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Influence of wine-making protocol and fining agents on the evolution of the anthocyanin content, colour and general organoleptic quality of Vinhão wines

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Abstract

This paper reports the evolution, over two years, of the anthocyanin content, colour and organoleptic quality of red wines prepared from a single batch of Vinhão grapes by means of three different protocols (maceration/fermentation with conventional pumping-over or in rotary vats, and fermentation after initial carbonic maceration), with and without the use of four different fining agents (polyvinylpolypyrrolidine, gelatin, egg albumin, and casein). Carbonic maceration led to lower anthocyanin levels and less intense coloration than the other two methods immediately following vinification, but during storage the carbonic maceration wines underwent less colour degradation than the others, so that after two years the colour density differences among the three were negligible. Wines treated with fining agents tended to have somewhat lower anthocyanin levels and, especially in the case of PVPP, less intense colouration than untreated wines, and their colour was at best only marginally more stable during storage, but they nevertheless generally achieved higher panel ratings for organoleptic quality than untreated wines, especially as regards taste.

Keywords: Phenolics; Colour; Red wine; Wine-making; Fining agents; Vinho verde

1. Introduction

The Vinhão wines of the Vinho Verde winegrowing region of northern Portugal are low-alcohol, acid young red wines that have traditionally been made by pressing the grapes immediately after harvest and fermenting the must for 5–8 days at 20–25 °C in the presence of skins, stems and seeds. This technique extracts high levels of anthocyanins – one of the most appreciated characteristics of these wines is their deep purplish red colour – but also tends to overextract tannins, leading to excessive astringency. Accordingly, wine-making protocols in which stems are removed prior to fermentation are being investigated. Since wine-making conditions also affect the initial colour of red wines (Dallas & Laureano, 1994; Sims, Eastridge, & Bates, 1995), it is necessary that new protocols introduced for Vinhão wines do not diminish their attractive colouration.

In this work, we investigated differences in the colour and other properties of Vinhão wines made by three different protocols. Furthermore, since the colour of Vinhão wines can suffer significant degradation during storage (Castillo-Sanchez, Arantes, & Maia, 1996), we investigated the evolution of these properties during two years storage and whether this evolution was influenced by the wine-making protocol or fining agents.

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2. Material and methods

2.1. Grapes and wines

Grapes of *Vitis vinifera* var. *Vinhão* were harvested by hand at technical maturity in an experimental vineyard in Arcos de Valdevez (northern Portugal) and were immediately transported to the winery and processed by one of the following three protocols (Fig. 1): destemming and crushing followed by fermentation for 1 week at 25 °C with conventional hourly pumping-over (PO); a variant in which the cap was immersed by rotation of a rotary vat every 12 h rather than by pumping-over (RV); and a protocol in which destemming and crushing were preceded by 2 weeks' carbonic maceration at 30 °C, but post-crushing fermentation time was reduced to 2 or 3 days at 25 °C (CM). In each case, fermentation, racking and pressing were followed by cold stabilization for 4 weeks at 10 °C, after which part of the wine was bottled 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 2 weeks' stabilization at 10 °C. Once bottled, all wines were stored at 10–12 °C, and after 8, 14, 20 and 26 months they were analysed and evaluated organoleptically as described below.

2.2. Analyses

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. Monomeric anthocyanin concentration was determined from the absorbance measured at 520 nm, using malvidine-3-glycoside chloride as standard (Ribereau-Gayon & Stonestreet, 1965). Hue was quantified as the ratio of absorbance at 420 and 520 nm (Glories, 1984). Alcohol content, pH and titratable acidity were determined by OIV methods (OIV, 1990).

2.3. Sensory evaluation

Wine samples labelled with three-digit code numbers which varied from tasting to tasting were rated on a

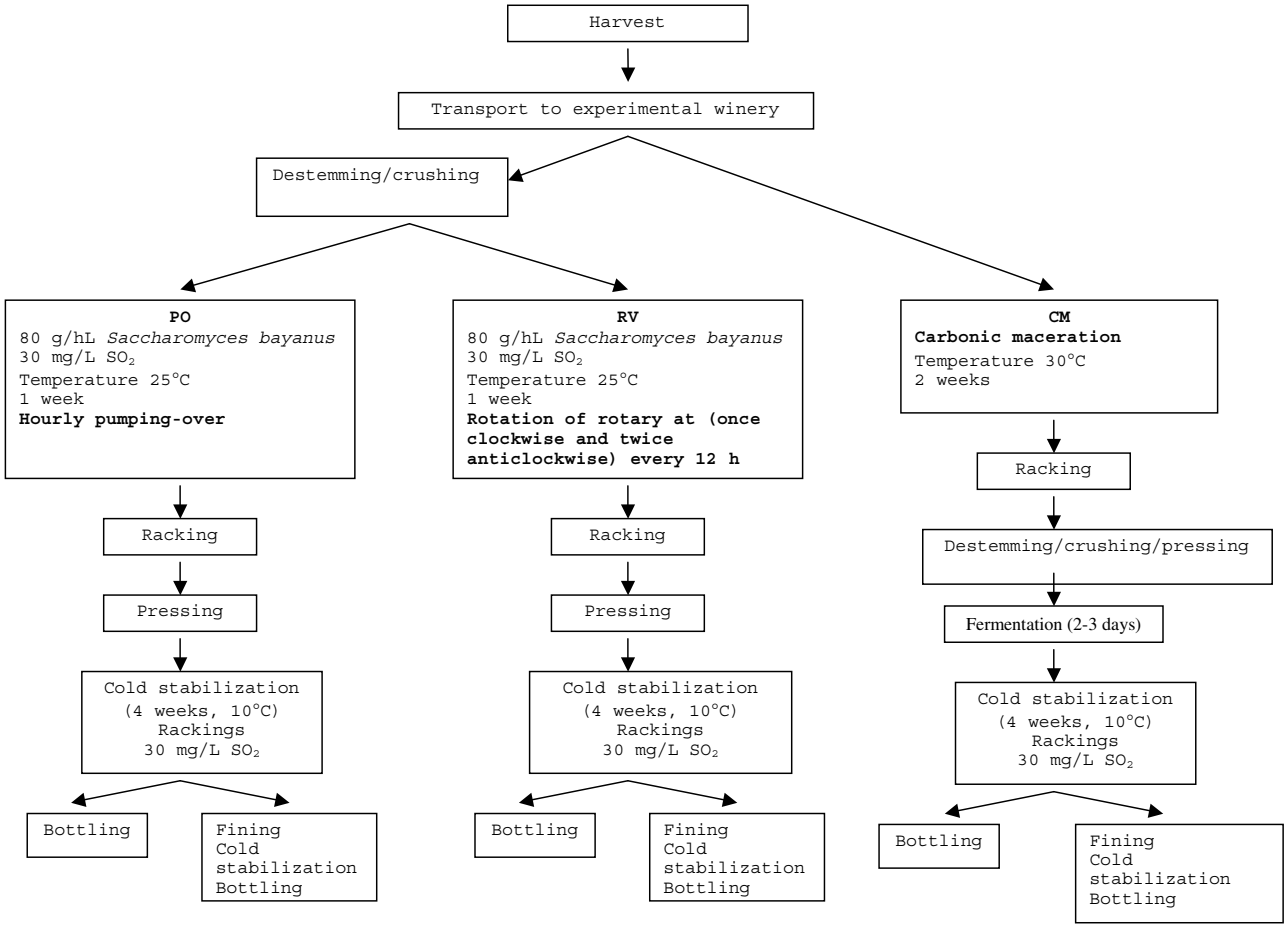

Fig. 1. Flow chart of the three wine-making processes employed in this study.

scale of 0–8 by a panel of eight specialists Vinho Verde winetasters for colour (under natural light against a white background), foam, aroma and taste. The aroma ratings reported below are means of ratings for three distinct aspects of aroma (limpidity, intensity and quality), and the taste ratings are means of ratings for five aspects of taste (limpidity, body and mouthfeel, harmony, length, and intensity of aftertaste).

3. Results and discussion

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

The protocol involving carbonic maceration (CM) produced wine with less total acidity than the other two methods, while pumping-over (PO) led to lower pH and higher alcohol content than the use of either carbonic maceration or rotary vats (RV) (Table 1). At bottling, the ordering of the wines by pH (PO < RV < CM) was nevertheless consistent with their total acidities (PO > RV > CM). Furthermore, their orderings by colour density (PO > RV > CM) and hue value (PO < RV < CM) (Table 2) were in keeping both with their pH ordering (since decreasing pH increases the proportion of anthocyanins in flavylium form (Dallas & Laureano, 1994; Gao, Girard, Mazza, & Reynolds, 1997)) and with their anthocyanin contents (PO > RV > CM). Thus, although the carbonic maceration 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 (Gomez-plaza, Gil-muñoz, Lopez-roca, Martinez-cutillas, & Fernandez-fernandez, 2001) and to the higher temperature used (Du Pleissis, 1973), these effects seem to have been outweighed by the effect of reducing post-crushing fermentation time to 2–3 days, which reduced the duration of intimate contact between skin and must. It is also possible that the higher temperature of the carbonic maceration process may have accelerated the polymerization of anthocyanins with each other and with other phenolics (Gomez-plaza, Gil-muñoz, Lopez-roca, & Martinez, 2000b; Mirabel, Saucier, Guerra, & Glories, 1999), which would have raised their hue value. The differences between the PO and RV wines as regards anthocyanin content, colour density and hue may be attributed to twice-daily vat rotation being a less efficient means of cap immersion than hourly pumping-over.

Table 1
Alcohol contents, pH and total titratable acidities of Vinhão wines made using three vinification protocols (PO, RV and CM; see text), immediately post-vinification and after 26 months' storage

Wine and storage time	pH	Alcohol (%)	Total Acidity (mg/L)
PO			
0 months	3.461	9.9	7.35
26 months	3.489	–	6.20
CM			
0 months	3.540	9.3	6.00
26 months	3.635	–	5.85
RV			
0 months	3.522	9.2	7.27
26 months	3.585	–	6.77

During the two years following bottling, all the unfined wines underwent a rise in pH and hue value and a fall in total acidity, anthocyanin content (by 75–80%) and colour density (by 23–43%) (see Tables 1 and 2 and Fig. 2). However, whereas the fall in anthocyanin content took place almost exclusively during the first 14 months following bottling, colour density only exhibited this behaviour in the case of CM wine, falling very linearly for PO and RV wines ($r^2 = 0.9961$ and 0.9490, respectively). Together with the rise in hue value, the fact that the colour density of PO and RV wines did not decrease so sharply during the first year as did their anthocyanin content suggests that at least in these cases the fall in anthocyanin content was not only due to the degradation of anthocyanins but also to their gradual polymerization (Gonzalez-Neves & Gomez-Cordoves, 1995; Santos-Buelga, Francia-Aricha, & Rivas-Gonzalo, 1996). During the second year of storage, in spite of there being comparatively little reduction in the anthocyanin content of any of the wines, hue value continued to rise, and this rise was in fact more rapid than during the first year in the cases of PO and RV wines, in which, moreover, colour density continued to fall. This behavior may have been due to the polymerization of phenolics that until then had been involved in copigmentation complexes with monomeric anthocyanins; if so, the difference in behaviour between CM wines on the one hand and PO and RV wines on the other may have been due to quantitative and qualitative differences in their initial monomeric phenolic contents (Auw, Blanco, O'keefe, & Sims, 1996).

It is noteworthy that the decreases in anthocyanin content and colour density during the two-year storage period were larger, the higher the initial values of these parameters, CM wine being the most stable in these respects and PO wine the least (Fig. 2). It is possible that these differences in initial values and stability may reflect differences in the extent to which anthocyanins copolymerized with flavan-3-ols such as catechin and procyanidins during fermentation (Gomez-Plaza et al., 2001; Mirabel et al., 1999; Okuda, Takayagani, Sato, & Yokotsuka, 2002). As a result of the differences in initial values and stability, at the end of the storage period all three wines had very similar monomeric anthocyanin contents and colour

Table 2
Colour densities, anthocyanin contents (mg/L) and hue values of Vinhão wines made using three vinification protocols (PO, RV and CM; see text), with[a] and without fining, immediately post-vinification and at various times after bottling (means of three determinations)

Time (months)	PO	POp	POa	POg	POc	CM	CMp	CMa	CMg	CMc	RV	RVp	RVa	RVg	RVc
Colour density															
0	28.1	–	–	–	–	15.7	–	–	–	–	22.8	–	–	–	–
8	25.4	23.8	25.8	25.4	25.7	13.1	11.5	11.9	12.6	12.5	22.4	19.2	22.2	20.9	21.0
14	22.5	20.4	20.0	23.1	23.3	12.6	12.3	12.1	12.8	12.7	19.9	18.1	18.5	19.5	18.7
20	19.4	16.7	17.7	17.7	17.3	12.3	11.4	12.4	12.4	12.2	18.1	15.6	16.2	17.0	15.9
26	16.4	15.5	15.7	15.3	15.8	12.0	11.3	11.6	11.9	11.8	15.2	15.0	15.1	15.0	15.1
Anthocyanins (mg/L)															
0	756	–	–	–	–	421	–	–	–	–	562	–	–	–	–
8	827	725	781	762	670	341	307	320	307	306	468	369	402	400	381
14	355	288	313	319	266	202	159	186	171	168	299	235	256	238	253
20	341	290	312	314	260	204	169	181	187	174	274	220	244	231	248
26	226	230	222	236	218	162	137	111	130	129	216	170	168	189	182
Hue															
0	0.442					0.556					0.480				
8	0.472	0.468	0.463	0.464	0.485	0.618	0.605	0.612	0.608	0.632	0.543	0.531	0.536	0.526	0.537
14	0.494	0.521	0.589	0.480	0.477	0.665	0.761	0.617	0.662	0.669	0.532	0.558	0.580	0.602	0.563
20	0.614	0.573	0.580	0.572	0.581	0.687	0.708	0.693	0.700	0.708	0.672	0.627	0.635	0.640	0.641
26	0.658	0.616	0.622	0.634	0.642	0.757	0.723	0.748	0.744	0.743	0.705	0.668	0.684	0.696	0.643

[a] Fining agents are indicated by a small letter following the abbreviation of the vinification protocol, as follows: p, polyvinylpolypyrrolidine (PVPP); a, albumin; g, gelatin; c, casein.

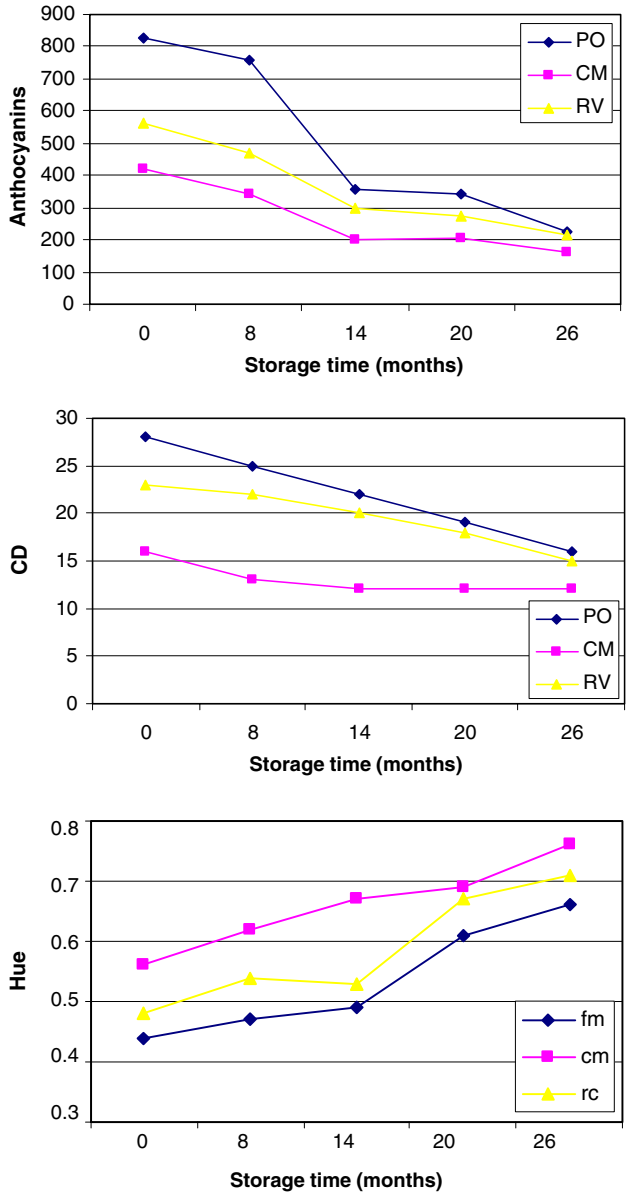

Fig. 2. Evolution, during 26 months' storage, of the anthocyanin contents, colour densities and hues of Vinhão wines made using three wine-making protocols (see Fig. 1).

densities. However, they differed less as regards changes in hue value, which spanned an interval of about 0.1 (with PO < RV < CM) both immediately after bottling and two years later.

The results of the sensory evaluation trials (Table 3) exhibit two main features. First, PO wine generally scored higher than the others for the various attributes evaluated. Second, all three wines maintained their organoleptic quality during the first 20 months of storage, but by month 26 all three showed marked deterioration of at least two of the four facets evaluated.

3.2. Effects of fining agents

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 8 months' storage and after 26 months were at best only marginally less brown (Table 2). After 14 months' storage, fining led to considerably higher hue values for several combinations of wine-making protocol and fining agent.

The colour density of fined wines was generally somewhat less than that of unfined wines during the first 20 months' storage, especially when the fining agent was PVPP, which can precipitate light-weight phenolics (Donner, Becard, & Irwin, 1993; Sims et al., 1995). After 26 months, however, the difference between fined and unfined wines was very small (3–6% for PVPP-treated wines).

Somewhat surprisingly, although PVPP caused more loss of colour than the other fining agents, it was only in RV wine that it caused a slightly greater loss of anthocyanins than the other agents. In CM wine, all the fining agents caused very similar anthocyanin losses, while in PO wine casein caused losses some 50% greater than those caused by PVPP. The three wines (PO, RV and CM) also differed as regards the evolution of fining-induced anthocyanin losses: for CM wine the difference between fined and unfined wines was generally about 30–40 mg/L at all evaluation times (so that anthocyanin losses seemed to have occurred largely within the first 8 months); for RV wine the difference with respect to the unfined wine gradually lessened, so that after 26 months it was about half the difference observed after 8 months; while for PO wine the difference observed after 8 months had essentially been eliminated by the end of the experiment.

The fining-induced loss of colour density and anthocyanin content was expected in view of previous reports of the same effects (Maury, Sarni-manchado, Lefebvre, Cheynier, & Moutounet, 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, Gil-muñoz, Lopez-roca, Hera-orts, and Martinez-cultillas (2000a), which attribute this increase to the fining agents preventing anthocyanins from being fixed on solids that are removed following fermentation.

In general, the sensory ratings of the fined wines were similar to or rather higher than those of the unfined wines, even as regards colour, although the attribute benefitting most from fining was taste. Notably, for PO wine (though not for the others) PVPP prevented the sudden fall in colour and taste ratings that the unfined wine suffered between months 20 and 26.

Table 3
Panel ratings of sensory characteristics of Vinhão wines made using three vinification protocols (PO, RV and CM; see text), with[a] and without fining, immediately post-vinification and at various times after bottling (means of three determinations)

Storage time (months)	Colour					Flavour[b]					Foam					Aroma[c]				
	PO	POp	POa	POg	POc	PO	POp	POa	POg	POc	PO	POp	POa	POg	POc	PO	POp	POa	POg	POc
8	7.0	7.1	7.4	7.6	7.1	5.9	6.0	6.5	6.8	6.3	5.3	6.3	6.1	6.2	6.4	5.2	6.1	5.9	6.2	6.2
14	7.1	7.2	7.5	7.4	7.2	5.7	5.5	5.6	5.6	5.2	5.5	6.2	5.9	5.9	5.2	5.4	5.5	5.4	4.8	3.3
20	7.0	7.3	7.6	7.7	7.3	5.5	5.4	5.2	5.3	5.2	5.8	6.1	6.2	6.0	5.2	4.5	5.3	5.2	4.6	3.4
26	5.8	7.3	6.2	5.6	6.1	4.2	5.2	4.8	4.8	5.0	5.2	6.0	6.0	5.8	5.1	3.5	4.2	3.5	3.4	3.0
	CM	CMp	CMa	CMg	CMc	CM	CMp	CMa	CMg	CMc	CM	CMp	CMa	CMg	CMc	CM	CMp	CMa	CMg	CMc
8	5.6	5.8	6.6	6.7	6.8	6.1	6.2	6.3	6.3	6.2	4.8	6.3	6.1	6.2	6.2	5.8	6.3	6.8	6.5	6.4
14	5.2	6.5	6.3	6.4	6.3	4.8	6.6	5.8	6.2	5.5	4.7	6.2	6.0	5.8	5.3	4.9	5.2	5.0	4.5	4.1
20	5.5	6.6	6.4	6.5	6.8	5.0	6.5	5.8	6.2	5.4	4.8	6.2	6.1	5.9	4.2	4.6	4.8	4.8	4.4	4.0
26	5.2	6.0	6.2	6.2	5.2	3.5	6.2	5.5	6.0	3.6	4.5	4.9	6.0	5.7	3.8	3.0	4.0	3.1	4.3	3.0
	RV	RVp	RVa	RVg	RVc	RV	RVp	RVa	RVg	RVc	RV	RVp	RVa	RVg	RVc	RV	RVp	RVa	RVg	RVc
8	6.2	5.9	6.0	5.9	6.1	6.0	5.5	5.6	5.5	5.8	6.1	5.9	5.8	5.8	5.9	4.9	5.1	5.3	5.3	5.6
14	5.9	5.7	5.6	5.8	5.9	4.5	5.4	5.6	4.9	4.2	5.2	5.8	5.8	4.9	4.5	4.8	4.8	4.5	4.9	4.2
20	5.9	5.8	5.7	5.8	5.9	4.6	5.5	5.6	4.9	4.3	5.2	5.7	5.7	4.9	4.7	4.7	4.8	4.6	4.9	4.2
26	6.4	6.3	6.1	6.1	6.2	3.5	4.9	4.5	4.4	3.9	4.5	5.0	4.8	4.7	4.0	3.1	4.1	3.1	3.2	4.0

[a] Fining agents are indicated by a small letter following the abbreviation of the vinification protocol, as follows: p, polyvinylpolypyrrolidine (PVPP); a, albumin; g, gelatin; c, casein.
[b] Means of five flavour attributes.
[c] Means of three aroma attributes.

4. Conclusions

The initial colour of Vinhão wine, and the stability of its colour during storage, is significantly influenced by the protocol employed in its making. Of the three techniques used in this study, PO afforded the wines with the greatest anthocyanin contents, highest colour densities and lowest hue values, while CM afforded wines at the opposite ends of the observed ranges of these variables. However, the anthocyanin content and colour density of CM wine were the most stable and those of PO wine the most unstable, so that after 26 months' storage all three wines had quite similar anthocyanin contents and colour densities. Differences in hue values were largely maintained, all three wines becoming gradually browner.

References

Auw, J. M., Blanco, V., O'keefe, S. F., & Sims, C. A. (1996). Effect of processing on the phenolics and the color of sauvignon, chambourcin and noble wines and juices. *American Journal of Enology and Viticulture, 47*, 279–286.

Castillo-Sanchez, J. X., Arantes, J. & Maia, M. O. (1996). Étude de l'Évolution des Composés Phénoliques des Vins du Nord du Portugal issues des Différentes Processus de Vinification. In *Polyphenols communications 96, 18th international conference on polyphenols* (Vol. I, pp. 55–56), July 15–18, 1996, Bordeaux

Dallas, C., & Laureano, O. (1994). Effects of pH, sulfur dioxide, alcohol content, temperature and storage time on colour composition of a young portuguese red table wine. *Journal of the Science of Food and Agriculture, 65*, 477–485.

Donner, L. W., Becard, G., & Irwin, P. L. (1993). Binding of flavonoids by polyvinylpolypyrrolidine. *Journal of Agricultural and Food Chemistry, 44*, 2402–2407.

Du pleissis, C. S. (1973). Browning of white wines. *Die Wynboer, 499*, 11–13.

Glories, Y. (1984). La Couleur des Vins Rouges. 2ª Partie. Mésure, Origine et Interprétation. *Connaissance de la Vigne et du Vin, 18*, 252–271.

Gomez-plaza, E., Gil-muñoz, R., Lopez-roca, J. M., Hera-orts, M. L., & Martinez-cultillas, A. (2000a). Effect of the addition of bentonite and polyvinylpolypyrrolidine on the colour and long-term stability of red wines. *Journal of Wine Research, 11*, 223–231.

Gomez-plaza, E., Gil-muñoz, R., Lopez-roca, J. M., & Martinez, A. (2000b). Colour and phenolic compounds of a young red wine. Influence of wine-making techniques, storage temperature, and length of storage time. *Journal of Agricultural and Food Chemistry, 48*, 736–741.

Gomez-plaza, E., Gil-muñoz, R., Lopez-roca, J. M., Martinez-cutillas, A., & Fernandez-fernandez, J. I. (2001). Phenolic compounds and colour stability of red wines: effect of skin maceration time. *American Journal of Enology and Viticulture, 52*, 266–270.

Gonzalez-neves, G. & Gomez-Cordoves, C. (1995). Relaciones entre el Color y la Composición Fenólica de un Vino Tinto Joven con Diferentes Adiciones de Anhidrido Sulfuroso y Acetaldehido. In: *Actas del XXI Congreso Mundial de la de la Viña y el Vino* (pp. 354–368)

Gao, L., Girard, B., Mazza, G., & Reynolds, A. G. (1997). Changes in anthocyanins and colour characteristics of pinot noir wines during different vinification processes. *Journal of Agricultural and Food Chemistry, 45*, 2003–2008.

Maury, C., Sarni-manchado, P., Lefebvre, S., Cheynier, V., & Moutounet, M. (2001). Influence of fining with different molecular weight gelatins on proanthocyanidin composition and perception of wines. *American Journal of Enology and Viticulture, 52*(2), 140–144.

Mirabel, M., Saucier, C., Guerra, C., & Glories, Y. (1999). Copigmentation in model wine solutions: occurrence and relation to wine aging. *American Journal of Enology and Viticulture, 50*, 211–218.

OIV. (1990). Office Internationale de la Vigne et du Vin. Recueil des Méthodes Internationales d'Analyse des Vins et des Moûts, Paris

Okuda, T., Takayagani, T., Sato, M., & Yokotsuka, K. (2002). Changes in radical scavenging activity of Japanese Cabernet Sauvignon Red Wines during ageing. *Journal of Wine Research, 13*, 93–100.

Ribereau-gayon, P., & Stonestreet, E. (1965). Le Dosage des Anthocyanines dans le Vin Rouge. *Bulletin de la Societe Chimique de France*, 2469–2562.

Santos-Buelga, C., Francia-Aricha, E. & Rivas-Gonzalo, J. C. (1996). Role of flavan-3-ol structure on direct condensation with anthocyanins. In *Polyphenols Communications 96, 18th international conference on polyphenols* (Vol. I, pp. 9–10), July 15–18, 1996, Bordeaux

Sarni-machado, P., Deleris, A., Avallone, S., Cheynier, V., & Moutounet, M. (1999). Analysis and characterization of wine: condensed tannins precipitated by proteins as fining agent in enology. *American Journal of Enology and Viticulture, 50*, 81–86.

Sims, C. A., Eastridge, J. S., & Bates, R. P. (1995). Changes in phenols, colour and sensory characteristics of muscadine wines by pre- and post-fermentation additions of PVPP, casein, and gelatin. *American Journal of Enology and Viticulture, 46*, 155–158.