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Research Article

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Micro-Oxygenation and Fining Agent Treatments: Impact on Color of Moroccan Red Press Wine

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Abstract

Red press wine is collected after pressing solid parts (seed and skin) of grapes pomaces. Higher pressure induces more colorful, astringent, bitter and rustic wines. This is caused by the presence of undesirable phenolic compounds. To overcome this problem, the most common practice used in wine industry is the oenological treatment which enhances the clarity, stability and the wine taste. In the present work, press wines were separately submitted to four different treatments: micro-oxygenation and the addition of three fining agents: gelatin, polyvinylpolypyrrolidone (PVPP) and pea protein. The phenolic total index decreased (8%) significantly ($P<0.05$) for gelatin treatment, the PVPP based formulation treatment led to the largest loss (8%) in color intensity, (12%) in redness (a*), (9%) in polymeric pigments and increasing to 9% lightness (L*). Unlike micro-oxygenation which has decreased (5%) the color intensity. For the monomeric anthocyanins, the greatest reduction of acylated and coumaroylated anthocyanins at bottling and glucoside anthocyanidins after five months of storage was observed for both basic PVPP and gelatin.

Keywords

Red press wine, Micro-oxygenation, Fining agents, Anthocyanins, Color intensity

Introduction

During red wine making, the color stabilization is a real key step. Indeed, extracting a maximum of color and a sufficient amount of tannins to stabilize it and provide body to the wines is quite an art [1]. Anthocyanins and their derivatives are the crucial pigments responsible for the red wine color. During wine aging, the concentration of monomeric anthocyanins declines constantly, while numerous more complex and stable anthocyanin derived pigments are formed [2]. It is usually supposed that these pigments are formed by direct condensation between anthocyanin and/or flavan-3-ols or by a aldehyde-mediated condensation resulting in oligomeric and polymeric pigments, sometimes referred to as pigmented polymers. Some authors have shown that anthocyanins can react as well with metabolites of the two successive fermentations including pyruvic acid, vinylphenol, glyoxylic acid, giving rise to a new family of anthocyanin-derived pigments, namely, pyrano-anthocyanins [2-4]. The color of wine is mainly assessed by spectrophotometric measurements that are converted into typical indexes claimed to reflect the composition and concentration of anthocyanins and polymeric pigments. The color evolution is influenced by several factors, including yeast metabolites, oxygen exposure and storage time [5]. In young red wines the original anthocyanins (also called monomeric anthocyanins in

opposition to polymeric anthocyanins) occur predominantly in a dynamic equilibrium among five major molecular forms, including the bisulfite addition flavene compound, the quinoidal base, the flavylium cation, the semimetal or carbinol pseudobase and the chalcone (cis and trans forms) [6]. The monomeric anthocyanin molecules are not very stable, so their concentration in wine decreases within the first few months of barrel aging. Despite the total apparent disappearance of monomeric anthocyanins (which occurs within few years), the wine keeps the red color which is due to combination reactions of monomeric anthocyanins with various compounds in the wine, especially tannins. The resulting structures are not very well characterized [7]. The red press wine is elaborated from the pressing of grape pomace after the maceration. It often presents some characteristic qualities like to be rich on phenolic compounds and colors than running wine. It can also give roundness, volume and structures to running wine at blinding step and it is used to increase the color of less colorful and tannic wine [8, 9]. The soft and fruit taste of wine are masked by press wine in the other hand. That is why the press wines are treated by oenological treatments (fining, micro-oxygenation) before the blinding with running wine [8]. In the practice, it is of great importance to obtain a good color and clearness of wines and to possibly keep them for a prolonged period. Fining agents are applied to obtain limpid and bright wines, they are intended to achieve clarity and improve color, flavor, and physical stability of wine, and they should serve to remove only the components that make wine unstable. The fining agents such as PVPP, gelatin, egg albumin and casein demonstrated reduction of phenolic levels and altered the color in some wines [10]. The use of plant-derived proteins as wine fining agents has become of much interest [11]. Micro-oxygenation is a technique that consists of introducing small and measured amounts of oxygen into wines with the objective of improving wine color characteristics and stability, aroma and texture [12]. The oxygen leads to different chemical reactions anthocyanins and tannins. All of these events result in the formation of more stable compounds that stabilize wine color since they partly resist discoloration by SO_2 and provide better color stability at wine pH [13, 14]. The main objectives of this work were to compare the impact of micro-oxygenation and of three types of fining agents (gelatin, PVPP-formulation and pea protein) on the chromatic characteristics and pigment composition of the red press wines after treatment, at bottling and after five months of storage on the basis of absorbance measurements and liquid chromatography on line with UV-visible detection.

Materials and Methods

Winemaking

In 2015 vintage: after the end of the alcoholic fermentation, the grape pomace of grapes *Vitis vinifera* var of Cabernet-Sauvignon is pressed with pressure degrees varying from 0 to 300 mbars in the cellar Château Rôslane. At the end of pressing, the wine is treated with clarification enzyme, centrifuged at 8000 tr/min and distributed on four tanks of 10 hectoliters, one called control (T_0) and the others treated with some commercial fining agents: Pork liquid gelatin (T_2) at a concentration of 0.6 mL/L, PVPP coupled with bentonite (T_3) (dose 0.8 g/L, powders) and formulation of pea protein bentoniteand polysaccharides (T_4) (dose 0.8 g/L, powders). Another tank of 25 hectoliters was used for micro-oxygenation trial (T_1), the air being delivered at 60 mL/L/month until the beginning of malolactic fermentation. Five months after the end of alcoholic fermentation, the samples were stored in bottles of 750 mL and analyzed [15].

Analysis

Chromatic characteristics

In this work we used the same chromatic measurement procedure described in the previous work [2, 16]. Absorbance measurements were made with a SAFAS UV mc2 spectrophotometer (Monaco) and color indices were deduced from these absorbance measurements. All the absorbance measurements were converted to a 10 mm light path cell and a dilution of 1 before calculating the indices. Absorbance values at 420, 520 and 620 nm were measured, 30 min after the addition of acetaldehyde, in a 1 mm light path cell. Hue was calculated as $A_{420\ nm}/A_{520\ nm}$ and color intensity (CI) as $A_{420\ nm} + A_{520\ nm} + A_{620\ nm}$, yellow ($A_{420\%}$), red ($A_{520\%}$) and blue color intensity ($A_{620\%}$) according to Glories [16] are: $A_{420\%} = (A_{420}/CI)*100$, $A_{520\%} = (A_{520}/CI)*100$ and $A_{620\%} = (A_{620}/CI)*100$. Wine pigments corrected of bisulphite at wine pH (PV) was defined as the absorbance at 520 nm, 30 min after addition of acetaldehyde. Color due to derivatives resistant to sulphite bleaching was determined at 520 nm in a 1 mm light path cell, 30 min after addition of a metabisulphite solution. Sulphites bleaching resistant pigments ($PRSO_2$) were then calculated. Total pigments at acidic pH (PpH<1) was determined from absorbance at 520 nm with a 10 mm light path, 4 h after a 100 fold dilution in HCl 1 M. The CIELab chromatic characteristics L* (lightness), a* (measure of redness) and b* (measure of yellowness) were determined directly by the software incorporated in spectrophotometer Konica Minolta apparatus (Japan).

Phenolic compounds

$PTI_{280\ nm}$, which estimates total phenol content, was determined according to the method described by Ribéreau-Gayon et al. [7]. Total anthocyanins were determined by spectrophotometry of wine diluted with ethanol and hydrochloric acid, making a reading of an aliquot with water ($A0_{520\ nm}$). Another type treated with $NaHSO_3$ ($A_{520\ nm}$), the formula is as follows: $[Ant] = (A0_{520\ nm} - A_{520\ nm})*875$ [17]. Polymeric pigments (PP) corresponded to pigments resistant to SO_2 bleaching and were estimated according to Glories [16]. Two samples were prepared to calculate $IC(SO_2)$ and $IC(H_2O)$. The first one was prepared as follows: 0.5 mL of wine, 4.5 mL of model wine (12% ethanol, 1.5 $g.L^{-1}$ tartaric acid and pH = 3.2), and 20 µL of Na_2SO_3. After 5 min, absorbance at 420 and 520 nm were read under 10 mm optical way: $IC(SO_2) = A_{420\ nm} + A_{520\ nm}$. The second one was prepared as follows: 0.5 mL of wine, 4.5 mL of model wine, and 20 µL of water; $IC(SO_2) = A_{420\ nm} + A_{520\ nm}$. $PP = IC(SO_2)/IC(H_2O)$ [18]. In this work we used the same procedure for the HPLC analyses previously described by Ducasse [18]. Monomeric anthocyanins were

analyzed by direct injection of wine samples into the HPLC system. HPLC-DAD analyses were performed using a Waters 2690 system equipped with an auto-sampler system, a Waters 996 photodiode array detector, and Millennium 32 chromatography manager software (Waters, Milford, MA). Separation was achieved on a reversed-phase Atlantis dC18 column (250 × 2.1 mm i.d., 5 µm packing) protected with a guard column of the same material (20 × 2.1 mm i.d., 3 µm packing) (Waters, Milford, MA). The elution conditions were as follows: 0.250 mL/min flow rate; oven temperature 30 °C; solvent A: water/formic acid (95/5 v/v); solvent B: acetonitrile/water/formic acid (80/15/5 v/v/v); elution began isocratically with 0% B during 5 min, then continued with linear gradients from 0% to 10% B for 20 min, 10% to 20% B for 15 min, 20% to 45% B for 15 min, 45% to 60% B for 15 min, 60% to 80% B for 5 min, followed by washing and re-equilibration of the column.

Sensorial analysis

The sensory evaluations of different samples of press wine were performed by a panel of five professional judges. The panel test was planned to compare the effect of four oenological treatments on color intensity of the corresponding red press wines after treatment at bottling and after five months of storage (before blinding with running wine). The scoring scale was from 1 to 6.

Statistical data analysis

The data are presented as means ± SD. The statistical calculations used are Analysis of variance one factor and the least significant difference (LSD) according to Student-Newman-Keuls. The five samples at bottling and after five months were compared to separate the means, and significance was accepted at the 5% level comparison of treatment means (LSD, 5% level). The statistical treatment was done using SPSS 17 statistics software.

Results and Discussion

Chromatic characteristics and sensorial analysis

According to Table 1, we showed the impact of the micro-oxygenation and fining agents on changes of chromatic characteristics of Cabernet-Sauvignon press wine. At bottling, it clearly showed that fining treatments (gelatin (T_2), PVPP based formulation (T_3) and pea protein (T_4)) reduced significantly the $PTI_{280\ nm}$, color intensity (CI), intensity of red color ($A_{520\%}$), redness (a*), yellowness compound (b*) and increased the lightness (L*). The latter is distinct from PVPP based formulation (T_3) (11%). The color intensity (CI) of press wine was significantly increased (5%) by micro-oxygenation treatment (T_1) compared to the control (T_0). The significant increase of hue intensity by all treatments indicated that red and blue color participation in wine are decreased, unlike yellow color which was increased. The CIELab method proved that in each fined wine, lightness (L*) increased significantly, which seemed to be correlated with less redness (a*), due to the removal of pigments [19]. This data are in accordance with the results obtained from polymeric pigments (Figure 1). After five months in the bottle, a further decrease in $A_{520\%}$, a* and L* were observed in all samples, the same happened for the color intensity (CI) except T_4 treatment. In addition, an increase in yellow ($A_{420\%}$), blue ($A_{620\%}$), hue and yellowness compounds (b*) were also observed in all samples. The observed differences at bottling step between samples were not maintained after five months of storage. The phenolic total index ($PTI_{280\ nm}$) was significantly lowered by adding fining agents (T_2, T_3 and T_4). In general, gelatin T_2 was the most fining agent that decreased the $PTI_{280\ nm}$. Owing to the capacity of the polyphenol compounds to establish hydrogen bonds with proteins, a part of the polyphenolic fraction is probably removed during the fining treatments [20]. The micro-oxygenation treatment (T_1) led to a significant increase of both colors (red and blue intensities), redness (b*) and the color intensity (CI) compared to the control (T_0). This rise of color intensity can be mostly attributed to an increase of free anthocyanins coming from the colorless anthocyanin bisulfite adducts [21]. The oxygen played an important role in the different processes that took place during winemaking process and the ageing of wine. It had an influence on the phenolic composition and indirectly affected the wine color [12]. Pea protein (T_4) led to more lightness (L*) and the yellow compound (b*) increased after storage. As illustrated on the Figure 2, all pigments are represented by the total pigments at acidic pH parameter (PpH<1). The latter is converted to a value 100 to estimate the percentage of both pigments PV (wine pigments at wine pH) and $PRSO_2$ (the pigments resistant to discoloration). For all the studied samples, the parameters proportion (PpH<1, PV, $PRSO_2$) evolved between the bottling step and the five months of storage. At bottling, the PV contributed to an average of 48% in all the five samples. After five months, the PV increased to an average of 73%. The highest values of PV at bottling and after five months were due to PVPP based formulation treatment (T_3). The press wine contained polyphenols, which include essentially anthocyanins, proanthocyanidins, phenolic acids, and new derived pigments generated from polyphenols during winemaking and the ageing process. Some of these new phenolic compounds are more stable pigments than free anthocyanins and enable to stabilize the color of the press wine [7, 8]. The increases of PV and $PRSO_2$ observed after five months of storage showed the existence of reactions between anthocyanins and/or other co-pigment. These reactions released more complex and stable anthocyanin derived pigments, such as various pyranoanthocyanins, polymeric anthocyanins produced from condensation between anthocyanin and/or flavan-3-ols directly or mediated by aldehydes. Such variations can result in the significant changes of the color [2, 8, 22]. The monitoring of total anthocyanins and the polymeric pigments parameter during the storage (Figure 2) showed a drop of total anthocyanins, and an increase of polymerized pigments. However, the control wine maintained a greater amount of total anthocyanins compared to the treated wines after five months. These results are due to the breakdown and/or the reactions of a part of anthocyanins with other molecules [7, 8]. The anthocyanins and large phenols, such as polymerized anthocyanins, are preferentially removed by fining agents. During five months, the greatest loss of total anthocyanins and polymerized pigments was observed by gelatin (T_2) and pea protein based formulation (T_4) fining treatment respectively.

Table 1: Impact of micro-oxygenation and agents used for fining on color changes of red press wines (mean=SD); a-d: Means within a line followed by the same letter are not significantly different (LSD, 5%) (n=2). T_0: control; T_1: micro-oxygenation; T_2: gelatin; T_3: polyvinylpolypyrrolidone based formulation; T_4: pea protein.

Parameters	Sig	T_0	T_1	T_2	T_3	T_4	Sig	T_0	T_1	T_2	T_3	T_4
Month			At bottling						5 months			
$PTI_{280\ nm}$	**	97.01 ± 0.04^c	97.01 ± 0.05^c	89.00 ± 0.05^a	89.02 ± 0.04^a	92.01 ± 0,05^b	*	99.12 ± 0.0^c	104.95 ± 0.0^d	90.17 ± 0.07^a	92.3 ± 0.2^b	90.33 ± 0.1^a
D420 (%)	n.s	32.74 ± 1^a	33.91 ± 0.1^a	34.74 ± 0.6^a	35.08 ± 0.1^a	35.70 ± 1^a	**	42.15 ± 0.4^b	38.6 ± 0.5^a	43.3 ± 0.4^c	44.0 ± 0.1^c	42.5 ± 0.1^b
D520 (%)	*	53.31 ± 1.2^b	51.67 ± 0.1^{ab}	49.98 ± 0.1^a	50.94 ± 0.4^a	50.41 ± 0.6^a	**	41.15 ± 0.04^a	44.66 ± 0.1^b	41.49 ± 0.1^a	41.43 ± 0.6^a	42.06 ± 0.3^a
D620 (%)	n.s	13.94 ± 0.2^a	14.43 ± 0.1^a	15.28 ± 0.7^a	13.99 ± 0.5^a	13.89 ± 0.4^a	**	16.67 ± 0.3^b	16.75 ± 0.6^b	15.25 ± 0.3^{ab}	14.65 ± 0.7^a	14.24 ± 0.2^a
CI	**	16.77 ± 0.3^b	17.71 ± 0.1^c	14.77 ± 0.1^a	14.34 ± 0.2^a	14.76 ± 0.2^a	**	14.93 ± 0.2^b	15.6 ± 0.04^c	14.1 ± 0.1^a	14.0 ± 0.1^a	15.01 ± 0.2^b
Hue	*	0.61 ± 0.0^a	0.66 ± 0.0^{ab}	0.70 ± 0.0^b	0.69 ± 0.0^b	0.71 ± 0.0^b	*	1.0 ± 0.01^{ab}	0.9 ± 0.01^a	1.0 ± 0.01^b	1.1 ± 0.01^b	1.0 ± 0.01^{ab}
L* (%)	**	56.23 ± 0.1^a	56.06 ± 0.1^a	60.70 ± 0.1^d	62.48 ± 0.1^c	61.18 ± 0.1^b	**	48.07 ± 0.1^a	50.25 ± 0.1^c	50.06 ± 0.1^c	48.5 ± 0.1^b	55.55 ± 0.1^d
a*	**	40.70 ± 0.1^d	40.51 ± 0.1^d	37.86 ± 0.1^c	35.64 ± 0.1^a	36.58 ± 0.1^b	**	30.27 ± 0.1^c	31.53 ± 0.1^d	29.34 ± 0.1^b	26.39 ± 0.1^a	30.77 ± 0.1^c
b*	**	2.56 ± 0.1^c	1.78 ± 0.1^a	2.47 ± 0.1^c	2.23 ± 0.1^b	1.84 ± 0.1^a	**	3.61 ± 0.1^b	4.19 ± 0.1^c	3.89 ± 0.1^b	2.88 ± 0.1^a	5.95 ± 0.1^d

$PTI_{280\ nm}$: Phenolic Total Index; CI: Colour Intensity; D420(%): yellow intensity; D520(%): red intensity; D620(%): blue intensity; L*(%): lightness; a*: redness; b*: yellowness; n.s: no significant (p>0.05); significant levels: *: p<0.05; **: p<0.01.

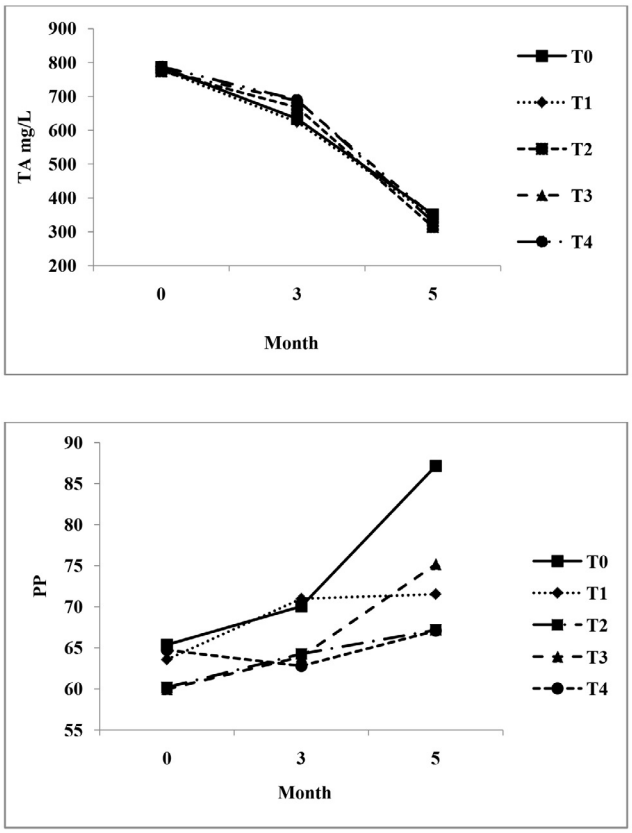

Figure 1: Evolution of total anthocyanins (TA) and polymerized pigments (PP) after five months of storage. T_0: Control; T_1: micro-oxygenation; T_2: Gelatin; T_3: polyvinylpolypyrrolidone based formulation; T_4: pea protein.

The result of sensorial analysis presented in Figure 3, showed that, all the different treatments had no significant impact on the variation of the color intensity during the two steps (bottling and the five months storage step). It was observed for all samples during the five months storage that there was a slight reduction of color intensity. This result is consistent with the change in the color intensity and redness (a*) measured by the spectrophotometer and CIElab (Table 1).

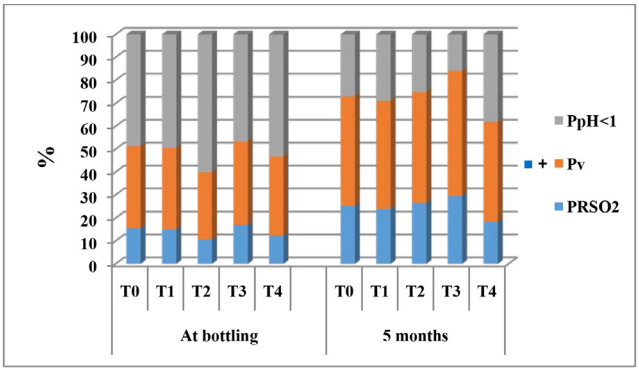

Figure 2: Chromatic characteristics and colour of micro-oxygenated and both fined/unfined red press wines at bottling and after five months of storage. (PpH<1): total pigments at acidic pH; (PV): wine pigments corrected of bisulphite at wine pH; (PRSO2): sulphites bleaching resistant pigments; T_0: control; T_1: micro-oxygenation; T_2: gelatin; T_3: polyvinylpolypyrrolidone based formulation; T_4: pea protein.

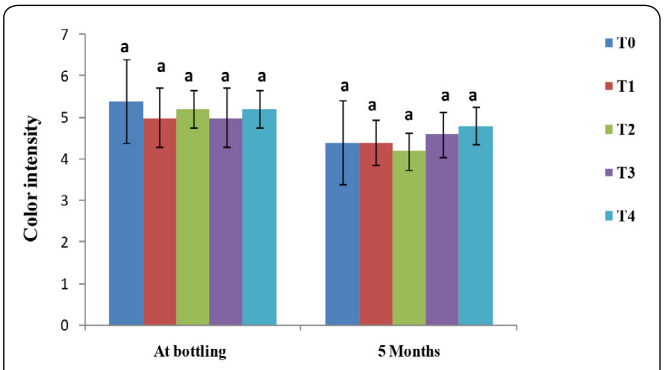

Figure 3: Sensorial colour intensity of micro-oxygenated and both fined/unfined red press wines at bottling and after five months of storage. Means followed by the same letter are not significantly different (LSD, 5%). T_0: Control; T_1: micro-oxygenation; T_2: gelatin; T_3: polyvinylpolypyrrolidonebased formulation; T_4: peaprotein.

Monomeric anthocyanins

The monomeric anthocyanins identified with their retention time by HPLC are given in Table 2. At bottling,

Table 2: Anthocyanin compounds identified with their retention time by HPLC-DAD analyses.

Pic	Retention time (min)	Compound
1	39,68	Delphinidine-3-*O*-glucoside
2	41,75	Cyanidine-3-*O*-glucoside
3	43,79	Petunidine-3-*O*-glucoside
4	45,56	Peonidine-3-*O*-glucoside
5	47,33	Malvidine-3-*O*-glucoside
6	48,99	Delphinidine-3-*O*-acetylglucoside
7	49,88	Cyanidine-3-*O*-acetylglucoside
8	52,36	Petunidine-3-*O*-acetylglucoside
9	55,19	Peonidine-3-*O*-acetylglucoside
10	56,72	Malvidine-3-*O*-acetylglucoside
11	65,19	Peonidine-3-*O*-coumaroylglucoside
12	65,67	Malvidine-3-*O*-coumaroylglucoside

These results are corroborated by McCloskey and Yengoyan [5] who have shown that acylated monoglucoside pigments breakdown faster than the other monoglucosides in wine. A part of the monomeric anthocyanins is supposed to be gradually incorporated into polymeric pigments which confer color stability to the wine. This result showed that $PRSO_2$ (pigments resistant to discoloration to sulfite) are increasing after five months of storage (Figure 2). The greatest decrease of malvidine-3-*O*-glucoside is conducted by both gelatin treatments (T_2) and basic PVPP formulation (T_3). The latter was coupled with bentonite. Several works [24] have described that the most remarkable effects on wine phenolic composition were produced by bentonite and gelatin, which significantly decreased anthocyanin and tannin concentrations respectively. Also the use of non-traditional fining agents, as vegetable proteins, may have less impact on the color and anthocyanin content of red wine.

Table 3: Influence of red press wine treatment on amount of monomeric anthocyanins (mean=SD) a-e: Means within a line followed by the same letter are not significantly different (LSD, 5%) (n=2). T_0: control; T_1: micro-oxygenation; T_2: gelatin; T_3: polyvinylpolypyrrolidone based formulation; T_4: pea protein.

Compound mg/L	Sig	T_0	T_1	T_2	T_3	T_4	Sig	T_0	T_1	T_2	T_3	T_4
			At bottling						5 months			
Del-3-glu	**	13.48 ± 0.20^a	13.56 ± 0.21^a	13.75 ± 0.20^{ab}	14.57 ± 0.10b^b	14.67 ± 0.21^b	**	7.34 ± 0.15^b	7.77 ± 0.91^{bc}	7.19 ± 0.77^{ab}	6.40 ± 0.11^a	8.44 ± 0.13^c
Cya-3-glu	n.s	1.58 ± 0.19^a	1.38 ± 0.15^a	1.29 ± 0.23^a	1.65 ± 0.18^a	1.65 ± 0.21^a	n.s	1.37 ± 0.54^a	1.16 ± 0.20^a	1.24 ± 0.65^a	1.30 ± 0.45^a	1.29 ± 0.20^a
Pet-3-glu	*	16.63 ± 0.21^a	16.55 ± 0.12^a	16.65 ± 0.30^a	18.75 ± 0.21^c	17.55 ± 0.20^b	***	7.62 ± 0.25^a	8.11 ± 0.21^b	7.29 ± 0.18^b	6.27 ± 0.23^a	9.15 ± 0.51^c
Peo-3-glu	*	6.14 ± 0.18^a	6.97 ± 0.31^b	6.60 ± 0.15^{ab}	7.70 ± 0.20^c	6.80 ± 0.20^b	n.s	0.72 ± 0.22^a	0.66 ± 0.20^a	0.63 ± 0.21^a	0.55 ± 0.16^a	0.68 ± 0.11^a
Mal-3-glu	***	139.33 ± 1.1^a	138.55 ± 0.97^a	142.75 ± 0.56^b	153.25 ± 0.64^d	148.65 ± 0.43^c	***	77.15 ± 0.91^c	83.85 ± 1.19^d	75.75 ± 0.51^b	64.95 ± 0.81^a	94.75 ± 0.96^e
Del-3-ac	*	8.40 ± 0.20^a	7.99 ± 0.14^a	9.74 ± 0.21^c	9.01 ± 0.70^b	9.78 ± 0.25^c	----	n.d	n.d	n.d	n.d	n.d
Cya-3-ac	***	5.00 ± 0.11^a	5.01 ± 0.10^a	8.90 ± 0.23^b	8.76 ± 0.35^b	9.02 ± 0.61^b	----	n.d	n.d	n.d	n.d	n.d
Pet-3-ac	n.s	5.26 ± 0.31^a	5.21 ± 0.51^a	5.24 ± 0.21^a	5.07 ± 0.18^a	5.27 ± 0.20^a	----	n.d	n.d	n.d	n.d	n.d
Peo-3-ac	*	3.07 ± 0.41^{ab}	3.23 ± 0.41^b	2.73 ± 0.50^{ab}	2.91 ± 0.34^{ad}	2.37 ± 0.65^a	*	1.56 ± 0.13^a	1.70 ± 0.21^b	2.30 ± 0.19^{ab}	2.80 ± 0.18^c	2.27 ± 0.21^{ab}
Mal-3-ac	***	32.69 ± 0.53^c	34.85 ± 0.21^d	30.15 ± 0.20^b	26.75 ± 0.32^a	34.95 ± 0.41^d	ns	4.04 ± 0.40^a	3.87 ± 0.47^a	3.82 ± 0.35^a	3.71 ± 0.41^a	3.81 ± 0.65^a
Peo-3-cou	n.s	2.14 ± 0.2^a	1.78 ± 0.61^a	1.60 ± 0.21^a	1.13 ± 0.20^a	1.93 ± 0.21^a	----	n.d	n.d	n.d	n.d	n.d
Mal-3-cou	***	17.13 ± 0.13^c	18.85 ± 0.46^d	16.05 ± 0.51^b	13.35 ± 0.10^a	21.15 ± 0.17^d	*	2.59 ± 0.91^{ab}	2.13 ± 0.51^a	2.83 ± 0.61^{ab}	3.16 ± 0.41^c	2.73 ± 0.56^{ab}
Total	*	250.85 ± 3.35^a	253.95 ± 4.2^a	255.47 ± 3.51^a	262.91 ± 3.52^{ab}	273.81 ± 3.75^b	**	102.4 ± 3.51^b	109.2 ± 3.90^c	101 ± 3.47^b	89.15 ± 2.76^a	123.13 ± 3.3^d

n.s: no significant (p>0.05); significant levels: *: p<0.05; **: p<0.01; ***: p<0.001; n.d: means not detected; Del-3-glu: Delphinidine-3-*O*-glucoside; Cya-3-glu: Cyanidine-3-*O*-glucoside; Pet-3-glu: Petunidine-3-*O*-glucoside; Peo-3-glu: Peonidine-3-*O*-glucoside; Mal-3-glu: Malvidine-3-*O*-glucoside; Anthocyanidin-3-ac: Anthocyanidin-3-*O*-acetylglucoside; Anthocyanidin-3-cou: Anthocyanidin-3-*O*-coumaroylglucoside.

the amount of malvidine-3-*O*-acetylglucoside and malvidine-3-*O*-coumaroylglucoside were lowered by both fining agents: gelatin (T_2) and PVPP based formulation (T_3) (Table 3). However, this amount was increased by the micro-oxygenation and pea protein fining agent (T_4). The glucoside derivative remained higher than control wine after the addition of the fining agents. Several works have shown that isinglass's and gelatin had the least effect on total monomeric anthocyanin amount, while the highest effect was observed by casein and potassium caseinate [23]. After five months of wine storage, the most stable anthocyanins were those containing glucoside derivatives, while the amounts of acylated and coumaroylated anthocyanins were considerably decreased.

Conclusion

This study reveals that all fining agent used (liquid gelatin, fining agent based on PVPP and pea protein) allow a good clarification of the Moroccan treated press wine in comparison with the untreated one. Each treatment has a distinct behavior on chromatic characteristics and anthocyanins composition, affecting both the monomers and the polymeric pigment. All fining agents decreased the pigmentation significantly. Fining agent PVPP based formulation seemed to eliminate more color. However, the micro-oxygenated press wine was more colored. On the sensory quality plan, the press wine remained very colored and its color is not altered by all treatments.

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Conflict of Interest

The authors declare no conflict of interest.

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