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Effect of the addition of enological stabilizers on the wine filterability index: application of simplex-centroid[1]

Efeito da adição de estabilizantes enológicos sobre o índice de filtrabilidade dos vinhos: aplicação do simplex-centroide

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ABSTRACT - The search for quality improvement in wine has intensified the use of products and additives in the different stages of winemaking, from the receipt of the grapes until the bottling. Among the additives employed at the end of the process, we can cite the arabic gum, mannoproteins and carboxymethylcellulose (CMC). However, the addition of these products most of the times, is performed immediately before the final filtration, which can lead to inefficiency due to membrane clogging and also causing the retention of these stabilizers. The objective of this work was to evaluate the effect of different enological stabilizers on the wine filterability index (FI) and its turbidity. The products were added to a white wine after tangential filtration (0.22 µm). The experimental design used was simplex-centroid. The maximum limits for each additive were established according to the current brazilian legislation, using the following products and doses: 0.3 g L^{-1} Arabic gum, 0.15 g L^{-1} of Mannoprotein and 0.1 g L^{-1} of CMC. The FI was measured using specific software connected to the Vessel Data Filterability Index, developed and owned by AEB Engineering. The addition of mannoprotein compromised the filterability index and increased the turbidity of the wine. The application of CMC and arabic gum does not resulted in an FI enhancement that makes filtration impossible, the same effect occurred with turbidity. The presence of CMC and/or arabic gum produced an antagonistic effect, reducing the filterability index that the mannoprotein alone would result.

Key words: Wine Stabilization. Filtration. CMC. Arabic Gum. Mannoprotein.

RESUMO - A busca pelo incremento de qualidade no vinho tem intensificado o uso de insumos e aditivos em diferentes etapas da vinificação, desde o recebimento da uva até o momento anterior ao envase. Entre os aditivos adicionados ao final do processo destacam-se a goma arábica, manoproteínas e carboximetilcelulose (CMC). Entretanto, a adição destes três insumos na maioria das vezes é realizada momentos antes da filtração final, podendo acarretar na ineficiência desta devido ao entupimento da membrana, e também, causando a retenção destes estabilizantes. O objetivo do trabalho foi avaliar o efeito de diferentes estabilizantes enológicos sobre o índice de filtrabilidade do vinho (FI) e sua turbidez. Os produtos foram adicionados a um vinho branco com passagem por filtração tangencial (0,22 µm). Utilizou-se do planejamento experimental simplex-centróide. Os limites máximos para cada aditivo foram estabelecidos a partir da legislação vigente, utilizando os seguintes produtos e doses: 0,3 g L^{-1} de Goma Arábica, 0,15 g L^{-1} de Manoproteína e 0,1 g L^{-1} de CMC. O FI foi mensurado através de software específico conectado ao aparelho Vessel Data Filterability Index, desenvolvido e pertencente a AEB Engineering. A adição de manoproteína comprometeu o índice de filtrabilidade e elevou a turbidez do vinho. A aplicação de CMC e Goma Arábica não promoveu aumento do FI que impossibilite a filtração, o mesmo efeito ocorreu com a turbidez. A presença de CMC e/ou Goma Arábica produziu um efeito antagônico, reduzindo o índice de filtrabilidade que a manoproteína isolada resultaria.

Palavras-chave: Estabilização de Vinhos. Filtração. CMC. Goma Arábica. Manoproteína.

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INTRODUCTION

The elaboration of wines has several steps, from the reception of the grapes to the bottling of the product. In most cases, with the completion of fermentation, the wine is not yet ready to be bottled, not being suitable for a good acceptance by consumers (EL RAYESS *et al*., 2011). The main issues to be treated are possible instabilities, such as the presence of tartaric salts and unstable proteins, turbidity, and sensory characteristics of the beverage. In this pre-bottling stage, procedures such as the use of cold treatment, stabilizers, fining, and filtrations are widely adopted.

Cold treatment is currently the most widely used form of tartaric stabilization, due to its high efficiency. However, it is possible that changes in the organoleptic qualities of the product, above all, on the color stability, intensified the use of enological additives that replace the conventional method, in addition are responsible for the increase of quality of the beverage, such as arabic gum (GUISE *et al.*, 2014; OBRADOVIC; HANCOCK, 2010), mannoproteins (GUADALUPE *et al.*, 2012; GUISE *et al.*, 2014) and carboxymethylcellulose (CMC) (GUISE *et al*., 2014; SOMMER *et al*., 2016).

Arabic gum is a polysaccharide consisting of hydroxyproline and arabinogalactan-protein. It is an exsudase gum, derived mainly from the exudate of two species of Acacia (BECHERVAISE; CARR; BIRD, 2016). It is used in enology to control iron casses and to stabilize the color of red wines, besides contributing as protective colloid for tartaric stabilization (OBRADOVIC; HANCOCK, 2010).

Among the main groups of polysaccharides present in the wine are the mannoproteins (GUADALUPE *et al.*, 2012), originating from cell walls of yeast *Saccharomyces cerevisiae*. Your properties, which aid in tartaric and protein stability, in addition to improvements in the quality of the product, make them widely used in the enological industry (RIBEIRO *et al.*, 2014), being added exogenously to wines.

CMC is widely used in the food, beverage, cosmetic and various other industries, in addition, studies report its preservative action on some bacteria (LIRA JUNIOR *et al.*, 2013). The additive, a complex mixture of polysaccharides of various sizes and molecular modifications, is incorporated into wine immediately prior to bottling and is responsible for tartaric stabilization (BOSSO *et al.*, 2010; GERBAUD *et al.*, 2010) in order to inhibit the formation of potassium crystals.

Pre-bottling filtration is recurrent in the enological industry and aim the removal of suspended particles that could compromise the visual quality of the product in the bottle and guarantee the microbiological stability. This practice is carried out, most of the time, after the incorporation of the aforementioned enological additives (RODRIGUES *et al.*, 2012). However, the additives are colloids, and the addition of this type of compound may adversely affect wine filtration (BOWYER; EDWARDS; EL RAYESS *et al*., 2011; EYRE, 2013).

The presence of polysaccharides, polyphenols and proteins are the main causes of membrane clogging (EL RAYESS *et al*., 2011). According to Bowyer, Edwards and Eyre (2013), there may be clogging and blocking of the filter medium with the passage of wines that already have high colloidal content or have added. In addition, Vernhet, Cartalade and Moutounet (2003) reported the possibility of excessive retention of colloids to affect the final quality of the product and risk of instability due to inadequate doses of stabilizers. In this way, the objective of this work was to evaluate the effect of the addition of three stabilizers, isolated and combined, on the wine filterability index and turbidity.

MATERIAL AND METHODS

Wine

For the experiment, a young white wine, *Vitis labrusca*, vintage 2017, with known physicochemical characteristics and filtered by tangential filtration, with a porosity of 0.22 µm, was used, eliminating any other factor that might interfere with the index at the time of bottling. The samples were conditioned at room temperature in Bordeaux style glass bottles (0.75 L). The physico-chemical characteristics of the wine are presented in Table 1.

Enological stabilizers

For the combinations evaluated, the following products, supplied by AEB Group (Brescia, Italy) were used: Arabic Gum (AG) from Acacia (*Acacia* spp.) (trade name: Arabinol LA) in liquid solution (density: 1.05 – 1.15 Kg L^{-1} , dry residue: 20% and pH: 4.5) containing 0.3 a o 0.5% Sulfur Dioxide (SO_2); Mannoprotein (trade name: BÂTONNAGE Body), in form of granulated owder (density: 0.5 Kg L^{-1}) containing from 11 to 13% in Nitrogen; Carboxymethylcellulose (CMC) (Trade name: New-Cel) in liquid solution (Dry residue: 4.5 to 5.5%, pH: 3.6 to 4.1, viscosity: 200 cps at 25 °C and 350 cps at 5 °C) containing about 0.2% Sulfur Dioxide (SO_2) and a ratio between the number of carboxylated groups and glucose units equal to 1.

Physicochemical analysis

The filterability index allows to be sure of the effect of the products on the filtration with greater accuracy, as the turbidity (measure in NTU) does not represent a clogging indicator and can be a bad

Table 1 - Physical-chemical characteristics of white wine 2017 vintage before addition of stabilizers

Analytical Variables[a]	
Density (g L^{-1})	994.2
Alcohol content (mL L^{-1})	110.0
Sugar content (g L^{-1})	1.2
Volatile acidity (g L^{-1} acetic acid)	0.3
Total acidity (g L^{-1} tartaric acid)	4.9
pH	3.39
Total SO_2 (mg L^{-1})	88
Free SO_2 (mg L^{-1})	25
Turbidity (NTU[b])	0.570
Filterability Index	11.01

[a]Means values obtained from two replicates. [b]NTU: Nephelometric Turbidity Units

attribute to represent the conditions of the liquid, since the compounds are soluble in wine, as explained by Bowyer, Edwards and Eyre (2012). The Index was measured using specific software connected to the Vessel Data Filterability Index, developed and owned by AEB Engineering (Italy). In the process, a volume of 0.6 L of each sample is filtered through a cellulose nitrate membrane, with a diameter of 25 mm and porosity of 0.65 µm, resulting in a filter area of 4.8 cm^2. A controlled pressure of 2 bar is exerted on the liquid and the filtered volume is weighed by a balance, and the Filterability Index is automatically calculated, as described by Bowyer, Edwards and Eyre (2012), Togores (2018) and Vernhet (2019). The formula for obtaining this parameter (FI) is described in Eq. 1, where T_{400} refers to the elapsed time for the filtration of 0.4 L and T_{200} to the time of 0.2 L filtered.

$$FI = T_{400} - 2\ T_{200} \quad (1)$$

The turbidity of the wines was measured directly on a nephelometric turbidimeter (Hanna Mark, model HI98703-02, Romania) with prior calibration with standard formazine solutions provided by Hanna Instrument. The turbidity was expressed in NTU (Nephelometric Turbidity Unit).

In order to monitor both characteristics of the wines, all analyzes were performed 2 h, 24 h and 14 days after the addition of the products.

Experimental design and statistical analysis

It is usual that the main suppliers of enological products offer mixtures of stabilizers for wines. However, scientific studies usually focus on the individual assessment of the stabilizers, or at most, on the combination of two. The development of a formulation involving more than two additives requires some experimental specificities (CORNELL, 2011). Being possible to analyze whether there is synergistic or antagonistic effect of the stabilizers on the filterability index and the behavior of the stabilizers when there is a time interval between the incorporation of the same in the wine and the filtration.

The experimental design of simplex-centroid mixture was used to evaluate the behavior of the mixtures formed by Arabic Gum (AG), Mannoprotein and Carboxymethylcellulose (CMC), added on a white wine. The design for three components was composed of 7 trials with two replicates at the central point and two replicates at the other points vertices and lateral. As control treatment, an additional treatment was performed in duplicate where the wine went all the similar processes, however, without receiving any stabilizers. The upper limits for each stabilizer were established from previous trials and by current brazilian legislation (BRASIL, 2016). Arabic gum, Mannoprotein and CMC doses were defined as 0.30 g L^{-1}, 0.15 g L^{-1} and 0.1 g L^{-1}, respectively. The proportions in each formulation, expressed in real concentrations and in pseudocomponents (CORNELL, 2011), are presented in Table 2.

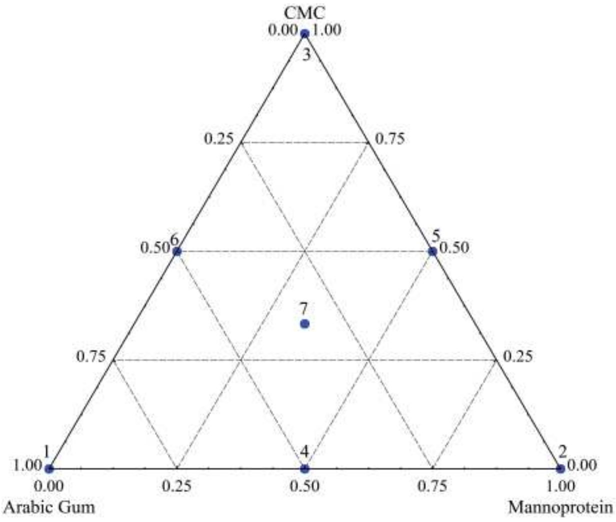
The mixexp package (LAWSON; WILLDEN, 2016) in the R software version 4.0.2 (R CORE TEAM, 2020) was used in the planning of the mixtures and analysis of the data obtained experimentally. In this process, AG, Mannoprotein and CMC were represented by the as input variables, for the system under study, designated by X_1, X_2 and X_3, respectively. Figure 1 shows the 3-component simplex-centroid experimental design, where the seven circles represent the AG/Mannoprotein/CMC mixtures

Table 2 - Matrix of the simplex-centroid experimental design with the pseudocomponents, the real concentrations and replicates for mixture of three stabilizers

Point	Test	Pseudocomponents			Real concentrations (g L^{-1})			Replicas
		X_1 Arabic Gum	X_2 Mannoprotein	X_3 CMC	X_1 Arabic Gum	X_2 Mannoprotein	X_3 CMC	
Vertice 1	1	1.0	0.0	0.0	0.300	0.000	0.000	2
Vertice 2	2	0.0	1.0	0.0	0.000	0.150	0.000	2
Vertice 3	3	0.0	0.0	1.0	0.000	0.000	0.100	2
Lateral 1	4	0.5	0.5	0.0	0.150	0.075	0.000	2
Lateral 2	5	0.5	0.0	0.5	0.150	0.000	0.050	2
Lateral 3	6	0.0	0.5	0.5	0.000	0.075	0.050	2
Center	7	0.33	0.33	0.33	0.100	0.050	0.033	2
Control	8	0.0	0.0	0.0	0.0	0.0	0.0	2

X_1 – Arabic Gum. X_2 – Mannoprotein. X_3 – Carboxymethylcellulose. $X_1+X_2+X_3=1$ or 100%

Figure 1 - Simplex-centroid experimental design for 3 compounds. CMC –Carboxymethylcellulose



prepared to obtain an appropriate response surface, using the expression of the quadratic model (CORNELL, 2011), shown in Equation 2. Mathematical models adjusted to each response were submitted to analysis of variance (ANOVA) to evaluate the significance ($p < 0.05$) and the adjusted coefficient of determination (adjusted R^2).

$$y = \sum_{i=1}^{q} \beta_i X_i + \sum \sum_{i<j}^{q} \beta_{ij} X_i X_j \qquad (2)$$

RESULT AND DISCUSSION

This study allowed to evaluate the individual and combined effect of the three stabilizers on the filterability index and the turbidity of the wine over time (2 h, 24 h and 14 days). According to the analysis of variance, the models adjusted for the variable filterability index response explained 97%, 99% and 99% of the variation, respectively (Table 3). For the turbidity, the adjusted models explain 99% of the variation (Table 4). These parameters were negatively influenced by the three stabilizers.

Quadratic equation modeling allows to infer that there is an antagonistic effect of the CMC and Arabic Gum stabilizers on the Filterability Index when added in combination with Mannoprotein. That is, in the presence of Arabic Gum together with Mannoprotein, the value of filterability index will be lower than using the Mannoprotein alone. As for turbidity, at the time of addition, and after 24 h, there is an antagonistic effect of CMC on Mannoprotein.

To better observe the region of ternary combination between the independent variables Arabic Gum, Mannoprotein and CMC, diagrams with contour lines were used. They show the contour regions of the response surface for the dependent variables obtained by the mathematical models.

All treatments containing enological additives increased the Filterability Index compared to the control treatment, which had a filterability index of 11.01. It can be observed that, just after the addition, the Filterability Index (Figure 2) of the wine rised according with the increase of mannoprotein concentration. This result was expected since previous studies have found that mannoproteins induced a decrease in microfiltration flow (VERNHET *et al.*, 1999).

Thus, all treatments containing Mannoprotein showed an increase in the Filterability Index, so that wines 2 h after the addition, were improper for filtration. According to the literature, the Filterability Index must be below 20 for the wine to be considered filterable (BOWYER; EDWARDS; EYRE, 2012; TOGORES, 2018).

Table 3 - Regression coefficients (β) and analysis of variance of the models adjusted to the filterability index after 2 h, 24 h and 14 days the addition of a mixture containing AG, Mannoprotein and CMC

Filterability Index	2 hours	24 hours	14 days
β_1 (Arabic Gum)	15.7215**	14.7034**	13.3244**
β_2 (Mannoprotein)	118.532**	113.0714**	65.4814**
β_3 (CMC)	13.6391*	10.5059**	10.1284**
β_{12}	-96.8363**	-169.3774**	-85.6841**
β_{13}	-5.4923	-10.9364	5.5479
β_{23}	-119.5213**	-85.1924**	-64.3821**
Significance of the model (p)	<0.001	<0.001	<0.001
R^2 adjusted	0.98	0.99	0.99

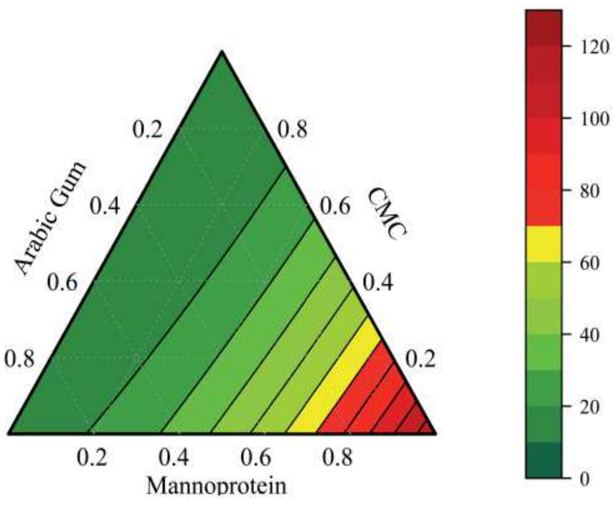
$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$; X_1 – Arabic Gum. X_2 – Mannoprotein. X_3 – Carboxymethylcellulose. *Significant coefficients ($p < 0.05$). **Significant coefficients ($p < 0.01$)

Table 4 - Regression coefficients (β) and analysis of variance of the models adjusted turbidity (NTU) after 2 h, 24 h and 14 days the addition of a mixture containing AG, Mannoprotein and CMC

Turbidity (NTUa)	2 hours	24 hours	14 days
β_1 (Arabic Gum)	0.8874**	0.8617**	0.8006**
β_2 (Mannoprotein)	2.2524**	1.7267**	1.3856**
β_3 (CMC)	0.8324**	0.5167**	0.5506**
β_{12}	0.4223*	0.1359	0.4171**
β_{13}	-0.0777	0.5759**	0.6071**
β_{23}	-0.7277**	-0.0341	0.1771
Significance of the model (p)	<0.001	<0.001	<0.001
R^2 adjusted	0.99	0.99	0.99

$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$; X_1 – Arabic Gum. X_2 – Mannoprotein. X_3 – Carboxymethylcellulose. *Significant coefficients ($p < 0.05$). **Significant coefficients ($p < 0.01$).[a]NTU: Nephelometric Turbidity Units

Figura 2 - Triangular diagram with contour lines is the experimental model (in terms of pseudocomponents) for filterability index in wines after 2 hours of addition. The area between points delimites the experimentally analyzed region. The real values for each test are shown in bold. CMC – Carboxylmethylcellulose



The addition of CMC and Arabic Gum, and their mixtures, did not promote changes that made the filtration unfeasible, with indices falling within the recommended range. Previous studies have indicated that the use of CMC will negatively affect the Filterability Index of wines, so that with 0.1 g L^{-1} the increase of the FI was 5 points and using a dose of 0.3 g L^{-1} the increase was approximately 25 points, making the operation impossible, so that the wine is not suitable for the final filtration (BOWYER; EDWARDS; EYRE, 2012). According to Togores (2018), the presence of protective colloids, such as Arabic Gum, can cause a great inconvenience, since it affects the sedimentation of substances by delaying the clarification, as well as can quickly clog the filtering surfaces, being a significant problem.

The turbidity is used as a way of assessing the level of particles in a wine (clarity), so it is a parameter to be appropriated for bottling. A commonly used value for bottling is < 1 NTU (BOWYER; EDWARDS; EYRE, 2012). Similar as the Filterability Index, all treatments containing enological stabilizers had an increased turbidity right after the addition incorporation of them (Figure 3), compared to the control treatment, that had a value of 0.570 NTU. As for FI, all treatments containing mannoprotein significantly increased the NTU of the wine, >1 NTU. This result is contrary to the expected, as the objective of this colloid is to protect against turbidity-causing instabilities (DUFRECHOU *et al.*, 2015; VAN SLUYTER *et al.*, 2015).

For this study, at the moment of the addition of the products, the Filterability Index is directly relationed to the turbidity of the samples. Authors argue that membrane clogging correlates more consistently with colloidal size than with turbidity, in which case the Filterability Index is a more reliable parameter for evaluating filtration (VERNHET; CARTALADE; MOUTOUNET, 2003).

Two hours (2 h) after the addition of the products, the mixture of CMC and Mannoprotein resulted in an antagonistic effect on turbidity, that is, the CMC overrides the effect that the mannoprotein would cause alone on the parameter. After 24 hours of addition, the mixture of Arabic Gum and CMC has a synergistic effect, that is, the combination of these two products results in an even greater turbidity. Previous studies report an increase in wine turbidity with the addition of different polysaccharides, among them, gum arabica, mannoprotein and CMC, due to protein aggregation (JAECKELS *et al.*, 2016).

On the incorporation of the products and the time elapsed to the analysis of the Filterability index (Table 5) and turbidity (Table 6), all stabilizers and their mixtures resulted in a reduction in the variable responses over time, especially in the treatments with mannoprotein after 14 days (50%). However, despite the reduction, the values are above standards, especially in the first hours after incorporation of the products (24 h) and also after 14 days, in the case of treatment with only mannoproteins. After 24 hours (Figure 4a) the center point (combination of the 3 products) resulted in an FI < 20, as well as after 14 days (Figure 4b) the mixture of Mannoprotein and Arabic Gum was also within the standards, showing that the wines were already suitable for filtration.

Figure 3 - Triangular diagram with contour lines is the experimental model (in terms of pseudocomponents) for turbidity (NTU) in wines after 2 hours of addition. The area between points delimites the experimentally analyzed region. The real values for each test are shown in bold. CMC – Carboxylmethylcellulose

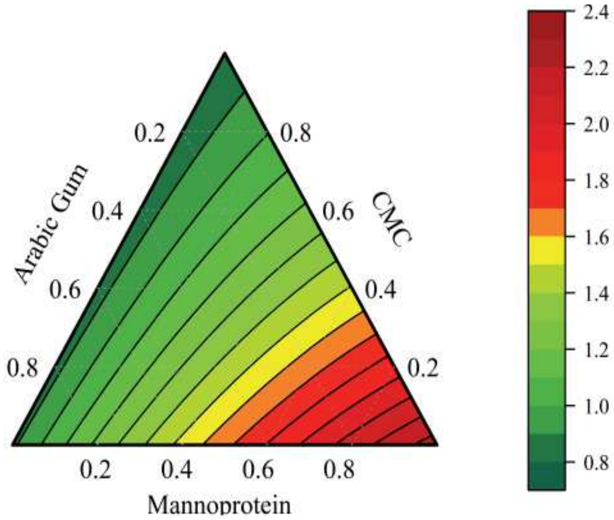


Table 5 - Results for the filterability index after 2 h, 24 h and 14 days the addition of a mixture containing AG, Mannoprotein and CMC

Filterability Index	2 hours	24 hours	14 days
Control	11.01 aC	10.69 aC	11.18 aB
X_1 (Arabic Gum)	15.42 aC	14.55 aC	13.45 aB
X^2 (Mannoprotein)	118.24 aA	112.91 aA	65.61 bA
X_3 (CMC)	13.34 aC	10.35 aC	10.26 aB
X_{12}	44.10 aB	22.17 bC	17.46 bB
X_{23}	37.39 aB	41.12 aB	21.19 bB
X_{13}	14.49 aC	10.50 aC	12.60 aB
X_{123}	21.98 aC	15.18 aC	14.75 aB

Values with different capital letters in the single columns (treatments) and different lower-case letters in the single rows (time) are statistically different. Tukey's test ($p < 0.05$). CMC – Carboxymethylcellulose

Table 6 - Results for the turbidity (NTU) after 2 h, 24 h and 14 days the addition of a mixture containing AG, Mannoprotein and CMC

Turbidity (NTUa)	2 hours	24 hours	14 days
Control	0.570 aE	0.575 aE	0.570 aE
X_1 (Arabic Gum)	0.885 aD	0.860 aD	0.800 aD
X_2 (Mannoprotein)	2.250 aA	1.725 bA	1.385 cA
X_3 (CMC)	0.830 aD	0.515 bE	0.550 bE
X_{12}	1.685 aB	1.335 bB	1.200 cB
X_{23}	1.370 aC	1.120 bC	1.015 cC
X_{13}	0.850 aD	0.840 aD	0.830 aD
X_{123}	1.260 aC	1.095 bC	1.040 bC

Values with different capital letters in the single columns (treatments) and different lower-case letters in the single rows (time) are statistically different. Tukey's test ($p < 0.05$).[a]NTU: Nephelometric Turbidity Units; CMC – Carboxymethylcellulose

Figure 4 - Triangular diagram with the level curves obtained by the experimental model (in terms of pseudocomponents) for the filterability index of wines after 24 hours (a) and 14 days (b). The area between points delimites the experimentally analyzed region. CMC – Carboxymethylcellulose

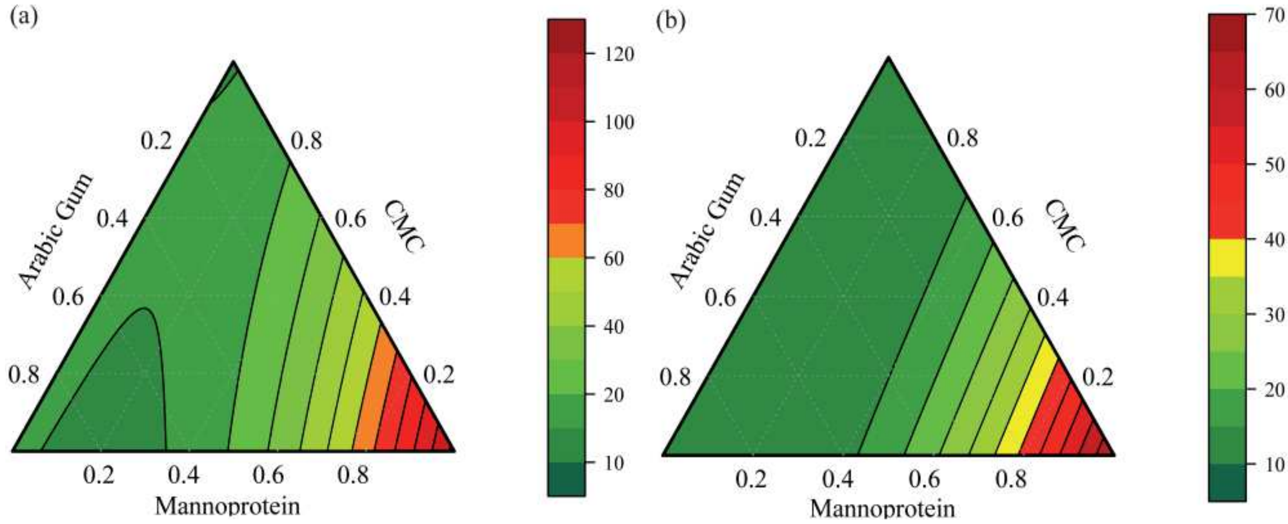
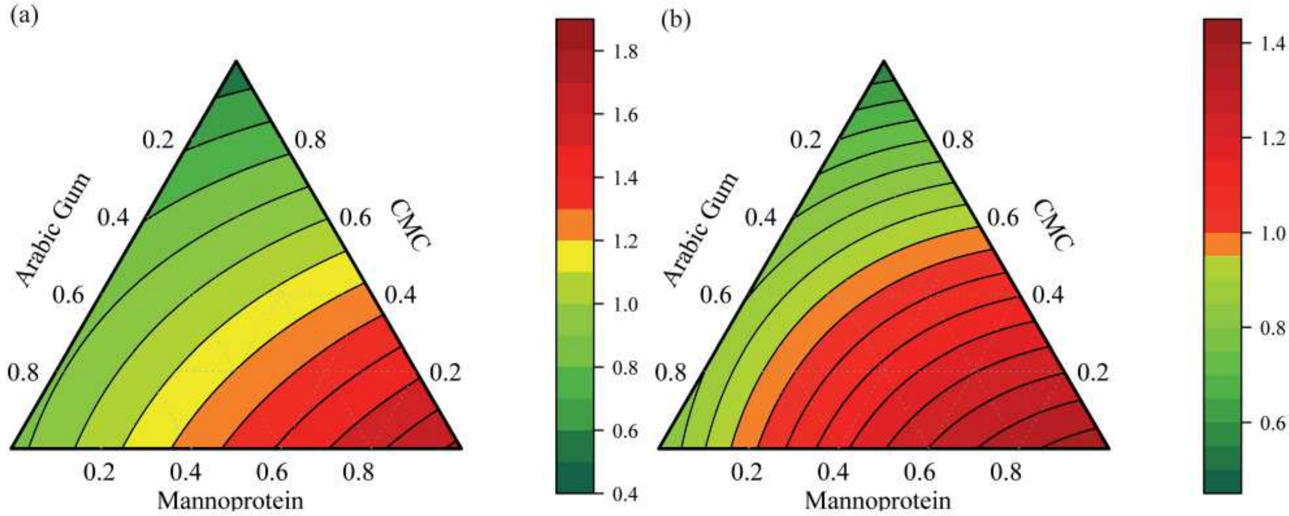


Figure 5 - Triangular diagram with the level curves obtained by the experimental model (in terms of pseudocomponents) for turbidity (NTU) of wines after 24 hours (a) and 14 days (b). The area between points delimites the experimentally analyzed region. CMC – Carboxymethylcellulose



For the turbidity, after 24 h, the treatment containing CMC did not present significant differences from the control (Figure 5a), being this the stabilizer of lesser influence on both analyzed variables. According to Peynaud and Blouin (2004), the CMC is stable over time, temperature rise, filtration and not present risks of an increased turbidity directly or indirectly. For 14 days (Figure 5b), all treatments containing mannoprotein remained turbid over the recommended value for bottling (> 1 NTU).

CONCLUSIONS

The simplex-centroid mixture design proved to be a good tool to evaluate interactions between wine stabilizers. All stabilizers increased the parameters evaluated in comparison to the control treatment. The addition of mannoprotein impaired the filterability index and increased the turbidity of the wine. The application of CMC and Arabic Gum did not result in an increase in FI that make that makes filtration impossible, and the same effect occurs with turbidity.

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