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Impact assessment of the reverse osmosis technique in wine alcohol management

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Abstract. This study aimed to assess the impact of reverse osmosis on stable isotope ratios, which serve as markers for wine authenticity, particularly in relation to ethanol content reduction. The research focused on Fetească albă, a white wine from the Cotnari vineyard, where varying amounts of permeate were extracted using reverse osmosis. Additionally, dilution experiments were conducted by adding 10% and 20% permeate to the control wine. Isotope analyses for $\delta^{18}O$ and $\delta^{13}C$ were performed using IRMS, while $(D/H)_I$ and $(D/H)_{II}$ values were measured with SNIF-NMR. No significant changes were observed in $\delta^{13}C_{CPDB}$ and $\delta^{18}O_{VSMOW}$ values. However, ANOVA revealed significant differences in $(D/H)_I$, $(D/H)_{II}$, and R values, indicating that reverse osmosis influenced the wine samples. Notably, the $(D/H)_I$ ratio differed significantly between permeate and retentate samples ($p<0.05$), a trend also confirmed for $(D/H)_{II}$ (ppm) between control/permeate and control/retentate ($p<0.05$), as well as for R values. Correlation analysis showed a significant relationship between $\delta^{13}C_{VPDB}$ ($r = 0.516$, $p<0.05$) and $(D/H)_I$ with added permeate ($r = 0.709$, $p<0.05$). Isotope parameters effectively distinguished differences in ethanol content between control and treated wines.

1. Introduction

The typicity of a wine reflects the terroir which is defined by several factors that are related with interactions between environment (soil and climate) and the vegetal material [1]. Weather conditions have a tremendous influence on the terroir factors [2], because all other factors are directly influenced by the quantity of precipitations or by temperatures or sun light exposure.

Since increasing temperatures are considered a stress in the photosynthetic activity and accumulation mechanisms, this will be a major impact on the grapes sugar quantity and total acidity.

Sugar content depends on photosynthetic activity and accumulation mechanisms and was found that heat stress (40 °C) generally increased glucose and fructose content. Like sugars, total acidity has been shown to reflect the degree of berry ripening [3]. Sugar accumulation and acid degradation are two indices that are directly correlated since levels of acids as tartaric acid can be modified by a faster accumulation of glucose under hot conditions (30/35 °C). In the same situation, malic acid is also affected and has a dynamic variation during grapes ripening because malic acid is heat sensitive (> 46 °C) and its degradation is explained by activity of phosphoenolpyruvate carboxylase which is optimum at about 38 °C [4].

Total acidity in grapes depends on levels of both tartaric and malic acid, whose pathways can be connected.

A high content of glucose and fructose in grapes results in increased ethanol levels in wine due to alcoholic fermentation, which enhances the sensation of astringency but diminishes the perception of aromatic compounds such as esters, higher alcohols, monoterpenes and thiolic compounds [5].

Since high alcohol content is a negative factor for wine quality, various techniques have been developed to reduce alcohol content and maintain a balance between alcohol levels and the sensory quality crucial for the specificity and typicity of wines. Depending on the stage within winemaking technologies, these steps can be pre-fermentation, during fermentation, or in the post-fermentation phase [6].

To reduce alcohol content during pre-fermentation phases, one method involves decreasing fermentable sugars to lower alcohol production, either through membrane separation or non-membrane methods. However, reducing sugars to achieve lower alcohol content carries the risk of resulting in wines with poor sensory qualities [7].

One of the most important alternatives is post-fermentation methods, such as reverse osmosis (RO) and osmotic distillation (OD), which can be used to remove alcohol. These methods can be applied to reduce alcohol content by up to 5% v/v without significantly impacting other sensory parameters [8].

Most of the studies involved in alcohol reduction were focused on the important parameters as phenolic composition, volatile and sensory characteristics but elimination of ethanol may lead to aspects regarding the wine origin and integrity since alcohol elimination could affect the balance of oxygen, hydrogen and carbon isotopes with impact in the origin of glycerol and sugars resulted only from fermentation [9].

Adulteration of wine can happen in many ways, e.g. addition of non-grape ethanol, addition of non-grape sugar, water or other unauthorized substances, undeclared mixing of wines from different geographical areas or countries, mislabelling of variety and age. It is therefore necessary to base the proof of authenticity of a wine on origin-specific parameters which do not undergo alterations during vinification or storage. Elimination of ethanol during the dealcoholisation process may lead to change the isotope composition of the samples, so in the situation of alcoholic corrected wines, this could lead to misinterpretation of the samples by concluding a potentially denaturation process [10].

The aim of the article is to assess the extent to which reverse osmosis treatment methods, applied to reduce the alcohol content of wines, can influence the typicity of the samples by inducing changes in the isotope ratios of carbon, oxygen, and hydrogen, which are part of the chemical structure of ethanol.

2. Materials and methods

2.1. Wine preparation procedures

The reverse osmosis procedure was applied to Feteasc̆ă albă variety obtained from the Cotnari Vineyard. The basic parameters of the wine included alcohol content with a value of 11.78 ± 0.52 % vol., pH of 3.15 ± 0.24, and total acidity (TA) of 8.16 ± 0.24 g/L tartaric acid and were associated with the control wine, as shown in Table 1.

For reverse osmosis (RO), the wines were pumped from a feed tank (with a volume of 1000 L) through a reverse osmosis system Flavy ML 60, Bucher Vaslin equipped with a series of 2 spiral-wound membranes, pore size of 1 nm. The flow rate was adjusted so that the working pressure used, multiplied by the temperature of the wine passing through the osmosis membranes does not exceed the value of 1200 cumulative units. The normal operating pressure is 65 bar. At the exit from the osmosis membranes, the retentate (V) was quantitatively passed through a heat exchanger to maintain a constant working temperature.

To generate retentate (V) and permeate (A) at the application of the osmosis method, 5 fractions were extracted from the wine, each having a volume of 100 L. The collection of the fractions was carried out successively: for the first extraction, 100 L of wine was extracted and for the second fraction, the process continued with another 100 L. The process continued in the same way until the fifth fraction was extracted. In total, 500 L were extracted through osmosis, for which the essential physicochemical parameters were tested. Representative samples were taken from the retentate after sampling (V1 – V5). Reconstituted wines were prepared by diluting the control sample with 10% osmosis water (D1) and 20% osmosis water (D2), respectively. All the samples were subjected to analysis according to the established protocol.

2.2. Instrumentation

Alcoholic strength by volume (OIV-MA-AS312-01B) was done at Dujardin Salleron DE2000, distillation unit and measurement with electronic densimeter from Anton Paar, model DMA5000.

pH was analysed according to method OIV-MA-AS313-15 by mean of a WTW inoLab 720 measuring device.

Analysis of the stable isotopes of hydrogen, carbon and oxygen were carried out in the Stable Isotope Laboratory of the National Research and Development Institute for Cryogenics and Isotopic Technologies - ICSI Rm. Valcea – ROMANIA.

Continuous Flow Isotope Ratio Mass Spectrometer Delta V Plus (Thermo Scientific) coupled to an elemental analyser Flash EA 1112 HT with GasBench II isotopic equilibration module (CF-IRMS) was used to determine the $^{18}O/^{16}O$ isotopic ratios of water extracted from wine and the $^{13}C/^{12}C$ isotopic ratios of ethanol extracted also from wine.

Site-specific natural isotopic fractionation by nuclear magnetic resonance (SNIF-NMR) was used to determine the ratios of the methyl-group $(D/H)_I$ and of the methylene-group $(D/H)_{II}$ values in the ethanol molecule, ethanol extract from wine. The calibration of the devices was performed with CO_2 4.5 type (99.995% purity) from a tank, which was previously calibrated with BCR 656 and VSMOW2. All isotopic values for the samples are calculated automatically by Isodat 3.0 acquisition software.

The relative distribution of deuterium in molecules I and II is expressed in factor R ($R=2(D/H)_{II}/(D/H)_I$ (OIV-MA-AS311-05/2011). For measurements we used an Ascend 400 Bruker spectrometer, with a selective deuterium probe-head with a frequency tuned to 61.42 MHz, a fluorine lock channel and an automatic sample changer.

The mean values and standard deviations are calculated with Eurospec (Eurofins-Nantes) and TopSpin Bruker softwares, from ten repetitive experiments with an exponential multiplying factor (LB) equal to 2.

2.3. Standards and reagents

For the quality control of our analysis, at the beginning and the end of each sequence, several standards were used. Laboratory standards used to determine $^{18}O/^{16}O$ from water extracted from wine were IA-R063 ($\delta^{18}O_{VSMOW}$= −0.41 ± 0.11 ‰) and IA-R064 ($\delta^{18}O_{VSMOW}$= −12.34 ± 0.13 ‰) provided by ISO Analytical Laboratory Standard UK.

The primary standards for $^{18}O/^{16}O$ stable isotope ratio were Vienna Standard Mean Ocean Water VSMOW2 ($\delta^{18}O_{VSMOW}$= 0 ± 0.02 ‰) and SLAP2 ($\delta^{18}O_{VSMOW}$ = −55.5 ± 0.02 ‰), for quality control was used GRESP ($\delta^{18}O_{VSMOW}$ = −33.4 ± 0.04 ‰).

For carbon 13 measurements a reference material BCR 656 and BCR 660 provided by the Institute for Reference Material and Measurements (IRMM) Belgium, with a certified value of $\delta^{13}C_{VPDB}$= −26.91 ± 0.07 ‰ and −26.72 ± 0.09 ‰ and two working standards (with values of $\delta^{13}C_{VPDB}$= −28.4 ± 0.3 ‰ and −24.5 ± 0.3 ‰, respectively).

2.4. Sample preparation and results calculation

Before the isotopic analysis, the wine samples were distilled on an Automated Distillation Control System (ADCS) with Cadiot column spinning bands to extract the ethanol and water. The Cadiot columns had temperatures probes and a solenoid valve which regulated the distillate flow. The alcoholic distillate had an alcoholic strength of at least 85% wt., and the loss of alcohol during each distillation was lower than 0.6 % vol, in according with official method OIV-MA-AS312-06:R2009 [11].

The results are reported in δ, notation in ‰:

$$\delta_{\frac{A}{B}}(‰) = \left(\frac{R_A}{R_B} - 1\right) x1000$$

The sign "‰" represent the unit for isotope ratio: negative δ values indicate lower abundances of the rare isotope than in the reference material and positive δ values the higher abundances. The $^{18}O/^{16}O$ ratios (reported as $\delta^{18}O_{VSMOW}$) are measured and reported to Vienna Standard Mean Ocean Water (VSMOW) with SD of ± 0.1 and $^{13}C/^{12}C$ ratio was measured and reported as $\delta^{13}C_{VPDB}$ relative to Pee Dee Belemnite (PDB).

3. Results and discussion

3.1. Evaluation of reversed osmosis efficiency

The efficiency of the extraction reverse osmosis method was demonstrated, as all permeate samples showed an alcohol content variation that was directly proportional to the volume extracted from the storage tank. Furthermore, the alcohol content of all partially dealcoholized wines, correlated directly with the number of extraction stages, showed that the application of reverse osmosis was suitable for the proposed objectives.

Table 1 presents the general parameters of the analysis samples (retentate, permeate, dilution). Significant differences for alcohol were observed between the control samples. Mean values were 11.78 ± 0.52% vol. control, 9.88 ± 0.36% vol. permeate and 11.96 ± 0.60% vol. retentate.

The same observation was made regarding the monitoring of the total acidity evolution in the wines, as well as the distribution of concentrations between the retentate and permeate. Compensation of TA values was confirmed by the values observed during the wine's reconstitution through *remontage* of the two phases. As expected, the average total acidity for permeate samples was 3.57 ± 0.30 g/L tartaric acid, compared to the control samples (8.16 ± 0.24 g/L tartaric acid) and retentate (8.56 ± 0.17 g/L tartaric acid), respectively.

No significant differences in total acidity were found between the control and reconstituted samples ($p>0.05$), except for the retentate samples, which had significantly lower values. The same correlation was observed in the comparative evaluation between alcohol content and acidity. Similar significant differences occurred between permeate, control, and retentate, since the solubility of potassium hydrogen tartrate depends on ethanol content [12].

Table 1. Quality parameters alcohol content (% vol.), pH and TA (total acidity (g/L tartaric acid equivalent) for control wine, permeate (osmosis extraction steps: A1 to A5), retentate (osmosis concentrates: V1 to V5) and reconstituted wines (dilution D1, D2).

	% vol	pH	TA (g/L)
Control wine	11.78±0.52	3.67±0.24	8.16±0.24
A1	8.87±0.25	3.68±0.09	1.63±0.25
A2	9.41±0.36	3.76±0.12	2.33±0.11
A3	9.75±0.12	3.69±0.05	3.45±0.08
A4	10.39±0.72	3.65±0.12	4.62±0.33
A5	10.99±0.36	3.61±0.08	5.86±0.75
V1	11.51±0.51	3.62±0.1	8±0.05
V2	11.52±1.25	3.68±0.24	8.94±0.12
V3	12.07±0.36	3.62±0.16	8.96±0.08
V4	12.21±0.8	3.67±0.48	8.99±0.21
V5	12.49±0.12	3.76±0.42	7.89±0.39
D1	11±0.28	3.78±0.1	6.72±0.15
D2	10.85±0.1	3.89±0.1	6.34±0.07

As the variation in ethanol exerted an influence on potassium bitartrate, it had a limited effect on total acidity but not on pH variations [13]. In this context, where the total acidity values were linear and dependent on ethanol concentration, the pH values showed a limited variation ranging between 3.61 ± 0.08 and, respectively, 3.76 ± 0.12. The variations from the control wine values were −0.07

and 0.08 for permeate, and −0.06 and 0.08 for the retentate. The values for the reconstituted wines were also within the same range (table 1).

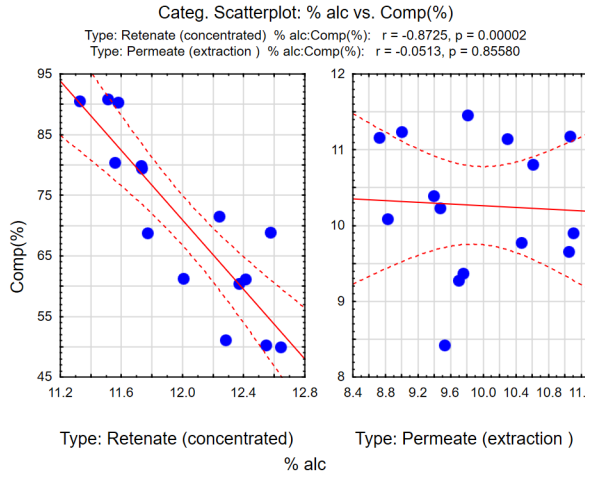
There were directly proportional variations with a high correlation coefficients regarding the transfer of ethanol following the reverse osmosis process and the acids contributing to the total acidity. Figure 1 shows the remarkable distribution of alcohol concentration (a) and acidity (b) between the two phases, which is further confirmed by the normalization of the values in the dilutions wines.

Regarding the variations in ethanol in relation to the extraction phases, a direct relation was observed between permeate and retentate. The correlation coefficient for retentate, the correlation coefficient was −0.872 ($p<0.05$). The coefficients were inversely proportional in the situation of retentate, and the variation occurred in the form of a decrease in the retentate samples correlated with constant concentrations in the permeate samples.

Regarding total acidity, the distribution was similar, titrimetric analysis conducted according to the OIV method [14] and revealed a direct correlation with the sampling number of extractions. In this regard, the total acidity value for sample A1 was 1.63 ± 0.25 g tartaric acid/L, while for A5 determined value was 5.86 ± 0.75 g/L tartaric acid. The value for the reference wine was approximately 8.16 ± 0.24 g/L tartaric acid. The extractions performed by removing reverse osmosis water led to a decrease in concentration in the remaining sample matrix (retentate).

Thus, for permeate, the correlation coefficient was insignificant since, as alcohol, total acidity maintained at values constant. An exception was observed for the retentates, which showed a value of −0.745 ($p<0.05$), indicating that the acid transfer rates in the permeate samples were much lower for the acids involved in total acidity. Lower transfer is showed by the correlation which has a lower value than the coefficient for alcohol modification (r= −0.872) resulting in the same constant transfer of acids in the permeate samples, as the presence of alcohol increased the affinity of several organic acids to water solution [15].

a)



b)

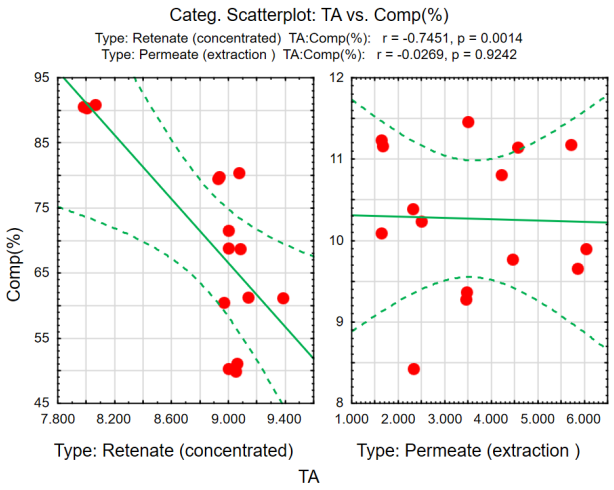


Figure 1. Variation of alcohol concentrations vs. percentage of permeate (a) and total acidity vs. retentate proportion (b) from wine.

3.2. Partial validation of the isotope parameters applied to reverse osmosis fractions

Official validated isotope methods for ethanol and water in wine samples and grape must were applied [11, 16, 17]. To ascertain whether the same methods can also be used for analysing the samples involved in the reversed osmosis, we assessed the repeatability of SNIF-NMR and IRMS analyses of ethanol from retentate, permeate and compared these values with those obtained for control wine and with those reported in the corresponding official methods.

The repeatability limit (r), that is, the value less than or equal to which the absolute difference between two results obtained under repeatability conditions may be expected to be, with a probability of 95% (calculated as 2.8 × sr), where sr is repeatability standard deviation [18].

To assess repeatability, the same samples were analysed according to methods requirements. Values of sr for $\delta^{13}C_{VPDB}$ and $\delta^{18}O_{VSMOW}$ for reconstituted wines (dilutions) and retentate-permeate are consistent with those for the wines reported in the OIV methods.

In the following table are presented R, $(D/H)_I$ and $(D/H)_{II}$ and $\delta^{13}C_{VPDB}$ values related to the ethanol obtained from wine and the $\delta^{18}O_{VSMOW}$ values for the water extracted from wine samples.

Table 2. Isotope parameters ($\delta^{13}C_{VPDB}$ [‰], $\delta^{18}O_{VSMOW}$ [‰], $(D/H)_I$ (ppm), $(D/H)_{II}$ (ppm), R for control sample (M), permeate (A1 – A5), retentate (V1 – V5) and dilutions (D1 – D2).

Av ± sr	$\delta^{13}C_{VPDB}$ [‰]	$\delta^{18}O_{VSMOW}$ [‰]	$(D/H)_I$ (ppm)	$(D/H)_{II}$ (ppm)	R
M	−25.84	2.69	100.3	125.5	2.501
	0.3	0.3	0.7	1	0.02
A1	−25.81	2.45	99.9	125.2	2.506
	0.3	0.3	0.8	1	0.021
A2	−25.8	2.51	100.1	125.6	2.509
	0.3	0.3	0.8	1	0.022
A3	−25.84	2.47	100.3	125.4	2.501
	0.3	0.3	0.8	1	0.021
A4	−25.79	2.47	100.2	125.9	2.514
	0.3	0.3	0.8	1	0.02
A5	−25.82	2.46	100.3	125.8	2.508
	0.3	0.3	0.9	1	0.022
V1	−25.83	2.55	100.4	125.6	2.502
	0.3	0.3	0.8	1	0.018
V2	−25.84	2.52	100.3	125.6	2.504
	0.3	0.3	0.8	1	0.021
V3	−25.77	2.59	100	125.4	2.507
	0.3	0.3	0.7	0.9	0.018
V4	−25.72	2.56	100.3	125.4	2.501
	0.3	0.3	0.8	0.9	0.019
V5	−25.70	2.63	100.2	125.4	2.504
	0.3	0.3	0.8	0.9	0.019
D1	−27.12	0.37	100.8	127.7	2.532
	0.3	0.3	0.8	1	0.019
D2	−27.14	0.48	100.4	127.4	2.538
	0.3	0.3	0.8	1	0.019

Validation data applied to the analysis $(D/H)_I$ and $(D/H)_{II}$ for the repeatability values are slightly higher here since repeatability standard deviations were less than 0.9 ppm for $(D/H)_I$ and 1 ppm for $(D/H)_{II}$. However, according to the OIV-MA-AS311-05 method [19], the repeatability limit for $(D/H)_I$ is 0.99, while for $(D/H)_{II}$, the repeatability limit is 1.75. In these conditions for the R, relative distribution of deuterium in molecules I and II, the repeatability limit is 0.01. The variations between the $(D/H)_I$ and $(D/H)_{II}$ values in wine, retentate-permeate samples, and control samples fell within these limits. As mentioned, values for R in the repeatability tests were higher than 0.01 ppm but were comparable to the average value between different types of samples and without any statistical difference in relation with control samples.

3.3. Distribution of isotope ratios between reverse osmosis fractions

The mean difference for $(D/H)_I$ between control wine and the samples produced by reversed osmosis ranged from −0.4 to 0.5 ppm and therefore values were below the repeatability limits of the official methods reported in Table 2.

The difference was not significant according to a paired t-test ($p < 0.001$), in line with previous findings. The wine samples had different $(D/H)_I$ values, ranging from 99.9 to 100.8 ppm and covered a large part of the typical variability of results corresponding with the type of wine and the usual vinification procedures which excluded use of potential denaturation products [20].

Some differences were not significant according to the ANOVA test (Tukey HSD test), which was applied to verify the means created for the groups in the study. However, the variability of the $(D/H)_{II}$ ratio in the comparative evaluation between the control sample and retentate/permeate can be mentioned. $(D/H)_{II}$ ratio in the comparison between the control sample and the retentate and permeate forms was confirmed by the p-values, which were lower than 0.05. A particular component is the evaluation of the $\delta^{18}O_{VSMOW}$ ratio, which did not show the same behaviour as the $(D/H)_{II}$ ratio [21].

Significant differences were not observed for the reconstituted samples (dilution) compared to the control, permeate, and retentate samples.

Among the grouping variables that structured the modification of ethanol concentrations, was the $\delta^{13}C_{VPDB}$ ratio for ethanol, which showed a significant distinct distribution in the reconstituted samples (dilution) compared to permeate ($p<0.05$) and for the reconstituted samples compared to retentate ($p<0.05$), as well as between the two types of extracts that were made in the study.

Monitoring the $\delta^{13}C_{VPDB}$ of ethanol obtained from wine also appears not to differ from that of control wine after extraction. In the context of significant differences, the $\delta^{13}C_{VPDB}$ ratio values for the reconstituted samples were −27.03 ± 0.39 ‰ compared to values of −25.79 ± 0.38 ‰ (retentate) and −25.91 ± 0.25 ‰ (permeate), respectively. The mean difference for $(D/H)_{II}$, which showed the same behaviour as $\delta^{13}C_{VPDB}$, is highlighted by the distribution of values between the reconstituted wines (dilution) (127.48 ± 1.41 ‰), retentate (125.03 ± 0.72 ‰) and permeate (125.22 ± 1.04 ‰) samples, with a range between −1.7 ‰ to 0.8 ‰, values that are below the repeatability limits of official methods. The differences were significant according to multivariate ANOVA tests ($p < 0.001$). Wine samples had different $(D/H)_I$ values, ranging from 99.97 to 100.82 ppm, covering much of the typical variability of the products. Further, evaluating the water from reverse osmosis for the $\delta^{18}O_{VSMOW}$ through reaction with CO_2, differentiation from oxygen present in ethanol was

performed, and it was concluded that with the dynamics of ethanol, there was no similar dynamic of osmosis water [22]. The $\delta^{18}O_{VSMOW}$ values ranged between 2.79 ± 0.59 ‰ (control sample) and 2.64 ± 0.24 ‰ (retentate), but compared to permeate.
An exemption was produced in dilution experiments that had values of 0.37 ± 0.3 and a 0.48 ± 0.3. Since the notation $\delta^{18}O_{VSMOW}$ [‰] refers to the ratio of heavy oxygen isotopes (Oxygen-18) to light oxygen isotopes (oxygen-16) in a sample, expressed in per mil deviation from the Vienna Standard Mean Ocean Water (VSMOW), this ratio is a valuable tool for understanding the origin and history of water molecules. In the context of wine, the value for $\delta^{18}O_{VSMOW}$ = 0.48 ± 0.3‰ indicates that the water extracted from the wine is enriched in heavy oxygen isotopes compared to VSMOW [23]. The dilution samples, formed by mixing retenate and permeate in different ratios, would have intermediate $\delta^{18}O_{VSMOW}$ values depending on the proportions of each component. The observed values of 0.48 ± 0.3 ‰ and 0.37 ± 0.3 ‰ suggest that the dilution process alter the isotopic composition of the water in the samples, which is expected since the retenate and permeate already have relatively similar $\delta^{18}O_{VSMOW}$ values.

The dynamics and distribution of the $\delta^{13}C_{VPDB}$ ratio showed high variability due to significant differences produced at the level of different experimental categories. A significant difference was found between the reconstituted sample and the two types of experiments (retentate/permeate). Due to the different distributions between the various experiments, the dynamics within the experiments are being evaluated to describe the direction of evolution. As long as the two experiments were developed through repeated extractions using directly proportional sample volumes, the distribution rate of ethanol content and organic acids could be determined, but the variation of the characteristic parameters of isotope ratios according to the extraction rates for permeate and retentate were also considered. Proportional correlations were considered for retentate in cases where the $\delta^{13}C_{VPDB}$ distribution showed an inverse variation with the extraction rate from the wine in this type of sample ($r = -0.95$, $p = 0.019$). As a confirmation of the variation in ethanol concentration, a direct proportional variation between the $\delta^{13}C_{VPDB}$ ratio and the distribution of $(D/H)_{II}$ was observed, which showed a direct correlation with $r = 0.912$ ($p < 0.05$), results that were in accordance with other findings [24].

Linear Discriminant Analysis was used to evaluate the discrimination between classes as function of distribution of isotope ratios between the component elements.

In this context, table 3 represents standardized coefficients for three canonical variables (Root 1, Root 2, and Root 3). These roots are the discriminant functions generated by the LDA, and the standardized coefficients indicate the relative contribution of each variable to the discriminant functions. These functions are combinations of the original variables, created to maximize the separation between predefined groups (permeate, control, retentate and reconstituted wines (dilutions)) [25].

Table 3. Standardized Coefficients for canonical variables.

Variable	Root 1	Root 2	Root 3
δ13CVPDB [‰]	−0.791	0.31	0.013
δ18OVSMOW [‰]	0.352	1.118	−0.257
(D/H)I (ppm)	0.318	0.503	−0.715
(D/H)II (ppm)	0.563	0.63	0.359
R	0.418	−0.321	−0.373
Eigenvalue	3.943	0.289	0.044
Cum. Prop	0.922	0.99	1

The eigenvalues indicate the amount of variance explained by each discriminant function since Root 1 explains the most variance (3.943), meaning it is the most important discriminant function. Root 2 explains a much smaller proportion of the variance (0.289), and Root 3 explains the least (0.044).

Relative to cumulative proportion Root 1 captures 92.2% of the total variance, Root 2 increases the cumulative proportion to 98.9% and this suggests that the first two roots explain almost all of the variance between the groups, while the third root adds very little additional information.

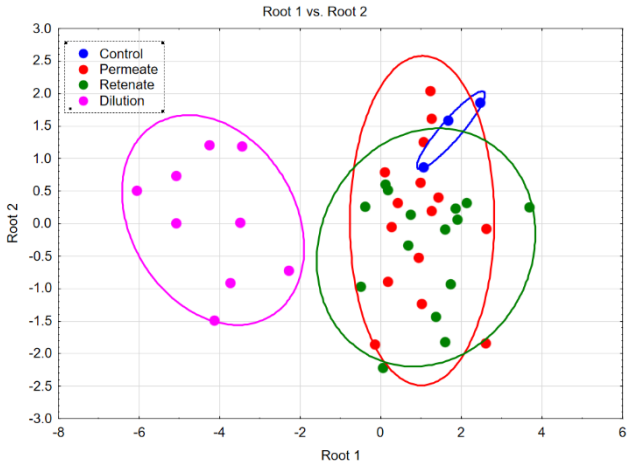


Figure 2. Linear discriminant analysis for isotope ratio distribution between discrimination classes.

According to figure 2. Root 1 is primarily influenced by $\delta^{13}C_{VPDB}$ (negatively) and $(D/H)_{II}$ (positively), with moderate contributions from the other variables. This discriminant function is likely capturing a major dimension of difference between type of wine, particularly driven by carbon isotope ratios and hydrogen concentrations which are characteristic to ethanol. In the case of root 2 is heavily influenced by $\delta^{18}O_{VSMOW}$ (strongly positive) and also has moderate contributions from the hydrogen variables. This discriminant function likely reflects a secondary dimension of variation, largely driven by the oxygen isotope ratio. The oxygen ratio is characteristic to water from wine, but since in the ad-hoc Tuckey test, parameters did not show any statistical differences, this is confirmed by the separation of factors.

In the absence of reference data for all regions and the specific properties imposed by the pedoclimatic effects on

the isotope ratios of the wines analysed, a series of threshold values were considered for the involved parameters. For $\delta^{18}O_{VSMOW}$ (water), a lower limit of −5 was established, for (D/H)I (ethanol), less than 96 ppm, and for $\delta^{13}C_{VPDB}$ [‰] (ethanol), greater than −22 ‰. For the reference regions, intervals were considered and drawn to play a role in the specific individualization of wines [26].

3.4. Evaluation of wines from the perspective of isotope ratios

In the case of samples that were subjected to alcohol concentration reduction by the addition of reverse osmosis water, it was found that the $\delta^{18}O_{VSMOW}$ values for the diluted sample showed the highest difference compared to the control sample, 0.29±0.054 ‰, while the values were correlated with those for the permeate, 0.35±0.05 ‰, and the retentate sample showed differences (0.147±0.05 ‰) with lower values. As noted in the literature, it is observed that samples with a high content of reverse osmosis water can influence the $\delta^{18}O_{VSMOW}$ values. Even in these conditions, the levels of the ratios were within the limits considered as part of the reference domain for wines from the Central European area, whose reference intervals for $\delta^{18}O_{VSMOW}$ water were between −4 and +3.

For wines from the EU, the $(D/H)_I$ parameter varies approximately between 98 and 104 ppm, and for $\delta^{13}C_{VPDB}$ ethanol values ranged between −30 ‰ and −24 ‰, which covers variability in both Southern and Central Europe regions [26].

The mean values with standard deviations of the $\delta^{13}C_{VPDB}$ were close relative to the control samples ($\delta^{13}C_{VPDB}$= 25.84 ± 0.3 ‰) same situation was registered for $(D/H)_I$ of 99.97 ± 0.54 ppm. The $(D/H)_{II}$ value of ethanol (125.68 ± 1 ppm), which is more correlated with the $\delta^{18}O_{VSMOW}$ value of water indicates a correspondingly higher standard deviation. The expression of all variables included in the retentate and permeate maintained the ratio values. Indeed, in the case of reconstituted (diluted) samples, it was found that the $(D/H)_{II}$ showed a higher value of 127.48 ± 1.41 ppm, which showed a deviation compared to the other types of samples; however, no series of samples fell outside the reference range. Diluted samples produced lower values for the $\delta^{13}C_{VPDB}$ and higher for $(D/H)_{II}$. Thus, in the conditions of sample dilution, an increase in these two parameters was observed, correlated with lower values for $(D/H)_I$, which presented $\delta^{18}O_{VSMOW}$ values but without a statistically significant difference [27].

4. Conclusions

Reversed osmosis is confirmed to be an efficient method applied in the post-fermentative process to reduce the alcohol concentration of a white wine. The reduction is confirmed by the variation of ethanol, but the main advantage is determined by the different transfer rate of acids that are involved in titrated acidity. The reduced transfer rate permits the optimization steps for the alcohol extraction and reintroduce of osmosis water back in the system with specific wine components as acids, sensory compounds, colour compounds etc.

The repeatability values for all parameters for retentate and permeate, control and dilutions are comparable to each other and to the values of the OIV reference method. The standard deviations values of water and concentrated samples are not different. The same was found with $\delta^{13}C_{VPDB}$ of dilutions and $\delta^{18}O_{VSMOW}$ of water along with $(D/H)_I$ and $(D/H)_{II}$ of ethanol from fermentation.

We conclude that isotope methods for analysing ethanol using the $\delta^{13}C_{VPDB}$ from ethanol obtained through fermentation, and $\delta^{18}O_{VSMOW}$ determined from water originating from fermented products can be applied to the analysis of samples from experiments set up for reverse osmosis processes.

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