Influence of Transmembrane Pressure on the Retention of Bioactive Compounds and Alcohol Content in Red Wine Nanofiltration

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Abstract: - Membrane nanofiltration technology is an efficient method for wine dealcoholization and concentration of various bioactive compounds and has a vast number of applications in wine production. To obtain optimal features of the obtained products, both the parameters and the mode of filtration, as well as the characteristics of the used filtration membrane, are of great importance. In this study, nanofiltration of the native Bulgarian red wine Mavrud via MaxiMem Prozesstechnik GmbH filtration system, completed with polymer membranes was performed. Rejection coefficients of ethanol and selected wine components were measured and their increase with applied pressure (10 to 50 bar) was observed. Cross-flow flat sheet nanofiltration with polymer membranes was performed in concentration and diafiltration mode. Partial dealcoholization (5-8% EtOH) was obtained with good preservation of the remaining composition. Depending on the degree of dealcoholization and the characteristics of the membranes, a loss of 0 to 24% anthocyanins, 8 to 19% polyphenols, and 2 to 30% ORAC activity was observed.

Key-Words: - dealcoholization, nanofiltration, polymeric membrane, reduced-alcohol wine, bioactive properties, transmembrane pressure, gas chromatography.

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1 Introduction

Pressure-driven membrane processes have found valuable applications in the pharmaceutical [1] and food industries [2], overcoming challenges such as imperfect membrane separation and technological approaches to its improvement (multistage membrane processes) [1]; interactions with the solvent during its transition from aqueous to organic (in pharmaceutical wastewater) [3], valorization of valuable bioactive substances (food wastes and by-products) [4], wine and must processing (clarification, control of must's sugar content, wine dealcoholization) [5]. Membrane separation processes in wine processing applied individually or in combination in the post-fermentation period have been successful in achieving the desired alcohol content while maintaining high product sensory characteristics, [6]. Nanofiltration membranes with appropriate properties can be effective in moderating the ethanol content of wine either for concentration (higher ethanol rejection is favorable) or dealcoholization (low ethanol rejection). The capability of the membrane to preserve the quality of the wine depends on the degree of dealcoholization, the membrane material and charactetistics (flux and rejection behavior), and the use of multi-stage processes. In partial dealcoholization, where wine with a reduced alcohol content of 1-2% is obtained, the membrane separation does not appreciably alter the color and the rest of the wine composition. In this respect, nanofiltration (NF) may have an advantage over reverse osmosis (RO) regarding aroma rejection, and less loss of anthocyanins and volatile acids. Diafiltration mode improves the ethanol removal selectivity and is used in wine dealcoholization [7], [8]. NF has the potential for implementation both in the production of low-alcohol (7% alcohol) and in dealcoholized wines (<0.5% alcohol), especially in combination with RO. Typically tight membranes (150-300 Da MWCO) are used concerning alcohol rejection and reduced loss of other valuable compounds; considering the membrane material and structure, the most common are asymmetric polymers, because of good flux, durability, and easier cleaning.

The present study investigates the effect of transmembrane pressure on the retention of bioactive compounds and alcohol while red wine nanofiltration in concentration and diafiltration modes.

2 Materials and Methods

Dry red wine Mavrud vintage 2020 was provided by the Bulgarian wine cellar, Harmanli, Bulgaria, and was used in a preliminary set of experiments on nanofiltration. Distilled water (water still GFL Type 2004, Germany) was used throughout the work. For cleaning procedures sodium hydroxide and citric acid from „Valerus Ltd.“ were used. The calibration of the Gas Chromatograph (GC) was done using standard ethanol-water solutions. Absolute ethanol, 99.9%, from Valerus Ltd.“ was used. An internal standard for the GC n-propanol p.a. from Firma Chempur was used.

Laboratory membrane filtration system MaxiMem Prozesstechnik GmbH (Figure 1) providing possibilities for performing all pressure-driven membrane processes, was used in the study. In the present investigation temperature regime with cooling was applied using the thermostatic cooling bath LAUDA Alpha RA 8.

The filtration unit was used with a polymeric rectangular flat sheet membrane with an active area of 215 cm^2. The commercial membrane Nadir® NP030 P supplied by MICRODYN-NADIR GmbH Advanced Separation Technologies was used. It is an asymmetric polyethersulfone membrane, MWCO 500 Da, that exhibits nanofiltration characteristics when exposed to high pressure. Results with red wine (Mavrud) nanofiltration were obtained using nanofiltration membrane Alfa Laval NF99HF (thin film composite polyester, MWCO 200 Da) as well. Transmembrane pressure is varied in the range of 10 to 50 bar. The cross-flow rate is 1.2 l/min respectively.

Fig. 1: Laboratory membrane filtration system MaxiMem Prozesstechnik GmbH

The analysis of the alcohol content of the permeate and retentate was carried out on a Gas Chromatograph (Agilent 8890, Agilent Technology, USA), with a flame ionization detector completed with Agilent CP-Wax 52 CB column (0,25 mm x 50m; df - 20μm). The method was designed specifically for the quantitation of ethanol in the wine filtrates. The GC-FID parameters for the alcohol analysis are given in Table 1.

Table 1. Gas Chromatography sampler parameters for analysis of ethanol in wine filtrates

Parameter	**Value**
Inlet temperature	250ºC
Injection mode	Split
Oven temperature	40ºC
Front SS Intel H2 heater	220 ºC
Septum purge flow	3 ml/min
Pressure	12,063 psi
Split ratio	10:1
Split flow	14 ml/min
Column flow	1,4 ml/min
Linear velocity	34,536 cm/sec
Front detector FID heater	240 ºC
Makeup N2 flow	15ml/min
H2 flow (Combined)	35ml/min

The data analysis for the calibration procedure was carried out using Agilent CG Software. The calibration was performed with model ethanol samples in the range of 2-20 vol%. with R^2=0,998. As an internal standard n-propanol was used.

The ethanol concentration for every studied sample was calculated as a mean average over three individual measurements.

The analysis of total polyphenols of the wine was performed via High-Performance Liquid Chromatography (HPLC) analysis of phenolic components according to [9] on an HPLC system (Agilent 1220, Agilent Technology, USA), with a binary pump and UV-Vis detector (Agilent Technology, USA). Separation was performed on an Agilent TC-C18 column (5 µm, 4.6mm × 250mm) at 25°C and a wavelength of 280nm was used.

Total anthocyanin (TA) content was determined by the pH differential method [10].

Oxygen Radical Absorbance Capacity (ORAC) was measured according to the method [11]. ORAC analyses were carried out on FLUOstar OPTIMA plate reader (BMG Labtech, Germany) with an excitation wavelength of 485nm and emission wavelength of 520 nm.

3 Results and Discussion

The results for Ethanol content (EtOH) and bioactive compounds: Total Polyphenols (TP), Total Anthocyanin content (TA), and Oxygen Radical Absorbance Capacity (ORAC) of the wine before and after the nanofiltration at various transmembrane pressure (10-50 bars) in both concentration and diafiltration mode with two different membranes Alfa Laval NF99HF and Microdyn Nadir NP 030P are summarized in Table 2.

As can be seen from Table 2, the concentration runs result in a similar degree of concentration regarding the polyphenols and anthocyanins, which have high rejection coefficients. The antioxidant activity follows this trend, proving that the bioactive compounds are retained. Figure 2 shows the concentration degree in nanofiltration with Alfa Laval membrane at V_p/V_f=0.49-0.5. No appreciable effect of the pressure is observed. Due to low flux and fouling the concentration runs with the membrane Nadir are performed at a limited pressure range (30-50 bar) and V_p/V_f=0.27-0.28, where a similar behavior of the concentration degree and the respective antioxidant activity is observed, although less pronounced.

The rejection coefficients of total polyphenols (TP) and anthocyanins (TA) are high for both membranes. They are determined by the measured concentrations at the beginning C_f and the average permeate end concentration C_p:

$$RTA/TP=(1-C_p/C_f).100, \%.$$

Table 2. Wine characteristics before and after nanofiltration

			INITIAL WINE				FINAL WINE			
Membrane	TMP bar	Operation mode	EtOH, vol %	TP, mg/l	TA, mg/l	ORAC, µmol/l	EtOH, vol %	TP, mg/l	TA, mg/l	ORAC, µmol/l
ALFA LAVAL NF 99 HF	10	CONCENTRATION	11.16	2881.8 ± 80.5	27.4	69894.6 ± 1383.1	10.51	4385.4 ± 145.0	46.8	107723.3 ± 1835.0
	20		13.30	2549.2 ± 34.8	22.4	48355.6 ± 602.3	12.24	4323.9 ± 38.7	41.7	97031.1 ± 706.4
	30		13.84	2040.3 ± 22.6	27.4	53787.5 ± 1133.5	10.99	4219± 58.0	38.7	109299.4 ± 1788.3
	40		11.63	2715.5 ± 19.3	28.1	85445.5 ± 852.8	12.13	4795.4 ± 80.5	44.1	121310.3 ± 485.9
	50		12.84	2731.5 ± 9.7	29.1	87879.2 ± 1049.3	11.76	4927.6 ± 9.7	51.1	130034.9 ± 1641.4
	20	DIAFILTRATION	12.79	2872.7 ± 1.6	32.1	80219.2 ± 1375.5	7.42	2624.4 ± 3.2	32.1	78154.0 ± 1816.2
NADIR NP 030P	30	CONCENTRATION	11.91	2835.0 ± 12.9	30.1	70162.6 ± 1411.1	11.18	3446.8 ± 11.3	32.1	103836.2 ± 3007.3
	40		12.99	2925.1 ± 12.0	27.4	103992.1 ± 3068.9	12.14	3401.2 ± 8.1	29.1	116088.0 ± 1586.2
	50		12.11	2663.1 ± 14.5	33.1	62360.3 ± 1378.0	11.77	3357.9 ± 9.7	37.4	91251.4 ± 1036.5
	40	DIAFILTRATION	13.92	2836.2 ± 8.1	28.7	89508.3 ± 335.7	5.08	2291.8 ± 9.7	21.7	62526.7 ± 272.1

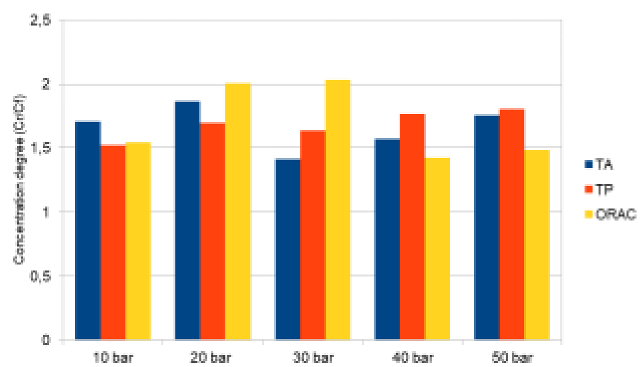

Fig. 2: Concentration degree in nanofiltration with Alfa Laval NF99HF membrane for pressure range 10-50 bars

Full rejection (RTA=100%) of anthocyanins is observed, with the rejection coefficients of polyphenols being over 97%. From this point of view, the two membranes can effectively separate polyphenols and anthocyanins from wine. The rejection coefficients have a slight tendency to increase with applied pressure (Figure 3), which is a commonly observed phenomenon and is usually associated with membrane compression at higher pressures. Furthermore, the rejection coefficients for the Alfa Laval membrane are higher, which is related to the smaller MWCO of this membrane (200 Da). It has also the advantage of an essentially high permeate flux.

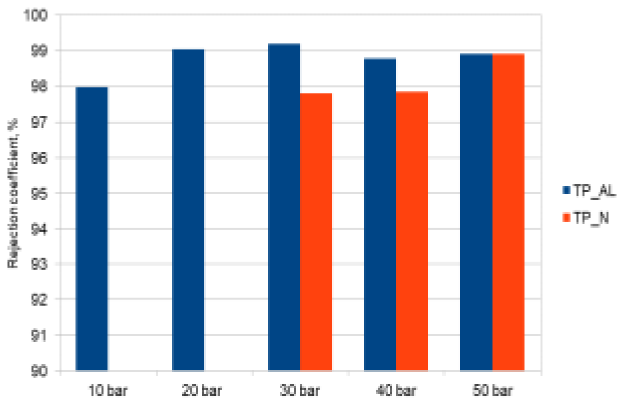

Fig. 3: Rejection coefficient for Total Polyphenols, obtained by Alfa Laval NF99HF (AL in blue) and Microdyn Nadir NP030 P (N in red color) membranes for pressure range 10-50 bars

Concerning the ethanol separation, the data for the concentration runs in Table 2 give no evidence for some ethanol concentration. For effective separation of alcohol from wine, it is important to have a large difference in the coefficients for ethanol compared to those for the other components. Constant volume diafiltration was performed at a pressure selected from the previous experiments. For the Alfa Laval membrane at 20 bar, rejection coefficients of the order of 10% were measured (REtOH=9.37-10.10%) and then proven in the diafiltration experiment. The obtained dealcoholization of 7.4% coincided with the calculated one (7.59% vs 7.42%) by the analytical solution [8] for the respective flux and ethanol rejection.

Moreover, the experimental results (Figure 4, dots) in a wider range of permeate volumes satisfactorily confirm the expected logarithmic dependence (Figure 4, dashed line, coefficient of determination R_2=0.813) of the degree of dealcoholization $(C_f-C_r)/C_f$ versus the number of penetrated diavolumes V_p/V_f.

Diafiltration with the Nadir membrane was performed at higher transmembrane pressure in order to overcome the disadvantage of the low permeate flux. The rejection coefficient for ethanol for this membrane is higher, steadily increasing with increasing pressure in the range of 18 to 25%. The measured flux and rejection in the concentration runs are not confirmed during diafiltration. The permeate flux has been higher – 4.12 l/(m^2.h) vs 3.45 l/(m^2.h) - and the obtained final ethanol concentration of 5.08% corresponds to 6.5% calculated value, which means that the true rejection coefficient has been considerably lower than the previously measured. Following the data in Table 2, the loss in TA, TP, and antioxidant activity has been satisfactorily low for the membrane Alfa Laval (0% in TA, 8% in TP, and 2% in ORAC capacity). The higher losses, observed with the Nadir membrane (up to 24% of TA, 19% TP, and 30% ORAC capacity) can be attributed to the stronger dealcoholization as well as the low permeate flux implying longer diafiltration time and associated losses. The higher losses, observed with the Nadir membrane (up to 24% of TA, 19% TP, and 30% ORAC capacity) can be attributed to the stronger dealcoholization, somewhat lower TA and TP rejection coefficients, as well as the low permeate flux implying longer diafiltration time and associated losses.

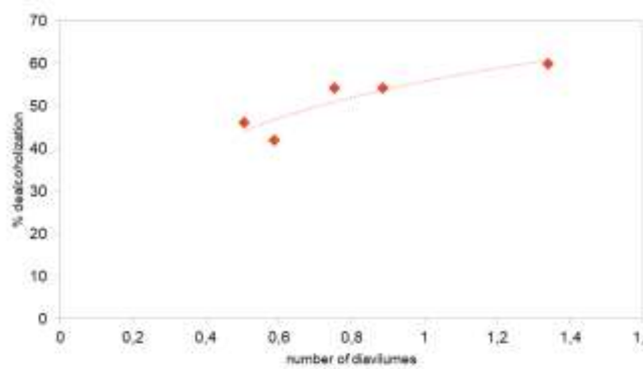

Fig. 4: Degree of dealcoholization $(C_f-C_r)/C_f$ versus the number of penetrated diavolumes V_p/V_f

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

- I. Tsibranska has carried out the simulation, statistics and optimization.
- J. Genova, M. Dencheva-Zarkova have organized and executed all the experiments.
- D. Yankov has edited the final manuscript.

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Conflict of Interest

The authors have no conflicts of interest to declare.