

Use of polyaspartates for the tartaric stabilisation of white and red wines and side effects on wine characteristics

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ABSTRACT

Aim: The stabilising efficacy against tartaric precipitations of polyaspartates-based products (PAs), in particular potassium polyaspartate (KPA), was tested with six different wines (three white and three red). Some side effects on wine characteristics (white wine colour stability, wine turbidity and filterability) were also studied.

Results and conclusions: All PAs showed good stabilising efficacy against tartaric precipitations according to the cold test. With the same test, the PAs were stable in wine for 1 year of storage, which was the total duration of the study. The dose of 100 mg/L was sufficient to stabilise the tested wines. No differences in filterability were observed in comparison with MTA (metatartaric acid). The hypothesised protective effect against colour browning in white wines was not observed.

Significance and impact of the study: The international wine trade requires stable wines. This paper provides information to support wineries in managing the use of KPA, as little information is available to date in the literature on this stabilising additive.

KEYWORDS

metatartaric acid, polyaspartates, potassium polyaspartate, tartaric stability, wine

INTRODUCTION

Potassium bitartrate (KHT) is the main salt present in wine. Its concentration is variable and depends on wine pH and on the content of tartaric acid and potassium. KHT has a low solubility in hydroalcoholic solutions and it can precipitate in wines during bottle ageing, causing the formation of a crystalline sediment (Usseglio-Tomasset *et al.*, 1992). Various oenological practices can be performed to prevent the formation of this deposit, which is not acceptable to most consumers. These practices consist in removing the excess KHT by precipitation at a low temperature (Ribéreau-Gayon *et al.*, 2006), or a part of bitartrate and potassium ions by electrodialysis (Moutounet and Escudier, 1991), or only potassium with cation exchange resins (Mourgue, 1993).

The use of certain additives that can inhibit the growth of KHT crystals or act as protective colloids is also authorised by the EU in order to prevent the appearance of precipitates in the bottle. Metatartaric acid (MTA) was the first additive to be authorised, followed by carboxymethylcellulose (CMC) and mannoproteins (MP), and more recently the use of potassium polyaspartate (KPA) (Commission delegated regulation EU 2017/1961-August 2, 2017).

Polyaspartates are homopolypeptides synthesised by thermal polymerisation of L-aspartic acid (ASP) or monoammonium malate, resulting in polysuccinimide (PSI), followed by hydrolysis (Bosso *et al.*, 2015). Polyaspartates are involved in the inhibition of calcium sulphate scale formation (Sarig and Shifrin, 1977) and some of them, such as the sodium salt of polyaspartate, are used to prevent fouling (by changing the crystal structure of calcium salts), as an additive in detergents, for the prevention of corrosion, as an adjuvant in the prevention of dental plaque, and as an excipient in certain drugs. Many works have been published on the stabilising properties of MTA, CMC and MP (Lubbers *et al.*, 1993; Crachereau *et al.*, 2001; Moine-Ledoux and Dubourdieu, 2002; Bosso *et al.*, 2010; Gerbaud *et al.*, 2010; Claus *et al.*, 2014; Guise *et al.*, 2014; Coulter *et al.*, 2015). However, to our knowledge only our previous work (Bosso *et al.*, 2015) has focused on the screening of various types of polyaspartates (PAs), different per producer, type of salt (sodium or potassium) and average molecular weight, added to white and red wines provided by commercial wineries. The molecular weights of the tested PAs ranged from 2 to 8 KDa. The products were added at a dose of 100 mg/L to the wines before bottling.

All PAs proved to have a good stabilising efficacy against KHT precipitation and a good stability in wine over time, unlike MTA. No differences in stabilising efficacy between the various PAs were observed soon after the addition, whereas some differences were noticed in the duration of their efficacy over time.

The aim of the present work was to verify the stabilising properties of PAs on tartaric precipitations, focusing mainly, but not exclusively, on potassium polyaspartate (KPA), whose use has recently been authorised in oenology. The trials were carried out with some white and red wines provided by commercial wineries, and with two wines (one white and one red) adjusted to two different levels of pH and alcoholic content, in order to modify the degree of tartaric instability by intervening on the dissociation degree (pH variation) and solubility (variation of the alcohol content) of KHT.

As reported above, PAs are obtained by polymerisation of L-aspartic acid. MTA, obtained by polymerisation of tartaric acid, proved to be particularly unstable in wine, where it is rapidly hydrolysed to tartaric acid, thus losing its stabilising efficacy. Storage trials were therefore conducted in order to verify the duration over time of the stabilising efficacy of PAs in wine (a medium with acidic pH). Other possible side effects of KPA were also evaluated, such as the impact on wine turbidity and filterability. Finally, as PAs are used by the industry as de-scaling agents of water pipes due to their chelating properties for the calcium ion (Mocanu *et al.*, 2012), we also wanted to verify whether a chelating effect for other cations, such as iron and copper, could have consequences on the evolution of the colour of white wine.

This paper reports the results of the study on a laboratory scale of some oenological properties of KPA that have been investigated during the STABIWINE project (“Use of biopolymers for sustainable stabilisation of quality wines” - Grant Agreement n.314903 - Seventh Framework Program. Theme: SME-2012-2. Research for SME associations), in parallel with the research on the food safety of the product.

The data collected during the project supported the planning of large-scale trials in commercial wineries whose results, together with those concerning the product safety, led to the EU authorisation of the use of KPA as an additive for the tartaric stabilisation of wines.

MATERIALS AND METHODS

1. Experimental trials

This work was aimed at determining the effect of adding some PAs, in particular KPA, to white and red wines sampled directly from the tank in commercial wineries. The following oenological properties of the additives were studied:

- effect on tartaric stability immediately after addition (different wines and different doses)
- effect on tartaric stability after 6 or 12 months of bottle ageing
- effect on wine filterability immediately after the addition
- effect on the colour of white wines

Due to the large number of tests, it was impossible to perform all of them simultaneously or delay them over time while still working with the same wines, with the risk of finding important changes in their composition and degree of tartaric instability. The study of the different oenological properties was therefore performed at different times with different wines. At the beginning of each experiment, the wines were in the condition of tartaric instability, but they were ready for bottling according to other parameters, such as protein stability for white wines and limpidity for all wines. Table 1 shows the additives used for the tests, and Table 2 summarises the experiments performed with the different wines.

The effect of KPA on tartaric stability was assessed for all the wines involved in study. All trials were performed in duplicate. For the storage tests the wines were bottled and stored in a thermostatic room at 20 °C.

In addition, for a white and a red wine (W1 and R3, Table 2, Experiment 4) the alcoholic degree and pH were artificially modified to verify whether the stabilising efficacy of KPA could be influenced by these parameters. In particular, a complete factorial design was planned by varying on two

levels the factors pH (3.00 and 3.70), alcoholic degree (12 and 15 % v/v) and dose of KPA (100 and 200 mg/L).

As regards the effect on the colour stability of white wines (Table 2, Experiment 2), the chelating effect of PAs for iron and copper was verified using two different products. For the first white wine (W1), the concentration of the two metals (iron and copper) was increased and adjusted to 5.0 and 0.8 mg/L, respectively. The control wine was compared with two treated with KPA (100 and 200 mg/L) and two treated with NaPA-10 (100 and 200 mg/L). For the second white wine (W2), the natural content of 1.5 mg/L iron and 0.075 mg/L copper was not corrected, and the control wine was compared with two treated with KPA at the doses of 100 and 200 mg/L. All trials were oxygenated (4 mg/L oxygen), then bottled in 750 mL bottles and stored in a thermostatic room at 20 °C.

2. Analytical methods

2.1. Free aspartic acid (Table 2, Experiment 1)

The free aspartic acid content was determined with HPLC after 12 months of bottle ageing according to the method proposed by Park *et al.* (2000) and modified by Bosso *et al.* (2015), based on a pre-column derivatisation reaction between ortho-phthalaldehyde (OPA) and 2-aminoethanol as derivatising agents, and with 2-methylaspartate as internal standard.

2.2. Dissolved oxygen (Table 2, Experiment 4)

The concentration of dissolved oxygen in the white wines was measured with a luminescence-based technology (NomaSense™ O₂ Trace, PreSens GmbH, Regensburg, Germany).

2.3. Filterability

The filterability test (Table 2, Experiment 5) consisted in measuring the time needed to filter

TABLE 1. Main characteristics of the PAs and MTA used in the experiment.

	Molecule	Composition	Molecular weight (KDa)
NaPA-3	Polyaspartic Acid	Liquid; sodium salt, 40 % p/p	3
KPA	Polyaspartic Acid	Liquid; potassium salt, 40 % p/p	5
NaPA-10	Polyaspartic Acid	Liquid; sodium salt, 40 % p/p	10
NaPA-15	Polyaspartic Acid	Liquid; sodium salt, 40 % p/p	15
MTA	Metatartaric Acid	powder	

TABLE 2. Summary of the experiments performed with the different wines.

Experiment	Studied effect	Wine provenience	Wines	Wine composition	Additives (see Table 1)	Doses	Analyses	Results in paragraph	
1	Tartaric stability and duration of the effect for 1 year of bottle aging	Terre da Vino - Barolo (CN), Italy	Dolcetto red wine (R1)	12.87 % v/v alcohol, total extract 26.3 g/L, pH 3.42, titratable acidity 6.26 g/L as tartaric acid	All PAs and MTA	0 and 100 mg/L	Cold test and conductivity test	After addition and 3, 6 and 12 months of bottle aging	3.1
							Free aspartic acid	after 12 months of bottle aging	
2	Effect on tartaric stability and colour of white wines	Azienda Zaccagnini Bolognanao Italy	Table white wine (W1)	12.00 % v/v alcohol, total extract 22.0 g/L, pH 3.46, titratable acidity 5.03 g/L as tartaric acid	KPA and NaPA-10	0, 100 and 200 mg/L	Dissolved oxygen	Before and after addition and until the total consumption	3.2
							Cold test and conductivity test free and total SO ₂ , colour, total polyphenols	After addition and after 6 months of bottle aging	
		Acetaldehyde	After 6 months of aging						
		Dissolved oxygen	Before and after addition and until the total consumption						
		Azienda Zaccagnini Bolognanao Italy	Pecorino and Riesling white wine (W2)	12.48 % v/v alcohol, total extract 20.7 g/L, pH 3.43 and titratable acidity 5.1 g/L as tartaric acid	KPA (100 and 200 mg/L)	0, 100 and 200 mg/L	Cold test and conductivity test	After addition	
							Free and total SO ₂ , colour, total polyphenols, acetaldehyde	After addition and after 6 and 12 months of bottle aging	
3	Effect of the dose on tartaric stability and turbidity	Experimental Cellar CREA-VE Asti Italy	Syrah red wine (R2)	13.23 % v/v alcohol, total extract 29.7 g/L, pH 3.39, titratable acidity 6.64 g/L as tartaric acid	KPA and MTA	KPA: 0, 100, 200, 500 and 1000 mg/L; MTA: 0 and 100 mg/L	Cold test, conductivity test and turbidity	After addition	3.3
4	Tartaric stability when varying wine composition (pH and % alcohol) and dose	Azienda Zaccagnini Bolognanao Italy	Table white wine (W1)	11.93 % v/v alcohol, total extract 20.6 g/L, pH 3.46, titratable acidity 5.10 g/L as tartaric acid	KPA	0, 100 and 200 mg/L	Cold test and conductivity test	After addition	3.4
							Cold test, conductivity test and turbidity	After addition	
5	Effect on filterability	Experimental Cellar CREA-VE Asti Italy	Cortese white wine (W3)	12.83 % v/v alcohol, total extract 23.6 g/L, pH 3.39, titratable acidity 6.10 g/L as tartaric acid	KPA and MTA	100 mg/L	Filterability test	After addition	3.5

increasing volumes of wine: 100 mL (T_{100}); 200 mL (T_{200}); 300 mL (T_{300}); 400 mL (T_{400}); and 500 mL (T_{500}). Cellulose acetate filters (Sartorius Minisart NML, 0.45 μm , diameter 28 mm) and filtering apparatus from Sartorius (Sartorius Stedim srl, Italy) were used. The filterability index (FI) and the modified filterability index (MFI) were calculated: $FI = T_{400} - 2T_{200}$ and $MFI = (T_{500} - T_{100}) - 2(T_{300} - T_{100})$. Filterability is generally considered good when $FI < 10$, acceptable when $10 < FI < 20$, and not acceptable when $FI > 20$.

2.4. Cold test

The tartaric acid content in the wines was determined with HPLC (Cane, 1990) before and after storage at $-4\text{ }^{\circ}\text{C}$ for 6 days. After cooling and before the analysis, the wines were filtered (0.45 μm) at a low temperature to avoid the solubilisation of the KHT salts. The concentration of tartaric acid (H_2T) was measured before and after the period of cold storage, and the difference between the two values due to the precipitation of KHT was calculated: ΔH_2T represents an index of the stability/instability degree of wines (Motta *et al.*, 2009). The wines are considered stable (no loss of tartaric acid by precipitation) when ΔH_2T is ≤ 0.1 (Bosso *et al.*, 2016).

2.5. Conductivity test (mini-contact test)

The tartaric stability of the wines was assessed by measuring the drop of electric conductivity ($\Delta\chi$ expressed as $\mu\text{S}/\text{cm}$) of 100 mL wine at $0\text{ }^{\circ}\text{C}$, 4 min after the addition of finely micronised KHT (10 g/L) as a precipitating agent: the higher the difference in conductivity, the higher the tartaric instability (Bosso *et al.*, 2016). The test was performed with Check Stab a-2001 Millennium (Delta Acque, Firenze, Italy). The measurements were taken with a probe made up of two platinum electrodes, which were periodically recalibrated with a 0.01 N KCl solution to ensure a high degree of reliability. The temperature was controlled to a resolution of $0.1\text{ }^{\circ}\text{C}$. The tartaric stability thresholds, at the described operating conditions, were equal to 150 and 100 $\mu\text{S}/\text{cm}$ for white and red wines, respectively.

2.6. Physicochemical analyses

Ethanol concentration, total extract, pH, titratable acidity, free and total SO_2 were determined according to EU methods (EEC Regulation 2676/90), and acetaldehyde was measured with a colorimetric method (Di Stefano and Ciolfi, 1982). For white wines, the absorbance at

420 nm (A_{420}) was measured with a quartz cuvette (10 mm o.p.) after filtration with a 0.45 μm polypropylene filter. CIELAB indices (cylindrical coordinates: L^* lightness, C^* chroma, h^* hue) were determined according to Piracci (1994). Wine turbidity was measured with Turbiquant 3000 IR and expressed as nephelometric turbidity units (NTU). The content of iron (Fe) and copper (Cu) was determined by atomic absorption spectroscopy with a Perkin Elmer 5100 PC AA spectrophotometer (Perkin Elmer, Norwalk, CT, USA) according to EU methods (EEC Regulation 2676/90). The content of total polyphenols was determined by spectrophotometry with the Folin-Ciocalteu method (Di Stefano *et al.*, 1989).

2.7. Statistical elaboration

Data was processed with ANOVA. The comparison between the mean values of at least three theses was performed with the Tukey's post hoc test. SPSS for Windows version 15.0 (SPSS Inc., Chicago II USA, 2004) was used.

RESULTS AND DISCUSSION

1. Experiment 1: Influence of different types of PAs on the tartaric stability of a Dolcetto red wine (R1) after the addition and during aging

After the addition of PAs and MTA all wines were stable to tartaric precipitations (Table 3). After 3 months of bottle aging the MTA trial became unstable, while all PAs trials remained stable. No statistically significant differences between the PAs trials were observed with the cold test, while according to the conductivity test the NaPA-10 and NaPA-15 trials had significantly lower $\Delta\chi$ values than the NaPA-3 and KPA trials.

After 6 months of bottle aging, the cold test showed that the PAs trials maintained their stability to tartaric precipitations, and the instability degree of the control and MTA trials remained the same. Conversely, the conductivity test showed an important increase in $\Delta\chi$ values for all wines, particularly for the PAs trials that resulted unstable with this test.

The analyses were repeated after 12 months of bottle aging: the $\Delta\chi$ values further increased for all wines, particularly for the PAs trials. Despite this, all PAs trials were still stable with the cold test. An increase in tartaric instability, determined with the cold test, was observed only for the control.

The good stability of PAs to acidic cleavage at room temperature ($20\text{ }^{\circ}\text{C}$) was confirmed by

TABLE 3. Mean values of the stability indexes ($\Delta\chi$ and ΔH_2T) after the addition of the products to the Dolcetto red wine and after 3, 6 and 12 months of bottle aging, and free aspartic acid content after 12 months of bottle aging. Comparison among different types of PAs and MTA (ANOVA and Tukey's test).

		Control	NaPA-3	KPA	NaPA-10	NaPA-15	MTA	F value	sig.
After bottling	ΔH_2T (g/L)	0.500 b*	0.049 a	0.035 a	0.057 a	0.083 a	0.047 a	15.0	**
	$\Delta\chi$ ($\mu S/cm$)	223 b	40.4 a	40.3 a	49.0 a	47.5 a	38.4 a	21.7	**
3 months	ΔH_2T (g/L)	0.57 b	0.06 a	0.03 a	0.02 a	0.01 a	0.6 b	379	***
	$\Delta\chi$ ($\mu S/cm$)	244 d	88 b	83 b	72 a	69 a	192 c	2016	***
6 months	ΔH_2T (g/L)	0.48 bc	0.16 ab	0.02 a	0.04 a	0.02 a	0.68 c	14.5	**
	$\Delta\chi$ ($\mu S/cm$)	280 d	151 c	148 bc	128 ab	118 a	275 d	436	***
12 months	ΔH_2T (g/L)	0.93 c	0.10 b	0.00 a	0.00 a	0.00 a	0.95 c	10731	***
	$\Delta\chi$ ($\mu S/cm$)	314 c	248 b	249 b	220 a	218 a	322 c	504	***
	Aspartic acid (mg/L)	1.55 a	5.10 d	3.30 b	3.85 bc	4.30 c	n.d.	342	***

Different letters along the line discriminate the trials significantly different from one another ($p < 0.05$, Tukey's test).

** , *** represent significance at $p \leq 0.01$ and $p \leq 0.001$, respectively.

the low increase of free aspartic acid content (monomer released after PAs hydrolysis) in the wines after 12 months of bottle aging: the largest difference in its concentration between the PAs trials and the control was only 3.55 mg/L (3.55 % by weight of the added amount of PAs) (Table 3).

These results were in agreement with a previous work (Bosso *et al.*, 2015): the PAs had a good stabilising efficacy against tartaric precipitations, which was maintained after 12 months of aging; conversely, the MTA trials were unstable 3 months after the additions. The increase of the $\Delta\chi$ parameter over time, which was also observed during further experiments (data not reported), could depend on the fact that during aging KPA interacts permanently with the wine's KHT crystals and loses the ability to react with the excess KHT that is added during the conductivity test. During a recent study on the mechanisms of action of some additives used to prevent the growth of KHT crystals in wines, Lankhorst *et al.* (2017) hypothesised that the effect of these additives is due to their adsorption on the surface of the crystals. In general, the cold test is considered the reference test to evaluate wine tartaric stability from an objective thermodynamic point of view, while the conductivity test is accepted by the industry due to its rapidity and ease of use, and this discrepancy in the results could lead to negative commercial consequences for wines containing KPA. Therefore, the conductivity test seems to be suitable for verifying the degree of tartaric stability/instability of the wines soon after the addition of KPA, but not always during wine aging. To date, no other works in the literature highlighted this behaviour of the mini-contact test with wines treated with potassium polyaspartate,

due to the current lack of research on the use of this additive in oenology.

2. Experiment 2: Effect of PAs on the tartaric stability and colour of two white wines (W1 and W2)

2.1. Effect on tartaric stability

Table 4 shows the results of the cold and conductivity tests performed for the W1 white wine.

After storage for 6 days at $-4^\circ C$ (cold test), the control was significantly unstable compared to the trials with the two PAs added at two different doses (stable). The same result was obtained with the conductivity test (the stability thresholds are reported in the Materials and methods section). No significant differences were observed between the two PAs and the two doses used with the cold test; on the contrary, there were statistically significant differences between the trials with 100 mg/L and the trials with 200 mg/L of PAs with the conductivity test.

After 6 months of bottle aging (Table 4), the results were unchanged: the PAs trials were stable and statistically different from the control (unstable), with both the cold test and the conductivity test. Moreover, with the cold test no differences were observed between the different PAs nor between the different doses, whereas with the conductivity test the wines with the higher dose and NaPA-15 had significantly lower $\Delta\chi$ values compared to the wines with the lower dose and KPA.

The W2 white wine was unstable ($\Delta H_2T = 0.15$ g/L and $\Delta\chi = 209$ $\mu S/cm$). After the addition of PAs, the wines were stable for both the cold test ($\Delta H_2T = 0.0$ g/L for both doses) and the conductivity

TABLE 4. W1 white wine. Mean values of the stability indexes ($\Delta\chi$ and ΔH_2T), free and total SO_2 , A420 and acetaldehyde after bottling and after 6 months of bottle aging. Effect of the type and dose of PAs (ANOVA and Tukey's test).

	Additives	$\Delta\chi$ ($\mu S/cm$)	ΔH_2T (g/L) (cold test)	Free SO_2 (mg/L)	Total SO_2 (mg/L)	A420	Acetaldehyde (mg/L)
After bottling	Control	433.4 b	0.670 b	17.28	92.00	0.115	n.d.
	KPA	131.3 a	0.015 a	17.44	92.40	0.117	n.d.
	NaPA-15	131.0 a	0.013 a	17.84	92.88	0.115	n.d.
After 6 months of bottle aging	Control	395.9 c	1.25 b	7.20	78.88	0.135	42.6 a
	KPA	116.8 b	0.04 a	6.56	73.13	0.135	45.4 b
	NaPA-15	86.8 a	0.07 a	6.96	77.76	0.138	43.4 ab
	Dose (mg/L)	$\Delta\chi$ ($\mu S/cm$)	ΔH_2T (g/L) (cold test)	Free SO_2 (mg/L)	Total SO_2 (mg/L)	A420	Acetaldehyde (mg/L)
After bottling	0	433.4 c	0.67 b	17.28	92.00	0.115	n.d.
	100	147.5 b	0.01 a	17.68	93.20	0.116	n.d.
	200	114.8 a	0.02 a	17.60	92.08	0.116	n.d.
After 6 months of bottle aging	0	395.9 c	1.25 b	7.20	78.88	0.135	42.6
	100	123.6 b	0.06 a	6.78	75.06	0.135	43.8
	200	80.0 a	0.05 a	6.74	75.83	0.138	45.1

*Different letters along the column, separately for each sampling, discriminate treatments significantly different from one another ($p < 0.05$, Tukey's test). n.d. = not determined

test ($\Delta\chi = 45.80$ and $37.10 \mu S/cm$ for the trials with 100 and 200 mg/L of KPA, respectively). The results of the cold test did not change after 12 months, while the $\Delta\chi$ values (conductivity test), especially for the PAs trials, increased ($\Delta\chi = 156.6$ and $111.7 \mu S/cm$ for the trials with 100 and 200 mg/L of KPA, respectively).

The poor reproducibility of the conductivity test during aging, tartaric stability being equal (when measured with the cold test), suggests that the conductivity test is not suitable for measuring over time the tartaric stability of PAs added wines.

2.2. Effect on colour stability and on wine oxidation process

Two experiments were carried out to assess the influence of PAs on the colour of white wines and the oxidative evolution, associated with the possible chelating effect for iron and copper. Therefore, all trials were oxygenated before bottling, at the dose of 4 mg/L, corresponding to an amount of oxygen 4-5 times greater than the dose that is normally dissolved in the wines during correct bottling operations.

At the beginning of the first experiment (W1 wine, Table 4), all trials were similar for the content of free and total SO_2 and colour intensity (A420). After

6 months of bottle aging, only small significant differences in acetaldehyde content were present. SO_2 and acetaldehyde concentrations are related to the wine oxidation process: the free and total SO_2 content tends to decrease by oxidation, while the acetaldehyde content increases (Fenton's reaction) (Elias and Waterhouse, 2010). The absence of important differences between the trials for these parameters suggests that PAs have no role in the wine oxidation process. During the first 15 days following the oxygen supplies, the concentration of dissolved molecular oxygen was also monitored (Supplementary material: Figure A). All wines showed a high oxygen consumption rate, similar for all trials. Danilewicz (2011) suggested that the consumption rate of molecular oxygen, soon after the oxygen supplies, is influenced by the presence of metals and in particular iron. In fact, the molecular oxygen reacts directly and rapidly with iron (Fe^{++}), reducing itself to peroxy radical capable of oxidizing phenols, whereas SO_2 and phenols participate later in the oxidation process by reacting with the peroxy radical that is formed by iron. Moreover, it seems that copper acts as a catalyser of the reaction between O_2 and Fe^{++} (Danilewicz, 2011). When increasing the concentration of iron and copper in wines, an increase was observed in the consumption rate

of dissolved oxygen and in the loss of free SO₂ (Danilewicz, 2007).

According to our results, a chelating effect of PAs for iron and copper should be excluded, because otherwise a decrease of the initial oxygen consumption rate should have been observed in the PAs trials, particularly with the higher dose. The same can be deduced by considering another step of the oxidation process, the Fenton's reaction, during which the hydrogen peroxide that is formed by reduction of the peroxy radical in the presence of phenols or SO₂ is further reduced to hydroxyl radical in the presence of Fe⁺⁺ (Elias and Waterhouse, 2010). A decrease in the rate of this reaction limits the production of acetaldehyde via oxidation of ethanol by the hydroxyl radical. Conversely, in our case the acetaldehyde content did not decrease, but rather it slightly increased in the PAs trials compared to the control.

For this first experiment, iron and copper were added to a wine (W1) in order to accelerate the oxidation reaction and to emphasise the possible differences due to the chelating effect of PAs for metals. The second experiment (W2), without addition of exogenous iron and copper, was performed to verify whether an excess of these metals could have had a masking effect on the chelating action of PAs.

A white wine was used with an original content of 1.5 and 0.075 mg/L of iron and copper, respectively (W2). KPA was added to the wine at two different doses (100 and 200 mg/L). The

results (Table 5) confirmed what was observed in the first experiment: no important differences were noticed for free and total SO₂, acetaldehyde and colour parameters after 1.5, 3, 6 and 12 months of bottle aging. Moreover, the addition of KPA did not slow down the oxygen consumption rate that, on the contrary, resulted increased compared to the control (Supplementary material: Figure B).

The presence of KPA did not influence white wine colour and its oxidation process during bottle aging. Unlike for PAs, some authors (Guise *et al.*, 2014) observed a stabilising effect of CMC on wine colour, variable with the type and dose of CMC, which was probably due to the removal of polyphenols. On the contrary, this effect was not observed during our experiments with PAs. No differences between the PAs trials and the control were observed soon after the addition of PAs nor after 6 months (first experiment) and 1 year (second experiment) of bottle aging. As an example, soon after the addition of PAs all wines of the first experiment had an average total polyphenols content of 110-111 mg/L, and after 6 months the average concentration dropped to 103 mg/L in the control and 98 and 101 mg/L in the trials with 100 and 200 mg/L of PAs, respectively.

3. Experiment 3: Effect of increasing doses of KPA on the tartaric stability and turbidity of a red wine

The Syrah red wine (R2 wine) was highly unstable, and after the cold test it had lost, by KHT precipitation, a large amount of tartaric acid

TABLE 5. W2 white wine. Mean values of free and total SO₂, colour parameters and acetaldehyde after bottling and during bottle aging. Study of the effect of KPA at two different doses (ANOVA test).

	Dose of KPA (mg/L)	Free SO ₂ (mg/L)	Total SO ₂ (mg/L)	A420	L*	c*	h*	Acetaldehyde (mg/L)
After bottling	0	21.76	144.0	0.120	98.29	8.73	-1.38	87.2
	100	20.80	144.0	0.125	98.09	8.94	-1.39	87.9
	200	21.76	144.0	0.120	98.22	8.87	-1.38	86.7
1.5 months	0	5.12	6.48	0.111	98.55	8.32	-1.355	110.0
	100	5.92	151.8	0.117	98.69	9.21	-1.355	109.9
	200	5.92	152.0	0.119	98.84	9.15	-1.350	109.9
3 months	0	5.92	146.4	0.121	98.76	9.46 b	-1.346 a	n.d.
	100	6.72	147.2	0.120	99.01	9.53 b	-1.330 a	n.d.
	200	7.52	145.3	0.114	99.38	9.15 a	-1.277 b	n.d.
6 months	0	5.44 a	106.9	0.140	98.37	10.6 a	-1.35	n.d.
	100	9.12 b	72.3	0.150	97.99	11.1 b	-1.36	n.d.
	200	8.16 ab	86.7	0.145	98.11	10.8 ab	-1.36	n.d.
12 months	0	9.92	110.7	0.170	98.07	12.35	-1.339 a	92.1
	100	9.38	115.1	0.170	98.01	12.71	-1.345 b	95.5
	200	11.36	113.8	0.170	98.11	12.69	-1.339 a	95.4

Different letters along the column, separately for each sampling, discriminate treatments significantly different from one another ($p < 0.05$, Tukey's test). n.d. = not determined

(1.23 g/L) equivalent to 40.6 % of the original content before the cold test (Table 6). The wine became stable with the addition of MTA or KPA at the lower dose (100 mg/L). Turbidity was 9.5 NTU for the control, and it decreased for the wines with 100, 200 and 500 mg/L KPA and 100 mg/L MTA. This experiment was aimed at verifying whether the addition of KPA at doses higher than the maximum authorised level could negatively affect the colloidal equilibrium of the wine, causing an increase in turbidity. The results showed that only doses 10 times higher than the maximum authorised level resulted in a statistically significant increase in turbidity.

4. Experiment 4: Effect of KPA on tartaric stability: influence of wine composition and dose

4.1. White wine (W1)

Table 7 reports the average results of the tartaric stability tests (cold test and conductivity test) for the white wines whose alcoholic degree and pH were artificially modified. All trials had the same content of tartaric acid and potassium.

The wines with pH 3.00 were more stable to KHT precipitations than those with pH 3.70. The pH influences the dissociation degree of tartaric acid: for the same concentration of tartaric acid a decrease of wine pH causes a decrease in the concentration of HT⁻ ions. Considering the pK values (pK = -logK, where K is the dissociation constant of an acid) of tartaric acid, that is pK₁ = 3.04 and pK₂ = 4.37 as reported by Usseglio-Tomasset (1995), it is possible to calculate the percentages of the three forms of tartaric acid (H₂T, HT⁻ and T²⁻) present in a wine at a given pH. In particular, the percentage of HT⁻ ions that form insoluble salts with K⁺ ions varies from 46.75 % to 69.80 % (50 % variation) of the tartaric acid present in wine when its pH varies from 3.00 to 3.70. After the addition of KPA all wines became stable, but the effect of KPA seemed weaker at low

pH values. However, as the wines were originally less unstable at a low pH than at a high pH, a correct evaluation was not possible.

To date, no studies have yet been conducted to define the mechanism of action of KPA in wines. Some authors (Lankhorst *et al.*, 2017) have recently studied the mechanisms of action of CMC, MTA and MP in a model solution and in a deionised white wine, spiked with KHT, with the use of a method based on dynamic light scattering. The method can detect the presence of wine colloids with sizes ranging from 10⁻³ to 10⁻¹ µm, which are already present in wine, and it can distinguish them from the KHT crystals. The results showed that these additives cannot prevent the nucleation of KHT crystals, but rather intervene by slowing down their growth.

Crachereau *et al.* (2001) studied the effect of CMC on the growth of KHT crystals in a supersaturated solution of the salt: CMC caused a slowing down of the KHT crystals growth and a modification of their shape. According to the authors, this effect was due to the interactions between the negatively charged CMC and the faces of the KHT crystals, positively charged by the accumulation of potassium ions, in competition with the bitartrate ions. It could be hypothesised that also the stabilising action of KPA depends on its negative charge; indeed, all additives used for the tartaric stabilisation of wines, including mannoproteins, are negatively charged. Wine pH could therefore influence the stabilising efficacy of KPA by modifying its negative charge, which decreases with decreasing pH.

The increase of the alcoholic degree did not influence the results of the conductivity test (Δχ), but conversely a negative and significant effect on tartaric stability was observed with the cold test (Table 7). Ethanol influences KHT solubility: when the ethanol content increases, the solubility of KHT in wines decreases. Considering as constant all the other chemical parameters, and applying

TABLE 6. Results of the tartaric stability tests and turbidity values of a Syrah red wine with different doses of KPA, and with MTA

	Control	KPA				MTA	Sig.
		100 mg/L	200 mg/L	500 mg/L	1000 mg/L	100 mg/L	
Wine turbidity (NTU)	9.52 c	6.47 a	6.35 a	6.37 a	11.88 d	8.38 b	***
Δχ (µS/cm)	198.7 d	77.0 c	59.7 b	48.8 ab	40.0 a	47.5 ab	***
ΔH ₂ T (g/L)	1.23 b	0.02 a	0.01 a	0.02 a	0.00 a	0.03 a	***

Different letters along the line discriminate treatments significantly different from one another (p < 0.05, Tukey's test). *** represents significance at p ≤ 0.001.

TABLE 7. Mean values of the tartaric stability parameters (cold test and conductivity test) as function of pH, alcohol content and dose of KPA in W1 white wine and R3 red wine, and ANOVA results.

		pH 3.00		pH 3.70		11.5%		15.0%		100 mg/L	200 mg/L	
White wine	pH effect	$\Delta\chi$ ($\mu\text{S/cm}$)	before addition	100.0 a	390.0 b	before addition	246.8 a	242.7 a	$\Delta\chi$ ($\mu\text{S/cm}$)	after addition	66.0 b	54.8 a
		$\Delta\text{H}_2\text{T}$ (g/L)	after addition	42.6 a	69.5 b	after addition	58.7 a	62.1 b	$\Delta\text{H}_2\text{T}$ (g/L)	after addition	0.05 a	0.04 a
	pH effect	$\Delta\text{H}_2\text{T}$ (g/L)	before addition	0.15 a	1.13 b	before addition	0.49 a	0.78 b	Dose effect	after addition	66.0 b	54.8 a
		$\Delta\chi$ ($\mu\text{S/cm}$)	after addition	0.038 a	0.053 a	after addition	0.05 a	0.04 a	Dose effect	after addition	0.05 a	0.04 a
Red wine	pH effect	$\Delta\chi$ ($\mu\text{S/cm}$)	before addition	32.4 a	76.9 b	before addition	50.5 a	58.8 b	$\Delta\chi$ ($\mu\text{S/cm}$)	after addition	47.0 b	41.6 a
		$\Delta\text{H}_2\text{T}$ (g/L)	after addition	31.3 a	57.3 b	after addition	44.2	44.5	$\Delta\text{H}_2\text{T}$ (g/L)	after addition	0.01	0.02
	pH effect	$\Delta\text{H}_2\text{T}$ (g/L)	before addition	0.003 a	0.16 b	before addition	0.03 a	0.16 b	Dose effect	after addition	0.01	0.02
		$\Delta\chi$ ($\mu\text{S/cm}$)	after addition	0.018	0.011	after addition	0.03	0.02	Dose effect	after addition	0.01	0.02

Different letters along the line discriminate treatments significantly different from one another ($p < 0.05$, ANOVA results).

the formulas proposed by Usseglio-Tomasset *et al.* (1992), it can be estimated that an increase of 3 % ethanol from 12 to 15 % v/v causes a 25 % reduction of the KHT solubility product in a wine with a saturation temperature ranging from 10 to 15 °C. During the cold test, the appearance and precipitation of KHT crystals in unstable wines are due to the formation of crystallisation nuclei (primary crystallisation) and to their subsequent growth. The formation of the crystallization nuclei depends on the supersaturation state of the wines, that in turn is influenced by the alcohol content. On the contrary, the alcohol content does not influence the conductivity test, during which exogenous KHT crystals are added as crystallisation nuclei, and the measured conductivity drop is only caused by the growth of the added KHT crystals. This is probably the reason for the differences in results observed between the two tests.

After the addition of KPA (independently of the dose) all wines became stable, regardless of their initial level of tartaric instability. Statistically significant differences between wines with different alcoholic degree were observed with the conductivity test, but they were modest and of no practical interest. In all cases, a dose of 100 mg/L was sufficient to stabilise the wines. No differences were observed with the cold test between the trials with different doses of KPA, whereas some significant differences, but without any practical interest, were noticed with the conductivity test.

4.2. Red wine (R3)

The experiment was repeated with a Montepulciano red wine (R3) with an initial low degree of tartaric instability. After the adjustment of wine pH, the trials acidified at pH 3.00 became stable, whereas those at pH 3.70 became more unstable than the original wine. As with the white wine, the increase in alcoholic degree caused an increase of tartaric instability, which in this case was significant with both the cold and the conductivity tests. After the addition of KPA, all wines became stable and no dose effect was observed (Table 7).

Finally, wine turbidity was measured before and after the addition of KPA. Statistically significant interactions between pH and alcohol content were observed (Supplementary material: Table A). The initial wine turbidity was on average 5 NTU; after the addition of KPA the turbidity remained virtually unchanged in the trials at pH 3.00, whereas it increased in the trials at pH 3.70, particularly in those with the higher alcoholic degree. The appearance of haze after

the addition of KPA was a rare event throughout the whole research, observed only in this case (R3 red wine) during this work. The appearance of haze observed with an increase of pH could be the consequence of electrostatic interactions between molecules with opposite charges. The destabilising effect of the high alcoholic degree was additive to the pH effect: the trials with the highest alcoholic degree were also the most unstable. However, the pH effect prevailed on the alcohol effect: at low pH no differences in turbidity were observed between the trials with different alcoholic degree.

5. Experiment 5: Effect of KPA on wine filterability

The filterability of the Cortese white wine (W3) and Barbera red wine (R4) was tested 48 hours after the addition of KPA and MTA. The addition of both additives did not modify wine filterability (Figure 1). For the control, MTA and KPA trials, the filterability index (FI) and the modified filterability index (MIF) were respectively 9, 6, 7 and 7, 6, 6 for the white wine (good filterability), and 17, 19, 18 and 20, 19, 20 for the red wine (values considered as acceptable according to the scale reported in section 2.2.3. above). The addition of KPA caused a similar effect as MTA, that is usually added to wines before filtration at bottling.

CONCLUSION

This paper concerns the study of the tartaric stabilising properties of polyaspartates (PAs), in particular of potassium polyaspartate (KPA). During a screening of different PAs added to a Dolcetto red wine, all products showed a good stabilising efficacy against tartaric precipitations that remained unchanged after 1 year of bottle aging, according to the cold test. The other experiments concerned mainly the study of the stabilising efficacy of KPA when varying the type of wine, its chemical composition (pH, alcoholic degree) and the dose of use.

Regardless of the initial degree of tartaric instability, the dose of 100 mg/L was sufficient to stabilise all tested wines. Moreover, an experiment was carried out with increasing doses of KPA, up to 1000 mg/L, and also in this case the minimum dose (100 mg/L) was sufficient to stabilise the wine, but an increase of wine turbidity was observed with the maximum dose (1000 mg/L).

The modification of wine composition (alcoholic degree and pH) influenced the degree of tartaric instability, which increased with the increase of

pH and alcoholic degree. Regardless of the initial degree of instability, the dose of 100 mg/L was sufficient to stabilise all wines.

Moreover, some experiments were performed to verify the influence of KPA on the oxidation process of white wines and on their colour evolution over time. The trend of the oxidation process was studied by monitoring the evolution of free and total SO₂, acetaldehyde and colour parameters until 6 or 12 months after the addition of PAs. The presence of PAs did not cause statistically significant variations of the studied parameters. The chelating effect of KPA for iron and copper, expected to cause a slowdown of the oxygen consumption rate, was not observed.

The use of additives for wine tartaric stabilisation is generally recommended at bottling, just before the final filtration. Like MTA, the addition of KPA did not modify wine filterability (0.45 µm filter).

Some aspects related to the interactions between PAs, the colouring substance and, in general, the colloidal matter of the red wines, have not been discussed here and will be the object of a future work.

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