VALORISATION VIA NANOFILTRATION OF THE ANTIOXIDANT RESIDUAL WATER FROM INDUSTRIAL HYDRODISTILLATION OF *ROSA DAMASCENA MILL.* PETALS

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ABSTRACT

The essential oil produced from R. damascena petals is one of the most valuable and important base materials in the flavour and fragrance industry. For each ton of processed rose flowers, less than 0.5 kg of essential oil and about 4 tons of residual water is generated. Due to the presence of polyphenolic compounds in the latter, its uncontrolled disposal has an adverse effect on the ecological balance. Moreover, valuable substances with biological activity are lost. The possibility to recover by nanofiltration a refined polyphenolic fraction, composed primarily of flavonoids, their glycosides and polyphenolic acids, contained in the waste residual water was demonstrated in the present work. The nanofiltrations were carried out with DL (Veolia) and NP030 (Mycrodin Nadir) commercial nanofiltration membranes. The DL membrane was proposed for process design due to its lower susceptibility to fouling by the target solutes. The experimentally determined membrane permeance for the DL membrane ranged from 1.8 to 0.5 LMH bar^{-1} during batch concentration at an operating pressure of 10 bar and degree of feed volume reduction in the range from 1 to 2. Since the target compounds are well known antioxidants, their concentrations in the studied materials were evaluated in terms of global antioxidant activity. The retentates were up to 2.00 times more potent antioxidants compared to the feed solutions. The experimentally determined membrane rejection coefficients for both membranes with respect to global antioxidant activity were in agreement with theoretically predicted values reported in a previous study, indicating efficient membrane fractionation.

Keywords: valorisation, Rosa damascene Mill., residual water, nanofiltration, polyphenolic fractions, antioxidant activity.

INTRODUCTION

R. damascena is a deciduous shrub and is an emblematic plant species for Bulgaria. Its large-scale industrial cultivation and high quality of the produced rose oil established worldwide bestowed Bulgaria with the name “the country of the oil rose”. The flowers have a strong and very pleasant fragrance. The essential oil produced from *R. damascena* petals is one of the most valuable and important base materials in the flavour and fragrance industry. A variety of commercial products, such as rose oil, rose water, dried flowers, rose concrete, and rose absolute are produced from the flowers of the oil rose. There is considerable interest in the development of new innovative products from *R. damascena* of industrial and practical importance due to its antimicrobial [1], antioxidant, analgesic, anti-inflammatory, antispasmodic, antidiabetic, and antidepressant properties [2].

Rose oil is usually produced by hydrodistillation of

fresh rose flowers, which remains the major technology ensuring high and sustainable quality of the obtained essential oil. As a result of the hydrodistillation of rose flowers, three fractions are generated: spent plant mass, aqueous condensate (hydrosol, hydrolat), and waste extract (residual water) [3]. The hydrosol contains small amounts of volatile aromatic substances. Therefore, it is further enriched in the essential oil components by distillation to allow the separation of rose essential oil as a product. The residual water is generally considered waste and is disposed of in the environment. For each ton of processed rose flowers, less than 0.5 kg of essential oil and about 4 tons of residual water is generated (Galen-N Ltd., Bulgaria, private communication) [5]. Many reports attribute the antioxidant properties of *R. damascena* to the content of phenolic compounds, representatives of different subclasses of the flavonoid family (flavan-3-ols, flavanones, flavonols, and flavones) [4, 5]. Despite the available information on the key biologically active and essential oil components in the rose plant, their distribution in the waste fractions and their quantitative content in these fractions are scarcely reported in the literature [3]. However, recent theoretical studies confirm that antioxidant components from the flavonoid family remain mainly in the residual water during the hydrodistillation process [3]. Due to the large quantities of this effluent, substantial amounts of waste extracts, rich in biologically active substances are released into the environment every year on the territory of Bulgaria. Their uncontrolled disposal can lead to contamination of the soil, as well as surface and groundwater. In areas with tradition in the production of essential oil from *R. damascena*, this becomes a serious environmental problem, due to the disturbance of the ecological balance. The studies aimed at the valorisation of this waste stream reported in the literature are scarce [5, 6]. A technology has been proposed, based on the adsorption of the polyphenolic compounds contained therein onto the surface of an activated resin, followed by their desorption with ethanol or water-ethanol mixtures. Hence, the residual water is separated into depleted and enriched in polyphenolic compounds fractions. The polyphenol-enriched fraction was shown to have a similar phytochemical profile to that of the residual water [5]. In a more recent study, freeze-dried and ground wastewater was extracted with 0.3 % aqueous solution of trifluoroacetic acid [6]. The liquid phase was further mixed with Amberlite XAD-7 ion-exchange resin to selectively adsorb the target biologically active polyphenolic fraction. Finally, the resin was rinsed with methanol containing TFA, and the extract was isolated using a rotary evaporator.

In recent years, separation processes at the molecular level, and nanofiltration in particular, have been the subject of intensive research regarding their application for the utilization of by-products in the agricultural and food industry [7 - 9]. Research trends in the field of nanofiltration and nanofiltration membrane formation highlight their application to wastewater treatment and clean water production [10]. Despite this intensive research and the proposed technological solution for the utilization of the waste extract in the production of *R. damascena* essential oil, the possibility of increasing the efficiency and eliminating the need to use organic solvents by integrating nanomembrane separation has not been explored.

The aim of the present work is to evaluate the possibility for valorisation of the effluent extract from the rose essential oil production via nanofiltration. For this purpose, a pretreated via microfiltration *R. damascena* residual extract from an industrial essential oil distillery in Bulgaria was fractionated using different nanofiltration membranes. The feasibility of the nanofiltration for concentrating the target polyphenolic compounds was demonstrated by analysing the antioxidant activity of the resulting permeate and retentate fractions.

EXPERIMENTAL

Materials

Residual water from the hydrodistillation of *R. damascena* petals was obtained from Galen N Ltd. (Zelenikovo, Bulgaria). The waste extract was obtained in sealed laboratory plastic bottles and kept frozen until use. The absolute ethanol used for the determination of antioxidant activity was purchased from Valerus Ltd. (Bulgaria), 2,2-diphenyl-1-pikryl-hidrazyl (DPPH•), and gallic acid from Sigma-Aldrich (UK). For nanofiltration two commercially available membranes were used: DL, polyamide thin-film composite (PA-TFC) from Veolia, France, with a molecular weight cut off (MWCO) of 150-300 Da; NP030 polyethersulfone (PES) from Microdyn Nadir, Germany, with a MWCO of 500 Da.

Nanofiltration

The nanofiltration experiments were carried out using a dead-end membrane filtration cell (METcell, Evonik Membrane Extraction Technology, London, UK). The cell allows for testing polymeric membrane samples with an active surface area of 54 cm^2 at operating pressure of up to 69 bar, applied by compressed nitrogen (Fig. 1). The maximum feed volume was 250 mL.

The stirring rate was kept constant at 350 rpm to minimize concentration polarization, according to the Instruction Manual for MetCells, Membrane Extraction Technology LTD, 2008, London, UK. The runs were carried out at a constant ambient temperature and transmembrane pressure of 10 bar, maintained by high purity nitrogen (99.996 %), supplied from a gas cylinder. Before nanofiltration, the feed solution was microfiltrated with cellulose acetate membrane filters with a pore size of 0.45 μm (Chemplus Scientific Ltd., China). Each nanofiltration experiment was carried out with a new membrane sample to avoid "memory" effects on the membrane properties. Before each nanofiltration, membrane conditioning was performed by initially permeating distilled water at 10 bar until a steady-state permeate flux is observed. The aim was to avoid the compression effect in the later stage of the experiments and to remove the conditioning agent used to preserve the membrane structure during storage. The rose residual water feed volume, $V_{F,}$ during the nanofiltration runs was 100.0 mL. During the batch concentration experiment the permeate from the bottom of the cell was continuously collected in a measuring cylinder. Accumulation of certain permeate volumes over time was measured until 50.0 mL of permeate were obtained and the filtration cell was depressurised. The volume of retentate remaining in the cell, $V_{R,}$ was consequently 50.0 mL. Under these conditions the degree of feed volume reduction (DFVR) coefficient, defined as V_F/V_R equals 2 at the end of each filtration experiment. Samples of feed, permeate, and retentate were taken for analysis of their antioxidant activity.

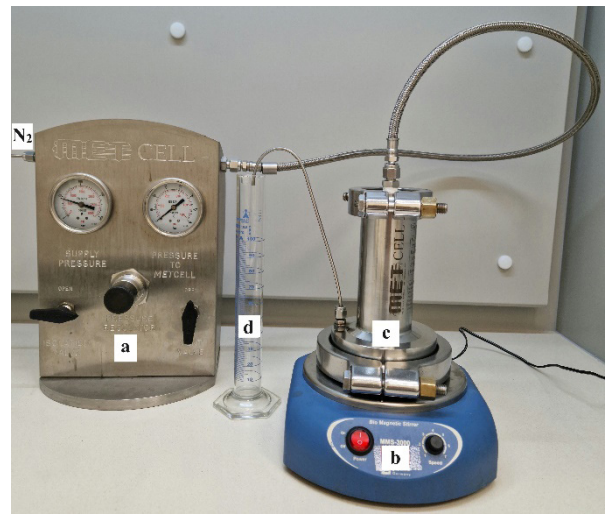

Fig. 1. Laboratory set-up for batch nanofiltration: a - pressure control unit; b - electromagnetic stirrer; c - nanofiltration cell (METcell); d - permeate vessel.

Antioxidant capacity determination

The DPPH radical scavenging assay is a fast, simple, inexpensive and widely used colorimetric method for the evaluation of antioxidant properties of natural extracts [11 - 14]. In ethanolic solution DPPH• shows a strong absorption band with a maximum at 517 nm [11 - 14], while its reduced form (DPPH) does not absorb significantly at this wavelength, allowing for quantitative colorimetric determination.

An ethanolic solution (99.9 %) of DPPH was prepared and its concentration was adjusted to approximately 0.1 mM so that the absorbance was between 0.9 - 0.8 at 517 nm. Single use polystyrene cuvettes (3 mL capacity, 10 mm optical path length) and a T70 UV/VIS Spectrometer (PG Instruments Ltd) were used to measure the absorbance. Appropriate dilutions of the studied fractions were prepared to ensure a moderate radical scavenging reaction rate and equilibria in the reaction mixture and thus reliable analysis. The quantities of the *R. damascena* feed solution and nanofiltration fractions in the samples subjected to antioxidant capacity assay were as follows. For DL membrane: Feed - 4.55, 1.92 and 0.98 mL L^{-1}; Permeate - 16.67, 8.33 and 4.55 mL L^{-1}; and Retentate - 2.38, 1.92 and 0.66 mL L^{-1}. For NP030 membrane: Feed - 4.55, 1.92 and 0.98 mL L^{-1}; Permeate - 25, 16.67 and 12.5 mL L^{-1}; Retentate - 4.55, 2.38, 1.92 and 0.66 mL L^{-1}. The control sample contained a solution of 1,5 mL DPPH and 0,05 mL 99.9 % ethanol. 0,05 mL of the tested sample, containing *R. damascena* feed solution or nanofiltration fraction, was added to 1.5 mL of DPPH• solution. Each measurement was performed in two replicates. After mixing of the antioxidant samples

with the DPPH• solution the absorbances of the test (A_s) and the control (A_c) solutions were measured at 517 nm at 15 minutes intervals for 1 hour to examine the reaction kinetics. The free radical scavenging activity of each sample was then evaluated as percent quenched or remnant DPPH• defined as (Equation 1 and 2):

$$\%\ \text{quenched DPPH}\bullet = \frac{A_c - A_s}{A_c}\ 100 \quad (1)$$

$$\%\ \text{remnant DPPH}\bullet = 100 - \%\ \text{quenched DPPH}\bullet \quad (2)$$

The antioxidant activity is characterized by the EC_{50} value, which represents the concentration necessary to reduce 50 % of the initial DPPH• present in the sample. The presented data are mean arithmetic values of the two replicates for %quenched and %remnant DPPH•. The deviation of the experimental values from the mean arithmetic was less than 3.5 % in all cases.

RESULTS AND DISCUSSION

The evolution of the permeate flux during the membrane conditioning and nanofiltration of *R. damascena* residual water with the DL and NP030 nanomembranes is shown in Fig. 2. The data for the permeate flux, $\bar{F}$, was evaluated based on the measurements of the cumulative permeate volumes, V_p, versus time, as described previously [15].

The results demonstrate that both membranes reach steady operation after permeation of at least 180 mL of distilled water at the operating pressure. For both membranes, the permeate flux drastically decreased during batch nanofiltration of *R. damascena* extract. The membrane permeance with the DL membrane decreases from 1.8 to 0.5 LMH bar^{-1} with increasing the degree of feed volume reduction during batch concentration in the range from 1 to 2, respectively. This decrease can be explained by the increased mass transfer resistance in the diffusion boundary layer at the membrane surface due to concentration polarisation and by the increased osmotic pressure difference between the permeate and retentate side during the membrane batch concentration. In the case of the NP030 membrane, the membrane permeance was well below 1 LMH bar^{-1}. For this reason, the PES membrane was considered less feasible for practical application with this system. However, its selectivity with respect to global antioxidant activity was further

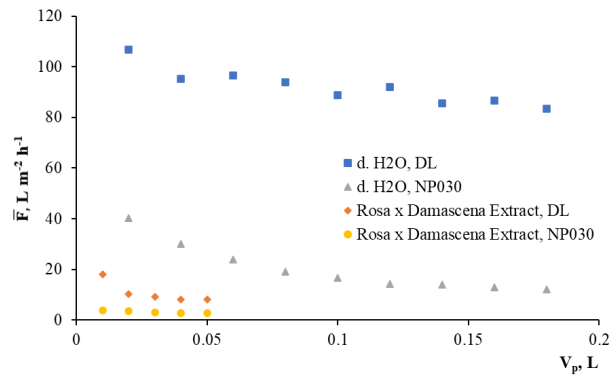

Fig. 2. Permeate flux in LMH (L m^{-2} h^{-1}) versus cumulative permeate volumes for DL and NP030 nanomembranes during conditioning with distilled water and batch filtration of *R. damascena* extract at 10 bar transmembrane pressure.

experimentally characterised along with the one of the polyamide TFC membrane for comparison with previously reported theoretical predictions [3].

Investigation on the antioxidant capacity of the waste *R. damascena* extract and its nanofiltration fractions were carried out to assess their potential applications. As shown in Fig. 3**,** all studied solutions react rapidly with DPPH• in less than 20 min, and then the reaction plateaued. This feature of the reaction kinetics was independent of the membrane applied for nanofiltration (Fig. 3a, b). This also applies to the gallic acid, used as a reference antioxidant compound. To eliminate the impact of the time, and to perform an accurate comparative study of the antioxidant potential of the products, the samples were allowed to react for a standard time of 60 min in all further measurements.

The data for the DPPH• quenching at different concentrations of gallic acid and of the *R. damascena* extract and its nanofiltration fractions obtained by NP030 (Fig. 4a) and DL (Fig. 4b) nanomembranes illustrate that all rose extract solutions possess significant antioxidant capacity. The experimental values for quenched DPPH, corresponding to the gallic acid solution are lower compared to the values for all rose extract solutions, which indicates that a millilitre of any extract solution is more potent antioxidant than a milligram of gallic acid. As expected, the feed and retentate fractions exhibit significantly higher radical scavenging activity compared to the permeates, which is due to the relatively high retention of the antioxidant constituents of the

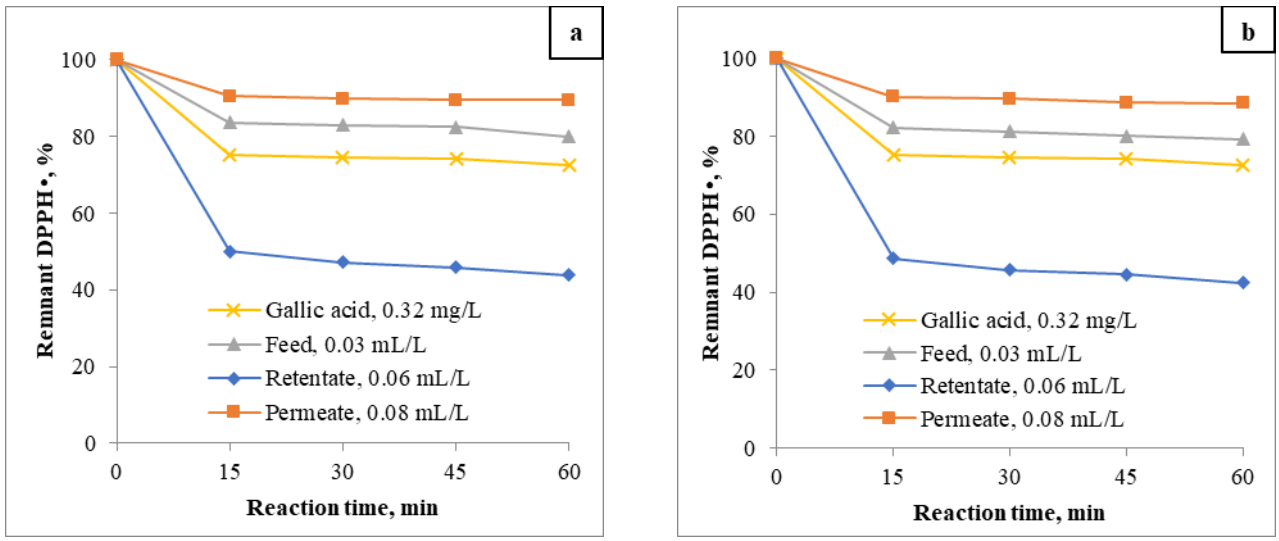

Fig. 3. Representative kinetics of radical scavenging reaction of gallic acid, *R. damascena* extract, and its nanofiltration fractions obtained with NP030 (a) and DL (b) membranes at indicated concentrations in the reaction mixture.

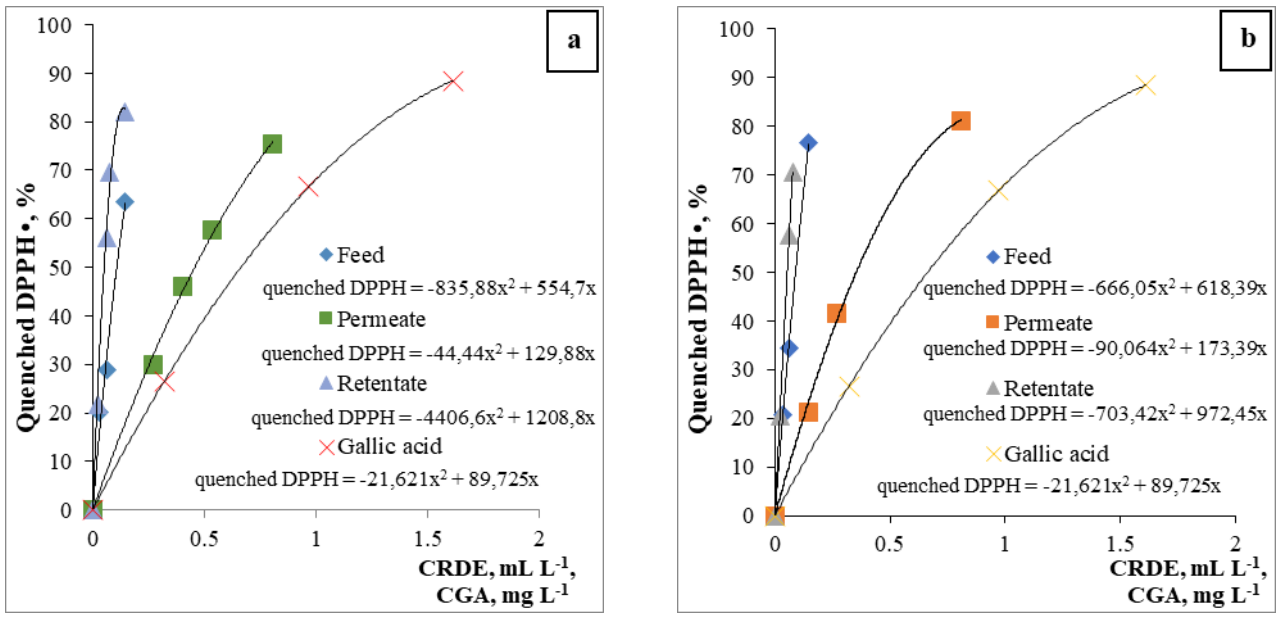

Fig. 4. Experimental data and curve fitting for DPPH• quenching in 60 min by *R. damascena* extract (RDE), its fractions filtrated by NP030 (a) and DL (b) nanomembranes and Gallic acid solution (GA) versus concentration of antioxidant in the samples.

solutions by the membrane. This retention is further quantified via the EC_{50} values.

The experimental data series in Fig. 4 were fitted by a second order polynomials with regression coefficients higher than 0.992 in all cases. The EC_{50} values of *R. damascena* extract and its nanofiltration fractions were calculated using these empirical equations and reported in Table 1. The EC_{50} values calculated at a reaction time of 60 min for each test solution, were used as a practical tool for quantitative assessment of the nanomembrane efficacy to concentrate the polyphenolic fraction contained in the *R. damascena* residual water.

The capability of the nanofiltration membranes to selectively retain the antioxidant components in the residual water was evaluated in terms of membrane rejection coefficients, where the reverse EC_{50} values, or the so-called efficacy indexes ($1/EC_{50}$), were used instead of the absolute concentrations of the target constituent.

Table 1. Data for antioxidant activity of the *R. damascena* fractions and the separation performance of the NP030 and DL membranes, at operating pressure of 10 bar.

№	Membrane	Sample	Antioxidant activity	Membrane rejection, %		Deviation from material balance, %
			$1/EC_{50}$	R_1	R_2	
1	NP030	Feed	9.09	72.5	82.0	6.54
		Permeate	2.50			
		Retentate	18.5			
2	DL	Feed	11.1	77.5	87.5	1.25
		Permeate	2.50			
		Retentate	20.0			

Table 1 summarizes the experimental membrane rejections, calculated in accordance with Eq. 3 and 4, as well as the deviation from the mass balance during the batch filtration (Eq. 5) [15].

$$R_1 = 1 - \frac{Ec_{50}F}{Ec_{50}P} \quad (3)$$

$$R_2 = 1 - \frac{Ec_{50}R}{Ec_{50}P} \quad (4)$$

$$Err = \frac{V_F\left(\frac{1}{Ec_{50}F}\right) - \left[V_P\left(\frac{1}{Ec_{50}P}\right) + V_R\left(\frac{1}{Ec_{50}R}\right)\right]}{V_F\frac{1}{Ec_{50}F}}\,100,\% \quad (5)$$

where V_F, VP, V_R are the volumes of the feed, permeate, and retentate fractions from the batch nanofiltration; $EC_{50}F$, $EC_{50}P$, $EC_{50}R$ are the concentrations of feed, permeate, and retentate solutions respectively in the reaction test solution, necessary to reduce 50 % of the initial DPPH• present. The values for the efficacy indexes (Table 1) indicate that the retentates at DFVR of 2 with NP030 and DL membranes are respectively 2.04 and 1.80 times more potent antioxidants compared to the feed solutions. The corresponding permeates are 7.40 and 8.00 times less active compared to the retentates. Obviously only small fraction of the antioxidant compounds is lost in the permeates.

During batch nanofiltration, the concentrations of the target compounds in the retentate and in the permeate increase with increasing the DFVR under the assumption for a constant membrane rejection coefficient [12]. Therefore, the rejection values calculated from Equations 3 and 4 are dependent on the DFVR coefficient and provide a range within which the real membrane rejection coefficients are contained [16]. Both membranes demonstrate sufficiently high rejection coefficients, which is in line with the data for the efficacy indexes of the nanofiltration fractions. In a previous theoretical study [3] the rejection coefficients of the NP030 and DL membranes were predicted based on regression models and the results supported a concept for application of the nanofiltration for production of a refined polyphenolic fraction, composed primarily of flavonoids, their glycosides and polyphenolic acids. This hypothesis deemed viable as under pH of the solutions over the pKa of the above antioxidant compounds, they are expected to be in dissociated form and the predicted membranes rejections were sufficiently high to permit their concentration. At the same time, low molecular weight and aromatic compounds contained in the extracts would be permeated through the membranes. In this way, the obtained refined polyphenolic fraction has a higher value for application as functional ingredients in different products [3]. Interestingly, the current experimental study is in a good agreement with these theoretical speculations. The range of the experimental rejection coefficients from 73 % to 88 % in this study corresponds well to the theoretically predicted in [3] values for individual key antioxidant constituents of the residual waters. The experimental results also reflect the somewhat higher predicted rejections for the DL membrane in comparison with the NP030 one. The deviation from the mass balance for the integral PES NP030 membrane (6.54 %), indicates that during the nanofiltration, a part of the *R. damascena* extract constituents were adsorbed within the membrane matrix. This „depot“ effect has been previously studied in the

context of application of the NP030 membrane for concentration or fractionation of other liquid systems containing polyphenolic compounds in both batch and steady-state operation modes [16, 17]. It has been demonstrated that this is a dynamic phenomenon, which affects the membrane process performance but does not necessarily prevent its viability. The results in this study showed a negligible „depot“ effect with the DL polyamide TFC membrane, which makes it a better choice for process design due to the lower impact of the concentration of the target solutes on its performance characteristics.

CONCLUSIONS

A previously published hypothesis for the valorisation of the residual water from the hydrodistillation of rose essential oil via nanofiltration is experimentally confirmed in this work. The aim of the membrane separation was to obtain a refined polyphenolic fraction in concentrated form with a composition that allows to be used as a functional ingredient in foods, cosmetics, and nutraceuticals.The conducted batch concentration experiments of *R. damascena* waste extracts with two commercially available nanofiltration membranes, NP030 (Microdyn Nadir) and DL (Veolia), revealed rejection coefficients with respect to global antioxidant activity in the range of 73 - 83 % and 78 - 88 % respectively, which corresponds to previously reported theoretically predicted values for individual key antioxidant constituents of the residual waters, such as flavonoids, their glycosides and polyphenolic acids. A significant amount of the antioxidant components adsorbed into the NP030 membrane matrix, contributing to the so called “depot” effect, which negatively impacts the membrane performance characteristics. In the case of the DL membrane this phenomenon was negligible and the practically viable membrane permeance in the range of 1.8 to 0.5 LMH bar^{-1} with degree of feed volume reduction during batch concentration in the range from 1 to 2, makes it a promising candidate for process design.

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