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Discrimination of aging wines with alternative oak products and micro-oxygenation by FTIR-ATR

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Summary

The use of alternative oak wood products (AOP), such as chips, cubes and staves, among other, from different geographical origins is a common practice for wine aging, where the micro-oxygenation (MOX, adding small doses of oxygen constantly over time) is essential to obtain a final wine more stable in time and with similar characteristics of barrel-aged wine. The aim of this work was to identify if spectroscopic techniques allow to discriminate wines aged with alternative oak products (chips and staves) from different oak woods (American, French and Spanish) and a floating micro-oxygenation (20 µg·L^{-1}) after 10 years of bottling and compared to those aged in barrels. The spectral information and analysis were performed in a FTIR-ATR, with 128 scans per spectrum at a spectral resolution of 8 cm^{-1} in the wavenumber range from 4,000 to 450 cm^{-1}. Principal component analyses of spectral information were performed using the Unscrambler® X. The results indicate that with this technique it is possible to clearly separate the wines aged by the three systems (chips, staves and barrels) in the case of American oak. In the case of French oak, wines aged in chips were clearly differentiated from wines aged in staves with those aged in barrels between the two. It is also possible to clearly separate aged wines with different Spanish oak systems. The application of FTIR-ATR appears to be a powerful technique for discriminating the quality of wines aged by different AOPs and wood barrels from different geographical origins.

K e y w o r d s : red wine; FTIR-ATR; aging system.

Introduction

Wine aging through the use of barrels is par excellence the technique most commonly used in wineries. However, the high costs linked to this technique, along with the long periods of time required have made other alternatives to wine aging, as the well-known alternative oak products (AOP's) kept in stainless steel tanks, to be taken into account to achieve the same properties, but with shortened times and reduced costs (Oberholster *et al.* 2015) and simplified traditional maturation of wines in oak barrels (Arapitsas *et al.* 2004). AOP's can be found in a variety of forms (chips, cubes, staves, etc) and their effects on wine depend on several factors: origin of the wood, seasoning, toasting process, amount of fragments added to the wine and time in contact with wine, among others (Del Álamo *et al.* 2010, Fernández de Simón *et al.* 2010a and b, Gallego *et al.* 2011, 2012).

When AOP are used, it is essential to take into account the oxygenation effect which occurs in barrels due to the diffusion of oxygen trough the wooden walls of the barrel to reproduce the behaviour, and hence, increasing the quality of the wine. This is why it is important to perform this process while adding oxygen (micro-oxygenation).The use of AOP's was approved by EU regulations (CE) No. 2165/2005 and (CE) No 1507/2006, which define the terms of use of oak fragments in wine. According to the oenological CODEX published by the International Organization of Vine and Wine, the wine aging from barrel is known as "Ageing in small capacity wooden containers (OENO 8/01)" and as "usage of pieces of oak wood in winemaking (OENO 9/01)" when AOP's are used.

Most of the studies related to the evolution of bottled wine do not usually cover times longer than two years (Fernández de Simón *et al.* 2006, Gallego *et al.* 2012, Oberholster *et al.* 2015, Avizcuri *et al.* 2016) or four years (Cassino *et al.* 2019). Those study time of more than 10 years are very scarce (Del Alamo Sanza *et al.* 2019). For this reason, it is interesting to go deeper into longer bottle aging times. Although the studies aimed at differentiation and classification of wines are relatively abundant, those to establish criteria to discriminate between wines aged in barrels and with AOP's are limited. Some of them have been based on the differences on volatile compounds (Hernández-Orte *et al.* 2014, Del Alamo Sanza *et al.* 2019), phenolic and anthocyanins (Del Álamo *et al.* 2008), basic (Astray *et al.* 2019) sensory (Espitia-López *et al*. 2015) or by means of an electronic panel test (Gay *et al.* 2010, Apetrei *et al.* 2012).

In the last decades, additional interest is being devoted to the vibrational spectroscopy methodologies (FTIR, NIR and RAMAN), successfully applied to wine, because differences in the composition of wines are reflected in

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the infrared spectra. Also, these techniques have desirable features, such as speed, automation, ease-of-use, cost-effectiveness, minimal or no sample preparation, non-sample destruction and absence of residues by not employing reagents nor solvents, what it makes environmental-friendly (Magdas *et al.* 2018). Thereby, numerous studies have concerned strategies for the characterization of wines based on compositional profiles treated with Fourier transform infrared (FTIR) spectroscopy as source of information: to monitor total phenolic compounds and antioxidant activity during the winemaking process (Versari *et al.* 2010, Preserova *et al.* 2015), to follow the aging process (Basalekou *et al.* 2017, Condurso *et al.* 2018, Fereiro-González *et al.* 2019), to discriminate between grape varieties and between wines aged in different types of containers (Basalekou *et al.* 2017), to quantify organic acids (Regmi *et al.* 2012), to determine the anthocyanins (Soriano *et al.* 2007) or to study the wine polysaccharides (Boulet *et al.* 2007), among others.

Thus, the aim of this work was to discriminate wines by FTIR-ATR after 10 years in the bottle and aged with alternative oak products (chips and staves), from different oak woods (American, French and Spanish) and a floating micro-oxygenation (20 μg·L^{-1}), compared to those aged in barrels. For this propose we work only with a single-variety grape to fixed only the effect of alternative oak products and oak woods species.

Experimental section

Wood and wine samples: Wine from a single red variety grape ('Tinta del País', Spanish appellation of origin 'Ribera del Duero') was aging during 195 d in different aging systems: a) traditional aging with oak barrels from *Q. alba* (American oak) and *Q. petraea* (French oak); and b) alternative aging with products from oak wood (AOP) with i) chips (1 × 0.5 cm^2, approximately) from *Q. alba* (American oak), *Q. petraea* (French oak) and *Q. pyrenaica* (Spanish); and ii) staves (100 × 8 × 1 cm^3) from *Q. alba* (American oak), *Q. petraea* (French oak) and *Q. pyrenaica* (Spanish), according to Del Álamo *et al.* 2010. Briefly, barrels were manufactured by INTONA (Navarra Spain) who also supplied the wood for the alternative oak products. The seasoning of the wood was carried out in the open air for 3 years in the usual way. After seasoning, the wood was cut in the two sizes previously indicated and toasted at medium intensity level (200 °C for 35 min), in an industrial-scale convection oven, with supports specially adapted to staves or special oven trays for chips. The AOP's dosage was calculated by the relation surface/volume of 225-L barrels determining the quantity of chips and staves necessary to reproduce the same relation in 210-L stainless steel tanks (Del Alamo Sanza *et al.* 2004). For the wine barrel aging, four barrels were manufactured from American and French oak wood. For wine aging with AOP's, two stainless steel 210-L tanks were used with a floating micro-oxygenation strategy and with a DO setpoint of 20 μg·L^{-1} (Del Álamo *et al.* 2010) using Eco2 device (Oenodev, France) and ceramic diffusers which included a different oxygen dosage according to wine demand to reach 20 μg·L^{-1} of dissolved oxygen.

During wood and bottle contact, all wines were aged in the same wine cellar where humidity (65-75 %) and temperature (15-16 °C) conditions were controlled. After wood contact, wines from each tank were bottled with natural cork closures (Salamantina de Corcho S.L., Machacón, Spain), and stored during ten years in the same wine cellar. For this work, four bottles of each treatment, being a total of thirty-two bottles, were opened and analysed in quadruplicate.

Spectral analysis by Fourier Transform Infrared Spectroscopic method with Attenuated Total Reflectance (FT-IR-ATR): Spectra were acquired with a Bruker spectrometer (Alpha) using a diamond single reflection attenuated total reflectance (FTIR-ATR). 4 spectra per sample were obtained with 128 scans per spectrum at a spectral resolution of 8 cm^{-1} in the range of 4,000 to 450 cm^{-1}.

The FTIR-ATR used is equipped with a flow-through cell with controlled temperature. The cleaning of the cell is done by the injection of water in the flow-through cell and the background is also measured with distilled water.

Chemometric analysis: To perform the Principal component analyses with spectral data the following pre-treatements were tested: standard normal variate (SNV), multiplicative scatter correction (MSC), normalize, centred and scaled, first derived Savitzky-Golay (1st derived), second derived Savitzky-Golay (2nd derived), baseline and several combinations of 2 pre-treatments, to identify the better one to use in the further analysis.

Principal component analyses (PCA) of spectral information were performed using the Unscrambler® X, version: 10.5.46461.632 (CAMO Software AS, Oslo, Norway) and OPUS®, version: 7.5.18 (Bruker Optik, Germany).

Results and Discussion

The average absorbance spectra of one of the red wine samples studied is plotted in Fig. 1, where the spectral information is in accordance with previous reports by other authors (Moreira *et al.* 2004). The IR region from 2990 to 3626 cm^{-1} has a very strong influence due to water present in the samples (Patz *et al.* 2004). However, in this case it has not been detected in the resulted spectra because the background was performed with water. The region from 1700 to 860 cm^{-1} corresponds to C-C and C-O vibrations in volatile compounds of the beverages and those from 1500 to 1716 cm^{-1} correspond to OH-stretching and to OH-bending (Shurvell 2001) is also influenced by the water, but also by other minor components. There is a noticeable IR information in the regions from 3000 to 2900 cm^{-1} and from 1500 to 800 cm^{-1}. The peaks between 2900-3000 cm^{-1} are due to the O-H stretching of the acid components and those at 1275 and 1326 cm^{-1} are related to C-O stretching in the acid molecules, corresponding the peak at 1275 cm^{-1} also to in-plane bending of O-H (Tarantilis *et al.* 2008). The peaks that appear between 1200 cm^{-1} and 1500 cm^{-1}

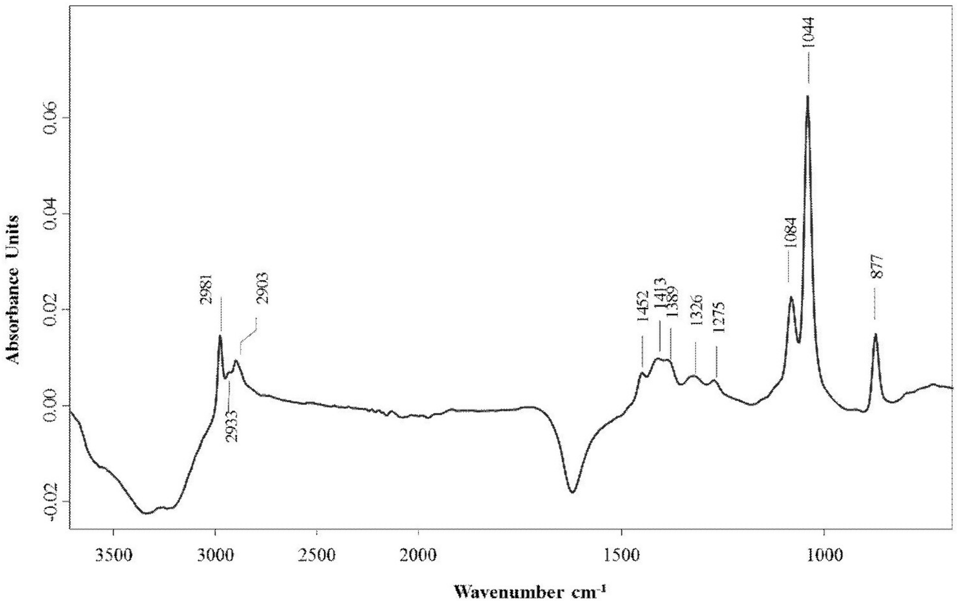

Fig. 1: FTIR-ATR average absorbance spectra of red wine samples.

are due to -CH groups in the organic acids (Bevin *et al.* 2006). The small peak at 1452 cm^{-1} was assigned to C-OH bending deformation. The much-closed peaks at 1084 and 1044 cm^{-1} correspond to the C-O stretch absorption bands and are important regions for ethanol and methanol quantification, respectively (Silverstein *et al.* 1991, Shurvell 2001, Anjos *et al.* 2016). These regions contain also important absorption bands for glucose and fructose concerning the C-C stretch, C-OH and C-O groups (Anjos *et al.* 2015). The sugar compounds in wine appeared in concentrations much lower than the other organic acids and also lower than ethanol and this overlap of spectral information could impede the separation between samples. The linking C = O, C-O and O-H from the carboxylic acid produce absorption bands similar to those of the same bonds in the organic acids present in wines and in the same region. The sulphur dioxide present in wines is dissociated to sulphate ions that show absorption bands of S = O and S-O that according to Silverstein *et al.* (1991) absorb at 1060-1150 cm^{-1}, and in this case could also contribute to the peak observed at 1084 cm^{-1}. Regmi *et al.* 2012 concluded that FTIR spectroscopy could discriminate between organic acids in wine and wine-derived products.

The statistical analysis for discrimination purposes in this study was performed in the following regions: 3000 to 2850 cm^{-1}; 1400 to 1200 cm^{-1}; 1000 to 950 cm^{-1} and the region that contain the peak at 877 cm^{-1}. The pre-treatment that give better results and consequently, used in all subsequent chemometric analyses, was the standard normal variate (SNV) plus second derived Savitzky-Golay with 15 smoothing points. The spectra were accepted to analysis when the difference between spectrum and mean sample spectrum is lower than 0.002 between four spectra. The validation method used was the cross validation method. The criteria used to classify the samples were a ratio of calibrated to validated residual variance of 0.5; ratio of validated to calibrated residual variance of 0.75 and residual variance increase limit of 6 %.

The regions previously mentioned were analysed and the results were plotted in the PCA's summarized in Figs 2, 3 and 4. One region (or more) yielding powerful results was selected in order to test the viability of FTIR technique to differentiate wines bottled after 10 years. The wines were previously aged with alternative oak products (chips and staves), from different woods of America (*Q. alba*), France (*Q. petraea*) and Spain (*Q. pyrenaica*) together with a floating microoxygenation (FMOX 20 $\mu g \cdot L^{-1}$), and simultaneously were also aged in American and French oak barrels.

Different comparisons have been performed to differentiate wines: a) among barrel or alternative oak products (AOP's), chips and staves, from American oak and French oak (Fig. 2); b) among oak chips from different botanical origin (American, French and Spanish) (Fig. 3a); c) among oak staves from different botanical origin (American, French and Spanish) (Fig. 3b); and d) among barrels from American or French origin (Fig. 4). In general, PCA's have demonstrated that in most of the classifications tested, it has been possible to differentiate wines after 10 years in bottle, previously aged with AOP's from different botanical origin and micro-oxygenated, compared to those aged in barrels.

Fig. 2 shows the score plot of two principal components for red wines aged with three different systems (chips + FMOX, staves + FMOX and barrel) for American and French oak. Fig. 2a) establishes the discrimination according to wines aged in barrels or AOP's (chips + FMOX or staves + FMOX) from American oak when the region 1400 to 1200 cm^{-1} was considered. In this analysis, the first two principal components accounted for 99 % of the total variance. The wine aged with American oak wood chips + FMOX is situated on the right part of the graph, well differentiated from the other two samples (aged with staves + FMOX and barrels) where the first component explained 98 % of the total variance. In case of wines aged in barrels and those from staves+FMOX, the second component

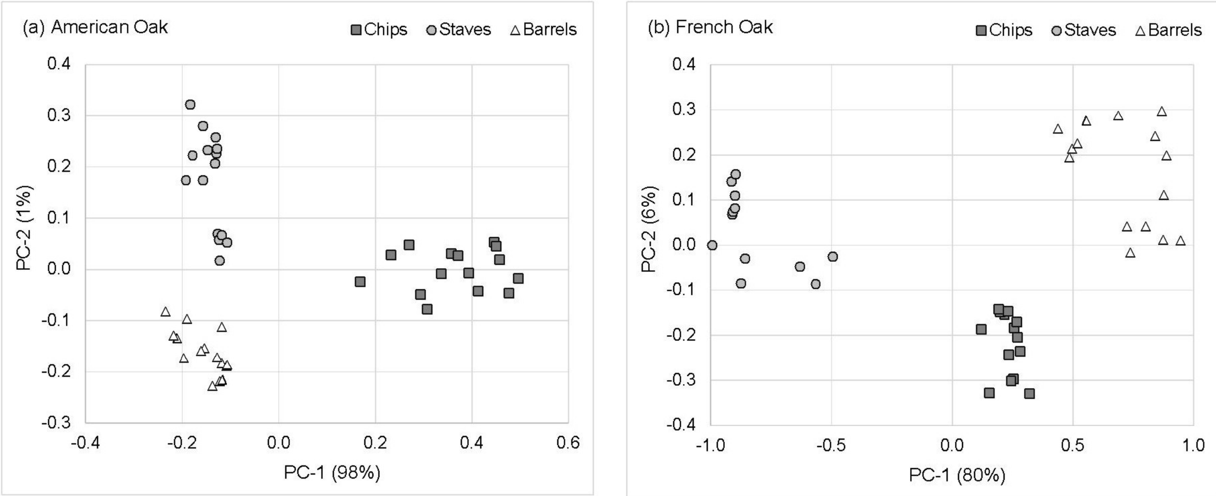

Fig. 2: Score plot of two principal components from raw spectral data acquired with FTIR-ATR data of red wines based on: (**a**) different systems for American oak from 1400 to 1200 cm^{-1} and (**b**) different systems for French oak from 3000 to 2850 cm^{-1}; 1400 to 870 cm^{-1}.

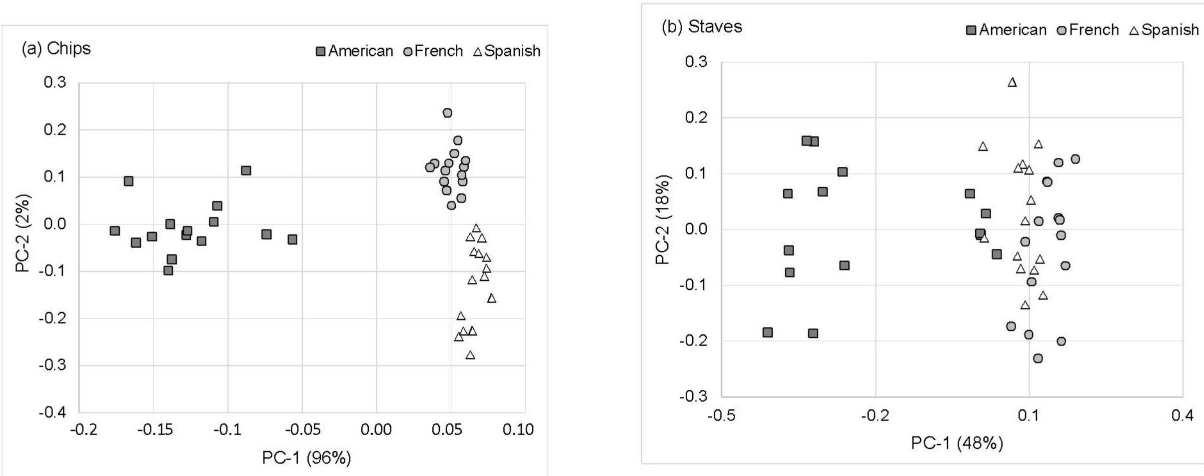

Fig. 3: Score plot of two principal components from raw spectral data acquired with FTIR-ATR data of red wines based on: (**a**) Chips+FMOX of different oak wood from 3000 to 2850 cm^{-1} and 1400 to 1200 cm^{-1} and (**b**) staves + FMOX of different oak wood from 1400 to 1200 cm^{-1}.

only explained 1 %. This result is in accordance with other experiments which observed that wines aged with staves were those that showed properties closest to those aged in barrels (Gutiérrez Afonso 2002, Del Álamo *et al.* 2008). When the same comparison was studied for French oak (Fig. 2b) it was shown that the first two principal components explained the 86 % of the total variance. In this case, the first component, which accounted for 80 % of the total variance, clearly discriminated the three wine aging systems. The samples from ageing with chips + FMOX are placed in an intermediate position between those aged with staves + FMOX and those aged in barrels. In this case, the regions used for the separation were 3000 to 2850 cm^{-1} and 1400 to 870 cm^{-1}. The last region encompasses the peaks of 1084 and 1044 cm^{-1}, which are indicative of an alcohol functional group (Agatonovic-Kustrin 2013). The basic parameters of these wines indicated that the alcohol degree of wines aged with French oak (chips + FMOX, staves + FMOX and barrels), showed significant differences (Del Alamo-Sanza *et al.* 2019), so the differentiation could be due in part to these differences, since ethanol is the alcohol more abundant in wines. When wines were studied by means of the oak botanical origin (*Q. alba, Q. petraea* and *Q. pyrenaica*) it was also possible to observe a differentiation both separately for chips + FMOX (Fig. 3a), staves + FMOX (Fig. 3b) and barrels (Fig. 4). Concerning the wines aged with chips + FMOX and when the regions 3000 to 2850 cm^{-1} and 1400 to 1200 cm^{-1} were considered (Fig. 3a), the three groups can be distinguished, which were separated by the two first components, explaining 96 % of the total variance by the first component and 98 % together with the second one. The first component clearly discriminated between the American oak wines and the other two, standing to the left of PC1 and showing a heterogeneous group of samples with PC1 values from -0.05 to -0.2. When wines aged with staves + FMOX were studied (Fig. 3b), the PCA resulting from the 1400 to 1200 cm^{-1} region was able to explain 66 % of the total variance, in fact, it did not allow a clear separation between the three wines, only those from American staves + FMOX seems to have a trend to

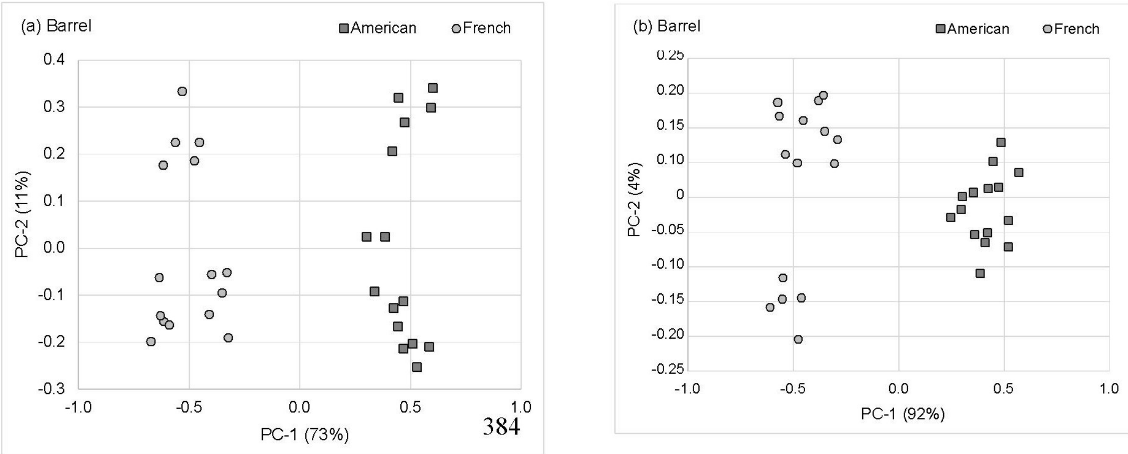

Fig. 4: Score plot of two principal components from raw spectral data acquired with FTIR-ATR data of red wines based on barrels of different oak wood: (**a**) from 3000 to 2850 cm^{-1}; 1400 to 1200 cm^{-1} and (**b**) from 1000 to 950 cm^{-1}.

have a good separation. It is interesting to note that wines aged with Spanish oak chips + FMOX or staves + FMOX showed characteristics similar to those aged with French oak, especially in the case of the use of staves.

Fig. 4 showed the differentiation of wines aged with American and French barrels. When the regions 3000 to 2850 cm^{-1} and 1400 to 1200 cm^{-1} were considered (Fig. 4a), the first component was able to explain 73 % of the total variance and allows the differentiation of wines from both types of barrels. However, when the region 1000 to 950 cm^{-1} was studied (Fig. 4b), the percentage of the total variance accounted by the first component was 92 %, representing a significant improvement in the differentiation with respect to the previous analysed region (Fig. 4a). This analysis also allows the differentiation of wines from both types of barrels, showing a greater homogeneity of wines aged in American oak barrels.

Conclusions

The results have demonstrated the possibility of using FTIR-ATR in conjunction with chemometric analysis to discriminate red wine 'Tinta del País' (Spanish appellation of origin 'Ribera del Duero') bottled after 10 years, which were aged with alternative oak products (chips and staves), from oak of different botanical origin (*Q. alba*, *Q. petraea* and *Q. pyrenaica*) and a floating micro-oxygenation, compared to those aged in American and French oak barrels.

The results presented in this work are promising and is a first approximation in the study for this wine, but more research is needed to understand whether another grape variety (red and white) would have the same results.

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