

Colour change of bottled white wines as a quality indicator

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ABSTRACT

Aim: The present work aimed to estimate the probability of bottled white wine exhibiting acceptable colour change during a certain predefined time using a predictive model based on a descriptive mathematically expressed theory.

Materials and methods: Nine mono-varietal Greek white wines (produced by three varieties) were bottled and stored at two different temperatures (20 and 30 °C). Their absorbance at 420 nm (A420) was recorded every three months for 12 months. Results were processed by applying the root cause analysis versus storage conditions. Furthermore, a predictive mathematical model was adapted to the experimental data based on the probability of the product showing browning-index values higher than a specific threshold during its shelf life.

Results: As expected, the absorbance at 420 nm increased with time, while the elevated storage temperature had a significant effect on colour alteration. The browning rate was dependent on grape variety. When storage temperature was 20 °C, a lower A420 increase was observed for Sauvignon blanc wine, while at 30 °C, browning was higher. Assyrtiko wines were more resistant to colour change at both temperatures. The probability of a bottled product maintaining an acceptable colour change by the end of one year, was used as a measure of its quality. A very successful fit was achieved between mathematical and experimentally obtained data for shelf life predictions.

Significance and impact of the study: The modelling of the experimental results, as well as the exploitation of this fitting to make predictions independently of the specific results, indicate that storage temperature will potentially impact the colour of wines over time, depending on the evolution of the absorbance values at 420 nm. For the wine sector, the selection of the appropriate storage conditions for a given grape variety is an important factor to take into consideration.

KEY WORDS

packaging, shelf life, browning, Assyrtiko, Malagouzia, Sauvignon blanc

INTRODUCTION

Wine is a complex matrix whose composition continuously changes during storage depending on many factors, such as exposure to oxygen and light, and storage temperature and duration (Pati *et al.*, 2019). While bottle storage is important for the improvement of red wine quality, for white wine it can result in colour (browning) and aroma alteration, which can contribute to an overall deterioration in quality and marketability (Kallithraka *et al.*, 2009). However, some white wines can derive short-term benefits from the development of a characteristic bottle bouquet.

In white wines, browning can be perceived as being either positive (in the case of sherries and sweet fortified wines, such as white Ports or Rivesaltes), or negative (for sparkling and dry young white wines). The brown colour increases both the colour intensity and browning index while decreasing brightness (Gonzales *et al.*, 1994).

Colour starts to change in the early stages of winemaking via enzymatic reactions (Ribéreau-Gayon *et al.*, 2006) caused by the active polyphenol oxidase, in which hydroxycinnamic esters play an important role. After fermentation, non-enzymatic oxidation usually occurs, which give rise to a brown colour progressively replacing the initial colour (generally pale-yellow) of white wines (Li *et al.*, 2008; Oliviera *et al.*, 2011; Pati *et al.*, 2006; Pati *et al.*, 2014). The most important constituents in white wines which are able to participate in oxidation reactions are hydroxycinnamates and flavanols. In particular, the oxidation of ortho-dihydroxyphenolic compounds (such as (+)-catechin and (-)-epicatechin) and hydroxycinnamic acids results in the formation of yellow-brown compounds due to the polymerisation of ortho-quinones (Cheynier *et al.*, 1989; Guyot *et al.*, 1996). Indeed, studies on wine model solutions have confirmed the formation of two types of yellow pigments, xanthylum salt pigments and ethyl-ester of xanthylum salts, showing visible absorption maxima at 440 and 460 nm respectively, both of which are derived from flavanol oxidation and polymerization (Es-Safi *et al.*, 2000). With an absorption maximum in the region of 400–500 nm, these pigments directly contribute to white wine browning during aging. This reaction, and thus the extent of browning, is accelerated in model wine solutions by adding transition metals, such as iron and copper, which probably act as catalysts to form intermediate oxidation products (Karbowiak *et al.*, 2010). Other constituents of the wine, such as SO₂

and ascorbic acid, are also of high importance in polyphenol oxidation; when added to wine, they are able to reduce the ortho-quinones (Singleton, 1987). Moreover, the presence of polysaccharides (released by inactivated dry yeasts) can have a positive effect on the colour of the wines. This colour protection could be due to the interaction of polysaccharides and certain phenolic compounds which are easily-oxidizable (hydroxycinnamic tartaric esters), as well as to reduced oxidation through higher oxygen consumption (Del Barrio Galan *et al.*, 2018).

Other parameters, both intrinsic and environmental, are key factors in determining the extent of browning oxidation in white wine. In addition to the effect of grape variety and the degree of maturity at time of harvest, temperature, oxygen content and pH increase the browning rate (as measured by the change in optical density at a wavelength of 420 nm) (Karbowiak *et al.*, 2010). An excess of ultraviolet and visible radiation also produces significant oxidative changes in the wine polyphenolic content during storage (Benítez *et al.*, 2002). For white wines, colour change is more dependent on light exposure than oxygen concentration at 20 °C, whereas at 45 °C their respective effects become equal (Cartagena *et al.*, 1994). High pH and high temperature are also found to increase the browning of wine. An increase in pH increases the concentration of phenolate ions relative to the phenol form, thus increasing oxidation rates by about nine times when pH is between 3 and 4 (Singleton 1987). However, it should be noted that the different factors (temperature, oxygen, pH, light) involved in the oxidation of white wines during storage have an effect on wine oxidation rate as a whole, and the isolated effects of each parameter are very difficult to study (Karbowiak *et al.*, 2010).

Wine oxidation, and consequently browning, are mainly driven by the ingress of oxygen through the wine stopper, which acts as a barrier to oxidation (Roullier-Gall *et al.*, 2016; Grouvisier-Urien *et al.*, 2018). This is the reason why several studies have focused on the gas barrier properties of the different types of stoppers comparing natural, agglomerated, synthetic closures and screw caps (Waters *et al.*, 1996; Lopes *et al.*, 2007; Oliveira *et al.*, 2013; Grouvisier-Urien *et al.*, 2018). It has been demonstrated that synthetic closures accelerate oxidation in wines in comparison with cork stoppers and screw caps (Mayr *et al.*, 2015; He *et al.*, 2013). Indeed, based on to the value of oxygen transfer rate (OTR), it appears that

TABLE 1. Oenological parameters of the wines right after bottling.

Samples*	Ethanol % (v/V)	Total acidity (g/L tartaric acid)	pH	Volatile acidity (g/L)	SO ₂ free (mg/L)	SO ₂ Total (mg/L)
A1	13.5±0.2	6.98±0.1	2.9	0.22±0.03	30.76±0.12	88.32±4.43
A2	14.3±0.1	6.89±0.2	3.0	0.39±0.05	28.16±3.62	83.20±1.81
A3	12.9±0.1	7.01±0.3	2.9	0.28±0.05	27.32±3.43	89.20±3.45
M1	13.5±0.2	5.50±0.2	3.3	0.24±0.06	28.28±3.44	98.20±4.12
M2	13.3±0.2	5.30±0.3	3.4	0.30±0.05	25.32±2.66	94.72±4.12
M3	14.0±0.2	5.65±0.2	3.3	0.26±0.04	24.85±3.22	89.55±2.95
SB1	13.6±0.1	6.25±0.2	3.2	0.29±0.04	28.10±3.60	96.56±4.32
SB2	14.0±0.2	6.40±0.1	3.1	0.31±0.06	25.60±2.80	97.84±4.56
SB3	13.9±0.3	6.20±0.1	3.2	0.28±0.04	26.88±3.40	97.60±4.50

*: A, M and SB are Assyrtiko, Malagouzia and Sauvignon blanc wines respectively.

synthetic stoppers exhibit the lowest barrier to gas transfer (Grouvisier-Urien *et al.*, 2018). Screw caps are the only closures that have a significant impact on the prevention of oxygen entrance; however, some authors have observed the development of undesirable reduced organoleptic characteristics due to insufficient oxygen ingress (He *et al.*, 2013; Lopes *et al.*, 2009). The two main factors that influence gas transfer rate are the thickness of the closure and the pressure gradient applied on each side of it. However, complications arise when making comparisons between types of closures as experimental conditions are usually not identical (closure size, time of contact, stopper compressed or not, bottleneck interface, etc.) (Grouvisier-Urien *et al.*, 2018).

Wine is a product that has the specificity of being sold without any specific indication of shelf life. This is especially true for white wines, which can be stored for a very long time while the optimum for their sensory quality is debatable. Since no strong consensus exists on the extent to which colour change during storage is 'acceptable' (to the consumers), it is rather complex to predict the shelf life of a white wine by assessing the ingress of oxygen into the bottles. Here, we describe a predictive model for the estimation of product quality, which is based on the probability of bottled white wine maintaining an acceptable colour in terms of its visual appearance. For this purpose, several parameters affecting the extent of colour change, such as wine variety and storage temperature, were considered.

MATERIALS AND METHODS

1. Wine samples and oenological parameters

Three different white grape varieties grown in Greece (Assyrtiko, Malagouzia and Sauvignon blanc) were used for the production of nine mono-varietal wines. In more detail, three wines from each one of the three indigenous varieties were produced at different wineries, from which different samples were taken and used in order to broaden the picture of the oxidative colour alterations that take place in wine. All samples were bottled in 750-ml glass bottles. The bottles were sealed using agglomerated type corks of certain oxygen permeability properties (i.e., DIAM P035=0.0015 cm³ O₂/day) (www.diam-closures.com) and stored in dark conditions at 20 °C and 30 °C. After 0, 3, 7 and 12 months of storage, three bottles of each varietal wine were removed and each one was analysed in three replicates. Several classical analytical parameters (free and total SO₂ contents, % vol, pH, titratable and volatile acidity) were determined after bottling according to the OIV methods (2009) (Table 1).

2. Colour intensity

The absorbance at 420 nm (A420) of the wine samples was measured using a 10-mm-path-length cuvette against the blank (distilled water) using a UV/VIS spectrophotometer (Jasco Corp., Tokyo, Japan) according to the official method of the International Organization of Vine and Wine (OIV) (OIV methods 2015).

3. Model description

It is fundamental to quantify the browning of bottled wine as a way of indicating product quality. Because quality deterioration can be depicted by the increment of browning, we have selected absorption at 420 nm as an index for quantifying the wine quality. Given the sparse experimental measurements, we chose to apply curve fitting by using third-order polynomials supported by cubic splines. As adsorption values evolve over time, it is necessary to define a quality threshold; i.e., an adsorption value that corresponds to the upper accepted value (limit) of browning. When browning is higher than this threshold, the quality is lower than the limit and the product has reached the end of its shelf life. Such thresholds can be defined through relevant experience and additional adjective factors.

The proposed model, in accordance with a concept previously described by Coutelieris and Kanavouras (2006) and Kanavouras and Coutelieris (2006), is able to estimate the critical time when the profile intersects the threshold line; it can thus predict the time when the product's quality will fall below the specified acceptable threshold. Thus, the probability of the product reaching the end of its shelf life is represented by the area between the curve formed by the actual values and the threshold line divided by the whole area under this curve. In this context, we are able to mathematically define the probability (P_{safe}) as:

$$P_{safe} = 1 - \frac{\int_{t_{cr}}^{12} I(t)dt}{\int_0^{12} I(t)dt} \quad (1)$$

where the integral on the nominator calculates the surface over the threshold line and under the curve, while the integral on the denominator calculates the whole area below the adsorption curve. In the above formula, $I(t)$ denotes the fitting function (polynomial) on the experimental measurements of absorbance at 420 nm and we have arbitrarily defined the maximum shelf life of a white wine to 12 months. Furthermore, t_{cr} denotes the critical time when the quality index attains the threshold value. This value is calculated numerically by solving the equation

$$I(t) = \text{threshold} \quad (2)$$

The numerical solution of the above equation has been obtained by applying a rather simple Newton-Raphson approach. Finally, both the integrals of the above formula (1) have been calculated by

using Newton–Cotes closed form. Note also that the whole concept is depicted in Figure 2 (see below).

RESULTS AND DISCUSSION

1. Effect of temperature

A rather distinct wine making protocol was followed by each winery for the wine production. Accordingly, characteristic differences in the colour evolution of the wines may have arisen as a result of having added different SO_2 compounds or extra antioxidant compounds, such as ascorbic acid (Elias *et al.*, 2009, Kilmartin *et al.*, 2001). Moreover, the chemical composition of the wine, as well as its phenolic content and the presence of metal ions (such as ethanol, tartaric acid, glycerol, remaining sugars and organic acid contents) may also have played an important role in both the rate and the extent of browning. Hydroxyl radicals, which are produced during wine oxidation, are non-selective and can react and oxidize almost any organic molecule found in wine (Waterhouse and Laurie, 2006; Oliveira *et al.*, 2011). Hence, for the same amount of oxygen present in the wine mass, the colour evolution of each wine can differ. The colour measurements obtained for the three different wines of each grape variety (produced in different wineries) were averaged in order to obtain more representative results and eliminate the effect of the different wine making protocols.

Figure 1 shows the evolution of averaged experimental data (A420) from Assyrtiko, Malagouzia and Sauvignon blanc wines according to the storage temperature. An acceptable fitting between the actual measured values and the mathematically obtained ones can be observed, confirming the validity of the model. For all the varieties and the temperatures considered, the values of R^2 were always lower than 0.93. As expected, the higher temperature (30 °C) resulted in higher browning rates in agreement with other published data (Kallithraka *et al.*, 2009; Recamades *et al.*, 2006). In order to be able to use a polynomial of 3rd degree in all cases for fitting experimental data, we accepted a lower agreement in some cases, such as for sauvignon at 30 °C. When the applied regression is linear, the indicator of the agreement between experimental results and simulations should be R-squared, rather than standard deviation or other descriptive statistics.

As soon as a wine is bottled, it has practically no more access to additional oxygen. However, in practice, oxygen continues to permeate

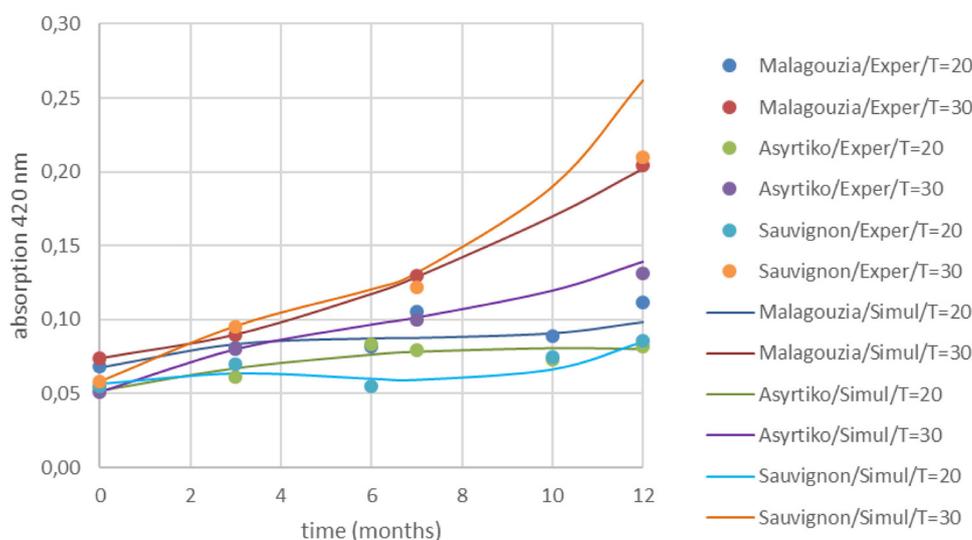


FIGURE 1. Average absorbance values at 420 nm of Assyrτικο, Malagouzia and Sauvignon blanc, wines stored at 20 °C and 30 °C over a period of twelve months.

into the bottle through the corks resulting in slow polymerisation and condensation tannin-anthocyanin reactions. These processes are partly reversible owing to acid hydrolysis reactions (Salas *et al.*, 2003). Experimental evidence indicates that storage conditions might have a strong effect on the content of wine phenols (Recamales *et al.* 2006). Parameters such as temperature and light influence the hydrolysis, oxidation and condensation reactions, which mainly take place during storage (Recamales *et al.*, 2006). The variable browning observed in this study could be attributed to the different storage temperatures. Such an observation most likely indicates a potentially solid dependency of oxidation on the elevated storage temperatures, indicating that wines are better protected from oxidation at lower temperatures. The elevated temperature employed presumably increased the rate of the reaction(s) involved, thus reducing the time during which measurable browning could occur (Fernandez-Zurbano *et al.*, 1995).

Regarding the impact of the grape cultivar on oxidation rates, significant differences were observed between the three varieties studied (Figure 1). The 12 month storage of wines at an elevated temperature (30 °C), resulted in higher A 420 values for Sauvignon blanc wines followed by Malagouzia wines, while the browning of Assyrτικο wines was the lowest. At 20 °C, Sauvignon blanc and Assyrτικο wines were characterised by less colour change in comparison to Malagouzia. Whether the reaction rates among the various reactions occurring in the wines are

similarly affected by temperature needs to be further investigated.

Given the permeability of the cork, as provided by the producer, ($P_{O_2}=0.0015 \text{ cm}^3/\text{day}$), the calculated amount of oxygen entering the bottles over a 12 month period is 0.315 cm^3 per 750ml of wine, or 0.420 cm^3 per liter of wine, (Kanavouras *et al.*, 2019). Accordingly, any alteration in colour may correspond to the respective increase of the oxygen in the wine mass.

Since any cork can provide a significantly limited oxygen permeation surface in comparison to the whole bottle surface, we can safely assume that the cork used in this study resulted in a limited oxidation acceleration with indistinct differences. Therefore, in terms of wine quality, the selection of packaging materials is highly dependent on the selection of the body materials, and apparently on the initially dissolved oxygen and the antioxidant additives and preservatives (Karbowiak *et al.*, 2010).

According to published results, the combination of oxygen dissolved at bottling and the oxygen transferred through closures has a significant effect on the development of browning after bottling. Compared to wines sealed with other types of closures, relatively more oxidation occurs in wines highly exposed to oxygen at bottling and sealed with synthetic closures highly permeable to oxygen, which has a sensorial impact on aroma, colour, low antioxidants and various volatile compounds (Oliveira *et al.*, 2020; Pati *et al.*, 2019).

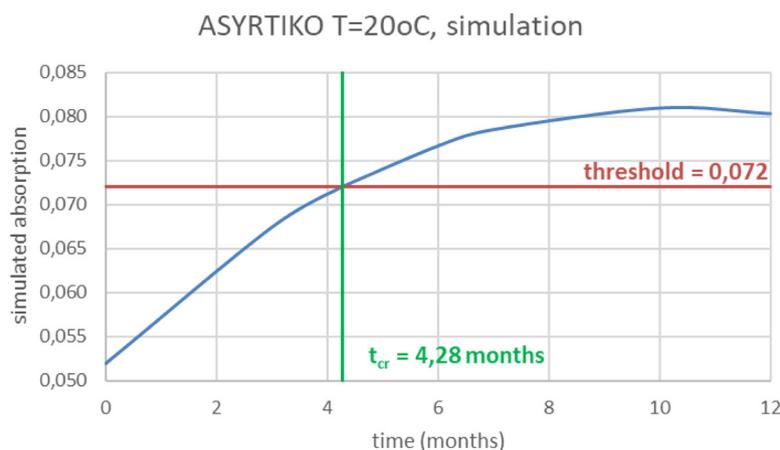


FIGURE 2. Graphical representation of the identification of critical time in equation (2) for Assyrtiko wines stored at 20 °C.

The justified engineering of the packaging properties within certain technological boundaries, could allow certain modifications to be made to the chemical composition of bottled wine. This requires a common interactive approach between the edible product and the packaging, which could synergistically contribute to a high-quality end product. It is also worth mentioning at this point that packaging materials and storage conditions play a significant role in the fine-tuning of product quality in order to ensure maximum potential consumer satisfaction, while strengthening and securing the product in a highly competitive modern environment.

2. Modelling colour change in white wines

The probability of bottled wine not reaching the end of its shelf life, P_{safe} , was introduced to wine research for the first time by our research team (Kanavouras *et al.*, 2019); a similar mathematical approach was employed to identify the characteristic volatile compounds that could be used as potential oxidation markers to establish the quality of the stored wines and to predict their shelf-life. This work has been further developed in the present study to predict the changes in a wine’s visual appearance, since, along with aromatic alterations, browning is considered an important parameter that directly affects white wine quality.

As briefly mentioned above, in order to estimate the time needed for the bottled wine to go over an arbitrarily defined and acceptable visual quality threshold, it is necessary to translate the microscopic-level measured values of A420 into a macroscopic quality index. This could be represented as the probability that the wine (P_{safe}) can maintain this predefined acceptable quality

(as far as its visual appearance is concerned) by the end of a defined period, customarily set to 12 months. Coutelieris and Kanavouras (2006) and Kanavouras and Coutelieris (2006) have shown this probability to be analogous to the fraction of the area between the A420 over time curve and above the arbitrarily defined acceptable quality threshold over the overall area of the evolution curve. By expressing the integrals of the areas, the P_{safe} values for a wine, can be obtained using equation (1).

Critical time (t_{cr}) values can be calculated using equation (2). First, the point where the threshold line intersects with the A420 evolution curve needs to be defined (usually arbitrarily) based on sensory studies or a change in specific quality characteristics (e.g., colour change). The perpendicular coordinate value represents the critical time, while the vertical coordinate is the threshold limit above which the absorption values are not considered acceptable. A graphical representation of the above is given for Assyrtiko wine in Figure 2 (for $T=20\text{ °C}$), where the blue line is the simulated absorption quantified in the y-axis. In more detail, t_{cr} for bottled Assyrtiko wines stored at 20 °C was calculated to be 4.28 months for a threshold of approximately 40 % more than its A420. However, as previously mentioned, this threshold was set arbitrarily due to the lack of a consensus regarding an acceptable colour change in white wines. For this reason, in this work P_{safe} has been calculated as a function of different threshold values.

Clearly, different thresholds correspond to different t_{cr} , and consequently to different P_{safe} values, as can be seen in Figures 3A and 3B.

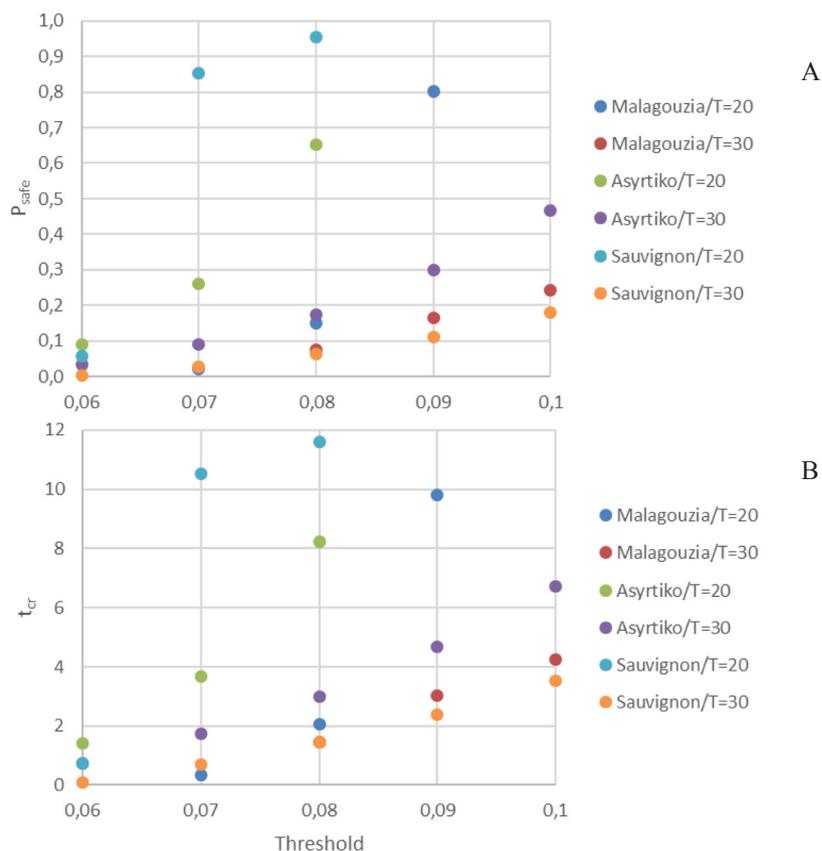


FIGURE 3. Effect of threshold value (A) and t_{cr} (B) on P_{safe} for the three wine varieties (Assyrtiko, Malagouzia, Sauvignon blanc) stored at 20 °C and 30 °C.

Figure 3A shows the impact of the threshold selection on the probability of each bottled wine maintaining an acceptable colour change by the end of its shelf-life during the study's defined time of 12 months of storage. As can be seen, the selected t_{cr} designates a significant effect of the variety on the P_{safe} . However, different wines responded differently to the oxygen transmitted through the cork and reaction outcome of the oxidation phenomena, as indicated by the evolution of A420. For example, if the threshold is set to be 0.07 absorption at A420, the highest P_{safe} is obtained for Sauvignon blanc wines at 20 °C, followed by Assyrtiko at 20 °C and Assyrtiko at 30 °C. The variety with the lowest P_{safe} was Sauvignon blanc at 30 °C. When the threshold is set to 0.08 absorption at A420, the highest P_{safe} is still obtained for Sauvignon blanc wines at 20 °C, and the lowest for Malagouzia and Sauvignon blanc wines at 30 °C. It seems that Sauvignon blanc wines are more sensitive to temperature changes compared to Assyrtiko wines. When the threshold value was set to 0.1 absorption at A420, only the wines stored at 30 °C were considered (it is not possible to reach the absorption value at A420 of the wines stored at lower temperature

within 12 months). In this case, the highest P_{safe} value was obtained for Assyrtiko wines, followed by Malagouzia, and the lowest was for Sauvignon blanc.

It is important to note, that similar behavior was observed when the impact of the threshold selection on the probability of each bottled wine maintaining an acceptable colour change by the end of its shelf life was considered (Figure 3B). Higher t_{cr} values were calculated for Sauvignon blanc wines at 20 °C for lower threshold values, but at higher thresholds for Assyrtiko at 30 °C. In more detail, Sauvignon blanc wines can be stored for longer at 20 °C without colour change going over the threshold; however, at 30 °C, the duration is much shorter for Sauvignon blanc in comparison with the remaining two varieties.

It is also worth noting that the relationship between P_{safe} and t_{cr} seems to be linear, which can mainly be attributed to the shape of the absorption curve, whose deviation from linearity is quite low. Obviously, this may not be valid for any other application, thus it can not be considered as a more general rule.

CONCLUSION

This present study investigated the impact of grape cultivar and storage temperature on bottled wines' colour using a mathematical model. A series of three characteristic grape varieties cultivated in Greece and bottled at different wineries were used. Regarding the impact of the grape cultivar, it was notable that significant differences could be determined among them for wines stored at 20 °C or 30 °C. Based on the analytical results, a lower A420 increase was observed for Sauvignon blanc wine at 20 °C, while at 30 °C, the same variety exhibited the highest browning. Assyrtiko wines were more resistant to colour change at both temperatures while Malagouzia wines exhibited the highest colour change at 20 °C and an intermediate browning at 30 °C. Whether the rates among the various reactions in the wines would be similarly affected by temperature in time still requires further investigation.

The modelling of the experimental results shows the impact of the threshold selection on the probability of each bottled wine maintaining an acceptable colour change by the end of its shelf-life (12 months). For low threshold values, the highest and lowest P_{safe} values were obtained for Sauvignon blanc wines at 20 °C and 30 °C respectively. For high threshold values, the highest P_{safe} value was obtained for Assyrtiko wines, followed by Malagouzia, and the lowest for Sauvignon blanc at 30 °C. A similar trend was observed when t_{cr} was considered. The mathematical treatment of the data indicates that storage temperatures should be taken into account when setting the quality threshold (colour change) of a wine from a given grape variety.

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