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Evaluation of similarities between natural and accelerated browning of fino sherry wines by chemometric techniques

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A new accelerated browning method that allows the prediction of the evolution of white wine was developed. The determination of similarities between natural browning phenomena and accelerated browning is a critical point for the introduction of this new method for control purposes. Since phenolic compounds are the main substances responsible for browning, their evolution in both the natural process and accelerated browning was investigated. HPLC is used to quantify the phenolic composition and about 40 peaks are obtained for each analysis. Since the amount of information obtained is fairly large, chemometric techniques (cluster analysis of cases and principal component analysis) were used to analyse the evolution of phenolic compounds. It was demonstrated that the effects of accelerated browning on samples of wine have the same characteristics as natural browning.

Introduction

The browning phenomenon in fino sherry wine causes serious economic losses for the wine-producing industry. The phenomenon is the result of the oxidation of phenolic compounds present in the wine.[1] Among the phenolic compounds in white wines, catechins and hydroxycinnamic acid derivatives are the most oxidizable compounds.[2] However, there are more compounds involved in the oxidation process.[3] Browning not only affects the colour of the wine but also produces changes in the wine's organoleptic properties, such as its aroma and its flavour.[4,5] The end result is deterioration of the wine, but over a period of time that cannot be determined by *a priori* means, usually over some 8–12 months. Numerous enological techniques are employed to prevent or slow the browning process but they have proved of limited use.[6]

Our research has been aimed at resolving the analytical problem presented by this economic loss. To this end, an accelerated browning method has been designed[7] which, on the one hand, allows the determination of the optimum period of time within which the wine should be consumed and on the other, it permits an objective evaluation of the efficiency of enological techniques used for the prevention or delay of browning.

Previous analytical methods carried out to evaluate the susceptibility of wine to browning were based on heating the sample.[8,9] However, the chemical reactions due to heating the wines are different to those produced during natural browning.[5]

The object of the work described here was to validate the trial method devised. This involved the comparison of the evolution of the compounds responsible for browning during the natural process with the results obtained from the accelerated process. Previous work has proved the validity of basing the characterisation of fino sherry wine on the determination of these phenolic compounds.[10]

The phenolic compounds are usually determined by HPLC,[11,12] which generates a considerable volume of data on each sample. Consequently, the comparison between the natural and accelerated processes is more appropriately carried out using chemometric techniques, as is customary in other analytical studies of foods and drinks which generate large amounts of data.[13,14] In our case, the techniques used were cluster analysis (CA) and principal components analysis (PCA).

Experimental

Samples

Two different methods of preservation of the wine samples were used for monitoring the natural browning process: type I, wine preserved in 750 ml bottles, with open necks covered with cotton-wool to avoid the introduction of foreign agents; and type II, wine preserved in 750 ml bottles, corked but with a 150 ml air chamber.

Fifty-four bottles from the same commercial lot were kept in order to have each sample in triplicate for every type of sample, plus 12 samples analysed before starting the assay and considered as not evolved samples.

Analysis of the polyphenolic composition

A volume of 100 ml of wine was extracted using 80 ml of diethyl ether by means of continuous rotary extraction, following the method of Brú *et al.*[15] 2,5-Dihydroxybenzaldehyde was used as an internal standard.

Chromatographic analysis was performed by HPLC in a Waters (Milford, MA, USA) chromatographic system (M-45 and 510 pumps, Model 717 automatic injector, UV-440 detector, Millenium 2.0 software) using a LiChrospher column (Merck, Darmstadt, Germany). UV detection at 280 nm was used. Gradient elution was applied according to the method reported by Guillén *et al.*[11] Briefly, two solvents were used: A (10% methanol–2% acetic acid in water) and B (90% methanol–2% acetic acid in water). The initial conditions were a flow rate of 1 ml min^{-1} and 100% A, reaching 85 : 15 A–B in 15 min and 50 : 50 A–B in 35 min; both changes were done using a convex gradient.

Peak heights were measured automatically and corrected by reference to the internal standard. Peak heights were used instead of peak areas because we have found that for these samples they produce better results, as there are several poorly resolved peaks. A typical chromatogram is shown in Fig. 1.

The accelerated browning was carried out using the methodology devised by the authors.[7] Briefly, 125 ml of wine were used for each analysis and 1.5 V was used as the electric potential difference between the sample and the reference solution. Two platinum electrodes were used and the absorbance (420 nm) was recorded during the application of the accelerated method.

The chemometric treatment was performed by means of the BMDP (Los Angeles, CA, USA) program, using 2M (cluster analysis) and 4M (principal component analysis) sub-routines.[16]

For CA, the nearest neighbour method was used as the linkage distance and Euclidean distances were used as the distance measure.

No preprocessing method was used prior to PCA.

Results and discussion

Modelling the natural evolution

The polyphenolic composition of fino sherry wine was studied during the course of the evolution of the browning phenomenon, specifically over 12 months with HPLC analyses being carried out after 0, 1, 2, 3, 8, 10 and 12 months.

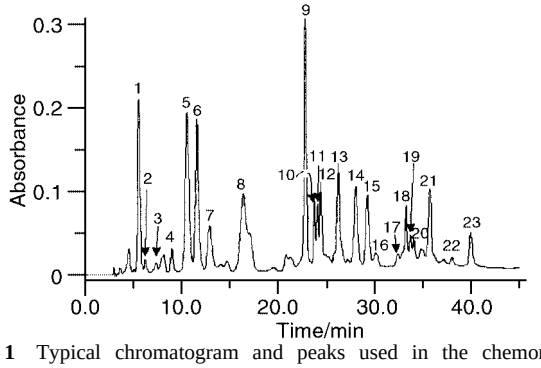

Fig. 1 Typical chromatogram and peaks used in the chemometric analysis.

The data that were submitted to chemometric treatment were the measured peak heights. Fig. 1 shows the peaks used.

The first treatment applied to the data was CA. This was intended to provide a visual indication of the natural evolution of the samples by determining the degree of similarity found among them. In Fig. 2, the result from the CA is shown. The resulting tree diagram shows a specific evolution over the course of the development of the phenomenon.

The observed evolution shows a very similar form for the samples with both preservation methods, types I and II, except for month 12, where there is a more marked variation for the type I samples. Therefore, samples from the same month were treated as from the same group in later analysis. There is a high degree of similarity between results for the early months; until the third month no clear differentiation is observed. The evolution proceeds in a constant way until month 12.

The same kind of evolution can be seen in Fig. 3, which presents the results of PCA applied to the same samples. Based on this, it can be concluded that the evolution in this case is centred on principal component 1 (PC1), which accounts for 46.77% of the total variance (PC2, 17.73%; PC3, 13.10%; PC4, 9.32%). It is this component which may be considered as effectively describing the browning phenomenon, since neither of the other two components represented shows any evolution related to this phenomenon under study.

The variables mainly contributing to PC1 are 2, 3, 8, 10, 15 and 21, all with loadings in excess of 0.7, together with variables 12 and 17, which show loadings of 0.60 and 0.70, respectively (Table 1).

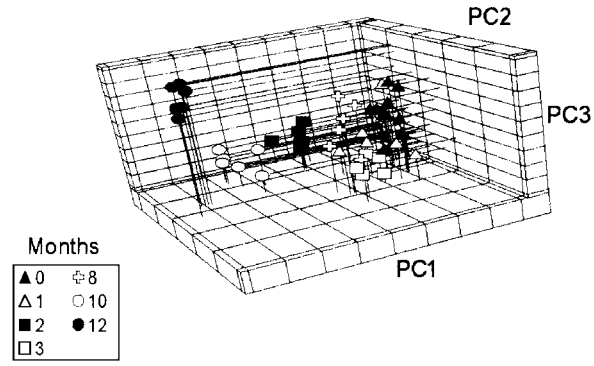

Fig. 3 Distribution in factorial space (PC1, PC2, PC3) for the natural evolved samples.

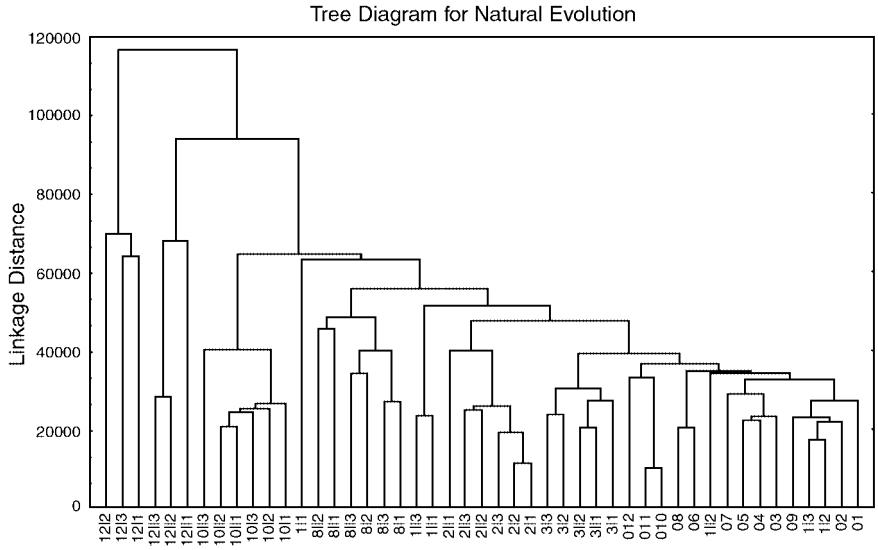

Fig. 2 Tree diagram derived from the cluster analysis results for natural evolved samples.

Hence these two analyses effectively characterised the evolution of the phenolic compounds during the course of the browning process.

Natural evolution and accelerated browning

In order to check the degree of similarity between the natural and accelerated browning processes, analyses were performed of the combined data corresponding both to the natural evolution previously described and to the method of accelerated browning applied to the samples taken from the successive stages of the natural process (accelerated browned samples: AB samples), specifically from months 0, 1, 2 and 3.

Fig. 4 shows the tree diagram corresponding to the result from the CA of the combined data. As can be observed, the samples subjected to accelerated browning show a basic similarity to the naturally evolved samples between months 8 and 10. It should also be emphasised that the results for these accelerated samples are integrated within the series of results for naturally evolved samples and do not appear as an independent separate group of results. This integration is significant, since it means that there is no detectable difference between the accelerated-browned samples and the naturally evolved samples exhibiting greater degrees of browning, based on phenolic compounds.

First, we can say that accelerated browning does not produce different kinds of changes to those produced during natural browning. If it were the case that accelerated browning caused the wine to evolve completely differently from the natural evolution, there would appear in the tree diagram a completely differentiated grouping of the accelerated samples in comparison with the natural samples.

Second, it is evident that the accelerated browning process really is an acceleration, resulting in samples that are essentially similar to those having evolved naturally over a much longer period of time and so showing a greater degree of browning. This is precisely the objective for developing the accelerated browning method. Furthermore, it is important to note that the results from the accelerated samples form a relatively compact group. Hence, independently of their original degree of browning, the final result is homogeneous. Therefore, the method appears to continue the natural browning process.

A similar result can be observed from the PCA of the combined data (Fig. 5), where the results corresponding to the accelerated samples are again seen to be integrated with those for the naturally browned samples, in this case for the months between 8 and 10–12.

In the graphical representation in Fig. 5, it can again be seen that the evolution of the natural phenomenon is best reflected in PC1, which here accounts for 36.64% of the total variance. In addition, the variables contributing most to PC1 of the PCA are basically the same as those in the case of the PCA for the naturally evolved samples alone. Specifically, in this analysis the variables 2, 3, 8, 10, 17 and 21 have loadings in excess of 0.7, whereas variable 12 has a loading of 0.63. In other words, this PC can be interpreted in the same way as before, that it, is the PC effectively representing the natural evolution of the samples (Table 2).

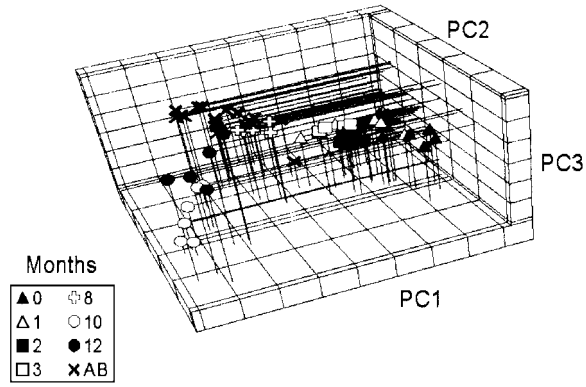

Fig. 5 Distribution in the factorial space (PC1, PC2, PC3) of all the samples, both natural evolved and accelerated browned.

Table 1 Loadings of the main variables in PC1 for natural evolved samples

Variable	2	3	8	10	12	15	17	21
Loading	−0.944	0.865	0.727	−0.816	0.603	0.704	0.697	0.854

Table 2 Loadings of the main variables in PC1 for both natural evolved and accelerated browned samples

Variable	2	3	8	10	12	17	21
Loading	−0.845	0.830	0.741	−0.814	0.627	0.751	0.767

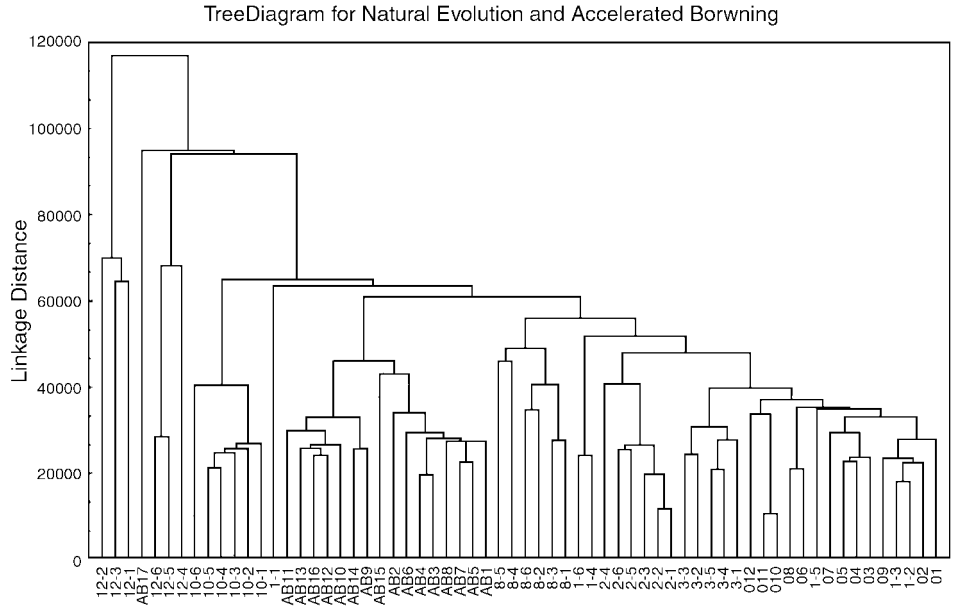

Fig. 4 Tree diagram derived from the cluster analysis results for both natural evolved and accelerated browned samples.

Hence the fact that, according to this factor, the accelerated samples are distributed among the months 8–12 means that the changes to which they have been subjected follow the same lines as the changes produced during the natural browning process.

Moreover, it should be emphasised that PC1, the factor concentrating the variability due to the evolution during the natural browning, remains the primary principal component, much more important than the other principal components (variance explained: PC1, 36.64%; PC2, 15.69%; PC3, 14.90%; PC4, 11.61%; PC5, 6.42%). This finding means that the changes associated with the evolution of the natural browning process remain the major cause of the differentiation between the samples. If the effects of accelerated browning were different from those of the natural process, these results would not have been obtained.

Conclusions

Based on the results obtained, it can be concluded that the effects of accelerated browning on samples of wine have the same characteristics as natural browning, with the final results of both processes being closely similar. In fact, the application of accelerated browning appears to complement the natural phenomenon, since after the accelerated process, samples that began with different initial degrees of browning all finish up in the same final condition. Looked at another way, it appears that a more prolonged and/or intense application of the acceleration process is necessary to reach a more intense final browning.

The method of accelerated browning may be used to evaluate the intrinsic susceptibility of wine to natural browning and the efficiency of enological techniques for the prevention of browning.

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