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Phenolic compounds of Moroccan red press wines: Influence of fining agents and micro-oxygenation treatments

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ABSTRACT

To improve the sensory quality of Moroccan red press wines, press wines were separately submitted to micro-oxygenation and three fining agents (gelatin, Polyvinylpolypyrrolidone (PVPP) based formulation and pea protein) treatments at the winery Château Roslane, Morocco. The results showed that each treatment has a distinct behavior in relation with interactions and precipitation monomeric flavan-3-ol and fractions apparent and total condensed tannins. The fining agent corresponding to the vegetable protein has a greater affinity with the fraction of oligomeric condensed tannins with apparent aDP: 3.2. However, gelatin and PVPP based fining agent have an affinity with the catechin and epicatechin as well as with the fraction of polymeric tannins (apparent aDP: 6.6). For the structural parameters of proanthocyanidins, no significant differences were found out in global aDP and percentage of prodelphinidin between the different treatments. The global percentage of galloylation appeared slightly affected, while the global percentage of oxidized proanthocyanidins increased (9%) significantly ($P < 0,05$) for micro-oxygenation treatment, unlike the fining agent has basic vegetable protein has decreased 36% the percentage of oxidation. For the Saliva Precipitation Index (SPI) and sensory analysis, the greatest reduction of SPI, bitterness and astringency was observed for both basic PVPP and vegetable protein fining agents.

1. Introduction

In the traditional red wine making process, the press wine constitutes 13–17% of red wine production (Vivas, 2007). It is collected after pressing of solid parts (seed and skin) of grapes pomaces after the end of alcoholic fermentation and before malo-lactic fermentation. Several types of press wine are collected: the more there is pressure, the more colorful, astringent, bitter and rustic the wine will be (Renouf & Murat, 2012). The technique of pressing allows the extraction of high levels of pigments that are responsible of one of the most appreciated characteristics of these wines (deep purplish red color). In controversy, it also tends to over-extract tannins, leading to excessive astringency and bitterness (Vivas, 2007; Renouf & Murat, 2012). The improvement of the quantity of running wine via its blending with press wine requires the improvement of the quality of the latter (Trione & Martinez., 2001). Indeed, the blending process is delicate because the press wine contains undesirable phenolics, astringency vectors and greenness, high level of turbidity and instability of color. In many cases, press wine grows separately; however, it is blending with free running wine at the end of winemaking stage (Renouf & Murat, 2012). Bitterness and astringency are two important attributes of wine flavor. Several authors (Lea, 1990; Peleg, Gacon, Schlich, & Noble, 1999; Vidal et al., 2003) reported that wine bitterness and astringency are due to tannins quantity and structures (molecular size and degree of galloylation), The perception of the astringency of condensed tannins seems increasing with tannin size and degree of galloylation (Vidal et al., 2003) which confers their ability to complex with proteins, probably because they have more interaction sites (Baxter, Lilley, Haslam, & Williamson, 1997; De Freitas & Mateus, 2001). Several studies have been devoted over the forty last year's to the qualitative and quantitative analysis of phenolic compounds by means of the major modern techniques but the

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analysis of tannins (proanthocyanidins) remains difficult due to their polymeric nature. Indeed, the determination of the quantitative and qualitative characteristics of tannins (subunits composition and mean degree of polymerization) proceeds by a chemical depolymerization prior to analysis by liquid chromatography. Owing to their antioxidant properties, tannins undergo oxidation in the course of wine making and wine aging, which results in the formation of additional linkages, either between different polymeric chains (intermolecular bonds), either within two subunits of the same polymeric chain (intramolecular bond) (Mouls & Fulcrand, 2015). These oxidative bonds are hard to break and resist to the chemical depolymerization under the reaction conditions. This results in an extended hump under the peaks of the chromatography profile that usually passes unnoticed and the oxidized tannins are not taken into account in the analysis of tannins. Consequently, only the part of tannins that are able to cleave into monomeric units (native tannins) are identified and quantified so far. For that reason, tannin analysis gives access to the "Apparent tannins". The press wines are often richer in tannins than running wines; these tannins are frequently perceived as harsh. To overcome this negative effect, press wines need an indispensable phase of maturation, during which the structures of tannins are modified. This maturation phase may be associated with micro-oxygenation and/or aging on lees. The latter improves wine mouth feel by oxidative polymerization of tannins and/or by the coating of tannins by yeast polysaccharides. Alternatively, protein-based fining agents (gelatin, casein …) can be used to quickly remove the fraction of the more aggressive tannins (Sarni-Manchado, Deleris, Avallone, Cheynier, & Moutounet, 1999). The various protein-based fining agents can behave differently, depending on their composition, their origin and their preparation condition (Cosme, Ricardo-da-Silva, & Laureano, 2008). More recently, some proteins of vegetable origin have also been investigated as possible wine fining agents (Marchal, Marchal-Delahaut, Lallement, & Jeandet, 2002). The main objectives of this work were to compare the effects of micro-oxygenation and of three types of fining agents (gelatin, PVPP -formulation and pea protein) on the bitterness and astringency of the corresponding red press wines after treatment, and on the structural characteristics of their proanthocyanidins. For that purpose, the wines were fractionated by low pressure chromatography into three main fractions (monomeric, oligomeric, and polymeric flavan-3-ols).

2. Materials and methods

2.1. Materials

A pneumatic type of wine press (Bucher Vaslin) is used for the elaboration of press wine. A system of micro-oxygenation (Visio 6) and a disk stack centrifuge RE50V (8000 Tr/min) were used in this experiment.

2.2. Winemaking

In 2014–2015 vintage. After the end of the alcoholic fermentation, the grape pomace of grapes *Vitisvinifera of Cabernet Sauvignon* is pressed with pressure degrees varying from 0 to 300 mbar in the cellar Château Roslane (Fig. 1). At the end of pressing, the wine is treated with clarification enzyme, centrifuged at 8000 tr/min and distributed on four tanks of 10 hL, 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, Polyvinylpolypyrrolidine (PVPP) coupled with bentonite (T_3) (dose 0.8 g/L, Powders)and formulation of vegetable pea protein of, bentonite and polysaccharides (T_4) (dose 0.8 g/L, Powders). Another tank of 25 hL was used for micro-oxygenation trial (T_1), the air being delivered at 60 mL/L/month until the beginning of malolactic fermentation. The fining agent doses were chosen after performing a sensory

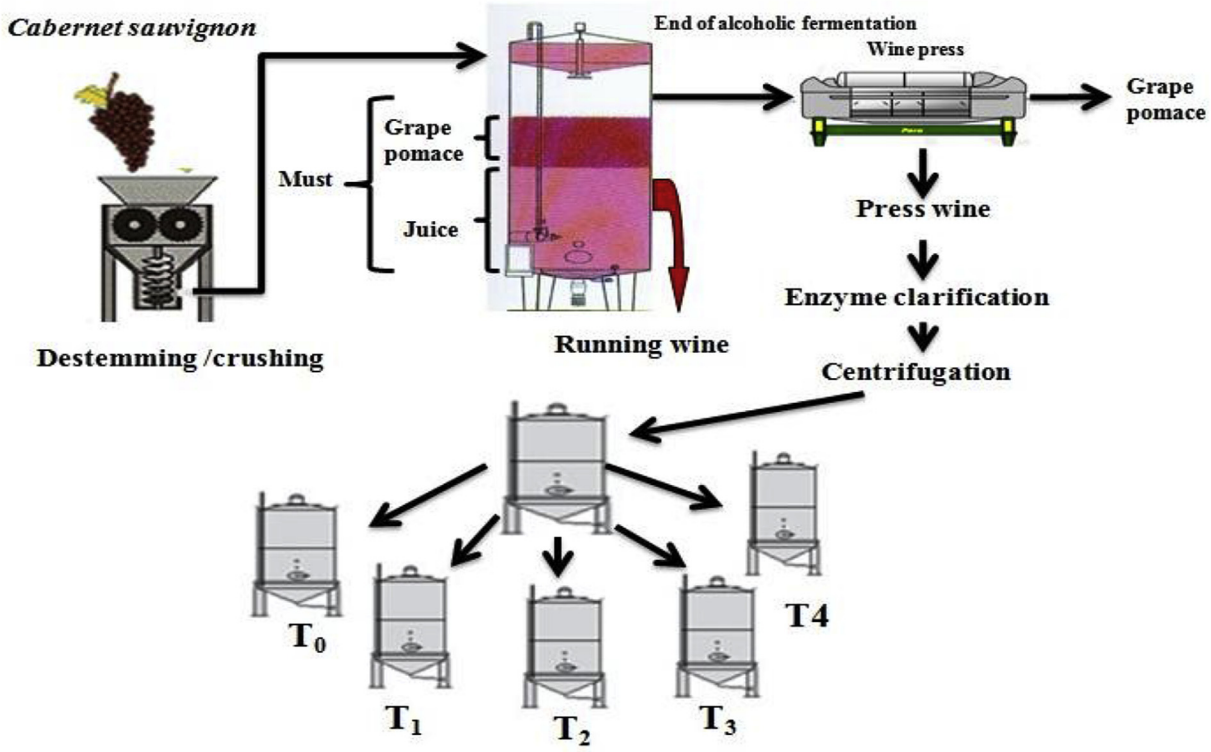

Fig. 1. Protocol of winemaking press wine Château Roslane.

evaluation (astringency and bitterness) of fining trials in bottles of 750 mL. Five months after the end of alcoholic fermentation, the samples were stored in bottles of 750 mL and analyzed.

2.3. Turbidity measurement

Nephelometric Turbidity Unit (NTU) is determined using a turbidimeter Hach 2100P.

2.4. Analysis of phenolic compounds

2.4.1. Extraction of press wine proanthocyanidins

5 mL of wine were concentrated to 2 mL and then directly injected on a Flash Chromatography system (PuriFlash 430, Interchim) and fractionated on a Toyo pearl TSK gel HW-50 (F) column (3.8,13 cm) (methyl acrylate copolymer in solution in 20% aqueous ethanol) (Mouls & Fulcrand, 2012). The solvents used for elution were the followings: solvent A (CH_3CH_2OH + 0.05%TFA); solvent B (H_2O/TFA, 99.95:0.05, v/v) and solvent C (CH_3COCH_3 + 0.05%TFA). The wine fractionation conditions are presented in Table 1. The five fractions studied in this work were separated according to the UV profile on line with the flash chromatography system.

2.4.2. General procedure for the chemical depolymerization

In this work, the thioglycolysis procedure was the same as described in the previous work (Mouls & Fulcrand, 2015). A 4 g/L solution of each dried sample was prepared in methanol.100 μL of the solution was placed in a 250 μL glass insert and 100 μL of thioglycolic acid solution (40 μL of thioglycolic acid with 4960 μL of methanol/concentrated HCl 95:5 v/v) was added. After sealing the glass insert with an inert cap, reactions were carried out at 90 °C for 6 min.

2.4.3. General procedure for UPLC ESI/MS analyses

In this work we used the same procedure for the UPLC ESI/MS analyses previously described by Mouls and Fulcrand (2015). Analyses were performed on an Analytical Reversed-Phase UPLC ESI-MS piloted by HyStar 3.2 software. Direct injection of undepolymerized samples (2 μL) into the UPLC system coupled to ESI-MS gives access to the monomer composition (Catechin, Epicatechin, Epigallocatechin and Epicatechin-3-*O*-gallate). The injection of samples after depolymerization gives access to the composition and quantity of apparent tannins. The liquid chromatography system was an Acquity UPLC (Waters, Milford, MA) equipped with a Photodiode Array Detector. The results are mean values of three determinations. The flow rate was 0.55 mL min^{-1} and the gradient conditions were solvent A (H_2O/CHOOH, 99/1, v/v); solvent B (CH_3CN/H_2O/CHOOH, 80/19/1, v/v/v); initial 0.1% B; 0–2 min, 25% B linear; 2–4 min, 35% B linear; 4–5 min, 35% B isocratic; 5–6 min, 40% B linear; 6–8 min, 99.9% B linear and 8–10 min, 99.9% B isocratic. Reversed-phase UPLC analysis of the products yielded by depolymerization allows determination of the structural composition of proanthocyanidins, which are characterised by the nature of their constitutive extension units (released as thioethers of thioglycolicacidmethyl ester) and terminal units (released as flavan-3-ols). It also allows calculation of their structural characteristics such as the average degree of polymerization (aDP), the fraction of prodelphinidins (% prod) and the fraction of galloylation (% gal) (Rigaud, Perezilzarbe, Ricardo Da Silva, & Cheynier, 1991; Preys et al., 2006; Cosme et al., 2008). After the integration of peak area of the extension and terminal monomeric units (flavan-3-ol with or without the nucleophilic reagent respectively) released from tannins by chemical depolymerization (Fig. 2), the % oxidation of tannins is calculated by the following formula:

$$\%\,\mathbf{oxidation} = \frac{\boldsymbol{\Omega}}{\boldsymbol{\Delta} + \boldsymbol{\Omega} * 100}$$

Δ: area of the peaks of flavan-3-ols, **Ω:** Area of the hump under the peaks of flavan-3-ols.

The total amount of tannins is actually the sum of the amount of apparent and oxidized tannins.

2.4.4. SPI (Saliva Precipitation Index) and PTI (Phenolic Total Index)

According to the protocol reported by Gambuti, Rinaldi, Pessina, and Moio (2006) and Rinaldi, Gambuti, and Moio (2012), the press wine was filtered through a 0.45 μm membrane, diluted and put in contact with the saliva at 37 °C. After cold centrifugation, all the wine tannins interact with the excess saliva proteins. The supernatant is recovered and then analyzed by electrophoresis. The proteins are denatured in the presence of β-mercaptoethanol and then remaining proteins are quantified. The reduction percentage of salivary is calculated. Four proteins are quantified in saliva. Proline-rich basic protein (PRPb) at 15 ± 2 kDa, the α-amylase (62 ± 3 kDa) and two proline-rich proteins glycosylated (PRPg 1 and 2) PRPg to 70 ± 3 kDa. The reduction of protein is calculated on the diluted saliva for each family. The results are expressed as a reduction from the saliva. PTI (Phenolic Total Index) is calculated based on the value of SPI and DO_{280nm}: SPI/DO_{280nm}

2.4.5. Sensory evaluation

The sensory evaluations of different samples of press wine were performed by a panel of five professional judges (oenologists). The sensory evaluation was performed three months after treatment and the tests were conducted at ambient temperature in individual boxes. Each sample was presented in a balanced random order in coded wine glasses. Judges were asked to rate the intensity of the perceived astringency and bitterness on a 0–5 scale. Judges rinsed glasses twice with de-ionized water between samples.

2.4.6. Statistical analysis

The data are presented as means ± SD (The means presented in the results are from the analyses), the statistical calculations (Analysis of variance, and PCA) and the least significant difference (LSD) according to Student-Newman-Keuls was used to compare and separate the means, and significance was accepted at the 5% level. Comparison of treatment means (LSD, 5% level) were done using the XLSTAT 2013 statistics software. Data collected for the taste astringency and bitterness was subjected to Friedman analysis software.

Table 1
Wine fractionation conditions.

Fraction	Number tube	Time Second	Flow rate mL/min	Solvents[a]		
				A	B	C
	1	00	20	0,0	100	0,0
	1	25	20	0,0	98	02
	2	01:00	20	0,0	95	05
	3-4-5	05:04	20	0,0	55	45
	5	05:07	14,06	0,0	55	45
I	6 → 23	39:50	13,1	0,0	45	55
II	24 → 29	45:05	15,3	45	25	30
III	30 → 34	51:30	18,0	45	25	30
IV	35 → 37	57:09	20,0	100	0,0	0,0
V	38→ rest	01:03:13	20,0	100	0,0	0,0
	38→ rest	01:03:13	5,0	100	0,0	0,0
	38→ rest	01:03:13	50,0	100	0,0	0,0

[a] A: (CH_3CH_2OH + 0.05% TFA); B: (H_2O/TFA, 99.95:0.05, v/v); C: (CH_3COCH_3 + 0.05% TFA).

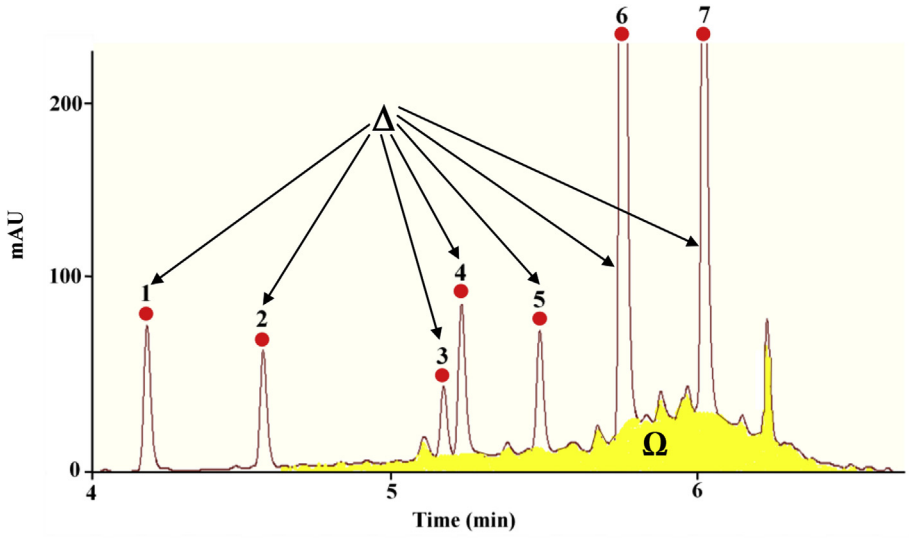

Fig. 2. A typical UPLC chromatogram of depolymerization products (Δ) from press wine tannins: peaks numbered and marked with a red dot; 1, catechin; 2, epicatechin; 3, epicatechin 3-*O*-gallate; 4, epigallocatechin-thiol; 5, catechin-thiol; 6, epicatechin-thiol; 7, epicatechin 3-*O*-gallate-thiol; (Ω) the hump stained in yellow corresponds to the unresolved oxidation products from the tannins. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results and discussion

3.1. Effect of fining treatments and micro-oxygenation on tannic apparent fractions profile

To evaluate the impact of treatments on the characteristics of tannins, the phenolics of the resulting press wines were fractionated by flash chromatography. All the flavanols, including monomers and tannins, were collected in four fractions (MF, FIII, FIV, FV). Fig. 3 displays the relative losses (%) of flavanols content in each fraction of the treated press wines compared to the control. It clearly shows that fining treatments (T_2, T_3 and T_4) mainly affect the monomeric composition of the resulting press wine compared to the control (T_0). The monomeric flavan-3-ols, generally associated with bitterness (Cosme, Ricardo-da-Silva, & Laureano, 2009), was significantly decreased (45–68%) in the press wine treated by the three fining agents compared to the control wine. Moreover, except the pea protein/bentonite/polysaccharide fining treatment (T4), all the other treatments (fining and micro-oxygenation) induced a significant loss (20–30%) of the largest apparent tannins (F5 fraction, aDP : 6.6 corresponding to the average value of T_0, T_1, T_2, T_3 and T_4 aDP values of fraction V). The treatment by gelatin (T_2) led to the largest loss (32%) in polymeric apparent tannins. However, T_4 treatment appeared to affect more specifically the oligomeric tannins recovered in the FIV fraction (F4 fraction, aDP : 3.2 corresponding to the average value of T_0, T_1, T_2, T_3 and T_4 aDP values of fraction IV) with a relative loss of 18%. These results are in accordance with previous reports, which suggest that the gelatins selectively remove proanthocyanidins with high degrees of polymerization (Sarni-Manchado et al., 1999). The treatment T_4 did not lower the concentration of these compounds significantly.

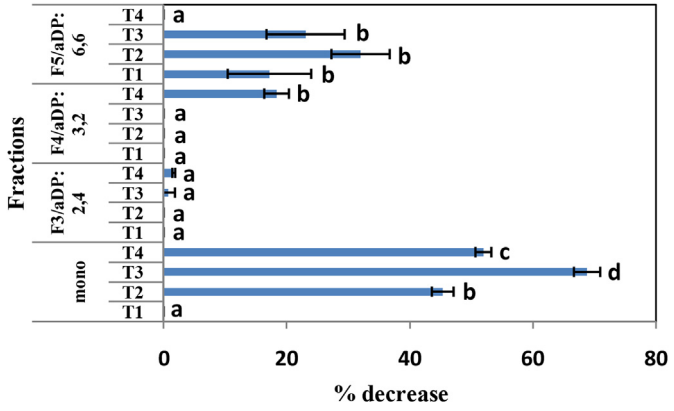

Fig. 3. Decrease of monomeric flavanol (MF) and the apparent tannic fractions (%) F3, F4, and F5, with the average degree of polymerization (aDP) 2,4; 3,2; and 6,6, respectively, after different treatments (The aDP of fractions 3, 4 and 5 corresponding to the average value of T_0, T_1, T_2, T_3 and T_4 aDP values of fraction FIII, FIV and FV respectively). The concentration (mg/L) of monomeric flavanol and condensed tannins in control wine: MF (139,8 ± 2,3), F3 (94,7 ± 1,8), F4 (56,3 ± 0,3), F5 (581,1 ± 30,1), respectively. Means within a column followed by the same letter are not significantly different (LSD, 5%) (n = 2).

3.2. Effect of fining treatment and micro-oxygenation on clarity and polyphenolic amount of press wine

The phenolic total index (PTI) and the Nephelometric Turbidity Unit (NTU) were lowered significantly by addition of all fining agents Table 2. These are in agreement with previous studies reported in the literature on fining (Cosme et al., 2008; Maury, Sarni-Manchado, Lefebvre, Cheynier, & Moutounet, 2003; Oberholster, Carstens, & duToit, 2013). In general, gelatin (T_2) was the fining agent that decreased the most the PTI and the NTU values. The fine characterization of the phenolic loss can be achieved by liquid chromatography analysis. For the monomeric catechin and epicatechin flavan-3-ols, the fining agents treatment promoted a greater decrease in catechinthan in epicatechin (Cosme et al., 2009). The greatest loss of catechin and epicatechin was observed by treatment (T_3) 69% and 68% respectively (Table 1). The content of apparent condensed tannins evaluated after chemical depolymerization decreased significantly by both treatments (T_2) and (T_3), and total tannins are significantly lowered by the three fining agent treatment. The T_2 and T_3 treatments appear to have an influence on the oxidized tannins as well, but less significantly than the T_4 treatment that seems to eliminate more oxidized tannins.

Table 2
Effect Micro-oxygenation treatments and fining agents on the physicochemical and polyphenolic quality of red press wines.

		T_0	T_1	T_2	T_3	T_4
	Turbidity (NTU)	95,95 ± 2,47^{c}	109,5 ± 2,12^{d}	34,8 ± 0,14^{a}	72,85 ± 0,91^{b}	70,7 ± 2,68^{b}
	PTI (280)	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}
	Tanins-T (mg/L)	2165,0 ± 93,80^{d}	2829,5 ± 49,54^{e}	1556,3 ± 28,46^{b}	1806,1 ± 19,93^{c}	1294,5 ± 19,54^{a}
	Tanins-A (mg/L)	732,92 ± 31,71^{b}	789,1 ± 13,82^{c}	611,69 ± 11,19^{a}	644,10 ± 7,11^{a}	754,04 ± 11,38^{bc}
Monomers	Cat (mg/L)	93,90 ± 1,95^{d}	99,08 ± 1,69^{e}	50,52 ± 0,90^{c}	29,51 ± 2,69^{a}	39,42 ± 0,57^{b}
	Epi (mg/L)	45.94 ± 0,36^{d}	45,38 ± 0,76^{d}	25,84 ± 0,58^{b}	14,07 ± 0,85^{a}	27,70 ± 0,37^{c}
	EGC (mg/L)	n.d	n.d	n.d	n.d	n.d
	ECG (mg/L)	n.d	n.d	n.d	n.d	n.d

PTI: phenolic total index, Tannins-T: Total tannins, Tannins-A: Apparent Tannins, Cat: Catechin, Epi: Epicatechin EGC: Epigallocatechin, ECG: Epicatechin-3-*O*-gallate. (mean ± SD), values followed by the same letter are not significantly different (LSD, 5%) (n = 2). n. d. means not detected.

3.3. Structural characterization of apparent proanthocyanidins (oligomeric and polymeric) of different fractions

The structural characteristics of wine proanthocyanidins obtained by reverse-phase UPLC-DAD-MS analyses of depolymerization products released by thioglycolysis are presented in Table 3. For majority of modalities, the analyzes of the amounts of apparent proanthocyanidins of each fraction showed that during the elution on flash chromatography, aDP and the percentage of galloylation increase. Conversely, the percentage of oxidation and the percentage of prodelphinidins decrease (Table 3). This suggests that the polymerized tannins containing more of epicatechin-3-*O*-gallate subunits are less prone to oxidation. The comparison between the different treatments is quite difficult to make, firstly because the composition of tannins in the treated press wines is likely different. These differences in tannins and flavanols composition may affect their fractionation on the flash chromatography system. Consequently, the distribution of oligomeric and polymeric proanthocyanidins into FIII, FIV and FV fractions may vary according to the sample of treated wine. So, the overall quantities of different parameters were calculated according to a generic method of laboratory (Table 3), with the following formula:

$$\textbf{Global}\ (\mathbf{X}) = \frac{\sum_{i=1}^{n}(\mathbf{Y}*\mathbf{X})}{\sum_{i=1}^{n}\mathbf{Y}}$$

Y: Expressed in mole for aDP and the rates of galloylation, prodelphinidin and in mass for oxidation.**X:** aDP and the rates of galloylation (% gal), prodelphinidin (% prod) and of oxidation (% oxi).

3.4. Effect of micro-oxygenation and fining treatments on global parameters of proanthocyanidins structures

The authors Maury, Sarni-Manchado, Lefebvre, Cheynier, and Moutounet (2001); Lea and Arnold (1978) reported that the aDP and % of galloylation influence the astringency. However, the calculated global parameters (aDP, percentage of prodelphinidin and percentage of galloylation) (Table 3), were not statistically different between the different treatments of the press wine, Conversely, the percentage of oxidation parameter is significantly affected by the different treatments: the micro-oxygenation (T_1) leads to a significant unsurprising increase of 9% in oxidation parameter. This increase is due to the large amount of oxygen introduced into the wine in the micro-oxygenation treatment period (pre-MLF). The effectiveness of micro-oxygenation depends significantly on SO_2 levels that are usually low at this winemaking stage in order to promote malolactic fermentation. The increase of oxidized tannins (9%) may be underestimated because a part of oxidized tannins likely become insoluble. The loss of tannins in FV of T_1 press wine compared to the control supports this hypothesis. Besides, the amount of total tannins (apparent tannins plus oxidized tannins) recovered by flash chromatography from the micro-oxygenation treatment is higher compared to the control wine. This could be explained by a larger adsorption on the Sephadex gel of the less oxidized tannins that can make more H-bondings than the high oxidized tannins. Actually, oxidation which corresponds to a loss of H_2 may reduce the number of OH phenolic groups interacting with the Sephadex gel.

T_4 treatment significantly reduces oxidation by 36% compared to the control. This finding deserves to be connected with the tannins composition of T_4 press wine that had specifically lost oligomers and oxidized tannins by T_4 treatment. As depicted in Fig. 4, the astringency intensity revealed by sensory analyses is significantly ($p < 0.05$) different between all enological treatments. These differences reflect both the quantitative and qualitative differences of tannins composition induced by treatments. The percentage of tannin oxidation is estimated for the first time in this study to take into account the part of tannins usually disregarded. It is noteworthy that the oxidized fraction of tannins is the largest fraction of total tannins and may contribute to wine flavor. This oxidized fraction may be formed in the winemaking process of press wine where a part of marc remains in contact with air during the alcoholic fermentation. However, the information obtained from tannin analysis is very partial and does not reflect the actual composition of tannins (Poncet-Legrand et al., 2010). Indeed, the authors reported that the tannin oxidation factor decreases the performance of depolymerization reactions used for the analysis of tannin (Vernhet, Carrillo, & Poncet-Legrand, 2014). Consequently, the relationships between the oxidized structures, their physicochemical properties and their impact on wine sensory quality are not yet established.

3.5. Impact of fining and micro-oxygenation treatment on the bitterness, astringency and the Saliva precipitation Index (SPI)

The results of the SPI and taste presented in Fig. 4 show that all different treatments have a significant impact on the reduction of the SPI of press wine over control. This result is due to oligomeric and polymeric apparent tannins (fraction 4, aDP: 3.2 and fraction 5, aDP: 6.6) probably associated with astringency and that were significantly removed. The two fining agents (T_2) and (T_3) are treatments ensuring the highest reductions of SPI 26% and 43%, respectively. This reduction in astringency is confirmed by tasting only for fining agent (T_3). Same results are obtained for this product with a dose of 0.3 g/L (Renouf & Murat, 2012). However, the wine treated by fining agent (T_2) is considered more astringent than wines treated by both fining agents (T_3) and (T_4) (Fig. 4). Taster juries recognized attenuation astringency by fining agent (T_3), probably due to the impact of PVPP on the amount of apparent proanthocyanidins (Table 2). The fining agent (T_4) was judged less astringent by tasters Fig. 4. This result is probably due to precipitation of a large amount of oxidized tannins by proteins pea. The

Table 3
Structural characterization of apparent oligomeric, polymeric and global proanthocyanidins. Average degree of polymerization (aDP), percentage of galloylation (% gal), percentage of prodelphinidin (% prodel) and percentage of oxidation (% oxi) are reported for press wine control and press wine control after different treatments (mean = SD). Values followed by the same letter are not significantly different (LSD, 5%) (n = 2).

	T_0			T_1			T_2			T_3			T_4			T_0	T_1	T_2	T_3	T_4
Fraction	III	IV	V	III	IV	V	III	IV	V	III	IV	V	III	IV	V	Global parameters calculated				
aDP	2.2±0.5	1.8±0.3	5.9±0.7	3.1±0.9	4±0.04	7±0.2	2.3±0.2	4.3±0.4	6.7±0.5	3±0.3	3.7±0.04	6.6±0.8	1.6±0.4	2.5±0.1	6.6±0.4	4.1±0.2^{a}	5.3±0.2^{a}	4.8±0.0^{a}	4.6±0.1^{a}	4.2±0.7^{a}
% gal	6.4±2.1	4.2±2	19±0.7	8.4±3	14.1±1.6	17.7±1	5.4±2	13±2.7	11±9	6±1	13.5±2.1	16±1.3	3±2.5	6±0.3	14±1	13.1±0.7^{a}	14±1.6^{a}	12±0.5^{a}	13±0.2^{a}	10±1.2^{a}
% Prod	31±0.2	26.3±0.06	10±6.5	38.1±0.8	17±0.6	22±2	23±3.3	19±0.3	20±0.8	35.7±2.7	18.7±1.2	22±1.4	26±1.6	11±0.6	19±4	17.5±3.9^{a}	20±1.4^{a}	21±1.2^{a}	22±1.0^{a}	18±0.6^{a}
% Oxi	84.3±0.3	80.4±0.2	56±0.5	71±0.5	66.8±10.6	75.4±3.2	85.2±0.7	66.1±5.3	48.6±6.5	81.7±2.9	69.4±1.4	55.4±1.6	75.3±12.3	65.4±0.04	23.1±1.1	66.2±0.4^{b}	72±1.2^{c}	60±1.1^{b}	64±0.03^{b}	41±3.6^{a}

*Proanthocyanidins not detected in fraction I and II.

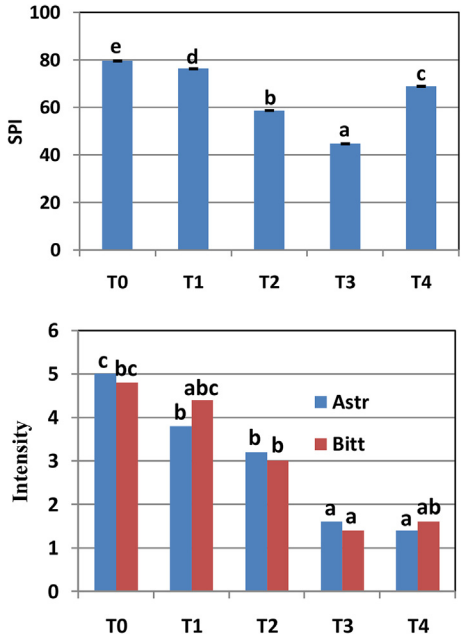

Fig. 4. The result of the Saliva precipitation Index (SPI) and bitterness and astringency Intensity of red press wine. Means within a column followed by the same letter are not significantly different (LSD, 5%).

vegetable sourced fining agent appears to possess similar qualities to animal protein in clarifying wines after treatment. For this reason, the dose of vegetable sourced fining agent required to treat the same red wines does not exceed 0.3 g/L, except for some very concentrated press wines for which the dose is 0.5 g/L (Navarre & Langlade, 2010). The efficiency of T_4 treatment may be due to the presence of polysaccharides in the formulation of the fining agent (mannoproteins) that interact with proanthocyanidins leading to soluble complexes remaining in the press wine, thus masking the note of astringency. This finding is in agreement with the results of Boulet et al. (2016). These authors actually showed that the polysaccharides decrease astringency of wine whereas oligosaccharides increase it. Escot, Feuillat, Dulau, and Charpentier (2001) have demonstrated that the wine structure was modified by the addition of mannoproteins which reduces astringency of red wine due to a higher tannin/mannoprotein complexation level. In addition, decrease of astringency in T_4 press wine may result from the precipitation of a large amount of oxidized tannins by pea proteins. The literature reports that flavan-3-ol monomers (Rossi & Singleton, 1966) have been known from a long time to contribute in bitterness. In our experiments, it is observed that fining treatments have a significant $P < 0.05$ impact on the reduction of bitterness (Fig. 4). The largest reduction are noted for (T_3) and (T_4) fining agents, 70 and 66% respectively which is in agreement with the analytical data obtained for the oligomeric flavan-3-ol (Table 2). The SPI value estimated for the micro-oxygenated press wine at a dose of 60 mL/L/month before malolactic fermentation is low compared to the control, which in accordance with the decrease of astringency evaluated by tasting. In this study, PCA was used to evaluate the relationship between the physicochemical and sensory characteristics of press wines (Fig. 5). Two experiments (Fig. 5A and B) were done; in the first one (Fig. 5A), the PCA was done from all the experimental data, including the sensory, physical chemical and analytical data of the press wines; in the second experiment (Fig. 5B), only the data showing statistical differences were taken into account to apply the PCA. The first axis (F1) of both PCA explained the largest part of variance (61 and 73,15% respectively).

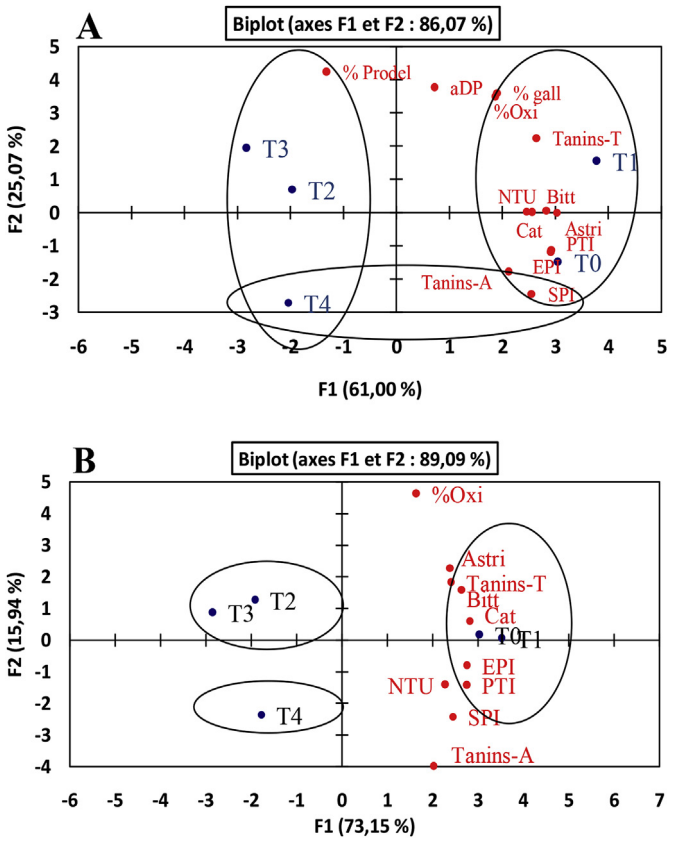

Fig. 5. Principal component analysis (PCA) of red press wines A: (PC1–61%, PC2–25.07%) and B (PC1–73.15%, PC2–15.94%). Relationship between press wines physicochemical and sensory characteristics (score and loading biplot). NTU: Nephelometric turbidity unit, PTI: Phenolic total index, Tannins-T: Total tannins, Tannins-A: Apparent Tannins, Cat: Catechin, Epi: Epicatechin, aDP: Average degre of polymerization, % gall: percentage of galloylation, % prodel: percentage of prodelphinidin, % oxi: percentage of oxidation, Bitt: Bitterness, Astri: Astringency and SPI: Saliva Precipitation Index.

As shown in Fig. 5A and B, the press wines are separated along this axis (F1) in two groups (T_0, T_1) and (T_2, T_3, T_4) according to their amount of tannins and their sensory attributes (T_0,T_1: richer in tannins and perceived as more bitter and astringent). This other group (T_2, T_3, and T_4) corresponding to all the fined press wines are less rich in tannins, astringent and bitter compared to the first group. The second component (F2, 16%, Fig. 5 B) distinguished two groups (T_0, T_1, T_2, T_3) and T_4 according to the quantitative and qualitative tannins characteristics. Indeed, T_4 is associated with high level of apparent tannins and less amount of oxidized and total ones. Unlikely, the other group (T_0, T_1, T_2, T_3) are associated with higher content of total and oxidized tannins and less level of apparent tannins. Additionally, the second component separated clearly the fined press wine in two groups, on one side (T_2, T_3) and on the other side T_4.

4. Conclusion

This study shows that all fining agent used (liquid gelatin, Fining agent based on Polyvinylpolypyrrolidone (PVPP) and protein of pea) allow very good clarification of the Moroccan treated press wine when compared with the untreated red press wine. After wine treatment, each treatment has a distinct behavior on flavanol composition, affecting both the monomers and condensed tannins. All fining agents decreased significantly the amount of monomers and apparent condensed tannins. Fining agent based on vegetable protein is the treatment that seems to eliminate more oxidized tannins 36% in comparison to the control. However, the tannins of micro-oxygenated press wine were more oxidized (9%). On the sensory quality plan, we noted that Press wine tastes (Astringency and bitterness) are better with a formulation has basic Polyvinylpolypyrrolidone (PVPP) and protein of pea than with liquid gelatins, which are the more commonly used fining agents. Fining agent based on vegetable protein is thus a suitable alternative solution to animal proteins used as fining agent, because it is able to clarify and diminish bitterness, astringency and oxidized tannins.

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