

NANOFILTRATION AS A SUSTAINABLE TOOL
FOR CONCENTRATING BIOACTIVE COMPOUNDS
IN MAVRUD RED WINE

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Abstract

In the present study, nanofiltration for concentrating total polyphenols, flavonoids, and anthocyanins in Mavrud red wine was performed. The Maxi-Mem membrane filtration system, employing the Microdyn Nadir[®] NP030 P nanofiltration membrane, was utilized for this purpose. The effects of transmembrane pressure (20–50 bar) and flow rate (1.2–3 L min⁻¹) on the contents of phenolics, flavonoids, and anthocyanins in the wine were evaluated. The radical scavenging capacity and total antioxidant activity of the wine and wine concentrates, were also assessed. All wine concentrates exhibited higher concentrations of bioactive compounds and antioxidant activity than the original wine. Overall best concentration results for the three groups of analyzed compounds were determined at 40 bar pressure and 1.2 L min⁻¹ flow rate, showing values of 3925.0 mg GAE L⁻¹, 1386.3 mg CE L⁻¹, and 158.0 mg ME L⁻¹. The highest antioxidant activity of 15.2 mmol TE L⁻¹ was reached at 40 and 50 bar pressure and 1.2 L min⁻¹ flow rate. The results indicate that nanofiltration is a sustainable tool for concentrating phenolics in red wine.

Key words: red wine, nanofiltration, concentration, polyphenols, flavonoids, anthocyanins

Introduction. Alcohol consumption has long been the subject of debate. Among alcoholic beverages, red wine stands out as having a protective role in

human health rather than leading to the risk of chronic degenerative diseases. Its low-to-moderate intake has been associated with lower risk of developing neurodegenerative diseases, occurrence of type 2 diabetes and inflammation, which can help to reduce the risk of related diseases [1]. The cardioprotective advantages of moderate wine consumption along with longevity and antiaging effect have also been highlighted [2]. These health benefits are mainly attributed to polyphenols which are the major bioactive components in red wine.

Numerous factors – internal (grape cultivar and origination) and external (climate, winemaking practices), have been shown to impact phenolic composition and/or concentration in wine. Thus, research has been undertaken to develop wines with specific quality and/or quantity criteria regarding polyphenols. Some authors have studied the influence of soil composition, variation in temperature and water availability on the concentration of phenolic compounds in grapes and, consequently, in wine [3,4]. Other researchers have investigated the effect of pre- or post-fermentative maceration on phenolic compounds in red wines [5,6]. Additionally, when viticultural or vinification strategies do not provide the desired amount of phenolics, membrane filtration such as nanofiltration (NF), can be used to meet the expectations. Several studies have applied NF to retain active compounds in wine and have confirmed its appropriate use for their concentration. IVIĆ et al. [7] investigated the concentration of phenolic compounds in conventional and ecological Cabernet Sauvignon red wines during nanofiltration with polyamide NF M20 membranes at different pressures, with and without cooling. The authors observed that phenolic retention depends not only on the process parameters, but also on the type of wine and the characteristics of the membrane. MASSOT et al. [8] also considered recent advances in nanomembrane processing as a great potential of nanofiltration in the field of oenology, where appropriate control of operating parameters could yield different separations for the same feed and the same membrane.

Red wine phenolics comprise a large group of compounds, including flavonoids and non-flavonoids. Many of the positive health-related properties listed above associated with the consumption of red wine have been correlated with its flavonoid content [9]. Anthocyanins, a sub-class of flavonoids, have been directly linked to the red colour, astringency, and bitterness of wine [10].

This study concerns the concentration of polyphenols, flavonoids, and anthocyanins in native Mavrud red wine by nanofiltration using the Microdyn Nadir[®] NP030 P membrane. The effects of transmembrane pressure (20–50 bar) and flow rate (1.2–3 L min⁻¹) were investigated during the process. The antioxidant activity of wine concentrates was also evaluated since polyphenols are considered potent antioxidants due to their free radical scavenging ability.

Experimental. Laboratory-scale cross-flow nanofiltration set-up. Nanofiltration experiments were carried out on a membrane filtration system MaxiMem (Prozesstechnik GmbH) presented elsewhere [1]. It was equipped with

a commercial flat sheet nanofiltration membrane Nadir[®] NP030 P (MICRODYN-NADIR). This was a polyethersulphone membrane (MWCO \sim 500 Da) with an active area of 215 cm². In all experiments, the initial feed volume ($V_f = 0.75$ L) and final permeate volume ($V_p = 0.76 \times V_f$) were kept constant. For effective maintenance of the filtration system, temperature regime with cooling was applied using the thermostatic bath LAUDA Alpha RA 8.

Mavrud red wine nanofiltration operated in concentration mode was conducted, where the retentate was recycled back to the feed tank, and the permeate was collected separately.

Materials. Mavrud red wine (13.0 vol.%, vintage 2020) was provided by the wine producer Arendatori Ltd. (Harmanli, Bulgaria). Folin–Ciocalteu reagent (2N solution), gallic acid, (+)-catechin hydrate (> 96.0%), sodium nitrite, aluminium chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95%) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich. Hydrochloric acid (37%) and sodium bisulphite (39%) were purchased from Merck. Ethanol (96%) and sodium hydroxide were domestic products (Valerus). All chemicals were of analytical grade.

Analytical methods. The colourimetric method of Folin–Ciocalteu was applied to determine the total polyphenol (TP) content in wine [11]. The measurements were carried out at a wavelength of 765 nm. Gallic acid, considered equivalent to most phenolics on a mass basis, was used as the reference standard. The results were expressed as mg of gallic acid equivalents per liter (mg GAE L⁻¹).

The aluminium complexation assay was applied to determine the total flavonoid (TF) content in wine [12]. The samples absorbance was measured at 510 nm against water blank. Since the content of catechins (flavan-3-ols) is prevalent in wine, catechin was used as the standard for the calibration curve and the TF content was expressed as mg of catechin equivalent per liter of wine (mg CE L⁻¹).

The total anthocyanin (TA) content in wine samples was determined by the modified method of Ribéreau–Gayon and Stonestreet [13]. The absorbance was measured at a wavelength of 520 nm. Usually, the main anthocyanin in red wines is malvidin-3-monoglucoside and its derivatives. Thus, TA content was expressed as mg of malvidin-3-monoglucoside equivalent per liter of wine (mg ME L⁻¹). The DPPH assay was used to determine the radical scavenging capacity (RSC) of the wine, following a modified protocol [14]. Aliquots of red wine and retentate were 10-fold diluted. 0.1 mM DPPH solution was prepared fresh daily. The absorbance of wine samples (A_{sample}) was measured at 517 nm after a reaction time 60 min against the absorbance of a control (A_{blank}) where ethanol was added instead of wine.

RSC was calculated by the following equation

$$(1) \quad \text{RSC (\%)} = 100 \times \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}}$$

Trolox, the commercial water-soluble analogue of vitamin E, was used to assess the total antioxidant activity (TAA) of the wine. Standard solutions of Trolox (0.05–1.5 mmol L⁻¹) were prepared for calibration and the results were expressed as mmol Trolox equivalent per liter of wine (mmol TE L⁻¹).

All colourimetric measurements were performed on a UV/VIS spectrophotometer (UV-1600PC, VWR, Belgium). Samples were analyzed in duplicates. The results were expressed as mean ± standard deviation.

Results and discussion. Effect of the operating parameters on the content of TP, TF and TA. Data on the content of bioactive compounds in the original Mavrud wine and the retentates obtained by nanofiltration are summarized in Table 1.

Table 1

Total content of phenolics, flavonoids and anthocyanins in original Mavrud wine and wine concentrates. Radical scavenging capacity and total antioxidant activity of original Mavrud wine and wine concentrates

	TP, mg GAE L ⁻¹	TF, mg CE L ⁻¹	TA, mg ME L ⁻¹	RSC, %	TAA, mmol TE L ⁻¹
Wine (bottle)	3041.2 ± 54.5	1020.4 ± 12.7	117.4 ± 3.7	77.4 ± 14.4	12.9 ± 1.3
Wine (concentrates)					
TMP*, bar					
20	3307.5 ± 62.9	1131.0 ± 49.5	133.3 ± 16.5	79.6 ± 6.3	13.3 ± 1.1
30	3572.5 ± 27.6	1244.7 ± 63.2	145.1 ± 10.7	88.6 ± 11.0	14.9 ± 2.1
40	3925.0 ± 51.5	1386.3 ± 31.5	158.0 ± 7.1	90.3 ± 10.2	15.2 ± 1.8
50	3942.5 ± 40.3	1212.0 ± 33.9	149.9 ± 9.7	90.1 ± 10.9	15.2 ± 2.0
Flow rate**, L min ⁻¹					
1.2	3572.5 ± 27.6	1244.7 ± 63.2	145.1 ± 10.7	88.6 ± 11.0	14.9 ± 2.1
2.0	3752.5 ± 60.1	1331.4 ± 47.2	155.4 ± 7.4	82.7 ± 9.3	13.9 ± 1.7
3.0	3922.5 ± 43.1	1313.7 ± 51.9	160.9 ± 5.5	82.4 ± 9.1	13.8 ± 1.7

Notes:

TMP – transmembrane pressure;

RSC – radical scavenging capacity;

TAA – total antioxidant activity;

* – at 1.2 L min⁻¹ flow rate; 18 ± 0.5 °C;

** – at 30 bar pressure; 18 ± 0.5 °C.

The average concentrations of TP, TF and TA in the Mavrud wine were determined to be 3041.2 mg GAE L⁻¹, 1020.4 mg CE L⁻¹ and 117.4 mg ME L⁻¹, respectively. The results showed that these values increased after nanofiltration in the obtained wine, and their contents depended on the investigated process parameters. The degree of concentration was evaluated according to Eq. (2) and presented in Fig. 1 and Fig. 2.

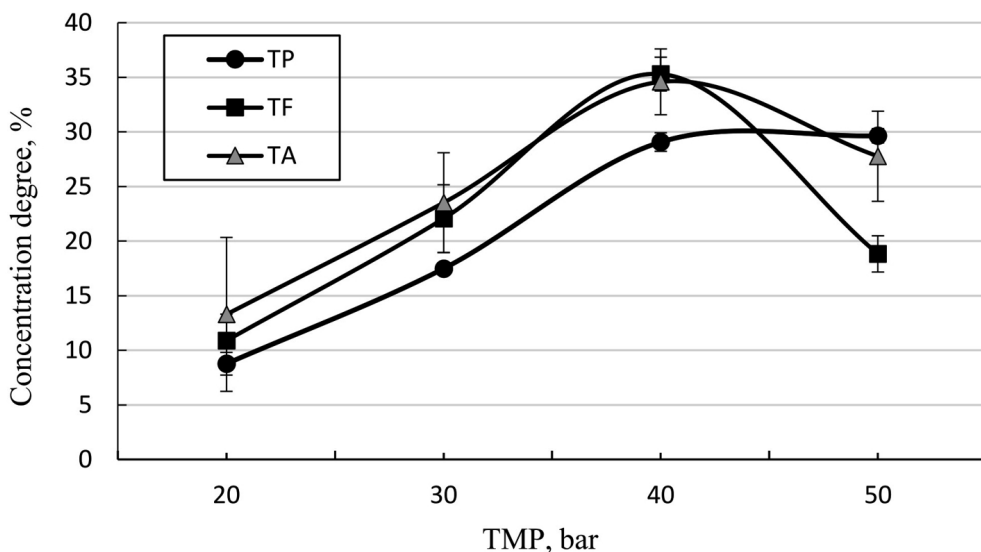


Fig. 1. Impact of transmembrane pressure on the selective retention of secondary metabolites in Bulgarian Mavrud wine during nanofiltration (1.2 L min⁻¹ flow rate; 18 ± 0.5 °C)

$$(2) \quad \text{Concentration degree (\%)} = 100 \times \frac{C_R - C_F}{C_F},$$

where C_R is the concentration of bioactive compounds in retentate, and C_F is the concentration of bioactive compounds in feed.

When considering the influence of transmembrane pressure (Fig. 1), there was a continuous concentration of TP, TF and TA in the retentates with increasing pressure up to 40 bars. With an increase in pressure within 20–40 bar, the concentration degree of polyphenols, flavonoids and anthocyanins was 19%, 22.5% and 19%, respectively. Further increase in pressure to 50 bar had no effect on TP concentration degree. Regarding flavonoids and anthocyanins, at a pressure of 50 bar, a decrease in their concentration in the retentate was observed, more noticeable for the first mentioned ones, with a 12.5% lower TF concentration degree compared to that at 40 bar pressure. Likely, some of these constituents aggregated at higher pressure within the pores and at the membrane surface, induced by permeation flux.

Variation of the flow rate (Fig. 2) had a less pronounced effect on the concentration degree of the bioactive compounds. For polyphenols and anthocyanins, increasing the flow rate in the range of 1.2–3 L min⁻¹ resulted in a 9.8% and 11.0% rise in TP and TA concentration degree, respectively. Even more, a uniform increase of approximately 4.5% in the degree of polyphenol concentration was observed both times the flow rate was increased. The trend in flavonoid concentration indicated that increasing the flow rate from 1.2 to 2 L min⁻¹ resulted

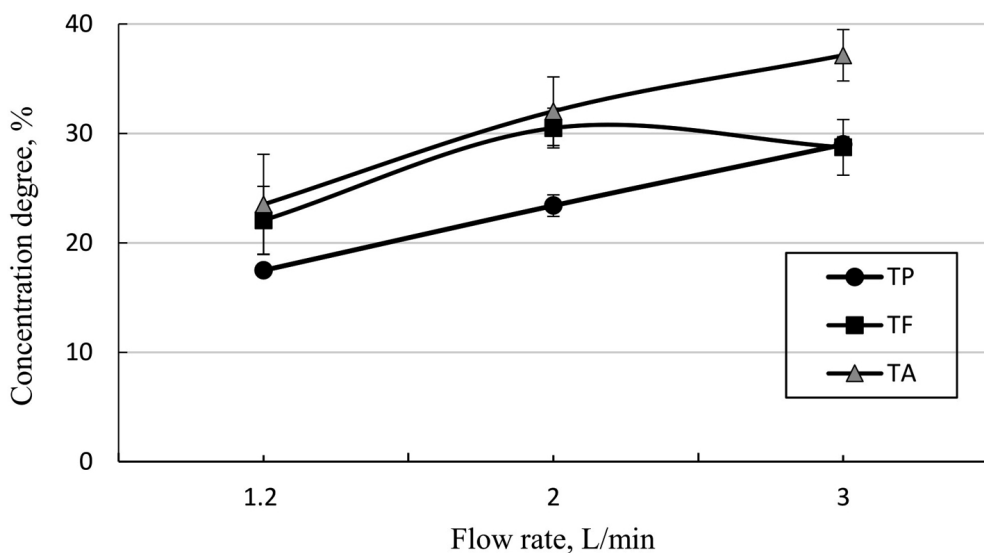


Fig. 2. Impact of flow rate on the selective retention of secondary metabolites in Bulgarian Mavrud wine during nanofiltration (30 bar pressure; 18 ± 0.5 °C)

in a 5.5% TF concentration degree. However, an additional rise in flow rate to 3 L min^{-1} led to a slightly lower concentration degree.

It can be concluded that the wine nanofiltration process at a TMP of 40 bar and a flow rate of 1.2 L min^{-1} with the examined membrane gave the best results for the concentration of all studied bioactive compounds (TP, TF, TA). For polyphenols, similarly good results were obtained with the following combinations of process parameters: 50 bar and 1.2 L min^{-1} , as well as 30 bar and 3 L min^{-1} . For anthocyanins, this referred to a set of 30 bar and 3 L min^{-1} . However, for a slower decline in permeate flux, correspondingly slower fouling of the membrane, and avoiding its compression, it is better to implement a higher cross-flow velocity while maintaining a moderate pressure.

Effect of the operating parameters on the antioxidant activity. The radical scavenging capacity determined by the DPPH assay and the total antioxidant activity of both the original wine and the retentates obtained by nanofiltration are presented in Table 1. The DPPH assay demonstrated an average of 77.4% RSC of the investigated Mavrud red wine, corresponding to $12.9 \text{ mmol TE L}^{-1}$ TAA. Regarding the effect of TMP, the difference in free radical scavenging efficiency between the initial wine and the wine concentrate at 20 bar was minimal. Increasing the pressure to 30 bar led to a 9% increase in RSC to 88.6%. With further increase, the percentage of RSC rose above 90%, resulting in TAA of $15.2 \text{ mmol TE L}^{-1}$ at 40 and 50 bar. The influence of the flow rate on antioxidant activity was less pronounced, ranging from 13.8 to $14.9 \text{ mmol TE L}^{-1}$. It can be concluded that the best results for antioxidant activity during wine nanofiltration

were obtained at 40 bar and 1.2 L min^{-1} , similar to the case when concentrating the bioactive components. Our findings are in agreement with those of Ivić et al. [15], who reported higher TAA values in all wine concentrates after nanofiltration compared to the original Cabernet Sauvignon red wine. However, the increase in pressure had little effect on the TAA of the retentates, with a slightly higher value at 55 bar.

A closer look at how the content of bioactive compounds can be related to antiradical activity is presented in Fig. 3. The course of change in TP content and TAA followed a similar trend with varying pressure, indicating that the antioxidant activity of Mavrud wine was in close relationship with total phenolics.

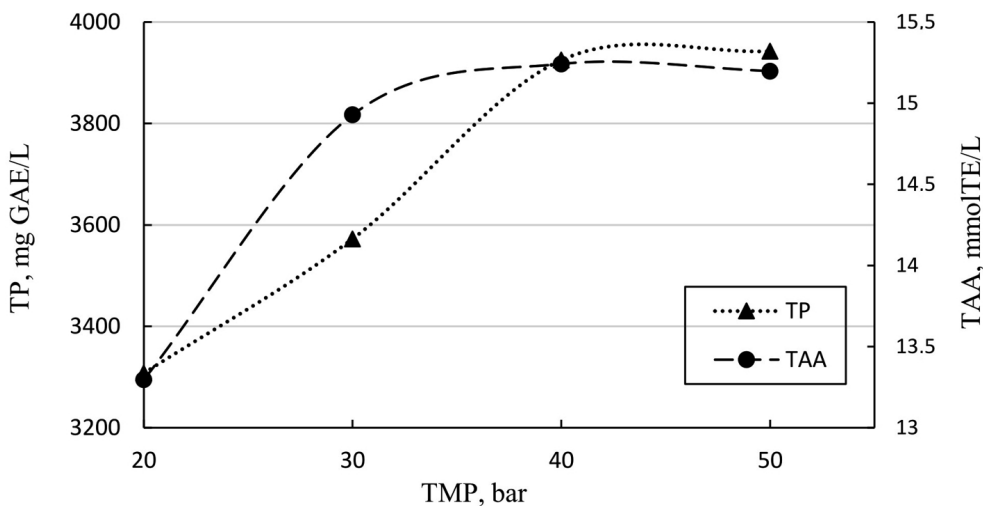


Fig. 3. Change in total phenolic content and total antioxidant activity with varying trans-membrane pressure

Conclusions. The performance of nanofiltration for concentrating biologically active compounds in Bulgarian red wine Mavrud was evaluated. The NF Nadir[®] NP030 P membrane used in the process effectively retained the polyphenols in the retentate. This resulted in higher concentrations of polyphenols, flavonoids, and anthocyanins in the obtained wine under all examined conditions. The best concentration outcomes for all valuable compounds were determined at 40 bar pressure and 1.2 L min^{-1} flow rate. All wine concentrates also exhibited higher antioxidant activity than the original wine. Moreover, the highest total antioxidant activity values for the bioactive compounds altogether were also achieved at 40 bar and 1.2 L min^{-1} , suggesting a close relationship between the antioxidant activity of Mavrud wine and its phenolics. The obtained wine, with concentrated phenolics and antioxidant properties, could provide therapeutic efficacy with reduced ethanol intake. This approach can position red wine as a starting material for “smart food”.

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