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RESEARCH ARTICLE

Behavior of Moroccan red press wine after the clarification process

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Abstract

The press wines represent a non-negligible part of the produced volumes, into practice these wines are integrated to run wine, but the assembly work is delicate, because it can affect the quality of the runinig wines. The aim of this work is to study the sensory and physicochemical behavior of Moroccan presswine varieties *Petit Verdot and Cabernet Sauvignon* after the clarification process (enzyme and gelatins) during five months before blinding with running wines. The statistical evaluation of the obtained results confirmed a significant ($p <0.05$) decrease of press wines turbidities and from the first seven weeks. The turbidities of press wine witch treated with enzyme stand lower than those treated only with gelatin. Also a significant difference in color intensity and the amount of anthocyanins extracted from the two grapes varieties. From the results obtained from the taste panel, the taste quality of press wine is improved as a result of the significant reduction ($p <0.05$) of bitterness and astringency intensity. Press wine was stored for five months, a significant reduction in total phenolic index ($p <0.05$), and the total anthocyanin ($p <0.05$) were shown. In contrast, low variation of wine color intensity, the quantity of tannins and intensity of astringency. The clarification process improves significantly sensory and filterability of Moroccan red press wine before blinding with runinig wines.

INTRODUCTION

The traditional red wine making process is found to contribute at about 13 to 17% of press wine of the total production of the red wine (Vivas, 2007). After recovering running wine, marcs and solid parts (seed and skin) of the grape were pressed by press wine. According to the scales pressure, several types of press wine are collected; so the more there is pressure, the more colorful, astringent, bitter and rustic the wine will be (Renouf et al., 2012). The improvement of the quantity of free run wine via its blending with press wine necessitates the improvement of the quality of the latter (Trione et al., 2001). Indeed, the blending process is delicate because the press wine contains undesirable phenolic, astringency vectors, greenness, turbidity levels and instability of color. In many cases, wine press grows separately; however, it is blending with free running wine at the end of winemaking stage (Renouf et al., 2012). The pressured wine is turbid with a lot of particle-laden of various origins: micro-organisms (Saccharomyces yeasts and bacteria), cell debris from the solid parts of the bay and aggregates (Vivas, 2007). Turbidity is sometimes stubborn and clarification is difficult because the grape pectin influences the clarification and stabilization of must and wine (Vivas, 2007). The pectin polysaccharides are found in wine at a rate raging between 2000 and 4000 $mg.L^{-1}$ (Ribereau-Gayon, 1998). It is found to be responsible for turbidity, viscosity and filter stoppages (Rensburg, 2000)**.** Pectinases are the enzymes with the most important technological effects. The commercial preparations of pectic enzymes are obtained from fungal sources (Ana, 2006). They enable the increase of the free running juice volume by decreasing its viscosity and improving the clarification of both juice and wine filtration. Moreover, when the mixture of cellulases, glucanases and hemicellulases takes place, pectinases speed up the natural process of wine making and improve the quality of the wine as well (Ana, 2006). Clarification operation by fining with gelatin or egg white, which is found to be similar to filtration, eliminates much of the micro-organisms (Vivas, 2007) and precipitates suspended particles responsible for haze. The gelatin fining has an impact on the tannins via its positive influence on the wine taste (Scotti, 1997).

There are few studies that talk about press wine particularly in the Moroccan context, including Meknès and other places. The aim of this work is to study the sensory and physicochemical behavior of press wines after the step of clarification (enzyme and gelatins) which takes place immediately after the pressing. Press wines used in this study come from two different grape varieties (*Cabernet Sauvignon* and *Petit Verdot*) and treated with enzymes and gelatins according to a factorial experimental design. The physicochemical analyzes (turbidity, color, polyphenols) and sensory (astringency and bitterness) were determined to the experiment monitor.

1. Materials and Methods

1.1. Materials

A pneumatic type of press wine (Bucher Vaslin) is used for the elaboration of different types of press wine. Experimental stainless steel tanks were used. The capacities of the tanks were 10 hectoliters. Also a disk stack centrifuge RE50V (8000 Tr/min) was used in this experiment.

1.2. Oenological products

Depectyl clarification (Dose 2 g/hl) is an enzyme prepared in the form of powder. It was produced by fermentation of selected strains of *Aspergillus niger*. These enzyme functions in the following enzymatic activities: Endo-and exo-polygalacturonase (EC 3.2.1.15)>29000 nkat/g, pectin methyl esterase (EC 3.1.1.11)>15 000 nkat/g and pectinlyase (EC 4.2.2.10)>1600 nkat/g (information provider). In this experiment, two types of animal gelatins have been used. The first one is cold soluble gelatin (G_1) with an amount of 20 g/hl of atomized powder. The second one is hot soluble gelatin (G_2) with an amount of 15 g/hl of large grains vary from 1 to 3 mm (manufacturer data).

1.3. Elaboration of press wine

In 2013 vintage, grapes *Vitis vinifera var*, particularly *Cabernet Sauvignon* and *Petit Verdot* which were grown in the vineyard of Boufkrane in the outskirts of Meknès, Morocco have been cultivated and transported to the cellar CHATEAU ROSLANE to be transformed into wine respecting the protocol below (Fig.1).

1.4. Experimental clarification treatments

After the end of the alcoholic fermentation, the grape pomace of each variety is pressed to elaborate different types of press wine. The first pressed juice was pressed with pressure degrees that varied from 0 to 300 mbar. However, the second was pressed under pressure degrees that varied from 300 to 1400 mbar (Step A of figure 1). The two press wines are then distributed into different tanks. Then, the control samples are collected for standard analysis before being stored in bottles for physicochemical and sensory analyzes (Step C of Figure 1). The experimental tanks were filled and treated at the same time via clarifying product (gelatins and enzymes) (Step B of Figure1). At the end of malolactic fermentation, the wine is centrifuged at 8000 tr / min. After the physicochemical and sensory analyzes, the samples were stored in glass bottles 75 cl (Step D of Figure 1) for five months before the last analysis.

A 2^{4-1} fractional factorial design comprising different factors, Type of grape: X_1 (*Petit Verdot* (-1), *Cabernet Sauvignon*(+1)); Type of the wine press: X_2 (ferst press wine(-1), second press wine (+1)); the enzyme treatment: X_3 (No enzyme (-1), Enzyme (+1)); gelatin type: X_4 (Cold soluble gelatin (-1), Hot soluble gelatin (+1)). The fractional design had the following alias relations: $X_1= X_2X_3X_4$, $X_2 =X_1X_3X_4$, $X_3= X_1X_2X_4$, $X_4 = X_1X_2X_3$, $X_1X_2=X_3X_4$, $X_1X_3=X_2X_4$, $X_2X_3= X_1X_4$ et $I= X_1X_2X_3X_4$ (Table 2) **(**Goupy,1990). This purpose of this experiment design was to evaluate the effects of these four factors on the physicochemical and sensory parameters of pressed wine.

1.5. Methods of analysis

The pH, alcoholic strength, total acidity, Reducing sugar, free and total SO_2 were determined according to the methods of the OIV (1990) table 1.

1.5.1. Turbidity measurement

The turbidity (NTU) is determined using a turbidimeter Hach 2100P

1.5.2. Phenolic compound and Color determination in wines

Absorbance measurements were determined with a UV–visible spectrophotometer ANACHEM 220 with 1cm path length glass cells. $OD_{280\ nm}$, which estimates total phenol content, was determined following the method described by (Ribereau et al., 1998). Color Intensity (CI) and Tint (T) were determined using the spectrophotometric absorbance of the wine at 420, 520, 620 nm (Glories, 1984).

Total anthocyanin: was determined by Spectrophotometry of wine diluted with ethanol and hydrochloric acid, making a reading of an aliquot with water **($A0_{520}$).** Another type treated with **$NaHSO_3$ (A_{520})**, the formula is as follows: **$[Ant] = (A0_{520} - A_{520})*875$** (Ribereau et al., 1965)**.**

Total tannins: Were determined by Spectrophotometry of wine diluted with water and hydrochloric acid and heated **(A_{550})**. Wine diluted in the same way but not heated **($A0_{550}$)** the formula is as follows**: $[Tan] = (A_{550} - A0_{550})*19:33$** (Ribereau et al., 2012).

1.5.3. Sensoriel evaluation

The sensory evaluations of different samples of press wine were performed by a panel of 6 professional judges; the first sensory evaluation was organized after centrifugation and the second after five months. The tests were conducted at ambient temperature in individual boxes. Each sample was presented in a balanced random order in coded wine glasses. Bitterness and astringency were rated sequentially to avoid confusion between the two properties. Judges were asked to rate the intensity of the perceived astringency on a 0-7 scale. At a later stage, panelists rated bitterness. Judges rinsed glasses twice with de-ionized water between samples.

1.5.4. Statistical analysis

The statistical calculations (calculation of coefficients, t test, analysis of variance, etc...) were done using the JMP6 software. Data collected for the taste, bitterness and astringency were subjected to Friedman analysis, multiple comparison and non-parametric procedures by the paired-samples T-test (before and after five months of storage) with the Wilcoxon signed ranks test using (Xlstat 2013) statistics softwar. The Significance of the results was established at $P < 0.05$.

2. Results and discussion

Table 1: Physicochemical and sensory characteristics of different control press wines (Step C, Fig.1).

Parameter	*Petit Verdot*		*Cabernet Sauvignon*	
	P_1^a	P_2^b	P_1^a	P_2^b
pH	3.81	3.83	3.89	3.85
Total acidity $g.L^{-1}$ H_2SO_4	3.61	4.02	4.01	4.13
Alcohol content	11.69	12.16	12.88	13.01
Volatile acidity $g.L^{-1}$ H_2SO_4	0.28	0.3	0.09	0.06
Reducing sugar $g.L^{-1}$	1.9	2.2	2.6	2.8
Free SO_2 $mg.L^{-1}$	26	24	29	28
Total SO_2 $mg.L^{-1}$	39	34	41	45
PTI DO_{280}^c	100,87	128,83	139,63	174,87
Total anthocyanin $mg.L^{-1}$	912	996	973,9	1232,3

Color intensity	19,2	23,7	23,8	26,9
Total tanins $g.L^{-1}$	5,58	5,99	6,97	8,40
Intensity of bitterness	4.83	6.17	5.17	6 ,00
Intensity of astringency	5.5	6.83	6.33	6.33

a, First press wine, b, Second press wine, c, Phenolic total indix (DO $_{280nm}$)

Table 2: Matrix Fractional experimental design 2^{4-1} and the responses

Run	I	$X_2X_3X_4$	$X_1X_3X_4$	$X_1X_2X_4$	X_3X_4	X_2X_4	X_1X_4	$X_1X_2X_3$	Turb[a]	PTI[b]	Ant[c]	CI[d]	Tan[e]	Bitt[f]	Astr[g]
	$X_1X_2X_3X_4$	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	X_4	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7
1	+1	-1	-1	-1	+1	+1	+1	-1	54,23	67,23	763,3	13	3,93	2,83	3
2	+1	+1	-1	-1	-1	-1	+1	+1	60,37	107,77	857,8	17,7	5,94	3,67	4,67
3	+1	-1	+1	-1	-1	+1	-1	+1	104,67	124,83	903,3	16,7	5,92	4,17	5,5
4	+1	+1	+1	-1	+1	-1	-1	-1	277,67	128,23	1220	25	6,54	5,00	4,83
5	+1	-1	-1	+1	+1	-1	-1	+1	20,97	77,5	740	12	4,19	2,33	3,33
6	+1	+1	-1	+1	-1	+1	-1	-1	26,6	113,43	1018	19,2	6,13	3,83	4,67
7	+1	-1	+1	+1	-1	-1	+1	-1	50,57	89,1	856,9	14,2	4,30	3,50	4,5
8	+1	+1	+1	+1	+1	+1	+1	+1	221,67	124,17	1090	24	6,03	4,83	5,33
con[h]	a_0	a_1	a_2	a_3	a_1a_2	a_1a_3	a_2a_3	$a_1a_2a_3$							

a, Turbidity , b, Phenolic total indix ($DO_{280\ nm}$), c, Total anthocyanin, d, Color intensity, e,Total tanins, f, Intensity of Bitterness, g, Intensity of Astringency, h, contraste

Table 3: Analysis of variance (ANOVA) for the experimental results of fractional design.

	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7
	F ratio							F value						
Model	14714	6,76	161,4	217,2	9,22	304	252,3	0,006*	0,28	0.060	0,051	0,246	0,043*	0,048*
	Contraste							P value						
Intercept	102,09	104,0	931,3	17,72	5,37	3,77	4,47	0.001	0,020	0.003	0,004	0,015	0,003	0,003
X_1	44,48	14,36	115,4	3,75	0,78	0,56	0,39	0.004**	0,147	0,027*	0,021*	0,104	0,025*	0,034*
X_2	61,55	12,55	86,43	2,25	0,32	0,60	0,56	0.003**	0,167	0.036*	0,035*	0,242	0,023*	0,024*
X_3	-22,14	-2,28	Ng	-0,37	-0,21	-0,14	Ng	0,008**	0,540	-------	0,204	0,352	0,964	------
X_4	-0,17	4,53	-33,56	Ng	0,14	Ng	0,22	0,666	0,408	0,093	--------	0,459	------	0,059
X_1X_2/X_3X_4	41,54	-4,75	22,21	0,77	-0,2	-0,02	-0,35	0,004**	0,393	0,140	0,101	0,366	0,537	0,037*
X_1X_3/X_2X_4	Ng	Ng	12,43	0,5	Ng	0,14	0,14	-------	-------	0,242	0,156	-----	0,098	0,091
X_2X_3/X_1x_4	-5,38	-6,96	-39,33	-0,5	-0,32	-0,06	-0,10	0,035*	0,287	0,080	0,156	0,243	0,220	0,128

X_1, Grape variety, X_2, Type of press wine, X_3, Enzymes, X_4 ,Type of gelatins, X_iX_j, Interaction,Y_1, Turbidity (NTU),Y_2, Phenolic total indice (DO $_{280nm}$), Y_3, Total anthocyanins (mgl^{-1}), Y_4 , Color intensity , Y_5 , Total tanins (g/l^{-1}), Y_6, Intensity of Bitterness, Y_7, Intensity of Astringency, ng , Neglected, * , Significant at $P < 0,05$, ** Significant at $P < 0,01$

Table 4: Ranking of different press wine according to the intensity of astringency and bitterness.

Friedman test *p*-value (bilateral)		Astringency <0.002**			Friedman test *p*-value (bilateral)		Bitterness <0.001**	
Samples	Mean of ranks	Groupes			Samples	Mean of ranks	Groupes	
1	1,833	A			1	1,833	A	
5	2,167	A	B		5	2,333	A	B
3	4,333	A	B	C	3	4,000	A	B
2	4,583	A	B	C	2	4,500	A	B
6	5,083	A	B	C	6	4,667	A	B
4	5,083	A	B	C	7	5,667	A	B
8	6,333		B	C	8	6,417		B
7	6,583			C	4	6,583		B

**Significant at $p < 0,01$

Table 5: Variation in phenols and sensory characteristics after five months of storage in bottles at cellar temperature (Step D figure 1).

	PTI DO280*	CI*	Total Anthocyanins	Tanins	Bitterness Intensity	Astringency Intensity
P value	0,0002**	0,08	0,002**	0,59	0,02*	0,06
Trial	t_0----------t_5	t_0----------t_5	t_0----------t_5	t_0----------t_5	t_0----------t_5	t_0----------t_5
1	67,23---61,43	13,0---16.56	763,3---752	3,93---4,76	2,83---2,8	3,00---4,5
2	107,77---100,77	17,7---18,69	857,8---741	5,94---5,91	3,67---4,0	4,67---4,5
3	124,83---114,70	16,7---14,89	903,3---770	5,92---5,90	4,17---5,8	5,50---6,0
4	128,23---115,23	25,0---23,30	1220---917	6,54---7,32	5,00---4,8	4,83---5,25
5	77,50---66,27	12,0---10,91	740,0---527	4,19---4,33	2,33---3,3	3,33---3,75
6	113,43---105,87	19,2---14,57	1018---582	6,13---4,10	3,83---4,8	4,67---4,5
7	89,10---87,10	14,2---16,23	856,9---609	4,30---4,89	3,50---4,3	4,50---6,0
8	124,17---113,93	24,0---23,93	1090---813	6,03---7,35	4,83---5,3	5,33---5,5
Mean	**104,0---95,60**	**17,7---17,41**	**931,0---713**	**5,30---5,50**	**3,77---4,3**	**4,40---5,0**

t_5, five months, CI, Color intensity, PTI, Phenolic total indix (DO $_{280nm}$), *, significant at $p<0.05$, **, significant at $p<0.01$

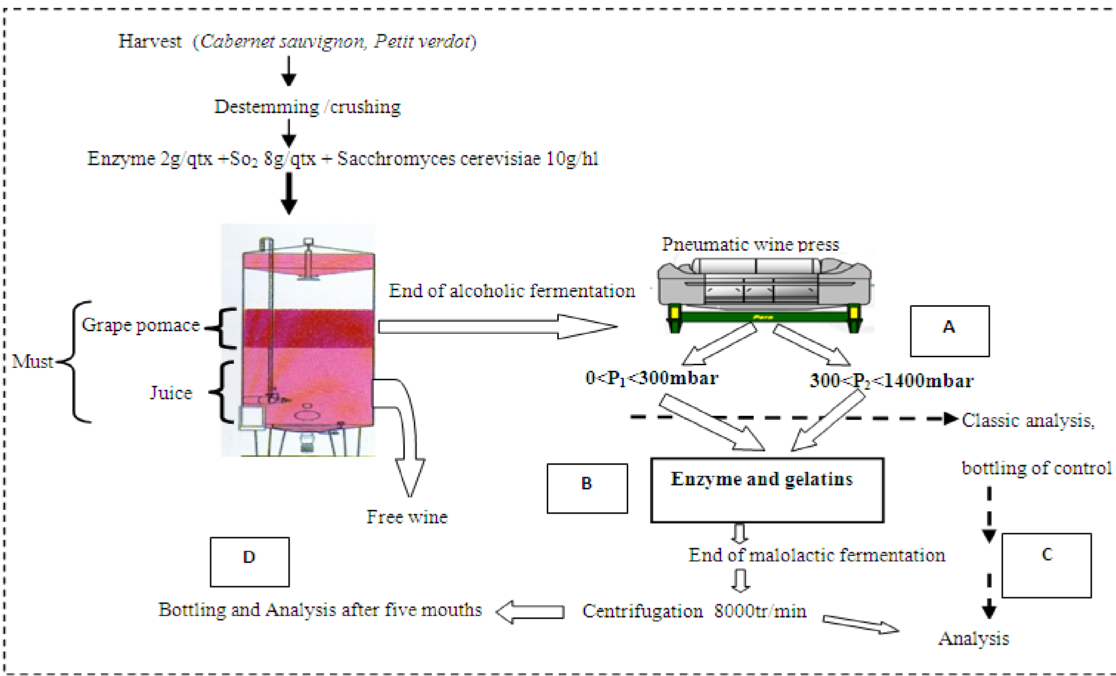

Figure 1: Protocol of winemaking press wine (First and seconds press wine) CHATEAU ROSLANE.

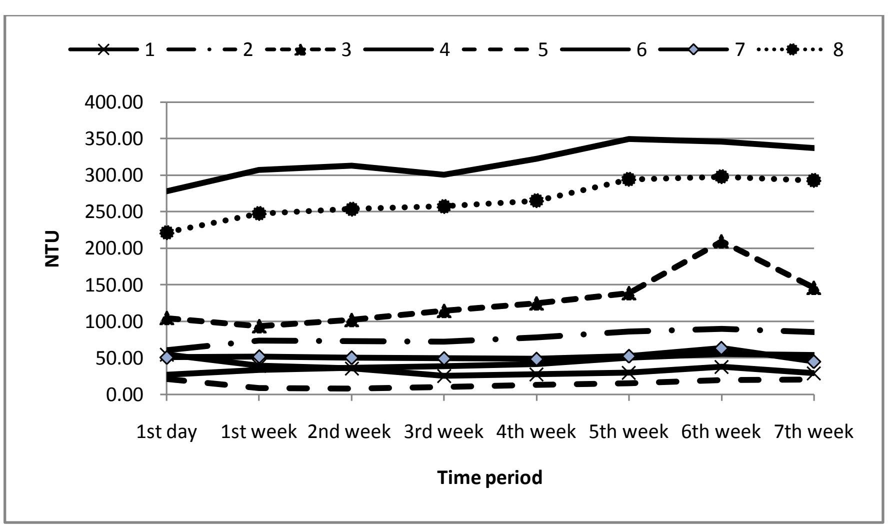

Figure 2: Evolution of the wine turbidity of the eight trials of experience stored up in bottles during the first seven weeks.

2.1. The multivariate analysis of clarification treatment of different types of press wine on the immediate turbidity

Following testing the significance of the effects (Test 't') (Table 3) we noted that the variation of type of grape (X_1) and the type of press wine (X_2) have a significant (sig $p < 0.05$) effect on the increase of the immediate turbidity (Y_1). To liberate the rest of the imprisoned juice, it is necessary to crumble the grape pomace and pressed again to higher levels. The latter press juice is more turbid as it presents high levels of turbidity (Vivas, 2007).The main polysaccharides responsible for turbidity, viscosity and filter stoppages are pectins, glucans (the major component of cellulose), and to a lesser extent, hemicellulose (mainly xylans) (Pretorius, 2000). The haze is essentially generated by interactions between polysaccharides and other compounds of wine preventing their sedimentation. Polysaccharides wine can originate from the walls of the skin of the pulp of grapes (Villetaz, 1981). The yeast *Saccharomyces cerevisiae* walls (glucans and mannoproteins) (Dubourdieu et al., 1981), it can be found glucan, secreted by *Botrytis cinerea* (Pretorius, 2000). The addition of pectinases lowers the viscosity of grape juice and causes cloud particles to aggregate into larger units, which sediments are removed easily by settling (Rensburg et al., 2000). The results obtained in the shows that the treatment of the press wines with pactinases (X_3), contributes to a significant (sig $p < 0.05$) effect on reducing immediate turbidity. The turbidities of press wine witch treated with enzyme (Trials n°5, 6, 7 and 8 of fig 2) stand lower than those treated only with gelatin (Trials n°1, 2, 3 and 4 of fig. 2). From the fifth week there was an increase of turbidity of different press wine.

2.2. Effect of alternative clarification on the phenolic composition and color of the press wine

We noted that all the variables have no significant effect on total phenolic index (Y_2) and tannins (Y_5). Regarding the color intensity (Y_4) and the quantity of anthocyanin (Y_3), we noted a significant (sig $p < 0,05$) effect of these two variables type of grape (X_1) and type of press wine (X_2) (Table 3). The difference in the amount of anthocyanins extracted from two varieties (*Petit Verdot* and *Cabernet Sauvignon*) reflects the difference in color intensity between the two varieties. Anthocyanins are responsible for the color of red grapes. They are located in the vacuoles of the cells of the skin. The color of wine depends on the wealth of each anthocyanins grape, which itself varies depending on the vintage of the order of 100 $mg.L^{-1}$ for *Pinot*, 1500 $mg.L^{-1}$ for *Syrah* or *Cabernet-Sauvignon* (Ribereau Gayon, 2012).

2.3. Effect of alternative clarification on sensory quality (astringency and bitterness) of press wine

On the one hand, the significant (sig: $p < 0,05$) difference in intensity of astringency (Y_7) and bitterness (Y_6) noted between the two press wine varieties (Table 3). On the other hand, there is no difference in totals tannins (Y_5) (Table 3), therefore, the molecular size of proanthocyanidins affects their relative bitterness and astringency level (Peleg, 1999). The composition and the structural characteristics of proanthocyanidins are dependent on the grape localization (seed or skin) and on the variety. In the literature, data concerning variety effect on Bordeaux wine grape phenolic composition are largely absent (Kleopatra et al., 2011). We noted a significant (sig $p < 0,05$) effect of variables type of press wine (X_2) (Table 3) on intensity of astringency (Y_7) and bitterness (Y_6). According to pressures exerted which does not give identical press wine in the composition. Because the grape pomace is a heterogeneous environment that presents a mixture of skins and seeds, and a very small fraction of vegetable debris. The juice imprisoning is in two forms: the first which is weakly retained in the pores of grape pomace and is collected by a simple static or dynamic dripping. To a pressing cycle, the first pressing is qualitative (Vivas, 2007) less astringent (group A and AB of table 4) and bitter (group A of table 4). To liberate the rest of the imprisoned juice, it is necessary to crumble the grape pomace and press it again to higher levels. The latter press juice is more astringent, bitter and herbaceous taste that is due to the solubilization of dry tannins in wine pressing. The great diversity of gelatins available in the market is a result of the collagen origin and the nature of the production process. Protein fining agents could be used to remove specific phenolic compounds and consequently astringency or bitterness of wines (Cosme, 2007). From the results of the sensory evaluation trials (table 3), we noted that the variables type of gelatin have no significant effect on intensity of astringency (Y_7) and bitterness (Y_6). There is a wealth of European data which shows that low Bloom strength gelatin (Low Molecular Weight) is optimum for fining (Calderon et al., 1968). This is probably due to the facility of use of Low Molecular Weight (LMW) gelatins when compared with high molecular weight (HMW) gelatins that need to be heated before incorporation in the wine. High Bloom Strength (HMW) gelatins used in fining have to be prepared as a dilute solution of gelatin. In fact, there is really no detectable difference in performance between the use of HMW and LMW gelatins (Marchal et al., 1993).

2.4. Interpretation of changes in physicochemical and sensory parameters

The eight samples of experimental design stored in bottles for five months (Step D Fig. 1) were analyzed. According to the non-parametric procedures (paired-samples T-test with the Wilcoxon) it was noted that there had been a significant decreases in total phenolic index (Sig, $p < 0.01$) and total anthocyanins (Sig: $p < 0.01$), while against intensity of color and the tanins were constant (Table 5). The anthocyanins molecules are not chemically very stable, relatively small fraction of anthocyanins disappears by degradation, oxidation, precipitation, or formation of other colorless compounds, such as castavinols which can act as a reserve of anthocyanins (Jackson, 2008; Monagas, 2009), and disappear after a few years then always the wine was red. The color intensity increases and moves to the purple color (Robinson, 1931) because the co-pigmentation moves also to balance the different forms of anthocyanins. The sensory level has no significant difference in astringency but bitterness increases significantly (Sig: $p < 0.05$) during the five months of storage.

Conclusion

Several factors influence the physicochemical and sensorial behavior of red press wine as the grape variety, the intensity of pressure, treatment with agent clarification (enzymes and gelatin). Before blinding with runinig wines, it is necessary to work at low pressures degrees, using enzymes of clarification to improve the filterability and fining with gelatins to reduce the astringency, bitterness and to stabilize wine color.

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