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Phenolic compounds and colour stability of Vinhão wines: Influence of wine-making protocol and fining agents

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Abstract

The purpose of this work is to study the evolution, over three selected harvests, of phenolic compounds and colour stability of red wines produced in the north of Portugal by means of two wine-making processes (conventional maceration/fermentation and fermentation after initial carbonic maceration), with and without the use of four different fining agents (polyvinylpolypyrrolidine, gelatine, egg albumin, and casein). In general, it was observed that wines obtained by conventional maceration/fermentation (PO) present the highest colour intensity and polyphenolic content (total and monomeric anthocyanins, flavan-3-ol monomers and polymers), and the lowest orange–red hue, immediately following vinification. Nevertheless, carbonic maceration (CM) afforded wines with most stability in colour density, for 26 months' storage. Different evolutions of anthocyanins and of flavan-3-ol monomers (catechins) and polymers (procyanidins) in PO and CM protocols during storage were observed. Decrease of anthocyanins and flavan-3-ol dimmers were, in general, more remarkable in PO wines. Monomers and trimers underwent a rise, especially in CM wines. Wines treated with fining agents tended to have somewhat lower anthocyanins levels, and especially in the case of PVPP, less intense colouration than untreated wines. Principal component analysis (PCA) revealed that catechins and some procyanidins are mainly responsible for the separation and evolution of wines during storage according polyphenolic composition. Partial least squares (PLS) regression models confirm that colour density in wines from PO and CM protocols explains a high proportion of the variance in hue, chemical age, IFC and anthocyanins and a prediction model has been built.

Keywords: Polyphenols; Colour; Red wine; Wine-making; Total and monomeric anthocyanins; Flavan-3-ol monomers and polymers; PCA; PLS

1. Introduction

The phenolic compounds are among the most important components of wines and are directly related not only to its colour, astringency, bitterness and oxidative level, but also to well-known health beneficial effects as antioxidants. Wine phenolics belong to two main groups, non-flavonoid (namely, hydroxybenzoic acid and hydroxycinnamic acid and their derivatives, stilbenes and phenolic alcohols) and flavonoid (namely, anthocyanins, flavan-3-ol monomers and polymers, flavonols and dihydroflavonols). Free anthocyanins, extracted from grapes, are responsible for the colour of young red wine. During storage and aging, wine colour changes from a bright red to a reddish-brown hue. This is attributed to the formation of new, more stable, polymeric pigments proceeding from reactions between anthocyanins and other phenolic compounds, including in particular, flavan-3-ol monomers and polymers. The reactions considered responsible for the formation of these changes included: acetaldehyde-mediated condensation, co-pigmentation and self-association (Boulton, 2001).

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The most important factors affecting the content of these compounds in wine are their concentration in grape, the winemaking technology used and their transformation during wine ageing process (Gao, Girard, Mazza, & Reynolds, 1997; Gómez-Plaza, Gil-Muñoz, López-Roca, & Martínez-Cutillas, 2000a; Gómez-Plaza, Gil-Muñoz, López-Roca, Martínez-Cutillas, & Fernández-Fernández, 2001; Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999; Ortega-Regules, Romero-Cascales, López-Roca, Ros-García, & Gómez-Plaza, 2006; Revilla & González-San José, 2003; Spranger et al., 2004; Sun, Spranger, Roque-do-Vale, Leandro, & Belchior, 2001; Sun & Spranger, 2005). Clarification techniques also affect wine quality. Fining is applied to obtain limpids and bright wines, eliminating substances in suspense as well as instability proteic. Also the fining is responsible for elimination of some phenolic compounds of colloidal nature, implicated on oxidation phenomena and the excess astringency of wine, given a contribution on the improvement of some organoleptic characteristics of wines. Fining agents such as PVPP, gelatin, egg albumin and casein have demonstrated reduction of phenolic levels and alters the colour in some wines (Donner, Becard, & Irwin, 1993; Gómez-Plaza, Gil-Muñoz, López-Roca, de la Hera-Ortis, & Martínez-Cutillas, 2000b; Maury, Sarni-Manchado, Lefebvre, Cheynier, & Moutounet, 2001; Sims, Eastridge, & Bates, 1995).

In this paper, we investigated over three harvests (2000, 2001 and 2002), the colour (colour density and hue), the major phenolic compounds (anthocyanins, flavan-3-ol monomers (catechins) and polymers (procyanidins)) and other properties, in Vinhão red wines, made by different winemaking technologies. Furthermore, we address the evolution of these properties during two years of storage and whether this evolution was influenced by the winemaking protocol or fining agents. This study is the second part of an extensive research. The first one, including the results obtained in one selected harvest (1999), was previously published (Castillo-Sánchez, Mejuto, Garrido, & García-Falcón, 2006).

2. Materials and methods

2.1. Grapes and wines

Vinhão (*Vitis vinifera*) grapes were hand-harvested from an experimental local vineyard located in Arcos de Valdevez, (Região dos Vinhos Verdes, North of Portugal), in 2000, 2001 and 2002 at technical maturity. All the grapes were transported to the experimental winery for immediate processing following two protocols (Castillo-Sánchez et al., 2006): destemming and crushing followed by fermentation for one week at 25 °C with conventional hourly pumping-over (PO) and a protocol in which destemming and crushing were preceded by two weeks' carbonic maceration at 30 °C, but post-crushing fermentation time was reduced to two or three days at 25 °C (CM). In each case, fermentation, racking and pressing were followed by cold stabilization for four weeks at 10 °C, after which part of the wine was bottled (control wine) and the remainder was divided into four parts that were each treated with one of the four fining agents: polyvinylpolypyrrolidine (PVPP, at a concentration of 1 g/l), albumin (0.2 g/l), gelatin (0.2 g/l) and casein (0.6 g/l). Before bottling, the fined wines were subjected to a further two weeks' stabilization at 10 °C. Once bottled, all wines were stored at 10–12 °C during 26 months.

2.2. Analysis

Immediately post-vinification and after 8, 14, 20, and 26 months, bottled storage in all wines the following analyses were carried out: routine analyses, determination of chromatic characteristics and phenolic compounds by spectrophotometry (total anthocyanins and folin–ciocalteau index). In order to confirm the results obtained, control wines from 2000 harvest were detected also by liquid chromatography. In this way, catechins, procyanidins and monomeric anthocyanins were immediately determined post-vinification and after 14 and 26 months' storage. Total anthocyanins by spectrophotometry and Folin–Ciocalteau index were also analyzed in the collected grapes of Vitis vinifera before each wine-making protocol.

2.2.1. Routine analyses

Alcohol content, pH and titratable acidity were determined by OIV methods (OIV, 1990).

2.2.2. Colour

Colour density was determined by measuring absorbance at 420, 520 and 620 nm in a 1 mm cell (Glories, 1984) using a Unicam 5625 spectrophotometer (Unicam Ltd., Cambridge, UK). Hue was quantified as the ratio of absorbance at 420 and 520 nm (Glories, 1984).

2.2.3. Phenolic compounds

2.2.3.1. Determinations by spectrophotometry. Total anthocyanin concentration was determined by the absorbance measured at 520 nm, using malvidine-3-glycoside chloride as standard (Ribereau-Gayon & Stonestreet, 1965). Chemical age (CA) of wines was determined as the ratio of absorbance at 520 and 280 nm (Somers & Evans, 1977). The total phenolics were determined by colorimetry with phosphotungstic-phosphomolybdic acid (OIV, 1990) at 750 nm. The results were expressed in units (dimensionless) of the index of the Folin–Ciocalteau (IFC).

In the collected grapes, total anthocyanins and IFC, were determined after fractioned extraction with methanol, water and acetone to separate phenolic compounds (Revilla, Escalona, Alonso, & Kovac, 1995).

Triplicate analyses for each determination were carried out and relative standard deviations (%) obtained were lower than 10%.

2.2.3.2. Determinations by high performance liquid chromatography (HPLC). The HPLC system used was a Merck L-6200. A pump equipped with a Waters 717 Plus auto sampler, a Konica UV–vis 2000 spectrophotometer and a Perkin–Elmer 730 integrator–registrator. Monomeric anthocyanins (delphidin-3-glucoside (D-3-Gl), cyanidin-3-glucoside (C-3-Gl), petudin-3-glucoside (P-3-Gl), peonidin-3-glucoside (Po-3-Gl), malvidin-3-glucoside (M-3-Gl), peonidin 3-(6-acetyl glucoside) (PeAc-3-Gl), peonidin-3-(6-coumaryl glucoside) (PoCu-3-Gl), malvidin-3-(6-acetyl glucoside) (MAc-3-Gl) and malvidin 3-(6-coumaryl glucoside) (MaCu-3-Gl)) were detected by direct injection after centrifugation and filtration of original extract, using formic acid, milli-Q water and acetonitrile as HPLC solvents (Dallas & Laureano, 1994). Catechins ((+)-catechin ((+)cat) and (−)-epicatechin ((−)-epi)) and procyanidins (B1 procyanidin (B1), B2 procyanidin (B2), B3 procyanidin (B3), B4 procyanidin (B4), T2 procyanidin (T2), C1 procyanidin (C1), B1-3-O-gallate procyanidin and B2-3-O-gallate procyanidin) were extracted by solid phase extraction, using polyamide columns. After removing phenolic acids with phosphate buffer, catechins were eluted with acetonitrile/water and procyanidins with acetone/water. Catechin and procyanidin fractions were evaporated till dryness under vacuum and the residue was redissolved in a methanol/water mixture as described before (Dallas, Ricardo-da-Silva, & Laureano, 1995; Ricardo da Silva, Rosec, Bourzeix, & Heredia, 1990). Finally, an aliquot was detected by HPLC, employing acetic acid, milli-Q water and acetonitrile, under gradient mode (Dallas et al., 1995; Ricardo da Silva et al., 1990). Quantification was performed using malvidin-3-glucoside for anthocyanins, (+)-catechin for catechins and B2 procyanidin for procyanidins. Duplicate analyses were carried out for each sample and relative standard deviations (%) obtained are shown in Table 1.

2.3. Chemometric study

The statistical methods used for data analysis of wine samples were principal component analysis (PCA) and partial least-squares regression (PLS-R). Unscrambler program for Windows version 9.1 was used for data processing (Camo Process AS, 2004).

3. Results and discussion

3.1. Phenolic Compounds in grapes

In order to compare the total anthocyanins and phenolic compounds from selected wines during 2000, 2001 and 2002 harvests, total anthocyanins and phenolic compounds were evaluated also, in the collected grapes of *V. vinifera var. Vinhão* before each wine-making protocol. Antocyanin content between 1835 and 2000 mg/l and IFC values between 156 and 243 mg/l were obtained for the three selected harvests. The anthocyanin levels detected were higher than those determined by other authors *V. vinifera var* such as cabernet franc, merlot, pinot noir, syrah, monastrell or cabernet sauvignon (Mazza et al., 1999; Ortega-Regules et al., 2006) because of the characteristic flesh purple colour of this grape

Table 1
Changes in concentration (mg/l) of individual anthocyanins, catechins and procyanidins in the selected wines of 2000 harvest after 26 months' storage ($n = 2$)

Compound		Time of analysis	Type of winemaking technology	
			PO	CM
Anthocyanins[a] (RSD < 8%)	D-3-Gl	0	17	10
		26	n.d.	4.1
		ER	**100**	**59**
	C-3-Gl	0	31	18
		26	n.d.	3.8
		ER	**100**	**79**
	P-3-Gl	0	21	12
		26	5.0	2.6
		ER	**76**	**79**
	Po-3-Gl	0	66	38
		26	15	8.2
		ER	**78**	**79**
	M-3-Gl	0	452	260
		26	130	56
		ER	**71**	**79**
	PeAc-3-Gl	0	29	17
		26	n.d.	10
		ER	**100**	**43**
	PoCu-3-Gl	0	41	24
		26	5.5	11
		ER	**87**	**54**
	MaCu-3-Gl	0	28	16
		26	7.3	8.5
		ER	**74**	**47**
Flavanol-3-ol Polymers[b] (procyanidins) (RSD < 10%)	B1	0	138	100
		26	99	91
		ER	**28**	**9.0**
	B2	0	60	32
		26	42	10
		ER	**30**	**69**
	B3	0	21	22
		26	10	7.0
		ER	**52**	**69**
	B4	0	47	16
		26	n.d.	17
		ER	**100**	**−6.3**
	T2	0	19	16
		26	23	31
		ER	**−23**	**−99**
Flavan-3-ol monomers[c] (Catechins) (RSD < 10%)		0	330	120
		26	290	260
		ER	**13**	**−93**
	(-)- Epicatechin	0	290	43
		26	**560**	**170**
		ER	−210	−385

n.d.: non-detecteable.
ER: extinction rate (%).
RSD (%): relative standard deviation in percentage.
Results expressed as:
[a] Malvidin-3-glucoside;
[b] Procyanidin B2;
[c] (+)-catechin.

variety called "tintoreira". Differences between grapes from the three sampling harvests were due to the climatic year (Mazza et al., 1999; Ortega-Regules et al., 2006; Revilla et al., 1995), observing the highest one in 2001.

3.2. Effects of wine-making protocol on the extraction of polyphenols

As was previously reported by the present authors in 1999 harvest (Castillo-Sánchez et al., 2006), the two selected protocols produced different colour densities and anthocyanin content immediately post-vinification. In general, CM protocol produced wines with less colour density and higher hue than the other selected winemaking procedure (PO). Therefore, PO extracts more pigmented phenolic compounds (between 38% and 44% for PO and between 15% and 28% for CM). These recoveries were calculated taking into account the concentration of phenolic compounds in grapes and wines for each harvest. Although the CM protocol might have been expected to increase the release of anthocyanins from the grape skin due to the longer overall time spent macerating and fermenting and to the higher temperature used (Gao et al., 1997; Lorincz, Kállay, & Pásti, 1998), these effects seem to have been overweighed by the effect of reducing post-crushing fermentation time to 2–3 days, which reduced the duration of intimate contact between skin and must. Similar results were obtained by Spranger et al. (2004) where they detected higher anthocyanin levels in classical fermentation Castelão red wines obtained than in CM Castelão red wines.

With regard to hue, the highest values were determined in CM wines. Reasonable levels due to the higher temperature of the carbonic maceration process, which could accelerate the polymerization of anthocyanins each other and with other phenolics. CA values also confirm this behaviour. Similar results were shown by Gao et al. (Gao et al., 1997) with pinot noir wines obtained with different fermentation temperatures.

In order to check and to explain the results obtained by spectrophotometry, the extracts of 2000 harvest were determined by HPLC. It is noteworthy that the anthocyanin levels obtained by HPLC are lower than the levels obtained by spectrophotometric methods. This difference, previously documented (Rivas-Gonzalo, Gutierrez, Hebrero, & Santos-Buelga, 1992), is attributed to the monomeric anthocyanins, detected by HPLC, in comparison with the total anthocyanins detected by spectrophotometry. Nevertheless, the results obtained by both techniques confirm the higher extraction efficiency of PO protocol.

As Table 1 shows, malvidin-3-glucoside was the domineering anthocyanin in PO and CM wines, account for 60% and 66% of all anthocyanins, respectively. Peonidine-3-glucoside was the second important one (9–10%) detected in PO wines, the rest of anthocyanins were delfinidine, cianidine and petunidine-3-glucoside, acetyl peonidine and cumarilade peonidine and malvidin. In CM wines, peonidine-3-glucoside and peonidine cumarilade-3-glucoside account for 9% and 10%, respectively, appearing the rest in lower concentrations (5%). Quantitatively anthocyanin levels are higher in PO and CM wines than other red wines like mencia, brancellao, (García-Falcón, Pérez-Lamela, Martínez-Carballo, & Simal-Gándara, 2007; Pérez-Lamela, García-Falcón, Simal-Gándara, & Orriols-Fernández, 2007), pinot noir (Mazza et al., 1999); monastrell (Gómez-Plaza et al., 2000a; Gómez-Plaza et al., 2001) castelão (Spranger et al., 2004) or tinta miuda (Sun et al., 2001; Sun & Spranger, 2005) (Table 1). Although there are quantitative differences between these wines, the qualitative profile is very similar, but in wines obtained in the present work malvidin 3-(6-acetyl glucoside) was not detected.

Procyanidins and catechins were also determined by HPLC in 2000 harvest, because these compounds are very important for the stabilisation of colour during the storage and aging of wines. As in anthocyanins, procyanidin and catechin levels in PO wines were much higher than in CM wines, and also higher than levels reported by other authors in *Vitis vinifera var* wines (monastrell, cabernet sauvignon, tinto fino, castelao, mencia, brancella or tinta roriz) (Dallas et al., 1995; Gómez-Plaza et al., 2000a; Gómez-Plaza et al., 2000b; Gómez-Plaza et al., 2001; Mayen, Mérida, & Medina, 1995; Revilla & González-San José, 2003).

Spranger et al. (2004) also detected higher catechins and procyanidin levels in Castelão red wines made by classical techniques. Otherwise, Sun and Spranger (2005) reported highest procyanidin levels in CM tinta miúda red wines.

In PO wines, for all flavan-3-ol polymers, B1 was the most important procyanidin (49%), followed by B2 (21%) and B4 (17%). B3 procyanidin and T2 trimer accounted for 10%. Epicatechin and catechin accounted for 29% and 70% of all flavan-3-ol monomers, respectively. In CM wines, B1 and B3 procyanidins and catechin are detected in higher rates than PO wines (55%, 12% and 88%, respectively), while B2 procyanidin and epicatechin in lower rates (17% and 12%). Only in PO wines, C1 procyanidin trimer and galloylated procyanidins were analyzed in very low concentration levels.

These results confirm the influence of the wine-making protocol in the extraction of phenolic compounds from grapes and also, in their sensorial characteristics of obtained wines (Boulton, 2001; Gómez-Plaza et al., 2000a; Gómez-Plaza et al., 2001; Spranger et al., 2004; Sun et al., 2001; Sun & Spranger, 2005).

3.3. Effects of wine-making protocol on the evolution of unfined wines

During storage, all the protocols present the same trend. They underwent a pH rise and hue value and a fall in total anthocyanin content, colour density and chemical age (Fig. 1).

When total anthocyanin content evolution and colour density are studied during storage in PO and CM wines,

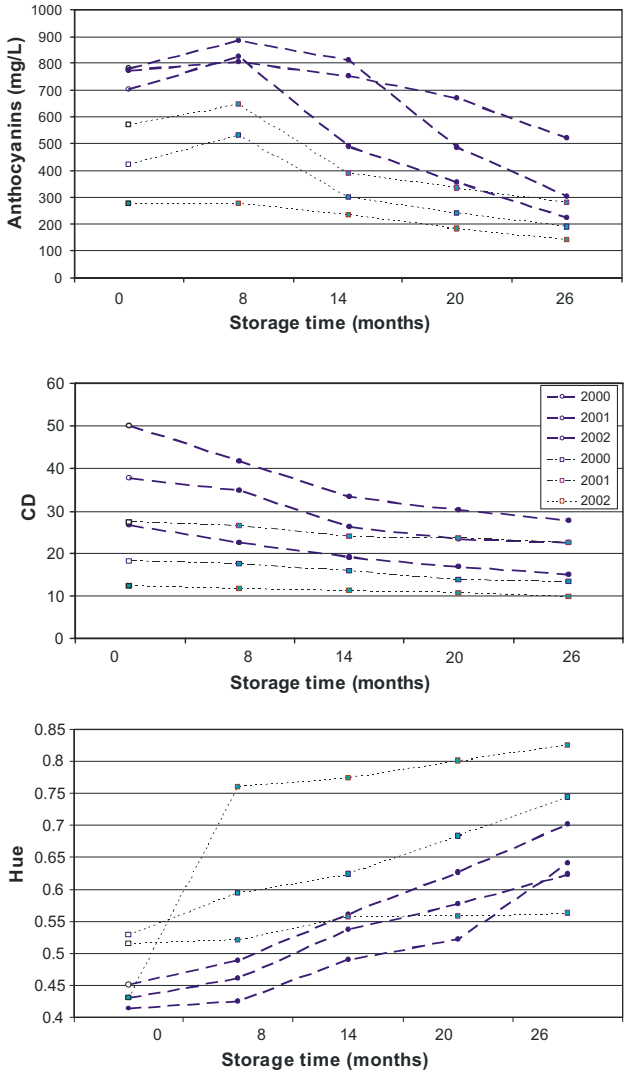

Fig. 1. Evolution during storage of total anthocyanin contents, colour densities and hues of PO and CM wines. ● PO protocol; ■ CM protocol.

no correlations are observed (Fig. 1). All wines underwent a fall of total anthocyanins, mainly in the first year, diminishing gradually till 26 months' storage. Otherwise the progressive fall of colour density during storage could be assumed as a linear function with an acceptable regression coefficients (MC: $r = 0.96$; C: $r = 0.98$) and they could be very useful to predict colour density in future harvests. The absence of parallelism in total anthocyanins and colour density evolution during storage make clear that the fall in anthocyanins was not only due to degradation of anthocyanins but also to their gradual polymerization with other phenolics (flavan-3-ol monomers and polymers, flavones, phenolic acids, ldots). These new compounds, which contribute an orange–red hue, are more stable to pH changes and they promote colour stabilization. The progressive hue rise during storage for all selected wines point out the evolution of violet–red to orange–red hue.

Although the colour density of CM wine is markedly different from the PO wine at the time of bottling (less red), after 26 month's storage this difference became smaller and therefore, CM wines would keep the colour density longer. This fact could be explained in own process of carbonic maceration, where other phenolics could be extracted and native phenolic compounds, apparently disappeared, would be converted to other phenolic forms and they would contribute to colour stabilization. In fact, hue values in CM wines are higher than in PO wines and therefore, orange–red hues corresponding to larger range of polymerization of phenolics (Glories, 1984). CA values also confirm these results.

Total monomeric anthocyanin content, determined by HPLC, decreases notably during the first 14 months' storage (63% for PO wines and 55% for CM wines) and more gradually during the following 12 months' storage (37% and 43%, respectively). As Figs. 2 and 3 show, anthocyanin levels in red young wines depends significantly on the type of winemaking technology, but in aged red wines, these differences become considerably lower. Differences between anthocyanin levels by HPLC and spectrophotometry in aged red wines are higher than in red wines at the time of bottling (Rivas-Gonzalo et al., 1992; Sun & Spranger, 2005). This phenomenon also reveals that during the storage, the anthocyanins are polymerized with other compounds and they cannot be determined by HPLC.

Extinction rates (ER) of all individual anthocyanins were significantly different among the different winemaking technologies. Delfinidine-3-glucoside, cyanidine-3-glucoside and peonidine-3-acetyl glucose were the more unstable anthocyanins in PO protocol, disappearing completely after 14 months of storage. Unlike other authors (Sun & Spranger, 2005), in this work malvidin-3-glucoside was the most stable anthocyanin after 26 months' storage. In CM protocol, cyanidine, petunidine, peonidine and malvidin-3-glucoside, were the more unstable ones (79%) contrasting with coumaryls and acetyl forms, which were the anthocyanins with more stability. No anthocyanin was 100% degraded like in PO wines.

As for anthocyanins, evolution of all individual flavan-3-ol monomers and polymers were significantly different among the different winemaking protocols (Fig. 2). PO wines underwent a rise in approximately 20% of total

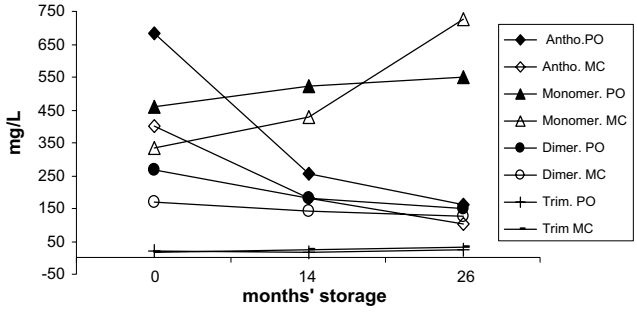

Fig. 2. Evolution of free anthocyanins (antho.), flavan-3-ol monomers (monomer), flavan-3-ol dimers (dimer) and flavan-3-ol trimers (trim.) during storage of 2000 harvest PO and CM wines.

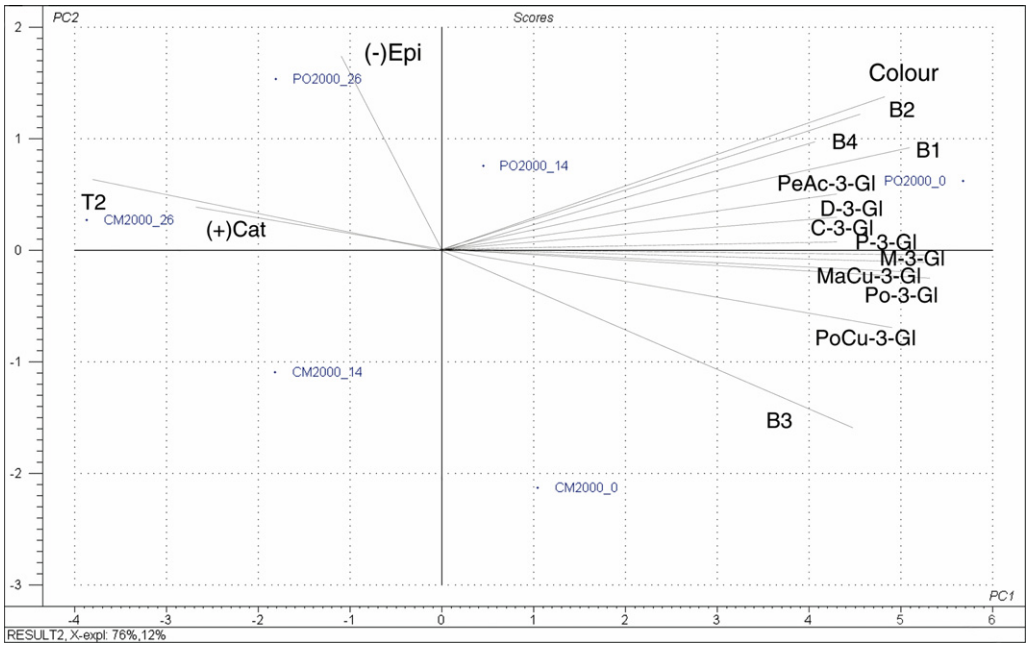

Fig. 3. Distribution of wines in the 2-D-co-ordinate system defined by the first two principal components. CM: wines from carbonic maceration; PO: wines from conventional protocol; 2000: harvest from 2000. Attribute loadings are shown as vectors. See Section 2.2.3 for definition of abbreviations.

monomeric flavonols, especially epicatechin. Procyanidins in PO wines underwent a decrease by 30% in the first 14 months' storage and by 40% after 26 months' storage. This decrease is due to the fall of flavan-3-ol dimers (procyanidins B1, B2, B3 and especially B4, which is completely degraded during the first 14 months' storage). On the other hand, the trimer T2 increased by 23% during the following two years of bottling. In CM wines, flavan-3-ol monomer levels underwent a rise more important than in PO wines (70% in front of 20% of PO wines). B1 procyanidin was the more stable procyanidin, decreasing only by 9%, followed by B2 and B3 procyanidins (decreasing 69%). The trimer T2 increased by 99%.

Contradictory results about the evolution of flavan-3-ols during storage have been published by some authors. Sun and Spranger (Sun & Spranger, 2005) confirmed the decrease of catechins and procyanidins during two years' of storage in PO and CM wines. Otherwise, other authors (García-Falcón et al., 2007; Gómez-Plaza et al., 2000a; Gómez-Plaza et al., 2000b; Gómez-Plaza et al., 2001; Mayen et al., 1995) observed a fall in catechins levels and a rise in procyanidins. These oscillations in flavan-3-ol monomers and polymers, in both CM and PO wines, would be the result of complex polymerization and depolymerization processes as well as some combinations with anthocyanins to give more stable pigments.

The different evolution of anthocyanin content, and especially of catechins and procyanidins in PO and CM protocols, combined to the capacity of CM protocol to extract some procyanidins not detected in PO wines (trimer C1, procyanidin B1-3-O-gallate and procyanidin B2-3-O-gallate) could explain the difference in colour stability. Decrease of dimmers in PO and CM protocols concomitant to the anthocyanins, does not mean degradation of these substances, in fact, there are condensations between these compounds, generating new colour stable substances.

The analysis and interpretation of the data given by numerous variables is difficult without the aid of multivariate statistical analysis. Principal component analysis (PCA) is a useful tool data inspection and reduction of dimensions. Performed on the wine data after 26 months' storage it makes it possible to reduce the structure of the data to two dimensions. The total variance explained by these two components amounts to 87% of the variability of the data. Fig. 3 shows the variables with the highest loadings in each principal component 1 and principal component 2. Clear separation was found between winemaking protocol and storage time. Wines at the end of the alcoholic fermentation and some wines from 14 months' storage are located in the positive part of principal component 1 where all the anthocyanins and almost all of procyanidins have the highest loadings. Wines from 26 months' storage have higher influence of epycatechins and some procyanidins and therefore, they are located in the negative part of PC1.

3.4. Effects of fining agents

The object of fining is to aid to create a product, which is near perfect in terms of taste, colour, bouquet and clarity. The fining method should not take away from any of these characteristics and should allow the clarified conditions to be maintained for as long as necessary before the wine is consumed. Any fining treatment preferably should

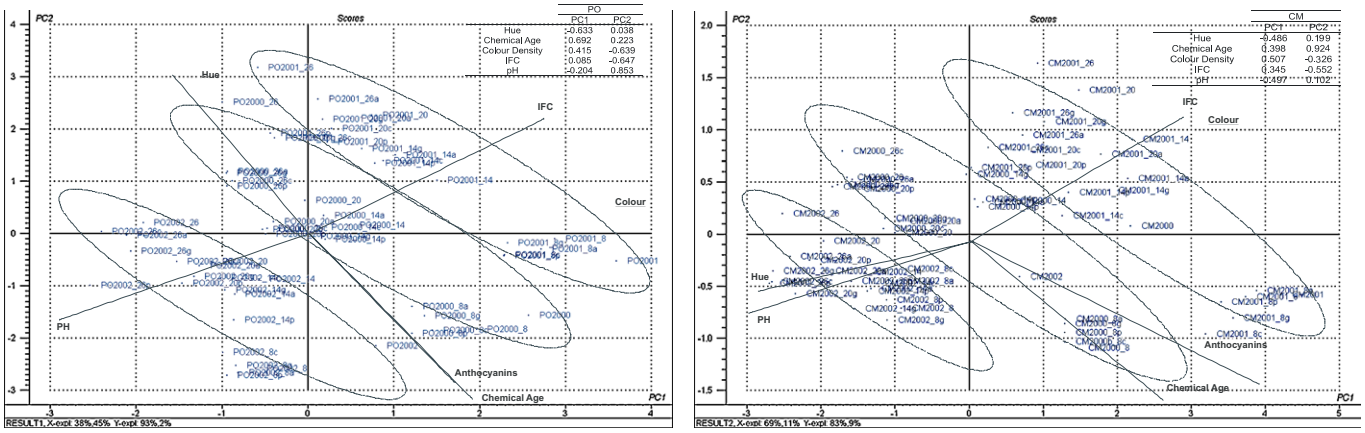

Fig. 4. Partial least-squares regression plots for CM and PO protocols, showing the *X* loading weights and the *Y* loadings. See Section 2.2.3. for definition of abbreviations.

have little or no effect on the essential aromatic and flavour compounds of wine. In this work, the fining selected agents were: polyvinylpolypyrrolidine (PVPP), albumin (A), gelatin (G) and casein (C). In this way only spectrophotometric determinations were carried out in order to check the effect of these agents on the evolution of colour density during storage.

The colour density and anthocyanin content of fined wines during storage, as well as in unfined wines, underwent a rise in pH and hue value, and a fall in chemical age, colour density and anthocyanin content. Only these last two parameters were different during storage in regard to unfined wines. Although fining agents can potentially improve the stability of wine colour, in this study the hues of fined wines did not differ significantly from those of the unfined wines after 26 months' storage.

The colour density in fined wines in the three selected harvests was generally somewhat less than in unfined wines during storage, especially when the fining agent was PVPP. Anthocyanin content changed also in fined wines and it was also PVPP the agent, which promoted an important fall of these pigmented polyphenols. The lower anthocyanin content in fined wines, especially when the fining agent was PVPP, verified the lower colour density. This fining induced the loss of colour density and anthocyanin content was expected in view of the previous reports of the same effects (Castillo-Sánchez et al., 2006; Maury et al., 2001; Sarni-Machado, Deleris, Avallone, Cheynier, & Moutounet, 1999; Sims et al., 1995). It would be interesting for future research to investigate whether pre-fermentation addition of fining agents to Vinhão wines causes the increase in anthocyanin content reported for other red wines by Gomez-plaza et al. (Gómez-Plaza et al., 2000b), which attribute this increase to the fining agents preventing anthocyanins from being fixed on solids that are removed following fermentation.

The method of regression by PLS has been used extensively in chemometrics, where they have found a wide field of application. Thus, it was used to find correlations between colour densities in wines (*Y* variables) with a series of values corresponding to hue, chemical age, IFC, pH and anthocyanins (*X* variables) for data obtained from CM and PO protocols. To attach a weighting to each variable, the data obtained were divided by the standard deviation of each series and later processed by means of PLS1 algorithm of Unscrambler program, utilizing the method of "cross validation". "Leverage correction" validation method may result in over-optimistic validation results. The selected algorithm was able to correlate a block of *X* variables with *Y* variable, giving a regression coefficient of 0.9693 and 0.9767 for a model with two principal components for CM and PO protocols. Fig. 4 shows a partial squares regression plots and also the regression line drawn against the prediction obtained in the calibration model using PLS.

Fig. 4 shows the contribution of each variable to PC1 and PC2, confirming the negative contribution of hue and pH values, because of the fall in anthocyanins, and the positive contribution of chemical age, IFC and anthocyanins.

Table 2
Predicted colour densities for the PO wines from 1999 harvest obtained by means of the regression models obtained by PLS1

Storage time (months)	Fining agent	Prediction	Real values	Deviation
0	–	32.6	28.1	3.17
8	–	30.6	25.4	3.71
	p[a]	30.4	23.8	4.69
	a	31.1	25.8	3.75
	g	30.8	25.4	3.82
	c	30.0	25.7	3.05
14	–	24.7	22.5	1.55
	p	22.2	20.4	1.28
	a	22.4	20.0	1.68
	g	23.5	23.1	0.291
	c	21.5	23.3	1.28

[a] Fining agents are indicating by the following abbreviation: p, polyvinylpolypyrrolidine (PVPP); a, albumin; g, gelatin; c, casein.

Finally, the PLS model was applied to determine the colour density of the set of samples from 1999 harvest, previously reported by the present authors (Castillo-Sánchez et al., 2006). As it can be observed when the results are compared with the real values (Table 2), although the results are appreciably different, the order of colour densities predicted is similar.

It can be concluded that PLS, using the normal routine parameters, hue, IFC, pH, chemical age and anthocyanins as predictive variables, can be used to determine the colour density of "vinhos verdes".

4. Conclusions

In the present paper, it has been demonstrated that the initial colour of Vinhão wine and its stability during storage, depend on the wine-making protocol employed. At the end of the alcoholic fermentation, PO afforded the wines with the greatest antocyanin, flavan-3-ol monomers and polymers contents, highest colour density and lowest hue values.

The levels of anthocyanins and flavan-3-ol dimer compounds decreased during storage of both selected protocols and they underwent a rise in flavan-3-ol monomers and trimers. However, colour density of CM wine was more stable than PO wines during the storage. This behaviour reveals that in CM wines polymerization and copigmentation reactions prevail over degradation of phenolic compounds.

All fined wines, especially with PVPP, presented lower anthocyanin levels and colour density than unfined wines.

PCA showed that the discrimination between wines from different protocols and time of storage is mainly due to the positive and negative correlation between phenolics and therefore, it would be possible to distinguish between wines from different protocol and storage time.

A prediction PLS-model was calculated to predict the colour densities of Vinha-o wines from PO and CM protocols.

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