

Evaluation of reverse osmosis and nanofiltration membranes in concentrating hawthorn fruit and anise seed extract

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ABSTRACT

This study aimed to assess the effectiveness of two reverse osmosis membranes (RO99 and X20) plus one nanofiltration membrane (NF270) at concentrating hawthorn fruit and anise seed extracts. Extracting the anise was done using water at a temperature of 37 °C over a period of 100 min. For hawthorn, ethanol-water (56%) was used as the solvent and extraction occurred at 55 °C for 80 min. The transmembrane pressure (TMP), temperature, and recirculation flow rate of the membrane separation process were monitored and set at 35 bar, 30 °C, and 400 l/h respectively. Using a spectrophotometer, the quantification of valuable compounds was examined. After studying the flow levels, it was discovered that the X20 membrane had the tiniest alterations in permeability, followed by RO99 and NF270. Moreover, in terms of efficiency, the X-20 outperformed RO-99 and NF-270 membranes, where TPC was increased (20 and 18-fold) for anise seed and hawthorn fruit extracts respectively, and TFC was increased 8-fold for both of the extracts. While using NF-270, TPC was increased only (11 and 6-fold), and TFC (4 and 2-fold) for anise seed and hawthorn fruit extracts respectively. For the antioxidant activity, the process using X-20 showed an improvement of around 12-fold for anise extracts and 15-fold for hawthorn extracts for antioxidant activity. In terms of brix, the anise extracts saw a 3-fold increase and the hawthorn extracts saw a 4-fold boost after going through the X-20 membrane concentration process. Additionally, the X-20 membrane exhibits the highest retention rates for both anise and hawthorn extracts and is least affected by fouling during the concentration process.

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KEYWORDS

Pimpinella anisum L. (anise), *C. rataegus monogyna* J. (hawthorn), reverse osmosis, nanofiltration

INTRODUCTION

The Hawthorn (*Crataegus. Monogyna* J.) is one of the most sought-after edible fruits in the world and can be used for making wines, jams, juices, sweet and tinned foods. Medicinal products and functional foods made from it are also effective at treating chronic heart failure and high blood pressure (Liu et al., 2010).

Pimpinella anisum L., otherwise known as anise. It belongs to the Apiaceae family of herbal spices. This family includes over 3,500 species, many of which are acknowledged to possess nutraceutical properties due to their nutritional and medicinal value. Anise is thus often used in the pharmaceutical, cosmetic, flavour, and perfume industries (Sayed-Ahmad et al., 2017).

The study of medical plants begins with pre-extraction and extraction procedures, which are the main steps in the processing of bioactive ingredients from plants. There are many methods used in these extraction and separation processes such as maceration, infusion, and percolation (Alsobh et al., 2022). The ensuing step towards getting these energized constituents is purification and concentration. During the last few years, scientists have focused on membrane technology as an eco-friendly way to refine natural extracts.

Despite all the benefits this technology provides, choosing the right membrane as well as the right operating conditions to prevent membrane fouling are still challenges. The suitable membranes for different kinds of separation are selected based on their pore sizes (Zin et al., 2020). The correlation between membrane efficiency and attributes such as molecular weight, width, pKa, and logP was explored. It is evident that the efficacy of a membrane is determined by the membrane type, the solute, and their combined effect. Temperature, pH, pressure, and concentration are also factors that can affect the degree of rejection (Li et al., 2010). Nanofiltration and reverse osmosis membranes are widely employed in the process of purifying water, specifically for the purpose of desalination. Yet, their application in different areas within the food and beverage industries continues to broaden, primarily as a means of concentrating extracts and juices (Alsobh et al., 2022). This study aimed to assess the effectiveness of nanofiltration and reverse osmosis membranes on the concentration of important components found in Anise seed and hawthorn fruit extracts.

MATERIALS AND METHODS

• Chemicals and reagents

The applied chemicals and reagents for the analysis were collected per following lists:

- Ethanol $\geq 99\%$, (Reanal, Budapest, Hungary),
- Methanol and sodium nitrite (Lach-ner, Neratovice, Czech Republic),
- Citric acid $\geq 95\%$ (Carl Roth GmbH, Karlsruhe, Germany),
- Aluminium chloride 99% and sodium carbonate (Merck, Darmstadt, Germany),



- Sodium acetate (VWR Chemicals, Leuven, Belgium),
- Gallic acid, L-ascorbic acid, quercetin $\geq 95\%$, 2,4,6-Tris(2-pyridyl)-s-triazine $\geq 99\%$, and Folin–Ciocalteu reagent (Sigma-Aldrich, St Saint Louis MO, USA).
- Raw materials and extraction methods

For this experiment, hawthorn fruit was gathered from various trees cultivated in Hungary. Before beginning the extraction process, the sticks were removed and the fruit was washed and cleaned, then wiped down to get rid of any surplus liquid. A GM 200 Pulverizer was used to cut up the fruit into smaller pieces. The extraction of phenolic compounds from these pieces was executed using an OS20-S Electric LED Digital Overhead Stirrer at 55 °C with ethanol 56% and a 10% sample-to-solvent ratio for 80 min. For the anise seed, the extractions were done using water instead as a solvent at 37 °C for 100 min.

Determination of total phenolic content (TPC)

The amount of total phenolic compounds was determined by the Folin-Ciocalteu colorimetric method, based on a procedure established by [Singleton and Rossi \(1965\)](#). Gallic acid was used as the reference standard phenolic compound. The total amount of phenols was colorimetrically quantified at 760 nm. A calibration curve was created using gallic acid dissolved in methanol. The total phenol content was expressed in milligrams of gallic acid equivalents per gram of dry-weight.

Determination of total flavonoids content (TFC)

A colorimetric method was utilized to quantify the flavonoids present. The absorbance of the mixture was assessed at a wavelength of 415 nm via spectrometry. A standard curve was established by making quercetin solutions with concentrations between 12.5 and 100 μg per milliliter in methanol. Total flavonoid content was revealed as quercetin equivalents (milligrams QUE/grams of dry weight).

- Determination of antioxidant activity (AA)

To define the antioxidant activity, a method developed by [Benzie and Strain \(1996\)](#) was used with a few modifications and ascorbic acid to create a calibration curve. Results were expressed in milliequivalents of ascorbic acid/g of dry weight ([Ribeiro et al., 2019](#)).

- Total suspended solids measurement

Total suspended solids (TSS) were measured according to the AOAC method, by filtering a known volume of a sample, drying the filter and captured solids, and then weighing the filter to determine the weight of the captured suspended solids in the sample.

- Membrane separation processes

The Trisep X20 advanced composite membrane, Alfa Laval RO99 membrane as RO membranes, and NF-270 membrane (which is composed of piperazine and benzenetricarbonyl tri-chloride), with a surface area of (0.18 m²) were examined, and the attributes of the used membranes are summarized in [Table 1](#). The cross-flow filtration was completed using DDS Filtration Equipment (LAB 20-0.72, Denmark). The transmembrane pressure (inlet and outlet)



Table 1. The attributes of the used membranes

Membrane	Type	Model	Permeate Flow m3/day (GPD)
X20	X20 Fully Aromatic Polyamide-Urea Advanced Composite Membrane	TRISEP 4040-X20-TSA	9.3 (2,450)
RO99	ACM Fully Aromatic Polyamide Advanced Composite Membrane	RO-3838/30-FF	4.9 (1,300)
NF-270	Polyamide Thin-Film Composite	NF270-2540	3.2 (850)

was 30 bars, the recirculation flow rate 400L/h, and the fluid temperature was kept at 35 °C. For each 200 mL of filtrate collected, the time was recorded to track progress, with samples taken after every 400 mL of permeates. Pure water flux measurements were conducted before and after concentration to measure membrane and fouling resistance. To remove the polarization layer completely, distilled water was used for rinsing once the concentration step concluded.

Based on the concentrations of permeate C_p (mg/L) and retentate C_R (mg/L), retention (%) can be estimated as following equation (Zin et al., 2020).

$$R = \left(1 - \frac{C_p}{C_R}\right) \times 100$$

The fouling index was calculated by comparing the pure water permeability PWP_a (L/m²h) before and pure water permeability PWP_b (L/m²h) after the filtration process as shown by the following equation (Mänttari et al., 2007):

$$\text{Foulin \%} = \left(1 - \frac{PWP_a}{PWP_b}\right) \times 100$$

Results and discussions

- Total phenolic compounds and flavonoids (TPC, TFC)

Total phenolic compounds and flavonoids beheld in the initial extracts of anise seed and hawthorn were (TPC: 28.12 ± 1.93 and 45.31 ± 0.8 mg GAE/g dw), (TFC: 7.56 ± 4.68 and 18.38 ± 0.41 mg QUE/g dw) individually. The examined compounds content increased during the concentration processes, and reached the maximum scavenged amount using X-20 membrane (TPC: 578.85 ± 1.81 and 833.61 ± 0.45 mg GAE/g dw) and (TFC: 62.8 ± 1.93 and 144.57 ± 1.58 mg QUE/g dw). Whilst less amount of TPC, TFC was found in each finale of NF-270 membrane (TPC: 208.5 ± 1.67 and 275.52 ± 2.99 mg GAE/g dw) and (TFC: 31.35 ± 1.23 and 39.31 ± 1.13 mg QUE/g dw) for anise seed and hawthorn extracts.

Significant differences ($P < 0.05$) between the concentrates from the different membranes can be observed in Tables 2 and 3. As mentioned in the tables, TPC of the concentrates from X-20 improved 20-fold for anise extracts and 18-fold for hawthorn extracts while TFC increased around 8-fold for both anise and hawthorn extracts. Meanwhile, the recovered amounts of TPC in NF-270 concentrates went up to 11- and 6-fold along with 4- and 2-fold of TFC for anise and hawthorn extracts, respectively. Our results are consistent with several studies which compared membrane efficiencies in the concentration of plant extracts, in which (Nunes et al., 2019) found that the reverse osmosis (BW30) membrane was most effective in concentrating extracts and



Table 2. Total phenolic content (TPC), Total flavonoids content (TFC), and antioxidant activity (AA)) for the different fractions of streams using the selected membranes X-20, RO-99, and NF-270 for anise seed extracts

		TPC mg GAE/g dw	TFC mg QUE/g dw	FRAP mg AAE/g dw
Initial		28.12 ± 1.93 ^d	7.56 ± 4.68 ^d	8.15 ± 0.44 ^d
Retentate	X20	578.85 ± 1.81 ^a	62.8 ± 1.93 ^a	99.07 ± 0.45 ^a
	RO99	438.40 ± 3.78 ^b	47.17 ± 0.46 ^b	79.47 ± 2.46 ^b
	NF270	208.5 ± 1.67 ^c	31.35 ± 1.23 ^c	43.25 ± 0.56 ^c
Permeates	X20	0.40 ± 0.45 ^g	0.16 ± 0.93 ^f	1.80 ± 0.99 ^f
	RO99	8.8 ± 0.47 ^f	2.03 ± 0.76 ^f	1.26 ± 0.7 ^f
	NF270	25.2 ± 0.53 ^e	5.10 ± 0.88 ^e	4.91 ± 0.33 ^e

^{a–g}: significant differences between TPC, TFC, and FRAP in Initial, Retentate, and Permeate.

Table 3. Total phenolic content (TPC), Total flavonoids content (TFC), and antioxidant activity (AA)) for the different fractions of streams using the selected membranes X-20, RO-99, and NF-270 for hawthorn fruit extracts

		TPC mg GAE/g dw	TFC mg QUE/g dw	FRAP mg AAE/g dw
Initial		45.31 ± 1.93 ^d	18.38 ± 0.41 ^d	31.12 ± 1.55 ^d
Retentate	X20	833.61 ± 0.45 ^a	144.57 ± 1.58 ^a	455.88 ± 0.65 ^a
	RO99	628.63 ± 1.22 ^b	117.27 ± 2.68 ^b	343.79 ± 3.87 ^b
	NF270	275.52 ± 2.99 ^c	39.31 ± 1.13 ^c	78.35 ± 1.45 ^c
Permeates	X20	0.22 ± 0.56 ^g	0.52 ± 0.2 ^g	6.03 ± 1.8 ^g
	RO99	9.00 ± 0.43 ^f	2.43 ± 0.21 ^f	7.47 ± 0.4 ^f
	NF270	27.03 ± 0.6 ^e	7.45 ± 1.01 ^e	20.4 ± 1.4 ^e

^{a–g}: significant differences between TPC, TFC, and FRAP in Initial, Retentate, and Permeate.

that total phenolics and flavonoids in the final concentrates were 15% higher than those achieved with NF270 or NF90. Li et al. (2010) reported a comparative study using nanofiltration and reverse osmosis membranes for phenol removal from synthetic wastewater. A low rejection rate was observed for nanofiltration (0.41–0.72) with maximum flux ($180 \text{ L m}^{-2} \text{ h}^{-1}$), whereas a high rejection rate was observed for reverse osmosis (0.81) with minimum flux ($60 \text{ L m}^{-2} \text{ h}^{-1}$)

• Antioxidant assay

The trend of antioxidant activity is inclined to increase during the concentration processes. The process using X-20 showed around 12-fold and 15-fold of antioxidant activity went up for anise extracts and hawthorn extracts respectively. Whereas, the lowest increase was during the NF-270 process, where the antioxidant activity increased 5-fold for anise extracts, while the increase did not exceed 2-fold for hawthorn extracts measured. On the other hand, it can be seen that there is a significant difference in FRAP values between the three membranes (Tables 2 and 3). Zin et al. (2020) indicated that the antioxidant activity increased around 4.5 times using (X20) membrane, vs around 4 times using RO99 for ethanol-water extracts of beetroot peel and Li et al. (2018) reported that using a nanofiltration membrane to concentrate grape seed extract increased the antioxidant activity around 2.24 times.



- Membrane fouling:

The results show X-20 presents the lowest fouling index, followed by RO-99. In addition, the cleaning step was able to remove the foulants from the reverse osmosis membranes surface and reinstate their efficacy. In comparison to NF-270 membrane, the contamination was irreversible.

Retention percentages were calculated from the concentration data of final permeates and retentates. In X20 membrane concentration processes, TPC and TFC retentions for both anise and hawthorn extracts were >99%, and for antioxidant activity were around 98% for both anise and hawthorn extracts individually. In the case of the RO99 membrane, retentions of TPC, TFC and AA were lower by about 2–4% for both anise and hawthorn extracts. In the NF-270 membrane, the retention of TPC, TFC, and AA was <90% for both anise and hawthorn extracts.

- Total suspended solids (TSS)

The results showed that membrane technology can be used to concentrate anise and hawthorn extracts. Where TSS increased using (X20) membrane to 3.11 °Brix for anise extract and 4.88 °Brix for hawthorn extract, while (2.41°Brix and 3.91°Brix was reached using RO99 membrane for anis and hawthorn extracts, respectively). The lowest TTS was reached using a nanofiltration membrane (NF270) with 1.91, and 3.17 °Brix. for anis and hawthorn extracts respectively. [Molnár et al. \(2012\)](#) reported that raspberry juice concentration with RO membranes had a final concentration of 25°Brix, which is 2.5 times higher than the original juice and twice higher than the final concentrated juice obtained NF membrane. [Hodúr et al. \(2009\)](#) mentioned that concentration by RO resulted in an increase of the TSS content to 4.28 °Brix; for NF the corresponding level was 8.88 °Brix.

CONCLUSIONS

This research paper has studied the potential of reverse osmosis and nanofiltration membranes to concentrate bioactive compounds from anise seed and hawthorn extracts. To our knowledge, no previous work has mentioned the concentration of these extracts before. For this study, two reverse osmosis (X20, RO-99), and one nanofiltration (NF270) were evaluated membrane in terms of its ability to recover out phenolic compounds and flavonoids. The X20 membrane was revealed as the most suitable for large-scale production due to its 99% removal of phenolic and flavonoid compounds. This resulted in an increase of 20 and 18 times for anise seed extract and hawthorn fruit extract, respectively, with regard to total phenolic content (TPC). The total flavonoid content (TFC) also experienced a boost of 8 times for both extracts. In comparison, nanofiltration membranes led to less than 80% removal of phenolic compounds and flavonoids. This only produced an 11-fold and 6-fold rise in TPC for anise seed and hawthorn fruit extracts, respectively. There were just 4-fold and 2-fold increases in TFC for those same extracts.

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