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Multivariate Evaluation of Changes Induced in Red Wine Characteristics by the Use of Extracting Agents

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The aim of the present work was to study the effect of both vintage and the use of four types of pectinolytic enzymatic preparations on the quality of red wine using factor analysis. We also studied the evolution of the factors obtained along two years of storage of the wine. The results obtained from the factor analysis revealed that the addition of pectinolytic enzymes enriched the phenolic composition and elicited an improvement in the visual aspect of the wine, which persisted during the storage period considered.

KEYWORDS: Multivariate evaluation; factor analysis; red wine; pectolytic enzymes; color; phenolic compounds

INTRODUCTION

It is well-known that the composition of wine is influenced by a broad spectrum of factors such as varietal factors, vintage, and production factors, including cultural and enological practices. Usually, a modification in the production process generates some changes in the chemical composition of the final product (*1*).

These changes are associated with changes in the properties of wine (*2*) and can be identified through chemometric modeling (*3*). Multivariate statistical analysis has proved its usefulness in the detection of both the most significant changes induced by each factor and the most representative factors or those factors which best differentiate wine samples.

Multivariate analysis has been mainly applied to differentiate wines by their geographic origin and the grape variety used (*4*−*7*) but few works have been carried out as regards the effects of enological practices (*8*−*10*). Among the different multivariate analyses, factor analysis is a very interesting tool because it permits one to define the latent variables that account for the linear relationships between the experimental parameters, and to interpret the vast numbers of data deriving from the studies. Factor analysis has been mainly used to characterize wines in terms of environmental parameters (*11*−*13*) rather than to study the effect of elaboration parameters (*14*).

The application of pectolytic enzymes is one such enological practice that modifies the chemical composition of wines. These enzymes induce changes in the classical enological parameters and contents in methanol (*15, 16*), the phenolic composition (*17, 18*), technological variables (filterability, turbidity) (*19, 20*), and the chromatic parameters of wines (*21, 22*). These effects have been found to have different incidences and intensities, depending on several factors such as the variety of grapes employed for the wine, the vintage, and the type of enzyme preparation used (*23, 24*).

In light of the above, the aim of the present work was to use factor analysis as a tool to study the overall modifications induced by the use of pectolytic enzymes. The study was carried out for two vintages and for different commercial products in order to establish, if possible, the effect of vintage and type of treatment.

Thus, two types of pectolytic preparations were used: clarifying and color-extracting pectolytic enzymes. The reason for the use of two types of enzymatic preparations was because the main use of clarifying agents is to facilitate the filtration process and to reduce the turbidity of wines. Despite this, some studies have shown that application of this type of preparation also produces an increase in the extraction of phenolic compounds and affords better chromatic characteristics (*25*). These effects are described as being typical of color-extracting enzymes, although the products used are more expensive than those used for clarification.

It is also well-known that the aspect or visual characteristics of wines, such as their color or turbidity, are directly correlated with wine quality. Accordingly, modifications of these characteristics induced by the use of pectolytic enzymes could demonstrate the advantages or disadvantages of these products in wine.

MATERIALS AND METHODS

Experimental Material. Grapes of the Tinto Fino (*Vitis vinifera*) variety grown in the same vineyard were harvested at commercial maturity (23−25 °Brix). The harvested grapes were quickly transported in plastic boxes (∼20 kg) to the laboratory where they were processed. The damaged grape clusters (broken or with visual microbial alterations) were separated in order to eliminate undesirable contamination. Then, groups of intact clusters were processed according to the next explained treatment.

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Vinification - Control. The grapes were de-stemmed and crushed, and 0.04 g/L of SO_2 was added. They were inoculated with 0.1 g/L of commercial yeast, *S. cerevisiae* and *S. bayanus* (Wormser Oenologie) which had been previously hydrated. The alcoholic fermentation was carried out in small steel tanks (100 L), with a temperature control of ≤25 °C. The end of fermentation was taken as lowering of reducing sugars concentration to <4 g/L.

At the end of the alcoholic fermentation the wine was strained off and the grape pomace was pressed ($P < 2$ kg/cm^2). All the resulting wine was mixed and transferred into another tank and kept at 1 °C for 24 h to facilitate the settling process. Afterward, the wine was centrifuged (10500*g* for 10 min at 5 °C) and the clarified wine was bottled in 250-mL green glass bottles and stored at 15 °C for two years.

Vinification - Enzymatically Treated. In these experiments, the only difference between treated samples and the control sample was the addition of pectolytic enzymes 1 h before the inoculation with yeast (the time suggested by the producers). During this time, the grape pomace was kept at 20 °C.

Four different commercial pectolytic enzymes were used at the maximum and minimum doses suggested by the producers. The type of enzymes and codes used were the following: (a) clarifying pectolytic enzymes, Zimopec PX1 (Perdomini SPA) 0.01 g L^{-1} (Z.1) and 0.03 g L^{-1} (Z.3), and Rapidase CX (DSM) 0.02 g L^{-1} (R.2) and 0.05 g L^{-1} (R.5); (b) color-extracting enzymes, Pectinase WL Extraction (Wormser Oenologie) 0.005 g L^{-1} (P.05) and 0.01 g L^{-1} (P.1), and Rapidase Ex. Colour (DSM) 0.02 g L^{-1} (Rex.2) and 0.05 g L^{-1} (Rex.5).

Two consecutive vintages (1998 and 1999) were used for this study. In both vintages each treatment (control and enzymatic vinification) was duplicated. In addition, analytical parameters were analyzed in duplicate in every sample.

Wine samples were taken at the end of the vinification process, and at 6-month intervals for two storage years.

Analytical Procedures. *Color Variables of Glories (26).* A direct measurement of wine absorbance to 420, 520, and 620 nm was carried out in a DU 650 Beckman spectrophotometer with a 1-mm quartz cell. The following variables were then calculated: color intensity (I), tonality (To), proportion of yellow (% Ye), proportion of red (% Rd), and proportion of blue (% Bl).

Families of Phenolic Compounds. Total polyphenols (TP), total anthocyanins (ACY), catechins (CAT), and proanthocyanidins (PRO) were assayed according to the traditional methods described in Paronetto (*27*).

Individual Phenolic Compounds by HPLC. Analyses were carried out using a HP 1100 Series, fitted with a 7125 Rheodyne injector (Fisons Instrument). The system was equipped with a diode array detector.

Anthocyanin compounds were analyzed using the LC−MS method proposed by Revilla et al. (*28*) and studied according to the following groups: anthocyanin monoglucosides (Acy-gls), acetic esters (Acy-ac), cinnamic esters (Acy-cin), including both coumaric and caffeic esters, pyruvic acid derivatives or vitisins A (Acy-py-gls), vitisin B and malvidin-catechin derivatives (Mv-cat).

Low-molecular-weight phenols and procyanidin compounds were extracted by column fractionation following the method proposed by Di Stefano and Cravero (*29*). Low-molecular-weight phenols were quantified using the method proposed by Bartolomé et al. (*30*). Individual low-molecular-weight phenols were grouped in phenolic aldehydes and acids. Flavan-3-ol derivatives were analyzed using the method proposed by Pérez-Magariño et al. (*31*) and quantified in the following groups: catechin and epicatechin monomers, dimers, trimers, tetramers, and galloyl derivatives.

Enological Variables. The determined parameters were reducing sugars, tritratable acidity (Ac_T), pH, individual acids (malic, lactic, and acetic), and volatile acidity (Ac_{vol}). Reducing sugars, tritratable acidity, and volatile acidity were quantified according to the OIVV Official Methods (*32*). °Brix was determined with a termostatted refractometer model CONVEX. pH was determined with a CRISON Micro pH-2000 pH meter. Individual malic, lactic, and acetic acids were determined with Boehringer-Manheim enzymatic tests (*33*).

Technological Variables. Total pectins, methanol, and turbidity were also measured. The methanol was quantified according to the cromotropic acid method (*32*). Total pectin was measured in wine alcohol insoluble residues after their acid (*34*). The Robertson method (*35*) was used to quantify the total pectin content which was expressed as mg/L of galacturonic acid. Turbidity was determined with a 2100P Hach turbidimeter and expressed as nephelometric units (NTU).

Statistical Analysis. The statistical analysis of the data was carried out by factor analysis using principal components. Factor analysis is a method of multivariate analysis that linearly transforms one set of variables into another set of fewer variables (factors) that conserve all the information of the original set, searches for associations among the variables, and is able to detect natural groups present in the samples (unsupervised method).

The maximum number of factors is equal to the number of initial variables; the number of factors chosen is determined by applying certain selection criteria such as choosing those factors that show an eigenvalue higher than unity or choosing as many factors as is necessary to explain at least 80% of the overall variance. Here we applied factor analysis by principal components and worked with standardized data with a view to avoiding the assignment of greater weight or influence to the variables with the highest absolute values. We chose the selection criterion of the number of factors showing an eigenvalue of greater than 1. The factors were subjected to varimax rotation, which minimizes errors and renders the factors orthogonal to one another, thus explaining the maximum variance of the data. Moreover, this type of rotation helps in the interpretation of the possible associations among variables (*36*).

Statistical analysis was performed using the Statgraphic Plus program for Windows (Manugistics Inc. 1995).

RESULTS AND DISCUSION

The number of original variables analyzed was 31 and the number of individuals included in the study was 360 (nine types of wine, in duplicate elaborated for two years, five sample collections, and two analyses per collection). The analysis was applied to all the data; that is, considering the data from the wines from both crops and analyzing each of them over two years of storage in semestral collections. The aim of the joint study was to examine the associations between the variables studied independently of the effect of vintage and age so that the variables would have as general a meaning as possible.

After the factor analysis, seven factors with an eigenvalue greater than unity were obtained, overall accounting for 83% of the total variance. Because the factors were subjected to varimax rotation, which aims at associating a variable with the factor in which most of the total variance is explained, it can be assumed that the variables associated with the same factor are also more or less interrelated or correlated with one another.

The loading of each variable in each factor after the varimax rotation is shown in **Table 1**, in which the coefficients with a value lower than 0.250 were eliminated in order to simplify the presentation of the results.

Factor 1, accounting for 38% of the total variance, was associated with a great number of variables studied, many of them with a high loading. The associated variables with highest loading were phenolic variables, such as total polyphenols and flavan-3-ol derivatives, with the exception of the trimers and epicatechin. These compounds evolve during the wine aging in a peculiar way, such as described in a previous paper (*37*). This fact explains why they were associated to other two different factors. Classic enological variables, such as reducing sugars, pH, and malic acid, and chromatic variables, such as the percentage of yellow and tonality, were other variables associated with significant loadings.

The derivatives of flavan-3-ol appear with a positive sign, whereas the color parameters (tonality and % of yellow) do so with a negative sign. These results are consistent with the data reported by Gómez-Cordovés and González SanJosé (*38*) who

Table 1. Factor Loading Matrix after Varimax Rotation

	factor 1	factor 2	factor 3	factor 4	factor 5	factor 6	factor 7
reducing sugars	**0.753**				0.468		
pH	**–0.627**	0.279		0.367	0.333		
turbidity	[a]		**0.902**				
volatile acidity	–0.251			**0.824**	0.252		
methanol					**0.851**		
malic acid	**–0.770**	0.333			0.334	0.252	
lactic acid				**0.724**			
tritatable acidity	–0.316	0.464		**–0.545**	0.364	0.321	
% yellow	**–0.787**	*–0.492*					
% red	*0.620*	**0.678**					
% blue	–0.390	**–0.786**	0.338				
intensity	*0.497*		**0.795**				
tonality	**–0.702**	*–0.538*	0.275				
total polyphenols	**0.841**	0.282					
total anthocyanins	–0.274	**0.883**					
total catechins	**0.867**						
proanthocyanidins	**0.906**						
phenol acids		**0.717**				0.306	0.249
phenol aldehydes		0.476					**0.683**
catechin	**0.802**						
epicatechin							**0.840**
dimer flavan-3-ol	**0.825**						
trimer flavan-3-ol	0.383					**–0.684**	
tetramer flav-3-ol	**0.840**						
galloyl flavan-3-ol	**0.556**						
Acy-gls		**0.929**					
Acy-acetic	0.327	**0.871**					
Acy-coumaric	0.336	**0.895**					
Acy-pyr		**–0.534**			0.406	*0.503*	
vitisin B	0.353	**–0.750**				0.259	
malvidin-cat deriv.		**–0.671**				*0.567*	
explained var.	37.9%	18.5%	7.6%	6.7%	5.1%	4.1%	3.3%

[a] Values lower than 0.25 are not shown in order to simplify the results exposition.

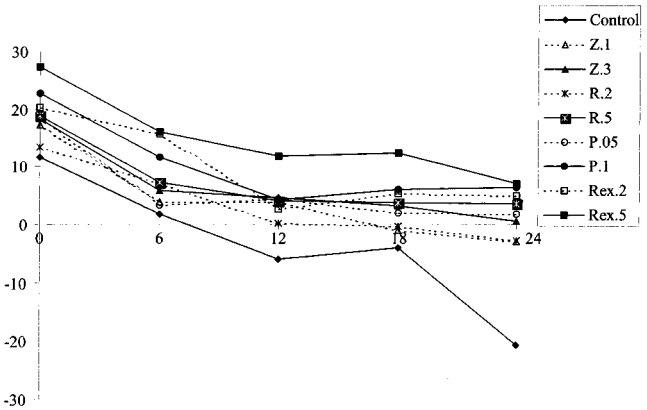

Figure 1. Evolution of factor 1 medium values throughout the storage period.

reported that the presence of derivatives of flavan-3-ol contributes to the stabilization of the red color.

The evolution of factor 1 along the storage period of the wines (**Figure 1**) shows that the wines treated enzymatically always had higher values in that factor. On one hand, this is due to a greater content of phenolic compounds resulting from the application of the enzymes, and, on the other, to the higher intensity and % of red values and the lower % of yellow and tonality values, the result of greater color stability.

From the above results it may be inferred that although the wines treated with color-extracting enzymes (Rex and P.1) initially had higher values in this factor than those elaborated with clarifying enzymes, after the first six months of storage only one product (and then at the maximum dose applied (Rex.5)) showed significant differences. At two years, hardly any differences between the treatments were detected.

Study of factor 2 brought to light some very interesting results. This factor explained 18.5% of the total variance, and grouped variables related to the anthocyanin composition of the wine, including newly formed pigments and chromatic variables such as the percentage of red and blue, as well as tonality and the percentage of yellow. The positive correlation established between total and individual anthocyanins with red shows that the greater extraction of these compounds will lead to a higher value of this color component, such that, logically, it established a negative correlation with tonality and yellow.

Additionally, the negative-sign coefficient of the percentage of blue and of the new anthocyanin pigments should be interpreted in terms of the notion that these new pigments stabilize the violet tonalities helping to maintain elevated values of the blue component and, in the case of some of them, inducing important bathochromic shifts (*39*). Moreover, the greater formation of new pigments implies that there will be fewer free monomer anthocyanins because they are formed from these latter.

The high positive value of the phenolic acids variable is probably due to the fact that the enzymatic preparations also favor their extraction.

The evolution of the second factor with time (**Figure 2**) again revealed a noteworthy difference between the wines obtained with enzymatic treatments and the control wine, the former showing higher values, above all after the first six months. Again, overall there were no large differences between the wines treated with extracting preparations and clarifying agents.

From the foregoing, it would be expected that from a certain point the treated wines would show lower values for factor 2 than the control wine. This, however, was not the case, owing to the strong weight of the blue component in the control wines. This was strongly increased because of the presence in suspen-

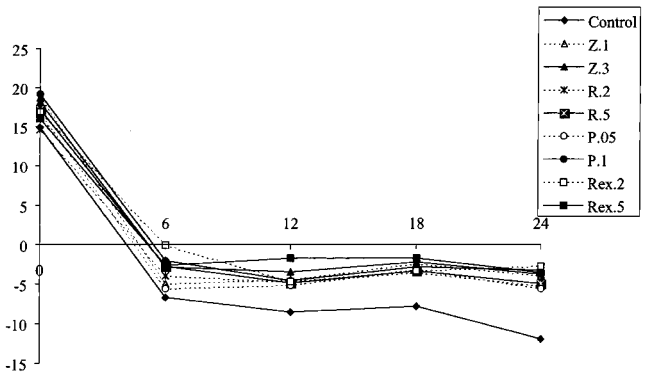

Figure 2. Evolution of factor 2 medium values throughout the storage period.

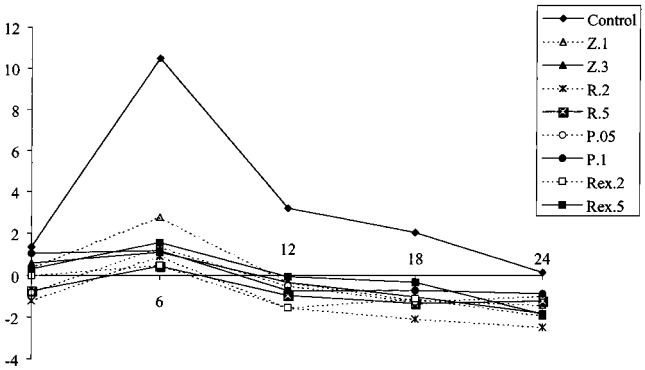

Figure 3. Evolution of factor 3 medium values throughout the storage period.

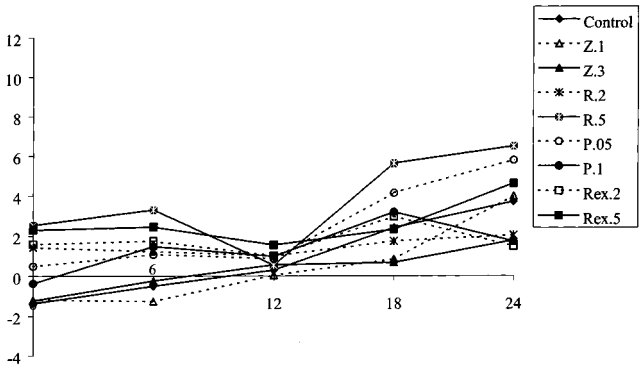

Figure 4. Evolution of factor 4 medium values throughout the storage period.

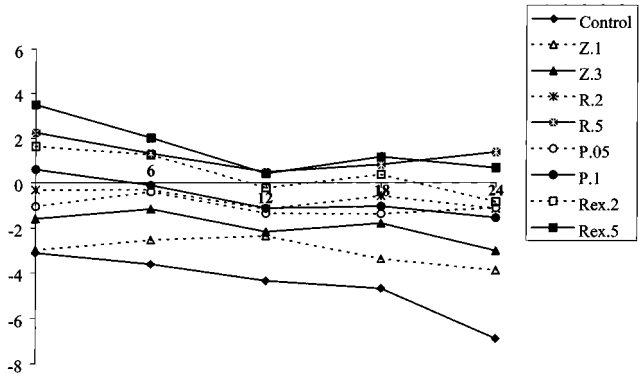

Figure 5. Evolution of factor 5 medium values throughout the storage period.

sion of very stable violet colloids, as mentioned below in the context of their relationship with factor 3.

Factor 3, accounting for 7.5% of the variance, was associated with two very important parameters as regards the visual aspect of the wine: namely, turbidity and intensity. Both parameters were strongly related, as when turbidity increased so did intensity. This was because a generalized increase in absorbance was observed across the whole of the spectrum. Although the wines were centrifuged prior to evaluating the chromatic parameters and turbidity, the particles remaining in suspension were responsible for the differences observed between the control wines and those treated enzymatically.

The evolution of this factor (**Figure 3**) shows that the application of enzymes elicits a marked reduction in turbidity and therefore causes the wines to have a lower apparent intensity. Thus, in all cases the enzymatically treated wines showed lower intensity values than the control wine. It should be noted that the increase in intensity of the control wine is due to the formation, during storage, of violet polymers that remain in suspension because of the higher levels of pectins. These pectins act as stabilizing colloids (*16*) until their volume and molecular weight increase to values that lead to their precipitation or that allow them to be eliminated.

These first three factors account for 64% of the variability and show that the variables most affected by the enzymatic treatment are those related to the phenolic composition, chromaticity, and visual aspect of the wine. Accordingly, treatment with enzymes will afford wines with a higher phenol content, including anthocyanic compounds, derivatives of flavan-3-ol, and low-molecular-weight phenols. This positively affects color, affording wines with a lower yellow component and tonality and greater stability of the red component. Furthermore, the addition of enzymes leads to a strong decrease in turbidity, which is low and stable over time.

The next four factors together explain only 19% of the total variance.

The fourth factor groups variables related to the acidity of the wine, and its evolution fluctuates only slightly. Increases in the acidity of certain samples in the later collections are mainly due to increases in the volatile acidity of some bottles, probably because the cork allowed the passage of excess oxygen (**Figure 4**).

Factor 5 is associated with a single variable with high loading: the methanol content. It is well-known that the addition of pectinolytic preparations may strongly affect the levels of ethanol, above all if such preparations show pectin−methylesterase activity, which induces the hydrolysis of the methoxy group of the pectins, releasing methanol into the medium (*16*). This is clearly reflected in the evolution of this factor (**Figure 5**), in which it may be seen that all the treated wines had higher levels of methanol. In this case, it was the R treatments (both extractive and clarifying) at the highest dose that led to the greatest release of methanol, whereas the Z treatments were those that caused the smallest increase in this parameter. These findings are consistent with the results of previous work in which greater pectin−methyl esterase activity was observed in R preparations than in Z preparations.

Factor 6 was associated, with a positive sign, with some of the new anthocyanin derivatives, although the variable with the highest loading and with a negative sign was the trimers of flavan-3-ol. The association of these compounds with one another could be considered occasional, forced by the mathematical model employed, because their qualitative modification during storage was more or less equivalent, although in the opposite sense since the first were formed while the second

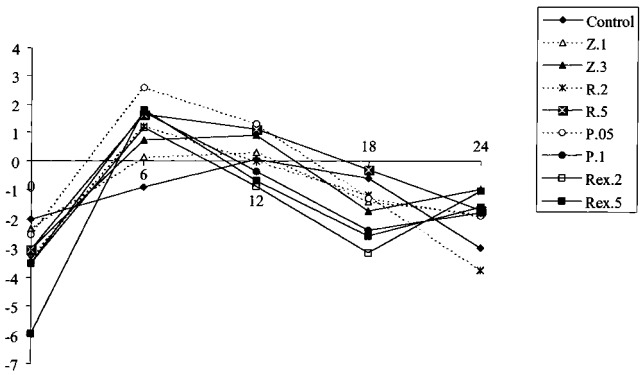

Figure 6. Evolution of factor 6 medium values throughout the storage period.

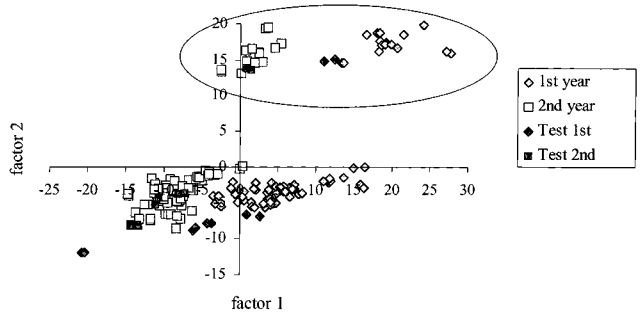

Figure 7. Sample distribution in the plain described by factors 1 and 2.

disappeared, probably because they continued to be polymerized or condensed with other compounds.

The evolution of this factor (**Figure 6**) was high during the first six months of storage, which is the period of maximum formation of the new anthocyanin pigments (*39*). In general, after the first six months of storage no large differences were found among the different wines for this factor.

Finally, factor 7 is associated with epicatechin and phenolic aldehydes, an association that may also be occasional, and that may have been forced by the mathematical model, which ensures that all the variables are associated with a single factor. Unlike the rest of the compounds studied, the levels of both compounds underwent small variations along the storage period, this factor thus persisting at more or less constant values along time.

As mentioned above, factor analysis has the advantage of being able to detect the presence of groups among the samples studied. Thus, on plotting the samples on the plane described by the first two factors (**Figure 7**), the initial wines (surrounded by an ellipse in the figure) were clearly separated from the stored wines. Both groups can essentially be separated by the factor 2 values, probably owing to the strong losses of anthocyanins during the first six months of storage, and the appearance and accumulation of new pigments during this time. Because these changes slow after 6 months, the stored wines are little differentiated from one another.

Moreover, it was possible to observe the separation of the samples as a function of the vintage of the wines, more easily visible in the initial samples than in the stored samples. Because the wines from the first vintage are generally situated to the right of those from the second vintage, factor 1 can be considered to be associated with the year of vintage.

Factor analysis of the results obtained here allow us to conclude that the parameters most modified by the addition of pectinolytic enzymatic preparations are the composition in phenols and the chromatic parameters of the wine; these variables are closely related to each other.

The use of enzymatic preparations leads to wines that are richer in phenolic compounds and with a better visual aspect and chromaticity. This improvement persists for at least the first two years of storage.

In principle, the differences found between the effects of the extraction and clarifying treatments do not seem to justify the greater production costs brought about by the use of the extraction treatments.

In this regard, it should be noted that the grape variety used in the present study is rich in readily extractable phenolic compounds, such that the results should be interpreted with caution when attempting to extrapolate them to other varieties. If the grape variety to be treated is poor in pigments or if these are difficult to extract, it is possible that the use of extracting preparations showing a relatively higher extraction potential could be justified.

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