EFFICACY ASSESSMENT OF SEVERAL CRYSTALLIZATION INHIBITORS USED FOR TARTARIC ACID STABILIZATION IN RED WINES

George Adrian COJOCARU, Arina Oana ANTOCE*

University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, Department of Bioengineering of Horti-Viticultural Systems, 59, Mărăști Blvd, District 1, 011464, Bucharest, Romania

*Corresponding author emails: arina.antoce@horticultura-bucuresti.ro; aantoce@yahoo.com

Abstract

Red wine tartaric acid stabilization is a challenging process, due to the presence of tannins which interfere with the tartrate crystallisation. For this reason, of the known methods for tartaric acid stabilization, the crystallization inhibition seems more appropriate for the red wines. This paper presents several treatments with new crystallization inhibitors (mannoproteins and potassium polyaspartate) applied for an unstable red wine, in the presence of colour stabilization agents (arabic gum, tannin, egg albumin). For the tested wine, known to be difficult to stabilize due to its rich extract, none of the two mannoproteins tested resulted in stability, while the potassium polyaspartate proved to be highly efficient. Kept at cold, the mannoprotein treated samples lead to an important deposit of flocculated proteins, in lower quantity in the case of additional treatment with tannin, while the wines containing potassium polyaspartate did not form deposit, irrespective of the additional colour stabilization treatment. The sensory analysis revealed some better characteristics of the wines treated with polyaspartate, such as reduced perception of bitterness and a slight increase in mouthfeel perception. The influence on colour stabilization of combined treatments is also discussed.

Key words: colour stabilization, mannoproteins; polyaspartate, sensory characteristics.

INTRODUCTION

Wine stabilization is a very important stage in wine production, its effectiveness being crucial for the overall quality and marketing strategies of the wine. Young red wines are released on the market soon after their production, if not properly stabilized, have high potential to precipitate both the colour matter and potassium bitartrate. In order to avoid these incidents, several types of treatments can be applied, compliance in with the recommendations of the International Organisation of Vine and Wine (OIV).

The classical stabilization method widely used against colour matter and potassium bitartrate precipitation is the cold treatment (OIV, 2019b 5/88 and 2/04). During cold stabilization, wines are kept for a period of time at very low temperatures, close to the wine freezing point, to remove in this way the unstable colour matter and to induce the crystallization and precipitation of potassium bitartrate. However, the method is time-consuming and expensive, thus alternative solutions are also available or under research.

Two modern methods for stabilization of wines against potassium bitartrate precipitation involve removing their salt-forming excess ions. First method applies cation exchangers to remove the potassium cations (OIV, 2019a, 43/2000; OIV, 2019b 1/93 and 447/2011), while the second uses electrodialysis to extract of potassium cations and tartrate anions in super-saturation (OIV, 2019a 29/2000; OIV, 2019b 1/93). These methods are very efficient, but still have the disadvantage of high costs for both equipment acquisition and operation.

The most economical methods for potassium bitartrate stabilization are the use of crystallization inhibitors, but these are not always efficient for a long period of time, due to their hydrolysis, and in certain cases they may interact with colour matter, which in red wines is causing precipitation, limiting the stabilizing effect benefits. Generally, these inhibitors are added in clarified wines just before bottling. One of the most common and oldest inhibitors for potassium bitartrate stabilization is the metatartaric acid, used in a dose of maximum 10 g/hl (OIV, 2019a 31/2000; OIV, 2019b 16/70), this being able to stabilize wines for a short time (several months) after bottling. The treatment with metatartaric acid induces stability only for a limited time due to its hydrolysis into tartaric acid, the rate being temperature-dependent (with higher rates at higher temperatures).

Aside of the metatartaric acid, which is the classical inhibitor, other new substances are approved. Using specific veast mannoproteins for potassium bitartrate stabilization is a possible option for winemakers (OIV, 2019a 26/2004; OIV, 2019b 4/01 and 15/05). The specific mannoproteins are obtained by enzymatic hydrolysis of yeast cell walls and purification bv ultrafiltration of those mannoprotein fractions with a molecular weight of about 40 kDa (Feuillat et al., 1998, Gerbaud et al. 1996; Moine and Dobordeau, 1996: Moine-Ledoux et al., 1997). These specific fractions have a stabilizing effect when added in a dose between of 15-25 g/hl, while exceeding these doses results in a reduced stabilizing (Moine-Ledoux et al., 1997). Other fractions of mannoproteins with different molecular weights attracted the attention of researchers as well, due to other important effects in winemaking technology, such as improvement of wine protein stability, which in turn leads to a decrease in bentonite doses required for protein stabilization of white wines (Dupin et al., 2000; Gonzalez-Ramos et al., 2008: Gonzalez-Ramos 2006 and Gonzalez 2006; Gonzalez-Ramos et al., 2009; Moine and Dobordeau, 1996; Waters et al., 1994). Other mannoproteins may be used for the stabilization of foam in sparkling wines (Nunez at al., 2005). Sensory effects are also produced by some mannoprotein fractions with edulcorant properties, thus increasing the sweetness and balancing sourness and bitterness (Moine, 2004). Having a proteic component, these complex macromolecules can interact with polyphenols acting as protective colloids, improving the stability of colour matter and reducing the perception of astringency (Fornairon et al., 2002; Trione and Martínez, 2001; Vidal et al., 2004; Escot et al., 2001).

Other inhibitors against potassium bitartrate precipitation are cellulose gums or sodium carboxymethylcellulose (OIV, 2019a 366/2009; OIV, 2019b 2/08). Due to the interaction with colour compounds, these treatments are allowed only for white and sparkling wines, where they can ensure a long term potassium bitartrate stability. The doses must be under 10 g/hl.

Potassium polyaspartate is the most recently approved additive for potassium bitartrate stabilization (OIV, 2019a 572/2017; OIV, 2019b 543/2016). The potassium polyaspartate can be used in all wines. Red wines must be stabilized against high colloidal instability before this treatment. The dose must not exceed 10 g/hl, not only because at higher doses the stability is not improved but also because in certain cases an increase in turbidity may occur.

For commercialisation of young red wines, aside of tartaric stabilization, colour matter stabilization is another important aspect to take into account. The classical cold stabilization may contribute also to colour stabilization, by removing certain unstable colloids and pigments. But, when cold stabilization is not used, along with alternative methods for tartaric acid stabilization, alternative methods for colour stabilization are needed.

addition (OIV, 2019a Tannins 6/2008. 352/2009, 554/2015 and 574-2017; OIV, 2019b 16/70) is mainly used to facilitate clarification of young wines by removing the excess of proteins through flocculation and sedimentation, with or without other fining processes. However, the tannins can bring other benefits as well. They are commonly added in low tannin red wines made from varieties with thin skins or with short maceration, to improve sensory properties and to stabilize the colour through interaction with free anthocyanins, which leads to many anthocyanin-derived pigments (García-Estévez et al., 2017: Cheynier et al., 2006).

Arabic gum (OIV, 2019a 27/2000; OIV, 2019b 12/72) is an additive which can be used to prevent the precipitation of colloidal colour matter in red wines or to avoid copper haze and protect wines against light iron haze. Treatment with arabic gum is done at bottling time, the maximum dose being 0.3 g/l.

Using combined stabilization treatments for both potassium bitartrate and colour matter stabilization is an appealing solution for a winemaker, as the time and work-load are reduced. However, in order to establish the combinations and dosages laboratory trials are needed to evaluate their efficiency. Also, sensory changes of final product should be evaluated as well. In this paper, some combined stabilization protocols are assessed, including newly approved products and some recommendations are formulated based on the findings.

MATERIALS AND METHODS

The red wine used for the trials was produced in 2018 from the Romanian grape variety Negru de Dragasani. The wine was made through classic technology at industrial scale at Negrini wine cellar, located in Dragasani, a traditional wine growing region of South Romania. The main wine parameters are (OIV, 2018): 13.8% vol. alc.; 5.9 g/l total acidity as tartaric acid: pH = 3.65; 0.18 g/l volatile acidity as acetic acid; 28.1 g/l non-reducing extract; free $SO_2 = 1.6 \text{ mg/l}$; total $SO_2 = 9.2 \text{ mg/l}$; 0.26 g/l malic acid; 1.39 g/l lactic acid; $T_{SAT} = 20.2$ (very unstable wine in relation to potassium bitartrate). The theoretical saturation temperature (T_{SAT}) was determined by measuring the difference in electrical conductivity of wine at 20°C before (C1) and after an excess of potassium bitartrate was added to wine (C₂). Knowing the average value of electrical conductivity (33 μ S/cm) necessary for a decrease of 1°C in theoretical saturation temperature, then the relation describing T_{SAT} is (Ribéreau-Gayon *et al.*, 2006; Würdig *et al.*, 1982):

$$T_{SAT} = T - \frac{C_2 - C_1}{33}$$

The values lower than 15 for red wines mean that the wine is stable; between 15 and 20, the wine is unstable; over 20, the wine is very unstable.

Once the malolactic fermentation finished, the wine was racked off sediments and sulphited with 60 mg/l SO₂ using 10% m/v K₂S₂O₅ solution. The young wine was treated first for colour stabilization by 3 different methods and after 7 days the samples were racked and treated by other 3 different methods, this time for potassium bitartrate stabilization. After 20 days the samples were racked again, sulphited by an additional 10 mg/l SO₂ and bottled. By combining the treatments for colour and potassium bitartrate stabilization, 9 experimental variants resulted. The control sample was only treated by the classical cold stabilization method. All variants (Table 1) were prepared in two sets and in triplicate. The bottled samples were stored for two months, one set of wines at cellar temperature of 12- 16° C and the second one at 4° C.

Codification	Technological variants for colour stabilization	Technological variants for potassium bitartrate stabilization
Control	cold stabilization treatment, 14 days at 0°C	
AG_MP1	30 g/hl arabic gum, [®] Araban Spray Dry,	20 g/hl mannoprotein, ®Mannostab, Laffort
AG_MP2	Vason extracted from Acacia verek	20 g/hl mannoprotein, ®MPA, Vason
AG_KPA		10 g/hl potassium polyaspartate, ®Zenith Uno, Enartis
TAN MP1	10 g/hl mixture of ellagic, gallic,	20 g/hl mannoprotein, ®Mannostab, Laffort
TAN MP2	catechinic and procyanidic tannins,	20 g/hl mannoprotein, ®MPA, Vason
TAN_KPA	[®] Premium Color SG, Vason	10 g/hl potassium polyaspartate, ®Zenith Uno, Enartis
ALBU MP1	20 g/hl micro-granulated egg albumin,	20 g/hl mannoprotein, ®Mannostab, Laffort
ALBU MP2	[®] Albuclar SG, Vason	20 g/hl mannoprotein, ®MPA, Vason
ALBU [_] KPA		10 g/hl potassium polyaspartate, [®] Zenith Uno, Enartis

Table 1. Experimental variants and the detailed treatments used for colour and potassium bitartrate stabilization all the table in the same page

A sensory assessment was performed on the first set of samples, by a team of five tasters who analysed taste and mouthfeel characteristics (sweetness, sourness, bitterness, astringency and body of wine) on intensity scales from 0 to 5. The second set of wines,

cold stored, was inspected for sediments, and if they were present, they were gravimetrically evaluated by using a drying balance (KERN MLB 50-3N), after being separated by centrifugation (Hettich Mikro 220R centrifuge, with 50 ml tubes, set for 10 minutes at 6000 rpm). The drying balance ran at 120° C and reading was done after the automatic shutoff, occurring for a change of weighing value < 1 mg within 60 seconds. The results were reported as g/l dry weight sediment.

The CIELab colour parameters and total polyphenols index were determined with WinAspect software version 2.2.7 and a computer-controlled double beam spectrophotometer Specord 250 from Analytik Jena AG.

RESULTS AND DISCUSSIONS

The CIELab parameters and total polyphenols for all performed stabilization treatments are presented in Table 2. The main results show some differences induced by certain treatments, such as an increase of lightness (parameter L) and a decrease of total polyphenol index in samples treated with egg albumin as a fining agent or that the arabic gum treatment induces an increase of yellowness in wine samples (*b* parameter). In order to establish the individual effect of the performed treatments, the influence of colour stabilization treatments (Table 3) or the influence of the potassium bitartrate stabilization treatments (Table 4) were statistically analyzed.

The effect of colour stabilization treatments (Table 3), without taking into account the effect of potassium bitartrate stabilization treatments. showed that tannin and arabic gum treatments seem to have the highest efficiency on colour stabilization. Even though the cold treatment along with egg albumin fining removed the pigment supposed to be unstable, the resulted wines remained unstable. The most efficient mechanism for colour stabilization is proven to be the interaction of tannins with anthocyanins, which prevents the loss of free and polymerized anthocyanins, which is here evidenced by an increase in redness (a parameter) and polyphenol index (TPI), along with a reduced yellowness (b parameter) comparing to arabic gum. The use of arabic gum as protective colloid works well with the mention that induces a slightly increase in yellowness (b parameter).

Comparing the wine samples stored at cellar temperature with those stored in the refrigerator, we can observe an increase in lightness (L parameter), which indicates a more advanced loss in pigments and other solid matter. However, in potassium bitartrate highly unstable wines, the phenomenon of tartaric acid co-precipitation with tannins, pigments and other molecules can also occur (Manns *et al.*, 2005; Vernhet *et al.*, 1999).

The mechanism of co-precipitation is not well understood but it is likely to appear because of hydrophobicity or surface charges on the crystal faces and could be dependent on the pH of the wine (Manns *et al.*, 2005; Vernhet *et al.*, 1999).

Considering the effect of colour stabilization treatments in our experimental samples, a highly unstable wine regarding potassium bitartrate leads to co-precipitation of crystals along with pigments (Figure 2), even though the wine is stable regarding colour matter (Table 3).



Figure 1. Principal Component Analysis bi-plot of CIELab parameters and Total Phenolic Index

The results indicated in Table 4, revealed that potassium polyaspartate is the only treatment efficient to prevent crystal formation in our red wine samples and because of its efficiency, the possibility of colour matter loss is avoided (Figure 2). Thus, the influence of potassium bitartrate stabilization technique is equally important for the colour matter stabilization, stabilizing the tartrate preventing also some pigment loss in red wines.

To highlight the effect of stabilization treatments on the CIELab parameters and total polyphenol index (TPI), the results from the Cellar stored samples are plotted in a Principle Component Analysis (PCA) diagram (Figure 1). As we can observe in the PCA bi-plot, the

parameter L is increased by subtractive techniques (egg albumin fining and cold treatment which remove part of the compounds from wine), while it is relatively constant in additive techniques. The positive value of parameter a indicates the shift of colour towards red, indicating a better colour appearance and stability, which can be obtained by tannin treatment followed by a period to form associations with anthocyanins. However, the tannin treatment increases the total polyphenols, which provide a better structure in red wines. The treatment with arabic gum is associated with similar effects as tannin, but the disadvantage of increasing the has parameter b, shifting the colour slightly towards yellow. A similar behaviour for CIELab and total polyphenol index was not mannoprotein present for or potassium polyaspartate treatments. The combined effect of stabilization (colour matter and potassium bitartrate) can be observed in Figure 2, where the dry weight of sediment was measured and analysed statistically.



Figure 2. Measured dry weight of sediment (potassium bitartrate and colour matter)

The efficacy of potassium polyaspartate on our red wine samples is undeniable, because no sediment was detected in those samples, proving the attainment of stability regardless of the treatment for colour stabilization. However, in the case of tannin treatment for colour stabilization, both mannoproteins showed a reduced co-precipitation while combined with egg albumin or arabic gum the co-precipitation effect is increased in a similar way as in control samples.

The results highlight a combined beneficial effect of both stabilization treatments on the final product stability (lower colour matter loss more efficient potassium bitartrate and stabilization). If one of these compounds is not well stabilized in the final product, a co-precipitation may occur and a visual sediment of crystals with pigments or tannin will appear at the bottom of bottles. The parameter L from CIELab method seems to be a good indicator for the assessment of colour stability in red wines, as an increase in its value after storage at 4°C indicates a loss in pigments with potassium bitartrate crystals.

One Way ANOVA applied to the dry weight of sediment (Figure 2) indicates that there are certain significant differences (p < 0.05) among experimental variants. Although both colour and tartaric acid stabilization treatments were combined in each variant the statistical analyses were run only for the treatment combination. independent thus treatment effects were not evaluated in this way. Even so, the results are strongly suggesting that, as compared to other treatments, tannin combined with MP's may lead to an increased stability, at least for a short period of time comparing with the other combinations.

However, the most efficient treatment for stabilization of colour matter and potassium bitartrate remains potassium polyaspartate (KPA) regardless of its combination with a treatment for color stabilization. Potassium polyaspartate (KPA) provided very good stability in our samples with no detected sediment in bottles (Figure 2).

The classical cold treatment for 14 days at 0°C did not provide the desired physico-chemical stability in our samples. A lower temperature of about -4°C probably would have been a better way to stabilize the potassium bitartrate and colour for this type of wine with a very unstable character.

Table 2. CIELab parameters and total polyphenols in samples stored in cellar against samples stored in refrigerator

Samples	*L1±sd	*a1±sd	*b1±sd	*TPI1±sd	*L2±sd	*a2±sd	*b2±sd	*TPI2±sd
Control	51.86±0.96	47.21±0.63	1.56 ± 0.17	57.21±3.71	53.87±0.22	45.97±0.17	1.68 ± 0.17	61.07±0.24
AG MP1	51.47±0.23	47.28±0.34	1.97 ± 0.08	63.13±2.39	52.84±0.19	46.22±0.13	1.83 ± 0.08	60.99±1.74
AG MP2	51.61±0.83	46.95±0.80	1.99±0.21	61.43±0.43	51.47±1.04	46.63±0.72	1.92±0.15	61.68±2.52
AG KPA	50.94±0.24	47.55±0.45	2.10 ± 0.18	61.24±0.9	51.52±0.44	46.71±0.4	2.19±0.24	59.21±1.80
TAN MP1	51.27±0.61	47.73±0.39	1.95±0.17	62.35±0.49	51.38±1.66	46.26±0.85	1.87±0.17	63.78±1.28
TAN MP2	51.21±0.61	48.16±0.28	1.67±0.21	62.81±0.79	51.48±0.77	47.24±0.42	1.71±0.19	61.73±2.45
TAN KPA	51.94±0.35	47.70±0.23	1.31±0.04	62.52±2.22	50.7±0.74	46.68±1.05	$1.54{\pm}0.03$	60.69±1.81
ALBU MP1	52.54±0.84	47.08±0.72	1.64±0.21	59.95±1.41	53.55±0.07	46.58±0.13	1.43 ± 0.04	59.57±1.56
ALBU MP2	52.82±0.13	47.12±0.44	1.27±0.18	59.37±0.79	53.65±0.46	46.29±0.46	1.39±0.06	58.63±3.03
ALBU_KPA	53.21±0.71	46.82±0.31	$1.04{\pm}0.35$	59.16±1.38	53.13±0.22	46.09±0.05	1.39 ± 0.03	59.18±0.55
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*L1, a1, b1, TPI1 - samples stored in cellar (12-16°C); L2, a2, b2, TPI2 - samples stored in refrigerator (4°C).

Table 3. Comparison of samples stored in cellar (12-16°C) against samples stored in refrigerator (4°C) as regards CIELab parameters and TPI of wines treated for colour matter stabilization (Test Tukey p < 0.05)

Treatment	L_1	*a1	*b1	*TPI1	*L2	*a2	* b 2	*TPI2
Control	51.86 ^{ab}	47.21 ^{ab}	1.56 ^{ab}	57.21°	53.87 ^b	45.97 ^a	1.68 ^b	61.07 ^a
Arabic gum	51.34 ^a	47.26 ^{ab}	2.02^{c}	61.93 ^{ab}	51.94 ^a	46.52 ^a	1.98°	60.63 ^a
Tannins	51.47 ^a	47.86 ^a	1.66 ^b	62.56 ^a	51.19 ^a	46.73ª	1.71 ^b	62.07 ^a
Albumin	52.85 ^b	47.01 ^b	1.32 ^a	59.49 ^{cb}	53.45 ^b	46.32 ^a	1.40 ^a	59.13ª

*L₁, a₁, b₁, TPI₁ - samples stored in cellar (12-16°C); L₂, a₂, b₂, TPI₂ - samples stored in refrigerator (4°C).

Table 4. Comparison of samples stored in cellar (12-16°C) against samples stored in refrigerator (4°C) as regards CIELab parameters and TPI of wines treated for potassium bitartrate stabilization (Test Tukey p < 0.05)

Treatment	L_1	*a1	* b 1	*TPI1	L_2	*a2	*b2	*TPI2
Control	51.86 ^a	47.21ª	1.56 ^{ab}	57.21 ^b	53.87 ^b	45.97ª	1.68 ^a	61.07 ^a
MP1	51.76 ^a	47.36 ^a	1.85 ^b	61.81ª	52.59ª	46.35 ^a	1.71 ^a	61.45 ^a
MP2	51.88ª	47.41ª	1.64 ^{ab}	61.20 ^a	52.20 ^a	46.72 ^a	1.68 ^a	60.68 ^a
KPA	52.03ª	47.36 ^a	1.48 ^a	60.97 ^a	51.79 ^a	46.49 ^a	1.71ª	59.69ª

*L₁, a₁, b₁, TPI₁ - samples stored in cellar (12-16°C); L₂, a₂, b₂, TPI₂ - samples stored in refrigerator (4°C).

The sensorial results (Figure 3, left) showed that arabic gum in combination with potassium bitartrate stabilizers brings substantial differences on bitterness, astringency and wine body. The body of wine samples was increased to a greater extent when arabic gum was combined with potassium polyaspartate, showing a lower effect and different behaviour regarding the MP1 and MP2 mannoproteins.

The perceived bitterness was very different regarding the two mannoproteins, MP1 being perceived with a lower bitterness than control samples, while MP2 increased the bitterness in the wine samples. The lowest bitterness perception was found in wines treated with potassium polyaspartate.

The astringency perception is reduced by potassium polyaspartate and MP1 comparing to control, while MP2 increased this perception. The sweetness appears to be less affected by the treatments involved, with a slight increase induced by all stabilizers for potassium bitartrate.

The sour taste is not affected by mannoproteins, while it is slightly masked by potassium polyasparatate treatments.



Figure 3. Sensory changes induced by the stabilization treatments: left - *arabic gum* in combination with mannoproteins (MPs) or polyaspartate (KPA); middle - *tannin* in combination with MPs or KPA; right - egg albumin with MPs or KPA

The results from the combination of tannin with potassium bitartrate stabilizers (Figure 3, middle) stand out for a similar behaviour on bitterness, astringency and wine body. The most notable effect was given by potassium polyaspartate which appears to mask the bitterness and sourness and lower astringency, while increasing the wine body.

The sensory results regarding egg albumin fining combined with potassium bitartrate stabilizers (Figure 3, right), appear to show reduced wine complexity because of various phenolic compounds removal form wine, causing the samples to be close together for most of sensorial parameters. An exception can be observed regarding potassium polyaspartate which has a clear influence on increasing wine body.

CONCLUSIONS

- *Arabic gum* ensures colour matter stability by its efficacy as a protective colloid, but this mechanism may not be enough if the wine is not stable regarding potassium bitartrate (KHT). Co-precipitation of potassium bitartrate with pigments and tannins may occur when no or an inadequate treatment to prevent crystallization was applied. Other induced effect is a slight increase of yellowness (parameter b) in the treated wines.
- *Tannin* provides the most interesting effect on colour matter, increasing the redness (parameter a) and total polyphenolic index (TPI), while keeping the yellowness (parameter b) similar to control samples. The structure of wines treated with tannin were improved, but this may lead to a longer maturation period required to attenuate the astringency. The co-precipitation phenomenon can also occur if the potassium bitartrate remains unstable.
- *Egg albumin* treatment is often used, but we find it not recommendable for quality wines, because it reduces the colour and structure, enhancing the acidic and vegetal sensations, also reducing the overall complexity. This treatment may be beneficial in certain oxidized wines of lower quality, where the reducing of yellowness (parameter b) is needed, but with the compromise of

reducing some desired phenolic compounds as well. Even if the unstable phenolic compounds are reduced by this treatment, the unstable potassium bitartrate coprecipitates with the rest, bringing an additional loss of pigments and tannins.

- Mannoproteins MP1 and MP2 may increase the body of wine, but they are not able to mask the sour taste. Astringency and bitterness perceptions depended on the type of mannoprotein and on other treatments combined with them. A certain degree of stability for short term can be observed when MP1 and MP2 mannoproteins were combined with tannin treatment, but in the end they can co-precipitate with pigments in the case of wines with highly unstable potassium bitartrate. The treatment with mannoproteins may be a solution for wines with a longer stabilization period as in the case of barrel matured wines, but may not be a good choice for young red wines.
- Potassium polyaspartate is providing positive features in young red wines with highly unstable potassium bitartrate. The structure of wine is improved and a more complex mouthfeel is perceived, lowering the sourness and astringency perceptions. It gives the best results in combination with tannins, but also in combination with arabic gum. Potassium polyaspartate was able to stabilize potassium bitartrate in highly unstable young red wine and to prevent coprecipitation, thus being recommended for use for these purposes.

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