Significance of CIELAB parameters in the routine analysis of red wines

Relevancia de los parámetros CIELAB en el análisis de rutina de vinos tintos

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ABSTRACT

Correlations have been sought among different parameters (total polyphenols, anthocyanins and flavonoids, monomeric anthocyanins, Zn, Cu, Mn and Fe) analysed in two consecutive vintages of a Tempranillo wine. A comparative study of those parameters with color characteristics (both CIELAB and Glories parameters) is presented for these wines. Principal Component Analysis (PCA) has showed the existence of a close relationship between CIELAB parameters and polyphenol concentrations, whereas no correlation could be found with classic color parameters (but in the expected case of Color Intensity, CI). An equation is proposed that allows the concentration of total polyphenols to be estimated as a function of total anthocyanins, cyanidin-3-glucoside and petunidin-3-glucoside concentrations. Chromatographic fractioning of wine samples evidenced the specific interaction of both petunidin-3-glucoside and cyanidin-3-glucoside with Fe, Zn and Cu.

Se han buscado las correlaciones entre los diferentes parámetros (polifenoles totales, antocianinas y flavonoides, antocianinas monoméricas, Zn, Cu, Mn y Fe) analizados en dos añadas de un vino Tempranillo. Se presenta un estudio comparativo de los parámetros de color característicos (tanto parámetros CIELAB como Glories) para estos vinos. El Análisis de Componentes Principales (PCA) ha puesto de manifiesto la existencia de una estrecha correlación entre los parámetros CIELAB y las concentraciones de

polifenoles, mientras que no se ha podido encontrar una correlación con los parámetros clásicos del color (excepto en el caso esperable de la Intensidad Colorante,IC). Se propone una ecuación que permite estimar la concentración de polifenoles totales en función de las concentraciones de antocianinas totales, cianidin-3-glucósido y petunidin-3-glucósido. El fraccionamiento cromatográfico de muestras de vino ha demostrado la existencia de interacciones tanto de petunidin-3-glucósido como de cianidin-3-glucósido con Fe, Zn y Cu.

Keywords: Red wine; Polyphenols; Metals; Fractionation; Complexation; Wine color; CIELAB, PCA.

Palabras clave: Vino tinto; Polifenoles; Metales; Fraccionamiento; Complejación; Color del vino; CIELAB; PCA

1.- INTRODUCTION

Phenolic compounds are known substances which play a relevant role in oenology. They are responsible for differences between white and red wines, especially for the color and taste of the latter. Many beneficial properties such as antioxidant, bactericide and cardiovascular protective have recently been associated with these compounds (German and Walzem, 2000). Given their importance, they have attracted the interest of researchers who have developed new methodologies for their quantification and analysis in both grape and ready to use wines (González-San José *et al.*, 1991; Hakansson *et al.*, 2003; Vivar-Quintana *et al.*, 2002; Masa Vázquez *et al.*, 2007); their evolution once bottled has also received attention (Monagas *et al.*, 2006).

Color is one of the most important organoleptic characteristics of a red wine, not only for it gives the first and immediate image of it, but for it acts also as an indicator of other aspects related to its quality. A red wine color depends mainly on its phenolic composition, especially on its anthocyanic fraction, and many authors have studied these parameters (Bordignon-Luiz *et al.*, 2007; Cliff *et al.*, 2007; Pérez-Magariño and González-San José, 2006; Torkangerpoll and Andersen, 2005).

Two methodologies are commonly accepted for the analysis of wine colour: the standard parameters defined by Glories (Glories, 1984) (colour intensity -CI-, hue –H-, brightness –B-, % Yellow, % Red and % Blue) and the CIELAB chromatic coordinates (L*: Lightness; H*: angular hue; a*: red-green colour

67 contribution; b*: yellow-blue colour contribution; C*: Chroma; s*: saturation)
68 defined in 1986 by the Comission Internationale de L'Éclairage (Central
69 Boureau of the Commission Internationale de L'Éclairage (CIE), 1986).
70 On the other hand, CIELAB parameters allow a more precise definition of the
71 chromatic properties of a wine (Almela *et al.*, 1996; Zamora, 2003) but still has
72 not been spread as a routine practice in wineries for evaluation of the wine
73 quality; this may be due –at least partially- to the fact that a concrete
74 relationship between chromatic coordinates and wine quality has not been
75 readily established.
76 The inorganic fraction of wine, albeit a minority (Eschnauer and Neeb, 1988),
77 is made up by constituents contributing to the development of the vine plant as
78 well as to the nutritional and organoleptic properties of a wine (Fernández
79 Pereira, 1988). A variety of analytical techniques have been used to determine
80 total metal concentrations in final wine (Eschnauer and Neeb, 1988;
81 Fernández Pereira, 1988; Clark and Scollary, 2000; Marengo and Aceto,
82 2003), Mn having been found as tracer of geographic location of grapes used
83 in the wine production (González *et al.*, 1988). Evidences have been reported
84 for the ability of certain metals to form complexes with polyphenols (Clark and
85 Scollary, 2000; Esparza *et al.*, 2005; Vestegaard *et al.*, 2005) which may result
86 in slight changes in the beverage coloration (Hidalgo Togores, 2003; Usseglio
87 Tomasset, 1998). Nevertheless, and on spite of the fact that future wine
88 properties depend to a good extent on processes taking place along its
89 maceration and vinification, few studies have been found for this life span of a

wine (Gil-Muñoz *et al.*, 1998; Gil-Muñoz *et al.*, 1999). Other studies (Chicón *et al.*, 2002; Gómez-Míguez *et al.*, 2007) are centered on the color change and its correlation with polyphenol and anthocyanin variations taking place on the very first days of winemaking (approximately covering the alcoholic pre-fermentation period only).

The aim of the present study is to find useful correlations between common parameters analyzed in a winery (such as total polyphenols, individual anthocyanins and metals) and colour parameters.

This has encouraged us to proceed to a characterization study embodying as many parameters as possible for the whole of vinification period (up to the moment in which the wine is either bottled or transferred to an oak barrel) for two consecutive controlled vintages of a *Vitis vinifera* Tempranillo wine.

Although polyphenolic and metal presence and fates are quite predictable, for they have been previously reported (Castiñeira Gómez *et al.*, 2004; Esparza *et al.*, 2004; Ribéreau-Gayon *et al.*, 2003), in this work we tried to collect and employ as many data as possible –including variability of crops- in order to reliably correlate them with chromatic factors.

2.- EXPERIMENTAL

2.1.- Wine samples

Vitis vinifera (Tempranillo variety) grapes harvested in a supervised experimental vineyard located at La Jeringa in the municipality of Olite

111 (Navarra, Spain) were used to produce the wine studied in this work. After
112 destemming and crushing, grape must was allowed to ferment in the presence
113 of 0.25 g L^{-1} yeast (80% *Saccharomyces cerevisae* Na33 and 20%
114 *Saccharomyces bayanus* EC 1118) and 0.08 g L^{-1} potassium metabisulphite.
115 Samples were taken from a single 10,000 L fermentation tank from day-1 up to
116 day-73 of alcoholic (12-15 first days) and malolactic fermentations for the first
117 vintage (2003) and from day-1 up to day-58 of both fermentation periods for
118 the second vintage (2004). Samples were collected daily for the first two
119 weeks and thereafter sampling was done once every two weeks
120 approximately.
121 Collected samples were frozen at -20ºC for a variable time between 15 and 40
122 days. Before analysis, samples were thawed and centrifuged for 5 min at
123 4,000 min^{-1} in a Biofuge Stratos (Heraus) apparatus refrigerated at 4ºC to
124 avoid any further fermentation to take place.
125 Total polyphenols, flavonoids and anthocyanins, as well as four monomeric
126 anthocyanins, were quantified for samples taken along two to three months of
127 winemaking for those two vintages. Concentrations of Zn, Cu, Fe and Mn
128 have been determined for the same samples. For statistical purposes, results
129 presented in this paper are completed with those reported elsewhere (Esparza
130 *et al.*, 2004) for the precedent vintage (2002), in order to ascertain a better
131 statistical significance. On the other part, this entire group of data is employed
132 together with chromatic parameters exhaustively studied for these three

133 vintages (Esparza *et al.*, 2006), in order to check the influence of the former on
134 the latter.
135 Furthermore, a fractionation study has been carried out for several samples in
136 different stages of vinification with variable polyphenolic and metallic
137 compositions, in order to assess which specific individual anthocyanin has the
138 ability to bind target metals.

139 *2.2.- Total polyphenol quantification*

140 A modification of the *Prussian blue* method (Price and Butler, 1977) has been
141 used. A 1.00 mL sample is filtered through a washed sea sand bed,
142 evaporated to dryness in a Büchi rotary evaporator R-200 with vacuum line V-
143 502 and re-dissolved in a 5.00 mL acetic acid/sodium acetate (Suprapur,
144 Merck) buffer solution at pH 4 containing 8% ethanol and 50% methanol. This
145 procedure was done by triplicate for each sample. Onto variable volumes
146 (0.065 to 0.15 mL) of these reconstituted aliquots, 0.15 mL of 0.1 M $FeCl_3$
147 (Panreac P.R.S.) plus 0.15 mL of 0.08 M $K_3Fe(CN)_6$ (Panreac, P.A.) were
148 added and made up to 10.00 mL.
149 After exactly 15 min, absorbance is measured at 720 nm vs. a reagent blank
150 using disposable Plastibrand®(Brand Gmbh, Wertheim, Germany) cuvettes of
151 1 cm length in a UV-VIS Spectrophotometer (model 1203, Shimadzu). Since a
152 time-dependent kinetics was observed, time was scrupulously offset and
153 spectroscopic measurement process was repeated in full 4 times for each of
154 the triplicate aliquots, so that in total we have 12 absorbance data for each

sample. Method was previously validated according to the Asociación Española de Farmacéuticos de la Industria (Spanish Association of Industry Pharmacist, AEFI) standard (Aguirre Ortega, 2001) for the studied samples. Resulting equation as average of 3 separate calibration graphs obtained for 5 gallic acid standards is:

$y = 0.060 + 0.282\ x$; $R^2 = 0.998$;

Detection limit (DL): 0.120 mg L^{-1}; Quantification limit (QL): 0.401 mg L^{-1}

where x is gallic acid concentration expressed in mg L^{-1} and y is the absorbance at 720 nm. Detection and Quantification limits have been calculated according to the classical procedures (MacDougall and Crummettl, 1980).

2.3.- Total anthocyanin quantification

Anthocyanin quantification is based on their absorbance at 520 nm in the 5.00 mL alcoholic buffer pH 4 re-constituted solutions filtered through a 0.45 µm Low Protein Binding Durapore (PVDF; Millex®-HV, Millipore, Ireland) filters. Measurements were made by triplicate for each of the three aliquots per sample.

Validation was done according to the same standards of AEFI; three independent calibration graphs obtained for seven standards of malvidin-3-glucoside yielded the following results:

$y = - 0.0044 + 3.273\ x$; $R^2 = 0.999$; DL: 0.006 mg mL^{-1}; QL: 0.021 mg mL^{-1}

where y is the absorbance at 520 nm and x is the malvidin-3-glucoside concentration in mg L^{-1}.

2.4.- Individual anthocyanin measurements

Four anthocyanins were measured, namely petunidin-3-glucoside (Pt-3-gluc), cyanidin-3-glucoside (Cy-3-gluc), malvidin-3-glucoside (Mv-3-gluc) and malvidin-3-glucoside acylated with *p*-cumaric acid (Mv-3-*p*-cm-gluc), following the experimental conditions published elsewhere (Esparza *et al.*, 2004).

2.5.- Total flavonoid quantification

Total flavonoid quantification was accomplished by following an adaptation of the method described in the German Pharmacopoeia (DAB 10, 2001). Aliquots of 2.00 mL of the sample re-dissolved in the 5.00 mL alcohol-containing buffer solution are mixed with 2.00 mL of 0.08 M $AlCl_3$ (Probus) and made up to the mark with water to 5.00 mL. After 30 min, absorbance vs. a blank is measured at 425 nm. Procedure was repeated twice for every triplicate.

In this case, three separate calibration plots were done from 9 quercetin standards giving rise to the final equation:

$$y = 0.0104\ x - 0.0065;\ R^2 = 0.996;\ DL: 2.54\ mg\ L^{-1};\ QL: 8.46\ mg\ L^{-1}$$

where x is stands for the quercetin concentration expressed in mg L^{-1} and y is the absorbance at 425 nm.

2.6.- Total metal quantification

2.6.1.-Sample digestion and preparation

An Ethos Plus microwave labstation (Milestone) with computer–controlled easywave software was used to digest all samples. Aliquots of 2.00 mL of centrifuged supernatant wine were treated with 6.00 mL of sub-boiling HNO_3 under the same experimental conditions previously employed (Esparza *et al.*, 2004). Once digested, samples were made up to 10.00 mL with ultra-pure water (Wasserlab G.R. Type I-reagent grade-water system, Millipore).

2.6.2.-Atomic Absorption Spectroscopic (AAS) measurements

Metals were quantified by AAS by using an acetylene-air flame in a Perkin-Elmer Atomic Absorption Spectrometer A Analyst 800. Experimental conditions for each metal are summarized in Table 1.

2.7.- Sample fractionation

A procedure published elsewhere (Esparza *et al.*, 2004), was used with the following modifications in order to improve sensitivity: column size was increased to 50.00 x 3.00 cm, volume sample was doubled to 6.00 mL and metal quantification was done by means of an Agilent 7500 Series ICP-MS instead of the ICP-AES used before.

Analyzed samples belong to day 2, 6 and 17 of vinification of vintage 2003. This choice guarantees matrices with variable ratios of metals and polyphenols. Around 30 fractions were collected for each sample.

3.- RESULTS AND DISCUSSION

3.1.- Organic fraction

Concentration of total anthocyanins (TA) and total polyphenols (TP) determined at different vinification stages are shown in Figure 1 for vintages 2003 (Figure 1.a) and 2004 (Figure 1.b). Besides, Figure 1.b. also depicts the total flavonoids (TF) analyzed for that year.

Vintage 2003 presents higher concentrations than vintage 2004. An exactly parallel pattern was observed for color, that is to say, CI was higher for vintage 2003 than for vintage 2004. Moreover, published results for vintage 2002 show highest proportion of TP (Esparza et al., 2004) and a corresponding highest CI as well. Since the parcel where grapes were cultivated, agricultural practices and fermentation processes are all tightly controlled and kept constants, the only factor governing the found differences might be the climatic conditions (humidity, temperature and sun exposition hours), in which a relevant influential factor could be the extreme night-low and day-high temperatures. Pérez-Magariño (Pérez-Magariño *et al.,* 2006) showed that harvesting day is yet another parameter affecting the polyphenol concentration found in wine. As a matter of fact harvesting dates have also varied in our study from one vintage to another, namely 2002/10/03, 2003/09/29 and 2004/10/19.

Tables 2 and 3 show the data obtained for individual anthocyanins. At first glance, their evolution follow that of the total anthocyanins, that is to say, values for vintage 2003 are higher than those for vintage 2004. In all instances Mv-3-gluc is the most abundant one. Besides, one can observe a sharp increase for all of them in the first days and a subsequent decrease as a consequence of well known condensation reactions with tannins (Ribéreau-Gayon, 2003).

When compared with published results for variety Syrah of *Vitis vinifera* (Gómez-Míguez *et al*, 2007), higher concentrations of individual anthocyanins are found for Tempranillo samples produced in the first 7 days.

3.2.- Statistical Analysis: Pearson correlation and Principal Component Analysis

A Pearson correlation matrix is presented in Table 4, in which above described data for vintages 2003 and 2004, and those previously found for vintage 2002 are collected together.

The correlation among all variables should be highlighted, best values being reached between Pt-3-gluc and Cy-3-gluc. This means that the synthesis, presence and fate of these two anthocyanins are closely related along all vinification processes. Regression analysis of available data has enabled us to produce a simple equation that allows prediction of Pt-3-gluc concentration as a function of the experimental value of Cy-3-gluc.

$$[\text{Pt-3-gluc}] = 1.124 \cdot [\text{Cy-3-gluc}] - 10.588;\ R^2 = 0.979 \qquad (1)$$

258 in which both species concentrations are expressed in terms of mg L^{-1}.

259 A further check of this good correlation was done by calculating the values of
260 Pt-3-gluc for all samples from Cy-3-gluc experimental values and plotting them
261 vs. the real experimental values measured for those same samples. Linear
262 regression coefficient was calculated to be 0.986 and the slope was close to
263 unity (0.983).

264 On the other hand, TP analysis is cumbersome with a derivatization reaction
265 that demands a careful timing of the spectroscopic measurements, while TA
266 quantification is straightforward and individual anthocyanins measurements
267 are more reproducible enabling the number of replicas to be low. It should be
268 kept in mind that the Prussian blue method here utilized for TP analysis was
269 chosen for it was found to be less interference prone than the Folin-Ciocalteu,
270 but at the same time it leads to less precise measurements what makes the
271 analysis of a larger number of replicas mandatory. These facts have prompted
272 us to try and look for a possible relationship to be established that would
273 permit the estimation of TP from the experimental value of TA and/or some of
274 the individual anthocyanins. Regression analysis provided us with the following
275 equation in which concentrations are expressed in g L^{-1}.

276 $[TP] = 1.307 \cdot [TA] + 50.026 \cdot [Pt\text{-}3\text{-}gluc] - 22.699 \cdot [Cy\text{-}3\text{-}gluc] + 8.750 \cdot 10^{-2}$; $R^2 = 0.925$ *(2)*

277 An excellent agreement between the estimated values of TP from the above
278 equation *(2)* and the experimentally measured concentrations was found
279 (slope: 1.0083; R^2:0.9785).

Chromatic characterization of the three aforementioned vintages have been previously studied and reported (Esparza *et al.*, 2006). It is now feasible to try and find whether definite relationships exist among those parameters and the organic fraction of the same wines here analyzed.

A PCA was done taking into account TP and TA values, as well as individual anthocyanin concentrations, together with chromatic parameters (both CIELAB and classic parameters) belonging to all analyzed samples. The required data check showed the following results:

- The determinant of the Pearson's correlation matrix is remarkably low: $8.415 \cdot 10^{-39}$.
- The Bartlett's sphericity test renders a p-value of 0.000 arising good expectations of a reliable factorial analysis.
- The Kaiser-Meyer-Olkin test yields a satisfactory value of 0.749.
- Calculated communalities are high as shown in Table 5.
- Statistical program collapses all data to two principal components covering the 87.2% of the variance (the first component covers the 47.5% while the second one explains the remnant 39.8%).

Rotated matrix of the 2 extracted components is shown in Table 6, where values less than 0.3 have been discarded in order to better appreciate the variable distribution in each of the components. For clarity purposes, a component plot in rotated space is presented in Figure 2.

From these results we can undoubtedly conclude that CI, L*, a*, C* and s* are related with polyphenolic composition of the red wine. If this is the case, then they

303 may be considered as wine quality indicators. The rest of the chromatic parameters
304 have not been found to adequately correlate with phenolic content of wines, and thus
305 would not be tracers of the quality of the wine.
306 Up to very recent times, CIELAB parameters were neither known nor expected to be
307 related with the quality of a wine. However, in view of presented results, we would
308 like to emphasize the feasibility of using most of CIELAB parameters (4 out of 6) to
309 assess the quality of a wine, understood as phenolic content. Furthermore, having in
310 mind that positive a* values represent a red fraction of color (Pérez-Magariño and
311 González-San José, 2002), we may assert that this parameter is a measurement of
312 the total contribution of anthocyanins to the red color of a wine.
313 On the other hand, classic parameters such as %Red, %Yellow and %Blue, Hue and
314 Brightness are not related with the absolute phenolic content, but the fact that they
315 do also measure color, indicates that they could help to characterize varietal and/or
316 geographical origin through the diverse ratio of present anthocyanins via their
317 characteristic contribution to the percentage of color.
318 In summary, the existence of a direct relationship between the quality of a wine and
319 the CIELAB parameters can be proposed: those wines with lower values of L* and
320 highest values of a*, C* and s*, should be the most appreciated for their quality.

321 *3.3.- Inorganic fraction*

322 Table 7 and Table 8 show the concentrations of the four analyzed metals on
323 supernatants obtained from samples along wine vinification for vintages 2003
324 and 2004. Results indicate that both Zn and Mn concentrations tend to remain
325 constant along the vinification period. Concentration of Fe increases as

vinification proceeds, while Cu concentration diminishes in the same interval of time for both vintages, independent of the initial and absolute values. It is well known that solubilities of Cu and Fe compounds in wine are opposite between themselves in either oxidant or reductive atmosphere (Tomasset, 1998) what agrees with our experimental results.

3.4.- Statistical analysis for metals, phenolic compounds and chromatic parameters

No outstanding significant correlations have been found when all these variables collected for three consecutive vintages have been treated in the Pearson matrix. Most conspicuous results for metals and polyphenols are the following:

- Both Fe and Cu are the only metals that appear associated with TA (Fe and TA:0.604**; Cu and TA: -0.570**).
- Only Cu presents a high and inverse significant correlation with TF (-0.879**).
- Cu and Zn show a certain relationship with individual anthocyanins such as Mv-3-gluc (-0.608** and -0.516**, respectively) and Cy-3-gluc (-0.521** and -0.415**, respectively). This fact corroborates the experimental finding reached previously in a fractionation study in which Zn and Cu appeared in the same chromatographic fraction as the Cy-3gluc (Esparza et al., 2004) for wines of vintage 2002.

- As far as metals and chromatic parameters are concerned, the relevant facts are summarized as follows: Clear and definite correlations exist only for Fe. This metal exerts a positive influence on Hue (H) (0.520**) and on % Blue (0.688**), whereas a negative effect is observed on %Red (-0.576**) and on Brightness (-0.568**). Consequently, an increase in Fe concentration would result in an increase of the blue color and in a relatively smaller decrease of the red color.

3.5.- Sample Fractionation

Open column chromatography on the Sephadex ® solid phase allowed a neat separation of all main detectable anthocyanins as depicted in Figure 3.

When those fractions were analyzed for metal content, it was found that Fe, Cu and Zn were mainly accumulated in those very same fractions in which petunidin-3-glucoside and cyanidin-3-glucoside appear, whereas no definite association was found for any of the metals with either malvidin-3-glucoside or malvidin-3-*p*-cm-glucoside. Figure 4 shows an example for the case of petunidin-3-glucoside with both Cu and Zn. Improved analytical procedures and techniques used enhanced an extension of previous results, so that now we have shown that cyanidin-3-glucoside interacts not only with Cu and Zn (Esparza *et al.*, 2004), but also with Fe; similarly we have also found that petunidin-3-glucoside interacts with the three metals. These results are consistent with the fact that both anthocyanins possess the cathecol group, through which strong metal complexation may take place (Brown *et al.*, 1998)

and undergo chromatographic separation without alteration. This assertion does not imply that another linkage path might not be through the less strong π aromatic ring (Esparza *et al.*, 2005).

3.6.- Metals added to vinification

A thorough study was undertaken in order to definitely and unambiguously check whether metal addition onto a wine would originate a color change, as previously advanced (Esparza *et al.*, 2006). For that purpose, care was taken to use experimental conditions that would not induce changes unrelated to metal additions.

Cu and Zn studies resulted negative. Since respective complexes do exist, we may conclude that they must be colorless, probably due to the fact that interaction may occur mainly through the colorless carbinol form of the anthocyanin (Esparza *et al.*, 2007).

As for the Fe study, results showed a parallel pattern to the preliminary reported results. Shortly, it can be confirmed that Fe addition results in an %Blue increase and in a decrease in both %Red and brightness. These experimental findings reflect the prediction derived from the above described statistical analysis.

4.- CONCLUSIONS

- PCA showed that a simple colorimetric measurement of L*, a*, c* and s* would serve as a quick guide to reveal the polyphenolic content of a given wine and, therefore, its quality.
- Pearson correlation analysis has proved to be a good tool to detect correlations of color with polyphenols and metal contents, but fails to fully describe the existing relationships between metals and polyphenols as experimentally found.
- Both petunidin-3-glucoside and cyanidin-3-glucoside were seen to strongly interact with Fe, Cu and Zn.
- a* is a measure of the total contribution of anthocyanins to the red color of a wine, whereas classical Glories %Red parameter remains unaffected by anthocyanins concentration.
- Supplement of Zn, Cu and Mn did not yield any substantial wine color change.

5.- ABBREVIATIONS USED

TP: Total polyphenols; TA: Total anthocyanins; TF: Total flavonoids;

Cy-3-gluc: cyanidin-3-glucoside; Pt-3-gluc: petunidin-3-glucoside;

Mv-3-gluc: malvidin-3-glucoside; Mv-3-*p*-cm-gluc: malvidin-3-*p*-cumaroil-glucoside;

CI: Color intensity. DL: Detection limit; QL: Quantification limit;

AEFI: Asociación Española de Farmacéuticos de la Industria (Spanish Association of Industry Pharmacist); PCA: Principal Components Analysis

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Table 1. Experimental conditions for the atomic absorption spectroscopic measurements of the assayed metals.

Tabla 1. Condiciones experimentales para la determinación de los metales por espectroscopía de absorción atómica

Experimental conditions	Metal			
	Zn	**Fe**	**Cu**	**Mn**
λ (nm):	213.9	248.3	324.8	279.5
Slit width:	0.7	0.2	0.7	0.2
Lamp current (mA):	15	30	15	20
Calibration interval:	0 – 0.3 mg L^{-1}	0 – 1.2 mg L^{-1}	0 – 0.6 mg L^{-1}	0 – 1.8 mg L^{-1}
Calibration line	$y = 0.307x + 1.86 \cdot 10^{-3}$	$y = 0.058x - 1.2 \cdot 10^{-4}$	$y = 0.0764x + 1.43 \cdot 10^{-4}$	$y = 0.067x + 5.1 \cdot 10^{-4}$
Correlation coefficient:	0.9999	0.9998	0.9999	0.9999
n:	4	4	4	4
Detection limit*:	0.002 mg L^{-1}	0.024 mg L^{-1}	0.007 mg L^{-1}	0.022 mg L^{-1}
Quantification limit*:	0.006 mg L^{-1}	0.082 mg L^{-1}	0.025 mg L^{-1}	0.075 mg L^{-1}

*Detection and Quantification limits have been calculated according to MacDougall et al (DAB 10, 2001).

Table 2 . Evolution of individual anthocyanins along the vinification process for the vintage 2003 (mean ± standard deviation).

Tabla 2. Evolución de las antocianinas individuales a lo largo del proceso de vinificación para la añada 2003 (media ± desviación estándar)

Vinification day	Individual anthocyanin content (mg L^{-1}) ± SD[a]			
	Pt-3-gluc	**Cy-3-gluc**	**Mv-3-gluc**	**Mv-3-*p*-cm-gluc**
1	10.37 ± 0.62	14.54 ± 0.11	62.27 ± 3.30	5.04 ± 1.19
2	21.84 ± 2.17	22.34 ± 0.51	81.94 ± 2.40	8.78 ± 0.34
3	31.41 ± 1.19	30.46 ± 1.46	130.06 ± 0.37	18.80 ± 1.16
4	57.37 ± 0.76	58.19 ± 0.22	215.00 ± 0.83	40.59 ± 0.28
5	54.07 ± 3.31	55.08 ± 1.14	214.65 ± 10.18	41.99 ± 0.11
6	80.62 ± 4.78	81.46 ± 6.03	308.93 ± 21.91	61.00 ± 1.39
7	55.11 ± 0.68	55.60 ± 1.67	210.24 ± 0.10	40.55 ± 0.62
8	43.34 ± 3.80	40.49 ± 1.80	151.22 ± 4.90	26.43 ± 0.60
9	75.61 ± 3.30	81.62 ± 4.50	307.73 ± 12.69	55.65 ± 1.60
10	55.64 ± 2.90	59.08 ± 5.44	225.30 ± 8.21	37.89 ± 0.26
11	44.13 ± 1.93	44.35 ± 0.05	161.32 ± 0.05	28.10 ± 0.58
16	27.32 ± 0.06	27.14 ± 1.45	102.00 ± 6.98	20.92 ± 8.98
25	43.36 ± 1.39	42.70 ± 3.38	164.40 ± 6.53	24.17 ± 0.02
31	61.11 ± 4.70	60.70 ± 1.45	237.80 ± 9.15	34.09 ± 0.51
38	39.09 ± 2.94	38.96 ± 1.40	151.24 ± 3.51	21.07 ± 0.57
45	23.79 ± 0.76	24.00 ± 3.17	102.11 ± 3.17	13.53 ± 0.92
51	35.16 ± 2.54	34.12 ± 0.73	134.04 ± 3.86	18.76 ± 0.26
59	35.96 ± 2.41	37.35 ± 1.61	144.57 ± 3.72	18.50 ± 0.32
67	32.66 ± 3.95	35.35 ± 4.44	140.70 ± 12.45	20.75 ± 0.11
73	36.15 ± 4.74	34.84 ± 1.26	144.21 ± 7.76	19.35 ± 0.59

a: data are calculated after two replicate measurements per analyzed sample
a: los datos fueron calculados como promedio de dos réplicas por muestra

Table 3. Evolution of individual anthocyanins along the vinification process for the vintage 2004 (mean ± standard deviation).
Tabla 3. Evolución de las antocianinas individuales a lo largo del proceso de vinificación para la añada 2004 (media ± desviación estándar)

Vinification day	Individual anthocyanin content (mg L^{-1}) ± SD[a]			
	Pt-3-gluc	Cy-3-gluc	Mv-3-gluc	Mv-3-*p*-cm-gluc
1	9.48 ± 1.29	11.08 ± 0.90	39.27 ± 1.45	9.40 ± 0.32
2	11.09 ± 0.83	15.63 ± 0.38	61.83 ± 5.60	9.97 ± 0.04
3	24.12 ± 0.13	34.45 ± 3.56	153.74 ± 13.29	25.50 ± 0.49
4	26.96 ± 1.12	42.06 ± 1.34	181.94 ± 1.82	29.40 ± 5.20
5	33.14 ± 1.42	48.50 ± 1.78	216.50 ± 8.53	28.54 ± 3.29
6	29.35 ± 0.19	49.14 ± 1.22	217.34 ± 0.67	25.23 ± 2.78
7	36.04 ± 1.90	55.04 ± 3.81	232.88 ± 1.00	30.90 ± 1.48
9	31.77 ± 1.93	44.20 ± 1.89	201.98 ± 4.53	23.89 ± 0.77
10	32.77 ± 1.31	49.36 ± 3.21	211.58 ± 9.75	25.08 ± 9.21
11	29.15 ± 0.65	44.87 ± 0.71	192.66 ± 1.38	20.17 ± 7.29
18	35.80 ± 0.56	55.85 ± 1.53	243.45 ± 4.49	27.39 ± 2.03
25	34.21 ± 0.12	49.63 ± 0.59	248.62 ± 5.93	30.44 ± 4.37
32	31.52 ± 0.79	48.66 ± 3.25	236.55 ± 4.92	29.06 ± 1.32
51	23.30 ± 0.09	37.20 ± 1.61	231.94 ± 0.26	25.16 ± 2.18
58	11.37 ± 0.65	15.22 ± 1.58	54.88 ± 5.74	17.00 ± 0.01

a: data are calculated after four replicate measurements per analyzed sample
a: los datos fueron calculados como promedio de cuatro réplicas por muestra

Table 4. Pearson correlation matrix of the phenolic fraction for vintages 2002, 2003 and 2004.
Tabla 4. Matriz de correlaciones de Pearson de la fracción fenólica correspondiente a las añadas 2002, 2003 y 2004.

	TP	TA	Pt-3-gluc	Cy-3-gluc	Mv-3-gluc	Mv-3-*p*-cm- gluc	TF
TP[a]	1						
TA[a]	0.881**	1					
Pt-3-gluc [b]	0.948**	0.848**	1				
Cy-3-gluc[b]	0.926**	0.828**	0.990**	1			
Mv-3-gluc [b]	0.811**	0.783**	0.911**	0.952**	1		
Mv-3-*p*-cm-gluc[b]	0.570**	0.486**	0.667**	0.695**	0.757**	1	
TF[c]	0.894**	0.886**	0.913**	0.932**	0.934**	0.878**	1

** Correlations are significant at the 0.01 level (2-tailed)
a :correlation data were calculated from 55 different samples;
b: correlation data were calculated from 52 different samples;
c: correlation data were calculated from 15 different samples
** Las correlaciones fueron significativas para un nivel 0,01 (2 colas)
Las correlaciones fueron calculadas a partir de (a): 55 , (b): 52 y (c):15 muestras diferentes.

Table 5. Communalities arising from the statistical study

Tabla 5. Comunalidades extraídas del análisis estadístico

Communalities	Extraction
TP	0.893
TA	0.899
Pt-3-gluc	0.896
Cy-3-gluc	0.887
Mv-3-gluc	0.845
Mv-3-*p*-cm-gluc	0.453
CI	0.905
T	0.984
% Yellow	0.989
% Red	0.979
% Blue	0.804
Brightness	0.982
L*	0.859
a*	0.928
b*	0.785
H*	0.755
C*	0.921
s*	0.934

Table 6. Component matrix rotated[a] according to Varimax method with Kaiser normalization
Tabla 6. Matriz de componentes rotada[a] por el método Varimax con normalización de Kaiser

	Component	
	1	**2**
TP	0.942	
TA	0.946	
Pt-3-gluc	0.944	
Cy-3-gluc	0.929	
Mv-3-gluc	0.875	
Mv-3-*p*-cm-gluc	0.646	
CI	0.936	
T		0.950
% Yellow		0.951
% Red		-0.961
% Blue		0.891
Brightness		-0.957
L*	-0.900	
a*	0.724	-0.636
b*		0.875
H*		0.867
C*	0.724	-0.630
s*	0.875	-0.409

[a]: rotation converged after 3 iterations
[a]: la rotación convergió tras tres iteraciones

Table 7. Total Zn, Fe, Mn and Cu concentrations along vinification time of vintage 2003. (mean ± standard deviation)
Tabla 7. Concentraciones totales de Zn, Fe, Mn y Cu a lo largo del tiempo de vinificación de la añada 2003 (media ± desviación estándar)

Vinification day	Metal content (mg L^{-1}) ± SD[a]			
	Zn	Fe	Mn	Cu
1	0.490 ± 0.008	0.568 ± 0.027	1.240 ± 0.056	0.965 ± 0.011
2	0.417 ± 0.008	0.337 ± 0.037	1.077 ± 0.038	0.493 ± 0.009
3	0.378 ± 0.014	0.350 ± 0.026	0.842 ± 0.033	0.363 ± 0.011
4	0.357 ± 0.012	0.310 ± 0.029	1.047 ± 0.030	0.195 ± 0.009
5	0.267 ± 0.022	0.545 ± 0.041	1.108 ± 0.021	0.280 ± 0.006
6	0.280 ± 0.021	0.612 ± 0.030	1.073 ± 0.033	0.310 ± 0.002
7	0.243 ± 0.022	0.600 ± 0.056	1.082 ± 0.023	0.232 ± 0.007
8	0.298 ± 0.015	0.595 ± 0.029	1.125 ± 0.016	0.187 ± 0.005
9	0.282 ± 0.017	0.735 ± 0.026	1.105 ± 0.033	0.203 ± 0.003
10	0.307 ± 0.017	0.917 ± 0.033	1.090 ± 0.023	0.268 ± 0.007
11	0.332 ± 0.008	0.802 ± 0.036	1.088 ± 0.022	0.422 ± 0.006
16	0.353 ± 0.016	0.760 ± 0.047	1.115 ± 0.022	0.437 ± 0.005
25	0.357 ± 0.012	1.773 ± 0.054	1.107 ± 0.019	0.365 ± 0.002
31	0.328 ± 0.007	1.727 ± 0.041	1.098 ± 0.028	0.148 ± 0.006
38	0.307 ± 0.008	1.613 ± 0.048	1.093 ± 0.024	0.147 ± 0.004
45	0.305 ± 0.010	1.570 ± 0.042	1.042 ± 0.024	0.140 ± 0.003
51	0.445 ± 0.016	1.673 ± 0.037	1.030 ± 0.028	0.147 ± 0.007
59	0.343 ± 0.013	1.960 ± 0.031	1.060 ± 0.031	0.150 ± 0.007
67	0.392 ± 0.022	1.707 ± 0.048	1.042 ± 0.049	0.148 ± 0.007
73	0.398 ± 0.017	1.580 ± 0.049	0.972 ± 0.044	0.092 ± 0.007

a: data are calculated after six replicate measurements per analyzed sample
a: los datos fueron calculados como promedio de seis réplicas por muestra

Table 8. Total Zn, Fe, Mn and Cu concentrations along vinification time of vintage 2004. (mean ± standard deviation)
Tabla 8. Concentraciones totales de Zn, Fe, Mn y Cu a lo largo del tiempo de vinificación de la añada 2004 (media ± desviación estándar)

Vinification day	Metal content (mg L^{-1}) ± SD[a]			
	Zn	**Fe**	**Mn**	**Cu**
1	0.446 ± 0.009	0.241 ± 0.024	0.745 ± 0.064	0.958 ± 0.122
2	0.295 ± 0.007	0.293 ± 0.037	0.593 ± 0.025	0.432 ± 0.004
3	0.256 ± 0.009	0.546 ± 0.079	0.651 ± 0.033	0.290 ± 0.007
4	0.230 ± 0.008	0.267 ± 0.045	0.664 ± 0.032	0.207 ± 0.003
5	0.201 ± 0.008	0.387 ± 0.032	0.744 ± 0.044	0.170 ± 0.006
6	0.531 ± 0.030	0.233 ± 0.032	0.633 ± 0.026	0.180 ± 0.005
7	0.337 ± 0.006	0.617 ± 0.026	0.760 ± 0.025	0.167 ± 0.010
8	0.270 ± 0.006	0.612 ± 0.029	0.714 ± 0.031	0.178 ± 0.007
9	0.225 ± 0.009	0.618 ± 0.025	0.725 ± 0.023	0.202 ± 0.009
10	0.182 ± 0.006	0.802 ± 0.052	0.479 ± 0.050	0.190 ± 0.004
11	0.280 ± 0.006	0.840 ± 0.051	0.480 ± 0.051	0.168 ± 0.005
18	0.254 ± 0.005	0.845 ± 0.044	0.593 ± 0.084	0.131 ± 0.007
25	0.233 ± 0.005	0.731 ± 0.030	0.641 ± 0.019	0.130 ± 0.010
32	0.233 ± 0.012	0.968 ± 0.032	0.670 ± 0.044	0.158 ± 0.008
51	0.233 ± 0.006	0.687 ± 0.018	0.679 ± 0.027	0.080 ± 0.007
58	0.202 ± 0.008	0.636 ± 0.031	0.789 ± 0.035	0.083 ± 0.009

a: replicate measurements ranged between 6 and 9 per analyzed sample
a: los datos fueron calculados como promedio de entre 6 y 9 réplicas por muestra

Figure 1:

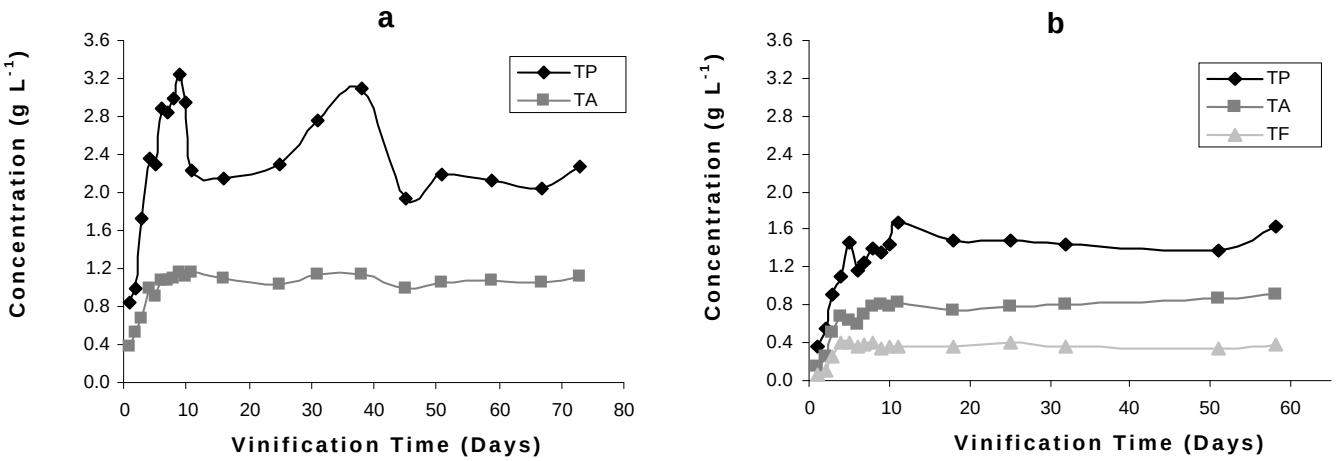

Figure 1: Total content of polyphenols (TP), anthocyanins (TA) and flavonoids (TF) for samples of (**a**) vintage 2003 and (**b**) vintage 2004 along their vinification.

Figura 1: Contenido total de polifenoles (TP), antocianinas (TA) y flavonoides (TF) para las muestras de las añadas 2003 (**a**) y 2004 (**b**) a lo largo de su vinificación

Figure 2:

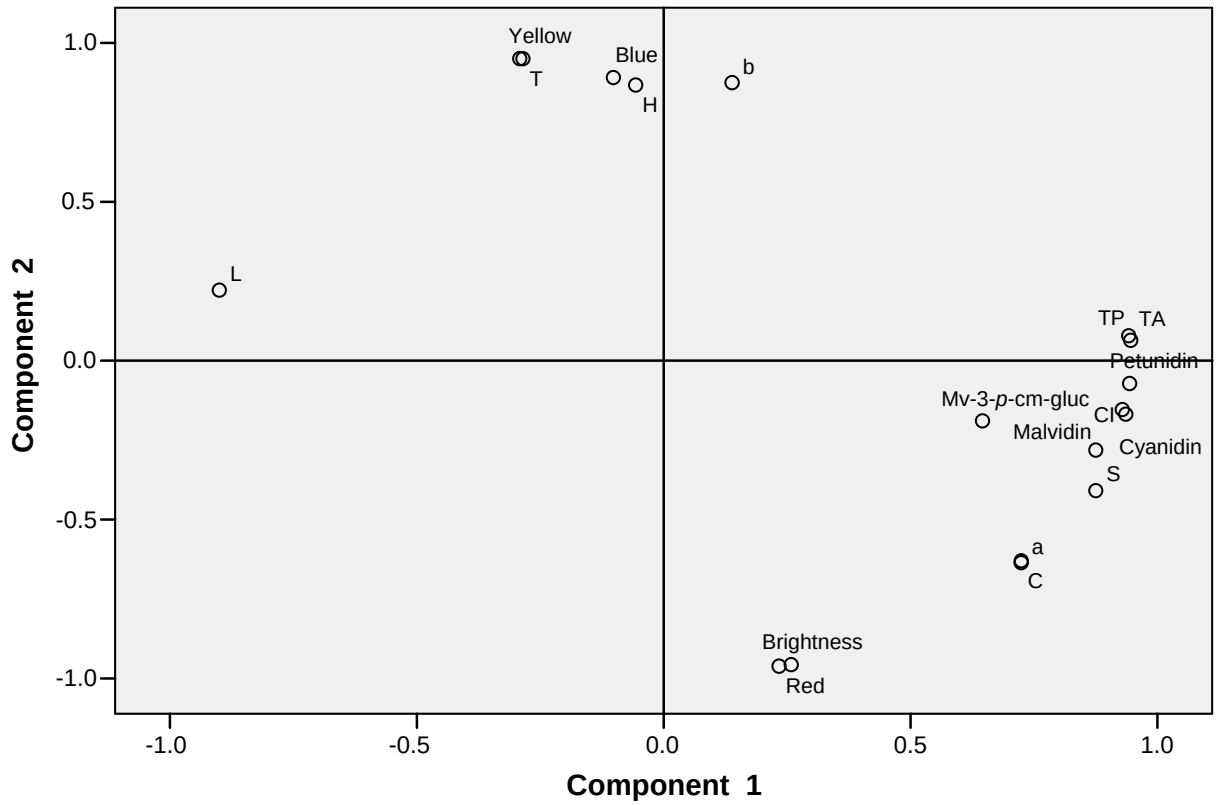

Figure 2: Component plot in rotated space of data collected in Table 5

Figura 2: Gráfico de componentes en espacio rotado de los datos incluidos en la Tabla 5

Figure 3:

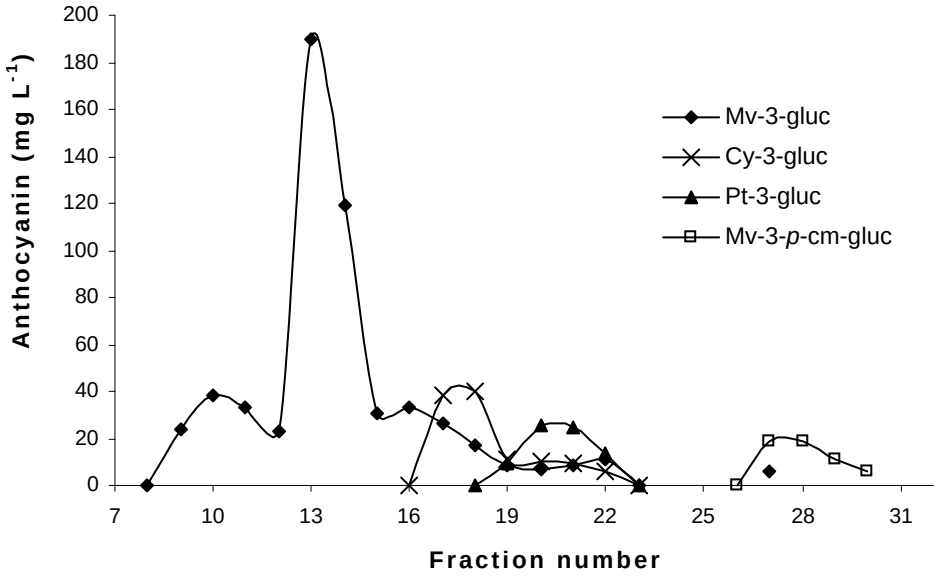

Figure 3. Chromatographic fraction profiles of the four monomeric analyzed anthocyanins for day-6 of vinification in wine of harvest 2003.

Figura 3: Distribución de antocianinas en las distintas fracciones cromatográficas obtenidas para el vino del sexto día de vinificación de la cosecha 2003.

Figure 4:

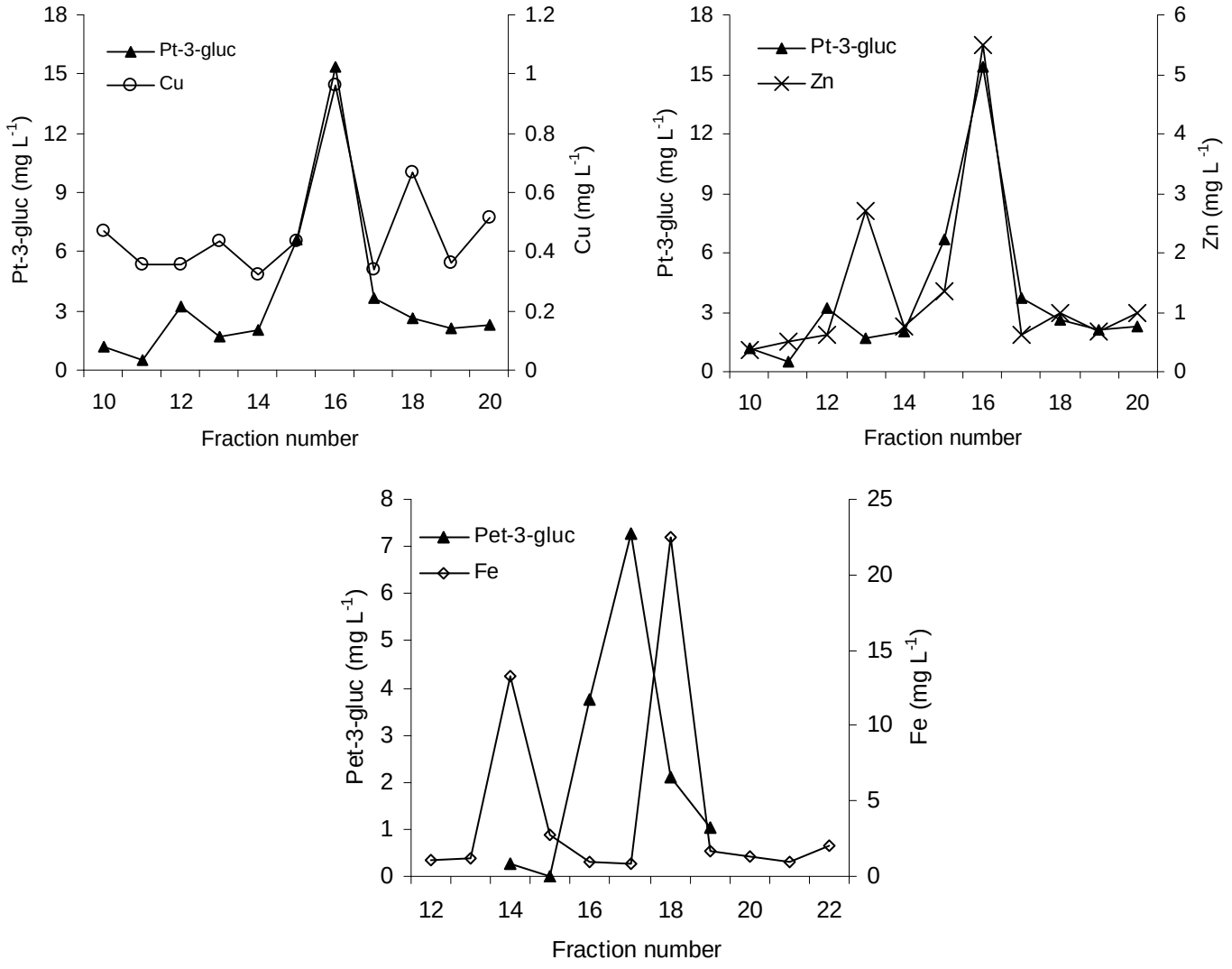

Figure 4. Distribution of petunidin-3-glucoside and Cu, Zn and Fe in the chromatographic fractions (vintage 2003).

Figura 4. Correlación entre metales (Fe, Cu y Zn) y petunidin-3-glucósido en las fracciones cromatográficas de la añada 2003.