

Nanofiltration process as non-thermal alternative to thermovinification in Pinot noir winemaking

Nioi Claudia^{1*}, Lisanti Maria Tiziana^{2*}, Lacampagne Soizic¹ and Pascal Noilet³,
Mietton Peuchot Martine¹ and Ghidossi Rémy¹

¹Université de Bordeaux, Institut des Sciences de la Vigne et du Vin, EA 4577, Unité de recherche Œnologie, 210, chemin de Leysotte, CS 50008, F-33882 Villenave d'Ornon, France

²Università degli Studi di Napoli Federico II, Dipartimento di Agraria-Sezione di Scienze della Vigna e del Vino, Viale Italia, 83100, Avellino, Italy

³Bucher-Vaslin, Rue Gaston Bernier - BP 28, 49290 Chalonnes sur Loire, France

*corresponding author: claudia.nioi@u-bordeaux.fr; mariatiziana.lisanti@unina.it

ABSTRACT

This study aimed to evaluate the potential of membrane technology to improve polyphenol extraction during red wine alcoholic fermentation of the Pinot noir grape variety. The effect of adding permeate obtained from *saignée* nanofiltration onto grape pomace during alcoholic fermentation on wine phenolic extraction was investigated. The process consisted in the addition of nanofiltration permeate (NF) from *saignée* onto grape pomace in three different steps during alcoholic fermentation, corresponding to volumic mass of 1.060, 1.030 and 1.000 g.cm⁻³ (treatments called NF1060, NF1030 and NF1000, respectively). This innovative extraction strategy was compared with the extraction by thermovinification (TV), a conventional method used to increase anthocyanin extraction in red winemaking. All trials were performed at microvinification scale (30 kg of grapes, in duplicate). Total polyphenol index, color intensity and total anthocyanins were measured during fermentation. In the finished wines, after malolactic fermentation, tannins, HCl index, gelatin index and dialysis index were determined and a sensory evaluation was made by an expert panel. The results show that nanofiltration treatments were able to extract the same amount of total polyphenols and reach the same color intensity as thermovinification. A higher extraction of anthocyanins (around 25 %) was obtained using NF1030 and NF1000, and a higher content of tannins was found in NF wine than TV wine, with a higher degree of polymerization in NF1060 and NF1030. The perceived astringency reflected the gelatin index values, with NF1030 wine the least astringent. The wine tasters agreed on a higher sensory appreciation for NF wines, especially NF1030. The results obtained in this study are a good incentive to promote research on nanofiltration processes as a non-thermal alternative to modulating polyphenol extraction during red wine fermentation.

KEYWORDS

extraction, phenolic compounds, nanofiltration, red winemaking

INTRODUCTION

Phenols are essential compounds for the quality of red wine, as they are responsible for important sensory qualities, color (anthocyanins) and mouth-feel characteristics (as flavanols for wine bitterness and astringency) as well as for red wine longevity (Kennedy *et al.*, 2006). Although the amount and nature of grape phenolic compounds mostly depend on cultivar and cultural practices (Downey *et al.*, 2006; Kennedy, 2008; Villano *et al.*, 2017), the winemaking process has an essential role in the phenolic composition of wine (Sacchi *et al.*, 2005). Indeed, according to some studies, around half of the phenolic compounds located in berry skins and seeds are extracted and diffuse into the fermenting must during the maceration step (Boulton *et al.*, 1999; Ortega-Regules *et al.*, 2009; Salmon, 2006). The contacting method used has an effect both on the rate and extent of extraction (Boulton *et al.*, 1999). In general, anthocyanins are extracted in the earliest stages of fermentation and are later re-adsorbed to various extents on grape pomace (Nagel and Wulf, 1979; Gao *et al.*, 1997), whereas tannins are generally extracted later and continue to increase in concentration until the end of maceration (Ribéreau-Gayon 1982; Spranger *et al.*, 2004). However, the rate and the extent of phenolic extraction during maceration cannot be predicted, because it depends on several operational parameters, such as solid-liquid ratio, temperature, alcohol production rate, and cap management techniques (Esti and Tamborra, 2006; Rolle *et al.*, 2009; Setford *et al.*, 2017). Moreover, grape variety and phenolic concentration, which may differ in different vintages or during the harvesting period, could influence polyphenol extraction in the winemaking process (Jackson and Lombard, 1993).

Several winemaking techniques - such as increasing fermentation temperature (Cacace & Mazza, 2003; O'Neill *et al.*, 2011), thermovinification (Sacchi *et al.*, 2005, El Darra *et al.* 2016), flash release (Morel-Salmi *et al.*, 2006; Doco *et al.*, 2007), must or grape freezing (Sacchi *et al.*, 2005), *saignée* (juicerunoff) (Harbertson *et al.*, 2009; Lukić *et al.*, 2017), enzymatic treatment (Mojsov, 2013) have been developed over the years to enhance and/or modulate the extraction of phenolic compounds. Nevertheless, most of these processes have the major disadvantage of high energy consumption, while the use of high temperatures should be carefully evaluated as it may affect both polyphenols and volatiles, responsible

for the sensory quality of red wines, to various extents (Fischer *et al.*, 2000; Girard *et al.*, 2001; FGeffroy *et al.*, 2018).

Recently, innovative non-thermal techniques, such as ultrasound-assisted extraction, pulsed-electric fields, microwaves, and high hydrostatic pressure, have been investigated to facilitate the degradation of grape skin cells and thus accelerating the release of phenolic compounds into the must (Delsart *et al.*, 2012; Delsart *et al.*, 2014; Tao *et al.*, 2014; Ferraretto and Celotti, 2016; El Darra *et al.*, 2016, Casassa *et al.*, 2019). Moreover, these techniques also find an interesting application for the extraction of bioactive molecules from by-products of the wine industry, especially polyphenols with antioxidant activity (Corrales *et al.*, 2008). A secondary useful effect of these applications is a decrease in the microbial population of grapes or must (Morata *et al.*, 2014; Morata *et al.*, 2015; Lisanti *et al.*, 2019).

To the best of our knowledge, among innovative physical techniques, the membrane technologies have never been applied to enhance grape polyphenols extraction in red winemaking.

A membrane separation process is a filtration technique based on the permeation of solute molecules through an inorganic or organic semi permeable membrane. In recent years, membrane processes have been successfully employed in winemaking with several advantages, such as low operating costs, saving time and energy (low temperatures, reduced wine loss, reduced wastes, automated process), and mild effects on phenolics, sensory active volatiles and organoleptic properties (Bánvölgyi *et al.*, 2006; Massot *et al.*, 2009; El Rayess and Mietton-Peuchot, 2016). At present, one of the most applied membrane process in wine industry is cross-flow microfiltration for wine microbiological stabilization (Massot *et al.*, 2009; El Rayess and Mietton-Peuchot, 2016). Other techniques, such as nanofiltration, reverse osmosis, membrane contactor or electrodialysis have many applications in winemaking, such as must concentration, must sugar reduction, wine partial dealcoholization, decrease in ethylphenol, acidification and deacidification of must and wine, and tartaric stabilization (Massot *et al.*, 2009; Lisanti *et al.*, 2013; El Rayess and Mietton-Peuchot, 2016).

In this study, we investigated the potentialities of nanofiltration technique as a non-thermal process to enhance polyphenol extraction during red winemaking. In particular, we were interested in

the vinification of Pinot noir; this grape cultivar that has a low anthocyanin content compared to other red cultivars and no acylated anthocyanins (Mazza *et al.*, 1999) so extraction and color stability are a challenge in the winemaking process and must be carefully managed (Parley *et al.*, 2001; Sacchi *et al.* 2005). The innovative process consisted in the application of nanofiltration to a fraction of the must (35 % of the initial volume, called *saignée*), separated by racking after destemming-crushing and tank filling. The obtained concentrate (the fraction not passing through the nanofiltration membrane), rich in phenolic compounds, was immediately reintroduced into the fermentation tank. The permeate (the fraction passing through the membrane), poor in phenolic compounds, was stored and later added to grape pomace in three different fermentation steps, corresponding to volumic mass of 1.060, 1.030 and 1.000 g.cm⁻³. This operation had two aims:

- i) increasing the phenolic concentration gradient between grape pomace and fermenting must in order to enhance the extraction according to Fick's diffusion law, where diffusion rate depends on solute concentration gradient (Setford *et al.* 2017);
- ii) favoring anthocyanin extraction in aqueous phase by diluting the fermenting must with the permeate (Ribéreau-Gayon *et al.*, 2006). The wines obtained at laboratory-scale by the NF process were compared with the wine obtained from the same grapes treated by thermovinification.

MATERIALS AND METHODS

1. Microvinifications

The wines were obtained by microvinification of Pinot noir grapes (Camas vineyards, Bordeaux, France). Two processes aimed to enhance polyphenol extraction were applied: nanofiltration of a fraction of must (NF) and the conventional thermovinification (TV). The microvinifications took place in our experimental cellar (I.S.V.V., Bordeaux, France) and were carried out in 25 L stainless steel tanks that followed the height (50 cm) to diameter (25 cm) ratio of standard industrial tanks. The grapes (30 kg for each microvinification) were constituted by randomly collecting bunches from a unique mass. Grapes were destemmed-crushed and sulphited (5 g.hL⁻¹). Table 1 shows the base chemical parameters of the obtained musts (sugars, total acidity, pH), determined just after crushing. Musts were transferred into stainless steel tanks to undergo alcoholic fermentation.

At this point, NF musts underwent the nanofiltration treatment (section 2.2), while TV musts were treated as described in Section 2.3. All musts were then inoculated with selected *Saccharomyces cerevisiae* yeast F33 (Laffort Oenologie S.A., France) at 20 g.hL⁻¹ and fermentations were carried out at controlled temperature (25-28 °C).

During alcoholic fermentation, volumic mass, temperature, pH, total and volatile acidity were monitored. Polyphenol extraction was monitored by the analysis of total polyphenol index, color index and total anthocyanins. All microvinifications were conducted in duplicate. Finished wines were racked off after 12 days, when all the trials reached a volumic mass of 0.995 g.cm⁻³ and reducing sugars were lower than 2 g.L⁻¹. Twenty mg.L⁻¹ of sulfur dioxide were added, stored at 15 °C in the tanks and then bottled at the end of the malolactic fermentation. The wines were not filtered and no fining agents were used. Tannins, HCl index and dialysis index were determined in the finished wines. Moreover, the wines were tasted by a panel of experts to evaluate the effect of the treatment on the sensory characteristics.

2. Nanofiltration treatments

Nanofiltration treatments were conducted in cross-flow mode filtration apparatus (Sepa CF II Membrane Cell System, GE Osmonics, Minnetonka, MN, USA). A polyamide membrane with a molecular weight cut-off range of 200-400 Da was used (NF, Alfa Laval, Saint Priest, France). A defined volume (9 L) of grape must (35 % of the initial volume, called *saignée*) was racked from the fermentation tanks soon after filling the tank. The turbidity of the *saignée* musts was between 100 NTU and 200 NTU for the different tanks. *Saignée* musts were filtered by the NF apparatus and the retentates were immediately reintroduced into the tank of origin. The permeates were stored at 10°C and totally reintroduced onto the grape pomace into the tank of origin in different steps of the alcoholic fermentation, when the volumic mass reached 1.060 g.cm⁻³ (sample NF 1060), 1.030 g.cm⁻³ (sample NF 1030) and 1.000 g.cm⁻³ (sample NF 1000) (Figure 1). The volumic mass was measured during alcoholic fermentation at a fixed time every day with a densimeter (DMA™ 35, Anton-Paar, Austria). During fermentation, a single punch down per day was performed by a stainless steel manual plunger, except on the days when the permeate was added, to maintain the gradient of concentration of phenolic compounds between grape pomace and permeate.

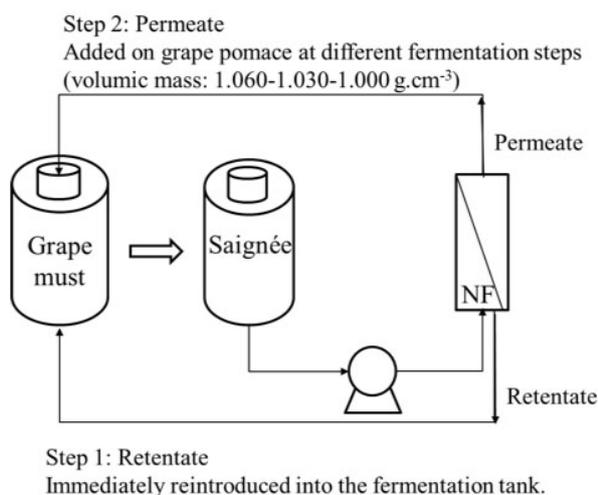


FIGURE 1. Process scheme of nanofiltration (NF) operational mode.

A part of the juice was removed before fermentation (saignée) and filtered by NF apparatus: the retentate was immediately reintroduced into the fermentation tank, while the permeate was added later to the grape pomace, at different steps of alcoholic fermentation in each sample (NF1060, NF1030, NF1000).

3. Thermovinification (TV)

Destemmed-crushed Pinot noir grapes were placed in 25 L stainless steel tanks and maintained at 80 °C for 30 min, by a water-bath system. The temperature was monitored by a Teflon-coated thermocouple (Thermocoax, Suresnes, France) inserted in the center of the tank. The rise in temperature of the grapes from room temperature (22 °C) up to 80 °C took 35 minutes. The grapes were then pressed by a hydraulic basket press and vinified in the liquid phase (see Section 2.1).

4. Physico-chemical analyses and spectrophotometric measurement

Wine standard physico-chemical parameters (sugar content, volumic mass, pH, total and volatile acidity) were determined according to the OIV official methods (OIV., 2015). Color intensity (CI) was calculated as the sum of absorbance at 420, 520 and 620 nm, determined according to Glories' method (Glories, 1984).

Total polyphenol index (TPI) was measured as absorbance at 280 nm (Glories, 1984). Tannins were determined according to Ribéreau-Gayon and Stonestreet (1966) and total anthocyanin content was calculated from the absorbance at 520 nm, at pH less than 1 (Ribéreau-Gayon and Stonestreet, 1965). Gelatin index (index of reactivity of phenolic molecules in wine toward gelatin), dialysis index and HCl index (index of polymerization of procyanidins) were obtained using the methods described by Ribéreau-Gayon (Ribéreau-Gayon *et al.*, 2006). These indexes are related to the reactivity and the polymerization state of tannins in wine. For all spectrophotometric measurements, a UV-Vis Spectrophotometer V-550/FP 750 (Jasco Corporation, Tokyo, Japan) was used.

All the analyses described in this paragraph were performed in duplicate on the two replicates of vinification (n=4) and the results are expressed as mean ± standard deviation. The existence of significant differences between each NF treatment and TV was estimated by calculating the 95 % confidence intervals (CI) of the difference between means. Following this approach, if the 95 % CI of the difference is 0, then there is no significant difference between the samples. If it is not 0, then there is a statistically significant difference between the samples (Knezevic, 2008).

5. Evaluation of sensory quality

Sensory analyses were conducted to estimate the sensory characteristics of TV and NF wines. Sensory analysis took place in a standard tasting room with individual booths air conditioned at 20 °C (ISO 8589:2007). Normalized glasses were used (ISO 3591:1977).

The wines were presented to a panel of eight expert wine tasters from the University of Bordeaux (I.S.V.V., France). They were asked to rate different sensory characteristics: acidity, astringency, bitterness and global aroma intensity on a five-point scale (from 1 = very weak to 5 = high intensity). The two replicates of microvinification were mixed and analyzed in duplicate.

TABLE 1. Base chemical parameters of the musts used for the different trials.

Must analysis	NF1060	NF 1030	NF1000	TV
Sugars (g.L ⁻¹)	245 ± 4	250 ± 5	250 ± 0.5	248 ± 1.2
Total acidity (g.L ⁻¹ of tartaric acid)	6.7 ± 0.3	6.6 ± 0.2	6.6 ± 0.3	6.9 ± 0.1
pH	3.3 ± 0.1	3.3 ± 0.1	3.4 ± 0.1	3.4 ± 0.1

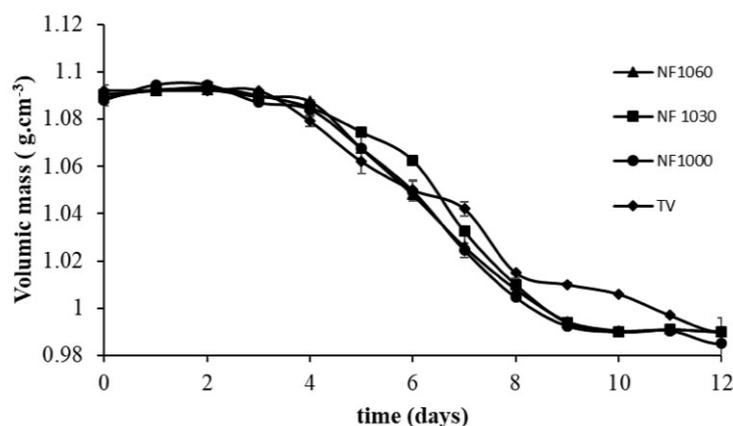


FIGURE 2. Fermentation kinetics. Must volumic mass of trials treated by nanofiltration (addition of permeate of saignée nanofiltrate NF at 1.060, 1.030 and 1.000 g.cm⁻³ of volumic mass) and by thermovinification (TV). Values are the mean ± standard deviations of duplicate analyses on each duplicate of microvinification (n = 4).

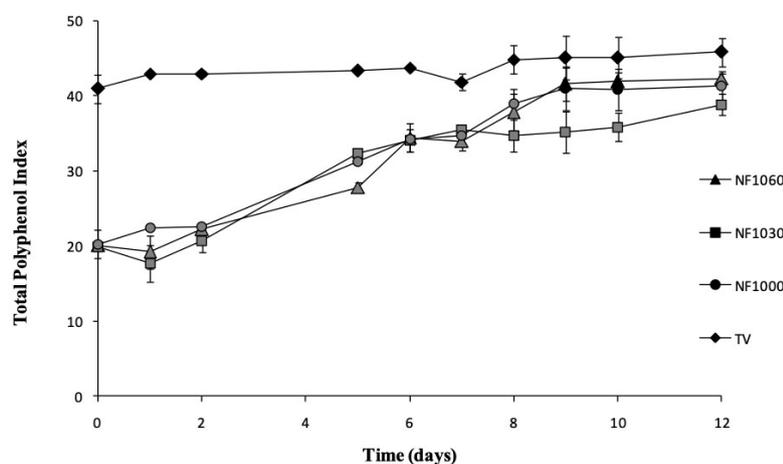


FIGURE 3. Total polyphenol index during alcoholic fermentation for the different treatments. NF 1060, 1060, 1000 = permeate from NF added at volumic mass of 1.060, 1.030, 1.000 g.cm⁻³, respectively. TV = thermovinification treatment. Values are the mean ± standard deviations of duplicate analyses on each duplicate of microvinification (n=4). The gray marker indicates that the mean value significantly differs from the corresponding value of TV (significance level 95 %).

RESULTS AND DISCUSSION

1. Fermentation kinetics

Table 1 shows the base chemical parameters determined on the musts used for the microvinifications. Each reported value corresponds to the mean ± standard deviation of the analyses made in duplicate on the two replicates of vinification (total number of analyses for each trial = 4).

Regarding sugars, total acidity and pH, the results show a good homogeneity among the grapes.

Volumic mass measurements during fermentation provide information concerning the progress of alcoholic fermentation (Figure 2). The kinetics showed a classical progress of alcoholic

fermentation and no fermentation stop was observed. The kinetics, until the eighth day, follow the same trend for all the trials. All the NF treatments reached the end of fermentation by the ninth day, whereas the TV trial had a slower fermentation rate in the last phases.

2. Effects of the treatments on the extraction kinetics of total polyphenols, total anthocyanins and color intensity

To evaluate the effect of the different treatments on the extraction of phenolic compounds, total polyphenol index, total anthocyanin concentration and color intensity were measured during fermentation. In NF microvinifications, the pomaces were in contact with the fermenting musts until the end of alcoholic fermentation, while TV wine fermentations were conducted in the

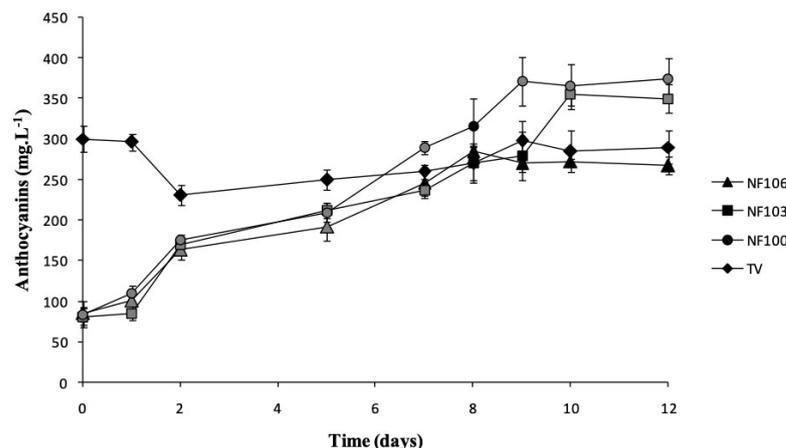


FIGURE 4. Anthocyanin concentrations of the experimental wines during fermentation.

NF = permeate from NF added at volumic mass of 1.060, 1.030, 1.000 g.cm⁻³; TV = thermovinification pre-treatment. Values are the mean \pm standard deviations of duplicate analyses on each duplicate of microvinification (n = 4). The gray marker indicates that the mean value significantly differs from the corresponding value of TV (significance level 95 %).

liquid phase, after the thermal treatment of must and the separation of skins by pressing. Figure 3 shows the evolution of the total polyphenol index (TPI).

As expected, the values for TPI were significantly higher in TV must at the beginning of fermentation, as the thermal treatment of the must in the presence of solid parts is aimed at damaging the skin cell membranes, thus accelerating the extraction of phenolic compounds, mainly anthocyanins (Sacchi *et al.*, 2005). The TPI value of the TV trial remained substantially constant during alcoholic fermentation.

The evolution of TPI for NF trials reflected a typical solid-liquid extraction phenomenon (Amendola *et al.*, 2010; Setford *et al.*, 2017). After a lag phase the TPI increased, following a sigmoidal shaped curve, until reaching a maximum TPI. This reflects Fick's second law and is in accord with two-terms extraction models with first and zero order terms proposed to fit experimental data for TPI (Amendola *et al.*, 2010; Zanoni *et al.*, 2010). The extraction kinetics of phenolic compounds, estimated by TPI, followed the same trend for the three NF treatments. Several factors may have concurred in modulating the diffusion of phenolics from solid parts of grapes in NF trials. On one hand, the factors normally involved in phenolic extraction from pomace are phenol concentration gradient between grape pomace and the fermenting must, and an increase in alcohol concentration during alcoholic fermentation. On the other hand, additional factors were added by the innovative process of an increase in the gradient of concentration of phenolics between pomace and the fermenting must by adding the permeate

poor in phenols (TPI of permeate = 4) on pomace, and the addition of an aqueous phase (permeate) on the pomace, thus favoring the extraction of anthocyanins. It is very difficult to establish how each single factor affected the entire process. It was positive that the TPI reached in NF1000 and NF1060 wines did not differ significantly from the value obtained by TV from the ninth day of fermentation until the end. The differences found on the twelfth day, although statistically significant, can be considered negligible from a technological point of view. On the basis of these results, NF treatments seem to promote the same enhancement of polyphenol extraction as thermovinification does (Sacchi *et al.*, 2005; Carew *et al.*, 2014; El Darra *et al.*, 2016), therefore the developed NF protocol may be a suitable non-thermal alternative.

Figure 4 shows the evolution of anthocyanin concentration. The kinetic for TV wine is a good reflection of the typical behavior reported in the literature, with a rapid initial extraction followed by a decrease in the first phases of fermentation (Ribéreau-Gayon *et al.*, 2006). The nanofiltration treatment NF1060 determined a final concentration of anthocyanins not significantly different from TV. The NF treatments with a later addition of the permeate (NF1030 and NF1000) determined instead an increase in anthocyanin concentration of about 20 % for NF1030 and 30 % for NF1000, compared to the TV treatment. This could be attributable to the addition of the aqueous phase at the final step of fermentation that could have enhanced the extraction of the last fractions of anthocyanins still located in the skins. Indeed, anthocyanins are favorably extracted in aqueous

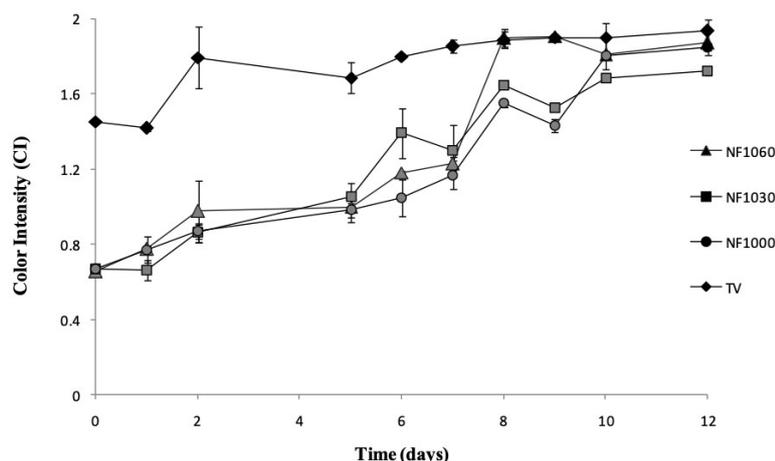


FIGURE 5. Evolution of color intensity (CI) of the experimental wines during alcoholic fermentation. NF = permeate from NF added at volumic mass of 1.060, 1.030, 1.000 g.cm⁻³, TV = thermovinification treatment. Values are the mean ± standard deviations of duplicate analyses on each duplicate of microvinification (n = 4). The gray marker indicates that the mean value significantly differs from the corresponding value of TV (significance level 95 %).

TABLE 2. Analysis of tannins (g.L⁻¹), gelatin, dialysis and HCl index at the end of malolactic fermentation.

End of AF	Tannins (g.L ⁻¹)	Gelatin index	Dialysis index	HCl index
NF 1060	4.93 ± 0.02 *	30.3 ± 5	24 ± 3	11.5 ± 2
NF 1030	6.61 ± 0.05 *	16 ± 3 *	18 ± 2 *	8 ± 1 *
NF 1000	6.32 ± 0.3 *	23.3 ± 3 *	16 ± 2 *	6 ± 1 *
TV	3.57 ± 0.04	34.5 ± 5	26 ± 3	10 ± 3

NF = permeate from NF added at volumic mass of 1.060, 1.030, 1.000 g.cm⁻³, TV = thermovinification pre-treatment. Values are the mean ± standard deviations of duplicate analyses on each duplicate of microvinification (n=4). * the mean value of NF treatment significantly differs from the corresponding value of TV (significance level 95 %).

media (Sacchi *et al.*, 2005; Ribéreau-Gayon *et al.*, 2006). Moreover, the poor addition of the permeate in anthocyanins (< 5 mg.L⁻¹) allows the restoration of a gradient of concentration between skins and the liquid phase, which drives the diffusion of anthocyanins, according to Fick's law (Setford *et al.* 2017), and that normally is very little in the last phases of fermentation. From the observation of the extraction curves, it is also evident applying the NF treatments meant the typical adsorption of anthocyanins on grape pomace during maceration did not occur, probably due to the shift of adsorption equilibrium between the liquid phase and cell membranes and vacuoles. It can be observed that from the ninth day of maceration-fermentation NF1000 treatment had reached its final level of extraction, while NF1030 had a substantial increase between the eighth and the ninth day. From the ninth day the alcoholic fermentation was concluded, making it possible to choose the treatment and the moment of racking

off in relation to the desired anthocyanin and tannin level of extraction.

Figure 5 shows the evolution of color intensity (CI). During fermentation, the kinetic of the CI is mainly due to the diffusion of free anthocyanins from grape skins to the fermenting must, however reactions of self-association and copigmentation also occur and contribute to global color intensity (Boulton, 2001; González-Manzano *et al.*, 2008). This could explain the difference in trends between total anthocyanin extraction and CI. From the second day of fermentation, the CI was constant for TV treatment, which was shown to enhance the red wine color (El Darra *et al.*, 2016). As for anthocyanin extraction, an increase of CI during fermentation was observed in all NF microvinifications, however in NF1060 (corresponding to the earliest addition of permeate) the CI abruptly reached TV value between the seventh and the eighth day, then remained stable until the end of fermentation. The

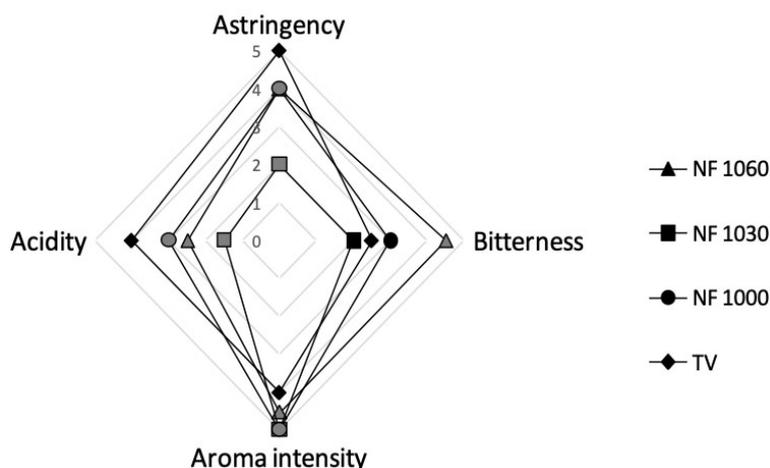


FIGURE 6. Sensory profiles of the experimental wines. NF = permeate from NF added at volumic mass of 1.060, 1.030, 1.000 g.cm⁻³, TV = thermovinification pre-treatment. The values are the means of the two replicates of microvinification mixed and analyzed in duplicate. The gray marker indicates that the mean value significantly differs from the corresponding value of TV (significance level 95 %).

higher anthocyanin concentrations in NF1000 and NF1030 was not reflected in CI, as NF1000 did not differ from TV and NF1030 had actually a lower CI.

On the twelfth day, the CI of NF1000 and NF1060 was not different in respect to TV, while NF1030 had a slightly lower value (decrease of 11 %). Also concerning the extraction of color, very important for wine from Pinot noir grapes that have poor color development and low pigment stability (Sacchi *et al.*, 2005), NF treatment seems to be a suitable alternative to TV.

3. Tannins, HCl, gelatin and dialysis index of finished wines

The obtained wines were analyzed to evaluate the impact of the different NF treatments on tannins in comparison with TV. The HCl index, dialysis index and gelatin index were determined, to gain information on the reactivity and the polymerization state of tannins in wine (Table 2). We observed that tannin concentration was higher in all NF wines, compared to TV wines, especially in NF1030 and NF1000. In both cases, tannin concentration was two times higher. The lower tannin content in TV wine was expected, due to less contact with the skins during fermentation (in alcoholic phase) in comparison with NF treatments (Table 2).

In addition to being responsible for astringency in red wine, tannins can combine with anthocyanins in forming co-pigments, thus determining color stabilization. A higher tannin content could be a positive feature for Pinot noir wine, for which color stability is a challenge, especially if aging is expected (Parley *et al.*, 2001; Sacchi *et al.* 2005).

Moreover, considering the data on anthocyanin extraction (see the previous paragraph), the moment of racking off may be chosen, in the last days of maceration, according to the desired ratio anthocyanin/tannin, in relation to wine style and expected shelf-life.

The HCl index reflects the state of polymerization of wine tannins. The highest mean HCl index was observed for NF1060, which did not significantly differ from TV, while NF1030 and NF1000 had a lower value (Table 2). The dialysis index is related to tannin structure. A high dialysis index indicates that there are some large size molecules, generally polymerized pigments that cannot pass through the pores of dialysis membrane. Wines obtained by the treatment NF 1060 had a dialysis index not significantly different from TV, while NF 1030 and NF1000 gave lower values (Table 2). The gelatin index is based on the capacity of tannins to react with proteins by stable combinations. At a first approach, this index may be correlated to the fraction of wine tannins capable of reacting with salivary proteins and thus eliciting astringency perception (Llaudy *et al.*, 2004).

Similar to what was found for HCl and dialysis indexes, NF 1060 wine was not significantly different from TV, while the other two NF treatments gave a lower gelatin index. In summary, we can say that the moment of addition of the permeate in NF treatments gives wines that differ by the macroscopic characteristics of the tannin fraction (degree of polymerization, reactivity toward proteins, fraction of polymerized pigments) and an earlier addition of the permeate (NF 1060 treatment) gives a wine comparable to

that obtained by TV. Further studies will be aimed at explaining these results from a molecular point of view.

Wine tasting was performed to assess the sensory outcome of the different extraction techniques (Figure 6). Sensory scores for astringency well reflected the values of gelatin index, indeed wines made by TV, NF1060 and NF1000 treatments had a more intense astringency. On the contrary, NF1030 wine had a less intense astringency according to the lowest mean gelatin index. The mean acidity of TV wine was perceived as the most intense, followed by NF1060 and NF1000, while NF1030 had a weak acidity. The high acidity may have contributed to enhanced astringency sensation in TV, NF1060 and NF1000 wines.

The global aroma intensity of the three NF wines was higher than that perceived in TV wine. This could be due to their higher tannin content, in fact previous studies showed that tannins increase the volatility of several esters and alcohols of wine (Mitropoulou *et al.*, 2011). Via a discussion at the end of the sensory sessions, a general consensus was found among judges on the greatest appreciation for the wines from nanofiltration treatments, most of all NF1030.

CONCLUSIONS

In this study a new process based on membrane nanofiltration was developed, with the aim of enhancing polyphenol extraction during alcoholic fermentation of Pinot noir wine. The process consists in the addition of nanofiltration permeate from *saignée* on pomace, at different steps of alcoholic fermentation (NF1060, NF1030, NF1000 corresponding to the addition at a volumic mass of 1.060, 1.030, 1.000 g.cm⁻³). The innovative process proved to be a suitable non-thermal alternative to thermovinification, in terms of extracted anthocyanins, tannins and wine color. The moment of addition of permeate on must had an impact on the quality of the extracted polyphenols, as wines obtained by the latest additions (NF1030 and NF1000) were richer in anthocyanins and had lower gelatin index, dialysis index and HCl index. Moreover, they resulted to be less astringent. Sensory analysis by an expert panel found a consensus of a greater appreciation for the wines from nanofiltration treatments, most of all NF1030. The process that was developed could satisfy the requirement for eco-sustainable processes. Indeed, the energy consumption associated with thermovinification is 11.6 kWh.hL⁻¹ (El Darra *et al.*, 2016), whereas the average

consumption in the nanofiltration process in agro-food treatments is lower than 0.8 kWh.hL⁻¹ (Gude, 2011).

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