Master of Science in Viticulture & Enology

Joint diploma "EuroMaster Vinifera" awarded by:

INSTITUT NATIONAL D'ETUDES SUPERIEURES AGRONOMIQUES DE MONTPELLIER
AND
INSTITUTO SUPERIOR DE AGRONOMIA DA UNIVERSIDADE DE LISBOA

Master thesis

Comparison between six different Carboxymethylcelluloses used as enological products for the tartaric stabilization of red wines

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2014-2016

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Lisbon, 23/11/2016

Abstract

The carboxymethylcellulose (CMC) is a cellulose derivative authorized in white and sparkling wines production as a tartaric salts crystallization inhibitor. Previous studies report negative effects of the product when added to red wines; more specifically, it has been seen that the CMC decreases the content of total phenols, flavonoid and non-flavonoid phenols, reducing the colour intensity; it interacts with the phenolic compounds, promoting turbidity and colorant matter precipitation.
In order to evaluate if these effects characterize all the red wines when in contact with the product, we studied in detail the impact of six different CMCs, coming from six Portuguese oenological companies on the same Portuguese red wine, Castelão variety. The research has been focused on the evaluation of the wine responses in terms of tartrate and colouring matter stability, turbidity, phenolic compounds, tannins' composition, tannin power, chromatic and sensory characteristics.
The CMC resulted as a strong inhibitor of tartaric salts crystallization, even after 5 months from the addition. It generally reported an increase in the colour intensity of the wines, as such as in the coloured anthocyanins concentration. Therefore, the CMCs treated wines revealed a stronger and powerful colour. No colouring matter precipitation occurred. The total phenols concentration of the CMCs added samples did not completely differ from the control wine, as such as the tannins' composition in terms of monomeric, oligomeric and polymeric fractions content. In terms of sensorial quality, the CMC treated wines did not reveal any important differences compared to the control.
The study revealed that the CMC represents a valid sustainable enological alternative to stabilize the red wines in terms of tartaric salts crystallization. The positive results achieved are in opposition with the ones obtained in most of the previous studies, opening new prospective and scenarios concerning the effects of the CMC utilization on red wines.

Key words: Carboxymethylcellulose, Tartaric stabilization, Red wines, Phenolic composition, Chromatic characteristics

Resumo

A carboximetilcelulose (CMC) é um derivado da celulose autorizada na produção de vinhos brancos e espumantes como inibidor da cristalização dos sais tartáricos. Estudos anteriores reportam alguns efeitos negativos do produto quando adicionado aos vinhos tintos; mais especificamente, tem sido observado que a CMC diminui o teor de fenóis, fenóis flavonóides e não flavonóides, reduzindo a intensidade da cor; o produto interage com os compostos fenólicos, promovendo a precipitação da matéria corante e turbidez.
Neste estudo foram avaliadas seis diferentes CMCs num vinho Português, casta Castelão. A investigação tem sido focada na avaliação das respostas do vinho em termos de estabilidade tartárica, matéria corante, compostos fenólicos, composição dos taninos, poder tanante, e das características cromáticas e sensoriais.
A CMC resultou como um forte inibidor da cristalização dos sais tartáricos. Geralmente, as CMCs reportaram um aumento na intensidade e nos pigmentos vermelhos. Portanto, os vinhos tratados com as CMCs reportaram uma forte cor vermelha e nenhuma precipitação da matéria corante ocorreu. A concentração de fenóis totais nas amostras com a adição da CMC não se demonstrou completamente diferente do vinho testemunha, como a composição dos taninos em termos de frações monoméricas, oligoméricas e poliméricas. Em termos de qualidade sensorial, os vinhos tratados com as CMCs não revelaram diferenças importantes em relação ao controlo.
O estudo revelou que a CMC representa uma alternativa enológica sustentável válida, para estabilizar os vinhos tintos em termos de cristalização dos sais tartáricos. Os resultados positivos alcançados estão em oposição com os obtidos na maioria dos estudos anteriores, abrindo novas perspectivas e cenários sobre os efeitos da utilização CMC em vinhos tintos.

Palavras-chave: Carboximetilcelulose , Estabilização tartárica , Vinhos tintos, Composiçao fenolica, Características cromáticas

Extended abstract

The carboxymethylcellulose (CMC) is a widely used oenological product and it represents one of the possible treatments applied in winemaking to stabilize the wines in terms of tartaric salts precipitations, mainly potassium bitartrate (KHT) and calcium tartrate (CaT). It is considered as a sustainable alternative to most of the stabilization treatments because of its advantages to be cheaper, less energy consuming, easier to apply compared to, for example, cold stabilization, ion exchange resins and electrodialysis.

However, its addition is only allowed to treat white and sparkling wines (Commission Regulation (EC) N° 606/2009). According to previous studies, the CMC utilization has been noticed to negatively affect the red wines' quality. More specifically, being a polysaccharide with protective colloidal characteristics, it has been seen that the CMC interacts with the phenolic compounds and with the unstable proteins, decreasing the content of total phenols, flavonoids and non-flavonoids, reducing the colour intensity and promoting turbidity, change in colour and colorant matter precipitation.

In order to evaluate if these responses can characterize all the red wines when in contact with the product, the effectiveness of six different CMCs on a Portuguese red wine, Castelão 2015, has been analysed.
The study, conducted over a 5- months period, represents a wide and complete research, considering that several analytical estimations, followed by a sensorial evaluation of the wines, have been performed.
The initial wine has been characterized considering the conventional oenological parameters described by the O.I.V. methods: pH, total acidity, volatile acidity, alcoholic strength, reducing substances content, SO_2 concentration (total and free).
After the addition of the products and one-week period of contact, the effects of the treatments on the following oenological parameters have been evaluated: i) the tartaric stability has been estimated using a modified mini-contact test described by Angele (1992); ii) the turbidity assessment has been performed applying a method exposed by the O.I.V. (1990); iii) the colouring matter stability has been tested referring to the method displayed by Claus et al. (2014); vi) the proanthocyanidins fractionation has been characterized following the Sun method (Sun et al., 1998); v) the tannin power has been evaluated using the method exposed by Freitas & Mateus (2001); vi) the colour intensity and tonality, the anthocyanins (total and coloured) content and the pigments (total and polymerized) content have been estimated according to the methodology described by Somers & Evans (1977); v) the phenols (total, flavonoids and non-flavonoids) content has been determined using the method shown by Singleton et al. (1971).
A sensory analysis has been set up to understand how the quality of the wine in terms of aroma and mouthfeel sensations could have been effected by the presence of the products.

The results obtained during the research have shown that, in opposition with most of the previous studies, the CMCs reported general positive effects on the wines' composition. It has been found that all the products were highly efficient as tartaric salts crystallization inhibitors, even after a 5-months contact period. The colour intensity, as such as the coloured anthocyanins and the polymerized pigments content, generally increased. Furthermore, no colouring matter precipitation occurred. However, the turbidity of the treated wines has slightly increased. Regarding the total phenols concentration of the CMCs added samples, it did not completely differ from the control wine, as such as the tannins' composition in terms of monomeric, oligomeric and polymeric fractions content.

Moreover, in terms of sensorial quality, the CMC appeared to not strongly affect the aromatic characterization of the wine and its mouthfeel sensation. All the treated wines have been described as similar to the control sample, with a slightly decreased aromatic intensity and a higher acidity concerning the taste. However, all the samples have been described with positive characteristics.

In conclusion, according to the results obtained in this study, it can be said that the CMC has been found to be a valid alternative to stabilize the red wines in terms of tartaric salts crystallization. Furthermore, the positive results achieved are in opposition with most of the previous studies, opening new prospective and scenarios concerning the effects of the CMC utilization on red wines.

Resumo alargado

A carboximetilcelulose (CMC) é um produto enológico amplamente utilizado e representa um dos possíveis tratamentos aplicados na produção de vinhos para estabilização em termos de precipitações de sais tartáricos, principalmente bitartarato de potássio (KHT) e tartarato de cálcio (CAT). Pelas suas vantagens de ser um produto mais económico, fácil de aplicar, e com menores custos associados, em comparação com outras tecnologias, como a estabilização a frio, eletrodiálise e resinas de troca iônica, a CMC é considerada como uma alternativa sustentável para a maioria dos tratamentos.

No entanto, a sua adição atualmente é apenas permitida para tratar os vinhos brancos e espumantes (Regulamento (CE) N° 606/2009). De acordo com estudos anteriores, a utilização da CMC tem sido associada a efeitos negativos na qualidade dos vinhos tintos. Mais especificamente, sendo um polissacarído com características coloidais de proteção, tem sido observado que a CMC interage com os compostos fenólicos e com as proteínas instáveis, diminuindo o teor de fenóis, flavonóides e não flavonóides, reduzindo a intensidade da cor e promovendo a turbidez, a mudança de cor e a precipitação da materia corante.

A fim de avaliar se estas respostas podem caracterizar todos os vinhos tintos, quando em contato com o produto, a eficácia de seis CMCs diferentes num vinho tinto Português, Castelão 2015, foi analisada.
O estudo, realizado ao longo de 5 meses, representa uma ampla e abrangente pesquisa, considerando a análise de vários parâmetros, seguida de uma avaliação sensorial dos vinhos. O vinho inicial caracterizou-se considerando os parâmetros enológicos convencionais utilizando os métodos da O.I.V.: pH, acidez total, acidez volátil, teor alcoólico, teor de substancias redutoras, concentração de SO_2 (total e livre). O efeito da aplicação da CMC no vinho foi avaliado após uma semana de contacto. Para cada CMC aplicada, foram avaliados os seguintes parâmetros enológicos: i) estabilidade tartarica: utilizou-se um teste de mini-contato modificado, descrito por Angele (1992); ii) turbidez: aplicou-se o método de referência da O.I.V. (1990); iii) estabilidade da matéria corante: aplicou-se o método apresentado por Claus et al. (2014); iv) fracionamento das proantocianidinas: aplicou-se o método Sun (Sun et al, 1998).; v) poder tanante: foi avaliado utilizando o método exposto por Freitas & Mateus (2001); vi) intensidade da cor, tonalidade, antocianinas (totais e coloridas) e os pigmentos (totais e polimerizados) foram estimados de acordo com a metodologia descrita por Somers & Evans (1977); vii) os fenóis (totais, flavonóides e não flavonóides) determinaram-se pelo método de Singleton et al. (1971). A análise sensorial efetuou-se, com o objetivo de perceber se a qualidade dos vinhos, em termos de aroma e sabor, tinham sido afetados pela adição das CMCs aplicadas.

Os resultados obtidos durante a pesquisa mostraram que, em oposição com a maioria dos estudos anteriores, os CMCs relataram efeitos positivos na composição dos vinhos. Verificou-se que todos os produtos foram altamente eficientes como inibidores da cristalização dos sais tartáricos, mesmo depois de um período de contacto de 5 meses. Em relação a intensidade da cor, as antocianinas coradas e o teor de pigmentos polimerizados, verificou-se um aumento. Além disso, não ocorreu precipitação da matéria corante, mas a turbidez dos vinhos tratados aumentou ligeiramente. A concentração de fenóis das amostras adicionadas com a CMC, não se verificou completamente diferente do vinho controlo, tal como a composição dos taninos em termos de conteúdo em fracções monoméricas, oligoméricas e poliméricas.

Além disso, em termos de qualidade sensorial, a CMC pareceu não afetar fortemente a caracterização aromática do vinho e o seu sabor. Todos os vinhos tratados têm sido descritos como semelhante ao controlo, com uma diminuição da intensidade aromática e uma acidez ligeiramente superior. No entanto, todas as amostras foram descritas com características positivas.

Em conclusão, de acordo com os resultados obtidos no presente estudo, pode afirmar-se que a CMC é uma alternativa válida à estabilização dos vinhos tintos em termos de cristalização dos sais tartáricos. Além disso, os resultados positivos alcançados estão em oposição com a maioria dos estudos anteriores, abrindo novos cenários e perspetivas sobre os efeitos da utilização da CMC nos vinhos tintos.

Acknowledgments

I wish to thank the following people and organisations for their contribution to this project: J.M. Ricardo da Silva and Mario Caldeira Andrade for supporting this study, following the researches; Daniel Duarte, Diana Faria, Francisco Amorim, Juliane Oliveira and the Instituto Superior de Agronomia for facilitating the analytical work; AEB Bioquìmica Portuguesa S.A., Agrovin S.A., Angelo Coimbra & Ca. Lda, Ecofiltra Lda, Proenol Indùstria Biotecnològica Lda, SAI, for supplying the products analysed; and Sociedade Agrìcola de Rio Frio S.A. for providing the wine used for the experiments. .

Furthermore, I would love to thank the jury (Professor Carlos Lopes, Professor Sofia Catarino and Professor Celotti Emilio) and all the Vinifera EuroMaster members: directors, secretaries and professors, to have given me the possibility to be part of this important experience and moreover to have followed me during the whole master period.

A special thanks goes to the people that always encourage and support my decisions: my family and my friends.

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I. INTRODUCTION

1. Introduction

The tartaric precipitation phenomena have been more and more studied in the past years, because of the increasing wine production and quality need and for the greater importance of wine business and marketing researches.

The tartaric precipitation event under natural conditions is an unreliable, unpredictable phenomenon that occurs during the entire period of the wine's production, from the fermentation to the conservation period. Its occurrence in bottled wines, in form of sediments, can have negative consequences to the final aspect of the wine. Even if the tartaric salts do not represent a risk for human's health and do not cause any organoleptic deterioration, some inexpert consumers doubt about the authenticity of the product, not appreciating it in terms of visual characteristics. Therefore, it can represent a cause of wine depreciation by some consumers.
In the less serious cases, the presence of few and small crystals can be observed, that especially in the red wines do not represent a risk. In other cases, abundant and important crystallizations can occur becoming really evident, especially in white and rosé wines. The problem is all the more serious when it comes to traditional methods, where the crystals can be a source of "garbage" (gushing), i.e. sparkling wines produced with Champenoise method. As an example, in Champagne, over 90% of the Chateaux stabilize their wines in terms of tartaric salts (www.institut-oenologique.com).

The tartaric precipitation phenomena have a common origin: the presence of tartaric acid (H_2T) and of the two cations potassium (K^{+2}) and/or calcium (Ca^{+}).
The tartaric acid is considered the "wine acid", being the specific acid of the grapes; its concentrations in the musts are variable from 2-3 g/l when they come from southern vineyards, to 6 g/l in the ones coming from northern vineyards (Ribéreau – Gayon et al., 2006).
The potassium (K^{+2}) is the major cation taken up by grapevines and, therefore, the principal cation present in the wine. Its concentration varies from 0.5 to 2 g/l (Boulton et al., 1996; Ribéreau – Gayon et al., 2006).
Regarding the calcium (Ca^{+}), according to Boulton et al. (1996) and Ribéreau – Gayon et al. (2006), its security limit concentration in wines is equal to 80 mg/l. A high presence of this cation is due to: i) deacidification of the must with calcium carbonate; ii) storage of the wine in concrete tanks; iii) addition of oenological products containing calcium (i.e. Ca bentonite); iv) filtration with equipment containing calcium; v) accidental contaminations.

The major physical instabilities related to tartrates precipitations are linked to potassium bitartrate (KTH) and calcium tartrate (CaT) presence. However, other salts can be responsible for the precipitation in a less extent, such as potassium tartrate, double potassium calcium tartrate and calcium tartromalate.
The reasons that could lead to tartaric precipitation phenomena are several (Boulton et al., 1996):

- Initial instability of the salts that is enhanced by high ethanol content, low temperatures used for storage and high pH values
- Incomplete stabilization in the cellar

- Use of non- representative samples for the stability tests
- Use of non- appropriate stability tests
- Removal of colloidal materials that were preventing the precipitation.

Despite the fact that some producers decide not performing any stabilization treatments to their wines, i.e. natural wines or high quality wines for niche markets, the choice of stabilization before bottling became almost imperative for most of the producers in the last years.
Nevertheless, it is known that the stabilization could lead to flat wines or could negatively affect the product in terms of sensorial characteristics; as a result, it is important to choose the more suitable treatment (i.e. length, concentration of the additives) to the wine.

One of the treatments used to stabilize the wines in terms of tartaric precipitations is the application of Carboxymethylcellulose (CMC), an additive with the property to be a salts crystallization inhibitor.
It is already widely used as food additive but only recently, in 2009 (Commission Regulation (EC) N° 606/2009), it has been allowed to treat white and sparkling wines. Several are the advantages that characterize this product: it is cheaper, less energy consuming, more sustainable and easy to apply compared to other methods (i.e. cold stabilization, use of ion exchange resins, electrodialysis) (Lasanta & Gòmez, 2012). In the paragraph 2.3.3.3. its synthesis, its application method, its oenological characteristics and finally its effects on wine will be reported.

In reference to the introductory part of the work, it was considered necessary, in the interests of a greater internal consistency, making the state on some issues, as reported below.
In the paragraph 2.1., considering the importance of potassium bitartrate (KHT) and calcium tartrate (CaT) in tartrates precipitation, the formation and the eventual sedimentation of these salts is exposed.
In the paragraphs 2.2. and 2.3., the different tests to assess the tartaric stability of a wine and the different methods to achieve it are exposed.
In the paragraph 2.4., the wine's phenolic composition is exposed.
In the paragraph 3, the research objectives are reported.

2. State of art

2.1. Tartaric instabilities phenomena

2.1.1. Formation of the salts

As reported by Lasanta & Gòmez (2012): “Different equilibriums related to the dissociation of tartaric acid (H_2T) and the precipitation of potassium bitartrate (K_2T) and calcium tartrate (CaT) coexist in wines”.

The dissociation of the tartaric acid is regulated by the thermodynamic equilibrium of its three forms: i) undissociated form (H_2T); ii) hydrogen tartrate form (TH^-) and iii) tartrate form (T^{2-}) (Boulton et al., 1996).

$$H_2T \leftrightarrow H^+ + TH^-$$

$$TH^- \leftrightarrow H^+ + T^{2-}$$

The ratio of each tartaric acid ion present in solution depends on the pH conditions and can be determined using the three expressions (Lasanta & Gòmez, 2012):

Where $c = [H_2T] + [HT^-] + [T^{2-}]$
K_1 and K_2 = *thermodynamic dissociation constants based on alcoholic strength and temperature conditions*

$$[H_2T] = \frac{c}{1 + \frac{K_1}{[H^+]} + \frac{K_1 \cdot K_2}{[H^+]^2}} \qquad [HT^-] = \frac{c}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}} \qquad [T^{2-}] = \frac{c}{1 + \frac{[H^+]}{K_2}\left(1 + \frac{[H^+]}{K_1}\right)}$$

Therefore, it appears clear that according to pH, alcoholic strength and temperature conditions, the tartaric acid is more or less salified with K^{+2} and Ca^+ in the five forms above mentioned (potassium bitartrate, calcium tartrate, potassium tartrate, double potassium calcium tartrate and calcium tartromalate).

2.1.2. Crystallization and precipitation kinetics

As reported by Boulton et al. (1996), the crystallization of KHT and CaT involves three phases:

1. Saturation phase
2. Nucleation phase
3. Growth phase

1. Saturation phase

The solubilisation of the KHT and CaT salts is regulated by the equilibriums:

$$KHT_{cryst} \leftrightarrow K^+ + HT^-$$

$$CaT_{cryst} \leftrightarrow Ca^{+2} + T^{2-}$$

1. $CP = [HT^-]_r\,[K^+]_r \qquad SP = [HT^-]_e \times [K^+]_e$
2. $CP = [T^{2-}]_r\,[Ca^{+2}]_r \qquad SP = [T^{2-}]_e \times [Ca^{+2}]_e$

Where *CP = concentration of the product*
r = real concentrations
SP = solubility product
e = concentrations obtained at the thermodynamic equilibrium of the:
1. *KHT/ dissolved KHT*
2. *CaT/dissolved CaT*

under temperature and pressure conditions of the wine.

When the concentration of the product of the real concentrations (CP) is higher than the solubility product (SP), the wine is defined as supersaturated. In a supersaturation condition, the excess salts precipitate until the CP equals the SP. In other words, the precipitation stops when the equilibrium between CP and SP is reached.

In the table below (table 1), the solubility of tartaric acid, potassium bitartrate and calcium tartrate in a) H_2O and b) hydro alcoholic solution (10% vol.) at 20 ° C is reported.

Table 1: Solubility of H_2T, KHT, CaT in a) H_2O and b) hydro alcoholic solution (10% vol.) at 20 ° C.

	Tartaric acid	**Potassium bitartrate**	**Calcium tartrate**
	$L(+)\text{-}C_4H_6O_6$	$KHC_4H_4O_6$	$CaC_4H_4O_6, 4H_2O$
Solubility in H_20 at 20 °C	4.9 g/l	5.7 g/l	0.53 g/l
Solubility in 10% vol. hydro alcoholic solution	Data not shown	2.9 g/L	Data not shown

Adapted from Ribérau – Gayon et al. (1998).

It is clear that the solubility of the potassium bitartrate drastically decreases in ethanol conditions.

3. Nucleation phase

This phase consists in the formation of a small crystal, known as nucleous. It represents an interface between solid and liquid phases, which is responsible of all the exchange reactions.The creation of the nucleous is a highly- energy required phase (Ribéreau-Gayon et al., 2006).

Two types of nucleation phenomena can occur:

- Spontaneous or primary nucleation: it corresponds to the spontaneous formation of a nucleous when TH^- and K^+, or T^{2-} and Ca^{+2} are present in the wine at the supersaturation limit.
- Induced or secondary nucleation: it corresponds to the formation of a nucleous induced by the presence of small particles in the wine. When the small particles have the same chemical nature as the salt, homogenous secondary nucleation occurs; on the contrary, when they have different nature as the salt, the phenomenon is known as heterogeneous secondary nucleation.

4. Growth phase

Once formed, the nuclei become stable and start growing. During the growth, the ions K^+ and TH^- or Ca^{+2} and T^{-2} are incorporated. Because of the higher presence of K^+ and/or Ca^{+2} cations on their surface, the crystals are defined as positively charged nuclei, attracting negatively charged molecules.
Higher the nucleous surface, higher the crystallization rate.

2.1.3. Factors affecting the crystallization of KHT and CaT

Several factors affect differently the crystallization kinetics of the two salts KHT and CaT.

- pH: the solubility of the two salts strongly depends on the pH. Higher is the pH, lower is the solubility because the dissociation of the acidic functions is higher (Sicheri, 2015).
  For the KHT, the crystallization is facilitated when the pH value is between 3.5 and 4 because of the maximal TH^- proportion (Boulton et al., 1996; Ribéreau–Gayon et al., 2006).
- Temperature: the KHT solubility, compared to the CaT, is more influenced by the temperature conditions. T decreases promote the KHT insolubilization (Boulton et al., 1996; Ribéreau–Gayon et al., 2006).
- Alcoholic strength: KHT and CaT solubility is inversely proportional to the alcoholic strength. According to Sicheri (2015), a wine with 12-13% vol. of alcohol dissolves half of the tartaric salts, compared to the initial must.
- Ionic strength: KHT and CaT solubility is directly proportional to the ionic strength of the wine (Boulton et al., 1996; Ribéreau – Gayon et al., 2006).
- Stirring: by increasing the nucleation speed, stirring increases the crystallization rate (Boulton et al., 1996; Ribéreau – Gayon et al., 2006).
- Colloidal composition of the wine: the colloids play an important role in the crystallization of the salts. They inhibit the crystallization. The inhibition effect is more important in red wines because of the higher concentration of colloidal particles, such as condensed tannins (Boulton et al., 1996; Ribéreau – Gayon et al., 2006).

2.2. Tests to assess the tartaric stability

During the years, the oenological research has worked worldwide trying to find tests that could assess the tartaric stability. Nowadays there are several analytical options available for evaluating cold stability, the two most commonly used methods are concentration product and conductivity.

2.2.1. Refrigerator test

The traditional and simplest test, called “Refrigerator test” is based on the storage of the wine (100 ml), taken before or after artificial cold stabilization, in a refrigerator for 4-6 days at 0° C and then inspected for crystals. If crystals are observed the wine is considered unstable. It is a simple, practical and easy-to-perform method that does not require special equipment.

Nevertheless, it is a long and qualitative process that does not provide information about the instability degree of the wines; therefore, studies have been conducted to find tests that could lead to faster and more precise results (quantitative) (Boulton et al., 1996; Ribérau-Gayon et al., 2006).

2.2.2. Conductivity tests

In the respect of what exposed above, the conductivity tests have been developed.

As reported in www.etslabs.com (2013): "All the conductivity methods are based on indirect measurement of potassium ion concentration changes. Potassium ions are primarily responsible for the electrical conductivity in wine. When potassium bitartrate crystals form, potassium ion concentration drops, along with the conductivity. This change is used to estimate a wine's cold stability. Conductivity test methods vary based on temperature, time, seeding method, and interpretation of results. A wine sample is chilled, and seeded with finely ground potassium bitartrate (KHT) crystals, also known as cream of tartar. Potassium bitartrate naturally present in the wine attaches to these added crystals, causing a corresponding drop in the wine's electrical conductivity. Large drops in a wine's conductivity indicate that it is more likely to form KHT crystals at cold temperatures and is less likely to be cold stable".

2.2.2.1. Mini- contact test

The first conductivity test used to assess the tartaric stability is represented by the Mini- contact test (Müller-Spath, 1979; Angele, 1992). This test consists in the addition of 4 g/l of potassium bitartrate, maintaining the wine at a temperature of 0° C for 2 hours, constantly agitating. The increase in weight of the KHT collected (exogenous KHT and endogenous KHT) is assessed. The test is based on homogeneous induced nucleation, faster than primary nucleation. As exposed by Boulton (1996), the test tends to overestimate the wine's stability. It was observed that after the 2 hours' contact, only 60-70% of the endogenous tartrate has crystallized.

Despite the fact that this is a simple and moderately reliable test, it is relatively long, it does not take into account the size of the seed tartrate particles and it defines the stability of the wine at 0 °C at the time of testing, making no confirmation about the colloidal reorganization during storage and wine aging.

2.2.2.2. Modified mini- contact test

This test is run seeding the wine with 10 g/l of KHT and measuring the drop in conductivity at 0 °C after a 5-10-minute time period. When conductivity drops by less than 5% over the test period, the wine is generally considered stable, although wineries often use a more stringent criterion of 3% (Angele, 1992; Ribérau-Gayon et al., 2006; www.etslabs.com, 2013). Wines falling within this 5% change window can be considered stable only at or above the specified conductivity test temperature. Lack of a standardized test temperature is a limitation of the conductivity tests. In addition, it does not take into account the particles' size and it is based on a relatively short test time.

This will be the test used during this study.

2.2.3. Saturation temperature test (Wurdig test)

The Wurdig test is also defined as “Saturation temperature test” because it is based on the concept that the lower is the saturation temperature of a wine, the more potassium bitartrate it is capable of dissolving at low temperature, the less supersaturated it is and therefore, the more stable it is considered in terms of tartaric precipitations (Ribérau-Gayon et al., 2006).

The Wurdig test consists in the assessment of the saturation temperature. The saturation temperature, according to Wurdig et al. (1982), Boulton et al. (1997) and Ribérau-Gayon et al. (2006), is determined using a two-step experiment:

1st step: the wine is cooled to a temperature of approximately 0 °C in a thermostat controlled bath equipped with sources of heat and cold. The temperature is then raised to 20 °C in 0.5 °C increments and the wine’s conductivity is measured after each temperature change.

2nd step: the wine is brought to a temperature close to 0 °C, 4 g/l of KHT crystals are added and the temperature is raised to 20 °C in 0.5 °C increments and the wine’s conductivity is measured after each temperature change.

As a result, the experiment gives two conductivity curves. As shown in the Figure 1, the intersection point of the two curves is considered as the Saturation Temperature (Ribérau-Gayon et al., 2006).

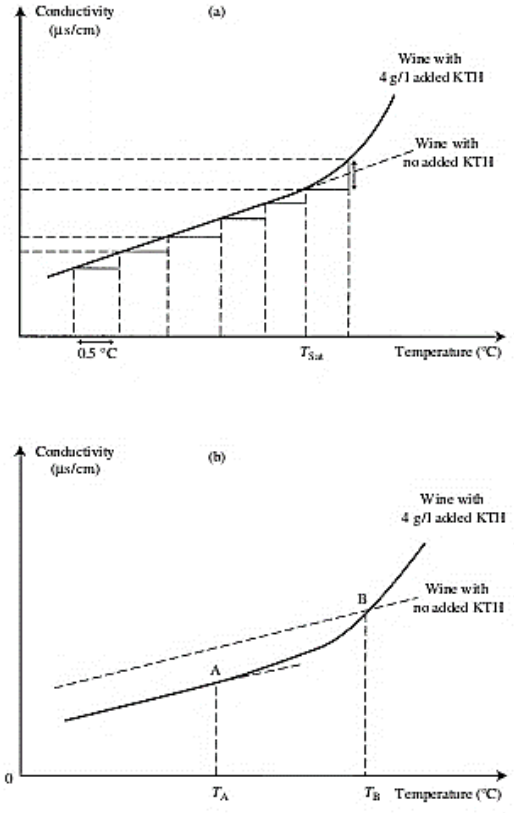


Fig. 1: Experimental determination of the saturation temperature of a wine by the temperature gradient method (Wurdig et al., 1982). a) Example of a wine that is not highly supersaturated, in which no induced crystallization occurs immediately after the addition of calcium potassium tartrate crystals; b) Example of a highly supersaturated wine, in which induced crystallization occurs immediately after the addition of calcium potassium tartrate crystals;
Extracted from Ribérau-Gayon et al. (2006).

Being the Wurdig test long, complex and difficult to perform, an adaptation of the method is used on the production scale. It consists in the utilization of two equations (the first for the white wines and the second for rosé and red wines), based on statistical studies on several hundreds of wines.

$$\mathbf{1^{st}: T_{sat} = 20 - \frac{(\Delta L) at\ 20°C}{29.3}}$$

It is only applicable to wines where the solubilization temperature of KHT is between 7 and 20 °C.

$$\mathbf{2^{nd}: T_{sat} = 29.91\ \frac{(\Delta L) at\ 30°C}{58.3}}$$

Where *ΔL = variation in the conductivity of a wine at 20°C or 30°C before and after the addition of 4 g/l of KHT*

Therefore, it appears clear that lower the saturation temperature, higher the wine tartaric stability.

2.3. Tartaric stabilization treatments

2.3.1. Evolution of the tartaric stabilization treatments

In the last decades, the tartaric stabilization treatments have been characterized by an important evolution.

In the second edition of the book "*Analyse et contrôle des vins*" (1958), Ribéreau – Gayon & Peynaud write: *"Dans le vins jeunes, blanc ou rouges, il peut se déposer sous l'action du froid du tartrate acide de potassium ou crème de tartre, sous forme de petits cristaux lamellaires ou massifs, faciles à reconnaître à leur aspect microscopique, à leur solubilité à chaud et à leur réaction acide. Leur chute est très rapide et permet de les séparer par un simple décantage. Pour prévoir cette précipitation, on expose un échantillon à basse température, au- dessous de 0° C et un voisinage du point de congélation du vin, en prenant soin d'ensemencer avec une trace de poudre de cristaux de bitartrate de potassium. On laissera au froid plusieurs semaines. On a intérêt à opérer sur un vin filtré au préalable".*

In few words, these two authors considered the cold treatment with addition of traces of potassium bitartrate powder as the only procedure to stabilize the wine in terms of KHT precipitation.

Being the cold stabilization a time and energy consuming process (Boulton et al., 1996; Ribéreau–Gayon et al., 2006; Lasanta & Gòmez, 2012), the research continuously worked on new treatments, cold treatment alternatives, aiming the reduction of environmental impact and time requirement. In other words, studies have been conducted trying to find faster and environmental sustainable options, in terms of energy saving.

For this reason, around the 1990s, the electrodialysis treatment has been developed (Gavach, 1992; Guerif, 1993), as such as the ion exchange resin one (Mourgues, 1993).

Furthermore, studies have been reported based on the addition of substances that prevent crystal precipitation: addition of metatartaric acid (MTA), carboxmelhylcellulose (CMC) and yeasts' mannoproteins (YMP) are the three treatments allowed.

Therefore, it appears clear that since 1950s an important evolution occurred, completely changing the tartaric stability treatments scene.

However, not all of these cold treatment alternatives are economical advantageous.

The tartaric stability can be achieved with:

- Physical methods i) using treatments based on the removal of the tartaric acid and the related salts, as the Cold treatment or ii) using treatments that remove cations K^+ and Ca^{2+}, necessary for the precipitation of the tartaric acid, as Ion exchange resins and Electrodialysis.
- Chemical methods, adding substances that prevent the crystals formation: metartaric Acid (MTA), yeasts mannoproteins (YMP), carboxymethylcellulose (CMC).

Nevertheless, while they are all effective against KHT precipitation, some of them do not have the same effects when the CaT instability is treated.

The CaT instability is more difficult to predict, control and avoid than KHT. Its nucleation time is longer; therefore, its precipitation tends to occur later, usually after various years in the bottles.

Furthermore, while the CaT crystallization can induce the KHT one, the contrary is not true (Ribéreau–Gayon et al., 2006).

2.3.2. Physical treatments

The physical treatments represent the most ancient methods developed to achieve the tartaric stability and, therefore, to remove the tartaric acid and the related salts.

The three physical treatments are represented by the cold treatment, the ion exchange resins system and the electodialysis.

The three treatments are based on the removal of the tartaric acid, the tartaric salts or the ions present in super-saturated form from the wine.

2.3.2.1. Cold treatment

As reported by Blouin (1982) and Lasanta & Gomez (2012): “Cold treatment is the most widely used technique for stabilization by cooling the wine to a temperature close to its freezing point and storing it for a time between 3 days and 3 weeks, being 1 week the most common”.

As already mentioned in the paragraph 3.3: “the KHT solubility, compared to the CaT, is more influenced by the temperature conditions. T decreases promote the KHT insolubilization”. Therefore, the cold treatments are not really effective for CaT (Maujean et al., 1984) and for some red and sweet wines with high colloidal content (Usseglio-Tommaset et al., 1980).

The cold stabilization can be performed with three possible procedures:

1. Slow cold stabilization, without tartrate crystal seeding
2. Rapid cold stabilization with tartrate crystal seeding: static contact process
3. Rapid cold stabilization: dynamic continuous contact process

These three treatments have a common base concept: cooling down the wine to a temperature near the freezing point to reduce the solubility of the tartrate salts far below their solubility constant and to force the crystallization, precipitation and successive separation of excess salts (Blouin, 1982, Lasanta & Gomez, 2012).

The freezing temperature of the wine is empirically determined according to the expression:

$$\textbf{Freezing temperature (°C)} = \frac{-[alcoholic\ strenght - 1]}{2}$$

Usually, it is between -4 °C and -5 °C.

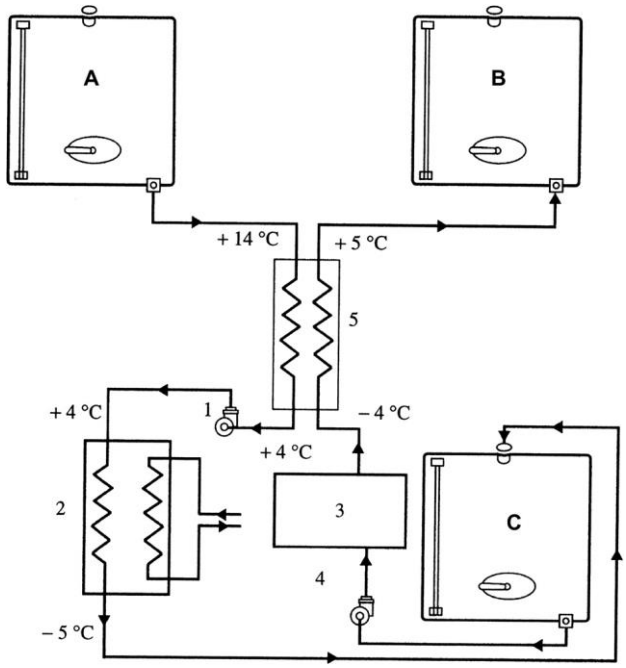


Fig. 2. Schematic diagram of a cold stabilization installation: A, untreated wine (+ 14 °C); B, treated wine (+ 5 °C); C, wine during stabilization (- 5 °C); 1, untreated wine pump; 2, treating wine at - 5 °C (refrigeration system and plate heat exchanger); 3 filter at the end of the cold treatment; 4, filtered wine pump; 5, heat exchanger for precooling wine to be treated by using it to warm treated wine.
Extracted from Ribéreau-Gayon et al. (2006).

1. Low cold stabilization, without tartrate crystal seeding

This process consists in cooling the wine close to the freezing temperature to induce spontaneous nucleation (endogenous KHT nucleous). The refrigeration of the wine is usually carried out by a cooling equipment (shown in Figure 2) composed by a heat interchanger in direct contact with the evaporation chamber (ultracooler), which instantly chills the wine. This thermal shock is necessary to increase the effectiveness of the treatment (Blouin, 1982; Gomez et al., 2004).

To achieve the goal, tanks with insulating layer ensuring a maximum temperature increase of 0.8 - 1.0 °C per week are used, or, otherwise, the tanks are located in a thermally insulated chamber with a cooling unit.

Therefore, it appears clear that this cold treatment method is a very slow process: 2-3 weeks are needed to achieve the tartaric stabilization.

Its effectiveness depends on wine composition, where the colloidal content plays an important role.

Furthermore, using this technique there is i) a risk of excessive oxidation as oxygen dissolves more rapidly at low temperature and ii) a decrease of the colour intensity due to the simultaneous precipitation of the polyphenols together with the KHT salts (Ribereau-Gayon et al., 2006).

2. Rapid cold stabilization with tartrate seeding: static contact process

In order to increase the effectiveness of the slow cold treatment, finely divided crystals of KHT can be added to the wine.

This technique was proposed by Müller-Spath in 1979 and it consists in the addition of a dose equal to 4 g/l of KHT and constant stirring. The temperature is kept between 0 and 1 °C and the optimum duration is 5 hours. In this way, the temperature of the treatment increases and time required is largely reduced. According to Blouin et al. (1979), the KHT can be reused at least twice for red wines and up to 8 times for white wines, although a reduction in the effectiveness in each reuse estimated in 20 µS/cm can characterize the treatments (Garcia et al., 1991). The size of the KHT crystals used should be between 50 and 100 µm, since crystals smaller than 10 µm do not have any effect (Blouin, 1982). According to this author, the contact method has similar results to the conventional treatment and does not produce any adverse effect on the sensorial characteristics of the wine.

The same method can be applied adding CaT crystals; this process also induces the precipitation of KHT (Minguez & Hernandez, 1998), although it is expensive and requires a duration between 2 and 7 days (Viaux et al., 1996; Müller et al., 1997; Lasanta & Gòmez, 2012).

The crystals added act as crystallization nuclei, therefore their action is to attract K^+ and TH^- or Ca^{+2} and T^{2-} on the surface (Blouin et al., 1979; Lasanta & Gòmez, 2012).

3. Rapid cold stabilization: dynamic continuous contact process

This treatment is a continuous KHT stabilization process.

The contact time of crystals (4 g/l) with wine (under agitation), is regulated by the volumetric flow rate of the crystallizer, and by the supersaturation state of wine (i.e. throughput= 60 hl/h; volume of crystallizer= 90 hl; treatment time = 1 h 30 min). As for the “Rapid cold stabilization with tartrate seeding: static contact process”, the used KHT crystals should have a size between 50 and 100 µm (generally 60 µm) (Blouin, 1982).

In the figure below (Figure 3), the scheme of a Rapid cold stabilization treatment in dynamic contact is shown.

This treatment, being continuous, is more demanding than the other processes in terms of operational control (Ribéreau-Gayon et al., 2006):

- The particle size of contact tartrate and the level in the crystallizer must be monitored by sampling after a few hours.
- A method of monitoring the effectiveness with a very short response time is required; if the treatment is insufficiently effective, wine can be recycled through crystallizer.

Despite the fact that this treatment requires close monitoring, it is also very efficient.

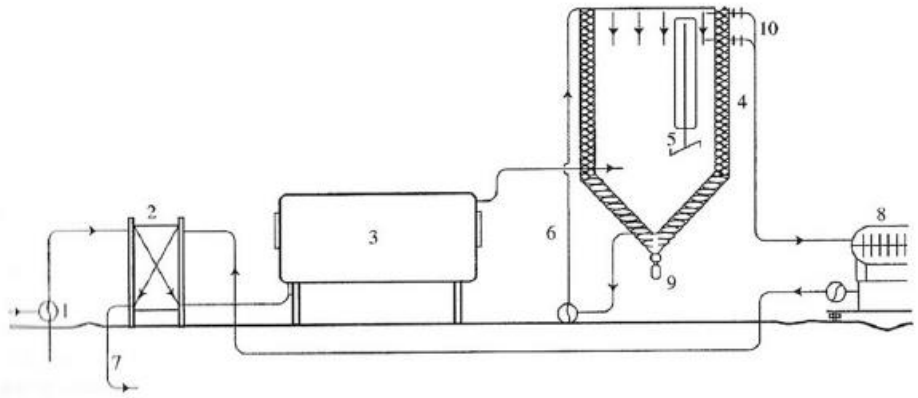


Fig. 3: Schematic diagram of a continuous cold stabilization system: 1-intake of wine to be treated; 2-heat exchanger; 3-refrigeration system; 4-insulation; 5-mechanical agitator; 6-recycling circuit (optional); 7-outlet of treated wine; 8-filter (earth); 9-drain; 10-overflow.
Extracted from Ribéreau- Gayon et al. (2006).

2.3.2.2. Ion exchange resins

Ion exchange resins treatment is based on an electro physical principle, as exposed by Lasanta & Gòmez (2012): the resins have a polymeric matrix and several covalently attached ionized functional groups; these groups can be electrically neutralized by mobile ions of opposite electrical charge or "against-ion" that can be exchanged with the ions of the wine.

There are two types of resins:

- Cationic resins that exchange cations having sulphonate ($-SO_3^-$) or carboxylic ($–COO^-$) as functional groups
- Anionic resins that exchange anions having $-NR^{3+}$, NHR^{2+}, $-NH_2R^+$ as functional groups.

Several studies (Mourgues, 1993; Mira et al., 2006), over the years, report that the possible ion resins exchange treatments are the following:

- Replacement of potassium by hydrogen in potassium bitartrate, with a cationic resin in acid cycle
- Replacement of the tartrate anion by a hydroxyl group with an anionic resin in basic cycle
- Mixed treatment: replacement of potassium by hydrogen and tartrate by hydroxyl with two resins, one cationic and another anionic.

However, the only ones authorized for wines treatment by the EU are the cationic resins in acid cycle (Commission Regulation (EC) N° 606/2009), and the most used are the ones with a sulphonate functional group ($-SO_3^-$).

The efficacy of the treatments depends on the characteristics of the resins (Ribéreau–Gayon et al., 2006): porosity, grain size, distensibility, valence of the ion exchanger (the ease of exchange increases with the valence of the exchanger ion: $Na^+ < Ca^{2+} < Al^{3+}$) and atomic number of the ion exchanger (the ease of exchange increases with the atomic number).

To be used in winemaking, the resins should be characterized by mechanical strength, total insolubility in wine and the absence of off-flavours, furthermore, they should be capable of being regenerated many times (Ribéreau–Gayon et al., 2006).
As shown by several researchers (Mourgues, 1993; Gòmez et al., 2002, Ribéreau–Gayon et al., 2006), the treatments with cationic resins in acid cycle effect the pH and the cations concentrations, decreasing their values. Thus, in recent years, this technique has been used not only for tartaric stabilization treatments, but also to modify the pH of red and white wines (Benítez et al., 2002; Walker et al., 2002; Mira et al., 2006; Bruijn et al., 2009; Lasanta et al., 2013; Ibeas et al., 2015).

Nevertheless, this property leads to the necessity of mixing treated wines with untreated wine in order to obtain an equilibrated and stable wine. Mourgues (1993) and Gòmez et al. (2002) suggest mixing 10–20% of treated wine with the rest. With a higher percentage of treated wine, as exposed in a study conducted by Ibeas et al. (2015), the taste persistence decreases, as well as the aroma fineness, the aroma intensity, the taste body and the taste equilibrium.

However, even if the use of the resins can have a slight effect on aromas and on the colour of red wines, decreasing total and individual anthocyanin (Walker et al., 2002; Mira et al., 2006), these treatments are effective; furthermore, being characterized by a reduction in time and energy consuming, they are seen as a sustainable physical alternative to cold treatments.

2.3.2.3. Electrodialysis

Several researchers have exposed the principle behind the electrodialysis treatments (Moutounet et al., 1994; Ribéreau – Gayon et al., 2006; Lasanta & Gòmez, 2012): “the ions present in the wine in a super-saturated form can move under the action of an electric field with the help of membranes permeable only of anions on one hand, and membranes permeable only to cations on the other hand”.

The Figure 4 represents the scheme of an electrodialysis equipment: a large number of elementary cells (up to 500) composes it; each cell has two compartments, a dilute chamber and a concentrate chamber, delimited by an anionic and a cationic membrane arranged alternately. Two electrodes are placed at the end of the stacking chambers; a difference of potential is applied to the electrodes, producing the migration of cations and anions from the wine to the electrolyte.
Hence, the wine flows parallel to the membrane in the dilution chambers, and the ions contained are moved into the adjacent chambers (concentrate), where are retained. Thus, the wine as it flows in the dilute chambers is progressively depleted in K^+ ions, whereas in the concentrate chamber, its concentration increases.

The treatment is stopped when the concentration of ions is reduced to the desired level.

The ions reduction is determined measuring the conductivity of the wine, more specifically using the deionization rate (DR) percentage. Therefore, the DR measures the intensity of the treatment; it is also used to regulate the intensity of the treatment (Lasanta & Gòmez, 2012).

As an example, in a study conducted by Moutonet et al. (1997), DR of 24.5%, 26.7% and between 8.4 and 13.2% were needed, respectively for white, rosé and red wines.

At the end of the treatment, the wine should be characterized by:

- A maximum alcohol content reduction of 0.1% vol
- A maximum pH reduxtion of 0.25
- A maximum volatile acidity reduction of 0.11 g/l of acetic acid.

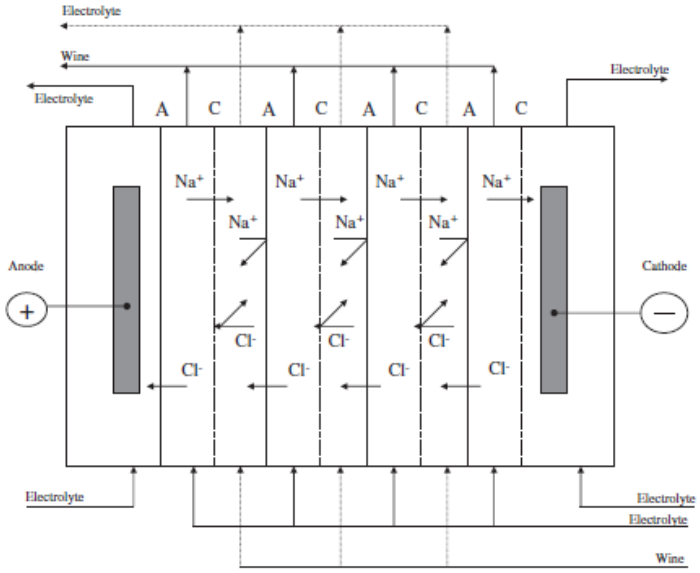


Fig. 4: Scheme of an electrodialysis equipment. A: Anionic membrane; C: Cationic membrane. Extracted from Guerif et al. (1993).

2.3.3. Chemical treatments

Tartaric stabilization could also be achieved by the addition of substances that prevent crystals precipitation, either by inhibiting their formation or by modifying their properties (i.e. shape) and making them soluble at lower temperatures.

The additives' action takes place in the nucleation phase of the crystallization kinetic.

2.3.3.1. Metatartaric acid (MTA)

Metatartaric acid has been the first additive used as tartaric stabilization treatment, concerning the chemical methods (Goertges & Stock, 2000).

MTA is the product of a partial esterification of tartaric acid produced by heating it at 170°C; this procedure is responsible for the prevention of the nuclei's growth. Preventing the nuclei's growth, the crystals stabilization and successive precipitation is blocked (Ribéreau-Gayon et al., 2006).

The esterification reaction of the tartaric acid is reversible; this means that tartaric acid may be formed again by hydrolysis, consecutively causing instability. It appears clear that the main drawback of a treatment with

MTA is represented by its low stability in the wine over time (Ribéreau-Gayon et al., 2006; Gerbaud et al., 2010).

Hence, the MTA effectiveness is strongly dependent on (Ribéreau-Gayon et al., 2006):

- The esterification rate: it is more affective at higher rates
- The temperature: it may last from one week at 30 °C to 2 years at 0°C, being from 1 year to 18 months at common cellars' temperatures (10°C – 18°C)

For these reasons, the MTA treatments are performed on wines to be consumed in a short period and stored at low temperatures (Ribéreau-Gayon et al., 2006; Lasanta & Gòmez, 2012).

According to Maujean et al. (1985), 10 g/hl of MTA is the maximum dose to make the wine stable in terms of KHT and CaT precipitations.

2.3.3.2. Yeast Mannoproteins (YMP)

Yeast mannoproteins are polysaccharides that present a protein portion about 100 kDa in apparent size. They are the major polysaccharide group present in wine, being part of the cell walls of *Saccharomyces cerevisiae.*

They can be present in wine for several reasons:

- Yeast's liberation during the fermentation (Escot et al., 2001; Ribéreau-Gayon et al., 2006; Quirós et al., 2010)
- Yeast's liberation during ageing sur lees by autolysis (Ribéreau-Gayon et al., 2006)
- They can be added to the wine as commercial preparation to i) prevent protein haze; ii) enhance and interact with some wine aromas; iii) soften the astringency and v) inhibit tartaric salts crystallization (Ribéreau-Gayon et al., 2006; Bouisson et al., 2007).

According to Gerbaud et al. (2007), when added to treat the tartaric instability, YMP act binding to nucleation points and preventing the expansion of the crystal structure, therefore affecting the crystal growth.

As exposed by Moine-Ledoux & Dubordieu (2002), the YMP application is not characterized by the MTA drawback: it is considered stable over time; in addition, as mentioned above, it can improve other features of wine quality, such as protein stability, polyphenolic stability and sensory attributes, especially aromas.

The application doses should be kept between 15 and 25 g/hl (Moine-Ledoux & Dubordieu, 1999).

2.3.3.3. Carboxymethylcellulose (CMC)

2.3.3.3.1. Synthesis

CMC is a cellulose derivative that is synthesised by the reaction of cellulose with chloroacetic acid in basic solution. It is obtained by the etherification of the free primary alcohol groups of the glucopyranose units linked by β (1-4) glycosidic linkages.

CMC is widely used as additive in food industry as it is not degraded or reabsorbed by humans.

2.3.3.3.2. Addition in wines and oenological characteristics

CMC use in wine for tartrate stabilization has been studied since the 1980s (Asvany, 1986; Gerbaud et al., 1996) as an economic and environmental sustainable alternative to cold stabilization.

CMC effect in wine is based on the inhibition of the tartaric salts crystallization. Its mechanism of action has been studied over the years, and according to the Institut Œnologique de Champagne, as soon as the crystals are created, CMC deposits on certain surfaces, altering their dimensions: two of the seven crystal faces are lost. The potassium or bitartrate ions can no longer increase the size of the salts and the crystal is flattered. Furthermore, being defined as a negatively charged molecule at the wine pH, CMC acts by binding with electropositive surface of the KHT crystals or by complexing potassium, decreasing the amount of free ions involved in the salts crystallization (Rodriguez-Clemente & Corea-Gorospe, 1988; Chachereau et al., 2001).

In other words, being the CMC (as MTA and MP) a protective colloid, it binds the crystals to the nucleation points, inhibiting the growth.

According to Ribéreau-Gayon et al. (2006), the CMC action and effectiveness are strongly linked to two properties known as DS (substitution degree) and DP (polymerization degree). The DS value expresses the number of carboxylate groups present on the molecule, therefore, the number of anchor sites involved in cation complexation (Lubbers et al., 1993): higher the DS, higher the CMC's effectiveness. When applied in winemaking, the CMC has to be characterized by a DS between 0.60 and 0.95 (OIV resolution Oeno 366-2009) (OIV, 2015a). The DP indicates the molecule's size and it has an important influence on the product's viscosity; in the specific, higher the DP and the molecule's weight, higher the viscosity and the facility to apply the product (Bosso et al, 2010).

Nevertheless, as exposed by Moutounet et al. (2010) and Guise et al. (2014), the ions composition characterizing the CMC plays an important role in its effectiveness: high concentrations of divalent cations, such as calcium, magnesium and iron could lead to an interfering effect on the CMC activity.

It can be added to the wine in two forms: i) granular/fibrous powder form; ii) liquid form: can be diluted with wine to the required volume of the product, which can then be added to the wine tank with homogenisation.

As reported in the OIV resolution (OIV-Oeno 366-2009), the solutions applied must contain at least 3.5% of CMC and the maximum legal dose allowed is equal to 100 mg/l; therefore, prior to use, the products in granular form are generally diluted in water to reach concentrations of 50 g/l or 100 g/l equal to 5% or 10% of

CMC; however, when in liquid forms, the products are already prepared and present concentrations of 50 - 100 g/l.

2.3.3.3.3. CMC effects on wines

The use of CMC is allowed since 2009 (Commission Regulation (EC) N° 606/2009), although until now only for white and sparkling wines to a maximum dose of 100 mg/l. The following, the effects on white, rosé and red wines are reported.

According to several researchers (Bosso et al., 2012; Greeff et al., 2012; Guise et al., 2014), the CMC's addition is effective preventing the formation of the crystals.

Guise et al. (2014) analysed the CMC's effect on white wines coming from two Portuguese wine regions: Douro Valley and Vinho Verde. They reported a positive effect of CMC, in terms of tartaric stabilization of the wines, as shown in the Figure 5.

During the study, Guise and the team used a mini contact test to assess the tartaric stability of the wines.

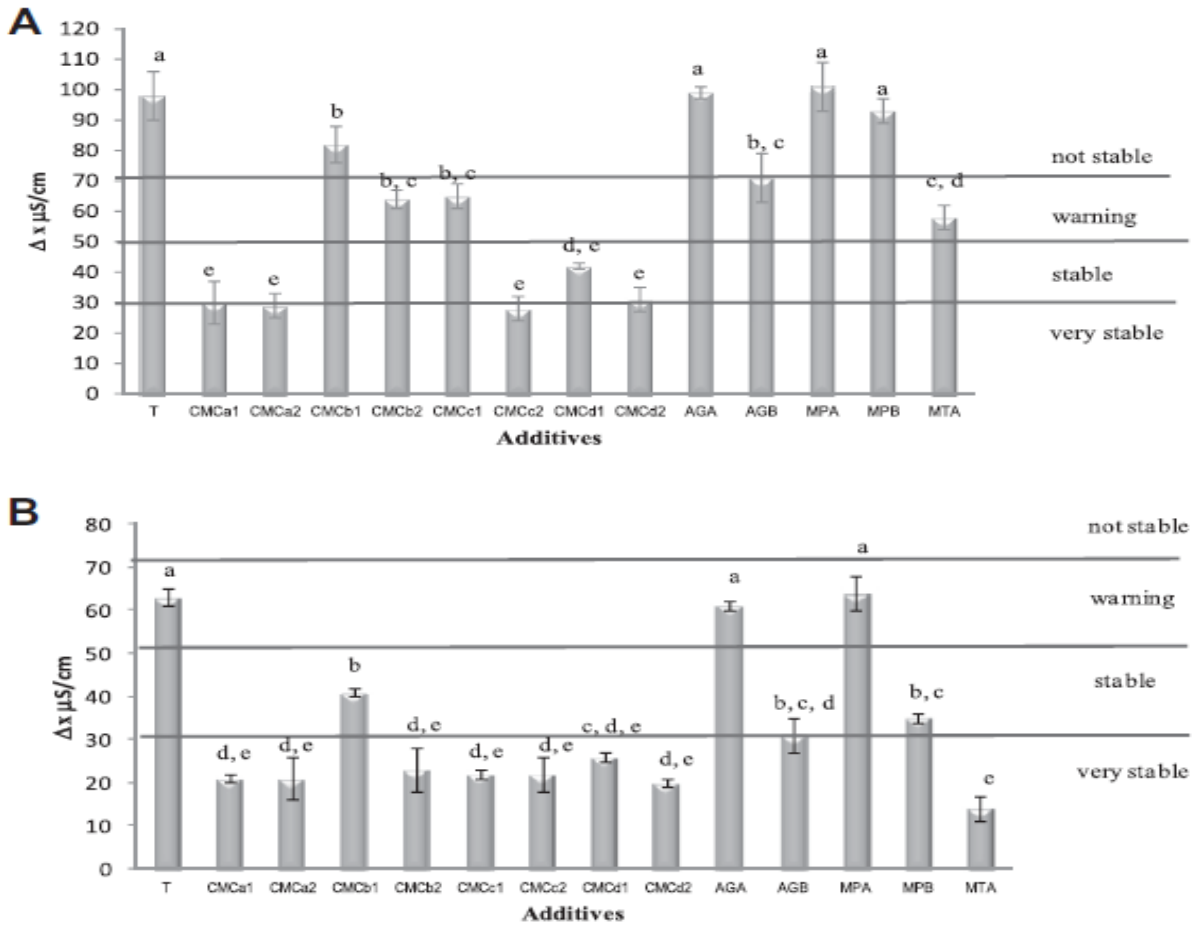


Fig. 5: Effect of oenological additives on wine tartaric stability of two white wines (A – Vinho Verde, B – Douro). Untreated wine (T), CMC solution at 20% (CMCa), CMC solution at 4% (CMCb), CMC solution at 5% (CMCc), CMC solid power (CMCd), Arabic gum (AGA) Arabic gum (AGB), Mannoprotein (MPA); Mannoprotein (MPB); Metatartaric acid (MTA), 1 – medium concentration, 2 – high concentration. The variation of electric conductivity (Dx), expressed in ls/cm, indicated the level of stability (Dx), < 30 very stable, 30–50 stable, 50–70 warning and >70 not stable. Means for each wine followed by the same letter are not significantly different (Tukey, $p < 0.05$).
Extracted from Guise et al. (2014).

According to the wines and the CMC's composition (potassium content, DS etc…), its addition can be more or less effective. It appears clear that, as represented in the Figure 5, all the CMCs treatments were effective for Douro Valley wines. This is due to the lower potassium concentration and higher calcium and magnesium content characterizing the wines.

On the contrary, some CMCs were not effective for Vinho Verde wines. Therefore, it is important to choose the right dose and type of CMC to add.

Moreover, it is important to consider the wine that has to be treated, in order to be conscious about the results and to not lose money. In that respect, Bosso et al. (2012) conducted a study on CMC's addition on Chardonnay and Pinot Blanc wines. As shown in the Figure 6, the effects "Dose of CMC" and "Kind of CMC" were more significant for Pinot Blanc than for Chardonnay, due to the lower pH and alcoholic content characteristics of the Pinot Blanc wines.

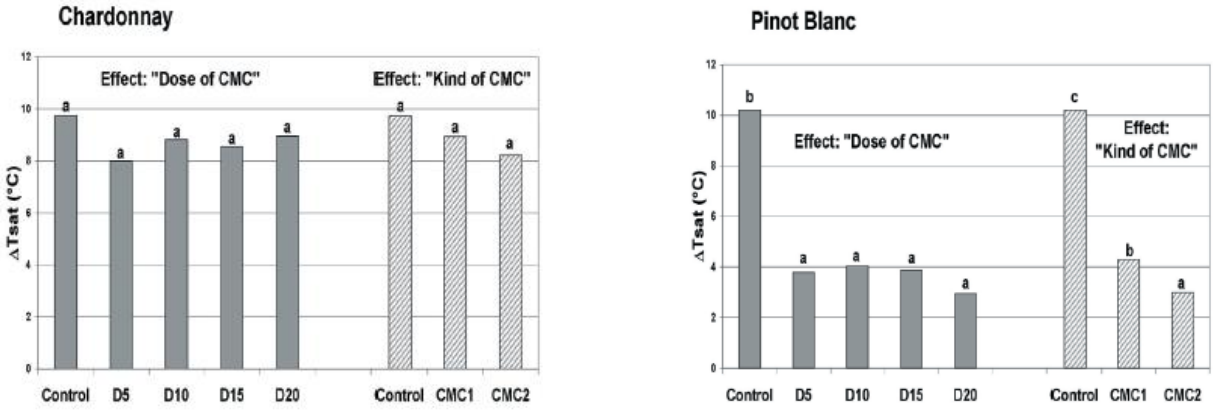


Fig. 6: Mean variation (n = 4) of the saturation temperature (ΔT_{sat}) after 10 d at -4 °C in the treatments added with different doses of CMC (Effect of the dose of CMC: Control=0 g/hl; D5=5 g/hl; D10=10 g/hl; D15=15 g/hl and D20=20 g/hl), and mean variation (n = 8) of the saturation temperature (ΔT_{sat}) in the control and in the treatments added with 2 types of CMC (Effect of the kind of CMC). 1st experiment. Different letters indicate significant statistical difference ($P \leq 0.05$), separately for the 2 studied factors (dose and kind of CMC).
Extracted from Bosso et al. (2012).

Greeff et al. (2012) evaluated the CMC's efficiency as a crystallisation inhibitor when added to different Pinotage wines (white wine with no skin contact and Blanc de Noir with skin contact for 6 hours) made from the same grapes. The CMC tested, as exposed by the team, "showed a good efficiency for most of the samples, with a higher dosage of CMC required to prevent a significant loss of K^+ concentration in Blanc de Noir wines". Furthermore, Greeff et al. (2012) analysed the effect of CMC's addition on aged commercial wines (Sauvignon blanc and Chenin blanc wines aged for 12 months): the results showed that K^+ concentration was significantly higher for the CMC-treated wines, compared with the control, proving the CMC's efficiency as a crystallisation inhibitor.

Nevertheless, Guise et al. (2014) showed that CMC generally decreases the content of total phenols, flavonoid and non-flavonoid phenols, reducing the colour intensity. More specifically, in the red wines, CMC interacts with the phenolic compounds, promoting turbidity, change in colour and colorant matter precipitation (Claus et al., 2014). It is for this reason that the CMC addition is not yet allowed for red wines treatments.

But at the same time, on the other hand, after the addition of CMC on Pinotage and Syrah red wines, Greeff et al. (2012) reported that: “No clear tendencies regarding CMC’s effectiveness in reducing K^+ and H_2T losses in the Pinotage red wines were observed (results not shown). However, in the Syrah wine the CMC treatment led to significant higher K^+ and H_2T concentrations in the wines stored at -4 and 15 °C as well as those stored at only 15 °C. However, visual observations in the two red wines were impaired by colour precipitate, which made accurate visual crystal formation assessments difficult. Thus, CMC seems to be an efficient crystal inhibitor in some South African red wines, which correlates with the findings of Moutounet et al. (2010) and Motta et al. (2009). However, more research is required to establish the effectiveness of CMC on the crystal formation in red wines, as this is not the case in all red wines. Colour and total phenol data showed decreases that were mostly insignificant, showing that the possible decreases in red wine colour by CMC is not a given in all red wines”.

According to the O.I.V. (Commission Regulation (EC) N° 606/2009), the addition of the CMC as tartaric salts crystallization inhibitor is not authorized for the production of commercial red wines. Being this research an academic work, the utilization of the product has been authorized by the Instituto Superior de Agronomia with the aim of verifying its influence on the chemical and sensorial composition of a Castelão variety wine.

Therefore, based on what exposed above, this research will help understanding the behaviour of the analysed red wine when treated with different CMCs coming from different suppliers.

Concerning rosé wines, no literature could be found regarding the efficiency of CMC as crystallisation inhibitor.

2.4. Main red wine’s phenolic compounds

The wine phenolic’ composition plays an important role in the description of the wines and, in the specific, in the analysis of the wine’s responses to the CMC addition.

The proanthocyanidins (PA), also known as condensed tannins or 3 -flavanols, are flavonoids compounds of extreme importance in enology. They have been studied over the years and some researches show their effects on the wines characteristics and on human health. According to their characteristics, specifically their degree of polymerization (DP) (Haslam, 1974; Porter and Woodruffe, 1984; Okuda et al., 1985; Haslam & Lilley, 1988; Masquellier, 1988; Robichaud & Noble, 1990; Rigaud et al., 1993), they are involved in several reactions:

- They impact the haze formation, interacting with proteins (Oh & Hoff, 1986; Yokotsuka & Singleton, 1987; Powers et al., 1988; Jouve et al., 1989; Ricardo da Silva et al., 1991b; Singleton, 1992) as such as they impact the colour stability (Timberlake & Bridle, 1976; Singleton & Trousdale, 1992)
- They impact astringency and bitterness (Haslam, 1974; Arnold & Noble, 1978; Arnold et al., 1980; Singleton, 1992), oxidation and browning (Oszmianski et al., 1985; Cheynier et al., 1988; Lee and Jaworski, 1988; Cheynier & Ricardo da Silva, 1991). Higher the concentration of catechins, higher the

bitterness; on the contrary, higher the concentration of procyanidins and polymerized tannins, higher the astringency

- They also influence the wines aging behavior (Haslam, 1980).

Furthermore, some studies showed their beneficial effects on arteriosclerosis (Masquellier 1982, 1988) and their radical-scavenging ability (Ricardo da Silva et al., 1991c).

Moreover, the tannins produce stable combinations with proteins, polymers and polysaccharides (Ribéreau-Gayon et al., 2006); these combinations are of extreme importance because they are responsible of the "aggressivity" of the wine. The index used to express the reaction between the tannins present in the wine and the salivary proteins (especially mucin), is the tannin power. As exposed by Kaushal (2014): "The result of this reaction is the formation of insoluble aggregates which can precipitate, blocking lubrication of palate leading to sensation of dryness and constriction"; therefore, when the value is too high, it is generally correlated with a non- balanced and "aggressive" wine.

Not only the tannins but also the anthocyanins and the reaction products between these two compounds are important parameters to study in the evaluation of the wine's response to the CMC addition.

The anthocyanins are flavonoids compounds known as the wine's coloured pigments; therefore, they are the compounds responsible for the colour of the red wines (Ribéreau-Gayon et al., 2006). According to the wine pH and SO_2 levels, the anthocyanins can show several structures and, as a consequence, different colours.
In the specific, the equilibrium between the four structures reported below is responsible for the colour of the anthocyanins (Ribéreau-Gayon et al., 2006):

- in acidic solutions of pH conditions around 3, they are characterized by a red colour, given by the higher presence of the red flavylium cation form
- in pH conditions between 3.2 and 3.5 the equilibrium switches to the colourless carbinol, responsible for a colour loss and for the decolouration of the anthocyanins
- in solutions with a pH higher than 4, the quinonic base form is the most relevant, responsible for a malva - blue colour
- In alkaline or neutral soutions, the colour switches into yellow, given by the chalcones forms.

Therefore, it appears clear that the anthoycanins responsible for the red colour are the ones under the flavylium cation form, also known as ionized or coloured anthocyanins.

Moreover, the various forms of anthocyanins can react between them or with other substances, such as tannins, producing, as a result, an increase in the colour intensity and a colour shift towards violet – blue.
The reaction between anthocyanins and flavanols (or condensed tannins) can occur in two different ways:

- directly: the anthocyanin and the tannin react forming either an A^+-P adduct or a P-A^+ adduct, according to the compounds position. In the case of the A^+-P adduct, the anthocyanin reacts under the flavylium cation form; in the case of the P-A^+ adduct, it is under the form of the carbinol base
- indirectly: the reaction between anthocyanin and flavanol is mediated by an acetaldehyde molecule.

These reactions' products, extremely important because of their strong contribution to the colour of the young red wines, are defined as co-pigmented compounds and they are much more stable compared to the free anthocyanins (Ribéreau-Gayon et al., 2006).

In conclusion, for all these reasons, the CMCs effect on the tannins and anthocyanins' composition will represent an important part of this study.

3. Research objectives

After what exposed above, the main goal of my research is to assess the effectiveness of six different CMCs to prevent tartaric precipitation in a Portuguese unstable red wine, vintage 2015, Castelão variety, coming from Sétubal Peninsula and to analyse its effects on tartrate and colouring matter stability, phenolic compounds, tannins' composition, anthocyanins' content, chromatic and sensory characteristics.
The six products come from six different Portuguese companies specialized in the production and commercialization of oenological products.

This will represent a fundamental and innovative study, considering that for the first time six different products have been analysed on only one unstable red wine. Moreover, this research will show a quite complete and diverse analytical approach in the CMC effects estimation, since potential influences on several important chemical characteristics and on the sensory quality of the red wine in exam have been evaluated.
Furthermore, being the CMC still not authorized in the production of red wines (Commission Regulation (EC) N° 606/2009), the results coming from this research will offer a starting point for future studies and experiments with the aim of verifying the eventual authorization of the addition of this additive also in red wines. Additionally, the results could provide important information to the wine industry to select appropriate solutions to optimize the tartaric stabilization process and thereby improving wine quality reducing the costs and being more sustainable.

II. MATERIALS AND METHODS

1. Wine's characteristics

The whole research has been performed using a Portuguese red wine coming from Herdade do Rio Frio, vintage 2015, Castelão variety. The vineyards are located in the Sètubal Peninsula, specifically in Pinhal Novo. The Sètubal Peninsula is characterized by a Mediterranean climate: hot and dry summers, pleasant but rainy winters and high humidity levels during the year.

Concerning the winemaking process, after the manual harvest, the grapes have been submitted to crushing and destemming processes and SO_2 and toasted French oak powder have been added.
After the addition of exogenous yeasts, the alcoholic fermentation (AF) started; its temperature has been kept around 18°C. During the fermentation process, délestage (once per day for four days) and remontage (five minutes per hour for three days) processes have been performed.
When the alcoholic fermentation stopped, the malolactic (MLF) one occurred; the temperature during the MLF has been kept at 18°C.

When the malolactic fermentation stopped, the SO_2 has been added and the wine has been transferred into a stainless steel tank to be stored.

2. CMCs addition and characteristics

Six different CMCs, coming from six different suppliers, have been analysed. In the research, they will be indicated with: CMC1, CMC2, CMC3, CMC4, CMC5, CMC6.
In table 1, the most important products' characteristics (concentration of CMC, DS, [SO_2] and pH) are reported.
Because of the different concentrations of CMC presented by the products, a dose equal to 0.2 g/l has been applied for CMC1, CMC3 and CMC5; while a dose of 0.1 g/l has been added for the CMC2 and CMC4. Regarding the CMC6, because of the lack of information regarding its concentration, a volume of product equal to 2 ml/l has been considered for the addition. The addition has been performed directly in the 0.75 l bottles and a contact period of 5 days at 16-18°C has been established.

Table 2: Main characteristics (concentration of CMC, DS, [SO_2] and pH) of the CMCs.

Colonna1	Concentration (%)	DS	[SO_2]	pH
CMC1	10	0.6-0.9	3.0 g/l ± 0.3	3.8±0.2
CMC2	5	0.6-0.9	2.0 g/l ± 0.5	3.8±0.2
CMC3	10	Data not known	2-4 g/l	3.7-4.7
CMC4	5	Data not known	≥10 mg/kg	Data not known
CMC5	10	>0.85	2 - 5 g/l	6.5-7.5
CMC6	Data not known	Data not known	Data not known	Data not known

Extracted from the CMCs technical brochures.

Concerning the visual density, the CMC4 is characterised by the highest density, followed by CMC1, CMC6, CMC5, CMC2 and CMC3, the least dense product. More detailed information is presented in the Annex 2: Technical brochures.

3. Analyses of conventional oenological parameters

Before the addition of the CMCs, the control wine has been analysed. The most important physicochemical parameters, such as Total Acidity (TA), Volatile Acidity (VA), Alcoholic Strength, pH, Total and Free SO_2 and Reducing Sugars have been determined, according to the O.I.V. methods, to characterize the wine.

Here below a short description of the methods used for the detection and their importance have been reported.

- TA: Method OIV-MA-AS313-01. It is based on a titration with an indicator, bromothymol blue, and comparison with an end-point colour standard.
- VA: Method OIV-MA-AS313-02. It is based on the separation of the volatile acids by steam distillation followed by a titration using standard sodium hydroxide. The acidity of free and combined sulfur dioxide distilled under these conditions should be subtracted from the acidity of the distillate. The acidity of any sorbic acid, which may have been added to the wine, must also be subtracted. Carbon dioxide is first removed from the wine.
- Alcoholic Strength: Method OIV-MA-AS312-01B. The method is based on the principle that the ethanol has a depressive effect on the boiling point of the wine and that, therefore, the difference in temperature between the boiling point of the wine and the boiling point of distilled water is related to the alcoholic content of the wine. The boiling point of water is determined first by filling the ebulliometer with distilled water and bringing it to the boiling point. This temperature is recorded as temperature at 0.0% alcohol. The boiling point of the wine sample is then determined by filling the boiling chamber with 50 ml of wine, filling the condenser with cold water (this prevents evaporation of the alcohol) and boiling. Once the thermometer is stable the temperature is recorded. The alcohol content of the wine is determined using an ebulliometry degree wheel in which the boiling point of the distilled water and wine sample are located and an alcohol content (volume/volume) is read off.
- pH: Method OIV-MA-AS313-15. The method is based on the difference in potential between two electrodes immersed in the liquid under test. One of the two electrodes has a potential that is a function of the pH of the liquid, while the other has a fixed and known potential and constitutes the reference electrode.
- Total and Free SO_2: Method OIV-MA-AS323-04A. The free sulfur dioxide is determined by potentiometric titration with iodide/iodate. The total sulfur dioxide is determined by potentiometric titration with iodide/iodate after alkaline hydrolysis. With a double platinum electrode and a LED indicator, it detects the electric current as soon as the oxidizing solution of iodide/iodate is in excess. The user controls the flow of this solution, leading to a change of LED signs that indicates the end of the measure.
- Reducing Substances: Method OIV-MA-AS311-01A. With this method the reducing substances, that include the reducing sugars, are detected. Reducing substances comprise all the sugars exhibiting ketonic and aldehydic functions and are determined by their reducing action on an alkaline solution of a copper salt. The concentration of cupric ion in excess is then determined by iodometry. A previous

clarification is performed treating the wine with neutral lead acetate to eliminate interference of other reducing compounds.

The determination of these parameters is of extreme importance because of their influence on the wine microbiology, taste and flavours. In other words, these characteristics are fundamental to indicate if the wine is stable in terms of microbiological and/or oxidative reactions or if an eventual spoilage is occurring. Furthermore, they explicate the response of the wine to the addition of the products (Ribéreau-Gayon et al., 2006).

4. Tartaric stability test: modified mini- contact test

Principle of the method: the crystallisation causes a decrease in the conductivity over the time; a big change in conductivity reveals a large tartrate precipitation and, hence, a high degree of instability (Angele, 1992).

The tartaric stability of the wines has been assessed seeding the wine with 10 g/l of KHT and measuring the drop in conductivity at 0 °C after a 5-minutes time period. As exposed in introductive part (paragraph 4.2.2.): "when conductivity drops by less than 5% over the test period, the wine is generally considered stable; wines falling within this 5% change window can be considered stable only at or above the specified conductivity test temperature".

The evaluation was performed using a Thermo Scientific Orion Star A212 Conductivity Benchtop Meter.

5. Turbidity analysis

Turbidity is defined as the reduction of the transparency of a liquid due to the presence of undissolved substances (OIV-MA-AS2-08: R2009). The turbidity is due to the diffusion of light (Tyndall effect) existing in any colloidal solution through which a light beam is shone (Ribéreau-Gayon et al., 2006). High turbidity values negatively affect the wine's aspect and, in some cases, the wine's flavours being linked to microbial problems, tartrate and colouring matter precipitations and metallic casse (Ribéreau-Gayon et al., 2006). Being the colloidal phenomena involved in the turbidity occurrence, it is important to evaluate the effect that the CMC, as a protective colloid, can have on the clarity of the wine.

Principle of the method: the light diffused by a standard formazine suspension, at a 90° angle to the direction of the incident beam, is determined using a nephelometer (HACH 2100 N). The unit of turbidity used is: NTU - Nephelometric Turbidity Unit.

The analyses have been performed after 2 and 5 months from the addition of the products to compare the effect of the products over the time.

6. Colouring matter stability

To assess the colouring matter stability of the wines, the following method has been used (Claus et al., 2014).

Principle of the method: being the coulouring matter perfectly soluble in water and ethanol 50%, if the deposits coming from previous centrifugation and storage at 4.0°C for 4 days are completely dissolved, it means that the colouring matter is unstable and prone to precipitate and vice versa.

1st step: fifty milliliters of each wine sample are clarified by centrifugation (20 000 × *g*, 30 min).

2nd step: the six products are added to the supernatants to achieve CMC concentrations of 2 ml/l and 1 ml/l. The control wine contains no CMC.

3rd step: haze formation is visually monitored after 4 days at 4.0°C.

4th step: after 4 days the samples are centrifuged (10 000 x *g*, 15 min).

5th step: if deposits are present, the samples are diluted in 10 ml of water or in 5 ml of ethanol 50%. The samples diluted in water, after the addition, are heated up to 40°C in a water bath.

6th step: if a complete solubilization of the deposits occurs, the research is positive. In the contrary, if the solubilization is not complete, the research is negative.

During this research, both dilution (in water and in ethanol) have been used. For the first repetition, the 2ml/L samples have been analysed after dilution in water and, on the contrary, the 1 ml/l samples have been analysed after a contact with ethanol 50%. For the second repetition, for each CMC's concentration (1 and 2 ml/l) and for each dilution substances (water and ethanol), the protocol has been repeated.

7. Wines' phenolic composition analyses

7.1. Characterization of wine proanthocyanidins

The method used to assess the tannins composition was developed and described by Sun et al. (1998).

Principle of the method: it is based on the separation of grape and wine proanthocyanidins (PA) on the basis of their polymerization degree (DP) using C_{18} Sep-Pak cartridges, followed by vanillin reaction in an acidic medium.

This method involves several steps:

I part

1st step: A volume of wine equal to 5 ml has been dealcoholized by rotary evaporation. During the evaporation the temperature has been maintained below 30°C, always around 26 – 28 °C. The volume of wine sample will be defined as V_sample.

2nd step: 20 ml of phosphate buffer (pH 7.0) have been added to the dried wine sample.

3rd step: The sample was then passed through the two preconditioned neutral Sep-Pak cartridges connected in series: the superior one is tC18 Sep-Pak and the inferior is C18 Sep-Pak.

An elution has been carried out with 10 ml of phosphate buffer solution (1/8) to eliminate phenolic acids.

4th step: After the cartridges were dried with N_2 for 1 hour, other two elutions have been carried out.

The first elution has been performed using 25 ml of ethyl acetate to elute catechins (F1) and oligomeric PA (F2), accompanied by some other small phenolic molecules.

The second elution has been performed with 15 ml of methanol to elute the polymeric PA and anthocyanins (in the cases of red wine or red grape skin extract) (F3).

5th step: For the separation of catechins from oligomeric PA, F1+2, previously dissolved in 3 ml of phosphate buffer solution (pH 7.0), has been evaporated to dryness under vacuum at 25 °C and then redeposited onto the same connected cartridges preconditioned.

6th step: After the cartridges were dried with N_2 for 1 hour, separation of catechins and oligomeric PA has been realized by sequential elution with 25 ml of diethyl ether (F1) and then with 15 ml of methanol (F2).

7th step: The three fractions have been evaporated and the dried residuals have been dissolved in a volume of methanol defined as V_rs.

II part

8th step: Two solutions have been prepared for the determination of each fraction:

A) 2 ml of the sample obtained in the I part + 5 ml of sulfuric acid – methanol + 5 ml of methanol.
B) 1 ml of the sample obtained in the I part + 2,5 ml of sulfuric acid – methanol + 2,5 ml of vanillina 1%. The vanillin was used for detection.

9th step: The absorbance (A_{F1}) at 500 nm, after 15 minutes at 30° has been determined for F1.

The maximum absorbance value at 500 nm ambient temperature (A_{F2} and A_{F3}) has been determined for F2 and F3.

III part

The concentrations of F1, F2 and F3 (expressed in mg/l) have been determined using the following formula:

$$[\boldsymbol{C}] = \frac{(\boldsymbol{V\_rs} \times \boldsymbol{A})}{(\boldsymbol{b} \times \boldsymbol{V\_sample})}$$

Where: *V_rs = Volume of methanol used to dissolve the three fractions*

A = Absorbance value obtained as explained above

b = inclination of the curve
b for F1 = 0,0081
b for F2 = 0,0046
b for F3 = 0,0037

V_sample = Initial volume of the sample (5 ml)

The analysis has been performed in quadruplicates.

7.2. Tannin power analysis

The tannin power is an important index used to evaluate the astringency (more specifically its level) of a wine.

The tannin power of the wines has been determined with a procedure described by Freitas and Mateus (2001) based on the concept that: “Procyanidin molecular structure contains several groups such as the aromatic rings and carbon – hydrogen skeleton of the pyranic ring which provide many sites of hydrophobic nature able to interact with proteins” (Kaushal, 2014). Here following, the procedure is described:

1st step: the wine sample has been diluted 1/50 with a wine model solution (hydro alcoholic solution:12 % (v/v); tartaric acid: 5 g/l; pH: 3.2) previously filtrated (0.45 µm). 4 ml of the diluted solution have been placed on a turbidity meter paper and the turbidity has been determined by using a nephelometer (HACH 2100 N). The obtained value will be designated as **d0**.

2nd step: after the measurement, 300 µl of a BSA solution (Bovine Serum Albumin 0.8 g/l) have been added to 8 ml of the solution prepared in the 1st step, agitating using a vortex. The solution has been stored in a dark place at ambient temperature for 45 minutes. Once ready, the turbidity of the solution has been determined by using a nephelometer (HACH 2100 N). The obtained value will be designated as **d**.

3rd step: the tannin power of the wine sample has been obtained by using the following formula:

$$\boldsymbol{Tannin\ power}\ (\frac{\boldsymbol{NTU}}{\boldsymbol{ml}}) = (\boldsymbol{d} - \boldsymbol{d0})/\mathbf{0.08}$$

The analysis has been performed in triplicates.

7.3. Chromatic characterization and other phenolic compounds analyses

The chromatic characteristics are important factors affecting the wine quality. Together with taste and flavours, they represent the sensorial properties of a wine (Ubigli, 2004). The chromatic characteristics of a wine are luminosity and chromaticity, which are correlated to: colour intensity, shade, ionization index, total phenolic index (TPI) and total anthocyanin content of the wine. The luminosity mainly depends on climatic conditions (rainfall events, sun, cold and hot temperatures, etc…), vineyard's management and soil characteristics. The chromaticity, instead, is determined by several oenological treatments and characteristics, such as: acidity and pH, oxidation conditions of the phenolic compounds, etc… (Ubigli, 2004).

The determination of the chromatic characteristics has been performed using the spectrophotometer method (Method OIV-MA-AS2-07B, Type IV method), proposed by Somers & Evans (1977).
Several parameters have been analysed taking into consideration the absorbencies (or optical densities) values at different wavelengths.
Here below all the procedures and formulas used for the calculation are exposed.

1st: The colour intensity (I):

$$\boldsymbol{I}\ (\boldsymbol{u.a.}) = (\mathrm{A420\ x\ k}) + (\mathrm{A520\ x\ k}) + (\mathrm{A620\ x\ k})$$

where: *A420 = absorbance at 420 nm of the wine*
A520 = absorbance at 520 nm of the wine
A620 = absorbance at 620 nm of the wine
K = correction factor = 10.
u.a. = absorbance unit

2nd: The tonality (T):

$$\boldsymbol{T}\ (\boldsymbol{u.a.}) = (\mathrm{A420\ x\ k})/\ (\mathrm{A520\ x\ k})$$

3rd: The total anthocyanin content. This parameter determination is of extreme importance, thinking that the anthocyanins are the pigments related to the red colour of the wines (Ribéreau-Gayon et al., 2006). The total anthocyanin content includes the colourless anthocyanins and the ionized anthocyanins, responsible for the colour. They have been analysed by measuring difference in absorbance of wine sample with introduction of hydrochloric acid (HCL) and sulfur dioxide (SO_2).
Here below the formula used for the measurement is reported.

$$\boldsymbol{ANT\_tot}\ (\boldsymbol{u.a.}) = 20\ \mathrm{x}\ [(\mathrm{A''520\ x\ k'}) - (5/3\ \mathrm{x}\ (\mathrm{A'520\ x\ k}))]$$

where: A'520 = absorbance at 520 nm of a solution made of wine in presence of SO_2; in other words, it is the absorbance at 520 nm after bleaching all free pigments with SO_2

A"520 = absorbance at 520 nm of a solution made of wine in presence of HCl; in other words, it is the absorbance at 520 nm after shifting all free pigments to the coloured flavylium form

k' = correction factor = 101

4th: The ionization index: it expresses the percentage of ionized anthocyanins of the total amount. As exposed above, the ionized anthocyanins are the ones strictly responsible for the wine colour. It has been calculated by measuring absorbance of wine sample with presence of HCl and SO_2 solution.
Here below the formula used for the measurement is reported.

$$\boldsymbol{Ionization\ index}\ (\%) = \frac{(\mathbf{A520\ x\ k}) - (\mathbf{A'520}\ \boldsymbol{x}\ \boldsymbol{k})}{(\boldsymbol{A}\text{"}\ \mathbf{520}\ \boldsymbol{x}\ \boldsymbol{k\prime}) - (\mathbf{5/3}\ \boldsymbol{x}\ (\boldsymbol{A\prime}\ \mathbf{520}\ \boldsymbol{x}\ \boldsymbol{k}))}\ \mathbf{x\ 100}$$

5th: The coloured anthocyanin content: it refers to the concentration of coloured anthocyanins among the total anthocyanin content. The coloured anthoycanins, also known as ionized anthocyanins, are the ones under the flavylium cation form. The flavylium cation form is the most present anthocyanins' structure in acidic solutions of pH conditions around 3 and it is characterized by a red colour.

$$\boldsymbol{ANT\_col}\ (\boldsymbol{u.a.}) = \mathbf{20\ x\ [(A520\ x\ k) - (A'520\ x\ k)]}$$

6th: The total pigments content: it includes polymerized and non- polymerized pigments. In other words, this parameter expresses the concentration of a wide range of molecules, such as phenolic compounds (i.e. flavonoids) and anthocyanins and, moreover, it refers to the substances resulting from the polymerization of the different phenolic compounds.

$$\boldsymbol{PIG\_tot}\ (\boldsymbol{u.a.}) = \mathbf{A"520\ x\ k'}$$

7th: The polymerized pigments content:

$$\boldsymbol{PIG\_pol}\ (\boldsymbol{u.a.}) = \mathbf{A'520\ x\ k}$$

8th: The polymerization index:

$$\boldsymbol{Polymerization\ index}\ (\%) = \mathbf{[(A"520\ x\ k')/\ (A'520\ x\ k)]\ x\ 100}$$

7.4. Quantification of flavonoid phenols and non-flavonoid phenols

9th: The total phenols content. It relates to flavonoids (catechins, epicatechins, flavonols, anthocyanins and condensed tannins) and non- flavonoids (phenolic acids, benzoic and cinamic acids, and their derivatives, stilbenes and other volatile phenols) substances. It is the result of the following formula:

$$\boldsymbol{PHEN\_tot}\ (\boldsymbol{u.a.}) = \mathbf{A280\ x\ k''}$$

where: *A280 = absorbance of the wine at 280 nm*

k'' = corretion factor = 100

10th: The non-flavonoids content. This determination is based on the absorbency measurement at 280 nm wavelength of the sample before and after the precipitation of the flavonoids through a reaction with formaldehyde under specific conditions of low pH and room temperature.

$$\boldsymbol{Non\_flav\ (u.a.) = \mathrm{A280\ x\ k}}$$

11th: The flavonoids content: it is the result of the difference between the total phenols and the non-flavonoids content.

$$\boldsymbol{Flavonoids\ (u.a.) = (PHEN\_tot) - (Non\_flav)}$$

Total phenols, non- flavonoids and flavonoids contents will be also expressed in mg/l of gallic acid. The curve used to the determination is reported below:

$$\boldsymbol{y = 0.0309x - 0.0169}$$

Therefore:

$$\boldsymbol{x = \frac{(0.0169 + y)}{0.0309}}$$

Where: y = absorbances values
x = value expressed in mg/l

All of these parameters have been determined to evaluate the response of the wine to the CMCs addition. As reported elsewhere, the main drawback of the CMCs utilisation in red wines is related to a decrease in the concentration of total phenols, flavonoid and non-flavonoid phenols, reducing the colour intensity. More specifically CMC interacts with the phenolic compounds, promoting colorant matter precipitation. It is for this reason that this research is mainly based on the CMC effects on chromatic characteristics and on the colouring matter stability.

All the analyses (1st – 11th) have been performed in triplicates.

8. Sensory evaluation

A formal sensory evaluation has been performed to understand how the sensorial quality of the wine could have been effected by the presence of the products. The tasting has been performed by four professional tasters.

The addition of the six CMCs to the wine has been performed the same day, to prevent eventual effects driven by contact - time reasons.

The tasting has been made comparing each sample with the control to evaluate eventual differences or similarities. Finally, a comparison between the sensorial properties of the treated wines has been implemented to estimate the behaviour of the same wine when in contact with six different products.

9. Statistical analyses

To evaluate the effects of the products on tannins composition, tannin power and chromatic characteristics, a one-way Analysis of Variance (ANOVA test) has been performed.

The one-way ANOVA is used to determine if there are any significant differences between the means of three or more independent groups; therefore, in this study it has been used to assess if all the treatments (control wine, CMC1, CMC2, CMC3, CMC4, CMC5, CMC6) have the same population mean or if at least one population mean differs from the others.
Technically, a one-way ANOVA test is based on two hypotheses:

- H0 (null hypothesis): all the treatments have the same population mean; therefore, no significant differences are found
- H1 (alternative hypothesis): at least one population mean differs from the others.

Using this test, the differences are significant when the *p-value* is below 0.05. When no significant differences were found the letters “ns” were used.

In conclusion, if the H0 is accepted and no differences have been found, the statistical analysis is considered concluded; if the H0 is rejected and the H1 is true, the differences are evaluated by a post-hoc test. For this study a Tukey post-hoc test has been used. The Tukey post-hoc test shows where the differences are located.

For the statistical analyses, the SPSS (Statistical Package for Social Science) software has been used.

III. RESULTS AND DISCUSSION

In this chapter, the results obtained for the different types of analyses carried out during the entire period of the research are exposed.

Several analyses have been performed during this study. The analyses included: the conventional oenological parameters measurement, a tartaric stability test, tannins' composition and tannin power estimation, chromatic characteristics assessment and colouring matter stability evaluation.

To study the results:

- A simple observation of the values coming from the conventional oenological analyses, from the modified mini-contact test and from the turbidity analyses has been made to estimate the wine's state, the CMCs' effects on the wines' clarity and the tartaric stabilization efficiency of the treatments
- Visual observations have been made to evaluate the colouring matter stability
- Statistical evaluations have been performed for tannins' composition, tannin power and chromatic characteristics.

1. Wine's conventional oenological characteristics

In the table below the characterization of the wine explained by its conventional oenological parameters is reported.

Table 3: Physicochemical characteristics of the Control wine.

	Total Acidity (TA) (g/l of tartaric acid)	Volatile Acidity (VA) (g/l of tartaric acid)	Alcoholic Strength (%/V)	pH	Total SO_2 (mg/l)	Free SO_2 (mg/l)	ReducingSubstances (g/l)
Wine							
1	4.65	0.32	13.6	3.52	60	28	2.14
2	4.65	0.36	13.6	3.53	62.5	32	2.2
Mean value	**4.65**	**0.34**	**13.6**	**3.53**	**61.2**	**30**	**2.17**

- TA of 4.65 g/l of tartaric acid is a quite good value for a red wine coming from a hot-temperate region as the wine in exam. It generally stands between 4 and 8 g/l (Ribéreau-Gayon et al., 2006).
- VA of 0.34 g/l of acetic acid reveals that the wine is stable in terms of microbiological spoilage. The result, combined with a tasting performed to analyse the sensory characteristics of the wine, shows no olfactory influence of acetic acid. The optimal values range between 0.4 and 0.5 g/l of acetic acid (Schneider, 2003).
- Free SO_2 of 30 mg/l and Total SO_2 of 61.2 show that the wine is protected against microbiological spoilage. The Free SO_2 optimal value generally ranges about 35 mg/l. Furthermore, being the Total SO_2 value lower than 150 mg/l (legal limit for red wines coming from the UE), it can be said that the wine is safe in terms of physiological effects on human's health (Ribéreau-Gayon et al., 2006).
- pH value of 3.53 is an optimal value for a red wine, beneficial for microbiologic stability (Ribéreau-Gayon et al., 2006).

- Reducing Substances of 2.17 g/l is a value that indicates that the wine is a dry wine (< 4 g/l) that completed the alcoholic fermentation, therefore a wine with no risks of microbiological spoilage (Ribéreau-Gayon et al., 2006).

We can therefore conclude that, being all the values in the optimal range, the wine is in a good state, with no risks of microbiological spoilage.

2. Effects of CMCs addition on tartaric stability

Values in Table 4 indicate the tartaric stability test results of the seven samples and, therefore, the tartaric stabilization efficiency of the six different CMCs.

Table 4: Results of modified mini-contact test applied on wines after 5 days and after 5 months from the treatment with carboxymethylcelluloses.

	Control	CMC1		CMC2		CMC3		CMC4		CMC5		CMC6	
		5 d	5 m	5 d	5 m	5 d	5 m	5 d	5 m	5 d	5 m	5 d	5 m
Wine	2170	2246	2258	2150	2341	2102	2164	2294	2149	2240	2273	2151	2460
Wine + KHT	2110	2307	2311	2276	2356	2155	2238	2300	2228	2293	2316	2237	2324
1st minute	2070	2297	2335	2278	2362	2154	2238	2298	2231	2306	2320	2243	2415
2nd minute	2054	2302	2334	2276	2355	2154	2234	2302	2232	2311	2322	2244	2409
3rd minute	2040	2305	2332	2277	2357	2153	2234	2302	2232	2314	2321	2245	2405
4th minute	2031	2307	2329	2276	2358	2152	2232	2302	2232	2316	2320	2244	2402
5th minute	2027	2306	2326	2276	2358	2152	2232	2302	2232	2317	2320	2243	2399
6th minute	2020	2307	2321	2276	2358	2152	2230	2302	2232	2317	2320	2243	2399
7th minute	2006	2307	2319	2276	2358	2152	2230	2302	2232	2317	2320	2243	2399
$\Delta_{conductivity}$	**7.6**	**-2.7**	**-2.7**	**-5.9**	**-0.7**	**-2.4**	**-3.0**	**-0.3**	**-3.9**	**-3.4**	**-2.1**	**-4.3**	**2.5**

5 d = 5 days; 5 m = 5 months.
Wine: wine conductivity; Wine + KHT: conductivity value of the wine + 10 g/l of KHT; 1st minute: conductivity value of the wine + 10 g/l after 1 minute, continuously agitating; 2nd minute: conductivity value of the wine + 10 g/l after 2 minutes, continuously agitating; 3rd minute: conductivity value of the wine + 10 g/l after 3 minutes, continuously agitating; 4th minute: conductivity value of the wine + 10 g/l after 4 minutes, continuously agitating; 5th minute: conductivity value of the wine + 10 g/l after 5 minutes, continuously agitating; 6th minute: conductivity value of the wine + 10 g/l after 6 minutes, continuously agitating; 7th minute: conductivity value of the wine + 10 g/l after 7 minutes, continuously agitating; $\Delta_{conductivity}$: drop in conductivity Δ(1- 9).
Results are expressed in conductivity units ($\mu S\ cm^{-1}$).

All the products improved the tartaric stability of the Control wine, being their drop in conductivity always below 5%. It can therefore be said that the CMC is a suitable KHT crystallization inhibitor, as already exposed by Bosso et al. (2012), Greeff et al. (2012) and Guise et al. (2014).

However, the values coming from the two analyses show some differences: i) the CMC1 reports the same increase in conductivity after 5 days and after 5 months from the addition (-2.7%); ii) for the CMC2 and the CMC5 the variation in conductivity has been found higher in the first than in the second experiment; the CMC3 and the CMC4 show an opposite behaviour compared to the previous two (the second experiments have reported higher variations in conductivity); however, these results do not show big differences between the two experiments; iii) the CMC6 is the only sample showing two completely different results: during the

second experiment, the drop in conductivity has been increased enormously. The big differences regarding the CMC6 treatment can maybe be explained by the product's composition and, consequently, by its effectiveness: as exposed in the paragraph 5.2., the CMC6 is maybe characterized by several impurities coming from its production, that contribute in the decrease of the efficiency over the time.
However, it is clear that the results do not follow a linear trend, making difficult a description of the general behaviour. Furthermore, it appears evident that the concentration of CMC characteristic of the products does not impact their effectiveness in terms of tartaric stability: the CMCs characterized by the same concentrations (CMC1, CMC3, CMC5 on one hand and CMC2 and CMC4 on the other) do not present the same results, revealing that concentration of the products and their efficiency are independent variables.

Nevertheless, the results reported in table 4 reveal an important effect: the six products were still effective as salts crystallization inhibitors after 5 months from the first experiments.

Further analyses could be helpful in the future to assess if the differences coming from the two analyses are driven by analytical aspects/particularities (i.e. slight differences in the samples temperature during the analyses) or by the products, their effectiveness and their properties.

3. Effects of CMCs addition on the turbidity

The effects of the CMCs addition on the turbidity is displayed in the table below (table 5).

These results are in line with the ones obtained by Moutounet et al. (2010), Claus et al. (2014) and Guise et al. (2014): the turbidity of the wine increased in all the treated samples, confirming that the CMC promotes the development of turbidity.
This is a normal behavior when the CMC is added on white wines characterized by intrinsic protein instability: as expressed by Claus et al. (2014), as a protective colloid, the CMC binds with the unstable proteins, increasing their molecular dimensions and making them forming the haze.
Nevertheless, it is widely known that the red wines do not suffer in terms of protein instability (Ribérau-Gayon et al., 2006); therefore, in these wines, the increase in turbidity can be generally explained by two factors: i) the presence of phenolic compounds that, binding with the colloidal portions present in solution (i.e. CMC), promote colouring matter precipitation (Ribérau-Gayon et al., 2006). However, being the wine used for the experiments already stabilized in terms of colour with the addition of French oak powder and, moreover, not having presented any colouring matter precipitations after the application of the CMCs (paragraph 4), it appears clear that the higher turbidity levels detected in the treated wines are not due to the CMC-phenolic compounds reactions; ii) the concentration of carbohydrate polymers/ protective colloids of the wine solution: when their content is much higher than the quantity needed to coat the unstable particles, they may cause a flocculation phenomenon known as depletion (Ribérau-Gayon et al., 2006). Being the CMC a cellulose derivative with protective colloidal properties, maybe the higher turbidity levels can be linked to this factor.
Even so, several observations can be made: i) it can be noticed that the turbidity values decreased during the time and that the differences between the turbidity value of the Control and the one of the treated samples are always lower than 3.5 NTU; ii) all the values reported in table 2 can be considered normal bearing in mind that

the wine was a young one not filtered before the products addition. As reported by Ribèreau-Gayon et al. (2006), a red wine is defined as clear when its turbidity value is below 2 NTU; on the contrary, it is considered turbid when its turbidity level is above 8 NTU, while between 2 and 8 NTU of turbidity it is described as cloudy. It can therefore be stated that, being all the values coming from the analyses performed after 5 months from the addition between 4 and 7 NTU, the wines are considered cloudy; iii) the CMC's concentrations of the products did not impact the turbidity of the wines: the products with higher concentrations of CMC (CMC1, CMC3 and CMC5) did not present higher turbidity values.

Table 5: Turbidity of the wines. Comparison between the values obtained after 2 and after 5 months from the treatment with carboxymethylcelluloses.

	After 2 months	After 5 months
Control	4.6	3.6
CMC1	4.8	4.3
CMC2	5.7	4.4
CMC3	7.6	5.0
CMC4	7.9	6.5
CMC5	8.7	7.0
CMC6	5.9	4.4

All the values are expressed in NTU.

4. Effects of CMCs addition on colouring matter stability

After being centrifuged, all the samples, except for the control wine, presented deposits.

After contact with ethanol 50% and water, the results show that in all the samples the deposits did not solubilize completely. As a result it can be said that, being the colouring matter not completely solubilized in water and ethanol 50%, it is stable and not prone to precipitate.

This result is of extreme importance if we think that the main drawback concerning the utilization of the CMC in red wines is represented by the fact that the CMC interacts with the phenolic compounds, promoting colouring matter precipitation, as exposed by Guise et al. (2014). One possible explanation to this effect can be related to the initial colour stabilization of the control wine: the French oak powder, applied during the pre-fermentative operations, is used in winemaking with the aim of facilitating the extraction of the colour during the future fermentation and, moreover, to stabilize it in terms of future colouring matter precipitations. It is therefore clear that the initial wine was already stabilized in terms of colouring matter precipitations.

Some previous studies (Greeff et al., 2012) are in line with this result reporting that this is not a trend characterizing all the red wines since some of them do not respond to the addition of the CMC making the colouring matter precipitating.

Nevertheless, because of the lack of information and studies regarding the eventual colouring matter precipitation following the CMC's addition on red wines, the results obtained during this work represent a strong support to the oenological research, opening new prospective and scenarios concerning the effects of the CMC utilization. However, it could be representative, for the future, to study the effects of the CMCs addition when added on colour unstable red wines.

5. Effects of CMCs addition on wines' phenolic composition

In the table below (table 6), the control wine is presented considering its phenolic and chromatic characteristics before the addition of the products. Absorbances values at 420, 520 and 620 nm, intensity, tonality, total and polymerized pigments, total and coloured anthocyanins, total phenols, non- flavonoids and flavonoids, tannin power, monomeric, oligomeric and polymeric fractions of the tannins as well as total tannins' composition, are important parameters to be considered for the evaluation of the wine status and, clearly, for the assessment of the wine's response to the addition of the product.

Table 6: Phenolic and chromatic characteristics of the control wine.

Variable	Mean
A420 (u.a.)	2.75
A520 (u.a.)	4.14
A620 (u.a.)	0.88
Intensity (u.a.)	7.77
Tonality	0.665
Total pigments(u.a.)	22.79
Polymerization index (%)	886.01
Polymerized pigments (u.a.)	1.99
Total anthocyanins (mg/l of malvidin 3-glucoside)	389
Ionization index (%)	11
Coloured anthocyanins (mg/l of malvidin 3-glucoside)	42
Total phenols (mg/l of gallic acid)	1774
Non- flavonoids (mg/l of gallic acid)	141
Flavonoids (mg/l of gallic acid)	1633
Tannin power (NTU/ml)	277.7
Monomers (mg/l)	21.2
Oligomers (mg/l)	56.8
Polymers (mg/l)	972.3
Total tannins (mg/l)	1050.2

The values come from the calculations exposed in the paragraph 5.4. of MATERIALS AND METHODS.

In the adapted table 7, six different young wines, vintage 1975, analysed by Somer and Evans (1977) are displayed. Being the Castelão wine sample results similar to the one reported in table 6, it can therefore be said that it shows common values for young wines, regarding total anthocyanins (389 mg/l), coloured anthocyanins (42 mg/l) and total phenols (1774 mg/l – 54.8 u.a.).

Table 7: Overview over chromatic characteristics of six young wines vintage 1975.

Wines	1	2	3	4	5	6
pH	3.5	3.52	4.07	4.13	3.75	3.70
Colour density	10.8	3.5	12	5.5	12.7	6.6
Color hue	0.54	0.64	0.67	0.86	0.59	0.75
Total anthocyanins (mg/l)	371	381	486	390	460	408
Ionised anthocyanins (mg/l)	97	28	78	21	109	36
Total phenolics (u.a.)	47	40	62	55	66	55

Adapted from Somers and Evans (1977).

5.1. Effects on tannin power

The table 8 shows the results coming from the tannin power analysis.

Table 8: Tannin power of the wines.

TREATMENT	Tannin power
Control	277.7 ± 0.5 ns
CMC1	255.7 ± 6.5 ns
CMC2	258.2 ± 7.1 ns
CMC3	252.2 ± 4.1 ns
CMC4	260.5 ± 8.7 ns
CMC5	251.4 ± 0.4 ns
CMC6	257.4 ± 4.3 ns

The values are expressed in NTU/ml.

There are some differences between the seven samples. The control wine, with a value of 277.7 NTU/ml reveals the highest tannin power, followed by the CMC4, CMC2, CMC6, CMC1 and CMC3. The sample CMC5, with 251.4 NTU/ml, is characterized by the lowest tannin power.

The table 8 clearly shows that those differences between the samples are not significative (ns).
However, it can be observed that the CMC treated samples, compared to the control wine, present always a lower value ranging from 260.5 NTU/ml for the CMC4 to 251.4 NTU/ml for the CMC5.
This result is explained taking into consideration the CMC composition: being this polysaccharide a protective colloid, the tannins reaction with the BSA solution (Bovine Serum Albumin 0.8 g/l) is lower when the CMC is present. Moreover, taking into consideration the concentrations of CMC characterizing the products applied, it is clear that, as expected, higher the CMC concentration (CMC1, CMC3 and CMC5), higher the protective colloid property and lower the tannins reaction with the BSA solution.

Being the tannin power a descriptor of the astringency level, supposedly it can be said that the control wine has a higher astringency level. More detailed information is reported in the paragraph 6.

In the Annex 1, tables 7 – 9, all the laboratory and statistical analyses performed for the tannin power evaluation are reported.

5.2. Effects on tannins (monomers, oligomers, polymers) composition

This study represents a complete research about the effects of the CMC on the wine's tannins composition. The quantification of monomeric, oligomeric and polymeric fractions in the wine after the product's addition has not been evaluated in previous researches. Therefore, this work will provide important results that could be used, in the future, as hypothetical approaches in the determination of the CMC influence on these fundamental wine's properties.

In the table 9 the results concerning the three proanthocyanidins fractions and the total tannins concentration, coming from the Sun method, are reported.

Table 9: Proanthocyanidins fractions in the wines.

TREATMENT	F1 - Monomers	F2 - Oligomers	F3 – Polymers	Total tannins
Control	21.2 ± 2.8 ns	56.8 ± 3.0 a	972.3 ± 28.9 ab	1050 ± 31.4 ab
CMC1	22.7 ± 0.5 ns	57.4 ± 1.2 ab	892.9 ± 21.6 a	973.0 ± 22.5 a
CMC2	23.8 ± 0.6 ns	73.4 ± 0.8 c	921.6 ± 15.4 ab	1018.8 ± 15.7 ab
CMC3	20.6 ± 0.5 ns	56.2 ± 0.9 a	956.8 ± 22.1 ab	1033.5 ± 20.7 ab
CMC4	20.7 ± 1.2 ns	70.9 ± 1.9 b	977.9 ± 13.1 ab	1069.5 ± 11.7 ab
CMC5	20.7 ± 1.9 ns	72.0 ± 2.3 c	906.8 ± 39.8 ab	999.5 ± 39.4 a
CMC6	25.5 ± 1.9 ns	94.7 ± 6.0 d	1007.7 ± 4.9 b	1127.9 ± 6.6 b

F1: Monomeric fraction; F2: Oligomeric fraction; F3: Polymeric fraction.
The values are expressed in mg/l of: monomers (F1), oligomers (F2), polymers (F3) and monomers + oligomers + polymers (TOTAL).

It is clear that there are some differences between the seven samples in all the fractions:

- CMC6 and CMC3 show respectively, the highest and the lowest values (25.5 mg/l and 20.6 mg/l) concerning the monomeric fraction concentration
- CMC6 and CMC3 show respectively, the highest and the lowest values (94.7 mg/l and 56.2 mg/l) concerning the oligomeric fraction concentration
- CMC6 and CMC1 show respectively, the highest and the lowest values (1007.7 mg/l and 892.9 mg/l) concerning the polymeric fraction and the anthocyanins concentration
- CMC6 and CMC1 show respectively, the highest and the lowest values (1127.9 mg/l and 973.0 mg/l) concerning the total tannins' concentration.

As reported in the table 9, the oligomeric and polymeric fractions and the total tannins are characterized by significant differences in the samples values; therefore, we can conclude that the hypothesis null is rejectable and that at least one population mean differs from the others in these three fractions.

For the oligomeric fraction (F2) we can observe that four subsets are formed: 1) CMC3, control and CMC1; 2) CMC1 and CMC4; 3) CMC4, CMC5 and CMC2; 4) CMC6. Each subset includes samples that show the same behaviour. Regarding this proanthocyanidins fraction, the control wine shows a lower value compared to all the CMCs treated samples, except for the CMC3. Additionally, the control completely differs from four samples, revealing that the CMC has a strong impact in the wine's oligomeric composition.

Concerning the polymeric fraction (F3) and the total tannins, the samples are subdivided into two groups. The two subsets for the F3 include: 1) CMC1, CMC5, CMC2, CMC3, control and CMC4; 2) CMC5, CMC2, CMC3, control, CMC4 and CMC6. The subsets concerning the total tannins are so formed: 1) CMC1, CMC5, CMC2, CMC3, control and CMC4; 2) CMC3, control, CMC4 and CMC6. It is clear that the first subset, for the two fractions, it is composed by the same samples; the difference between the two fractions characterizes the second subset. More precisely, the five samples CMC5, CMC2, CMC3, control and CMC4 are present in both subsets concerning the F3; on the contrary, regarding the total tannins' composition (TOTAL), only three samples are present in both groups, specifically CMC3, control and CMC4. This can be referred to the fact that in the total tannins' composition, monomers and oligomers have a strong impact.

The CMCs treated wines show lower polymers and total tannins' concentrations compared to the control (expect for the CMC4 and CMC6 samples). Nevertheless, the control wine does not completely differ from any CMCs treated one; therefore, it is clear that the CMC, during this study, did not reveal a strong impact on the polymeric fraction and on the total flavanols content.

Differently from the oligomeric, polymeric fractions and from the total tannins concentration, the significance of the differences for the monomeric fraction reveals that all the treatments have the same population mean. Consequently, it can be said that the different CMCs do not impact the monomers concentration. However, several observations can be made:

- As expected, being null the significance of the differences between the seven treatments, they all belong to the same subset. This can be due to the fact that the variability of the repetitions in the control, CMC6, CMC5 and CMC4 is high, as reported in the figure 7 (F_MONO), eliminating the differences in all the other treatments. The variability in the repetition for each treatment is represented by the height of the lines. This variability can be referred to the differences in the repetitions' values: as reported in the table 1, annex 1, the quadruplicates concerning the monomeric fraction present a high variability (i.e. for the control wine the values range from 0.1 mg/l to 0.2 mg/l)
- The treatments CMC1 and CMC2 slightly differ from the CMC3, showing higher values.

The detailed results coming from the statistical analyses and the quadruplicates' values obtained during the experiments are reported in the Annex 1, tables 1 - 6.

In the figure 7, everything exposed above is visually shown.

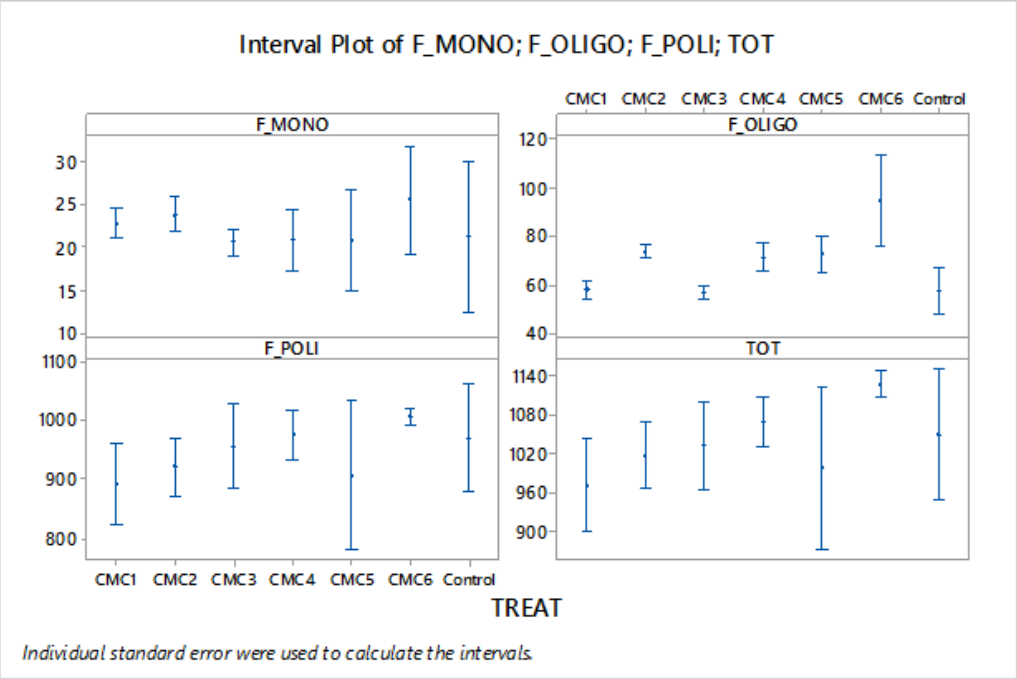


Fig. 7: Interval plot of monomeric, oligomeric, polymeric fractions and of total tannins' concentration.

In conclusion, being the behaviour of the treated samples not completely different compared to the control wine, we can assume that the CMC, in general, does not strongly impact the tannins' composition.

On the other hand, it is not possible to observe a linear trend in the different products, making difficult a description of their influence on the wine in terms of reaction with the tannins' fractions. The only exception is characterized by the CMC6: this sample has always the highest value in all the fractions. For this reason, we can say that the CMC6 has a strong influence on the tannins' composition of the wine, increasing the concentrations of all the values. A possible explanation to the effect of the CMC6 to the tannins' structure can be related to the product's composition: the CMC is a plant cell wall derivative, coming from the synthesis of the cellulose. According to Chen (2014), the cellulose is characterized by the presence of condensed tannins; therefore, the synthesis of the CMC can lead to the presence of some impurities coming from the cellulose which, in some cases, can alter some wine analytical and sensorial characteristics. Specifically, some substances, such as the condensed tannins present in the plant cell walls, can be released to the wine with the effect of an increasing tannins' concentration in the wine in exam. To better understand if the CMC's composition could have an impact on the proanthocyaninds concentration, a total phenols quantification of all the six products has been performed. In the table inserted (table 10) below, the results are displayed.

Table 10: Total phenols quantification of the carboxymethylcelluloses.

Total phenols	**CMC1**	**CMC2**	**CMC3**	**CMC4**	**CMC5**	**CMC6**
1	6.0	3.0	1.8	0.6	2.7	43.8
2	4.9	3.0	1.9	0.6	2.2	44.3
3	5.3	3.0	2.0	0.7	2.2	45.0
Mean value	**5.4**	**3.0**	**1.9**	**0.6**	**2.3**	**44.4**

The values are expressed in u.a. They are the results of the following calculation: A280*k; here k represents the correction factor (25).

Considering the results in table 10, it is clear that the total phenols' concentration of the CMC6 influenced the tannins' composition of the treated wines, making monomeric, oligomeric and polymeric fractions increasing. Even though, this effect has been evident only for the oligomeric fraction, characterized by an important difference in the significance.

In terms of sensorial quality, a high concentration of condensed tannins coming from the CMC could strongly influence bitterness and astringency; in the specific, higher the concentration of monomers, higher the bitterness; on the contrary, higher the concentration of procyanidins and polymerized tannins, higher the astringency. Therefore, it can be said that the CMC6 treated wine could be characterized by a higher astringency level compared to the other samples. However, the astringency level is strictly related to the tannin power; for this reason, the results coming from the sensorial analysis, exposed in the paragraph 6, will help understanding how the proanthocyaninds' composition influences the wine's aromas and tastes characteristics.

In the sub-paragraph 5.3.3., the CMCs' total phenols concentration effects on the wines' flavonoids and non-flavonoids composition is exposed.

5.3. Effects on chromatic characteristics

The chromatic characteristics analyses are of extreme importance if we think that previous studies (Guise et al., 2014) have reported that the CMCs utilisation in red wines reports a decrease in the concentration of total phenols, flavonoid and non-flavonoid phenols, reducing the colour intensity.

This paragraph will expose the results concerning the chromatic characteristics analyses performed during the period of the experiments.

All the results evaluated below are referred to more detailed analyses exposed in the tables 10 – 29 of the Annex 1.

To better evaluate the CMC effects on the chromatic characteristics, this paragraph has been divided into three sub-paragraphs. The first one will expose the CMCs effect on absorbances (at 420, 520 and 620 nm), intensity and tonality; the second one the CMCs effect on anthocyanins and pigments content and the third one the CMCs effect on total phenols.

5.3.1. CMCs effects on the absorbances at 420, 520 and 620 nm, colour intensity and tonality

The first parameters analysed, the absorbances at 420,520 and 620 nm are reported in the table inserted below (table 11).

Table 11: Absorbances at 420,520 and 620 nm of the wines.

TREATMENT	A420	A520	A620
Control	2.75 ± 0.01 a	4.14 ± 0.00 a	0.88 ± 0.00 a
CMC1	2.90 ± 0.01 b	4.27 ± 0.01 c	0.90 ± 0.00 ab
CMC2	2.97 ± 0.00 c	4.32 ± 0.00 d	0.90 ± 0.00 ab
CMC3	3.22 ± 0.01 d	4.89 ± 0.01 f	1.00 ± 0.00 c
CMC4	2.96 ± 0.00 c	4.25 ± 0.00 c	0.90 ± 0.01 ab
CMC5	3.22 ± 0.00 d	4.80 ± 0.01 e	0.98 ± 0.00 c
CMC6	2.95 ± 0.01 d	4.19 ± 0.00 b	0.91 ± 0.00 b

A420: Absorbance at 420 nm. A520: Absorbance at 520 nm. A620: Absorbance at 620 nm.
All the values are expressed in u.a. (absorbance unit).

For each absorbance value, a different number of subsets is formed: four for the absorbance at 420 nm, five for the absorbance at 520 and three for the absorbance at 620 nm. Therefore, it is clear that the absorbances respond in a different way according to the product: at the wavelength value of 620 nm, the products show a similar behaviour; on the contrary, at 520 nm almost all the samples differ from each other, revealing a high heterogeneity; at 420 nm, the samples respond with an intermediate behaviour.

Even with these differences, the three absorbances are characterized by a common property: the control wine shows always the lowest values (2.75 u.a. at 420 nm, 4.14 u.a. at 520 nm and 0.88 u.a. at 620 nm) and the CMC3 always the highest (3.22 u.a. at 420 nm, 4.89 u.a. at 520 nm and 1.00 u.a. at 620 nm).

This result is of extreme importance if we think that the control wine always differs from the treated samples in a significant way.

In other words, we can observe that the CMC addition causes an increase in the absorbances values.

In the table 12, the effect of the CMCs on the two chromatic characteristics of intensity and tonality is reported.

Table 12: Colour intensity and tonality of the wines.

TREATMENT	Colour Intensity	Tonality
Control	7.77 ± 0.02 a	0.665 ± 0.001 ab
CMC1	8.08 ± 0.01 b	0.679 ± 0.002 b
CMC2	8.18 ± 0.01 c	0.687 ± 0.000 d
CMC3	9.11 ± 0.03 e	0.659 ± 0.000 a
CMC4	8.11 ± 0.01 b	0.696 ± 0.000 e
CMC5	9.00 ± 0.01 d	0.671 ± 0.002 b
CMC6	8.05 ± 0.01 b	0.705 ± 0.002 f

The values come from the calculations exposed in the paragraph 5.4. of MATERIALS AND METHODS.
The MEAN values of the 3 repetitions are expressed in u.a. for the colour intensity. The tonality has no units.

The intensity, described as the sum of the absorbances at the three wavelengths, shows the lowest value for the control wine (7.77 u.a.) and the highest for the CMC3 (9.11 u.a.), as expected.
This is an important result, in opposition with most of the studies conducted before (Guise et al., 2014) that reported a decrease in the colour intensity of the red wines after the CMC addition.
Therefore, we can conclude that, as already exposed by Greeff et al. (2012), not all the red wines respond to the CMC addition in the same way: The Castelão wine showed an increase in the colour intensity when the six different CMCs have been added. This observation is of extreme importance if we think that this can be a starting point for further analyses on different red varieties.

Concerning the tonality, the trend is different: the CMC3 treated wine shows the lowest value (0.659) while the CMC6 the highest one (0.705). The control wine does not differ completely from the CMC3 and the CMC5, while it varies in a significant way from all the other treated samples. Furthermore, the control wine shows always a lower tonality value compared to the CMCs treated samples, except for the CMC3. Hence, it can be said that during the current study the CMC influenced the tonality of the wine, making it generally increasing (expect for the CMC3 treatment).

5.3.2. CMCs effects on anthocyanins' and pigments' content

In the table 13, total and coloured anthocyanins content, ionization index, total and polymerized pigments' concentration and polymerization index results are reported.

Table 13: Total anthocyanins and total pigments content, polymerization and ionization indexes of the wines.

TREATMENT	Total anthocyanins	Ionization index	Coloured anthocyanins	Total pigments	Polymerization index	Polymerized pigments.
Control	389 ± 12 bc	11 ± 0 ab	42 ± 0 a	22.79 ± 0.64 bc	886.01 ± 22.10 b	1.99 ± 0.01 a
CMC1	389 ± 2 bc	11 ± 0 ab	45 ± 0 b	22.79 ± 0.13 bc	884.86 ± 7.11 ab	1.99 ± 0.00 a
CMC2	381 ± 3 b	12 ± 0 b	45 ± 0 b	22.46 ± 0.18 b	914.72 ± 11.27 bc	2.03 ± 0.01 ab
CMC3	381 ± 0 b	14 ± 0 c	55 ± 0 d	22.59 ± 0.03 b	940. 38 ± 1.58 bc	2.10 ± 0.01 c
CMC4	419 ± 6 c	10 ± 0 a	45 ± 0 b	24.27 ± 0.32 c	825.56 ± 12.95 a	1.98 ± 0.01 a
CMC5	343 ± 2 a	15 ± 0 d	53 ± 0 c	20.70 ± 0.10 a	1029.32 ± 5.77 d	2.11 ± 0.01 c
CMC6	360 ± 5 ab	11 ± 0 ab	42 ± 0 a	21.49 ± 0.29 ab	971.36 ± 7.02 cd	2.07 ± 0.01 bc

The values come from the calculations exposed in the paragraph 5.4. of MATERIALS AND METHODS.
The values are expressed as following: Total anthocyanins and coloured anthocyanins– mg/l of malvidin 3-glucoside; total and polymerized pigments – u.a. (absorbance unit); ionization and polymerization index - %.

Concerning these parameters, several observations can be made:

- The differences between the samples are significant for all the parameters
- For the total anthocyanins' content, the CMC5 shows the lowest value (343 mg/l) and the CMC4 the highest one (419 mg/l)
- For the ionization index, the CMC4 reports the lowest value (10%) and the CMC5 the highest one (15%)
- For the coloured anthocyanins' content, the CMC6 is characterized by the lowest value (42 mg/l) and the CMC3 by the highest one (55 mg/l)
- For the total pigments' content, the CMC5 reveals the lowest value (20.70 u.a.) and the CMC4 the highest one (24.27 u.a.)
- For the polymerization index and the polymerized pigments, the CMC4 shows the lowest values (respectively 825.56% and 1.98 u.a.) and the CMC5 the highest ones (respectively 1029.32% and 2.11 u.a.)
- Observing the control wine behaviour, it is clear that it differs completely from some samples in all the parameters: from the CMC5, CMC6 and CMC4 for the total anthocyanins' content; from the CMC4, CMC1, CMC2, CMC5 and CMC3 for the coloured anthocyanins' content; from the CMC5, CMC6 and CMC4 for the total pigments; from the CMC4, CMC3, CMC6, CMC3 and CMC5 for the polymerized pigments, showing the strong influence that the CMCs have on the different parameters.

In conclusion, it can be said that the CMCs analysed in this study reported a general increase in the ionization index (except for the CMC4) and, therefore, in the coloured anthocyanins' content (except for the CMC6). The same behaviour characterizes the polymerization index, and, consequently, the polymerized pigment: the CMCs show a general increase in these two parameters (except for the CMC4 and for the CMC1).
Consequently, it is clear that the CMCs studied influenced the red pigments composition of the wine, resulting in a rise of its red colour. In total opposition with the previous results (Guise et al., 2014), but in line with the ones obtained during this study, this consequence completely changes the CMC scenario.

Moreover, in addition with the results exposed above about colour intensity, tonality and colouring matter precipitation, this is in line with what Greeff et al. obtained in 2012, showing that not all the red wines respond to the CMC addition with a colour reduction. Hence, more studies and researches are needed to better understand the behaviour of the red wines when the CMC is added as a tartaric stabilization product.

5.3.3. CMCs effects on total phenols

In the table below (table 14) the results concerning the total phenols analyses, are shown.

Table 14: Total phenols content (flavonoids and non- flavonoids) of the wines.

TREATMENT	Total phenols	Non- flavonoids	Flavonoids
Control	1774 ± 4 b	141 ± 0 ab	1633 ± 5 b
CMC1	1740 ± 16 b	144 ± 0 bc	1596 ±16 b
CMC2	1802 ± 30 b	193 ± 0 d	1608 ± 30 b
CMC3	1779 ± 10 b	197 ± 1 d	1582 ± 10 b
CMC4	1751 ± 11 b	148 ± 1 c	1603 ± 10 b
CMC5	1623 ± 4 q	145 ± 0 bc	1478 ± 4 a
CMC6	1786 ± 17 b	137 ± 1 a	1649 ± 17 b

The values come from the calculations exposed in the paragraph 5.4. of MATERIALS AND METHODS.
All the values are expressed in mg/l of gallic acid.

Compared to the characteristics exposed elsewhere (paragraphs 4.3.1. and 4.3.2.), the effect of the CMCs on the phenols content reflects a different behaviour. In the specific, it is not possible to observe a linear trend characterizing the three parameters.

For the total phenols content the CMC5 sample shows the lowest concentration (1623 mg/l), followed by the CMC1, CMC4, control, CMC3, CMC6 and CMC2 samples. Moreover, two subsets are formed, completely separating the CMC5 treated wine from the rest of the samples. The control wine, with a concentration of total phenols equal to 1774 mg/l, does not differ from the CMC1, CMC2, CMC3, CMC4 and CMC6 treatments. Furthermore, the control sample is characterized by an intermediate value (1774 mg/l), clearly showing that the treatments do not have a strong influence on the total phenols content.
However, this result is of extreme importance if we think that the previous studies (Guise et al., 2014) reported a decrease in the total phenols content after the CMC addition on red wines.

For the non- flavonoids content, four subsets are formed but only the CMC6 treated wine shows a lower value compared to the control. All the other CMCs treated samples are characterized by higher values, reflecting that these products induced an increase in the non-flavonoids concentration.

In opposition, concerning the flavonoids content, the CMCs treated samples are characterized by the lowest values; the only exception is represented by the CMC6. This result is in line with the ones already exposed above regarding total tannins and total anthocyanins concentration. The flavonoids compounds are composed by flavonols, flavononols, condensed tannins and athocyanins. Therefore, if the tannins and anthocyanins content is higher for the control wine than for most of the CMCs treated samples, it appears clear that the flavonoids content reflects the same behaviour. However, as reported in the table 14, the control wine completely differs only from the CMC5, meaning that the treatments did not completely change the control wine's flavonoids structure.
Therefore, it appears clear that the CMCs' total phenols composition, exposed in the table 9 (sub-paragraph 5.1), does not have a significant impact on the wines' phenols characterization; even if the CMC6 treated wine shows the highest tannins' concentration and flavonoids' content, it does not affect the phenols' composition in a significant way.

In conclusion, it can be said that the CMCs effect on total phenols is not as important as for the other chromatic parameters above exposed (except for the non- flavonoids compounds). However, these results, differently from the one obtained by Guise et al. (2014), do not reveal a negative effect of the CMCs addition on the general phenols asset of the wine, showing that further researches and studies are needed to better describe the wines red behaviour after carboxymethylcellulose is added.

Finally, it has been noticed that the different CMC concentrations characterizing the products did not have an important impact on the chromatic characteristics: it has not been found any correlation between these parameters.

6. CMCs impact on the sensorial characteristics

The sensory evaluation has been considered necessary to conclude this complex and diverse study.
To study the sensorial effects caused by the CMC addition, each product has been compared to the control wine.
The control wine showed a clear, powerful red colour, with a good chromatic intensity. In the aromas, an important fruity character, accompanied with woody and chocolate notes has been detected. Concerning the taste, good acidity, sweetness and astringency levels have been perceived. No bitterness has been found. The control wine has been considered as a well-balanced wine.

When compared to the control wine, the treated samples did not display significant differences: they have been all considered similar to the initial wine. They were characterized by a powerful and intense colour, however with more violet- blue notes. It has been noticed, as expected, that the aroma intensity slightly decreased after the products' addition. Even though, the treated wines appeared with a higher fruity character with no woody and chocolate notes perceived. Regarding the mouthfeel sensations, the treated wines showed a lower astringency, correlated with a higher acidity. These results are linked to the CMC's nature: being the CMC a polysaccharide, it binds with the tannins, reducing their impact on the astringency, and with the aromatic compounds, making more difficult their perception (Lubbers et al., 1993; Vidal et al., 2004; Carvalho et al., 2006; Chalier et al., 2007). Furthermore, no bitterness has been found after the product's addition, like in the control wine.

To confirm if the higher acidity perceived during the tasting was linked to the intrinsic wines' characteristics, an analysis of the total acidity has been performed for the wines treated with the CMCs. The results, displayed in the table 15 show that all the samples added with the CMC are characterized by a significantly higher total acidity level, reflecting the sensation perceived by the tasters.

Table 15: Total acidity and pH of the wines.

	Control	**CMC1**	**CMC2**	**CMC3**	**CMC4**	**CMC5**	**CMC6**
Total acidity (g/l of tartaric acid)	4.65	4.8	4.8	4.65	5.25	5.1	4.8
pH	3.57	3.62	3.59	3.62	3.61	3.62	3.61

As exposed by Rodriguez- Clemente & Correa- Gorospe (1988): “during the KHT precipitation, the decrease in the concentration of K^+ ions in wine is greater than that of the HT^-. This may be due to the negatively charged impurities (such as protective colloids: CMC) absorbed onto the crystal faces and which allow them to act as traps for the K^+ ions”. Therefore, the CMC in this case acts as a “complexing potassium ions substance”, that makes the amount of free ions available for the crystals growth decreasing (Cabrita et al., 2016); in other words, the CMC retains the minerals and liberates the tartaric anions. This behaviour can maybe explain the increase in the total acidity levels when CMC is present. Bearing in mind this effect related to the CMC addition, it can be stated that the application of this product can probably improve the sensorial quality of some wines (i.e. the ones characterized by low acidity levels). It is clear that, to understand if this effect occurs in all the wines treated with CMC and if it can really improve their quality, further researches are needed.

Furthermore, it has been noticed that the increase in total acidity is not related to the pH level, that did not show important differences after the CMCs’ addition, comparing it to the control.

Moreover, being all the treated wines (the CMC6 treatment included) perceived to have a lower astringency value compared to the control, it can be said that the tannins’ composition in terms of oligomeric and polymeric fractions did not impact the wine astringency, and that, therefore, the tannin power had a stronger impact on this mouthfeel sensation.
However, among all the samples, the CMC3 treated wine has been perceived as the one more similar to the initial wine; moreover, it showed to have the same total acidity level as the control.

In conclusion, it can be said that the results obtained confirmed the positive effects elsewhere exposed: the CMC did not negatively impact the wine characteristics.

7. Economic impact of the CMC utilization

Being the economic impact an important aspect considered when oenological products and/or techniques are used, this paragraph will represent a short overview regarding the economic influence that the several tartaric stabilization treatments are characterized by.

In the Table 16, the costs of tartrate stabilisation are reported (Lasanta & Gòmez, 2012).

Table 16: Costs of tartrate stabilization.

	Method	Direct costs (€/hl)	Amortization (€/hl)	Total costs [f] (€/hl)	Rate [e] (%)
Gomez et al., 2002 [a]	Cold treatment	0.76	0.19	0.95	100
	Ion exchange	0.07	0.04	0.11	11.58
	Electrodialysis	0.56	0.58	1.14	120
Low et al., 2008 [b,c]	Cold treatment	1.38	0.67	2.05	100
	Cold treatment with seeding	3.74	0.69	4.43	216.10
	Semi continue cold treatment	1.99	0.72	2.71	132.20
	Continue cold treatment	2.60	0.66	3.26	159.02
	Electrodialysis	3.1	1.57	4.68	228.29
Rondeau, 2011 [d]	MTA	0.07	-	0.07	7.40
	CMC	0.7	-	0.7	73.68
	MP	3.0	-	3.0	315.78

[a] Adapted from Gòmez et al. (2002).
[b] Adapted from Low et al. (2008).
[c] Currency at April 7, 2012: 0.787 €/AUD.
[d] Extracted from Rondeau (2011).
[e] Rate considering cold treatment as 100.
[f] The amortization presupposes a service life of 10 years.

Extracted from Lasanta & Gòmez (2012).

It is clear that the total costs of tartrate stabilisation completely differ from one technique to the other:

- They are equal to 0.95 €/hl (Gomez et al., 2002) and 2.05 €/hl (Low et al., 2008) using the cold treatment; these costs increase when cold treatment with seeding, semi continue cold treatment and continue cold treatment are used (respectively 4.43 €/hl, 2.72 €/hl and 3.26 €/hl)
- Regarding the electrodialysis, they are equal to 1.14 €/hl (Gomez et al., 2002) and 4.68 €/hl (Low et al., 2008)
- Concerning the additives, MTA and CMC are lower- priced than cold treatment and MP is higher-priced (respectively 0.07 €/hl, 0,7 €/hl and 3.0 €/hl).

As exposed by Lasanta & Gòmez (2012): “the costs in the second paper (Low et al., 2008) are higher than the first (Gomez et al., 2002) because of the differences in executions time and location”.

Therefore, it can be said that, according to the values reported in table 10, the ion exchange is the cheapest technology and electrodialysis the most expensive, being all the modalities of cold treatment more expensive than this one and that, concerning the additives, the MTA is the cheapest and the MP the most expensive. Moreover, the MTA and the CMC are cheaper than all the other techniques, except the ion exchange resins.

For all these reasons we can say that the utilisation of CMC in the wine industry for red wines tartaric stabilisation can be useful to reduce the companies' costs. Furthermore, being the CMC a non- energy requiring technique, it can be an opportunity to reduce the environmental impact. Therefore, the CMC can be used as a sustainable alternative to cold treatment.

IV. CONCLUSIONS

Considering the aims of my research, it can be said that the responses of the wine to the CMCs addition have been positive and that the different CMCs reported a strong influence on the wine's characteristics, even if in different ways.

The first and important result revealed that the carboxymethylcellulose resulted as a strong inhibitor of potassium bitartrate salts crystallization, making possible further analyses and experiments about colour, colouring matter stability and chromatic characteristics.
In this regard, the addition of this product reported an important influence on the colour and on the chromatic characteristics of the initial wine, with a general increase in colour intensity, coloured anthocyanins and polymerized pigments' content.
Furthermore, the total phenols concentration of the CMCs added samples did not completely differ from the control wine, as such as the tannins' composition in terms of monomeric, oligomeric and polymeric fractions content.
In addition to this, the studied CMCs revealed a fundamental, unexpected and of extreme importance effect that completely changes the CMC scenario regarding its utilization on red wines: no colouring matter precipitation occurred in the four months of the experiments.
However, the CMC reported an increase in the wine turbidity.

In terms of sensorial quality, the treated wines have been all considered similar to the initial wine. In general, it has been noticed that the CMCs added samples were characterized by a powerful colour, however with more violet- blue notes compared to the control; an intense fruity and woody character, even if with an intensity slightly decreased after the products' addition; regarding the mouthfeel sensations, the treated wines showed a lower astringency, correlated with a higher acidity. No bitterness has been found after the product's addition, like in the control wine. Among all the samples, the CMC3 treated wine has been perceived as the one more similar to the initial wine.

Together with these results, it has also been seen that the CMC, compared to the other tartaric stabilization treatments is characterized by lower costs and higher sustainability. This, in line with the higher environmental and economic consciousness characterizing the winemaking and viticulture processes, represents a fundamental aspect that, in the next future, will have more and more importance.

For all of these reasons it can be said that, being the CMC still forbidden in the production of red wines, this study has been of extreme importance in the evaluation of the wines' response to the addition of the products; the results will offer a starting point for future studies and experiments with the aim of verifying the eventual authorization of the addition of this additive also in red wines.
It is clear that further researches are needed to better evaluate and understand how the red wines react to the CMC application. It could be helpful, in the future, to analyse this product on different red wines, coming from different regions and/or countries with different weather conditions, viticulture and wine making processing (i.e. not stabilized in terms of colouring matter precipitations).

It is important to say that we decided to stop the analyses after five months because of timing reasons, but it would be interesting and helpful to evaluate the colouring matter stability after one year-contact between the wine and the CMC. This would be supportive to my research to better understand the response of the wine to the product's addition and to establish the CMC's long term effect. Additionally, it could be representative to analyse the effect of the CMCs when added at the same CMC concentration and dose, and to estimate which CMC produces the highest stability. This can be a fundamental trial to evaluate eventual differences in the sensorial characterization of the wine. Moreover, it could be necessary to study the effects of the CMC's addition on the sensory characteristics basing on a wider panel of tasters, more detailed evaluations and statistical analyses.

V. REFERENCES

- **Angele, L. (1992).** *STABISAT: Tartaric stability control and production management*. Revue des Œnologues, 65, 43-47.
- **Arnold, R. M., Noble, A. C. (1978).** *Bitterness and astringency of grape seed phenolics in a model wine solution.* American Journal of Oenology and Viticulture, 29, 150-152.
- **Arnold, R. M., Noble, A. C., Singleton, V. L. (1980).** *Bitterness and astringency of phenolic fractions in wine.* Journal of Agricultural and Food Chemistry, 28, 675-678.
- **Asvany, A. (1986).** *Resultats recents de l'utilisation de carboxymethylcellulose*. 15eme session du groupe d'expert technologie du vin O.I.V., doc.1425/87.
- **Benítez, J. G., Marcías, V.M. P., Pazo, J. A. S., & Rodríguez, L. P. (2002b).** *Industrial development of proton exchange for tartrate stabilization of sherry wines*. European Food Research and Technology, 214, 418–422.
- **Benítez, P., Castro, R., & Barroso, C. G. (2002a).** *Removal of iron, copper and manganese from white wines through ion exchange techniques: effects on their organoleptic characteristics and susceptibility to browning*. Analytica Chimica Acta, 458, 197–202.
- **Blouin, J. (1982).** *Les techniques de stabilisation tartrique des vins par le froid*. Connaissance de la Vigne et du Vin, 16, 63 - 77.
- **Blouin, J., Guimberteau, G., & Auduit, P. (1979).** *Prévention des précipitations tartriques dans les vins par le procédé par contact*. Connaissance de la Vigne et du Vin, 13, 140 - 169.
- **Bosso, A., Salmaso, D., Faveri, E., Guaita, M., & Franceshi, D. (2010).** *The use of carboxymethylcellulose for the tartaric stabilization of white wines, in comparison with other oenological additives*. Vitis, 49, 95–99.
- **Boulton, R.B., Singleton, V.L., Bisson L.F., & Kunkee R.E. (1996).** *Principles and practices in winemaking*. Chapman & Hall, New York.
- **Bruijn, J., Valdebenito, A., Loyola, C., Serra, I., Salazar, F., & López, F. (2009).** *Continuous stabilization of Chardonnay with ion-exchange resin: influence on protein and phenolic profiles of wine*. Chilean Journal of Agricultural Research, 69, 54–59.
- **Carvalho, E., Mateus, N., Plet, B., Pianet, I., Dufourc, E., & De Freitas, V. (2006).** *Influence of Wine Pectic Polysaccharides on the Interactions between Condensed Tannins and Salivary Proteins.* Journal of Agriculture and Food Chemistry, 54, 8936 – 8944.
- **Chalier, P., Angot, B, Delteil, D., Doco, T., & Gunata, Z. (2007).** *Interactions between aroma compounds and whole mannoprotein isolated from* Saccharomyces cerevisiae *strains.* Food Chemistry, 100, 22 – 30.
- **Chen, H. (2014).** *Chemical Composition and Structure of Natural Lignocellulose*. Biotechnology of Lignocellulose: Theory and Practice, 2, 25 - 71.

- **Cheynier, V., & Ricardo da Silva, J. M. (1991).** *Oxidation of grape procyanidins in model solutions containing trans-caffeoyltartaric acid and polyphenol oxidase.* Journal of Agriculture and Food Chemistry, 39, 1047-1049.
- **Cheynier, V., Osse, C., Rigaud, J. (1988).** *Oxidation of grape juice phenolic compounds in model solutions.* Journal of Food Science, 53, 1729-1729.
- **Claus, H.**, **Tenzer, R. M., Sobe M. S., König H., & Fröhlich J. (2014).** *Effect of carboxymethylcellulose on tartrate salt, protein and colour stability of red wine.* Australian Journal of Grape and Wine Research, 20, 186–193.
- **Crachereau, J. C., Gabas, N., Blouin, J., Hébrard, B., & Maujean, A. (2001).** *Stabilisation tartrique des vins par la carboxyméthylcellulose*. Bulletin de l'OIV, 841–842.
- **European Union (2009).** *Commission Regulation (EC) N 606/2009 of 10 July 2009 laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions*. L 193, pp. 1-59.
- **Freitas de Victor, Mateus N. (2001)**. *Nephelometric study of salivary protein–tannin aggregates.* Journal of the Science of Food and Agriculture. 82,113-119.
- **Garcıa, J. M., Alcantara, R., & Martın, J. (1991).** Evaluation of wine stability to potassium hydrogen tartrate precipitation. American Journal of Oenology and Viticulture, 42, 336 - 340.
- **Gavach, C. (1992).** *L'électrodialyse : principes*. Revue Française d'Œnologie, 137, 53 - 58.
- **Gerbaud, V., Gabas, N., Blouin, J., & Crachereau, J. C. (2010).** *Study of wine tartaric acid salt stabilization by addition of carboxymethylcellulose (CMC): comparison with the "protective colloids" effect*. Journal International des Sciences de la Vigne et du Vin, 44, 231 - 242.
- **Gerbaud, V., Gabas, N., Blouin, J., Pellerin, P., & Moutounet, M. (1997).** *Influence of wine polysaccharides and polyphenols on the crystallization of potassium hydrogen tartrate*. Journal International des Sciences de la Vigne et du Vin, 31, 65 - 83.
- **Gerbaud, V., Gabas, N., Laguerie, C., Blouin, J., Vidal, S., Moutounet, M., et al. (1996).** *Effect of wine polysaccharides on the nucleation of potassium hydrogen tartrate in model solutions.* Transaction of the Institution of Chemical Engineers, 74, 782 - 790.
- **Goertges, S., & Stock, R. (2000).** *Crystals in wine. Crystal stabilization and stability control*. Deutsche-weinmagazin, 2, 24 - 28.
- **Gomez, J., Palacios, V. M., Sanchez, J. A., & Perez, L. (2002*). Industrial development of proton exchange for tartrate stabilization of sherry wines*. European Food Research and Technology, 214, 418 - 422.
- **Gomez, J., Palacios, V. M., Szekely, P., Veas, R., & Perez, L. (2002*). Estimacion de los costes directos de la estabilizacion tartarica mediante tratamiento por frıo, intercambio protonico y electrodialisis*. Tecnologıa del Vino, 45-49, Julio-Agosto.
- **Gomez, J., Palacios, V., Veas, R., Valcarcel, M., & Perez, L. (2004).** *Characterisation, control and improvement of the cold treatment of Sherry wines*. Food Control, 15, 111 - 116.

- **Greeff, A.E., Robillard, B., du Toit, W.J. (2012).** *Short- and long-term efficiency of carboxymethylcellulose (CMC) to prevent crystal formation in South African wine*. Food Additives & Contaminants: Part A, 29, 1374 – 1385.
- **Guerif, G. (1993).** *L'électrodialyse appliquée à la stabilisation tartrique des vins*. Revue des Œnologues, 69, 39 - 42.
- **Guise, R., Filipe-Ribeiro, L., Nascimento, D., Bessa, O., Nunes, F.M., Cosme, F. (2014).** *Comparison between different types of carboxymethylcellulose and other oenological additives used for white wine tartaric stabilization*. Food Chemistry, 156, 250 – 257.
- **Haslam, E. (1974).** *Polyphenols-proteins interactions*. Biochemistry Journal, 139, 285-288.
- **Haslam, E. (1980).** *In Vino Veritas. Oligomeric procyanidins and the aging of red wines.* Phytochemistry, 19, 1577-1582.
- **Haslam, E., Lilley, T. H. (1988).** *Natural acan not be separated in the same conditions stringency in foodstuffs-molecular interpretation*. Critical Reviews in Food Science and Nutrition, 27, 1 - 40.
- **Ibeas, V., Correira, A.C., & Jordão, A.M. (2015)**. *Wine tartrate stabilization by different levels of cation exchange resin treatments: impact on chemical composition, phenolic profile and organoleptic properties of red wines*. Food Research International, 69, 364 – 372.
- **Jouve, C., Cabanis, J. C., Bourzeix, M., Heredia, N., Rosec, J. P., Vialatte, C. (1989).** *Teneurs en catéchines et procyanidols de vin blancs et rosé ; effets du collage par la caséine*. Revue Française d'Œnologie, 117, 14-20.
- **Khairnar, K. (2014).** *Effect of different organic amendments on soil quality, vines growth, grape production and wine quality of mechanically pruned vineyards.* European Master of Science in Viticulture and Enology thesis.
- **Lasanta, C., & Gómez, J. (2012).** *Tartrate stabilization of wines*. Trends in Food Science & Technology, 28, 52–59.
- **Lasanta, C., Caro, I., & Pérez, L. (2013).** *The influence of cation exchange treatment on the final characteristics of red wines*. Food Chemistry, 138, 1072–1078.
- **Lee, C. Y., & Jaworski, A. (1988).** *Phenolics and browning potential of white grapes grown in New York*. American Journal of Enology and Viticulture, 39, 337-340.
- **Low, L. L., O'Neill, B., Ford, C., Godden, J., Gishen, M., & Colby, C. (2008).** *Economic evaluation of alternative technologies for tartrate stabilisation of wines*. International Journal of Food Science and Technology, 43, 1202-1206.
- **Lubbers, S., Léger, B., Charpentier, C., & Feuillat, M. (1993).** *Effet colloïde protecteur d'extraits de parois de levures sur la stabilité tartrique d'une solution hydro alcoolique modèle*. Journal International des Sciences de la Vigne et du Vin, 27, 13–22.
- **Masquellier, J. (1982).** *L'alimentation et la consommation de vin*. Proceedings, C. R. Symposium Int., Verona: Italy, 147-155.
- **Masquellier, J. (1988).** *Physiological effects of wine. His share in alcoholism.* Bulletin O.I.V., 61, 554 - 578.

- **Maujean, A., Malherbe, V., & Dupont, A. (1984).** *Traitement par le froid artificiel des vins de Champagne. I. Contrôles et traitements par le froid artificiel des vins de Champagne. Efficacité des traitements selon la nature du collage des vins.* Connaissance de la Vigne et du Vin, 18, 49 - 65.
- **Maujean, A., Sausy, L., & Vallee, D. (1985).** *Détermination de la sursaturation en bitartrate de potassium d'un vin. Quantification des effets colloïdes-protecteurs*. Revue Française d'Œnologie, 100, 39 - 50.
- **Minguez, S., & Hernandez, P. (1998).** *Tartaric stabilization of red, rose, and white wines with L (+)-calcium tartrate crystal seeding*. American Journal of Enology and Viticulture, 49, 177 - 182.
- **Mira, H., Leite, P., Ricardo-Da-Silva, J., & Curvelo-Garcia, A. S. (2006).** *Use of ion exchange resins for tartrate wine stabilization*. Journal International des Sciences de la Vigne et du Vin, 40, 223 - 246.
- **Moine-Ledoux, V., & Dubourdieu, D. (1999).** *An invertase fragment responsible for improving the proteins stability of dry white wines*. Journal of the Science of Food and Agriculture, 79, 537 - 543.
- **Moine-Ledoux, V., & Dubourdieu, D. (2002).** *Role yeast mannoproteins with regard to tartaric stabilisation of wines*. Bulletin O.I.V., 75, 471 - 482.
- **Motta S, Bollito A, Guaita M, Petrozziello M, Panero L, Bosso A. (2009).** *Use of carboxymethylcellulose and other additives in the tartaric stabilization of red wines/ impiego della carbossimetilcellulosa e uso di altri additivi nella stabilizzazione tartarica dei vini rossi*. Bulletin O.I.V., 82, 367–377.
- **Mourgues, J. (1993).** *Utilisation des résines échangeuses d'ions. Revue des Œnologues*, 69, 51- 54.
- **Moutounet M, Bouissou D, Escudier JL. (2010).** *Effets de traitement de stabilization tartrique de vins rouges par une gomme de cellulose (carboxymethylcellulose)*. Infowine. 2, 1826–1590.
- **Moutounet, M., Escudier, J. L., & Saint-Pierre, B. (1994).** *L'électrodialyse, adaptation à la stabilisation tartrique des vins. In Les acquisitions récentes dans les traitements physiques du vin. Incidences sus la composition chimique et les qualités organoleptiques des vins.* Bernard Doneche, 103 - 115.
- **Moutounet, M., Saint Pierre, B., Battle, J. L., & Escudier, J.L. (1997).** *Le stabilisateur tartrique : principe et description du procédé.* Revue Française d'Œnologie, 162, 15 – 17.
- **Müller, T., Scholten, G., & Friedrich, G. (1997).** *Simultaneous potassium bitartrate and calcium tartrate of wines by means of a contact process with calcium tartrate*. Mitteilungen Klosterneuburg, 47, 74 - 84.
- **Müller-Spath (1979).** *La stabilisation du tartre avec le procédé par contact*. Revue Française d'Œnologie, 73, 41c.
- **Oh, H. I., Hoff, J. E. (1986).** *Effect of condensed grape tannins on the in vitro activity of digestive proteases and activation of their zymogens.* Journal of Food Science, 51, 577-580.
- **Okuda, T.; Mori, K.; Hatano, T. (1985).** *Relationship of the structures of tannins to the binding activities with hemoglobin and methylene blue*. Chemical and Pharmaceutical Bulletin, 33, 1424-1433.

- **Oszmianski, J., Sapis, J. C., Macheix, J. J. (1985).** *Changes in grape seed phenols as affected by enzymatic and chemical oxidation in vitro*. Journal of Food Science, 50, 1505-1506.
- **Porter, L. J.; Woodruffe, J. Haemanalysis (1984).** *The relative astringency of proanthocyanidin polymers*. Phytochemistry, 23, 1255-1256.
- **Powers, J. R., Nagel, C. W., Weller, K. (1988).** *Protein removal from a wine by immobilized grape proanthocyanidins.* American Journal of Enology and Viticulture, 39, 117-120.
- **Ribéreau-Gayon, J., & Peynaud, E. (1958).** *Analyse et contrôle des vins. (2eme Ed.).* Libraire Polytechnique Ch. Béranger. Chapitre 17, p. 355.
- **Ribéreau-Gayon, P., Dubordieu, D., Doneche, B., & Lonvaud, A. (2006).** *Handbook of enology, (2nd Ed.)*. Chichester: John Wiley & Sons.
- **Ricardo da Silva, J. M., Cheynier, V., Souquet, J. M., Moutounet, M., Cabanis, J. C., Bourzeix, M. (1991b).** *Interaction of grape seed procyanidins with various proteins in relation to wine fining.* Journal of the Science of Food and Agriculture, 57, 111-125.
- **Ricardo da Silva, J. M., Darmon, N., Fernàndez, Y., Mitjavila, S. (1991c).** *Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds.* Journal of Agricultural and Food Chemistry, 39, 1549-1552.
- **Rigaud, J., Escribano-Bailon, M. T., Prieur, C., Souquet, J. M., Cheynier, V. (1993).** *Normal-phase high-performance liquid chromatographic separation of procyanidins from cacao beans and grape seeds.* Journal of Chromatography, 654, 255-260.
- **Robichaud, J. L.; Noble, A. C. (1990).** *Astringency and bitterness of selected phenolics in wine.* Journal of the Science of Food and Agriculture, 53, 343-353.
- **Rodriguez-Clemente, R., & Correa-Gorospe, I. (1988).** *Structural, morphological and kinetic aspects of potassium hydrogen tartrate precipitation from wine and ethanolic solutions*. American Journal of Enology and Viticulture, 39, 169–178.
- **Rondeau, N. (2011).** *Are there any option to cold treatment?* data current at April 7, 2012. http://www.in-enologia-veritas.com/2011/01/¿existen-alternativas-a-la-estabilizacion-por-frio/.
- **Schneider, V. (2003).** *Flüchtige Säure – ein Gärproblem*. Das Deutsche Weinmagazin, 24, 10 – 12.
- **Sicheri, G. (2015).** *Enologia: con elementi di chimica viticolo – enologica*. Capitolo 5, 270.
- **Singleton, V. L. (1992).** *Tannins and the qualities of wines. In Plant Polyphenols Synthesis, Properties, Significance;* Plenum Press: New York, 859-880.
- **Singleton, V. L., & Trousdale, E. K. (1992).** *Anthocyanin-tannin interactions explaining differences in polymeric phenols between white and red wines.* American Journal of Enology and Viticulture, 1, 63 - 70.
- **Somers, T.C., Evans, M.E. (1977)**. *Spectral evaluation of young Red wines: Anthocyanin Equilibria, Total Phenolics, Free and Molecular SO_2, Chemical Age.* Journal of the Science of Food and Agriculture, 28, 279-287.

- **Sun, B., Leandro C., da Silva, J.R., Spranger, I. (1998).** *Separation of Grape and Wine Proanthocyanidins According to Their Degree of Polymerization.* Journal of Agricultural and Food Chemistry, 46, 1390-1396.
- **Timberlake, C. F., Bridle, P. (1976).** *Interaction between anthocyanins, phenolic compounds and acetaldehyde and their significance in red wines.* American Journal of Enology and Viticulture, 27, 97-105.
- **Ubigli, M. (2004).** *I profili del vino*. *Alla scoperta dell'analisi sensoriale.*
- **Usseglio-Tomasset, L., Bossia, P. D., Delfini, C., & Ciolfi, G. (1980).** *I vini Recioto e Amarone della Valpolicella*. Vini d'Italia, 125, 85 - 97.
- **Viaux, L., Gabas, N., & Robillard, B. (1996***). Induction de la cristallisation de l'hydrogenotartrate de potassium par le tartrate de calcium*. Revue Franc¸ aise d'Œnologie, 157, 5 - 9.
- **Vidal, S., Francis, L., Williams, P., Kwiatkowski, M., Gawel, R., Cheynier, V., & Waters, E. (2014).** *The mouth-fell properties of polysaccharides and anthocyanins in a wine like medium.* Journal of Agricultural and Food Chemistry, 85, 519 – 525.
- **Walker, T., Morris, J., Threfall, R., & Main, G. (2002).** *pH modification of Cynthiana wine using cationic exchange*. Food Chemistry, 50, 6346 - 6352.
- **Wurdig G., Muller T. and Fiedrich G. (1982).** *Bulletin O.I.V.*, 613, 220**.**
- **Yokotsuka, K., Singleton, V. L. (1987).** *Interactive precipitation between grape peptides from gelatin and specific grape tannin reactions in wine-like model solutions.* American Journal of Enology and Viticulture, 38, 199-206.

Websites

- www.etslabs.com (2013). *Cold stability tests for wine*. Technical Bulletin.
- www.institut-oenologique.com (2011). *The alternative to tartaric stabilisation.*

VI. ANNEXES

Annex 1

Laboratory and statistical results

1. Tannins (monomers, oligomers, polymers) composition analyses results

Table 1: Sun method results. Monomeric, oligomeric, polymeric fractions and total tannins' concentration.

	Control	CMC1	CMC2	CMC3	CMC4	CMC5	CMC6
F1 A500							
1	0.163	0.173	0.205	0.157	0.178	0.133	0.174
2	0.163	0.195	0.180	0.176	0.165	0.152	0.184
3	0.234	0.184	0.192	0.166	0.143	0.191	0.230
4	0.126	0.184	0.192	0.166	0.186	0.196	0.238
MEAN	**0.17**	**0.18**	**0,19**	**0.16**	**0.17**	**0.17**	**0.21**
Vrs (ml)	5	5	5	5	5	5	5
b	0.0081	0.0081	0.0081	0.0081	0.0081	0.0081	0.0081
Vsample (ml)	5	5	5	5	5	5	5
F1 – monomers	**21.17**	**22.72**	**23.77**	**20.56**	**20.74**	**20.74**	**25.52**
F2 A500							
1	0.294	0.264	0.328	0.248	0.349	0.305	0.371
2	0.275	0.264	0.347	0.269	0.306	0.358	0.415
3	0.236	0.25	0.337	0.258	0.323	0.331	0.456
4	0.24	0.278	0.337	0.258	0.326	0.331	0.5
MEAN	**0.26**	**0.26**	**0.34**	**0.26**	**0.33**	**0.33**	**0.43**
Vrs (ml)	5	5	5	5	5	5	5
b	0.0046	0.0046	0.0046	0.0046	0.0046	0.0046	0.0046
Vsample (ml)	5	5	5	5	5	5	5
F2 – oligomers	**56.79**	**57.39**	**73.37**	**56.20**	**70.87**	**72.05**	**94.67**
F3 A500							
1	0.744	0.627	0.654	0.748	0.708	0.682	0.756
2	0.688	0.656	0.71	0.668	0.751	0.585	0.741
3	0.767	0.656	0.682	0.708	0.712	0.715	0.740
4	0.679	0.704	0.682	0.708	0.724	0.702	0.746
MEAN	**0.72**	**0.67**	**0.68**	**0.71**	**0.72**	**0.67**	**0.74**
Vrs (ml)	25	25	25	25	25	25	25
b	0.0037	0.0037	0.0037	0.0037	0.0037	0.0037	0.0037
Vsample (ml)	5	5	5	5	5	5	5
F3 – polymers	**972.26**	**892.91**	**921.62**	**956.76**	**977.93**	**906,76**	**1007,70**
TOTAL	**1050.23**	**973.01**	**1018.76**	**1033,51**	**1069,54**	**999,55**	**1127,90**

The F1 – monomers, F2 – oligomers and the F3 – polymers results are obtained from the calculations exposed in the paragraph 5.4. of MATERIALS AND METHODS.
The TOTAL (total tannin's concentration) is the sum of the three fractions (F1 + F2 + F3).

Table 2: One- way ANOVA test results for monomeric, oligomeric, polymeric fractions and total tannin's concentration.

Univariate Tests

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
F_MONO	Contrast	87.052	6	14.509	1.475	.242 (ns)
	Error	177.062	18	9.837		
F_OLIGO	Contrast	4553.558	6	758.926	21.230	.000
	Error	643.451	18	35.747		
F_POLI	Contrast	42230.947	6	7038.491	3.101	.029
	Error	40861.748	18	2270.097		
TOT	Contrast	61247.947	6	10207.991	4.427	.006
	Error	41503.593	18	2305.755		

The F tests the effect of the TREATMENTS (the control wine and the 6 CMCs). This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Tables 3 and 4: Tukey post-hoc tests results for monomeric and oligomeric fractions.

F1 - CATECHINS

Tukey HSD

TREAT	N	Subset 1
CMC3	4	20.5 a
CMC4	4	20.7 a
CMC5	4	20.7 a
Control	4	21.2 a
CMC1	4	22.7 a
CMC2	4	23.8 a
CMC6	4	25.5 a
Sig.		.324

F2 - OLIGOMERS

Tukey HSD

TREAT	N	Subset 1	2	3	4
CMC3	4	56.2 a			
Control	4	56.8 a			
CMC1	4	57.4 ab	57.4 ab		
CMC4	4		70.9 bc	70.9 bc	
CMC5	4			72.08 c	
CMC2	4			73.4 c	
CMC6	4				94.7 d
Sig.		1.000	.063	.996	1.000

Tables 5 and 6: Tukey post-hoc tests results for polymeric fraction and total tannin's concentration.

F3 – POLYMERS

Tukey HSD

TREAT	N	Subset	
		1	2
CMC1	4	892.9 a	
CMC5	4	906.7 ab	906.7 ab
CMC2	4	921.6 ab	921.6 ab
CMC3	4	956.7 ab	956.7 ab
Control	4	972.3 ab	972.2 ab
CMC4	4	977.9 ab	977.9 ab
CMC6	4		1007.7 b
Sig.		.208	.090

TOTAL

Tukey HSD

TREAT	N	Subset	
		1	2
CMC1	4	973.0 a	
CMC5	4	999.5 a	
CMC2	4	1018.7 ab	1018.7 ab
CMC3	4	1033.5 ab	1033.5 ab
Control	4	1050.2 ab	1050.2 ab
CMC4	4	1069.5 ab	1069.5 ab
CMC6	4		1127.9 b
Sig.		,120	,060

2. Tannin power analyses results

Table 7: Tannin power analyses results.

	Control	CMC1	CMC2	CMC3	CMC4	CMC5	CMC6
d0 (vino)							
1	2.34	2.23	2.2	2.62	2.31	2.67	2.52
2	2.46	2.44	2.03	2.8	2.82	2.05	2.47
3	2.16	2.56	2.3	2.25	2.46	2.45	2.53
d (BSA)							
1	24.6	23	22.5	22.3	24.4	22.8	22.7
2	24.6	23.6	23.8	23.6	22.5	22.2	22.8
3	24.4	22	22.2	22.3	23.2	22.5	23.8
Tannin power (NTU/ml)	**277.67**	**255.71**	**258.21**	**252.21**	**260.46**	**251.37**	**257.42**

The Tannin power results are obtained from the calculations exposed in the paragraph 5.4. of MATERIALS AND METHODS.

Table 8: One- way ANOVA test results for the tannin power parameter.

Tests of Between-Subjects Effects

Dependent Variable: Tannin power (NTU/ml)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	1410172.172	7	201453.167	2300.268	.000
TREAT	1410172.172	7	201453.167	2300.268	.000 (ns)
Error	1226.094	14	87.578		
Total	1411398.266	21			

The F tests the effect of the TREATMENTS (the control wine and the 6 CMCs). This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Table 9: Tukey post-hoc test results for the tannin power.

Tannin power (NTU/ml)

Tukey HSD

TREAT	N	Subset	
		1	2
CMC5	3	251.4 a	
CMC3	3	252.2 b	252.2 ab
CMC1	3	255.7 ab	255.7 ab
CMC6	3	257.4 ab	257.4 ab
CMC2	3	258.2 ab	258.2 ab
CMC4	3	260.4 ab	260.4 ab
Control	3		277.7 b
Sig.		.887	.058

3. Chromatic characteristics analyses results

3.1. Absorbances at 420, 520 and 620 nm, intensity and tonality results

Table 10: Absorbances at 420, 520, 620 nm, intensity and tonality analyses results.

		Control	CMC1	CMC2	CMC3	CMC4	CMC5	CMC6
A420	**1**	2.76	2.9	2.97	3.24	2.96	3.22	2.94
	2	2.74	2.91	2.96	3.21	2.96	3.22	2.95
	3	2.76	2.9	2.97	3.22	2.95	3.22	2.97
Average		**2.75**	**2.90**	**2.97**	**3.22**	**2.96**	**3.22**	**2.95**
A520	**1**	4.15	4.27	4.33	4.91	4.25	4.8	4.19
	2	4.13	4.26	4.31	4.87	4.25	4.77	4.18
	3	4.14	4.29	4.32	4.89	4.24	4.82	4.19
Average		**4.14**	**4.27**	**4.32**	**4.89**	**4.25**	**4.80**	**4.19**
A620	**1**	0.89	0.9	0.9	1.01	0.91	0.99	0.9
	2	0.87	0.9	0.89	099	0.91	0.98	0.91
	3	0.88	0.9	0.9	1	0.89	0.98	0.92
Average		**0.88**	**0.90**	**0.90**	**1.00**	**0.90**	**0.98**	**0.91**
Intensity		**7.77**	**8.08**	**8.18**	**9.11**	**8.11**	**9.00**	**8.05**
Tonality		**0.67**	**0.68**	**0.69**	**0.66**	**0.70**	**0.67**	**0.71**

Intensity and tonality values are obtained from the calculations exposed in the paragraph 5.4. of MATERIALS AND METHODS.

Table 11: One- way ANOVA test results for the absorbances (420, 520, 620 nm) analyses.

Univariate Tests

ABSORB		Sum of Squares	df	Mean Square	F	Sig.
A420	Contrast	.521	6	.087	834.656	.000
	Error	.001	12	.000		
A520	Contrast	1.668	6	.278	2123.309	.000
	Error	.002	12	.000		
A620	Contrast	.040	6	.007	96.512	.000
	Error	.001	12	6.825E-005		

The F tests the effect of the TREATMENTS (the control wine and the 6 CMCs). This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Table 12: Tukey post-hoc test results for the Absorbance analyses at 420 nm.

ABSORBANCE AT 420 nm

Tukey HSD

TREAT	N	Subset			
		1	2	3	4
Control	3	2.753 a			
CMC1	3		2.903 b		
CMC6	3			2.953 c	
CMC4	3			2.957 c	
CMC2	3			2.967 c	
CMC5	3				3.220 d
CMC3	3				3.223 d
Sig.		1.000	1.000	.684	1.000

Table 13: Tukey post-hoc test results for the Absorbance analyses at 520 nm.

ABSORBANCE AT 520 nm

Tukey HSD

TREAT	N	Subset					
		1	2	3	4	5	6
Control	3	4.140 a					
CMC6	3		4.187 b				
CMC4	3			4.247 c			
CMC1	3			4.273 c			
CMC2	3				4.320 d		
CMC5	3					4.797 e	
CMC3	3						4.890 f
Sig.		1.000	1.000	.142	1.000	1.000	1.000

Table 14: Tukey post-hoc test results for the Absorbance analyses at 620 nm.

ABSORBANCE AT 620 nm

Tukey HSD

TREAT	N	Subset		
		1	2	3
Control	3	.8800 a		
CMC2	3	.897 ab	.897 ab	
CMC1	3	.900 ab	.900 ab	
CMC4	3	.903 ab	.903 ab	
CMC6	3		.910 b	
CMC5	3			.983 c
CMC3	3			1.000 c
Sig.		.053	.473	.251

Table 15: One- way ANOVA test results for intensity and tonality analyses.

Univariate Tests

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
INTENSITY	Contrast	4.759	6	.793	1357.880	.000
	Error	.007	12	.001		
TONALITY	Contrast	.005	6	.001	134.362	.000
	Error	7.531E-005	12	6.276E-006		

The F tests the effect of the TREATMENTS (the control wine and the 6 CMCs). This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Table 16: Tukey post-hoc test results for the intensity analyses.

INTENSITY

Tukey HSD

TREAT	N	Subset				
		1	2	3	4	5
Control	3	7.77 a				
CMC6	3		8.05 b			
CMC1	3		8.08 b			
CMC4	3		8.11 bc	8.11 bc		
CMC2	3			8.18 c		
CMC5	3				9.00 d	
CMC3	3					9.11 e
Sig.		1.000	.236	.055	1.000	1.000

Table 17: Tukey post-hoc test results for the tonality analyses.

TONALITY

Tukey HSD

TREAT	N	Subset					
		1	2	3	4	5	6
CMC3	3	.659 a					
Control	3	.665 ab	.665 b				
CMC5	3		.671 b				
CMC1	3			.679 c			
CMC2	3				.687 c		
CMC4	3					.696 d	
CMC6	3						.705 e
Sig.		.135	.104	1.000	1.000	1.000	1.000

3.2. Anthocyanins' content (total and coloured), total and polymerized pigments' content and total phenols analyses results

Table 18: Anthocyanins' content (total and coloured), total and polymerized pigments' content and total phenols analyses results.

	Control	CMC1	CMC2	CMC3	CMC4	CMC5	CMC6
Total anthocyanins (mg/l)	389.29	389.29	381.34	381.69	419.36	343.77	360.97
Ionization index (%)	11.01	11.69	11.99	14.60	10.79	15.63	11.75
Coloured anthocyanins (mg/l)	42.87	45.53	45.73	55.73	45.26	53.73	42.4
Total pigments (Abs)	22.79	22.79	22.45	22.59	24.27	20.70	21.49
Polymerized pigments (Abs)	1.99	1.99	2.03	2.10	1.98	2.11	2.01
Polymerization index (%)	884.78	884.78	914.54	940.39	825.24	1029.27	971.18
Total phenols (Abs)	54.80	53.77	55.67	54.97	5410	50.13	55.18
Total phenols (mg/l)	1774.01	1740.57	1802.06	1779.40	1751.36	1622.98	1786.31
Non-flavonoids (Abs)	4.35	4.44	5.97	6.08	4.56	4.47	4.23
Non-flavonoids (mg/l)	141.22	144.34	193.64	197.42	148.12	145.31	137.55
Flavonoids (Abs)	50.45	49.32	49.70	48.88	49.54	45.66	50.95
Flavonoids (mg/l)	1632.79	1596.22	1608.41	1581.98	1603.24	1477.67	1648.76

The values are obtained from the calculations exposed in the paragraph 5.4. of MATERIALS AND METHODS.

Table 19: One- way ANOVA test results for anthocyanins' content (total and coloured), ionization index, total and polymerized pigments' content, polymerization index analyses.

Univariate Tests

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
ANT_tot (mg/L)	Treat	10191.425	6	1698.571	15.612	.000
	Error	1523.200	14	108.800		
Ionization %	Treat	62.126	6	10.354	88.686	.000
	Error	1635	14	.117		
ANT_col (mg/L)	Treat	497.638	6	82.940	483.815	.000
	Error	2.400	14	.171		
PIG_tot (Abs)	Treat	22.622	6	3.770	13.428	.000
	Error	3.931	14	.281		
PIG_pol (Abs)	Treat	.050	6	.008	27.698	.000
	Error	.004	14	.000		
Polimerization %	Treat	78960.226	6	13160.038	33.418	.000
	Error	5513.133	14	393.795		

The F tests the effect of the TREATMENTS (the control wine and the 6 CMCs). This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Table 20: One- way ANOVA test results for total phenols (non-flavonoids and flavonoids) analyses.

Univariate Tests

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
PHEN_tot (mg/L)	Treat	65059.346	6	10843.224	13.999	.000
	Error	10844.097	14	774.578		
NON_flav (mg/L)	Treat	11906.645	6	1984.441	602.874	.000
	Error	46.083	14	3.292		
Flav (mg/L)	Treat	55401.876	6	9233.646	12.217	.000
	Error	10581.234	14	755.802		

The F tests the effect of the TREATMENTS (the control wine and the 6 CMCs). This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Tables 21 and 22: Tukey post-hoc test results for total anthocyanins and ionization index analyses.

Total anthocyanins (mg/l)

Tukey HSD

TREAT	N	Subset		
		1	2	3
CMC5	3	343 a		
CMC6	3	360 ab	360 ab	
CMC2	3		381 b	
CMC3	3		381 b	
CMC1	3		389 bc	389 bc
Control	3		389 bc	389 bc
CMC4	3			419 c
Sig.		.499	.085	.062

Ionization index (%)

Tukey HSD

TREAT	N	Subset			
		1	2	3	4
CMC4	3	10 a			
Control	3	11 ab	11 ab		
CMC1	3	11 ab	11 ab		
CMC6	3	11 ab	11 ab		
CMC2	3		12 b		
CMC3	3			14 c	
CMC5	3				15 d
Sig.		.064	.063	1.000	1.000

Table 23: Tukey post-hoc test results for the coloured anthocyanins analyses.

Coloured anthocyanins (mg/l)

Tukey HSD

TREAT	N	Subset			
		1	2	3	4
CMC6	3	42 a			
Control	3	42 a			
CMC4	3		45 b		
CMC1	3		45 b		
CMC2	3		45 b		
CMC5	3			53 c	
CMC3	3				55 d
Sig.		.749	.749	1.000	1.000

Table 24 and 25: Tukey post- hoc test results for the total and polymerized pigments analyses.

Total pigments (Abs)

Tukey HSD

TREAT	N	Subset		
		1	2	3
CMC5	3	20.70 a		
CMC6	3	21.49 ab	21.49 ab	
CMC2	3		22.45 b	
CMC3	3		22.59 b	
Control	3		22.79 bc	22.79 bc
CMC1	3		22.79 bc	22.79 bc
CMC4	3			24.27 c
Sig.		.607	.139	.073

Polymerized pigments (Abs)

Tukey HSD

TREAT	N	Subset			
		1	2	3	4
CMC4	3	1.98 a			
CMC1	3	1.99 ab	1.99 ab		
Control	3	1.99 ab	1.99 ab		
CMC2	3		2.03 bc	2.03 bc	
CMC6	3			2.07 cd	2.07 cd
CMC3	3				2.10 d
CMC5	3				2.11 d
Sig.		.958	.200	.284	.092

Table 26: Tukey post-hoc test results for the polymerization index analyses.

Polimerization index (%)

Tukey HSD

TREAT	N	Subset			
		1	2	3	4
CMC4	3	825.56 a			
CMC1	3	884.86 ab	884.86 ab		
Control	3		886.01 b		
CMC2	3		914.72 bc	914.72 bc	
CMC3	3		940.38 bc	940.38 bc	
CMC6	3			971.36 cd	971.36 cd
CMC5	3				1029.32 d
Sig.		.051	.074	.066	.059

Tables 27,28,29: Tukey post-hoc test results for the total phenols, flavonoids and non- flavonoids analyses.

Total phenols (mg/l)

Tukey HSD

TREAT	N	Subset	
		1	2
CMC5	3	1622 a	
CMC1	3		1740 b
CMC4	3		1751 b
Control	3		1774 b
CMC3	3		1779 b
CMC6	3		1786 b
CMC2	3		1802 b
Sig.		1.000	.181

Flavonoids (mg/l)

Tukey HSD

TREAT	N	Subset	
		1	2
CMC5	3	1478 a	
CMC3	3		1582 b
CMC1	3		1596 b
CMC4	3		1603 b
CMC2	3		1608 b
Control	3		1633 b
CMC6	3		1649 b
Sig.		1.000	.114

Non-flavonoids (mg/l)

Tukey HSD

TREAT	N	Subset			
		1	2	3	4
CMC6	3	137 a			
Control	3	141 ab	141 ab		
CMC1	3		144 bc	144 bc	
CMC5	3		145 bc	145 bc	
CMC4	3			148 c	
CMC2	3				193 d
CMC3	3				197 d
Sig.		.181	.111	.161	.161

Annex 2

Technical brochures

1. Ecofiltra: CELSTAB

CELSTAB®
STABILITY OF WINES

ESTABILIZAÇÃO

CELSTAB®

Solução de goma de celulose (CMC/E466) (Resolução OIV 366/ 2009).

Apta à elaboração de produtos destinados ao consumo humano directo, no quadro do uso regulamentado em enologia. Conforme o Regulamento CE n.º 606/200 e o Food Chemical Codex.

ESPECIFICIDADES

CELSTAB® é um polímero de celulose altamente purificado, de origem vegetal, com baixo grau de polimerização e viscosidade. A sua formulação líquida, com uma concentração de 100 g/L facilita a sua incorporação no vinho.

APLICAÇÕES E PROPRIEDADES ENOLÓGICAS

CELSTAB® está destinado à estabilização dos vinhos face às precipitações de bitartarato de potássio. A sua acção traduz-se por uma inibição das fases de nucleação e do crescimento dos microcristais (através de uma desorganização da superfície dos sais responsáveis pela formação dos cristais).

CARACTERÍSTICAS FÍSICAS

Aspecto	líquido
Cor	amarelo pálido

ANÁLISES QUÍMICAS

solução pH 1%	3,8 ± 0,2	SO_2	3 g/L ± 0,3
Grau de substituição	0,6 - 0,9	Chumbo	< 2 ppm
Glicolato livre	< 0,4%	Cádmio	< 1 ppm
Sódio	< 12,4%	Mercúrio	< 1 ppm
Cloreto de Sódio	< 0,5%	Arsénico	< 3 ppm

PROTOCOLO DE UTILIZAÇÃO

DOSE DE UTILIZAÇÃO

Dose utilização recomendada: 10 cL/hL (Dose máxima legal: 100 mg/L).

No caso de utilização em vinhos tintos ou rosés, existe um risco elevado de interacção do CELSTAB® com a matéria corante que pode provocar a formação de uma turvação e/ou um precipitado.

Preconização para os vinhos com forte instabilidade tartárica:

- Testes preliminares em laboratório para verificar a dose de utilização.
- Testes de estabilidade para validar ou não a eficácia do tratamento.

LAFFORT
l'œnologie par nature

APLICAÇÃO

- Diluir **CELSTAB®** em duas vezes o seu volume de vinho.
- Para os vinhos tranquilos, a incorporação far-se-á antes da última filtração **com a ajuda de uma bomba doseadora ou de um ENODOSEADOR**, nos vinhos perfeitamente colados e clarificados. Certificar-se que a homogeneização é perfeita.
- **Recomenda-se que a incorporação seja feita no mínimo 48 horas antes da filtração.**
- **Para os vinhos efervescentes**, a incorporação é feita na operação de tiragem (menos perdas de vinho) ou por adição no licor de expedição (neste caso, filtrar a solução de **CELSTAB®**).

CONDIÇÕES ENOLÓGICAS

- **Proteínas:**

  - O uso de **CELSTAB®** far-se-á nos vinhos estáveis no que respeita às casses proteicas (no caso de se adicionarem taninos tardiamente, recomenda-se refazer o teste de estabilidade proteica).

  - **CELSTAB®** reage formando uma turvação nos vinhos tratados com Lysozyme (Lisozima).
- **É possível a interacção com a matéria corante de certos vinhos tintos e rosés.**

CONSERVAÇÃO

- Armazenar num local ameno, seco, isento de odores, na sua embalagem de origem intacta, dentro do limite da DLUO indicada.
- Data de limite de utilização óptima D.L.U.O. (embalagem fechada): 2 anos.
- Embalagem aberta: utilizar rapidamente.

ACONDICIONAMENTO

Lata de 1,05 kg e 5,25 kg.

Bidão de 21 kg.

Contentor de 1050 kg.

REPRESENTANTE

ECOFILTRA

Rua do Mirante, 64 – Parque Industrial de Grijó – 4415-493 Grijó
Telef: 22 741 84 50 - Fax: 22 741 84 59 - ecofiltra@ecofiltra.pt

IMPORTANT

As medidas ou condições de utilização estão fora do nosso controlo. A LAFFORT® não será responsável no caso de insucesso do tratamento e do aparecimento de cristais de ácido tartárico.

LAFFORT
l'œnologie par nature

CS 61 611 – 33072 BORDEAUX CEDEX - FRANCE– Tél.: +33 (0)5 56 86 53 04 – www.laffort.com

2. Angelo Coimbra: Vinoprotect

FICHA TÉCNICA

ANGELO COIMBRA & CA. LDA.

Vinoprotect®

REVISÃO:	08/07/2016
VERSÃO:	NP-25/02/2013 NP-15/07/2013

Solução de goma de celulose – Carboximetilcelulose (CMC) – E466

Vinoprotect ® é uma goma de celulose destinada à estabilização tartárica dos vinhos tranquilos ou efervescentes.

VANTAGENS ENOLÓGICAS

As gomas de celulose ou CMC são utilizadas na produção de alimentos e bebidas. Publicações referem-nas como um "substituto de pectina". Estes coloides são produzidos a partir da celulose que é um polímero natural de D-glucose, na qual a ligação entre as unidades de glucose é do *tipo ß, 1:4*. As gomas de celulose constituem uma vasta gama de produtos que apresentam caracteres físico-químicos variados, o que em enologia é pouco conveniente, pelo que se selecionou uma goma de celulose específica eficaz, neutra em gosto e de fácil utilização.

Derivado de fibras vegetais, este coloide protetor apresenta-se sob a forma de pó granular ou fibroso branco, ligeiramente higroscópico e inodoro.

Este produto específico para o vinho foi selecionado tendo em conta dois fatores: GS – Grau de Substituição e GP – Grau de Polimerização, parâmetros que determinam a viscosidade e a solubilidade do produto em água. ***Vinoprotect®*** é o melhor compromisso entre estes dois critérios e a sua capacidade de estabilizar o vinho face aos riscos de precipitação tartárica, ao pH e à temperatura do vinho.

Princípio de ação: ***Vinoprotect®*** permite manter uma dispersão uniforme de dois ou mais componentes no vinho e impede a nucleação ou o contacto direto entre os cristais de tartarato.

UTILIZAÇÃO

DOSE DE APLICAÇÃO:

- De 14 a 20 cl/hl ou 7 a 10g/hl, conforme a instabilidade do vinho.
- Dose máxima legal: 20 cl/ hl (10 g/hl)

A solução é estabilizada através de SO_2.

MODO DE APLICAÇÃO:

- ***Vinoprotect®*** apresenta-se sob forma líquida (preparação a 5%) para uma utilização mais fácil.

- **Utilização antes da filtração final:** Introduzir ***Vinoprotect®*** 24 h antes do enchimento em vinhos colados e pré-filtrados se necessário. Diluir em vinho e adicionar com a ajuda de uma bomba doseadora ao longo de uma remontagem.

- **Utilização após a filtração final:** Introduzir ***Vinoprotect®*** depois da última filtração com a ajuda de uma bomba doseadora.

- **Vinhos efervescentes: *Vinoprotect®*** é aplicado na tiragem ou no "dégorgement".

- Antes da utilização, consultar um enólogo.

PRECAUÇÕES GERAIS:

Aplicar ***Vinoprotect®*** nos vinhos estabilizados em relação a proteínas.

Não aplicar em vinhos estabilizados com lisozima.

Vinhos brancos: *Vinoprotect®* não afecta o IC (Índice de colmatagem). Utilizar sobre vinhos desprovidos de proteínas.

Vinhos roséss e tintos: *Vinoprotect®* pode provocar um aumento da viscosidade, pela associação a taninos a baixa temperatura e consequentemente causar uma quebra na cor e problemas de filtração. Devem ser efetuados ensaios prévios.

Vinhos fortemente instáveis: de preferência efetuar testes de validação de eficácia nestes vinhos para verificar a dose de aplicação e a eficácia do tratamento.

De uma maneira geral, ***Vinoprotect®*** é um coloide. As interações com os coloides e outras substâncias (taninos, proteínas, etc.), cuja natureza e quantidade variam de vinho para vinho, são possíveis. Tais complexos podem afetar a filtrabilidade e causar, para além de custos suplementares, uma perda considerável do produto, pelo que a aplicação de tratamentos e controlos adequados permitem, na maior parte dos casos, uma utilização eficaz do tratamento.

ESPECIFICAÇÕES

Aspeto	Líquido	**Arsénio**	<3 ppm
Cor	Amarelo muito pálido	**Cádmio**	<1 ppm
pH	3,8 ± 0,2	**Chumbo**	<2 ppm
Peso molecular	30 000 – 50 000	**Sódio**	<12,4%
SO_2	2,0 ± 0,5 g/l	**Mercúrio**	<1 ppm
Ácido Cítrico	4 g/l	**Glicolato de Sódio livre**	<0,4%
Grau de substituição	0,6 – 0,9	**Cloreto de Sódio**	<0,5%
Cinzas (sobre extrato seco)	5 – 10g/100g		

ACONDICIONAMENTO E CONSERVAÇÃO

Embalagens de 5 kg e 20 kg.
Contentor de 1000 kg.

Conservar na embalagem original hermeticamente fechada, em local apropriado, seco, fresco (5 a 25ºC) e inodoro. Respeitar o prazo de validade de 2 anos.

RESPONSABILIDADE

Na medida em que as condições de utilização se encontram fora de controlo, declina-se qualquer responsabilidade por precipitações que possam surgir após o tratamento com ***Vinoprotect®.***

LEGISLAÇÃO / SEGURANÇA ALIMENTAR

- Não é, nem contém, Organismos Geneticamente Modificados (OGM), assim como não é obtido inteira ou parcialmente, a partir de substratos geneticamente modificados, não sendo, pois, abrangido pelos requisitos de etiquetagem.
- Alergénios: contém anidrido sulfuroso (2 ± 0,5 g/l).
- Não foi submetido a qualquer tipo de tratamento ionizante.
- Não contém hormonas.
- Não contém pesticidas.
- Não contém nanomateriais.
- Em conformidade com Codex Enológico Internacional.
- Em conformidade com Regulamento (CE) nr. 606/2009.

3. Agrovin: ESTABICEL

AGROVIN

Estabilizantes Ficha técnica

ESTABICEL

Preparación líquida de goma de celulosa para la estabilización tartárica de vinos

CARACTERÍSTICAS

Estabicel es una goma de celulosa de origen vegetal, purificada y seleccionada por su grado de sustitución, grado de polimerización y baja viscosidad.

APLICACIÓN

Estabilización frente a la precipitación de sales de ácido tartárico, debido a la inhibición del fenómeno de nucleación. Su formulación liquida facilita su empleo en el vino.

CUALIDADES ORGANOLÉPTICAS

La aplicación de **Estabicel** no modifica ningún aspecto sensorial del vino.

COMPOSICIÓN

Solución de goma de celulosa (CMC sódica/E466) al 10% estabilizada con SO_2.

Alérgeno: Contiene sulfitos.

DOSIS

Vino terminado	50-100 ml/hl

Límite de utilización: 100 ml/hl.

MODO DE EMPLEO

1. Diluir **Estabicel** en 2-4 veces su volumen en vino.

2. Añadir al volumen total de vino preferentemente con bomba dosificadora antes de la última filtración. Asegurar la homogeneización. Se aconseja una temperatura del vino mayor de 14ºC

3. Esperar al menos 24 horas antes del embotellado.

Estabicel se emplea sobre vino clarificado, filtrado y antes de la microfiltración y embotellado.

En vinos espumosos se añade en la mezcla 24 horas antes de realizar el tiraje, o en el degüelle, con el licor de expedición.

Precauciones de trabajo:

1. En vinos de fuerte inestabilidad tartárica, se recomienda realizar test previos de estabilidad para verificar la eficacia del tratamiento.

2. **Estabicel** no protege frente a la caída de materia colorante. La aplicación en tintos y rosados debe hacerse sobre vinos estables en materia colorante para evitar la precipitación de ésta con el tiempo. La utilización de goma arábiga GOMASOL PRO puede mejorar la estabilidad de materia colorante de vinos tintos y rosados.

3. No aplicar **Estabicel** en vinos inestables frente a proteínas o tratados con lisozima.

Dadas las particulares condiciones de estabilidad de cada vino, AGROVIN no se responsabiliza de la aparición de sales de ácido tartárico tras el tratamiento.

ASPECTO FÍSICO

Gel transparente ligeramente viscoso de color amarillo pálido.

PRESENTACIÓN

Envases de 5, 22 y 1100 Kg.

PROPIEDADES FISICOQUÍMICAS Y MICROBIOLÓGICAS

SO_2 [mg/l]	2000-4000
Densidad 20ºC [g/cc]	1,030-1,060
Viscosidad [cP]	50-350
pH	3,7-4,7

MODO DE CONSERVACIÓN

Conservar en el envase de origen, en lugar fresco y seco, ausente de olores.

Una vez abierto debe emplearse lo antes posible.

Consumo preferente: antes de 1 año a partir del envasado.

4. Proenol: CRISTAB GC

ESTABILIZAÇÃO
Estabilização Tartárica

Cristab GC®

Características/ Propriedades

Goma pura de celulose

- CRISTAB GC® é uma goma de celulose. As gomas de celulose são naturais e extraídas da celulose da madeira. A madeira utilizada é proveniente de florestas de crescimento sustentado.
- CRISTAB GC® assegura a estabilização tartárica em vinhos gaseificados e tranquilos. Foi seleccionada devido à sua eficácia na estabilização de vinhos tendo em conta as precipitações de bitartarato de potássio e tartarato de cálcio, assim como pela sua neutralidade organoléptica.
- CRISTAB GC® apresenta características altamente específicas, (grau de substituição, grau de polimerização e viscosidade) tornando-a particularmente bem adaptada e eficaz para assegurar a estabilidade tartárica dos vinhos.
- CRISTAB GC® mantém a eficácia ao longo do tempo: testes realizados em vinhos efervescentes mostraram que após 4 anos da adição de CRISTAB GC® a estabilização tartárica dos vinhos ainda é assegurada.
- CRISTAB GC® está disponível em pó ou na forma de um líquido claro e viscoso, com uma concentração de 50g/L.
- CRISTAB GC® actua tanto na formação de cristais (nucleação) como no potencial crescimento de micro-cristais que estão presentes no vinho.

Legislação

A União Europeia autoriza a adição de gomas de celulose para assegurar a estabilização tartárica "apenas com vinhos e com todas as categorias de vinho efervescente ou semi-efervescente" (regulamento nº606/209). Dose máxima legal autorizada: 10g/hL (100mg/L).

Dose de aplicação

Dose em pó: 10g/hL (100mg/L).
Dose Líquido: 200mL/hL (2mL/L)

Modo de utilização

Preparação da Solução:

- Dissolva CRISTAB GC® em água quente (a uma temperatura entre os 40°-50°C).
- Misture a água com uma hélice ou agitador.
- Polvilhe CRISTAB GC® na água agitando sempre, a solução obtida é altamente emulsionada: é recomendável que a solução seja preparada no dia anterior ao tratamento para permitir que as bolhas de ar desapareçam.
- Dependendo do método de agitação, faça uma solução de 50g/L (1kg em 20L de água) ou 25g/L (1kg em 40L de água).

Instruções para utilização:

- Dilua a solução obtida, ou o CRISTAB GC® líquido, com vinho para facilitar a aplicação.

Em vinhos efervescentes:

- **No engarrafamento**: adicione CRISTAB GC® à mistura.
- **No *dégorgement*** adicione CRISTAB GC® à mistura de vinho/açúcar (licor de expedição). Assegure que a mistura esta bem homogeneizada antes de a utilizar.

Em vinhos tranquilos:

CRISTAB GC® pode ser introduzido antes da filtração, não é colmatante. Incorpore em todo o vinho com uma bomba doseadora ou uma DOSACOL.

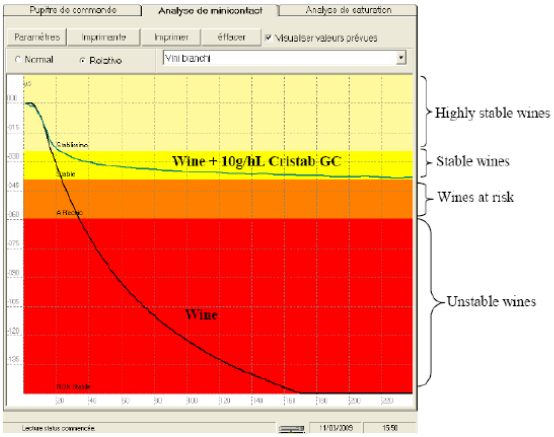


Figura 1: Eficácia do CRISTAB GC® em relação à estabilização tartárica.

O I&D avaliou a estabilização tartárica dos vinhos e os resultados obtidos estão representados no quadro da figura 1, com 4 zonas de estabilização.

A figura 1 mostra que o vinho utilizado durante os ensaios era instável (curva a preto) e que um tratamento de 10g/hL com CRISTAB GC® estabilizou o vinho em relação às precipitações tartáricas (curva a verde).

Os testes preliminares permitem:

- Determinar a dose ideal (teste de estabilidade após o tratamento: 6 dias a -5°C).
- Avaliar as interacções da goma de celulose com as cores do vinho tinto e rosé.
- Assegurar que o tratamento é suficientemente eficaz em vinhos altamente instáveis, realizando um teste de estabilidade (armazenar a -5°C durante 6 dias).

CRISTAB GC® é utilizado em vinhos previamente estabilizados, com atenção às casses de proteínas. Tal como o ácido metatartárico o CRISTAB GC® reage com a lisozima.

Embalagem

- Embalagem em Pó: 1Kg.
- Embalagem em Pó: 5Kg.
- Embalagem Líquida: 5L.
- Embalagem Líquida: 20L.
- Embalagem Líquida: 1000L.

Qualidade, Segurança-Ambiente

Rastreabilidade: O número de lote em todas as embalagens de CRISTAB GC® permite que o produto seja rastreado (origem do produto) e controlado (do produtor ao consumidor).

Segurança-Ambiente:

- A manipulação de CRISTAB GC® não representa qualquer perigo ao consumidor.
- CRISTAB GC® em solução líquida é estabilizado com SO_2.
- Não contém Organismos Geneticamente Modificados, não foi produzido a partir dos mesmos e não inclui substâncias com origem nos referidos organismos.
- Não tratado por radiação ionizante.
- O CRISTAB GC® em pó contém as substâncias alérgicas referidas na Directiva 2007/68/CE. O CRISTAB GC® líquido contém sulfitos ≥ 10mg/kg.
- Está conforme os Regulamentos CE 479/2008 e 606/2009.
- Está conforme o Codex Enológico Internacional, versão em vigor.

Armazenamento

Embalagem selada de origem: Ao abrigo da luz, num local seco e isento de odores. Proteger das baixas temperaturas (produto em solução liquida).
Embalagem aberta: utilizar rapidamente.

5. SAI: SAIStab CMC10

Qualidade & Inovação em Enologia

SAIstab®CMC10

Carboximetilcelulose para uso enológico

- Ficha Técnica -

Descrição:

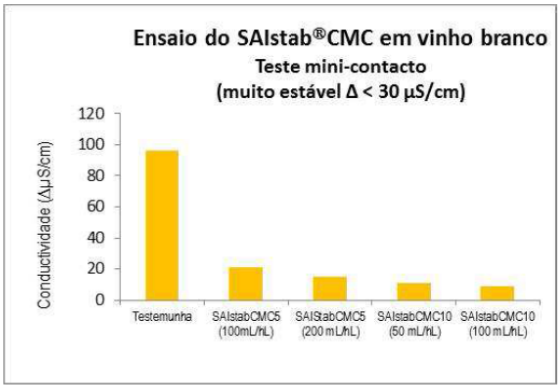
SAIstab®CMC10 é uma solução aquosa de carboximetilcelulose sódica, com um baixo teor de polimerização, de baixa viscosidade, específica para aplicação em Enologia.

SAIstab®CMC10 previne a formação e crescimento dos cristais de bitartarato de potássio. É utilizado como estabilizante tartárico de longo prazo (3 a 4 anos).

A adição de **SAIstab®CMC10** aos vinhos é de fácil incorporação graças à sua baixa viscosidade.

Embalagens:

SAIstab®CMC10 está disponível em embalagens de 1Kg, 5Kg, 25Kg, 220Kg e 1000Kg



Características físico-químicas:

Cor: Incolor

Odor: Sulfuroso

pH: 6.5 - 7.5

Turbidez: <50

SO2 (%): 0.2 - 0.5

Grau de substituição (%): >85

Aplicação:

A adição do **SAIstab®CMC10** pode ser efectuada antes ou após a filtração mas tendo sempre em atenção que o vinho deve estar estabilizado ao nível proteico e corante.

O **SAIstab®CMC10** pode reagir com macromoléculas como as proteínas e mesmo com partículas corantes instáveis provocando turvação.

De modo a prevenir estas situações, é recomendável o ensaio prévio em Laboratório de modo a verificar a estabilidade do vinho e a dose de aplicação mais correta que garanta o efeito pretendido.

Se possível utilizar uma bomba doseadora ou um sistema *venturi*, de modo a garantir uma boa homogeneização. Caso tal não seja possível, dissolver a quantidade necessária de CMC em cerca de 10% do vinho a tratar e adicioná-lo lentamente ao restante vinho. Remontar cuidadosamente de modo a garantir uma boa homogeneização.

Nota: Uma homogeneização incorreta poderá causar problemas de filtração e de estabilidade.

Dose de aplicação:

De 50 a 100mL/hL

100mL/hL (100mg/L de CMC é a dose máxima legal)

Armazenamento:

Manter o **SAIstab®CMC10** na sua embalagem original, em local fresco e seco afastado da luz direta do sol.

SAI
Rua José Bragança Tavares, 78
4580-593 Paredes
Tel/Fax: 255 783 066
E-mail: geral@sai.com.pt

A informação contida nesta ficha técnica corresponde ao atual estado do conhecimento e experiência do fabricante pelo que o seu uso deve ser restrito à informação aqui presente

Somente para uso profissional e enológico. Reg 606/2009

TTC167-B

6. AEB: New-cel

AEB New-cel
Carboxymethlycellulose (CMC)

***New-cel* Introduction:**

AEB New-cel is an organic carboxymethlycellulose (CMC) polymer soluble in water, used to achieve tartrate stability in wines without the necessity for traditional cold stabilization.

The stabilization of tartrate precipitations represents one of the most significant cost implications for any winery. Currently, the traditional method of cold stabilization of a wine involves refrigeration of the entire volume of wine to close to -4°C for a period of at least 72hrs (or longer if -4°C cannot be achieved) followed by filtration. This process is a significant contributor to a winery's carbon footprint and electricity budget and is a process compounded by the warm climate location and exposed nature of many of Australia's inland winemaking facilities.

To add to the significant costs involved, the non-specific nature of traditional cold stabilization may have unintended impacts on wine organoleptic characteristics, as it is possible that compounds which are affected by cold filtration can be removed by this process e.g. colour and mouthfeel components.

New-cel was developed by ***AEB Group*** based on in-house research in which we have gauged that the ideal formulation for the most effective wine stabilization occurs when the CMC polymer contains a ratio between the number of carboxylated groups and glucose units equal to 1. This allows the ultimate efficacy in forming a chemical barrier between the crystals of potassium bi-tartrate preventing their enlargement. In wines, tartaric acid and potassium normally forms crystal structures with 7 sides, these progressively enlarge starting from micro-formations, until they become visible as small "glass-like" deposits in the wine. The long polymeric chains of ***New-cel*** act as colloidal protectors and wrap the crystal structure with a protective film and deform them making their growth impossible.

***New-Cel* in Red Wines:**

New-cel will prevent tartrate deposits in red wine regardless of whether or not the colour components are cold stable, however, it will not normally prevent the cold instability cause by unstable colour present in red wine. Unstable wine colour can cause interference with the mode of action of CMC if the wine contains unstable colour prior to its addition and post-CMC-addition in both cases causing cloudiness and an increase in turbidity. It is important to stabilize the red wine colour to prevent clouding of the wine by cold unstable colour precipitation. Colour can be partially stabilized by micro-oxygenation over time, chilling the wine and filtration prior to CMC addition or the addition of gum Arabic (***Arabinol 30***) prior to the addition of ***New-cel***.

***New-cel* Composition:**

Carboxy-methyl-cellulose (E466) stabilized in sterile, deionized water.
Citric Acid (E330).
Potassium Bisulfite (E228) - 100 g/hL of New-cel bring about 2 mg/L of SO2.
Ascorbic acid (E300).

***New-cel* Utilization:**

- Wines must be protein stable and turbidity <1ntu.
- Directly dissolve the solution into the wine while pumping over.
- Recommended Rate: 100-150g/hL. It is important to bench trial the dosage rate and test the cold stability before adding ***New-cel*** in the cellar.