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INNOVATIVE EXTRACTION AND CONCENTRATION TECHNIQUES FOR BIOACTIVE COMPOUNDS OF HAWTHORN FRUIT AND ANISE SEED

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1 INTRODUCTION

Plants and their active ingredients have attracted people for many years. People have used plants to treat many diseases and relieve pain, and using plants for these purposes is as old as humanity. Moreover, the connection between people and their search for drugs in nature dates from the far past. For centuries, plants have been of great interest to humans as flavors, fragrances, dyes, preservatives, and pharmaceuticals. Today, medicinal plants are of great importance due to their significant properties as a great source of therapeutic phytochemicals that may lead to new drug development. Much research indicates that most phytochemicals from plant sources such as phenols and flavonoids have a positive effect on health and cancer prevention, treatment of diabetes, and cardiovascular, in addition to their role against bacteria and pathogens.

Extraction is the first step of any medicinal plant study and plays a significant and crucial role in the final result and outcome. Extraction methods are sometimes referred to as ‘‘sample preparation techniques’’. There are many factors affecting extraction processes the most common are matrix properties of the plant part, solvent, temperature, pressure, and time. As a result of an increased understanding of the chemical nature of the diverse bioactive molecules, and the huge technological and technical improvements in bioactive compounds extraction and analysis, pharmaceuticals, food additives, and even on natural pesticides sectors have become interested in bioactive molecules from natural sources.

Bioactive compounds can be found and characterized in various plant parts such as leaves, stems, flowers, and fruits. Extraction of plant materials can be done by various extraction procedures. Non-conventional methods, which are more environmentally friendly due to decreased use of synthetic and organic chemicals, reduced operational time, and better yield and quality of extract, have been developed. Today, non-conventional techniques are used to enhance the overall yield and selectivity of bioactive components from plant materials such as ultrasound, pulsed electric field, enzyme digestion, extrusion, microwave heating, ohmic heating, supercritical fluids, and accelerated solvents. At the same time conventional extraction methods, such as Soxhlet, maceration, infusion, percolation, and decoction.

The second steps in obtaining these active substances are purification and concentration; for instance, the crude extracts from solvent extraction are unusable immediately, and intensive treatment such as purification or refining

is required. Achieving the usability of a plant-based material involves concentrating on the desired products and removing unwanted materials alongside separating products from an organic solvent. Therefore, making an extracted plant material usable is, generally, the most challenging aspect of producing natural compounds. The conventional purification approaches include distillation, evaporation to remove solvents, or the usage of additives such as caustic for oil refining processes. Distillation requires a significant amount of energy. Adding chemicals such as caustics to crude extracts can also lead to undesirable results, including molecular cross-linking and rearrangements resulting in a decrease in the formation of toxic compounds. Furthermore, from an environmental point of view, conventional processes of obtaining active substances from plants consume large amounts of water and chemicals and create heavily contaminated effluents (Sereewatthanawut et al., 2018).

In recent years, researchers have paid a lot of attention to membrane technology, and they have considered it an environmentally benign technology for purifying natural extracts. For two decades, researchers have used various membrane-based technologies to separate, restore and concentrate bioactive compounds (such as phenolic compounds, anthocyanins, carotenoids, antioxidants, and polysaccharides) from Agri-Food products and their derivatives (such as wastewater), clarification and concentration of natural extracts, recovery of odors from natural and processed products, production of non-alcoholic beverages. In other words, membrane technologies represented a viable alternative to conventional techniques due to the low operating and maintenance costs, moderate operating conditions of temperature and pressure, ease of control and expansion, and highly selective separation. In particular, pressure-driven membrane processes, such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO).

1.1 Objectives

Hawthorn (*Crataegus. monogyna* jacq.) is one of the most popular edible fruits and has been used to make wines, sweet or tinned foods, as well as jams and juices. It is also used to make medicinal products and functional foods for the treatment of chronic heart failure and high blood pressure. The antioxidant capacity of phenolic compounds present in different parts of the hawthorn has been reported in several studies. Research on anise seed (*Pimpinella anisum* L.) content and its biological activities has shown antidiabetic, antimicrobial, analgesic, and antioxidant properties due to its content of lipids and fatty acids,

proteins, amino acids, and reducing sugars. For this reason, it is taken into consideration as a promising supply of phenolic compounds. The major focal aim of this study is to valorize polyphenolic compounds from hawthorn fruit and anise seed. Accordingly, the following factors are to be inquired about:

- Extracting active compounds from plants requires a technique that takes into account the nature of the plant, the properties of the target compounds, and their association with plant tissues.
- Several factors influence the extraction of bioactive compounds, including the solvent type and polarity, the particle size of the plant materials, the solvent-to-solid ratio, the extraction temperature, and the extraction duration.
- A membrane technology is considered to be an environmentally friendly and effective method of concentrating plant extracts.
- Selecting the appropriate membrane to concentrate any plant extract is an important step in achieving the highest possible concentration while maintaining the membrane's properties and preventing contamination.
- Hawthorn and anise are considered medical herbal plants and sources of bioactive compounds.

2 MATERIAL AND METHODS

2.1 Heat-assisted-extraction (HAE)

The HAE process was carried out by (OS20-S Electric LED Digital Overhead Stirrer). The hawthorn fruits *(C. monogyna* Jacq.*)* were extracted with an aqueous ethanol solution, while pure water was used as a solvent for the extraction of anise seed *(Pimpinella anisum* L.*)*. Response surface methodology based on central composite design (RSM-CCD) was employed to analyze the influence of HAE parameters (independent variables) on the extraction yields of target compounds (response variables) and to optimize them. The RSM-CCD consisted of 20 randomized experimental runs including six replicates in the center point. Independent variables and experimental ranges for HAE were for hawthorn fruit: ethanol concentration (10 - 90 % v/v), extraction temperature (30 – 60 °C), and extraction time (10 – 90 min), for anise seed: extraction temperature (25 – 55 °C), extraction time (20 – 100 min), and sample to solvent ratio (2 – 10 g/100 mL).

With the aim of finding the solvent which extracts the highest content of polyphenol and flavonoid compounds extraction from anise seed, seven solvents were examined: absolute ethanol, absolute methanol, absolute isopropanol, ethanol (50 % v/v), methanol (50 % v/v), isopropanol (50 % v/v) and pure water. Extraction was carried out using (HAE) at 40 °C for 20 minutes with 10 g/100 mL of the sample-to-solvent ratio.

In order to evaluate and compare phenol and flavonoid content and antioxidant activity of three species of hawthorn fruit (*C. monogyna* Jacq., *C. pinnatifida* Bge, and *C. crus-galli* L.). the extraction was carried out using the HAE method. The extraction processes were performed at 45 °C, by using ethanol 50 % v/v as a solvent with 10 g of the fruit in 100 mL solvent for 50 min.

2.2 Microwave-assisted-extraction (MAE)

MAE experiments were performed with the central composite design with three numeric factors at three levels which consisted of twenty randomized runs with six replicates in the central point. Investigated independent MAE factors were microwave power (100, 450, and 800 W), extraction time (20, 70, and 100 seconds), and sample-to-solvent ratio (2, 7, and 12 g/100 mL). (MAE) extraction was carried out using (Specs Electrolux EMM 2005) oven using ethanol-aqueous solution (60 % v/v) for hawthorn fruit, while pure aqueous solution was used for anise seed. The microwave treatments were

performed with intermittent mode (40 s on 20 s off, 20 s on 20 s off) and ice water was used to cool the sample between microwave treatments, which prevented the superheating effect and evaporation loss.

2.3. Ultrasound-assisted-extraction (UAE)

The ultrasound-assisted extraction (UAE) was carried out by power ultrasound (3.5 W/cm^2, 20 kHz) produced by a generator (Weber ULC 400 Premium Ultrasonic Generator). A three-level design with three variables was utilized to obtain the optimized extraction condition. The RSM-CCD consisted of 20 randomized experimental runs including six replicates in the center points. Independent variables and experimental ranges for UAE were for hawthorn fruit: ethanol concentration (20, 30, and 40 % v/v), extraction time (5, 10, and 15 min), and sample-to-solvent ratio (2, 7, and 12 g/100 mL), for anise seed: ethanol concentration (0, 10, and 20 % v/v), extraction time (5, 10, and 15 min), and sample-to-solvent ratio (2, 7, and 12 g/100 mL). To stabilize the heat distribution throughout the treatments, an icy water bath was used maintaining the temperature around 25 ºC.

2.4 Anthocyanidins extraction

In order to know the effect of extracting solvent and methods on the anthocyanidins extraction process from hawthorn fruit *(C. monogyna* Jacq.), three methods: ultrasound, microwave, and heat-assisted extraction together with three solvents (methanol, ethanol, and isopropanol) have been compared. The working solvents were prepared by mixing 80 % (v/v) of each organic solvent, 19.9 % (v/v) of water, and 0.1 % (v/v) of hydrochloric acid (HCl). After that, each solvent was diluted with pure water to a concentration of 50 % (v/v) before being used for extraction.

The HAE process was performed by (OS20-S Electric LED Digital Overhead Stirrer) at 65 °C for 30 min by using the prepared solvents and 10 g of the fruit in 100 mL of the solvent. Microwave extractions were accomplished by a microwave oven (Specs Electrolux EMM 2005) at 800 W of microwave power. Pulse mode, and ice water was used to cool the sample between microwave treatments to avoid superheating and evaporation of the solvent. 40 s on 20 s off followed by 20 s on 20 s off (till the time was up (10 min)). The UAE was performed by power ultrasound (3.5 W/cm^2, 20 kHz) produced by a generator (Weber ULC 400 Premium Ultrasonic Generator) with a treatment time of 30 min.

2.5 Membrane separation

To determine the best membrane to concentrate hawthorn fruit and anise seed extracts, the cross-flow filtration process was performed by DDS Filtration Equipment (LAB 20-0.72, Denmark). RO membranes of low fouling type Trisep X-20 advanced composite membrane (Microdyn), thin film composite Alfa Laval RO99 membrane, and NF 270 membrane made from piperazine and benzenetricarbonyl trichloride with active surface areas of 0.18 m^2 were evaluated. The transmembrane pressure difference was 30 bars and the recirculation flow rate was 400 L/h maintaining the temperature of the stream at 35 °C, the filtration processes were completed once the volumetric reduction ratio (VRR) reached 3. The extraction was accomplished according to the best conditions that were determined previously by a single batch type extractor which was designed with a thermostat water bath (LAUDA COLINE E100) and (OS20-S Electric LED Digital Overhead Stirrer). For hawthorn, the extraction conditions were 55 °C, with 56 % v/v ethanol solvent and 10 g fruit in 100 mL of the solvent for 80 min. For anise seed, the extractions were completed using pure water as solvent at 37 °C for 100 min.

Later, based on the previous evaluation of the efficiency of the three membranes and the capability of the experimental set-up, the X-20 membrane, and operating variables were selected within the following ranges: temperature 25 – 45 °C, and TMP 20 – 40 bar. The flow rate was set up at 600 L/h, and the volumetric reduction ratio VRR at 4. The response surface methodology (RSM) was applied to evaluate the effects of reverse osmotic filtration parameters and optimize various conditions for different responses. Central composite design (CCD) was applied and included 11 randomized runs with 3 replicates in the central point.

In every concentration process, pure water flux measurements were performed before and after the concentration step in order to estimate membrane resistance and fouling resistance. After the concentration, distilled water was used for rinsing and removing the polarization layer completely.

The different extracts were analyzed by Spectronic GENESYS 5 (MILTON ROY, U.S.A) spectrophotometer, in which TPC was analyzed by Folin's method; TFC was analyzed by aluminium chloride assay; AA was determined by (FRAP) ferric reduction antioxidant power, (DPPH) 2,2-diphenyl-1-picryl-hydrazyl-hydrate, and (ABTS) 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) methods; TMA was determined using the pH-differential

method. All measurements were triplicated and averaged. Dry wight-based percentages were calculated.

3 RESULTS

For hawthorn fruit extraction, heat-assisted extraction was carried out using three variables, as mentioned in the materials and methods section (2.1). RSM with CCD has been developed to approach the optimum process condition through the interaction between the different variables and the experimental outcomes. The supreme amounts of TPC (80.65 ± 6.08 mg GAE/g dw) were recovered under the operating variables of 50 % v/v of ethanol concentration, 40 °C, and 90 min of extraction time, the highest amounts of TFC (19.93 ± 1.68 mg QUE/g dw) were obtained at 90 % v/v of ethanol concentration, 60 °C, and 90 min of extraction time. While like TPC the highest AA was found in the extract of HAE at 50 % v/v of ethanol concentration, 50 °C, and 45 min of extraction time, where the values were 35.29 ± 3.12 mg AAE/g dw, 24.43 ± 2.4 %, and 51.58 ± 5.09 % for FRAP, DPPH, and ABTS respectively. The positive linear effect of solvent concentration, extraction temperature, and extraction time was found to be significant for all response variables. However, the quadratic effect of extraction temperature was only found to produce significant ($p < 0.0001$) negative effect on TPC and ($p < 0.5$) on FRAP antioxidant activity. The interaction effect of the three studied variables was not found to be significant for all responses. The ANOVA for the lack of fit test indicates that the model could adequately fit the experimental data ($p < 0.05$) for all response variables.

MAE experiments were performed with the central composite design with three numeric factors at three levels which consisted of twenty randomized runs (materials and methods section 2.2). The operating condition optimized predominantly by the model depending on the maximum yields of targeted compounds, where the highest experimental values of extracted (TPC: 54.11±5.93 mg GAE/g dw), (TFC: 12.82 ± 1.55 mg QUE/g dw), and antioxidants (24 ± 3.11 mg AAE/g dw) by FRAP were observed in the extract of MAE at 450 W for 70 s with 7 g/100 mL of sample-to-solvent ratio. 21.61 ± 2.74 % and 43.75 ± 5.12 % of DPPH and ABTS were found in the extract of MAE at 800 W for 120 s with 12 g/100 mL of sample-to-solvent ratio. The obtained regression coefficients demonstrated a positive linear effect of microwave power, extraction time, and sample-to-solvent ratio were found to be significant for all response variables. In addition, the quadratic effect of microwave power and extraction time was found to produce a negative significant effect on all the responses. The interaction effect of the three studied variables was only found to be significant for DPPH and ABTS. The

ANOVA for the lack of fit test indicates that the model could adequately fit the experimental data ($p < 0.05$) for all response variables.

In the case of (UAE), RSM with a three-level design with three variables was utilized to obtain the optimized extraction condition. The independent variables were ethanol concentration (% v/v) extraction time (min), and sample-to-solvent ratio (g/100 mL) as was mentioned in materials and methods section (2.3). The operating condition optimized predominantly by the model depending on the maximum yields of targeted compounds was; 30 % v/v ethanol concentration, 10 min of extraction time, and 12 g/100 mL of sample-to-solvent ratio. Under this processing condition, the experimental outcomes were TPC (87.1 ± 5.42 GAE mg/g dw), TFC (29.87 ± 2.09 mg QUE/g dw), FRAP (38.78 ± 2.51 mg AAE/g dw), DPPH (33.79 ± 2.26 %), and ABTS (66.15 ± 6.91 %) respectively in the evaluated range. The obtained regression coefficients demonstrated a linear effect of solvent concentration, extraction time, and sample-to-solvent ratio were found to be significant for all response variables. In addition, the quadratic effect of solvent concentration, and extraction time was found to produce a negative significant effect on all the responses. The interaction effect of the three studied variables was found to be significant for all the responses except the TFC. The ANOVA for the lack of fit test indicates that the model could adequately fit the experimental data ($p < 0.05$) for all response variables.

The efficiency of the extraction method from hawthorn fruit was in order UAE > HAE > MAE. In addition, using UAE reduced used-ethanol concentration by around 50 % compared to both other extraction methods, and reduced the extraction time by 90 % compared to HAE, also UAE was carried out at room temperature.

- In order evaluation and compare phenol and flavonoid content and antioxidant activity of three species of hawthorn fruit (*C. monogyna* Jacq., *C. pinnatifida* Bge, and *C. crus-galli* L.). the extraction was carried out using the HAE method at 45 °C, by using ethanol 50 % v/v as a solvent and 10 g of the fruit in 100 mL solvent) for 50 min. The total levels of phenols and flavonoids in extracts of hawthorn species were in the following order (*C. crus-galli* L. > *C. pinnatifida* Bge. > *C. monogyna* Jacq.). Total phenols in the extracts were ranked from 54.66 ± 0.62 to 86.83 ± 0.34 mg GAE/g dw, the total flavonoids ranged from 11.85 ± 0.41 to 32.67 ± 0.42 mg QUE/g dw, and AA by FRAP ranged from 76.67 ± 0.14 to 99.83 ± 0.04 mg AAE/g dw. According to the results of MANOVA,

there is a significant difference between the three species of hawthorn at the 95 % confidence interval. As well as according to the bivariate correlation test, there was a positive correlation between the antioxidant activity index and the total content of phenolic and flavonoids of ethanolic extracts ($r = 0.982$, $r = 0.895$) respectively.

- With the aim to know the effect of extracting solvents and methods on the anthocyanins extraction process from hawthorn fruit, three methods: ultrasound-, microwave-, and heat-assisted extraction together with three solvents (methanol, ethanol, and isopropanol) have been compared. The results showed that the maximum amount of TMA (0.152 ± 0.002 mg CGE/g dw) was obtained via UAE technique using methanol solvent while was (0.125 ± 0.007 mg CGE/g dw, 0.107 ± 0.007 mg CGE/g dw) using MAE and HAE as the extraction methods and methanol as solvent. Likewise, Hawthorn fruit extracts prepared with the ultrasonic method with various solvents were characterized with darker colour compared with both method of microwave and conventional with the same solvents, as the L* values were noted less for UAE with methanol (42.14 ± 0.19), ethanol (43.89 ± 0.23), and isopropanol 45.83 ± 0.015) solvents respectively. Increased a* (redness) and decreased b* (blueness to yellowish) characteristics indicated the red colour of the hawthorn fruit extract with a purple shade. Escalated a* values of UAE were 24.56 ± 0.45, 22.94 ± 1.16, and 20.09 ± 0.29 for methanol, ethanol, and isopropanol indicating more intense colour than MAE (18.05 ± 0.55, 17.78 ± 0.02, and 17.25 ± 0.6) and HAE (8.3 ± 0.2, 6.91 ± 0.14, and 3.25 ± 0.5). The extracts using methanol solvent via UAE showed significantly ($p < 0.05$) greater amounts of TPC (49.14 ± 0.38 mg GAE/g dw) and TFC (18.38 ± 0.19 mg QUE/g dw) compared to other extraction methods and applied solvents. Whilst, the lowest TPC (24.76 ± 0.27 mg GAE/g dw) and TFC (7.06 ± 0.48 mg QUE/g dw) were found using Isopropanol solvent and HAE. the percentage of inhibition of methanolic extracts of hawthorn using UAE, MAE, and HAE was slightly higher ($p < 0.05$) than that of ethanolic and isopropanol extracts by all of AA (FRAP, DPPH, and ABTS) assays. In addition, the UAE extraction method outperformed both MAE and HAE using the same solvents. AA values of the fruit extracts by UAE with methanol solvent are as follows: (FRAP = 250.24 ± 1.46 mg AAE/g dw, DPPH = 157.32 ± 0.39 %, and ABTS = 200.28 ± 0.39 %) while those values decreased to 240.13 ± 0.82 mg AAE/g dw (FRAP); 153.42 ± 0.95 and 83.33 ± 1.17 % measured by DPPH and (ABTS) via MAE. Followed

by, the least amounts of AA were detected by methanolic HAE as FRAP = 162.32 ± 0.93 mg AAE/g dw, DPPH = 130.05 ± 1.0 %, and ABTS = 151.46 ± 0.9 %, respectively. In addition, the Pearson correlation analysis approach established a strong positive linear correlation between TPC, TFC, and radical scavenging assays (DPPH, ABTS) of Hawthorn extracts [TPC-DPPH: r = 0.924, TPC-ABTS: r = 0.95], [TFC-DPPH: r = 0.929, TFC-ABTS: r = 0.946]. Meanwhile, the correlation was lower between the bioactive compounds and radical scavenging assay (FRAP) [TPC-FRAP: r = 0.627, TFC-FRAP: r = 0.595].

For anise seed extraction, The extraction variables for HAE extraction from anise seed included extraction temperature (A, °C), extraction time (B, min), and sample-to-solvent ratio (C, g/100 mL) as mentioned in the material and methods section (2.1). The utmost amounts of TPC (39.66 ± 3.37 mg GAE/g dw) and AA (FRAP: 5.69 ± 0.41 mg AAE/g), (DPPH: 5.92 ± 0.47 %), and (ABTS: 1.47 ± 0.12 %) were obtained at 40 °C with 6 g/100 mL of the sample-to-solvent ratio after 100 min of the extraction time. Meanwhile, the highest amount of TFC (8.78 ± 0.65 mg QUE/g dw) was examined at 25 °C, 10 g/100 mL of sample-to-solvent ratio after 100 min of the extraction time. The positive linear effect of extraction time, and a negative linear effect of extraction temperature were found to be significant for all response variables, while the effect of sample-to-solvent ratio (C) was only found to TFC. The quadratic effect of extraction temperature was found to produce significant ($p < 0.001$) negative effects on TFC, FRAP, DPPH, and ($p < 0.0001$) on TPC and ABTS, and the quadratic effect of sample-to-solvent ratio was only found to be negatively significant to TFC, FRAP, and DPPH. The interaction effect of extraction time and sample-to-solvent ratio was found to be significant for TFC ($p < 0.001$), and the interaction effect of extraction temperature and extraction time has a negative effect on FRAP ($p < 0.05$). The ANOVA for the lack of fit test indicates that the model could adequately fit the experimental data ($p < 0.05$) for all response variables.

For (MAE), the optimization experiment was applied using response surface methodology (RSM) to optimize three critical operating variables-three levels (microwave power, extraction time, and sample-to-solvent ratio) in order to achieve the maximal content of particular groups of bioactive compounds, as mentioned in the material and methods section (2.2). The supreme amounts of bioactive compounds from anise seed were recovered under the operating variables of 450 W of microwave power, 120 s of irradiation time, and 7 g/100 mL of the sample-to-solvent ratio. The recovered

amounts of TPC, TFC, FRAP, DPPH, and ABTS are 50.54 ± 3.26 mg GAE/g dw, 21.67 ± 1.62 mg QUE/g dw, 11.16 ± 0.39 mg AAE/g dw, 17.36 ± 1.47 %, and 4.23 ± 0.42 % respectively. The obtained regression coefficients demonstrated a positive linear effect of microwave power, extraction time, and sample-to-solvent ratio were found to be significant for all response variables. In addition, the quadratic effect of microwave power, and sample-to-solvent ratio (C^2) was found to produce a negative significant effect on all the responses. The interaction effect of the three studied variables was only found to be significant for ABTS. The ANOVA for the lack of fit test indicates that the model could adequately fit the experimental data ($p < 0.05$) for all response variables.

UAE was performed using 20 kHz and 3.5 W/cm^2 (Weber ULC 400 Premium Ultrasonic Generator) with different solvent concentrations and extraction times as was mentioned in section (2.3) of material and methods. The highest values of recovered TPC (43.26 ± 1.65 mg GAE/g dw), TFC (16.24 ± 0.69 mg QUE/g dw), and antioxidants (8.62 ± 0.33 mg AAE/g dw) by FRAP, and 2.64 ± 0.13 % by ABTS were observed at 10 % v/v of ethanol concentration for 10 min of the extraction time with 12 g/100 mL of sample-to-solvent ratio. While 14.98 ± 0.85 % of DPPH was found in the extract at 20 % v/v of ethanol concentration for 15 min of the extraction time with a 12 g/ 100 mL of sample-to-solvent. The obtained regression coefficients demonstrated a linear and quadratic effect of solvent concentration, and extraction time on all the responses, while sample-to-solvent ratio has only a linear significant effect for all response variables except ABTS which was not influenced by sample-to-solvent ratio. The interaction effect of the ethanol concentration and extraction time was found to be only significant for DPPH. The ANOVA for the lack of fit test indicates that the model could adequately fit the experimental data ($p < 0.05$) for all response variables.

The efficiency of the extraction method from anise seed was in order MAE > UAE > HAE. In addition, the results show that increasing ethanol concentration by up to 14 % can enhance the extraction of flavonoids by around 50 % using UAE compared to using pure water and HAE, and reduced the time by around 90 %.

- With the aim of finding the solvent which extracts the highest content of polyphenol and flavonoid compounds extraction from anise seed, seven solvent were examined: absolute ethanol, absolute methanol, absolute isopropanol, ethanol (50 % v/v), methanol (50 % v/v), isopropanol (50 %

v/v) and pure water as mentioned in the materials and methods section. The total phenolic and flavonoid content (TPC), (TFC) of the seed extracts was measured using Folin-Ciocalteu's colorimetric method. TPC ranged from 17.57 ± 0.65 GAE/g dw to 43.84 ± 0.39 GAE/g dw, while TFC ranged from 8.69 ± 0.85 QUE/g dw to 17.22 ± 0.82 QUE/g dw. In addition, there are significant differences in the content of phenolic and flavonoids using different solvents, where the highest amount of phenolics and flavonoids were found in 50% methanol extract followed by absolute methanol, while the lowest amount of phenolics was in the absolute Isopropanol extract. Accordingly, the percentage of inhibition of extracts of anise using absolute methanol and methanol (50 % v/v) was slightly higher ($p < 0.05$) than that of ethanolic, isopropanolic, and wateric extracts by all AA (FRAP, DPPH) assays. Obtained results showed that there is a high correlation between total phenolics and flavonoid contents with ferric ion reduction [TPC-FRAP: r = 0.989, TFC-FRAP: r = 0.886] and [TPC-DPPH: r = 0.994, TFC-DPPH: r = 0.867].

- **For membrane concentrations**, RO membranes of low fouling type Trisep X-20 advanced composite membrane (Microdyn), thin film composite Alfa Laval RO99 membrane, and NF270 membrane made from piperazine and benzenetricarbonyl trichloride with active surface areas of 0.18 m^2 were applied. 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: 64.31 ±1.81 and 92.62 ± 0.45 mg GAE/g dw) and (TFC: 20.93 ±1.93 48.19 ± 1.58 mg QUE/g dw). Whilst less amount of TPC, TFC was found in each finale of NF-270 membrane (TPC: 34.74 ±1.67 and 45.92 ± 2.99 mg GAE/g dw) and (TFC: 10.45 ± 1.23 and 19.65 ±1.13 mg QUE/g dw) for anise seed and hawthorn extracts.

TPC of the concentrates from X-20 improved 2.3-fold for anise extracts and 2-fold for hawthorn extracts while TFC increased around 2.5-fold for both anise and hawthorn extracts. Meanwhile, the recovered amounts of TPC in NF-270 concentrates went up to 1.3 and 1- fold along with 1.4 and 1-fold of TFC for anise and hawthorn extracts, respectively. likewise, the process using X-20 showed around 2-fold and 2.4-fold of antioxidant activity (FRAP) went

up for anise extracts and for hawthorn extracts whereas around antioxidant activity measured by the DPPH method increased 1.5-fold for both anise and hawthorn extracts. The lowest increase was during NF-270 process, where the antioxidant activity increased 1.2-fold and 1-fold by the FRAP and DPPH methods for anise extracts, while the increase did not exceed 1-fold for hawthorn extracts measured by both methods.

In addition, the flux of anise extract reached 4.61 (L/(m^2·h)) at VRR = 3 after about 57 minutes of concentration time using (NF270) membrane whereas the permeate fluxes of 5.5 (L/(m^2·h)) and 9.7 (L/(m^2·h)) were revealed after 57 minutes and 42 minutes of concentration times by RO99 and X-20 membranes. While for hawthorn extracts it took more than 1.5 hours for the permeate flux to reach VRR = 3 with a flux of 3.02 (L/(m^2·h)) using an NF-270 membrane. In the case of X-20 membrane, one hour time was enough to reach the same level of VRR with a flux velocity of 6.6 (L/(m^2·h)). X-20 shows the lowest fouling index, followed by RO99. In addition, the cleaning step was able to remove the foulants from the reverse osmosis membranes surface and reinstate their efficacy. In comparison to NF270 membrane, the contamination was irreversible. At the same time, using an X-20 membrane, TPC and TFC retentions for both anise and hawthorn extracts were > 99 %, and for antioxidant activity were around 98 % (using both of FRAP and DPPH assay) for 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 NF270 membrane, the retention of TPC, TFC, and AA was < 90 % for both anise and hawthorn extracts.

Optimization of membrane concentration processes, two operating variables were selected based on the capability of the experiment within the following ranges: temperature 25 – 45 °C, and TMP 20 – 40 bar for both hawthorn fruit and anise seed as was mentioned in section (2.5) of materials and methods.

The operating condition optimized by the model depended on the maximum recovery of targeted compounds from **hawthorn fruit extracts** and the highest permeate flux was found at (T = 35 °C and TMP = 40 bar). Under this processing condition, the recovered outcomes are TPC (117.51 ± 3.62 mg GAE/g dw), TFC (19.65 ± 0.44 mg QUE/g dw), FRAP (63.93 ± 2.51 mg AAE/g dw), DPPH (20.86 ± 0.36 %), ABTS (74.22 ± 1.5 %), and permeate flux (6.6 4 ± 0.52 (L/(m^2·h))) individually. In comparison the lowest fouling index (27.71 ± 1.20 %) was found at (T= 45 °C and TMP = 40 bar), the lowest

membrane resistance and fouling resistance ($1.66 \cdot 10^{14} \pm 8.62 \cdot 10^{12}$ 1/m, and $2.45 \cdot 10^{13} \pm 4.25 \cdot 10^{12}$ 1/m) were found at (T = 25 °C and TMP = 20 bar, T= 35 °C and TMP = 20 bar) respectively in the evaluated range. According to p values of regression coefficients, the linear term of the temperature had a negative significant ($p < 0.01$) influence on TPC, TFC, FRAP, and DPPH, and had more effect on ABTS ($p < 0.001$), while the quadratic term of temperature had a highly negative significant influence ($p < 0.0001$) on all responses. Likewise, the linear term of applied transmembrane pressure TMP had a highly positive effect ($p < 0.0001$) on all responses. While the quadratic term of TMP had a higher effect on ABTS ($p < 0.001$), and less effect on TPC, TFC, FRAP, and DPPH ($p < 0.01$, and $p < 0.05$). The linear coefficients of TMP were found to be the most significant effect to increase the permeate flux ($p < 0.001$), followed by the linear effect of temperature ($p < 0.05$). On the other hand, the quadratic coefficient of temperature produces a decrease in the permeate flux with a significant effect ($p < 0.05$). The interaction factors and the quadratic effect of TMP do not produce a significant effect ($p > 0.05$) in the permeate flux. Meanwhile, TMP was found to be the most significant effect in decreasing the fouling index ($p < 0.0001$), followed by temperature) ($p < 0.001$), and then the interaction factor between TMP and temperature factor ($p < 0.01$), meanwhile, the quadratic term of temperature has a significant effect to increase the fouling index ($p < 0.01$). While the quadratic term of TMP does not produce a significant effect ($p > 0.05$) in the fouling index. The quadratic coefficients of temperature (A^2), and the interaction factors (AB) were found to have a significant effect in increasing the fouling resistance ($p < 0.0001$). On the other hand, the linear coefficient of temperature (A) produces a decrease in the fouling resistance with a significant effect ($p < 0.01$). The linear and quadratic coefficients of TMP (B, and B^2) do not produce a significant effect ($p > 0.05$) in the fouling resistance. A linear model was obtained for membrane resistance, and was found that the temperature (A) was have the greatest effect on the increasing membrane resistance ($p < 0.0001$), while TMP (B) had no significant effect on it ($p > 0.05$)

In the case of **anise seed extracts**, the best conditions were found at (T = 35 °C and TMP = 40 bar), where the amount of recovered TPC, TFC, and their Antioxidant activity by FRAP, DPPH, and ABTS under these conditions are (92.86 ± 3.33 mg GAE/g dw), (9.73 ± 0.35 mg QUE/g dw), (13.75 ± 0.46 mg AAE/g dw), (8.87 ± 0.32 %), and (4.4 ± 0.21 %) respectively, as well as the highest permeate flux (15.06 ± 1.33 (L/(m^2·h))), In comparison the lowest fouling index (23.79 ± 2.40 %) was found at (T = 35 °C and TMP = 40 bar), the lowest membrane resistance and fouling resistance ($1.06 \cdot 10^{14} \pm 9.38 \cdot 10^{12}$

1/m, and $4.78 \cdot 10^{13} \pm 8.53 \cdot 10^{12}$ 1/m) were found at (T = 25°C and TMP = 20 bar, T= 35°C and TMP= 30 bar) respectively in the evaluated range. According to p values of regression coefficients, the linear term of the temperature had a negative significant ($p < 0.01$) influence on TPC, and all AA assays, and had more effect on TFC ($p < 0.001$), while the quadratic term of temperature had a highly negative significant influence ($p < 0.0001$) on TPC, TFC, and AA assays except for ABTS which was less affected with ($p < 0.001$).The linear term of applied transmembrane pressure TMP had a highly positive effect ($p < 0.0001$) on TPC, TFC, and AA assays except for ABTS which was less affected by TMP ($p < 0.001$). Likewise, the quadratic term of TMP had the lowest effect on the responses ($p < 0.05$ and $p < 0.01$). The linear coefficients of TMP were found to be the most significant effect to increase the permeate flux ($p < 0.001$), followed by the linear effect of temperature ($p < 0.05$). On the other hand, the quadratic coefficient of temperature produces a decrease in the permeate flux with a significant effect ($p < 0.01$). The interaction factors and the quadratic effect of TMP do not produce a significant effect ($p > 0.05$) in the permeate flux. In the same time, TMP and temperature was found to be the most significant effect in decreasing the fouling index, followed by the quadratic effect of temperature that produces an increase in the fouling index ($p < 0.01$). The interaction factor between TMP and temperature factor and the quadratic term of TMP does not produce a significant effect ($p > 0.05$) in the fouling index. The quadratic coefficients of temperature (A^2), and the interaction factors (AB) were found to have the highest significant effect in increasing the fouling resistance ($p < 0.001$), as well as, TMB (B) produces a small increasing effect on the fouling resistance with ($p < 0.05$).On the other hand, the linear coefficients of temperature (A) and the quadratic coefficient of TMP (B^2) produce a decrease in the fouling resistance. A linear model was obtained for membrane resistance, and was found that temperature (A) was found to have a positive effect on the increasing membrane resistance ($p < 0.001$), While TMP (B) had no significant effect on it ($p > 0.05$).

4 CONCLUSIONS AND RECOMMENDATIONS

The major purpose of this dissertation is to optimize bioactive compounds extraction processes from hawthorn fruit and anise seed using three extraction techniques heat, microwave, and ultrasound-assisted extraction. In addition, to concentrate the extracts using reverse osmosis and nanofiltration membranes.

For hawthorn fruit extraction:

- With HAE and EW solvent, the supreme amounts of TPC (80.65 ± 6.08 mg GAE/g dw) were recovered under the operating variables of 50 % v/v of ethanol concentration, 40 °C, and 90 min of extraction time, the highest amounts of TFC (19.93 ± 1.68 mg QUE/g dw) were obtained at 90 % of ethanol concentration, 60 °C, and 90 min of extraction time. While like TPC the highest AA was found in the extract of HAE at 50 % (v/v) of ethanol concentration, 50 °C, and 45 min of extraction time, where the values were 35.29 ± 3.12 mg AAE/g dw, 4.25 ± 2.5 %, and 51.58 ± 5.09 % for FRAP, DPPH, and ABTS respectively in the evaluated range.
- In the case of MAE extracts, the highest experimental values of extracted TPC (54.11 ± 5.93 mg GAE/g dw), TFC (12.82 ± 1.55 mg QUE/g dw), and antioxidants (24 ± 3.11 mg AAE/g dw) by FRAP were observed in the extract of MAE at 450 W for 70 s with 7 g/100 mL of sample-to-solvent ratio. 21.61 ± 2.74 % and 43.75 ± 5.12 % of DPPH and ABTS were found in the extract of MAE at 800 W for 120 s with 12 g/100 mL of sample-to-solvent ratio in the evaluated range.
- From twenty experimental runs with UAE extraction, the maximum amounts of recovered amounts of TPC, TFC, FRAP, DPPH, and ABTS are 87.1 ± 5.42 GAE mg/g dw, 29.87 ± 2.09 mg QUE/g dw, 38.78 ± 2.51 mg AAE/g dw, 33.79 ± 2.26 %, and 66.15 ± 6.91 %, respectively in the evaluated range at the processing conditions of 30 % (v/v) of ethanol concentration, 10 min of extraction time, and 12 g/100 mL of sample-to-solvent ratio
- The models show that the highest TPC and TFC (87.1 ± 5.42 mg GAE/ g and 29.57 ± 2.09 mg QUE/g of dw, respectively) can be obtained from the extract of UAE, likewise, the

antioxidant activity was compatible with the obtained TPC, and TFC, where the highest AA can be obtained using UAE by all the assays. Accordingly, the efficiency of the extraction method from hawthorn fruit was in order UAE > HAE > MAE. In addition, using UAE reduced used-ethanol concentration by around 50 % compared to both other extraction methods, and reduced the extraction time by 90 % compared to HAE, also UAE was carried out at room temperature.

- The extraction of phenolic compounds process from the three species of hawthorn fruit (*C. monogyna* Jacq., *C. pinnatifida* Bge, and *C. crus-galli* L.) showed that the levels of phenols and flavonoids in extracts of hawthorn species were in the following order (*C. crus-galli* L. > *C. pinnatifida* Bge. > *C. monogyna* Jacq.). Total phenols in the extracts were ranked from 54.66 ± 0.62 to 86.83 ± 0.34 mg GAE/g dw and total flavonoids ranged from 11.85 ± 0.41 to 32.67 ± 0.42 mg QUE/g dw, and AA by FRAP ranged from 76.67 ± 0.14 to 99.83 ± 0.04 mg AAE/g dw.

- Among three extraction methods and three different solvents used to extract anthocyanin from hawthorn (*C. monogyna* Jacq.), the maximum amount of TMA (0.152 ± 0.002 mg CGE/g dw) was obtained via UAE technique using methanol solvent, while were (0.125 ± 0.007 mg CGE/g dw, 0.107 ± 0.007 mg CGE/g dw) using MAE and HAE as the extraction methods and methanol as solvent. Likewise, Hawthorn fruit extracts prepared with the ultrasonic method with various solvents were characterized with darker color compared with both method of microwave and conventional with the same solvents, as the L* values were noted less for UAE with methanol (42.14 ± 0.19), ethanol (43.89 ± 0.23), and isopropanol (45.83 ± 0.015) solvents respectively. Increased a* (redness) and decreased b* (blueness to yellowish) characteristics indicated the red color of the hawthorn fruit extract with a purple shade. Escalated a* values of UAE were 24.56 ± 0.45, 22.94 ± 1.16, and 20.09±0.29 for methanol, ethanol, and isopropanol indicating more intense color than MAE (18.05 ± 0.55, 17.78 ± 0.02, and 17.25 ± 0.6) and HAE (8.3 ± 0.2, 6.91 ± 0.14, and 3.25±0.5). The extracts using methanol solvent via UAE showed significantly ($p < 0.05$) greater amounts of TPC (49.14 ± 0.38 mg GAE/g dw) and TFC (18.38 ± 0.19 mg QUE/ g dw) compared to other extraction methods and applied solvents. Whilst, the lowest TPC (24.76 ± 0.27 mg GAE/g dw) and TFC (7.06 ± 0.48 mg QUE/g dw) were found using

Isopropanol solvent and HAE. The percentage of inhibition of methanolic extracts of hawthorn using UAE, MAE, and HAE was slightly higher ($p < 0.05$) than that of ethanolic and isopropanolic extracts by all of AA (FRAP, DPPH, and ABTS) assays. In addition, the UAE extraction method outperformed both MAE and HAE using the same solvents. AA values of the fruit extracts by UAE with methanol solvent are as follows: (FRAP = 250.24 ± 1.46 mg AAE/ g dw, DPPH = 157.32±0.39 %, and ABTS = 200.28 ± 0.39 %) while those values decreased to 240.13 ± 0.82 mg AAE/g dw (FRAP); 153.42 ± 0.95 and 83.33 ± 1.17 % measured by DPPH and (ABTS) via MAE. Followed by, the least amounts of AA were detected by methanolic HAE as FRAP=162.32 ± 0.93 mg AAE/g dw, DPPH = 130.05 ± 1.0 %, and ABTS=151.46 ± 0.9 %, respectively. In addition, the Pearson correlation analysis approach established a strong positive linear correlation between TPC, TFC, and radical scavenging assays (DPPH, ABTS) of Hawthorn extracts TPC-DPPH: $r = 0.924$, TPC-ABTS: $r = 0.95$ [TFC-DPPH: $r = 0.929$, TFC-ABTS: $r = 0.946$]. Meanwhile, the correlation was lower between the bioactive compounds and radical scavenging assay (FRAP) [TPC-FRAP: $r = 0.627$, TFC-FRAP: $r = 0.595$].

For anise seed extraction:

- Among the twenty experimental runs by HAE extraction and PW solvent, the utmost amounts of TPC (39.66 ± 3.37 mg GAE/g dw) and AA (FRAP: 5.69 ± 0.41 mg AAE/g), (DPPH: 5.92 ± 0.47 %), and (ABTS: 1.47 ± 0.12 %) were obtained at 40 °C with 6 g/100 mL of sample-to-solvent ratio after 100 min of the extraction time. Meanwhile, the highest amount of TFC (8.78 ± 0.65 mg QUE/g dw) was examined at 25 °C, 10 g/100 mL sample-to-solvent ratio after 100 min of the extraction time in the evaluated range.
- With MAE extraction, the supreme amounts of bioactive compounds from anise seed were recovered under the operating variables of 450 W of microwave power, 120 s of irradiation time, and 7 g/100 mL of sample-to-solvent ratio. The recovered amounts of TPC, TFC, FRAP, DPPH, and ABTS are 50.54 ± 3.26 mg GAE/g dw, 21.67 ± 1.62 mg QUE/g dw, 11.16 ± 0.39 mg AAE/g dw, 17.36 ± 1.47 %, and 4.23 ± 0.42 % respectively in the evaluated range.
- In the case of UAE extracts, the highest experimental values of extracted TPC (43.26 ± 1.65 mg GAE/g dw), TFC (16.24 ±

0.69 mg QUE/g dw), and antioxidants (8.62 ± 0.33 mg AAE/g dw) by FRAP, and 2.64 ± 0.13 % by ABTS were observed in the extract of UAE at 10 % (v/v) of ethanol concentration for 10 min of the extraction time with 12 g/100 mL sample-to-solvent ratio in the evaluated range. While 14.98 ± 0.85 % of DPPH was found in the extract at 20 % (v/v) of ethanol concentration for 15 min of the extraction time with a 12 g/100 mL sample-to-solvent ratio in the evaluated range.

- The models show that the highest TPC and TFC (49.9 ± 3.26 mg GAE/g and 20.86 ± 1.62 mg QUE/g of dw, respectively) can be obtained using MAE. Likewise, the antioxidant activity was compatible with the obtained TPC, and TFC, where the highest AA can be obtained using MAE extracts by all the assays. Accordingly, the efficiency of the extraction method from anise seed was in order MAE > UAE > HAE. In addition, the results show that increasing ethanol concentration by up to 14 % (v/v) can enhance the extraction of flavonoids by around 50 % using UAE compared to using pure water and HAE, and reduced the time by around 90 %.

- To determine the best solvent for anise seed (*Pimpinella anisum* L.) the extraction was carried out using seven solvents and the HAE extraction method. TPC ranged from 17.57 ± 0.65 GAE/g dw to 43.84 ± 0.39 GAE/g dw, while TF ranged from 8.69 ± 0.85 QUE/g dw to 17.22 ± 0.82 QUE/ g dw. The highest amount of phenolics and flavonoids were found in 50 % (v/v) methanol extract followed by pure methanol, while the lowest amount of phenolics was in the absolute Isopropanol extract. The percentage of inhibition of extracts of anise using absolute methanol (FRAP: 12.37 ± 1.06 mg AAE/g dw; DPPH: 9.01 ± 0.06) and methanol 50 % (v/v) (FRAP: 13.35 ± 0.52 mg AAE/g dw; DPPH: 10.81 ± 0.25) was slightly higher ($p < 0.05$) than that of ethanolic, isopropanolic, and wateric extracts by all AA (FRAP, DPPH) assays. In addition, the obtained results showed that there is a high correlation between total phenolics and flavonoid contents with ferric ion reduction [TPC-FRAP: $r = 0.989$, TFC-FRAP: $r = 0.886$] and [TPC-DPPH: $r = 0.994$, TFC-DPPH: $r = 0.867$].

Extracts concentration

In order to concentrate hawthorn fruit and anise seed extracts, three types of membranes were examined (RO99, X-20, and NF 270). the examined compounds content increased during the concentration processes and reached the maximum scavenged amount using X-20 membrane (TPC: 64.31 ± 1.81 and 92.62 ± 0.45 mg GAE/g dw) and (TFC: 20.93 ± 1.93 and 48.19 ± 1.58 mg QUE/g dw). Whilst less amount of TPC, TFC was found in each finale of NF-270 membrane (TPC: 34.75 ± 1.67 and 45.92 ± 2.99 mg GAE/g dw) and (TFC: 10.45 ± 1.23 and 19.65 ± 1.13 mg QUE/g dw) for anise seed and hawthorn extracts. TPC of the concentrates from X-20 improved 2.3-fold for anise extracts and 2-fold for hawthorn extracts while TFC increased around 2.5-fold for both anise and hawthorn extracts. Meanwhile, the recovered amounts of TPC in NF 270 concentrates went up to 1.3 and 1-fold along with 1.4 and 1-fold of TFC for anise and hawthorn extracts, respectively. Likewise, the process using X-20 showed around 2-fold and 2.4-fold of antioxidant activity (FRAP) went up for anise extracts and for hawthorn extracts whereas around antioxidant activity measured by the DPPH method increased 1.5-fold for both anise and hawthorn extracts. The lowest increase was during NF 270 process, where the antioxidant activity increased 1.2-fold and 1-fold by the FRAP and DPPH methods for anise extracts, while the increase did not exceed 1-fold for hawthorn extracts measured by both methods.

In addition, the flux of anise extract reached 4.61 ($L/(m^2 \cdot h)$) at VRR = 3 after about 57 minutes of concentration time using (NF 270) membrane whereas the permeate fluxes of 5.5 ($L/(m^2 \cdot h)$) and 9.7 ($L/(m^2 \cdot h)$) were revealed after 57 minutes and 42 minutes of concentration times by RO99 and X-20 membranes. While for hawthorn extracts it took more than 1.5 hours for the permeate flux to reach VRR = 3 with a flux of 3.02 ($L/(m^2 \cdot h)$) using an NF 270 membrane. In the case of X-20 membrane, one hour time was enough to reach the same level of VRR with a flux velocity of 6.6 ($L/(m^2 \cdot h)$).

X-20 shows the lowest fouling index, followed by RO99. 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. At the same time, using an X-20 membrane, TPC and TFC retentions for both anise and hawthorn extracts were > 99 %, and for antioxidant activity were around 98 % (using both of FRAP and DPPH assay) for 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.

To optimize the concentration processes of the extracts, 11 experiments were run for both hawthorn fruit and anise seed extracts using an X-20 membrane.

- In the case of hawthorn, the highest amounts of recovered TPC, TFC, and their antioxidant activity by FRAP, DPPH, and ABTS are (117.51 ± 3.62 mg GAE/g dw), (19.65 ± 0.44 mg QUE/g dw), (63.93 ± 2.51 mg AAE/g dw), (20.86 ± 0.36 %), and (74.22 ± 1.5 %) respectively, as well as the highest final permeate flux (6.64 ± 0.52 (L/(m^2·h)) were found at (T= 35 °C and TMP = 40 bar). In comparison, the lowest fouling index (27.71 ± 1.20 %) was found at (T = 45 °C and TMP = 40 bar), the lowest membrane resistance and fouling resistance ($1.66 \cdot 10^{14} \pm 8.62 \cdot 10^{12}$ 1/m, and $2.45 \cdot 10^{13} \pm 4.25 \cdot 10^{12}$ 1/m) were found at (T = 25 °C and TMP = 20 bar, T= 35°C and TMP = 20 bar) respectively in the evaluated range..
  In the case of anise, the highest amount of recovered TPC, TFC, and their Antioxidant activity by FRAP, DPPH, and ABTS are (92.86 ± 3.33 mg GAE/g dw), (9.73 ± 0.35 mg QUE/g dw), (13.75 ± 0.46 mg AAE/g dw), (8.87 ± 0.32 %), and (4.4 ± 0.21 %) respectively, as well as the highest final permeate flux (15.06 ± 1.33 (L/(m^2·h))). In comparison the lowest fouling index (23.79 ± 2.40 %) was found at (T = 35 °C and TMP = 40 bar), the lowest membrane resistance and fouling resistance ($1.06 \cdot 10^{14} \pm 9.38 \cdot 10^{12}$ 1/m, and $4.78 \cdot 10^{13} \pm 8.53 \cdot 10^{12}$1/m) were found at (T = 25°C and TMP = 20 bar, T= 35°C and TMP= 30 bar) respectively in the evaluated range.

Recommendations

- Hawthorn extraction requires further exploration and the application of different ultrasound intensities and carries out more comparisons between heat-assisted and microwave-assisted extraction. It is recommended that a RSM approach be used with a wider set of setup variables for all the extraction methods.
- Quantitative analysis of bioactive compounds should be performed using HPLC or GC in order to compare the different species of hawthorn.

- Further study can be applied for anthocyanin extraction and use more safety acids instead of HLC like (acetic acid, citric acid, and tartaric acid).
- The RSM approach with wider setup variables is encouraged for anise extraction to optimize the extraction of bioactive compounds, especially the extraction time.
- Implementing microfiltration as a preliminary step before nanofiltration and reverse osmosis processes is recommended. Microfiltration efficiently removes suspended solids and macromolecules, improving product quality and reducing fouling potential by eliminating larger foulants. This pre-treatment optimizes downstream membrane performance and extends membrane lifespan, enhancing overall filtration efficiency and product quality.
- The scanning of other types of membranes that are subjected to higher limits of pressures and temperatures is recommended with the extent of the variables studied in the RSM approach.
- Studying the possibility of the application of hawthorn extract and anise seed in food products especially in dairy products and beer is recommended.
- Attempts could be made to encapsulate or prepare nanomaterials from these extracts.

5 NEW SCIENTIFIC RESULTS

From my dissertation, I have found out:

1) Within the evaluation range, the best extraction conditions differed between the two plants, which aligns with the understanding that plant matrices influence extraction methods and conditions. Ultrasound-assisted extraction using an ethanol-aqueous solution of approximately 60 % (v/v) proved to be the most effective method for extracting polyphenol compounds from hawthorn fruit. Maximum amounts of phenolic and flavonoid compounds, along with their antioxidant activity, were achieved with a 30 % (v/v) ethanol concentration, 10 minutes of extraction time, and a sample-to-solvent ratio of 12 g/100 mL. Ultrasound-assisted extraction (UAE) reduced ethanol consumption by approximately 50% compared to both heat-assisted and microwave-assisted extraction methods, while also reducing extraction time by 90 % compared to heat-assisted extraction. Moreover, UAE was conducted at room temperature.
   In contrast, microwave-assisted extraction (using 450 W of microwave power, 120 seconds of irradiation time, and a 7 g/100 mL sample-to-solvent ratio) with pure water proved to be more effective for extracting polyphenols from anise seed compared to ultrasound-assisted and heat-assisted extraction methods. Furthermore, increasing ethanol concentration by up to 14% (v/v) can enhance flavonoid extraction by around 50% using UAE compared to using pure water, while also reducing extraction time by approximately 90%.
2) In the study of heat-assisted extraction, it was found that increasing temperatures, ethanol concentration, and extraction time could significantly enhance the extraction of total flavonoids from hawthorn fruit. The maximum amount of flavonoids was obtained at 90 % (v/v) ethanol, 60 °C, and 50 minutes of extraction time. Conversely, the maximum amount of phenolic compounds was obtained at 50 % (v/v) ethanol, 45 °C, and 90 minutes within the evaluation range. Additionally, the results indicated that the yield of flavonoids from anise seed tended to increase with higher material-to-solvent ratios at lower temperatures.
3) For the extraction of total monomeric anthocyanins from hawthorn (*C. monogyna Jacq*.), ultrasound-assisted extraction (3.5 W/cm^2, 20 kHz, for 30 minutes at 25 °C) demonstrated superior extractability

compared to microwave-assisted extraction (10 minutes at 800 W with 50 % duty cycle) and heat-assisted extraction (30 minutes at 65 °C). This superiority is attributed to thermal degradation, which may occur due to the unstable and rapid decomposition of anthocyanin compounds under the high heat of microwave irradiation or the elevated temperature used in heat-assisted extraction. Furthermore, methanol extracts exhibited the highest content of anthocyanins, phenolic compounds, and flavonoids, and demonstrated the highest antioxidant activity across three scavenging assays (FRAP, DPPH, ABTS) when compared to ethanol and isopropanol extracts.

4) Reverse osmosis and nanofiltration membranes demonstrate high efficiency in concentrating extracts from both hawthorn fruit and anise seed. Among these membranes, the thin-film polyamide (X-20) membrane outperforms the polyester thin-film composite (RO99) and polyamide thin-film composite (NF 270) membranes, especially at 30 bar, 35 °C, 400 L/h and VRR = 3, exhibiting superior retention of phenolic and flavonoid compounds while showing the lowest fouling index. Additionally, the trend of antioxidant activity tends to increase during the concentration processes. Specifically, the process using the X-20 membrane resulted in approximately 2-fold and 2.4-fold increases in antioxidant activity (FRAP) for anise and hawthorn extracts, respectively, while antioxidant activity measured by the DPPH method increased by 1.5-fold for both extracts. Furthermore, it is important to employ multiple methods to assess the antioxidant activity of extracts. Significant differences in FRAP values were observed among the final extracts from different membranes, whereas no significant differences ($p > 0.05$) were observed in DPPH values between the reverse osmosis membranes X-20 and RO99.

5) Using the thin-film polyamide X20 reverse osmosis membrane at processing conditions of 35 °C temperature, 40 bars pressure, a recirculation flow rate of 600 L/h, and VRR = 4, the concentration of phenolic compounds from hawthorn fruit and anise seed extracts significantly increased. The final concentrations obtained were approximately 2.7-fold higher for phenolic compounds from hawthorn fruit and 3.5-fold higher from anise seed compared to the crude extracts. Additionally, the total flavonoid content in hawthorn and anise extracts increased by 2.2-fold and 2.4-fold, respectively, after the filtration process. The reverse osmosis membrane filtration processes for hawthorn fruit and anise seed extracts successfully rejected these

compounds at 99 % efficiency in the concentrates, leading to a substantial increase in their antioxidant activity.

6 LIST OF PUBLICATIONS AND CONFERENCES

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Conference abstract publications

1- **Alsobh. A**, Vatai. G , and Bánvölgyi. S optimize and compare heat, microwave, and ultrasound-assisted extraction techniques to obtain bioactive compounds from anise (*pimpinella anisum*) seed using response surface methodology (RSM). 21 st wellmann international scientific conference, 2024, pp 65.

2- **Alsobh. A**, Vatai. G , and Bánvölgyi. S .Recovery of bioactive anthocyanin pigments from hawthorn fruit by infusion, microwave, and ultrasound-based extraction techniques. 4th Young Researchers' International Conference on Chemistry and Chemical Engineering (YRICCCE IV), Debrecen, 2023, pp.39.

3- **Alsobh. A**, Vatai. G , and Bánvölgyi. S. Optimization and comparison of heat, microwaves, and ultrasound-assisted extraction techniques to obtain polyphenol compounds from hawthorn fruit (*crataegus monogyna* jacq.). 29th International Symposium on Analytical and Environmental Problems (ISEP), Szeged, pp.72 (2023).

4- **Alsobh. A**, Vatai. G , and Bánvölgyi. S. Identification of bioactive compounds using rp-hplc analysis of some hawthorn species extracts (*crataegus pinnatifida* bge., *c. monogyna jacq*., *c. crus-galli*. L) and antioxidant activity evaluation, 28th International Symposium on Analytical and Environmental Problems (ISEP), pp.72 (2022).

5- **Alsobh. A**, Vatai. G , and Bánvölgyi. S. Optimization of ultrasound-assisted extraction of antioxidant compounds from Hawthorn using response surface methodology, 4th FoodConf(conference of food science and technology),), 10 - 11 June 2022, Budapest, Book of abstracts edited by: Lilla Szalóki-Dorkó, Ildikó Batáné Vidács, Pradeep Kumar, Andrea Pomázi, Attila Gere, ISBN 978-615-01-5422-0, pp.

6- **Alsobh. A**, Vatai. G , and Bánvölgyi. S. Anise (*Pimpinella anisum*), chemical composition, medicinal and food uses, a review, XXV. Tavaszi Szél Konferencia 2022 Absztraktkötet Szerkesztette: Molnár Dániel és Molnár Dóra, ISBN 978-615-82054-8-1, pp. 715.

7- **Alsobh. A**, Vatai. G , and Bánvölgyi. S. Hawthorn (CRATAEGUS): medicinal benefits and extraction methods, review , (2022) A Lippay János – Ormos Imre – Vas Károly

(LOV) Tudományos Ülésszak tanulmányai = Proceedings of János Lippay – Imre Ormos – Károly Vas (LOV) Scientific Meeting. MATE Budai Campus, Budapest. ISBN 978-963-269-988-2, pp. 40.

8- **Alsobh. A**, Vatai. G , and Bánvölgyi. S. Recent Developments in the Application of Membrane Technologies for Concentration of Plant Extracts, BIOSYSFOODENG 2021 4th June, 2021. LURDY CONFERENCE AND EVENT CENTRE, BUDAPEST, HUNGARY, E462.