In: Recent Advances in Wine Stabilization… ISBN: 978-1-63484-883-1
Editors: António Manuel Jordão and Fernanda Cosme 

*Chapter 2*

# RECENT DEVELOPMENTS IN WINE TARTARIC STABILIZATION

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## ABSTRACT

Tartrate precipitation is still a relevant subject in Enology, being one of the most common problems of wine physical-chemical instability. Potassium bitartrate and calcium tartrate precipitations are undesirable phenomena which can occur in bottled wines, especially when these are stored at low temperatures. The occurrence of tartrate salt crystals (potassium hydrogen tartrate – KHT and calcium tartrate – CaT) in bottles has severe consequences in the final aspect of the wine and therefore on the consumer's acceptance, making tartrate wine stabilization virtually mandatory before bottling. Currently, several solutions to prevent this haze are available: subtractive methods including the conventional cold treatments that promote the cristalization of KHT, removal of potassium and calcium ions either by electrodialysis or ion exchange resins; and additive methods such as the addition of carboxymethylcellulose, mannoproteins or metatartaric acid. For monitoring the KHT stability, several analytical methods have been developed based on conductivity evaluation, namely the mini-contact test and the saturation temperature measurements (*TS*). These methods will also be revisited, aiming to raise awareness of their utility as tools in quality control of wines. This review addresses tartrate precipitation subject and the most recent preventive solutions available, pointing out the advantages and drawbacks of each one, and its impact on the final characteristics of the wine.

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**Keywords:** wine tartaric stabilization, cation exchange resins, metatartaric acid, carboxymethylcellulose, mannoproteins

## 1. INTRODUCTION

One of the most common problems of bottled wine instability is the appearance of sediments of potassium bitartrate, and in a less extent, calcium tartrate. These two tartrate salts are naturally presents in grape juice, usually at saturated levels, and their crystallization naturally occurs during alcoholic fermentation, mainly due to the presence of ethanol and decrease of temperature at its final stage, and continues during wine storage. Although this is a natural phenomena of physical-chemical stabilization of young wines, tartrate precipitation in bottled wine is understood as a quality fault, especially in white wines that are generally stored at low temperature. Indeed, these sediments possess no problems concerning human health but their appearance leads to important economic losses because it may change consumer's perception on wine quality. Thus, tartrate stabilization of wines is highly recommended and is a common practice before the bottling and commercialization of the major part of quality wines.

In a recent review, Lasanta and Goméz (2012) explained the mechanism behind tartaric precipitation. In summary, different equilibriums related to the dissociation of tartaric acid ($H_2T$) exist in wines:

$$H_2T \rightarrow H^+ + TH^- \text{ and } TH^- \rightarrow H^+ + T^{2-}$$

Hence, the total molar concentration of tartaric acid is:

$$c = [H_2T] + [TH^-] + [T^{2-}]$$

On the other hand, the solubility of the two salts is described by the following equilibriums:

$$KHT_{cryst} = K^+ + HT^- \text{ and } CaT_{cryst} = Ca^{2+} + T^{2-}$$

Tartrate stability could be evaluated comparing the thermodynamic constants of these equilibrium equations with the real solubility constants of each salt obtained by multiplying the molar concentrations of the ions. But this theoretical consideration does not take into account that there are compounds in wines affecting the equilibriums and inhibiting the growing of KHT and CaT crystals. In fact, the extent to which precipitation of crystals can occur in a certain wine remains unknown, although it is well known that the solubility product for potassium bitartrate depends on several factors such as alcohol content, pH, temperature and the concentration of other cations and anions, besides $K^+$ and $Ca^{2+}$. The presence of metals like magnesium, the so-called "complexing factors", sulphates, proteins, gums, polyphenols and others affect the formation and precipitation of KHT.

Aiming to prevent this instability, there are several methods to perform a wine stabilization, based on different principles: the removal of some tartaric acid (cold stabilization) or the removal of the cations that are necessary to the precipitation of the tartaric acid in the form of crystals of potassium bitartrate and calcium tartrate (electrodialysis and ion exchange); or using additives (metatartaric acid, mannoproteins or carboxymethylcellulose) to prevent the crystals to be formed.

The most traditional one is cold stabilization that consists of cooling the wine at a temperature near the freezing point for several days to induce KHT precipitation before bottling. The freezing temperature of the wine is empirically determined according to the expression:

$$\text{Freezing temperature } (^{o}C) = -\,(\text{alcoholic strength} - 1)/2$$

However, its effectiveness depends on wine composition (colloidal content plays an important role (Usseglio-Tomasset et al., 1980) since this process does not allow a precise control of the final KHT concentration and is not effective for CaT (Maujean et al., 1985). Other drawbacks could be ascribed to this technique, namely time and energy consumption, significant losses of wine which are discharged together with the precipitated KHT, and a decrease on color intensity due to a partial and simultaneous precipitation of polyphenols together with the KHT salts (Gómez-Benítez et al., 2003). Rodrigues et al. (2012a) showed that cold static tartaric stabilization also promotes a decrease in the high molecular weight mannoproteins, that are one of the major polysaccharide groups found in wines (Feuillat, 2003) playing a crucial role in several important interactions and properties of wines.

In order to increase the effectiveness of the cold treatment, Muller-Spath (1979) proposed adding finely divided crystals of KHT to act as crystallization nuclei (Blouin et al., 1979), thus enhancing the growth of KHT crystals and increasing the efficiency of this method being this technique known as “contact method”. Cold stabilization can be performed in a static way (with or without KHT crystal seeding) or by a dynamic continuous process, which enables a reduction in time. Despite the drawbacks that can be assigned to this technique, the overall quality of the resulting wines is very good.

Electrodialysis (ED) is based on ion electrical migration. In ED the wine circulates in rectangular channels confined by cation and anion selective membranes and, by the action of an external electric field, the ions are forced to migrate to the electrodes giving rise to a wine stream depleted in ions (Strathman, 1986). ED uses permeable membranes selective to the ionic species, both cationic and anionic, so it is to be expected, in some extension, also a removal of anions, such as sulphate ions. A major advantage of ED is that this technique does not interfere with the other wine compounds playing a major role in its organoleptic properties (Gonçalves et al., 2003). Several studies with Portuguese wines addressing the comparison of the organoleptic characteristics of wines treated by ED and conventional cold stabilization processes showed that no significant differences were found in color, aroma and taste between the wines treated by the two processes. (Gonçalves et al., 1998; Cameira dos Santos et al., 2000).

In spite of these aforementioned methods could be a valid alternative to achieve wine tartaric stabilization, this review is focused mainly on additives and ion exchange resins.

## 2. ADDITIVES

Tartrate stability could also be achieved by chemical methods, adding substances that prevent crystal precipitation, either by inhibiting their formation or by modifying their properties and making them soluble at a lower temperature. The first compound developed on an industrial scale for preventing tartrate precipitation was metatartaric acid (MTA), being for a long period of time the product most widely used for this purpose. More recently, carboxymethylcellulose (CMC) and mannoproteins (MP) extracted from yeasts have been suggested as stabilizers and after numerous studies and tests their use in Enology is currently widespread. In comparison with physical based approaches the use of additives presents relevant advantages, namely those related to cost of energy and initial investment in specific equipment.

### 2.1. Metatartaric Acid

The effect of metatartaric acid (MTA) in opposing the growth of the submicroscopic nuclei of crystals and in retarding tartaric precipitation in the bottle is well known (Goertges and Stock, 2000). More precisely, the presence of its molecules during the tartrate crystal building prevents the feeding phenomenon.

MTA, also known as ditartaric acid, is a polymerized substance formed from the intermolecular esterification of L-tartaric acid, between an acid function of one molecule and a secondary alcohol function of another molecule (Ribéreau-Gayon et al., 2006). This esterification reaction is promoted at a temperature of 150-170 °C under atmospheric pressure or under a reduced pressure in order to obtain an esterification rate higher than the theoretical equilibrium rate (33%). This reaction is reversible as tartaric acid may be formed again by hydrolysis.

More exactly, MTA is a mixture of polymers with different molecular weight. Its primary constituents are the ditartaric monoester and diester in variable proportions, mixed with variable amounts of non-esterified tartaric acid, pyruvic acid (representing 1 to 6% w/w of MTA) and small quantities of poorly known polyester acids. It is available in crystalline form or in powder form with white or yellow color. This additive shows high solubility in water and alcohol being rapidly hydrolyzed in aqueous solution at 100 °C. As MTA is highly hygroscopic it should be stored in dry conditions.

MTA effectiveness in preventing tartaric precipitation is determined by the rate of esterification. Many MTA preparations with distinct anti-crystallizing properties, depending on the esterification rate, can be found in the market. For enological application, a minimum rate of 32% of esterification is established by the International Organization of Vine and Wine (OIV) through the International Oenological Codex (resolution Oeno 31/2000) (OIV, 2015a).

Several laboratory tests are described for assessing the effectiveness of a MTA preparation. As an example of a high practicability test, Ribéreau-Gayon et al., (2006) reported a procedure carried out on a saturated potassium bitartrate solution distributed by 10 mL test tubes added with increasing levels of MTA preparations with different esterification levels. The precipitation of bitartrate was induced by ethanol (1 mL, 96% vol.) and the preparation leaved overnight at 0°C. It was observed that only 1.6 mg of the preparation with an esterification number of 40.8 was required to inhibit crystallization, while 4.0 mg of the preparation with an esterification number of 26.6 was necessary.

The main drawback of MTA is its low stability in wine as it hydrolyzes over time generating tartaric acid (Lubbers et al., 1993; Gerbaud et al., 2010), losing its protector effect, increasing the acidity and enhancing the tartrate instability. Furthermore, its effectiveness for calcium tartrate stability is lower than for potassium bitartrate (Postel, 1983). It was observed that total hydrolysis of a 2% MTA solution took three months at 23°C and 10 months at 5°C, reinforcing the importance of preparing the MTA solutions immediately before its application to wine (usually a concentrated solution, at 200 g $L^{-1}$, in cold water). Moreover, the same phenomenon occurs in wine representing a serious problem concerning MTA effectiveness. It is well known that pH and temperature strongly influences the rate of hydrolysis: ranging from 1 week at 30°C to 2 years at 0°C, being from 1 year to 18 months at usual temperatures in wine cellars (usually between 10°C and 18°C) (Ribéreau-Gayon et al., 1977). For these reasons, of major enological importance, MTA use is only effective in wines intended to be consumed within a short period of time, normally within 12 months.

Generally MTA is applied after fining operation, in order to eliminate the risk of partial removal due to flocculation. It is especially affected by bentonite and potassium ferrocyanide treatments, while high-temperature bottling has little or no negative effect. On the other hand, in an incidental manner, a slight opalescence may be observed after treatment, particularly when MTA with high esterification rate have been used. To avoid this phenomenon, it is recommended its addition before the final clarification.

The treatment of wine to prevent the precipitation of potassium hydrogen tartrate and calcium tartrate using these products is regulated by the OIV through the International Code of Oenological Practices (resolution 16/70) (OIV, 2015b). According to this reference document the following prescriptions should be followed: a) the addition should be carried out immediately before bottling; b) the dose to be used should not be higher than 10 g $hL^{-1}$; c) the duration of protection depends on the storage temperature of the wine, because the acid hydrolyses is slow in the cold, but is rapid under hot conditions; d) the MTA should comply with the prescriptions of the International Oenological Codex (Oeno 31/2000) (OIV, 2015a).

Recently we have developed a study on wine tartaric stabilization by applying different treatments, namely ion exchange resins for removal of cations involved in the crystallization, cold stabilization for removal of tartaric acid and finally MTA as protector colloid. The results of this study, shown in Figure 1, are discussed later in this chapter.

## 2.2. Carboxymethylcellulose

Carboxymethylcellulose, also named as cellulose gum, CMC or sodium CMC among other designations, is a derivative from cellulose used as additive (E466) in food industry

since the forties of last century, mainly because of its emulsifier properties. It is produced by chemical modification of cellulose, the most abundant polysaccharide in nature. The CMC for enological use is prepared exclusively from wood by chemical treatment with alkali and monochloroacetic acid or its sodium salt (OIV resolution Oeno 366-2009) (OIV, 2015a).

The chemical modification of cellulose is carried out in a two-stage process consisting of a treatment of the cellulose with sodium hydroxide to obtain the alkali-cellulose complex, followed by an etherification reaction between the alkali-cellulose complex and monochloroacetic acid with formation of CMC. More exactly, this additive is obtained by etherification of the free primary alcohol groups of the glucopyranose units linked by β (1-4) glycosidic bonds (Ribéreau-Gayon et al., 2006).

A CMC is characterized by the degree of etherification of its alcohol functions, known as the degree of substitution (DS), and by the average number of glucopyranose units per polymer unit, known as its degree of polymerization (DP). The effectiveness of CMC as protector colloid is straightly related with the aforementioned characteristics.

The DS value indicates the number of glucopyranose units that have been etherified by sodium chloroacetate in an alkaline medium in relation to total glucopyranose units. The theoretical maximum of the DS value for cellulose/CMC is 3.0, but the range for commercially available CMC grades is generally in the range 0.4 to 1.5 (Heinze and Koschella, 2005). The CMC effectiveness as protective colloid is strongly related to DS value, increasing with this parameter. This is explained by the fact that DS value determines the number of anchor sites involved in cation complexation (Lubbers et al., 1993). According to OIV monography on carboxymethylcelluloses (OIV resolution Oeno 366-2009) (OIV, 2015a), the DS of a CMC for wine treatment must be comprised between 0.60 and 0.95. According to this document, only the CMC showing a DS between 0.6 and 1.0 are completely soluble.

The molecular weight of CMC is rather dispersed, ranging from 17,000 and 300,000 Da. The CMC viscosity, an important characteristic concerning its facility of use, is determined by the DP, increasing with molecular weight. In addition, the viscosity also varies according to the cation. Divalent cations, such as calcium, magnesium and iron, decrease this rheological characteristic.

Pure CMC pKa is around 4.3 and at wine pH, about 20% of the carboxymethyl groups carry negative charges in solution (Gerbaud et al., 2010).

CMC inhibits tartaric precipitation through a protective colloid effect. It acts as a negatively charged polymer at wine pH interacting with the electropositive surface of potassium bitartrate crystals, significantly reducing their growth rate and modifying the shape of potassium bitartrate crystals (Crachereau et al., 2001). Furthermore, CMC can also act by complexing potassium ions, decreasing the amount of free ions available for the crystals edification (Rodriguez-Clemente and Correa-Gorospe, 1988).

It was demonstrated that low-viscosity CMC are effective in preventing tartrate crystallization at doses remarkably lower (12-250 times) than those currently used in the food industry (Crachereau et al., 2001). Moreover, it was claimed that the effectiveness of CMC at a dose of 2 g $hL^{-1}$ is equivalent to 10 g $hL^{-1}$ MTA treatment, with the advantage that CMC has a very stable effect, namely to heating, which is not the case of MTA (Gerbaud et al., 2010). The same authors observed that the inhibitory effect is maintained at 2°C, when the crystallization risk is increased, stating that in that case, 3 to 5 times higher concentrations are

required to achieve the same effect than at 11.5°C. Furthermore, the CMC efficiency is directly related to its concentration.

Recently, Guise et al. (2014) have studied the impact of different types of CMC's at two concentrations in two white wine samples (Douro valley and Vinho Verde region) on tartrate stability, physical-chemical composition and sensory characteristics, and have compared its effectiveness with other enological additives. While all CMC's and MTA stabilized the wines, arabic gums and mannoproteins did not stabilized. CMC's had no significant effect on tartaric acid, potassium, calcium and sensory attributes.

CMC are available in the form of granules or fibrous powder, blank or slightly yellowish or greyish, slightly hygroscopic, odorless and tasteless. Due to its hygroscopic properties this additive must be stored in dry conditions. Solutions can be prepared prior to use but must contain at least 3.5% CMC (OIV resolution Oeno 366-2009) (OIV, 2015a). However, the stability of CMC under solution form is low, which can be an important drawback, requiring a careful stock management. CMC should be used immediately before bottling, as any physical-chemical modification induced by treatments like acidification or de-acidification can compromise its protective effect.

It should be noted that, as proteins can interact with CMC, protein stability of wine must be assured previously to wine treatment. Another serious limitation of CMC is that this additive is not recommended for red wine treatment as it can promote the colorant matter precipitation, constituting a very important restriction to its use in red wines.

After several years of studies on tartaric stabilization by CMC addition and discussion of the respective results, CMC was approved in the European Union in 2009 as an oenological product. The treatment with CMC is regulated by the OIV through the International Code of Oenological Practices (OIV resolution Oeno 2/08) (OIV, 2015b). However, the use of this additive in wines under the scope of tartaric stabilization is limited to white and sparkling wines. Moreover some prescriptions are established: a) it can be used at doses up to a maximum of 100 mg $L^{-1}$, b) bearing in mind its incorporation, granulated form or less viscous products are preferred, c) the CMC should comply with the prescriptions of the International Oenological Codex (OIV resolution Oeno 366-2009) (OIV, 2015a).

## 2.3. Mannoproteins

Mannoproteins (MP) are the main polysaccharides of microbiological origin in wine, released from *Saccharomyces cerevisiae* cell walls during winemaking by different mechanisms, including yeast autolysis, that occur both during alcoholic fermentation and during ageing on yeast lees.

These polymers are naturally present in significant amounts in wines, especially in red wines, and their concentration depend on the winemaking process. MP are, after arabinogalactan-proteins from grapes, the second most abundant polysaccharides in wines, achieving 200 mg $L^{-1}$ and representing more than 30% of total polysaccharides of wine (Waters et al., 1994; Gerbaud et al., 1997; Gonçalves et al., 2002).

A systematic characterization of MP in terms of chemical composition and molecular structure was carried out by several research teams mainly during the nineties, revealing its natural diversity (Pellerin and Brillouet, 1992; Waters et al., 1994; Gonçalves et al., 2002). MP are almost pure mannans (D-mannose content represent 80 to 90% of the total sugar

content) including small amounts of D-glucose, N-acetylglucosamine and proteins (10 to 20%), and represent several fractions over a wide range of molecular weights (20 to 2000 kDa), with an average value of 250 kDa (Gonçalves et al., 2002; Ribéreau-Gayon et al., 2006). The molecular structures of MP consist of a peptide chain linked to D-mannose units in α-(1→6), α-(1→2) and α-(1→3) (Saulnier et al., 1991; Waters et al., 1994).

MP can exhibit a negative charge at wine pH which explains their capacity to establish electrostatic and ionic interactions with other wine compounds. The charge density depends on MP content in phosphate groups (Vernhet et al., 1996).

Yeast MP are located on the wall external layer, where they are covalently bound to an amorphous matrix of β-1,3-glucans. Their release can occur during alcoholic fermentation in the yeast growing phase and after yeast autolysis by the action of the exogenous β-1,3-glucanase enzyme on the yeast walls (Feuillat, 2003). These last MP are similar to those released during alcoholic fermentation but they have less protein content (Saulnier et al., 1991).

The differences in terms of composition and structure provide MP various properties in the wine. Among their excellent enological properties, yeast MP contribute to several aspects of wine quality by protecting against protein haze (Waters et al., 1994), soften astringency by combining phenolic compounds from grapes and wood (Riou et al., 2002), interacting with aroma compounds, stimulating growth of malolactic bacteria, adsorbing ochratoxin A, stabilizing tannins (Rodrigues et al., 2012b) and, interfering in filterability and fouling of filter membranes. Moreover, MP act as natural inhibitors of KHT crystallization, preventing the occurrence of precipitates in wine (Lubbers et al., 1993; Dubourdieu and Moine-Ledoux, 1997) since they affect the rate of crystal growth by binding to nucleation points and preventing expansion of the crystal structure (Gerbaud et al., 1997).

It is well known that the eventual removal of these protective colloids by drastic fining or filtration can affect wine sensory characteristics and tartaric stabilization, as they reduce the effectiveness of physical stabilization treatments, especially cold stabilization. By the contrary, the traditional practice of barrel-aging white wines on yeast lees for several months frequently gives them high stability, suggesting an important role of yeast autolysis MP.

Besides its natural occurrence in wines, MP can be added directly to wine as commercial preparations. Currently, there are several oenological additives in the market containing MP in their composition with the aim of preventing potassium tartrate precipitation. The MP preparations are obtained by digesting yeast walls with an industrial preparation of β-(1-3) and β-(1-6)-glucanases, allowed in winemaking as a clarifying enzyme for improving the filterability of wines produced from botrytized grapes (Ribéreau-Gayon et al., 2006). Heat-extracted MP does not have the same stabilizing effect. On the other hand, the inhibiting effect of MP extracted from yeast on tartrate crystallization is not related to the invertase fragment responsible for protein stabilization. Moine-Ledoux and Dubourdieu (2002) demonstrated that the crystallization inhibitory activity is due to a particular highly-glycosylated MP of approximately 40 kDa. Considering that MP properties in wine strongly depend on its chemical composition and structure, it is very important to know the composition of the commercial preparations and check the efficiency of these products.

The stabilizing effect of MP is stronger than that of MTA, and may delay the appearance of crystals for a month in relation to an untreated wine (Moine-Ledoux and Dubourdieu, 2002). Furthermore, MPs are stable over time in contrast to MTA. It was observed that using

a dose of 25 g $hL^{-1}$ of MP, wines remain stable even after having been stored at -4°C for six days (Moine-Ledoux and Dubourdieu, 2002). In fact, MPs are very efficient inhibitors at a concentration of 20 g $hL^{-1}$, in most cases. Nevertheless, for highly saturated wines, where a higher concentration is needed to achieve the same inhibitory effect, MP flocculation may occur that counteracts the expected effect (Gerbaud et al., 2010). MP effectiveness and the optimal dose, which is specific according to the characteristics of the wine being treated, must be determined by preliminary tests that should include the assessment of protein stability. It is well established that the use of excess doses of this additive is inefficient.

The treatment of wines by using MP from yeast wall degradation to improve stability only with regards to tartaric salts and/or proteins in the case of white or rose wines is described by OIV (Resolution Oeno 4/01; 15/05) (OIV, 2015b). The MP should comply with the prescriptions of the International Oenological Code. According to this reference document (Resolution Oeno 26/2004) (OIV, 2015a), MPs are extracted from *Saccharomyces cerevisiae* yeast cell walls by physical-chemical or enzymatic methods. MPs are offered in powder form, usually microgranulated, white or beige in color, odorless, or in a colloidal solution, yellow in color, translucid. For solution preparations, the concentration of MP and the content of sulphur dioxide must be indicated.

Concerning the cost estimation of additives stabilization methods, MTA and CMC are lower priced than cold treatment (traditional treatment) and MP is expensive, what constitutes an important drawback of this product.

However, in addition to tartaric stabilization, the wine treatment with MP can significantly contribute to improve its overall sensory quality. Lastly, being naturally found in wine in contrast with MTA and CMC, MP addition is easily understood and above criticism in terms of wine quality including authenticity.

## 3. ION EXCHANGE RESINS

More recently, the application of a substitution process promoted by ion exchange resins, using cation exchangers, authorized since 2009 by Council Regulation (EC) No 606/2009 (EU, 2009), opened a new field of tartaric stabilization and pH adjustment in wines. The principle of this technique is the use of a cation-exchange resin in the protonated form, where the potassium ions in the wine are replaced by the protons from the resin. Commercial available resins for ion exchange equipment are based on a polymeric structure of styrene-divinylbenzene containing functional groups of sulphonic acid. Typically, the operation involves mixing a certain amount of wine treated by resins with the rest of the untreated wine. The amount of wine treated ranges usually from 10 to 20%, depending on the initial wine characteristics and must be evaluated in each case, in order to achieve the full potential of this technique.

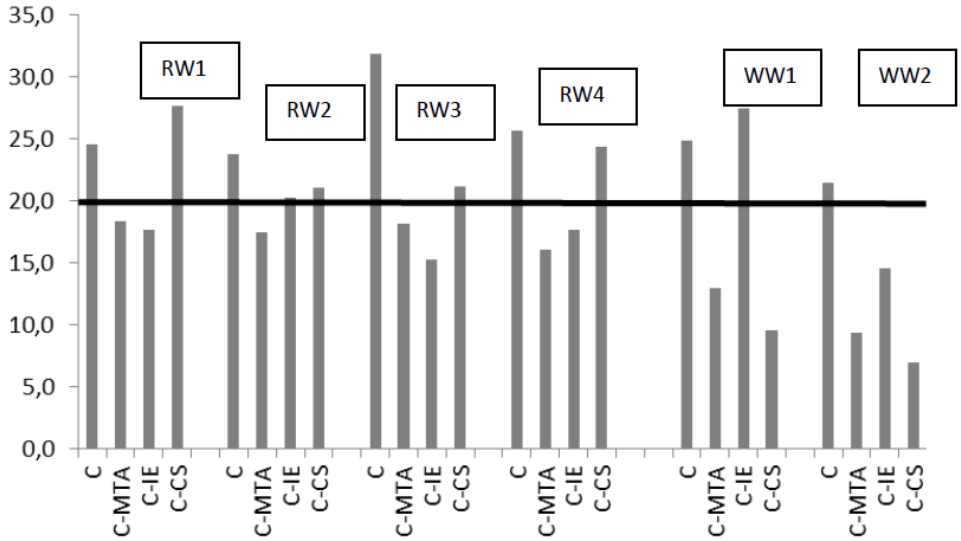
Only tartrate stabilization of wines by cation exchange is authorized by OIV (Oeno 1/93, Oeno 447/20119 (OIV 2015b) although other two ways can be performed to achieve tartrate stabilization: the use of an anion exchange resin replacing tartrate or other anions for $OH^-$, and a mixed treatment using a cation exchange and a anion one, replacing potassium cations and tartrate anions by $H^+$ and $OH^-$ (Mira et al., 2006; Lasanta et al., 2013). In fact, the OIV resolution concerning this subject (Oeno 443/2012) (OIV 2015b), although recognizing the

existence of cation exchangers and anions exchangers, only accept the use of cation exchanger with the purpose of stabilize the wine regarding to tartrate precipitation. Other two important effects/objectives can also be achieved: to lower pH of wines with low fixed acidity and high cations content, and to avoid metallic hazes.

Although tartaric stabilization is the main goal when ion exchange resins are used in wines, several other chemical aspects of wines are modified. Potassium ions are removed from the wines, allow achieving tartaric stabilization, but other cations like calcium, iron, manganese and copper are also removed (Benítez et al., 2002), although in a less extension. The consequence of this removal is related with a possible reduction of the susceptibility of browning that may affect wine characteristics, especially white wines. In fact, this is a non-enzimatic oxidation process of polyphenols containing a cathecol ring or a galloyl group, like (+)-catechin, (-)-epicatechin, gallocatechin, gallic acid and its esters, and caffeic acid, which are the most readily wine oxidized constituents (Oliveira et al., 2011). The process of its oxidation to semiquinones radicals and benzoquinones while oxygen is reduced to hydrogen peroxide is mediated by the redox cycle of $Fe^{3+}/Fe^{2+}$ and $Cu^{2+}/Cu^{+}$ (Oliveira et al., 2011). This cations removal can also be considered a positive side effect since removal of iron or copper can act as a preventive tool for metallic precipitations. In fact, these metals capture the interest of enologists not only as sources of wine instability, but by being central to the whole of wine chemistry (Danilewicz, 2003). In this sense, the removal of cations from the wine will not only promote the tartaric stability but also can prevent metallic precipitations involving ferric and cupric ions.

The wine tartaric stability that can be achieved by the use of cationic resins is essentially related to diminish $K^{+}$ content, meaning the initial amount of tartaric acid remains the same (Ibeas et al., 2015), and thus lowering the pH of the final wines which can be seen as a way to contribute to wine stability and durability over time. In general this variation in the pH of wines, *c.a*. 0.2 units is just enough to alter the anthocyanin equilibrium toward the flavylium forms resulting in wines with higher colour intensity and lower hue, according to the known effect that pH has on the colour of red wines (Heredia et al., 1998). Nevertheless, a decrease in phenolic compounds can also be observed. In a recent study Ibeas et al. (2015) reported that the content of individual anthocyanins decreased significantly with the increase in the percentage of wine treated with cation exchange resin. Lasanta et al. (2013) also observed a decrease in both anthocyanin and tannin content of the wines treated with a cation exchange resin. These authors also reported minor significate differences regarding volatile compounds, a small decrease in certain aromatic compounds, although in some cases these compounds were present at concentrations below their odour threshold, thus not imparting any positive or negative attribute to the wine aroma.

While the available literature on the effect of using cation exchange resins in wines is scarce, it appears to be consensual that from the chemical point of view this process is beneficial to wine characteristics, but the same statement cannot be done regarding sensorial characteristics. In fact, the real impact of treating wines with cation exchange resins in the organoleptic characteristics of the final wines is not yet clear. In reality, either due to an insufficient amount of articles related to this subject, or from the insufficient number of wines reported in the few literature existing, it becomes clear that research on volatile and aromatic composition of wines arising from treatments through cation exchange resins is necessary, as well data on sensory evaluation of the wines.




Legend: **C**- control wine; **C-MTA** – wine with metatartaric acid; **C-IE** – wine treated with ion exchange resin; **C-CS** – wine treated with cold stabilization; **RW** – red wine; **WW** – white wine.

Figure 1. Tartaric stabilization results by minicontact test results (μS $cm^{-1}$).

## 4. Determination of the Tartrate Stability

The simplest method to verify the efficacy of a stabilization treatment is to observe the stability of a wine sample stored at low temperature (Brugirard and Rochard, 1992). But this system is slow, difficult to reproduce and subjective. Most accurate methods to verify tartrate stability are based on conductivity techniques, namely saturation temperature measurements and/or minicontact test. However, it should be noted that both tests, saturation temperature determination (*Ts*) and minicontact test (*Mc*) are particularly suitable to assess the wine stability concerning KHT. In fact, there are still some difficulties/limitations concerning CaT. The saturation temperature (*Ts*) for KHT of the wine represents the wine saturation level of this salt, signifying low values of *Ts* high stability of the wines. *Ts* can be determined measuring the electrical conductivity during a cycle of increasing temperatures of two samples, a control and another one with added KHT, being *Ts* the temperature at which the conductivities of the two samples match up (García et al., 1991). The problem with this method is that many times, *Ts* doesn't correspond to the real stability temperature because of the large metastability of KHT and the presence of crystal growth inhibitors (Maujean et al., 1985), leading to differences ranging to differences from 5 to 12.5ºC in white wines and from 10 to 21.1ºC in red wines, according to Berta (1993). Results obtained by us show that these differences can range from 6.0ºC and 21.5ºC for white wines and from 8ºC to 18ºC for rose wines, while red wines always present higher values, above 20ºC. Also it is worthwhile to notice that cold stabilization led to smaller values of *Ts*, and that the addition of metatartaric acid increases the *Ts* values both in white and rose wines, while for the red wines its behavior is different, since no differences in *Ts* values where found either with mannoproteins or metatartaric acid addition or cold stabilization.

The minicontact test (*Mc*) measures the decrease in the conductivity of a wine kept at low temperature in contact with KHT (Angele, 1992). The wine is kept at 0 ºC in the presence of 4 g $L^{-1}$ of KHT to induce precipitation of this salt, which is quantified by conductivity measuring and plotting conductivity versus time, extrapolating up to an infinite time, and obtaining this way the maximum decrease in conductivity (*Mc*), which is the measure of the real stability. This author proposed that *Mc* higher than 40-45 μS $cm^{-1}$ indicates high risk of KHT sedimentation, and *Mc* values lower than 20-25 μS $cm^{-1}$ indicates stable wines. Moutounet et al. (2010) proposed to use the percentage of conductivity decrease instead of absolute values, considering stable wines when it's lower than 3%. The values obtained for *Mc* depend on the wine chemical characteristics in spite of the tartaric stabilization method used. Results obtained in our laboratory shows that for wines subjected to cold stabilization *Mc* values ranges from 8.2 to 27.8 μS $cm^{-1}$ for white wines, from 26.6 to 55.7 μS $cm^{-1}$ for rose wines and from 30.0 to to 55.7 μS $cm^{-1}$ for red wines. With the addition of 10 g $hL^{-1}$ of metatartaric acid, lower values where obtained, ranging from 4.6 to 11.1 μS $cm^{-1}$ in white wines, from 8.1 to 15.8 μS $cm^{-1}$ for rose wines and from 18.9 to 39.4 μS $cm^{-1}$ for red wines.

In another experiment conducted by us, we compared the use of cold stabilization, metatartaric acid addition and ion exchange resins in the tartaric stabilization of four red (RW1- RW4) and two white (WW1- WW2) different wines, respectively (Figure 1). Control wines (C) presented a variation in conductivity higher than 20 μS $cm^{-1}$, thus a real risk of tartaric precipitation can occurs. These remarks are made assuming that a drop in conductivity before and after KHT being added equal or smaller than 20 μS $cm^{-1}$ (Angele, 1992), means a very stable wine. The addition of metatartaric acid (C-MTA) induced a variation in conductivity of wines always smaller than 20 μS $cm^{-1}$, meaning that this technique can indeed have a positive effect on tartaric stabilization of wines. Cold stabilization (CS) gave origin to stable wines only when applied to white wines. Regarding the effect of the ion exchange resin treatment, only in white wine 1, seems not to be an effective technique. However this result can be easily explained if we take into consideration the amount of wine treated by ion exchange resin in relation of the total volume of wine. In WW1 only 10% of total volume of wine was treated by the ion exchange resins, while 15% was used for WW2. Regarding red wines the percentage of total wine treated by the ion exchange resins was 15% except for RW2 (12.5%). These results highlight the need to previously determine the percentage of wine to be treated by resins in order to achieve tartaric stability in total volume, as we stated before.

## CONCLUSION

Tartrate stability of wines must be ensured before bottling in order to prevent the appearance of crystals in the bottom of bottles. This phenomenon is especially important to prevent in white wines, because consumers generally drink white wine cold, and the low temperature promotes the growth of tartrate crystals and their precipitation.

There are several techniques that can be applied to wines aiming to achieve stable wines, based on different principles: removing chemical entities implied in precipitation of tartrates or adding substances preventing tartrate precipitations. All the techniques available for

winemakers have positive and negative impacts in wine characteristics, and choose one technique over another should be a conscientious decision.

An important final remark is that winemakers and oenologist must keep in mind that all consideration concerning tartrate stability of wines, either the techniques to achieve wines stability or the methods to verify the tartaric stabilization of a given wine, are in fact, devoted to tartaric precipitations involving potassium cations. The role of the calcium cation in the precipitation of tartrates is still not yet fully understood, and unlike HTK precipitations, CaT precipitations are not so well predictive. The use of severe filtration systems just before bottling wines and the consequent removal of naturally occurring protective colloids in wines may be pointed out as an explanation to justify a late appearance of some CaT in bottled wines in spite of being considered stabilised at bottling time.

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