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Dissipation of Three Fungicides and Their Effects on Anthocyanins and Color of Monastrell Red Wines

N. Briz-Cid[1] (nbriz@uvigo.es)

R. Rial-Otero[1] (raquelrial@uvigo.es)

M.A. Camara[2] (mcamara@um.es)

J. Oliva[2,*] (josoliva@um.es)

J. Simal-Gándara[1,*] (jsimal@uvigo.es)

[1] Nutrition and Bromatology Group, Department of Analytical and Food Chemistry, CITACA, Faculty of Food Science and Technology, University of Vigo – Ourense Campus, E32004-Ourense, Spain.

[2] Department of Agricultural Chemistry, Geology and Pedology, Faculty of Chemistry, University of Murcia, Campus de Espinardo, Murcia, Spain.

*** Corresponding authors:** José Oliva (josoliva@um.es), and Jesús Simal-Gándara (jsimal@uvigo.es)

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Abstract:

The effect of fungicides on fermentation is of paramount importance to control the quality and safety of wines. In this work, the quality (oenological parameters, color, phenolic content, antioxidant activity, and fungicide residues) of wines from Monastrell grapes fortified with iprovalicarb, mepanipyrim and tetraconazole fungicides was evaluated. Along of the winemaking process, initial residues of mepanipyrim and tetraconazole were removed in more than a 90 % while dissipation of iprovalicarb was around 73 %. Significant statistical differences were found in presence of iprovalicarb and mepanipyrim residues especially at the highest concentration assayed. For both fungicides, an increase of the volatile acidity (between 4 and 8.6 times), the lactic acid content (between 8.6 and 20.5 times), the percentage of polymeric anthocyanins (between 1.3 and 1.7 times) and also a slight increase of the total phenolic index and the total anthocyanins content determined by spectrophotometry was observed. On the contrary, the total monomeric anthocyanins content decreased about 16.3 and 28.6 % in presence of iprovalicarb and mepanipyrim, respectively. These results could be related with the addition of SO_2 to the grape must and a higher development of acetic acid or lactic bacteria in presence of these fungicides. The color of the final wines was also different in comparison with the control, with a higher yellow component, color intensity, tonality and hue angle, because of pH changes in the medium. Tetraconazole fermentations had a more similar trend to the control wine, probably due to the lower concentration of this fungicide in the grape must at the initial time. No effects on the antioxidant activity was observed for anyone of the target fungicides. A multivariate statistical analysis was done to view interrelationships between different variables (color and anthocyanins profile). The obtained model allowed to separate wines according to the fungicide treatment applied.

***Keywords*:** fungicides; dissipation; winemaking process; anthocyanins; antioxidant activity.

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1. Introduction

The control of vine diseases is one of the most important factor to obtain quality grapes and, consequently, quality wines. The treatments with fungicides during the season are the most effective methods to fight against them. Iprovalicarb is a valinamide carbamate fungicide used against *Plasmopara viticola* in viticulture practices and its target site of action is the cellulose synthase of cell wall. Mepanipyrim is an aniline-pyrimidine used against *Botrytis cinerea* and affects the methionine biosynthesis. Tetraconazole is used to fight against *Uncinula necator* in grapevines. It belongs to the triazoles chemical group and acts avoiding sterol biosynthesis on the membrane (FRAC, 2018).

The concentration of fungicide residues in grapes depends on active substance, formulation, applied dose, time interval from application, and climatological conditions (Whitmyre, Ross, Lunchick, Volger, & Singer, 2004). Diverse studies have found residues of fungicides at trace levels on vinification grapes after harvest (González-Rodríguez, Cancho-Grande, & Simal-Gándara, 2009a; González-Rodríguez, Cancho-Grande, Torrado-Agrasar, Simal-Gándara, & Mazaira-Pérez, 2009b; González-Rodríguez, Cancho-Grande, & Simal-Gándara, 2011), although levels in the range of 0.5-2.5 mg kg^{-1} were reported in others (Cabras & Angioni, 2000; Cabras et al., 2001; Rial-Otero, Cancho-Grande, Simal-Gándara, 2003). Some oenotechnological processes (crushing, pressing, racking, clarification and filtration) can influence the dissipation of fungicides along the winemaking process reducing their levels (Cabras et al 1998; Cabras & Angioni, 2000; Oliva, Payá, Cámara, & Barba, 2007a, Oliva, Payá, Cámara, & Barba, 2007b; González-Rodríguez et al., 2009b).

Color of red wines is an appreciate quality factor and is highly affected by a different distribution of the monomeric and polymeric anthocyanins. Phenolic compounds are considered the origin of color, taste and astringency (tannins), and have nutritional interest due to their antioxidant properties (Cheynier, 2001; Waterhouse, 2002). The presence of

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fungicide residues on grape must may modify the color and the phenolic composition of wines (Briz-Cid, Figueiredo-González, Rial-Otero, Cancho-Grande, & Simal-Gándara, 2014; Briz-Cid, Figueiredo-Gonzalez, Rial-Otero, Cancho-Grande, & Simal-Gándara, 2015; Mulero et al., 2015; Briz-Cid, Castro-Sobrino, Rial-Otero, Cancho-Grande, & Simal-Gándara, 2018; Castro-Sobrino et al., 2018). However, this field remains quite unexplored until now.

In the present study, the effect of three fungicides (iprovalicarb, mepanipyrim and tetraconazole) on the chromatic characteristics (color and phenolic composition) of Monastrell red wines of the Designation of Origin (DO) Jumilla (Southeast of Spain) was evaluated. Monastrell grapes are the more representative of DO Jumilla, growing on over 80 % of the cultivated area (Regulatory Council of DO Jumilla, 2018). Monastrell grapes were separately fortified with the three fungicides at levels corresponding to two and five times their Maxima Residue Level (MRL) in grapes established in the EU regulation (Regulation (EC) Nº 396/2005 and its later modifications for these fungicides including Commission Regulations (EU) Nº 34/2013, 777/2013 and 2016/486), simulating critical agricultural practices. The dissipation of fungicides during the winemaking process and their effect on several oenological parameters and the antioxidant activity was also studied. Finally, several statistical analyses were performed in order to understand the obtained results.

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2. Materials and methods

2.1. Chemicals and standards

Analytical standards of the fungicides iprovalicarb, mepanipyrim and tetraconazole (Pestanal grade) were purchased from Supelco (Bellefonte, PA, USA). Standard of malvidin-3-*O*-glucoside chloride was purchased from Extrasynthese (Genay, Lyon, France). Solvents (residue analysis grade) were ethyl acetate, ultra-pure water and methanol from Sigma Aldrich (St. Louis, MO, USA), and ethanol from Scharlau (Barcelona, Spain). Trifluoroacetic acid was also purchased from Sigma Aldrich. Strata C18-E (2 g, 12 mL size) cartridges from Phenomenex (Torrance, CA, USA) were used for anthocyanins extraction.

2.2. Wine samples

Red grapes, *Vitis vinifera* var. Monastrell from Jumilla (Murcia, Spain), were harvested in 2016. Total amino acids concentration in Monastrell grapes was 6.5 g kg^{-1} and that of free amino acids lower than 4 g kg^{-1}; being glutamic acid (2.2 g kg^{-1}), proline (1.1 g kg^{-1}) and arginine (1.0 g kg^{-1}) the main amino acids. Grape characterization showed the following results: sugar content of 13.5 %; pH 3.27; total acidity of 4.7 g L^{-1}; 2.41 g L^{-1} of malic acid; and <0.01 g L^{-1} of gluconic acid.

Vinifications were made, in triplicate, with 8 kg of destemmed and crushed grapes in presence of fungicide residues: three with mepanipyrim, three with tetraconazole and three with iprovalicarb standard solutions at two concentrations levels corresponding to two and five times their Maxima Residue Level (MRL) on grapes (Regulation (EC) Nº 396/2005 and later modifications). A control vinification (without fungicides) was also carried out, in triplicate, for comparative purposes. Twenty one vinifications were carried out.

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Vinifications were performed as follows: crushed grapes (8 kg) were transferred to the fermentation vessels and sulphites (80 g kg^{-1}) were added. Fermentation process was lead in presence of *Saccharomyces cerevisiae* var. *bayanus* Lalvin T73™ (25 g HL^{-1}) from Lallemand Wine (Montreal, Canada). During the maceration time (10 days at 18 °C), a daily homogenization was performed to guarantee polyphenols extraction. Density and temperature were measured every day to control for delays or stoppages in the fermentation. The obtained must was pressed, and left to ferment for another 4 days. After 7 days of sedimentation, wines were transferred to other clean vessels and the lees were discarded. A clarification step was developed with bentonite (40 g HL^{-1}) and gelatin (8 g HL^{-1}) and, after 6 days wine were filtered (0.45 μm) and bottled.

2.3. Fungicide residues analysis

Fungicides were extracted from the matrix following a QuEChERS multiresidue method that use acetonitrile as extraction solvent (Martínez et al., 2015; Payá, et al., 2007). The obtained extract was acidified with formic acid and then, directly injected into the liquid chromatograph. LC-MS/MS analyses were performed following the chromatographic conditions described by Cermeño, Martínez, Oliva, Cámara & Barba (2016).

2.4. Wines characterization

2.4.1. Oenological parameters

Alcoholic degree, total acidity, volatile acidity, pH, malic and lactic acid content, glucose/fructose ratio, dry extract and total polyphenols index (TPI) were measured using an Enological Multiparametric Analyzer (FTIR-Vis-UV MultiSpec). The clarified wine samples were placed into the autosampler in 10 mL test tubes. Samples were suctioned and passed through an inert filter to prevent the entry of higher particles into the system (greater than 30

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µm). A degasser prevented air or carbon dioxide from entering into the measuring cells. Before the measurement, thermostatization of the sample at 25 ºC took place. After the measurement by the equipment, an automatic cleaning of the system took place. All wines were analyzed in duplicate.

2.4.2. Color determination

Chromatic characteristics were determined by means of a Beckman Coulter DU730 Life Science UV/Vis spectrophotometer (California, USA). After wines centrifugation (15 min at 3000 rpm) in a Rotina 35R centrifuge (Hettich Zentrifugen, Tuttlingen, Germany), spectrophotometric measures were taken using quartz cells from Hellma (Müllheim, Germany) of 1 mm and 1 cm of path length for undiluted and diluted samples, respectively. The visible spectrum (200-800 nm) was recorded ($\Delta\lambda$ = 2 nm) to calculate the colorimetric indices (% yellow, % red, % blue, tonality and color intensity) according to Glories (1984). All measurements were carried out in triplicate. A hydro-alcoholic solution (12 % ethanol) was used as blank. CIELab parameters (lightness (L^*), color components (a^* and b^*), Chroma (C_{ab}^*) and hue angle (h_{ab})) were also determined (OIV, 2016).

2.4.3. Phenolic composition and distribution

Total anthocyanins content and their distribution into the monomeric, polymeric and copigmented fractions were determined according to Boulton (1996). Monomeric anthocyanins profile was determined according to Briz-Cid et al. (2014). All the anthocyanins (malvidin, petunidin, peonidin, delphinidin and cyanidin derivatives) were quantified as malvidin-3-*O*-glucoside by a standard calibration curve, with adsorption being measured at 520 nm.

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2.4.4. Antioxidant activity

Samples were analyzed following the method reported by Brand-Williams, Cuvelier, & Berset (1995). The scavenging activity of the free radicals, using the free radical reaction DPPH˙, was evaluated. The results were expressed as equivalent mM mL^{-1} of Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a vitamin E analogous (Mulero, et al., 2015).

2.5. Multivariate statistical analysis

Partial Least Squares Regression (PLS2) analysis was used for relating CIELab color parameters (Y matrix) and anthocyanins concentrations (X matrix), through a linear multivariate model, performed with The Unscrambler software from CAMO. A cluster analysis to group variables (color parameters and phenolic concentrations) based on the squared Euclidean similitude distance by Ward's method (Ward, 1963), together with a discriminant analyses to separate wines were also performed with the statistical software package Statgraphics Centurion XVI from StatPoint Technologies Inc.

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3. Results and discussion

3.1 Fungicide dissipation

Dissipation with winemaking depended on target fungicide (**Table 1**): 97 % (in mass units) for mepanipyrim, 91-92 % for tetraconazole, and 72-74 % for iprovalicarb. Since the MRL for tetraconazole in grapes is four times lower than for the other two fungicides, their residual levels in clarified wines was decreasing as follows: 2.6-6.0 mg L^{-1} for iprovalicarb > 0.2-0.7 mg L^{-1} for mepanipyrim > 0.2-0.5 mg L^{-1} for tetraconazole.

Between 33-38 % (in mass units) of the residues of iprovalicarb and mepanipyrim, and 65-72 % of tetraconazole, were eliminated in the grape pomace after pressing. Cabras et al. (1998) already reported a high affinity of tetraconazole for the solid matter, whereas an inverse behavior was found for iprovalicarb (González-Rodríguez et al., 2009a). An additional 2-6 % was removed with the lees, whereas the clarification step had an important impact on mepanipyrim residues (a decrease of 22-24 % with respect to the wine before clarification).

For mepanipyrim, the sum of the amounts (mg) in the must-wine after pressing and grape pomace was lower than the initial amount in crushed grapes. The aqueous degradation of mepanipyrim in aqueous solutions (Calza, Medana, Baiocchi, Branca, & Pelizzetti, 2004; Anfossi, Sales, & Vanni, 2006) and in a grape juice analog (López-Fernández, Pose-Juan, Yáñez, Rial-Otero, & Simal-Gándara, 2018) could be the explanation of this finding.

Taken into account that the acceptable daily intake (ADI) for iprovalicarb, mepanipyrim and tetraconazole is 0.015, 0.012 and 0.004 mg kg^{-1} bw per day, respectively (EFSA 2008, 2015, 2017), in the case of a reference person weighing 70 kg, the maximum allowable intake would be 1.05, 0.84 and 0.28 mg day^{-1}. Thus, a moderate consumption of wine (a cup of 150 mL) represents an intake lower than 25 % of the ADI for those fungicides dissipated in a higher

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extension (mepanipyrim and tetraconazole) and 37.14 and 85.57 % of the ADI for iprovalicarb at 2MRL and 5MRL, respectively (**Table 1**).

3.2 Oenological parameters

With the evolution of must density during fermentation, it was proved that all vinifications had a regular course. Therefore, the initial fungicide levels in grapes do not inhibit yeast metabolism. Global parameters obtained for wines in presence and absence of fungicides are showed in **Table 2a**. As it can be seen in this table, statistical significant differences were found in all oenological parameters in presence of fungicides, especially in presence of iprovalicarb and mepanipyrim residues at the highest concentration assayed (5MRL). The volatile acidity of these wines (expressed as acetic acid concentration) was 8.6 times higher than that observed in the control wine. High acetic acid values in wines are considered a fault and are typically associated with the respiratory metabolism of ethanol by acetic acid bacteria (Dzialo, Park, Steensels, Lievens, & Verstrepen, 2017). However, the effect of lactic acid bacteria should be also taken into account. Lactic acid bacteria can metabolize malic acid, sugars and citric acid to produce lactic acid. However, the development of heterofermentative bacteria, such as those from the genus *Leuconostoc* and *Oenoccocus*, produce carbon dioxide, ethanol and acetic acid in addition of lactic acid (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2006). In this work a secondary malolactic fermentation happened spontaneously in presence of fungicide residues, registering a higher extension of the process for iprovalicarb and mepanipyrim. Consequently, a deacidification of high acid wines, by transformation of the malic acid into lactic acid, was observed (Liu and Pilone, 2000). Although, in general, bacterial development occurs after yeast development, the presence of fungicide residues could have helped to bacteria development by extending the lag phase of yeast. Under these conditions, with high sugar levels on the medium, an important increment of the volatile

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acidity during the malolactic fermentation could be observed (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2006).

3.3 Color changes

Most important color changes respect to the control wine were observed for iprovalicarb and mepanipyrim, especially at 5MRL (**Table 2a**). In the CIELab bidimensional space, iprovalicarb and mepanipyrim wines at 5MRLs showed a higher yellow component (**Figure 1**). A significant increment of the yellow component and a decrease of the red and blue components were also confirmed by Glories. An increment in the saturation (C_{ab}^*), in the hue angle (h_{ab}) and the tonality was also found for those wines. These results are in concordance with those reported by Briz-Cid and coworkers (2015), who observed that mepanipyrim had a high influence in the color of Tempranillo and Graciano wines. These values could be explained taken into account the lower pH values registered for these wines during the alcoholic fermentation. Malolactic fermentation increase the pH of the grape must about 0.1 - 0.2 pH units (Zamora, 2003). At this point must be stated that the pH values showed in **Table 1** corresponds with the pH of the final wines after malolactic fermentation. Some authors point out that h_{ab}, C_{ab}^* and color intensity values increased at more acidic pH independently of the aging time (Kountoudakis et al., 2011).

Colorimetric differences can be considered as visually detectable when the value of the Euclidean distance ($\Delta E_{ab}^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$) is higher than 3.0 CIELab units (Martínez, Melgosa, Pérez, Hita, & Negueruela, 2001). As it can be seen in **Table 2a**, values higher than 11 were obtained for iprovalicarb and mepanipyrim at 5MRL. Therefore, these wines were perceived as different.

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3.4 Phenolic characterization

Iprovalicarb and mepanipyrim produced significant increments on the total phenolic index and the total anthocyanins content measured by spectrophotometry, being these increments fungicide concentration dependent (**Table 2a and 2b**). For both fungicides, a different distribution of the monomeric and polymeric anthocyanins was obtained. The monomeric anthocyanins content decreased about 1.5-3.8 times with respect to the control wine while the content of polymeric forms was 1.3-1.6 times higher (**Table 2b**). For tetraconazole at 5MRL, a similar trend was observed although in a lower extension.

In addition, significant differences were also found in the total monomeric anthocyanins content determined by HPLC in the presence of iprovalicarb and mepanipyrim residues at 5MRL (**Table 2b**). Decreases of about 16.3 and 28.6 %, in the total monomeric anthocyanins content with respect to the control wine were observed for iprovalicarb and mepanipyrim, respectively. The most affected anthocyanins were the malvidin (decreases between 14.6 and 29.0 %) and petunidin (decreases between 28.1 and 35.5 %) derivatives. No significant differences were found for the other fungicide treatments (**Figure 2**). Differences on the anthocyanins content between the spectrophotometric and the chromatographic methods were observed due to only free anthocyanins are determined by HPLC while the contribution of other pigments is possible by spectrophotometry (Rivas-Gonzalo, Gutierrez, Hebrero & Santos-Buelga, 1992). Since vinifications were done at the same time and in the same way and also compared with a control vinification (without fungicides), the behavior observed should be attributed only to the presence of fungicide residues in the must.

Similar results were found in Tempranillo and Graciano wines treated with mepanipyrim, with decreases in total monomeric anthocyanins content of 24.7 – 36.4 % (Briz-Cid et al., 2015). Low monomeric anthocyanins concentrations in wines in presence of other fungicides

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(metrafenone, boscalid kresoxim-methyl, famoxadone and trifloxystrobin) was also reported (Briz-Cid et al., 2014; Mulero et al., 2015).

The addition of SO_2 to the grape must and the presence of acetic acid or lactic acid bacteria could explain the higher percentage of polymeric anthocyanins, the decrease of monomeric anthocyanins especially malvidin derivatives and the increment of the color intensity in presence of iprovalicarb and mepanipyrim at 5MRL. The addition of SO_2 to the grape must induce acetaldehyde excretion during the yeast proliferation (Herraiz, Martin-Alvarez, Reglero, Herraiz & Cabezuelo, 1989; Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2006). In addition, acetic acid bacteria can oxidize ethanol to acetaldehyde (Lafon-Lafourcade & Ribèreau, 1984; Drysdale & Fleet, 1989). The presence of acetaldehyde was associated to rapid polymerization process between anthocyanins and catechin or tannins with increased color intensity and stability (Bakker, 1986; Liu & Pilone, 2000). In fact, condensation of malvidin-3-*O*-glucoside with acetaldehyde was demonstrated in model solutions (Timberlake & Bridle, 1976).

3.5 Antioxidant activity

Fungicide residues in the must did not induce changes in the antioxidant activity of the wines in comparison to the control (11.54 mM mL^{-1} of Trolox ± 0.32) even at the highest concentration assayed (5MRL). Mulero et al. (2015) also observed that the antioxidant activity of Monastrell red wines was not altered by the presence of quinoxyfen, fluquinconazole or famoxadone residues. However, the antioxidant activity found in those wines were lower than the reported in this vintage.

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3.6. Relationship between anthocyanins and color for differentiation amongst the wines

With the aim of linking anthocyanin concentrations with color parameters, the following multivariate statistics were done. A PLS2 was used to correlate anthocyanin profiles with color data. As it can be seen in **Figure 3**, a two-factor model explaining 98 % of the variance in X (anthocyanin profiles) and 71 % of that in Y (color data) was obtained. The model was evaluated via the root mean square error for predictions (RMSEP), which was lower than 10 for Y values. The scores plot in **Figure 3a** shows how the target wines can be separated in different quadrants. PC2 can separate control wines at the bottom from the rest. To separate the treated wines it is necessary PC1; with its help, wines can be separated in the upper PC2 half in the following order form right to left: Mepan 5 > Iprov 5 > Mepan 2 = Iprov 2 = Tetra 2 > Tetra 5. All variables inside the central ellipsoid in **Figure 3b** can be correlated amongst them ($r > 0.700$). Color parameters of H (hue angle), b^*, IC, tonality and yellow% were negatively correlated with mainly CYAN and PETU derivatives and red%. All these trends were connected to Iprovalicarb and Mepanipyrim treatments at 5MRL. The rest of treated wines were more similar to control wines. A cluster analysis of the anthocyanins concentrations and the color data (**Figure 4a**) allowed obtaining, by cutting the dendrogram at a linkage distance of 450, four main clusters or groups for the same correlated variables than in **Figure 3b**.

Since the anthocyanin profiles and color data can be used to separate wines, a discriminant analysis was performed (**Figure 4b**). Iprovalicarb and mepanipyrim treatments at 5MRL can be separated from the rest of treatments using the linear discriminant function 1 (85 % of variance), mainly because of the weight of the hue angle and b*. For the discrimination between iprovalicarb 5MRL and mepanipyrim 5MRL the discriminant function 2 (9 % of the variance) can be used, with the weights of cyanidin glucoside and delphinidin coumaryl being higher for iprovalicarb 5MRL.

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4. Conclusions

High fungicide dissipation rates along of the winemaking process were obtained for .mepanipyrim and tetraconazole (higher than 91 %) while around 27 % of the iprovalicarb concentration added to the must remain in the final wine. Those wines obtained in presence of mepanipyrim and iprovalicarb residues, especially to the highest concentration assayed (5MRL), were different to the control wine. For both an increase on the volatile acidity, the lactic acid content, the total phenolic index and the percentage of polymeric anthocyanins was observed, while a decrease of the total monomeric anthocyanins was found. The color of these wines was also different (higher yellow component, color intensity, tonality and hue angle) in comparison with the control wines. Since vinifications were done at the same time and in the same way and also compared with a control vinification (without fungicides), the behavior observed should be attributed only to the presence of fungicide residues in the must that affected the yeast development and allowed a rapid development of bacteria. This is also in concordance with the high concentration of both fungicides in the must after pressing in comparison to tetraconazole. By PLS2, for the differentiation of iprovalicarb and mepanipyrim wines at 5MRL, it was found a negative correlation between the decrease in the major petunidin and the minor cyanidin derivatives and red color (%), and the increase of color parameters of hue angle, b^*, IC, tonality and yellow color (%). The discriminant function 1 allowed separating iprovalicarb and mepanipyrim treatments at 5MRL from the rest of wines, whereas the discriminant function 2 allowed the discrimination between iprovalicarb at 5MRL and mepanipyrim at 5MRL.

Conflict of interest

The authors have declared no conflict of interest.

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Table 1. Fungicide concentrations (ppm) found in the different liquid and solid phases separated along the winemaking process (average ± standard deviation) with the final dissipation (% in mass units) determined in the clarified wine.

Step	Iprovalicarb		Mepanipyrim		Tetraconazole	
	10 mg/kg (5MRL)	4 mg/kg (2MRL)	10 mg/kg (5MRL)	4 mg/kg (2MRL)	2.5 mg/kg (5MRL)	1 mg/kg (2MRL)
Must-wine after pressing (5.3 L)	6.44±0.21	3.19±0.08	1.79±0.04	0.68±0.03	0.92±0.18	0.37±0.03
Grape pomace (2.2 kg)	12.12±2.39	5.56±0.08	12.41±1.40	5.04±0.28	5.90±0.45	2.63±0.46
Wine (3.5 L)	6.23±0.31	2.86±0.43	0.87±0.05	0.33±0.01	0.46±0.04	0.20±0.01
Lees (0.5 kg)	9.26±1.56	3.17±0.03	3.22±0.08	1.34±0.06	1.59±0.14	0.78±0.09
Clarified wine (3.5 L)	5.99±0.13	2.60±0.05	0.68±0.04	0.25±0.02	0.47±0.04	0.21±0.03
ADI (%)	85.6	37.1	12.1	4.5	25.2	11.3
Fungicide dissipation (%)	71.6	73.8	97.3	97.0	90.8	91.8

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Table 2. Oenological and color parameters of the obtained wines (a) in presence and absence of fungicides (average ± standard deviation), and mean values ± standard deviation (n=3) of the anthocyanins composition and monomeric anthocyanins content (b) for the control and the treated wines.

(a)

	CONTROL	IPROVALICARB 2MRL	IPROVALICARB 5MRL	MEPANIPYRIM 2MRL	MEPANIPYRIM 5MRL	TETRACONAZOLE 2MRL	TETRACONAZOLE 5MRL
Oenological parameters							
Alcoholic grade (% vol)	$13.73^{a} \pm 0.63$	$13.95^{ab} \pm 0.07$	$13.83^{a} \pm 0.05$	$14.03^{ab} \pm 0.03$	$14.04^{ab} \pm 0.03$	$14.27^{b} \pm 0.07$	$14.34^{b} \pm 0.04$
Acidity (g/L tartaric acid)	$6.17^{a} \pm 0.22$	$6.48^{b} \pm 0.06$	$6.85^{c} \pm 0.05$	$6.61^{b} \pm 0.08$	$6.53^{b} \pm 0.07$	$6.15^{a} \pm 0.03$	$6.18^{a} \pm 0.05$
Volatile acidity (g/L acetic acid)	$0.42^{a} \pm 0.05$	$1.66^{b} \pm 0.03$	$3.62^{c} \pm 0.06$	$1.67^{b} \pm 0.04$	$3.63^{c} \pm 0.14$	$0.66^{d} \pm 0.02$	$1.10^{e} \pm 0.01$
pH	$3.43^{a} \pm 0.02$	$3.46^{b} \pm 0.01$	$3.49^{c} \pm 0.01$	$3.45^{c} \pm 0.01$	$3.46^{c} \pm 0.01$	$3.42^{a} \pm 0.01$	$3.45^{c} \pm 0.01$
Malic acid (g/L)	$1.96^{a} \pm 0.16$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.08^{b} \pm 0.09$	$0.00^{b} \pm 0.00$	$1.57^{c} \pm 0.06$	$0.85^{d} \pm 0.10$
Lactic acid (g/L)	$0.34^{a} \pm 0.04$	$3.03^{b} \pm 0.10$	$6.98^{c} \pm 0.18$	$2.93^{b} \pm 0.15$	$6.91^{c} \pm 0.23$	$0.79^{d} \pm 0.03$	$1.90^{e} \pm 0.08$
Glucose/fructose (g/L)	$0.18^{a} \pm 0.16$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.01^{b} \pm 0.01$	$0.00^{b} \pm 0.00$
Dry extract (g/L)	$24.05^{a} \pm 0.58$	$27.45^{b} \pm 0.35$	$32.70^{c} \pm 0.54$	$27.07^{cd} \pm 0.55$	$33.52^{e} \pm 0.29$	$24.55^{a} \pm 0.37$	$26.30^{d} \pm 0.21$
Total Phenol Index (TPI)	$44.29^{a} \pm 0.57$	$45.48^{ab} \pm 0.80$	$47.36^{cd} \pm 0.48$	$45.73^{b} \pm 0.97$	$48.33^{d} \pm 0.24$	$44.37^{a} \pm 0.66$	$46.41^{bc} \pm 0.94$
Colorimetric indexes							
% yellow	$32.48^{a} \pm 0.25$	$32.92^{b} \pm 0.31$	$34.25^{c} \pm 0.06$	$33.26^{b} \pm 0.23$	$34.20^{c} \pm 0.19$	$32.94^{b} \pm 0.15$	$33.15^{b} \pm 0.16$
% red	$54.13^{a} \pm 0.24$	$53.73^{b} \pm 0.28$	$53.23^{d} \pm 0.13$	$53.65^{bc} \pm 0.19$	$53.32^{cd} \pm 0.08$	$53.90^{ab} \pm 0.23$	$54.02^{ab} \pm 0.27$
% blue	$13.39^{a} \pm 0.39$	$13.35^{a} \pm 0.48$	$12.52^{c} \pm 0.11$	$13.10^{ab} \pm 0.08$	$12.47^{c} \pm 0.12$	$13.16^{ab} \pm 0.16$	$12.83^{bc} \pm 0.26$
Tonality	$60.00^{a} \pm 0.46$	$61.27^{bc} \pm 0.57$	$64.34^{d} \pm 0.24$	$61.99^{c} \pm 0.63$	$64.14^{d} \pm 0.44$	$61.10^{b} \pm 0.50$	$61.36^{bc} \pm 0.50$
Color intensity	$12.09^{ab} \pm 0.22$	$12.90^{cd} \pm 0.46$	$12.98^{cd} \pm 0.37$	$12.80^{c} \pm 0.64$	$13.46^{d} \pm 0.23$	$11.87^{a} \pm 0.18$	$12.54^{bc} \pm 0.20$
CIELab space							
a*	$50.46^{a} \pm 0.17$	$50.24^{a} \pm 0.38$	$49.22^{b} \pm 0.62$	$50.19^{ab} \pm 0.98$	$49.98^{ab} \pm 0.27$	$49.91^{ab} \pm 0.42$	$50.84^{a} \pm 0.64$
b*	$5.33^{a} \pm 0.36$	$9.18^{b} \pm 0.59$	$16.34^{c} \pm 0.82$	$9.73^{b} \pm 0.78$	$17.21^{c} \pm 0.18$	$5.72^{a} \pm 0.29$	$8.47^{b} \pm 0.90$
L*	$45.97^{ab} \pm 0.79$	$44.53^{a} \pm 1.08$	$45.78^{ab} \pm 0.78$	$45.03^{a} \pm 1.59$	$44.77^{a} \pm 0.67$	$46.90^{b} \pm 0.52$	$45.65^{ab} \pm 0.33$
C_{ab}*	$50.68^{ab} \pm 0.22$	$51.07^{abc} \pm 0.43$	$51.87^{cd} \pm 0.84$	$51.13^{abc} \pm 1.10$	$52.86^{d} \pm 0.23$	$50.24^{a} \pm 0.40$	$51.55^{bc} \pm 0.70$
h_{ab}	$6.03^{a} \pm 0.40$	$10.35^{bc} \pm 0.62$	$18.36^{d} \pm 0.67$	$10.96^{c} \pm 0.68$	$19.00^{d} \pm 0.24$	$6.54^{a} \pm 0.35$	$9.45^{b} \pm 0.96$
ΔE_{ab}*		4.12	11.08	4.50	11.95	1.15	3.18

^{a,b,c,d,e}: statistical differences according to the *ANOVA* test ($p < 0.05$)

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(b)

Wines	CONTROL	IPROVALICARB 2MRL	IPROVALICARB 5MRL	MEPANIPYRIM 2MRL	MEPANIPYRIM 5MRL	TETRACONAZOLE 2MRL	TETRACONAZOLE 5MRL
Anthocyanins by UV/Vis							
Monomeric (%)	41.52^{a} ± 1.89	27.76^{b} ± 2.83	13.41^{c} ± 1.45	24.90^{b} ± 0.79	10.72^{c} ± 1.18	39.30^{a} ± 1.14	34.02^{d} ± 1.43
Copigmented (%)	15.14^{a} ± 2.08	14.17^{a} ± 1.40	15.52^{ab} ± 2.17	18.80^{b} ± 0.81	14.76^{a} ± 2.55	15.26^{a} ± 1.86	14.66^{a} ± 1.62
Polymeric (%)	42.22^{a} ± 2.45	56.80^{b} ± 0.70	71.06^{c} ± 0.83	56.30^{b} ± 1.06	72.82^{c} ± 0.48	45.44^{d} ± 0.83	51.32^{e} ± 0.99
TOTAL Anthocyanins (absorbance units)	6.36^{ab} ± 0.12	6.71^{ac} ± 0.11	6.86^{cd} ± 0.21	6.73^{c} ± 0.37	7.09^{d} ± 0.16	6.23^{b} ± 0.11	6.66^{ac} ± 0.16
Monomeric anthocyanins by HPLC							
Malvidin derivatives							
malvidin-3-*O*-glucoside	60.20^{abc} ± 4.19	51.61^{cd} ± 5.41	50.14^{d} ± 2.56	54.35^{bcd} ± 2.12	40.56^{e} ± 3.89	60.53^{ab} ± 4.10	66.34^{a} ± 6.38
malvidin-3-*O*-(6-*O*-acetyl)glucoside	0.87^{ab} ± 0.06	0.78^{a} ± 0.09	0.85^{ab} ± 0.04	0.83^{ab} ± 0.06	0.81^{ab} ± 0.08	0.87^{ab} ± 0.08	0.94^{a} ± 0.12
vitisin A	2.39^{ab} ± 0.17	2.28^{ab} ± 0.14	2.32^{ab} ± 0.22	2.25^{ab} ± 0.21	2.39^{a} ± 0.34	1.98^{b} ± 0.09	2.13^{ab} ± 0.11
vitisin B	1.92^{a} ± 0.12	2.63^{b} ± 0.30	2.48^{b} ± 0.11	2.33^{ab} ± 0.17	2.64^{b} ± 0.39	2.26^{ab} ± 0.10	2.45^{b} ± 0.26
subTOTAL (mg L^{-1}) (%)	**65.38^{abc} ± 3.99**	**57.30^{cd} ± 5.30**	**55.80^{d} ± 2.57**	**59.75^{bcd} ± 2.10**	**46.40^{e} ± 4.34**	**65.64^{ab} ± 4.11**	**71.85^{a} ± 6.73**
Petunidin derivatives							
petunidin-3-*O*-glucoside	13.94^{ab} ± 0.49	11.38^{cd} ± 1.25	10.64^{cd} ± 0.72	11.88^{ac} ± 0.40	9.47^{d} ± 1.46	13.86^{ab} ± 0.56	14.92^{b} ± 1.89
petunidin-3-*O*-(6-*O*-acetyl)glucoside	2.53^{a} ± 0.35	1.98^{b} ± 0.28	1.19^{c} ± 0.05	1.16^{c} ± 0.03	1.15^{c} ± 0.20	1.19^{c} ± 0.03	1.17^{c} ± 0.03
subTOTAL (mg L^{-1}) (%)	**16.46^{a} ± 0.56**	**13.36^{bc} ± 1.52**	**11.83^{cd} ± 0.71**	**13.05^{bc} ± 0.43**	**10.61^{d} ± 1.75**	**15.05^{ab} ± 0.58**	**16.09^{a} ± 1.91**
Delphinidin derivatives							
delphinidin-3-*O*-glucoside	7.50^{ab} ± 0.19	6.66^{bc} ± 0.71	6.04^{cd} ± 0.34	6.86^{abc} ± 0.32	5.46^{d} ± 0.82	7.88^{ae} ± 0.31	8.82^{e} ± 0.93
delphinidin-3-*O*-(6-*O*-p-coumaroyl)glucoside	3.89^{ab} ± 0.53	3.51^{abc} ± 0.69	3.36^{abc} ± 0.26	3.28^{bc} ± 0.36	2.82^{c} ± 0.35	3.66^{abc} ± 0.51	4.24^{a} ± 0.68
delphinidin-3-*O*-(6-*O*-acetyl)glucoside	0.94^{a} ± 0.03	0.83^{a} ± 0.05	0.91^{a} ± 0.04	0.82^{a} ± 0.07	0.91^{a} ± 0.13	0.90^{a} ± 0.08	0.92^{a} ± 0.04
subTOTAL (mg L^{-1}) (%)	**12.34^{abc} ± 0.72**	**11.00^{bcd} ± 1.33**	**10.31^{cd} ± 0.60**	**10.95^{bcd} ± 0.42**	**9.20^{d} ± 1026**	**12.34^{ab} ± 0.68**	**13.98^{a} ± 1.53**
Peonidin derivatives							
peonidin-3-*O*-glucoside	5.40^{ab} ± 0.26	5.59^{ab} ± 0.65	5.34^{ab} ± 0.21	5.57^{ab} ± 0.27	4.84^{a} ± 0.74	6.01^{bc} ± 0.34	6.63^{c} ± 0.63
peonidin-3-*O*-(6-*O*-acetyl)glucoside	0.70^{a} ± 0.09	0.42^{b} ± 0.06	0.41^{b} ± 0.03	0.42^{b} ± 0.02	0.30^{c} ± 0.02	0.60^{a} ± 0.05	0.60^{a} ± 0.06
peonidin-3-*O*-(6-*O*-p-coumaroyl)glucoside	0.05^{ab} ±≤ 0.01	0.05^{ab} ± 0.01	0.05^{a} ±≤ 0.01	0.05^{ab} ±≤ 0.01	0.05^{ab} ±≤ 0.01	0.05^{ab} ±≤ 0.01	0.04^{b} ±≤ 0.01
subTOTAL (mg L^{-1}) (%)	**6.15^{ab} ± 0.27**	**6.06^{ab} ± 0.69**	**5.80^{ab} ± 0.23**	**6.03^{ab} ± 0.26**	**5.19^{a} ± 0.74**	**6.66^{bc} ± 0.35**	**7.27^{c} ± 0.68**
Cyanidin derivatives							
cyanidin-3-*O*-glucoside	1.50^{ab} ± 0.02	1.51^{ab} ± 0.15	1.49^{ab} ± 0.06	1.60^{a} ± 0.07	1.29^{b} ± 0.15	1.85^{c} ± 0.09	2.12^{d} ± 0.19
cyanidin-3-*O*-(6-*O*-acetyl)glucoside	0.74^{ab} ± 0.05	0.84^{a} ± 0.12	0.69^{bc} ± 0.09	0.69^{bc} ± 0.03	0.57^{cd} ± 0.04	0.48^{d} ± 0.06	0.53^{d} ± 0.04
cyanidin-3-*O*-(6-*O*-p-coumaroyl)glucoside	0.54^{a} ± 0.06	0.46^{ab} ± 0.04	0.41^{bc} ± 0.02	0.45^{abc} ± 0.02	0.38^{c} ± 0.04	0.49^{a} ± 0.03	0.53^{a} ± 0.06
subTOTAL (mg L^{-1}) (%)	**2.78^{a} ± 0.06**	**2.80^{a} ± 0.22**	**2.59^{a} ± 0.11**	**2.74^{a}** ± 0.08	**2.25^{b} ± 0.14**	**2.82^{a} ± 0.12**	**3.17^{c} ± 0.25**
TOTAL monomeric anthocyanins (mg L^{-1})	**103.11^{ab} ± 4.70**	**90.52^{bc} ± 8.57**	**86.33^{c} ± 4.01**	**92.53^{bc} ± 2.47**	**73.64^{d} ± 7.58**	**102.60^{ab} ± 5.22**	**112.36^{a} ± 10.87**

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Figure captions

Figure 1. CIELab space for the control and treated wines (left), showing how iprovalicarb and mepanipyrim treatments at 5MRL showed a higher yellow component, giving as a result brighter wines (right).

Figure 2. Results correspond to mean ± standard deviation (n=3) of anthocyanin compounds (mg L^{-1}) extracted from the control and treated wines.

Figure 3. Two-dimensional PLS2: (a) scores plot for control and treated wines, together with (b) correlations between the loadings of X (anthocyanin profiles in blue) and Y variables (color parameters in red).

Figure 4. Dendrogram grouping variables according to the squared Euclidean similitude distance by Ward's method (a). Four groups can be detected by cutting the dendrogram at a linking distance of about 450; from left to right, they are the same correlated variables than in Fig. 3b: upper-left, down-left, down-right, and upper-right quartiles, which is the one with the color parameters more affected by iprovalicarb and mepanipyrim at 5MRL. The rest of treated wines were more similar to control wines. And discriminant biplot (b) for the classification variables of anthocyanin and color values.

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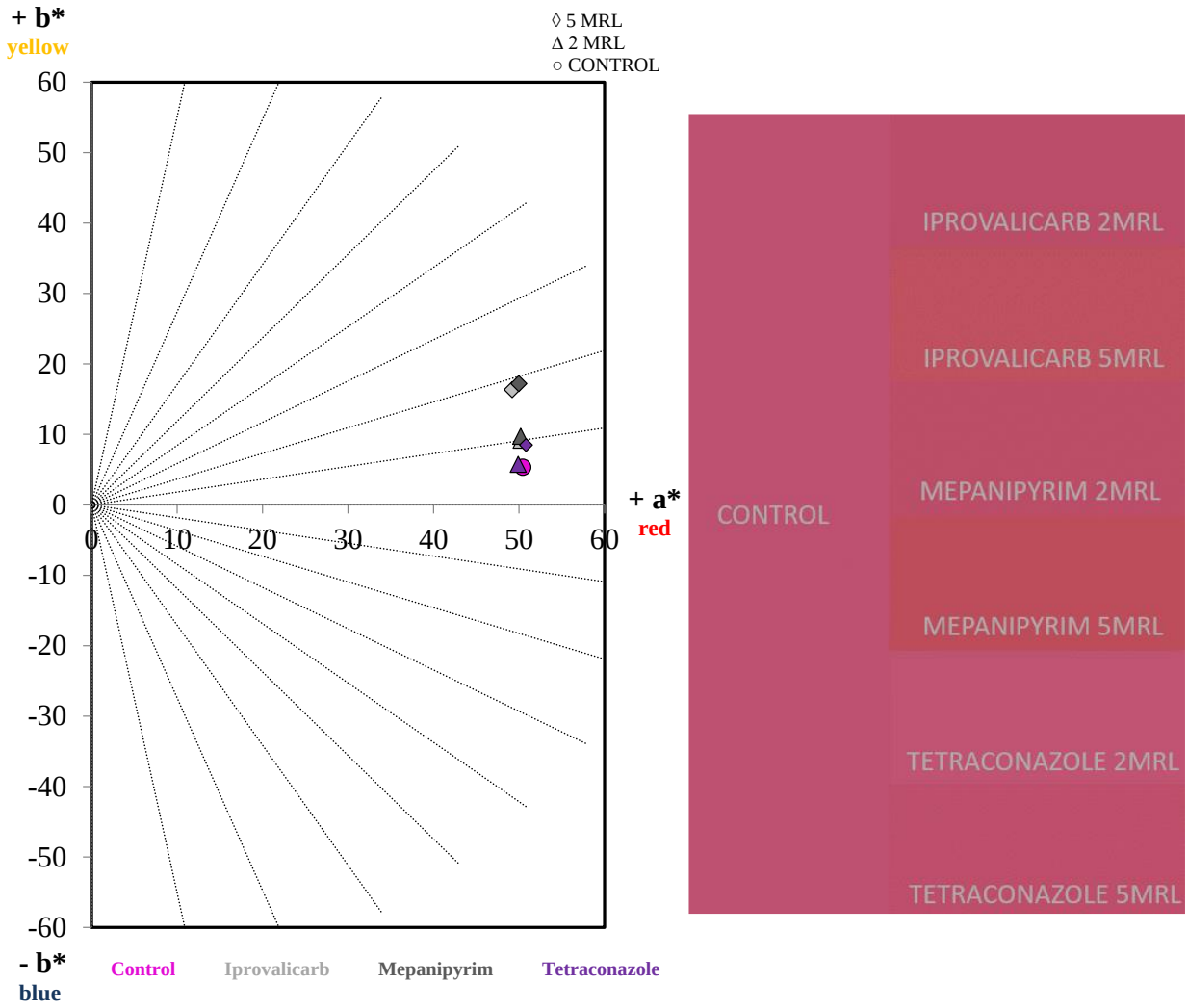

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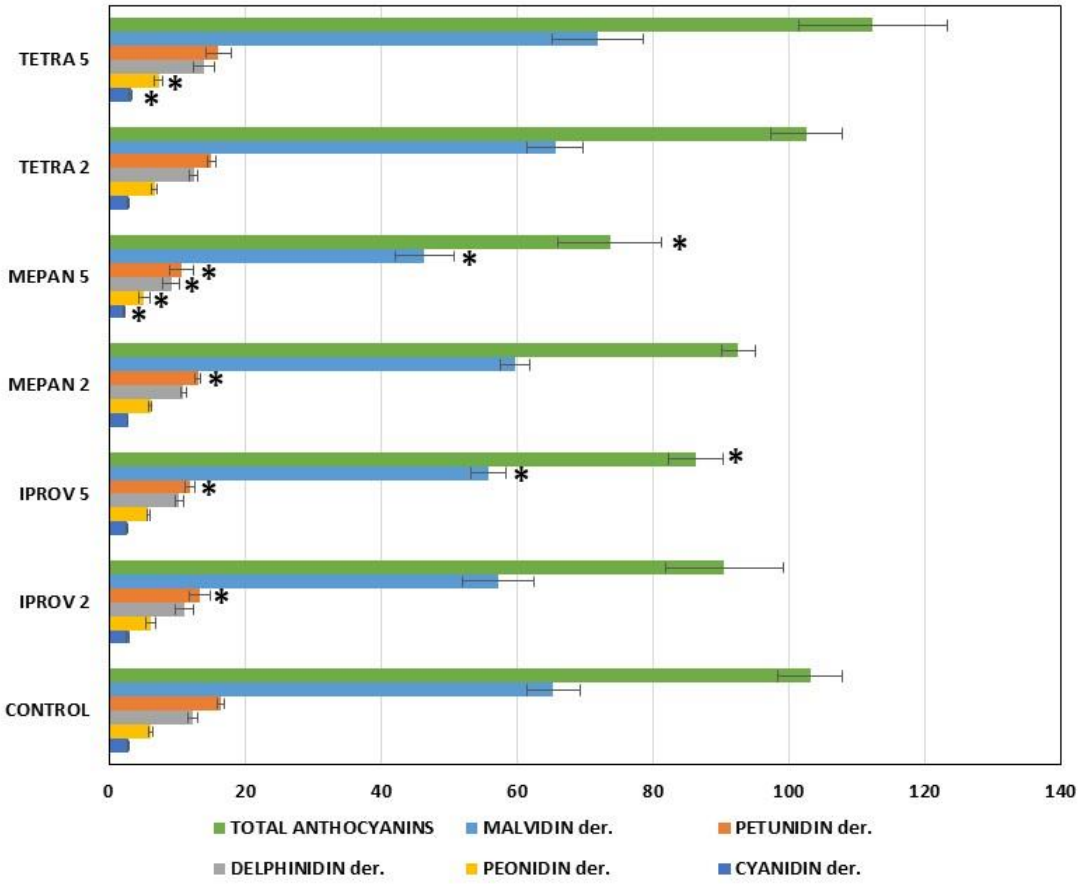

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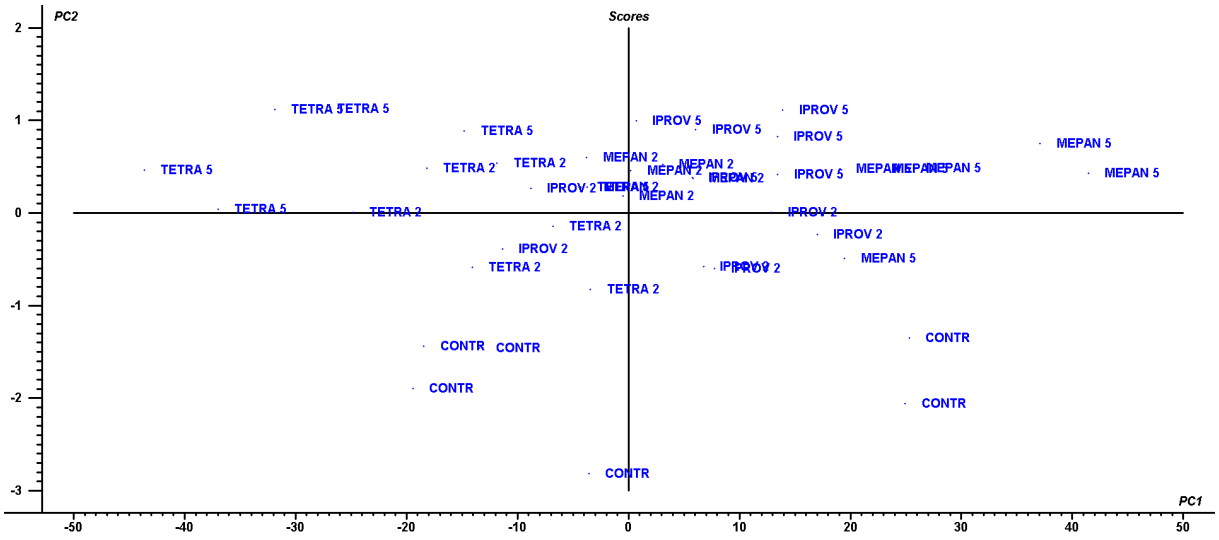
(a)



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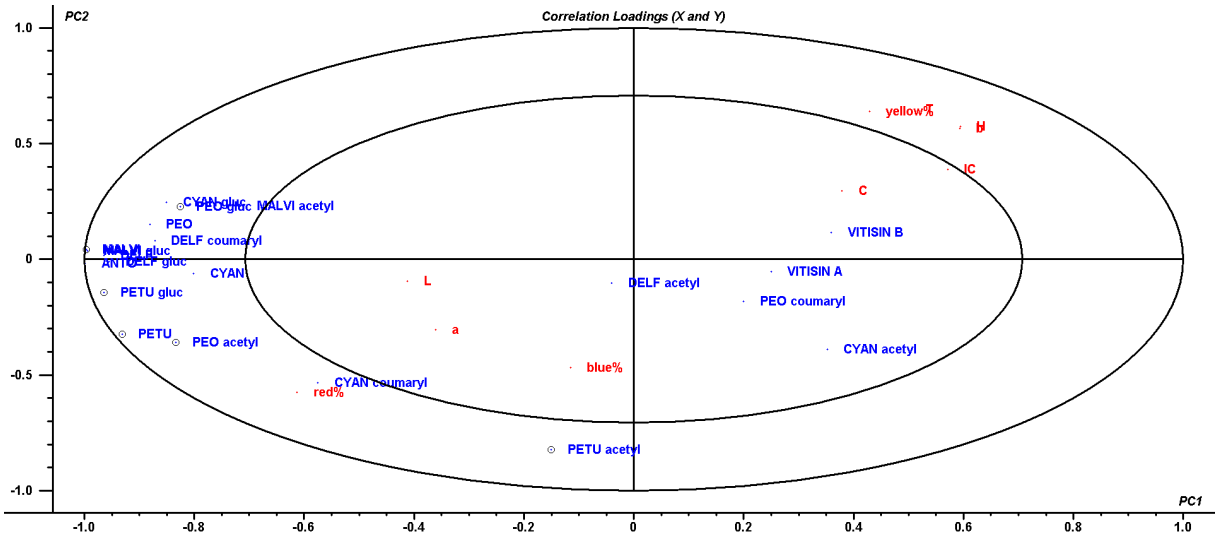

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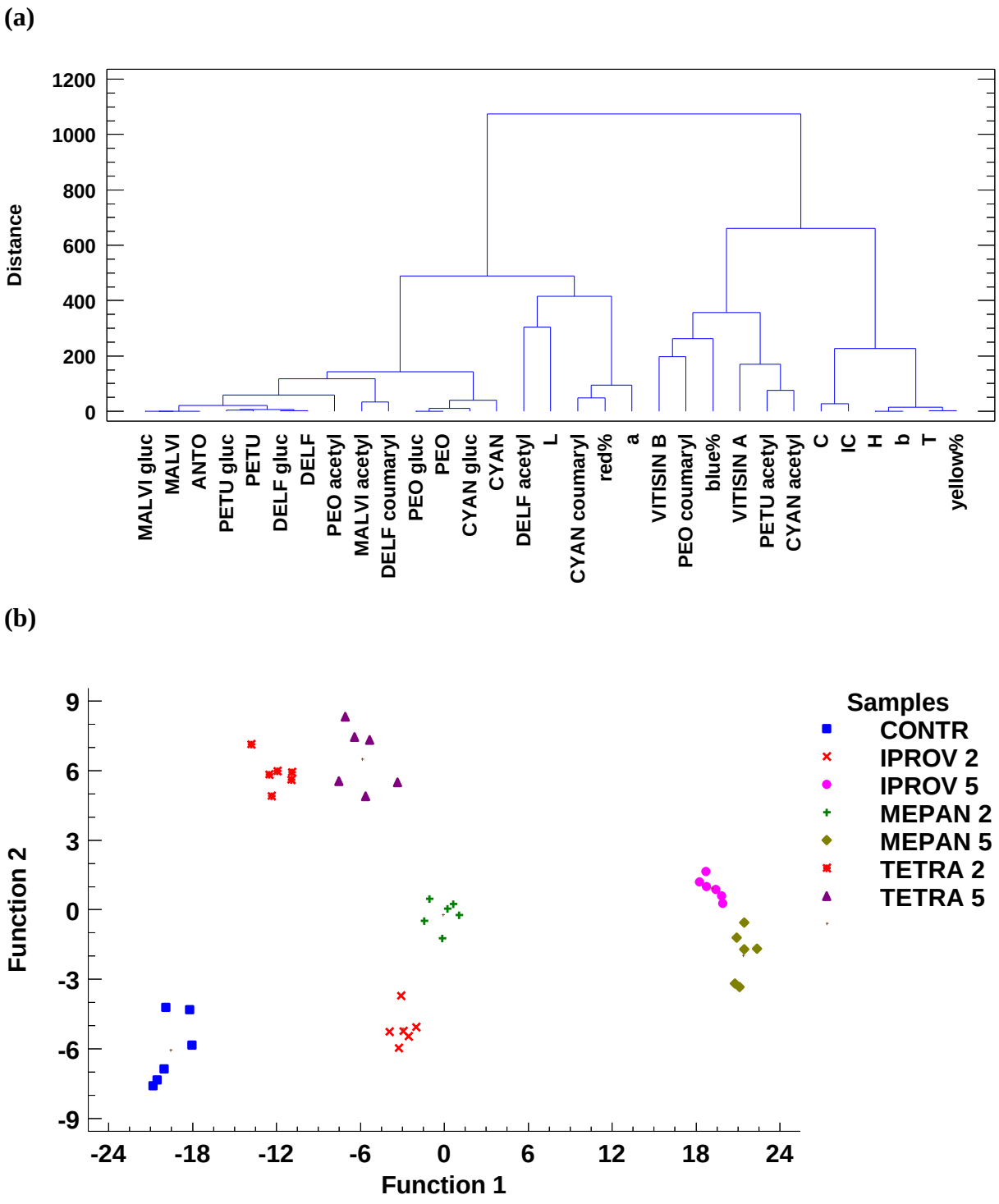

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