

Tangential-flow membrane clarification of Malvar (*Vitis vinifera* L.) wine: incidence on chemical composition and sensorial expression

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ABSTRACT

Malvar white wine (*Vitis vinifera* L.) was cold settled (CSW) and clarified by tangential-flow membrane filtration (TFMF). A 500 kDa molecular mass cut-off membrane was used. Filtration flux of 49-48 L/hm² was achieved at transmembrane pressure of 0.7 bar. The treatment produced a completely clarified wine with turbidity of 0.11 NTU, but also a 10.3 % loss of proteins, which could be related to the decrease of some flavour compounds. The CSW and the membrane filtered wines (MFW) were assessed by means of their aroma and phenolic composition, as well as their sensory properties. The results showed that the general physicochemical parameters and most of the analysed phenolic compounds were not or slightly (up to 7.6 %) affected by the TFMF process. Nevertheless, the treatment produced an important loss of some key aroma compounds: up to 43 % of fatty acid and alcohol esters and up to 26 % of higher alcohols. Most affected were aroma species with higher molecular masses and lower polarities. Sensory analysis confirmed the global decrease in wine aroma. TFMF treatment produced also an increase of 52 % of the wine benzaldehyde content.

KEYWORDS

Malvar wine, clarification, tangential-flow filtration, volatile composition, phenol composition, chromatography

ABBREVIATIONS

BSA: bovine serum albumin, CSW: cold settled wine, Δ NTU: net turbidity after heat test, HPLC: high performance liquid chromatography, log P: octanol-water partition coefficient, L_p : membrane hydraulic permeability, MFW: membrane filtered wine, NTU: nephelometric turbidity units, OAV: odour activity value, Q_f : feed flow, OT: odour threshold, PS: polysulfone, P_{TM} : transmembrane pressure, PVDF: polyvinylidene fluoride, TFMF: tangential flow membrane filtration, TPI: total phenol index

INTRODUCTION

Clarification is an essential unit operation of winemaking. It improves not only appearance of wines, but also is very important for their physicochemical and microbial stability. This statement is especially heightened for white wines, as they have to be clear, with turbidity lower than 1 NTU (Ribéreau-Gayon *et al.*, 2006a). Post-fermentation clarification is usually carried out at various stages, which can include natural or forced settling, fining and/or filtration. As natural settling can extend too long in time, forced settling (cold or centrifugal) is preferred for industrial production. Alternatively, fining with bentonites, proteins (egg whites, gelatine, caseinates, etc.), silica gel or tannins contributes also to speed up the settling treatment (Ribéreau-Gayon *et al.*, 2006b; Lira *et al.*, 2015). In most of the cases, clarification ends up with a stage of filtration. The classic treatment includes a combined filtration with cellulose-based filter plates or diatomaceous earths and fine polishing and microbial stabilization of the wine with 0.45 to 1.2 µm pore sizes pleated membrane filters (Hidalgo Togores, 2002). In all cases, these filter aids are useful until they clog, which means that their use generates wastes and subsequently, environmental problems.

An alternative to these treatments with a growing acceptance in the winemaking industry is the tangential-flow (TFMF) or cross-flow membrane filtration. In this case, the generated turbulent wine feed flow drags insoluble particles from the membrane surface and returns them back to the feed vessel, avoiding their deposition on the membrane surface and/or pores. In the practice, some fine particles and/or colloidal material build up a fine gel layer on the membrane surface (concentration polarization) (Baker, 2004) that produces a subsequent decrease of filtration flux and membrane fouling (El Rayess *et al.*, 2011). Nevertheless, membranes employed in tangential-flow filtration are useful during long periods (months and years) thanks to the recovery of the membrane permeability after chemical cleaning (Prodanov *et al.*, 2013). Nowadays, membrane manufacturers offer a wide range of membrane materials (organic polymer and mineral sintered layers), designs (spiral wound, hollow fibre or capillary, multichannel, etc.) and pore sizes, which allow many options of choice for any concrete application. However, this diversity

also produces some confusion of the users because of the specificity of each application and the difficulties to predict their effect. In most cases, for clarification of white wines, producers offer hydrophilic membrane materials (cellulose esters, polyvinylidene fluoride (PVDF), polysulfone (PS), polyethersulfone, etc.), compact membrane designs (hollow fibre or spiral wound) and membranes with pore sizes between 0.2 and 5 µm (El Rayess *et al.*, 2011).

For white wine clarification, some ultrafiltration membranes are also available. In general, membranes with smaller pore sizes (0.1 to 0.22 µm) give better clarification effects (turbidities < 0.5 NTU) and more stable filtration fluxes, as the sizes of their pores are considerably smaller than the sizes of the most abundant particles in wines and grape-derived products (Serrano *et al.*, 1992; Ribéreau-Gayon *et al.*, 2006a; Prodanov *et al.*, 2013). The problem of such fine clarification is that an important part of the colloidal matter can be removed also (Ulbricht *et al.*, 2009; El Rayess *et al.*, 2011; Oberholster *et al.*, 2013), leading to decrease of wine flavour intensity. It is noteworthy to mention that most bibliographic studies dealing with CFMF consider only sensory or general physicochemical evaluation of the clarification (Feuillat *et al.*, 1987; Flores *et al.*, 1991; Serrano *et al.*, 1992; Buffon *et al.*, 2014) or problems related to membrane stability: fouling, foulants or membrane materials (Vernhet *et al.*, 1999; Ribéreau-Gayon *et al.*, 2006a). Studies related to the effect of TFMF on wine flavour component concentrations and the intensity of their perception are scarce and in the cases where they were available, data about the TFMF process were not available (Serrano *et al.*, 1984; Flores *et al.*, 1991; Serrano and Paetzold, 1994). That is why the main aim of this study was to evaluate the junction of process parameters, aroma and phenolic composition and sensory evaluation of a young white wine before and after fine clarification with one of the membranes with tightest pores available on the market, the 500 kDa molecular mass cut-off Romicon membrane.

Another objective of this study was the chemical characterization of a wine, proceeding from the little known cv. Malvar (*Vitis vinifera* L.) grapes (VIVC 7254). This is a white variety, which produces fruity wines (Santos *et al.*, 2004). Its cultivation is admitted only into the denominations of origin 'Vinos de Madrid' and 'La Mancha'. Due to the scarce information

about this variety in the literature, its winemaking qualities and visual similarities to Airén (VIVC 157), the most widely grown variety of the same region, it was almost unknown before the last two decades (Huerta *et al.*, 1998; Santos *et al.*, 2004). Now, this variety is genetically characterised and recognized as autochthone (Ibáñez *et al.*, 2003; Cabello *et al.*, 2012). In this sense, the present study is the first most consistent effort for chemical characterisation of an authentic Malvar wine.

MATERIALS AND METHODS

1. Grapes

An amount of 1424 kg of technologically ripe grapes (22.3 % of total soluble substances) from *Vitis vinifera* L., cv. Malvar was harvested at the experimental vineyard of the Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), farm 'El Encín', Alcalá de Henares (Madrid), Spain. Grapes were handpicked and placed in rigid stackable plastic boxes in amounts of 18 to 20 kg/box to prevent grape smashing. After harvesting, they were stored overnight in a cooling chamber at 5 °C for maintaining their quality until crushing.

2. Chemicals and reagents

SO₂ and analytical grade N₂ (99.9999 %) were purchased from Carbueros metálicos (Cornellà de Llobregat, Spain), tartaric acid with 99.5 % purity was from TIDSA (Fuentidueña de Tajo, Spain) and NaOH and NaClO₃ were from Scharlab (Barcelona, Spain).

Demineralized water with electrical conductivity of 7 to 8 µS/cm was obtained by a reverse osmosis unit from Tesacua S.L. (Madrid, Spain). MilliQ-grade water was produced in a MilliQ® Integral 3 purification system (Merck Millipore, Billerica, USA).

Fermichamp trade marc *Saccharomyces cerevisiae* yeasts were acquired from Oenobrand (Montpellier, France).

3. Winemaking

The 1424 kg of grapes were crushed and destemmed in a model NDC18 crusher (Della Toffola, Treviso, Italy). The obtained grape mash was squeezed in a pilot pneumatic press (Eno Mundi, Zaragoza, Spain) for 3 h and SO₂ was added to it to obtain 80 mg/L of total SO₂. An amount of 250 kg of grape pomace and 1050 L

of grape must were obtained. The grape must (with a density ($\rho_{20^{\circ}\text{C}}$) of 1.095 g/mL, total acidity of 4.21 g of tartaric acid/L and pH of 3.84) was further refrigerated to 4 °C and kept at this temperature for overnight for settling coarse solids. About 750 L of the clear part of the must were transferred to a 1000 L stainless steel vessel and 1.5 g/L of tartaric acid were added to them to increase acidity. Next, 150 g of *Saccharomyces cerevisiae* yeasts (Fermichamp from Gist-brocades, France) were activated in 1 L of diluted with water must and inoculated in the must to initiate fermentation. Fermentation was carried out at 16-18 °C until the total reducing sugar content decreased to 1.6 g/L (i.e. 29 days). Then the clear part of the wine was transferred (first racking) to a variable volume tank with floating lid and was left to settle for another 26 days. Subsequently, the clear part of the wine was transferred to another vessel (second racking) and was left to settle at 4 °C. Two additional rackings were done at the 65th and the 80th days of winemaking and after that, the clarified wine was stored at 12 to 16 °C for 5 months.

4. Tangential-flow membrane filtration

Wine clarification was carried out by a low pressure laboratory TFMF unit, provided with a 100 L feed tank, variable flow peristaltic pump (Masterflex I/P, model 7591-07, Cole-Parmer Instrument Co., Vernon Hills, Illinois, USA), inlet pressure gauge (0 to 5 bar), 1" external diameter single cartridge of hollow fibre ultrafiltration membranes (Romicon™, Koch Membrane Systems, Wilmington, MA, USA), outlet pressure gauge (0 to 5 bar), needle pressure control valve and a 100 L recipient vessel. Membranes were from hydrophilic polysulfone, 500 kDa nominal molecular mass cut off, 0.09 m² of total filtration surface, 1.09 mm inner fibre diameter and 0.45 m of fibre length. Completely new membranes were used in this treatment.

Before starting filtration, the membrane cartridge was conditioned by consecutive washings with demineralised water at 20, 50 and 20 °C. An amount of 100 L of the cold settled Malvar wine (CSW) was submitted to fine clarification by the already described TFMF unit. Filtration was carried out by the batch concentration mode, recycling the retentate in the feed tank and collecting the filtrate into separate vessel. The feed flow (Q_f) was set at 660 L/h (linear velocity

of 2.9 m/s), transmembrane pressure (P_{TM}) of 0.7 bar and temperature of 14 to 20 °C (no external refrigeration). Filtration was stopped when 55 L of clarified wine were processed, that corresponds to 17 h of filtration.

5. Wine sampling

The clarified (membrane filtered) wine (MFW) and 10 L of the unfiltered CSW were bottled two days after filtration and stored at the IMIDRA's wine cellar at 11-15 °C until their instrumental and sensorial analyses. All analyses were completed in the next three months.

6. Membrane chemical cleaning

After filtration, membranes were washed subsequently with water at 20 °C for 30 min, and at 50 °C for 30 min. Regeneration was carried out with NaOH solution (pH 11) at 50 °C for 1 h, NaOH solution (pH 11) + 0.1 % sodium hypochlorite (NaClO) at 50 °C for 30 min. NaOH was removed by displacement with water at 20 °C until pH became neutral.

7. Membrane hydraulic permeability determination

Membrane hydraulic permeability (L_p) was determined before (L_p^0) and during filtration (L_p^F), as well as after chemical cleaning (L_p^C) by plotting the corresponding water (wine) flux values, measured at Q_f of 660 L/h and 20 °C, versus the applied T_{MP} , according to Cassano *et al.* (2011).

8. Physicochemical analysis

Hydrogen ion activity (pH) was measured directly by a FE20/EL20 pH-meter (Mettler Toledo, Spain). Total soluble substances (TSS) were determined by direct measurement of wine samples by hand-held refractometer Atago (model HSR500, Japan) in the interval of 0 to 32 % (°Brix). Turbidity (haze) was determined by a model D-112 turbidimeter in the interval of 0 to 800 NTU (Dinko Instruments, Barcelona, Spain). Titratable acidity was determined according to the method of the International Organisation of Vine and Wine (OIV) (OIV, 2015). Total SO₂ was determined iodometrically as a sum of the free and combined SO₂, according to OIV (OIV, 2009a). Total reducing sugars were determined according to the method of OIV (OIVb). Alcohol concentration was measured by aerometry, according to the method of OIV (OIV, 2009c). Total phenol index (TPI)

of the clarified wine was determined directly by measurement of the absorption of diluted (1/100) with water samples at 280 nm. TPI of non-clarified wine samples were measured after filtration of the diluted (1/100) samples through a PVDF membrane filter with a 0.45 µm pore size. Octanol-water partition coefficient (log P) was calculated by the software MOE (Chemical Computing Group ULC). Total protein content was assessed by Bradford's method using Coomassie brilliant blue reagent as described in Salazar *et al.* (2007) and was expressed as mg/L of bovine serum albumin (BSA) (Bio-Rad Laboratories Inc, Richmond, CA, USA).

Protein stability was carried out by heat test of both MFW and CSW wines, according to Lira *et al.*, (2015). In brief, 10 mL of wine samples were filtered through a regenerated cellulose membrane filter with a pore size of 0.45 µm (Whatman, England) and their turbidities were measured by turbidimetry. Samples were then heated at 80 °C for 2 h in a water bath. After incubation, they were cooled under tap water, placed in ice and left in refrigerator for overnight at 4 °C. Finally, samples were homogenized by gentle shaking and their turbidity was measured again. Wines were considered to be protein unstable when the difference between haze before and after heating (net haze) was greater than 2 NTU.

Aroma compounds were analysed by Purge&Trap-GC-MS following the methodology developed by Aznar and Arroyo (2007). The trap (Vocarb™ 3000) contained different adsorbents. Firstly, the samples were diluted with milliQ-grade water 1/20 (v/v) to avoid saturation of the trap by ethanol and possible problems due to foaming. An aliquot of 5 mL was purged with helium at flow rate of 40 mL/min during 20 min and desorbed at 250 °C during 10 min. Twenty one volatile compounds were analysed: 10 esters, 7 alcohols, 2 acids, 1 aldehyde and 1 lactone. Identification of the compounds was performed by comparison of their LRI (linear retention index) value and mass spectrum with those of pure reference compounds. Quantification was carried out by external calibration curves, for each of these compounds. Odour threshold (OT) values for the identified aroma compounds were taken from Guth, 1997, López *et al.*, 2002; Aznar *et al.*, 2003; Chavez *et al.*, 2007 and Escudero *et al.*, 2007. Odour activity values (OAV) were quantified, dividing

the concentration of each aroma compound by its OT value.

Individual phenolic compounds were determined by HPLC, according to Fernández de Simón *et al.*, 2003.

Losses for each measured parameter before and after TFMF were calculated according to the next equation:

$$\text{Loss (\%)} = 100 (C_{\text{CSW}} - C_{\text{MCW}}) / C_{\text{CSW}},$$

where C_{CSW} is the concentration of the measured parameter in the cold settled wine before TFMF and C_{MCW} is the concentration of the same parameter in the filtrate or the membrane clarified wine (MCW).

Results were reported as mean value \pm standard deviation (SD) of triplicate analysis of each sample.

9. Descriptive sensory analysis

Descriptive sensory analysis was carried out by a panel with ten trained judges from IMIDRA. This panel has been formed since 2003 by twenty five tasters after forty training sessions (seven months). Selection and formation of tasters was carried out according to the international standard ISO 8586:2012. Seventeen flavour descriptors were generated using 38 flavour compounds as described by Arroyo *et al.* (2009) and Balboa-Lagunero *et al.* (2011). All of them were presented to the panellists at threshold concentration, as well as with increasing concentrations in a control synthetic wine. Then, tasters were trained with the same compounds added to real wines from Airen grapes at different concentrations. Today, the IMIDRA's sensory panel is formed by nine permanent and five variable tasters. Taste sessions are carried out usually once to twice per week.

Sensory evaluations were realized under ISO standards related to methodology (ISO 3972:2011), sensory analysis vocabulary (ISO 5492:2008) and tasting room (ISO 8589:2007). Amounts of 20 to 30 mL of wine were served in coded standard wine tasting glasses at temperature of around 18 °C. Judges expressed their evaluation of each olfative (fruity, banana, alcoholic, off-flavour, oxidised aroma, microbial aroma and overall aroma intensity and quality) and taste (alcoholic character, acidity, fruitiness, vegetal/grass, bitter, body, salty and global taste

quality) descriptor within a scale from 1 (low intensity) to 10 (high intensity). Analysis was carried out at duplicate.

10. Statistical analysis

All analytical data were subjected to analysis of variance ANOVA (SPSS, Chicago, Illinois, USA), using the Duncan's test for the estimation of significant differences between samples with probability of 99.5 %.

RESULTS AND DISCUSSION

1. Winemaking

In general, the main physicochemical parameters of the studied Malvar wine accomplish the established for wines from denomination of origin 'Vinos de Madrid' specific characteristics (Huerta *et al.*, 1998; Gil *et al.*, 2006; Cordero-Bueso *et al.*, 2013; Cordero-Bueso *et al.*, 2016): dry wine with moderate acidity, relatively low pH and phenolic content and moderate to high fruity flavour (Table 1). However, what determines the singularity of this grape variety from technological point of view was its high susceptibility to oxidation. This phenomenon can be perceived easily, as it has a direct effect on wine colour and aroma: colour changes from yellow/greenish to green/grey and the characteristic fruity odour decreases, whereas some unpleasant oxidative flavours arise. Once started oxidation, it is difficult to stop it. That is why much care should be taken to avoid it. Generally, settling and clarification should be carried out in the shortest possible time, at as possible as low temperatures, avoiding manipulations causing aeration and concentrations of free SO₂ lower than 30 mg/L.

2. Tangential-flow membrane clarification

2.1. Effect on the main process parameters

The kinetics of the filtration flux during the membrane clarification process is shown in Figure 1.

Filtration started at flux of 52 L/hm² and finished in 17 h at 48 L/hm². This is a relatively low filtration rate, which can be attributed mostly to the low operating P_{TM} applied in this treatment. Literature data (Serrano *et al.*, 1992) show fluxes of 57 to 170 L/hm² (at P_{TM} of 0.7 to 1.3 bar) during clarification of white wines and refer to considerably higher initial flux declines of newly used membranes with losses of more

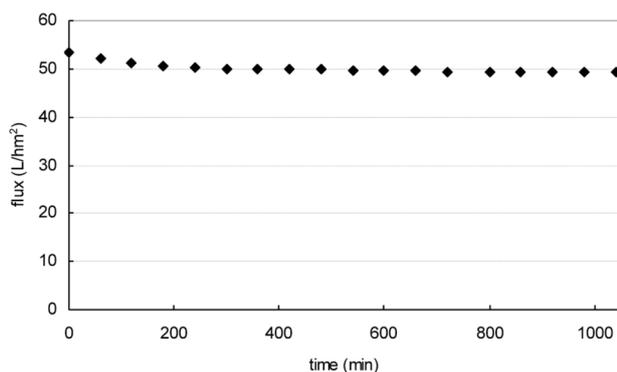


FIGURE 1. Permeate flux kinetics of Malvar wine during clarification by the 500 kDa hollow fibre membrane (Q_f 660 L/h, P_{TM} 0.7 bar, temperature 14 °C).

than 30 L/hm² in 1 h (El Rayess *et al.*, 2011). The observed very small initial flux decline of the filtered here wine can be attributed mostly to the low load of suspended solids, resulting after the exhaustive cold settling carried out before fine filtration (the cold settled wine turbidity was 24 NTU, Table 1).

Figure 2 shows that the hydraulic permeability of the studied membrane decreased down to 139 L/hm²bar during the filtration process, what means more than 75 % loss of permeability. After chemical cleaning, hydraulic permeability grew up to 516 L/hm²bar, what corresponds to a considerable loss of 20 % of the initial water flux. The chemical regeneration procedure was repeated again, but it did not improve the hydraulic permeability of the membrane, which means that this loss was irreversible.

TABLE 1. Main physicochemical parameters of the studied cold settled (CSW) and membrane filtered (MFW) wines.

Compound	CSW	MFW
pH	3.34 ± 0.02 ^a	3.31 ± 0.02 ^a
Total acidity (g/L)	5.6 ± 0.2 ^a	5.2 ± 0.1 ^b
Reducing sugars (g/L)	1.6 ± 0.4 ^a	1.6 ± 0.2 ^a
TPI	5.74 ± 0.01 ^a	5.47 ± 0.02 ^b
Ethanol (% v/v)	12.84 ± 0.03 ^a	12.81 ± 0.04 ^a
Glycerol (g/L)	7.85 ± 0.02 ^a	7.86 ± 0.04 ^a
Free SO ₂ (mg/L)	45 ± 3 ^a	42 ± 2 ^a
Total proteins (mg/L of BSA)	9.56 ± 0.15 ^a	8.58 ± 0.12 ^b
Turbidity (NTU)	24.0 ± 0.3 ^a	0.11 ± 0.07 ^b
Net turbidity after heat test (ΔNTU)	2.20 ± 0.08 ^a	0.88 ± 0.02 ^b

^bDifferent letters within the same row indicate significant differences ($p \leq 0.05$).

3. Quality of clarification

Quality of clarification should be understood as the extent of removal of the solid phase from a suspension and is related directly to the turbidity of the filtrate. Good clarification of white grape musts and wines is considered when their turbidity's are lower than 1 NTU (Ribéreau-Gayon *et al.*, 2006a). In this sense, the used here 500 kDa polysulfone membrane produced an excellent clarification of the treated wine, giving a turbidity of 0.11 NTU (Table 1).

Nevertheless, quality criteria should consider also a maximum retention of the sensory properties and the genuine chemical composition of the clarified wine. In this context and with reference to the basic physicochemical parameters of the wine, the 500 kDa membrane did not produce any significant effect on most of the measured parameters (Table 1). Only minor diminishing (up to 5.5 %) of the total wine acidity and total phenol index (TPI) were found, due most probably to the loss of some volatile acids, such as acetic and hexanoic acids (Table 2) and some hydroxycinnamic acids (*trans*-Fertaric and Caffeic), (Table 3), possibly associated to the 10.3% loss of the wine protein (colloidal) fraction.

The treatment produced also a significant drop of the heat induced turbidity (ΔNTU) (from 2.2 to 0.88 NTU), which is indicative for some haze stabilization effect (ΔNTU < 2). Nevertheless, this finding has not big relevance for the wine protein stability, as the unfiltered wine (CSW) had a ΔNTU value very close to the established limit (2.2). Indeed, the accumulated experience with

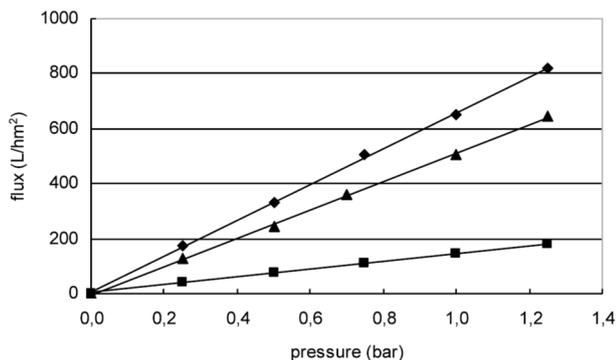


FIGURE 2. Hydraulic permeability of the 500 kDa hollow fibre membrane before (L_p^0) and during filtration (L_p^F) and after chemical cleaning (L_p^C) (Q_f 660 L/h, temperature 20 °C).

Malvar winemaking at the IMIDRA's wine cellar shows that these wines have high protein haze stability and usually do not need bentonite clarification (not published data).

Most relevant was the effect of this treatment on the sensory properties of the studied wines. Figure 3 shows the incidence of the TFMF process on the main olfactive parameters and Figure 4, on the main taste parameters.

Visual evaluation of the unfiltered Malvar wine showed that it was veiled to clear with moderately intense yellow/greenish colour (data not shown). The olfactive phase revealed a high fruity aroma with a clear predominance of banana, and a little bit lower intensities of pineapple, apples and pears. Alcohol odour also was moderately appreciable. Negative odour characteristics, such as oxidation, chemical and microbial off-flavours were found at very low levels. The taste phase showed a predominant fruity flavour with clear expression of banana aroma on the background of pineapple, apple and pear nuances. Alcohol taste was moderate and acidity, balanced. Probably, the most important particularity of this wine was the low intensity salty taste, which appeared at a late stage of the tasting, together with low intensity bitterness. These fine salty and bitter tastes are very specific for this variety and can be used, together with the high banana aroma, for its differentiation. Four of the ten tasters detected also the presence of acetic acid aroma in the wine.

On the other hand, the TFMF process produced a noticeable decrease of the global aroma quality and intensity (11.4 to 14.5 %, respectively), which was expressed mainly with loss of

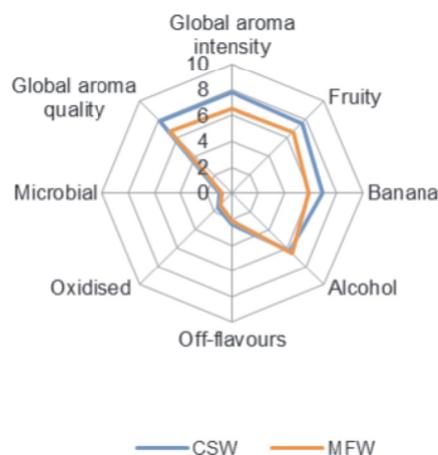


FIGURE 3. Main olfactive parameters of the studied cold settled (CSW) and membrane filtered (MFW) wines.

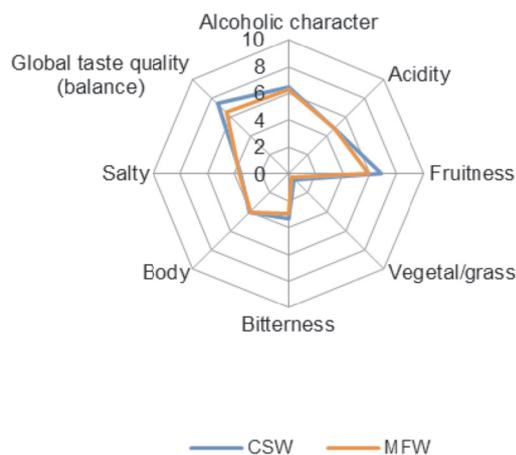


FIGURE 4. Main taste parameters of the studied cold settled (CSW) and membrane filtered (MFW) wines.

fruitiness (15.3 %) and especially, with the main fruity components: banana (13.2 %), apple and pineapple aroma (data not shown in this figure). However, the process produced also some positive effects, reducing at small extents some of the negative olfactive parameters, mainly those related to oxidation (Figure 3), vegetable/herbaceous (Figure 4) and acetic acid odours (Table 2), but they were at very low detection levels. With respect to the rest of the studied taste parameters, the filtration process decreased the global quality and fruitiness of the wine, but this effect was not so clearly defined by judges, as those corresponding to the aroma. In general, the 500 kDa polysulfone membrane produced an

TABLE 2. Aroma composition of Malvar wine before (cold settled wine, CSW) and after membrane filtration (membrane clarified wine, MCW), assessed by CG-MS, aroma compound loss (AC_{loss}) after filtration, odour threshold (OT), odour activity values of CSW and MCW (OAV_{CSW} and OAV_{MCW}), OAV loss (OAV_{loss}) after filtration, octanol-water partition coefficient of the studied molecules ($\log P$ (o/w)) and their molecular masses (MM).

Compound	Aroma description	CSW (mg/L)	MCW (mg/L)	AC_{loss} (%)	OT (mg/L)	OAV_{CSW} (-)	OAV_{MCW} (-)	OAV_{loss} (%)	$\log P$ (o/w)	MM (Da)
Ethyl butyrate	fruity, apple	0.334 ± 0.011	0.298 ± 0.016	11	0.020	17	15	11	1.44	116
Ethyl isovalerate	fruity, banana	0.003 ± 0.001	0.002 ± 0.014	16	0.003	0.92	0.77	16	1.80	130
Isoamyl acetate	banana, sweet, fruity	6.058 ± 0.511	4.060 ± 0.323	33	0.030	202	135	33	1.94	130
Ethyl hexanoate	fruity, pineapple	0.465 ± 0.045	0.302 ± 0.038	35	0.005	93	60	35	2.33	144
Hexyl acetate	sweet, fruity	0.176 ± 0.005	0.108 ± 0.007	39	0.002	88	54	39	2.47	144
Ethyl octanoate	fruity, banana, pear	0.402 ± 0.066	0.228 ± 0.056	43	2	201	114	43	3.21	172
Ethyl decanoate	grape	0.095 ± 0.034	0.060 ± 0.029	37	0.200	0.48	0.30	37	3.90	200
Ethylphenyl acetate	floral, lilac	0.403 ± 0.012	0.237 ± 0.015	41	0.250	1.6	0.95	41	2.27	164
Diethyl succinate	fruity, melon	2.207 ± 0.460	1.740 ± 0.389	21	1250	0.002	0.001	21	1.26	174
Ethyl lactate	fruity, buttery	15.2 ± 0.4	12.8 ± 0.3	9.0	150	0.1	0.09	16	0.17	118
S esters		25.3	19.8	22						
1-Propanol	soap, fatty	61.1 ± 1.8 ^a	63.6 ± 2.1 ^a	-4.1	314	0.19	0.20	-4.1	0.33	60
2-Methyl propanol	soap, fatty	14.3 ± 1.2 ^a	14.6 ± 0.1 ^a	-2.1	40	0.36	0.37	-2.1	0.91	74
1-Butanol	soap, fatty	0.766 ± 0.031 ^a	0.752 ± 0.027 ^a	1.8	160	0.005	0.005	1.8	0.84	74
Isoamyl alcohol	rancid, fusel cheese	147 ± 4 ^a	146 ± 5 ^a	0.5	30	4.89	4.87	0.5	1.22	88
1-Hexanol	vegetal, grass, bitter	1.53 ± 0.05 ^a	1.51 ± 0.04 ^a	1.1	8	0.19	0.19	1.0	1.88	102
2-Phenyl ethanol	floral, roses	25.4 ± 0.8	17.3 ± 1.0	32	14	1.81	1.23	32	1.50	122
S higher alcohols		250	244	8.9						
Acetic acid	vinegar	443 ± 11	383 ± 15	13	200	2.2	1.9	13	-0.08	60
Hexanoic acid	acid, cheese, fatty	4.63 ± 0.32	3.73 ± 0.28	19	0.420	11	8.9	19	1.72	116
S carboxylic acids		448	387	13						
Linalool	citrus, floral, fresh	0.004 ± 0.001	0.004 ± 0.001	18	0.015	0.29	0.23	18	2.13	154
γ -Butyrolactone	sweet, caramel	4.24 ± 0.08 ^a	4.35 ± 0.013 ^a	-2.6	20	0.21	0.22	-2.6	-0.64	86
Benzaldehyde	bitter, almond	0.113 ± 0.019	0.171 ± 0.025	-5.2	3000	0.04	0.06	-5.2	1.48	106

^a The same superscript in the same row means that there is no significant difference between values ($p \leq 0.05$).

appreciable reduction of the fruity flavour of the studied Malvar wine.

4. Incidence on aroma composition

Aroma compounds have key contribution to the sensory properties of wines. Twenty one volatile compounds were identified and quantified in both, the cold settled (CSW) and membrane clarified (MCW) Malvar wines by GC-MS analysis (Table 2). Among them, most diverse and important for the aroma expression of the studied wines was the group of the fatty acid and alcohol esters with a total amount of 25.3 mg/L. The calculated odour activity values (OAV) showed that Isoamyl acetate and Ethyl octanoate had the main importance to the global aroma, contributing with sweet, fruity, banana and pear aromas. These results agree with the aromatic notes found during the sensory analysis. The Ethyl hexanoate, Hexyl acetate and Ethyl butyrate had an important additional contribution to the main aroma bouquet with fresh apple, pineapple, strawberry and herbaceous connotations. The ethylphenyl acetate had a relatively low concentration, but enough to give some floral notes to the whole aroma. An important number of higher alcohols were also determined. They were found at higher amounts (250 mg/L), but had lower contribution to the global wine aroma because of their lower OAVs. Among them, only the Isoamyl alcohol and 2-Phenylethanol had olfactive significance, giving fusel and floral connotations, respectively. Other quantitatively important components, but with lower odour significance were the Hexanoic and Acetic acids. The first one is known to impart unwanted fatty aroma to wines. Its occurrence generally is related to the partial hydrolysis of Ethyl hexanoate and at higher values is indicative for the initiation of processes of oxidation. The low values determined here for Hexanoic acid validate the good state of the wine and mean that it has no contribution to the wine aroma. Acetic acid was major volatile compound with an amount of 443 mg/L. Even its presence is usually associated with acetic acid bacteria activity, amounts lower than 500 mg/L has still pleasant odour contribution to the wine aroma. Expert judges refuse wines with contents of acetic acid higher than 600 mg/L (Eder, 2006).

With respect to the membrane clarified wine (MCW), it can be seen that the TFMF produced an important loss of most of the determined aroma compounds. Most affected was the group

of fatty acid and alcohol esters, which have the highest contribution to the global wine aroma. Among them Ethyl octanoate, Ethylphenyl acetate, Hexyl acetate, Ethyl decanoate, Ethyl hexanoate, Isoamyl acetate, Diethyl succinate and Ethyl isovalerate decreased within the interval of 43 to 16 %. The aromatic alcohol 2-Phenylethanol and the terpene Linalool should be included also into this group because of their considerable loss of 32 and 18%, respectively and because of their positive aid to wine aroma. The loss of these compounds can be directly related to the decrease of the perception of fruitiness and rather banana, apple, pear and pineapple connotation of the treated wine and confirmed quantitatively the results already obtained from the sensory analysis. Contrary to this general effect, the loss of 13 and 19 %, respectively, of acetic and hexanoic acids could be considered if not positive, at least, not negative, as both compounds contribute to the wine unwanted odours. Nevertheless, it should be mentioned also, that a considerable decrease of these acids could be important for the global aroma equilibrium, because they act also, as a counterpart in the hydrolysis of the corresponding to them esters (Bertrand, 1981; Edwards *et al.* 1990). However, it can also be seen that the filtration process did not affect most of the higher aliphatic alcohols, increasing thus their contribution with soap, fatty, rancid and fusel over the weakened fruity aromas of the membrane clarified wine. It is interesting to note that these compounds have the smallest molecular masses (up to 102 Da, Table 2), which explain quite reasonably the obtained results.

5. Incidence on phenol composition

Phenolic compounds have important contribution to the sensory (acid and bitter taste), mouthfeel (astringency) and functional (redox) properties of wines. Fourteen phenolic compounds were identified and quantified in the studied here Malvar wines by reversed phase HPLC analysis (Table 3).

As it can be seen from Table 3, the total amount of individual phenolic compounds in the studied Malvar wine was relatively low: 46.5 mg/L (20.7 mg/L (Table 3) + 25.8 mg/L for 2-Phenyl ethanol and Ethylphenyl acetate, Table 2). This is most probably due to the proper variety and/or to the winemaking process carried out in this trial, in which only free running grape juice was used for fermentation. Most diverse was the

TABLE 3. Phenolic contents of the cold settled (CSW) and membrane clarified (MFW) Malvar wines and their loss after TFMF, assessed by HPLC.

Compound	CSW (mg/L)	MFW (mg/L)	Loss (%)
Gallic acid	0.646 ± 0.011 ^a	0.648 ± 0.015 ^a	- 0.31
<i>cis</i> -Caftaric acid	n.q.	n.q.	
Protocatechuic acid	0.398 ± 0.015 ^a	0.393 ± 0.010 ^a	1.19
<i>trans</i> -Caftaric acid	3.50 ± 0.02 ^a	3.51 ± 0.03 ^a	- 0.29
Grape reaction product	n.d.	n.d.	
Methyl gallate	0.254 ± 0.019 ^a	0.260 ± 0.022 ^a	- 2.38
p-Hydroxyphenylacetic acid	0.334 ± 0.009 ^a	0.331 ± 0.010 ^a	0.81
<i>cis</i> -Coutaric acid	n.q.	n.q.	
Tyrosol	11.4 ± 0.2 ^a	11.1 ± 0.2 ^a	2.62
<i>trans</i> -Coutaric acid	0.685 ± 0.008 ^a	0.678 ± 0.014 ^a	1.09
Catechin	0.523 ± 0.008 ^a	0.517 ± 0.009 ^a	1.34
<i>trans</i> -Fertaric acid	0.523 ± 0.006 ^a	0.483 ± 0.012 ^b	7.63
Caffeic acid	1.029 ± 0.013 ^a	0.983 ± 0.017 ^b	4.42
4-hydroxy-3,5-dimethoxybenzoic acid	0.040 ± 0.004 ^a	0.038 ± 0.003 ^a	4.34
<i>trans</i> -Resveratrol-5-glucoside	0.097 ± 0.012 ^a	0.100 ± 0.011 ^a	- 2.94
2,4-Dimethoxybenzoic acid	0.359 ± 0.007 ^a	0.353 ± 0.010 ^a	1.80
Tryptophol	0.910 ± 0.017 ^a	0.869 ± 0.014 ^b	4.55
Total quantified phenols	20.7	20.3	

The same superscript in the same row means that there is no significant difference between values ($p \leq 0.05$), n.q. – not quantifiable, n.d. – not detected.

group of tartaric esters of the hydroxycinnamic acids, *trans*-Caftaric, *trans*-Coutaric and *trans*-Fertaric acids with a total amount of 4.708 mg/L and the Caffeic acid (1.029 mg/L). Peaks of not negligible highness, corresponding to *cis*-Caftaric and *cis*-Coutaric acids were also found (chromatograms not shown), but they were not quantified because of the lack of adequate reference substances. *trans*-Caftaric acid was major hydroxycinnamic acid derivative, which is well established for *Vitis vinifera* white wines. However, the amount of all hydroxycinnamic acid derivatives was relatively low (Singleton *et al.*, 1985). The quantitative prevalence of the hydroxycinnamic acid esters over the hydroxycinnamic acids and the lack of grape reaction products were indicative for the good preservation state of the wine in spite of its relatively low amount of SO₂ (Table 1).

Major phenolic compounds in the cold settled wine were 2-Phenylethanol and Tyrosol with concentrations of 25.4 and 11.4 mg/L, respectively (Tables 2 and 3). These compounds, together with Tryptophol are present in wines as end-products of the metabolism of the amino

acids Phenylalanine, Tyrosine and Tryptophan by *Saccharomyces cerevisiae*, respectively (Hazelwood *et al.*, 2008). 2-Phenylethanol contributes to aroma with pleasant floral notes and it has been found that is a main component of Malvar wines with concentrations of up to 45.3 mg/L (Santos *et al.*, 2004; Cordero-Bueso *et al.*, 2013; Cordero-Bueso *et al.*, 2016). The presence of Tyrosol has not been described until now in Malvar wines. Concentrations of up to 22 mg/L have been determined in other white wines (Hernanz *et al.*, 2009; Darias-Martín *et al.*, 2008), which are related to increased bitterness. In the case of the studied here Malvar wine, tasters determined the bitter component as specific and pleasant. Four benzoic acids, Gallic, Protocatechuic, 4-Hydroxy-3,5-dimethoxybenzoic acid (Syringic) and 2,4-Dimethoxybenzoic were also found with a total amount of 1.446 mg/L, typical for these compounds in white wines.

With respect to the phenolic components analysed by HPLC (Table 3), the TFMF had much less negative effect than those analysed by

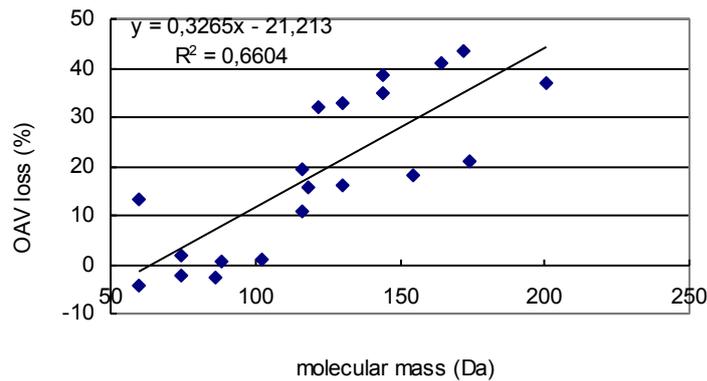


FIGURE 5. Correlation between loss of odour activity values and mass of the Malvar wine aroma compounds.

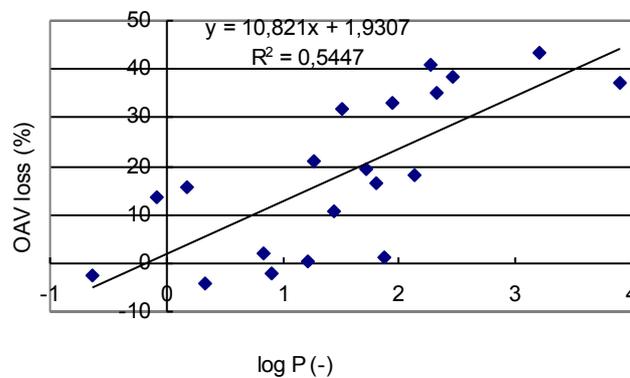


FIGURE 6. Correlation between loss of odour activity values and polarity of the Malvar wine aroma compounds.

GC. Minimal, but significant losses in the interval of 7.63 to 4.42 % were registered only for the *trans*-Fertaric and Syringic acids and Tryptophol. No correlation was found between the loss of phenols and their molecular masses or polarities.

6. Global effects

Thus, the obtained analytical results suggest that the used 500 kDa molecular mass cut-off membrane produced important losses of the most important aroma compounds of the treated Malvar wine. It is surprising that so open membrane was capable to retain so important part of the wine aroma and it is not easy to find conclusive explanation of this effect. Hereby we offer several features that can contribute to the searching for more consistent reply of this problem:

- If we take in consideration the global effect of the treatment, it becomes obvious that the most affected wine components were the volatile

compounds. Phenolic compounds (Table 1) were much less affected, if at all. This finding suggests that, at least one part of these volatile compounds can be lost during the filtration process by simple evaporation. At the concrete conditions of operation carried out in this work, the concentrate was recycled into the feed tank at open air and a high flow (600 L/h). These conditions of operation caused an intensive movement of the retentate stream during the 17 h of treatment, which can contribute to the loss of some of the volatile wine constituents. At industrial conditions of filtration, this process is carried out in closed installations and should not affect the treated wine.

- Even white wines contain relatively small macromolecular (colloidal) fraction (6 % of all wine constituents, according to Singleton *et al.*, 1988), it is known that it has high capability to bind (not covalently) small aroma molecules (Muñoz-González *et al.*, 2013), which can be retained together with the macromolecules into

the concentrate stream. The 10.3 % loss of proteins found in this study, which are main components of wine colloids, confirms this possibility. In a previous study on clarification of anthocyanin-rich grape pomace extracts (Prodanov *et al.*, 2013) it was found also, that the same 500 kDa PS membrane produced retention of 14 to 25% of the total condensed tannin content, which are main component of the colloidal fraction of this extract.

- The permeability of each component of the wine throughout the membrane depends of various factors. One of the most important is their molecular mass. Even the analysed volatiles differed between them in very small molecular mass interval (60 to 200 Da), plotting losses of odour activity values vs. masses of the Malvar wine aroma compounds (excluding Benzaldehyde) (Table 2) shows an acceptable correlation ($R^2=0.68$) (Figure 5). This finding could explain also the highest loss of some compounds with lower molecular masses in front of those with higher molecular masses.

- Other factor of great influence on the permeability of each wine component is their polarity. Plotting losses of odour activity values vs. polarities of the Malvar wine aroma compounds (excluding Benzaldehyde) (Table 2) gives lower, but still acceptable correlation ($R^2=0.54$) and could explain, at least in part, the higher loss of some less polar compounds in front of those with higher polarities (Figure 6).

7. Specific effects

However, the most surprising result (Table 3) was the 52 % increase in the Benzaldehyde content in the filtered wine. That means that the filtration process has catalysed the synthesis of this compound or it was released from any other precursor from the wine components or microbial metabolites. It is known that Benzaldehyde is produced during the alcohol fermentation and usually is present in wines at small amounts (up to 0.5 mg/L). It is responsible for bitter almond odours in wines. Delfini *et al.* (1991) found that several yeasts were capable to transform benzyl alcohol to benzaldehyde and other derivatives. Benzyl alcohol is also present in wines and musts at very low concentrations (Delfini and Formica, 2001), but it can also derive from external sources. Blaise and Brun (1986) found an important increase in the Benzaldehyde content of wines kept in tanks

coated with epoxy resin that release benzyl alcohol. It seems that this finding was one of the main reasons for the abandonment of this type of tank coating in France (Delfini, *et al.*, 1991). In other study, Lomascolo *et al.* (2001) increased 21 folds the biotransformation rate of L-phenylalanine to benzaldehyde by the use of a *Trametes suaveolens* strain and a styrene/divinylbenzene copolymer (HP20 resin) as a catalyst. All these data suggest that some polymers used actually in the food industry are chemically active in contact with some food constituents and can induce catalysis of some uncontrolled reactions. We have no any additional experimental material to give more precise explanation of this phenomenon, but the result is quite clear and unambiguous. Moreover, we have other experimental results on wine treatment by membranes with different polymer materials that affirm the appearance of the same effect (not published data). It is understandable that the final amount of benzaldehyde found in the filtered wine is into the normally accepted limits, but what is important here to highlight is that these results put in doubt the inertness of some polymers, such as polyesters used widely in the food industry, as well as in the manufacture of membrane cartridges. Further experiments are needed to make clearer the mechanisms of this phenomenon.

CONCLUSIONS

Malvar *Vitis vinifera* grapes gave a moderate to high fruity wine with intense connotations of banana, apple, pear and pineapple aromas and specific low intensity salty and bitter aftertaste. Twenty one volatile and fourteen phenolic compounds, important for the the sensory properties of the wine were identified and quantified. Isoamyl acetate and Ethyl octanoate had the main importance to the global aroma, contributing with sweet, fruity, banana and pear aromas. Ethyl hexanoate, Hexyl acetate and Ethyl butyrate contributed to the main aroma bouquet with fresh apple, pineapple, strawberry and herbaceous connotations and Ethylphenyl acetate, with some floral notes. Among the determined higher alcohols, only Isoamyl alcohol and 2-Phenylethanol had olfactive significance. 2-Phenylethanol, Tyrosol and a group of tartaric esters of the hydroxycinnamic acids, *trans*-Caftaric, *trans*-Coutaric and *trans*-Fertaric were the major phenolic compounds. These compounds are known for their high antioxidant potential. Probably due to their

presence, one of the main technological characteristics of this wine was its proneness to fast oxidation, which requires special care during clarification and storage. As far, as we are aware, this study is the first attempt for more consistent chemical characterisation of an authentic Malvar wine.

The use of a 500 kDa molecular mass cut-off PS membrane produced a completely clarified wine with turbidity of 0.11 NTU, but also a 10.3% loss of proteins, which could be related to the decrease of some flavour compounds. Quite good filtration flux of 49 to 48 L/hm² was obtained at relatively low transmembrane pressure (0.7 bar), but 20 % loss of the initial membrane water flux was achieved after only 17 h of operation. The general physicochemical wine parameters and most of the phenolic compounds were not affected significantly, or only in part, by the treatment. Nevertheless, the TFMF produced high loss of the most important aroma compounds: up to 43 % of fatty acid and alcohol esters and up to 26 % of higher alcohols, which led to a global decrease in the wine aroma. Most affected were aroma species with higher molecular masses and lower polarities. The obtained results suggest that the use of this membrane for clarification of moderate to highly aromatic wines is inappropriate. However, its good filtration skills may be used in the clarification of less aromatic or protein unstable wines, as this membrane showed also good abilities for haze stabilization. An additional possible inconvenient of the use of this membrane raised from the induced formation of benzaldehyde after contacting the wine with the membrane.

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