EFFECT OF USING GLUTATHIONE-ENRICHED INACTIVE DRY YEAST PREPARATIONS ON THE PHENOLIC COMPOSITION OF ROSÉ GRENACHE WINES DURING WINEMAKING

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Abstract

Aim: To know the effect of the addition of a commercial glutathione-enriched Inactive Dry Yeast (G-IDY) oenological preparation on the phenolic profile and colour parameters of rosé Grenache wines.

Methods and results: A Control wine (Cont-W) and a wine with the G-IDY preparation (G-IDY-W) were industrially manufactured. The evolution of the phenolic composition (anthocyanins and non-anthocyanins) and colour of both types of wines was evaluated during winemaking and their shelf-life (after 1, 2, 3 and 9 months of bottle aging). Results revealed that wines manufactured with the G-IDY preparation showed differences in both their phenolic composition and colour characteristics with respect to the control wines, particularly after 9 months of aging. These differences were more evident in the anthocyanin than in the non-anthocyanin compounds.

Conclusions: The G-IDY wines showed a greater decrease of the anthocyanins from grape origin, probably due to the formation of anthocyanin-polysaccharide complexes, and a higher concentration of some anthocyanin-derived pigments. These changes can be related to the slower colour evolution determined in wines produced using G-IDY preparations.

Significance and impact of the study: The addition of the G-IDY preparation during winemaking modifies the anthocyanin composition of the resulting wines, which seems to provoke a slower colour evolution during their shelf-life.

Key words: Grenache rosé wines, glutathione, Inactive Dry Yeast preparations, anthocyanins, non-anthocyanin compounds, colour

Résumé

Objectif : Étudier l'effet de l'ajout des dérivés de levures sèches inactives riches en glutathion (G-IDY) sur le profil phénolique ainsi que les paramètres de couleur des vins rosés au cours des neuf mois d' élevage.

Méthodes et résultats: Deux types de vinification des raisins de Grenache ont été réalisés industriellement pour obtenir un vin témoin (Cont-W) et un vin où a été réalisée une addition de G-IDY sur le moût au début de la fermentation alcoolique (G-IDY-W). L'évolution de la composition phénolique (anthocyanes et non-anthocyanes) et la couleur des deux types de vins ont été évaluées lors de la vinification et de la durée de conservation (après un, deux, trois et neuf mois de vieillissement en bouteilles). Les résultats ont révélé que les vins obtenus avec la préparation G-IDY montraient des différences à la fois dans leur composition phénolique et les caractéristiques de couleur par rapport aux vins témoins, en particulier après neuf mois de vieillissement. Ces différences étaient plus marquées pour les anthocyanes que pour les composés non-anthocyanes.

Conclusions: Les vins G-IDY ont montré une diminution plus importante des anthocyanes du raisin, probablement due à la formation de complexes entre les anthocyanes et les polysaccharides, et une concentration plus élevée de certains pigments dérivés d'anthocyanes. Ces changements peuvent être liés à l'évolution plus lente de la couleur, déterminée dans les vins produits en utilisant les préparations G-IDY.

Signification et impact de l'étude : L'ajout de la préparation G-IDY lors de la vinification modifie la composition anthocyanique des vins qui en résultent, ce qui semble provoquer une évolution plus lente de la couleur au cours de leur durée de conservation.

Mots clés: vin rosé de Grenache, glutathion, préparations de levures sèches inactives, anthocyanes, composés non-anthocyanes, couleur

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INTRODUCTION

Currently, Inactive Dry Yeast (IDY) preparations are widely used in the winemaking industry. These preparations are obtained by growing *Saccharomyces cerevisiae* yeasts in a sugar rich medium. Afterwards, yeasts are autolysed and dried, the final product being obtained in a powder form (Pozo-Bayón *et al*., 2009a). Therefore, the composition of these preparations may include a soluble fraction coming from the yeast cytoplasm (amino acids, peptides, etc.) and/or an insoluble fraction corresponding to the yeast cell walls (Pozo-Bayón *et al*., 2009b). Depending on their composition, IDY preparations are recommended for different applications in the winemaking industry (Pozo-Bayón *et al*., 2009a). They are mainly used to enhance malolactic and alcoholic fermentations, acting as nutrients (Pozo-Bayón *et al*., 2009a). Other IDY preparations are, however, devoted to being used for enhancing wine organoleptic properties. For example, some of them are specifically formulated to reduce the astringency of some wines. These preparations are characterized by a large amount of polysaccharides that can be released in the wine and form polysaccharide-proanthocyanidin complexes, reducing the number of free proanthocyanidins available to interact with salivary proteins and minimizing the loss of lubrication caused by the formation of these complexes (Escot *et al*., 2001 ; Feuillat, 2001). In addition, the presence of polysaccharides in wines can have an effect on wine viscosity, compensating for the loss of lubrication produced by the precipitation of proanthocyanidin salivary protein aggregates, thereby decreasing wine astringency and improving wine mouth-feel and body properties.

Since colour is one of the most important sensory characteristics of wines, there are some IDY preparations than can preserve the colour of wines during storage. This is because of the ability of yeast mannoproteins to interact with tannins and anthocyanins, thereby avoiding or minimizing polyphenol aggregation and precipitation (Escot *et al*., 2001), which usually occurs during wine aging. Besides the above-mentioned action mechanisms, some other IDY preparations may influence colour stability because of their involvement in the oxidation status of white and rosé wines. These preparations contain higher amounts of glutathione (GSH) than the IDY preparations used for other winemaking applications (Andújar-Ortiz *et al*., 2012). GSH is a yeast intracellular tripeptide (γ-L-glutamyl-L-cysteinylglycine) of non-proteinaceous origin with known antioxidant properties. Lavigne-Cruège *et al*. (2003) have shown that the addition of GSH before bottling reduces wine oxidation and colour deterioration and prevents the loss of varietal aroma compounds. It has also been shown that GSH added to oxygenated musts avoids must browning and leads to wines of acceptable and stable colour (Vaimakis and Roussis, 1996). This can be explained by the ability of GSH to react with *o*-quinones resulting from the oxidation of must and wine polyphenols, giving rise to the formation of the grape reaction product (2-S-glutathionyl caftaric acid) (GRP) (Cheynier *et al*., 1990), a less reactive compound, whose formation might prevent the progress of must and wine oxidation reactions.

However, the effect of the addition of glutathione-enriched IDY (G-IDY) preparations on the phenolic composition and colour of wines has not been studied so far. Only some recent works have studied the effect of adding a commercial mannoprotein-rich IDY preparation on the colour and on the total content of anthocyanins, hydroxycinnamic acids and proanthocyanidins of red (Guadalupe *et al*., 2007, 2008, 2010 ; Nikfardjam and Pickering, 2008) and white (Barrio-Galán *et al*., 2011) wines. In these studies, the mannoproteins released from the IDY preparations neither maintained the polyphenols in colloidal dispersion nor ensured colour stability (Guadalupe *et al*., 2007, 2008, 2010) in red wines, as previously suggested (Escot *et al*., 2001). In fact, these authors suggested the formation of coaggregates mannoprotein-tannin and mannoprotein-pigment that precipitated. On the other hand, in white wines, the addition of mannoprotein-rich IDY preparations led to a reduction of the colour intensity and the browning of the wines immediately after the treatment (Barrio-Galán *et al*., 2011). However, in both types of wines, it has been shown that the treatment with commercial yeast derivative products gives rise to wines with better sensorial characteristics than non-treated wines (Guadalupe *et al*., 2007 ; Barrio-Galán *et al*., 2011)

As previously mentioned, the G-IDY preparations are specifically formulated to enhance the stability of the colour of white and rosé wines during aging. However, as there are no previous studies that confirm these effects, the objective of this work was to discover the real effect of the addition of a G-IDY preparation on the phenolic composition (anthocyanic and non-anthocyanic compounds) and colour characteristics of industrially manufactured rosé Grenache wines during winemaking and bottle aging.

MATERIALS AND METHODS

1. Industrial winemaking

Industrial vinifications from monovarietal Grenache rosé grapes (2008 vintage) were performed in a cellar from the D.O.. Navarra, Spain. To do so, two 10,000 L tanks were filled with the same must. G-IDY wines (G-IDY-W) were prepared by directly adding the recommended dosage (20 g·HL^{-1}) of the commercial G-IDY powder to the must (Agrovín, Alcázar de San Juan, Spain), whereas Control wines (Cont-W) were made from

the same must but without G-IDY. To carry out the alcoholic fermentation, the same active dry yeast was used for both vinifications. General parameters of must and wines after alcoholic fermentation (probable alcohol degree of musts, total acidity, volatile acidity, alcohol degree of wines) were supplied by the winemakers (Table 1). From these determinations, it was concluded that the finished wines had values considered to be in the normal range for these types of wines. Afterwards, all the wines were stabilized and clarified in their own cellar and sent to our laboratory where they were kept at 12 ºC in the dark for 9 months. Analysis of the phenolic composition and colour of the must and wines was carried out once alcoholic fermentation was completed and during wine aging (after 1, 2, 3 and 9 months of aging in the bottle).

2. Analysis of anthocyanic compounds by RP-HPLC-DAD and RP-HPLC-ESI-MS

The analysis of anthocyanic compounds was carried out by RP-HPLC according to Monagas *et al*. (2005a) with some modifications. The equipment used was a Waters liquid chromatograph (Milford, MA) equipped with a 600-MS controller, a 707 Plus automatic injector and a 996 diode array detector (DAD) (RP-HPLC-DAD). Data were treated using the Waters Empower program (version 5.0). Separation was carried out in an RP Waters Nova-Pak C18 column (250 mm x 4.6 mm i.d.; 4 µm). Phase A was composed of water: formic acid (90: 10, v/v) and phase B was composed of methanol (HPLC grade). The flow rate was 0.7 mL·min^{-1}, and gradient was applied as follows: 15-20 % B linear from 0 to 5 min, 20-50 % B linear from 5 to 70 min, 50-100 % B linear from 70 to 75 min, followed by washing (methanol) and re-equilibration of the column from 75 to 105 min. Wine samples (190 µL), previously filtered through a 0.45 µm-membrane, were injected into the column. Detection of anthocyanic compounds was achieved between 260 and 600 nm, and quantification was carried out at 530 nm by external standard and expressed as mg·L^{-1} of malvidin-3-glucoside (Extrasynthèse, Genay, France). Determinations were made in duplicate.

In addition to their UV spectra, the identification of some phenolic compounds was also carried out by mass spectrometry coupled to HPLC. A Hewlett-Packard series 1100 (Palo Alto, CA) chromatography system, equipped with a DAD and a quadrupole mass spectrometer (Hewlett-Packard series 1100 MSD) with an electrospray interface (RP-HPLC-ESI-MS), was used. The gradient and the column used were the same as previously described for the RP-HPLC-DAD analysis, but using a flow rate of 0.5 mL·min^{-1}. The ESI source was operated in positive mode using the following parameters: drying gas (N_2) flow and temperature, 10 L·min^{-1} and 350 ºC, respectively; nebulizer pressure, 55 psi; and capillary voltage, 4000 V. Mass spectra were obtained using in-source collision-induced dissociation mass spectrometry, scanning negative ions from m/z 100 to 2000 using the following fragmentation program: from m/z 0 to 200 (150 V) and from m/z 200 to 2000 (300 V).

3. Analysis of non-anthocyanic compounds by RP-HPLC-DAD

In the analysis of non-anthocyanic compounds the same column and chromatograph as described above were used, following the protocol previously described by Monagas *et al*. (2005b). Briefly, a gradient consisting of solvent A (water/acetic acid, 98: 2, v/v) and solvent B (water/acetonitrile/acetic acid, 78: 20:2, v/v/v) was applied as follows: 0-80 % B linear from 0 to 55 min, 80-90 % B linear from 55 to 57 min, 90 % B isocratic from 57 to 70 min, 90-95 % B linear from 70 to 80 min, 95-100 % B from 80 to 90 min, followed by washing (methanol) and re-equilibration of the column from 90 to 120 min. Diode array detection (DAD) was performed from 220 to 360 nm. Wine samples (25 µL), previously filtered through a 0.45 μm-membrane, were injected into the column. Quantification was carried out by external standard calibration curves calculated at 280 nm. Pure phenolic compounds were purchased from Sigma (St. Louis, MO, USA) (gallic acid, caffeic acid, resveratrol and (+)-catechin), Extrasynthèse (Genay, France) (quercetin-3-*O*-glucoside and methyl ferulate), Fluka (Buchs, Switzerland) (-)-epicatechin and *p*-coumaric acid) and Aldrich (Steinheim, Germany) (tyrosol). Due to the lack of commercial standards, hydroxycinnamic derivatives were quantified using the free acid (caffeic and *p*-coumaric acids) calibration curve and procyanidins

Table 1 - Evolution of global composition during the winemaking of rosé Grenache wines

		pH	TA[a]	PAG[b]	AG[c]	VA[d]
Must		3.20	3.70	13.09	-	-
Cont-W[e]	After alcoholic fermentation	3.13	4.20	-	13.80	0.16
G-IDY-W[f]	After alcoholic fermentation	3.18	4.00	-	13.60	0.22

[a] Total acidity (g. L^{-1} sulphuric acid), [b] Probable alcohol degree (must, % v/v), [c] Alcohol degree (wines, % v/v), [d] Volatile acidity (g.L^{-1} acetic acid),
[e] Cont-W: control wine tank, [f] G-IDY-W: wine supplemented with the glutathione-enriched IDY preparation

Table 2. Concentration of anthocyanic compounds (mean±standard deviation, mg.L^{-1}) determined in the must, Control wines (Cont-W) and wines supplemented with the G-IDY preparation (G-IDY-W) immediately after alcoholic fermentation (0m) and 1, 2, 3 and 9 months of aging (1m, 2m, 3m and 9m, respectively)

	Must	Cont-W					G-IDY-W				
		0m	1m	2m	3m	9m	0m	1m	2m	3m	9m
Anthocyanins of grape origin											
Delphinidin-3-glucoside	13.50±0.02	1.38c±0.00	1.50^{e}±0.02	1.46^{d}±0.01	1.34^{b}±0.01	1.26^{a}±0.01	2.26^{d}±0.02	2.04^{c}±0.04	2.04^{c}±0.03	1.72^{b}±0.01	1.46^{a}±0.01
Cyanidin-3-glucoside	4.62±0.02	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
Petunidin-3-glucoside	12.10±0.01	2.76^{bc}±0.02	2.99^{c}±0.27	2.93^{bc}±0.05	2.62^{ab}±0.01	2.37^{a}±0.02	3.59^{d}±0.01	3.28^{c}±0.10	3.20^{c}±0.06	2.64^{b}±0.02	2.12^{a}±0.01
Peonidin-3-glucoside	24.30±0.04	1.40^{b}±0.01	1.46^{c}±0.02	1.49^{c}±0.02	1.37b±0.00	1.27^{a}±0.01	2.01d±0.00	1.82^{c}±0.06	1.87^{c}±0.02	1.65^{b}±0.01	1.34a±0.00
Malvidin-3-glucoside	79.70±0.24	33.22^{c}±0.44	38.10^{e}±0.07	35.92^{d}±0.03	31.41^{b}±0.06	27.92a±0.00	35.63^{e}±0.07	34.93^{d}±0.32	32.92^{c}±0.04	26.45^{b}±0.27	20.22^{a}±0.02
Peonidin-3-(6-*p*-acetyl)-glucoside	1.07±0.07	0.43c±0.0.00	0.44d±0.00	0.44^{d}±0.01	0.39b±0.00	0.35a±0.00	0.44^{c}±0.01	0.43^{c}±0.01	0.42c±0.00	0.37b±0.00	0.31a±0.00
Malvidin-3-(6-*p*-acetyl)-glucoside	3.24±0.01	1.38^{c}±0.02	1.63^{d}±0.05	1.66^{d}±0.05	1.26^{b}±0.01	1.05^{a}±0.01	1.54c±0.00	1.52^{c}±0.04	1.46^{c}±0.12	1.12^{b}±0.01	0.82^{a}±0.02
Peonidin-3-(6-*p*-coumaroyl)-glucoside	1.44±0.01	0.51e±0.00	0.48d±0.00	0.47^{c}±0.01	0.41b±0.00	0.40a±0.00	0.51e±0.00	0.42^{d}±0.01	0.40c±0.00	0.34b±0.00	0.29^{a}±0.01
Malvidin-3-(6-*p*-coumaroyl)-glucoside	5.26±0.00	1.70^{d}±0.01	1.74^{e}±0.01	1.60^{c}±0.02	1.36b±0.00	1.25a±0.00	1.75^{e}±0.01	1.44^{d} ±0.02	1.30^{c}±0.01	1.02^{b}±0.01	0.77^{a}±0.01
Malvidin-3-(6-caffeoyl)-glucoside	0.72±0.03	0.30^{b}±0.01	0.32c±0.00	0.38^{d}±0.01	0.30b±0.00	0.29^{a}±0.01	0.31^{cd}±0.01	0.30c±0.00	0.31d±0.00	0.26^{b}±0.01	0.23^{a}±0.01
Anthocyanin-derived pigments											
Vitisin A	n.d.	0.38^{a}±0.01	0.46^{c}±0.01	0.42b±0.00	0.40ab±0.00	0.42^{b}±0.01	0.29^{a}±0.01	0.41^{c}±0.04	0.37bc±0.00	0.36b±0.00	0.35^{b}±0.01
Vitisin B	n.d.	0.18a±0.00	0.22^{b}±0.01	0.23bc±0.00	0.23c±0.00	0.23c±0.00	0.24a±0.00	0.23a±0.00	0.25^{b}±0.01	0.38c±0.00	0.45d±0.00
Malvidin-3-glucoside-ethyl-epicatechin (I)	n.d.	0.18a±0.00	0.20b±0.00	0.22c±0.00	0.21^{c}±0.01	0.22^{c}±0.01	0.34e±0.00	0.21^{d}±0.01	0.22^{c}±0.01	0.25b±0.00	0.29a±0.00
Malvidin-3-glucoside-ethyl-epicatechin (II)	n.d.	0.29^{b}±0.01	0.26a±0.00	0.27^{a}±0.01	0.26a±0.00	0.26a±0.00	0.30b±0.00	0.27a±0.00	0.28a±0.00	0.33c±0.00	0.49^{d}±0.01
Malvidin-3-glucoside-ethyl-epicatechin (III)	n.d.	0.22b±0.00	0.21a±0.00	0.21ab±0.00	0.21a±0.00	0.21a±0.00	0.26^{c}±0.02	0.22a±0.00	0.22^{ab}±0.01	0.24bc±0.00	0.26c±0.00
Total anthocyanic compounds	146	44.54^{c}±0.48	50.24^{e}±0.15	47.91^{d}±0.03	42.01^{b}±0.07	37.72^{a}±0.01	49.71^{e}±0.09	47.76^{d}±0.51	45.50^{c}±0.27	37.37^{b}±0.25	29.63^{a}±0.04

Supercripts denote statistical differences among values within the same line for each type of wine ($p < 0.05$); n.d.: not detected; tr: trace values

were quantified using the (-)-epicatechin calibration curve. Analyses were carried out in duplicate.

4. Analysis of neutral polysaccharides

The concentration of neutral polysaccharides was determined according to Segarra *et al*. (1995). This spectrophotometric methodology allowed the quantification of neutral polysaccharides by adding phenol and sulphuric acid to the wine samples. After 30 minutes of reaction, the absorbance was determined at 490 nm. Results were expressed in $mg \cdot L^{-1}$ mannose. Analyses were carried out in duplicate.

5. Determination of colour parameters

Parameters of colour were determined according to Glories (1984). To do so, the absorbance of musts and wines was measured at three wavelengths using cuvettes of 1-mm path length: 420, 520 and 620 nm. Afterwards, colour parameters were assessed following this formula: Tint = A_{420}/A_{520}; % Yellow = $(A_{420}/X) \cdot 100$; % Red = $(A_{520}/X) \cdot 100$; % Blue = $(A_{620}/X) \cdot 100$, where $X = A_{420} + A_{520} + A_{620}$. Analyses were carried out in duplicate.

6. Statistical analysis

The statistical methods used for data analysis were cluster analysis (Ward's method from standardized variables), to discover the natural wine sample groupings in relation to the two key factors of the study (type of wine and sampling time), and one-way ANOVA, to test the effect of aging time on each type of wine.

STATISTICA program version 7.1 (StatSoft Inc., 2005, www.statsoft.com) was used for data processing.

RESULTS AND DISCUSSION

1. Phenolic composition of control and G-IDY wines after winemaking and its evolution during aging

Thirty phenolic compounds were identified in must and wines, corresponding to a wide range of anthocyanic compounds (n = 15) and non-anthocyanic phenolic compounds (n = 15). Tables 2 and 3 show the concentrations of phenolic compounds determined in the must, in the wines after alcoholic fermentation (0 month) and in the wines during aging (after 1, 2, 3 and 9 months) in both types of vinifications (Control and G-IDY). In an attempt to obtain a preliminary view of the main causes of variation in the phenolic profile of the wines, cluster analysis was carried out taking into consideration the concentrations of phenolic compounds determined in all the samples (n = 30). The variables were previously standardized, the squared Euclidean distance was taken as a measure of proximity between two samples, and Ward's method was used as the linkage rule. Figure 1 shows the dendrogram obtained. As can be seen, there were two large groups of wines, one corresponding to Control wines and G-IDY wines after ≤ 2 months of aging, while the other group included the G-IDY wines after 3 and 9 months of aging. In addition, within the first group (Control wines and ≤ 2 months G-IDY wines), two new groups, one corresponding to all the Control wines and the other one including the G-IDY wines, could be distinguished. These results clearly showed an effect of the wine type and of the aging time on the phenolic composition of the wines.

In order to check the influence of the aging time on the concentration of phenolic compounds, a one-way ANOVA was carried out on each type of sample. These results are shown in Tables 2 and 3, and as can be seen, differences in the evolution of anthocyanic and non-anthocyanic compounds were found during wine aging in both types of samples. These differences are described as follows:

2. Anthocyanic compounds

A total of 15 anthocyanic compounds were determined in the wines (Table 2 and Figure 2). Most of the anthocyanins usually found in grapes were identified, including 3-*S*-*O*-monoglucosides (delphinidin-, petunidin-, peonidin- and malvidin-3-glucoside) and 3-*O*-acylated monoglucosides (peonidin-3-[6-acetyl]-, malvidin-3-[6-acetyl]-, malvidin-3-[6-caffeoyl]-, peonidin-3-[6-*p*-coumaroyl]- and malvidin-3-[6-*p*-coumaroyl]-glucoside). In addition, some anthocyanin-derived

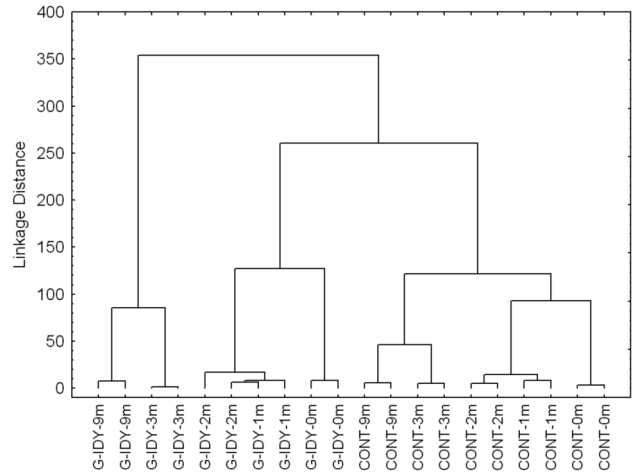

Figure 1 - Dendrogram resulting from applying cluster analysis to the data corresponding to the concentration of phenolic compounds determined in the Control wines (Cont) and in the wines supplemented with the G-IDY preparation (G-IDY) after alcoholic fermentation (0m) and during aging at 1, 2, 3 and 9 months (1m, 2m, 3m and 9 m, respectively).

Table 3. Concentration of non-anthocyanic compounds (mean±standard deviation, mg.L-1) determined in the must, Control wines (Cont-W) and wines supplemented with the G-IDY preparation (G-IDY-W) immediately after alcoholic fermentation (0m) and 1, 2, 3 and 9 months of aging (1m, 2m, 3m and 9m, respectively)

	Must	Cont-W					G-IDY-W				
		0m	1m	2m	3m	9m	0m	1m	2m	3m	9m
Hydroxybenzoic acid											
Gallic acid	4.67±0.34	4.20^{b}±0.01	4.34^{bc}±0.02	4.43^{c}±0.08	3.77^{a}±0.16	4.13^{b}±0.03	4.50^{b}±0.18	4.27^{b}±0.13	4.48^{b}±0.08	3.72^{a}±0.13	4.32^{b}±0.23
Hydroxycinnamic acids											
Trans-caftaric acid	38.80±1.23	24.34^{b}±0.02	26.34^{d}±0.16	26.24^{d}±0.04	23.13^{a}±0.10	25.81^{c}±0.07	25.13^{b}±0.12	26.02^{bc}±0.60	26.46^{c}±0.03	23.15^{a}±0.01	26.51^{c}±0.59
Caffeic acid	1.52±0.14	4.02^{a}±0.17	5.0.00b±0.53	5.19^{b}±0.33	4.53^{ab}±0.29	5.32^{b}±0.30	3.94^{ab}±0.11	3.76^{a}±0.02	4.06^{ab}±0.03	3.76^{a}±0.13	4.26^{b}±0.36
Trans-p-coumaric acid	1.57±0.02	1.88^{a}±0.06	1.91^{a}±0.01	2.24^{b}±0.18	2.34^{bc}±0.14	2.60^{c}±0.09	1.94^{a}±0.09	2.48^{b}±0.07	2.42^{b}±0.17	2.29^{b}±0.03	2.61^{b}±0.19
Coutaric acid	7.25±0.14	2.86^{b}±0.02	2.87^{b}±0.14	2.93^{b}±0.03	2.59^{a}±0.02	2.86^{b}±0.14	3.31^{b}±0.19	3.31^{b}±0.19	3.38^{b}±0.11	2.93^{a}±0.02	3.24^{ab}±0.06
Stilbenes											
Cis-Resveratrol	n.d.	0.27c±0.00	0.22^{b}±0.01	0.21ab±0.00	0.20^{a}±0.01	0.20a±0.00	0.26^{c}±0.01	0.18ab±0.00	0.18ab±0.00	0.17^{a}±0.01	0.19b±0.00
Trans-Resveratrol	2.51±0.03	1.87^{d}±0.02	1.32^{b}±0.04	1.43^{c}±0.01	1.24^{a}±0.02	1.41^{c}±0.03	1.80^{e}±0.05	1.36^{b}±0.03	1.44c±0.00	1.22a±0.00	1.52^{d}±0.02
Cis-Resveratrol glucoside	0.77±0.01	0.40b±0.00	0.42^{c}±0.01	0.41^{bc}±0.01	0.37^{a}±0.01	0.41bc±0.00	0.45^{ab}±0.02	0.44^{ab}±0.02	0.49^{b}±0.05	0.41^{a}±0.01	0.47^{ab}±0.03
Trans-Resveratrol glucoside	2.38±0.05	1.76^{b}±0.03	1.46^{a}±0.03	1.46^{a}±0.03	1.43^{a}±0.01	1.48^{a}±0.01	1.64^{b}±0.06	1.54^{ab}±0.04	1.51^{a}±0.03	1.47^{a}±0.05	1.47^{a}±0.02
Phenolic alcohol											
Tyrosol	n.d.	14.58^{a}±0.62	16.50^{ab}±0.49	16.35^{ab}±0.89	16.55^{ab}±1.38	17.67^{b}±1.15	11.32^{b}±0.60	8.84^{a}±0.56	11.04^{b}±0.41	12.83^{c}±0.20	11.11^{b}±0.53
Flavanols											
(+)-Catechin	20.10±1.93	11.43^{b}±1.23	10.98^{b}±0.27	9.77^{b}±0.68	9.56^{b}±0.47	7.31^{a}±1.02	12.44^{c}±0.29	9.31^{b}±0.72	8.57^{b}±0.13	7.23^{a}±0.08	6.64^{a}±0.63
(-)-Epicatechin	9.17±0.27	6.49^{a}±0.41	6.30^{a}±0.40	6.08^{a}±0.29	8.87^{b}±0.77	10.24^{c}±0.55	9.01^{b}±0.55	6.20^{a}±0.37	6.14^{a}±0.20	9.48^{b}±0.30	13.39^{c}±0.26
Trimeric procyanidin	20.20±1.59	14.53^{a}±0.71	15.77^{a}±0.47	15.69^{a}±0.80	13.88^{a}±1.27	15.90^{a}±1.62	17.37^{b}±0.81	14.46^{a}±1.13	13.57^{a}±0.96	14.60^{a}±0.12	13.34^{a}±1.63
Flavonol											
Quercetin-3-*O*-glucoside	4.25±0.10	1.11^{ab}±0.04	1.33^{bc}±0.03	1.56^{c}±0.18	0.89^{a}±0.04	1.45^{c}±0.11	1.37^{a}±0.06	1.31^{a}±0.05	1.66^{b}±0.11	1.84^{b}±0.06	1.75^{b}±0.07
Methyl Ferulate	n.d.	3.41^{a}±0.01	3.64^{c}±0.11	3.60^{bc}±0.04	3.48^{ab}±0.05	3.59^{bc}±0.03	3.41b±0.00	3.33^{ab}±0.04	3.26^{a}±0.08	3.29^{a}±0.04	3.24^{a}±0.02
Total Non-anthocyanins	113	93.15^{a}±2.53	98.38^{b}±0.91	97.62^{ab}±0.09	92.83^{a}±0.30	10.00.39b±3.60	97.89^{b}±0.58	86.80^{a}±2.15	88.66^{a}±0.17	88.38^{a}±0.21	94.06^{b}±2.63

Supercripts denote statistical differences among values within the same line for each type of wine ($p < 0.05$); n.d.: not detected

pigments, which can be formed from different types of condensation reactions during winemaking and aging (Monagas and Bartolomé, 2009), were found. Specifically, and as can be seen in Figure 2, the anthocyanin-derived pigments identified in the wines corresponded to vitisins A and B, formed by the condensation of malvidin-3-glucoside with pyruvic acid and acetaldehyde, respectively (Bakker and Timberlake, 1997). Besides these compounds, three isomers of malvidin-3-glucoside-ethyl-epicatechins (Figure 2) (named I, II and III in Table 2) were also detected in the Control and G-IDY wines. These compounds are formed by condensation reactions between malvidin-3-glucoside and flavanols in the presence of acetaldehyde (Bakker *et al*., 1993).

During alcoholic fermentation, a decrease in the concentration of most of the anthocyanins of grape origin was found (Table 2). However, a slightly higher concentration of these compounds was found in G-IDY-W than in Cont-W after alcoholic fermentation (0 month, in Table 2). During aging in the bottle, the concentration of grape anthocyanins also decreased in both types of wines. However, it is important to underline the faster decrease of anthocyanins in the G-IDY wines compared to the Control wines. This was particularly evident in the case of the major anthocyanin, malvidin-3-glucoside, whose concentration decreased by more than 16 % in the Control wines while it decreased by 43 % in the G-IDY wines. Consequently, the concentrations of malvidin-3-glucoside after 9 months of aging were 20.22 and 27.92 $mg \cdot L^{-1}$ in G-IDY-W and Cont-W, respectively.

The loss of anthocyanins during wine aging could be due, among other factors, to their interaction, and formation of insoluble aggregates, with polysaccharides released by the G-IDY (Guadalupe *et al*., 2007, 2008). To check this hypothesis, the content of neutral polysaccharides was determined in both types of wines (Cont-W and G-IDY-W) after alcoholic fermentation and during aging (1, 2, 3 and 9 months). Figure 3 shows the concentration of neutral polysaccharides determined in the wine samples. As can be seen, the content of polysaccharides in the wines after alcoholic fermentation (0 m) was higher in the G-IDY wines (2929 $mg \cdot L^{-1}$ mannose) than in the Control wines (2128 $mg \cdot L^{-1}$mannose), probably due to the release of neutral polysaccharides by the IDY preparations in the first stages after the addition of these preparations (Pozo-Bayón *et al*., 2009b; Barrio-Galán *et al*., 2011). However, the differences in the evolution of polysaccharides during the aging of both types of wines are worth emphasizing. Whereas the polysaccharide content remained stable in the Control wines, it decreased during aging in the G-IDY wines. This resulted in non-significant differences in the polysaccharide content in the two types of wines at 9 months (Figure 3). The decrease of polysaccharides observed in the G-IDY wines coincided with a substantial reduction in the anthocyanin content, suggesting the formation and precipitation of mannoprotein-polyphenol complexes, as has been previously indicated (Guadalupe *et al*., 2007, 2008, 2010).

Table 2 also shows the evolution of the anthocyanin-derived pigments. The use of HPLC-MS allowed the identification of these minor compounds in the wines. As can be seen, the concentrations of the pyranoanthocyanin pigments vitisins A and B (m/z 561.3 and 517.3,

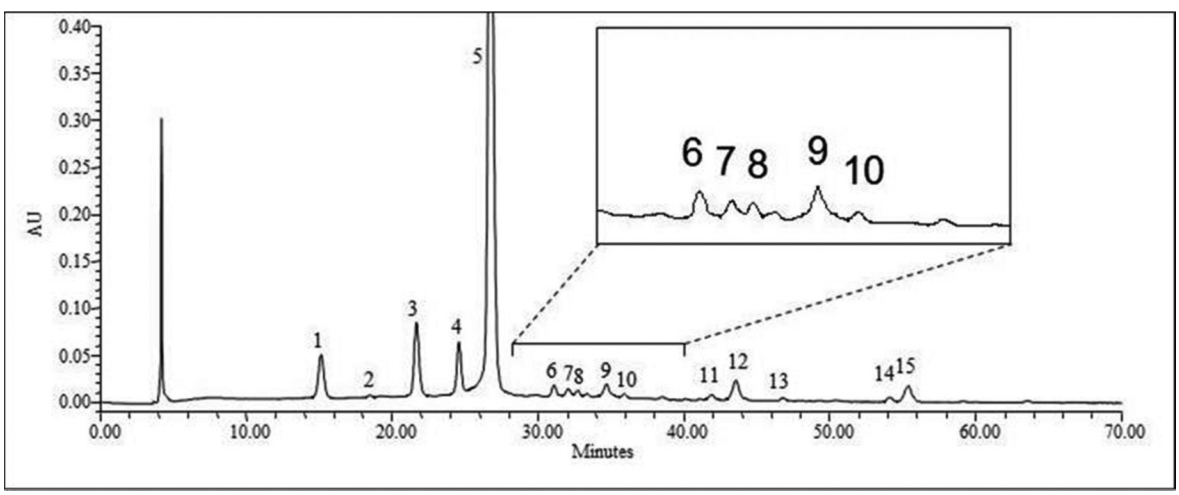

Figure 2. Chromatogram showing the main anthocyanic compounds identified in the G-IDY wine. 1 - Delphinidin-3-glucoside, 2 - Cyanidin-3-glucoside, 3 - Petunidin-3-glucoside, 4 - Peonidin-3-glucoside, 5 - Malvidin-3-glucoside, 6 - Vitisin B, 7 - Vitisin A, 8 - Malvidin-3-glucoside-ethyl-epicatechin (I), 9 - Malvidin-3-glucoside-ethyl-epicatechin (II), 10 - Malvidin-3-glucoside-ethyl-epicatechin (III), 11 - Peonidin-3-(6-acetyl)-glucoside, 12 - Malvidin-3-(6-acetyl)-glucoside, 13 - Malvidin-3-(6-caffeoyl)-glucoside, 14 - Peonidin-3-(6-p-coumaroyl)-glucoside, 15 - Malvidin-3-(6-p-coumaroyl)-glucoside.

respectively) were low in both types of wines (0.18-0.46 mg·L^{-1}), although higher than those previously found in base Grenache wines for rosé sparkling wines (Pozo-Bayón *et al*., 2004). One can see that vitisin A was formed during alcoholic fermentation in the same concentration in both types of wines and remained stable during aging. However, the concentration of vitisin B was higher in G-IDY-W than in Cont-W after alcoholic fermentation ($p < 0.05$). In addition, an increase of 88 % in the concentration of this compound in the 9-month G-IDY wine compared to the 0-month G-IDY wine (after alcoholic fermentation) was found. Interestingly, the concentration of vitisin B remained stable in the Control wines throughout the aging period. These differences in pyranoanthocyanin concentrations could influence wine colour, as it has been reported that pyranoanthocyanins might exhibit orange-red hues (Bakker and Timberlake, 1997). Moreover, because of their structure properties, these pigments are very stable, present greater resistance to colour bleaching by sulfur dioxide, and express more colour at higher pH values than their precursor anthocyanins (Bakker and Timberlake, 1997).

The concentrations of the other three anthocyanin-derived pigments identified in the samples, i. e., the three isomers of malvidin-3-glucoside-ethyl-epicatechin (m/z 809.51), are also included in Table 2 and shown in Figure 2. The isomer I was found at a higher concentration in G-IDY-W (0.34 mg·L^{-1}) than in Cont-W (0.18 mg·L^{-1}) (Table 2). As with vitisins, differences in the concentration of these compounds could also affect wine colour, as these compounds might exhibit a more violet hue compared to the parent anthocyanins (Timberlake and Bridle, 1976), although it is important to bear in mind that the concentrations of these compounds in rosé wines are very low compared to red wines.

3. Non-anthocyanic compounds

A total of 15 non-anthocyanic phenolic compounds were identified in musts and wines, including hydroxybenzoic acid (gallic acid), hydroxycinnamic acids (trans-caftaric, caffeic, *trans-p*-coumaric and trans-coutaric acid), stilbenes (cis-resveratrol, trans-resveratrol, cis-resveratrol-3-*O*-glucoside, *trans*-resveratrol-3-*O*-glucoside), phenolic alcohol (tyrosol), flavanols [(+)-catechin, (-)-epicatechin and trimeric procyanidin], flavonol (quercetin-3-*O*-glucoside) and methyl ferulate (Figure 4). All of them were detected in must, with the exception of cis-resveratrol, tyrosol and methyl ferulate, which are formed during alcoholic fermentation. In general, the differences in the concentration of these compounds between both types of wines after alcoholic fermentation were less noticeable than those observed for the anthocyanic compounds.

As can be seen in Table 3, *trans*-caftaric acid was the major phenolic compound, both in must and wines. The concentration of this compound was slightly higher in the must (38.8 mg·L^{-1}) than in the wines after alcoholic fermentation (above 25.2 mg·L^{-1}). The decrease of trans-caftaric and coutaric acids during alcoholic fermentation could be due, at least in part, to their oxidation by grape polyphenol oxidases in the must, which could result in the formation of the corresponding *o*-quinones (Singleton, 1987). These *o*-quinones can oxidize other compounds such as (-)-epicatechin and (+)-catechin, resulting in the enzymatic browning of white musts and wines (Fernández-Zurbano *et al*., 1998 ; Sioumis *et al*., 2006). In previous works, it was suggested that *o*-quinones might react with GSH when these compounds are present in the must, producing 2-S-glutathionyl caftaric acid (GRP) (Cheynier *et al*., 1990), thereby avoiding the progress of the oxidation reactions and reducing must browning. The ability of this type of G-IDY preparation to release reduced GSH into synthetic wines has recently been proven (Andújar-Ortiz *et al*., 2012). However, no clear effect of the addition of G-IDY preparation was found in the flavanol content of these wines. In fact, while G-IDY wines after alcoholic fermentation showed higher concentration of (-)-epicatechin (9.01 mg·L^{-1}) than Control wines (6.49 mg·L^{-1}), in the case of (+)-catechin, a similar concentration in both types of wines after alcoholic fermentation (11.43 and 12.44 mg·L^{-1} for Cont-W and G-IDY-W, respectively) was found.

In addition, very few differences in the evolution of non-anthocyanic compounds during aging between both types of wines were observed. Only the concentration of tyrosol and trimeric procyanidin (Table 3) was different.

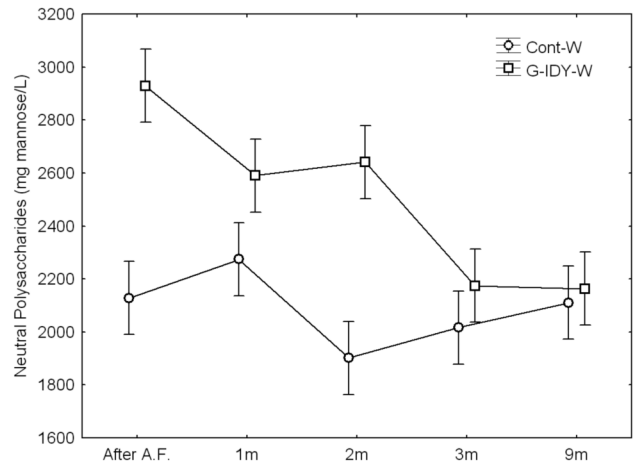

Figure 3. Concentration of neutral polysaccharides in the Control wines (Cont-W) and wines supplemented with the G-IDY preparation (G-IDY-W) after alcoholic fermentation and during aging at 1, 2, 3 and 9 months.

The concentration of tyrosol, a compound of fermentative origin, was higher in Cont-W than in G-IDY-W after alcoholic fermentation; however, in Cont-W it increased during aging, whereas in G-IDY-W it remained stable. These differences seemed to be more related to the different activity of yeasts during alcoholic fermentation. With regard to trimeric procyanidin, its concentration decreased through the shelf-life of G-IDY wines, whereas in Control wines it was stable. Similarly to anthocyanins, the decrease of procyanidins during the aging of G-IDY wines could also be related to the formation of mannoprotein-tannin aggregates, as has been previously suggested (Guadalupe *et al*., 2008, 2010).

4. Colour composition of Control and G-IDY wines after winemaking and its evolution during aging

Tint and percentages of yellow, red and blue were determined in must, in wines after alcoholic fermentation and in wines during aging (Figure 5). As can be seen in the figure, in must and wines red was the major component (about 60 %), followed by yellow (about 30 %), while the blue component was the minor one (<10%).

In general, the differences in colour parameters between both types of wines were not as pronounced as the differences in their phenolic composition. Specifically, Control and G-IDY wines underwent a similar decrease in the red component after 2 months of aging (Figure 5a). As a consequence, an increase in the yellow component (Figure 5b), and therefore in tint (Figure 5c), was observed, which is in line with previous works (Gómez-Cordovés *et al*., 1995). The increase in tint can be related to the evolution of the colour of rosé wines during aging (Salinas *et al*., 2005). It is important to underline that after 9 months of aging, tint increased less in G-IDY-W than in Cont-W, which might indicate a slower colour evolution in G-IDY-W than in Cont-W.

Finally, the blue component increased in all the wines during aging (Figure 5d). However, G-IDY-W showed a higher percentage of the blue component than Cont-W, which could be due, among other factors, to the higher concentration of some anthocyanic pigments that might exhibit a violet colour at wine pH, such as malvidin-3-glucoside-ethyl-epicatechin (isomer II), found at a higher concentration in the G-IDY wines.

Interpretation of wine colour in terms of their chemical composition is not an easy task. Several studies have proven statistical relationships between colour parameters and grape anthocyanins (Ho *et al*., 2001). Anthocyanin-derived pigments, in spite of their relative low concentration, have also been included in mathematical models for wine colour parameters, although this inclusion was dependent of grape variety (Monagas *et al*., 2006). The colour contribution of non-anthocyanic phenolic compounds involved in the anthocyanin copigmentation phenomena (i. e., hydroxycinnamic acids, flavanols and flavonols) is also difficult to quantify, as additional environmental factors are involved (pH, SO2 concentration, etc.). All this might explain why the phenolic concentration differences observed between Control and G-IDY wines might not lead to significant changes in wine colour.

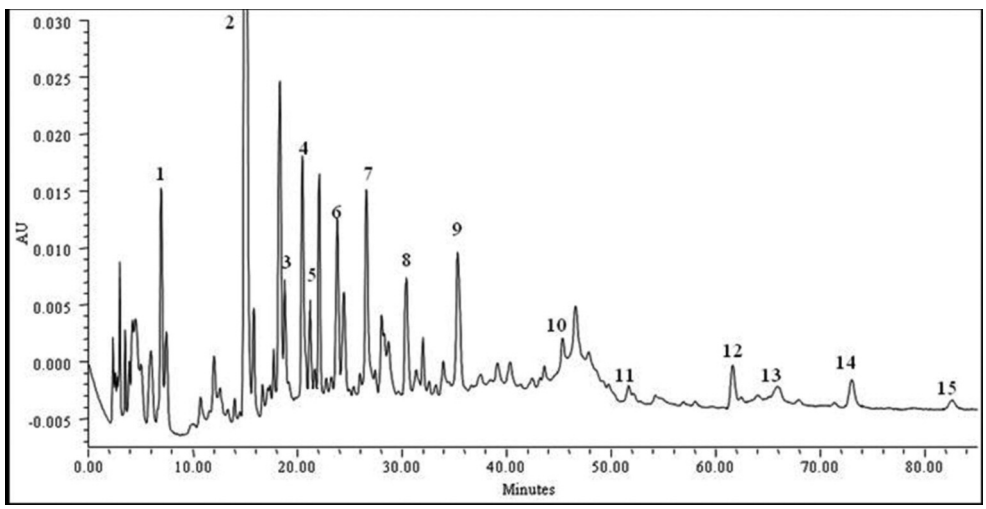

Figure 4. Chromatogram of non-anthocyanic compounds identified in wines treated with G-IDY. 1 - Gallic acid, 2 - Caftaric acid, 3 - Tyrosol, 4 - Coutaric acid, 5 - (+)-Catechin, 6 - ProcyanidinT2, 7 - Caffeic acid, 8 - (-)-Epicatechin, 9 - *p*-Coumaric acid, 10 - *trans*-Resveratrol-3-O-glucoside, 11 - Quercetin-3-O-glucoside, 12 - *cis*-Resveratrol-3-*O*-glucoside, 13 - *trans*-Resveratrol, 14 - Methyl ferulate, 15 - *cis*-Resveratrol.

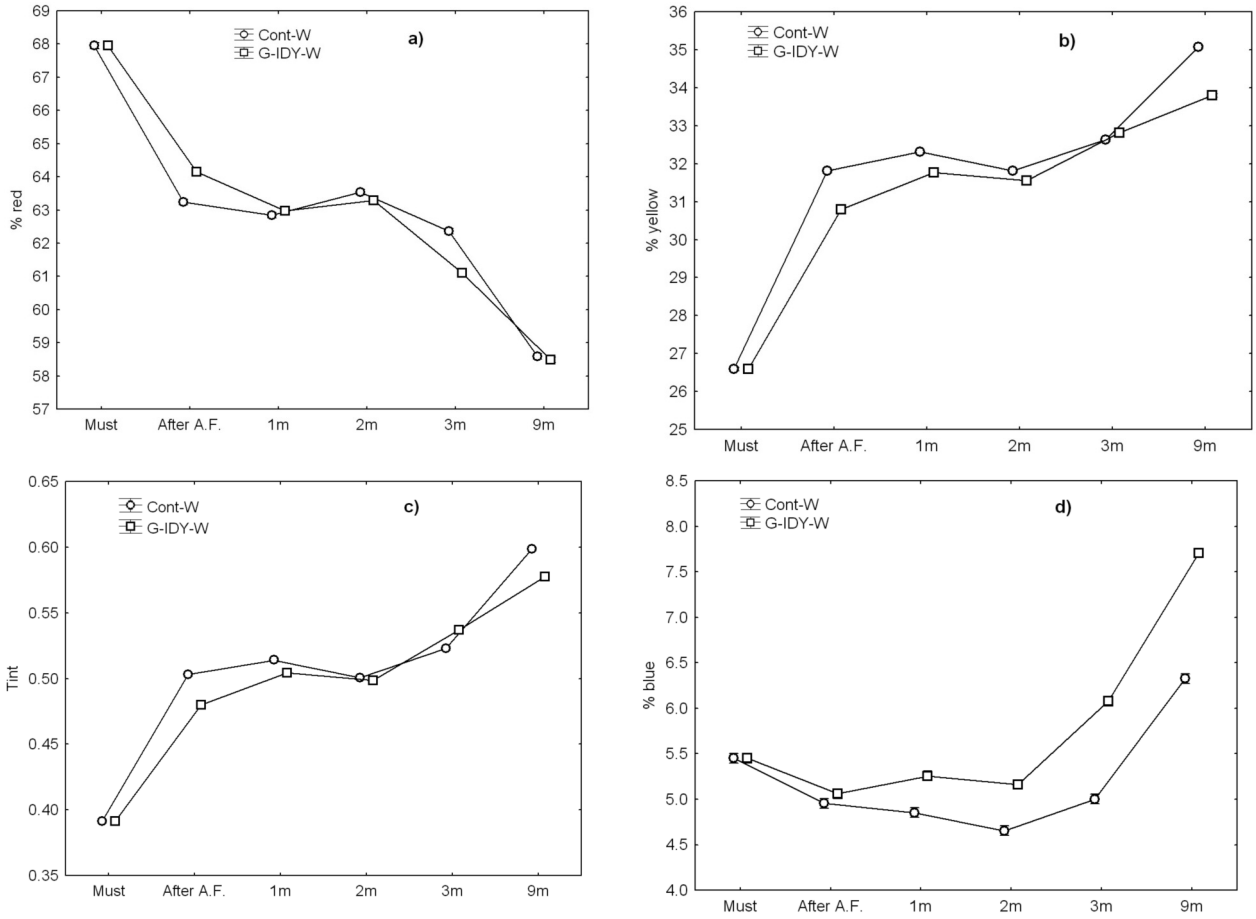

Figure 5. Evolution of colour parameters a) % red, b) % yellow, c) % tint and d) % blue determined in must, Control wines (Cont-W) and wines supplemented with the G-IDY preparation (G-IDY-W) after alcoholic fermentation and during aging at 1, 2, 3 and 9 months.

CONCLUSIONS

The use of a glutathione-enriched IDY preparation during the industrial winemaking of Grenache rosé wines can provoke changes in the phenolic composition and colour characteristics of wines, which are more evident in the advanced stages of aging (after 9 months of aging). These changes are more remarkable for anthocyanic than for non-anthocyanic compounds. Specifically, a higher decrease of anthocyanins of grape origin, probably due to the extra polysaccharide supplementation from the IDY preparation, which might form insoluble anthocyanin-polysaccharide complexes, and a higher concentration of some pyranoanthocyanins have been observed. These changes could be related to the slower colour evolution determined in wines produced with G-IDY preparations, and consequently, this result is in agreement with the enhancement of the colour stability of wines claimed by the IDY manufacturers. It is important to underline that the colour stabilization in young wines, such as the rosé wines in this study, could extend their shelf-life. However, no clear effect linked to the GSH released from these preparations has been found. The fate of this compound during wine fermentation and further winemaking steps needs to be more thoroughly investigated in order to optimise the formulation of IDY preparations for specific winemaking applications.

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