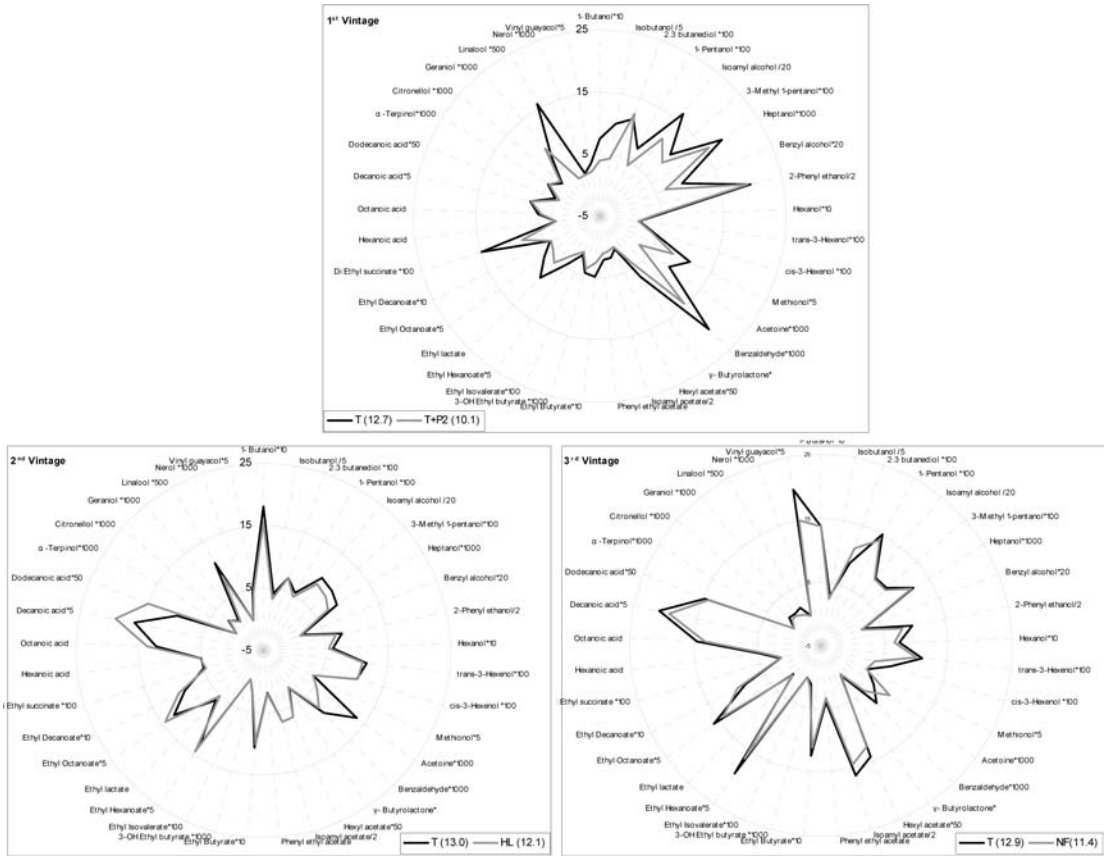

Figure 5. Mean volatile profiles of the control wines and the RAD wines more similar to control wine of each assay. T = control wines; T+P2 = wine obtained after mixing control must (T) with P2 permeate; HL and NF wines obtained after mixing control must (T) with HL and NF permeates, respectively. (Legend values) = mean value of alcohol degree of each showed wine.

not to be enough to justify the observed differences. From these facts, it seems that the accurate processing of the musts is the main reason to explain the good results obtained in the third assays.

Comparing the volatile composition of the RAD wines, slight differences were detected between them. Individual data did not point out any beneficial effect of the double process compared to the single process. To confirm this fact, all of the volatile data were analyzed all together by factorial analysis. Five factors showed eigenvalues higher than unity and all together explained the 91.8% of the total variance (Table 7). The graphical representation of the score values of each studied wine in the plane defined by the first two factors showed clearly the differences and similarities among wines (Figure 3). Once more, the score values of control wines were different from the other ones, but both RAD wines showed equivalent grades of similarity with control wine (similar distances between centroids). It could be interesting to consider that both types of RAD wines differed from the control wine in F1 values, but only the UF+NF wines differed from the control wines also in F2 values. This fact is mainly associated, as in previous assays, with the retention of aroma compounds or of their precursors by the membranes. So, the lower F1 scores of the RAD wines were associated with their lower levels of free terpenes, which could be retained by membrane. Furthermore, the higher F2 scores of UF+NF wines were associated with their lower levels of isoamyl alcohol, 3-methyl-1-pentanol and heptanol, data well correlated with the lowest levels of amino acids detected in UF +NF permeates (Table 5).

Differences among Wines Due to Vintage Factor. Enological parameters of the initial must of each vintage showed similar industrial maturity degree of grapes (°Brix and levels of sugar), but different degree of technological maturity (sugar and acidity relationship).[7] Values of the sugar and acidity relationship ranged from 29 (second vintage) to 59 (third one). These values were especially due to the high titratable acidity of grape of the second vintage and the lowest values of this parameter in grapes of the third vintage. This fact could be due to climatic differences among years. It is well-known that high temperatures produce a rapid decrease of acid levels and accelerate the accumulation of sugars, while low temperatures reduce the accumulation of sugars but maintain high values of acidity.[6] The loss of acidity is a negative quality factor, and grape picking should be carried out before this occurs. Furthermore, white grape harvest should be carried out before levels of aromatic precursors, as terpenoids, go down.[42] High temperatures during summer of the third vintage produced rapid reduction of acid levels, while the moderately warm summer of the second one preserved the levels of acids.

Important differences among the volatile composition of control wines of each studied vintage were detected (Figure 4). Others authors have described similar vintage effects in white wines.[43−45] Different reasons have been cited to explain the observed differences. Among them, the effect of parameters associated with fermentation conditions, such as the yeast involved, should be also considered.[46] These facts seem not to be applied in this study due to fermentation conditions, including inoculated yeast, being the same during the three

vintages. Frequently, climate factors are correlated with volatile differences, due to the fact that they affect the grape maturity and then their composition.[42,45] Furthermore, the influence of climate conditions on the levels of nitrogenous compounds of grapes and musts has been previously described.[47] Some of the differences observed among volatile profiles of control wines of each vintage were well correlated with the amino acid composition of the initial musts. For example, the highest values of isoamyl acetate of the control wine made with the grapes of the third vintage were well correlated with the strongly higher levels of leucine of the initial musts of this assay. Leucine has been described as a precursor of isoamyl acetate.[39] Similar results were found with phenylalanine, amino acid precursor of phenylethyl acetate,[39] and octanoic acid, the levels of which increased with the increase in levels of serine, threonine, and glutamic acid.[38] From the last comments and considering the different degree of technological maturity of grapes from each studied vintage, it seems to be possible that the differences in volatile composition of the control wines of the third vintages could be associated with climate conditions, which were different in the three studied vintages.

Summarizing all the obtained results and considering all the previous discussions, it seems possible to assert that nanofiltration techniques could be a good technique to reduce the levels of sugars of musts, and then to adjust their probable alcoholic degree. However, in order to minimize the effect of membrane retention on volatile composition of the obtained wines, an appropriate type of membrane should be selected and process conditions should be well established and controlled (Figure 5).

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Notes

The authors declare no competing financial interest.

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