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Innovative extraction and concentration techniques for bioactive compounds of hawthorn fruit and anise seed

Doctoral (PhD) dissertation by

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ABBREVIATIONS AND SYMBOLS

List of abbreviations

Δt time required to collect the filtrate (h)

ΔV volume of filtrate (L)

…….

α_1 slope of pure water flux curve before the concentration

α_2 slope of pure water flux curve after the concentration

α_3 slope of TPC calibration curve

α_4 slope of TFC calibration curve

α_5 slope of AA calibration curve

a * redness or greenness

A active surface area of membrane (m^2)

AA antioxidant activity (mg ASE/g DW)

Abs_0 the absorbance of the control

Abs_1 the absorbance in the presence of the test

ABTS 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)

AAE ascorbic acid equivalent

ANOVA analysis of variance

b * blueness or yellowness

C_0 feed concentration (mg/g)

C_p concentration of permeate (mg/g)

C_R concentration of retentate (mg/g)

C.V. % coefficients of variation

CCD central composite design

CGE cyanidin-3-glucoside equivalent

CIE commission internationale de l'eclairage

CE conventional extraction

DPPH 2,2-diphenyl-1-picryl-hydrazyl-hydrate

DF dilution factor

DFA desirability function approach

df degree of freedom

dw dry weight

DOE design of experiments

EW ethanol-water mixture

E-100 absolute ethanol

E-50 ethanol 50 % (v/v)

EAE enzyme assisted extraction

FRAP ferric reduction antioxidant power

GAE gallic acid equivalent

HAE Heat- assisted- extraction

HSD honestly significant difference

L path length (cm)

L * lightness

MAE Microwave-assisted extraction

M-100 absolute methanol

M-50 methanol 50 % (v/v)

MANOVA multivariate analysis of variance

MF microfiltration

NF nanofiltration

PW pure water

PWP_a pure water permeability after effluent filtration L /($m^2 \cdot h \cdot bar$)

PWP_b pure water permeability before effluent filtration L /($m^2 \cdot h \cdot bar$)

PLE pessurized liquid extraction

QUE quercetin acid equivalent

RO reverse osmosis

R percentage of retention (%)

R_m membrane resistance (1/m)

R_f fouling resistance (1/m)

R^2 coefficient of determination

RSM response surface technology

S the actual amount of sample (µL)

SS sum of squares

Std. Dev. standard deviation

S-100 absolute isopropanol

S-50 isopropanol 50 % (v/v)

S-to-S sample to solvent ratio

SFE supercritical fluid extraction

TMP applied transmembrane pressure difference (Pa)

TFC concentration of total flavonoid content (mg QUE/g dw)

TPC concentration of total phenolic compounds (mg GAE/g dw)

TMA Total monomeric anthocyanin content

TS the total amount of sample with added chemical solutions (μL)

UAE ultrasound-assisted extraction

UF ultrafiltration

V_0 feed volume (m^3)

V_P volume of permeate (m^3)

V_R volume of retentate (m^3)

VRR volume reduction ratio (m^3 /m^3)

List of symbols

ε is the molar absorptivity coefficient (26,900 1/cm/mol)

……..

J permeate flux of pure water (L/(m^2 · h))

J_v the volumetric permeate water flux (L/(m^2 · h))

M_w is the molecular weight of anthocyanin (449.2 g/mol)

μ permeate viscosity (Pa · s)

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 (Petrovska, 2012). For centuries, plants have been of great interest to humans as flavors, fragrances, dyes, preservatives, and pharmaceuticals (Đilas et al., 2009). 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 (Venugopal and Liu, 2012), treatment of diabetes (Chan et al., 2012), and cardiovascular diseases (Vasanthi and ShriShriMal, 2012), in addition to their role against bacteria and pathogens (Ullah et al., 2020).

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 (Azmir et al., 2013).

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 odours from natural and processed products, production of non-alcoholic beverages (Castro-Muñoz et al., 2020a). 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) were discussed by (Conidi et al., 2018).

1.1 Hypothesis

- 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.

1.2 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:

- Response surface methodology (RSM) has been applied to predict the most important parameters for the effective extraction of bioactive compounds from hawthorn fruit and anise seed.

- Updated extraction techniques such as ultrasound and microwave have been used to boost the extractability of polyphenolic compounds from those plants and antioxidant activities compared to conventional solid-liquid extraction.
- Determine the effect of the solvent types on the yield of the extracted compounds.
- Investigate whether membrane technology can be utilized to facilitate the concentration of bioactive compounds from hawthorn fruit and anise seed extracts.
- Assess the potential application of the nanofiltration membranes (NF) and reverse osmosis membranes (RO) to effectively retain phenolic compounds, while at the same time concentrating the extracts under the specific operating conditions.

2 LITERATURE REVIEW

2.1 Bioactive compounds

The history of plant's used for mankind is as old as the start of humankind. Initially, people used natural and cultivated plants for their nutritional purposes but after the discovery of medicinal properties, this natural flora became a useful source of disease cure and health improvement across various human communities. Egyptian papyrus showed that coriander and castor oil were useful for medicinal applications, cosmetics, and preservatives through thousands of recipes (Vinatoru, 2001); in Romania, the use of medicinal herbs has been known since antiquity. Where a thousand therapeutic uses of herbal plants were described by several scholars namely Hippocrates, Theophrastus, Celsus, Dioscorides, and many others (Paulsen, 2010). For example, the herb 'Motherwort' (*Leonurus cardiaca*) was mentioned by Herodotus (5^{th} century B.C.) in his writings about people living north of the Danube River. In the 19^{th} century, herbal products were introduced in the romanian pharmacopeia, and in 1904 the first institute of medicinal herbs was established in Cluj city (Vinatoru, 2001). The philosopher and physician Avicenna (980-1037A.D.) was best known for his document Canon Avicenna, also called Canon of Medicine, describing systematically the knowledge of medicine and pharmacology of that period. In this period they created a system for pharmacies, that amongst other remedies sold several known today as camphor, mastix, rhubarb, saffron, and aloe.

Today, herbal products serve various purposes including medicinal use, dietary supplementation, cosmetics, and culinary applications. They are derived from whole plants or plant parts, such as leaves, roots, or flowers, and are marketed and regulated as medicinal products, dietary supplements, medical devices, or cosmetics with specific labeling requirements. In Europe, numerous herbal plants have been extensively studied and utilized. Some notable examples include:

White mulberry (*morus alba* L.) is a fast-growing deciduous plant belonging to the Moraceae family, thriving in various climates from tropical to moderate regions. Introduced to Europe in the 11^{th} century alongside silkworm caterpillars (Przeor, 2022). Scientific studies suggest that extracts from white mulberry fruits may have hepatoprotective effects against liver cancer, while leaf extracts exhibit potential in lowering postprandial glucose levels and managing diabetes. Additionally, the antibacterial properties of mulberry leaves and their antioxidant activity contribute to inhibiting atherosclerosis (Przeor, 2022). In human studies, oral administration of mulberry leaf powder in doses ranging from 0.8 g to 1.2 g has shown significant reductions in postprandial glycemia and insulin secretion (Kimura et al., 2007).

Fenugreek is an annual herbal plant with fine seeds from the Leguminosae family. Both the seeds and leaves of fenugreek appear in literature as an ingredient of food and as medicine (Wani and Kumar, 2018). Nutraceutical properties of fenugreek include blood purification; sweat-inducing effects, supporting the removal of toxins; cleaning the lymphatic system; maintaining mucous membranes in good condition; removing excess mucus from the throat; relieving colds, bronchial problems, flu, asthma, rhinitis, constipation, sinusitis, pneumonia, and laryngitis (Wani and Kumar, 2018). One report suggested that 21 g/d of fenugreek per 60 kg adult, human is the recommended intake limit to prevent accidental overdose by oral administration (Singletary, 2017).

Ceylon cinnamon belongs to the *Lauraceae* family. The antioxidative and antibacterial activity of an extract derived from cinnamon has been demonstrated in recent years (Wang et al., 2018). Among the best-known herbs and spices in terms of antioxidant content, researchers indicate that cinnamon (77 mM per 100 g of antioxidant) has less antioxidative properties than only several other plants, which include allspice, cloves, and peppermint (Singh et al., 2016). Studies have shown that the median lethal dose value (LD50) of orally administered cinnamon in animals is 1850 ± 37 mg/kg. Hence, this is equivalent to a human dose of 11.4 ± 0.2 g/kg (Ranasinghe et al., 2017).

Ginger is one of the oldest spice and medicinal plants. In Europe, ginger is very widespread and is often used in combination with East cuisine. In the past, in traditional medicine, ginger was used as an ingredient with carminative, expectorant, and astringency properties. The studies conducted so far indicate that the beneficial qualities of ginger rhizomes are due to, among other things, its hypoglycaemic, hypocholesterolemic, antiarthritic, antirheumatic, and antioxidant activity (Przeor, 2022). Dosage of ginger varied greatly between primary studies, with0.5 – 2 g/d being most commonly administered (Crichton et al., 2022).

The general perception that herbal remedies or drugs are very safe and devoid of adverse effects is not only untrue, but also misleading. Herbs have been shown to be capable of producing a wide range of undesirable or adverse reactions some of which are capable of causing serious injuries, life-threatening conditions, and even death. Numerous and irrefutable cases of poisoning have been reported in the literature (Ekor, 2014).

The use of herbal plants in ancient times actually illustrates the history of bioactive molecules. In the past, people had no idea about bioactive molecules but the use of these compounds was by using extracts or powder of medicinal plants as the main active ingredient. Today, bioactive molecules are defined as secondary metabolites. Every living body, from one-cell bacterium to million-cell plants, processes diverse chemical compounds for their survival and subsistence. All compounds of biological systems can be divided into two broad arenas. One is primary metabolites, which are the chemical substances aimed at growth and development, such as carbohydrates, amino acids, proteins, and lipids. Another is secondary metabolites, which are a group of compounds other than primary metabolites believed to help the plant to increase their overall ability to survive and overcome local challenges by allowing them to interact with their surroundings. Typically, bioactive compounds of plants are produced as secondary metabolites (Harborne, 1993; Bernhoft, 2010).

In other words, secondary metabolites are those metabolites that are often produced in a phase subsequent to growth, have no function in growth (although they may have survival function), are produced by certain restricted taxonomic groups of microorganisms, have unusual chemical structures, and are often formed as mixtures of closely related members of a chemical family (Azmir et al., 2013). The production of secondary metabolites in different species is mainly selected through the course of evaluation and the particular needs of that species. For example, the synthesis of aroma by floral species attracts insects for their pollination and fertilization, and the synthesis of toxic chemicals has evolved toward pathogens and herbivores to suppress the growth of neighboring plants (Azmir et al., 2013). Among secondary metabolites, some of these substances affect biological systems which are considered bioactive. Thus a simple definition of bioactive compounds in plants is secondary plant metabolites eliciting pharmacological.

2.1.1 Classification and synthesis of bioactive compounds

Classification of bioactive compounds in different categories is still inconsistent rather it depends upon the intention of the particular classification. According to (Croteau et al., 2000), bioactive compounds of plants are divided into three main categories: (a) terpenes and terpenoids (approximately 25,000 types), (b) alkaloids (approximately 12,000 types) and (c) phenolic compounds (approximately 8000 types). The general structures of different categories of bioactive compounds are given in Fig. 1.

To date, we know that there are four major pathways for the synthesis of secondary metabolites or bioactive compounds: (1) shikimic acid pathway, (2) malonic acid pathway, (3) mevalonic acid pathway (MVA), and (4) non-mevalonate (MEP) pathway (Chung et al., 2016). Alkaloids are produced by aromatic amino acids (which come from the shikimic acid pathway) and by aliphatic amino acids (which come from the tricarboxylic acid cycle). Phenolic compounds are synthesized through the shikimic acid pathway and the malonic acid pathway. Through the mevalonic acid pathway and MEP pathway, terpenes are produced, which form their basic C 5 unit, isopentenyl diphosphate (IPP).

Number of natural products	
With nitrogen	
•Alkaloids (1)	12,000
•Non-protein amino acids (2)	700
•Amines (3)	100
•Cyanogenic glycosides (4)	60
•Glucosinolates (5)	100
•Alkamides	150
Without nitrogen	
•Monoterpenes (incl. Iridoids) (6)	2,500
•Sesquiterpenes (7)	5,000
•Diterpenes (8)	2,500
•Triterpenes, Saponins, Steroids (9)	5,000
•Tetraterpenes	500
•Phenylpropanoids, coumarins, lignans	2,000
•Flavonoids (10)	4,000
•Polyacetylenes, fatty acids, waxes (11)	1,000
•Polyketides (12)	750
•Carbohydrates	>200

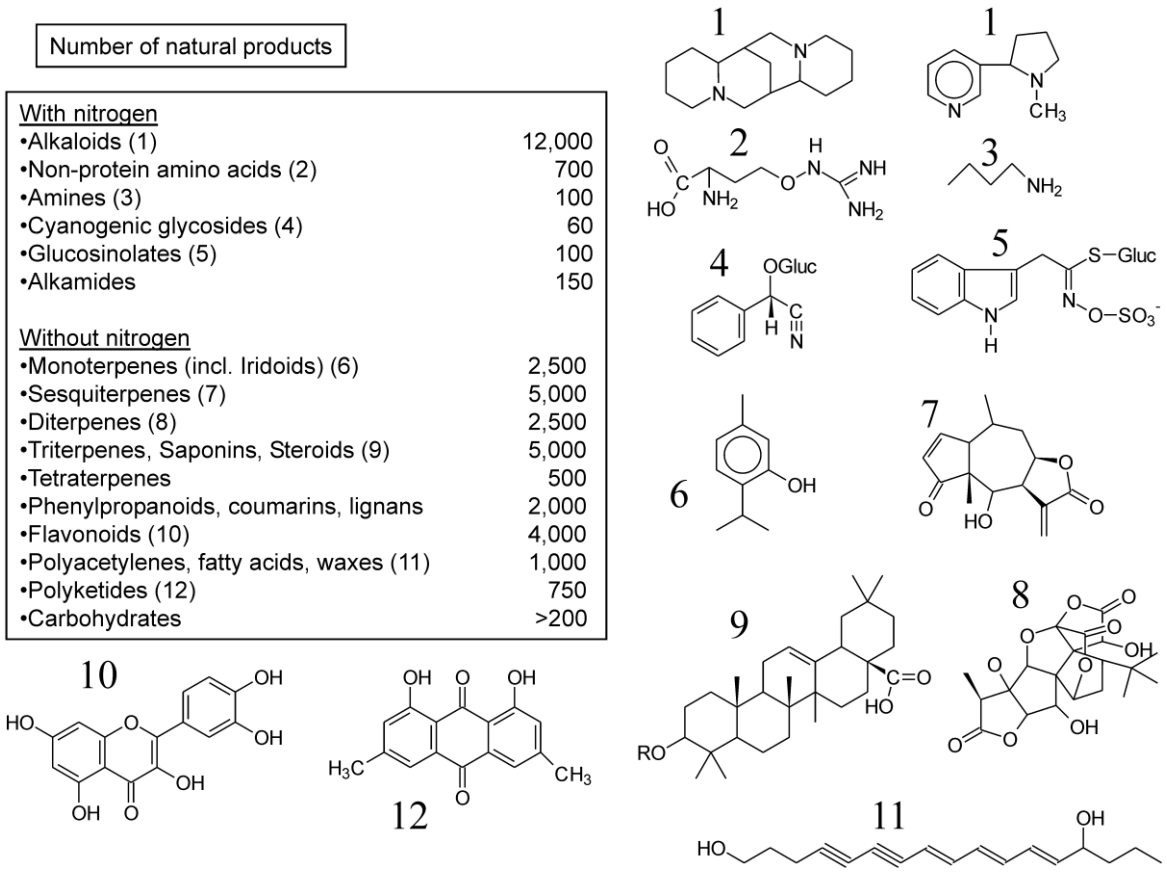


Fig 1. Structural diversity of plant secondary metabolites (Wink, 2003)

2.1.2 Extraction of bioactive compounds

Extraction is the crucial first step to obtaining bioactive compounds from plants. Considering the great variations among bioactive compounds and the huge number of plant species, it is necessary to build up a standard and integrated approach to extract and screen out these compounds. The basic operation of extraction includes steps, such as pre-washing, drying

of plant materials or freeze drying, and grinding to obtain a homogenous sample which often improves the kinetics of analytic extraction, and also increases the contact of the sample surface with the solvent system. In addition, proper actions must be taken to assure that potential active constituents are not lost, distorted, or destroyed during the preparation of the extract from plant samples.

Different solvent systems are used to extract the bioactive compound from natural products. The extraction of hydrophilic compounds uses polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in ratio of 1:1 are used. In some instances, extraction with hexane is used to remove chlorophyll. Furthermore, plant extracts are also prepared by conventional extraction techniques like maceration or percolation of fresh green plants or dried powdered plant material in water and/or organic solvent systems. Non-conventional extraction (emerging technologies) which include solid-phase microextraction, supercritical-fluid extraction, pressurized-liquid extraction, microwave-assisted extraction, ultrasonic-assisted extraction solid-phase extraction, and surfactant-mediated techniques. These latest technologies possess certain advantages such as reduction in organic solvent consumption and in sample degradation. These techniques are considered as green extraction of natural products and could be a new concept to meet the challenges of conventional techniques and help to protect both the environment and consumers. Additionally they enhance the competition of industries to be more ecologic, economic, and innovative. Fig. 2 shows the advantage of these emerging techniques.

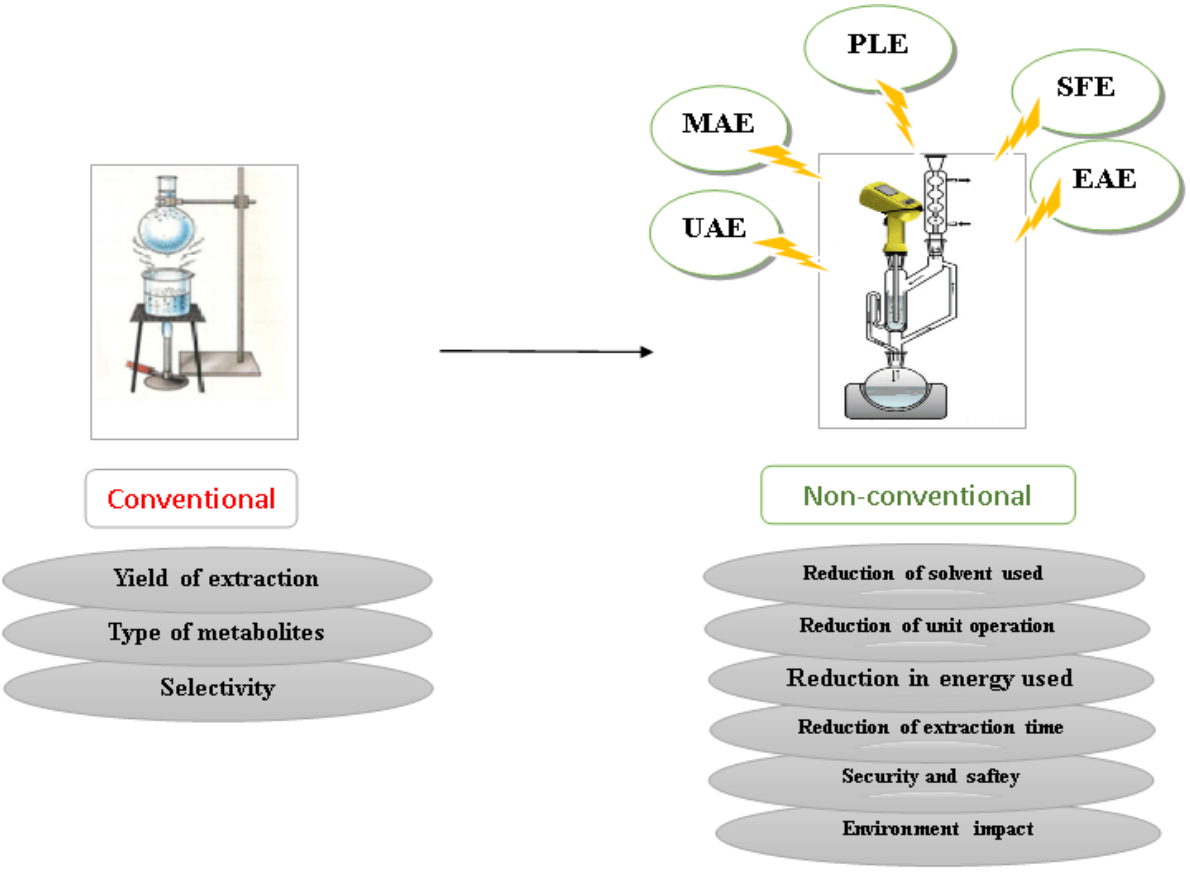


Fig 2. Advantages of conventional and non-conventional extraction techniques

2.1.2.1 Conventional extraction techniques

In order to obtain bioactive compounds from plants, several classical techniques are applied such as (1) soxhlet extraction, (2) maceration, and (3) hydrodistillation. Most of these techniques are based on the extracting power of different solvents in use and the application of heat and/or mixing.

The soxhlet extractor was first proposed by German chemist Franz Ritter Von Soxhlet (1879). which was originally used to determine fat in milk (De Castro and Priego-Capote., 2010) but now it is not limited to this only. In its classical implementation, the sample is placed in a thimble holder that is gradually filled with condensed fresh extractant (term used to refer to the solvent used for extraction) from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solute from the thimble-holder and unloads it back into the distillation flask, thus carrying the extracted analytes into the bulk liquid. This operation is repeated until extraction is complete. Operationally, soxhlet extraction is thus a continuous–discrete technique. In fact, since the extractant acts stepwise, the assembly operates as a batch system; however, the extractant is recirculated through the sample, so the system also operates continuously somehow. Soxhlet extraction has widely been used for extracting valuable bioactive compounds from various natural sources, usually combined between soxhlet and high-pressure, ultrasound-assisted extraction, or microwave-assisted extraction.

Maceration is an old method used for medicinal preparation. It is considered a wide and low-cost way to get natural products from plant materials, and it is considered a method of solid-liquid extraction. Maceration generally consists of several steps. After grinding of plant materials into small particles which increase the surface area for proper mixing with solvent. These materials are placed in a closed vessel and the solvent is added. It is allowed to stand for a long time (varying from hours to days) with occasional shaking. Sufficient time is allowed for the solvent to diffuse through the cell wall to solubilize the constituent present in the plant. The process takes place only by molecular diffusion. After the desired time, the liquid is strained off but the marc which is the solid residue of this extraction process is pressed to recover a large amount of occluded solutions. The obtained strained and the press-out liquid are mixed and separated from impurities by filtration. Occasional shaking in maceration facilitates extraction in two ways; (a) increase diffusion, and (b) remove the concentrated solution from the sample surface to bring new solvent to the menstruum for more extraction yield.

Hydrodistillation is a traditional method for the extraction of bioactive compounds and essential oils from plants, which do not involve organic solvents and it can be performed before dehydration of plant materials. There are three types of hydrodistillation: water distillation, water and steam distillation, and direct steam distillation (Vankar, 2004). In hydrodistillation, first, the plant materials are packed in a still compartment; second, water is added in sufficient amounts and then brought to a boil. Alternatively, direct steam is injected into the plant sample. Hot water and steam act as the main influential factors in freeing bioactive compounds of plant tissue. Indirect cooling by water condenses the vapor mixture of water and oil. Condensed mixture flows from a condenser to a separator, where oil and bioactive compounds separate automatically from the water. Hydrodistillation involves three main physicochemical processes; Hydrodiffusion, hydrolysis, and decomposition by heat. At a high extraction

temperature, some volatile components may be lost. This drawback limits its use for thermo labile compound extraction (Rasul., 2018).

2.1.2.2 Non- Conventional extraction techniques

To overcome the limitations of conventional extraction methods, new and promising extraction techniques are introduced. These techniques are referred as nonconventional extraction techniques. Some of the most promising techniques are ultrasound-assisted extraction, enzyme-assisted extraction, microwave-assisted extraction, pulsed electric field-assisted extraction, supercritical fluid extraction, and pressurized liquid extraction**.**

- **Ultrasound-assisted extraction (UAE)**

Ultrasounds were discovered at the end of the XIXth century. These acoustic waves have been applied to food processing as a new green technology, but also in the pharmaceutical, nutraceutical, and cosmetic fields. These waves, at certain frequencies and amplitudes, create cavitation bubbles which, when they reach a non-stable point, release high temperature and high pressure by imploding. In addition to that, the generation and collapse of cavitation bubbles induce shear force and turbulence within the fluid which results in the breakdown of the cell walls contributing to releasing the bioactive compound (Vilkhu et al., 2008). Ultrasound increases the solvent absorption of the pomace thereby enhancing the accessibility of solvent to the bioactive compounds to be extracted (Pingret et al., 2012). Ultrasound also increases the swelling index of the plant tissue matrix which helps in both the desorption and diffusion of solutes resulting in increased extraction (Dezhkunov et al., 2004). The increase in the extraction yield by UAE can't be attributed to a single mechanism but due to the combined effect of all the mechanisms. Several parameters can modulate ultrasonic waves. The two main ones are the frequency (Hz) and the amplitude (MPa). Power (W) is the amplitude over time and intensity is power over surface area (W/m). These parameters change ultrasonic waves and can interact differently with plant samples.

In general, UAE has been performed using ultrasonic baths and ultrasonic probes, which are based on a piezoelectric transducer as a source of ultrasound power. In the ultrasonic bath, the solid matrix is dispersed in the solvent in a stainless steel tank connected to a transducer. Ultrasonic probes consist of a probe or horn connected to a transducer. The probe is immersed in an extraction vessel and delivers ultrasound in the media with minimum energy loss. Ultrasonic baths are more economical and easy to handle, but their low reproducibility restricts their use in the extraction process. For this reason, probe-based systems are commonly preferred compared to bath systems due to the higher ultrasonic intensity (tip of the probe) and are used as a powerful tool for the extraction of bioactive compounds (Kumar et al., 2021).

- **Microwave-assisted extraction (MAE)**

The use of microwave energy in chemical laboratories was first described in 1986 by Gedye, and Ganzler described it in (1990) in the extraction of biological matrices for the preparation of analytical samples. MAE is a younger technique than UAE by some 35 years. Nevertheless, many laboratories have studied the enormous potential of this nonconventional energy source for synthetic, analytical, and processing applications. So far, the use of dielectric heating in synthesis and extraction has been documented by over 7000 and 2000 articles respectively (Vinatoru et al.,2017).

Microwaves are electromagnetic fields in the frequency range from 300 MHz to 300 GHz. They are made up of two oscillating fields that are perpendicular such as an electric field and a magnetic field. Electromagnetic energy is converted to heat following ionic conduction and dipole rotation mechanisms (Jain et al., 2009). During the ionic conduction mechanism heat is generated because of the resistance of the medium to flow ion. On the other hand, ions keep their direction along field signs which change frequently. This frequent change of directions results in collision between molecules and consequently generates heat.

The main parameter for microwave-assisted extraction is temperature. It is possible to change the temperature by changing the irradiation duration and power. In practice, the temperature can be set by regulating the irradiation power in order to maintain the right temperature. Conversely, it is also possible to use power intensity directly. The efficiency of microwave heating at a given frequency and temperature depends on the ability of the material to absorb electromagnetic energy and to dissipate heat and can be measured by $\tan \delta = \varepsilon'' / \varepsilon'$, which is the dielectric loss tangent. Where: ε', is the dielectric constant and is proportional to the amount of energy absorbed; ε'', is the dielectric loss or loss factor and indicates the ability of a medium to dissipate input dielectric energy as heat (Vinatoru et al.,2017).

The microwave-assisted extraction (MAE) methods can be classified into solvent-free extraction methods (usually for volatile compounds extraction) and solvent extraction methods (usually for non-volatile compounds extraction). The extraction mechanism of microwave-assisted extraction is supposed to involve three sequential steps described by Alupului (2012): first, separation of solutes from active sites of sample matrix under increased temperature and pressure; second, diffusion of solvent across sample matrix; third, release of solutes from sample matrix to solvent.

- **Pressurized liquid extraction (PLE)**

In 1996, Richter et al. first described PLE, which is also known as pressurized fluid extraction (PFE), enhanced solvent extraction (ESE), high-pressure solvent extraction (HPSE), or, most popularly, by the Dionex trade name, accelerated solvent extraction (ASE). PLE is regarded as a reasonably uncomplicated, exhaustive extraction technique, which is easy to learn and provides quantitative recoveries with little time spent on method development. This technique applies pressure in order to heat the extraction solvent above its boiling point. Thus, it enhances extraction efficiency by reaching higher temperatures than conventional extraction (maceration, soxhlet, etc.). Higher temperatures lead to a higher solvent solubility capacity, to a lower viscosity improving penetration of the solvent into plant cells, and to a reduction in solute-matrix interactions. All these effects lead to an improvement in the extraction yield, and may then lead to a decrease in selectivity (Lefebvre et al.,2021).

PLE works at high temperatures (usually up to 200°C) and high pressure (usually up to 200 bar) to extract quickly with low volumes of organic solvents, and it provides recoveries similar to other techniques. Parameters that significantly affect these recoveries are the extraction solvent, the temperature, the pressure, the static extraction time, the number of cycles, and the sample weight. Other parameters (e.g., purge time and flush volume) have shown little influence on the final recoveries (Nieto et al., 2010).

- **Supercritical fluid extraction (SFE)**

The application of supercritical fluid for extraction purposes started with its discovery by Hannay and Hogarth (1879) but the credit should also be given to Zosel who presented a patent for the decaffeination of coffee using SFE (Zosel, 1964). Since this beginning, the supercritical fluid technique has attracted wide scientific interest and it has been successfully used in environmental, pharmaceutical, and polymer applications and food analysis (Azmir et al., 2013).

A supercritical state is a distinctive state and can only be attained if a substance is subjected to temperature and pressure beyond its critical point. The critical point is defined as the characteristic temperature (Tc) and pressure (Pc) above which distinctive gas and liquid phases do not exist. Supercritical fluid possesses gas-like properties of diffusion, viscosity, and surface tension, and liquid-like density and solvation power. These properties make it suitable for extracting compounds in a short time with higher yields a wide variety of solvents is available for use as SFs, including carbon dioxide, nitrous oxide, ethane, propane, n-pentane, ammonia, fluoroform, sulphur hexafluoride, and water. Carbon dioxide is currently the solvent of choice, as it can easily reach supercritical conditions and has clear advantages (e.g., low toxicity, inflammability and cost, and high purity) over other fluids. However, the use of carbon dioxide is restricted by its inadequate solvating power for highly polar analytes, which can, to some extent, be boosted by using an appropriate modifier (Zougagh et al.,2004).

A basic SFE system consists of the following parts: a tank of mobile phase, usually CO_2, a pump to pressurize the gas, a co-solvent vessel and pump, an oven that contains the extraction vessel, a controller to maintain the high pressure inside the system and a trapping vessel. Usually, different types of meters like flow meters, and dry/wet gas meters could be attached to the system (Azmir et al., 2013).

- **Enzyme assisted extraction (EAE)**

Cell wall components like lignins, celluloses, and proteins pose mechanical barriers to routine extraction. Enzyme-assisted extraction (EAE) is used to overcome these barriers, enabling the extraction of bioactive compounds from various plants (Rodríguez et al.,2020). Enzyme-assisted extraction (EAE) is a method that uses enzymes to break down covalent bonds in the presence of water, increasing material permeability. This process can be used as a standalone method or a pretreatment for conventional extractions. Enzymes are highly specific and can be effective at low temperatures, moderate pH, and short times without expensive equipment. Enzymatic pretreatment can significantly improve extraction efficacy, and it can be performed in water or a buffer, eliminating the need for additional organic solvents (Krakowska-Sieprawska et al., 2021).

2.2 Hawthorn (*Crataegus monogyna* Jacq.)

Hawthorn, a common name of all plant species in the genus *Crataegus*, which belongs to the Rosaceae family, *Crataegus* is native to northern temperate zones, including those of North America, East Asia, Central Asia, and Europe. Common names for hawthorns may include, mayblossom, quickthorn, whitethorn, haw hazels, gazels, halves, hagthorn, and bread and cheese tree (Alirezalu et al., 2020; Güven et al., 2006).

Hawthorns are as thorny large shrubs or small trees. The hawthorn tree prefers the forest margins of lower and warmer areas (Güven et al., 2006). Usually multi-branched 2 – 5 m shrubby trees can reach a height of up to 10 m (Yanar et al., 2011). Hawthorn trees have bright to dark green leaves with margins that range from nearly entire to serrate to deeply lobed. The bushes or trees produce dense clusters of white flowers, often with a characteristic trimethylamine scent, which end with berries that range in colour from yellow through bright red to black, each containing one to three or five seeds, depending on the species (Edwards et al.,2012). Most of the species ripen their fruit in early to mid-autumn (Chang et al., 2002). Fig. 3 and Fig. 4 show the tree, flowers, and fruits of hawthorn.

Fig 3. Hawthorn (*C. monogyna Jacq*.) tree and flowers (source: ireland's wildlife website)

C. pinnatifida Bge. *C. monogyna* Jacq. *C. crus-galli* L.

Fig 4. Hawthorn fruits (source: alamy website)

2.2.1 Physical and chemical properties of hawthorn fruits

The physical and chemical properties such as the size distribution of hawthorn fruits are given in Tables 1, 2, and 3 according to the results of (Özcan et al., 2005):

Table 1. The physical properties of hawthorn fruits (Özcan et al., 2005)

Properties	Values	Properties	Values
Diameter (mm)	19.34 ± 0.18	Fruit density (kg/m^3)	1065.98 ± 28.18
Length (mm)	14.39 ± 0.12	Bulk density (kg/m^3)	466.06 ± 3.39
Mass (g)	3.03 ± 0.06	Volume (mm3)	3083.3 ± 261.41
Geometric mean diameter (mm)	17.52 ± 0.15	Velocity (m/s)	6.81 ± 0.32
Sphericity	1.22 ± 0.01	Hardness (N)	7.47 ± 0.46
Projected area (cm^2)	4.19 ± 0.22	Porosity (%)	56.28 ± 0.97

Table 2. Chemical properties of hawthorn fruits (Özcan et al., 2005)

Properties	Values	Properties	Values
Moisture (%)	64.26	Non-soluble HCl ash (%)	0,0012
Crude protein* (%)	2.48	pH	3,38
Crude oil (%)	0.87	Acidity (%)	1,98
Crude cellulose (%)	4.67	Water-soluble extract (%)	32,31
Crude energy (kcal/g)	34.02	Alcohol-soluble extract (%)	20,36
Ash (%)	2.28		

Table 3. Mineral contents of hawthorn fruits (Özcan et al., 2005)

Metal	content	Metal	content
Ca (mg/kg)	3046.37 ± 199.90	Cr (ppm)	1.10 ± 0.15
P (mg/kg)	1477.88 ± 53.15	Fe (ppm)	32.77 ± 2.46
K (mg/kg)	13,531.96 ± 501.38	Li (ppm)	1.62 ± 0.02
Mg (mg/kg)	1502.55 ± 120.06	Ni (ppm)	1.10 ± 0.03
Na (mg/kg)	312.18 ± 13.39	Pb (ppm)	0.71 ± 0.45
Al (ppm)	33.05 ± 2.49	Se (ppm)	0.56 ± 0.14
B (ppm)	22.50 ± 0.69	V (ppm)	5.86 ± 0.40

2.2.2 Chemical components of hawthorn leaves, flowers, and fruits

Sugars and sugar alcohols

Sugar content and composition significantly impact fruit flavor and acceptability. Sugar alcohols contribute to sweetness and health effects. *Crataegus* species have different sugars like glucose, sucrose, fructose, and xylose, while some hawthorn species have higher sugar alcohol content like sorbitol and myo-inositol. Table 4 displays the sugar content in some speices of hawthorn (Liu et al., 2010; Bignami et al., 2003).

Table 4. Some of sugars and sugar alcohols previously quantified in *Crataegus* fruits (Özcan et al., 2005)

Speice	Glucose Concentration (mg/g)	Sucrose Concentration (mg/g)	Fructose Concentration (mg/g)	Sorbitol Concentration (mg/g)	myo-inositol Concentration (mg/g)
C. pinnatifida	72.0	_	77.6	76.9	2.0
C. pinnatifida var. major	116.7	56.1	134.0	76.7	1.3
C. azarolus	67.3	62.4	72.6	23.0	–
C. opaca	9.5	4.3	18.0	2.7	0.1

Organic and phenolic acids

Crataegus species contains various acids, including malic, citric, succinic, ascorbic, tartaric, quinic, protocatechuic, 3- and 4-hydroxybenzoic, salicylic, and syringic acids (Glew et al., 2003). Citric acid is the most abundant, followed by malic and citric acids which have functions beyond plant primary metabolism, such as aluminum tolerance and phosphorus uptake regulation (Drouillon & Merckxin 2003). Ascorbic and tartaric acids act as antioxidants, protecting plants from reactive oxygen species and preventing oxidative stress-related diseases (Davey et al.,2000). Table 5 presents the quantities of specific acids found in *Crataegus* species based on a review of several studies by (Edwards et al., 2012).

Table 5. Organic and phenolic acid concentrations reported in *Crataegus* species (Edwards et al., 2012)

Acid	Speice	Concentration (mg/g) in the fruit
Malic	*C. aestivalis*	14.2 – 15.5
	C. azarolus	11.9 – 22.7
	C. germanica	1.26 – 5.729
	C. pinnatifida	Nd – 7.0
	C. pinnatifida. var. major	Nd – 7.2
Citric	*C. aestivalis*	0.2 – 1.9
	C. azarolus	1.9 – 6.4
	C. opaca	1.0 – 5.4
	C. pinnatifida	7.8 – 57.8
	C. pinnatifida. var. major	33 – 48.4
Succinic	*C. azarolus*	0.275 – 0.435
	C. monogyna	0.271– 0.359
	C. pinnatifida	Nd – 0.057
Ascorbic	*C. hupehensis*	13
	C. pinnatifida var. major	15.6
Tartaric	*C. cuneata*	12
	C. scabrifolia	21.9
	C. pinnatifida	16.3
	C. pinnatifida. var. major	11.7
3-hydroxybenzoic	*C. germanica*	0.0086 – 0.1439
4-hydroxybenzoic	*C. germanica*	0.3386 – 0.7837
Salicylic	*C. germanica*	0.0079 – 0.0506

Nd: not detected

Terpenes

Terpenes are natural products with properties similar to other secondary metabolites. They are found in plants and act as toxins, repellents, and attractants. With regards to the genus *Crataegus*, oleanolic acid and ursolic acid have previously been quantified in *C. pinnatifida*. These acids have anti-inflammatory, gastroprotective, and hypoglycemic properties in humans (Tian et al., 2010). Euscapic and corosolic acids have also been isolated from hawthorn but not quantified.

Essential oils

Essential oils are produced primarily of terpenoids and phenylpropanoids. Essential oils, found in plants, have antifungal, antiviral, and antibacterial properties, and are used in various industries such as perfumes, cosmetics, dentistry, agriculture, food preservation, and natural remedies (Bakkali et al., 2008). Few studies have been done regarding the essential oil composition of hawthorn. The most extensive study to date was carried out by (Kovaleva et al., 2009), in which the essential oil composition of the flowers of three North American species of hawthorn *(C. jackii, C. flabellata, and C. robesoniana*) was investigated by GC–MS analysis followed by NIST–MS library searches. In total, 46 compounds were tentatively identified, 19 of which were common to all three species. The compounds included several monoterpenoids, sesquiterpenoids, norterpenoids, and triterpenoids.

Phenylpropanoids

Phenylpropanoids include a number of organic compounds, all of which are derived from the amino acid phenylalanine. Phenylpropanoids may further be divided into a number of other compound classes. In hawthorn, many compounds from each of these classes have been identified, quantified, and tested for their pharmacological activity such a hydroxycinnamic acids, lignans, flavonoids. Table 6 presents the quantification of total flavonoids and total phenols in *Crataegus* species based on selected studies.

Table 6. Total flavonoids and total phenols quantified in *Crataegus* species (Edwards et al.,2012)

	Species	Concentration (mg/g)		
		Fruit	flower	leave
Total flavonoids	C. aronia var. aronia	–	3.17 – 5.358	9.13
	C. azarolus	Nd – 0.81	–	1.10 –1.50
	C. azarolus var. azarolus	–	3.45 – 3.464	–
	C. microphylla	–	6.2 – 12.8	7.2 – 20.4
	C. monogyna	4.46 –147.3	10.4 – 1026.6	24.95 – 28.60
	C. pentagyna	23.68	4.1-18.4	–
	C. pseudoheterophylla	–	7.8	5.5 – 7.58
Total phenols	C. azarolus var. aronia	4.985 – 6.543	9.31 – 10.14	–
	C. azarolus	1.85 – 23.0	–	–
	C. azarolus var. azarolus	7.79 – 8.107	0.45 – 9.913	–
	C. germanica	3.517 – 5.64	–	–
	C. monogyna	16.42 – 57.07	9.7-98.89	–
	C. pentagyna	92.12	–	–
	C. pinnatifida	19.44	–	–
	C. pinnatifida var. major	248.8	–	11.2 – 022.8
	C. scabrifolia	–	–	7 – 7.8

2.2.3 Health-beneficial properties of hawthorn

The evaluation of phytochemical composition is the first step for the determination of the beneficial health properties of a plant, several of the literature from in vitro and in vivo studies mentioned the health properties of the hawthorn. Where many beneficial properties have been

attributed to hawthorn, including anticancer, anti-HIV, anti-diabetic, and anticoagulant activity, cardioprotective effects, hepatoprotective effects, antihyperglycemic and antihyperlipidemic activities, wound healing effects, antimicrobial effects, gastroprotective effects, treatment of metabolic syndrome, regulation of cholesterol homeostasis, anti-atherosclerosis effects, anti-aging effects by (Nazhand et al., 2020).

Nazhand et al. (2020) summarized various health-promoting activities of hawthorn based on numerous in vitro studies, animal studies, and clinical trials. Tables 7 and 8 present selected studies from their review.

Table 7. In vitro and in animals reported activities for hawthorn (Nazhand et al., 2020)

	Activity	effects
In vitro	Anticancer	Pinnatifidanin BVI extracted from hawthorn had a preventive effect against Mrc5 human lung cells.
	Antioxidant	Naturally occurring compounds from ethanolic and aqueous extracts of *C. monogyna* showed antioxidant and hydrogen peroxide scavenging properties.
	Anti-inflammatory	Aqueous hawthorn fruit extract inhibited the expression of ILInterleukin-6, Interleukin-1β, Tumor necrosis factor-α and cyclooxygenase-2 genes, and prevented NO formation in RAW 264.7 cells.
Studies in animals	Anticataract potential	*C. pinnatifida* leaf extracts used three times a day reduced the level of malondialdehyde and increased serum levels of catalase and superoxide dismutase in rats with selenite-induced cataracts.
	Dyslipidemia therapy effect	*C. pinnatifi* fruit extract (250 mg/kg) for 7 days in high-fat-diet-fed mice with hyperlipidemia reduced blood lipid and lipid degradation by enhancing the hepatic expression of peroxisome proliferator-activated receptor α.

Table 8. Examples of studies in humans involving hawthorn (Nazhand et al., 2020)

Activity	Administration	Main Findings
Anti-inflammatory effect	Patients with diabetes (n = 37) received hawthorn vinegar (20 mL) diluted with water (40 mL) after meals for a month.	The treatment reduced serum levels of triglyceride, LDL, cholesterol and glucose, as well as decreased glycated hemoglobin, blood pressure and body weight.
Anti-hypertensive effect	Patients (n = 21) randomly received 1000 mg, 1500 mg and 2500 mg of hawthorn extract twice per day for four days.	The treatment lowered blood pressure.
Antihypertensive effect	The administration of hawthorn hydroalcoholic extract in subjects with primary mild hypertension.	A reduction in diastolic and systolic blood pressure after four months.

2.2.4 Applications of hawthorn in food products

Fresh hawthorn fruit can be eaten directly. With the continued development of science and technology hawthorn fruit has been processed into many types of products for example:

- Traditional hawthorn products

There are many products made from hawthorn on the market, with more than 150 types of products sold. Traditional hawthorn products in China mainly include sugar gourd, hawthorn cakes, hawthorn preserves, canned hawthorn, hawthorn chips, and hawthorn roll.

- Beverages

Pectin extracted from hawthorn wine residue was used to produce yogurt and was able to improve the firmness, denseness, and viscosity of stirred yogurt and increased the sensory acceptability of the yogurt, which offered the possibility of developing hawthorn wine by-products (Jiang et al., 2020). a study investigated the use of hawthorn juice in water kefir production, (it is a non-dairy-based kefir made by fermenting sucrose solution with yeasts). Results show a synergistic relationship between juices and water kefir grains, with acceptable microbial, physicochemical, and sensory profiles and high antioxidant activity. These new types of fermented products could offer a functional food alternative for those avoiding dairy products (Ozcelik et al., 2021).

- Brewing products

Hawthorn has long been used in wine production and vinegar production, with studies focusing on improving the properties and benefits of hawthorn wine. Hawthorn vinegar has volatile aromatic compounds and bioactive phenolic compounds, providing nutritional and health benefits, and increasing its use and consumption (Özdemir et al., 2022). In another study, beer with dotted hawthorn fruit (*Crataegus* punctate) juice was prepared and analyzed, resulting in the best scores in sensory analysis for aroma, taste, and overall quality, with the highest volatile compounds content compared to the control beer (Gasiński et al., 2020).

- Bakery products

Hawthorn berrie extracts have been used to improve the quality of wheat bread. The results show the potential of reduction of technological cycle duration by 60-90 min without degradation of the quality of finished products. In another study, the impact of wild-grown fruits (elderberry, sea-buckthorn, rowan, and hawthorn) was evaluated on the nutritional properties of wheat-flour bread. The bread enriched with wild-grown fruits had higher nutritional value due to significantly higher contents of fat and dietary fiber as well as ash in comparison with the control bread (Borczak et al., 2016).

- Meat products

Nowadays, new strategies for reducing the formation of heterocyclic aromatic amines (HAAs) mutagenic/carcinogenic have attracted researchers' attention. Meat preparation methods may have a significant role in HAA formation, and in particular, the addition of antioxidant compounds prior to cooking might be an effective way for decreasing the levels of these carcinogens. The inhibitory effect of different levels of hawthorn extract on the formation of heterocyclic aromatic amines (HAAs) in beef and chicken breast cooked by either pan-cooking or oven-cooking had been studied. The results showed that hawthorn extract could be

used as a mitigating agent in meat preparation since it is efficient in HAA inhibition as well as it did not lead to any change in structure, appearance, color, and odor (Tengilimoglu-Metin et al., 2017). The antioxidant and antibacterial effects of phenolics in hawthorn have also been investigated in a variety of commercial foods such as lamb burgers, frankfurters, and pork liver.

- Jam and sugar products

Since hawthorn is a fruit, it has been used to produce jam. Hawthorn jams on the market at present are broadly divided into two types: an ordinary hawthorn jam and compound jam with hawthorn flavor, such as hawthorn leaf flavonoid jam healthy jam, but also further enhances the use of hawthorn and hawthorn by-products. Also, Hawthorn candy products can change the sour and astringent taste of the hawthorn itself and expand the consumer market for hawthorn. Combining hawthorn with fondant not only enriches its taste but also adds nutritional health functions to fondant.

2.3 Anise *(Pimpinella anisum* L.*)*

The *Pimpinella anisum* has common names in different countries such as Anis vert (France); Anise seed (Japan); Anise and Star anise (the USA); Annesella (Italy); Anisa, Badian, Kuppi, Muhuri, Saunfand Sop (Iran and India); Boucage anis, Petit anise (North Africa), and anise (England).

The genus *Pimpinella* belongs to the Umbelliferae family. This family is characterized by umbellate inflorescences, small flowers, two mericarps' fruits with one seed and common stem, and the carpophore. They are also divided into two groups as follows: Anesum Ludwing DC. (*P. anisum* L.) and Tragoselinum Mill. DC (*P. peregrine* L., *P. saxifrage* L., *P. major* L.) and divided into three parts according to the appearance of the fruit (Nasır et al., 2021). Anise is an annual plant that reaches an average height of 30–50 cm. The plant is completely covered with fine hairs. The root is thin and spindle-shaped, the stem up, stalk-round, grooved, and branched upward. In midsummer, the thin stems are topped with umbrella-shaped clusters of tiny white flowers, which are heavy enough to make the stems flop. They turn into seedlike fruits. Anise is a cross-pollinating species and is genetically heterogeneous. The fruit is an ovoid-pear-shaped schizokarp somewhat compressed at the side. The two-part fruits separate heavily. The carpophore is almost two piece up to the base. Commercially available aniseed usually contains the whole fruits and occasionally parts of the fruit stalk The fruits with the style-foot are 3 – 5 mm long, 1.5 – 2.5 mm wide and 2 – 4 mm thick. Vittae (oil ducts) are almost always present embedded in the fruit wall on the dorsal surface, sometimes in or directly beneath the ridges. The fruits are downy. Their colour is greyish-green to greyish-brown (Özgüven et al., 2012). Fig. 5 and Fig. 6 show anise plant and seed.

Anise is cultivated in Turkey, Egypt, Spain, Russia, Italy, India, Greece, Northern Africa, Argentina, Malta, Romania, and Syria. Anise is primarily exported from Turkey, and also from Egypt and Spain in particular. *P. anisum* cultivation requires a warm and long frost-free growing season of 120 days. The plant needs a hot summer to thrive and for seeds to ripen. The small white flowers bloom in midsummer, and seed maturity usually occurs one month after pollination. Anise seeds are harvested between the end of July to the beginning of September, depending on the cultivation areas. Seed yields up to 500 – 1000 kg/ha have been achieved (Özgüven et al., 2012).

Fig 5. Anise *(Pimpinella anisum* L.) plant (source: sarah raven website)

Fig 6. Anise *(Pimpinella anisum* L.) seed (source: alamy website)

2.3.1 Chemical constituents of anise seed and oil

Aniseed contains 1.5 – 6.0 mass % of a volatile oil consisting primarily of trans-anethole and also as much as 8–11 mass % of lipids rich in fatty acids, such as palmitic and oleic acids, as well as approximately 4 mass % of carbohydrates, and 18 mass % of protein (Shojaii, and Abdollahi Fard., 2012).

The essential oil is the essence of the anise seed, which has been used in the pharmaceutical, food, perfumery, and flavoring industries. Anise oil was obtained using various extraction

methods and was analyzed by GC, GC-MS, and HPLC in several studies. The studies show that the major component of anise oil is trans-anethole (75 – 90 %), other constituents include coumarins (umbelliferone, umbelliprenine, bergapten, and scopoletin), lipids (fatty acids, betaarmyrin, stigmasterol, and its salts), flavonoids (flavonol, flavone, glycosides, rutin, isoorientin and isovitexin), protein, and carbohydrate. The composition of *Pimpinella anisum* essential oil from various origins is detailed in Table 9, while Table 10 presents the properties of aniseed oil

Table 9. Composition of *Pimpinella anisum* essential oil (%) of various origins (Khubeiz,& Zahraa, 2020; Saibi et al., 2012)

Components	Algeria	Turkey	Portugal	Syria
linalool	0.3	0.8	–	–
Terpinene 4-ol	–	0.6	–	–
tmethyl chavicol	–	0.8	–	–
α-terpineol	–	1.0	–	–
Estragol	1.9	–	2.2	0.29
Anisaldehyde	–	0.5	1.9	–
Cis-anethole	0.5	0.1	Tr	–
Trans-anethole	92.4	89.5	92.5	96.11
Methy eugenol	–	0.6	–	-
γ-himachalene	1.1	–	–	1.83
Yingiberene	0.3	–	–	0.53
Anesic acid	–	0.5	–	–
Anisylacetone	0.3	0.2	–	–
Anisyl alcohol	–	0.1	–	–
O-isoeugénol	1.9	0.2	–	–
Trans-Pseudoisoeugenyl-2-methybutyrate	–	–	0.1	–
1 butanoic acid, 2-methyl-, 4-methoxy- 0.3 - - - 2-(3-methyloxiranyl)phenyl ester	0.1	–	–	–

Table 10. Properties of aniseed oil (Yadav et al., 2015)

Parameter	Results
Color	Pale yellow
Specific gravity	0.987
Saponification value	168.3
Acid value	2.55
Iodine value	99
Refractive index	1.55
Odor	Sweet like Anethole

Total phenolic compounds and total flavonoids of anise seed extracts were determined, also, the fractionation and determination of phenolic compounds in many studies. Table 11 displays the phenolic compounds found in anise seeds, as determined by (Sakr et al., 2019) using HPLC

Table 11. Phenolic compounds in anise seeds extract (Sakr et al., 2019)

Phenolic compounds	Content (mg/100g) d.w	Phenolic compounds	Content (mg/100g) d.w
Catechein	71.68	Salycilic acid	7.07
Epicatechein	11.38	Pyrogallol	6.29
Caffeine	3.58	P-Coumaric acid	5.01
Caffeic acid	44.52	Salvianolic acid	1.84
Ellagic acid	10.64	Protocatchuic	1.98
Cinnamic acid	24.26	Chlorogenic acid	11.85
Rosmarinic acid	4.88	Coumarin	1.03
Catechol	21.91	Gallic acid	60.75
Alpha-Coumaric	18.74	3,4,5-methoxy-cinnamic	0.753
Ferulic acid	36.63		

2.3.2 Potential health benefits, medicinal uses of anise

Anise is one of the oldest species, used by ancient Greeks, Romans, and Arabs. Aniseed and its essential oil have been used in folk medicine for a wide range of therapeutic uses, such as a diuretic, mild expectorant, tranquilizer, stomachic, antifungal, antibacterial, anticonvulsant, carminative, milk secretion inducer, antispasmodic, expectorant, and intestinal purifier, among others (Rocha, & Fernandes, 2016).

The use of aniseed in folk medicine encouraged many researchers to investigate the medical properties of essential oil anise. Many studies have proven the health and medical effects of anise oil including antibacterial, antioxidant, and anti-cancer activity. (Shojaii, and Abdollahi Fard et al., 2012) summarized the medical effects and benefits of anise seed and oil in various studies, as presented in Table 12.

2.3.3 Usage and applications in food science and industry

Aniseed has been used as a spice since ancient times, being one of the most important plant species used in the culinary field. Today, the dry ripe fruits of anise are used in order to obtain its essential oil, which also plays an important role in food technology, being used as flavoring in many products, such as bread, cakes, candies, and beverages. For example, Turkish raki and Greek Ouzo are aniseed spirits used as aperitifs (Aćimović et al., 2015).

Essential oils including anise oil are usually used as "green products" to avoid spoilage and contamination of food by microorganisms without the use of synthetic preservatives. And these oils exhibit their potential in meat preservation, meat products, fish dishes, rice, and fruits (Burt, 2004).

In the meat industry, Apiaceae including anise (*Pimpinella anisum* L.) are favorable spices. Numerous meat products are recognized as products with added spices. In the dairy industry, they could be successfully used as safe and natural anti-pathogen sources in the production of

cheese and yogurt. With respect to the characteristic aroma, seed, and oil are used to add flavor to pickled vegetables, especially cucumber. Moreover, the oil and the extract from Apiaceae seed can be added to fatty oil (Aćimović et al., 2015).

Table 12. The pharmacological effects of (*Pimpinella anisum* L) (Shojaii, and Abdollahi Fard et al., 2012)

System	Effect	Preparation
Organism	Antibacterial	- Aqueous and 50% (v/v) methanol extract. - Ethanol extract. - Essential oil and methanol extract (in combination with Thymus vulgaris). - Aqueous decoction
	Antifungal	- Essential oil. - Fluid extract. - Methanol extract.
	Insecticidal	- Essential oil. - p-Anisaldehyde from aniseed oil.
	Antiviral	- Essential oil. - Lignin-carbohydrate-protein complexes from hot water extract.
Muscle	Muscle relaxant of tracheal chain.	- Aqueous extract. - Ethanolic extract. - Essential oil
	Antispasmodic and relaxant of anococcygeus smooth muscle.	- Hydroalcoholic extract (60 % ethanol)
Nervous system extract	Anticonvulsant	- Essential oil. - Methanol extract of seeds. - Aqueous extract of leaves and stem extract.
	Analgesic	- Essential oil. - Fixed oil
	Conditioned place aversion in morphine dependence.	- Essential oil
Gastrointestinal	Antiulcer	- Aqueous suspension
	Palliation of nausea. Laxative.	- Essential oils of aniseeds, foeniculum vulgar, Anthemis nobilis, and Mentha piperita Phytotherapic compound of anise and foeniculum vulgar, Sambucus nigra, Cassia angustifolia.
	Increase glucose absorption from the jejunum.	- Essential oil.
Renal	Reduce volume of urine by increase activity of the renal Na+-K+-ATPase.	- Essential oil.
Endocrine	Antidiabetic Hypolipidemic	- Seed powder.
Immune system	Antioxidant	- Ethanol and Water extract. - Essential oil. -Anise tea. - Oleoresin. - Ethyl acetate fraction of ethanol extract.
	Increase of β-carotene, vitamins A, C.	- Seed powder

2.4 Membrane technology

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 (Castro-Muñoz et al., 2020a). 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) (Conidi et al., 2018).

2.4.1 Microfiltration (MF) and ultrafiltration (UF)

Microfiltration (MF) membranes are used to retain colloidal particles as large as several micrometers. MF overlaps conventional filtration for the separation of small particles. Regarding MF and UF pores' size, microfiltration membranes (MF) have the largest pores, and ultrafiltration (UF) membranes are the next largest. Furthermore, UF and MF look similar, and in fact, they are more alike than they are different. Nonetheless, their different historical background kept them very distinct to practitioners and membrane manufacturers (Eykamp, 1995).

MF is one of the oldest pressure-driven membrane applications practiced commercially, where it comes second after dialysis (Eykamp, 1995). MF can remove micrometer-sized matter, such as suspended particles, major pathogens, large bacteria, proteins, and yeast cells based on the principle of physical separation (Anis et al., 2019a). Microfiltration grew out of the discovery of nitrocellulose in 1846. Later on, cellulose nitrate membranes were reported by Frick in 1855. Early cellulose nitrate membranes were prepared by dipping a test tube in a collodion solution. Surprisingly, some old materials are still used today (Eykamp, 1995). Microfiltration (MF) membranes are of average pore size between 0.1 and 10 μm where pores are distributed uniformly throughout the membrane. Moreover, MF is done under a pressure gradient of 1-3 bar following the sieving mechanism (Pal, 2020). The wide range of pore size in these films has allowed them to be applied in many fields such as desalination (Anis et al., 2019b), wastewater treatment (Saini et al., 2019; Katayon et al., 2007), and in the food field, especially in the milk and juice concentration and clarification fields (Rouquié et al., 2019; García et al., 2013; Elwell & Barbano, 2006), in the purification of pharmaceuticals (Wang and Huang, 2019), and as downstream processing in biotechnology (Wang et al., 2019).

Historically speaking, the first real ultrafiltration membrane was born in the early 1960s (Fritzmann et al., 2007). Ultrafiltration (UF) is a process in which a high molecular weight component is rejected by using a fine porous membrane. This process aims at separating water and fine solution from macromolecules and colloids (Mohamad et al., 2013). Ultrafiltration is also one of the membrane separation techniques that separates, purifies, and concentrates solutions between microfiltration and nanofiltration. Furthermore, ultrafiltration membranes reject the molecular weight 500~500000 Da. The approximate diameter of the pore is about

0.001~0.1μm, the operating pressure difference is generally 0.1~0.8MPa, and the diameter of the separated component is about 0.005~10μm (Li et al., 2018). Numerous polymer membranes have been developed for ultrafiltration applications. Polyethersulfone (PES) has been developed as a commonly employed material in ultrafiltration processes for protein separation. Due to its mechanical strength and physicochemical stability, polysulfone (PSF) is an excellent UF membrane material because of its film and membrane forming properties, and high mechanical and chemical stability (Mohamad et al., 2013).

Although the first aim of developing ultrafiltration membranes was purifying water, it has been applied in many fields since its inception. Among the first applications of ultrafiltration are the recovery of protein and its concentration from cheese whey (Matthews., 1984). Another application in the food industry is gelatin filtration (Simon et al., 2002), egg processing (Datta et al., 2009; Kim & Nakai, 1998), and also, commercial ultrafiltration which was applied to clarify the fruit juice (Urošević et al., 2017). Additionally, the ultrafiltration applications within biotechnology downstream processing are also increasing.

In comparison to the conventional processes, microfiltration and ultrafiltration can bring the following benefits: separation can be done without changing the temperature and pH of the solution and without chemical additives, thus reducing production costs and solving the problem of waste treatment, improving the product quality, and reducing labor costs (Urošević et al., 2017). However, filtration processes face the problem of membrane contamination, depending on the ratio of particle size to that of the membrane pore. Thus, particles may completely block, partially close, or internally constrict pores.

2.4.2 Nanofiltration (NF)

The term nanofiltration (NF) first appeared commercially by FilmTec (now Dow Chemical Company) in the mid-1980s to describe a new line of membrane products with properties between UF and RO membranes. Since the term NF was not known in the 1970s, such membrane was initially categorized as either loose/open RO, intermediate RO/UF, or tight UF membrane (Burggen et al., 2008; Schäfer et al., 2005). Regarding its features, the molecular weight cut-off (MWCO) of the NF membrane is about 200-1000 Dalton (Da), which corresponds to pore sizes between 0.5 and 2 nm. Furthermore, Unlike UF and RO membranes which generally carry no charge on their surface, NF membrane often carries positive or negative electrical charges (Strathmann, 2011). In most cases, NF membranes are negatively charged in neutral or alkaline conditions and positively charged in highly acidic conditions. Given this, the separation of NF membrane is governed by three distinct mechanisms, namely the steric hindrance (or size sieving), electrostatic (Donnan) exclusion, and dielectric exclusion.

The first generation of NF membranes was manufactured in the early 1970s from cellulose acetate (CA) or its derivatives. These membranes were manufactured based on the well-known dry and wet phase inversion technique of Sorian Loeb (Cohen, H., Loeb, 1977; Loeb et al., 1964). However, the poor biological and chemical stability of cellulose-based membranes has limited the range of industrial applications since these membranes have always suffered from constant changes in water flow and solute rejection during operation. Because of these reasons, a second-generation NF membrane was developed based on non-cellulosic materials. This film

is a thin film composite (TFC) that consists of three different layers; a selective layer of ultra-thin polyamide (PA), a small porous inner layer on the upper surface, and the third one is a polyester non-woven underlayment (Lau et al., 2012). Generally, the overall structure should have good resistance to acids, bases, oxidation and reduction, high pressures, and sometimes resistance to high temperatures (Bruggen et al., 2009). Since the first appearance of these films, significant efforts have been devoted to improving their properties and composition. This, in turn, has led to the production of the NF films with different separation properties, allowing applications for various industrial processes, and today there are many manufacturers of NF membranes.

As NF membranes differ in many aspects such as materials, morphology, transfer/separation mechanism, and applications, the characterizations of the membrane pore structure; pore radius, pore density, pore shape, pore length, and tortuosity are essential in light of understanding the process. Therefore, characterization methods are major to support the interpretation of dissolved transport, membrane fouling, etc. Several characterization methods that are based on direct automated observation and experimental methods have been applied too. Moreover, various methodologies have been used to investigate this characterization like the gas absorption-desorption technique, also known as Brunauer - Emmett - Teller (BET), which allows direct measurement of the pore size distribution. Reverse surface impregnation combined with Transmission Electron Microscopy (TEM) allows direct measurement of pore size and distribution. Atomic force microscopy (AFM) allows direct measurement of pore size, distribution, surface roughness, topography, and force interactions between the membrane and colloids. In addition, many methods and methodologies studied the chemical composition and physical properties of these membranes (Khulbe & Matsuura, 2021; Mohammad et al., 2015).

Regarding the usage of NF, it has been mainly applied in the procedures of the drinking water purification process, such as treatment and softening water (Ursino et al., 2018; Bruggen & Vandecasteele, 2003) and removing micro-pollutants (Lipp et al., 2010), removing sulfate and electrolytes from seawater (Park et al., 2016; Bhattacharya & Ghosh, 2004), and separating heavy metals from contaminated water (Castro-Muñoz et al., 2020b). Furthermore, using NF membranes in a non-aqueous has also held strong potential in several industrial applications since the 1990s. Due to the lower energy costs involved in the organic solvent membrane processes, a growing interest in applications including solvent recovery in the petrochemical and oleochemical industries (Shi et al., 2019), rrecovery of polyphenols and valuable components from Agro-Food By-Products (Cassano et al., 2018), as well as separation and purification of valuable products in the pharmaceutical industry can be observed (Martínez et al., 2012). Generally, the trend in nanofiltration research has increased since 2007. Besides, nanofiltration membranes continue to see an increasing interest in their use as a separation tool (Oatley-Radcliffe et al., 2017).

In addition to all the previous applications, the membrane technologies (MF, UF, NF, MD, OD) have found an application in the concentration and recovery of active substances from plant extracts. (Alsobh et al., 2021) summarized studies on the application of membrane technologies (MF, UF, NF, MD, OD) for concentrating and recovering active substances from plant extracts, as shown in Tables 13 and 14.

Table 13. Application of microfiltration and ultrafiltration membranes in the concentration of plant extracts (Alsobh et al., 2021)

Membrane type	Material	Pore size	Applied pressure	Temp	Raw material	Targeted product	Findings/results
Microfiltration	Ceramic	0.2-1.4 µm	50-200 kPa	50-60 ºC	Watermelon juice	Lycopene	41 times more concentrated. 34 times pure than the initial juice.
Microfiltration	Hollow Fiber	150 kDa	35-172 kPa		Tea leaves	Total polyphenol	80 % of the total polyphenol content. 75 % of the EGCG purity in permeability.
Ultrafiltration	Millipore type GS 0.22 Millipore Type HA 0.45	22 nm, 45 nm	5 bar	23 ºC	Grape seed	Polyphenol	maximum amounts of polyphenols. (11.4 % of total seed weight) were obtained.
Ultrafiltration	FSM0, 15PP (Fluoro Polymer) UV050 (polyvinylidene) UP150 (polyethersulphone)	0.15µm 50 kDa 150 kDa	12-30 bar	–	Grape seed extract	Polyphenols	very high retentions of phenols (87-91 %)
Ultrafiltration	polysulfone (PS) polyethersulfone (PES)	100 kDa 50 kDa	1-2 bar/step 1 0.8,1.5,3 bar/step2	50 ºC	pulp of papaya	Lycopene	lycopene retention was higher than 90 % . The best UF performance was obtained with the PS 100 membrane, pressure 1 bar

Table 14. Application of nanofiltration and reverse osmosis membranes in the concentration of plant extracts (Alsobh et al., 2021)

Membrane type	Material	Pore size	Applied pressure	Temp	Raw material	Targeted product	Findings/results
Nanofiltration	Polyamide	400,700,1000 Da	–	–	Grape seed	Phenolic	procyanidin rejection was 96.36 ± 0.87 %. The antioxidant activity was increased around 2.24 times
Nanofiltration	polysulfone with SBA-15-NH2 SelRO MPF-36, Koch membrane	600-1500 Da	10 bar	–	Geranium robertianum and Salvia officinalis	Phenolics, flavonoids, and antioxidants	>70% rejection rate of polyphenols and flavonoids; >88 % antioxidants. 85.9 % rejection rate of polyphenols and flavonoids, >90 % antioxidants
Nanofiltration	DuramemTM 200	200,300,500 Da	20,40 bar	50 ºC	Rosemary extracts	Phenolics and antioxidants	complete rejection of rosmarinic acid and other antioxidant components
Nanofiltration	NP010, NP030, TFC-S, NF200, Desal DL, Desal DK	200-1000 Da	0-40 bar	20 ± 2ºC	Artichoke brines	Flavonoids and caffeoylquinic acids	<92 % rejections of total caffeoylquinic acids, flavonoids, and cynarin
Forward Osmosis	A flat membrane module (developed by Osmotek, Inc., Corvallis, OR)	–	–	27±2 °C	Rose petals	Anthocyanin	The forward osmotic concentration using the membrane resulted in minimum degradation of anthocyanin
Reverse Osmosis	RO 99 and X20	–	average 40 bar	30 ºC	Beetroot peel extracts	Betalains, phenolics, and antioxidants	> 90 % betalains recovery; > 99 % phenolics and antioxidants recovery

2.4.3 Integrated membrane processes

Many research studies have integrated membrane unit operations into combined systems, resulting in benefits such as reduced energy consumption and improved product quality, processability, and selectivity. Table 15 presents examples of these integrated studies.

Table 15 Integrated membrane processes for concentrating bioactive compounds: selected studies

Study Description	Membrane Types and Processes	Key Findings	Reference
Concentration of Oleuropein from Olive Leaf Extract	- Microfiltration (0.2 nm pores) - Ultrafiltration (MWCO 5 kDa) - Nanofiltration (MWCO 300 Da)	Based on the content of solute in feed and retentate fractions of NF membrane, oleuropein was concentrated approximately 10 times to reach 1685 mg/100 g extract.	(Khemakhem et al., 2017)
Concentration of Pectin Extract from Red Currant Marc By-products	- Reverse osmosis (Two flat-sheet RO membranes: ACM2-TRISEP and SG composite) - Nanofiltration (One flat-sheet NF membrane: DL)	RO increased TSS content to 4.28 Brix. NF increased TSS content to 8.88 Brix. Membrane and fouling resistances affected gel resistance.	(Hodúr et al., 2009)
Production of Concentrated Sage Extract	- Reverse osmosis (Flat sheet ACM2 membranes) - Osmotic distillation	Osmotic distillation retained > 90 % of polyphenol content, flavonoid content, and antioxidant activity	(Torun et al., 2014)
Phenolic compound purification from Jamun Seed Extract	Ultrafiltration (MWCO 25, 50, 100 kDa) - Nanofiltration (MWCO 1000, 400, 250 Da)	- Operating conditions influenced flux, recovery, and purity of polyphenol extract.	(Balyan & Sarkar, 2016)
Anthocyanin Concentration Using Hybrid Membrane Processes	- Reverse Osmosis (Polyamide membrane) - Ultrafiltration (PVDF membrane) - Osmotic membrane -Distillation (PP membrane)	- Hybrid process increased anthocyanin concentration 25-fold to 980 mg/100 ml. - Enhanced color attributes observed with membrane processes.	(Patil and Raghavarao, 2007)
Yerba Mate Extract Clarification and Concentration	- Sequential microfiltration (Polyethersulfone) - Reverse osmosis (Polyamide-Polyethersulfone)	- Clarified extracts with reduced turbidity and stable polyphenol content. - UF1 membrane exhibited optimal performance.	(Santos et al., 2020)

2.5 The response surface methodology (RSM)

The response surface methodology (RSM) is a widely used mathematical and statistical method for modelling and analyzing a process in which the response of interest is affected by various variables, and the objective of this method is to optimize the response. The parameters that affect the process are called independent variables, while the responses are called dependent variables.

The main goals of the RSM study are to understand the topography of the response surface including the local maximum, local, minimum, and ridgelines, and find the region where the most appropriate response occurs (Bradley, 2007). The RSM investigates an appropriate approximation relationship between input and output variables and identifies the optimal operating conditions for a system under study or a region of the factor field that satisfies the operating requirements. There are two main experimental designs used in response surface methodology Box Behnken designs (BBD) and central composite designs (CCD). In recent years, central composite rotatable design (CCRD) and face central composite design (FCCD) have also been applied to optimization studies (Aydar, 2018).

The experimental data are evaluated to fit a statistical model (Linear, Quadratic, Cubic, or 2FI (two-factor interaction)). The coefficients of the model are represented by constant terms, A, B, and C (linear coefficients for independent variables), AB, AC, and BC (interactive term coefficient), and A^2, B^2, and C^2 (quadratic term coefficient). Correlation coefficient (R^2), adjusted determination coefficient (Adj-R^2), and adequate precision are used to check the model adequacies; the model is adequate when its p value < 0.05, lack of fit p value > 0.05, R^2 > 0.9, and AdeqPrecision > 4. Differences between means can be tested for statistical significance using analysis of variance (ANOVA) (Aydar, 2018).

Stages in the application of RSM as an optimization technique are as follows: (1) the selection of independent variables of major effects on the system through screening studies and the delimitation of the experimental region, according to the objective of the study and the experience of the researcher; (2) the choice of the experimental design and carrying out the experiments according to the selected experimental matrix; (3) the mathematic–statistical treatment of the obtained experimental data through the fit of a polynomial function; (4) the evaluation of the model's fitness; (5) the verification of the necessity and possibility of performing a displacement in direction to the optimal region; and (6) obtaining the optimum values for each studied variable (Bezerra et al., 2008).

The simplest model which can be used in RSM is based on a linear function. For its application, it is necessary that the responses obtained are well-fitted to the following equation:

$$y = \beta_0 \sum_{i=1}^{k} \beta_i x_i + \varepsilon \quad (1)$$

where k is the number of variables, β0 is the constant term, βi represents the coefficients of the linear parameters, xi represents the variables, and ε is the residual associated to the experiments.

To evaluate curvature, a second-order model must be used. Where a central point in two-level factorial designs can be used for evaluating curvature. The next level of the polynomial model should contain additional terms, which describe the interaction between the different

experimental variables. This way, a model for a second-order interaction presents the following terms:

$$y = \beta_0 \sum_{i=1}^{k} \beta_i x_i + \beta_0 \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (2)$$

where βij represents the coefficients of the interaction parameters.

In order to determine a critical point (maximum, minimum, or saddle), it is necessary for the polynomial function to contain quadratic terms according to the equation presented below:

$$y = \beta_0 \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \beta_0 \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (3)$$

where βii represents the coefficients of the quadratic parameter.

2.5.1 Advantages and disadvantages of RSM

Advantages of RSM

- A relatively small number of trials can yield a tremendous amount of knowledge in a cost effective manner (Reji and Kumar, 2022).
- Can be used to determine the interaction effects of the independent input parameters.
- The data-driven model equation can be utilized to illustrate the different combinations of independent input factors that affect the outcome of a process/product.
- Both experimental and numerical responses can be approximated using RSM (Raissi and Farsani, 2009).
- To maintain a high level of efficiency in terms of cost, time, and any other restrictions.
- Compared to the Taguchi and one factorial method, the RSM technique appears to be more promising in mathematical modelling for forecasting responses (Myers et al., 2002).

Disadvantages of RSM

- It cannot be utilized to explain why an interaction has developed (Aydar, 2018).
- This method necessitates the selection of appropriate operating parameter ranges, and the optimization result is limited to specific scales.
- RSM is not good at foretelling prospective outcomes for a system operated outside the range of a particular study (Reji and Kumar, 2022).
- RSM cannot operate with larger models.
- The more responses you receive, the more likely you will receive poor optimization results (Cassettari et al., 2013)

3 MATERIALS AND METHODS

3.1 Preparing of hawthorn fruit and anise seed for the extraction processes

The process flowcharts detailing the preparation and extraction steps for hawthorn fruit and anise seed are illustrated in Fig. 7 and Fig. 8. Raw materials were selected based on the harvest season. Hawthorn fruit was harvested from various trees across different regions in Hungary between September and October. Approximately 6 kg of *Crataegus monogyna* Jacq. were collected during the seasons of 2021. Additionally, around 30 g of each *Crataegus pinnatifida* Bge. and *Crataegus crus-galli* L. were obtained from the university garden during the 2021 season for comparative purposes between the species. After removing the sticks, the fruits were washed, cleaned, and wiped to remove the water, the fruit has been naturally air-dried in the shade until the moisture content of the dry fruit is 8.5 % and saved until the extraction. Before extraction, the dried fruit was ground into various portions using a GM 200 pulverizer manufactured by Retsch GmbH (Haan, Germany) to enhance extraction efficiency. This process aimed to increase the active surface area of the fruit, facilitating improved contact with the solvent during extraction. For the grinding step, approximately 30 grams of dried fruit was added to the machine's chamber, and the machine was operated at 2000 rpm for two minutes to achieve thorough grinding.

The anise seeds, totaling approximately 6 kg, were sourced from Syria, where they are typically harvested and dried between February and March. Subsequently, these seeds were ground into different portions using a GM 200 pulverizer, following the same process, quantity, and speed as the hawthorn.

3.2 Heat-assisted extraction (HAE)

Response surface methodology based on central composite design (RSM-CCD) was applied 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 points. Independent variables and experimental ranges for HAE were for hawthorn fruit: A – ethanol concentration (10 – 90 % v/v), B – extraction temperature (30 – 60 °C), and C – extraction time (10 – 90 min) (Table 16), for anise seed A – extraction temperature (25 – 55 °C), B – extraction time (20 – 100 min), and C – sample-to-solvent ratio (2 – 10 g/100 mL) (Table 17). Experimental ranges of independent variables were selected according to the literature data. The total phenolic content, total flavonoid content, and antioxidant activity were set as response variables. Each response variable was fitted to the quadratic model (Eq. 4):

$$y = \beta_0 + \beta_A A + \beta_B B + \beta_C C + \beta_{AB} AB + \beta_{BC} BC + \beta_{AC} AC + \beta_{AA} A^2 + \beta_{BB} B^2 + \beta_{CC} C^2 \quad (4)$$

where Y represents extraction yields of target compounds; A, B, C are selected extraction parameters; β_0 - intercept, β_A, β_B, β_C, - linear, β_{AB}, β_{AC}, β_{BC} - interaction, and β_{AA}, β_{BB}, β_{CC} - quadratic regression coefficients.

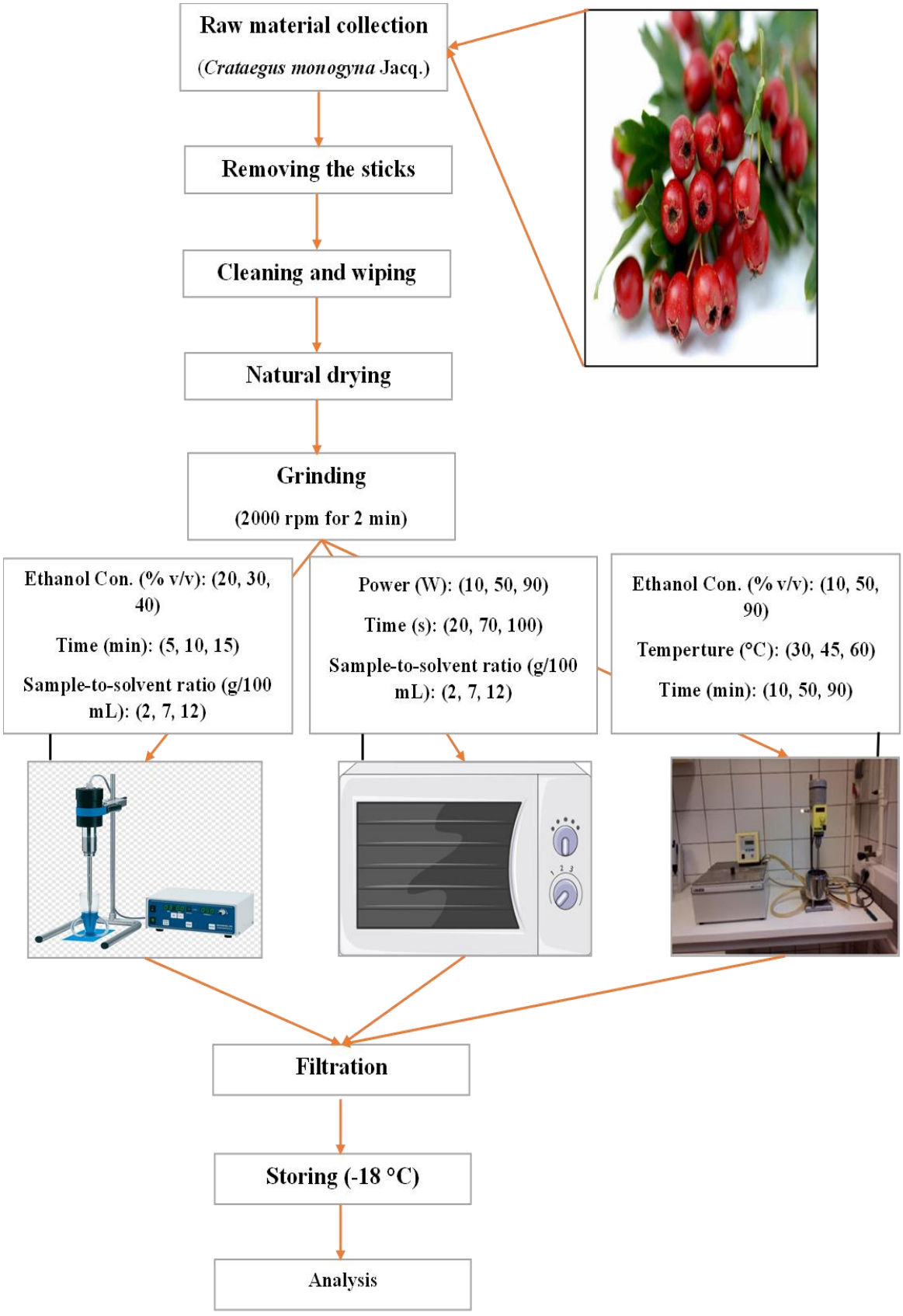


Fig 7. Flowsheet of hawthorn fruit extraction processes

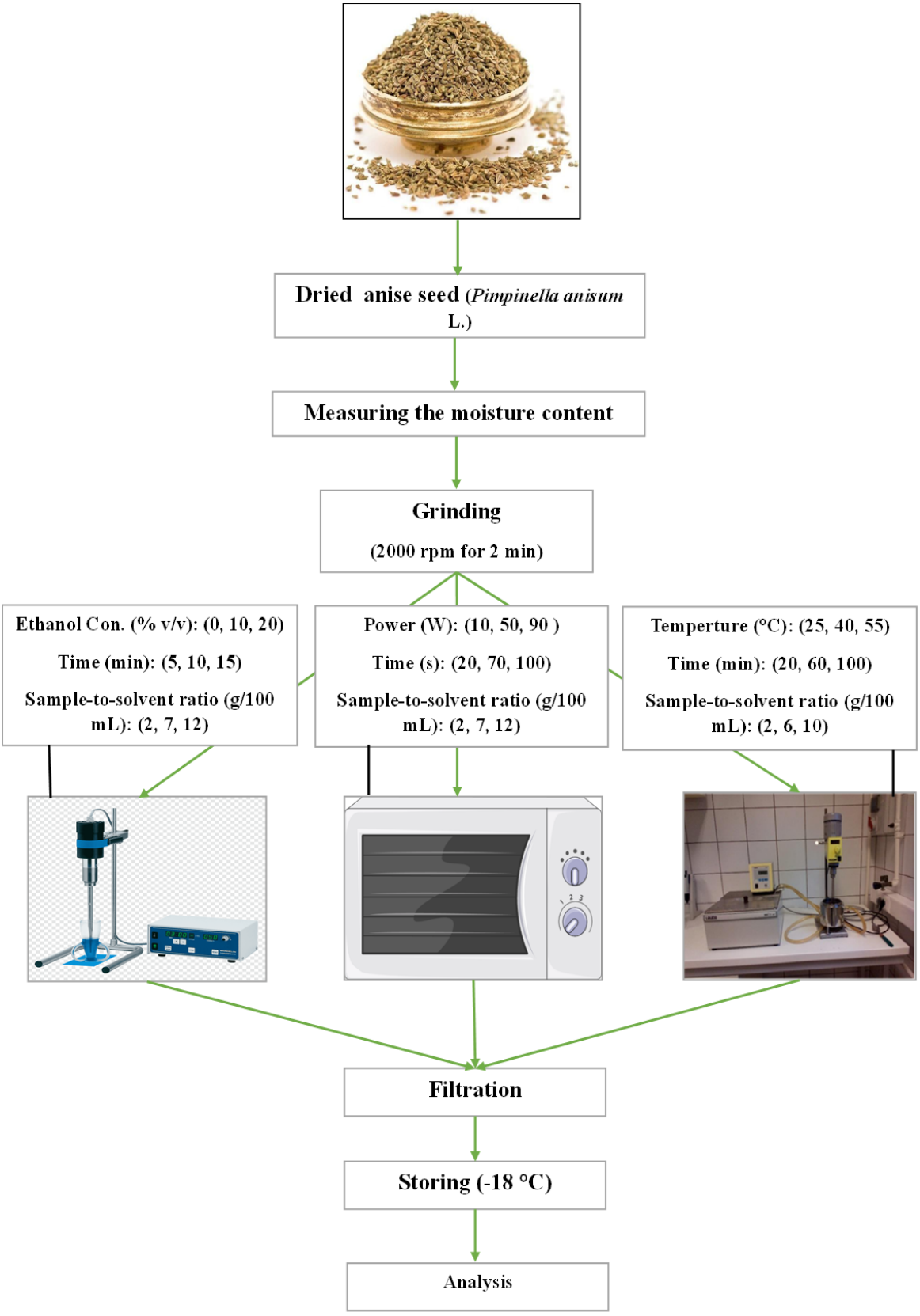


Fig 8. Flowsheet of anise seed extraction processes

Table 16. The experimental domain used in central composite design (CCD) for HAE extraction from hawthorn fruit

Input factors	Variables	Factor Levels		
		-1	0	1
Ethanol concentration (% v/v)	A	10	50	90
Extraction temperture (°C)	B	30	45	60
Extraction time (min)	C	10	50	90

Table 17. The experimental domain used in central composite design (CCD) for HAE extraction from anise seed

Input factors	Variables	Factor Levels		
		-1	0	1
Extraction temperture (°C)	A	25	40	55
Extraction time (min)	B	20	60	100
Sample-to-solvent ratio (g/100 mL)	C	2	6	10

The HAE process was carried out by (OS20-S Electric LED) digital overhead stirrer manufactured by Scilogex LLC (Connecticut, USA). The powdered hawthorn fruit (10 g) was extracted with aqueous ethanol solution in a double-walled tank connected to a (Lauda Ecoline E100 Immersion) thermostat manufactured by LP. Lauda (Lauda-Königshofen, Germany) to keep the temperature at a constant value (Fig. 9). Ethanol concentration, extraction temperature, and extraction time were adjusted to the experimental design requirements for each run. The powdered anise seed was extracted with an aqueous solution, and extraction temperature, extraction time, and sample-to-solvent ratio were adjusted according to the experimental design. The solid-solvent mixture was filtered and the obtained extracts were stored in a freezer at -18 °C until spectrophotometric analysis.

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

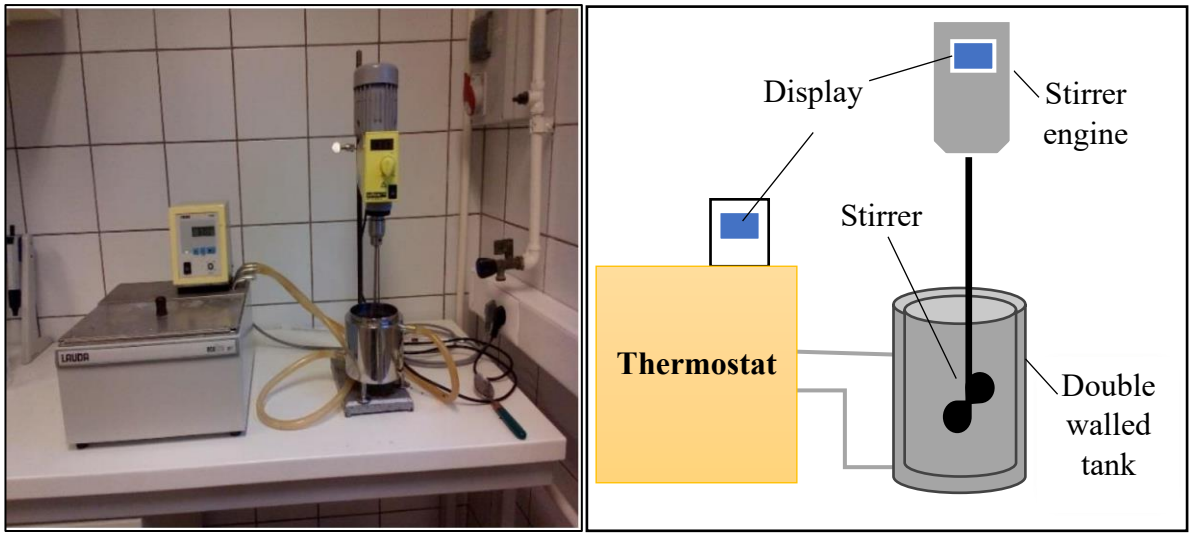


Fig 9. Picture of the experimental equipment and flow sheet of the HAE extraction equipment

3.2.1 Extraction of bioactive compounds from anise seed using different solvents

With the aim of finding the solvent which extracts the highest content of polyphenol and flavonoid compounds 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 (OS20-S Electric LED) stirrer and a double-walled tank connected to a (Lauda Ecoline E100 Immersion) thermostat stander (Fig 9). The powdered anise seed (10 g) was placed in a double-walled tank. The extraction processes were performed at 40 °C for 20 minutes with 100 ml of the solvents. Obtained solutions were filtered in a volumetric flask. Obtained extracts were stored in a freezer at -18 °C until the analysis. The quantification of specific bioactive compounds was performed spectrophotometrically. Where TPC was analyzed by Folin's method; TFC was analyzed by aluminum chloride assay; and AA was by FRAP (mg/g dw), and DPPH (%) methods.

3.2.2 Extraction of bioactive compounds from three species of hawthorn fruit

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, using the equipment which had been mentioned in Fig 9. The extraction processes were performed at 45 °C, by using ethanol 50 % v/v as a solvent (10 g of the fruit in 100 mL of solvent) for 50 min. To test the difference between the species, each measurement of total phenolic content, total flavonoid content, and antioxidant activity was repeated three times for each extract, and the obtained results were presented as mean ± standard deviation.

3.3 Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) was performed in mono-mode at a fixed frequency. 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) (Table 18). The response variables were fitted to the following second-order polynomial model (Eq. (4)) which is generally able to describe the relationship between the responses and the independent variables.

(MAE) extraction was carried out using (Specs EMM 2005) manufactured by Electrolux (Nuremberg, Germany) 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. The solid-solvent mixture was filtered and the obtained extracts were stored in a freezer at -18 °C until spectrophotometric analysis.

The quantification of specific bioactive compounds was performed spectrophotometrically. Where TPC was analyzed by Folin's method; TFC was analyzed by aluminum chloride assay; and AA was by FRAP (mg/g dw), DPPH (%), and ABTS (%) methods.

Table 18. The experimental domain used in central composite design (CCD) MAE extraction from both hawthorn fruit and anise seed

Input factors	Variables	Factor Levels		
		-1	0	1
Micowave Power (W)	A	100	450	800
Extraction time (s)	B	20	70	100
Sample-to-solvent ratio (g/100 mL)	C	2	7	12

3.4 Ultrasound-assisted extraction (UAE)

RSM Modelling was performed with Design Expert Software Trial Version 11.0.3 Trial (Stat-Ease, USA). A three-level design with three variables was applied to obtain the optimized extraction condition. The independent variables were ethanol concentration (% v/v), extraction time (min), and sample-to-solvent ratio (Tables 19 and 20). The response variables were total phenolic, flavonoid, and antioxidant activity. The quadratic was regarded as a model of the design in a total of 20 run samples. The multiple regression analysis was performed by the following second-order polynomial quadratic Eq. (4).

Table 19. The experimental domain used in central composite design (CCD) for UAE extraction from hawthorn fruit

Input factors	Variables	Factor Levels		
		-1	0	1
Ethanol concentration (%)	A	20	30	40
Extraction time (min)	B	5	10	15
Sample-to-solvent ratio (%)	C	2	7	12

Table 20. The experimental domain used in central composite design (CCD) for UAE extraction from anise seed

Input factors	Variables	Factor Levels		
		-1	0	1
Ethanol concentration (%)	A	0	10	20
Extraction time (min)	B	5	10	15
Sample-to-solvent ratio (g/100 mL)	C	2	7	12

The ultrasound-assisted extraction (UAE) was carried out by power ultrasound (3.5 W/cm^2, 20 kHz) produced by a generator (ULC 400) premium ultrasonic generator manufactured by Weber Ultrasonics AG (Karlsbad, Germany) (Fig. 10). The powdered plant (hawthorn fruit or anise seed) sample (10 g) was placed in a flask with the solvent. Ethanol concentration, extraction time, and sample-to-solvent ratio requirements of the experimental design for each run. To stabilize the heat distribution throughout the treatments, an icy water bath was used maintaining the temperature around 25 ºC. The solid-solvent mixture was filtered and the obtained extracts were stored in a freezer at -18 °C until spectrophotometric analysis.

The quantification of specific bioactive compounds was performed spectrophotometrically. Where TPC was analyzed by Folin's method; TFC was analyzed by aluminum chloride assay; and AA was by FRAP (mg/g dw), DPPH (%), and ABTS (%) methods.

Fig 10. The ultrasound waves generator

3.5 Statistical modelling and data calculation of HAE, MAE, and UAE extraction

The statistical analysis of the obtained results was provided by applying an analysis of variance (ANOVA) with a significance level of $p \leq 0.05$. The model adequacy was evaluated considering the model p-value, lack-of-fit testing, the coefficient of variation (C.V.%), and the coefficient of determination (R^2). The desirability function approach (DFA) was applied for the optimization of the extraction conditions. Optimization parameters were adjusted to "in range"

for all independent variables and to "maximize" for all response variables simultaneously. The RSM-CCD experimental design generation, analysis of experimentally obtained data, modelling, and optimization of extraction conditions were carried out using the software Design Expert 11.0.3 Trial (Stat-Ease, USA).

The coefficient of variation (C.V.%) indicates the relative dispersion of the experimental points from the predictions of the models. The CV% is a useful measure for comparing the variability of different datasets, especially when the datasets have different units or scales. A lower CV% indicates that the data points are close to the mean and there is less variability, while a higher CV% suggests greater variability relative to the mean (Maran and Manikandan, 2012).

The desirability function approach (DFA) searches for a combination of factor levels that jointly optimize a set of responses by satisfying the requirements for each response in the design. The scale of the desirability function ranges between 0 (completely undesirable response) and 1 (fully desired response). The individual desirabilities (d) for each response are obtained by specifying the goals—minimize, maximize, or target the response—and the boundaries required for each one (Maran and Manikandan, 2012).

Experimental validations of models were performed by comparing the predicted and experimentally obtained extraction yields under the optimized conditions. Extractions under defined optimal conditions were performed in triplicate and the values obtained were presented as mean ± standard deviation.

3.6 Anthocyanidins extraction by different solvents and extraction methods

In order to know the effect of extracting medium and methods on the anthocyanidins extraction process from hawthorn fruit, three methods: ultrasound, microwave, and heat-assisted extraction together with three solvents (methanol, ethanol, and isopropanol) have been compared.

To prepare the working solvents, 80 % (v/v) of each organic solvent, 19.9 % (v/v) of water, and 0.1 % (v/v) of hydrochloric acid (HCl) were mixed together. After that, each solvent was diluted with pure water to a concentration of 50 % (v/v) before being used for extraction.

- Heat-assisted extraction (HAE)

The extraction processes were performed by (OS20-S Electric LED) stirrer at 65 °C for 30 min by using the prepared solvent (10 g of the fruit in 100 mL of solvent).

- Microwave-assisted extraction (MAE)

All microwave extractions were accomplished by microwaves oven (Specs Electrolux EMM 2005). For the treatment of microwave, pulse mode, and cooling in between with icy water were performed 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)) was considered based on the pretest. The treatment was performed at 800 W of microwave power.

- Ultrasound-assisted extraction (UAE)

The UAE was performed by power ultrasound (3.5 W/cm^2, 20 kHz) produced by a generator (ULC 400) premium ultrasonic generator with treatment time of 30 min. To stabilize the heat distribution throughout the treatments, an icy water bath was used maintaining the temperature around 25 ºC.

3.7 Membrane separation

Despite the several advantages of this technology, there are still some challenges for eg. how to choose the appropriate membrane for the process; how to optimize the operational conditions; how to prevent massive membrane fouling; etc. Suitable membranes for different purposes of separation are chosen according to their pore sizes (Zin et al., 2020), as well as factors such as molecular weight, molecular width, solute dissociation constant (pK_a), hydrophobicity (log *P*) on the membrane efficiency were studied. It is clear that the membrane efficiency depends on membrane type, solute, and the mutual interaction between them. Temperature, pH, pressure, and concentration also have an influence on rejection (Li et al., 2010). To determine the optimal membrane to concentrate hawthorn fruit and anise seed extracts, RO membranes of low fouling type Trisep X-20 advanced composite membrane (Microdyn), thin film composite Alfa Laval RO-99 membrane, and NF 270 membrane made from piperazine and benzenetricarbonyl trichloride with active surface areas of 0.18 m^2 were evaluated. Fig. 11 shows the flowsheet of the membrane concentration processes.

After determining the optimal conditions to extract both hawthorn fruit and anise seed, 3 liters of extract from each material was prepared for every subsequent extraction process. The extraction was accomplished by a single batch type extractor which was designed with a thermostat water bath (lauda Ecoline E100 Immersion) and (OS20-S Electric LED) stirrer. For hawthorn, the extraction conditions were 55 °C, with 56 % v/v ethanol solvent (10 g fruit in 100 mL solvent) for 80 min. For anise seed, the extractions were completed using pure water as solvent at 37 °C for 100 min. After cleaning and preparing both of them as mentioned in section (3.1).

Cross-flow filtration process was performed by DDS Filtration Equipment (LAB 20-0.72, Denmark) (Fig. 12). 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. Table 21 presents the parameters of the filtration processes. During concentration, the time required to collect each 200 mL of filtrate was recorded, with sample collections performed at every 400 mL of permeate. Following separations, analytical measurements were conducted. Pure water flux measurements were taken both before and after the concentration step to estimate membrane resistance and fouling resistance.

After each concentration process, the membranes underwent thorough cleaning. First, they were treated with a 0.2 N NaOH solution for 10 minutes and then rinsed with distilled water for 20 minutes. Next, a second cleaning step was performed using a 0.2% citric acid solution for 10 minutes, followed by a final rinse with distilled water for another 10 minutes. These cleaning procedures were conducted under a transmembrane pressure (TMP) of 1 bar and at a temperature of 25 °C.

Table 21. Filtration processes parameters

X-20 membrane:	Polyamide/ MICRODYN	Equipment:	DDS
RO99 membrane:	Polyester/Alfa Laval	Surface of one membrane:	0.018 m^2
NF 270 membrane:	Polyamide/DOW	Pieces of membrane:	10
Temperature:	35 °C	Membrane Surface:	0.18 m^2
TMP:	30 bar	Initial liquid volume:	3000 mL

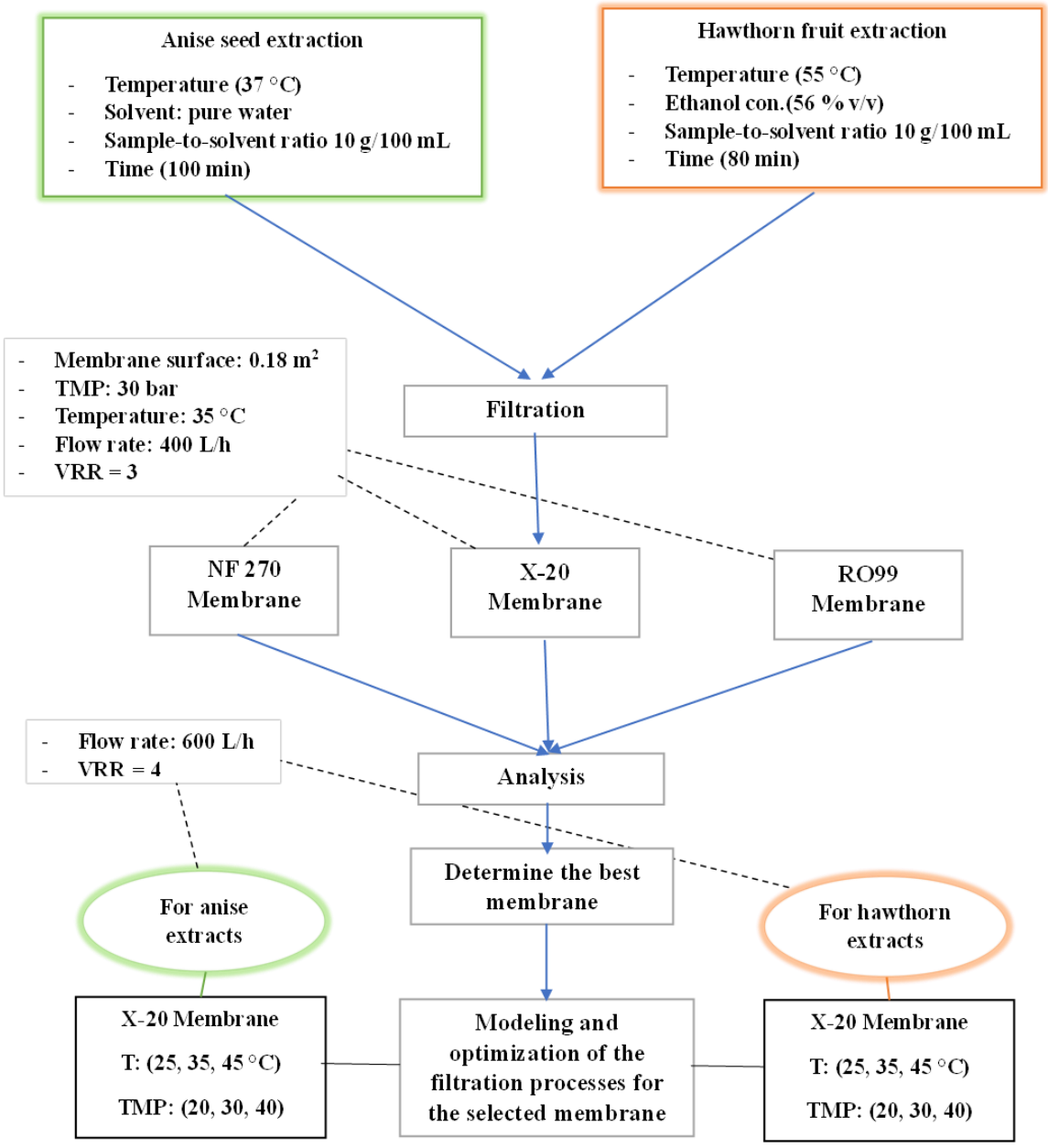


Fig 11. Flowsheet of the membrane concentration processes

The driving force in pressure-driven membrane separation is of course the pressure, or the pressure difference between the upstream and the downstream of the membrane, or between the feed and the permeate. This is referred to as transmembrane pressure TMP. And the permeate flux J ($m^3/m^2 \cdot s$) through a porous membrane is often described as the applied transmembrane pressure driving force (TMP), divided by the resistance to mass transfer, R_m (1/m) , and the permeate dynamic viscosity, μ (Pa · s) (Miller et al., 2014).

$$J = \frac{TMP}{\mu \cdot R_m} \qquad (5)$$

In the filtration of real feeds, concentration polarization, and membrane fouling occur to add additional resistances to the membrane and hence to the permeate to pass through. Therefore, the resistance of the fouling resistance R_F and resistance due to cake deposition (cake deposition) R_C need to be considered. The equation is expressed as follows:

$$J = \frac{TMP}{\mu \cdot (R_m + R_f + R_C)} \qquad (6)$$

Membrane resistance (R_m) and fouling resistance (R_f) can be derived from Darcy's Law:

$$R_m = \frac{1}{\mu \cdot a_1} \qquad (7)$$

$$R_f = \frac{1}{\mu \cdot a_2} - R_m \qquad (8)$$

where α_1 and α_2 means slope of pure water flux curves before and after measurement versus transmembrane pressure difference.

Permeate fluxes of the cross-flow membrane filtration tests at each operating condition were determined by measuring the permeate volume collected over a certain period in terms of a litter per square meter per hour ($L/(m^2 \cdot h)$) and using the following equation (9):

$$J_V = \frac{\Delta V}{A \cdot \Delta t} \qquad (9)$$

where J_v is the volumetric permeate water flux, A is the effective area of the membrane for permeation, and V is the volume of permeation over a time interval Δt.

According to the research of (Bánvölgyi et al., 2009) Volume Reduction Ratio (VRR) was approved by feed volume V_0 (m^3) and volume of retentate V_R (m^3) or volume of permeate V_P (m^3) following the Eq. (10), and based on the concentrations of permeate C_p (mg/L) and retentate C_R (mg/L), retention (%) can be estimated as follows Eq. (11)

$$VRR = \frac{V_0}{V_R} = \frac{V_0}{V_0 - V_P} \qquad (10)$$

$$R = \left(1 - \frac{C_P}{C_R}\right) \cdot 100 \qquad (11)$$

The fouling index was calculated by comparing the pure water permeability before and after the filtration at room temperature (20 - 25 °C) as shown by Eq. (12) (Mänttäri et al., 2007):

$$\text{Fouling index} = \left(1 - \frac{PWP_a}{PWP_b}\right) \cdot 100 \qquad (12)$$

where PWP_a pure water permeability after effluent filtration, L /(m^2·h·bar) PWP_b pure water permeability before effluent filtration, L /(m^2·h·bar).

Total phenolic content (TPC), total flavonoids content (TFC), and antioxidant activity (AA) for the different fractions of streams (retentate and permeates) were measured, and permeate flux, membrane fouling, and retention percentages were calculated.

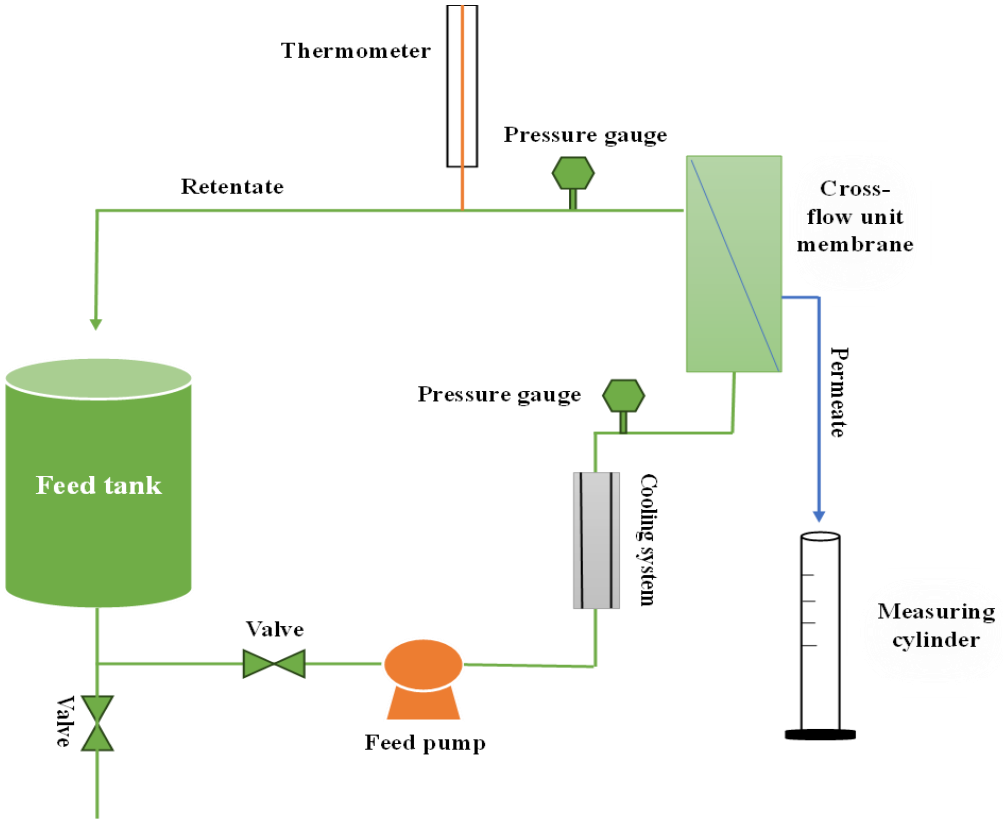


Fig 12. Scheme of cross-flow membrane filtration

3.7.1 Optimization of membrane process using (RSM)

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 response surface methodology (RSM) was applied to evaluate the effects of reverse osmotic filtration parameters and optimize various conditions for different responses. Table 22 summarizes the studied variables: temperature (A), and transmembrane pressure (B). Central composite design (CCD) was applied and included 11 randomized runs with 3 replicates in the central point. The recirculation flow rate was 600 L/h and the filtration processes were completed once the volumetric reduction ratio (VRR) reached 4. The total phenolic content, total flavonoid content, antioxidant activity in the final obtained retentate, final permeate flux, fouling index,

membrane resistance, and fouling resistance were set as response variables. All the coefficients of the different polynomial equations were tested for significance with an analysis of variance (ANOVA), and a polynomial model of the second degree was established to evaluate and quantify the influence of the variables.

Table 22. The experimental domain used in central composite design (CCD) for concentration processes

Input factors	Variables	Factor Levels		
		-1	0	1
T (◦C)	A	25	35	45
ΔP (bar)	B	20	30	40

Cross-flow filtration process was performed by DDS Filtration Equipment (LAB 20-0.72, Denmark) connected to a SPECK type NP10/15 -104 high pressure pump. Temperature and transmembrane pressure were adjusted to the experimental design requirements for each run. During the concentration, the time required to collect each 200 mL of filtrate was recorded and the sample collections were performed at every 600 mL of permeates. After separations, the analytical measurements were carried out. 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.

3.8 Analytical measurements

3.8.1 Determination of total phenolic content (TPC)

Total phenolic content was estimated by the Folin-Ciocalteu colorimetric method based on the method of Singleton and Rossi (1965), using gallic acid as the standard phenolic compound. Briefly, 20 µl (3 replicates) of the extract was mixed with 450 µl distilled water and 2.5 mL of 0.2 N Folin-Ciocalteu reagent. The mixture was allowed to stand for 1 minute, then 2 mL of aqueous Na_2CO_3 7% (w/v) was added. After incubation for 5 min at 50 °C temperature, absorbance was measured at 760 nm versus a prepared blank. A standard curve was generated using gallic acid in methanol. Total phenol values were expressed as gallic acid equivalents (mg GAE/g dw), and TPC was calculated according to the Eq. (13).

$$TPC = \frac{A \cdot TS \cdot DF}{S \cdot a_3} \qquad (13)$$

where A is the measured absorbance; TS is the total amount of sample with added chemical solutions (µL); DF is the dilution factor; S is the actual amount of the sample (µL); α_3 is the slope of the calibration curve.

3.8.2 Determination of total flavonoids content (TFC)

The colorimetric method described by (Zin et al., 2021; Floegel et al., 2011) was used to measure flavonoids. Briefly, 0.5 mL of each plant extract was mixed with 4 mL of distilled water and 0.3 mL of 10 % (w/v) aluminium chloride, After 5 min 0.3 mL of 5 % (w/v) sodium nitrite ($NaNO_2$) solution was added and mixed and allowed the mixture 1 min before adding 2

mL of 1 M sodium hydroxide (NaOH) was added and made it up to 10 mL with distilled water. The absorbance of the reaction mixture was measured at 510 nm using a spectrophotometer. A standard curve was generated by preparing quercetin solutions in methanol. Total flavonoids were expressed as quercetin equivalents (mg QUE/g dw), and TFC was calculated according to the Eq. (14).

$$TFC = \frac{A \cdot TS \cdot DF}{S \cdot a_4} \qquad (14)$$

where A is the measured absorbance; TS is the total amount of sample with added chemical solutions (µL); DF is the dilution factor; S is the actual amount of the sample (µL); α_4 is the slope of the calibration curve.

3.8.3 Determination of Antioxidant activity (AA)

3.8.3.1 FRAP assay

Antioxidant activity was determined spectrophotometrically according to the method described by Benzie and Strain (1996), with modifications. Ascorbic acid was used for the calibration curve, and FRAP reagent was prepared from 250 mL 0.3 M acetate buffer, 25 mL TPTZ 10 mM solution, and 25 mL 20 mM ferric chloride. 20 µl of extract (3 repeats) and 1500 mL of FRAP-prepared reagent were added to the test tube. After homogenization, the mixture was incubated at room temperature for 5 min in the dark. The absorbance was measured at 593 nm against a blank. Results were expressed in milliequivalents of ascorbic acid/g dw, The calculation was done using the Eq. (15).

$$AAE = \frac{A \cdot TS \cdot DF}{S \cdot a_5} \qquad (15)$$

where A is the measured absorbance; TS is the total amount of sample with added chemical solutions (µL); DF is the dilution factor; S is the actual amount of the sample (µL); α_5 is the slope of the calibration curve.

3.8.3.2 DPPH assay

The antioxidant activity of the extracts and standards was determined by the radical scavenging activity method using the (2,2-diphenyl-1-picrylhydrazyl) described by (Blois 1958; Zin, M.M. and Bánvölgyi., 2021). Briefly, 0.1 mL aliquots of solutions of the extracts or standards at different concentrations were added to 3.9 mL of a DPPH methanolic solution, which was prepared with 22 mg of DPPH dissolved in 50 mL of pure methanol, after a 30 mins incubation period at room temperature in the dark, the absorbance was measured at 517 nm. The radical scavenging activity was calculated as follows:

$$DPPH\% = \left(\frac{Abs_0 - Abs_1}{Abs_0}\right) \cdot 100 \qquad (16)$$

where Abs_0 was the absorbance of the control and Abs_1 was the absorbance in the presence of the test sample at different concentrations.

3.8.3.3 ABTS assay

ABTS [2, 2'-Azinobis (3-Ethylbenzothiazoline-6-Sulphonic Acid)], this assay was carried out using the modified method of Re et al. (1999). The ABTS stock solution was prepared by reacting ABTS aqueous solution (7 mM) with 2.45 mM aqueous solution of potassium persulfate in equal quantities; the mixture was allowed to stand in the dark at room temperature for 12-16 hrs before use. The working solution of ABTS was obtained by diluting the stock solution in methanol to give an absorbance of 0.70 ± 0.02 at 734 nm. Then, 1.0 mL of ABTS solution was mixed with 1 mL of the aqueous extracts. The mixture was then incubated at room temperature for exactly 5 min in the dark. The control was prepared by mixing 1.0 mL of ABTS solution with 1 mL of distilled water. The absorbance was measured against a blank at 734 nm using a spectrophotometer. The percentage of scavenging activity of each extract on ABTS was calculated as following equation (17):

$$ABTS\ \% = \frac{Abs_0 - Abs_1}{Abs_0} \cdot 100 \qquad (17)$$

where Abs_0 was the absorbance of the control and Abs_1 was the absorbance in the presence of the test sample at different concentrations.

3.8.4 Total monomeric anthocyanin content (TMA)

The total monomeric anthocyanin content (TMA) in the crude extracts was determined using the pH-differential method described by Giusti and Wrolstad (2001) with some modifications. Two buffer solutions were prepared, one with potassium chloride (0.025 M KCl) with a pH of 1.0 and the other with sodium acetate (0.4 M CH_3COONa) with a pH of 4.5. The pH of the buffer solutions was adjusted with 0.1 N hydrochloric acid (HCl). The absorbance was read at 530 nm and 700 nm using a spectrophotometer. All samples were analyzed three times. TMA was calculated using Equation (18), and the results were expressed as mg cyanidin-3-glucoside (CGE)/ g of dry weight (dw).

$$TMA = \frac{A \cdot M_W \cdot DF \cdot L}{\varepsilon} \qquad (18)$$

where $A = [(A_{530} - A_{700})_{pH\ 1.0} - (A_{530} - A_{700})_{pH\ 4.5}]$, M_w is the molecular weight of anthocyanin (449.2 g/mol), DF is the dilution factor, ε is the molar absorptivity coefficient (26,900 1/cm/mol), and L is the path length cuvettes (1 cm).

3.8.5 Colour value analysis

Various colour systems can be used for instrumental colour analyses. The system proposed by the International Commission on Illumination (CIE) in 1976, based on three-dimensional colour space the three axes are L*, a*, and b* (Fig. 13). The L* value is a measure of the lightness of an object and is quantified on a scale such that a perfect black has an L* value of zero and a perfect reflecting diffuser an L* value of 100. The a* value is a measure of redness (positive a*) or greenness (negative a*). The b* value is a measure of yellowness (positive b*) or blueness (negative b*). The a* and b* co-ordinates approach zero for neutral colours (white, greys) and increase in magnitude for more saturated or intense colours (Joiner, 2004).

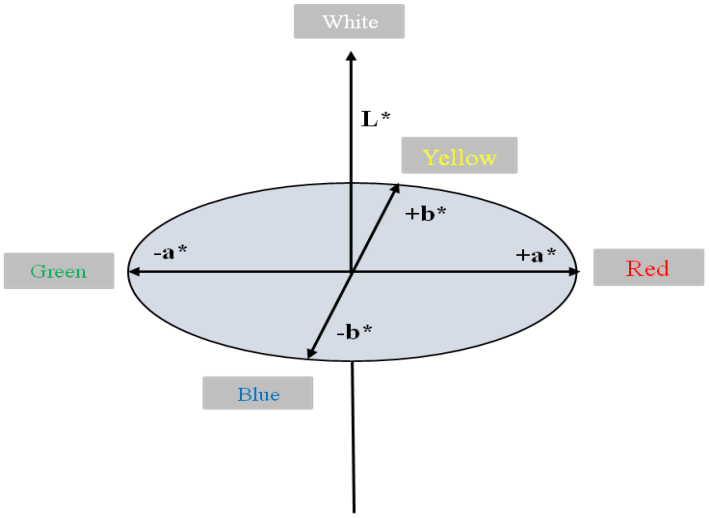


Fig 13. CIE Lab colour space

The colour characterization of hawthorn fruit extracts was measured through the CIE system using a Minolta Chroma meter CR- 400. The results reported are the average of at least 5 replications

3.9 Statistical analysis

Evaluation of statistical analysis was accomplished by the central composite design of Design-Expert Software Trial Version 11.0.3, based on the analysis of variance (ANOVA). Likewise, significant level differences were performed by one-way, two-way, and Multivariate (ANOVA) and Tukey's HSD tests were carried out to determine significant differences ($p \leq 0.05$) between the means by Statistical Product and Service Solutions statistics (SPSS IBM version 27.0).

4 RESULTS AND DISCUSSION

4.1 Heat-assisted extraction (HAE)

An optimization experiment was conducted using response surface methodology (RSM) to enhance the extraction of polyphenols and antioxidant activity. The extraction variables for hawthorn fruits included ethanol concentration (A, %), extraction temperature (B, °C), and extraction time (C, min). For anise seed, the extraction variables comprised 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 (3.2). The significance test of model fit was performed by the central composite design of Design-Expert software trial version 11.0.3, based on analysis of variance (ANOVA). RSM with CCD has been developed to approach the optimum process condition through the interaction between the different variables and the experimental outcomes. 2^k factorial design which is an orthogonal design was applied to fit the multiple linear regression model. Experimental runs were randomized to avoid the effects of extraneous factors which might present. Data distribution was transformed by the quadratic function.

4.1.1 Hawthorn fruits

4.1.1.1 RSM of hawthorn fruits EW extracts

The experimental outcomes of the recovered total phenolics, and flavonoid compounds and their respective antioxidant activities from the hawthorn fruit (*Crataegus monogyna* Jacq.) with EW solvent via HAE were denoted in (Appendix-Table 1). Twenty treatments (runs) were conducted according to CCD including replications in the center point. The model fixations for all responses were performed by the quadratic model function. The influence of each factor on the response was investigated by holding the other process variables constant. Response surface 3D polts were generated for each response.

4.1.1.2 Fitting the model

Optimization of the extraction process was carried out by applying a second-order polynomial equation. The model shows a high significance and a good fit with the experimental data of TPC and TFC content and has less variation around the mean (R^2 values 0.91, 0.85), respectively (Appendix-Table 2). The antioxidant activities (FRAP, DPPH, and ABTS) show the model is significant and the quality of fit to the second order polynomial equation checked using the coefficient of determination (R^2), which was 0.88, 0.84, and 0.82, respectively. The regression coefficients for dependent variables were obtained by multiple linear regressions.

The positive linear effect of solvent concentration (A), extraction temperature (B), and extraction time (C) was found to be significant for all response variables. However, the quadratic effect of extraction temperature (B^2) was only found to produce significant ($p < 0.0001$) negative effect on TPC and ($p < 0.05$) 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 (Appendix-Table 2). The predicted values and actual values which can be correlated by the coded and actual equations built by the model were

depicted in (Appendix-Fig. 1). The results indicated a good correlation between experimental and predicted data.

4.1.1.3 Effect of extraction variables on total phenolics content (TPC)

The model showed a high significant ($p < 0.0001$) value with the experimental data, whereas analysis of variance (ANOVA) showed a significant linear and quadratic effect of ethanol concentration and temperature (A; $p < 0.01$) and (B; $p < 0.0001$), (A^2; $p < 0.05$), and (B^2; $p < 0.0001$), while the extraction time has only linear effect (C; $p < 0.01$) on TPC (Appendix-Table 2). Based on regression coefficient (β) values, extraction temperature (B) showed a major positive effect followed by the effect of extraction time (C) and then ethanol concentration (A), while the quadratic effect of extraction temperature (B^2) showed a negative effect ($p < 0.0001$), and the quadratic effect of solvent concentration (A^2) was negative as well with ($p < 0.05$). The non-significant variables were removed and the fitted second-order polynomial equation showed as:

$$TPC = -166.13 + 0.77 \cdot A + 8.19 \cdot B + 0.19 \cdot C - 0.006.A^2 - 0.083 \cdot B^2 \qquad (19)$$

where A – ethanol concentration (% v/v) in the range (10 – 90 % v/v), B – temperature (°C) in the range (30 – 60 °C), C – extraction time (min) in the range (10 – 90 min).

The non-significant value of lack of fit ($F = 0.68$) showed the model is fitted to the spatial influence of the variables to the response with a good prediction ($R^2 = 0.91$) (Appendix-Table 2). The curvature observed in the 3D plot of TPC is attributed to the quadratic relationship with ethanol concentration and extraction temperature (Fig. 14). Furthermore, variations in ethanol concentration and temperature lead to an increase in TPC from level (-1) to level (0). This increase occurs because higher temperatures enhance solute solubility and diffusion coefficients, as well as soften plant tissue. However, beyond this point, further increases in ethanol concentration and temperature result in a decrease in TPC due to thermal degradation. Meanwhile, TPC continues to increase with longer extraction times.

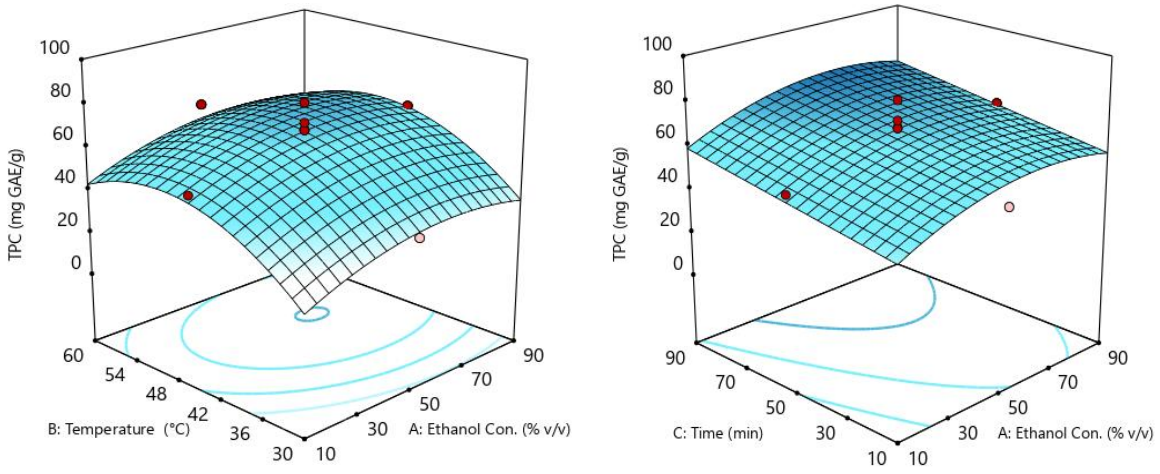


Fig 14. 3D response surface of TPC influenced by individual factors in the EW and HAE extracts of hawthorn fruit

4.1.1.4 Effect of extraction variables on total flavonoids content (TFC)

Linear effect of ethanol concentration (A), extraction temperature (B), extraction time (C), and quadratic ethanol concentration (A^2) showed a significant effect on TFC. Among these,

TFC depends more on (A), (B) ($p < 0.0001$) followed by (A^2; negative effect; $p < 0.001$) then (C; $p < 0.05$) having regression coefficient (β) values as mentioned in (Appendix-Table 2). The non- significant factors were removed and the fitted second-order polynomial equation showed here as:

$$TFC = -3.41 + 0.28 \cdot A + 0.19 \cdot B + 0.035 \cdot C - 0.002 \cdot A^2 \qquad (20)$$

where A – ethanol concentration (% v/v) in the range (10 – 90 % v/v), B – temperature (°C) in the range (30 – 60 °C), C – extraction time (min) in the range (10 – 90 min).

The non-significant value of lack of fit (F = 0.86) showed the model is fitted with good prediction ($R^2 = 0.85$) (Appendix-Table 2). The 3D plot of the TFC arises shows the quadratic dependence on ethanol concentration (Fig. 15). Where TFC increased as ethanol concentration increased in the levels (-1), (0), and decreased with the increasing of concentration in the level (1).

This effect of the extraction temperature for both TPC, and TFC can be due to the reason that increasing temperature extraction above certain values may promote possible concurrent degradation of phenolic compounds which were previously mobilized at lower temperature or even the decomposition of residual phenolics remaining in the plant matrix. The effect of the ethanol concentration can be because the polarity of the extraction solvents and the solubility of each component in the solvents impact the recovery of phenolic contents in various samples (Mokrani and Madani, 2016).

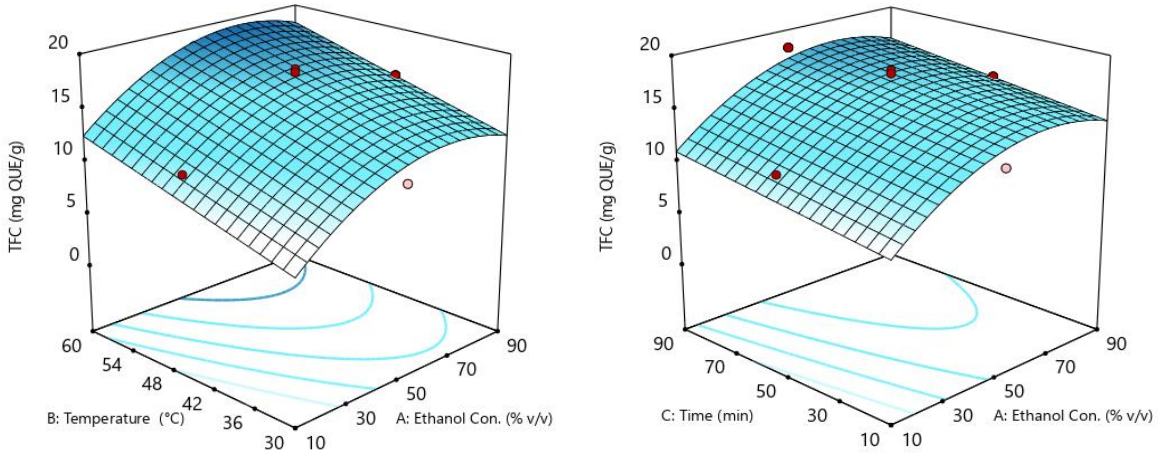


Fig 15. 3D response surface of TFC influenced by individual factors in the EW and HAE extracts of hawthorn fruit

4.1.1.5 Effect of extraction variables on antioxidant activity (AA)

The antioxidant activity was measured by FRAP, DPPH, and ABTS assays and the linear effect of ethanol concentration (A), extraction temperature (B), and extraction time (C) was found to significantly affect AA for all assays (Appendix-Table 2). Among all antioxidant assays tested, the effect of extraction temperature (B) significantly affects the AA. However, a negative effect of quadratic ethanol concentration (A^2) and extraction temperature (B^2) was also found significant in FRAP (Fig. 16). While only the quadratic effect of ethanol concentration (A^2) was found in DPPH and ABTS (Fig. 17 and Fig. 18). (Belwal et al., 2016) mentioned the quadratic effect of solvent concentration on antioxidant activity. The non-

significant factors were removed and fitted second-order polynomial equation for Antioxidant activity (FRAP, DPPH, and ABTS) showed as:

$$FRAP = -55.85 + 0.38 \cdot A + 2.86 \cdot B + 0.086 \cdot C . 0.003 \cdot A^2 - 0.028 \cdot B^2 \quad (21)$$

$$DPPH = -6.80 + 0.43 \cdot A + 0.26 \cdot B + 0.057 \cdot C - 0.0038 \cdot A^2 \quad (22)$$

$$ABTS = -12.50 + 0.93 \cdot A + 0.49 \cdot B + 0.11 \cdot C - 0.0084 \cdot A^2 \quad (23)$$

where A – ethanol concentration (% v/v) in the range (10 – 90 % v/v), B – temperature (°C) in the range (30 – 60 °C), C – extraction time (min) in the range (10 – 90 min).

The non-significant value of lack of fit showed the models are fitted with the good prediction (Appendix-Table 2). The 3D plots illustrate a clear positive correlation between total phenolic and flavonoid compounds and their antioxidant activity. Ethanol concentration exhibits a quadratic effect on all responses, whereas temperature shows a quadratic effect specifically on FRAP. This difference could be attributed to variations in the assays and their underlying mechanisms.

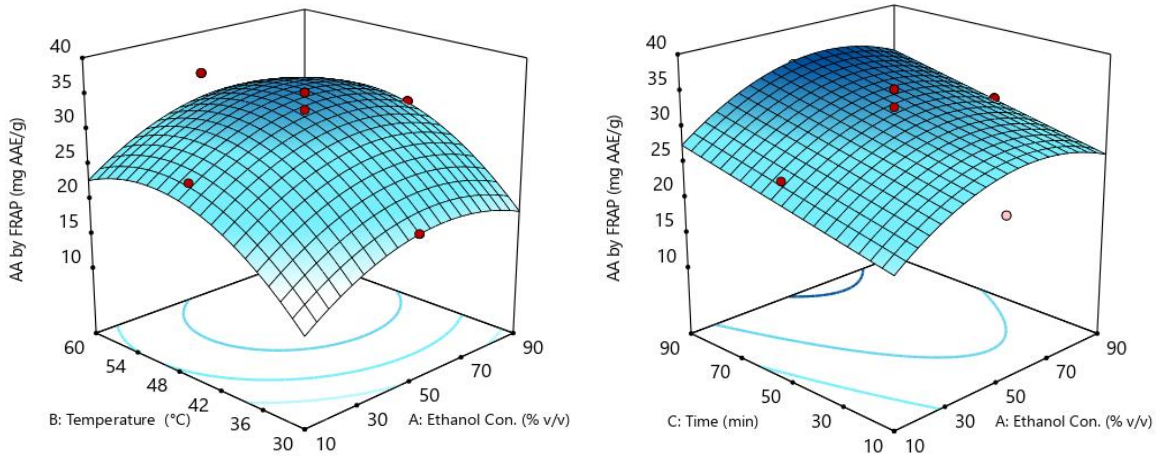


Fig 16. 3D response surface of FRAP influenced by individual factors in the EW and HAE extracts of hawthorn fruit

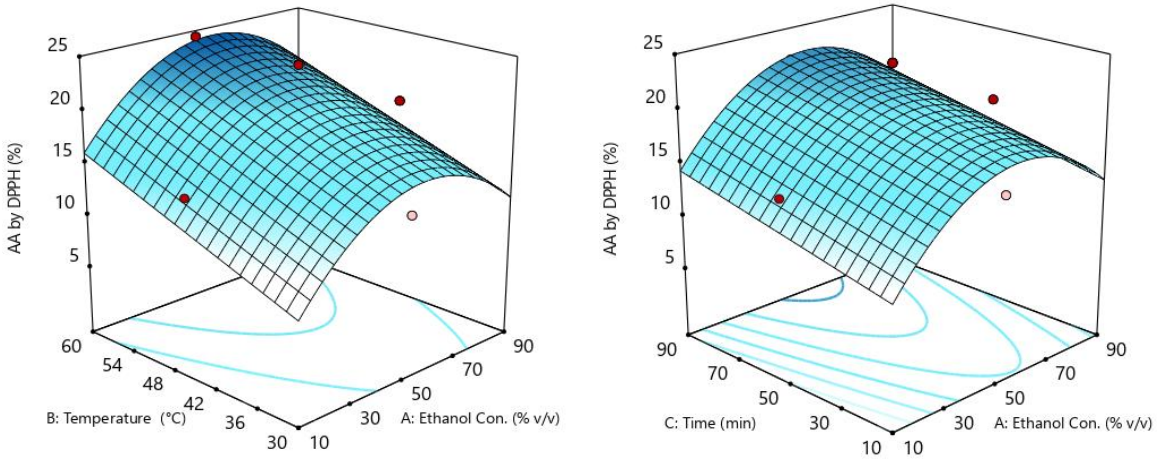


Fig 17. 3D response surface of DPPH influenced by individual factors in the EW and HAE extracts of hawthorn fruit

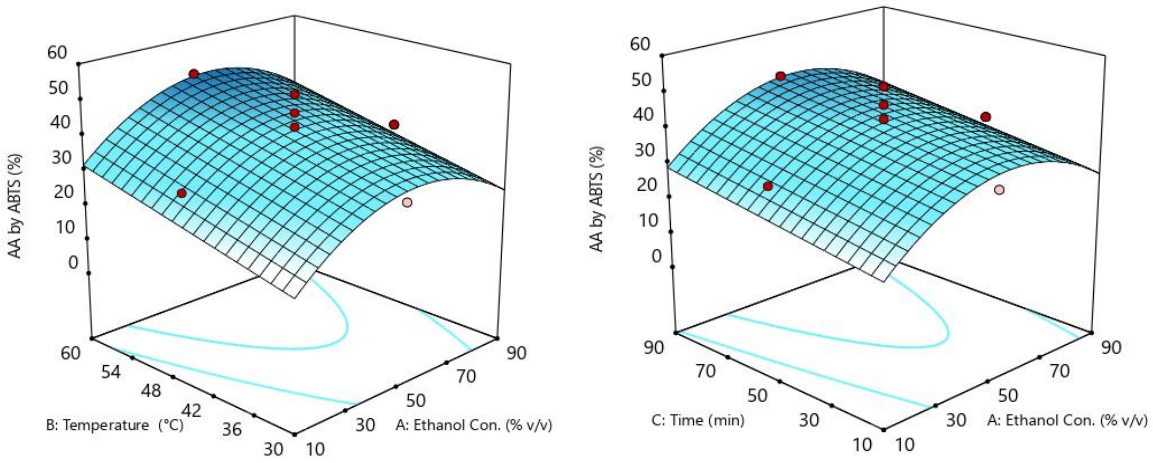


Fig 18. 3D response surface of ABTS influenced by individual factors in the EW and HAE extracts of hawthorn fruit

4.1.1.6 Determination and experimental validation of optimal conditions

The optimal conditions were determined by maximizing the (DFA) of the responses using Design Expert Software Trial Version 11.0.3. These optimal conditions were used for the extraction process and later the responses were determined and validated according to the above-mentioned procedure. The best conditions for determining TPC and TFC, and antioxidant activity (FRAP and DPPH, and ABTS) in a single experiment were – ethanol concentration (62.5 %), extraction temperature (60 °C), and extraction time (90 min) in the evaluated range, with overall desirability value of 0.98 which indicates that the selected combination of factors led to outcomes that closely align with the desired objectives of the experiment (Fig. 19). Under these conditions, the experimental values were in agreement with the predicted values with the coefficient of variance C.V. % range from 11.40 to 14.62 % (Appendix-Table 2).

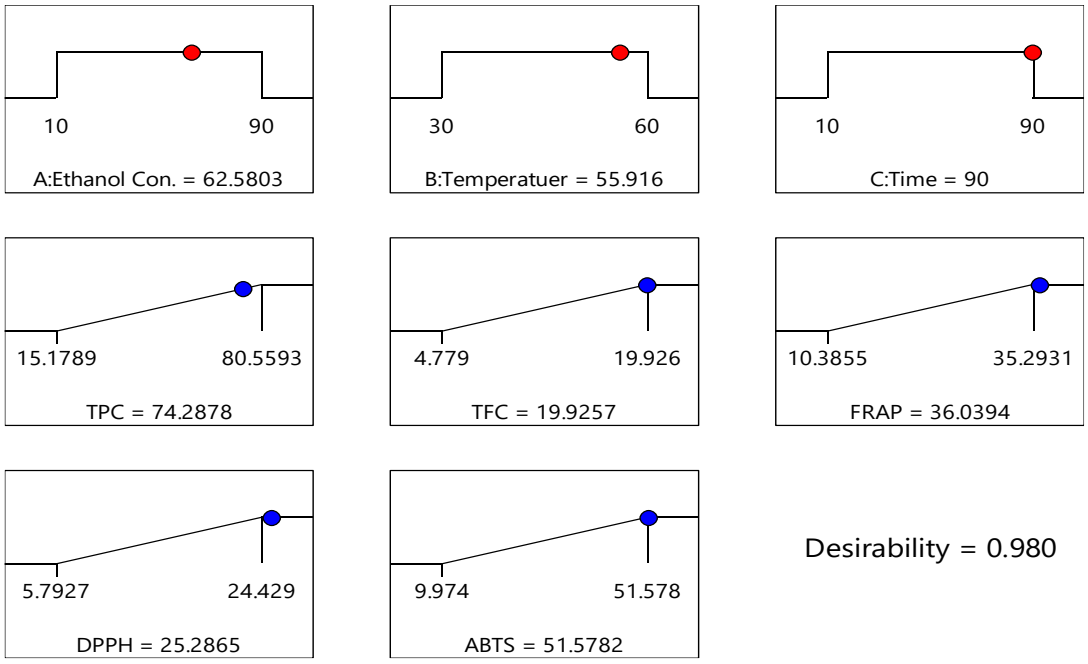


Fig 19. The desirability test based on the optimized values of targeted compounds in hawthorn EW and HAE extracts

4.1.2 Anise seed

4.1.2.1 RSM of anise seed PW extracts

The experimental outcomes of the recovered total phenolics, and flavonoid compounds and their respective antioxidant activities from the anise seed (*Pimpinella anisum* L.) with PW solvent via HAE were denoted in (Appendix-Table 3). Twenty treatments (runs) were conducted according to CCD including replications in the center point. The model fixations for all responses were performed by the Quadratic model function. The influence of each factor on the response was investigated by holding the other process variables constant. Response surface 3D graphs were generated for each response.

4.1.2.2 Fitting the model

Optimization of the extraction process was carried out by applying second-order polynomial equation. The experimental data are shown in (Appendix-Table 3). The model shows high significant and good fit with the experimental data of TPC, and TFC content and has less variation around the mean (R^2 values 0.90, 0.92), respectively (Appendix-Table 4). The antioxidant activities (FRAP, DPPH, and ABTS) show the model is significant and the quality of fit to the second-order polynomial equation checked using the coefficient of determination (R^2), which were (0.91, 0.88, and 0.90), respectively.

The regression coefficients for dependent variables were obtained by multiple linear regressions as shown in (Appendix-Table 4). The positive linear effect of extraction time (B), and a negative linear effect of extraction temperature (A) 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 (A^2) 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 (C^2) was only found to be negatively significant to TFC, FRAP, and DPPH. The interaction effect of extraction time and sample-to-solvent ratio (BC) was found to be significant for TFC ($p < 0.001$), and the interaction effect of extraction temperature and extraction time (AB) 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 (Appendix-Table 4). The predicted values and actual values which can be correlated by the coded and actual equations built by the model were depicted in (Appendix-Fig. 2). The results indicated a good correlation between experimental and predicted data.

4.1.2.3 Effect of extraction variables on total phenolics content (TPC)

The model showed a high significant ($p < 0.0001$) value with the experimental data, whereas analysis of variance (ANOVA) showed a significant ($p < 0.0001$) (Appendix-Table 4). Based on regression coefficient (β) values, extraction time (B) showed a major positive effect ($p < 0.0001$) followed by the negative effect of extraction temperature (A; $p < 0.001$), likewise the quadratic of extraction temperature (A^2) showed a negative effect ($p < 0.0001$), while TPC was not affected by the sample-to-solvent ratio (C). The non-significant variables were removed and the fitted second-order polynomial equation showed as:

$$TPC = -60.60 + 4.27 \cdot A + 0.18 \cdot B + 0.45 \cdot C - 0.056 \cdot A^2 \qquad (24)$$

where A – temperature (°C) in the range (25 – 55 °C), B – extraction time (min) in the range (20 – 100 min), C – sample-to-solvent ratio (g/100 mL) in the range (2 – 10 g/100 mL).

The non-significant value of lack of fit (F = 0.54) showed the model is fitted to the spatial influence of the variables to the response with a good prediction (R^2 = 0.90) (Appendix-Table 4). The 3D plot of the TPC arises shows the quadratic dependence on extraction temperature (Fig. 20). Where TPC increased as ethanol concentration increased in the levels (-1), (0), and decreased with the increasing of concentration in the level (1). While TPC increased as extraction time and sample-to-solvent ratio in all levels. This result is based on the mass transfer principle, in which the concentration gradient between the solid and the solvent is considered to be the driving force for mass transfer, as well as increase in extraction time can led to the mass transfer improved with penetration of solvent into the plant matrix (Al-Farsi and lee, 2008).

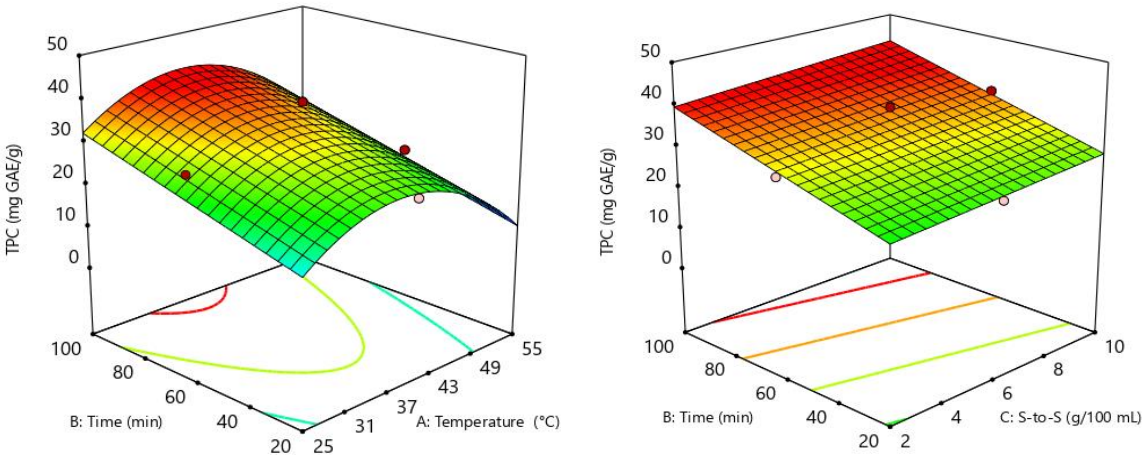


Fig 20. 3D response surface of TPC influenced by individual factors in the PW and HAE extracts of anise seed

4.1.2.4 Effect of extraction variables on total flavonoids content (TFC)

The model showed a high significant ($p < 0.0001$) value with the experimental data. And linear effect of extraction temperature (A), extraction time (B), the sample-to-solvent ratio (C), quadratic extraction temperature (A^2), quadratic sample-to-solvent ratio (C^2), and the interaction between extraction time and sample-to-solvent ratio (BC) showed a significant effect on TFC. Among these, TFC depends more on (B) ($p < 0.0001$) followed by the interaction (BC; $p < 0.001$) then the effect of the sample ratio (C; $p < 0.05$). And there was a negative effect of (A^2, and C^2) with ($p < 0.001$) having regression coefficient (β) values as mentioned in (Appendix-Table 4). The non- significant factors were removed and the fitted second-order polynomial equation showed here as:

$$TFC = -4.23 + 0.35 \cdot A + 0.013 \cdot B + 0.69 \cdot C + 0.0049 \cdot BC - 0.0049 \cdot A^2 - 0.068 \cdot C^2 \quad (25)$$

where A – temperature (°C) in the range (25 – 55 °C), B – extraction time (min) in the range (20 – 100 min), C – sample-to-solvent ratio (g/100 mL) in the range (2 – 10 g/100 mL).

The non-significant value of lack of fit (F = 1.19) showed the model is fitted with good prediction (R^2 = 0.92) (Appendix-Table 4). Fig. 21 shows the effect of extraction variables on the TFC content. Increases in extraction temperature and sample-to-solvent ratio from level (-

1) until level (0) produce an increase in the TFC content. In level (1) the increase in extraction temperature and sample-to-solvent ratio decreased TFC content. (Singh et al., 2022) reported the same results, and mentioned that the effect of the sample-to-solvent ratio could be due to sufficient solvency of the TFC in a larger volume of extraction solvent. Additionally, the simultaneous degradation of TFC may also be promoted by increasing temperature extraction above certain values.

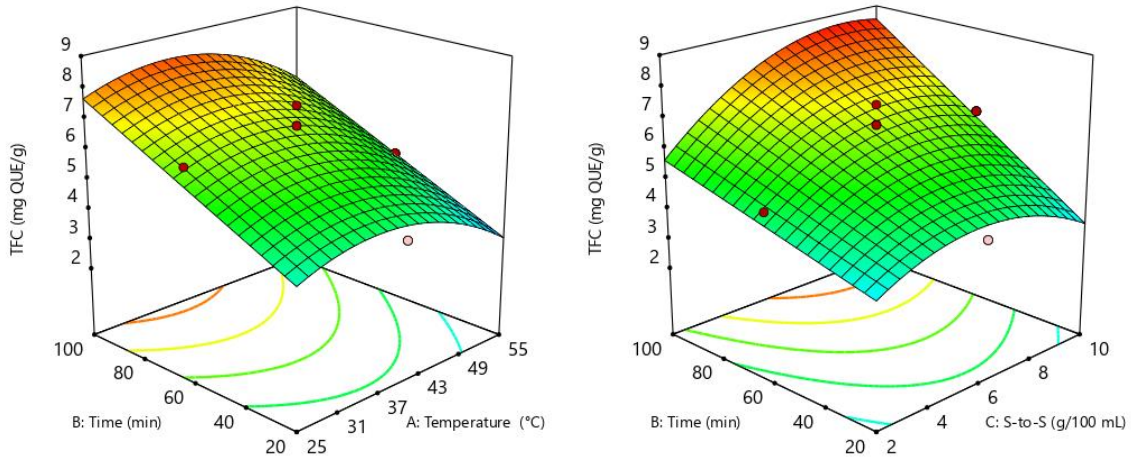


Fig 21. 3D response surface of TFC influenced by individual factors in the PW and HAE extracts of anise seed

4.1.2.5 Effect of extraction variables on antioxidant activity (AA)

The antioxidant activity was measured by FRAP, DPPH, and ABTS assays. The linear effect of extraction temperature (A) and extraction time (B) were shown to significantly affect AA for all assays, while sample-to-solvent ratio (C) has no significant effect (Appendix-Table 4). Among all antioxidant assays tested, the effect of extraction time (B) significantly affects the AA for all assays ($p < 0.0001$). However, a negative effect of interaction between extraction temperature and extraction time (AB) was only found significant in FRAP. The negative effect of quadratic extraction temperature (A^2) was found in FRAP and DPPH with ($p < 0.01$) and with ($p < 0.0001$) for ABTS (Fig. 22, Fig. 23, and Fig. 24). And a negative effect of quadratic sample-to-solvent ratio (C^2) was only found to be significant to FRAP and DPPH with ($p < 0.05$). The non-significant factors were removed and fitted second-order polynomial equation for antioxidant activity (FRAP, DPPH, and ABTS) showed as:

$$FRAP = -4.15 + 0.27 \cdot \mathrm{A} + 0.053 \cdot \mathrm{B} - 0.55 \cdot \mathrm{C} - 0.0006 \cdot \mathrm{AB} - 0.0033 \cdot A^2 - 0.041 \cdot C^2 \quad (26)$$

$$DPPH = -4.85 + 0.36 \cdot \mathrm{A} + 0.02 \cdot \mathrm{B} - 0.57 \cdot \mathrm{C} - 0.0046 \cdot A^2 - 0.047 \cdot C^2 \quad (27)$$

$$ABTS = -2.072 + 0.14 \cdot A + 0.0068 \cdot B + 0.017 \cdot C - 0.0019 \cdot A^2 \quad (28)$$

where A – temperature (°C) in the range (25 – 55 °C), B – extraction time (min) in the range (20 – 100 min), C – sample-to-solvent ratio (g/100 mL) in the range (2 – 10 g/100 mL).

The non-significant value of lack of fit showed the models are fitted with the good prediction.

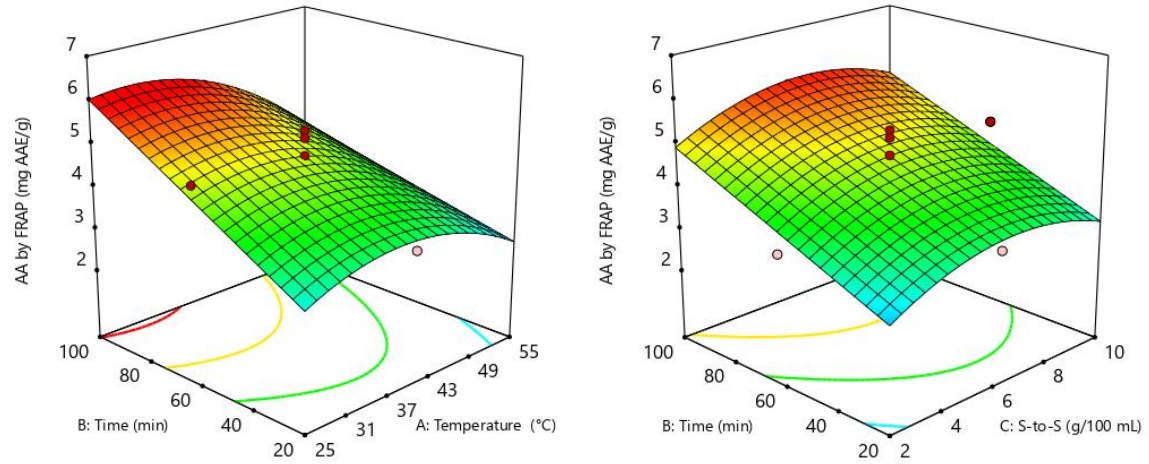


Fig 22. 3D response surface of FRAP influenced by individual factors in the PW and HAE extracts of anise seed

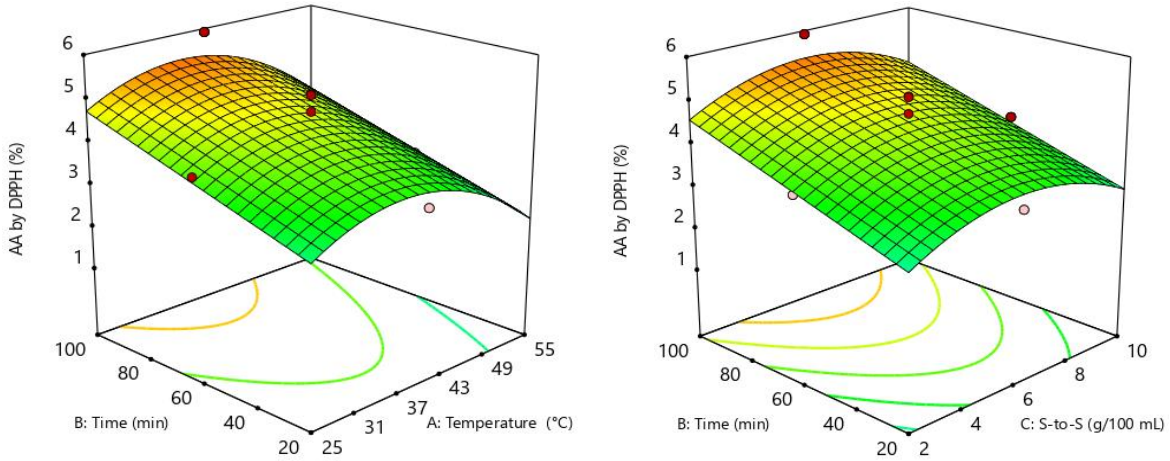


Fig 23. 3D response surface of DPPH influenced by individual factors in the PW and HAE extracts of anise seed

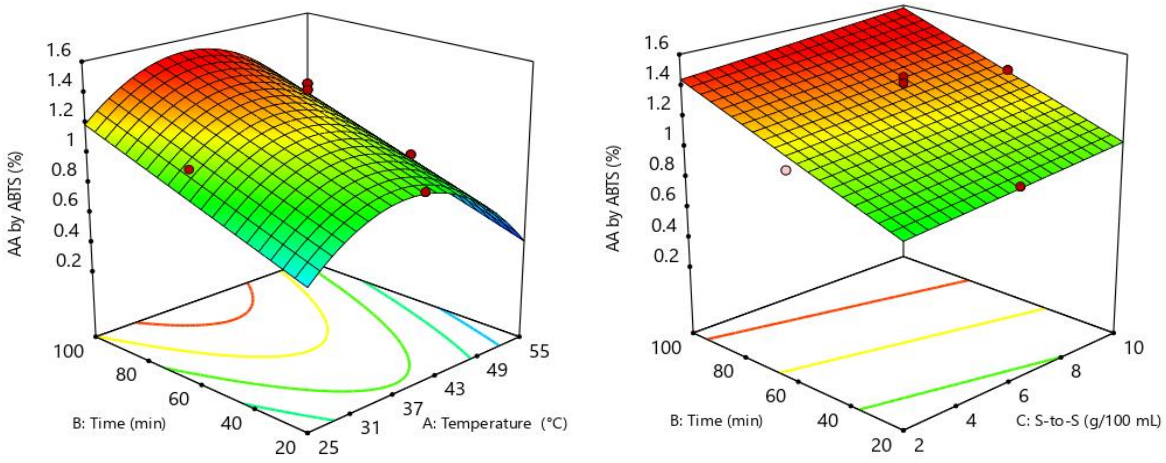


Fig 24. 3D response surface of ABTS influenced by individual factors in the PW and HAE extracts of anise seed

4.1.2.6 Determination and experimental validation of optimal conditions

The optimal conditions were determined by maximizing the desirability function approach (DFA) of the responses using Design Expert Software Trial Version 11.0.3. These optimized conditions were subsequently used for the extraction process, and the responses were later determined and validated according to the specified procedure. Specifically, the optimal conditions for determining total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (FRAP, DPPH, and ABTS) in a single experiment were an extraction temperature of 36.67°C, extraction time of 100 minutes, and sample-to-solvent ratio of 7.3 g/100 mL within the evaluated range. The overall desirability value of 0.96 suggests that the experimental conditions achieved a near-ideal balance or performance across all desired responses (Fig. 25). Under these optimal conditions, the experimental values closely matched the predicted values, with the coefficient of variance (C.V.%) ranging from 10.33% to 13.01% (Appendix-Table 4)

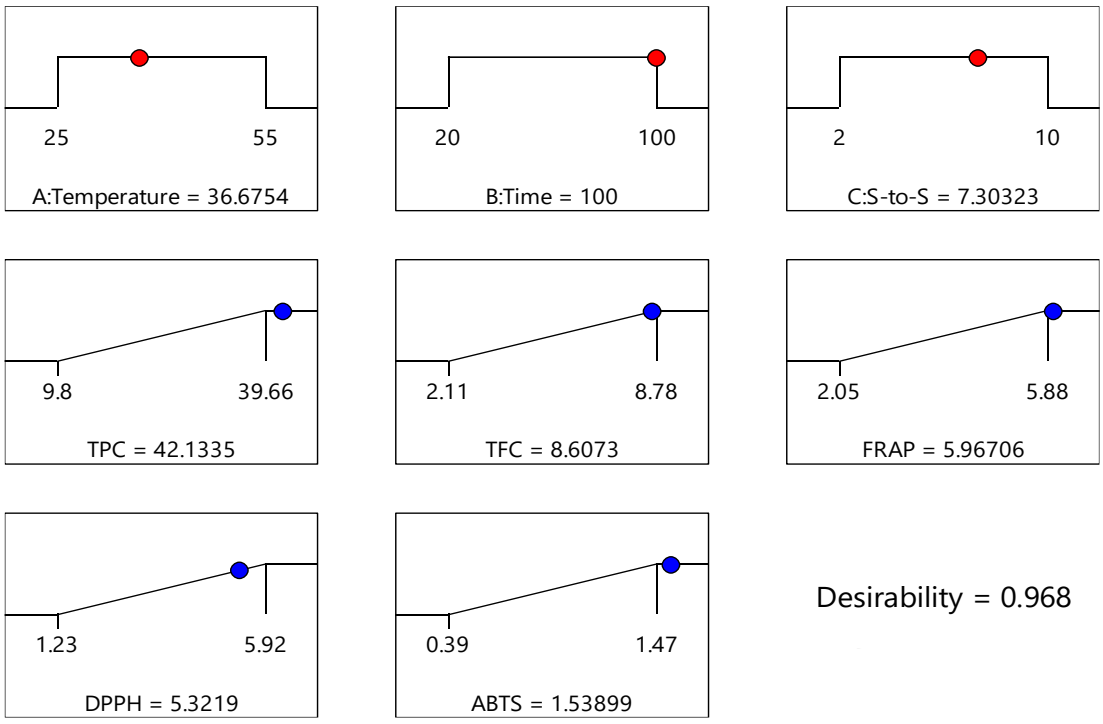


Fig 25. The desirability test based on the optimized values of targeted compounds in anise PW and HAE extracts

4.2 Microwave-assisted extraction (MAE)

An optimization experiment was conducted using response surface methodology (RSM) to enhance the extraction of compounds from hawthorn fruit and anise seed. This involved varying three critical operating variables (microwave power, extraction time, and sample-to-solvent ratio) across three levels. The aim was to maximize the content of specific compound groups (TPC and TFC) and antioxidant activity (FRAP, DPPH, and ABTS) using microwave-assisted extraction (MAE) with a 60% ethanol-water solvent for hawthorn and pure water for anise, as described in the Materials and Methods section (3.3). The significance of the model fit was assessed using the central composite design of Design-Expert Software Version 11.0.3, based on analysis of variance (ANOVA).

4.2.1 Hawthorn fruits:

4.2.1.1 RSM of hawthorn fruits EW extracts

The experimental outcomes of the recovered total phenolics, and flavonoid compounds and their respective antioxidant activities from the hawthorn fruit (*Crataegus monogyna* Jacq.) with EW solvent via MAE were denoted in (Appendix-Table 5). Twenty treatments (runs) were conducted according to CCD including replications in the centre point. 2^k factorial design which is an orthogonal design was applied to fit the multiple linear regression model. Experimental runs were randomized to avoid the effects of extraneous factors which might present. The model fixations for all responses were performed by the quadratic model function. The influence of each factor on the response was investigated by holding the other process variables constant. Response surface 3D graphs were generated for each response.

4.2.1.2 Fitting the model

The regression coefficients of the model for each response and the analysis of variance (ANOVA) results are summarized in (Appendix-Table 6). According to the high values of the coefficient of multiple determination (R^2) (0.91 and 0.89) for TPC, TFC, and (0.87, 0.88, and 0.90) for FRAP, DPPH, and ABTS, the applied second-order model is shown a high significance and a good fit with the experimental data. The obtained regression coefficients demonstrated a positive linear effect of microwave power (A), extraction time (B), and sample-to-solvent ratio (C) were found to be significant for all response variables. In addition, the quadratic effect of microwave power (A^2), and extraction time (B^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 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 (Appendix-Table 6). The predicted values and actual values which can be correlated by the coded and actual equations built by the model were depicted in (Appendix-Fig. 3). The results indicated a good correlation between experimental and predicted data.

4.2.1.3 Effect of extraction variables on total phenolics content (TPC)

The TPC in obtained hawthorn liquid extracts varied from 2.29 to 54.11 mg GAE/g dw. The lowest yield of TPC was obtained on the lowest level of microwave power (100 W), extraction time (20 min), and sample-to-solvent ratio (2 g/100 mL), while TPC was found to be at the middle level of the three studied variables.

According to (β) values of regression coefficients (Appendix-Table 6), the linear term of extraction time (B) had a highly significant ($p < 0.0001$) influence, followed by the effect of microwave power (A; $p < 0.001$), then the sample-to-solvent ratio (C; $P < 0.01$), while the quadratic term of microwave power (A^2) and extraction time (B^2) had negative significant influence with ($p < 0.01$ and $p < 0.001$) on TPC. All other effects are insignificant were removed and the fitted second-order polynomial equation showed as:

$$TPC = -37.081 + 0.107 \cdot \mathrm{A} + 1.041 \cdot \mathrm{B} + 1.12 \cdot \mathrm{C} - 0.000092 \cdot A^2 - 0.0056 \cdot B^2 \quad (29)$$

where A – microwave power (W) in the range (100 – 800 W) , B – extraction time (s) in the range (20 – 120 s), C – sample-to-solvent ratio (g/100 mL) in the range (2 – 12 g/100 mL).

The non-significant value of lack of fit (F = 1.16) showed the model is fitted to the spatial influence of the variables to the response with a good prediction (R^2 = 0.91). The graphs in Fig. 26 show that the TPC increased with the increase of microwave power in levels (-1) and (0) up to 600 W, and then slightly decrease with increasing microwave power in the level (+1). Likewise, the content of polyphenols in hawthorn extracts increased as the extraction time increased in levels (-1) and (0) then it started to decrease when the extraction time was longer than 99 seconds.

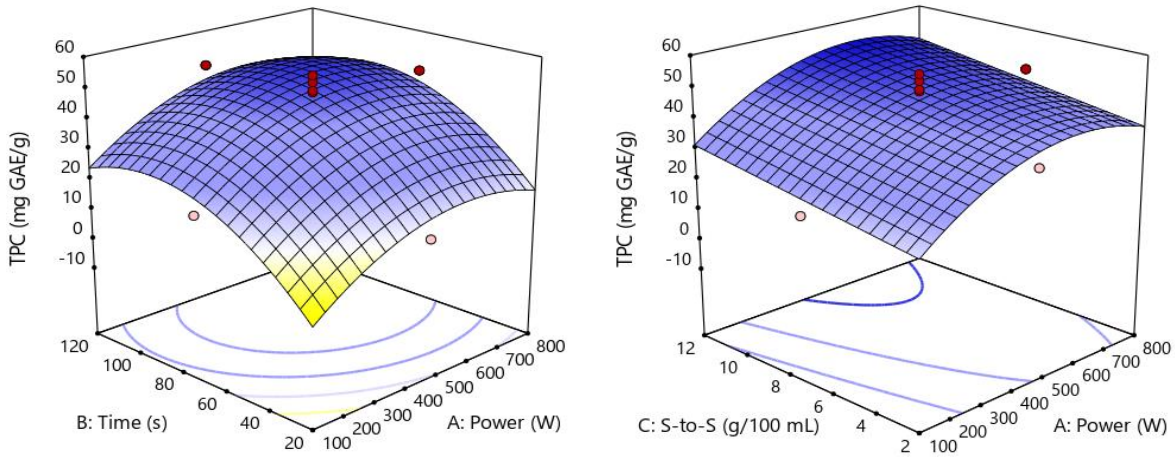


Fig 26. 3D response surface of TPC influenced by individual factors in the EW and MAE extracts of hawthorn fruit

4.2.1.4 Effect of extraction variables on total flavonoids content (TFC)

Experimental results of TFC obtained under different MAE conditions are presented in (Appendix-Table 5). The highest value of TFC (12.82 g QUE/g dw) was obtained with 7 g/100 mL of the sample ratio, 450 W, and 70 seconds. However, the lowest of TFC (0.45 mg QUE/g dw) was observed using 2 g/100 mL of the sample-to-solvent ratio, 100 W, and 20 second extraction. (Appendix-Table 6) shows regression coefficients, and it can be seen that the linear term of extraction time (B; $p < 0.001$) had a highly significant effect, followed by the effect of the microwave power ($p < 0.01$) then the sample-to-solvent ratio (C; $p < 0.05$), in which the effect of all these variables is less than their effect on TPC. While the quadratic term of extraction time (B^2) had a higher negative significant influence on TFC ($p < 0.001$) compared to its effect on TPC, in contrast to the quadratic term of microwave power (A^2) which has less effect on TFC ($p < 0.05$) compared its effect on TPC. The second-order polynomial model that predicts of TFC from hawthorn is given with the next equation after removing the non-significant variables:

$$TFC = -7.77 + 0.0361 \cdot A + 0.16 \cdot B + 0.27 \cdot C - 0.000034 \cdot A^2 - 0.00084 \cdot B^2 \quad (30)$$

where A – microwave power (W) in the range (100 – 800 W) , B – extraction time (s) in the range (20 – 120 s), C – sample-to-solvent ratio (g/100 mL) in the range (2 – 12 g/100 mL).

The non-significant value of lack of fit (F = 1.48) showed the model is fitted to the spatial influence of the variables to the response with a good prediction (R^2 = 0.89). The 3D response surfaces in Fig 27. show the quadratic effect of microwave power and extraction time. Where the TFC increased with the increase of microwave power in levels (-1) and (0) up to 600 W, and then slightly decrease with increasing microwave power until 800 W. While the content of

flavonoids in hawthorn extracts kept increasing as the extraction time increased in all levels until 105 seconds then started to decrease.

The effect of microwave power on TPC and TFC can be explained as the power increase improves the solubility and diffusion of the target compounds out of the plant matrix. Reduction in the yields beyond 600 W may be attributed to the thermal degradation of phenolic compounds in the plant sample at higher microwave power levels. While the decline of the yield after a certain time might be related to the effect of degradation emanating from over-exposure to microwave irradiation (Yingngam et al., 2020; Alara et al., 2018).

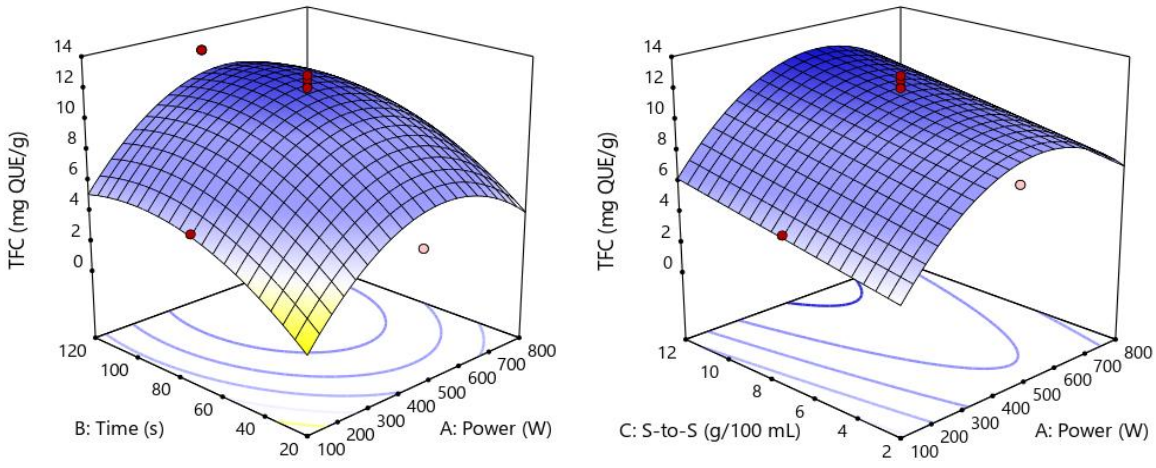


Fig 27. 3D response surface of TFC influenced by individual factors in the EW and MAE extracts of hawthorn fruit

4.2.1.5 Effect of extraction variables on antioxidant activity (AA)

The AA value for hawthorn extracts obtained by MAE was in the range (FRAP: 1.42 to 25.04 mg AAE/g dw, DPPH: 0.82 to 21.61 %, ABTS: 2.71 to 43.75 %) (Appendix-Table 5). The lowest antioxidant activity with the three assays was observed on the lower level of studied variables, the highest value of FRAP was at the middle level of the variables, while the highest values of DPPH and ABTS were observed on the high level of microwave power (800 W), extraction time (120 seconds), and sample-to-solvent ratio (12 g/ 100 mL).

According to the regression coefficients (β), the antioxidant activity (three assays) was significantly influenced by the linear term of extraction time (B), and the term of microwave power (A) has a highly significant effect on (DPPH and ABTS) with ($p < 0.001$) while its effect was less on FRAP values ($p < 0.05$), and the sample ratio (C) has the highest effect on ABTS ($p < 0.01$). Also, the interaction of the microwave power and sample ratio has a significant effect on DPPH and ABTS ($p < 0.05$), on the other hand, the quadratic terms of microwave power (A^2) and extraction time (B^2) show a negative significant effect on the values of all assays of antioxidant activity. The second-order polynomial model that predicts the antioxidant activity is presented with the following equations after removing the non-significant variables:

$$FRAP = -12.97 + 0.061 \cdot A + 0.32 \cdot B + 0.53 \cdot C - 0.000059 \cdot A^2 - 0.0017 \cdot B^2 \quad (31)$$

$$DPPH = -8.037 + 0.033 \cdot A + 0.29 \cdot B - 0.18 \cdot C + 0.0013 \cdot AC - 0.000035 \cdot A^2 - 0.0015 \cdot B^2 \quad (32)$$

$$ABTS = 16.67 + 0.056 \cdot \mathrm{A} + 0.66 \cdot \mathrm{B} - 0.056 \cdot \mathrm{C} + 0.0023 \cdot \mathrm{AC} - 0.000055 \cdot A^2 - 0.0033 \cdot B^2 \quad (33)$$

where A – microwave power (W) in the range (100 – 800 W) , B – extraction time (s) in the range (20 – 120 s), C – sample-to-solvent ratio (g/100 mL) in the range (2 – 12 g/100 mL).

The non-significant value of lack of fit showed the models are fitted with good prediction (Appendix-Table 6). The 3D response surfaces in (Fig. 28, Fig. 29, and Fig. 30) show the effect of all the studied variables on AA, which are in line with the change in TPC and TFC.

These data suggest that applying microwave power for a short time may be the most effective way to extract phenolic compounds and increase their antioxidant activity from anise seed using MAE. However, higher microwave power and increasing the time may lead to thermal degradation of the phenols.

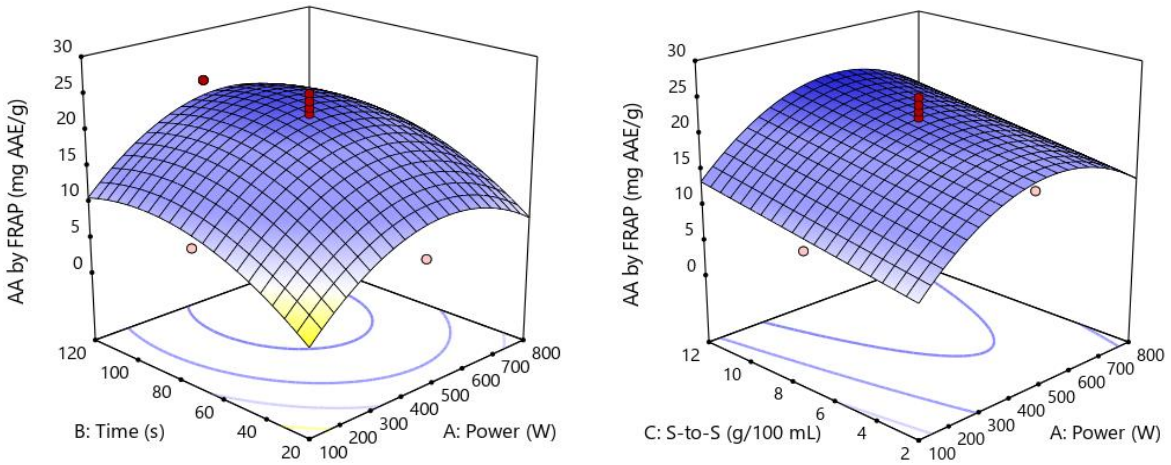


Fig 28. 3D response surface of FRAP influenced by individual factors in the EW and MAE extracts of hawthorn fruit

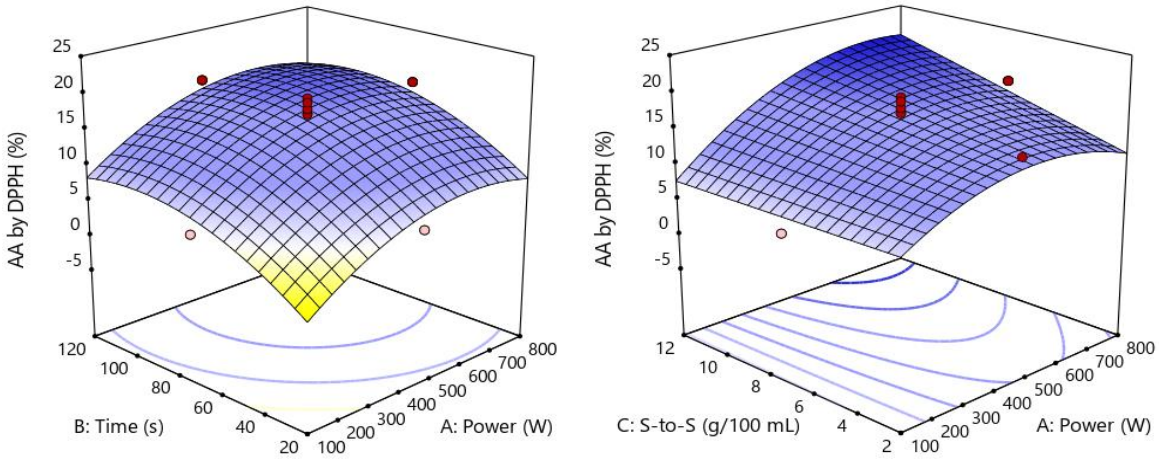


Fig 29. 3D response surface of DPPH influenced by individual factors in the EW and MAE extracts of hawthorn fruit

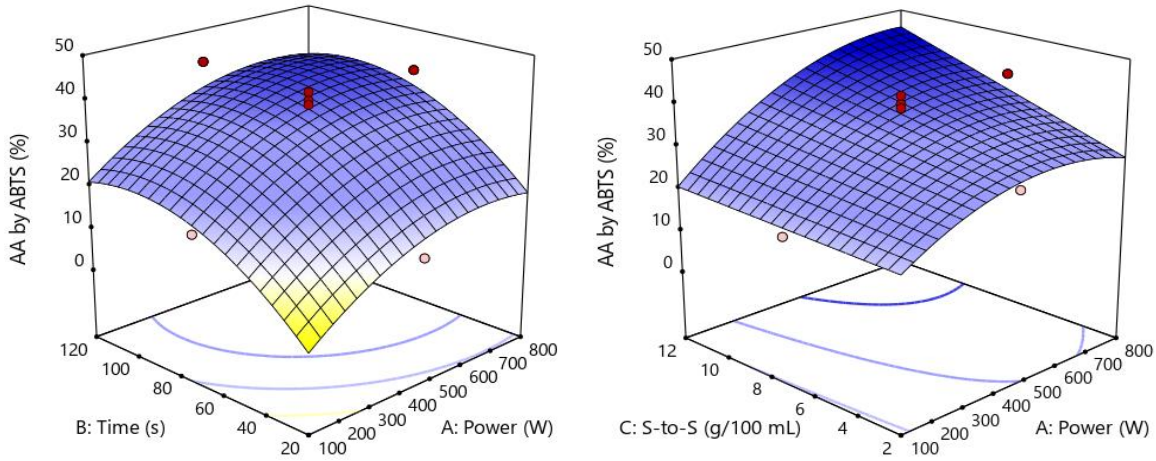


Fig 30. 3D response surface of ABTS influenced by individual factors in the EW and MAE extracts of hawthorn fruit

4.2.1.6 Determination and experimental validation of optimal conditions

In order to optimize the extraction of bioactive compounds from hawthorn fruit using MAE as an extraction method, the following constraints have taken (1) microwave power (100, 450, and 800 W), (2) extraction time (20, 70, and 100 seconds), and (3) sample to solvent ratio (2, 7, and 12 g/100 mL) respectively, were set for maximum desirability. By applying the desirability function approach (DFA), the optimum level of various parameters was obtained at – microwave power (600 W), extraction time (96 seconds), and sample-to-solvent ratio (12 g/100 mL) in the evaluated range, with overall desirability value of 0.99 where this value close to 1 signifies that the combination of factor levels chosen for the experiment successfully optimized the responses according to the defined criteria (Fig. 31). As a result of these optimal conditions, the experimental values were in agreement with the predicted values with the coefficient of variation C.V. % range from 18.01 to 22.43 % (Appendix-Table 6).

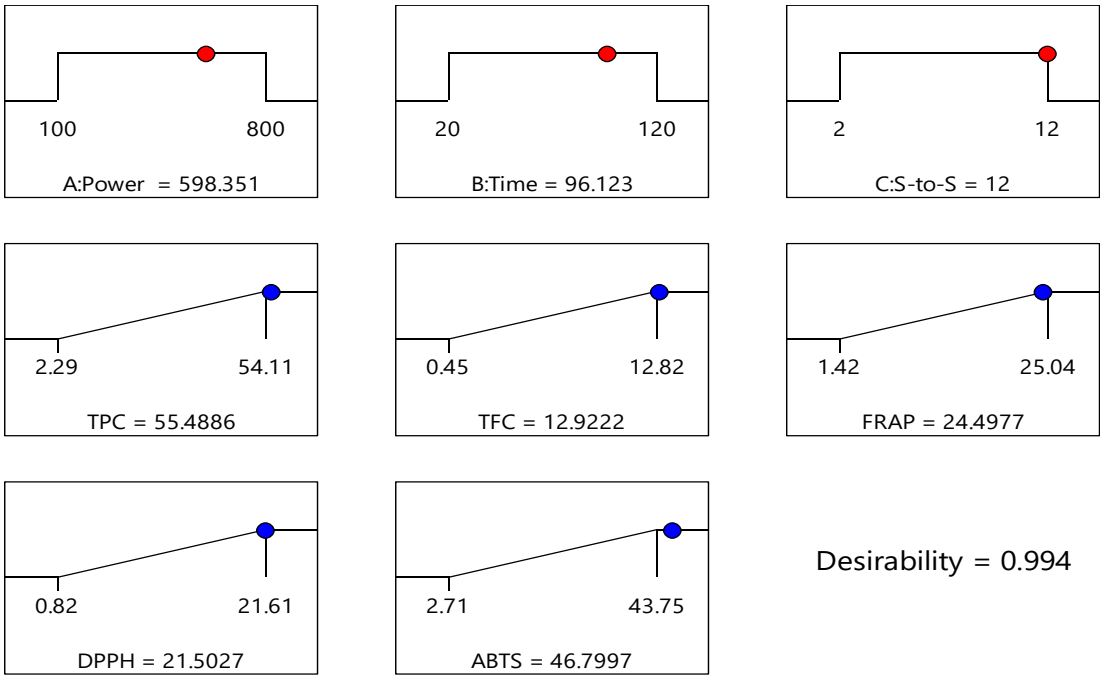


Fig 31. The desirability test based on the optimized values of targeted compounds in hawthorn EW and MAE extracts

4.2.2 Anise seed

4.2.2.1 RSM of anise seed PW extracts

The experimental outcomes of the recovered total phenolics, and flavonoid compounds and their respective antioxidant activities from the anise seed (*Pimpinella anisum* L.) with PW solvent via MAE were denoted in (Appendix-Table 7). Twenty treatments (runs) were conducted according to CCD including replications in the center point. 2^k factorial design which is an orthogonal design was applied to fit the multiple linear regression model. Experimental runs were randomized to avoid the effects of extraneous factors which might present. The model fixations for all responses were performed by the quadratic model function. The influence of each factor on the response was investigated by holding the other process variables constant. Response surface 3D graphs were generated for each response

4.2.2.2 Fitting the model

The regression coefficients of the model for each response and the analysis of variance (ANOVA) results are summarized in (Appendix-Table 8). According to the high values of the coefficient of multiple determination ($R^2 = 0.92$) for both TPC, TFC, and (0.96, 0.90, and 0.87) for FRAP, DPPH, and ABTS, the applied second-order model is shown a high significance and a good fit with the experimental data. The obtained regression coefficients demonstrated a positive linear effect of microwave power (A), extraction time (B), and sample-to-solvent ratio (C) were found to be significant for all response variables. In addition, the quadratic effect of microwave power (A^2), 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 (Appendix-Table 8). The predicted values and actual values which can be correlated by the coded and actual equations built by the model were depicted in (Appendix-Fig. 4). The results indicated a good correlation between experimental and predicted data.

4.2.2.3 Effect of extraction variables on total phenolics content (TPC)

The TPC in obtained anise liquid extracts varied from 15.66 to 50.54 g GAE/g dw. The lowest yield of TPC was obtained on the highest level of microwave power (800 W) and the lowest level of extraction time (20 seconds), and sample-to-solvent ratio (2 g/100 mL), while TPC was found to be highest on the high level of the extraction time (120 seconds) and the middle level of microwave power (450 W) and sample-to-solvent ratio (7 g/100 mL).

According to p values of regression coefficients (β) (Appendix-Table 8), the linear term of extraction time (B) concentration had a highly positive significant ($p < 0.0001$) influence, followed by the negative effect of microwave power (A) ($p < 0.01$), while the sample-to-solvent ratio has a positive significant effect on TPC with ($p < 0.05$). The quadratic term of microwave power (A^2) had a highly negative significant influence ($p < 0.0001$) on TPC, and the quadratic term of sample-to-solvent ratio (C^2) had a negative effect as well with ($p < 0.05$). The non-significant variables were removed and the second-order polynomial model used to express the TPC content as a function of independent variables is shown below:

$$TPC = +6.57 + 0.065 \cdot A + 0.13 \cdot B + 3.48 \cdot C - 0.000084 \cdot A^2 - 0.21 \cdot C^2 \qquad (34)$$

where A – microwave power (W) in the range (100 – 800 W) , B – extraction time (s) in the range (20 – 120 s), C – sample-to-solvent ratio (g/100 mL) in the range (2 – 12 g/100 mL).

The non-significant value of lack of fit (F = 0.34) showed the model is fitted to the spatial influence of the variables to the response with a good prediction (R^2 = 0.92). The graphs in Fig. 32 show the quadratic effect of microwave power and the sample-to-solvent ratio. The TPC increased with the increase of microwave power in the level (-1), and started to decrease in levels (0) and (+1) . While TPC increased as the sample-to-solvent ratio increased in levels (-1) and (0) then started to decrease in level (1). In addition, the content of polyphenols in anise extracts increased as the extraction time increased up to 120 seconds.

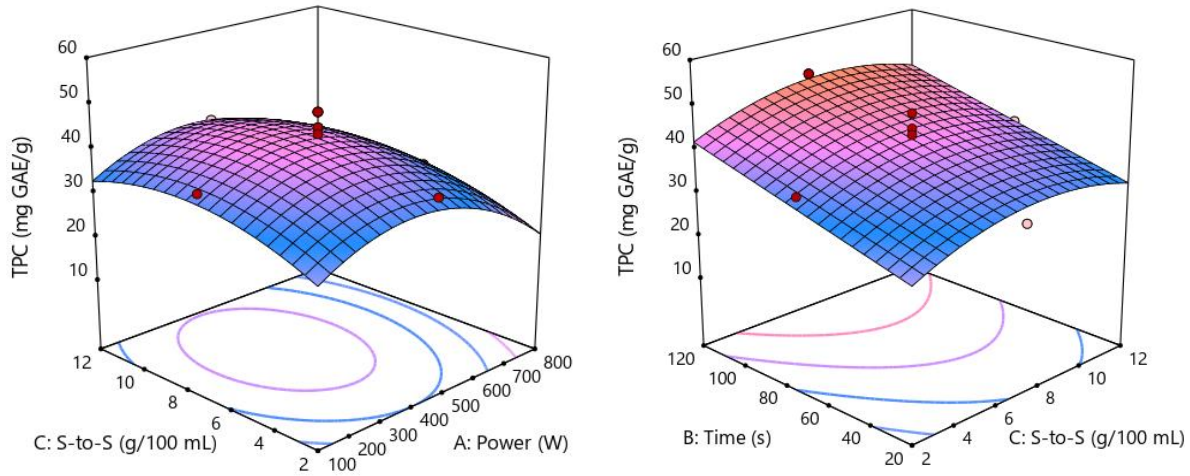


Fig 32. 3D response surface of TPC influenced by individual factors in the PW and MAE extracts of anise seed

4.2.2.4 Effect of extraction variables on total flavonoids content (TFC)

Experimental results of TFC obtained under different MAE conditions are presented in (Appendix-Table 7). The highest value of TFC (21.67 g QUE/g dw) was obtained with 450 W of microwave power, 7g/100 of the sample-to-solvent ratio, and 120 seconds. However, the lowest of TFC (5.01 g QUE/g dw) was observed at 800 W of microwave power, 2 g of the sample-to-solvent ratio, and 20 seconds of the extraction time. The regression coefficients values (β) in (Appendix-Table 8) shows that the linear terms of extraction time (B), and sample-to-solvent ratio (C) had the same positive significant effect ($p < 0.01$), and microwave power (A) had a negative significant effect ($p < 0.01$) on TFC. While the quadratic term of microwave power (A^2) and sample-to-solvent ratio (C^2) had a high negative significant influence with ($p < 0.0001$ and $p < 0.001$) on TFC. After removing the non-significant variables, the second-order polynomial model that predicts the content of TFC from anise is given with the next equation:

$$TFC = -1.068 + 0.034 \cdot A + 0.038 \cdot B + 2.56 \cdot C - 0.000044 \cdot A^2 - 0.15 \cdot C^2 \qquad (35)$$

where A – microwave power (W) in the range (100 – 800 W) , B – extraction time (s) in the range (20 – 120 s), C – sample-to-solvent ratio (g/100 mL) in the range (2 – 12 g/100 mL).

The non-significant value of lack of fit (F = 0.53) showed the model is fitted to the spatial influence of the variables to the response with a good prediction (R^2 = 0.92). The graphs in Fig. 33 shows the quadratic effect of microwave power and the sample-to-solvent ratio. Like TPC, the total flavonoid content increased with the increase of microwave power in the level

(-1), and started to decrease in levels (0) and (+1). While TFC increased as the sample-to-solvent ratio increased in levels (-1) and (0) then started to decrease in level (1). And the content of polyphenols in anise extracts increased as the extraction time increased up to 120 seconds.

The impact of microwave power on the extraction of total phenolic content (TPC) and total flavonoid content (TFC) likely involves reaching an optimal power level, beyond which reductions and degradation reactions occur, resulting in decreased compound content in the extracts. Conversely, the sample-to-solvent ratio influences the sufficient solvation of target compounds, with larger ratios of extraction solvent yielding higher compound yields.

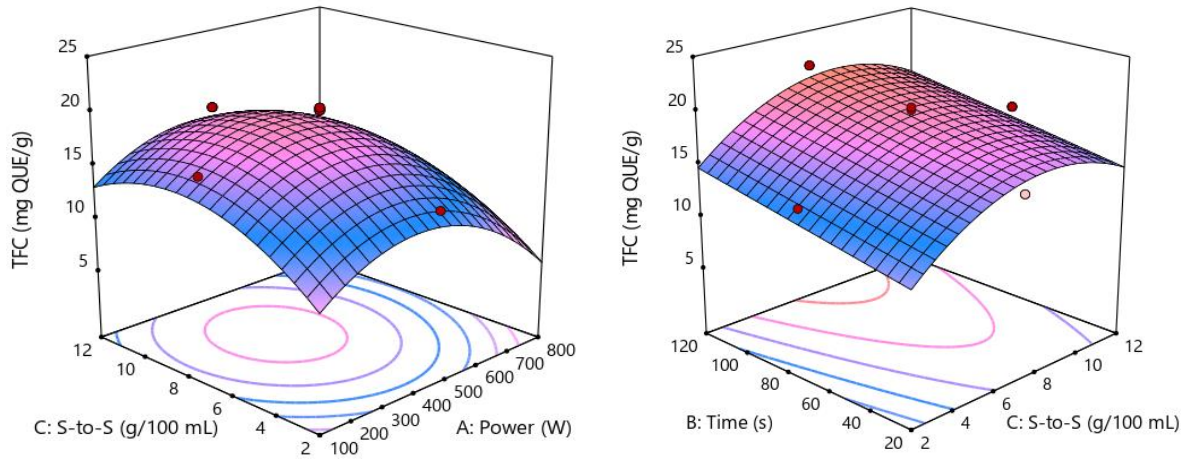


Fig 33. 3D response surface of TFC influenced by individual factors in the PW and MAE extracts of anise seed

4.2.2.5 Effect of extraction variables on antioxidant activity (AA)

The AA value for anise extracts obtained by MAE was in the range (FRAP: 4.91 to 11.16 mg AAE/g dw, DPPH: 3.78 to 17.36 %, ABTS: 1.15 to 4.23 %) (Appendix-Table 7). The lowest antioxidant activity with the three assays was observed on the highest level of microwave power (800 W) and the lowest level of extraction time and sample-to-solvent ratio ((20 seconds, 2 g/100 mL), the highest value of FRAP and DPPH was at the middle level of the microwave power and sample-to-solvent ratio and the highest level of extraction time, while the highest values of ABTS were observed on the lowest level of microwave power (100 W), and the highest level of extraction time and sample-to-solvent ratio (120 seconds, 12 g/100 mL).

According to the regression coefficients (β), FRAP and ABTS were negatively and significantly influenced by the linear term of microwave power (A) ($p < 0.001$), while DPPH was less affected by (A) with ($p < 0.01$), in contrast, the term of extraction time (B) had a higher positive effect on FRAP and DPPH with ($p < 0.0001$, and $p < 0.001$), and lower effect on ABTS with ($p < 0.05$), while the term of sample-to-solvent ratio (C) had a highly significant effect on FRAP ($p < 0.0001$) and equal effect on DPPH and ABTS ($p < 0.01$). On the other hand, a high negative significant effect of the quadratic terms of microwave power (A^2) was found on FRAP ($p < 0.001$), while its effect was less on DPPH and ABTS ($p < 0.05$), as was effect of sample-to-solvent ratio (C^2) highly significant on FRAP and DPPH ($p < 0.0001$, and $p < 0.001$) and less effect on ABTS ($p < 0.05$), in additional ABTS was significantly influenced by the interaction of the extraction time and sample-to-solvent-ratio (BC) ($p < 0.05$). (Lin et al., 2020) reported different effects of the studied parameters on the response variable (FRAP and DPPH),

and explained that can be due to the different mechanisms of the two methods and the correlations of TPC and TFC with antioxidant activity assays.

The second-order polynomial model that predicts the antioxidant activity is presented with the following equations after removing the non-significant variables:

$$FRAP = +3.031 + 0.0067 \cdot \mathrm{A} + 1.18 \cdot \mathrm{B} + 2.56 \cdot \mathrm{C} - 9.10 \cdot 10^{-6} \cdot A^2 - 0.069 \cdot C^2 \quad (36)$$

$$DPPH = -1.034 + 0.012 \cdot A + 0.042 \cdot B + 2.75 \cdot C - 0.000019 \cdot A^2 - 0.17 \cdot C^2 \quad (37)$$

$$ABTS = +1.77 + 0.0021 \cdot A + 0.0021 \cdot B + 0.38 \cdot C + 0.0015 \cdot BC - 4.51 \cdot 10^{-6} A^2 - 0.028 \cdot C^2 \quad (38)$$

where A – microwave power (W) in the range (100 – 800 W) , B – extraction time (s) in the range (20 – 120 s), C – sample-to-solvent ratio (g/100 mL) in the range (2 – 12 g/100 mL).

The non-significant value of lack of fit showed the models are fitted with good prediction (Appendix-Table 8), The 3D response surfaces in (Fig. 34, Fig. 35, and Fig. 37) show the effect of all the studied variables on AA.

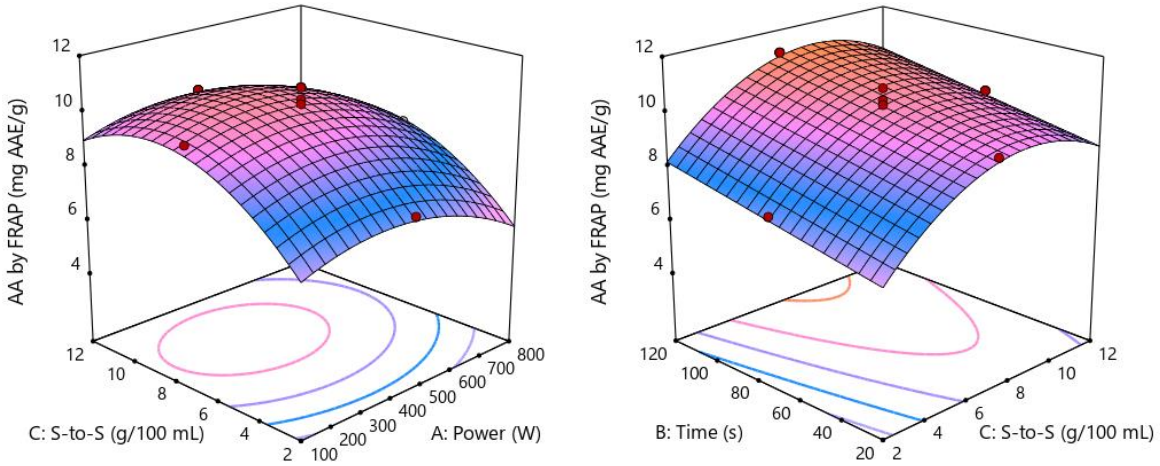


Fig 34. 3D response surface of FRAP influenced by individual factors in the PW and MAE extracts of anise seed

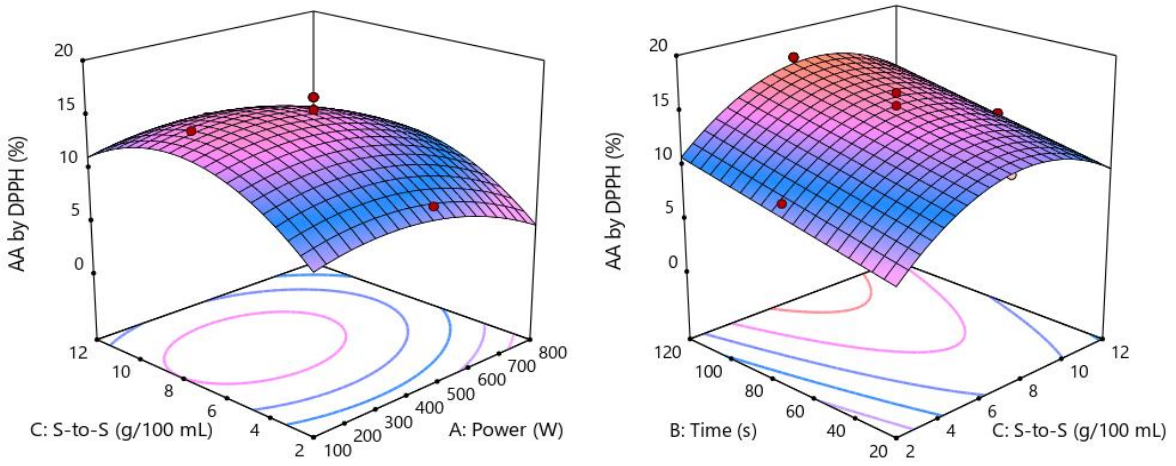


Fig 35. 3D response surface of DPPH influenced by individual factors in the PW and MAE extracts of anise seed

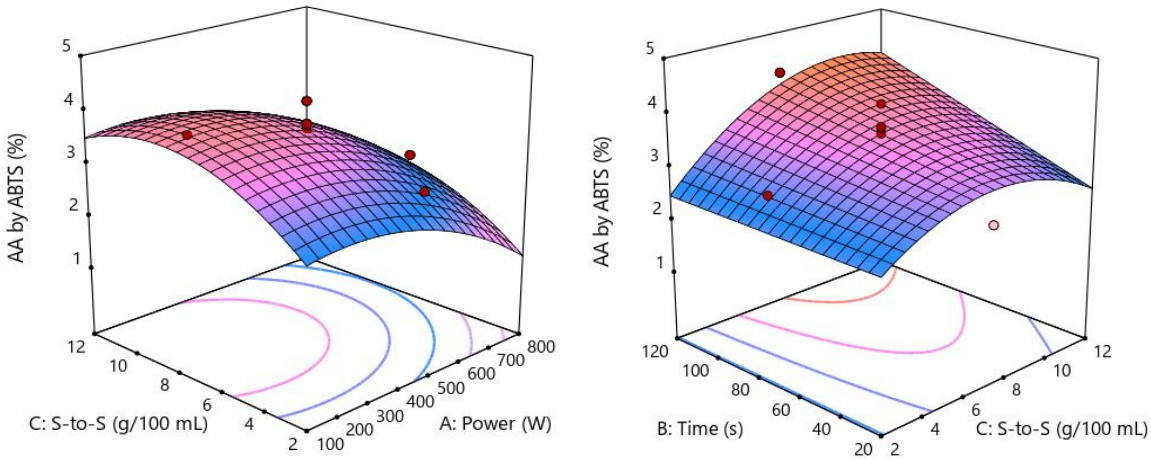


Fig 36. 3D response surface of ABTS influenced by individual factors in the PW and MAE extracts of anise seed

4.2.2.6 Determination and experimental validation of optimal conditions

To optimize the extraction of bioactive compounds from anise seed using MAE as an extraction method, the following constraints have taken (1) microwave power (100, 450, and 800 W), (2) Extraction time (20, 70, and 100 seconds), and (3) sample to solvent ratio (2, 7, and 12 g/100 mL) respectively, were set for maximum desirability. By applying the desirability function approach (DFA), the optimum level of various parameters was obtained a microwave power (480 W), extraction time (120 seconds), and sample-to-solvent ratio (8 g/100 mL) in the evaluated range, with overall desirability value of 0.99 where this value close to 1 signifies that the combination of factor levels chosen for the experiment successfully optimized the responses according to the defined criteria (Fig. 37). Under these optimized conditions, the experimental results closely aligned with the predicted values, demonstrating good agreement with a coefficient of variation C.V. % range from 4.38 to 14.11% (Appendix-Table 8).

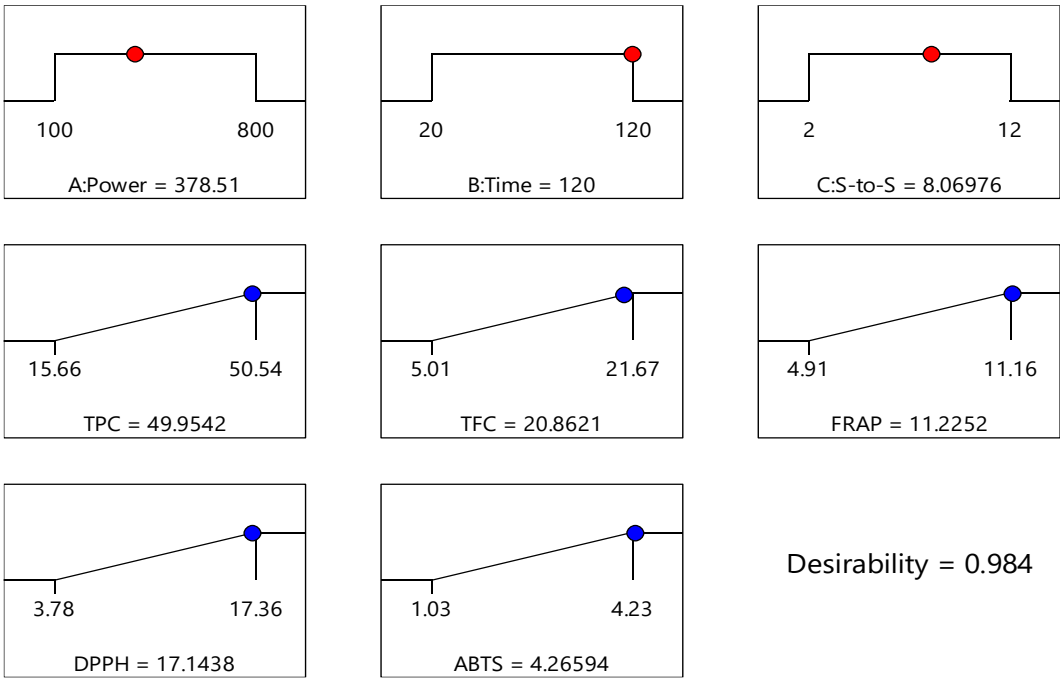


Fig 37. The desirability test based on the optimized values of targeted compounds in anise PW and MAE extracts

4.3 Ultrasound-assisted extraction (UAE)

Extraction of herbs using an ultrasound-assisted process is considered as one of the most inexpensive and simplest existing extraction systems and could be suitably operated rapidly for large-scale preparations. The application of ultrasound helps develop interesting and novel methodologies in food processing; these methodologies are often complementary to classical methods. Accordingly, ultrasonic waves have been used to assist in the extraction of bioactive compounds from hawthorn fruit. UAE was performed using 20 kHz and 3.5 W/cm^2 (ULC 400) premium ultrasonic generator with different solvent concentrations and extraction times as was mentioned in section (3.4) of material and methods.

4.3.1 Hawthorn fruits

4.3.1.1 RSM of hawthorn fruits EW extracts:

In order to optimize the antioxidant ability of the extracts of hawthorn fruit, RSM was conducted with a central composite rotatable design (CCD) based on three variables, and three levels were generated. Based on the single-factor experiments, three principal factors (concentration of ethanol, ultrasonication extracting time, and sample-to-solvent ratio). Twenty experimental runs and the recovered total phenolics, and flavonoid compounds and their respective antioxidant activities from the hawthorn fruit (*Crataegus monogyna* Jacq.) obtained are illustrated in (Appendix-Table 9). 2^k factorial design which is an orthogonal design was applied to fit the multiple linear regression model. Experimental runs were randomized to avoid the effects of extraneous factors which might present. The model fixations for all responses were performed by the quadratic model function. The influence of each factor on the response was investigated by holding the other process variables constant. Response surface 3D graphs were generated for each response.

4.3.1.2 Fitting the model

The regression coefficients (β) of the second-order polynomial model and the analysis of variance (ANOVA) results are summarized in (Appendix-Table 10). According to the low p-values (< 0.0001), the model obtained was statistically significant. Besides, the determination coefficient value (R^2) was (0.96 ,0.93) for TPC and TFC and (0.95, 0.93 , 0.90) for FRAP, DPPH, and ABTS as antioxidant activity assays, which implied a strong correlation between the predicted results and the actual results. The obtained regression coefficients demonstrated a linear effect of solvent concentration (A), extraction time (B), and sample-to-solvent ratio (C) were found to be significant for all response variables. In addition, the quadratic effect of solvent concentration (A^2), and extraction time (B^2) was found to produce a negative significant effect on all the responses. The interaction effect of the studied variables (AB) 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 (Appendix-Table 10). The predicted values and actual values which can be correlated by the coded and actual equations built by the model were depicted in (Appendix-Fig. 5). The results indicated a good correlation between experimental and predicted data.

4.3.1.3 Effect of extraction variables on total phenolics content (TPC)

The extraction of TPC was significantly influenced by all the studied variables. The highest of TPC (87.1 mg GAE/g dw) in the hawthorn fruit extracts was obtained using (30 % v/v)

ethanol concentration and (12 g/100 mL) sample-to-solvent ratio after 10 min of the extraction process. While the lowest content was found at the low level of the extraction variables (25.11 mg GAE/g dw) (Appendix-Table 9). According to (β) values of regression coefficients (Appendix-Table 10), the linear term of extraction time (B) had a highly positive significant ($p < 0.0001$) influence, followed by the linear effect of ethanol concentration (A; $p < 0.001$) and the linear effect of sample-to-solvent ratio (C; $p < 0.001$). The quadratic term of ethanol concentration (A^2) and extraction time (B^2) had a negative significant influence with ($p < 0.01$ and $p < 0.0001$) on TPC, and the interaction of ethanol concentration and extraction time (AB) was found to be significant ($p < 0.05$) as well. All other effects are insignificant were removed and the fitted second-order polynomial equation showed as:

$$TPC = -139.44 + 6.29 \cdot A + 17.44 \cdot B + 1.85 \cdot C + 0.108 \cdot AB - 0.109 \cdot A^2 - 0.88 \cdot B^2 \quad (39)$$

where A – ethanol concentration (% v/v) in the range (20 – 40 % v/v) , B – extraction time (min) in the range (5 – 15 min), C – sample-to-solvent ratio (g/100 mL) in the range (2 – 12 g/100 mL).

Analysis of variance for predicted models implied that the model was highly significant ($p < 0.0001$) with a good coefficient of determination ($R^2 = 0.96$). In addition, lack-of-fit (F-value 1.68, $p > 0.05$) was not significant. The 3D plot shows that the TPC increased as increasing the ethanol concentration from level (-1) to level (0), then the content decreased with the Increasing ethanol concentration at level (+1). Likewise was the effect of the extraction time where TPC increased during the first 11 min (levels (-1) and (0)) of the extraction process and slightly started to decrease (Fig. 38).

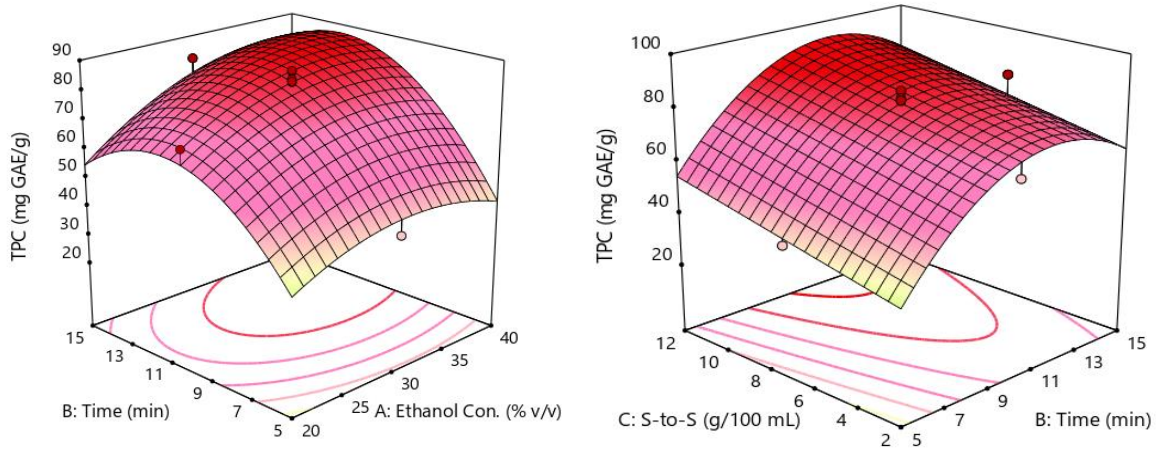


Fig 38. 3D response surface of TPC influenced by individual factors in the EW and UAE extracts of hawthorn fruit

4.3.1.4 Effect of extraction variables on total flavonoids content (TFC)

The extraction of TFC was significantly influenced by all the studied variables. The highest of TFC (29.87 mg QUE/g dw) in the hawthorn fruit extracts was obtained using (30 % v/v) ethanol concentration and (12 g/100 mL) sample-to-solvent ratio after 10 min of the extraction process. While the lowest content was found at the low level of the extraction variables (6.46 mg QUE/g dw) (Appendix-Table 9).

(Appendix-Table 10) shows regression coefficients (β) values of regression coefficients, and it is shown the linear term of extraction time (B) had a highly positive significant ($p < 0.0001$) influence, followed by the effect of linear term of ethanol concentration (A; $p < 0.001$) and the linear effect sample-to-solvent ratio (C; $p < 0.001$). The quadratic term of ethanol concentration (A^2) and extraction time (B^2) had a negative significant influence with ($p < 0.05$ and $p < 0.0001$), while the interaction of ethanol concentration and extraction time (AB) was not found to be significant on TFC. After removing insignificant variables the model obtained for the extraction of TFC was as follows:

$$TFC = -56.96 + 2.36 \cdot A + 7.081 \cdot B + 0.58 \cdot C - 0.034 \cdot A^2 - 0.31 \cdot B^2 \quad (40)$$

where A – ethanol concentration (% v/v) in the range (20 – 40 % v/v) , B – extraction time (min) in the range (5 – 15 min), C – sample-to-solvent ratio (g/100 mL) in the range (2 – 12 g/100 mL).

Analysis of variance for predicted models implied that the model was highly significant ($p < 0.0001$) with a good coefficient of determination ($R^2 = 0.93$). In addition, lack-of-fit (F-value 1.56, $p > 0.05$) was not significant. As shown in Fig. 39, the effect of the studied parameters on the TFC was like their effect on TPC where TFC increased with increasing ethanol concentration in levels (-1) and (0), while further increasing the ethanol concentration led to a significant decrease in the TFC in level (+1), as well as, TFC increased during the first 11 min (levels (-1) and (0)) of the extraction process and slightly started to decrease.

The effect of the ethanol concentration can be because of the polarity of the extraction solvents as was mentioned before which play an important role in allowing easier solvent penetration in the cells/ tissue for maximum solubility of compounds. Additionally, during ultrasonic extraction, the prolonged ultrasonic time increases the solvent temperature, which can cause phenolics to decompose, as well as increase solvent loss through vaporization, which directly affects mass transfer (Pan et al., 2012) reported similar results.

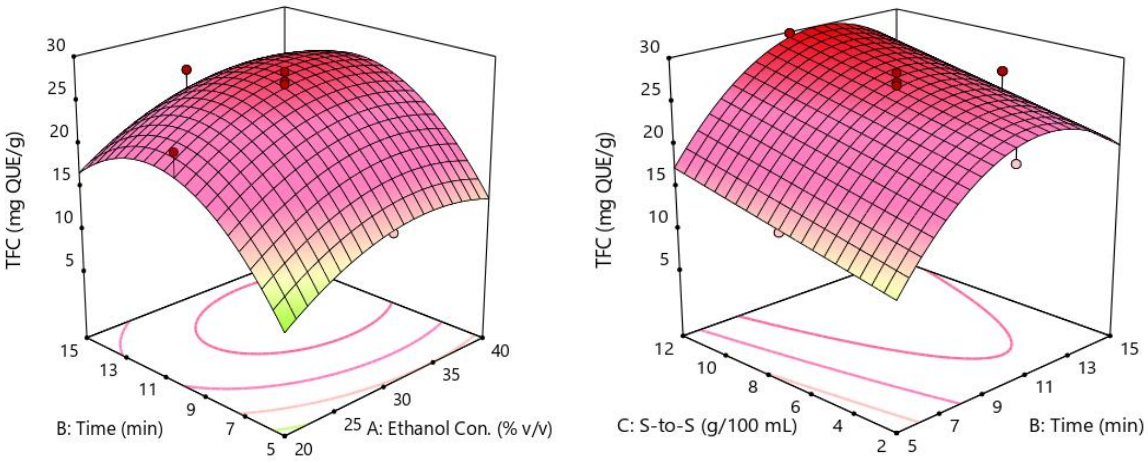


Fig 39. 3D response surface of TFC influenced by individual factors in the EW and UAE extracts of hawthorn fruit

4.3.1.5 Effect of extraction variables on antioxidant activity (AA)

The total antioxidant activity of hawthorn fruit was determined using FRAP, DPPH, and ABTS radical scavenging assay, the lowest antioxidant activity with the three assays was observed on the lower level of studied variables (FRAP =10.74 mg AAE/g dw, DPPH = 12.23

%, and ABTS = 15.14 %), the highest value was at the middle level of the ethanol concentration and extraction time (30 % v/v, and 10 min) with the higher level of the sample ratio (12 g/100 mL) (Appendix-Table 9).

According to the regression coefficients (β), the antioxidant activity (three assays) was significantly influenced by the linear effect of extraction time (B) ($p < 0.001$), and the term of ethanol concentration (A) has a significant effect on all assays with ($p < 0.01$). while the effect of the sample-to-solvent ratio has a higher effect on FRAP ($p < 0.001$) compared to its effect on DPPH and ABTS ($p < 0.01$, and $p < 0.05$). The interaction of the ethanol concentration and extraction time has a significant effect on all assays ($p < 0.05$), on the other hand, the quadratic terms of ethanol concentration (A^2) and extraction time (B^2) show a negative significant effect on the values of all assays of antioxidant activity. The second-order polynomial model that predicts the antioxidant activity is presented with the following equations after removing the non-significant variables:

$$FRAP = -62.79 + 2.87 \cdot A + 8.038 \cdot B + 0.71 \cdot C + 0.039 \cdot AB - 0.049 \cdot A^2 - 0.406 \cdot B^2 \quad (41)$$

$$DPPH = -44.12 + 2.27 \cdot A + 5.67 \cdot B + 0.57 \cdot C + 0.037 \cdot AB - 0.042 \cdot A^2 - 0.296 \cdot B^2 \quad (42)$$

$$ABTS = -129.89 + 7.15 \cdot A + 10.28 \cdot B + 1.13 \cdot C + 0.12 \cdot AB - 0.12 \cdot A^2 - 0.603 \cdot B^2 \quad (43)$$

where A – ethanol concentration (% v/v) in the range (20 – 40 % v/v) , B – extraction time (min) in the range (5 – 15 min), C – sample-to-solvent ratio (g/100 mL) in the range (2 – 12 g/100 mL).

Analysis of variance for predicted models implied that the model was highly significant ($p < 0.0001$) with a good coefficient of determination ($R^2 = 0.95$, 0.93, and 0.90) for FRAP, DPPH, and ABTS respectively. Moreover, lack-of-fit was not significant for all assays (Appendix-Table 10). The 3D response surfaces in (Fig. 40, Fig. 41, and Fig. 42) show the effect of all the studied variables on AA, which are in line with the changes of extracted TPC and TFC according to the correlation between the phenolic compounds and their antioxidant activity.

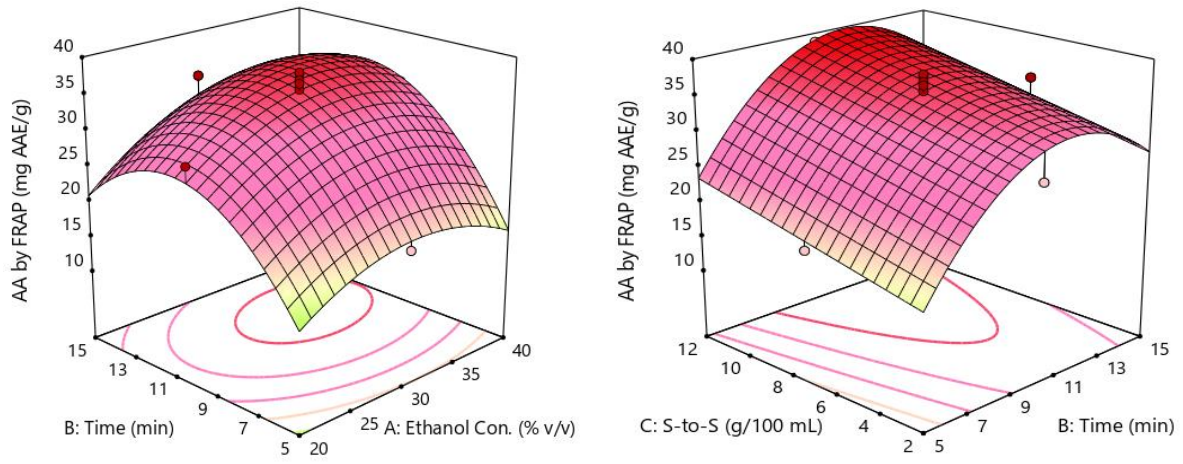


Fig 40. 3D response surface of FRAP influenced by individual factors in the EW and UAE extracts of hawthorn fruit

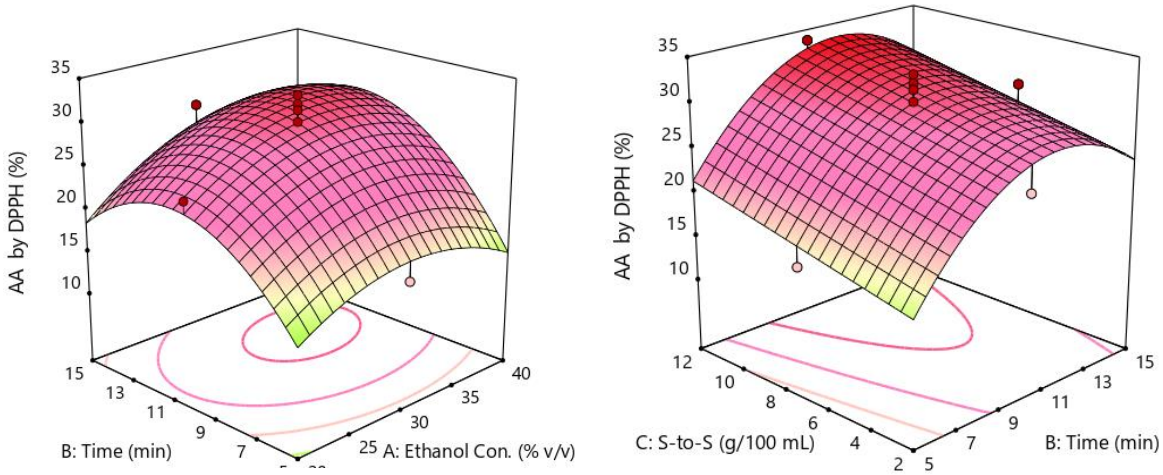


Fig 41. 3D response surface of DPPH influenced by individual factors in the EW and UAE extracts of hawthorn fruit

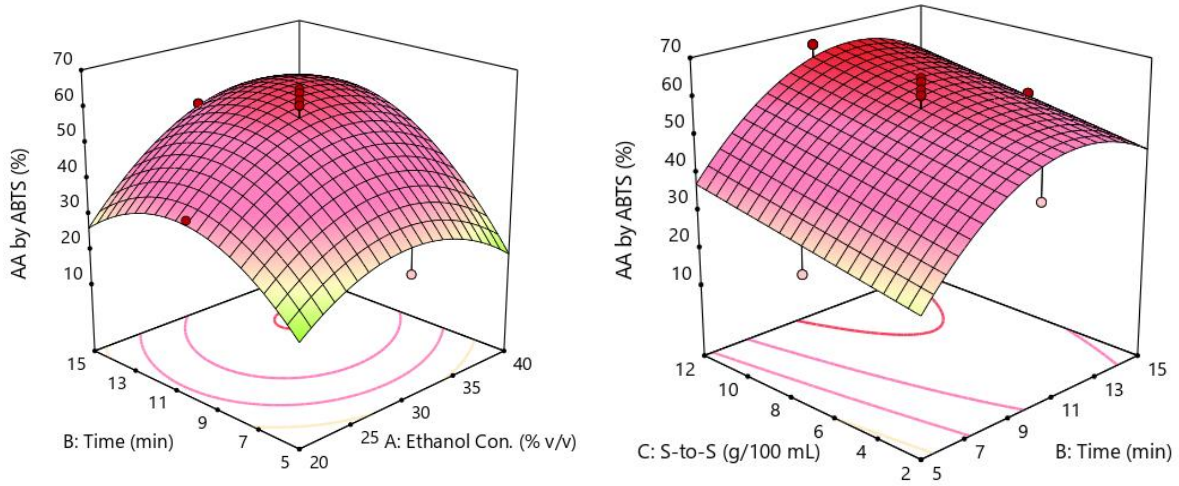


Fig 42. 3D response surface of ABTS influenced by individual factors in the EW and UAE extracts of hawthorn fruit

4.3.1.6 Determination and experimental validation of optimal conditions

In order to determine the best set of ethanol concentration, extraction time, and sample-to-solvent ratio for the extraction process from hawthorn fruit using UAE as an extraction method. The desirability function approach (DFA) was applied using Design Expert Software Trial Version 11.0.3. The best level of various parameters was obtained at the ethanol concentration (33.5 % v/v), extraction time (12 min), and sample-to-solvent ratio (12 g/100 mL) in the evaluated range, with an overall desirability value of 0.99 suggests that the experimental conditions achieved a near-ideal balance or performance across all desired responses (Fig.43). Under these optimal conditions, the experimental values agreed with the predicted values with the coefficient of variation C.V. % ranging from 8.29 to 16.09 % (Appendix-Table 10).

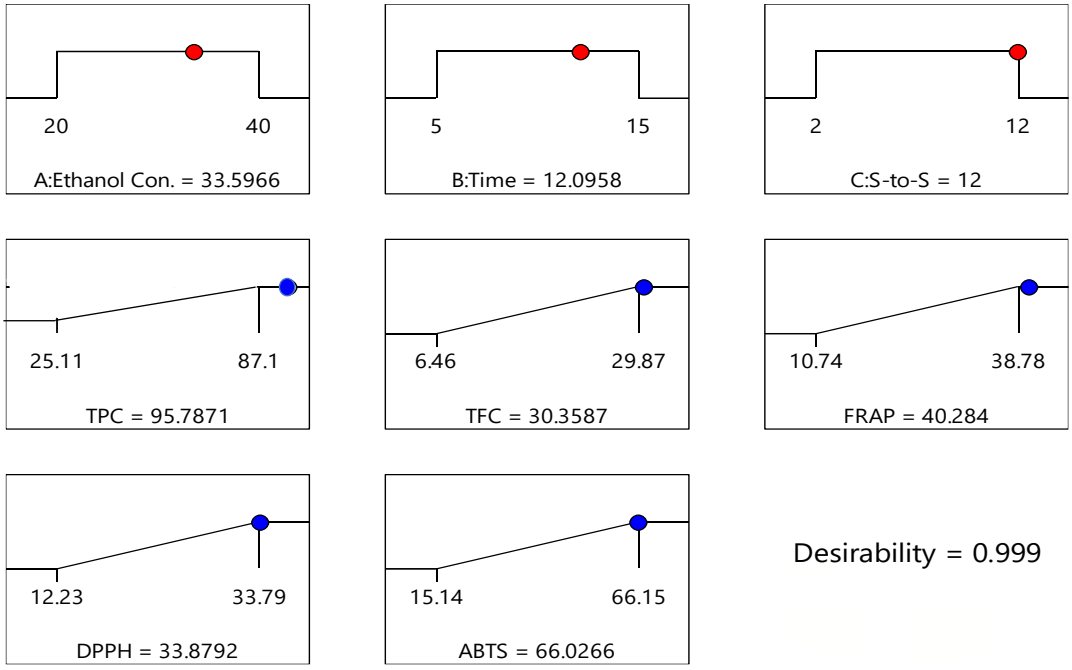


Fig 43. The desirability test based on the optimized values of targeted compounds in hawthorn EW and UAE extracts

4.3.2 Anise seed

4.3.2.1 RSM of anise seed EW extracts:

In order to optimize the antioxidant ability of the extracts of anise seed, RSM was conducted with a central composite rotatable design (CCD) based on three variables, and three levels were generated. Based on the single-factor experiments, three principal factors (concentration of ethanol, ultrasonication extracting time, and sample-to-solvent ratio). Twenty experimental runs and the recovered total phenolics, and flavonoid compounds and their respective antioxidant activities from the anise seed (*Pimpinella anisum* L.) obtained are illustrated in (Appendix-Table 11). 2^k factorial design which is an orthogonal design was applied to fit the multiple linear regression model. Experimental runs were randomized to avoid the effects of extraneous factors which might present. The model fixations for all responses were performed by the quadratic model function. The influence of each factor on the response was investigated by holding the other process variables constant. Response surface 3D graphs were generated for each response.

4.3.2.2 Fitting the model

The regression coefficients (β) of the second-order polynomial model and the analysis of variance (ANOVA) results are summarized in (Appendix-Table 12). According to the low p-values (< 0.0001), the model obtained was statistically significant. Besides, the determination coefficient value (R^2) was (0.95 ,0.94) for TPC and TFC and (0.95, 0.90, 0.90) for FRAP, DPPH, and ABTS as antioxidant activity assays, which implied a strong correlation between the predicted results and the actual results. The obtained regression coefficients demonstrated a linear and quadratic effect of solvent concentration (A), and extraction time (B) on all the responses, while sample-to-solvent ratio (C) has only a linear significant effect for all response. The interaction effect of the ethanol concentration and extraction time (AB) 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 (Appendix-Table 12). The predicted values and actual values which can be correlated by the coded and actual equations built by the model were depicted in (Appendix-Fig. 6). The results indicated a good correlation between experimental and predicted data.

4.3.2.3 Effect of extraction variables on total phenolics content (TPC)

All the studied variables had a significant effect on the of TPC, which ranged from (17.9 to 43.26 mg GAE/g dw). The highest TPC in the anise seed extracts was obtained using (10 % v/v) ethanol concentration and (12 g/100 mL) sample-to-solvent ratio after 10 min of the extraction process. While the lowest content was found at the low level of the extraction variables. According to (β) values of regression coefficients (Appendix-Table 12), The linear term of the extraction time (B) had a highly positive effect on TPC, and the quadratic term (B^2) had a negative significant effect ($p < 0.0001$) on it. Likewise, the linear term of ethanol concentration has a high positive effect ($p < 0.0001$), and the quadratic term has a negative effect ($p < 0.01$). At the same time, the sample-to-solvent ratio has a high positive effect with ($p < 0.0001$). All other effects are insignificant were removed and the fitted second-order polynomial equation showed as:

$$TPC = -6.42 + 1.026 \cdot A + 6.17 \cdot B + 0.62 \cdot C - 0.036 \cdot A^2 - 0.27 \cdot B^2 \qquad (44)$$

where A – ethanol concentration (% v/v) in the range (0 – 20 % v/v) , B – extraction time (min) in the range (5 – 15 min), C – sample-to-solvent ratio (g/100 mL) in the range (2 – 12 g/100 mL).

Analysis of variance for predicted models implied that the model was highly significant ($p < 0.0001$) with a good coefficient of determination ($R^2 = 0.95$). In addition, lack-of-fit (F-value 2.71, $p > 0.05$) was not significant. The 3D plot shows that the TPC slightly increased as increasing the ethanol concentration from level (-1) to level (1). While TPC increased during the first 11 min (levels (-1) and (0)) of the extraction process and slightly started to decrease as the time extraction increased at level (+1) as a result of decomposition of phenolics cmpounds and the loss of solvent by vaporization as was mentioned before. The increasing the sample-to-solvent ratio up to 8 g/100 mL enhanced the TPC due to increased surface area, thereby enhancing the mass transfer rate until the dissolution process reaches its equilibrium state (Fig. 44).

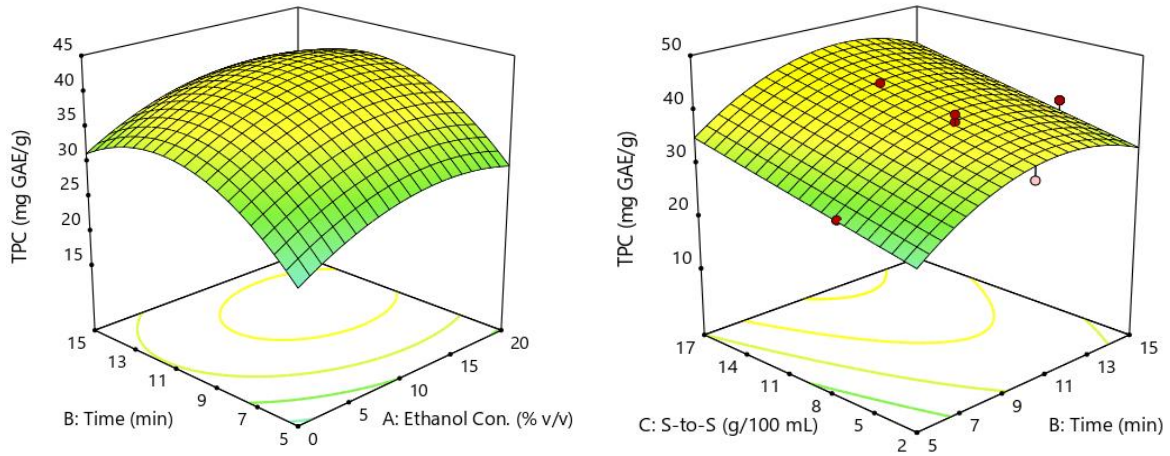


Fig 44. 3D response surface of TPC influenced by individual factors in the EW and UAE extracts of anise seed

4.3.2.4 Effect of extraction variables on total flavonoids content (TFC)

The extraction of TFC was significantly influenced by all the studied variables and regard (6.27 to 16.24 mg QUE/g dw). Like TPC The highest TFC in the anise seed extracts was obtained using (10 % v/v) ethanol concentration and (12 g/100 mL) sample-to-solvent ratio after 10 min of the extraction process. While the lowest content was found at the lowest level of the extraction variables. (Appendix-Table 12) of regression coefficients (β) values shows that the linear term of the extraction time (B) had a highly positive significant ($p < 0.0001$) effect on TFC, while the quadratic term (B^2) has a negative significant effect ($p < 0.0001$) on it. Likewise was the effect of the ethanol concentration where the linear term had a positive effect (A) and the quadratic term (A^2) had a negative significant effect with ($p < 0.05$). Additionally, the sample-to-solvent ratio is highly positive ($p < 0.0001$), insignificant variables were removed the model obtained for the extraction of TFC and the modle was as follows:

$$TFC = -1.51 + 0.32 \cdot A + 2.097 \cdot B + 0.28 \cdot C - 0.0109 \cdot A^2 - 0.0905 \cdot B^2 \quad (45)$$

where A – ethanol concentration (% v/v) in the range (0 – 20 % v/v) , B – extraction time (min) in the range (5 – 15 min), C – sample-to-solvent ratio (g/100 mL) in the range (2 – 12 g/100 mL).

Analysis of variance for predicted models implied that the model was highly significant ($p < 0.0001$) with a good coefficient of determination ($R^2 = 0.94$). In addition, lack-of-fit (F-value 0.98, $p > 0.05$) was not significant. As shown in Fig. 45, the TFC increased with increasing ethanol concentration from level (-1) to level (1). In addition, TFC keeps increasing during the first 13 min of the extraction process before the phenolic compounds began to decompose as result of the heating.

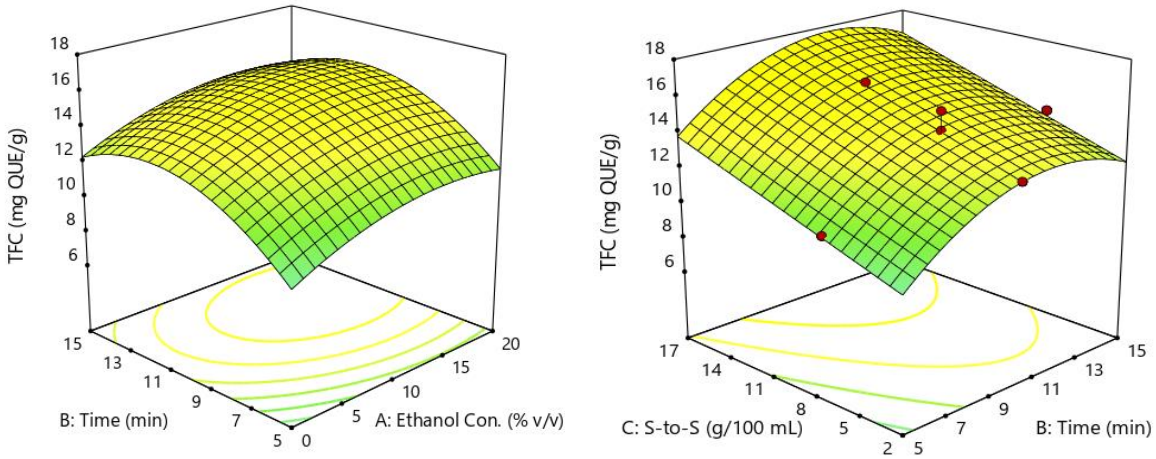


Fig 45. 3D response surface of TFC influenced by individual factors in the EW and UAE extracts of anise seed

4.3.2.5 Effect of extraction variables on antioxidant activity (AA)

AA was determined using FRAP, DPPH, and ABTS radical scavenging assay, FRAP values ranged between (3.81 – 8.62 mg AAE/g dw), DPPH (5.76 -14.98 %). and ABTS (1.01 – 2.46 %). The lowest antioxidant activity with the three assays was observed on the lower level of studied variables, the highest value was at ethanol concentration (10 % v/v), sample-to-solvent

ratio (12 g/100 mL), and after 10 min of the extraction time for FRAP and ABTS, while it found the higher level of the studied variables (Appendix-Table 11).

According to the regression coefficients (β), the antioxidant activity (three assays) was significantly influenced by the positive linear effect of extraction time (B) ($p < 0.0001$) for FRAP and DPPH and ($p < 0.001$) for ABTS, while its quadratic term has the higher negative effect on FRAP ($p < 0.0001$) followed by ABTS ($p < 0.001$) then DPPH ($p < 0.05$). And ethanol concentration (A) shows a linear and quadratic significant effect on FRAP, DPPH, and ABTS. Also, the sample-to-solvent ratio has a higher effect on FARP ($p < 0.0001$) compared to its effect on DPPH and ABTS ($p < 0.01$, and $p < 0.001$). In addition, the interaction of the ethanol concentration and extraction time (AB) was found to be significant only on DPPH ($p < 0.05$). The second-order polynomial model that predicts the antioxidant activity is presented with the following equations after removing the non-significant variables:

$$FRAP = -1.19 + 0.18 \cdot A + 1.24 \cdot B + 0.11 \cdot C - 0.0065 \cdot A^2 - 0.055 \cdot B^2 \quad (46)$$

$$DPPH = +1.97 + 0.18 \cdot \mathrm{A} + 1.093 \cdot \mathrm{B} + 0.22 \cdot \mathrm{C} + 0.018 \cdot \mathrm{AB} - 0.013 \cdot A^2 - 0.046 \cdot B^2 \quad (47)$$

$$ABTS = -0.29 + 0.046 \cdot A + 0.31 \cdot B + 0.038 \cdot C - 0.0015 \cdot A^2 - 0.013 \cdot B^2 \quad (48)$$

where A – ethanol concentration (% v/v) in the range (0 – 20 % v/v) , B – extraction time (min) in the range (5 – 15 min), C – sample-to-solvent ratio (g/100 mL) in the range (2 – 12 g/100 mL).

Analysis of variance for predicted models implied that the model was highly significant ($p < 0.0001$) with a good coefficient of determinations (R^2 = 0.95, 0.90, and 0.90) for FRAP, DPPH, and ABTS respectively. Moreover, lack-of-fit was not significant for all assays (Appendix-Table 12). The 3D response surfaces in (Fig. 46, Fig. 47, and Fig. 48) show the effect of all the studied variables on AA which is consistent with the changes in TPC and TFC during the extraction experiments.

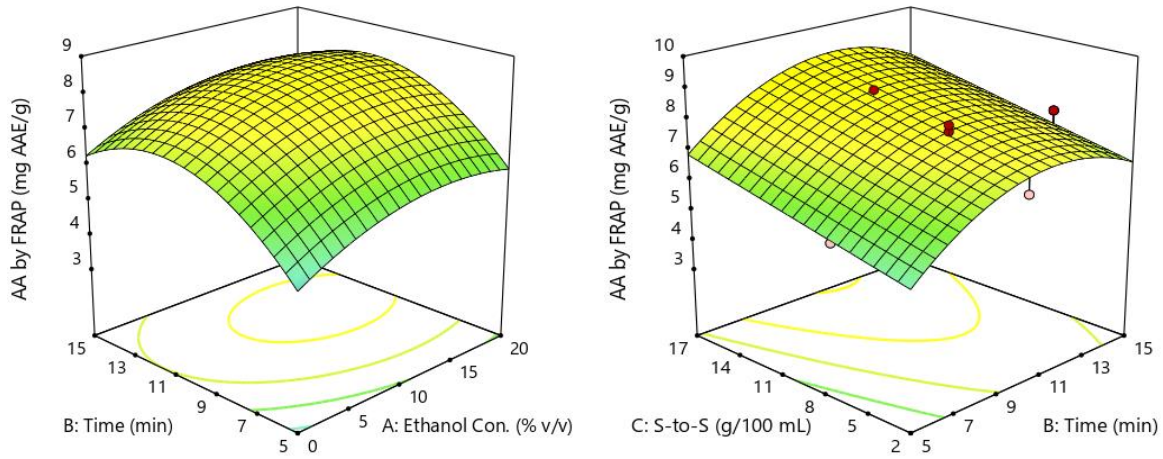


Fig 46. 3D response surface of FRAP influenced by individual factors in the EW and UAE extracts of anise seed

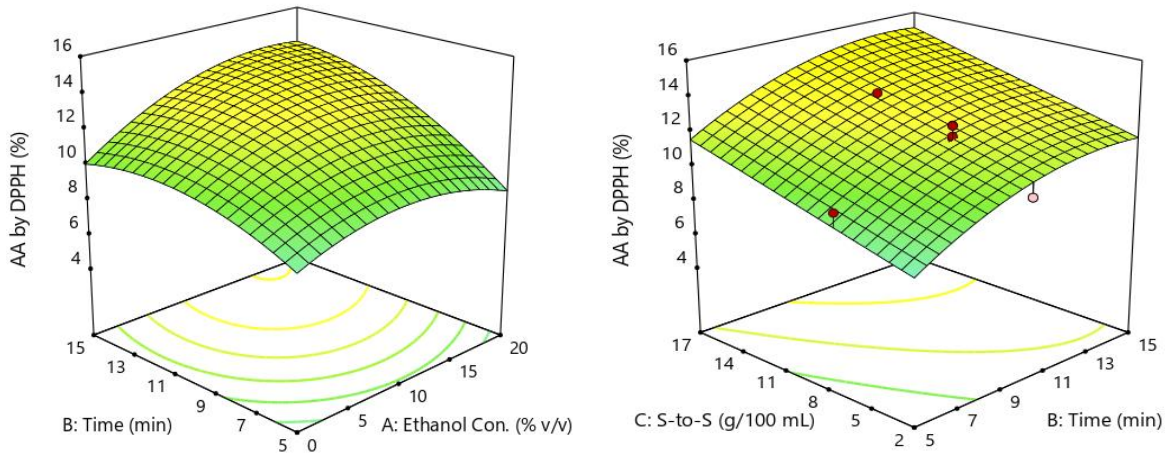


Fig 47. 3D response surface of DPPH influenced by individual factors in the EW and UAE extracts of anise seed

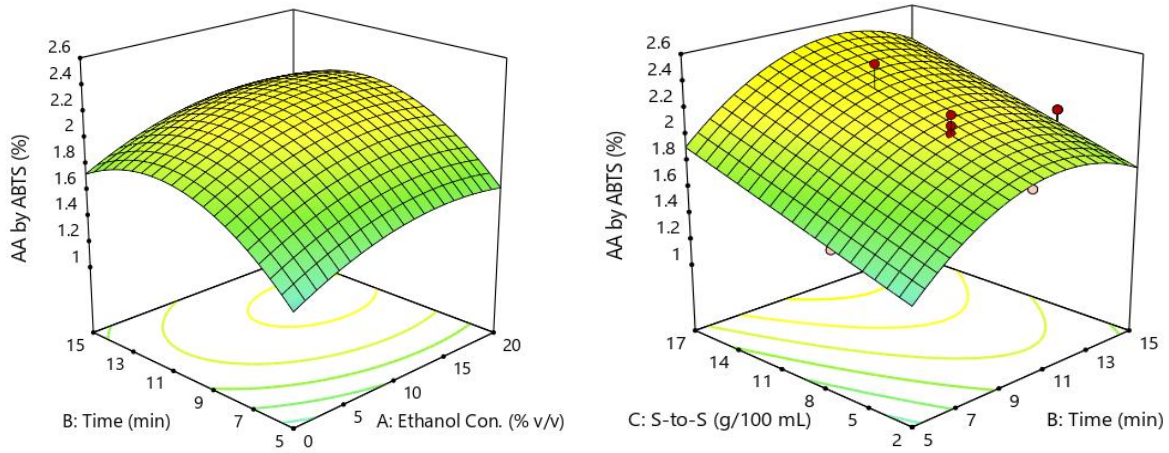


Fig 48. 3D response surface of ABTS influenced by individual factors in the EW and UAE extracts of anise seed

4.3.2.6 Determination and experimental validation of optimal conditions

To determine the optimal extraction parameters for anise seed using UAE as an extraction method, such as ethanol concentration, extraction time, and sample-to-solvent ratio. The desirability function approach (DFA) was applied using Design Expert Software Trial Version 11.0.3. The best level of various parameters was obtained at ethanol concentration (14.8 % v/v), extraction time (12 min), and sample-to-solvent ratio (12 g/100 mL) in the evaluated range, with an overall desirability value of 0.99 suggests that the experimental conditions achieved a near-ideal balance or performance across all desired responses (Fig. 49). Under these ideal conditions, the experimental values closely matched the predicted values, exhibiting a coefficient of variation C.V. % range from 4.58 to 7.78% (Appendix-Table 12).

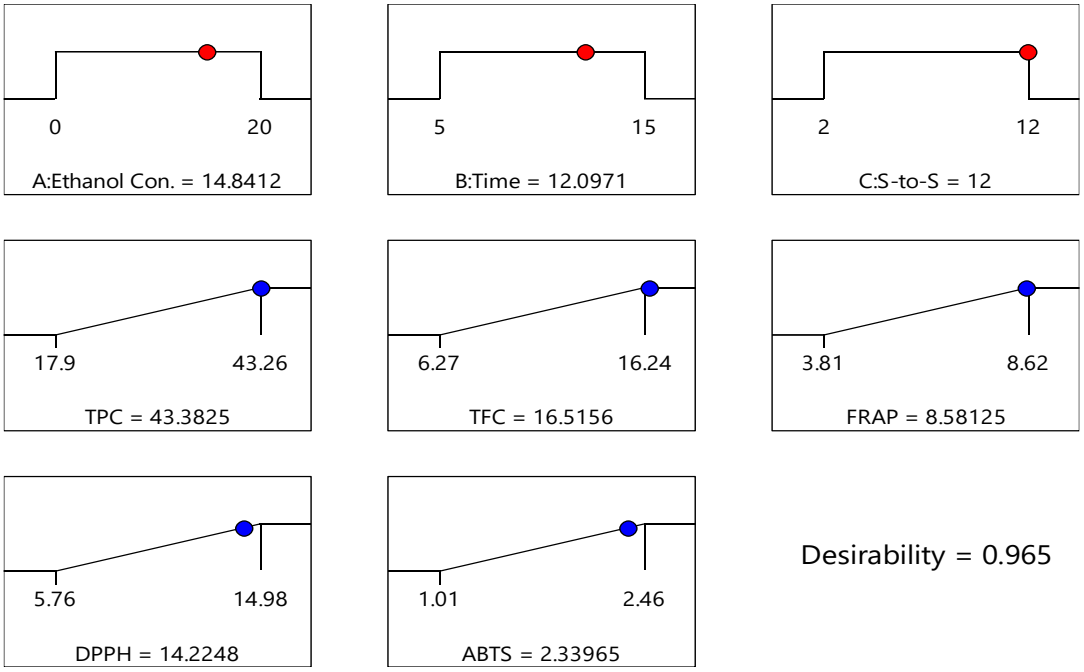


Fig 49. The desirability test based on the optimized values of targeted compounds in anise EW and UAE extracts

4.4 Comparison of extraction methods

Three extraction methods (HAE, MAE, and UAE) have been used to extract the bioactive compounds from both hawthorn fruit and anise seeds. A comparison was made between these methods to determine the optimal extraction methods and conditions for hawthorn fruit and anise seed.

4.4.1 Comparison of hawthorn fruit extraction methods

The extraction efficiency of various extraction methods UAE, MAE, and HAE for maximizing the recovery of bioactive compounds from hawthorn fruit was evaluated and results are presented in (Appendix-Tables 1, 5, and 9). These methods have been used for the extraction of phenolics and flavonoid compounds. Analysis of extracts obtained from different extraction methods was done by using chemical methods and a UV-VIS spectrophotometer. RSM has been used to maximize the extracts of TPC and TFC and the antioxidant activity in these extracts.

The models show that the highest TPC and TFC (95.78 ± 5.42 mg GAE/ g and 30.35 ± 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 % (v/v) compared to both other extraction methods, and reduced the extraction time by 90 % compared to HAE, also UAE was carried out at room temperatures. The extraction depends on the penetration and interaction of solvent with the plant materials, solubility, and diffusion of the compounds in the medium, and harvesting of the targeted solute. Different extraction methods exhibited a varied

degree of solubility and diffusivity that affected the total obtained bioactive compounds and their antioxidant activity.

4.4.2 Comparison of anise seed extraction methods

The extraction efficiency of various extraction methods UAE, MAE, and HAE for maximizing the recovery of bioactive compounds from anise seed was evaluated and results are presented in (Appendix-Tables 3, 7, and 11). These methods have been used for the extraction of phenolics and flavonoid compounds. Analysis of extracts obtained from different extraction methods was done by using chemical methods and a UV-VIS spectrophotometer. RSM has been used to maximize the extracts of TPC and TFC and the antioxidant activity in these extracts.

The models show that the highest TPC and TFC (49.9 ± 3.26 mg GAE/g dw and 20.86 ± 1.62 mg QUE/g 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 %.

It can be noticed that the effect of microwave power is the same on the yield of TPC and TFC from hawthorn fruit and anise seed. Although microwave extraction was the optimal method for extracting the bioactive compounds from anise, the decline starts early in the case of anise seed. The nature of the plant cells may cause this, as the plant cell walls tend to absorb microwave energy and cause an increase in internal temperature. As a result, phenolic compounds are leached out of the plant materials due to cell disruption. Additionally, some bioactive compounds form free radicals under the ultrasound frequency, which could be common in anise seed

4.5 Comparing several solvents for the extraction of phenolic compounds from anise seeds

The extraction of the powdered seed of anise (*Pimpinella anisum* L.) was carried out using seven solvents (absolute ethanol, absolute methanol, absolute isopropanol, ethanol (50 % v/v), methanol (50 % v/v), isopropanol (50 % v/v) and pure water) and the HAE extraction method as mentioned in section (3.2.1). All experiments were conducted three times independently and the data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Tukey's HSD tests were carried out to determine significant differences ($p \leq 0.05$) between the means by Statistical Product and Service Solutions Statistics (SPSS IBM version 27.0).

4.5.1 Phenolic and flavonoid content

Fig. 50 shows the total phenolic and flavonoid content (TPC), (TFC) of the seed extracts measured using Folin-Ciocalteu's colorimetric method. TPC ranged from 17.57 ± 0.65 mg GAE/g dw to 43.84 ± 0.39 mg GAE/g dw, while TFC ranged from 8.69 ± 0.85 mg QUE/g dw to 17.22 ± 0.82 mg QUE/g dw. 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 methanol 50 % v/v (M-50) extract followed by absolute methanol (M-100), while the

lowest amount of phenolics was in the absolute isopropanol (S-100) extract. There was no significant difference in TPC using ethanol 50 % v/v (E-50) and water, whereas there is a significant difference in TFC. Therefore, pure water will be used as a solvent for the next extraction processes from anise seed.

The results are consistent with those (Chung, 2009) who indicated that methanol extracts of star anise showed the highest polyphenol content followed by water and ethanol extracts. On the other hand, alcohol/water solutions showed a better influence on the extractability of phenolic compounds from grape marc and pomace in comparison to the mono-component solvents (Spigno et al., 2007; Pinelo et al., 2009).

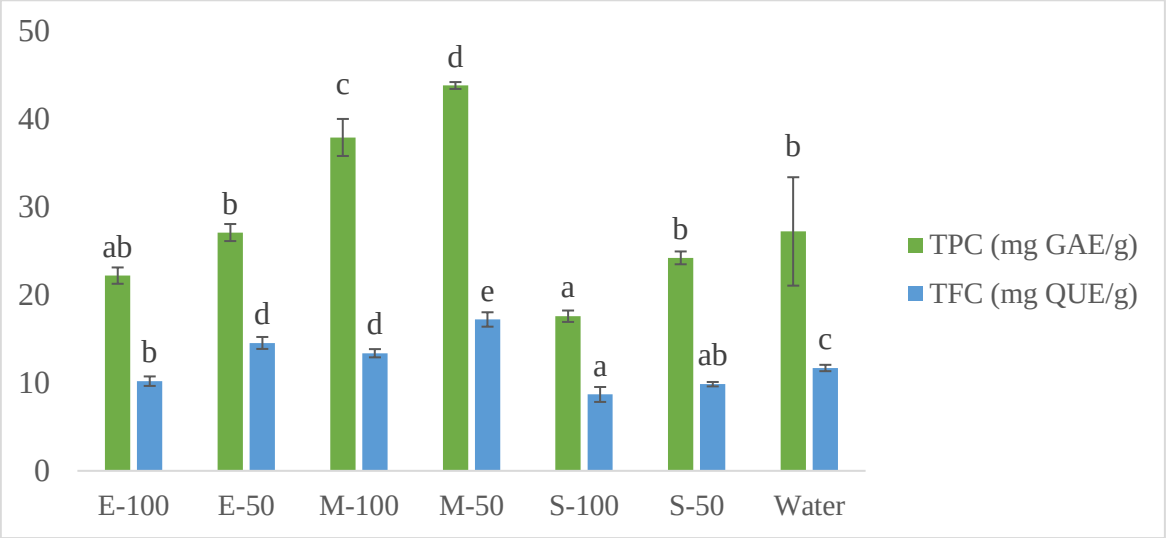


Fig 50. Total phenolics content and total flavonoid content of anise seed obtained with different solvents. (E-100) absolute ethanol, (E-50) ethanol 50 % (v/v), (M-100) absolute methanol, (M-50) methanol 50 % (v/v), (S-100) absolute isopropanol, (S-50) isopropanol 50 % (v/v)

a, b,… Values are means (n = 3) ± SD. Values with the same superscript letter are not statistically significant at the 95 % level

4.5.2 Antioxidant Activity

There are a huge variety of antioxidants contained in plants. Therefore, measuring the antioxidant capacity of each compound separately becomes very difficult. Several methods have been developed to estimate the antioxidant capacity of different plant materials. Usually, those methods measure the ability of antioxidants, in a particular plant material, to scavenge specific radicals, by inhibiting lipid peroxidation or chelating metal ions. For anise seed extracts, two different methods have been used to evaluate the antioxidant capacity of the extracts, they are ferric reducing/antioxidant power assay (FRAP assay) and DPPH free radical-scavenging assay.

As shown in Fig. 51, the percentage of inhibition of extracts of anise using absolute methanolic (M-100) and methanolic 50 % v/v (M-50) was slightly higher ($p < 0.05$) than the extracts that were obtained by other solvents by all AA (FRAP, DPPH) assays. In addition, there were no significant differences in the antioxidant capacity of aqueous and ethanolic extracts using the FRAP assay, while there were significant differences using DPPH where aqueous extracts outperformed the ethanolic extracts.

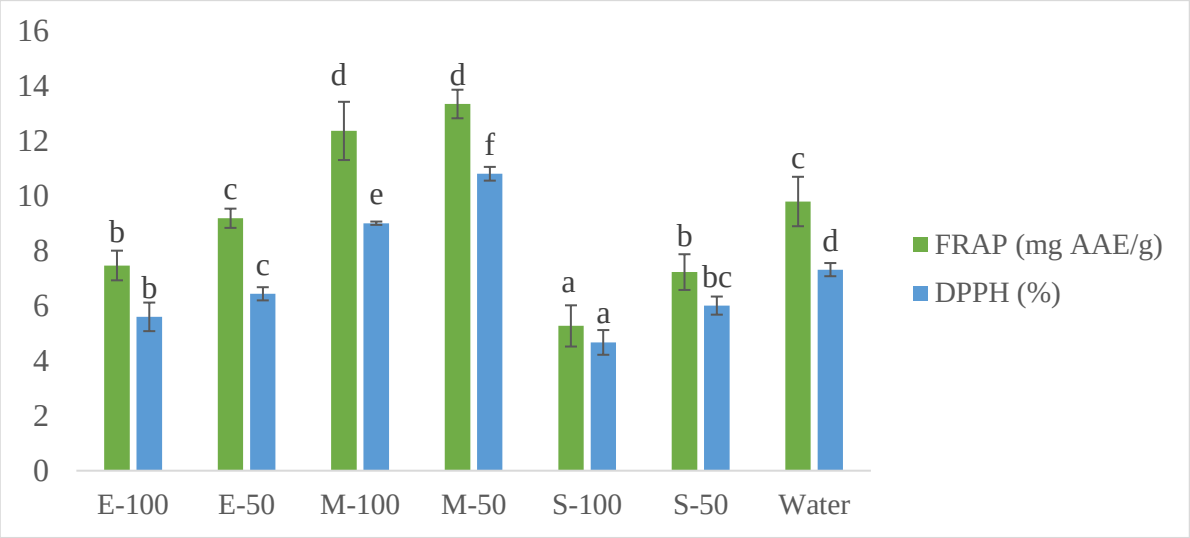


Fig 51. Antioxidant capacity of anise seed extracts obtained with different solvents. (E-100) absolute ethanol, (E-50) ethanol 50 % (v/v), (M-100) absolute methanol, (M-50) methanol 50 % (v/v), (S-100) absolute isopropanol, (S-50) isopropanol 50 % (v/v).

a, b,… Values are means (n = 3) ± SD. Values with the same superscript letter are not statistically significant at the 95 % level

4.5.3 Correlation between TPC, TFC, and different AA assays

The correlation between the bioactive compounds and antioxidant activity was also investigated in anise seed obtained extracts by several solvents. The 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].

4.6 Comparison of three different species of hawthorn fruit

The extraction of phenolic compounds process was performed from the three species of hawthorn fruit (*C. monogyna* Jacq., *C. pinnatifida* Bge, and *C. crus-galli* L.) as mentioned in section (3.2.2). To test the difference between the species, each measurement of total phenolic content, total flavonoid content, and antioxidant activity was repeated three times. Data sets were tested for normal distribution using the Shapiro-Wilk test of normality, and Levene's test was employed to examine the homogeneity of variances. MANOVA was conducted to evaluate significant differences between the mean values of studied species, and a bivariate correlation test was used to test the relation between antioxidant activity and each of the total content of phenolic and flavonoid using the statistical package (SPSS 27) (IBM, Armonk, USA).

4.6.1 Total phenolic compounds, total flavonoids and antioxidant activity

The mean values of the total content of phenols and flavonoids and antioxidant activity in extracts of hawthorn species are presented in Table 23. It can be seen that 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 and total flavonoids ranged from 11.85 ± 0.41 to 32.67 ± 0.42 mg QUE/g dw.

Alirezalu et al. (2020) reported that the total phenol content of several hawthorn species (*Crataegus* spp.) ranged from 21.19 to 65.06 mg GAE/g dw and the total flavonoid content ranged from 2.44 to 6.08 mg QUE/g dw, with antioxidant activity of 0.32 – 1.84 mmol Fe^{++}/g. Çalişkan et al. (2012) reported that hawthorn (*Crataegus* spp.) collected from the eastern Mediterranean region of Turkey had a total phenolic content ranging from 26.6 to 57.1 mg GAE/g dw and antioxidant activity of 42.7 to 82.9 mg ASE/g dw. For species (*Crataegus monogyna* Jacq.), the ethanol extract had a TPC of 101.01 mg GAE/g dw and a TFC of 48.27 ± 0.26 mg RU/g dw (Dekić et al., 2020). On the other hand, TPC contained approximately 45 mg GAE/g dw and TFC contained 62 mg RE/g dw in the ethanol extract of *pinnatifida* Bge. (Zhang et al., 2020). In comparison, our results are consistent with other similar studies, and the difference can be due to the different applied extraction conditions.

Table 23. Total phenolic, flavonoid compounds and antioxidant activity of EW extracts

Species	TPC (mg GAE/g) dw	TFC (mg QUE/g) dw	FRAP (mg AAE/g dw)
C. monogyna Jacq.	54.66 ± 0.62	11.85 ± 0.41	76.67 ± 0.14
C. pinnatifida Bge	76.33 ± 0.40	20.83 ± 0.17	96.34 ± 0.06
C. crus-galli L.	86.83 ± 0.34	32.67± 0.42	99.83 ± 0.04

According to the results of MANOVA, there is a significant difference between the three species of hawthorn at the 95 % confidence interval (Table 24). 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. These results indicate that the phenolic compounds could be the main contributor to the antioxidant properties of these shrubs.

Table 24. Multivariate analysis of variance (MANOVA) results of antioxidant activity and the total phenolics and flavonoids of extracts

Source of variation	Effect	Sum of Squares	df	Mean Square	F	Sig.
Species	TPC	1625.04	2	812.522	3629.67	0.000*
	TFC	650.08	2	325.041	2556.25	0.000*
	Antioxidant activity	936.03	2	468.017	55716.33	0.000*

*Significant at the 0.05

4.7 Comparison of anthocyanin extraction methods and solvents from hawthorn fruit

The anthocyanins extraction process from hawthorn fruit was performed using three methods: ultrasound-, microwave-, and heat-assisted extraction together with three solvents (methanol, ethanol, and isopropanol) as mentioned in section (3.6). All experiments were conducted three times independently and the data were expressed as mean ± standard deviation (SD). Two-way analysis of variance (ANOVA) and Tukey's HSD tests were carried out to determine significant differences ($p \leq 0.05$) between the means by Statistical Product and Service Solutions statistics (SPSS IBM version 27.0).

4.7.1 Effect of extraction methods and applied solvent types on TMA content

As plotted in Fig. 52, both the extraction methods and solvent have significant effects on the total anthocyanins content. 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.

Similar results were discussed by other studies such as; UAE with 1:30 solvent to liquid ratio was an effective method of extraction from saffron bio-residues with advantages like lower extraction time and higher extraction yields compared to conventional solid-liquid extraction (CSLE) and MAE (Da Porto and Natolino, 2018). Furthermore, a significant difference ($p < 0.0001$) was observed in the anthocyanin content of Australian blueberry among the three extraction methods of UAE, the Geno grinder, and the Dounce tissue grinder. In which, the UAE produced the highest yield of anthocyanins. In addition, the anthocyanins concentration using UAE to extract blood fruit mounted up by 6.19 % to 10.28 % as compared to that of conventional extraction (CE) (Sasikumar et al., 2021).

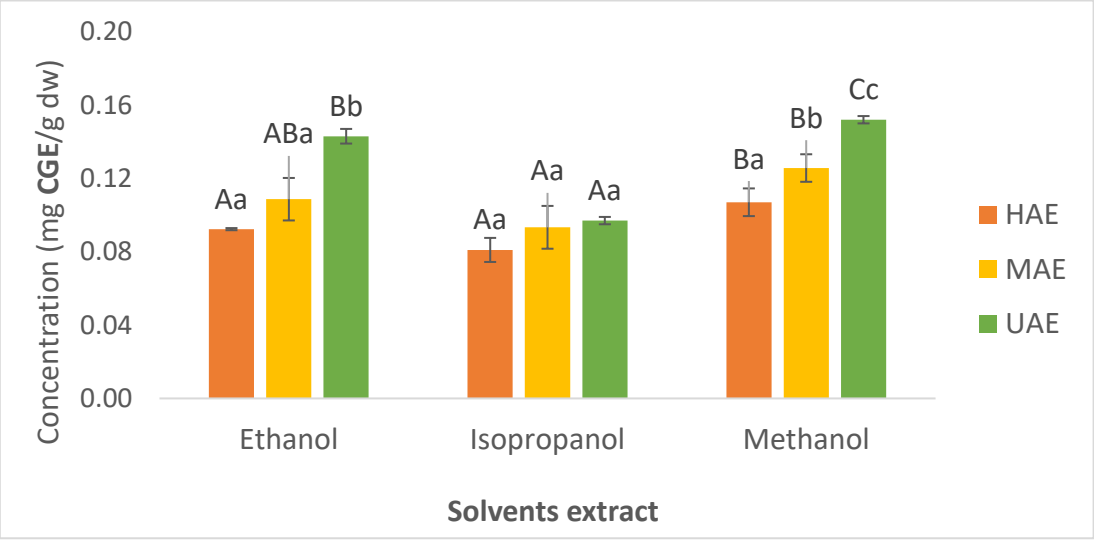


Fig 52. TMA of the extracts expressed in mg CGE/g dw obtained with different extraction methods and solvents

Upper cases for e.g. A, B, C… = significant differences between solvent with each extraction method

Lower cases for e.g. a, b, c… = significant differences between extraction methods with each solvent

4.7.2 Effect of extraction methods and applied solvents on the color values

The effect of extraction methods and solvents on the colour attributes, namely, L*, a*, and b* for hawthorn fruit extracts, was measured with the CIE method. 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 (Fig 53).

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), (Fig. 54) shows the color differences between the samples. These results are accommodated with Sasikumar and co-workers (2021) who claimed that the blood fruit extracts prepared with the ultrasonic method with various solvents were characterized with darker color compared with that of CE with the same solvents. Likewise, Sharma et al. (2021) reported a significant difference ($p < 0.05$) observed in all coloring attributes (L*, a*, b*, and ΔE*) in all the pumpkin (peel and pulp) extracts obtained from green extraction (ultrasonic and microwave-assisted extractions using corn oil) and conventional extraction. Similar results were stated by Nguyen and Pirak (2019) for UAE in contrast to conventional extraction (CE) of white dragon fruit peel.

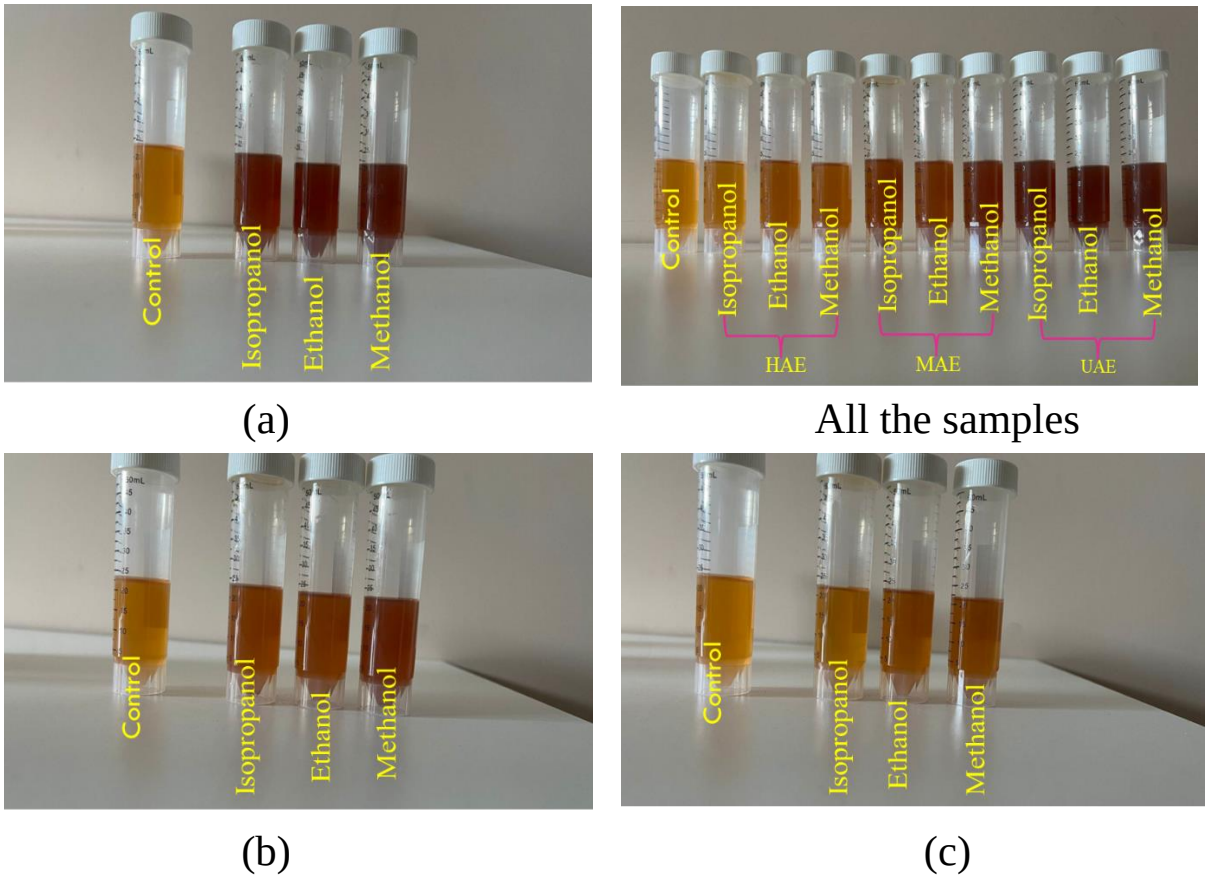
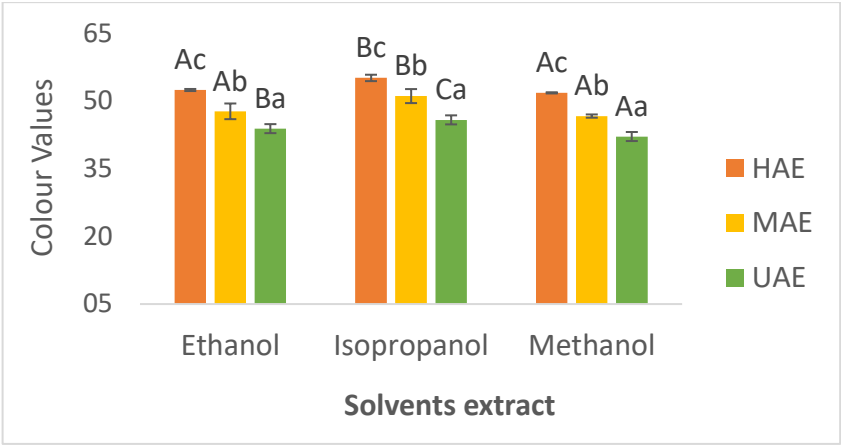
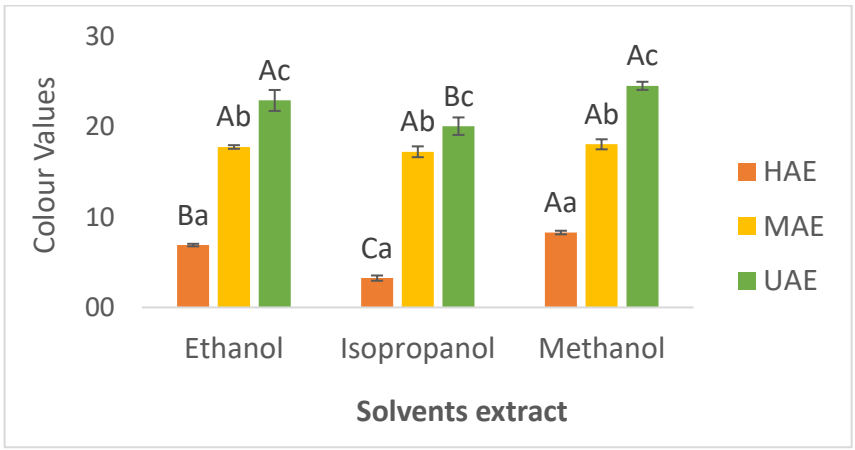


Fig 54. Hawthorn extracts by different extraction methods and solvents (a: UAE, b: MAE, c: HAE). The first sample from the left is the control sample followed by isopropanol, ethanol, and methanol samples

(a)



(b)



(c)

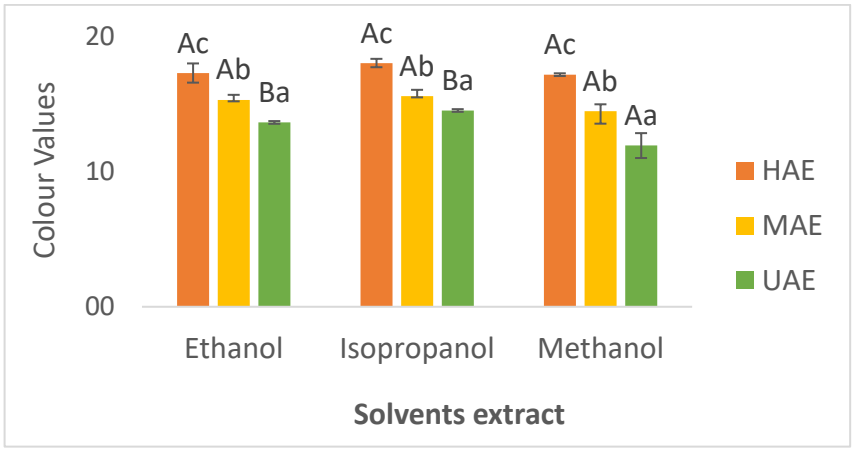


Fig 53. Comparison of color values of hawthorn extract obtained with different extraction methods and solvents; (a) L *, (b) a*, (c) b*

Upper cases for e.g. A, B, C… = significant differences between solvent with each extraction method.

Lower cases for e.g. a, b, c…= significant differences between extraction methods with each solvent.

4.7.3 Effect of extraction methods and applied solvent types on TPC and TFC

The combined effects of different extraction techniques and applied solvent types on extracted TPC and TFC were depicted in Fig. 55. As can be seen in the figure, 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. It can be due to the cavitation effect and strong shear forces produced by ultrasound which increases the efficiency of the extraction process by providing better mass transfer, increasing the permeability of the plant tissue, releasing the intracellular material, and improving analytes solubility and solvent penetration (Altemimi et al., 2015). The effect of different solvents can be attributable to the higher solubility of these compounds in methanol than the other solvents tested because the yield of extraction depends on the varying polarity of the solvents and the nature of the bioactive compounds in each plant (Do et al., 2014). For example, the results revealed that methanol exhibited the optimal solvent to extract the bioactive components from *S. buxifolia* branches ($p < 0.001$) since the highest content of phenolics (13.36 mg GAE/g dw), flavonoids (1.92 mg QE/g dw), alkaloids (1.40 mg AE/g dw), and terpenoids (1.25 % w/w) were obtained by using this solvent (Truong et al., 2019). On the other hand, (Do et al, 2014) declared that the best solvent for bioactive compounds extraction from *Limnophila* aromatic was ethanol compared to methanol and aqueous acetone. In this case, either extraction techniques play a bigger role than the types of applied solvent or the combinatorial effects of emerging techniques.

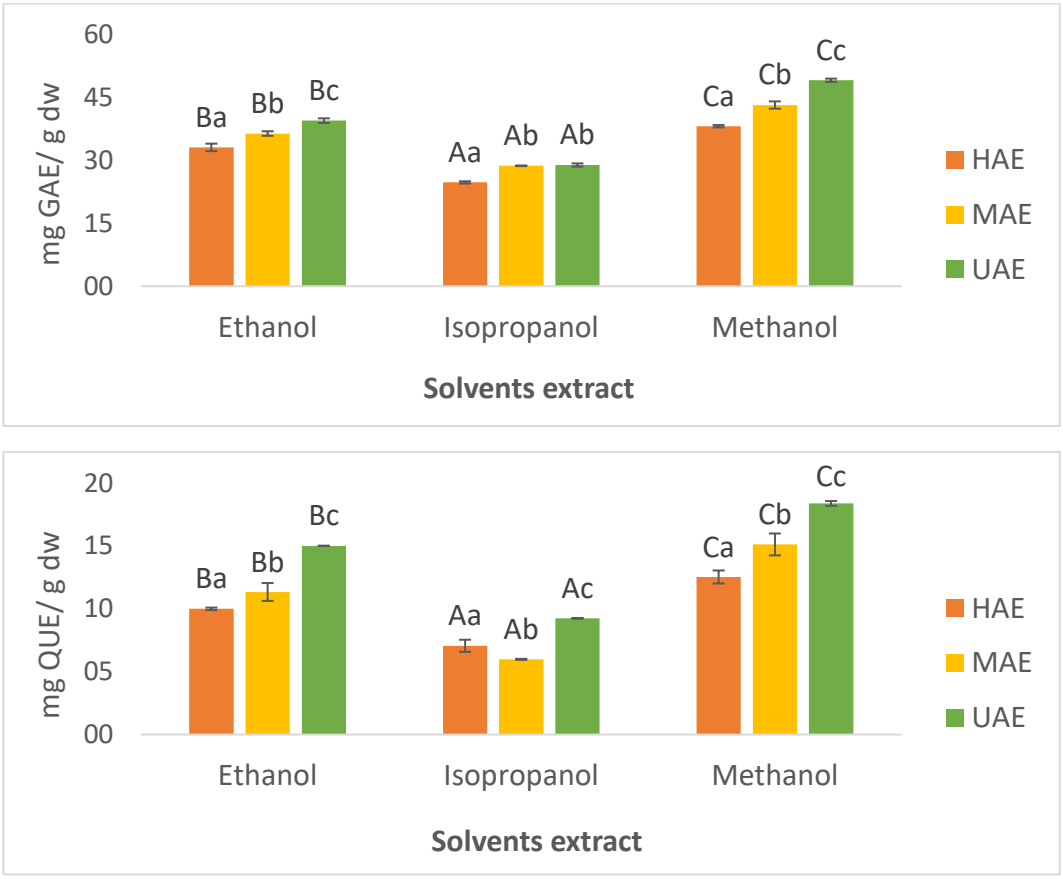


Fig 55. Total phenolic content and total flavonoids content of hawthorn extract using different extraction methods and solvents, TPC (a), TFC (b)

Upper cases for e.g. A, B, C… = significant differences between solvent with each extraction method.

Lower cases for e.g. a, b, c…= significant differences between extraction methods with each solvent.

4.7.4 Effect of extraction methods and applied solvent types on AA

As listed in Table 25, 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. This could be due to the fact that during UAE, the generation and the collapse of cavitation bubbles enhance the extraction process (Da Porto et al., 2013). AA values of the fruit extracts by UAE with methanol solvent were higher than MAE extracts, followed by HAE as shown in Table 25, which can be ascribed to the polarity of methanol which can disrupt cell walls and cause degradation resulting in the release of phenolic compounds. There is a positive correlation between the concentration of phenolic compounds and their antioxidant activity which explains the results. According to the former researchers, the antioxidant capacity of methanol extract was observed to be outweighed over other solvents of red currant, black currant, and grape extract (Lapornik et al., 2005). Grape seed extracts obtained from seeds defatted by ultrasound (US) resulted the highest in polyphenol concentration (105.20 mg GAE/g flour) and antioxidant activity (109 EqαToc/g flour) compared to soxhlet extraction (Da Porto et al., 2013). The antioxidant capacity of gac peel extract obtained by the UAE was also significantly higher than of the conventional extraction using the same ratio of solvent to material (Chuyen et al., 2018).

Table 25. Values of FRAP, DPPH, and ABTS resultant from hawthorn extracts

AA	Extraction methods	Methanol	Ethanol	Isopropanol
FRAP (mg AAE/g dw)	HAE	162.32 ± 0.93 ^{Ca}	155.97±0.35 ^{Ba}	132.14 ± 0.82 ^{Aa}
	MAE	240.13± 0.82 ^{Cb}	234.22±0.24 ^{Bb}	211.71 ± 0.65 ^{Ab}
	UAE	250.24±1.46 ^{Cc}	242.21±0.54 ^{Bc}	220.53 ± 0.52 ^{Ac}
DPPH (%)	HAE	130.05±1.0 ^{Ca}	122.01±1.64 ^{Ba}	98.22 ± 0.45 ^{Aa}
	MAE	153.42±0.95 ^{Cb}	135.3±0.5 ^{Bb}	115.07±1.24 ^{Ab}
	UAE	157.32±0.39 ^{Bc}	156.22±1.53 ^{Bc}	123.38± 1.43 ^{Ac}
ABTS (%)	HAE	151.46±0.9 ^{Ca}	143.29±1.42 ^{Ba}	123.43±0.69 ^{Aa}
	MAE	183.33±1.17 ^{Cb}	171.06±1.09 ^{Bb}	134.44±0.88 ^{Ab}
	UAE	200.28±0.39 ^{Cc}	182.4±0.9 ^{Bc}	141.16±1.21 ^{Ac}

Upper cases for e.g. A, B, C… = significant differences between solvent with each extraction method.

Lower cases for e.g. a, b, c… = significant differences between extraction methods with each solvent.

4.7.5 Correlation between TPC, TFC, and different AA assays

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: $r = 0.595$]. It can be due to the differences in the principles of the AA assays where the FRAP assay depends on the reduction of a ferric tripyridyl-triazine Fe $(TPTZ)^2$ (III) complex to the ferrous tripyridyl-triazine Fe $(TPTZ)^2$(II) by an antioxidant at a low pH of 3.6. The FRAP assay measures the reducing capability based on ferric ions, which is not relevant to antioxidant activity mechanistically and

physiologically, and let alone the total antioxidant capacity. On the basis of these facts, one should be aware of selecting a method to estimate antioxidant activity and use more than one to have a complete idea (Ou et al., 2002). It has been previously reported that antioxidant capacity determined by in vitro assays differs. Ou and coworkers (2002), analyzed different types of vegetables using FRAP and the oxygen radical uptake capacity (ORAC) assay, and did not find agreement among the analyzed vegetables. As well as, differences were observed between the two radical scavenging assays (DPPH and ABTS) (Wootton-Bearda et al., 2010).

4.8 Membranes evaluation in concentrating hawthorn fruit and anise seed extracts

Based on our previous experiment which determined the optimal conditions to extract both hawthorn fruit and anise seed, 3 liters of extracts were prepared for every subsequent concentration process from both hawthorn and anise. The extraction was conducted by a single batch type extractor which was designed with a thermostat water bath (Lauda Ecoline E100 Immersion) and (OS20-S Electric LED) stirrer. For hawthorn, the extraction conditions were 55 °C, with 56 % v/v ethanol solvent (10 g fruit in 100 mL solvent) for 80 min. For anise seed, the extractions were completed using pure water as solvent at 37 °C for 100 min as was mentioned in the material and methods section (3.7). 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 applied. Cross-flow filtration process was performed by DDS Filtration Equipment (LAB 20-0.72, Denmark) connected to a SPECK type NP10/15 -104 high pressure pump. 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, as mentioned in section (3.7). All experiments were conducted three times independently and the data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Tukey's HSD tests were carried out to determine significant differences ($p < 0.05$) between the means by Statistical Product and Service Solutions statistics (SPSS IBM version 27.0).

4.8.1 Total phenolic compounds and flavonoids (TPC, TFC)

Quantification of the total content of phenolic and flavonoid compounds, and antioxidant activity in initial feeds, retentates, and permeates were conducted by spectrophotometric analysis. The results in each sample for every membrane are shown in Tables 26 and 27.

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. As can note in Fig. 56 and Fig. 57 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: 34.74 ± 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. It is due to the loose/open pores of the NF membrane, which lead to quick passing of permeate and less rejection to the target bioactive compounds.

Significant differences ($p < 0.05$) between the concentrates from the different membranes can be observed in Table 26 and Table 27. As mentioned in the tables, 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.

Table 26. Total phenolic content (TPC), Total flavonoids content (TFC),and antioxidant activity (AA)) for the different fractions of streams using the selected membranes X-20, RO99, and NF 270 for anise seed extracts

		TPC mg GAE/g dw	TFC mg QUE/g dw	FRAP mg AAE/g dw	DPPH %
Initial		28.12 ± 1.93 ^{d}	7.56±4.68 ^{d}	8.15± 0.44 ^{d}	5.70 ± 0.4 ^{c}
Retentate	X20	64.31 ± 1.81 ^{a}	20.93 ±1.93 ^{a}	16.51 ±0.45 ^{a}	9.21 ±1.11 ^{a}
	RO99	48.71 ±3.78 ^{b}	15.72 ± 0.46 ^{b}	13.24 ±2.46 ^{b}	9.03 ± 1.98 ^{a}
	NF270	34.74 ±1.67 ^{c}	10.45 ± 1.23 ^{c}	10.81± 0.56 ^{c}	6 ± 0.87 ^{b}
Permeates	X20	0.04 ±0.45 ^{g}	0.05±0.93 ^{f}	0.3 ±0.99 ^{f}	0.14±0.76 ^{e}
	RO99	0.97±0.47 ^{f}	0.67 ±0.76 ^{f}	0.21 ± 0.7 ^{f}	0.21 ±0.73 ^{e}
	NF270	4.2±0.53 ^{e}	1.7 ± 0.88 ^{e}	1.22 ±0.33^{e}	1.10 ±0.65 ^{e}

Different letters indicate significant differences between the Initial extract, final retentate, and permeates at $p < 0.05$. Mean values with the same superscript letters are similar and no significant differences were observed between these samples.

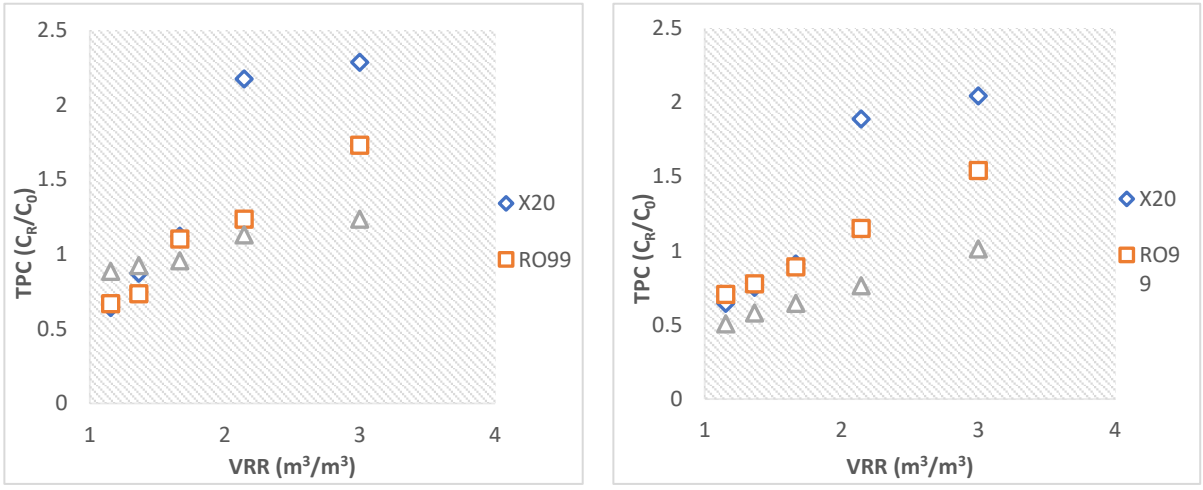
Table 27. Total phenolic content (TPC), Total flavonoids content (TFC),and antioxidant activity (AA)) for the different fractions of streams using the selected membranes X-20, RO99, and NF 270 for hawthorn fruit extracts

		TPC mg GAE/g dw	TFC mg QUE/g dw	FRAP mg AAE/g dw	DPPH %
Initial		45.31 ± 1.93 ^{d}	18.38 ±0.41d	31.12 ±1.55 ^{d}	18.00±1.02 ^{b}
Retentate	X20	92.62± 0.45 ^{a}	48.19±1.58 ^{a}	75.98 ± 0.65 ^{a}	28.1 ± 0.92 ^{a}
	RO99	69.84 ±1.22 ^{b}	39.09±2.68 ^{b}	57.29 ± 3.87 ^{b}	27.92 ± 1.36 ^{a}
	NF270	45.92±2.99 ^{c}	19.65±1.13 ^{c}	26.17 ±1.45 ^{c}	19.3 ± 3.01^{c}
Permeates	X20	0.02 ± 0.56 ^{g}	0.17 ± 0.2 ^{g}	1.01±1.8 ^{g}	0.23 ± 0.32 ^{f}
	RO99	1 ± 0.43 ^{f}	0.81 ± 0.21 ^{f}	1.24± 0.4 ^{f}	0.53 ± 1.2 ^{e}
	NF270	4.5±0.6 ^{e}	3.57 ± 1.01 ^{e}	10.2 ± 1.4 ^{e}	3.45 ± 1.4 ^{d}

Different letters indicate significant differences between the Initial extract, final retentate, and permeates at $p < 0.05$. Mean values with the same superscript letters are similar and no significant differences were observed between these samples.

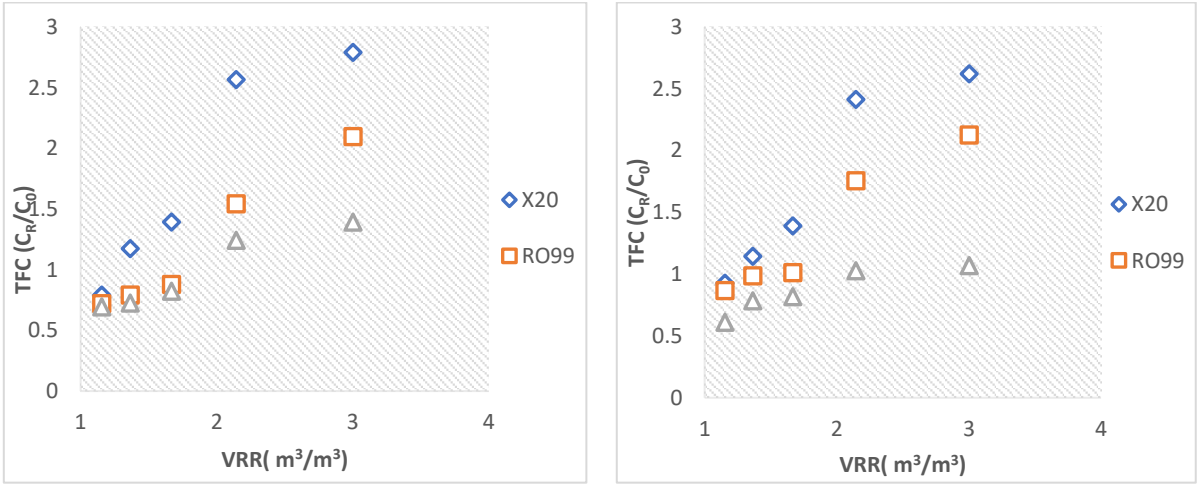
Our results are consistent with several studies which compared membrane efficiencies in the concentration of plant extracts. Nunes and co-authors (2019) revealed that the reverse osmosis

(BW30) membrane was the most effective for extracts concentration, and TPC and TFC of the concentrate from BW30 were significantly higher (around 15%) than those achieved with NF 270 and NF 90. Likewise, Li and co-authors (2010) reported a comparative study using nanofiltration and reverse osmosis membranes for phenol removal from synthetic wastewater. They employed three nanofiltrations (NF 90, NF 97, and NF 99) and two reverse osmosis (RO98pHt and RO99) membranes at phenol levels below 1000 ppm and it was pointed out that nanofiltration showed low rejection (0.41 – 0.72) with maximum flux 180 (L/(m^2·h)). Along the line, reverse osmosis recorded high rejection (0.81) with minimum flux 60 (L/(m^2·h)). Moreover, it was stated that at natural pH, rejection selectivity between phenolic solutes and dicarboxylic acids was higher for nanofiltration (NF-90) membrane compared to reverse osmosis (TFC-HR) membrane (López-Muñoz et al., 2010).



(a) (b)

Fig 56. Total phenolic compound content with VRR during concentration by NF 270 , RO99 and X-20 membranes, anise (a), hawthorn (b)



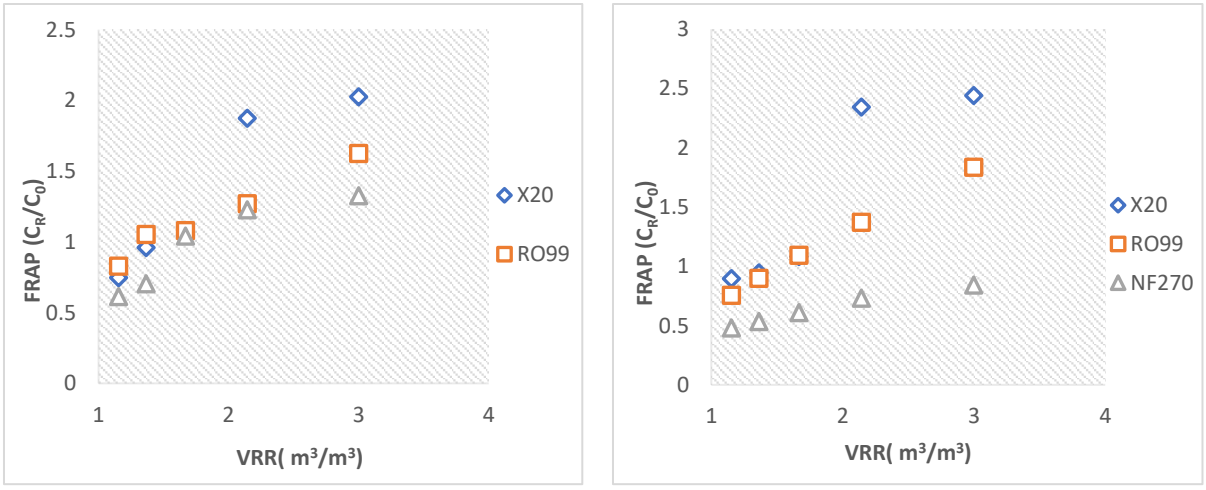
(a) (b)

Fig 57. Total flavonoids compound content with VRR during concentration by NF 270 , RO99 and X-20 membranes, anise (a), hawthorn (b)

4.8.2 Antioxidant activity

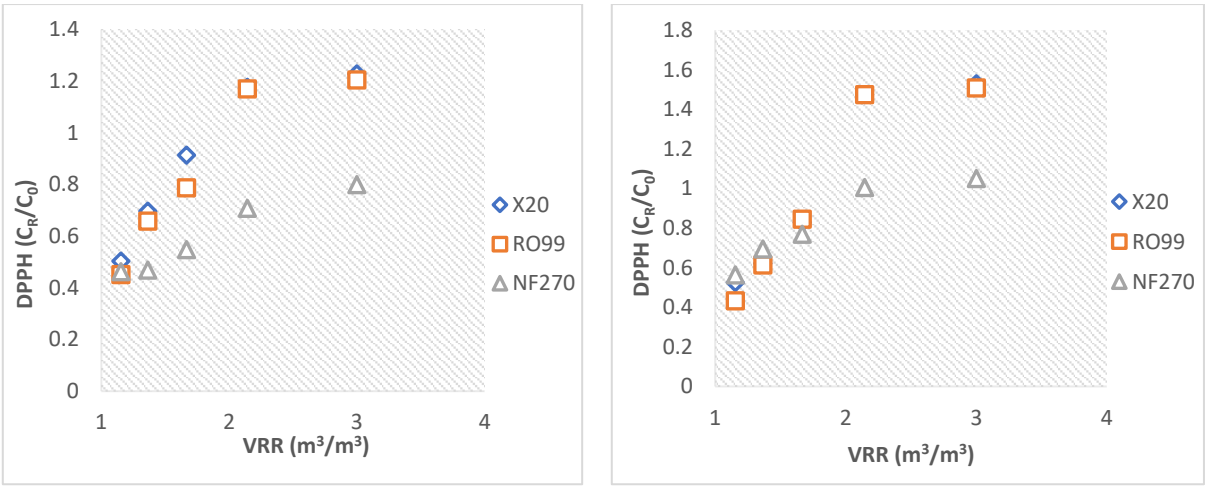
The amounts of antioxidants in the retentates imply that the membranes were quite effective in the concentration of different types of compounds that exhibit antioxidant properties. As plotted in Fig. 58 and Fig. 59, the trend of antioxidant activity is inclined to increase during the concentration processes. In which, the process using X-20 membrane 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. This is in line with the observed TPC and TFC that were retained with the different studied membranes.

On the other hand, it can be seen that there is a significant difference in FRAP values between the three membranes, while no significant differences ($p > 0.05$) were observed among the DPPH values between the reverse osmosis membranes X-20 and RO99 (Tables 26 and 27). Arend et al. (2017) reported some differences in antioxidant activity values obtained by different methods can be due to the inability of such methods to determine the total antioxidant activity of the sample. Since different mechanisms and compounds are involved, the reaction rates can change, and consequently, the antioxidant activity results. Moreover, it was observed that there is a significant difference in ABTS values although no significant difference in DPPH values was discovered in a nanofiltration process. Likewise, Nunes, et al (2019) reported similar results between FRAP and DPPH.



(a) (b)

Fig 58. FRAP values with VRR during concentration by NF 270 , RO99 and X-20 membranes, anise (a), hawthorn (b)



(a) (b)

Fig 59. DPPH values with VRR during concentration by NF 270 , RO99 and X-20 membranes, anise (a), hawthorn (b)

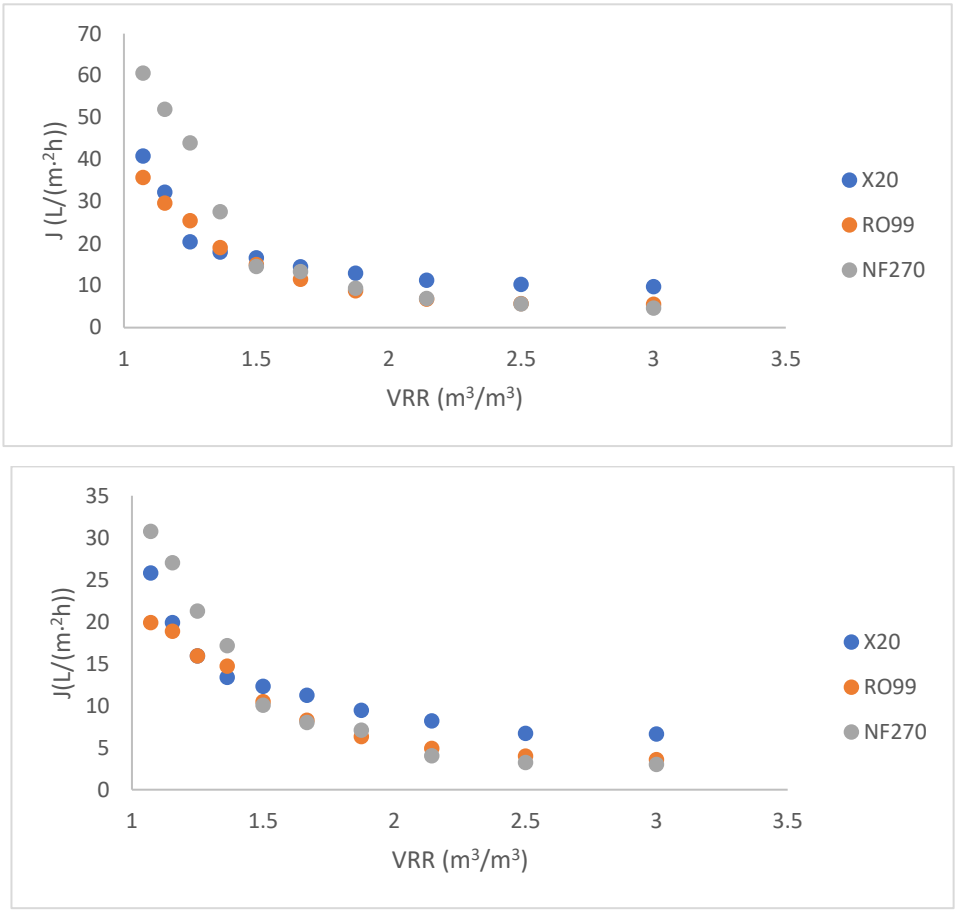
4.8.3 Permeate flux measurement

A reduction in permeate flux can be attributed to two primary mechanisms: The concentration of membrane-retained compounds and fouling phenomena. When the concentration of retained compounds within the membrane increases, it can lead to a decline in permeate flux. This mechanism is often associated with concentration polarization, where retained solutes form a dense layer near the membrane surface, reducing the effective driving force for filtration. Fouling is another significant factor contributing to flux decline (Le-Clech et al., 2006; Van der Bruggen et al., 2001).

Fig. 60 represents the permeate flux as a function of the volume reduction ratio (VRR) factor at TMP = 30 bar and T = 35 °C for the investigated membranes. As presented in the figure the volumetric permeate flux profiles for the three membranes differed from each other, which can be attributed to the "tightness" of their rejection layers. For NF 270, the permeate flux was the highest one at the beginning of the filtration for both anise and hawthorn extracts until VRR =1.5, but quickly dropped to values similar to those obtained with the other membranes (X-20 and RO99). Similar results have been observed by Nunes and co-workers (2019). Additionally, the flux of anise extract reached 4.61 (L/(m^2·h)) at VRR = 3 after about 57 minutes of concentration time using (NF 270) membrane whereas the permeate fluxs 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. This is in line with the expectation since the X-20 membrane is the lowest fouling membrane, so it was expected that the final flux would be the highest compared to other membranes.

As can be seen in Fig. 60, the reduction of permeate flux was manifested with elevated process time at fixed transmembrane pressure. The concentration process of hawthorn extracts was quite slower than anise extract, this could be due to the difference in phenolic compounds content in the extracts along with the type and the position of functional groups of these compounds (Arsuaga et al., 2011). 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)).



(a)

(b)

Fig 60. Permeate flux values changing with VRR during the concentration process by X-20, RO99, and NF 270, anise (a), hawthorn (b).

4.8.4 Membrane fouling

Membrane fouling is a serious drawback for membrane separations as it leads to reduced flow through the membrane. During the membrane processes of current work, the fouling of NF 270 membrane was faster compared to the reverse osmosis membranes. It might be due to the relatively looser polymeric structure of such membranes and the loose pores (open) which triggered penetration of foulants into them more easily, increasing hydraulic resistances and a corresponding reduction of their permeate fluxes. On contrary, for the reverse osmosis membranes, their tighter rejection layer caused the foulants accumulation on their surface instead of penetrating the membranes, thus the permeate flux remains relatively constant (Nunes et al., 2019).

Fig. 61 shows the fouling index which is a membrane-related parameter, which compares the water permeabilities of a membrane before and after filtration. The lower the fouling index, the better the long-term operational stability of a membrane. (Tang et al., 2011) grouped the factors affecting the fouling propensity into three categories, which consisted of 1) Membrane properties (morphology): The performance of the membrane is closely related to membrane morphology, such as surface porosity, membrane material, membrane molecular weight cut-off, membrane hydrophobicity, pore size, pore distribution, etc. 2) Feedwater composition: Feedwater usually consists of multiple components such as soluble organics, particulates, colloids, and a variety of electrolytes. These components may interact with each other and potentially alter the fouling behaviour in membrane filtration processes. In addition to the components of feedwaters, the chemical effects of salt content, pH, and ionic strength also affect the quality of the feed stream. 3) Hydrodynamic conditions: Hydrodynamic conditions such as crossflow velocity and flux strongly affect membrane fouling. Typically, higher membrane flux and lower crossflow velocity would induce more severe fouling than lower flux and higher crossflow velocity. This is attributable to crossflow influences on the mass transfer rate over the membrane surface.

As shown in Fig. 61 there is a significant disparity in the fouling index among the selected membranes, where 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, where the chemical cleaning step was not able to remove the deposits, and it was clear in the measurement of water flux after the cleaning. This is mainly due to the size of the pores, as the nanofiltration membrane has open pores compared to the reverse osmosis membranes, and this leads to internal contamination, as molecules are able to enter the pores of the membrane, while particles collect on the surfaces of the reverse osmosis membranes, causing external contamination that can be removed or reduced.

Along the line, the fouling of all the membranes was higher during anise extracts concentration compared to the hawthorn extracts. This can be attributed to the fact that anise extracts contain particles that could not be removed in previous filtration processes (traditional vacuum filtration) and even after centrifugation, which accelerated the membranes fouling.

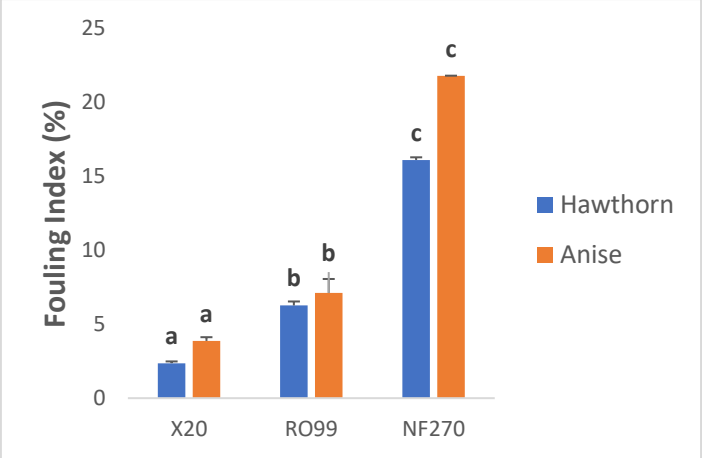
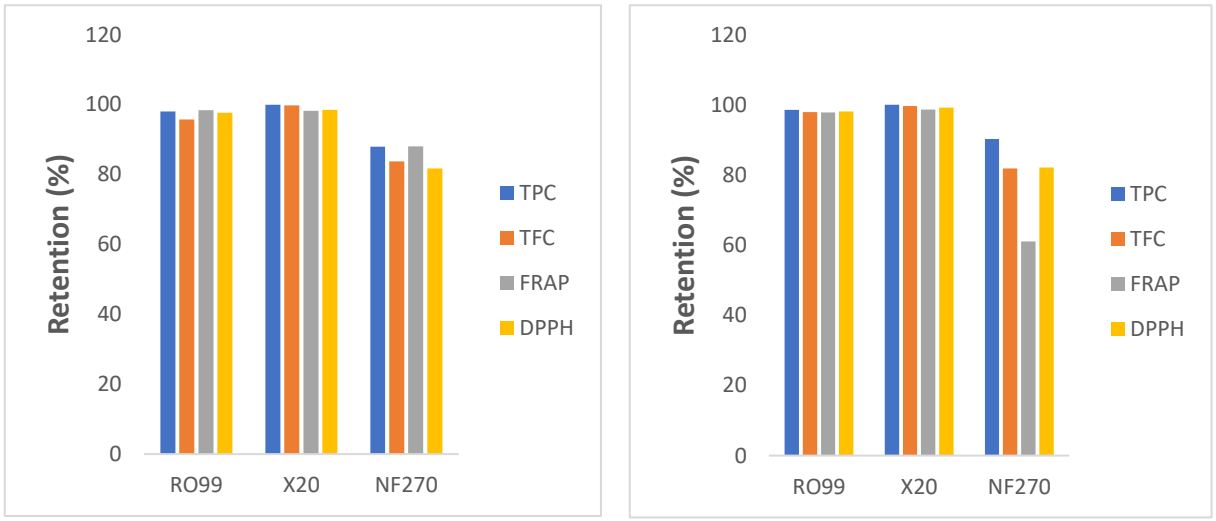


Fig 61. Fouling index (%) of X-20, RO99 and NF 270 membranes

Permeate and retentate concentrations were used to calculate retention percentages. Fig. 62 shows the significant differences in retention percentages (rejection) between the three membranes. TPC and TFC retention were > 99 % for both anise and hawthorn extracts in X-20 membrane concentration processes. Antioxidant activity retention was around 98% (using both 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. It is due to the difference between the pore size of the membranes, where decreasing the membrane pore size causes increased retention of TPC and antioxidant capacity of the retentate (Tsibranska et al., 2011), in addition to the difference in the fouling index between the membranes and the interactions established among solutes and the membrane construction material at a molecular level. On the other hand, the correlation between the retention of TPC, TFC, and antioxidant capacity has also been confirmed in several studies (Trigueros et al., 2022).



(a) (b)

Fig 62. Retention percentages of X-20, RO99 and NF 270 membranes, anise (a), hawthorn (b)

4.9 An RSM-based optimization of the concentration processes using an X-20 membrane

In the RO process, several parameters, including the type of osmotic agent, membrane pore size, concentration, flow rate, and temperature of both the osmotic agent and feed, affect the permeate flux and the concentration of total soluble solids. Based on the capability of the experimental set-up, two operating variables were selected within the following ranges: temperature 25 – 45 °C, and TMP 20 – 40 bar as was mentioned in section (3.7.1). The response surface methodology (RSM) was applied to evaluate the effects of reverse osmosis filtration parameters and optimize various conditions for different responses. Central composite design (CCD) was studied using two numeric factors on three levels. The CCD included 11 randomized runs with 3 replicates in the central point. The total phenolic content, total flavonoid content, and their antioxidant activity were measured in the final obtained retentate. The recirculation flow rate was 600 L/h, permeate flux response variables were defined as the

final flux at VRR = 4 and the fouling index was calculated by measuring water flux before and after every filtration experiment.

4.9.1 Hawthorn fruit extract concentration

The experimental outcomes of the recovered total phenolics, and flavonoid compounds and their respective antioxidant activities from the hawthorn fruit (*Crataegus monogyna* jacq), in addition to final permeate flux and fouling index were denoted in (Appendix-Table 13). 11 treatments (runs) were conducted according to CCD including replications in the center point. The model fixations were performed by the quadratic model function for TPC, TFC, AA, final permeate flux, the fouling index, fouling resistance (R_f), and linear model function for membrane resistance (R_m). The influence of each factor on the response was investigated by holding the other process variables constant. Response surface 3D graphs were generated for each response. The predicted and actual values that can be correlated by the coded and actual equations built by the model are depicted in (Appendix-Fig. 7 and Appendix-Fig. 8). The results indicated a good correlation between experimental and predicted data.

4.9.1.1 Effect of operating conditions on TPC, TFC, and AA

The model showed a high significant ($p < 0.0001$) value with the experimental data of TPC, TFC, and AA, whereas analysis of variance (ANOVA) showed significant linear and quadratic effects of (temperature and TMP). The non-significant values of lack of fit showed the models are fitted to the spatial influence of the variables to the response with the good prediction ($R^2 = 0.99$) for both TPC and TFC, and ($R^2 = 0.98, 0.99, 0.99$) for FRAP, DPPH, and ABTS respectively. According to (p values) of regression coefficients (Appendix-Table 14), 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 (A^2) had a highly negative significant influence ($p < 0.0001$) on all responses. Likewise, the linear term of applied transmembrane pressure TMP (B) had a highly positive effect ($p < 0.0001$) on all responses. While the quadratic term of TMP (B^2) 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 non-significant factors were removed and fitted the second-order polynomial equation for TPC, TFC, and AA assays as follows:

$$TPC = -393.36.25 + 31.06 \cdot A - 5.08 \cdot B - 0.45 \cdot A^2 + 0.11 \cdot B^2 \quad (49)$$

$$TFC = -45.79 + 3.78 \cdot A - 0.46 \cdot B - 0.05 \cdot A^2 + 0.01 \cdot B^2 \quad (50)$$

$$FRAP = -213.03 + 17.19 \cdot A - 3.1 \cdot B - 0.25 \cdot A^2 + 0.07 \cdot B^2 \quad (51)$$

$$DPPH = -25.6 + 2.7 \cdot A - 0.35 \cdot B - 0.04 \cdot A^2 + 0.008 \cdot B^2 \quad (52)$$

$$ABTS = -217.55 + 18.13 \cdot A - 3.5 \cdot B - 27 \cdot A^2 + 0.07 \cdot B^2 \quad (53)$$

where A – temperature (°C) in the range (25 – 45 °C), B – TMP (bar) in the range (20 – 40 bar).

The lack of fit test indicates that the model could adequately fit the experimental data ($p < 0.05$) for all response variables (TPC, TFC, and AA assays) (Appendix-Table 14). The 3D plot shows the effect of the studied parameters on the TPC, TFC, and AA assays (Fig. 63 and Fig. 64). The curvature in the 3D plot arises due to the quadratic dependence on temperature and TMP. The TPC, TFC, and their antioxidant activity increased with the temperature between levels (-1) and (0), after this point, an increase in temperature produces a decrease in all the

responses, due to the heat sensitive compounds which may be destroyed when heated at higher temperature. In addition, The TMP produces an increase in the response flux from level (-1) to level 1.

A similar behaviour was observed in the concentration of phenolic compounds from bergamot juice by nanofiltration membranes, where the high operating pressure led to a high rejection of phenolic compounds (Conidi, and Cassano, 2015), and in concentrate anthocyanins from roselle extract by ultrafiltration and nanofiltration membranes (Cissé et al., 2011). On the other hand, according to (Sánchez-Arévalo et al., 2021) when the TMP was increased from 5 to 10 bar, rejection of phenolic compounds increased but more increase in TMP up to 15 bar caused a decrease in phenolic compounds rejection in the nanofiltration process. In contrast, the detention of polyphenol compounds has been not influenced by TMP in the concentration of pomegranate juice by ultrafiltration and nanofiltration membranes (Conidi et al., 2017).

The variations in particle sizes and the pore size of the membrane are most likely the cause of the discrepancies in the outcomes. As well as, the increase in applied pressure causes in an increase in the driving force which affects the fouling phenomenon, and concentration polarization which can explain the effect of TMP (Jiang et al, 2018).

The temperature has a crucial role in the permeate fluxes, membrane fouling, and pores size a side with its the role in the diffusion of phenolic compounds (Gupta et al., 2003), therefore it is hard to explain the exact effect of the temperature, Additionally, increasing temperature above a certain value may lead to the evaporation of the solvents and promote possible concurrent degradation of phenolic compounds (Mokrani and Madani, 2016) which affects the retention of these compounds.

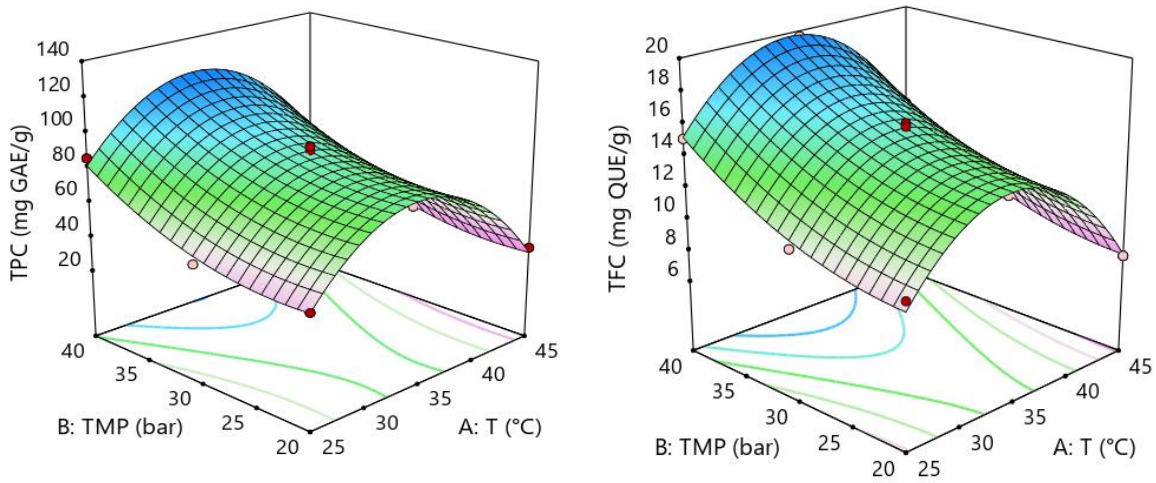


Fig 63. 3D response surface of TPC and TFC in the final retentate influenced by individual factors in the concentration of hawthorn extracts by X-20 membrane

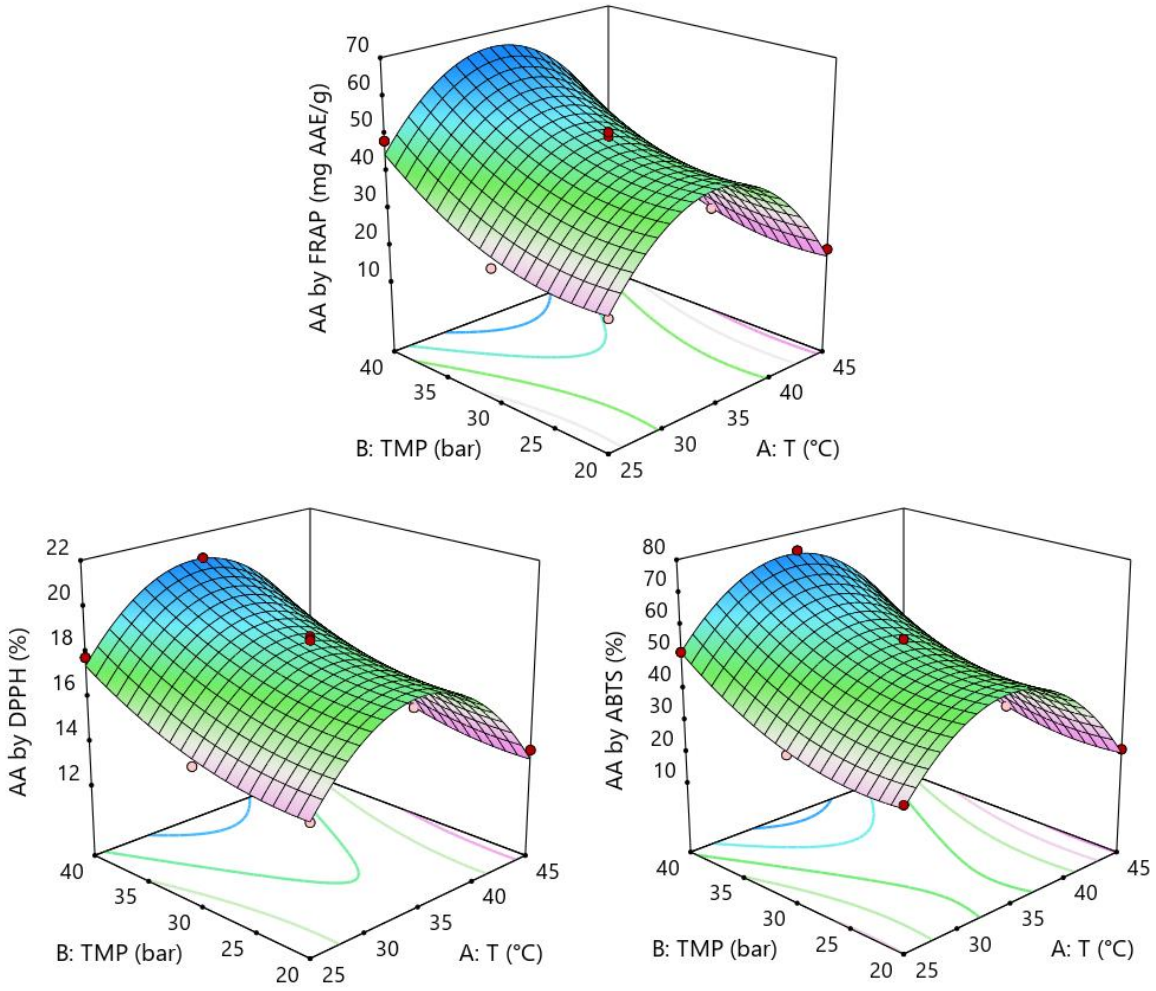


Fig 64. 3D response surface of FRAP, DPPH and ABTS in the final retentate influenced by individual factors in the concentration of hawthorn extracts by X-20 membrane

4.9.1.2 Effect of operating conditions on final permeate flux

The results of the quadratic model of final permeate flux are given in (Appendix-Table 14). ANOVA analysis and the statistic test factor, F-value, were used to evaluate the significance of the model at the 95 % confidence level. The F-value and p-value of the model were 23.54 and 0.0005, respectively, indicating the model is statistically significant. There is only a 0.05 % chance that such F-value could occur due to noise. Also, values of $p < 0.05$ indicate model terms are significant. The goodness of fit of the model was expressed by R^2 which was found to be 0.91 %. On the other hand, the p-value for lack-of-fit in the ANOVA is greater or equal to 0.05 ($p = 0.1111$, F-ratio 8.29), and the model appears to be adequate for the observed data at the 95.0 % confidence level.

The linear coefficients of TMP (B) were found to be the most significant effect in increasing the final permeate flux ($p < 0.001$), followed by the linear effect of temperature (A) ($p < 0.05$). On the other hand, the quadratic coefficient of temperature (A^2) produces a decrease in the final permeate flux with a significant effect ($p < 0.05$). The interaction factors (AB) and the quadratic effect of TMP (B^2) do not produce a significant effect ($p > 0.05$) in the final permeate flux, therefore, these factors will not be included in the regression model equation of the final permeate flux, which is presented as follows:

$$J = -14.75 + 0.78 \cdot A + 0.15 \cdot B - 0.0105 \cdot A^2 \quad (54)$$

where A – temperature (°C) in the range (25 – 45 °C), B – TMP (bar) in the range (20 – 40 bar).

3D response surface in Fig. 65 of final permeate flux shows the linear effect of (TMP) on the final permeate flux at all levels, while the temperature has a quadratic effect on final permeate flux, where final permeate flux increased with the temperature at level (-1) and (0), then started to decrease at level (1).

These results are consistent with the results were indicated by (Figueroa et al., 2011) where mentioned the TMP has the highest significant impact on boosting the permeate flux, followed by the correlation coefficient of temperature in the concentration of orange press liquor by ultrafiltration. In contrast, (Aloulou et al., 2022) found that the applied TMP had no influence on the permeate flux during the ceramic ultrafiltration membrane treatment of tuna cooking liquid and the temperature has the highest effect. This proves that raw materials and membrane properties, as well as the relation between them, have an impact on the concentration process.

4.9.1.3 Effect of operating conditions on fouling index

The results of the quadratic model of the fouling index are given in (Appendix-Table 14). The F-value and P-value of the model were 93.25 and $p < 0.0001$, respectively, indicating the model is statistically significant. There is only a 0.01% chance that such F-value could occur due to noise. Also, values of $p < 0.05$ indicate model terms are significant. The goodness of fit of the model was expressed by R^2 which was found to be 0.98 % indicating that only 1.6 % of the variability in the response could not be explained by the model. In addition, the p-value for lack-of-fit in the ANOVA is greater or equal to 0.05 ($p = 0.4317$, F-ratio 1.53), and the model appears to be adequate for the observed data at the 95.0% confidence level.

TMP (B) was found to be the most significant effect in decreasing the fouling index ($p < 0.0001$), followed by temperature (A) ($p < 0.001$), and then the interaction factor between TMP and temperature factor (AB) ($p < 0.01$), meanwhile, the quadratic term of temperature (A^2) has a significant effect to increase the fouling index ($p < 0.01$). While the quadratic term of TMP (B^2) does not produce a significant effect ($p > 0.05$) in the fouling index. (Figueroa et al., 2011) found that the interaction between TMP and temperature has a significant impact on increasing the fouling index, while the quadratic impact of transmembrane pressure causes a reduction in the fouling index. The following quadratic regression equation describes the fouling index in relation to the process variables:

The quadratic regression equation describing the effect of the process variables on the fouling index in terms is reported in the following:

$$Fouling\ index = +77.24 - 1.36 \cdot A + 0.22 \cdot B - 0.0301 \cdot AB + 0.027 \cdot A^2 \quad (55)$$

where A – temperature (°C) in the range (25 – 45 °C), B – TMP (bar) in the range (20 – 40 bar).

The effect of different variables on the fouling index is shown in Fig. 65. An interaction of TMP and temperature was observed from the warping of the 3D fouling index plot. The temperature produces a decrease in the fouling index from level (-1) to (0) after this point, an increase in temperature produces an increase in the fouling index.

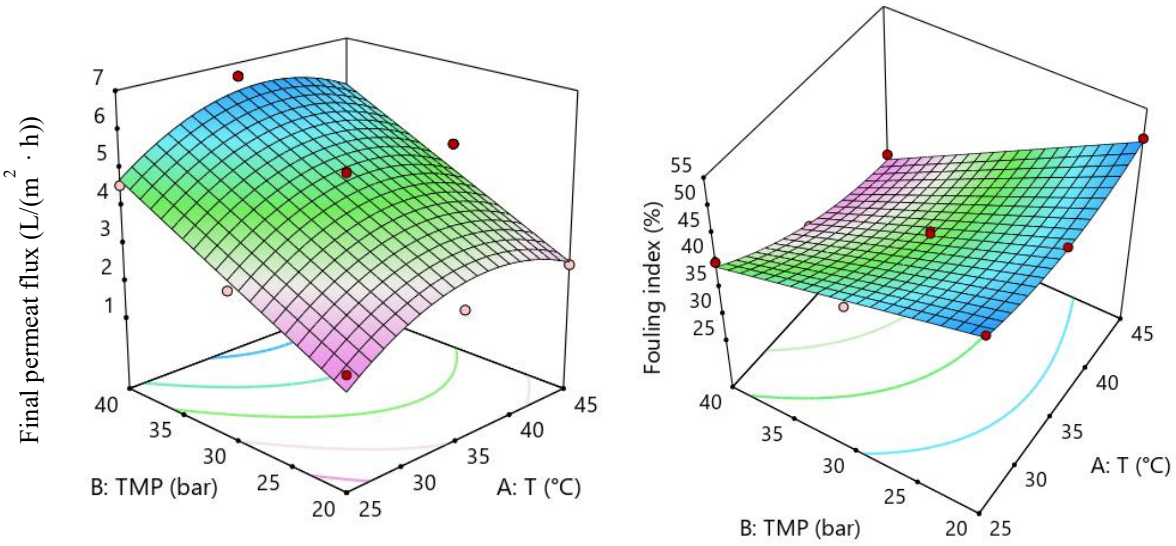


Fig 65. 3D response surface of final permeate flux and fouling index influenced by individual factors in the concentration of hawthorn extracts by X-20 membrane

4.9.1.4 Effect of operating conditions on membrane resistance (R_m) and fouling resistance (R_f)

The results of the linear model of the membrane resistance are given in (Appendix-Table 14). The F-value and p-value of the model were 49.9 and $p < 0.0001$, respectively, indicating the model is statistically significant. There is only a 0.01 % chance that such an F-value could occur due to noise. Also, values of $p < 0.05$ indicate model terms are significant. The model's goodness of fit was expressed by R^2, which was 92.58 % indicating that 7.42 % of the variability in the response could not be explained by the model. In addition, the p-value for lack-of-fit in the ANOVA is greater or equal to 0.05 ($p = 0.059$, F-ratio 16.08), and the model appears to be adequate for the observed data at the 95.0 % confidence level.

Temperature (A) was found to have the greatest effect on the increasing membrane resistance ($p < 0.0001$), while TMP (B) had no significant effect on it ($p > 0.05$) (Fig. 66). Following is the linear regression equation that describes the effect of the process variables on the membrane resistance (R_m):

$$R_m = +8.91 \cdot 10^{13} + 3.51 \cdot 10^{12} \cdot A + 5.83 \cdot 10^{10} \cdot B \qquad (56)$$

where A – temperature (°C) in the range (25 – 45 °C), B – TMP (bar) in the range (20 – 40 bar).

(Van den Brink et al., 2011) mentioned that the temperature did not affect the R_m of the homemade PVDF membranes. Membrane resistance is related to the structure and properties of the membrane, unfortunately, there is no more study mentioned about the effect of operation parameters on R_m.

(Appendix-Table 14) shows the results of the quadratic model of the fouling resistance. The F-value and p-value of the model were 305.39 and $p < 0.0001$, respectively, indicating the model is statistically significant. There is only a 0.01% chance that such an F-value could occur due to noise. Also, values of $p < 0.05$ indicate model terms are significant. The model's goodness of fit was expressed by R^2, which was 99.67 % indicating that only 0.33 % of the variability in the response could not be explained by the model. In addition, the p-value for

lack-of-fit in the ANOVA is greater or equal to 0.05 (p = 0.1, F-ratio 9.01), and the model appears to be adequate for the observed data at the 95.0 % confidence level.

The quadratic coefficients of temperature (A^2), and the interaction factors (AB) were found to have a significant effect in increasing the fouling resistance ($p < 0.0001$) which explains the curvature in the 3D plot of R_f (Fig. 66). 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. Following the removal of non-significant factors, the second-order polynomial equation for fouling resistance was fitted as follows:

$$R_f = +1.38 \cdot 10^{15} - 5.46 \cdot 10^{13} \cdot A - 2.26 \cdot 10^{13} \cdot B + 6.38 \cdot 10^{11} \cdot AB + 4.92 \cdot 10^{11} \cdot A^2 \quad (57)$$

where A – temperature (°C) in the range (25 – 45 °C), B – TMP (bar) in the range (20 – 40 bar).

The study of UF membrane in wastewater treatment found that the interaction factors between the temperature and TMP significantly affect fouling resistance (Zhang et al., 2019). Additionally, (Alresheedi et al., 2019) found that the fouling resistance decreased with the increase of the feed (water) temperature in ceramic ultrafiltration membranes which is consistent with our results which can be seen in the 3D plot of R_f (Fig.66).

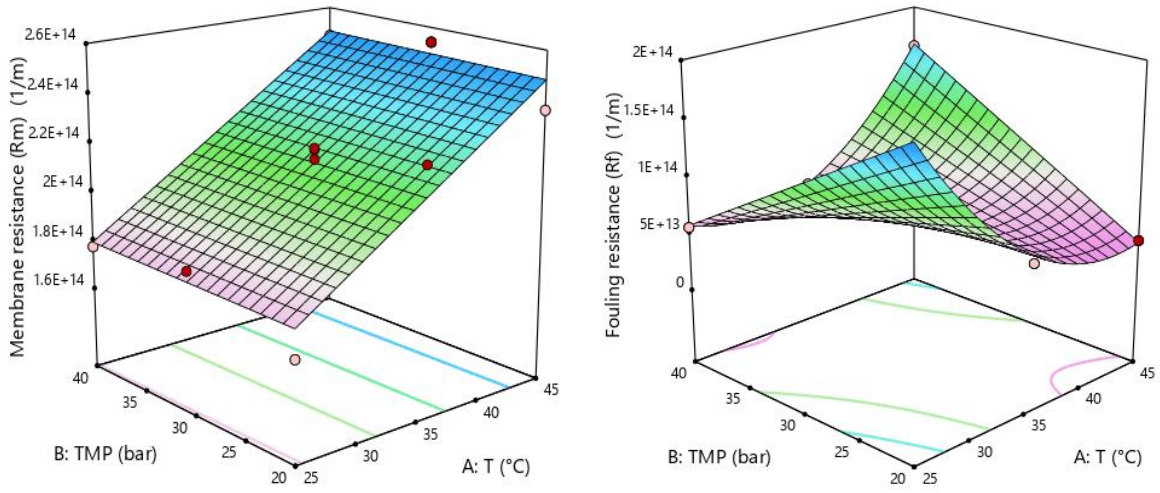


Fig 66. 3D response surface of membrane resistance (R_m) and fouling resistance (R_f) influenced by individual factors in the concentration of hawthorn extracts by X-20 membrane

4.9.1.5 Optimization of multiple responses

As was mentioned, the desirability function is widely used to determine a combination of variables to optimize multiple responses. This process aims to find operating conditions giving the maximum bioactive compounds yield and final permeate flux and the minimum fouling index simultaneously. These best conditions were determined using Design Expert Software Trial Version 11.0.3. The optimal conditions of the concentration process of hawthorn fruit extracts were (T = 35 °C, TMP = 40 bar) in the evaluated range (Fig. 67). The desirability of 0.89 suggests that the experimental conditions are performing very well and are close to achieving the desired objectives, there may still be room for further improvement in certain aspects. Under these conditions, the experimental values agreed with the predicted values with the coefficient of variation C.V. % ranging from 2.15 to 12.86 % (Appendix-Table 14).

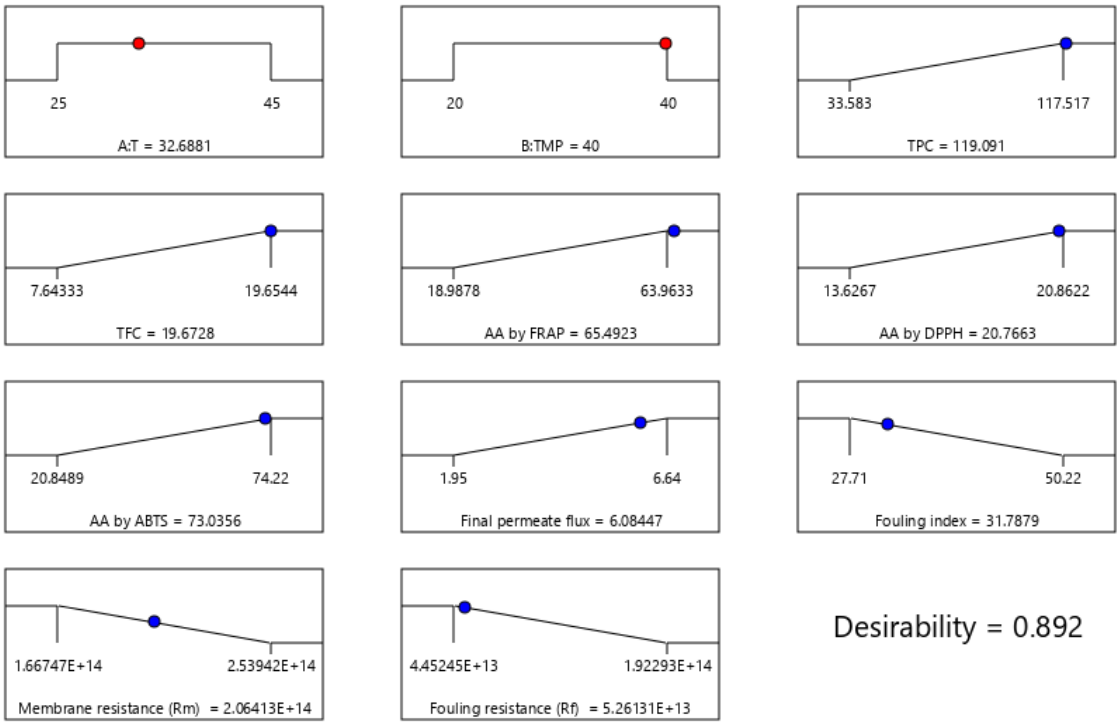


Fig 67. The desirability test based on the optimized values of targeted compounds during membrane concentration of hawthorn extracts

4.9.2 Anise seed extract concentration

The experimental outcomes of the recovered total phenolics, and flavonoid compounds and their respective antioxidant activities from the anise seed *(Pimpinella anisum* L.), in addition to final permeate flux and fouling index were denoted in (Appendix-Table 15). 11 treatments (runs) were conducted according to CCD including replications in the center point. The model fixations were performed by the quadratic model function for TPC, TFC, AA, final permeate flux, the fouling index, fouling resistance (R_f), and linear model function for membrane resistance (R_m). The influence of each factor on the response was investigated by holding the other process variables constant. Response surface 3D graphs were generated for each response. The predicted values and actual values which can be correlated by the coded and actual equations built by the model were depicted in (Appendix-Fig. 9 and Appendix-Fig. 10). The results indicated a good correlation between experimental and predicted data.

4.9.2.1 Effect of operating conditions on TPC, TFC, and AA

Optimization of the extraction process of bioactive compounds was carried out by applying a second-order polynomial equation. The experimental data are shown in (Appendix-Table 15). The model shows high significance ($p < 0.0001$) and a good fit with the experimental data of TP, and TF content and has less variation around the mean (R^2 values 0.98) for both TPC and TFC. The antioxidant activities (FRAP, DPPH, and ABTS) show the model is significant and the quality of fit to the second-order polynomial equation checked using the coefficient of determination (R^2), which were (0.98, 0.98, and 0.96), respectively.

According to (p values) of regression coefficients (Appendix-Table 16), 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 (A^2) 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 (B) 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 (B^2) had the lowest effect on the responses ($p < 0.05$ and $p < 0.01$). This result can be explained by the fact that increasing the temperature improves the penetration effect, decreasing the compounds' rejection. While the effect of TMP can be due to its effect on the driving force and shear rate.

The non-significant factors were removed and fitted the second-order polynomial equation for TPC, TFC, and AA assays as follows:

$$TPC = -245.84 + 21.42 \cdot A - 5.1 \cdot B - 0.3 \cdot A^2 + 0.11 \cdot B^2 \quad (58)$$

$$TFC = -16.44 + 1.52 \cdot A - 0.26 \cdot B - 0.023 \cdot A^2 \cdot + 0.007 \cdot B^2 \quad (59)$$

$$FRAP = -29.75 + 2.65 \cdot A - 0.49 \cdot B - 0.04 \cdot A^2 + 0.011 \cdot B^2 \quad (60)$$

$$DPPH = -21.67 + 1.83 \cdot A - 0.32 \cdot B - 0.027 \cdot A^2 + 0.007 \cdot B^2 \quad (61)$$

$$ABTS = -6.86 + 0.72 \cdot A - 0.23 \cdot B - 0.01 \cdot A^2 + 0.005 \cdot B^2 \quad (62)$$

where A – temperature (°C) in the range (25 – 45 °C), B – TMP (bar) in the range (20 – 40 bar).

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 (TPC, TFC, and AA) (Appendix-Table 16). The 3D plot shows the effect of the studied parameters on the TPC, TFC, and AA (Fig. 68 and Fig. 69). The curvature in the 3D plot arises due to the quadratic dependence on temperature and TMP. The TPC, TFC, and their antioxidant activity increased with the temperature between levels (-1) and (0), after this point, an increase in temperature produces a decrease in all the responses. In addition, the TMP produces an increase in the response flux from level (-1) to level (1).

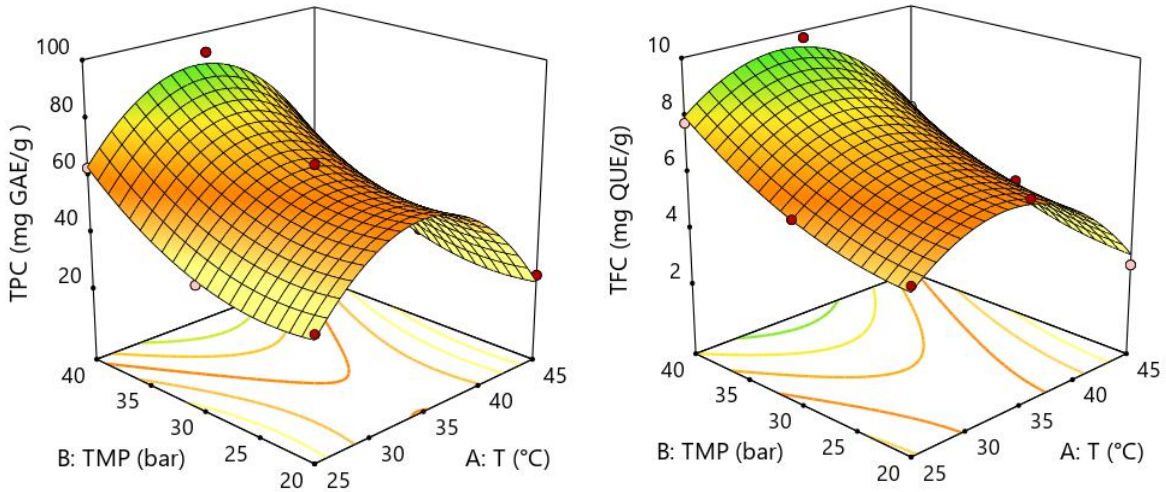


Fig 68. 3D response surface of TPC and TFC in the final retentate influenced by individual factors in the concentration of anise extracts by X-20 membrane

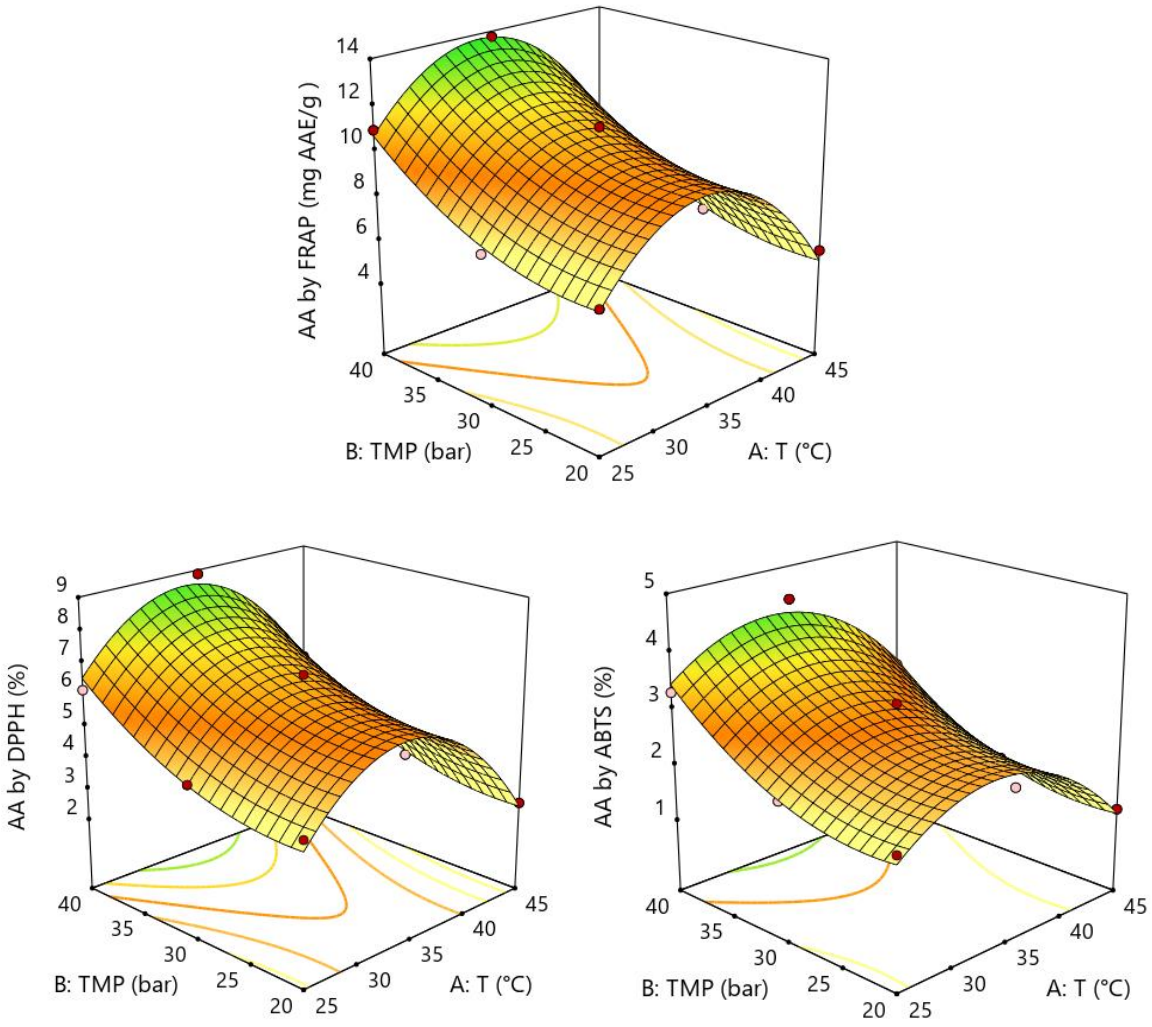


Fig 69. 3D response surface of FRAP, DPPH, and ABTS in the final retentate influenced by individual factors in the concentration of anise extracts by X-20 membrane

4.9.2.2 Effect of operating conditions on final permeate flux

The R-squared statistic indicates that the model explains 91.5 % of the variability in the final permeate flux. The lack-of-fit test is designed to determine whether the selected model is adequate to describe the observed data, or whether a more complicated model should be used. The test is performed by comparing the variability of the current model residuals to the variability between observations at replicate settings of the factors. Since the p-value for lack-of-fit in the ANOVA is greater or equal to 0.05 ($p = 0.1332$, F-ratio 6.79), the model appears to be adequate for the observed data at the 95.0 % confidence level. Meanwhile, the F-value and p-value of the model were 25.22 and 0.0004, respectively, indicating the model is statistically significant. There is only a 0.04 % chance that such F-value could occur due to noise.

The linear coefficients of TMP (B) were found to be the most significant effect to increase the final permeate flux ($p < 0.001$), followed by the linear effect of temperature (A) ($p < 0.05$). On the other hand, the quadratic coefficient of temperature (A^2) produces a decrease in the final permeate flux with a significant effect ($p < 0.01$). The interaction factors (AB) and the quadratic effect of TMP (B^2) do not produce a significant effect ($p > 0.05$) in the final permeate flux,

therefore, these factors will not be included in the regression model equation of the final permeate flux, which is presented as follows:

$$J = -43.48 + 2.33 \cdot A + 0.39 \cdot B - 0.311 \cdot A^2 \qquad (63)$$

where A – temperature (°C) in the range (25 – 45 °C), B – TMP (bar) in the range (20 – 40 bar).

3D response surface in Fig. 70 of final permeate flux shows the linear effect of (TMP) on the final permeate flux for all the values investigated, and the curvature in the 3D plot of the final permeate flux arises due to the quadratic dependence on temperature.

4.9.2.3 Effect of operating conditions on fouling index

The R-squared statistic indicates that the model as fitted explains 82.32 % of the variability in the fouling index. The p-value for lack-of-fit was equal to 0.407 (F-ratio 1.72). The F-value and p-value of the model were 10.86 and 0.005, respectively, indicating the model is statistically significant and fitted is completely adequate to represent the experimental data. TMP (B) and temperature (A), was found to be the most significant effect to decrease the fouling index, followed by the quadratic effect of temperature (A^2) that produces an increase in the fouling index ($p < 0.01$). The interaction factor between TMP and temperature factor (AB) and the quadratic term of TMP (B^2) does not produce a significant effect ($p > 0.05$) in the fouling index. The quadratic regression equation describing the effect of the process variables on the fouling index is reported in the following:

$$Fouling\ index = +111.36 - 3.86 \cdot A - 0.36 \cdot B + 0.051 \cdot A^2 \qquad (64)$$

where A – temperature (°C) in the range (25 – 45 °C), B – TMP (bar) in the range (20 – 40 bar)

The effect of different variables on the fouling index is shown in Fig. 70. The response surface of the fouling index is plotted against two operating variables. It shows the quadratic effect of temperature, where the fouling index decreased with temperature up to 37 and then started to increase with the increasing temperature.

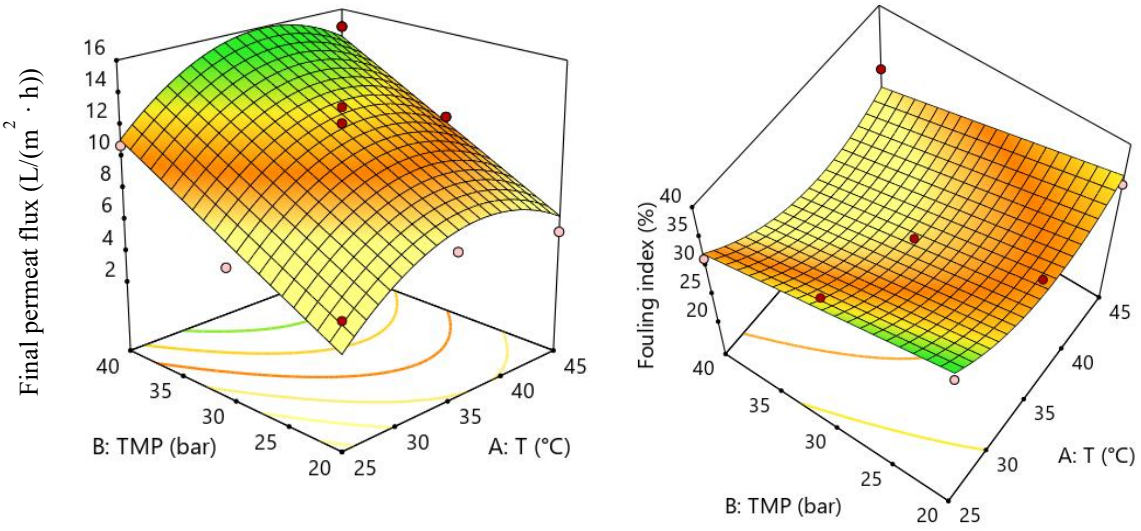


Fig 70. 3D response surface of final permeate flux and fouling index influenced by individual factors in the concentration of anise extracts by X-20 membrane

The observed outcomes can be attributed to several factors. Firstly, an increase in transmembrane pressure (TMP) amplifies the driving force, thereby resulting in higher permeate flux and a decrease in the fouling index. Secondly, the impact of temperature is closely linked to cake resistance. A decrease in temperature can induce cake shrinkage, leading to the formation of a denser cake with higher specific cake resistance. This change can directly influence the fouling index. Furthermore, variations in the fouling index with temperature can be attributed to alterations in fouling behavior at different temperature levels.

4.9.2.4 Effect of operating conditions on membrane resistance (R_m) and fouling resistance (R_f)

The F-value and p-value of the model were 22.90 and $p < 0.001$, respectively (Appendix-Table 16), indicating the model is statistically significant. There is only a 0.05 % chance that such an F-value could occur due to noise. Also, values of $p < 0.05$ indicate model terms are significant. The model's goodness of fit was expressed by R^2, which was 85.13 %. In addition, the P-value for lack-of-fit in the ANOVA is greater or equal to 0.05 ($p = 0.079$, F-ratio 11.48), and the model appears to be adequate for the observed data at the 95.0% confidence level.

The linear term of 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 ($p > 0.05$) (Fig. 71). Following is the linear regression equation that describes the effect of the process variables on the membrane resistance (Rm):

$$R_m = +5.64 \cdot 10^{13} + 2.45 \cdot 10^{12} \cdot A - 5.22 \cdot 10^{11} \cdot B \qquad (65)$$

where A – temperature (°C) in the range (25 – 45 °C), B – TMP (bar) in the range (20 – 40 bar)

(Appendix-Table 16) shows the results of the quadratic model of the fouling resistance. The F-value and p-value of the model were 54.08 and $p < 0.001$, respectively, indicating the model is statistically significant. There is a 0.31 % chance that such an F-value could occur due to noise. Also, values of $p < 0.05$ indicate model terms are significant. The model's goodness of fit was expressed by R^2, which was 98.45 %. Indicating that only 1.55 % of the variability in the response could not be explained by the model. In addition, the p-value for lack-of-fit in the ANOVA is greater or equal to 0.05 ($p = 0.08$, F-ratio 11.37), and the model appears to be adequate for the observed data at the 95.0 % confidence level.

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 ($p < 0.001$), which explains the curvature in the 3D plot of R_f (Fig. 71). 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. Following the removal of non-significant factors, the second-order polynomial equation for fouling resistance was fitted as follows:

$$R_f = +1.056 \cdot 10^{15} - 6.19 \cdot 10^{13} \cdot A + 1.66 \cdot 10^{12} \cdot B + 3.91 \cdot 10^{11} \cdot AB + 7.49 \cdot 10^{11} \cdot A^2 - 2.35 \cdot 10^{11} \cdot B^2 \qquad (66)$$

where A – temperature (°C) in the range (25 – 45 °C), B – TMP (bar) in the range (20 – 40 bar).

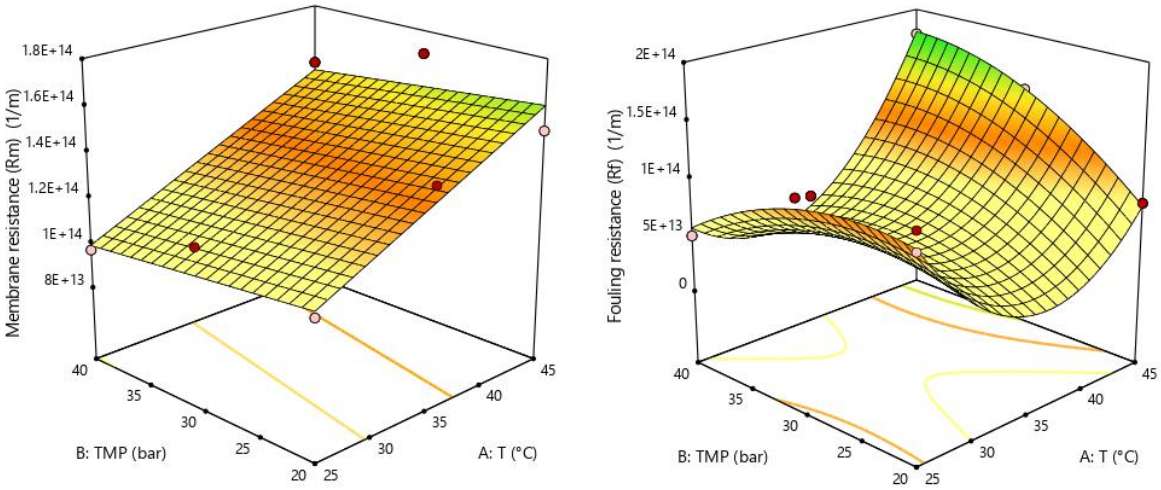


Fig 71. 3D response surface of membrane resistance (R_m) and fouling resistance (R_f) influenced by individual factors in the concentration of anise extracts by X-20 membrane

4.9.2.5 Optimization of multiple responses:

In order to find operating conditions giving the maximum bioactive compounds yield and final permeate flux and the minimum fouling index simultaneously in the concentration process of anise seed extracts. These optimal conditions were determined using Design Expert Software Trial Version 11.0.3. The optimal conditions of this process were (T = 34 °C, TMP= 40 bar) in the evaluated range (Fig. 72). The desirability value of 0.91 suggests that the achieved outcomes are highly satisfactory and leave little room for significant improvement. The results are close to the ideal, with minor areas where further optimization might be possible. Under these conditions, the experimental values agreed with the predicted values with the coefficient of variation C.V. % ranging from 5.08 to 13.15 % (Appendix-Table 16).

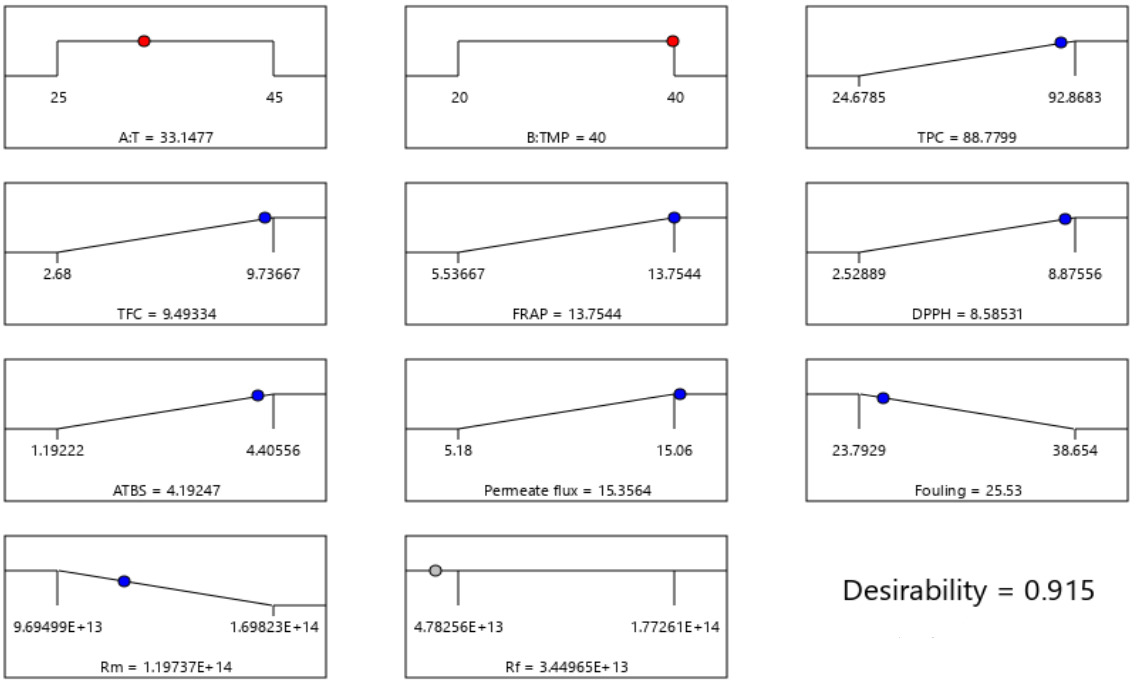


Fig 72. The desirability test based on the optimized values of targeted compounds during membrane concentration of anise extracts

5 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.

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, 24.43 ± 2.4 %, and 51.58 ± 5.09 % for FRAP, DPPH, and ABTS respectively in the evaluated range. The actual values calculated from the regression equation modelled by RSM were as follows: TPC (74.28 mg GAE/g dw), TFC (19.92 mg QUE /g dw), and AA by FRAP method (36.03 mg AAE/g dw) DPPH method (25.28 %), and ABTS method (51.57 %).

- 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. Meanwhile, the calculated scavenged amounts of the respective compounds via the RSM model were 55.49 mg GAE/g dw (TPC), 12.92 mg QUE/g dw of (TFC), 24.5 mg AAE/g dw of (FRAP), and 21.5 % of (DPPH), and 46.8 % of (ABTS).

- 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. In the meantime, the RSM model estimated the scavenged amounts of TPC, TFC, FRAP, DPPH, and ABTS as 95.78 mg GAE/g dw, 30.35 mg QUE/g dw, 40.28 mg AAE/g dw, 33.87 %, and 66.02 %, respectively.

- The models show that the highest TPC and C (95.78 ± 5.42 mg GAE/ g and 30.35 ± 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].

<u>For anise seed extraction</u>:

- 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. From the RSM model, the amounts of targeted bioactive compounds estimated by the actual equations were 42.13 mg GAE/g dw of TPC, 8.60 mg QUE/g dw of TFC, 5.96 mg AAE/g dw of FRAP, 5.32 % of DPPH, and 1.53 of ABTS individually.

- 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 4.2 ± 0.42 % respectively in the evaluated range. The actual values calculated from the regression equation modelled by RSM were as follows: TPC (49.95 GAE mg/g dw), TFC (20.86 mg QUE/g dw), and AA by FRAP method (11.22 mg AAE/g dw), DPPH method (17.14 %), and ABTS method (4.26 %).

- 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 of sample-to-solvent ratio in the evaluated range. Meanwhile, the calculated scavenged amounts of the respective compounds via the RSM model were 43.16 mg GAE/g dw (TPC), 16.5 mg QUE/g dw of TFC, 8.85 mg AAE/g dw of (FRAP), and 14.22 of (DPPH), and 2.33 % of (ABTS).

- The models show that the highest TPC and TFC (49.95 ± 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 optimal 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: 34.74 ± 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 ($L/(m^2 \cdot h)$) 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. Meanwhile, the calculated amounts of the respective values via the RSM model were TPC:119.09 mg GAE/g dw, TFC: 19.67 mg QUE/g dw, FRAP: 65.49 mg AAE/g dw, DPPH:20.76 %, ABTS: 73.03 %, final permeate flux: 6.084 (L/(m^2·h)), fouling index: 31.78 %, membrane resistance ($2.064 \cdot 10^{14}$ 1/m), and fouling resistance ($5.26 \cdot 10^{13}$ 1/m).

- 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. The actual values calculated from the regression equation modelled by RSM were as follows TPC: 88.78 mg GAE/g dw, TFC: 9.49 mg QUE/g dw, FRAP:13.75 mg AAE/g dw, DPPH: 8.58 %, ABTS: 4.19 %, final permeate flux: 15.35 (L/(m^2·h)), fouling index: 25.53 %, membrane resistance ($1.19 \cdot 10^{14}$ 1/m), and fouling resistance ($3.44 \cdot 10^{13}$ 1/m).

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.

6 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 sample-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.

7 SUMMARY

Bioactive compounds are described as important components of the secondary metabolism in plants, exerting several biological effects in both humans and animals. Accordingly, a piece of cumulative evidence is supporting the consumption of these compounds to improve health status, thus reducing the risk of developing cardiovascular, inflammatory, neurological, and metabolic diseases, and cancer. Several compounds can be listed among these bioactives, such as polyphenols, glucosinolates/isothiocyanates, carotenoids and other terpenopids, alkaloids, dietary fiber, and medium-chain monoglycerides. due to these facts, bioactive compounds received attention, and several techniques and solvents have been used to extract these compounds from plant matrices. The biggest challenge in the process of getting bioactive compounds is recovering these compounds from the solvents after extracting them, As the traditional approaches based on fumigation or chemical additions cause loss and change in these compounds. One of the promising solutions to avoid these losses is membrane technology, which has proven its efficiency as a green technology in the concentration pf polyphenolic compounds.

The major concept of this dissertation is to determine the optimal techniques and conditions to extract phenolic and flavonoid compounds from hawthorn fruit (*Crataegus monogyna* jacq.) and anise seed (*Pimpinella anisum* L.) and optimize the concentration process of the obtained extracts using membrane technology. Heat, microwaves, and ultrasound-assisted extraction techniques were applied for the extraction. Twenty experimental runs were achieved with the RSM modelling tool with three different operational setups with every technique. heat-assisted extraction was completed by changing the ethanol concentration, extraction temperature, and extraction time in three-levels for the extraction of hawthorn fruit, whilst pure water was used as a solvent for anise seed extraction, the effect of the extraction temperature, extraction time, and sample-to-solvent ratio were changed during the extraction process. With microwave-assisted extraction, microwave power, irradiation time, and sample-to-solvent ratio, were applied for the extraction purpose. Meanwhile, the concentration of ethanol, extraction time, and sample-to-solvent ratio were used as variables for ultrasound-assisted extraction for both hawthorn fruit and anise seed. The content of phenolic, flavonoid, and antioxidants were determined for every extract sample. The subsequent investigation was focused on the membrane technology (reverse osmosis and nanofiltration) for the concentration of the extracts obtained using HAE from hawthorn and anise. Two RO membranes and one nanofiltration membrane were examined to determine the optimal membrane. Subsequently, RSM with two variables in three-levels were used to optimize the concentration process of X-20 membrane.

Within the study ranges, microwave irradiation has shown privilege by boosting the extractability of phenolics, flavonoids, and relative antioxidant activities from anise seed. Besides, the ultrasonic application is typified to be crucial in assisting the extraction of the mentioned bioactive compounds from hawthorn. The solvent characteristics played a major role in the yield of extracted bioactive compounds and their antioxidant activity. Furthermore, the membrane concentration has been found to offer a good method for the recovery of bioactive compounds from hawthorn fruit and anise seed. Ultimately, the outcomes of the current investigation had led to the conclusion that for these two plants, the conventional extraction way could be replaced by thermal, otherwise, non-thermal emerging technologies, and use the reverse osmosis membranes to improve the whole process of getting bioactive compounds.

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FURTHER APPENDICES (A.2)

Appendix-Table 1. Experimental outcomes of extracted compounds from hawthorn fruit by EW solvent and HAE

Run	Ethanol (% v/v)	T (°C)	Time (min)	TPC (mg GAE/g dw)	TFC (mg QUE/g dw)	FRAP (mg AAE/g dw)	DPPH (%)	ABTS (%)
1	50	60	50	65.86	18.27	34.06	24.43	49.55
2	50	45	50	80.56	18.63	35.29	24.25	51.58
3	10	30	10	15.18	4.78	10.39	5.79	9.97
4	50	45	90	71.21	18.28	34.85	22.02	46.51
5	50	45	50	67.91	15.79	30.86	19.03	42.48
6	50	45	10	49.19	12.67	22.86	15.43	32.69
7	10	60	10	31.65	10.55	19.63	13.16	26.67
8	90	30	90	38.95	12.59	20.34	13.77	27.22
9	90	45	50	65.24	15.45	29.90	18.15	34.53
10	50	45	50	68.02	14.62	32.81	19.01	36.25
11	50	45	50	71.44	18.33	35.24	24.22	46.46
12	10	30	90	33.36	9.37	17.49	11.45	23.69
13	90	60	90	65.07	19.93	33.82	21.39	45.06
14	10	45	50	54.36	12.07	27.40	15.10	33.63
15	50	45	50	59.55	15.61	29.50	19.35	39.64
16	50	30	50	35.60	11.27	20.51	13.55	31.18
17	50	45	50	68.14	14.74	29.87	19.54	39.40
18	90	60	10	49.77	17.89	25.44	18.32	35.49
19	90	30	10	29.53	11.09	13.76	7.38	17.58
20	10	60	90	45.36	11.16	20.16	14.58	27.09

Appendix-Table 2. ANOVA for hawthorn fruit EW extract and HAE

Source	Estimated coefficient					F values				
	TPC	TFC	FRAP	DPPH	ABTS	TPC	TFC	FRAP	DPPH	ABTS
Intercept	67.50	15.82	31.85	20.08	41.57	29.78****	21.38****	19.85****	19.14****	17.66****
A-Solvent	6.87	2.90	2.82	1.89	3.88	12.76**	29.66****	8.17*	6.24*	5.81*
B-Temperature	10.51	2.87	5.06	3.99	7.42	29.89****	29.01****	26.32***	27.80****	21.22***
C-time	7.86	1.44	3.46	2.31	4.72	16.73**	7.26*	12.27**	9.31**	8.57*
AB										
AC										
BC										
A^2	-9.66	-3.33	-4.96	-6.17	-13.48	8.08*	19.58***	8.08*	33.20****	35.01****
B^2	-18.73		-6.32			30.93****		13.14**		
C^2										
Residual (SS)	517.26	42.57	136.34	86.09	389.23					
Lack of Fit(SS)	286.00	26.96	102.65	52.51	234.32	0.68	0.86	1.69	0.78	0.75
Multiple R^2	0.91	0.85	0.88	0.84	0.82					
Adjusted R^2	0.88	0.81	0.83	0.79	0.78					
Predicted R^2	0.85	0.73	0.73	0.72	0.69					
Std. Dev.	6.08	1.68	3.12	2.40	5.09					
Mean	53.30	14.15	26.21	17.00	34.83					
C.V. %	11.40	11.90	11.91	14.10	14.62					

Significant codes: '****' $p < 0.0001$, '***' $p < 0.001$, '**' $p < 0.01$ '*' $p < 0.05$

Appendix-Table 3. Experimental outcomes of extracted compounds from anise seed by PW solvent and HAE

Run	T (°C)	Time (min)	Sample-to-Solvent (g/100 mL)	TPC (mg GAE/g dw)	TFC (mg QUE/g dw)	FRAP (mg AEE/g dw)	DPPH (%)	ABTS (%)
1	40	60	6	39.52	7.43	5.31	5.11	1.46
2	40	60	6	33.23	6.78	4.73	4.71	1.23
3	25	100	2	29.35	4.54	4.63	4.11	1.1
4	40	100	6	39.66	7.87	5.69	5.92	1.47
5	40	60	6	30.37	5.98	4.46	3.78	1.11
6	25	20	10	15.15	2.32	2.05	2.03	0.55
7	55	100	2	21.84	4.22	3.51	2.87	0.78
8	40	60	6	39.47	6.77	5.14	5.09	1.42
9	40	60	10	36.64	6.22	4.78	3.92	1.32
10	40	60	6	31.06	6.03	4.53	3.78	1.11
11	55	60	6	20.61	4.78	3.07	2.98	0.78
12	40	60	2	30.81	5.12	5.33	3.66	1.08
13	25	100	10	35.57	8.78	3.88	3.67	1.28
14	55	20	10	12.34	2.43	2.17	2.07	0.45
15	40	60	6	33.42	5.87	4.44	4.32	1.21
16	40	20	6	25.45	4.25	3.41	3.33	0.98
17	55	20	2	9.8	2.11	2.05	1.23	0.39
18	25	60	6	30.65	6.54	4.86	4.01	1.12
19	25	20	2	13.51	2.97	2.63	2.56	0.55
20	55	100	10	23.89	5.93	3.32	3.03	1.01

Appendix-Table 4. ANOVA for anise seed PW extract and HAE

Source	Estimated coefficient					F values				
	TPC	TFC	FRAP	DPPH	ABTS	TPC	TFC	FRAP	DPPH	ABTS
Intercept	33.96	6.45	4.72	4.51	1.24	33.41****	24.28****	20.72****	19.93****	32.30****
A-Temperature	-3.58	-0.57	-0.59	-0.42	-0.12	11.27**	7.58*	17.52**	8.00*	9.68**
B- time	7.41	1.73	1.07	0.84	0.27	48.38****	70.00****	57.24***	31.85****	50.55****
C-Sample ratio	1.83	0.67	0.21	0.03	0.07	2.95	10.61**	2.09	0.04	3.44
AB			-0.4					6.46*		
AC										
BC		0.79					11.58**			
A^2	-12.69	-1.11	-0.76	-1.05	-0.44	71.04****	9.26**	9.25**	16.06**	65.54****
B^2										
C^2		-1.10	-0.67	-0.76			9.10**	7.19*	8.31*	
Residual (SS)	170.06	5.53	2.39	3.09	0.22					
Lack of Fit(SS)	88.49	3.63	1.70	1.26	0.11	0.54	1.19	1.73	0.38	0.46
Multiple R^2	0.90	0.92	0.91	0.88	0.90					
Adjusted R^2	0.87	0.88	0.87	0.83	0.87					
Predicted R^2	0.83	0.75	0.79	0.79	0.83					
Std. Dev.	3.37	0.65	0.41	0.47	0.12					
Mean	27.62	5.35	4.00	3.61	1.02					
C.V. %	12.19	12.20	10.33	13.01	11.86					

Significant codes: '****' $p < 0.0001$, '***' $p < 0.001$, '**' $p < 0.01$ '*' $p < 0.0$

Appendix-Table 5. Experimental outcomes of extracted compounds from hawthorn fruit by EW solvent and MAE

Run	Power (W)	Time (s)	Sample-to-Solvent (g/100 mL)	TPC (mg GAE/g dw)	TFC (mg QUE/g dw)	FRAP (mg AAE/g dw)	DPPH (%)	ABTS (%)
1	450	20	7	12.67	4.13	7.64	6.21	12.33
2	450	70	12	47.12	10.42	18.87	13.56	32.27
3	450	70	7	41.32	10.06	20.07	17.25	39.87
4	800	120	2	33.46	5.69	10.69	11.09	30.31
5	450	70	7	40.11	9.62	18.81	11.12	28.69
6	100	20	2	2.29	0.45	1.42	0.82	2.71
7	450	120	7	48.45	12.69	22.92	17.82	42.26
8	800	70	7	46.65	7.53	15.74	17.54	40.28
9	450	70	7	54.11	12.82	24	18.71	38.86
10	450	70	7	48.56	12.11	25.04	19.32	33.15
11	800	20	12	26.12	6.02	15.56	12.95	30.76
12	800	20	2	4.34	3.26	4.27	2.06	4.6
13	100	70	7	20.24	5.04	9.09	5.59	17.65
14	100	20	12	5.23	1.24	3.48	2.35	6.69
15	450	70	2	35.09	8.16	17.1	15.72	28.11
16	800	120	12	47.56	11.31	19.11	21.61	43.75
17	450	70	7	49.19	12.03	23.11	17.8	34.27
18	450	70	7	51.87	12.57	22.3	17.1	41.78
19	100	120	2	19.27	3.45	9.74	7.34	17.7
20	100	120	12	24.76	5.75	12.78	8.12	20.27

Appendix-Table 6. ANOVA for hawthorn fruit EW extract and MAE

Source	Estimated coefficient					F values				
	TPC	TFC	FRAP	DPPH	ABTS	TPC	TFC	FRAP	DPPH	ABTS
Intercept	45.69	10.88	20.85	16.22	34.83	29.54****	22.79****	18.14****	15.38****	18.77****
A-Power	8.63	1.79	2.89	4.10	8.47	21.21***	13.39**	8.60*	22.46***	27.30***
B-time	12.29	2.38	4.29	4.16	9.72	42.94****	23.71***	18.99***	23.07***	35.98****
C-Sample ratio	5.63	1.37	2.66	2.16	5.03	9.03**	7.90*	7.30*	6.20*	9.64**
AB										
AC				2.39	4.13				6.08*	5.20*
BC										
A^2	-11.33	-4.23	-7.20	-4.24	-6.68	11.69**	23.94***	17.13**	7.67*	5.43*
B^2	-14.21	-2.10	-4.33	-3.79	-8.35	18.40***	5.92*	6.21*	6.13*	8.49*
C^2										
Residual (SS)	492.01	33.42	135.52	97.45	341.40					
Lack of Fit(SS)	332.44	24.28	107.35	53.94	220.35	1.16	1.48	2.12	0.77	1.14
Multiple R^2	0.91	0.89	0.87	0.88	0.90					
Adjusted R^2	0.88	0.85	0.82	0.82	0.85					
Predicted R^2	0.81	0.77	0.71	0.75	0.75					
Std. Dev.	5.93	1.55	3.11	2.74	5.12					
Mean	32.92	7.72	15.09	12.20	27.32					
C.V. %	18.01	20.02	20.62	22.43	18.76					

Significant codes: '****' $p < 0.0001$, '***' $p < 0.001$, '**' $p < 0.01$ '*' $p < 0.05$

Appendix-Table 7. Experimental outcomes of extracted compounds from anise seed by PW solvent and MAE

Run	Power (W)	Time (s)	Sample-to-Solvent (g/100 mL)	TPC (mg GAE/g dw)	TFC (mg QUE/g dw)	FRAP (mg AAE/g dw)	DPPH (%)	ABTS (%)
1	450	20	7	31.67	15.56	9.61	12.45	2.62
2	450	70	12	39.23	17.62	9.71	11.99	3.24
3	450	70	7	41.54	18.18	9.96	13.79	3.63
4	800	120	2	24.46	6.58	6.81	6.83	1.03
5	450	70	7	41.11	17.42	9.89	12.79	3.44
6	100	20	2	18.34	5.82	6.15	4.82	2.54
7	450	120	7	50.54	21.67	11.16	17.36	4.23
8	800	70	7	28.65	11.71	8.51	9.29	2.55
9	450	70	7	43.32	17.98	10.28	15.53	3.76
10	450	70	7	35.34	15.09	9.33	12.75	2.86
11	800	20	12	19.88	8.4	7.22	4.99	1.64
12	800	20	2	15.66	5.01	4.91	3.78	1.15
13	100	70	7	38.19	17.25	10.01	16.52	4.15
14	100	20	12	26.11	10.6	7.93	9.12	2.74
15	450	70	2	37.43	14.25	7.56	9.92	3.15
16	800	120	12	31.56	10.69	8.6	10.98	2.76
17	450	70	7	48.23	20.38	10.88	16.68	4.18
18	450	70	7	44.76	20.13	10.45	14.36	3.73
19	100	120	2	36.32	10.87	7.18	8.44	2.24
20	100	120	12	38.56	14.59	9.92	12.94	4.23

Appendix-Table 8. ANOVA for anise seed PW extract and MAE

Source	Estimated coefficient					F values				
	TPC	TFC	FRAP	DPPH	ABTS	TPC	TFC	FRAP	DPPH	ABTS
Intercept	42.40	18.61	10.23	14.63	3.62	31.75****	33.78****	71.82****	26.26****	14.55****
A-Power	-3.73	-1.67	-0.51	-1.60	-0.68	13.08**	10.64**	17.77***	11.86**	25.71***
B-time	6.98	1.90	0.79	2.14	0.38	45.74****	13.73**	41.44****	21.28***	7.97*
C-Sample ratio	2.31	1.94	1.08	1.62	0.45	5.03*	14.25**	78.00****	12.25**	11.36**
AB										
AC										
BC					0.38					6.44*
A^2	-10.31	-5.34	-1.12	-2.39	-0.55	31.93****	34.70****	26.75***	8.49*	5.49*
B^2										
C^2	-5.40	-3.89	-1.74	-4.34	-0.71	8.75*	18.37***	65.15****	28.00***	9.00*
Residual (SS)	149.04	36.86	2.08	30.11	2.32					
Lack of Fit(SS)	56.39	18.05	0.67	17.99	1.36	0.34	0.53	0.26	0.82	0.89
Multiple R^2	0.92	0.92	0.96	0.90	0.87					
Adjusted R^2	0.89	0.90	0.95	0.87	0.81					
Predicted R^2	0.86	0.85	0.94	0.79	0.72					
Std. Dev.	3.26	1.62	0.39	1.47	0.42					
Mean	34.55	13.99	8.80	11.27	2.99					
C.V. %	9.44	11.60	4.38	13.02	14.11					

Significant codes: '****' $p < 0.0001$, '***' $p < 0.001$, '**' $p < 0.01$ '*' $p < 0.5$.

Appendix-Table 9. Experimental outcomes of extracted compounds from hawthorn fruit by EW solvent and UAE

Run	Ethanol concentration (% v/v)	Extraction time (min)	Sample-to-Solvent (g/100 mL)	TPC (mg GAE/g dw)	TFC (mg QUE/g dw)	FRAP (mg AAE/g dw)	DPPH (%)	ABTS (%)
1	40	15	12	84.18	24.47	31.7	28.52	58.09
2	40	15	2	68.55	22.43	29.12	24.76	48.45
3	40	5	12	51.41	16.98	21.09	18.65	24.98
4	40	5	2	26.66	8.73	12.1	13.14	18.69
5	20	15	12	56.74	17.11	23.29	20.42	29.65
6	20	15	2	36.09	11.56	16.32	14.56	20.18
7	20	5	12	36.48	11.77	16.99	16.09	23.07
8	20	5	2	25.11	6.46	10.74	12.23	15.14
9	30	10	7	86.7	27.11	37.96	32.16	63.84
10	30	10	7	83.03	26.85	36.42	30.13	60.45
11	30	10	7	86.29	28.32	37.76	33.13	64.91
12	30	10	7	74.15	23.32	31.09	25.81	42.44
13	30	10	7	82.7	25.15	35.61	29.73	60.31
14	30	10	7	83.85	27.22	36.89	31.45	61.87
15	40	10	7	76.48	25.01	32.81	26.73	42.7
16	20	10	7	70.78	23.01	29.77	25.07	38.82
17	30	15	7	82.06	25.31	33.62	28.74	52.96
18	30	5	7	42.58	14.11	18.55	16.17	24.29
19	30	10	12	87.1	29.87	38.78	33.79	66.15
20	30	10	2	66.7	21.76	27.77	24.08	42.6

Appendix-Table 10. ANOVA for hawthorn fruit EW extract and UAE

Source	Estimated coefficient					F values				
	TPC	TFC	FRAP	DPPH	ABTS	TPC	TFC	FRAP	DPPH	ABTS
Intercept	81.96	26.46	35.84	30.01	57.00	47.76****	39.80****	39.31****	28.52****	18.49****
A-Solvent	8.21	2.77	2.97	2.34	6.61	22.95***	17.53***	14**	10.79**	9.13**
B-time	14.54	4.28	5.46	4.07	10.32	72.01****	41.89****	47.26****	32.6***	22.26***
C-Sample ratio	9.28	2.93	3.58	2.87	5.69	29.34***	19.55***	20.33***	16.2**	6.77*
AB	5.43		1.97	7.42	6.41	8.03*		4.92*	5.41*	6.87*
AC										
BC										
A^2	-10.93	-3.48	-4.96	-4.02	-12.95	13.02**	8.85*	12.47*	10.18**	11.23**
B^2	-22.24	-7.78	-10.16	-7.47	-15.09	53.91****	44.23****	52.42****	35.08***	15.24**
C^2										
Residual (SS)	381.57	61.31	81.94	66.12	621.41					
Lack of Fit(SS)	278.20	45.23	49.78	32.88	276.78	1.68	1.56	0.97	0.62	0.50
Multiple R^2	0.96	0.93	0.95	0.93	0.90					
Adjusted R^2	0.94	0.91	0.92	0.90	0.85					
Predicted R^2	0.89	0.84	0.88	0.86	0.80					
Std. Dev.	5.42	2.09	2.51	2.26	6.91					
Mean	65.38	20.83	27.92	24.27	42.98					
C.V. %	8.29	10.05	8.99	9.29	16.09					

Significant codes: '****' $p < 0.0001$, '***' $p < 0.001$, '**' $p < 0.01$ '*' $p < 0.05$

Appendix-Table 11. Experimental outcomes of extracted compounds from anise seed by EW solvent and UAE

Run	Ethanol Concentration (% v/v)	Extraction time (min)	Sample-to-Solvent (g/100 mL)	TPC (mg GAE/g dw)	TFC (mg QUE/g dw)	FRAP (mg AAE/g dw)	DPPH (%)	ABTS (%)
1	20	15	12	36.82	14.53	7.33	14.98	2.08
2	20	15	2	34.78	13.25	6.99	12.65	1.91
3	20	5	12	30.9	12.34	6.17	8.23	1.69
4	20	5	2	24.92	9.78	4.99	7.83	1.35
5	0	15	12	31.25	12.78	6.11	10.34	1.72
6	0	15	2	25.8	9.84	5.27	8.45	1.36
7	0	5	12	26.17	10.75	5.29	9.05	1.45
8	0	5	2	17.9	6.27	3.81	5.76	1.01
9	10	10	7	41.23	15.76	8.15	12.92	2.23
10	10	10	7	38.61	14.18	7.68	12.22	2.15
11	10	10	7	39.82	14.69	7.94	12.34	2.09
12	10	10	7	38.18	13.75	7.51	11.34	1.93
13	10	10	7	38.53	14.11	7.8	12.95	1.98
14	10	10	7	38.83	14.5	7.76	11.87	1.87
15	20	10	7	38.21	14.09	7.66	10.82	2.01
16	0	10	7	35.17	13.36	7.03	11.01	1.93
17	10	15	7	38.22	14.1	7.62	12.12	2.04
18	10	5	7	28.94	11.02	5.62	10.09	1.52
19	10	10	12	43.26	16.24	8.62	13.65	2.46
20	10	10	2	33.57	13.2	6.71	10.23	1.86

Appendix-Table 12. ANOVA for anise seed EW extract and UAE

Source	Estimated coefficient					F values				
	TPC	TFC	FRAP	DPPH	ABTS	TPC	TFC	FRAP	DPPH	ABTS
Intercept	39.27	14.61	7.82	12.21	2.08	56.91****	41.35****	51.74****	19.83****	25.49****
A-Solvent	2.93	1.1	0.56	0.99	0.15	31.61****	25.06***	28.31***	13.51***	15.08**
B-time	3.80	1.43	0.74	1.67	0.21	53.13****	42.67****	49.44****	42.59****	26.72***
C-Sample ratio	3.14	1.43	0.57	0.94	0.19	36.27****	42.43*	29.53****	17.69**	22.31***
AB				3.80					9.92**	
AC										
BC										
A^2	-3.66	-1.1	-0.65	-1.36	-0.15	15.77**	8.01*	12.10**	8.15*	4.70*
B^2	-6.77	-2.26	-1.38	-1.17	-0.35	53.90****	34.01****	54.09****	6.03*	23.29***
C^2										
Residual (SS)	38.13	6.75	1.57	9.43	0.23					
Lack of Fit(SS)	31.65	4.31	1.33	7.52	0.13	2.71	0.98	3.04	2.45	0.78
Multiple R^2	0.95	0.94	0.95	0.90	0.90					
Adjusted R^2	0.94	0.91	0.93	0.86	0.87					
Predicted R^2	0.89	0.86	0.88	0.70	0.81					
Std. Dev.	1.65	0.69	0.33	0.85	0.13					
Mean	34.06	12.93	6.80	10.94	1.83					
C.V. %	4.85	5.37	4.92	7.78	6.98					

Significant codes: '****' $p < 0.0001$, '***' $p < 0.001$, '**' $p < 0.01$ '*' $p < 0.05$.

Appendix-Table 13. Experimental outcomes of recovered total phenolics, and flavonoid compounds and their respective antioxidant activities from the hawthorn fruit, permeate flux, fouling index, membrane resistance, and fouling resistance

Run	T (°C)	TMP (bar)	TPC (mg GAE/ g dw)	TFC (mg QUE/ g dw)	FRAP (mg AAE/ g dw)	DPPH (%)	ABTS (%)	Permeate flux $L/(m^2 \cdot h)$	Fouling index (%)	Membrane resistance (R_m) 1/m	Fouling resistance (R_f) 1/m
1	35	40	117.51	19.65	63.96	20.86	74.22	6.64	29.72	$2.07 \cdot 10^{14}$	$6.23 \cdot 10^{13}$
2	35	30	90.06	15.98	49.43	18.73	53.65	4.92	37.37	$2.15 \cdot 10^{14}$	$6.50 \cdot 10^{13}$
3	25	40	85.67	15.08	48.31	17.79	51.76	4.56	40.06	$1.77 \cdot 10^{14}$	$5.62 \cdot 10^{13}$
4	45	20	33.58	7.64	18.98	13.62	20.84	2.45	49.92	$2.36 \cdot 10^{14}$	$4.45 \cdot 10^{13}$
5	35	20	80.14	13.72	44.98	17.44	49.25	2.36	47.22	$2.25 \cdot 10^{14}$	$6.111 \cdot 10^{13}$
6	25	30	46.73	10.65	25.07	14.75	31.99	2.85	43.33	$1.83 \cdot 10^{14}$	$9.08 \cdot 10^{13}$
7	25	20	44.65	10.3	24.28	14.38	30.86	1.95	50.22	$1.66 \cdot 10^{14}$	$1.92 \cdot 10^{14}$
8	45	30	37.14	9.17	19.97	13.86	22.86	4.8	36.45	$2.53 \cdot 10^{14}$	$2.95 \cdot 10^{14}$
9	45	40	66.01	13.42	35.27	15.84	40.63	5.07	27.71	$2.47 \cdot 10^{14}$	$1.64 \cdot 10^{14}$
10	35	30	92.33	15.83	50.62	18.53	55.75	4.54	38.85	$2.19 \cdot 10^{14}$	$6.87 \cdot 10^{13}$
11	35	30	92.72	16.07	50.76	18.60	55.82	4.89	39.35	$2.19 \cdot 10^{14}$	$6.76 \cdot 10^{13}$

Appendix-Table 14 (a). ANOVA reverse osmosis concentration of hawthorn fruit extracts (TPC, TFC, FRAP, DPPH, and ABTS)

Source	Estimated coefficient					F-values				
	TPC	TFC	FRAP	DPPH	ABTS	TPC	TFC	FRAP	DPPH	ABTS
Intercept	89.94	15.76	49.22	18.5	54.67	143.67****	165.53****	90.37****	109.43****	294.80****
A-Temperature	-6.72	-0.97	-3.91	-0.59	-5.05	20.70**	28.88**	14.55**	16.58**	67.53***
B-TMP	18.47	2.75	9.88	1.51	10.94	156.28****	232.86****	93.08****	105.28****	317.45***
AB										
A^2	-45.34	-5.54	-25.12	-4.01	-26.63	397.64****	399.58****	253.85****	314.65****	793.82****
B^2	11.55	1.24	6.83	0.83	7.68	25.82**	19.99**	18.75**	13.52*	66.05***
Residual (SS)	78.58	1.17	37.78	0.78	13.58					
Lack of Fit (SS)	74.43	1.14	36.72	0.76	10.53	8.97	18.80	17.23	18.46	1.73
Multiple R^2	0.99	0.99	0.98	0.99	0.99					
Adjusted R^2	0.98	0.99	0.97	0.98	0.99					
Predicted R^2	0.96	0.96	0.93	0.94	0.98					
Std. Dev.	3.62	0.44	2.51	0.36	1.5					
Mean	71.51	13.41	39.24	16.77	44.33					
C.V. %	5.06	3.29	6.39	2.15	3.39					

Appendix-Table 14 (b). ANOVA reverse osmosis concentration of hawthorn fruit extracts (permeate flux, fouling index, membrane resistance, and fouling resistance)

Source	Estimated coefficient				F-values			
	Permeate flux	Fouling index	Membrane resistance (R_m)	Fouling resistance (R_f)	Permeate flux	Fouling index	Membrane resistance (R_m)	Fouling resistance (R_f)
Intercept	4.67	38.50	$+2.14 \cdot 10^{14}$	$+6.49 \cdot 10^{13}$	23.54***	93.25****	49.90****	305.39****
A-Temperature	0.49	-3.26	$+3.51 \cdot 10^{13}$	$-9.99 \cdot 10^{12}$	5.26*	44.28***	99.77****	22.04**
B-TMP	1.59	-8.31	$+5.84 \cdot 10^{11}$	$-2.54 \cdot 10^{12}$	54.38***	288.75****	0.027	2.15
AB		-3.01		$+6.38 \cdot 10^{13}$		25.28**		899.82**
A^2	-1.05	2.78		$+4.92 \cdot 10^{13}$	10.98*	14.67**		297.56**
B^2								
Residual (SS)	1.94	8.61	$5.94 \cdot 10^{26}$	$7.25 \cdot 10^{25}$				
Lack of Fit (SS)	1.85	6.49	$5.82 \cdot 10^{26}$	$6.53 \cdot 10^{25}$	8.29	1.53	16.08	9.01
Multiple R^2	0.91	0.98	92.58	99.67				
Adjusted R^2	0.87	0.97	90.72	99.35				
Predicted R^2	0.75	0.91	82.80	92.86				
Std. Dev.	0.53	1.20	$8.62 \cdot 10^{12}$	$4.25 \cdot 10^{12}$				
Mean	4.09	40.02	$2.14 \cdot 10^{14}$	$8.68 \cdot 10^{13}$				
C.V. %	12.86	2.99	4.03	4.90				

Significant codes: '****' $p < 0.0001$, '***' $p < 0.001$, '**' $p < 0.01$ '*' $p < 0.05$.

Appendix-Table 15. Experimental outcomes of recovered total phenolics, and flavonoid compounds and their respective antioxidant activities from the anise seed, permeate flux, fouling index, membrane resistance, and fouling resistance

Run	T (°C)	TMP (bar)	TPC (mg GAE/ g dw)	TFC (mg QUE/ g dw)	FRAP (mg AAE/ g dw)	DPPH (%	ABTS (%)	Permeate flux ($L/m^2.h$)	Fouling index (%)	Membrane resistance (R_m) 1/m	Fouling resistance (R_f) 1/m
1	35	40	92.86	9.73	13.75	8.87	4.4	15.06	23.79	$1.16 \cdot 10^{14}$	$5.23 \cdot 10^{13}$
2	35	30	61.9	7.09	10.28	6.62	3.1	13.15	29.79	$1.22 \cdot 10^{14}$	$4.78 \cdot 10^{13}$
3	25	40	63.02	7.76	10.92	6.14	3.29	10.75	31.51	$9.69 \cdot 10^{13}$	$4.99 \cdot 10^{13}$
4	45	20	24.67	2.68	5.53	2.52	1.19	5.26	33.76	$1.49 \cdot 10^{14}$	$7.88 \cdot 10^{13}$
5	35	20	55.68	6.43	9.29	5.38	2.32	6.53	35.68	$1.41 \cdot 10^{14}$	$1.53 \cdot 10^{14}$
6	25	30	36.54	5.73	7.21	4.4	2.09	5.57	38.21	$1.16 \cdot 10^{14}$	$1.16 \cdot 10^{14}$
7	25	20	36.4	5.03	6.86	4.12	1.98	5.18	38.65	$1.06 \cdot 10^{14}$	$1.08 \cdot 10^{14}$
8	45	30	27.23	4.44	5.72	2.88	1.42	10.56	30.08	$1.69 \cdot 10^{14}$	$1.51 \cdot 10^{14}$
9	45	40	47.32	6.12	8.7	5.2	2.62	14.82	30.60	$1.53 \cdot 10^{14}$	$1.77 \cdot 10^{14}$
10	35	30	64.28	6.87	11.06	6.4	2.66	12.15	27.94	$1.28 \cdot 10^{14}$	$5.17 \cdot 10^{13}$
11	35	30	64.11	6.92	11.06	6.34	2.72	12.12	25.89	$1.24 \cdot 10^{14}$	$5.46 \cdot 10^{13}$

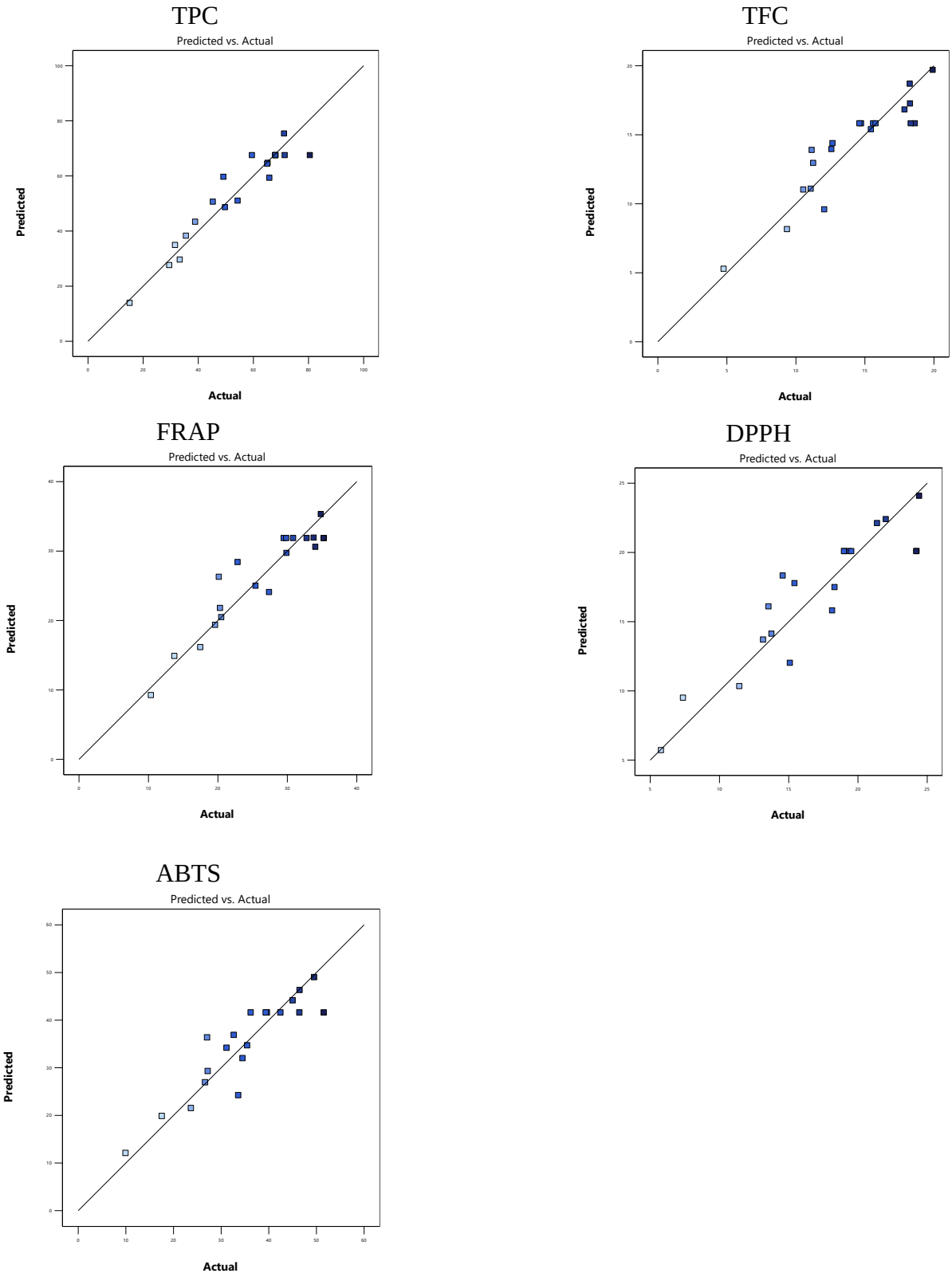
Appendix-Table 16 (a). ANOVA reverse osmosis concentration of anise seed extracts (TPC, TFC, FRAP, DPPH, and ABTS)

Source	Estimated coefficient					F-values				
	TPC	TFC	FRAP	DPPH	ABTS	TPC	TFC	FRAP	DPPH	ABTS
Intercept	63.41	7.13	10.63	6.42	2.84	90.23****	66.22****	76.25****	77.79****	41.26***
A-Temperature	-6.12	-0.88	-0.84	-0.67	-0.35	20.29**	37.37***	19.59**	26.20**	16.15**
B-TMP	14.41	1.58	1.95	1.37	0.8	112.46****	119.87****	105.79****	106.70****	82.45***
AB										
A^2	-31.48	-2.3	-3.91	-2.72	-1.08	226.60****	107.49**	179.46****	178.19****	63.18***
B^2	10.91	0.69	1.15	0.76	0.52	27.20**	9.82*	15.60**	14.19 **	14.83**
Residual (SS)	66.47	0.74	1.29	0.62	0.28					
Lack of Fit (SS)	62.94	0.72	0.89	0.58	0.17	8.91	13.06	1.10	6.70	0.77
Multiple R^2	0.98	0.98	0.98	0.98	0.96					
Adjusted R^2	0.97	0.96	0.97	0.97	0.94					
Predicted R^2	0.93	0.91	0.93	0.92	0.88					
Std. Dev.	3.33	0.35	0.46	0.32	0.21					
Mean	52.19	6.26	9.13	5.36	2.53					
C.V. %	6.38	5.64	5.08	6.04	8.56					

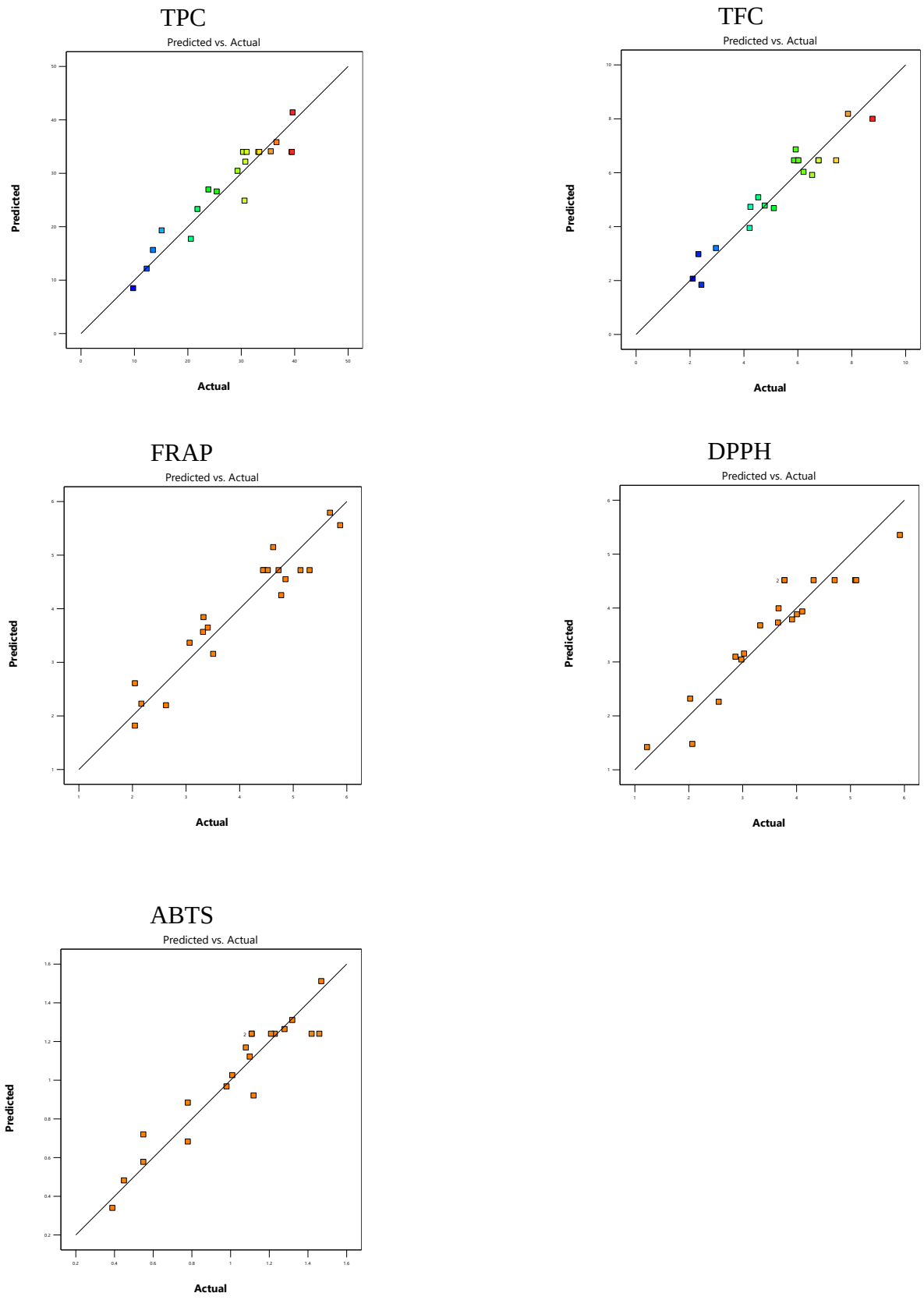
Appendix-Table 16 (b). ANOVA reverse osmosis concentration of anise seed extracts (permeate flux, fouling index, membrane resistance, and fouling resistance)

Source	Estimated coefficient				F-values			
	Permeate flux	Fouling index	Membrane resistance (R_m)	Fouling resistance (R_f)	Permeate flux	Fouling index	Membrane resistance (R_m)	Fouling resistance (R_f)
Intercept	11.80	28.62	$+1.29 \cdot 10^{14}$	$+5.44 \cdot 10^{13}$	25.22****	10.86**	22.90***	54.08***
A-Temperature	1.52	-2.32	$+2.54 \cdot 10^{13}$	$+2.20 \cdot 10^{13}$	7.88*	5.61*	43.95***	40.03**
B-TMP	3.94	-3.70	$-5.22 \cdot 10^{12}$	$+1.23 \cdot 10^{13}$	52.83***	14.24**	1.86	9.36*
AB				$+3.91 \cdot 10^{13}$				83.98***
A^2	-3.11	5.18		$+7.49 \cdot 10^{13}$	14.96**	12.73 **		143.69***
B^2				$-2.35 \cdot 10^{13}$				14.94*
Residual (SS)	12.36	40.33	$7.04 \cdot 10^{26}$	$2.18 \cdot 10^{26}$				
Lack of Fit (SS)	11.67	32.71	$6.85 \cdot 10^{26}$	$2.91 \cdot 10^{26}$	6.79	1.72	11.84	11.37
Multiple R^2	0.92	0.82	0.8513	0.9854				
Adjusted R^2	0.88	0.75	0.8142	0.9672				
Predicted R^2	0.75	0.52	0.7049	0.7866				
Std. Dev.	1.33	2.40	$9.38 \cdot 10^{12}$	$8.53 \cdot 10^{12}$				
Mean	10.10	31.45	$1.29 \cdot 10^{14}$	$8.88 \cdot 10^{13}$				
C.V. %	13.15	7.63	7.24	9.61				

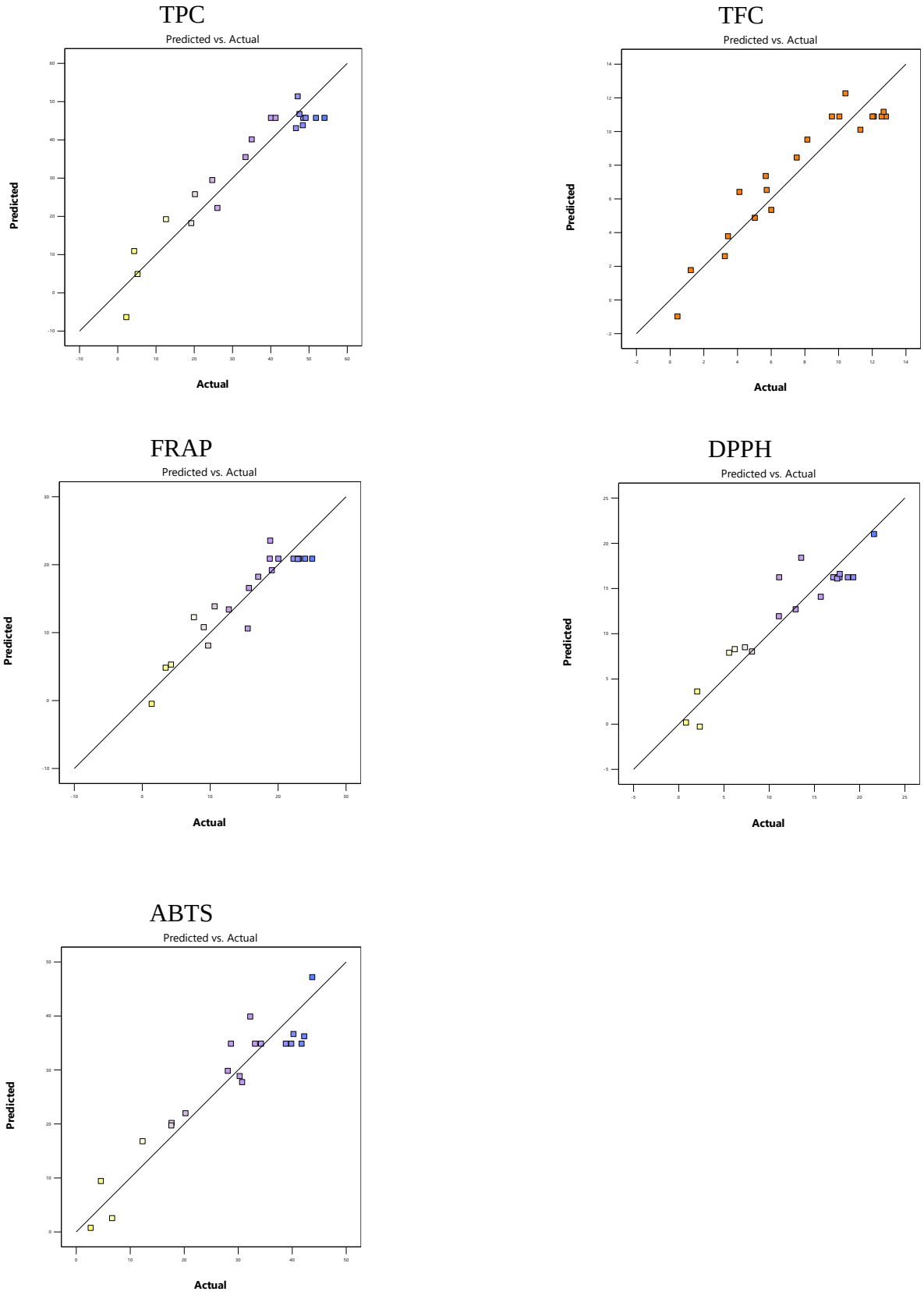
Significant codes: '****' $p < 0.0001$, '***' $p < 0.001$, '**' $p < 0.01$ '*' $p < 0.05$



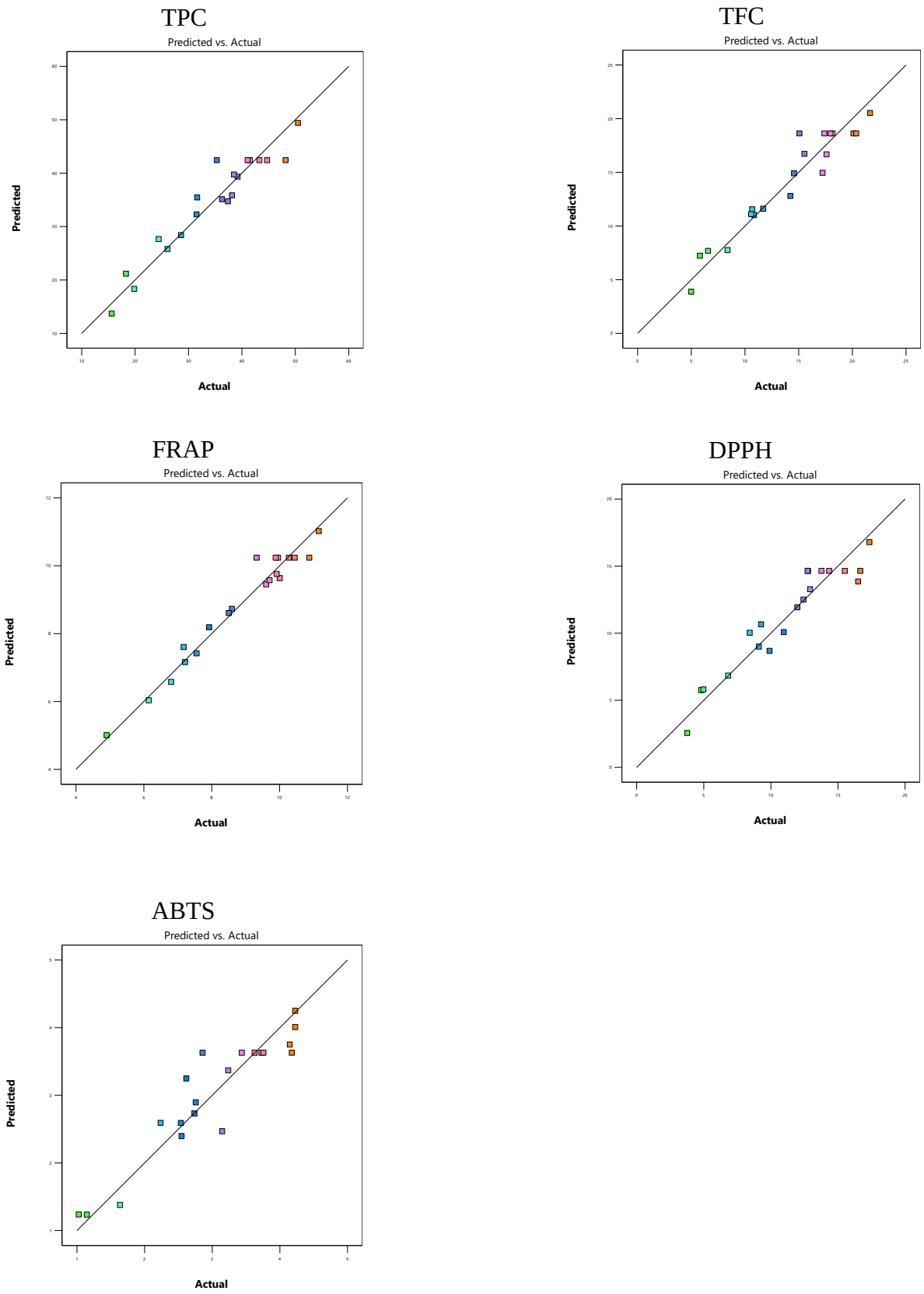
Appendix-Fig 1. Correlation between the predicted values and actual values calculated by the model according to the regression equations (hawthorn fruit EW extracts) (HAE)



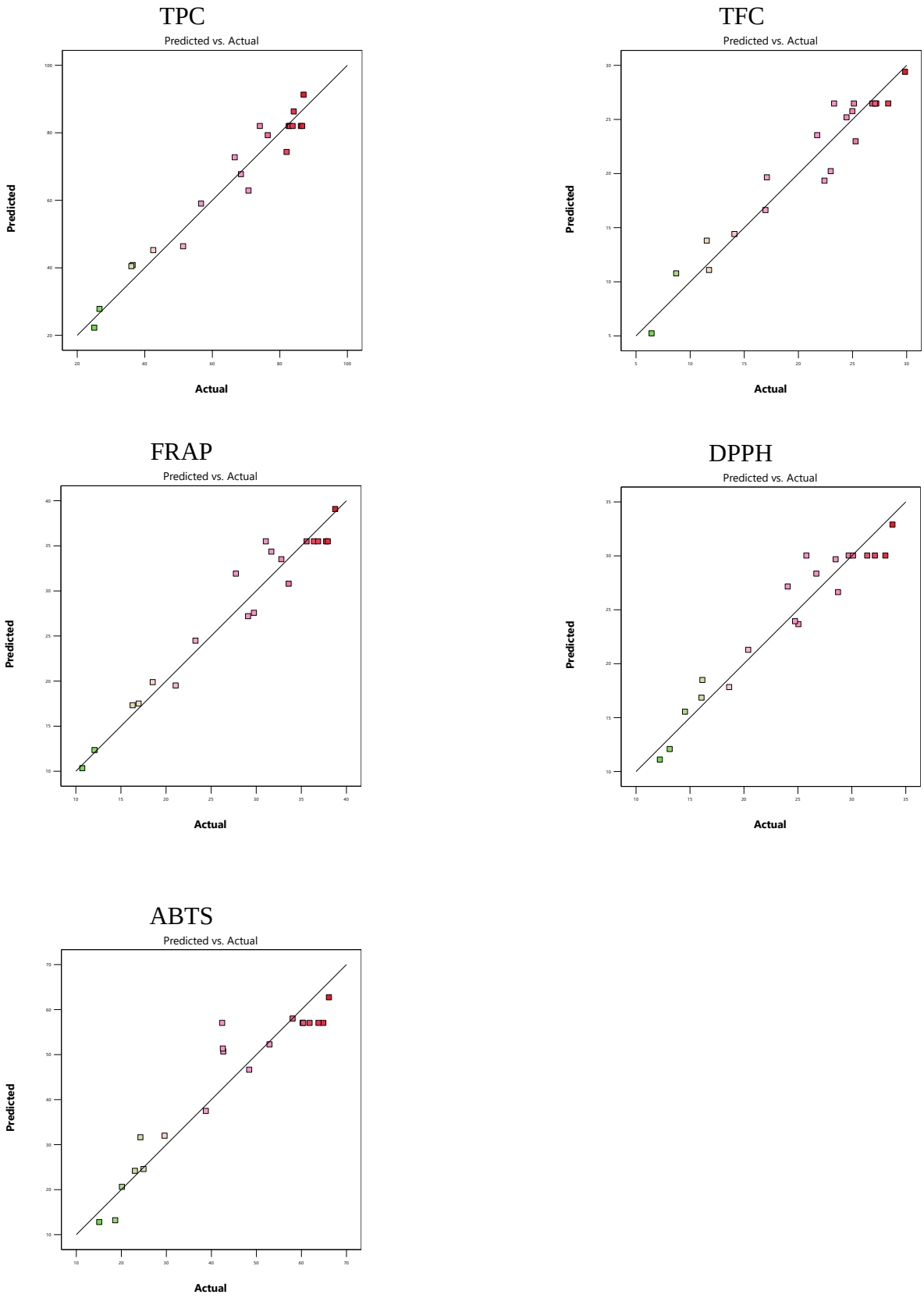
Appendix-Fig 2. Correlation between the predicted values and actual values calculated by the model according to the regression equations (anise seed PW extracts) (HAE)



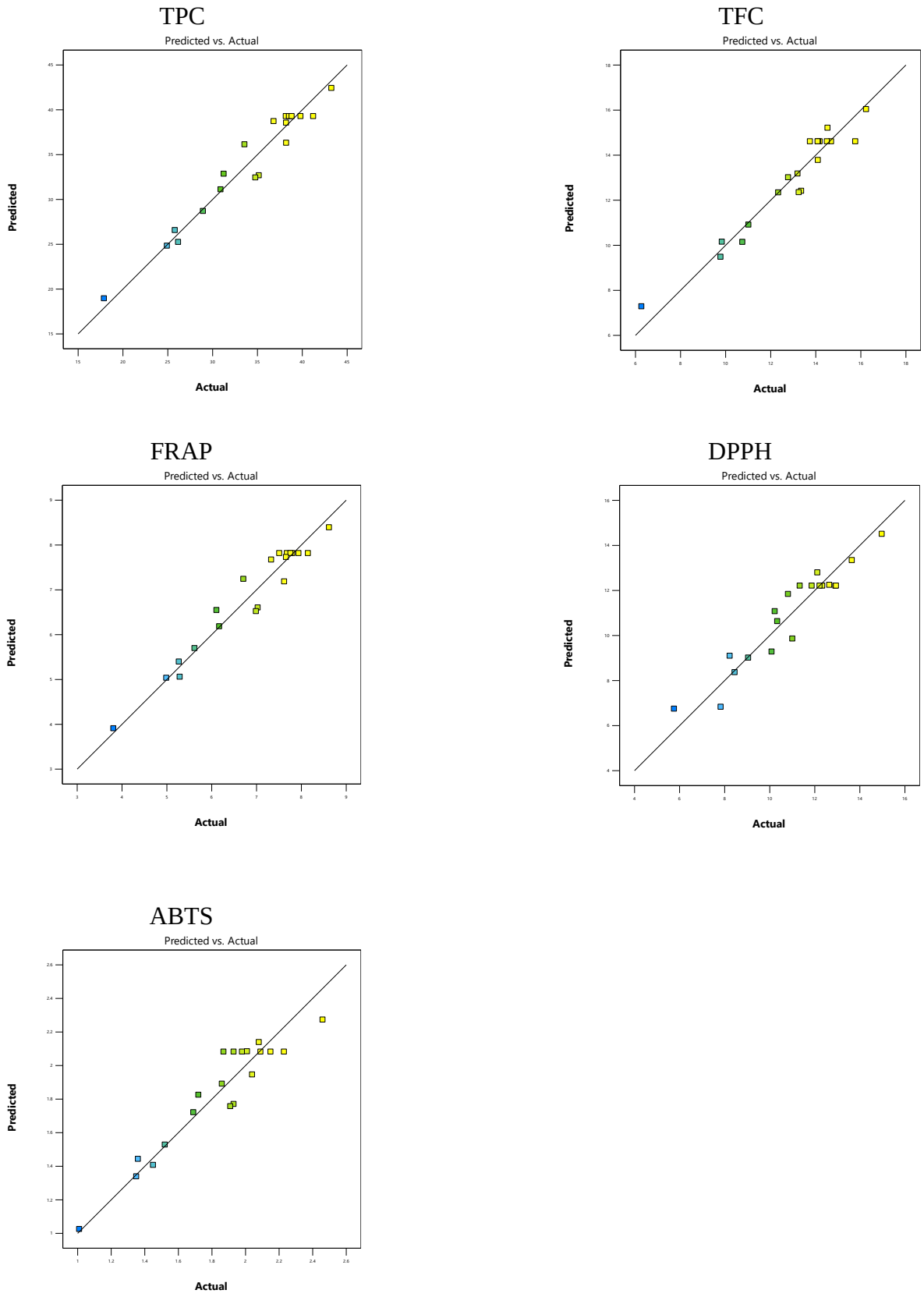
Appendix-Fig 3. Correlation between the predicted values and actual values calculated by the model according to the regression equations (hawthorn fruit EW extracts) (MAE



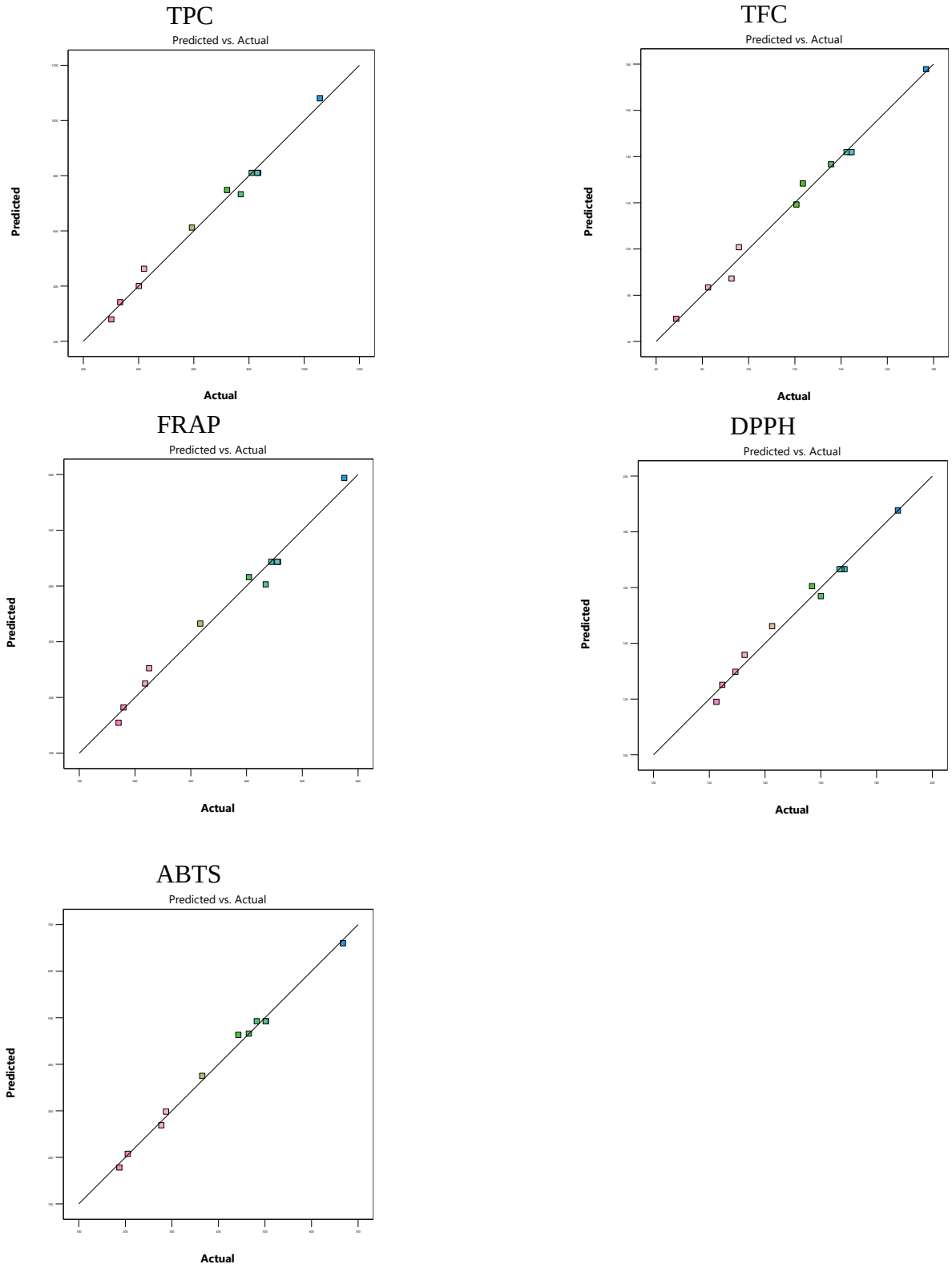
Appendix-Fig 4. Correlation between the predicted values and actual values calculated by the model according to the regression equations (anise fruit PW extracts) (MAE)



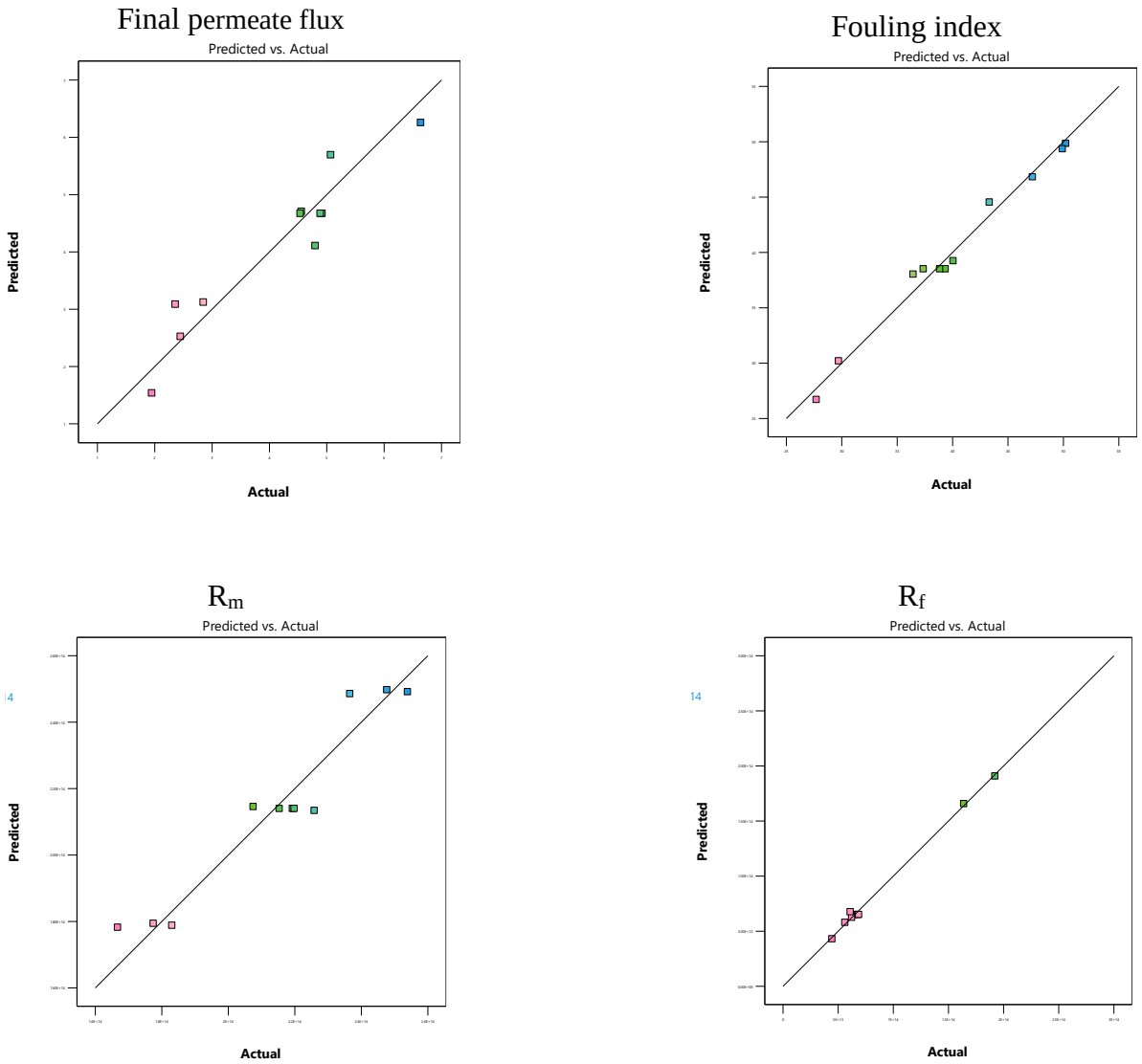
Appendix-Fig 5. Correlation between the predicted values and actual values calculated by the model according to the regression equations (hawthorn fruit EW extracts) (UAE)



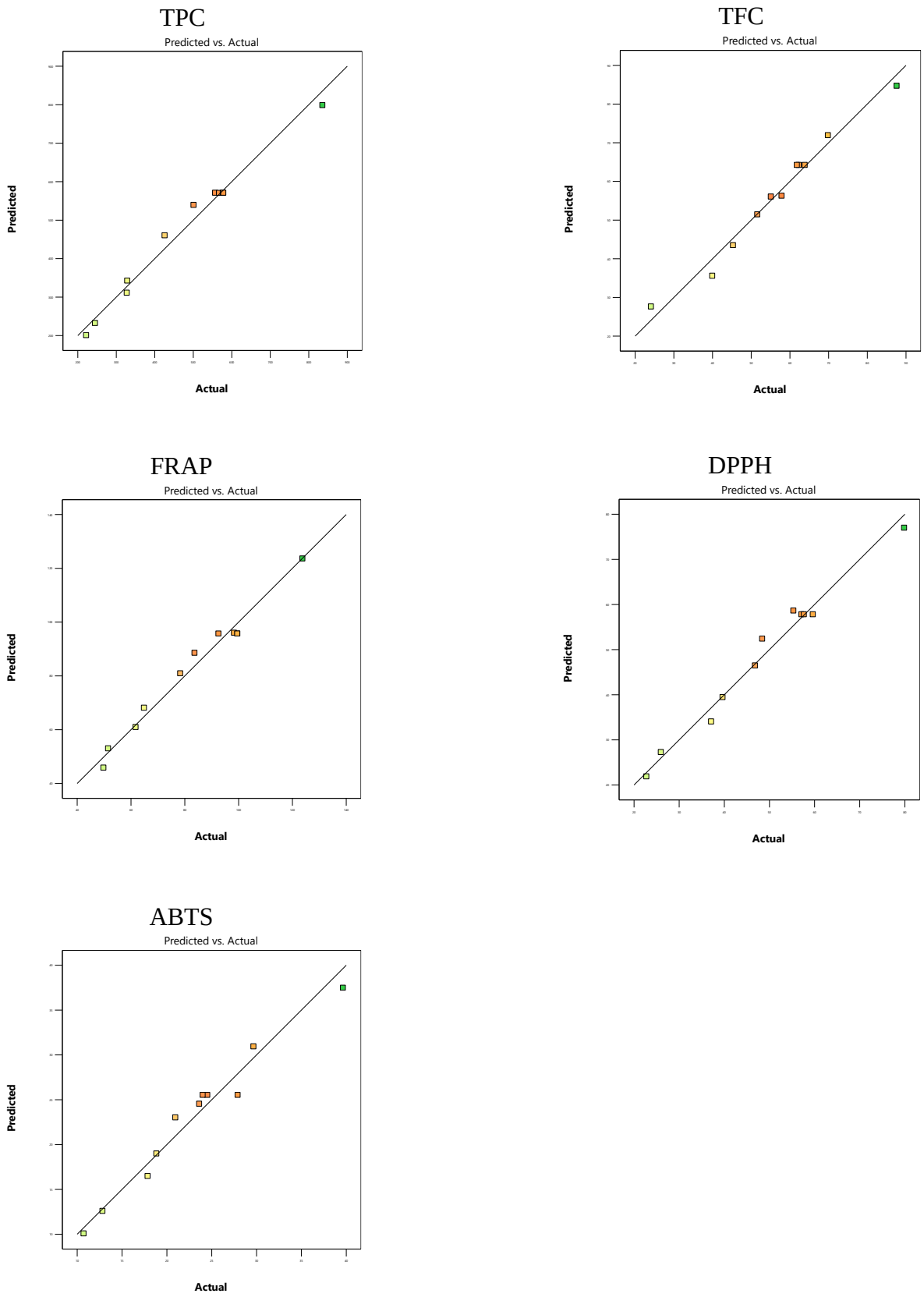
Appendix-Fig 6. Correlation between the predicted values and actual values calculated by the model according to the regression equations (anise seed EW extracts) (UAE)



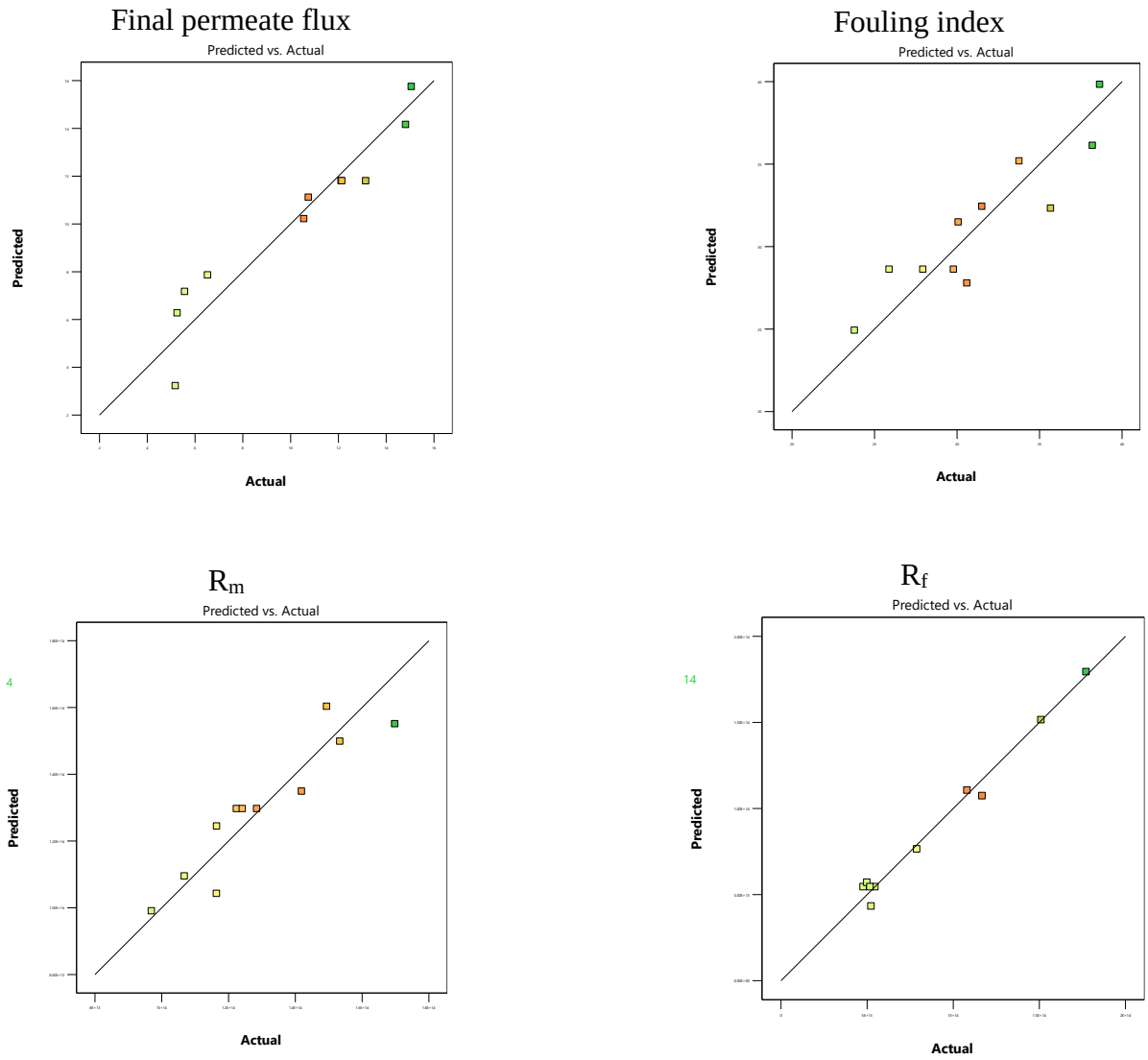
Appendix-Fig 7. Correlation between the predicted values and actual values calculated by the model according to the regression equations (Reverse osmosis concentration of hawthorn fruit extracts) for (TPC, TFC, FRAP, DPPH, and ATBS in the final retentate)



Appendix-Fig 8. Correlation between the predicted values and actual values calculated by the model according to the regression equations (Reverse osmosis concentration of hawthorn fruit extracts) for (final permeate flux, fouling index, , membrane resistance R_m, and fouling resistance R_f)



Appendix-Fig 9. Correlation between the predicted values and actual values calculated by the model according to the regression equations (Reverse osmosis concentration of anise seed extracts) for) (TPC, TFC, FRAP, DPPH, and ABTS in the final retentate)



Appendix-Fig 10. Correlation between the predicted values and actual values calculated by the model according to the regression equations (Reverse osmosis concentration of anise seed extracts) for (final permeate flux, fouling index, membrane resistance R_m, and fouling resistance R_f)

8 ACKNOWLEDGEMENT

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