

PRELIMINARY RESULTS ON TARTARIC STABILIZATION OF RED WINE BY ADDING DIFFERENT CARBOXYMETHYLCELLULOSES

RESULTADOS PRELIMINARES SOBRE ESTABILIZAÇÃO TARTÁRICA DE VINHO TINTO POR ADIÇÃO DE CARBOXIMETILCELULOSES

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(Received 14.03.2018. Accepted 14.05.2018)

SUMMARY

Despite being natural phenomena of physical-chemical stabilization of young wines, tartrate precipitations in bottled wines are often understood as a quality fault. Carboxymethylcellulose (CMC) is an inhibitor of tartaric salts crystallization, authorized as oenological additive, which application is currently limited to white and sparkling wines by OIV. The aim of this exploratory research was to study the effect of different CMC on a (tartaric) unstable red wine, in terms of tartrate stability, colouring matter stability, turbidity, comprehensive phenolic composition, and chromatic characteristics. All the CMC tested were able to inhibit potassium bitartrate crystallization in the red wine, promoting its tartaric stability. Slight increase in the colour intensity, coloured anthocyanins concentration, and turbidity of the wine were observed. No colouring matter precipitation was verified. The study seems to indicate that the CMC can be an efficient alternative to other tartaric stabilization treatments, also in red wines, opening new prospective and scenarios concerning the use of CMC in this type of wines.

RESUMO

Os precipitados de origem tartárica em vinhos engarrafados são normalmente percebidos como defeito da qualidade, não obstante resultarem de fenómenos naturais de estabilização físico-química, típicos de vinhos jovens. A carboximetilcelulose (CMC) é um inibidor da cristalização do ácido tartárico, autorizada como aditivo em Enologia, cuja aplicação está limitada a vinhos brancos e vinhos espumantes pela OIV. O presente estudo, exploratório, teve por objectivo avaliar o efeito de diferentes CMC em vinho tinto (inicialmente instável sob o ponto de vista tartárico), ao nível da sua estabilidade tartárica, estabilidade da matéria corante, turbidez, composição fenólica e características cromáticas. Todas as CMC ensaiadas proporcionaram a inibição da cristalização do bitartrato de potássio no vinho tinto, promovendo a sua estabilidade tartárica. Foram observadas ligeiras modificações na intensidade da cor, concentração em antocianinas coradas e turbidez. Não se verificou precipitação de matéria corante. Este estudo sugere que a CMC pode constituir uma alternativa enológica eficiente para estabilização tartárica também em vinhos tintos, abrindo novos cenários e perspectivas no que respeita à utilização de CMC neste tipo de vinhos.

Key words: tartaric stabilization, carboxymethylcelluloses, red wine, turbidity, phenolic composition.

Palavras-chave: estabilização tartárica, carboximetilceluloses, vinho tinto, turbidez, composição fenólica.

INTRODUCTION

The tartaric precipitation event under natural conditions is an unreliable, unpredictable phenomenon that occurs during the entire period of wine production, from the alcoholic fermentation to

the aging stage. According to pH, alcoholic strength and temperature, the tartaric acid is more or less salified with K^+ and Ca^{2+} ions as potassium bitartrate and calcium tartrate, respectively, and, in a less extent, as potassium tartrate, double potassium calcium tartrate and calcium tartromalate. It is

therefore clear that this phenomenon is related to the presence of tartaric acid (H_2T) and of the two ions potassium (K^+) and/or calcium (Ca^{2+}). More specifically, potassium bitartrate (KHT) crystallization and successive precipitation occurrence in wine involves saturation, nucleation and growth phenomena (Boulton *et al.*, 1996). Occurrence in bottled wines, in form of sediments, can lead to important economic losses. Although these sediments do not represent a risk for human health, they can change consumer perception of wine quality.

Different techniques are applied in winemaking to achieve the tartaric stabilization and to avoid the tartaric salts precipitations in bottled wines (Cabrita *et al.*, 2016). Physical approaches are represented by the cold treatments, electro dialysis and ion exchange resins: their action is based on the removal of potassium bitartrate or K^+ , Ca^{2+} and HT^- ions present in super-saturated wine. However, even if widely used, these techniques are characterized by several disadvantages: cold treatments and electro dialysis are generally expensive processes and the use of cation exchangers is not recommended when the instability is due to excessive tartaric acid content (Bosso *et al.*, 2010; Lasanta and Gómez, 2012; Cabrita *et al.*, 2016).

For these reasons, during the past years, economically advantageous and more sustainable methods have been studied and developed; they are based on the addition of substances that prevent the crystals precipitation by inhibiting their formation and making them stable at lower temperatures. These treatments are the addition of metatartaric acid (MTA), yeast mannoproteins (YMP), carboxymethylcellulose (CMC), and more recently potassium polyspartate (Resolution OIV-OENO 543-2016) (OIV, 2017a). Nonetheless, the MTA action is not stable over time and the YMP are characterized by high costs (Ribéreau-Gayon *et al.*, 2006; Gerbaud *et al.*, 2010; Lasanta and Gómez, 2012; Bosso *et al.*, 2015; Cabrita *et al.*, 2016).

The CMC is already widely used as food additive since the forties of last century but in 2009 it has been allowed to treat the wines with a maximum dose of 100 mg/L (European Union, 2009; OIV, 2017b), and its eventual increase to 200 mg/L is being discussed within OIV. However, having in mind potential negative effects of CMC on the quality of red wines, the International Organisation of Vine and Wine restricts the use of CMC for tartaric stabilization to white and sparkling wines (OIV Oeno 2/08) (OIV, 2017b). The advantages that characterize this product are several: it is relatively low in price, less energy

consuming and easy to apply compared to cold stabilization, use of ion exchange resins, and electro dialysis, does not require initial investment in specific equipment (Lasanta and Gómez, 2012); however, it is only efficient when protein stability of wine is assured, because proteins can interact with CMC (Ribéreau-Gayon *et al.*, 2006).

The CMC is a plant derivative produced by a treatment of the wood cellulose with sodium hydroxide, followed by an etherification reaction between the product previously obtained (alkali-cellulose complex) and monochloroacetic acid. More exactly, it is obtained by etherification of the free primary alcohol groups of the glucopyranose units linked by β (1-4) glycosidic linkages (Ribéreau-Gayon *et al.*, 2006; Cabrita *et al.*, 2016). Its mechanism of action, by inhibiting KHT crystallization, has been described: characterized by an electronegative charge, CMC binds the KHT crystals (electropositively charged) making the growth of some of their seven faces slower and flattening their surfaces (Rodríguez-Clemente and Correa-Gorospe, 1988; Crachereau *et al.*, 2001). The CMC action and effectiveness are strongly linked to two properties known as substitution degree (DS) and polymerization degree (DP) (Ribéreau-Gayon *et al.*, 2006). The DS value indicates the number of carboxylate groups present in the molecule, therefore, the number of anchor sites involved in the cation complexation: higher the DS, higher the CMC efficiency (Lubbers *et al.*, 1993). For wine treatment purposes, only CMC characterized by a DS between 0.60 and 0.95 are allowed (Resolution OIV-Oeno 366-2009) (OIV, 2017a). The DP indicates the molecule's size and it has an important influence on the product viscosity; the higher the DP and the molecule weight, the higher the viscosity and more difficult is the distribution of the product in the wine (Ribéreau-Gayon *et al.*, 2006). Also, the mineral composition of the CMC, especially their potassium concentration can influence CMC effectiveness on wine tartaric stabilization (Guise *et al.*, 2014).

The CMC is available in granular/ fibrous powder form or in liquid form. The solutions applied must contain at least 35 g/L of CMC; 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 of CMC; however, when in liquid forms, the products are already prepared and generally present concentrations of 50-100 g/L (European Union, 2009; OIV, 2017a).

According to previous studies, the CMC use has been noticed to negatively affect the quality of red wines (Moutounet *et al.*, 2010; Claus *et al.*, 2014). More

specifically, it has been seen that it interacts with the phenolic compounds, decreasing the content of total phenols by precipitation, flavonoids and non-flavonoids, reducing the colour intensity and promoting the increase of turbidity, changes in colour and colouring matter precipitation (Moutounet *et al.*, 2010; Claus *et al.*, 2014). Recently, it was claimed that the colour loss of red wines after CMC addition is probably the result of protein-bridged precipitation of anthocyanins and not a direct interaction between polyphenols and CMC (Sommer *et al.*, 2016).

Nevertheless, on the basis of a comprehensive review of the literature, information on the influence of CMC on detailed phenolic composition of red wines was not found. For these reasons, a study was developed to assess the efficiency of five different CMC, when added to an unstable red wine, in terms of tartrate stability. In addition, their effect on colouring matter stability, turbidity, phenolic composition, tannins composition, anthocyanins, and chromatic characteristics were evaluated, which is a novelty. To the best of our knowledge, for the first time the effects of five different commercial CMC on red wine have been analysed within the same study. Having in mind the still limited use of CMC in red winemaking, the results coming from this research will offer a starting point for future studies and experiments with the aim of improving the application of this additive also in red wines.

MATERIAL AND METHODS

Wine and carboxymethylcelluloses

The experiment was carried out using a red wine of Castelão grape variety (*Vitis vinifera* L.), 2015 vintage, from a winery in Península de Setúbal region, Portugal. After manual harvest, the grapes were crushed, destemmed, added with sulphur dioxide (50 mg/L) and with untoasted French oak (*Quercus sessiliflora* Salisb.) powder (1 g/L). Alcoholic fermentation was carried out at 18 °C for 10 days, by inoculation of a commercial yeast strain of *Saccharomyces cerevisiae*. During the maceration period of alcoholic fermentation, *délestage* (once per day, during four days) and pumping over (5 min/h during three days) operations were performed. Running off and pressing of the mash took place after seven days of maceration, before the end of alcoholic fermentation. Free-run and press wines were combined and stored at room temperature in a stainless steel tank. After spontaneous malolactic fermentation the wine was racked and treated with sulphur dioxide (50 mg/L).

For characterization purposes, general physical and chemical analysis, namely total acidity (TA), volatile acidity (VA), pH, alcoholic strength by volume, total and free sulphur dioxide and reducing substances was determined according to the OIV methods (OIV, 2017c). The physicochemical characteristics of wine, before CMC addition, were as follows: alcoholic strength by volume 13.6 % vol, total acidity 4.65 g/L (expressed in tartaric acid), volatile acidity 0.34 g/L (expressed in acetic acid), pH 3.53, total SO₂ 61 mg/L, free SO₂ 30 mg/L, reducing substances 2.2 g/L. For all these analytical parameters, the corresponding values are in accordance with usual values for a red wine (Ribéreau-Gayon *et al.*, 2006).

Five different CMC, in liquid form, from five different Portuguese suppliers were used in the experiment. In Table I, the most important characteristics of the CMC, namely concentration, DS, concentration of sulphur dioxide, and pH, are reported.

TABLE I

Characteristics of the carboxymethylcelluloses (CMC) additives used in the experiment

Características das carboximetilceluloses (CMC) utilizadas no ensaio experimental

Product	Content (g/L)	Degree of substitution (DS)	SO ₂	pH
CMC1	100	0.6–0.9	3.0 g/L ± 0.3	3.8 ± 0.2
CMC2	50	0.6–0.9	2.0 g/L ± 0.5	3.8 ± 0.2
CMC3	100	unknown	2–4 g/L	3.7–4.7
CMC4	50	unknown	≥ 10 mg/kg	unknown
CMC5	100	>0.85	2–5 g/L	6.5–7.5

Information extracted from the CMCs technical brochures.

Experimental conditions

The experimental trial was carried out with one control wine, without CMC addition and designated as Control, and five treated wine samples designated as CMC1, CMC2, CMC3, CMC4 and CMC5. The addition of the five CMC was performed directly in 0.75 L bottles. For each treatment, one replication was carried out. A dose equal to 0.2 g/L was applied for CMC1, CMC3 and CMC5; while a dose of 0.1 g/L was added for the CMC2 and CMC4, in accordance with the manufacturers' recommendations. These doses, which can be considered high, were used to amplify and consequently to detect potential CMC effects on colloidal fraction of the wine. The first analytical controls were carried out five days after CMC addition in the wine. During the entire period of the experiment, the samples were kept at temperature between 16–18 °C, under low light exposure.

Wine stability tests

Tartaric stability

The tartaric stability of the six wine samples (Control and CMC treated wines) was assessed five days after the CMC addition and repeated after five months by the modified mini-contact test (Mira *et al.*, 2006; Ribéreau-Gayon *et al.*, 2006; Claus *et al.*, 2014). This test consists in measuring the drop of electric conductivity ($\mu\text{S}/\text{cm}$) of 100 mL wine at 0 °C, in a period of 7 minutes after the addition of “finely” micronized KHT (10 g/L) as a precipitating agent: the higher the difference in conductivity, the higher the tartaric instability. If the drop in conductivity is over 5% (measured before adding potassium bitartrate), the wine is considered unstable (3% for white wines). As alternative criterion, if the decrease of conductivity is higher than 40-45 $\mu\text{S}/\text{cm}$ the risk of precipitation is considered high. Evaluation was performed with a Thermo Scientific Orion Star A212 Conductivity Benchtop Meter instrument.

Colouring matter stability

To assess the colouring matter stability of the samples, the method described by Claus *et al.* (2014) has been readapted and applied. It is based on the following principle: being the colouring matter perfectly soluble in distilled water (40 °C) or ethanol solution (50% vol), if eventual deposits coming from previous centrifugation and storage at 4 °C for four days are completely dissolved, it means that the colouring matter is unstable and prone to precipitate and vice versa.

This method was applied to the wine treated samples as described: 50 mL of each sample was clarified by centrifugation (20.000 g, 30 min); the haze formation was visually monitored after four days at 4 °C; after four days the samples were centrifuged (10.000 g, 15 min) and if deposits were present, they were diluted in 10 mL of water or 5 mL of ethanol (50% vol) (the samples diluted in water were heated up to 40 °C in a water bath, after the addition); if a complete solubilisation of the deposits occurred, the test was positive and vice versa. The analyses were carried out after 10 days from the CMC addition, the method was performed in duplicate and both dilution in water and ethanol was used.

Turbidity analysis

Having in mind the CMC polymer structure which gives it “protective colloid” characteristics (Ribéreau-Gayon *et al.*, 2006) and being the turbidity explained by colloidal phenomena, it is important to evaluate the effect of CMC on the wines clarity. The turbidity (NTU) of the treated wines was assessed by

measuring the light diffused by a standard formazine suspension, at a 90° angle to the direction of the incident beam. The analysis was performed in triplicate, after two and five months from the addition, using a HACH 2100 N nephelometer instrument (OIV, 2017c).

Wine colour and phenolic composition analyses

Characterization of wine proanthocyanidins (PA)

Tannins composition was assessed as described by Sun *et al.* (1998). The method is based on the separation of the wine PA on the basis of their degree of polymerization (DP) using a C₁₈ Sep-Pak cartridges, followed by a vanillin reaction in an acidic medium. The analyses were carried out after five days from the CMC addition and performed in quadruplicate.

Tannin power analysis

The tannin power measurement gives information on the astringency of a wine. This parameter was determined by applying an analytical procedure described by De Freitas and Mateus (2001) based on the following principle: 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. The analysis was carried out, in triplicate, after 15 days from the CMC addition.

Chromatic characteristics, anthocyanins and pigments analyses

Colour intensity and shade were determined using the spectrophotometric method described by OIV (2017c). The intensity of colour is given by the sum of optical densities calculated for 1 cm optical path and radiations of wavelengths 420, 520 and 620 nm. The shade is expressed as the ratio of absorbance at 420 nm to absorbance at 520 nm.

Total and coloured anthocyanins, total and polymerized pigments were determined using the spectrophotometric method proposed by Somers and Evans (1977). Red wine colour is an integration of contributions from monomeric anthocyanins and polymeric pigments. Because of gross differences between the two fractions in their responses to pH change and to sulfur dioxide addition, approximate measures of the states of anthocyanin equilibria in young red wines can be routinely made.

Chromatic characteristics, anthocyanins and pigments analysis were carried out, in triplicate, after three days from the CMC addition.

Flavonoids, non-flavonoids and total phenols

Flavonoids and non-flavonoids were quantified applying the method described by Kramling and Singleton (1969). This method is based upon the determination of the phenol content before and after precipitation and removal of the flavonoids through reaction with formaldehyde under selected conditions. Analyses were performed after five days from the CMC addition, in triplicate.

Statistical analyses

Tannins composition, tannin power, chromatic characteristics and phenols data were processed by one-way ANOVA test and a Tukey post-hoc test, to study the effect of the additives. Using the ANOVA test, the differences were considered significant when the *p*-value was below 0.05. SPSS for Windows, version 15.0 (SPSS Inc., Chicago IL USA, 2004) was used.

RESULTS AND DISCUSSION

Influence of carboxymethylcelluloses addition on wine stability

Wine tartaric stability

The results regarding the influence of the different CMC on wine tartaric stability, evaluated by the adapted mini-contact test, after five days and five

months from the treatment, are shown in Table II. Decision on stability was taken based on the drop in conductivity, expressed as percentage, as it is independent of the absolute values of initial and final conductivity. For Control wine, the drop of conductivity was of 164 $\mu\text{S}/\text{cm}$ and higher than 5%, more exactly 7.6%, evidencing the wine instability in respect to tartaric precipitation. The CMC treated wines exhibited a low-conductivity difference in the test, always quite lower than 5%, demonstrating that all the tested CMC were able to promote the tartaric stabilization of the wine. These results confirm that CMC can be a suitable KHT crystallization inhibitor also for red wines, as already exposed by a few studies developed with this type of wines (Moutounet *et al.*, 2010; Greef *et al.*, 2012; Claus *et al.*, 2014).

In this work, and for each CMC, one level of addition was tested: 100 g/L (CMC1, CMC3 and CMC5) and 200 g/L (CMC2 and CMC4). Independently of the dose applied all the CMC resulted in the tartaric stabilization of the wine. It is well known that other factors besides concentration, especially DS, play a main role in CMC efficiency. Unfortunately information disclosed by suppliers concerning DS of the tested CMC is not accurate enough to establish a relation between the CMC DS and its efficiency as KHT crystallization inhibitor.

TABLE II

Tartaric stability test results on the red wine treated with carboxymethylcelluloses (CMC)

Resultados do teste de estabilidade tartárica no vinho tinto tratado com carboximetilceluloses (CMC)

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

5d: after five days from the CMC addition; 5 m: after five months from the CMC addition; Initial wine: Conductivity value of the initial wine; Wine + KHT: conductivity value of the wine + 10 g/L of KHT; from 1st min to 7th min results correspond to conductivity value of the wine + 10 g/L of KHT after respectively 1 to 7 minutes, continuously agitating; Results are expressed in conductivity units ($\mu\text{S}/\text{cm}$); $\Delta_{\text{conductivity}}$: variation in conductivity, expressed as %.

It should be highlighted that the five CMC were still efficient as salts crystallization inhibitors after five months from the addition to the red wine, evidencing

short and medium term excellent efficiency. However, further research is needed to establish the efficiency of CMC in red wine, as it depends on wine

chemical composition (Guise *et al.*, 2014). In particular, in future studies it would be important to include wines with different degrees of tartaric instability.

Wine colouring matter stability

For all the samples, with exception of Control, after being centrifuged, a deposit was observed. After contact with ethanol (50% vol) and water, and for all the samples, the deposits did not solubilize completely. As a result, it can be concluded that, being the colouring matter not completely solubilized in water and ethanol, it was stable and not prone to precipitate. This result is of extreme importance having in mind that likewise turbidity development, the potential interaction with phenolic compounds, promoting colouring matter precipitation, is a limitation concerning the use of CMC in red wines (Moutounet *et al.*, 2010; Claus *et al.*, 2014). Nevertheless, the present observations are in accordance with a previous study evidencing that the colouring matter precipitation by CMC addition is not a trend for all the red wines (Greef *et al.*, 2012).

This result may be explained by the colouring matter stability of the red wine used in the experiment. Among other purposes, the application of oak powder (ellagitannins) during the pre-fermentative phase is carried out to promote the colour stabilization of red wines: its addition favors, when oxygen is present, the formation of pigments more coloured than the native anthocyanins (Pechamat *et al.*, 2014).

On the basis of a recent experiment in wine-like model solution (Sommer *et al.*, 2016) it seems that proteins play an important role in colour loss: it was observed that the colour loss associated with CMC additions required the presence of proteins and cannot be observed with CMC and anthocyanins alone. However, it is known that red wines, resulting from the alcoholic fermentation in the presence of the solid parts of the berry (skins and seeds), which is the case of the wine used in this experiment, show low content of free proteins as they are precipitated by tannins (Ribéreau-Gayon *et al.*, 2006).

Having in mind the scarce studies and lack of knowledge regarding the potential colouring matter precipitation following the CMC addition to red wines, the results obtained in this work represent a valuable contribution to the enological research, opening new prospective and scenarios concerning the effects of the CMC utilization. Further studies should be carried out, namely testing the effects of the CMC on colour stable and unstable wines.

Wine turbidity

The increase of turbidity has been appointed as a major drawback of CMC use in red wines (Moutounet *et al.*, 2010; Greef *et al.*, 2012; Claus *et al.*, 2014). Following, the results concerning the turbidity of Control and treated wines, measured after two and five months from the CMC addition, respectively, are reported: Control: 4.6 – 3.6 NTU; CMC1: 4.8 – 4.3 NTU; CMC2: 5.7 – 4.4 NTU; CMC3: 7.6 – 5.0 NTU; CMC4: 7.9 – 6.5 NTU; CMC5: 8.7 – 7.0 NTU. The turbidity of the wine increased with CMC addition, confirming previous observations that CMC promotes the development of turbidity (Moutounet *et al.*, 2010; Greef *et al.*, 2012; Claus *et al.*, 2014; Guise *et al.*, 2014; Sommer *et al.*, 2016).

According to the reference values proposed by Ribéreau-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 turbidity values determined after five months from the addition between 4 and 7 NTU, the wines are considered cloudy. Finally, as expected, the turbidity in the Control and in all the treated wines, kept at temperature between 16-18 °C, decreased during the time. Still, all the treated wines presented slight higher values of turbidity than Control.

This effect is expected after CMC addition in white wines characterized by intrinsic protein instability, as described by Claus *et al.* (2014). CMC binds with the unstable proteins, increasing their molecular dimensions and making them forming the haze. Nevertheless, in general red wines do not suffer protein instability; therefore, in these wines, the increase in turbidity can be explained by two phenomena: i) the presence of phenolic compounds that, binding with the colloidal portions present in solution (i.e. CMC), promote colouring matter precipitation (Ribéreau-Gayon *et al.*, 2006; Moutounet *et al.*, 2010). However, the wine used for the experiments did not present colouring matter precipitation after the application of the CMC, therefore suggesting 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. Being the CMC a cellulose derivative with “protective colloid” characteristics (Ribéreau-Gayon *et al.*, 2006), the increase of turbidity can be

explained by this occurrence. However, explanations regarding this phenomenon remain open for further investigation.

Previous studies reported the increase of wine turbidity in accordance with increasing CMC doses (Moutounet *et al.*, 2010; Greef *et al.*, 2012). In this work, for each CMC a single dose was added to the wine, thus it is not possible to discuss the effect of CMC dose on wine turbidity. Nevertheless, a higher increase of turbidity was observed in the wine treated with the CMC4 (0.1 g/L) in comparison with the wine treated with the CMC1 (0.2 g/L), indicating that CMC characteristics play an important role, and probably more relevant than the addition dose.

Despite the development of turbidity after CMC addition, the difference between Control wine and the wine showing the highest value (CMC5) was lower than 3.5 NTU, at 5 months from the CMC addition. These increments are quite low in comparison with the values reported in the literature (Moutounet *et al.*, 2010; Greef *et al.*, 2012; Sommer *et al.*, 2016), in some cases higher than 100 NTU, thus being considered as slight increases.

Previously, it was observed that the increase of turbidity depends on the red wine, CMC

characteristics and concentration, time and temperature of conservation (Moutounet *et al.*, 2010). Our results, of slight increases of turbidity after CMC addition, which are probably explained by the wine characteristics, seem to corroborate this.

Effect of carboxymethylcelluloses addition on colour and wine phenolic composition

The effect of the different CMC on proanthocyanidins (PA) composition, tannin power and chromatic characteristics are following reported.

Wine proanthocyanidins (PA) composition

Table III shows the results concerning flavanols, PA fractions (oligomeric and polymeric) and total tannins concentration. No significant differences were observed between Control and CMC treated wine in terms of total tannins concentration, and monomeric flavanols and polymeric fractions. Regarding the oligomeric fraction, significant higher concentrations were found in CMC2 (73.4 mg/L), CMC4 (70.9 mg/L) and CMC5 (72.0 mg/L) treated wines in comparison to Control (56.8 mg/L). Nevertheless, overall, it seems clear that the CMC additives used in this study did not strongly impact the tannins composition of the wine.

TABLE III

Effect of carboxymethylcelluloses (CMC) on monomeric flavanols, oligomeric and polymeric proanthocyanidins
Efeito das carboximetilceluloses (CMC) nos flavanóis monoméricos, proantocianidinas oligoméricas e poliméricas

Treatment	Monomeric flavanols	Oligomeric proanthocyanidins	Polymeric proanthocyanidins	Total tannins
Control	21 ± 3 ns	57 ± 3 a	972 ± 29 ab	1050 ± 31 ab
CMC1	22.7 ± 0.5 ns	57 ± 1 ab	893 ± 22 a	973 ± 23 a
CMC2	23.8 ± 0.6 ns	73.4 ± 0.8 c	922 ± 15 ab	1019 ± 16 ab
CMC3	20.6 ± 0.5 ns	56.2 ± 0.9 a	957 ± 22 ab	1034 ± 21 ab
CMC4	21 ± 1 ns	71 ± 2 bc	978 ± 13 ab	1070 ± 12 ab
CMC5	21 ± 2 ns	72 ± 2 c	907 ± 40 ab	1000 ± 39 a

Mean values ± standard deviation values, from four analytical replicates, are expressed in mg/L. In each column, means followed by the same letter are not significantly different at a 0.05 level of significance; ns – without significant difference.

Wine tannin power

The assessment of tannin power in control and CMC added wines gives information on the influence of each CMC on wine astringency. The control wine showed the highest value of tannin power, 277.7 NTU/mL, followed by the wines treated with CMC4, CMC2, CMC1, CMC3 and CMC5 with, respectively, 260.5, 258.2, 255.7, 252.2 and 251.4 NTU/mL. The differences between the samples were not significant (results not shown); however, it could be observed that the CMC treated samples, compared to the Control, presented always lower values.

Recently, within a bench-trial experiment to evaluate the dynamics of the reaction between CMC, proteins and tannins in a model solution (Sommer *et al.*, 2016) it was observed that by adding BSA (Bovine Serum Albumin) to solutions already containing tannins and CMC, there was no interaction between tannins and CMC independently of CMC concentration, and only after protein addition a significant haze was observed. On the contrary, our results indicate that the reaction of tannins with the BSA solution (0.8 g/L) tend to be lower when CMC was present. Moreover, the observations suggest that as higher the CMC

concentration (CMC1, CMC3 and CMC5), lower the tannins reaction with the BSA solution.

Wine chromatic characteristics, anthocyanins and pigments

The evaluation of CMC effect on wine chromatic characteristics is of utmost importance because previous studies have reported that CMC use in red wines decreases their concentration of total phenols, flavonoids and non-flavonoids, reducing the colour intensity (Moutounet *et al.*, 2010; Claus *et al.*, 2014). The effect of the tested CMC additives on colour intensity and tonality of the wine is displayed in Table IV.

TABLE IV

Effect of carboxymethylcelluloses (CMC) on wine colour intensity and tonality

Efeito das carboximetilceluloses (CMC) na intensidade da cor e tonalidade do vinho

Treatment	Colour Intensity (u.a.)	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 ± 0.001 d
CMC3	9.11 ± 0.03 e	0.659 ± 0.001 a
CMC4	8.11 ± 0.01 b	0.696 ± 0.000 e
CMC5	9.00 ± 0.01 d	0.671 ± 0.002 b

Mean values ± standard deviation values from three analytical replicates. In each column, means followed by the same letter are not significantly different at a 0.05 level of significance.

For colour intensity, the lowest and highest values were found in Control (7.77 u.a.) and CMC3 (9.11 u.a.). It should be highlighted that all the CMC treated wines showed significant higher values of colour intensity in comparison with Control. This is somehow a surprising result, in opposition with some of the studies conducted before that reported a decrease in the colour intensity of red wines after CMC addition (Moutounet *et al.*, 2010; Claus *et al.*, 2014). Therefore, it can be concluded that, as already exposed by Greeff *et al.* (2012), not all the red wines interact with CMC in the same way: the Castelhão wine showed an increase in the colour intensity after CMC addition. This observation is of extreme importance and represents a starting point for further analyses on different red varieties.

It is well known that the development of colour characteristics is complex. Co-pigmentation of anthocyanins is of critical importance in understanding the variation in colour and pigment concentration between wines, and in all reactions involving the anthocyanins during wine aging. It

accounts for 30 to 50% of the observed colour of young red wines, as well as resulting in higher absorbance values (hyperchromic shift). Co-pigmentation is primarily influenced by the levels of several specific, non-coloured phenolic components or cofactors (Boulton, 2001). CMC addition may have promoted differences in the relative proportion of the cofactors among Control and added wines, resulting in distinct colour intensities. However, this is only a hypothesis justifying further investigation.

Concerning the tonality of the wines, effect of CMC addition was also observed. Significant differences were verified between the control wine and the wines treated with CMC2 and CMC4, with Control showing the lowest value (0.665). Hence, it can be stated that the CMC influenced the tonality of the wine, resulting in its increase in these treatments.

The results concerning total and coloured anthocyanins, and total and polymerized pigments content are reported in Table V. For all these parameters, significant effect of CMC addition was observed. Regarding total anthocyanins content, the lowest (343 mg/L) and highest (419 mg/L) values were found in CMC5 and CMC4 samples, respectively. In respect to coloured anthocyanins, the concentrations ranged between 42.9 mg/L (Control) and 55.7 mg/L (CMC3). It should be stressed that all the CMC treated wines showed higher concentration of coloured anthocyanins in comparison with Control. For total pigments content, the CMC5 revealed the lowest value (20.70 u.a.) and the CMC4 the highest one (24.27 u.a.). Finally, for polymerized pigments, slight but significant differences were observed between samples, with values ranging from 1.98 u.a. (CMC4) to 2.11 u.a. (CMC5). In particular, the addition of CMC3 and CMC5 products resulted in higher concentrations of coloured anthocyanins and polymerized pigments, supporting the observations concerning colour intensity, and revealing an important influence of these specific CMC on wine characteristics.

In conclusion, all the CMC products used in this study resulted in higher content of coloured anthocyanins contents and in some cases in higher content of polymerized pigments as well. Consequently, it is clear that the studied CMC influenced the red pigments composition of the wine, resulting in the rise of its red colour. These results contradict previous observations reported on this topic, consequently changing the CMC scenario in what concerns its application in red wines (Claus *et al.*, 2014). Moreover, in addition with the results exposed above about colour intensity, tonality and colouring matter precipitation, this corroborates some

results obtained by previous researchers, showing that not all the red wines respond to the CMC addition with a colour reduction (Greef *et al.*, 2012). Hence, further studies are required to better understand the

behaviour of the red wines when the CMC is added as a tartaric stabilization product.

TABLE V

Effect of carboxymethylcelluloses (CMC) on total and coloured anthocyanins, total and polymerized pigments

Efeito das carboximetilcelulosas (CMC) nas antocianinas totais, antocianinas coradas, pigmentos poliméricos e pigmentos totais

Treatment	Total anthocyanins (mg/L of malvidin 3-glucoside)	Ionization index (%)	Coloured anthocyanins (mg/L of malvidin 3-glucoside)	Total pigments (u.a.)	Polymerization index (%)	Polymerized pigments (u.a.)
Control	389 ± 22 bc	11.0 ab	42.9 ± 0.3 a	23 ± 1 bc	8.9 b	2.00 ± 0.02 a
CMC1	389 ± 5 bc	11.7 ab	45.5 ± 0.3 b	22.8 ± 0.2 bc	8.9 ab	2.00 ± 0.02 a
CMC2	381 ± 7 b	12.0 b	45.7 ± 0.2 b	22.5 ± 0.3 b	9.2 bc	2.03 ± 0.01 ab
CMC3	381 ± 1 b	14.6 c	55.7 ± 0.2 d	22.6 ± 0.1 b	9.4 bc	2.10 ± 0.01 c
CMC4	419 ± 11 c	10.8 a	45.2 ± 0.5 b	24.3 ± 0.6 c	8.3 a	1.98 ± 0.02 a
CMC5	343 ± 4 a	15.6 d	53.7 ± 0.6 c	20.7 ± 0.2 a	10.3 d	2.11 ± 0.01 c

Mean values ± standard deviation values from three analytical replicates. In each column, means followed by the same letter are not significantly different at a 0.05 level of significance.

Effect on flavonoids, non-flavonoids and total phenols

The results concerning the effect of CMC addition on flavonoids, non-flavonoids and total phenols content of wine are displayed in Table VI. CMC addition to wine had significant effect on total phenols content of wine, but with exception of CMC5 sample, showing the lowest value (1623 mg/L), no significant differences were observed between Control and CMC treated wines. It should be highlighted the importance of this result, having in mind that previous studies

reported a decrease in the total phenols content after the CMC addition on red wines (Claus *et al.*, 2014). For non-flavonoids, the CMC treated samples were characterized by higher values than Control, reflecting that these products induced an increase in the non-flavonoids concentration. CMC2 and CMC3 promoted the highest increases, close to 50 mg/L, most probably explained by their chemical composition.

TABLE VI

Effect of carboxymethylcelluloses (CMC) on total phenols, flavonoids and non-flavonoids contents

Efeito das carboximetilcelulosas (CMC) nos teores de fenóis totais, flavonóides e não-flavonóides

Treatment	Total phenols (mg/L of gallic acid)	Non- flavonoids (mg/L of gallic acid)	Flavonoids (mg/L of gallic acid)
Control	1774 ± 4 b	141.2 ± 0.8 ab	1633 ± 5 b
CMC1	1740 ± 16 b	144 ± 1 bc	1596 ± 16 b
CMC2	1802 ± 30 b	194 ± 1 d	1608 ± 30 b
CMC3	1779 ± 10 b	197 ± 2 d	1582 ± 10 b
CMC4	1751 ± 11 b	148 ± 3 c	1603 ± 10 b
CMC5	1623 ± 4 a	145.3 ± 0.7 bc	1478 ± 4 a

Mean values ± standard deviation values from three analytical replicates. In each column, means followed by the same letter are not significantly different at a 0.05 level of significance.

In opposition, concerning the flavonoids content, all the CMC treated samples tend to show lower values than Control, even if only for CMC5 a significant difference was observed. This result is in line with the

ones already exposed above regarding total tannins and total anthocyanins concentration. The flavonoids are composed by flavonols, flavononols, condensed tannins and anthocyanins. Therefore, having in mind

the high concentration of the tannins and anthocyanins in the Control in comparison with the CMC5 sample, it seems clear that, as expected, the flavonoids content follows the same trend. With exception of CMC5 additive, it appears clear that CMC phenolic composition did not have a relevant influence on phenolic composition of wine. In fact, CMC effect on total phenols of wines was not as important as for the chromatic parameters above exposed. The results did not reveal a negative effect of the CMC addition on the general phenols asset of the wine, strongly indicating that further studies are needed to better understand the interaction between CMC and red wines.

CONCLUSIONS

This study suggested that CMC is able to prevent potassium bitartrate salts crystallization in red wine. All the tested CMC promoted the tartaric stability of a red wine, initially unstable. Nevertheless, the treatment with CMC influenced the wine characteristics. It was observed significant effect on chromatic characteristics, more precisely an increase in colour intensity, coloured anthocyanins and, for some treatments, in polymerized pigments content, contradicting previous studies. On the other hand, total phenols concentration and tannins composition in terms of monomeric and polymeric fractions of the treated wines did not completely differ from the Control. No colouring matter precipitation occurred during the five months of the experiment which, in addition to the before mentioned results, changes the CMC scenario regarding its application in red wines. However, the CMC resulted in slight increases of turbidity, which can be a drawback depending on the wine initial turbidity and intended final characteristics.

These results, in addition to CMC sustainability both in environmental and economic terms, reinforce its importance as an alternative to other stabilization treatments. For all of these reasons it can be concluded that, being the CMC yet generally restrict

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to the production of white and sparkling wines, this is a valuable study concerning the use of CMC for tartaric stabilization of red wines. The results offer a starting point for future studies and experiments with the aim of spurring a higher application of this additive also in red wines.

Yet, it is clear that further research is needed to better understanding of the interaction mechanisms and effects of CMC on red wines. It would be essential to assess the tartaric stability and colouring matter stability after a longer period of contact between the wine and the CMC: this would be supportive to this research to better understand the response of the wine to the product addition and to establish the CMC long term effect. Moreover, it would be crucial in the future, to test the effect of CMC on different red wines in terms of tartaric and colouring matter stability. At last, the evaluation of the CMC effect on wine sensory characteristics, with basis on a wide panel of tasters, would be of vital importance.

ACKNOWLEDGMENTS

The authors acknowledge the staff of the Laboratory of Enology of Instituto Superior de Agronomia (Diana Faria and Daniel Duarte) for technical support; AEB Bioquímica Portuguesa S.A., Agrovin S.A., Angelo Coimbra & Ca. Lda, Ecofiltra Lda, Proenol Indústria Biotecnológica Lda, SAI, for supplying the CMC; and Sociedade Agrícola de Rio Frio S.A. for providing the wine regarding the study development. Furthermore, Elisabetta Pittari thanks to all the Vinifera EuroMaster members: directors, secretaries and professors, for the opportunity to develop this project.

This work was supported by the Portuguese National Funding Agency for Science and Technology through the grant SFRH/BPD/93535/2013 and through the research centre LEAF (UID/AGR/04129/2013).

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