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TECHNICAL NOTE/NOTA TÉCNICA

EFFECTS OF STERILISING FILTRATION ON MICROBIOLOGICAL STABILITY, CHEMICAL COMPOSITION AND SENSORY PROPERTIES OF RED WINE

EFEITOS DA FILTRAÇÃO ESTERILIZANTE NA ESTABILIDADE MICROBIOLÓGICA, COMPOSIÇÃO QUÍMICA E CARACTERÍSTICAS SENSORIAIS DO VINHO TINTO

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SUMMARY

It was studied the effects of the sterilising filtration on the microbiological stability, chemical composition and sensory properties of a red wine that has not undergone malolactic fermentation and that needed to be bottled. The filtration was performed in a pilot-scale system composed by a set of membranes in series: a prefiltration membrane of 5 μm (fiberglass), followed by the sterilising membranes of 1 μm (fiberglass), 0.6 μm (fiberglass) and 0.45 μm (polyethersulfone). The results obtained showed that the levels of microorganisms decreased considerably after the sterilising filtration, without detection of microorganisms at the wines filtered with the membranes of 0.6 μm and 0.45 μm. The total phenolic content and the chromatic characteristics of the filtered wines were affected by the procedure under study, varying over the time of storage. After two months of storage, the filtered wine cannot be distinguished from the non-filtered wine by their phenolic content, being the wine filtered with the 0.6 μm membrane more similar to the control group. Except for lightness, there was a remarkable positive evolution of the chromatic characteristics of the wine, especially in the wine filtered with 0.45 μm. This suggests that two months after filtration the wines have actually reached its balance, along with the required microbiological stability. From the sensory point of view, there were no significant effects on the colour intensity, aroma quality, flavour quality and overall quality of the filtered wines after one month of storage. After two months of storage, there was a significant increment on colour intensity, which is in agreement with the effects observed in the chromatic characteristics. Although there were no significant differences among wines in the other sensory properties, there was a preference, corresponding to high quality, for the wine filtered with 1 μm membrane, followed by the wine filtered with 0.6 μm membrane.

RESUMO

Foram estudados os efeitos da filtração esterilizante na estabilidade microbiológica, na composição química e nas características sensoriais de um vinho tinto, para ser engarrafado, e no qual não ocorreu a fermentação maloláctica. A filtração foi efectuada num sistema piloto, constituído por um conjunto de membranas dispostas em série: uma membrana de pré-filtração de 5 μm (fibra de vidro), seguida de membranas esterilizantes de 1 μm (fibra de vidro), 0.6 μm (fibra de vidro) e 0.45 μm (polietersulfona). Os resultados obtidos demonstraram que os níveis de microrganismos diminuíram consideravelmente após a filtração esterilizante, sem detecção de microrganismos nos vinhos filtrados com membranas de 0.6 μm e 0.45 μm. O teor de compostos fenólicos e as características cromáticas dos vinhos filtrados foram significativamente afectados pelo processo em estudo, variando ao longo do tempo de conservação do vinho. Após dois meses de conservação, o vinho filtrado não se diferenciava do vinho não filtrado no teor de compostos fenólicos, sendo o vinho filtrado com membrana de 0.6 μm o que mais se assemelhava ao vinho não filtrado. À excepção da luminosidade, foi observada uma evolução francamente positiva nas características cromáticas do vinho, especialmente no filtrado com membrana de 0.45 μm. Este facto sugere que, dois meses após a filtração, os vinhos retomaram o seu equilíbrio, a par da requerida estabilidade microbiológica. Do ponto de vista sensorial, no final do primeiro mês de conservação, não foram verificados efeitos significativos na intensidade da cor, na qualidade do aroma, na qualidade do sabor e na qualidade global dos vinhos filtrados. Após dois meses de conservação, constatou-se a existência de um aumento significativo da intensidade da cor, coerente com os efeitos observados nas características cromáticas. Apesar da inexistência de diferenças significativas nas demais características sensoriais, foi revelada preferência, correspondente a uma maior qualidade, relativamente ao vinho filtrado com membrana de 1 μm, seguido pelo vinho filtrado com membrana de 0.6 μm.

Key words: Sterilising filtration, red wine, microbiological stability, chemical composition, sensory properties.
Palavras-chave: Filtração esterilizante, vinho tinto, estabilidade microbiológica, composição química, características sensoriais.

INTRODUCTION

According to the Resolution OENO 1/90 of the International Organization of Vine and Wine (OIV, 1990) the sterilising filtration is authorized for wines as a mean to obtain their microbiological stability for bottling, but membrane should have a maximum average pore diameter of 0.65 μm.

For sterilising filtration several types of filters have been introduced, and membrane filters are increasingly adopted owing to their easy handling and good pressure stability without an excessive flattening of the sensory profile of the wine (Peri *et al.*, 1988; Caldeira and Ricardo-da-Silva, 1995; Asano *et al.*, 2007).

This kind of wine filtration has always been a controversial issue due to its effect on wine chemical and sensory characteristics, especially in red wine. However, few scientific and technical literature on this specific subject are available (Peri *et al.*, 1988; Arriagada-Carrazana *et al.*, 2005; Malfeito-Ferreira, 2010) although there are several references in biotechnological and pharmaceutical fields (McBurnie and Bardo, 2004; Richard *et al.*, 2006; Rajniak *et al.*, 2008; Heusslein and Brendel-Thimmel, 2010).

Nevertheless, sterilising filtration is a crucial step in

winemaking process to ensure a timely transition to bottling and commercialization of wine, namely for wines that present very high level of microorganisms such as yeasts (including *Dekkera/Brettanomyces* yeasts), lactic acid and acetic acid bacteria, or those that have not undergone malolactic fermentation (MLF).

MLF, the enzymatic decarboxylation of L-malic acid to L-lactic acid and carbon dioxide (Davis *et al.*, 1985), is carried out by lactic acid bacteria, normally *Oenococcus oeni*, and secondarily by *Lactobacillus* spp. and *Pediociccus* spp. (Lonvaud-Funel, 1999; Cañas *et al.*, 2008a). In ideal conditions MLF follows alcoholic fermentation within a few days. MLF starts when the population of lactic acid bacteria reaches 10^6 CFU/mL after a fast growth rate which may begin sooner or later after the end of alcoholic fermentation. The Lag phase between the two fermentations essentially depends on temperature, pH, and ethanol content, the main factors determining bacterial growth in wine (Lonvaud-Funel, 1999), as well as the winemaking technology (Moreno-Arribas *et al.*, 2008). During red wine production, the MLF is a desirable phenomenon since it contributes to the desacidification of wine, enhanced microbiological stability, and also promotes multiple transformations on the chemical characteristics and the sensory properties of wines. After MLF the wines become enriched in several volatile and non-volatile compounds - alcohols, acids, esters, aldehydes and ketones, norisoprenoids, terpenes, volatile phenols, phenolic aldehydes and phenolic acids – that contribute to improve their aromatic complexity, colour stability, mouthfeel and give a long after-taste (Étievant *et al.*, 1989; Lonvaud-Funel, 1999; Liu, 2002; Ugliano and Moio, 2005; Alcaide-Hidalgo *et al.*, 2007; Cabrita *et al.*, 2008; Cañas *et al.*, 2008a,b; Moreno-Arribas *et al.*, 2008). Since the generation time of bacteria in some wines is long, even using inoculation with commercial preparatives of lactic acid bacteria, the multiplication becomes evident most often during bottling (Lonvaud-Funel, 1999). In this case, the volatile acidity may increase at an unacceptable level, and the wine is gaseous and cloudy. Their economic impact is significant. Most often *Lactobacillus Hilgardii* and *Lactobacillus fructivorans* are involved. These species are normally destroyed during wine production. However, some strains demonstrate abnormal tolerance to the medium, especially to the ethanol concentration (Lonvaud-Funel, 1999). The importance of the problem may be roughly related to the vintage which determines the wine composition, including notably pH, ethanol, phenolic compounds and the diversity of the initial lactic acid bacteria population (Lonvaud-Funel, 1999; Cabrita *et al.*, 2008; Moreno-Arribas *et al.*, 2008).

Therefore, when MLF does not occur during the winemaking process it should be suppressed before bottling by the elimination of lactic acid bacteria from wine, which can be achieved by a sterilising filtration.

The objective of this work was to study the effects of the sterilising filtration on the microbiological stability, chemical composition (total phenolic index and chromatic characteristics) and sensory properties (colour intensity, aroma quality, flavour quality and overall quality) of a red wine without MLF and that needed to be bottled. All of the analyses were performed only one month and two months after the filtration because practical observations show that a certain period of time is needed to red wine restored its balance (Luis Coimbra, personal communication).

MATERIALS AND METHODS

Wine

A sample of red wine (25 L) produced at INIA-Dois Portos winery in 2009 harvest and stored during 18 months in a stainless steel tank was used to perform the essay. The wine had the following analytical characteristics: ethanol content – 14.7 % v/v; total acidity – 7.0 g/L tartaric acid; volatile acidity – 0.58 g/L acetic acid; pH – 3.34; reducing sugars – 2.2 g/L; total SO_2 – 72 mg/L; free SO_2 – 10 mg/L; MFL – negative.

Filtration essay/Experimental design

The filtration process was performed in a pilot-scale system, designed by Multifiltra (Cacém, Portugal). The system consists of a set of Capsules in series. Four types of DEMICAP Capsules (Parker - Domnick Hunter) were used: a 5 μm of fiberglass Capsule for prefiltration followed by 1 μm (fiberglass), 0.6 μm (fiberglass) and 0.45 μm (polyethersulfone), as shown in Figure 1. The differential pressure for each filter was less than 10 mbar.

For the non-filtered wine (control group) and the wines filtered by each capsule of 1 μm, 0.6 μm and 0.45 μm, sampling was made in triplicate and thus total samples were twelve.

Chemical and sensory analyses were carried out one month (T1) and two months (T2) after the filtration essay, during which the wines were kept in bottles. The counting of microorganisms by culture was performed after one month of the filtration essay.

All of the wines (non-filtered and filtered) were centrifuged at 10,000 rpm for 10 min at 5 ºC before the chemical analysis.

Counting of microorganisms by culture

It were followed the procedures recommended by the "Compendium of International Methods of Analysis of Wine and Musts" (OIV, 2008). All of the procedures were made in aseptic conditions using a laminar flow chamber.

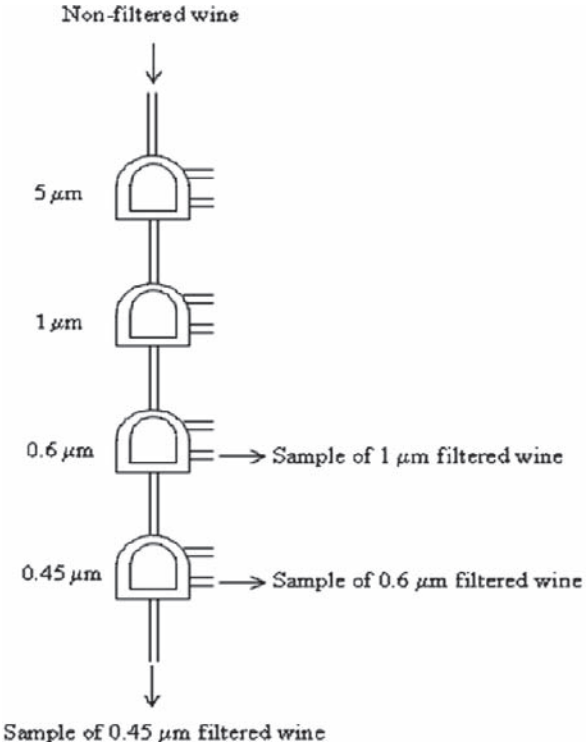

Figure 1 – Scheme of the filtration system
Esquema do sistema de filtração

Membrane filtration method was used for plate counting of yeasts, lactic acid bacteria and acetic acid bacteria.

The culture media used were YEPD for yeasts, MRS for lactic acid bacteria and Kneifel medium for acetic acid bacteria. The inhibitors added were: byphenil and chloramphenicol for yeasts; cycloheximide for acetic acid bacteria and lactic acid bacteria.

100 mL of each sampled wine were filtered through cellulose mixed esters membrane of 0.45 μm of pore size.

Colony forming units were counted after six days of incubation at 25 ºC for yeasts and acetic acid bacteria, and after ten days of incubation at 25 ºC for lactic acid bacteria.

Determination of total phenolic index

The total phenolic index (TPI) of the red wine was determined by measuring the absorbance at 280 nm (Ribéreau-Gayon, 1970). Wine was diluted with water.

Determination of chromatic characteristics

The colour parameters (CIELab) were determined with a Varian Cary 100 Bio spectrophotometer (Palo Alto, USA) and a 10-mm glass cell, by measuring the transmittance of the red wine every 10 nm from 380 to 770 nm, using a D_{65} illuminant and a 10º standard observer. The colour parameters measured were: lightness (L*); saturation (C*); chromaticity coordinates (a* and b*). Coordinate a* takes positive values for reddish hue and negative values for greenish hue, whereas coordinate b* takes positive values for yellowish hue and negative values for bluish hue.

Sensory evaluation

The sensory analysis of wines was carried out by a panel of 11 tasters, previously selected and trained.

The tasters were asked to make a raking test (Sauvageot, 1981), scoring the colour intensity, the aroma quality, the flavour quality and the overall quality of the wines, taking as reference the non-filtered wine (identified as such). In this case, the ranking test is useful to find out which is the most appreciated wine from a range, as well as the rank order of the wines under study.

The wine samples were blind tasted, in balanced orders to eliminate first-order carry-over effects (Williams, 1949), presented as 30 mL to the panel in wine tasting glasses (ISO 3591:1977), at room temperature, in a tasting room equipped with white light source. Water was provided for mouth rinsing between samples.

Statistical analysis

The one-way analysis of variance (ANOVA) was performed to evaluate the effects of filtration on the total phenolic index and chromatic characteristics.

Calculation of least significant difference (LSD) was applied for comparison of the different averages. All the calculations were carried out using Statistica vs '98 edition (Statsoft Inc., Tulsa, USA).
The statistical analysis of the data resulting from sensory analysis was based on the calculation of Friedman's coefficient. Results are expressed as average ranking score for each wine.

RESULTS AND DISCUSSION

The results of microorganisms counting by culture (Table I) show that the non-filtered wine (control) presented a very high level of microorganisms namely yeasts, and acetic acid bacteria. After sterilising filtration with different types of membranes the levels of microorganisms decreased considerably. The best results were obtained with the membranes of 0.6 μm and 0.45 μm, for which no microorganisms were detected at the sampled wines.

Concerning the chemical composition and the chromatic characteristics of the wines before and after filtration, the results of analysis of variance were shown in Table II. They reveal that a month after the sterilising filtration the red wine characteristics were still affected: total phenolic content (very significantly) and chromatic characteristics (significantly). However, after two months the effect becomes non-significant on the total phenolic content and highly significant on the chromatic characteristics. Peri *et al.* (1988) reported the opposite effect on the total phenolics of a red wine but resulting from the filtration with a polysulfone membrane of 0.2 μm. Arriagada-Carrazana *et al.* (2005), performing the filtration of a red wine with a prefiltering polypropylene cartridge of 1.2 μm and a polyvinylidene diffluoide cartridge of 0.65 μm, verified an average decrease of 10% in the total phenolic content in the filtered wine. This decrease is in accordance with the changes observed in the concentration of anthocyanins, tannins and colour intensity index (sum of absorbance at 620 nm, 520 nm and 420 nm). Similar results were obtained by Bruetschy *et al.* (1997) in a red wine filtered through a membrane of 0.65 μm.

After one month of storage the differences induced by the sterilising filtration on the phenolic composition were only found for 0.6 μm and 0.45 μm in relation to the non-filtered wine. It is important to point out that the filtration with 0.6 μm membrane induced a very significant increase on the total phenolic index, while the filtration with 0.45 μm membrane originated its decrease. Regarding the chromatic characteristics, the wine filtered with 0.1 μm membrane could not be distinguished from the non-filtered one, but the 0.6 μm and 0.45 μm membranes caused a significant decrease on lightness (increase of colour intensity), saturation and chromaticity coordinates (decrease of reddish and yellowish hues) of the red wine. These results are in agreement with those obtained by Arraigada-Carrazana *et al.* (2005) for the hue of our filtered wine with 0.6 μm membrane. Except for the lightness, the sterilising filtration exerted a negative influence on the colour of the wine after one month of storage.

After two months of storage, the filtered wine cannot be distinguished from the non-filtered wine by their phenolic content, being the wine filtered with 0.6 μm more similar to the control group. On the other hand, there was an increase on lightness (decrease on colour intensity), saturation and chromaticity coordinates (higher reddish and yellowish hues). Except for the lightness, there was a remarkable positive evolution of the chromatic characteristics of the wine, especially in the wine filtered with 0.45 μm. This suggests that two months after filtration the wine have actually reached its balance, along with the required microbiological stability.

In spite of the differences found in the chemical composition, from the sensory point of view the results obtained (Table III) show the absence of significant effects of the sterilising filtration on the colour intensity, aroma quality, flavour quality and overall

TABLE I

Plate count results obtained for the wines before and after filtration (one month).
Resultados de contagens em placa obtidos para os vinhos antes e após filtração (um mês)

Modality	Yeasts	Lactic acid bacteria	Acetic acid bacteria
Control	TNTC	Inconclusive[a]	TNTC
1 μm	1.1×10^1 CFU	3.0×10^1 CFU	4.0×10^0 ECFU [b]
0.6 μm	< 1.0 CFU	< 1.0 CFU	< 1.0 CFU
0.45 μm	< 1.0 CFU	< 1.0 CFU	< 1.0 CFU

TNTC – Too Numerous To Count/100 mL; CFU - Colony forming units/100 mL; ECFU – Estimated CFU/100 mL; [a] Development of yeast's colonies hampered the counting of lactic acid bacteria colonies; [b] Counting on the 3rd day of incubation because a mucilaginous mass presented by the colonies hampered their counting in the 6th day of incubation; Control – Non-filtered wine; 1 μm - Filtered wine collected after the capsule of 1 μm; 0.6 μm - Filtered wine collected after the capsule of 0.6 μm; 0.45 μm - Filtered wine collected after the capsule of 0.45 μm.

quality of the wines after one month of storage. Nevertheless, our results show that the wine filtered with 0.6 μm membrane presented the highest scores, except for colour intensity. Peri *et al*. (1988) verified unfavourable effects in red wine sensory properties caused by the filtration with membranes of 0.2 μm since it substantially reduces the wine colour intensity due to the removal of polymeric anthocyanins. Arriagada-Carrazana *et al.* (2005) report significant sensory differences between the unfiltered and filtered samples of red wine. The main differences were found in flavour and body, with no noticeable difference in colour.

After two months of storage, there is a significant increment on the colour intensity caused by the sterilising filtration, especially in the wine filtered with 0.6 μm membrane, which is in agreement with the effects observed in the chromatic characteristics. Although there is no significant difference among wines in the other sensory properties, there is a preference, corresponding to high quality, for the wine

TABLE II

Characteristics of the wines before and after filtration (one month and two months)

Características dos vinhos antes e após filtração (um mês e dois meses)

Modality	TPI	L* (%)	C*	a*	b*
			T1		
Effect	**	*	*	*	*
Control	54.92 ± 0.73 *b*	7.17 ± 0.21 *b*	38.72 ± 0.51 *b*	36.70 ± 0.42 *b*	12.35 ± 0.36 *b*
1 μm	55.25 ± 0.18 *bc*	7.19 ± 0.00 *b*	38.77 ± 0.00 *b*	36.74 ± 0.00 *b*	12.39 ± 0.00 *b*
0.6 μm	55.60 ± 0.05 *c*	6.80 ± 0.00 *a*	37.81 ± 0.01 *a*	35.95 ± 0.01 *a*	11.73 ± 0.00 *a*
0.45 μm	54.24 ± 0.19 *a*	6.70 ± 0.00 *a*	37.53 ± 0.01 *a*	35.71 ± 0.01 *a*	11.55 ± 0.00 *a*
			T2		
Effect	*ns*	***	***	***	***
Control	55.59 ± 0.90	6.18 ± 0.04 *a*	35.93 ± 0.11 *a*	34.32 ± 0.10 *a*	10.65 ± 0.07 *a*
1 μm	54.66 ± 1.14	7.35 ± 0.02 *b*	39.14 ± 0.05 *b*	37.04 ± 0.04 *b*	12.66 ± 0.04 *b*
0.6 μm	55.04 ± 1.40	7.44 ± 0.03 *b*	39.38 ± 0.07 *b*	37.23 ± 0.06 *b*	12.83 ± 0.05 *b*
0.45 μm	54.06 ± 1.43	7.77 ± 0.07 *c*	40.08 ± 0.15 *c*	37.78 ± 0.12 *c*	13.40 ± 0.12 *c*

Mean value ± standard deviation (three replications); [a,b,c] Mean values in the same column with different letters are significantly different at 0.05* or 0.01** level of significance; TPI – Total phenolic index; L* – Lightness; C* - Saturation; a*, b* - Chromaticity coordinates; Control – Non-filtered wine; 1 μm - Filtered wine collected after the capsule of 1 μm; 0.6 μm - Filtered wine collected after the capsule of 0.6 μm; 0.45 μm - Filtered wine collected after the capsule of 0.45 μm; T1 – After one month of storage; T2 – After two months of storage.

TABLE III

Mean values and results of the sensory analysis of wines before and after filtration (one month and two months)

Valores médios e resultados da análise sensorial dos vinhos dois meses após a filtração (um mês e dois meses)

Modality	Colour intensity		Aroma quality		Flavour quality		Overall quality	
	T1	T2	T1	T2	T1	T2	T1	T2
Effect	*ns*	**	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Control	3.0	1.6	2.9	2.5	2.6	2.4	2.6	2.3
1 μm	3.2	3.7	2.9	3.6	3.4	3.6	3.2	3.4
0.6 μm	2.9	3.8	3.4	3.1	3.7	3.3	3.4	3.3
0.45 μm	3.0	2.4	2.8	3.0	2.6	3.1	2.8	3.2

Ranking test: minimum = 1, maximum = 3; Friedman's coef. tab (3 gl; 99,5%); calc < tab indicate not significant difference (ns); calc > tab indicate very significant difference (**); Control – Non-filtered wine; Filtered wine collected after the capsule of 1 μm; 0.6 μm - Filtered wine collected after the capsule of 0.6 μm; 0.45 μm - Filtered wine collected after the capsule of 0.45 μm; T1 – after one month of storage; T2 – After two months of storage.

filtered with 1 μm membrane, followed by the wine filtered with 0.6 μm membrane. In addition, under these experimental conditions, the wine filtered with 1 μm presents the best sensory evolution over the time. According to Bruetschy *et al.* (1997) the influence of filtration on the sensory properties of the red wine decreases over the time.

CONCLUSIONS

In the experimental conditions, the sterilising filtration applied to a red wine that has not undergone malolactic fermentation and that needed to be bottled seems to be a suitable technical procedure to ensure the microbiological stability, without negative effects on wine composition and therefore on wine quality. Furthermore, from the sensory point of view there were no significant differences between the non-filtered and the filtered wines, being those filtered with 1 μm and with 0.6 μm the most appreciated.

However, before the use of this technique in oenological conditions, it is recommended that the technician/winemaker makes a previous test work to further confirm filter function for an application specific qualification, and evaluates its effects on the chemical composition and sensory properties of the wine. The type of filters used in the essay should be the same that to be used in the oenological practices. Indeed, measurements of flux and throughput per unit of membrane area can be used for initial estimates of cartridge requirements for the industrial filtration process. Small scale devices should contain a minimum of membrane area to conserve valuable bioprocess fluid and scale linearly with their corresponding large scale devices. There are a number of factors that can confound scaling predictions, if they are not carefully measured and controlled. These factors include differences in flow geometries between small and large scale devices, pressure losses associated with plumbing and elevation, variability in fluid properties, and variability in membrane properties (Giglia *et al.*, 2010).

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