Adjuvants and additives for the colloidal stabilization of red wines without the use of cold

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Abstract. The presence of turbidity and precipitates in the bottle causes depreciation of wine by consumers. Colloidal instability is at the basis of these phenomena. The cold treatment is a technique widely used to stabilize wines, as regards both the tartaric and the colloidal precipitations. However, it is an energy-consuming technique. In view of a sustainable management of winery practices, the work aimed at evaluating the possibility of using some enological products for the colloidal stabilization of wines without cold treatment. 8 adjuvants (sodium and calcium bentonite, hot and cold soluble gelatin, chitosan, isinglass, PVI/PVP, carboxymethylcellulose) and 4 additives (kordofan gum, 2 different mannoproteins, a natural polysaccharide polymer) were compared. The trial was performed with a Barbera (2019) and a Montepulcianod'Abruzzo (2020) wines, stabilized against tartaric precipitations without cold, but with colloidal instability. Three days after the treatments the wines were racked, filtered (3 μm) and bottled. After bottling and after 6 months of bottle aging, the wines were analyzed. Colloidal stability test (48 hours at 4°C) and shock test (1 and 7 days at 40 °C) were performed. As regards Barbera, the treatment with sodium bentonite (50 g/hL) or the addition of a mannoprotein (15 g/hL) allowed to stabilize the wine. For Montepulciano, having a higher colloidal instability than Barbera, the cold stabilization resulted necessary because no treatment was effective. All treatments had a modest impact on the color.

1 Introduction

Red wines contain colloids, macromolecules, and aggregates of molecules, that are aggregated proteins, polysaccharides and polyphenolic compounds [1] verified the presence in some red wines of colloids made up of aggregates of polysaccharides, proteins and phenolic compounds and others in which proteins were absent.

When the colloidal dispersions are stable over time, the wines remain limpid, but if they become unstable aggregates are formed which can remain in suspension (cloudy wines) or precipitate (appearance of deposits). The precipitation of colloids is one of the causes of the loss of color.

The permanence at low temperature (cold stabilization) is the most used technique for the stabilization of wines as regards both the tartaric and colloidal precipitations, but this is an energy-consuming technique. In view of a sustainable management of cellar practices, this work concerned the evaluation of the possibility of using some enological products (adjuvants and additives) for the colloidal stabilization of wines not subjected to cold stabilization.

The test involved 2 wines, a Barbera from the 2019 harvest and a Montepulcianod' Abruzzo from the 2020 harvest.

The clarification treatments with adjuvants were aimed at removing the unstable colloidal compounds, in particular polyphenols in aggregate form and/or proteins, while the addition of additives aimed at integrating the medium wine with protective colloids.

2 Materials and methods

The experiment was conducted with a Barbera wine from the 2019 harvest and a Montepulcianod' Abruzzo wine from the 2020 harvest, both unstable to colloidal precipitation. Before the test, the wines were stabilized against tartaric precipitation without the use of cold, respectively with the use of electrodialysis (Barbera) and exchange resins (Montepulcianod' Abruzzo).

The tests were carried out 3 months after the end of the alcoholic fermentation, with the wines that had completed the MLF. Barberahad an alcohol content of 12.77%, a pH of 3.62 and a titratable acidity of 4.95 g/L. Montepulcianod' Abruzzohad an alcohol content of 14.67%, a pH of 3.55 and a titratable acidity of 7.23 g/L.

Table 1. List of adjuvants, dose and method of use.

Adjuvants		Dose	Methods of use
Calcium bentonite	**BenCa**	50 g/hL	10% suspension in water
Sodium bentonite	**BenNa**	50 g/hL	10% suspension in water
Carboxymethylcellulose	**CMC**	10 g/hL	Dispersion in little wine
Nebulized gelatin	**Gel_fr**	10 g/hL	10% solution in cold water
Gelatin Gold	**Gel_cal**	10 g/hL	2% solution in hot water
Isinglass	**Ittcolla**	2 g/hL	1% solution in water
Chitosan	**chitosano**	10 g/hL	5% suspension in water
PVI/PVP	**PVIPVP**	20 g/hL	Dispersion in little wine

Eight theses were prepared, treated respectively with calcium bentonite (Ben_Ca), sodium bentonite (Ben_Na), carboxymethylcellulose (CMC), cold soluble gelatin/ nebulized gelatin (Gel_fr), hot soluble gelatin/ gelatin Gold (Gel_cal), isinglass (Ittcolla), chitosan and PVI/PVP

(PVIPVP). The doses of use of the enological products are reported in Table 1.

Three days after the treatment the wines were decanted, filtered with a 3 µm filter and bottled. Another 4 theses, in duplicate, were treated respectively with Kordofan gum (GommaK), Mannoprotein1 (Mannopr1), Mannoprotein2 (Mannopr2) and with a natural polysaccharide polymer (PolNat).

Table 2. List of additives, dose and method of use.

Additives		Dose	Methods of use
Kordofan gum	GommaK	25 g/hL	20% solution in water
Mannoprotein 1	Mannopr1	15 g/hL	Dispersion in little wine
Mannoprotein 2	Mannopr2	15 g/hL	Dispersion in little wine
Natural polimer	PolNat	10 g/hL	2% solution in water

The doses and methods of use of the additives are reported in Table 2. Forty eight hours after the additions, the wines were filtered with a 3 µm filter and bottled. Finally, a control thesis was prepared with the not treated wine, it was filtered at 3 µm and bottled. All tests were performed in duplicate as 10 L volumes. The bottles were stored in a thermostated room at a temperature of 20°C. The wines were analyzed 48 hours after bottling and after 6 months of bottle aging.

The analyses concerned the content of total anthocyanins and flavonoids [2], and wine color. The turbidity of the control wine was also evaluated and the presence of deposits in the bottle was verified. The colloidal stability test [3] and a shock test were performed.

The colloidal stability test consists in measuring the initial turbidity (NTU_0) of the wines, filtering them at 0.45 µm if the turbidity exceeds 2 NTU, and then storing them at 4°C for 48 h. The measurement is repeated at the end of the test (NTU_t): if the variation in turbidity ($\Delta NTU = NTU_t - NTU_0$) exceeds 2 NTU, the wine is considered unstable. The shock test consists in measuring the initial turbidity (NTU_0) of the wines, filtering them at 0.45 µm if the turbidity exceeds 2 NTU, and then storing them at 40°C for 24 h and for 7 days. The measurement is repeated at the end of the test (NTU_t): if the variation in turbidity ($\Delta NTU = NTU_t - NTU_0$) exceeds 2 NTU, the wine is considered unstable.

3 Results and Discussion

The effectiveness of some clarification treatments on the colloidal stabilization of red wines was compared. Some classic clarifiers were tested, used for the removal of the main constituents of the colloidal fraction, proteins and polyphenolic compounds (polymeric pigments and condensed tannins), such as bentonites (sodium and calcium), gelatins (hot soluble and cold soluble), isinglass and a mixture PVI/PVP.

Chitosan and carboxymethylcellulose (CMC) were also used. In this test, CMC was included among the enological adjuvants, despite being an additive for the prevention of tartaric precipitations, in order to verify whether its use caused the removal of the unstable colloidal fraction. [4] found that the addition of this product resulted in the formation of turbidity and the appearance of deposits in some red wines, and [5] verified that this instability was due to the interaction of CMC with the protein fraction of wines.

3.1 Barbera trial

3.1.1 Adjuvants

Table 3 shows the results of the colloidal instability test performed with the control theses and with the theses treated with clarifiers after bottling and after 6 months of storage.

Table 3. Barbera wine. Average values of turbidity, colloidal stability and shock tests for the control wine and the theses treated with adjuvants, after bottling and after 6 months of bottle aging. ANOVA and Tukey's test results. Different letters along the column discriminate the theses significantly different from one another (*p*<0.05; Tukey's test).

	Theses	Turbidity (NTU)	Colloidal stability test (ΔNTU)	Shock test (ΔNTU 24h)	Shock test (ΔNTU 7d)
After bottling	Control	0.96 b	29.2 b	1.34 b	10.56 b
	BenCa	0.21 a	11.20 ab	0.09 c	3.18 a
	BenNa	0. 39 a	1.69 a	0.00 c	0.02 a
	CMC	0.28 a	113.8 c	2.40 a	20.46 c
	Gel_fr	0.06 a	27.30 b	0.30 c	13.75 b
	Gel_cal	0.06 a	13.05 ab	0.17 c	12.41 b
	Ittcolla	0.13 a	25.0 ab	0.74 bc	13.26 b
	chitosano	0.38 a	22.9 ab	0.74 bc	10.89 b
	PVIPVP	0.34 a	20.2 ab	0.89 bc	11.99 b
	F	6.8	53.5	17.3	43.2
	sign	**(1)	***	***	***
After 6 months of bottle aging	Control	2.30 b	34.70 c	6.10 d	6.09 ab
	BenCa	0.40 a	3.22 a	0.10 a	10.28 abc
	BenNa	0.26 a	1.77 a	0.00 a	0.03 a
	CMC	12.10 c	94.92 d	0.00 a	15.60 bc
	Gel_fr	0.61 a	10.62 ab	1.88 ab	11.05 bc
	Gel_cal	0.60 a	1.12 a	1.65 ab	14.49 bc
	Ittcolla	0.85 a	19.79 b	3.19 bc	17.32 c
	chitosano	0.83 a	18.54 b	4.88 cd	10.57 bc
	PVIPVP	0.40 a	9.40 ab	3.11 bc	9.41 abc
	F	829	161	19.9	7.9
	sign	***(1)	***	***	**

(1) Significance: ** and *** represent significance at $p \leq 0.01$ and 0.001 respectively.

All theses treated with clarifiers, with the exception of the CMC theses, showed a lower degree of instability than the control, however only the one treated with sodium bentonite (BenNa thesis) was perfectly stable. Following in effectiveness, there were hot soluble gelatin and calcium bentonite. The CMC thesis had the highest degree of instability, however the appearance of turbidity was not immediate and the wines were clear after bottling; only later, during storage, they showed a progressive increase in turbidity (Table 1). On the contrary, the theses treated with the other clarifiers after 6 months of storage were clear, with turbidity values lower than the control.

The selectivity of action of the clarifiers on total anthocyanins and flavonoids and on color was verified. The results are reported in Table 4.

Table 4. Barbera wine. Total anthocyanins and flavonoids content and color parameters for the control wine and the theses treated with adjuvants, after bottling and after 6 months of bottle aging. ANOVA and Tukey's test results. Different letters along the column discriminate the theses significantly different from one another (p<0.05; Tukey's test).

	Theses	Total anthocyanins (mg/L)	Total Flavonoids (mg/L)	Color intensity (u.a.)	Color hue	$E520_{corr}$
After bottling	Control	318 d	1717 ab	6.7 e	0.61 a	4.8 f
	BenCa	294 a	1690 a	5.8 abc	0.65 bc	4.4 b
	BenNa	294 a	1731 ab	5.7 a	0.65 c	4.2 a
	CMC	314 bcd	1729 ab	6.1 cd	0.65 bc	4.7 de
	Gel_fr	316 cd	1732 ab	6.1 cd	0.64 abc	4.7 d
	Gel_cal	303 abc	1663 a	5.7 ab	0.62 ab	4.5 c
	Ittcolla	303 ab	1795 bc	6.1 d	0.63 abc	4.8 ef
	chitosano	320 d	1836 c	6.0 bcd	0.64 bc	4.7 def
	PVIPVP	291 a	1717 ab	5.9 abcd	0.64 bc	4.5 c
	F	24.2	10.0	33.8	8.4	186
	sign	***(1)	***	***	**	***
After 6 months of bottle aging	Control	191 ab	1181	5.9	0.71	3.9 d
	BenCa	193 ab	1227	5.3	0.74	3.6 ab
	BenNa	182 a	1213	5.5	0.73	3.5 a
	CMC	202 b	1215	5.5	0.72	3.8 cd
	Gel_fr	194 ab	1201	5.85	0.71	3.7 bcd
	Gel_cal	191 ab	1153	5.5	0.71	3.7 abc
	Ittcolla	190 ab	1174	5.77	0.72	3.8 cd
	chitosano	190 ab	1216	5.9	0.72	3.8 cd
	PVIPVP	195 ab	1211	5.8	0.71	3.8 cd
	F	4.7	1.4	4.3	1.8	14.5
	sign	*(1)	ns	*	ns	***

(1) Significance: *, ** and *** and ns represent significance at $p \leq 0.05$, 0.01, 0.001 and not significant, respectively.

After the treatments with adjuvants, the reductions in the total anthocyanins content were limited overall, ranging from 0 (chitosan) to 8.5% (PVIPVP). The removals caused by bentonites were equal to 7.5%. No significant reduction of the total anthocyanin content was observed, neither in the chitosan thesis, nor in the CMC and cold soluble gelatin theses. No decreases in the total flavonoid content were observed in any treated thesis.

The treatments with clarifiers resulted in a modest, but significant reduction in color intensity for all theses and a modest, but significant increase in hue for some theses. The losses in color intensity ranged from 0.6 to 1.0 u.a. For all theses, except for those treated with isinglass and chitosan, the value of the $E520_{corr}$ parameter (which expresses the intensity of the red color corrected of the bleaching effect of SO_2) was significantly lower than the control thesis.

After 6 months of storage, the total anthocyanin and flavonoid content and the color intensity dropped in all wines. No treated thesis was significantly different from the control for the total anthocyanins and flavonoids content and for the color intensity and hue. Only the theses treated with bentonites and with cold soluble gelatin (Gel_fr) had $E520_{corr}$ values significantly lower than the control; the differences, however, were modest and not visually appreciable.

3.1.2 Additives

The effect on colloidal stability of some enological additives was studied. A Kordofan gum, 2 mannoproteins and a natural polysaccharide polymer were tested. The tests were performed in parallel with the clarification tests.

Table 5 shows the results of the colloidal stability tests performed after bottling and after 6 months of storage. The addition of a mannoprotein (Mannopr2) stabilized the wine, while the addition of Kordofan gum was effective immediately after bottling, but its stabilizing effect was reduced during storage.

Table 5. Barbera wine. Average values of turbidity, colloidal stability and shock tests for the control wine and the theses with additives, after bottling and after 6 months of bottle aging. ANOVA and Tukey's test results. Different letters along the column discriminate the theses significantly different from one another (p<0.05; Tukey's test).

	Theses	Turbidity (NTU)	Colloidal stability test (ΔNTU)	Shock test (ΔNTU 24h)	Shock test (ΔNTU 7d)
After bottling	Control	0.47 ab	29.20 c	1.34 c	10.6 c
	GommaK	1.39 c	2.93 a	0.46 b	16.9 d
	Mannopr1	1.28 c	14.71 b	0.49 b	9.7 c
	Mannopr2	1.1 bc	0.73 a	0.00 a	3.1 b
	PolNat	0.30 a	9.13 ab	0.10 a	1.2 a
	F	13.3	21.8	165	522
	sign	**(1)	**	***	***
After 6 months of bottle aging	Control	2.40 c	24.10 c	6.08 c	6.09 ab
	GommaK	1.93 b	5.70 ab	1.20 ab	22.81 c
	Mannopr1	1.04 ab	13.40 b	4.02 bc	7.64 b
	Mannopr2	1.81 b	0.99 a	0.00 a	2.92 a
	PolNat	0.77 a	32.30 c	0.26 a	3.07 a
	F	9.1	20.4	23.0	176
	sign	***(1)	***	**	***

(1) Significance: ** and *** represent significance at $p \leq 0.01$ and 0.001 respectively.

All theses were then subjected to the shock tests (Table 5). After permanence at 40°C for 7 days, a strong increase in turbidity (ΔNTU > 10) was observed in the control thesis. Among the theses treated with additives, the one treated with the natural polysaccharide polymer (PolNat) remained stable after the shock test (ΔNTU < 2), and the Mannopr2 thesis, the only one stable after the colloidal stability test, showed an increase in turbidity slightly above the stability threshold (ΔNTU=3.1). The results of the shock test repeated after 6 months of bottle aging confirmed what observed after bottling: after storage at 40°C for 7 days, the Mannopr2 and PolNat theses showed the lowest increases in turbidity: 2.9 and 3.1 ΔNTU, respectively, values that were close to the stability threshold of 2 NTU.

As for the tests with clarifiers, the total anthocyanins and total flavonoids content and some color parameters were determined for the wines treated with additives (Table 6).

Table 6. Barbera wine. Total anthocyanins and flavonoids content and color parameters for the control wine and the theses with additives, after bottling and after 6 months of bottle aging. ANOVA and Tukey's test results. Different letters along the column discriminate the theses significantly different from one another (p<0.05; Tukey's test).

	Theses	Total anthocyanins (mg/L)	Total Flavonoids (mg/L)	Color intensity (u.a.)	Color hue	$E520_{corr}$
After bottling	Control	296 a	1442 a	7.1 c	0.61 abc	4.6
	GommaK	316 c	1480 ab	6.5 a	0.61 abc	4.7
	Mannopr1	305 ab	1450 a	6.7 b	0.62 bc	4.6
	Mannopr2	320 c	1503 b	6.3 a	0.63 c	4.7
	PolNat	309 bc	1507 b	6.5 a	0.60 a	4.6
	F	23.3	13.2	93.9	14.0	3.3
	sign	**(1)	**	***	**	ns
After 6 months of bottle aging	Control	182 a	1196	6.2	0.72	3.8
	GommaK	199 b	1200	5.85	0.72	3.7
	Mannopr1	196 ab	1193	6.0	0.71	3.7
	Mannopr2	210 b	1228	5.75	0.72	3.75
	PolNat	197 ab	1178	5.75	0.72	3.75
	F	6.6	4.0	4.8	0.64	2.4
	sign	**(1)	ns	ns	ns	ns

(1) Significance: ** and *** and ns represent significance at p≤0.01, 0.001 and not significant, respectively.

The theses treated with additives, except for the Mannopr1 thesis, showed total anthocyanins concentrations significantly higher than the control, even if the differences were quantitatively modest. The theses were very similar to each other in terms of total flavonoid content. Significant differences between theseswere found for the color intensity: the control thesis was distinguished from all the other theses treated with additives for the significantly more intense color. It is likely that the observed color differences depended on the SO_2 content (presence of SO_2 in preparations containing added additives). In fact, there was no difference between the theses for the color intensity when measured after the correction of the bleaching effect of SO_2 ($E520_{corr}$). After 6 months of bottle aging the theses were still significantly different from each other for the total anthocyanin content, albeit with modest differences: in particular, the Mannopr2 and GommaK theses presented a significantly higher total anthocyanin content than the control. As for the total flavonoid content, the differences between the theses were modest. Finally, no significant differences were found for the color parameters (color intensity and hue and $E520_{corr}$).

3.2 Montepulciano trial

The same test was repeated with a Montepulciano wine from the 2020 harvest.

3.2.1 Adjuvants

Table 7 shows the results of the stability tests and turbidity for the wines treated with adjuvants, performed after bottling and after 6 months of bottle aging.

Table 7. Montepulciano wine. Average values of turbidity, colloidal stability and shock tests for the control wine and the theses treated with adjuvants, after bottling and after 6 months of bottle aging. ANOVA and Tukey's test results. Different letters along the column discriminate the theses significantly different from one another (p<0.05; Tukey's test).

	Theses	Turbidity (NTU)	Colloidal stability test (ΔNTU)	Shock test (ΔNTU 24h)	Shock test (ΔNTU 7d)
After bottling	Control	0.45	274 ab	0.03	0.89
	BenCa	0.93	196 ab	0.00	0.60
	BenNa	0.22	172 a	0.08	0.60
	CMC	0.24	453 b	0.02	0.58
	Gel_fr	0.23	250 ab	0.01	0.45
	Gel_cal	0.29	158 a	0.03	0.22
	Ittcolla	0.49	261 ab	0.03	0.18
	chitosano	0.37	269 ab	0.01	0.11
	PVIPVP	0.73	236 ab	0.00	0.00
	F	2.7	3.0	0.6	1.1
	sign	ns (1)	ns	ns	ns
After 6 months of bottle aging	Control	0.65 ab	161 c	0.09 ab	0.56 a
	BenCa	0.51 ab	118 abc	0.24 ab	0.24 a
	BenNa	0.39 ab	101 ab	0.19 ab	0.37 a
	CMC	0.31 a	226 d	0.40 ab	5.80 b
	Gel_fr	0.44 ab	95 ab	0.00 a	0.96 a
	Gel_cal	0.68 ab	94 ab	0.00 a	0.05 a
	Ittcolla	0.35 ab	130 bc	0.68 b	1.34 a
	chitosano	0.78 b	75 a	0.05 a	1.52 a
	PVIPVP	0.42 ab	85 ab	0.04 a	0.57 a
	F	4.1	27.0	4.3	8.0
	sign	* (1)	***	*	**

(1) Significance: *, ** and *** and ns represent significance at p≤ 0.05, 0.01, 0.001 and not significant, respectively.

The initial wine (Control) was highly unstable with the colloidal stability test (48 h at 4 °C) (ΔNTU=274). The treatment with clarifiers resulted in an average (but not significant) reduction in the degree of instability, particularly in the theses treated with hot soluble gelatin (Gel_cal) and bentonites (BenNa and BenCa). However, all theses after the clarifying treatment were still highly unstable. Despite the results of the stability test, all wines were perfectly clear and stable after the shock test. After 6 months of storage, the wines remained clear, and the colloidal instability detected after bottling was confirmed, as well as the shock test stability.

As for the first test, the selectivity of action of the clarifiers on the total anthocyanins and flavonoids content and on the color was verified. The results are reported in Table 8. After the treatments with adjuvants, no significant reductions in the total anthocyanin content were observed in the clarified theses compared to the control. The highest average removals of total anthocyanins were observed after the treatment with sodium bentonite (BenNa) and were equal to about 2.3%. No decreases in total flavonoid content were observed in any clarified thesis compared to the control.

The presence of statistically significant differences between the theses was observed for the color intensity and the $E520_{corr}$ parameter. Only for this last parameter, the control thesis had significantly higher values than the theses treated with bentonites (BenCa and BenNa), PVI/PVP, hot soluble gelatin (Gel_cal) and CMC (reductions between 0.3 and 0.8u.a.). After 6 months of storage the total anthocyanin and flavonoid content dropped, and only the BenNa thesis showed a significantly lower total anthocyanin content than the control thesis. Some theses (Gel_fr, BenNa, Gel_cal, Ittcol and CMC) were distinguished from the control for the lower content of total flavonoids, and other theses (BenCa, PVIPVP, BenNa, CMC and Gel_Ca) for the lower color intensity and the lower values of the $E520_{corr}$parameter. The differences were always modest.

Table 8. Montepulciano wine. Total anthocyanins and flavonoids content and color parameters for the control wine and the theses treated with adjuvants, after bottling and after 6 months of bottle aging. ANOVA and Tukey's test results. Different letters along the column discriminate the theses significantly different from one another (*p*<0.05; Tukey's test).

	Theses	Total anthocyanins (mg/L)	Total Flavonoids (mg/L)	Color intensity (u.a.)	Color hue	$E520_{corr}$
After bottling	Control	794 ab	3610 ab	21.6 abc	0.545	17.65 de
	BenCa	788 ab	3640 b	20.8 a	0.55	16.9 ab
	BenNa	776 a	3600 ab	20.9 ab	0.55	16.75 a
	CMC	809 ab	3675 b	21.3 abc	0.545	17.4 c
	Gel_fr	787 ab	3432 a	21.6 abc	0.54	17.45 cd
	Gel_cal	805 ab	3549 ab	21.7 bc	0.535	17.35 c
	Ittcolla	818 b	3693 b	21.8 b	0.54	17.75 e
	chitosano	822 b	3717 b	21.9 c	0.54	17.35 c
	PVIPVP	812 ab	3677 b	21.2 abc	0.55	17.05 b
	F	4.8	7.1	7.1	3.1	57.0
	sign	* (1)	**	**	ns	***
After 6 months of bottle aging	Control	457 bcd	2692 de	20.4 d	0.63 bc	13.1 c
	BenCa	446 ab	2656 cd	19.4 a	0.64 c	12.5 a
	BenNa	432 a	2603 a	19.8 abc	0.64 bc	12.5 a
	CMC	446 ab	2639 bc	19.9 abc	0.63 abc	12.7 ab
	Gel_fr	453 bc	2596 a	20.3 cd	0.62 a	12.9 bc
	Gel_cal	465 cd	2619 ab	19.8 abc	0.63 a	12.6 ab
	Ittcolla	452 bc	2624 ab	20.3 cd	0.63 abc	12.9 bc
	chitosano	472 d	2726 f	20.0 bcd	0.62 ab	12.9 bc
	PVIPVP	464 cd	2697 ef	19.7 ab	0.63 abc	12.7 ab
	F	18.3	58.7	11.1	6.2	12.4
	sign	*** (1)	***	**	**	***

(1) Significance: *, ** and *** and ns represent significance at $p\leq$ 0.05, 0.01, 0.001 and not significant, respectively.

3.2.2 Additives

As observed for the adjuvants, the additives did not allow to obtain wines stable against colloidal precipitations (Table 9). The added products that determined a significant but insufficient reduction in the degree of instability were, in increasing order of effectiveness, Kordofan gum (GommaK), a mannoprotein (Mannopr2) and the natural polysaccharide polymer (PolNat). Despite the strong colloidal instability, the control thesis was clear after bottling, while the addition of additives caused a slight increase in turbidity in all theses (presence of significant differences compared to the control only for the Mannopr2 thesis). As observed for the theses treated with adjuvants and the control, all theses added with additives were perfectly clear and stable after the shock test. After 6 months, an increase in turbidity was observed in all theses, particularly in the PolNat thesis. The wines were all highly unstable with the colloidal instability test and stable with the shock test.

Table 9. Montepulciano wine. Average values of turbidity, colloidal stability and shock tests for the control wine and the theses with additives, after bottling and after 6 months of bottle aging. ANOVA and Tukey's test results. Different letters along the column discriminate the theses significantly different from one another (*p*<0.05; Tukey's test).

	Theses	Turbidity (NTU)	Colloidal stability test (ΔNTU)	Shock test (ΔNTU 24h)	Shock test (ΔNTU 7d)
After bottling	Control	0.47 a	321 c	0.03	0.58
	GommaK	1.77 ab	235 b	0.00	0.69
	Mannopr1	1.48 ab	333 c	0.00	0.00
	Mannopr2	3.51 b	197 b	0.00	0.12
	PolNat	1.03 a	66 a	0.00	0.00
	F	5.4	84.6	1.0	2.5
	sign	* (1)	***	ns	ns
After 6 months of bottle aging	Control	2.81 a	259	0.085	0.19
	GommaK	3.70 a	393	0.00	0.00
	Mannopr1	3.14 a	75	0.00	0.00
	Mannopr2	3.13 a	309	0.00	0.00
	PolNat	14.29 b	428	0.00	0.00
	F	31.7	1.6	1.0	3.6
	sign	*** (1)	ns	ns	ns

(1) Significance: * and *** and ns represent significance at $p\leq$0.05, 0.001 and not significant, respectively.

Table 10. Montepulciano wine. Total anthocyanins and flavonoids content and color parameters for the control wine and the theses with additives after bottling and after 6 months of bottle aging. ANOVA and Tukey's test results. Different letters along the column discriminate the theses significantly different from one another (*p*<0.05; Tukey's test).

	Theses	Total anthocyanins (mg/L)	Total Flavonoids (mg/L)	Color intensity (u.a.)	Color hue	$E520_{corr}$
After bottling	Control	796 ab	3652 ab	22.5	0. 54	16.6 ab
	GommaK	823 b	3752 b	22.6	0. 54	17.0 ab
	Mannopr1	808 b	3689 ab	22.4	0. 54	16.9 ab
	Mannopr2	809 b	3671 a	22.1	0. 54	17.0 ab
	PolNat	768 a	3545 a	22.2	0. 54	16.3 a
	F	10.1	5.8	0.8	0.2	3.1
	sign	* (1)	*	ns	ns	ns
After 6 months of bottle aging	Control	470 ab	2861 a	20.3	0.64	13.0 b
	GommaK	499 ef	2953 bcde	19.9	0.63	12.9 ab
	Mannopr1	477 abc	2896 ab	20.1	0.64	12.9 ab
	Mannopr2	483 bcd	2917 abcd	20.0	0.64	13.0 b
	PolNat	462 a	2962 cde	20.1	0.64	12.7 a
	F	17.8	10.0	1.1	1.2	7.8
	sign	** (1)	*	ns	ns	*

(1) Significance: *, ** and ns represent significance at $p\leq$0.05, 0.01 and not significant, respectively.

After bottling, no thesis was significantly different from the control for the content in total anthocyanins and flavonoids and for the color parameters (Table 10), while after 6 months in the bottle, compared to the test, the GommaK thesis had a content in total anthocyanins and flavonoids significantly higher, while the PolNat thesis a significantly lower value of $E520_{corr}$.

4 Conclusions

The Barbera wine (2019) which had a medium-low degree of colloidal instability was stable, without the use of cold, after a clarification treatment with sodium bentonite or after the addition of one of the 2 tested mannoproteins. The use of these 2 oenological products avoided the appearance of cloudiness even when the wine was stored for a short period under unfavorable thermal conditions (shock test at 40°C for 1 and 7 days), such as those that could accidentally occur during transport over long distances. The stabilizing effectiveness of mannoproteins depends on the type of product used: the best results were obtained with a mannoprotein with a molecular weight of 40-60 kDa.

On the contrary, no clarifying treatment and no additives among those tested during this experiment were instead able to stabilize the Montepulcianod'Abruzzo wine (2020), which had a high degree of colloidal instability. At the moment, in case of high colloidal instability a preliminary low temperature treatment should be always advisable to achieve the colloidal stability.

Overall, the treatments with clarifiers did not markedly affect the polyphenolic component and the color of the wines, and the differences between the treated theses and the control tended to further decrease during storage.

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