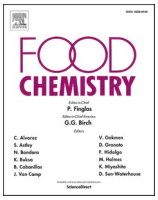
Food Chemistry 386 (2022) 132861

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Is pinking susceptibility index a good predictor of white wines pinking phenomena?

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ARTICLE INFO

Keywords:
Pinking
White wine
Anthocyanins
Pinking susceptibility index

ABSTRACT

The concentration of anthocyanins in white wines from different grape varieties (Prosecco, Sauvignon Blanc, Pinot Grey, Chardonnay, Síria) from diverse countries (Italy, Moldova, and Portugal) was determined. Anthocyanins, mainly malvidin-3-*O*-glucoside, were detected in all wines (from 0.7 to 704 μg/L). No correlation between anthocyanins concentration and the Pinking Susceptibility Index (PSI) was observed contrarily to the colour of wines exposed to oxygen ($r = 0.871$, $p < 0.00005$). The oxidation of wines with hydrogen peroxide resulted in the formation of various compounds. PSI was correlated with compounds absorbing in the 400–480 nm region, probably more related to the browning than the pinking phenomenon. The lack of correlation between the PSI and anthocyanins concentration in white wines can be due to the different chemical compositions of white wines that yield various compounds after oxidation that might not be related to the natural wine pinking phenomenon.

1. Introduction

Pinking is the emergence of pink tones in white wines exclusively produced from white grape varieties. Pinking is observed when white wines are produced under reducing conditions (Du Toit, Marais, Pretorius, & Du Toit, 2006; Filipe-Ribeiro, Andrea-Silva, Cosme, & Nunes, 2022; Gabrielli, Fracassetti, Romanini, Colangelo, Tirelli, & Lambri, 2021; Jones, 1989; Nel, du Toit, & van Jaarsveld, 2020; Simpson, 1977; Van Wyk & Louw, 1976). Pinking usually occurs after bottling and storage of white wines, but its appearance has also been described after alcoholic fermentation or even as soon as the grape must is extracted (Simpson, Miller, & Orr, 1982; Simpson, Bennett, & Miller, 1983; Singleton, Trousdale, & Zaya, 1979). The appearance of pinking has been described worldwide from wines produced from *Vitis vinifera* L. white grape varieties such as Chardonnay, Chenin Blanc, Crouchen, Muscat Gordo Blanco, Palomino, Riesling, Sauvignon Blanc, Sémillon, Sultana, Thompson Seedless, and Síria (Andrea-Silva et al., 2014; Du Toit et al., 2006; Filipe-Ribeiro et al., 2022; Nel et al., 2020; Simpson, 1977). Besides the change in colour of white wines by the occurrence of the pinking phenomena, not appreciated by the consumers, industry, and regulation entities, also results in white wine aroma change, probably resultant from the oxidation. Although no differences occur in the taste of white wines when pinking appears were described by a trained panel (Nel, du Toit, & van Jaarsveld, 2021).

Pinking is due to oxidative changes in white wines when exposed to oxygen (Gabrielli et al., 2021; Simpson, 1977; Singleton et al., 1979). Mainly observed in young wines produced under reducing conditions, it occurs after exposure to oxygen, related to the white grape variety and harvesting year. The cause of pinking in white wines is still controversial. Jones (1989) described that the pink colour in white wines is caused by at least ten compounds and polymeric material. Several hypotheses have been raised for the origin of the pink colouration in white wines. One hypothesis is that the slow dehydration of leucoanthocyanidins (flavan-3,4-diols) to their corresponding flavenes (flav-3-en-3-ol) under a highly reductive medium and then quick oxidation to their corresponding coloured flavylium cations (cyanidin) upon exposure to oxygen (Singleton, 1972; Zoecklein, Fugelsang, Gump, & Nury, 1995) causes the appearance of the pink colouration in white wine. Also, the slow acid catalysis cleavage of interflavan bonds of certain proanthocyanidins present in grape skins to their corresponding carbocation intermediate, which turns into flavylium cations following an oxygen exposure (Simpson, 1977), has been hyphotised as the pinking origin.

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https://doi.org/10.1016/j.foodchem.2022.132861
Received 22 October 2021; Received in revised form 27 March 2022; Accepted 28 March 2022
Available online 30 March 2022

The pink colour's appearance in white wines has been attributed to the formation of unknown red coloured compounds after oxidation of the 2-*S*-glutathionyl caftaric acid (Van Wyk et al., 1976). The more recent hypothesis is that the pinking of white wines is due small amounts of anthocyanins in white grapes, extracted during the winemaking process, remaining up in the final wines when using reducing conditions. Initially, anthocyanins react with hydrogen sulphite ions rendering the colourless flavene-4-sulfonate. When the sulphur dioxide concentration in wines lowers, there is an increase in the anthocyanins flavylium form due to the dissociation of flavene-4-sulfonate, which, when above a particular concentration of anthocyanins flavylium form turn the white wines pink (Andrea-Silva et al., 2014; Cosme et al., 2019). Anthocyanins in white wines made from white grapes were clearly shown for the Portuguese Síria white wines (Andrea-Silva et al., 2014). Nevertheless, although anthocyanins have been detected in the skins of the white grape varieties Chardonnay, Sauvignon Blanc and Riesling (Arapitsas, Oliveira, & Mattivia, 2015), the presence of anthocyanins in wines made from other white grape varieties from other locations has not been shown.

The most common method for measuring the white wine pinking susceptibility is by inducing white wine oxidation through the addition of hydrogen peroxide and by measuring the increase in absorbance at 500 nm (Simpson, 1977; Nel et al., 2020). Nevertheless, this also increases in the absorbance at 420 nm due to browning (Simpson, 1977; Nel et al., 2020). Therefore, the exposure of white wines to more or less oxidative conditions generally increases both optical densities and can result in possible errors in the interpretation of the result. A solution to correct this problem was developed by Simpson (1977) by extrapolating a baseline and measuring the difference in the optical density at 500 nm and the baseline value at the same wavelength after forced oxidation. The use of CIEL*a*b* and first derivative spectroscopy effectively eliminated the background, although its correlation with the classic Simpson method was not very good (Minute, Giotto, Filipe-Ribeiro, Cosme, & Nunes, 2021).

Therefore, the purpose of this work was to investigate the existence of anthocyanins in white wines made from different white grape varieties and grown locations and critically evaluate the most common method used for predicting pinking appearance in white wines: the Pinking Susceptibility Index (PSI). Anthocyanins were concentrated by SPE (Andrea-Silva et al., 2014). Also, the products formed by hydrogen peroxide oxidation of the same wines were isolated using this method. The correlation between the PSI and the whole visible spectra was studied by multivariate statistical methods, PCA and PLS analysis, to evaluate the spectral regions in the visible spectra most important to the measured PSI.

2. Material and methods

2.1. Wine sample choice

The wines used in this study are from GiottoConsulting srl client companies from Italy and Moldova. Wines collected in Portugal were also used (Supplementary material Table S.1.) based on the susceptibility to pinking observed by the producers over the years. All wines used in this study were produced and analysed in the same year and didn't present visible pinking when analysed. The production processes are the standard ones established by the different companies and considered the best possible solutions concerning the quality of the final wines. Considering the actions assumed to protect wines from oxidation, particularly during grape must extraction, the production processes performed for the wines can be grouped into "classic" and "reductive" production processes (Supplementary material Table S.1.). In the "classic" production process, the grapes were not pressed in a hyper-reductive atmosphere and were protected with the addition of potassium metabisulphite and nitrogen in the press. Grape musts were floated and fermented in stainless steel or concrete tanks. In the "reductive" production process, wines were produced in more extreme reductive environments beginning from cold grapes (dry ice used) and using inertised presses. Then grape musts were clarified by flotation or cold decantation and fermented in stainless steel or concrete tanks. Regardless of the production process adopted at all stages of vinification, free sulphur dioxide was carefully monitored to avoid the oxidative phenomenon. The chemical parameters of wines (Supplementary material Table S.2.) were determined by Fourier Transform Infrared spectroscopy using an FT-120 (Foss).

2.2. Pinking susceptibility index of white wines

The Pinking susceptibility index (PSI) of white wines was evaluated according to Simpson (1977) by induced oxidation of white wines for 24 h through the addition of hydrogen peroxide and thoroughly described in Minute et al. (2021). PSI of white wines was quantified by: (1) measuring the absorbance at 500 nm before and after the induced oxidation and multiplying by 1000; (2) by extrapolating the baseline at 500 nm using an exponential fitted equation to the absorbances at 650, 625, 600, 420, 410 and 400 nm and subtracting this theoretical baseline at 500 nm to the value obtained at 500 nm and multiplying by 1000. According to Simpson (1977), if the value achieved is higher than 5, the wine is susceptible to pinking. Nevertheless, a value of 3 has been recently suggested (Nel et al., 2021).

2.3. Derivative spectroscopy

The main properties of derivative spectroscopy, similar to classic spectroscopy, are the dependence of the derivative value on concentration and its additivity. The shape and intensity of the resulting derivative spectrum depend on the half-height width of the peaks in the zero-order spectra. Broad zero-order peaks are quenched, while narrow peaks are amplified by generating of higher orders of derivatives. Derivative spectroscopy increases methods sensitivity and selectivity compared to the classical zero-order derivative spectroscopy based on the same colour system. The increase in selectivity of the derivative spectroscopy results from the fact that differentiation permits obtaining a higher amount of information contained in the original absorption spectrum. Due to this, it is possible to take advantage of the differences in the position of the peaks (different A.max) and the peak half-width (different L values) (Talsky, Mayring, & Kreuzer, 1978; Kus, Marczenko, & Obarski, 1996). For obtaining the first derivative and second derivative spectra, Savitzky–Golay algorithm (Savitzky, & Golay, 1964; Steinier, Termonia, & Deltour, 1972) was used with 20 points smoothing and a third-order polynomial using Origin 8 software (Origin, OriginLab Corporation, Northampton, MA, USA).

2.4. Chromatic characteristics

Wine's chromatic characteristics after induced oxidation by the addition of hydrogen peroxide were measured using the International Commission on Illumination (CIE) using the L*, a*, b* coordinates (L* - lightness, a* - redness/ greenness, and b* - yellowness/ blueness) according to methods described by OIV (2021). The absorption spectra of wine samples were acquired from 380 to 780 nm using a quartz cell with a 1 cm path length. All analyses were performed in duplicate.

2.5. Isolation of pinking compounds by reversed phase solid phase extraction

The pinking compounds present in the white wine samples were purified and concentrated by reversed-phase (C-18) solid-phase extraction (Supelclean LC-18 SPE tube, 1 g of solid phase, and 6 mL volume). Briefly, the C-18 SPE column was conditioned four times with 5 mL of methanol followed by four times with 5 mL of a 0.1 M HCl solution. Then 100 mL of white wine samples, previously adjusted to pH 1 with 3 M

HCl, were applied. The column was washed four times with 5 mL of 0.1 M HCl solution, and the elution was performed with four times 5 mL of methanol and pooled. Methanol was removed under vacuum at 35 °C (Andrea-Silva et al., 2014). The residue was dissolved in 1 mL of a methanol/water (1:1 v/v) solution containing 20 ppm of sulphur dioxide and analyzed as soon as possible by HPLC. All analyses were performed in duplicate.

2.6. Determination of the phenolic compounds by high-performance liquid chromatography with photodiode array detector (RP-HPLC-DAD)

Phenolic compounds present in white wines water/methanolic extract were analysed by RP-HPLC-DAD (Ultimate 300 solvent delivery system equipped with a PD-100 UV–vis diode array detector, Dionex, USA). Separation was performed by gradient elution on an ACE 5 C-18 column (5 μm particle size, 250 mm length, 4.6 mm diameter, Advanced Chromatography Technologies, Scotland). Conditions of HPLC analysis were as follows: solvent A was a mixture of 95:5 water/formic acid (v/v), and solvent B was methanol. A linear gradient analysis for a total run time of 80 min was used as follows: starting from 5% solvent B during 2 min, increase to 80% solvent B over 68 min and then isocratic for 8 min, decreased to 5% solvent B over 2 min, and finally isocratic for 5 min. A flow rate of 1.0 mL/min was used. The injection volume was 50 μL, and the column temperature was 35 °C during the run. The detection was performed from 250 to 600 nm with a photodiode array detector (PDA-100, Dionex) (Guise et al., 2014). All analyses were performed in duplicate.

Anthocyanins identifications (cyanidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, and malvidin-3-*O*-glucoside) were made by injection of commercial standards and comparison of their retention times and UV–vis spectra. Other anthocyanins were identified by comparing the elution order and UV–vis spectra reported in the literature. Calibration curves of malvidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, and cyanidin-3-*O*-glucoside were used for the quantification of these anthocyanins. The results of delphinidin-3-*O*-acetylglucoside, petunidin-3-*O*-acetylglucoside, peonidin-3-*O*-acetylglucoside, cyanidin-3-*O*-acetylglucoside, malvidin-3-*O*-acetylglucoside, delphinidin-3-*O*-coumaroylglucoside, petunidin-3-*O*-coumaroylglucoside, peonidin-3-*O*- coumaroylglucoside, malvidin-3-*O*-coumaroylglucoside, cyanidin-3-*O*-coumaroylglucoside were expressed as respective glucoside equivalents (Filipe-Ribeiro, Milheiro, Matos, Cosme, & Nunes, 2017a; Filipe-Ribeiro, Milheiro, Matos, Cosme, & Nunes, 2017b).

2.7. Principal component analysis (PCA)

PCA is an unsupervised multivariate method used to reduce the dimensionality of data sets containing a high number of interrelated variables while retaining as much as possible the original variability. The second derivative visible spectra of white wines induced to oxidise by the addition of hydrogen peroxide were analysed by PCA to extract the highest sources of variability of the original data. Additionally, relations between the wavelengths were explored. PCA was performed using the STATISTICA 10 software (Statsoft. OK, USA).

2.8. Partial least squares (PLS)

PLS analysis was employed to access what wavelengths in the second derivative spectra of white wines oxidised by the addition of hydrogen peroxide were related with the PSI using the a* chromatic parameter. The number of components, and the quality of the PLS model, were evaluated by the goodness-of-fit parameter (R^2X), the proportion of the variance of the response variable that is explained by the model (R^2Y), and the predictive ability parameter (Q^2), which was calculated by 7-fold internal cross-validation (STATISTICA 10 software, Statsoft, OK, USA). R^2X and R^2Y represent the fraction of the variance of X and Y matrixes, respectively, and Q^2 is the model's predictive accuracy.

3. Results and discussion

3.1. Anthocyanins in white wines

To have further insight into the presence of anthocyanins in white wines other than those already described for the Portuguese Siria monovarietal white wines (Andrea-Silva et al., 2014), 14 white wines from Italy (5 wines), Moldova (4 wines), and Portugal (5 wines) produced exclusively from Chardonnay (wine ChIt4), Sauvignon Blanc (wines SBIt3, SBMo6, SBMo8, SBMo8, and SBPt14), Prosecco (wine PrIt1 and PrIt2), Pinot Grey (wine PGMo4), and Síria white grape varieties (wine SrPt10 and SrPt11), and three blend white wines (wine BlIt9, BlPt12, and BlPt13) were analysed by HPLC-DAD after preconcentration by solid-phase extraction (Andrea-Silva et al., 2014), resulting in a concentration factor of 100.

Wines were selected according to grape varieties and regions where the producers described the appearance of pinking in previous harvesting years. Table 1 and Figs. 1–3A show that all wines analysed contained anthocyanins ranging from 0.7 μg/L for wine ChIt4 to 704 μg/L for wine BlIt5. The blend wine from Nothern Italy (BlIt5) and Pinot Grey from Moldova (PGMo9) presented the highest levels of anthocyanins of all wines analysed, followed by Sauvignon Blanc from Moldova (SBMo6) and the two Siria white wines from Portugal (SrPt10 and SrPt11, Table 1). All wines contained malvidin-3-*O*-glucoside as the most abundant anthocyanin (0.3 to 484 μg/L), with wines PGMo9 and BlIt5 showing a higher diversity of anthocyanins that include cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, petunidin-3-*O*-acetylglucoside, peonidin-3-*O*-acetylglucoside, malvidin-3-*O*-acetylglucoside, delphinidin-3-*O*-coumaroylglucoside, petunidin-3-*O*-coumaroylglucoside, and malvidin-3-*O*-coumaroylglucoside. For wines, PrIt1, PrIt2, SBIt3, BlPt12, and SBPt14 malvidin-3-*O*-glucoside was the only anthocyanin detected after concentration (Table 1). These results show that low levels of anthocyanins are present in white wines from other grape varieties other than Síria and grown in different locations. The wines analysed in this study were sent by industrial producers for measuring PSI, and it was not possible to make any connection of wines anthocyanins concentration to the grape variety and grown location as the previous history of the white wines before analysis could not be fully disclosed (for example, if the wines were previously fined or not).

The PSI of the white wines was determined according to the classic method of Simpson (1977) by induced oxidation by the addition of hydrogen peroxide and incubation for 24 h at room temperature in the dark (Table 2). The PSI was measured by: the difference in the absorbance at 500 nm before and after oxidation, extrapolation of the baseline at 500 nm after oxidation, and measuring the CIEL*a*b* chromatic coordinates of the wines after oxidation (Minute et al., 2021). There was no significant correlation between the initial concentration of anthocyanins in white wines and the PSI. Wine SBMo6 was one of the wines presenting the lowest concentration of anthocyanins showed the highest PSI when measured by the classic method of Simpson (Simpson, 1977). On the other hand, the wine BlIt5 that showed the highest anthocyanins concentration, was one of the wines showing the lowest PSI (Table 2). These results can have two interpretations: 1) the pinking of white wines can be caused by other compounds present in white wines other than anthocyanins; 2) the method of predicting pinking susceptibility of white wines developed by Simpson (1977) can be inaccurate in predicting the pinking phenomenon. To further explore this point, the wines were exposed to oxygen in a glass for 24 h (Andrea-Silva et al., 2014) and the wine's CIEL*a*b* chromatic parameters were determined (Table 2). There was a lower increase in the CIEL*a*b* parameters when the wines were exposed to oxygen when compared to the same wines forced to oxidise using hydrogen peroxide. There was no correlation between the a* values of the wines oxidised by the addition of hydrogen

Table 1
Anthocyanin composition (μg/L) of white wines (mean. ± SD).

	C3G	Pet3G	Peo3G	M3G	D3AcG	C3AcG	Pet3AcG	Peo3AcG	M3AcG	D3CoG	C3CoG	Pet3CoG	M3CoG	Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	
PrIt1				4.2 ± 0.1										4.2 ± 0.1
PrIt2				1.6 ± 0.1										1.6 ± 0.1
SBIt3				0.9 ± 0.1										0.9 ± 0.1
ChIt4				0.4 ± 0.0	0.2 ± 0.1				0.1 ± 0.0					0.7 ± 0.1
BlIt5	0.6 ± 0.1	6.1 ± 0.7	90.9 ± 12.9	484 ± 58	7.3 ± 1.2		4.5 ± 1.5	6.7 ± 1.1	176 ± 26		0.5 ± 0.0	6.0 ± 1.0	18.7 ± 2.3	704 ± 33
SBMo6			2.2 ± 0.1	14.4 ± 3.0	0.3 ± 0.0				0.9 ± 0.4	0.4 ± 0.0		0.8 ± 0.0	0.1 ± 0.1	19.3 ± 3.6
SBMo7				3.0 ± 0.3	0.6 ± 0.1				0.8 ± 0.2			0.4 ± 0.0	0.2 ± 0.1	4.7 ± 0.9
SBMo8				10.2 ± 1.5	0.8 ± 0.0				2.3 ± 0.5	0.2 ± 0.0			0.5 ± 0.1	14.1 ± 2.1
PGMo9	0.9 ± 0.0	19.2 ± 2.1	50.9 ± 12.5	460 ± 92	3.9 ± 0.8					3.1 ± 0.2		4.4 ± 0.1		542 ± 108
SrPt10				10.4 ± 1.3	0.3 ± 0.0		0.4 ± 0.0		1.0 ± 0.2				0.7 ± 0.2	12.8 ± 1.3
SrPt11			2.0 ± 0.8	15.4 ± 1.7	0.5 ± 0.0				2.6 ± 0.2	0.2 ± 0.1		0.5 ± 0.1	0.5 ± 0.1	20.0 ± 0.8
BlPt12				1.9 ± 0.2										1.9 ± 0.2
BlPt13			0.5 ± 0.0	6.2 ± 0.3		0.2 ± 0.0							0.2 ± 0.0	7.2 ± 0.4
SBPt14				0.9 ± 0.0										0.9 ± 0.0

Cyanidin-3-*O*-glucoside (C3G), peonidin-3-*O*-glucoside (Peo3G), petunidin-3-*O*-glucoside (Pet3G), malvidin-3-*O*-glucoside (M3G), petunidin-3-*O*-acetylglucoside (Pet3AcG), malvidin-3-*O*-acetylglucoside (M3AcGlc), cyanidin-3-*O*-acetylglucoside (C3AcG), delphinidin-3-*O*-acetylglucoside (D3AcG), peonidin-3-*O*-acetylglucoside (Peo3AcG), cyanidin-3-*O*-coumaroylglucoside (C3CoG), petunidin-3-*O*-coumaroylglucoside (Pet3CoG), malvidin-3-*O*-coumaroylglucoside (M3CoG), delphinidin-3-*O*-coumaroylglucoside (D3CoG).

peroxide and the wines oxidised by exposure to oxygen ($r = 0.113$, Supplementary material Fig. S.1A.). This lack of correlation might be due to the incomplete conversion of the pinking compounds present when the wines were exposed to oxygen. Also, this lack of correlation can be due to the time used to oxidise the wines by exposure to oxygen. Nevertheless, it is interesting to note a significant correlation between the a* values of the wines exposed to oxygen and the logarithm of the concentration of anthocyanins in wines ($r = 0.871$, $p < 0.00005$, Table 1 and Supplementary material Fig. S.1B.). These results show that 76% of the variation observed in the colour of white wines exposed to oxygen for 24 h measured by the a* value can be explained by the variation in the white wines anthocyanins concentration on a logarithmic scale.

3.2. *Absorption species in wines after forced oxidation – unsupervised exploratory pattern recognition by principal component analysis (PCA)*

The pinking phenomenon is the appearance of a pink-red blush in wines after oxidation, resulting in a change in their visible spectra. Therefore, the visible spectra of white wines after oxidation by the addition of hydrogen peroxide were measured (Supplementary material Fig. S.2A). PCA was used for the unsupervised multivariate exploratory data analysis to recognise patterns in the sample distribution and relationships between variables and possible classes. The second derivative spectra were used for the analysis to eliminate baseline problems in the visible spectra (second derivative spectra in the 400–600 nm range, Supplementary material Fig. S.2B). PCA of the second derivative spectra yielded five principal components explaining >80% of the total variance in the original data set (Table S3. in Supplementary material).

Loading values >+0.75 and <−0.75 are highlighted in red in Fig. 4B (first PC) and Supplementary material Fig. S.3. (second PC). The loadings express how well the new PCs correlate with the original variables. Fig. 4B shows that the first PC, which explains 40.0% of the total variance, correlates positively with the wavelength in the range 496 to 508 nm and negatively with the wavelength in the range 400 to 436 nm, 445 nm, 552 to 580 nm, and 592–596 nm. The second PC, which explains 16.8% of the total variance, shows a positive correlation with wavelengths in the range 532–536 nm (Supplementary material Fig. S.3). None of the variables was decisive for the remaining PCs (PC3, PC4, PC5, PC6 and PC7). The scatter plot of the sample scores (Fig. 4A) shows that the samples are ordered with increasing PSI values according to negative PC1 scores. There was observed a significant correlation between the PSI measured by the a* chromatic coordinate (redness) of the wine samples after hydrogen peroxide oxidation and the PC1 scores ($r = -0.967$, $p < 0.00001$, Fig. 4C). The PC1 scores were also significantly correlated with the other two PSI calculated by measuring the absorbance at 500 nm before and after oxidation ($r = -0.869$, $p < 0.00006$) and after baseline correction ($r = -0.779$, $p < 0.001$). Nevertheless, the correlation coefficient obtained for the a* chromatic values was significantly higher ($p < 0.012$) than that obtained for the other two PSI (Supplementary material Fig. S.4.). Therefore, these results suggest that the PSI of white wines measured by the classical, baseline-corrected method and a* values are related with compounds that absorb in the range 400 to 436 nm, 445 nm, 552 to 580 nm, and 592 to 596 nm. On the contrary, samples with positive PC1 scores, that is, samples presenting low PSI, are related to compounds absorbing maximally at the 496 to 508 nm range. As the pinking phenomenon is the appearance of pink colour in white wines after oxidation, the relation of the PSI with wavelengths related to the brown colouration (in the 400 nm range) suggests that probably the values of PSI obtained by forced oxidation are seriously affected by the brown colouration even when the measurement is performed at 500 nm. These results agree with the observation of Romanini, Colangelo, Lucini, and Lambri (2019), that described that wines showed increased pinking (a*) when they were more browned (A420nm).

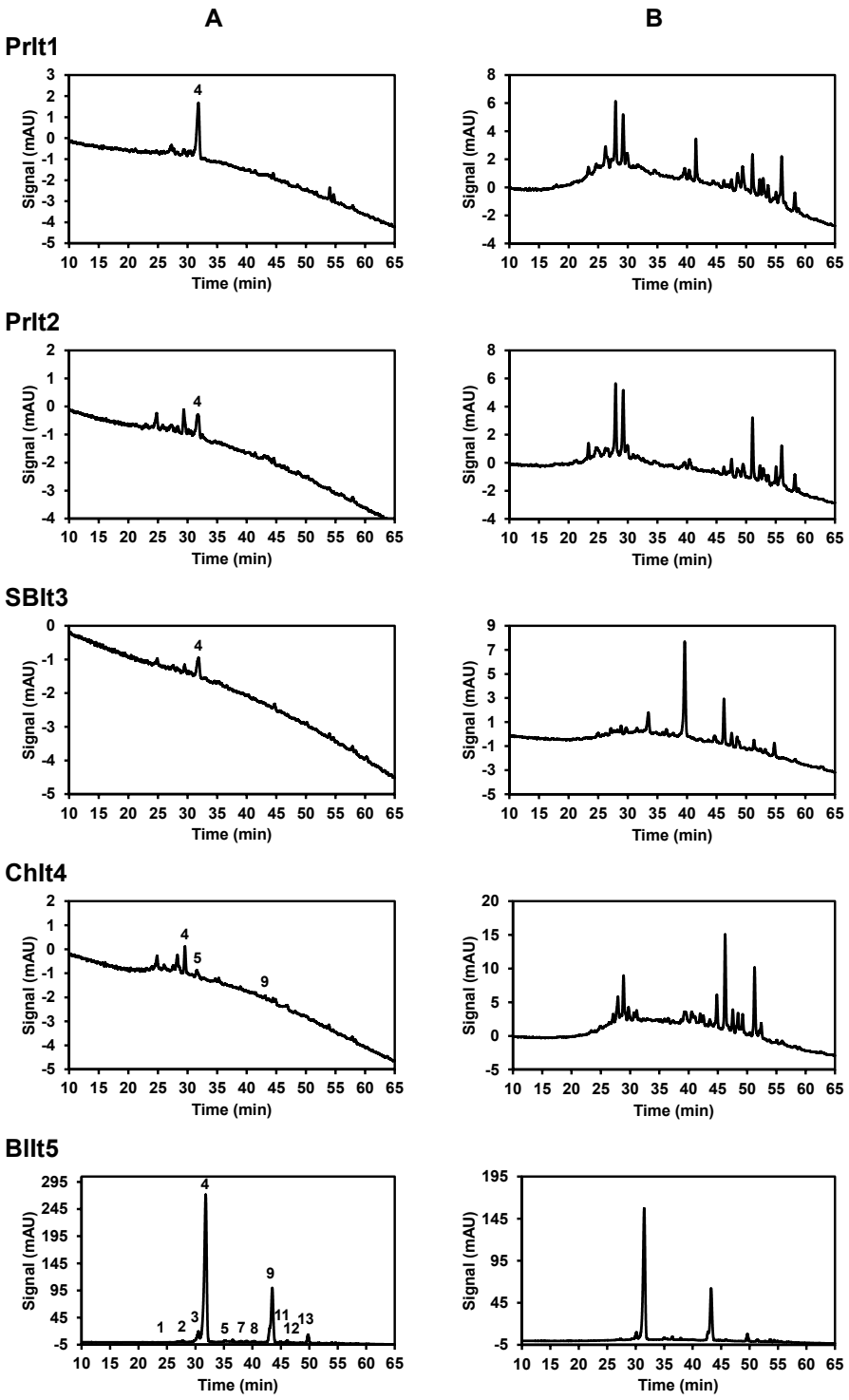

Fig. 1. HPLC-DAD chromatograms at 525 nm of white wines from Italy enriched by SPE before (A) and after (B) induced oxidation with hydrogen peroxide during 24 h at room temperature in the dark. 1 - Cyanidin-3-*O*-glucoside; 2 - petunidin-3-*O*-glucoside; 3 - peonidin-3-*O*-glucoside; 4 - malvidin-3-*O*-glucoside; 5 - delphinidin-3-*O*-acetylglucoside; 6 - cyanidin-3-*O*-acetylglucoside; 7 - petunidin-3-*O*-acetylglucoside; 8 - peonidin-3-*O*-acetylglucoside; 9 - malvidin-3-*O*-acetylglucoside, 10 - delphinidin-3-*O*-coumaroylglucoside; 11 - cyanidin-3-*O*-coumaroylglucoside; 12 - petunidin-3-*O*-coumaroylglucoside; 13 - malvidin-3-*O*-coumaroylglucoside.

3.3. Absorption species in wines after forced oxidation – Supervised feature extraction by Partial least squares regression (PLS-R)

To have a deeper understanding of the relation between the chromophores developed during the oxidation of white wines with hydrogen peroxide and the PSI measured by the a* chromatic coordinate, a Partial Least Squares (PLS1) regression of the standardised a* values (Y) on the standardised second derivative spectra of the white wines after oxidation with hydrogen peroxide (X) was performed. Three latent variables were retained in the PLS1 model based on the Q^2 statistics. The regression curve is shown in Fig. 4D. For the PSI, 74.9% of the variance in the three PLS components related to second derivative spectra after oxidation with hydrogen peroxide explained 99.0% of the PSI measured by the a* chromatic value. The first weight loading vector can contain useful qualitative spectral information since it is the first-order approximation to the pure component spectrum of the analyte (Fig. 4E). Thus, w1 can be useful for making band assignments and determining which spectral regions are more relevant to a particular

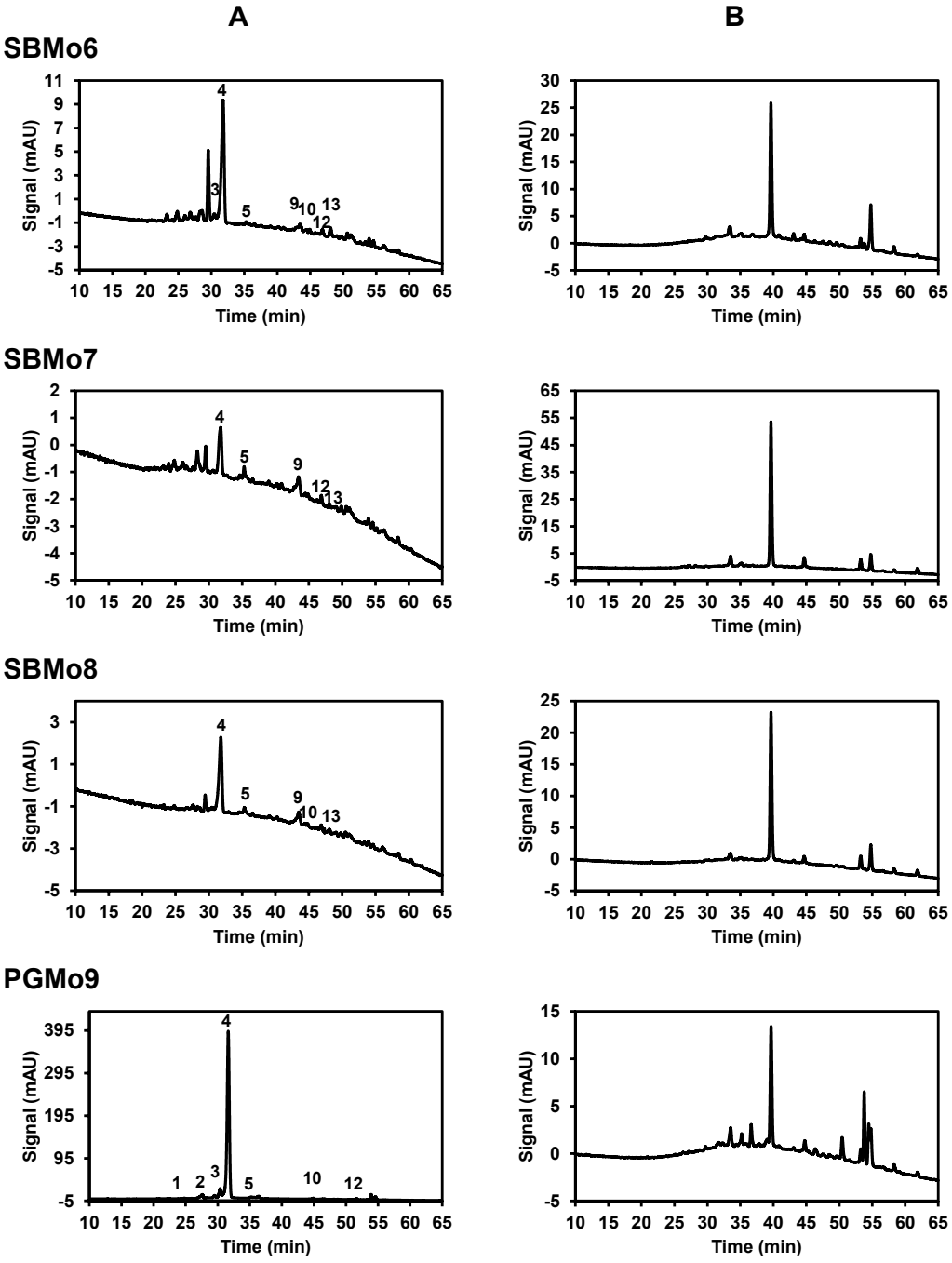

Fig. 2. HPLC-DAD chromatograms at 525 nm of white wines from Moldova enriched by SPE before (A) and after (B) induced oxidation with hydrogen peroxide during 24 h at room temperature in the dark. 1 - Cyanidin-3-*O*-glucoside; 2 - petunidin-3-*O*-glucoside; 3 - peonidin-3-*O*-glucoside; 4 - malvidin-3-*O*-glucoside; 5 - delphinidin-3-*O*-acetylglucoside; 6 - cyanidin-3-*O*-acetylglucoside; 7 - petunidin-3-*O*-acetylglucoside; 8 - peonidin-3-*O*-acetylglucoside; 9 - malvidin-3-*O*-acetylglucoside, 10 - delphinidin-3-*O*-coumaroylglucoside; 11 - cyanidin-3-*O*-coumaroylglucoside; 12 - petunidin-3-*O*-coumaroylglucoside; 13 - malvidin-3-*O*-coumaroylglucoside.

analyte (Haaland, & Thomas, 1988). Fig. 4E shows that the white wines' PSI was related to the chromophores absorbing at wavelengths in the range 400 to 445 nm and 488 to 508 nm and 552 to 596 nm. The same trend was obtained for the standardised regression coefficients (Supplementary material Fig. S.5A) and the VIP (variables importance in the projection of the PLS1 model, Fig. 4F). The VIP summarises a variable's contribution to the model is calculated as a weighted sum of the squared correlations between the PLS1 components and the original variables (Farres, Platikanov, Tsakovski, & Tauler, 2015). These results agree with the PCA results, further supporting that the highest contribution to the PSI was the formation of brown coloured compounds absorbing at the wavelengths in the range 400 to 445 nm and 488 to 508 nm.

The inclusion of the conventional oenological parameters (Supplementary material Table S.2.) in the analysis did not increase the model prediction ability.

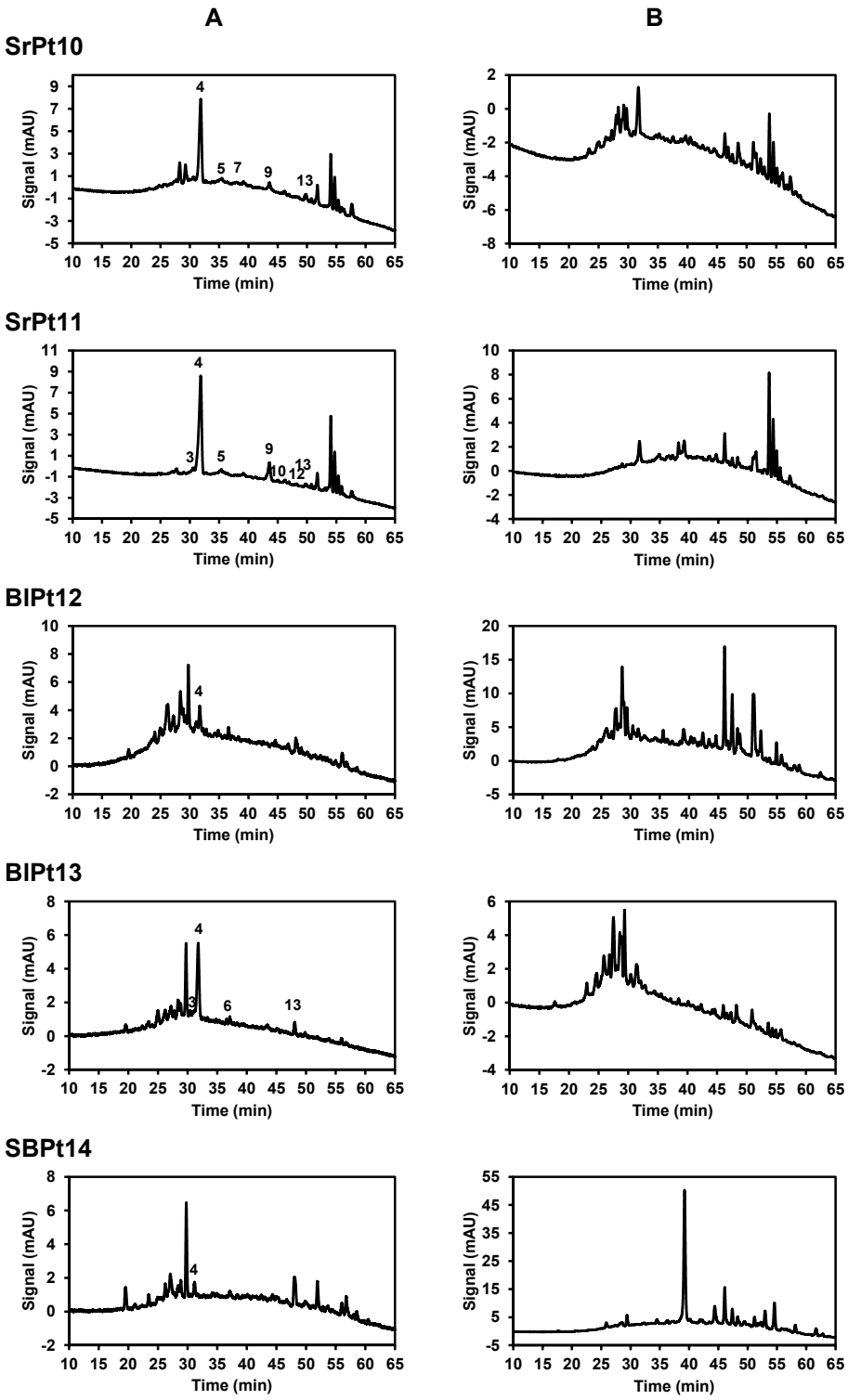

Fig. 3. HPLC-DAD chromatograms at 525 nm of white wines from Portugal enriched by SPE before (A) and after (B) induced oxidation with hydrogen peroxide during 24 h at room temperature in the dark. 1 - Cyanidin-3-*O*-glucoside; 2 - petunidin-3-*O*-glucoside; 3 - peonidin-3-*O*-glucoside; 4 - malvidin-3-*O*-glucoside; 5 - delphinidin-3-*O*-acetylglucoside; 6 - cyanidin-3-*O*-acetylglucoside; 7 - petunidin-3-*O*-acetylglucoside; 8 - peonidin-3-*O*-acetylglucoside; 9 - malvidin-3-*O*-acetylglucoside, 10 - delphinidin-3-*O*-coumaroylglucoside; 11 - cyanidin-3-*O*-coumaroylglucoside; 12 - petunidin-3-*O*-coumaroylglucoside; 13 - malvidin-3-*O*-coumaroylglucoside.

3.4. Absorption species in wines after forced oxidation – HPLC analysis of the products obtained by forced oxidation

To support the findings discussed previously, the compounds formed after oxidation of the wines with hydrogen peroxide were isolated by the same SPE method applied to the wines before oxidation (Fig. 1B, 2B, and 3B). For all wines, except for BlIt5, the anthocyanins were almost totally transformed during the oxidation with hydrogen peroxide. There were detected several compounds absorbing at 500 nm, although with different nature and intensity depending on the wine. A total of 47 compounds yield peaks with significant areas in wines after oxidation with hydrogen peroxide (area higher than 1.0 units in at least one of the wines analysed); nevertheless, none of them was detected in all wines (Supplementary material Table S.4.). The number of compounds detected ranged between 10 for SBMo8 and 32 for BlPt12. Total areas at 500 nm ranged from 11.93 for PrIt1 to 85.04 for BlIt5 (Supplementary material Table S.4.). Compounds with retention times 39.33, 46.24, and 53.86 min were detected in 86% of the wines after oxidation with

Table 2
Pinking susceptibility index and chromatic characteristics of white wines after induced oxidation of white wines with hydrogen peroxide (mean ± SD).

Sample	Addition of hydrogen peroxide					Exposure to oxygen 24 h		
	$Abs_{500nm\ bef.}$-$Abs_{500nm\ aft.}$	$Abs_{500nm\ bas.\ cor.}$	L*	a*	b*	L*	a*	b*
PrIt1	23.6 ± 0.5	9.2 ± 0.2	97.6 ± 0.01	1.00 ± 0.02	4.89 ± 0.01	98.9 ± 0.01	−0.26 ± 0.02	4.31 ± 0.01
PrIt2	11.8 ± 0.4	14.9 ± 0.5	97.9 ± 0.01	0.62 ± 0.02	5.53 ± 0.00	99.0 ± 0.01	−0.41 ± 0.02	4.15 ± 0.01
SBIt3	76.4 ± 0.6	32.8 ± 0.3	94.6 ± 0.02	2.58 ± 0.02	11.46 ± 0.00	99.3 ± 0.01	−0.41 ± 0.01	2.85 ± 0.01
ChIt4	118.5 ± 1.0	49.0 ± 0.4	93.8 ± 0.03	5.74 ± 0.05	10.26 ± 0.05	99.2 ± 0.02	−0.59 ± 0.02	3.93 ± 0.02
BlIt5	42.3 ± 0.2	14.5 ± 0.1	96.5 ± 0.01	2.43 ± 0.01	4.69 ± 0.01	98.2 ± 0.01	0.59 ± 0.01	4.79 ± 0.01
SBMo6	237.5 ± 1.5	121.1 ± 0.8	87.9 ± 0.05	9.43 ± 0.06	22.33 ± 0.03	98.6 ± 0.06	0.039 ± 0.06	4.32 ± 0.03
SBMo7	147.3 ± 1.4	18.7 ± 0.2	91.3 ± 0.04	4.09 ± 0.04	20.19 ± 0.01	98.2 ± 0.04	0.13 ± 0.01	4.71 ± 0.01
SBMo8	203.5 ± 1.4	91.3 ± 0.6	89.6 ± 0.04	5.83 ± 0.04	23.40 ± 0.02	99.0 ± 0.04	−0.57 ± 0.01	4.43 ± 0.02
PGMo9	232.8 ± 5.1	29.6 ± 0.6	88.0 ± 0.03	5.98 ± 0.13	27.26 ± 0.05	98.2 ± 0.03	0.46 ± 0.02	4.26 ± 0.04
SrPt10	14.9 ± 0.3	9.3 ± 0.2	96.4 ± 0.01	0.90 ± 0.02	8.83 ± 0.05	98.2 ± 0.20	−0.14 ± 0.03	3.68 ± 0.03
SrPt11	0.0 ± 0.1	3.7 ± 0.2	96.5 ± 0.01	−0.35 ± 0.02	8.06 ± 0.05	98.5 ± 0.07	−0.12 ± 0.01	3.28 ± 0.01
BlPt12	126.7 ± 0.9	69.2 ± 0.5	96.3 ± 0.01	7.06 ± 0.05	14.26 ± 0.02	97.6 ± 0.10	−0.55 ± 0.04	6.76 ± 0.04
BlPt13	27.1 ± 0.3	36.4 ± 0.4	100.1 ± 0.01	3.88 ± 0.04	7.27 ± 0.02	97.5 ± 0.09	−0.30 ± 0.02	3.45 ± 0.02
SBPt14	195.0 ± 1.6	25.3 ± 0.2	93.4 ± 0.03	4.85 ± 0.04	26.05 ± 0.05	98.0 ± 0.06	−0.78 ± 0.01	4.45 ± 0.01

L* (lightness); a* (redness/ greenness); and b* (yellowness/ blueness).

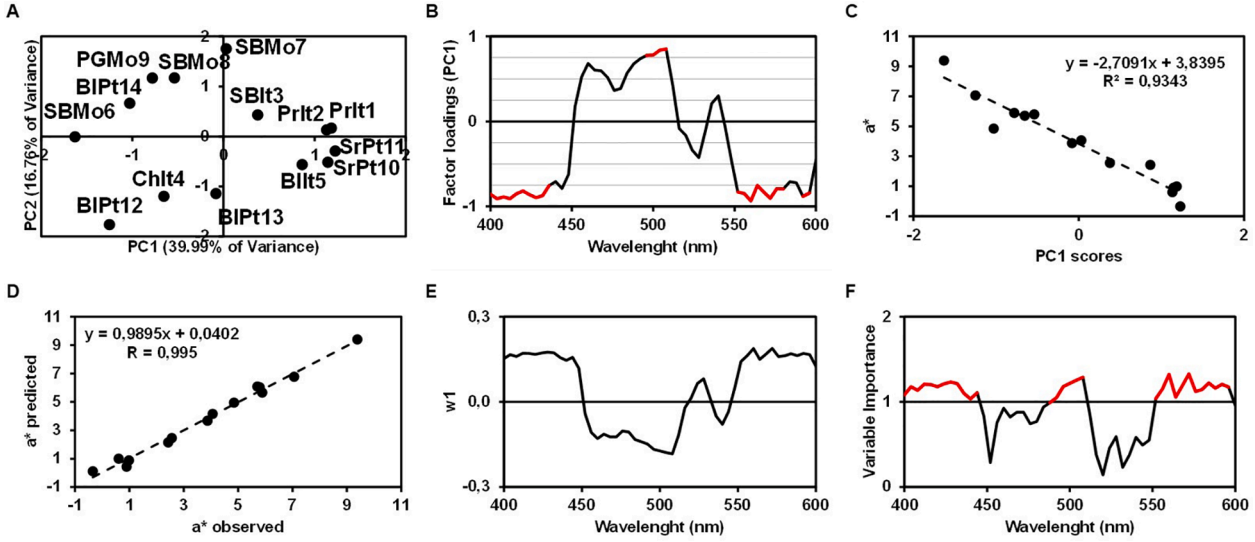

Fig. 4. Sample scores projection on the first and second principal component (A) and variable loading on the first principal component (B). Correlation between PC1 samples socres and a* values of the wines after induced oxidation by addition of hydrogen peroxide (C). Calibration curve obtained after PLS1 regression (D) first PLSl weight loading vector, w1 (E) and VIP scores (F).

maximum areas of 29.95, 9.25, and 3.58, respectively (Supplementary material Table S.4.). The compounds with retention times 44.82 and 53.20 min were the second most frequent compounds detected (79% of the wines with maximum areas of ~2, Supplementary material Table S.4.). Of the compounds with areas higher than 1.0 units, excluding the anthocyanins present in wine BlIt5, their maximum wavelengths ranged from 408 nm to 511 nm (Supplementary material Table S.4.). Nevertheless, most of the compounds showed maximum wavelengths lower than 490 nm (55%), between 490 and 499 nm (21%), and only 23% of the compounds detected showed an absorbance maximum higher than 500 nm. The most abundant compound, peak at 39.33 min, showed an absorption maximum at 463 nm. Peaks with retention times 46.24 min and 51.27 min showed absorption maxima at 468 nm (Supplementary material Table S.4. and Supplementary material Fig. S.6.). No correlation between total area at 500 nm and PSI, either measured by the a* chromatic value (r = 0.318), the difference in absorbances at 500 nm before and after oxidation (r = 0.258), or the absorbance at 500 nm after baseline correction (r = 0.042), was observed. To study the relationship between the compounds formed after oxidation of white wines with hydrogen peroxide and the PSI measured by the a* chromatic values, a PLS1 regression of the standardised a* chromatic values (Y) on the area of each compound detected after oxidation of the white wines with hydrogen peroxide (X) was performed. Only one latent variable was retained based on the Q2 statistics. The regression curve obtained is shown in Supplementary material Fig. S.7a. For the PSI, 24.4% of the variance in the first PLS component related to the abundance of each compound detected after hydrogen peroxide oxidation explained 47.2% of the PSI measured by the a* chromatic value. Fig. S.7b (Supplementary material) shows that the compounds with retention times of 54.79, 33.47, 29.26, 56.00, and 52.40 min had a VIP higher or equal to 1.5. These compounds were present only in 50, 50, 29, 21, and 36% of the wines analysed with maximum areas of 4.83, 1.56, 2.17, 1.69, and 2.48. Although the maximum wavelength of some of these compounds was near 500 nm (518 nm for compound detected at 33.47 min, 505 nm for compound detected at 29.26 min, 500 nm for compounds detected at 56.00 min, and 495 nm for compound detected at 52.40 min), for the compound detected at 54.79 min the maximum wavelength was at 462 nm (Supplementary material Fig. 7c to g). Nevertheless, as mentioned before, the model only accounts for 47.2% of the PSI variance observed. Altogether these results confirm that the PSI was highly correlated with the absorption at other wavelengths in the range 420 to 480 nm, suggesting again that the PSI, either measured by the classical Simpson method, after baseline correction, or using the a* chromatic value might not be

adequate for measuring the natural tendency of wines to acquire a pink colour.

4. Conclusions

The existence of anthocyanins in white wines from Chardonnay, Sauvignon Blanc, Prosecco, and Pinot Grey grape varieties, was described for the first time. Malvidin-3-*O*-glucoside was the major anthocyanin present in all wines in a concentration ranging from 0.3 to 403 μg/L. There was no correlation between the concentration of anthocyanins in white wines and the PSI measured after oxidation of white wines with hydrogen peroxide, contrarily to the a* chromatic value when the wines were oxidised by exposure to the atmosphere. After oxidation with hydrogen peroxide, several compounds were detected absorbing at 500 nm (a total of 47). The compounds formed were not the same in all wines, and the most abundant compound formed in the different wines was not always the same. The compounds showing the highest absorption at 500 nm showed a maximum absorption wavelength around 460–480 nm. Multivariate analysis of the second derivative spectra of wines after hydrogen peroxide oxidation, performed by PCA and PLS analysis, show that PSI was mainly correlated with compounds showing absorbance in the range 400–480 nm, probably being more related to the browning than to the pinking phenomenon. Although it cannot be excluded that anthocyanins are not the only compounds responsible for the pinking phenomenon of white wines and that the wines used in this study were obtained from different producers for which the exact winemaking process and wine treatments are not known in detail, the lack of correlation between the concentration of anthocyanins of white wines and PSI can be due to the different chemical composition of white wines that yield different compounds after oxidation with hydrogen peroxide. These compounds formed by hydrogen peroxide oxidation might not be related to the natural pinking phenomenon of white wines.

CRediT authorship contribution statement

Ana Carolina Gonçalves: Methodology, Validation, Formal analysis, Investigation. **Fabrizio Minute:** Methodology, Validation, Formal analysis, Investigation. **Federico Giotto:** Methodology, Validation, Formal analysis, Investigation, Funding acquisition. **Luís Filipe-Ribeiro:** Methodology, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Visualization, Supervision. **Fernanda Cosme:** Methodology, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Visualization, Supervision. **Fernando M. Nunes:** Methodology, Validation, Formal analysis, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We appreciate the financial support provided to CQ-VR – Chemistry Research Centre – Vila Real (UIDB/00616/2020 and UIDP/00616/2020) by FCT – Portugal and COMPETE. The financial support of the project AgriFood XXI (NORTE-01-0145-FEDER-000041) co-financed by the European Regional Development Fund through NORTE 2020 (Programa Operacional Regional do Norte 2014/2020) is also acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.132861.

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