

T. R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

**ULTRASOUND-ASSISTED EXTRACTION OF POLYPHENOLS
FROM GALANGAL FOLLOWED BY MEMBRANE
SEPARATION PROCESSES**

VERANUR GÜNGÖR
A THESIS SUBMITTED FOR THE DEGREE OF
MASTER OF SCIENCE
DEPARTMENT OF CHEMICAL ENGINEERING

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THESIS SUPERVISOR
PROF. DR. MURAT ÖZDEMİR
II. THESIS SUPERVISOR
PROF. DR. COŞKUN AYDINER

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T.C.
GEBZE TEKNİK ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ

HAVLICANDAN ULTRASON-DESTEKLİ
POLİFENOL EKSTRAKSİYONU
ARDINDAN MEMBRAN PROSESLERİ İLE
AYRIŞTIRILMASI

VERANUR GÜNGÖR
YÜKSEK LİSANS TEZİ
KİMYA MÜHENDİSLİĞİ ANABİLİM DALI

DANIŞMANI
PROF.DR. MURAT ÖZDEMİR
II. DANIŞMANI
PROF. DR. COŞKUN AYDINER

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JÜRİ

ÜYE

(TEZ DANIŞMANI) : Prof. Dr. Murat ÖZDEMİR

ÜYE

: Prof. Dr. Mehmet MELİKOĞLU

ÜYE

: Prof. Dr. İbrahim DOYMAZ

ONAY

Gebze Teknik Üniversitesi Fen Bilimleri Enstitüsü Yönetim Kurulu'nun

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SUMMARY

Polyphenols from galangal were extracted by ultrasound-assisted extraction (UAE), and with screening experiments, the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (DPPH and ABTS), were evaluated. The most effective intervals were determined as the temperature of 20-60 °C, time of 10-60 minutes, and solvent/solid ratio of 10-30. Other parameters have been taken as constant for ethanol/water ratio of 50%, the power of ultrasound of 50%, and cycle of ultrasound of 5. UAE parameters were optimized using the response surface method (RSM), Box-Behnken experimental design. Temperature, time and solvent/solid ratio were determined as factors and their effects on the independent variables which are total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (DPPH and ABTS) were evaluated. The correlation of the studies and the independent parameters in the optimization study were evaluated using Pearson Correlation. Optimum conditions were determined using the desirability function of 0.77, at 47.5°C, 52.5 min, and 30 solvent/solid ratio.

The optimized galangal extract was concentrated by direct ultrafiltration (UF) and nanofiltration (NF) and their combination, and the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (DPPH and ABTS), total solids, total dissolved solids, total suspended solids total carbohydrate contents, color, turbidity and clarity were evaluated. SEM images and contact angles of the treated membranes were also evaluated. The concentrating ability of the membranes was evaluated and the most successful membrane was determined.

Key Words: Galangal, Ultrasound-Assisted Extraction, Optimization, Antioxidant Activity, Total Polyphenol Content, Membrane Process.

ÖZET

Havlıcandan ultrases-destekli polifenol ekstraksiyonu yapıldı ve ön çalışmalarda sıcaklık, süre, etanol/su oranı, çözücü/katı oranı, ultrases güç seviyesi ve ultrases sıklığı parametrelerinin toplam fenolik içeriği (TPC), toplam flavonoid içeriği (TFC) ve antioksidan aktiviteye (DPPH ve ABTS) etkisi değerlendirildi. En etkin sıcaklık aralığı 20-60 °C, en etkin süre aralığı 10-60 dakika, en etkin çözücü/katı oranı 10-30 olarak; etanol/su oranı %50, ultrases güç seviyesi %50 ve ultrases sıklığı 5 olarak belirlendi. Yanıt yüzey yöntemi (RSM), Box-Behnken deneysel tasarımı kullanılarak ultrases-destekli ekstraksiyon parametreleri optimize edildi. Sıcaklık, süre ve çözücü/katı oranı faktör olarak belirlendi ve bağımsız değişkenler olan toplam fenolik içeriği (TPC), toplam flavonoid içeriği (TFC) ve antioksidan aktiviteye (DPPH ve ABTS) etkisi değerlendirildi. Ön çalışmaların ve optimizasyon çalışmasındaki bağımsız parametrelerinin birbirleri ile korelasyonu Pearson Korelasyonu kullanılarak değerlendirildi. Optimum koşullar, 47,5 °C, 52,5 dakika ve 30 katı çözücü oranı olarak, arzu edilebilirlik fonksiyonu kullanılarak belirlendi ve ultases-destekli ekstraksiyonun etkinliği gösterildi.

Optimize edilen havlıcan ekstraktı, direkt ultrafiltrasyon (UF) ve nanofiltrasyon (NF) ve bunların bir arada kullanılması ile konsantre edildi ve toplam fenolik içeriği (TPC), toplam flavonoid içeriği (TFC), antioksidan aktivite (DPPH ve ABTS), toplam katı, toplam çözünmüş katı, toplam askıda katı, toplam karbonhidrat içerikleri, renk, bulanıklık ve berraklıktaki değişimler değerlendirildi. İşlem görmüş membranların SEM görüntüleri ve temas açıları değerlendirildi. Membranların konsantre etme kabiliyetleri değerlendirildi ve en başarılı olan membran belirlendi.

Anahtar Kelimeler: Havlıcan, Ultrases-Destekli Ekstraksiyon, Optimizasyon, Antioksidan Aktivite, Toplam Polifenol İçeriği, Membran İşlemi.

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LIST of ABBREVIATIONS and ACRONYMS

<u>Abbreviations and Acronyms</u>	<u>Explanations</u>
A_0	: Absorbance of the DPPH solution
A_f	: Absorbance of feed
A_m	: Effective membrane area in m ³
A_s	: Absorbance of the sample solution
CA	: Cellulose acetate
C_f	: Amount of compound in permeate
C_p	: Amount of compound in feed
CV	: Coefficient of variance
J_i	: Initial permeate flux in L/m ³ .h
J_s	: Steady-state permeate flux in L/m ³ .h
J_v	: Volumetric permeate flux in L/m ³ .h
m_A	: Weight of the dried sample + dish as mg
m_B	: Weight of the dish as mg
R^2	: Coefficient of determination
Adj. R^2	: Adjusted coefficient of determination
R_c	: Retention coefficient
TC_r	: Total carbohydrate of retentate in mg GE/ml extract
TC_p	: Total carbohydrate of permeate in mg GE/ml extract
TDS_r	: Total dissolved solids of retentate in mg/ml
TDS_p	: Total dissolved solids of permeate in mg/ml
TS_r	: Total solids of retentate in mg/ml
TS_p	: Total solids of permeate in mg/ml
T_p	: Turbidity of permeate in NTU
T_r	: Turbidity of retentate in NTU
V_f	: Feed volume in ml
V_r	: Retentate volume in ml
β_0	: Coefficients of the intercept
β_i	: Coefficients of the linear
β_{ii}	: Coefficients of the quadratic

β_{ij}	: Coefficients of the interaction
a^*	: Lightness component or luminance from black (0) to white (100)
b^*	: Chromatic components from green (-120) to red (+120)
L^*	: Chromatic components from blue (-120) to yellow (+120)
C^*	: Chroma color
H^*	: Hue angle
X_i	: Independent variable
X_j	: Independent variable
X_1	: Independent variable (temperature)
X_2	: Independent variable (time)
X_3	: Independent variable (solvent/solid ratio)
ABTS	: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
ANOVA	: Analysis of variance
BBD	: Box-Behnken experimental design
DPPH	: 2,2-Diphenyl-1-picrylhydrazyl
FAO	: Food and Agriculture Organization
FRAP	: Ferric reducing antioxidant power
GAE	: Gallic acid equivalent
GE	: Glucose equivalent
MF	: Microfiltration
MWCO	: Molecular weight cut-off
NF	: Nanofiltration
NTU	: Nephelometric turbidity unit
PA	: Polyamide
PS	: Polysulfone
PTFE	: Polytetrafluoroethylene
RO	: Reverse osmosis
ROS	: Reactive oxygen species
RSM	: Response surface methodology
s/s ratio	: Solvent/solid ratio
SEM	: Scanning electron microscopy
t	: Filtration time in min

TC	: Total carbohydrates
TC _p	: Total carbohydrates of permeate
TC _f	: Total carbohydrates of feed
TDS	: Total dissolved solids
TDS _p	: Total dissolved solids of permeate
TDS _f	: Total dissolved solids of feed
TS	: Total solids
TS _p	: Total solids of permeate
TS _f	: Total solids of feed
TSS	: Total suspended solids
TSS _p	: Total suspended solids of permeate
TSS _f	: Total suspended solids of feed
TE	: Trolox equivalent
TEAC	: Trolox equivalent antioxidant capacity
TFC	: Total flavonoid content
TPC	: Total phenolic content
TS	: Total solids
UAE	: Ultrasound-assisted extraction
UF	: Ultrafiltration
V	: Total volume of the permeate in m ³
VRF	: Volume reduction factor
Y	: Output variable

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1. INTRODUCTION

Fruits, vegetables and herbs are the main parts of balanced nutrition. United Nations Food and Agriculture Organization (FAO) reported that the importance of plant-derived products in the nutrition and food industry is increasing. [Bakht et al., 2019]. Changes in the consumer market for industrialized foods make consumers to prefer functional foods that have beneficial effects on human health and welfare compared to food products containing synthetic antioxidants, chemical preservatives and additives [Tabio-Garcia et al., 2021].

Herbal products have been extensively used in traditional medicine and treatments from past ages to the present [Ramirez-Godinez et al., 2017]. Besides traditional medicine, herbal products are widely used in spices, flavoring, beverages, dyeing, fragrances and cosmetics. Herbs stand out with their high polyphenols and vitamins A, C and E [Kiselova et al., 2006]. Since extracts from herbs and spices delay oxidative degradation, they improve food quality and nutritional value, and thereby increasing the demand by the consumers [Wojdylo et al., 2007].

Zingiberaceae ginger family is an herbal plant family which has over 1300 species of aromatic flowering plants especially found in tropical and subtropical regions Southeast Asia. Galangal is a thick, creeping reddish-brown rhizome and used in Chinese medicine since early ages [Basri et al., 2017]. Galangal is a rhizomatous species of Zingiberaceae ginger family, mostly known its culinary and medicinal properties [Zhou et al., 2018]. There are two types of galangals which are greater galangal known as *Alpinia galanga* and lesser galangal named as *Alpinia officinarum*. Galangal stands out with its flavonoid called "galangin" which has been reported to be used in the treatment of some cancer types, cardiovascular diseases, diabetes, obesity, immunity and infections [Bhuyan and Basu, 2017], [Rampogu et al., 2021].

Polyphenols are generally more hydrophilic and can be extracted by organic solvents including ethanol, methanol, acetone, ethyl acetate, acetone or mixture of two or more solvents with water [Mojer et al., 2016], [Qui et al., 2010]. Conventional solvent extraction has some disadvantages such as low extraction yield, more solvent consumption, more energy requirement and long extraction time. Conventional solvent extraction also requires high temperatures which damage or reduce the bioavailability of polyphenols [Wen et al., 2018]. Green extraction methods such as ultrasound-

assisted extraction, pulsed electric field extraction, supercritical fluid extraction and microwave-assisted extraction have been developed to minimize negative effects of the conventional extraction. Among green extraction methods, ultrasound-assisted extraction is prominent because it has high extraction yields and reduces the use of solvent, energy, time and environmental pollution problems without requiring high temperatures [Chemat et al., 2017]. Process optimization is crucial to obtain optimum extraction conditions to maximize extraction yield with the highest antioxidant capacity [Singh et al., 2022]. Response surface methodology (RSM) is an effective and a useful statistical tool to optimize a variety of processes where the RSM has reduced number of experimental runs, is faster and provides valuable information regarding the system being optimized [Ozdemir, 1999], [Ozdemir et al., 2008]. Removal of extraction solvent to obtain a concentrated extract with a higher phenolic content can be achieved by membrane separation techniques which maintain antioxidant activity without destroying the phenolic compounds [Mulder, 2000].

1.1. Hypothesis

Multi-response optimization techniques can be employed to optimize extraction conditions of ultrasound-assisted extraction of polyphenols from galangal where membrane processes can be used to obtain a concentrated extract with a higher phenolic content and antioxidant activity.

1.2. Objectives

The objectives of this thesis are to i) investigate the effects of ultrasound-assisted extraction conditions (temperature, time, ethanol/water ratio, solvent/solid ratio, ultrasound power and ultrasound cycle) on extraction efficiency, total polyphenol content, total flavonoid content and antioxidant activity of galangal, ii) determine optimum ultrasound-assisted extraction conditions (temperature, time and solvent/solid ratio) for total polyphenol content, total flavonoid content, antioxidant activity and extraction efficiency to obtain extracts with the highest polyphenol content and antioxidant activity, and iii) obtain concentrated extracts with a higher phenolic content and antioxidant activity with the use of a membrane having the best efficiency.

2. METHODOLOGY

Figure 2.1 shows schematic diagram of the methods described in this study.

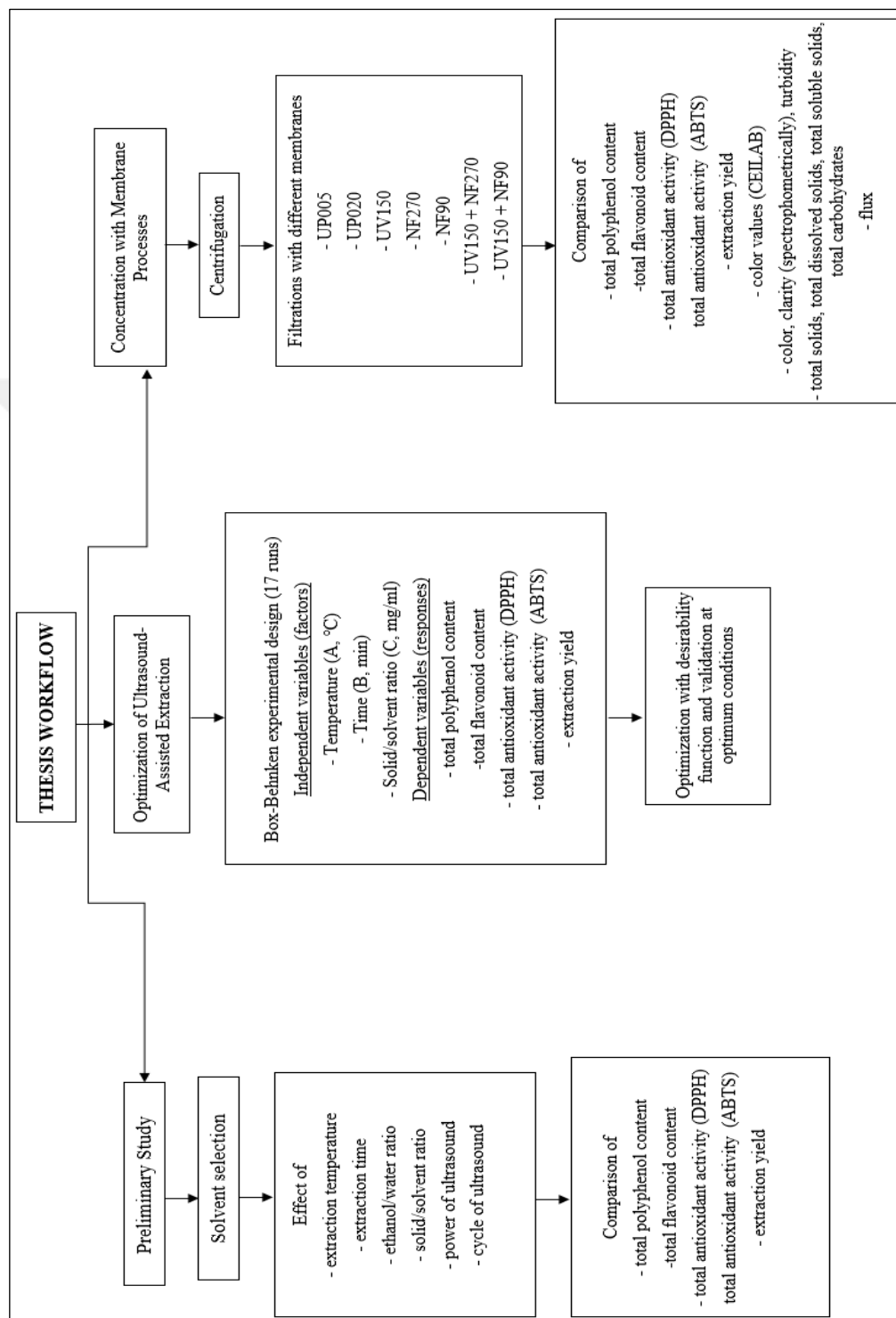


Figure 2.1: The schematic diagram of the methods described in this study.

3. OPTIMIZATION of ULTRASOUND-ASSISTED EXTRACTION of POLYPHENOLS from GALANGAL

Over the years, it has been known that there is a strong relationship between nutrition and human health. Consumers tend to choose their foods not only for their taste and pleasure but also for positive metabolic effects in the long run [Wen et al., 2018].

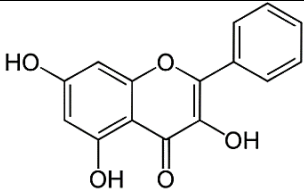
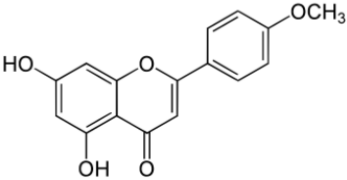
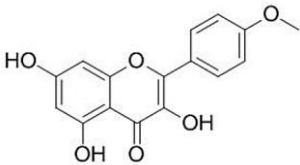
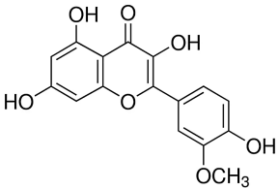
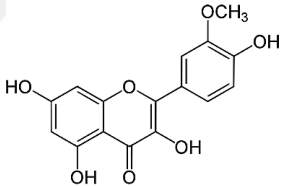
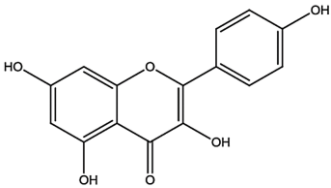
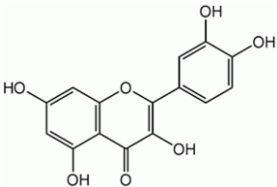
Phenolic compounds are water-soluble bioactive compounds that are naturally found in fruits, vegetables and plants. They contain one or more hydroxyl groups with aromatic rings. Phenolic compounds have the high antioxidant capacity and have free radical scavenging capability. It has been reported that polyphenols are effective in the control or prevention of metabolic syndrome and various chronic diseases in the elderly, and high biological activities are mainly due to strong antioxidant capacity, metal chelation and antiradical activities [Li et al., 2019]. Reactive oxygen species, which are unstable forms of oxygen and react quickly with other molecules, cause damage to lipids, proteins and DNA. Reactive oxygen species have adverse effects on immunological processes and pathophysiological mechanisms of inflammatory diseases [Dzialo et al., 2016]. It is known that antioxidants prevent lipid oxidation in biological systems and take part in defense mechanisms [Izadiyan and Hemmateenejad, 2016]. Flavonoids, a subgroup of phenolic compounds, are low molecular weight compounds consisting of two aromatic rings linked by three carbon atoms formed by a heterocyclic oxygen bond. Flavonoids are divided into different subclasses such as anthocyanins, anthocyanidins, flavones, flavonols, isoflavones, flavanones and flavanols (proanthocyanidins and catechins) according to the type of heterocyclic added [Cassano et al., 2017].

Antioxidant activity of phenolic compounds is related to their structure, conjugated double bonds and the existence of functional groups [Dzialo et al., 2016]. Phenolic compounds inhibit the formation of free radicals that occur in auto-oxidation processes. Phenolics show activity by chelating metal cations or donating electrons or hydrogen atoms [Amarowicz et al., 2004]. The antioxidant activity of phenolic compounds is usually determined by measuring the ability of a synthetic-colored radical or redox-active compound to scavenge radicals via a spectrophotometer using a suitable standard [Floegel et al., 2011].

Galangal (*Alpinia officinarum*) is a type of ginger species belonging to the Zingiberaceae family widely grown in the tropic regions of Southeast Asia, China, Indonesia and Thailand [Attokaran, 2017]. Galangal contains essential fatty acids and a high amount of polyphenols. Featured flavonoids in galangal are galangin [Eumkeb et al., 2010], 3-O-methyl galangin [Tao et al., 2006], and kaempferide and kaempferide-3-O- β -glucoside [Eumkeb et al., 2010]. Bioactive compounds commonly found in galangal are given in Table 3.1.

Extraction of bioactive compounds is one of the most important unit processes to separate target molecules for chemical analysis and synthesis of new compounds [Ojha et al., 2020]. The solubility of phenolic compounds is influenced by some factors such as extraction temperature, extraction time, solid to solvent ratio, type of solvent etc. The diversity of the structures of phenolic compounds determines the solubility characteristics of phenolic compounds in solvents with different polarities [Zlotek et al., 2016]. Solvent extraction of bioactive compounds at high temperatures primarily suffers from low antioxidant activity, low extraction yield, more solvent consumption, more energy requirement and long extraction time. Therefore, alternative extraction methods to conventional solvent extraction are developed to overcome the disadvantages of conventional solvent extraction [Lasano et al., 2019]. One of the most favorable extraction methods used in the extraction of bioactive compounds is ultrasound-assisted extraction which requires small capital cost, reduces waste and environmental pollution problems, provides high extraction yields, shortens extraction time, and has less energy and solvent consumption [Kumar et al., 2021] [Zekovic et al., 2017].

Table 3.1: Bioactive compounds commonly found in galangal.

Phenolic compound	Chemical structure	Molecular weight (g/mol)
Galangin		356.30
Acacetin		284.26
Kaempferide		300.26
Isohamnetin		316.26
3-o-methyl galangin		284.26
Kaempferol		286.23
Quercetin		302.24

The choice of solvent used in ultrasound-assisted extraction is important in obtaining higher extraction yields. The solubility of phenolic compounds in any solvent largely depends on the nature of the solvent used. Since phenolic compounds contain higher amounts of polar substances, they dissolve better in polar solvents. The

compatibility of phenolic compounds with the solvent system is directly related to the principle of "like dissolves like" [Thoo et al., 2010]. Polar solvents are divided into two groups which are protic and aprotic solvents. Protic polar solvents have dissociable hydrogen atoms, high dielectric constants, high polarity, and are capable of forming hydrogen bond. Water, ethanol, methanol and isopropyl alcohol were some commonly used protic polar solvents to extract bioactive compounds. On the other hand, aprotic polar solvents do not have dissociable hydrogen atoms, and they are unable to form hydrogen bonding which limits the solubility. Ethyl acetate, acetone, dichloromethane and tetrahydrofuran were examples to some of aprotic polar solvents used in polyphenol extraction. Polyphenols solubilizes better in polar protic solvents than aprotic solvents. Polar protic solvents interact better with functional groups of polyphenols and the presence of hydroxyl ions of them [Athmouni et al., 2015]. The polarity of the polyphenols to be dissolved must be compatible with the polarity of the solvent used.

High diversity of phenolic compounds creates differences in solubility of polyphenols; some of the phenolic compounds are more soluble in some solvents. For example, anthocyanins and proanthocyanidins dissolve better in water whereas hydroxybenzoic acids, catechin and epicatechin dissolve better in ethanol and isoflavanols and flavones dissolve better in methanol [Horzic et al., 2012]. *Asparagus officinalis* roots were extracted by protic and aprotic solvents where the highest amount of total phenolics was obtained with protic solvents including ethanol (83.6 mg GAE/ml extract), methanol (79.6 mg GAE/ml extract), water (77.1 mg GAE/ml extract) and isopropyl alcohol (76.9 mg GAE/ml extract) while the lowest amount of total phenolics was obtained with aprotic solvents such as acetone (65.2 mg GAE/ml extract) and ethyl acetate (29.2 mg GAE/ml extract) [Zhang et al., 2018]. Extraction of phenolic compounds in different protic solvents can enhance the extraction of polyphenols because the interaction of different polar groups in different protic solvents are different [Chirinos et al., 2007].

Water is categorized as a GRAS (Generally Recognized as Safe) substance in the list of FDA (Food and Drug Administration) [Web 1, 1977]. However, the efficiency of water to dissolve polyphenols is lower than organic solvents when it is used alone. In addition, phenolic compounds are more soluble in less polar organic solvents than water [Ballesteros et al., 2014]. Therefore, using a single solvent cannot effectively extract all phenolic compounds from a sample with maximum efficiency

[Mokrani and Madani, 2016]. Methanol/water (70%), ethanol/water (70%) and methanol/water (70%) were found as the best solvents for the extraction of polyphenols from grape marc, red currant and black currant marc, respectively [Lapornik et al., 2005]. The highest polyphenol yield was obtained with ethanol/water (40%) in the extraction of polyphenols from chestnut tree wood [Gironi and Piemonte, 2011]. Ethanol/water (50%) mixture was found the best solvent in the extraction of polyphenols from artichoke waste.

Increasing the extraction temperature can enhance the extraction of phenolic compounds by increasing the diffusion rate and mass transfer. Heating weakens the interactions between the phenolic compounds and polysaccharides or proteins and facilitates their solubility in a solvent [Mokrani et al., 2016]. The total phenolic content increased from 1400 to 2100 mg GAE/100 g dry weight when the extraction temperature was raised from 25 to 65 °C in phenolics extraction from *Orthosiphon stamineus* flowers [Chew et al., 2011]. However, high temperatures can damage the antioxidant activity of phenolic compounds. It was reported that the extraction rate of thermally stable phenolic compounds at increasing temperatures may be lower than the decomposition rate of the phenolic compounds solubilized at lower temperatures [Liyana-Pathirana and Shahidi, 2005]. It should be noted that some polyphenols are sensitive to thermal degradation, depending on the physicochemical and biochemical properties of the polyphenol compounds in the extract [Dzah et al., 2020]. Therefore, an optimum temperature yields for the extraction of polyphenols from a specific substrate. Total phenolic and total flavonoid contents increased from 18.16 to 32.38 mg GAE/g and 10.56 to 20.88 mg QE/g, respectively, by increasing the temperature from 30 to 60 °C in the extraction of polyphenols from Koreeb (*Dactyloctenium aegyptium*) seeds flour. When the temperature was raised to 70 °C, the total phenolic and total flavonoid contents decreased to 27.29 mg GAE/g and 20.64 mg QE/g, respectively. Similarly, the maximum DPPH antioxidant activity of the extract (82.22%) was attained at 60 °C and slowly decreased to 80.45% at 70 °C [Ahmed et al., 2020]. The extraction of bioactive compounds from peach (*Prunus persica* L.) revealed that the highest total phenolic content (363 mg GAE/100 g) was obtained at 25 °C, but the total phenolic content decreased to 284 mg GAE/100 g by increasing the temperature to 70 °C [Mokrani and Madani, 2016].

Extraction time is important for achieving high extraction yields without wasting too much energy because it takes a particular time for phenolics to dissolve in a

solvent. A decrease in total phenolic content due to prolonged time is explained by Fick's second law of diffusion where an equilibrium is reached between the concentrations of dissolved solid matrix and solution after a certain period of time [Mokrani and Madani, 2016]. Besides, extending the extraction time may increase the possibility of oxidation and lead to deterioration or loss of polyphenols extracted [Chew et al., 2021]. The deterioration of phenolic compounds reduces their ability to scavenge free radicals. It was shown that the total phenolic content of *Allium cepa* extract decreased when the extraction time was increased from 50 to 60 min [Fan et al., 2022]. The maximum extraction yield of polyphenols from okra (*Abelmoschus esculentus* L.) leaves was attained at the extraction time of 30 min when the extraction time was extended to 70 min, a lower extraction yield was obtained [Olawuyi et al., 2020].

The solvent/solid ratio changes the equilibrium constant and solubility, thereby increasing the total phenolic content. A high solvent/solid ratio enhances the contact between phenolic compounds and extraction solvent, thus increasing the diffusion rate of polyphenols [Tan et al., 2011]. The yield of polyphenols in rosehips increased almost 2-folds when the solvent/solid ratio was increased from 5 to 10 ml/g [Predescu et al., 2016]. Total phenolic compounds of date seeds increased by increasing s/s ratio from 2.5 g/100 g to 5.5 g/100 g when the s/s ratio increased from 10 to 60 ml/g [Al-Farsi and Lee, 2008]

Ultrasound is a sound wave with a frequency over 20 kHz, which is the highest frequency that the human ear can detect. Ultrasound mechanism is influenced by mechanical, thermal and cavitation effects [Wen et al., 2018]. Mechanical and thermal effects of ultrasound are used together to extract valuable compounds in plant materials, while the cavitation effect causes cell disruption and facilitates intracellular mass transport [Bakht et al., 2019]. Ultrasound cavitation has been described as a unique physical phenomenon caused by the propagation of powerful ultrasound waves. Ultrasonic waves consist of a series of compression and dilution cycles; they can propagate through a solid, liquid or gaseous medium, causing the prevailing of molecules from their original position [Kumar et al., 2021]. Cavitation is due to negative pressure in the solvent, which has a specific critical value by creating microscopically small voids or bubbles [Newman et al., 2001] [Ojha et al., 2020]. In other words, this negative pressure overcomes the attractive forces that bind the molecules together and the repulsive forces that pull the molecules apart, creating

cavitation bubbles. These bubbles grow and coalesce together and then collapse by forming a hot spot during the compression step. This hot spot speeds up the biochemical reactions around it [Kumar et al., 2021]. When the ultrasound is applied to the material surface, cavitation bubbles are collapsed asymmetrically, thus causing surface erosion which breaks down cell wall and exudate its contents, including the bioactive compounds to the extraction solvent [Khadhraoui et al., 2018], [Wen et al., 2018], [Kumar et al., 2021].

Ultrasound-assisted extraction can be done either in an ultrasound bath or with an ultrasound probe [Kumar et al., 2021]. In the ultrasound bath, ultrasound waves usually pass through two different media while the ultrasonic probe transmits ultrasound waves directly to the extraction medium. Therefore, more intense cavitations, higher extraction rates, lower extraction times and higher solvent recovery can be obtained with the ultrasound probe compared to the ultrasound bath [Dzah et al., 2020], [Mellado et al., 2019]. Phenolic compounds in walnut shells were extracted with an ultrasonic bath and ultrasonic probe under the same conditions, and more than 2-folds increase in extracting phenolic compounds was obtained when the ultrasonic probe was used [Dzah et al., 2020].

Recently, ultrasound-assisted extraction has becoming one of the leading non-thermal extraction methods in the extraction of bioactive compounds from plant-based foods. Bioactive compounds in grape seeds, which is by-product of winemaking process, were extracted with two separate extraction techniques; maceration for 12 h and the ultrasound-assisted extraction for 15 min. The total phenolic content of grape seed extract increased by 18% when the ultrasound-assisted extraction was used [Da Porto et al., 2013]. Comparison of ultrasound-assisted extraction and solvent extraction of orange peel polyphenols revealed that the antioxidant activity of ultrasound-assisted extract was 54% while the antioxidant activity of solvent extracted extract was 42% [Khan et al., 2010]. Almost 2-folds increase in total phenolic content of extracts of sage by products was obtained when the ultrasound-assisted extraction was employed [Zekovic et al., 2017]. Similarly, the total phenolic content of ultrasound-assisted waste spent coffee grounds was almost two times higher than the total phenolic content of solvent extracted waste spent coffee grounds [Al-Dhabi et al., 2017], [Mussatti et al., 2011]. The ultrasound-assisted extract of *Limonium sinuatum* flowers showed higher antioxidant activity than the extract of conventional solvent extraction [Xu et al., 2017]. The ultrasound-assisted extraction of phenolic compounds

from *Picea abies* bark yielded extracts with higher total phenolic content and antioxidant activity than the solvent extraction [Chmelova et al., 2020]. The total phenolics, total flavonoids, total tannins and antioxidant activities of *Berberis jaeschkeana* fruit polyphenols were comparatively higher than those of extracts obtained by conventional solvent extraction [Belwal et al., 2022].

Ultrasound power and ultrasound frequency or cycle are two crucial factors affecting the extraction yield in ultrasound-assisted extraction [Kumar et al., 2021]. The extraction yield of polyphenols from mashed tea leaves increased up to 16% when the ultrasound power was increased from 25 to 125 W [Van et al., 2017]. An increase of 9.5% in the extraction yield of polyphenols from *Acer truncatum* leaves was obtained when the ultrasound power level was increased from 150 to 240 W [Dzah et al., 2020]. The extraction yield of goji berry increased from 21.4% to 30.9% when the ultrasound power was increased from 100 to 255 W while the ultrasound power reached to 460 W, the extraction yield decreased to 28.2% [Skenderidis et al., 2016]. As the ultrasound power increases, the extraction efficiency of bioactive compounds increases to a point and then increasing the ultrasound power starts to generate free radicals which damage the phenolic compounds. Therefore, the ultrasound power needs to be adjusted depending on the food item from which bioactive compounds are extracted. Ultrasound frequency or cycle creates thermal (continuous) and non-thermal (pulse) effects which affect permeability of cell membranes [Zeng et al., 2014]. Total anthocyanins increased from 0.81 to 1.00 mg/g by increasing the ultrasound cycle from 2 to 5 during ultrasound-assisted extraction of grapes [Carrera et al., 2012]. Increasing the ultrasound frequency from 37 to 80 kHz increased the extraction yield of spinach extracts from 13.4% to 15.2% [Altemimi et al., 2015]. The total polyphenol content of *F. vesiculosus* seaweed increased from 422.7 to 453.9 mg GAE/g dry sample when the ultrasound frequency was risen from 35 to 130 kHz [Ummat et al., 2020]. Increasing the ultrasound frequency can have adverse effects on phenolic compounds. Higher ultrasound frequencies can break down food matrices and facilitate the extraction of a specific phenolic compound while limiting the extraction of other bioactive compounds. It was reported that the anthocyanin content of raspberry puree increased from 282 mg/l to 319 mg/l, but the total phenolic content of the raspberry puree decreased from 1454 mg/l to 1161 mg/l when the ultrasound frequency was increased from 20 to 490 kHz [Golmohamadi et al., 2013]. Therefore, the ultrasound frequency or cycle that is appropriate for the ultrasound-assisted extraction of bioactive

compounds from a food product being studied needs to be determined for the recovery of phenolic compounds.

Galangal is one of the most important members of culinary spices, it has very high antioxidant capacity and contains a high amount of polyphenols. Although several studies exist on the investigation of ultrasound-assisted extraction of phenolic compounds in culinary spices, there is a need to evaluate the effect of ultrasound-assisted extraction conditions toward optimization and obtain concentrated extracts from galangal through membrane processes. Therefore, the objectives of this study are to i) investigate the effects of ultrasound-assisted extraction conditions (temperature, time, ethanol/water ratio, solvent/solid ratio, ultrasound power and ultrasound cycle) on extraction efficiency, total polyphenol content, total flavonoid content and antioxidant activity of galangal, ii) determine optimum ultrasound-assisted extraction conditions (temperature, time and solvent/solid ratio) for total polyphenol content, total flavonoid content, antioxidant activity and extraction efficiency to obtain extracts with the highest polyphenol content and antioxidant activity, and iii) obtain concentrated extracts with a higher phenolic content and antioxidant activity with the use of a membrane having the best efficiency.

3.1. Materials and Methods for Ultrasound-Assisted Extraction of Polyphenols from Galangal

3.1.1 Materials

Galangal (*Alpinia officinarum*) powder was purchased from a local herbalist (Arifoğlu, İstanbul, Turkey) as freshly grounded and vacuum packed where each pack contained 25 grams of galangal powder. They were stored in a dark and dry place until extraction. Ethanol (99.9%), methanol (99.9%), isopropyl alcohol (99.7%), ethyl acetate (99.7%), acetone (99%), dichloromethane (99.8%), tetrahydrofuran (99.9%), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (100%), Folin-Ciocalteu reagent (<99%), glucose (99.5%) and quercetin (>95%) were supplied from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid (100%), sodium carbonate (99.9%), aluminum chloride (>99.5%) and sulfuric acid (>97%) were purchased from Merck (Darmstadt, Germany). Phenol (>99.5%) was supplied from Riedel-de Haen (Seelze, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (<97%), potassium

persulfate (>99%) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (>98%) were provided by Thermo-Fisher Scientific (Massachusetts, MA, USA). sodium nitrite (>97%) and sodium hydroxide (>97%) were purchased from Tekkim Chemical Company (Bursa, Turkey). Deionized water was used for the preparation of extraction solvents and solutions. All chemicals used were in analytical grade.

3.1.2. Sample Preparation

Galangal powder was sieved through a vibrating sieving machine equipped with up to eight stainless steel sieves and capable of separating particles down to 10 μm (Arslan Elek, İstanbul, Turkey). The powder under a Tyler mesh size of 50 (300 μm) was used in the extraction experiments. Particle size distributions of powders before and after the ultrasound-assisted extraction were also determined by a laser diffraction particle size analyzer (Mastersizer 2000, Malvern Instruments Co., Worcestershire, UK). Extraction trials were performed in a three-neck round-bottom glass flask (500 ml) placed in a temperature-controlled water bath (Labo B100-H23, Labo Co., İstanbul, Turkey) working with an accuracy of ± 0.1 $^{\circ}\text{C}$ (Figure 3.1). An ultrasonic generator equipped with a controllable power pulsed horn-type titanium probe (13 mm diameter) (200 W, 20 kHz, Bandelin HD 2200 Sonopuls, Bandelin Electronic GmbH & Co. KG, Germany) was used for ultrasound-assisted extraction trials. The amplitude control of the device was capable of providing ultrasonic vibrations in the probe to any desired level in the range of 10-100%. The loop allows to be set to any desired level in the range of 1 (0.1 second sonication – 0.9 second rest in every 1 second) to 9 (0.9 second sonication – 0.1 second rest in every 1 sec).

An ultrasound probe (horn) was placed at the middle neck of the extraction cell and immersed up to 1.5 cm above the bottom of the cell. A condenser was assembled to the right side neck to prevent solvent loss at high temperatures, and a digital thermometer was immersed half way in the liquid to monitor the temperature of the extraction medium. Samples of galangal powder (5-20 g) and extraction solvent (200 ml) were subjected to sonication between ultrasonic powers of 10 and 90%, and ultrasonic cycles of 1 and 9.

Extraction temperature, extraction time and solvent/solid ratio were changed from 20 to 60 $^{\circ}\text{C}$, 10 to 60 min and 10:1 to 30:1 (v/w). The extracts were consecutively

filtered through filter papers #1240 (14-18 μm) and #1244 (2-4 μm) (Filter-Lab, Filtros Anioia, S.A., Barcelona, Spain). Clear extracts were used for further analysis.

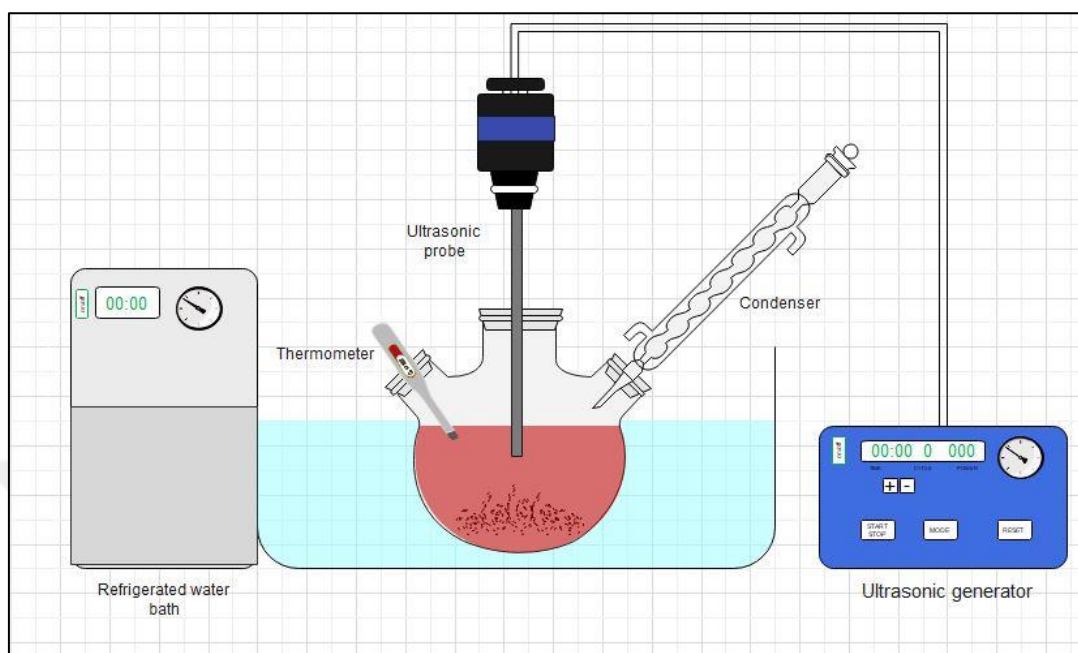


Figure 3.1: Schematic representation of the ultrasound-assisted extraction system.

3.1.3. Determination of Total Phenolic Content (TPC)

The total phenolic contents (TPC) of galangal extracts were analyzed colorimetrically using the Folin-Ciocalteu method with some modifications [Singleton et al., 1999]. Sample extracts were filtered by 0.45 μm PTFE (politetrafluoroetilen) syringe filter, and then filtrates were diluted with deionized water followed by stirring with a magnetic stirrer (Heidolph MR Hei-Tec, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) at room temperature for 2 min. Diluted extracts (0.5 ml) were mixed with 0.3 ml of Folin-Ciocalteu reagent and 1.5 ml of deionized water. The solutions were kept at room temperature for 3 min. After 3 min, 1 ml of 7% Na_2CO_3 solution was added to the solutions, and they were gently stirred for 30 sec. The solutions were left to stand for 1 hour in the dark at room temperature. After 1 hour, absorbances of the resulting blue-colored solutions were measured at 765 nm with a UV-VIS spectrophotometer (Perkin Elmer Lambda 35, Shelton, CT, USA). The TPC was quantified by using the standard curve of gallic acid ($R^2=0.9968$) and expressed as mg gallic acid equivalent (GAE) per gram of dry sample. All determinations were

performed in triplicate and average values of the measurements were reported. Absorbance readings were made against the deionized water as blank.

3.1.4. Determination of Total Flavonoid Content (TFC)

The total flavonoid contents (TFC) of galangal extracts were determined using the aluminum chloride colorimetric assay with some modifications [Rebollo-Hernanz et al., 2021]. Sample extracts were filtered by 0.45 μm PTFE syringe filter, and then filtrates were diluted with deionized water followed by stirring with a magnetic stirrer at room temperature for 2 min. Diluted extracts (1 ml) were mixed with 50 μl of 5% NaNO_2 , they were kept at room temperature for 5 min. Subsequently, 50 μl 10% AlCl_3 was added, and the solutions were left to stand at room temperature for 6 min. Then 0.5 ml of 1 M NaOH were added to the solutions, and they were gently stirred for 30 sec. Absorbances of the resulting orange-colored solutions were measured at 510 nm with a UV-VIS spectrophotometer (Perkin Elmer Lambda 35, Shelton, CT, USA). The TFC was quantified by using the standard curve of quercetin ($R^2=0.9833$) and expressed as mg quercetin equivalent (QE) per gram of dry sample. All determinations were performed in triplicate, and average values of the measurements were reported. Absorbance readings were made against the deionized water as blank.

3.1.5. Determination of Antioxidant Activity by DPPH and ABTS Methods

Antioxidant activities of galangal extracts were determined using DPPH and ABTS radical scavenging activity methods. Antioxidant activities were determined by DPPH free radical scavenging activity method by slight modifications [Köse et al., 2015]. Sample extracts were filtered by 0.45 μm PTFE syringe filter, and then filtrates were diluted with deionized water followed by stirring with a magnetic stirrer at room temperature for 2 min. An aliquot of aqueous extracts (0.25 ml) was mixed with 3.75 ml of 1 mM DPPH solution. The solutions were left to stand for 30 min in the dark at room temperature. After 30 min, absorbances of violet-colored solutions were measured at 517 nm with a UV-VIS spectrophotometer (Perkin Elmer Lambda 35, Shelton, CT, USA). The DPPH free radical scavenging activity (%) was expressed as

$$DPPH \text{ free radical scavenging activity (\%)} = \left(\frac{A_0 - A_s}{A_0} \right) \times 100 \quad (3.1)$$

where A_0 is the absorbance of the DPPH solution and A_s is the absorbance of the sample solution (aqueous extract). Three separate measurements were done, and results were given as the average of three separate measurements.

Antioxidant activities were also determined by ABTS-Trolox equivalent antioxidant capacity (TEAC) assay following the method of Thaipong et al. (2006) by slight modifications [Thaipong et al., 2006]. ABTS⁺ solution was prepared by mixing equal amounts of 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The stock solution was kept for 12-16 hours in the dark at room temperature. Before it was used, the stock solution was diluted with methanol until an absorbance of 1.1 ± 0.02 units at 734 nm was obtained in the reading of UV-VIS spectrophotometer. ABTS⁺ solution was prepared freshly for each assay. Extracts were filtered by 0.45 μ m PTFE syringe filter, and then filtrates were diluted with deionized water followed by stirring with a magnetic stirrer at room temperature for 2 min. An aliquot of aqueous extracts (0.1 ml) was mixed with 3.9 ml of ABTS solution. The solutions were left to stand for 2 hours in the dark at room temperature. After 2 hours, absorbances of blue-colored solutions were measured at 734 nm with a UV-VIS spectrophotometer (Perkin Elmer Lambda 35, Shelton, CT, USA). The ABTS antioxidant capacity was quantified by using the standard curve of Trolox ($R^2=0.9886$) and expressed as mM Trolox equivalent per ml extract. All determinations were performed in triplicate and average values of the measurements were reported. Absorbance readings were made against the deionized water as blank.

3.1.6. Determination of Total Solids (TS)

The total solids content of the samples was determined using AOAC (2005). Extracts (2 ml) were placed in completely dehumidified, cleaned and weighed glass petri dishes, and dried in an oven (Ecocell 55, MMM Medcenter Einrichtungen GmbH, München, Germany) at 103-105 °C until the constant weight was attained. Results were expressed in mg of total solids per ml of extract. Measurements were done in triplicate and average values were reported.

3.1.7. SEM Analysis

Images of high-resolution scanning electron microscopy of the dried galangal powder (particle size $\leq 300 \mu\text{m}$) used in the extraction experiments and the dried powder of ultrasound-assisted extract at optimum conditions were taken with a scanning electron microscope (Philips XL30 FEG, Oregon, USA) operating at a voltage of 5 kV. Sample surfaces were sputter coated with a thin layer of gold using a sputter coater (Quorum SC7620, Quorum Technologies, East Sussex, UK).

3.1.8. Experimental Design for Screening and Optimization Studies

Screening experiments were conducted to determine important factors and their levels in ultrasound-assisted extraction of galangal. For this purpose, extraction temperature (20-70 °C), extraction time (5-60 min), solvent type, solvent/solid (s/s) ratio (5:1-40:1), ultrasound power (10-90%) and ultrasound cycle (1-9) were chosen as important parameters. Deionized water, ethanol, ethanol/water (50%), methanol, methanol/water (50%), acetone, isopropyl alcohol, ethyl acetate, dichloromethane and tetrahydrofuran were used as solvents. Effects of the factors on extraction yield were total polyphenol content, total flavonoid content and antioxidant activity of galangal were investigated. The information obtained from the screening experiments were used in the selection of experimental design for the optimization studies.

A three-level three factors Box-Behnken experimental design of RSM (Response Surface Methodology) software (Design Expert, version 12 Stat-Ease, Inc., Minneapolis, MN, USA) was used to investigate the effect of independent variables (factors) on dependent variables (responses) (Table 3.2). The independent variables were extraction temperature (X_1), extraction time (X_2) and solvent/solid (s/s) ratio (X_3). The responses were TPC, TFC, and DPPH and ABTS radical scavenging activities. The complete design had 17 randomized trials with five replications at the center. The responses were fitted to the commonly used second-order polynomial model given below:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^k \beta_{ij} X_i X_j \quad (3.3)$$

where Y is the response, X_i and X_j are independent variables, k is the number of independent variables ($k = 3$), and β_0 , β_i , β_{ii} and β_{ij} are the regression coefficients of the intercept, linear, quadratic and interaction terms, respectively.

Table 3.2: Experimental design including independent variables (coded and uncoded) and their levels.

Runs	Coded Variables			Uncoded Variables		
	X_1	X_2	X_3	Temperature (°C)	Time (min)	Solvent/solid ratio (ml/g)
1	0	+1	+1	40	60	30
2	0	0	0	40	35	20
3	-1	0	-1	20	35	10
4	0	0	0	40	35	20
5	-1	-1	0	20	10	20
6	0	0	0	40	35	20
7	0	-1	+1	40	10	30
8	+1	0	-1	60	35	10
9	+1	-1	0	60	10	20
10	0	-1	-1	40	10	10
11	-1	0	+1	20	35	30
12	0	0	0	40	35	20
13	-1	+1	0	20	60	20
14	0	0	0	40	35	20
15	0	+1	-1	40	60	10
16	+1	0	+1	60	35	30
17	+1	+1	0	60	60	20

3.1.9. Statistical Analysis

At least triplicate measurements were taken for each experimental point. Experimental data for screening experiments were analyzed by one-way analysis of variance (ANOVA) followed by post hoc analysis using Tukey's multiple range test with a significance level (α) of 0.05 to determine significant differences between mean values using SPSS Statistics 22 software (SPSS Inc., Chicago, IL, USA).

The multiple regression analysis for each response was done with Design-Expert software (version 12.0, Stat-Ease, Inc., Minneapolis, MN, USA) to fit a second-degree polynomial, check whether significant relationship exists for linear, quadratic and interaction effects, and determine regression coefficients. Model diagnostics were performed by generating normal probability plot, and the plots of residual versus predicted values, and observed versus predicted values. The levels of significance of all terms in the model equations were determined by calculating the F-value ($p \leq 0.05$). The correlation analysis was done by Pearson multiple range test as described in Taylor (1990) using SPSS Statistics 22 software (SPSS Inc., Chicago, IL, USA). Differences were considered significant at the significance level of $p \leq 0.05$. Model adequacies were evaluated using the model analysis, lack of fit test, coefficient of determination (R^2) and coefficient of variance (CV%). Three-dimensional (3D) plots were generated to graphically visualize how the responses changed with respect to the factors and to obtain graphical representation of the response surface. Desirability function as the numerical optimization technique was used to predict optimum conditions. Experiments at the optimum conditions were carried out with three replications to validate the models developed by comparing the experimental data with the predicted values.

3.2. Results and Discussion of Screening Experiments for Ultrasound-Assisted Extraction of Polyphenols from Galangal

Ten different solvents (deionized water, ethanol, ethanol/water (50%), methanol, methanol/water (50%), acetone, isopropyl alcohol, ethyl acetate, dichloromethane and tetrahydrofuran) were investigated to determine the best solvent for ultrasound-assisted extraction of phenolic compounds. Affinities of different solvents on total

phenolic content of galangal are in the order of ethanol/water (50%) > methanol/water (50%) > methanol > ethanol > tetrahydrofuran > acetone > isopropyl alcohol > water > ethyl acetate and > dichloromethane (Figure 3.2). The increased affinity of phenolic compounds in polar protic solvents and the phenolic compound-solvent compatibility are the main reasons for the differences in extraction efficiencies of polyphenols. The principle of "like dissolves like" governs the compatibility of phenolic compounds with the solvent system [Thoo et al., 2010]. Ballesteros et al. (2014) suggested that the phenolic compounds are more soluble in polar protic solvents, which are less polar than water [Ballesteros et al., 2014]. The differences in the affinities of different solvents against phenolic compounds could be attributed to polarities of solvents.

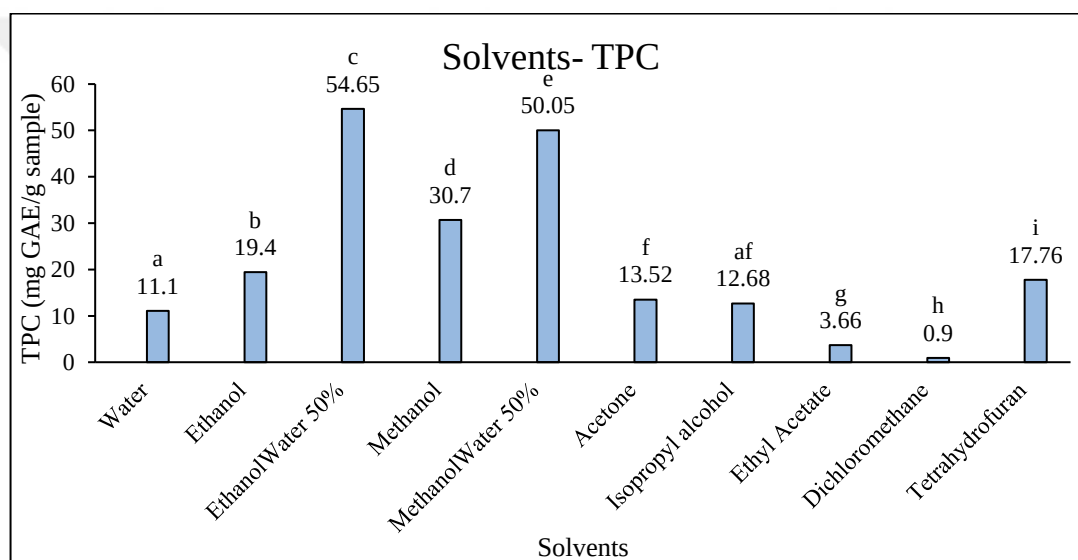


Figure 3.2: Effect of solvent type on total phenolic content (TPC) of galangal.

The highest TPC (54.65 mg GAE/g sample) was obtained for the ethanol/water (50%) solution because ethanol has higher hydrogen bonding ability than methanol although both solvents have hydroxyl groups. Polar-protic solvents with high dielectric constants in mixture form are reported to be better solvents than a single polar-protic solvent in extracting polyphenols [Durling et al., 2007], [Do et al., 2014]. Ethanol is also classified as GRAS (Generally Recognized as Safe) by the FDA (Food and Drug Administration), therefore it can be used in food-related applications. Rabelo et al. (2016) studied the extraction of polyphenols from artichoke solid wastes, and found that ethanol/water extractions of 25%, 50% and 75% yielded higher TPC than the extraction with water [Rabelo et al., 2016]. Junior et al. (2021) extracted phenolic

compounds from ciriguela (*Spondias purpurea L.*) peel and determined that ethanol/water solutions gave the highest polyphenols [Junior et al., 2021]. *Eucalyptus camaldulensis* leaves were extracted with ethanol, methanol, acetone and their 50% mixtures with water, and all the solvent mixtures gave higher phenolics than the extraction with a single solvent [Gharekhani et al., 2012].

The effect of extraction temperature on the TPC, DPPH, ABTS and TFC of ultrasound-assisted extraction of galangal was studied at the extraction time of 20 min, ethanol/water ratio of 50%, solvent/solid ratio of 10, ultrasound power of 50% and ultrasound cycle of 5 (Figure 3.3).

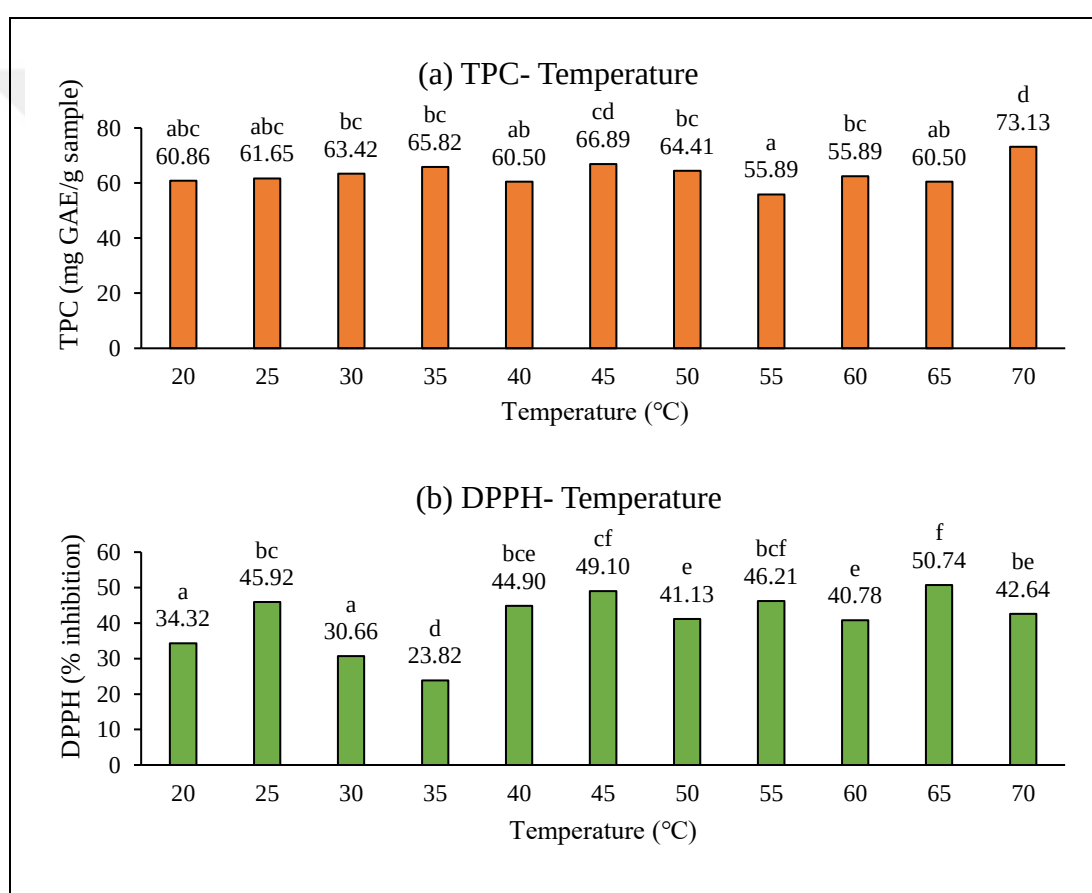


Figure 3.3: Effect of temperature on (a) TPC, (b) DPPH, (c) ABTS and (d) TFC of galangal at the extraction time of 20 min, ethanol/water ratio of 50%, solvent/solid ratio of 10, ultrasound power of 50% and ultrasound cycle of 5.

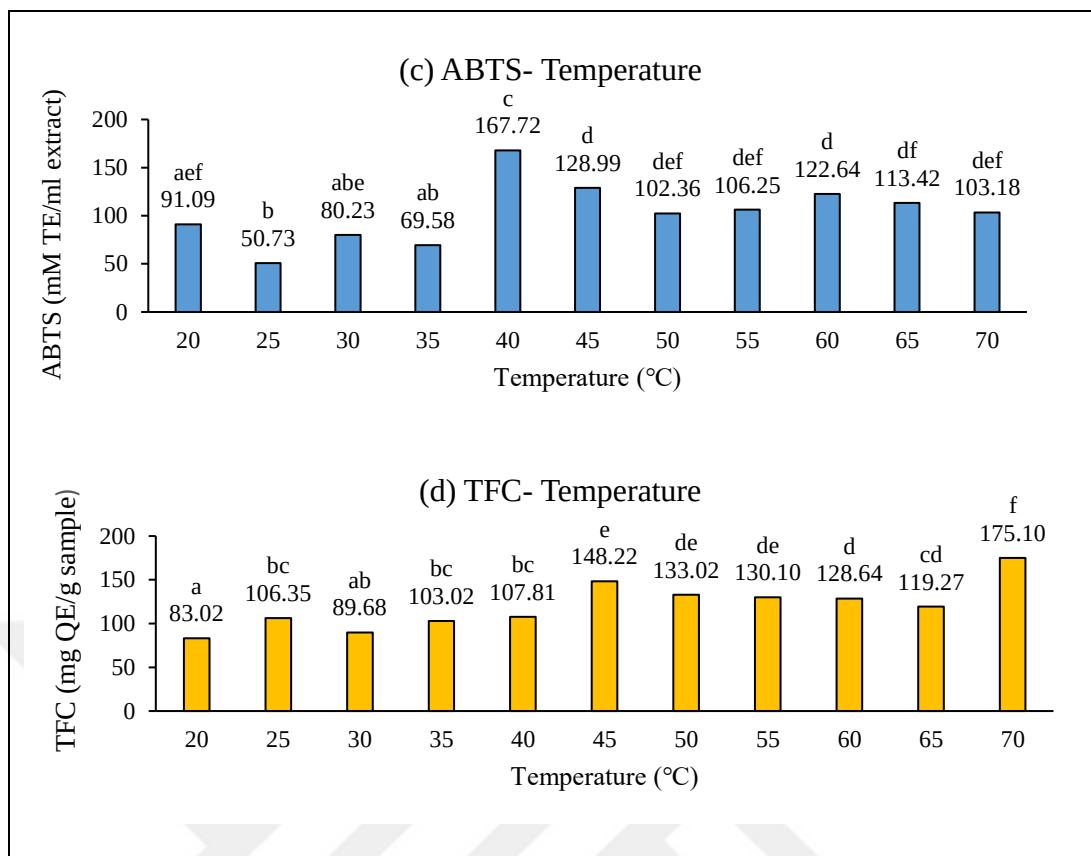


Figure 3.3: Continued.

Temperature was a significant effect ($p \leq 0.05$) on TPC (Figure 3.3a). TPC increased from 20° to 35 °C. Heating breaks the bonds between phenolic chemicals and polysaccharides and proteins, allowing them to be released into the solvent. Increasing temperature improves the extraction of phenolic compounds up to a certain temperature, but the amount of phenolics starts to decrease due to the decomposition of some polyphenolic compounds in the extract [Mokrani and Madani, 2016], [Alara et al., 2021]. The same trend was also observed in our study where increasing the temperature increased the decomposition of some phenolic compounds previously extracted at lower temperatures. Phenolic compounds extracted at low temperatures degrade as the temperature rises, and additional phenolic compounds begin to be extracted [Zhang and Lee, 2021]. Although the highest DPPH antioxidant activity was obtained at 65 °C, there was no significant difference ($p > 0.05$) between the DPPH values at 55 and 65 °C (Figure 3.3b). Jokic et al. (2009) also observed the same trend where DPPH antioxidant activities of soybeans were not significantly different ($p > 0.05$) at 60 and 70 °C [Jokic et al., 2009]. The highest ABTS antioxidant activity was achieved at 40 °C (Figure 3.3c), and there was no significant difference ($p > 0.05$) in

the ABTS antioxidant activity values of the extracts after 45 °C. According to Pearson correlation analysis, DPPH and ABTS were not strongly correlated ($r = 0.468$, $p > 0.05$) with each other, and it is well established that they did not show parallel results due to the different responses of phenolic compounds against radicals of DPPH and ABTS methods [Lachman et al., 2007], [Andres et al., 2020]. Only organic solvents (especially alcohols) can dissolve DPPH, which is a significant limitation of DPPH method in determination of hydrophilic antioxidants. ABTS can be dissolved not only in organic solvents, but also in aqueous solutions. This makes the determination of both hydrophilic and lipophilic components of phenolic compounds possible [Thoo et al., 2010], [Andres et al., 2020]. There was no significant difference ($p > 0.05$) in TFC values of galangal extracts between 45 and 55 °C (Figure 3.3d). Although the highest TFC and TPC values were attained at 70 °C, the extracts exhibited lower DPPH and ABTS values compared to the DPPH and ABTS of extracts at 45 °C. It was also reported that TPC and TFC antioxidant activities of *Orthosiphon stamineus* extracts increased linearly when the temperature was increased from 25 to 65 °C, but DPPH and ABTS values of the extracts decreased linearly [Chew et al., 2011]. These results indicate that the phenolic compounds extracted lose their antioxidant activity with increasing the extraction temperature. These findings are in conformation with our results in which as the extraction temperature rises, the antioxidant activity of the extracted phenolic compounds decreases. According to Pearson correlation analysis, TFC was moderately correlated with TPC ($r = 0.574$, $p > 0.05$) and DPPH ($r = 0.503$, $p > 0.05$). Furthermore, TPC showed negative correlation with DPPH ($r = -0.187$, $p > 0.05$) and ABTS ($r = -0.091$, $p > 0.05$). These correlations suggest that bioactive compounds affecting antioxidant activity were mostly flavonoids [Hirano et al., 2001]. Similar findings between TFC and DPPH were also reported by Vo et al. (2022) [Vo et al., 2022].

The effect of extraction time on the TPC, DPPH, ABTS and TFC of ultrasound-assisted extraction of galangal was studied at the extraction temperature of 25 °C, ethanol/water ratio of 50%, solvent/solid ratio of 10, ultrasound power of 50% and ultrasound cycle of 5 (Figure 3.4).

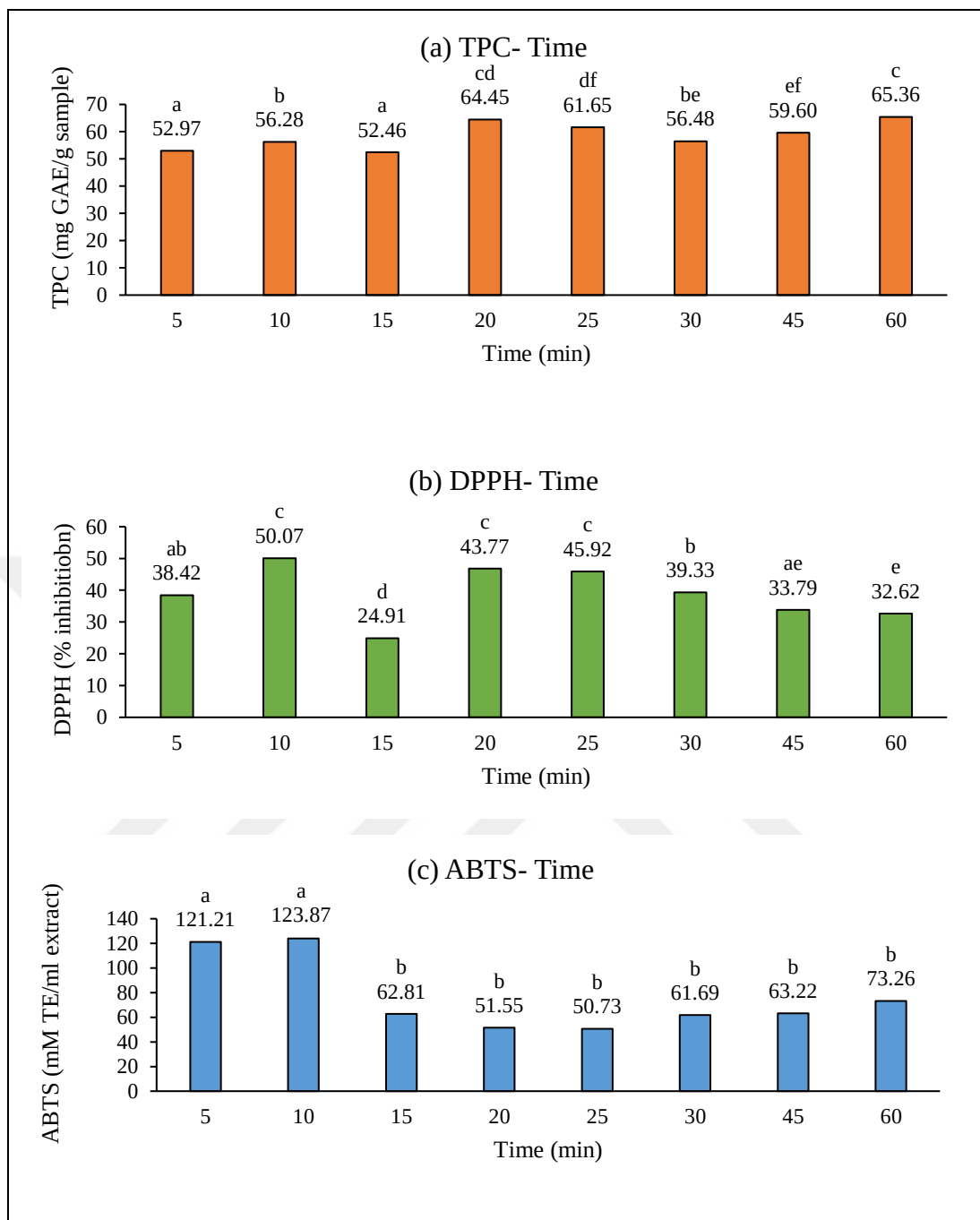


Figure 3.4: Effect of time on (a) TPC, (b) DPPH, (c) ABTS and (d) TFC of galangal at the extraction temperature of 25 °C, ethanol/water ratio of 50%, solvent/solid ratio of 10, ultrasound power of 50% and ultrasound cycle of 5.

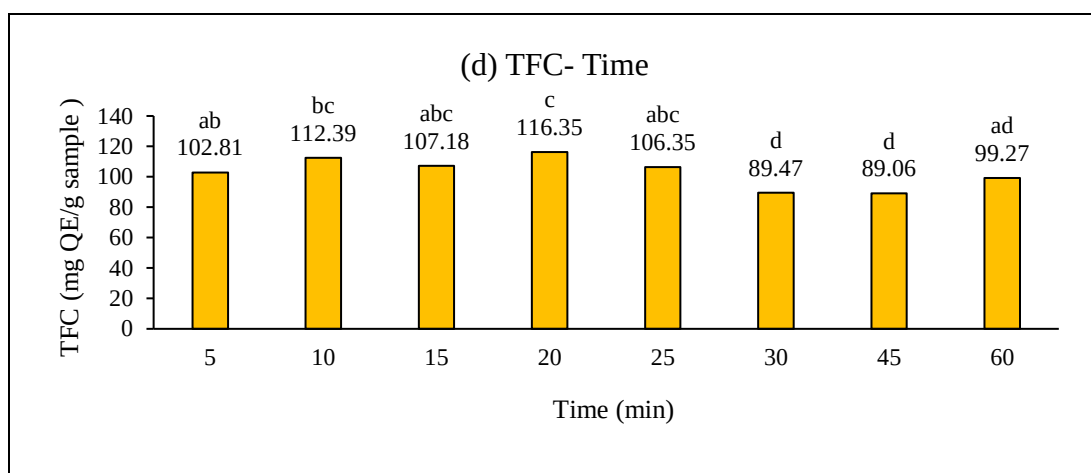


Figure 3.4: Continued.

Time was a significant ($p \leq 0.05$) effect on TPC (Figure 3.4a). Although the highest TPC was obtained at 60 min, there was no significant ($p > 0.05$) difference between the TPC values at 20 and 60 min. The difference in TPC values throughout the extraction period can be explained by the chain reactions between phenolic compounds depending on the extraction time which causes degradation of polyphenols that were already extracted. In other words, during extraction, other phenolic compounds start to be extracted while previously extracted phenolic compounds degrade [Mokrani and Madani, 2016]. Similar trends in TPC with the extraction time were also observed in the extraction of *Opuntia ficus indica* powder [Mahdep et al., 2021]. DPPH increased from 5 to 10 min where it reached a maximum value of 50% (Figure 3.4b). There was no significant difference ($p > 0.05$) among DPPH values of galangal extracts at extraction times of 10, 20 and 25 min. The maximum ABTS was attained at 10 min and as similar to the trend of DPPH, the ABTS values started to decrease after 10 min (Figure 3.4c). DPPH and ABTS of koreeb (*Dactyloctenium aegyptium*) seed flour extracts showed a similar trend observed in our study, and it was concluded that the long extraction times did not have a considerably positive effect on antioxidant activity [Ahmed et al., 2019]. Pearson correlation analyses showed that TPC was weakly correlated with DPPH ($r = 0.281$, $p > 0.05$) and negatively correlated with ABTS ($r = -0.497$, $p > 0.05$). This result can be attributed to the fact that the antioxidant activity ability does not depend only on phenolic compounds, but also the other compounds, such as vitamin E or C, which can be found in galangal [Rusea et al., 2014], [Rachkeeree et al., 2018]. On the other hand, non-parallel observations

between the phenolic and flavonoid content of the extracts and their antioxidant activity may be due to the differences in the amount and quality of the phenolic compounds in different extracts [Mokrani and Madani, 2016]. DPPH and ABTS antioxidant activities had a weak correlation with each other ($r = 0.232$, $p > 0.05$) because of the reason that the DPPH radical scavenging activity analysis is appropriate for hydrophobic compounds while ABTS is for both hydrophilic and lipophilic compounds. In these conditions, this can be explained by the fact that galangal has a higher proportion of hydrophobic phenolic compounds [Kim and Lee, 2020]. TFC exhibited a similar trend to TPC, and the highest TFC was obtained at 20 min, but there was no significant ($p > 0.05$) difference among the TFC values at 5, 10, 15 and 25 min (Figure 3.4d). Thoo et al. (2010) found that prolonged extraction times did not improve TFC of *Morinda citrifolia* extracts [Thoo et al., 2010]. According to Pearson coefficients, TFC had a weak correlation ($r = 0.092$, $p > 0.05$) with TPC. This finding indicates that the majority of phenolic compounds that dissolve in a specific period of time are non-flavonoids [Mekinic et al., 2014].

The effect of ethanol/water ratio on the TPC, DPPH, ABTS and TFC of ultrasound-assisted extraction of galangal was studied at the extraction temperature of 25 °C, extraction time of 20 min, solvent/solid ratio of 10, ultrasound power of 50% and ultrasound cycle of 5 (Figure 3.5).

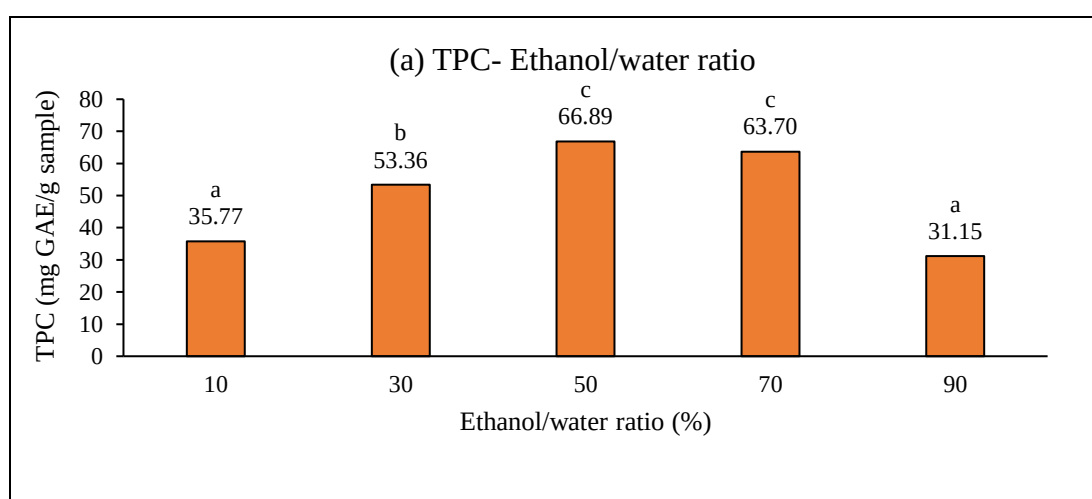


Figure 3.5: Effect of ethanol/water ratio on (a) TPC, (b) DPPH, (c) ABTS and (d) TFC of galangal at the extraction temperature of 25 °C, extraction time of 20 min, solvent/solid ratio of 10, ultrasound power of 50% and ultrasound cycle of 5.

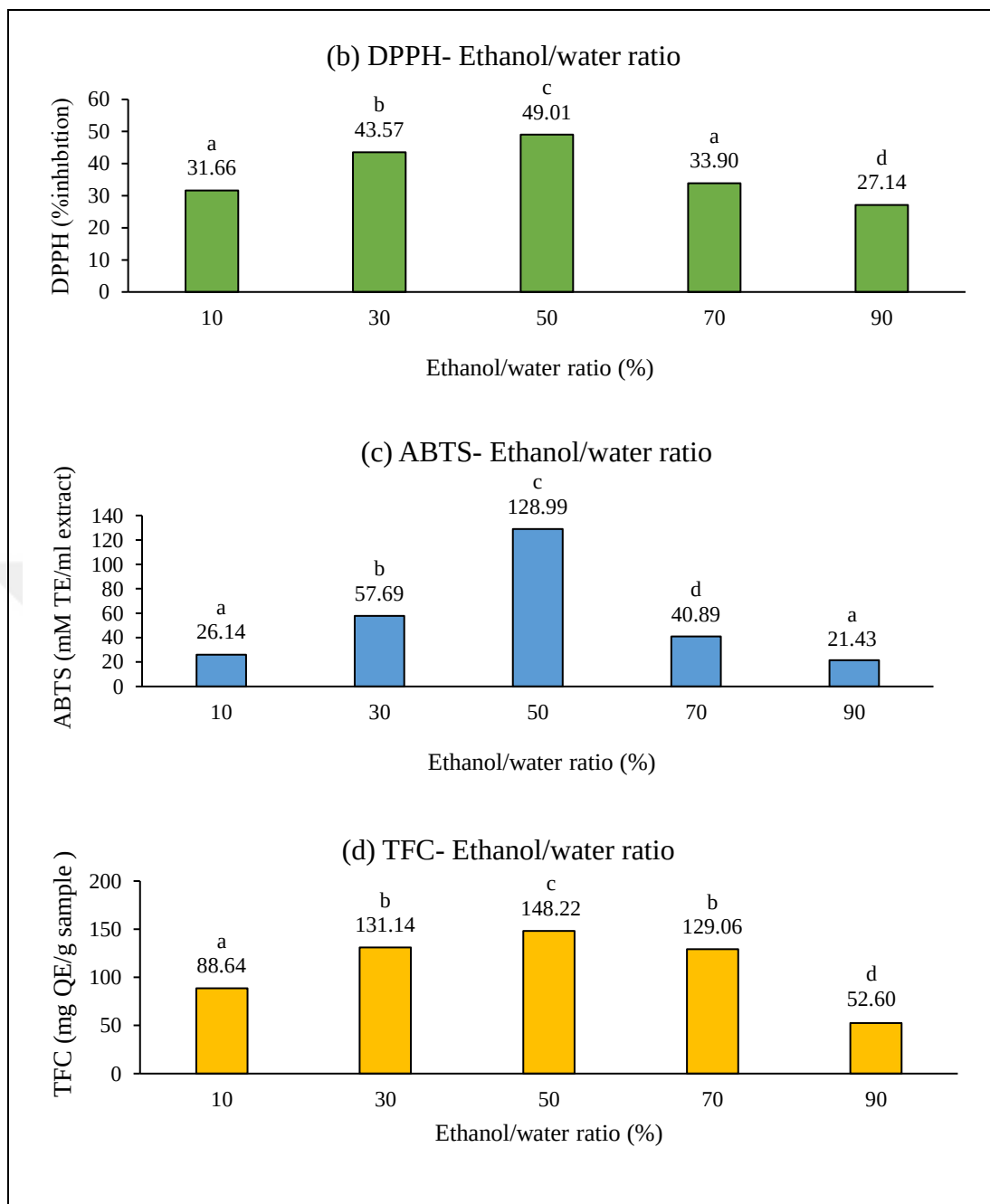


Figure 3.5: Continued.

The TPC increased significantly from 10% to 50% and reached the maximum value of 66.89 mg GAE/g sample, then decreased from 70% to 90% to its minimum value of 31.15 (Figure 3.5a). There wasn't a significant difference ($p > 0.05$) between 50% and 70%, which is similar to the phenolic compounds extracted from pistachio (*Pistacia vera* L.) [Özbek et al., 2020].

DPPH, ABTS and TFC increased from 10% to 50% ethanol/water ratio and reached the maximum values (148.22 mg QE/ g sample, 49.01 %inhibition and 128.99

mM TE/ml extract, respectively) and then decreased similar with TPC. For all the analyses, 50% was significantly higher ($p \leq 0.05$) than other concentrations. (Figures 3.5b, 3.5c, 3.5d). TPC was strongly correlated with TFC ($r = 0.944$, $p \leq 0.05$) which confirms that at different ethanol/water ratios, most of the TPC consists of flavonoids which are sub-branch of phenolic compounds. DPPH and ABTS were strongly correlated with each other ($r = 0.914$, $p \leq 0.05$) and this correlation indicates that both methods can be used to evaluate this antioxidant activity of the galangal extracts in specified ethanol/water ratios. Similarly, Merecek et al. (2017) compared antioxidant activity methods and reported a strong correlation ($r = 0.820$) between DPPH and ABTS antioxidant activity methods on spring barley extracts [Merecek et al., 2017].

Phenolic compounds donate their electrons from their hydroxyl molecules to reactive oxygen species, enabling them to neutralize free radicals. Therefore, a compatibility between phenolic compounds and antioxidant activities is expected. The strong correlation of TPC with DPPH ($r = 0.766$, $p > 0.05$) and ABTS ($r = 0.747$, $p > 0.05$) and also TFC with DPPH ($r = 0.874$, $p > 0.05$) and ABTS ($r = 0.761$, $p > 0.05$) were observed. These likely indicate the free radical scavenging ability of phenolic compounds; the higher this correlation, the stronger the power of this cleaning activity [Li et al, 2019]. Antioxidant activity of guava fruit extracts was compared with different methods, and a strong correlation between TPC and DPPH ($r = 0.86$) and ABTS ($r = 0.97$) were reported [Thaipong et al., 2006]. Osman et al. (2021) found strong correlation between TPC and DPPH ($r = 0.78$) and ABTS ($r = 0.860$) antioxidant activity values which shows that the antioxidant properties in the sample are due to the enormous contribution of phenolic compounds [Osman et al., 2021]. Several studies in the literature support these findings as the best solvent for phenolic compounds is ethanol/water ratio of 50%.

A study by Saphier et al. (2017) determined the TPC from chia seeds by 50% ethanol/water ratio as 1.99 mg GAE/g dry weight better than the other solvents (water, 20% ethanol/water ratio, 80% ethanol/water ratio and ethanol as 0.339- 1.49 mg GAE/g dry weight) [Saphier et al., 2017]. The TPC results of coffee pulp were 40.73 mg GAE/g dry weight with 50% ethanol/water ratio as solvent, while other percentage mixtures were 26.12-29.69 mg GAE/g dry weight [Kusumocahyo et al., 2020].

The effect of solvent/solid ratio on the TPC, DPPH, ABTS and TFC of ultrasound-assisted extraction of galangal was studied at the extraction temperature of 25 °C, extraction time of 20 min, ethanol/water ratio of 50%, ultrasound power of 50% and ultrasound cycle of 5 (Figure 3.6).

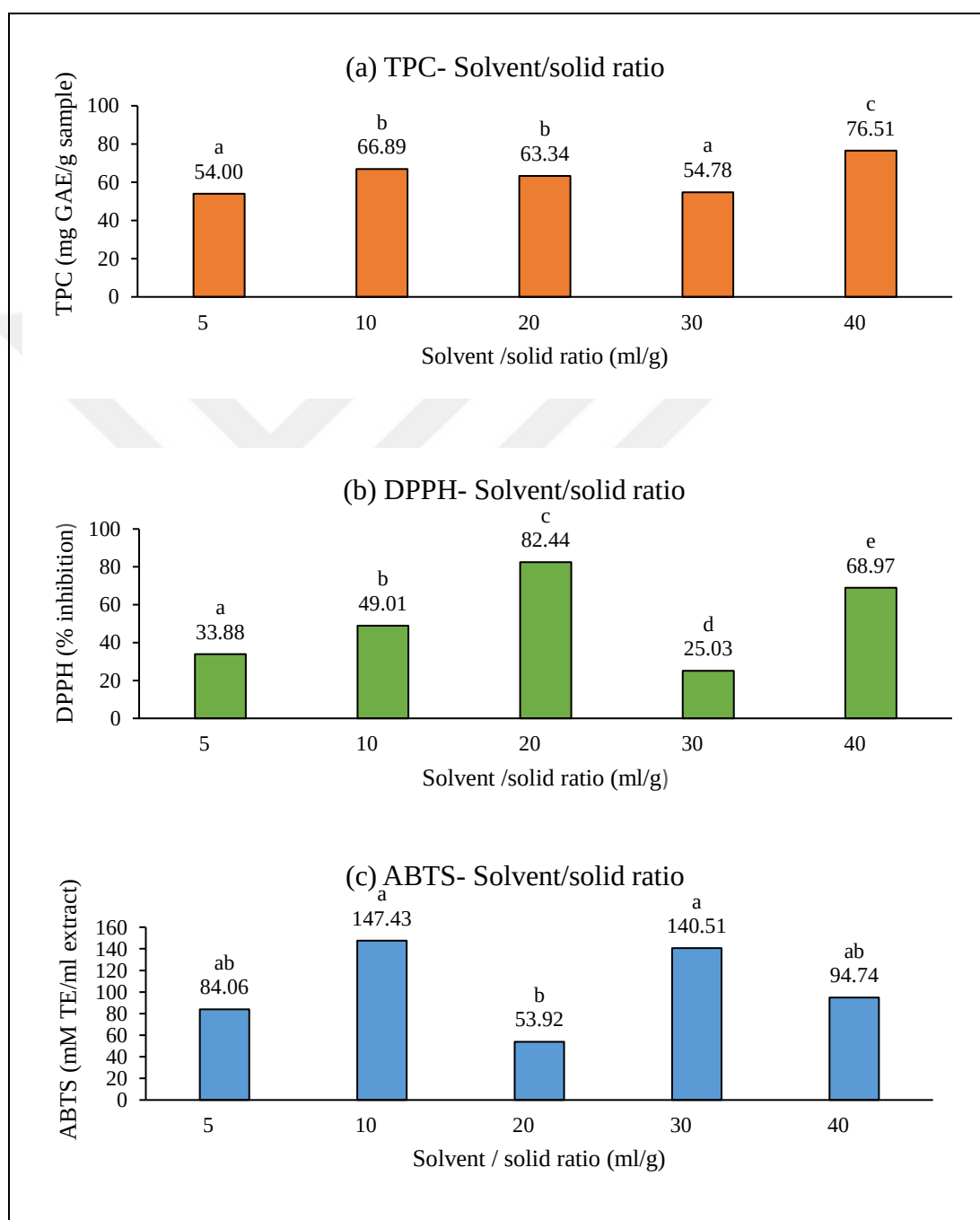


Figure 3.6: Effect of solvent/solid ratio on (a) TPC, (b) DPPH, (c) ABTS and (d) TFC of galangal at the extraction temperature of 25 °C, extraction time of 20 min, ethanol/water ratio of 50%, ultrasound power of 50% and ultrasound cycle of 5.

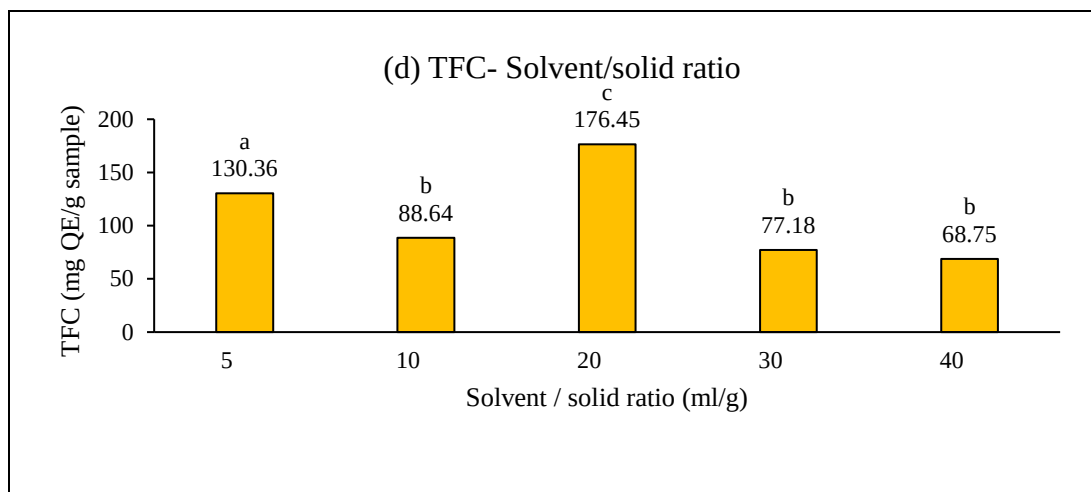


Figure 3.6: Continued.

There was significant difference between 5 and 10 s/s ratio ($p \leq 0.05$) for TPC (Figure 3.6a), DPPH (Figure 3.6b) and TFC (Figure 3.6d). TPC and ABTS (Figure 3.6e) were the highest at 40 s/s ratio (76.21 mg GAE/g sample and 22.50%, respectively), which means the solid-solvent interaction was more effective when the s/s ratio increases and ABTS-responded phenolic compound were more solubilized.

TFC for 40 solvent/solid ratio was the lowest 68.75 mg QE/g sample value for all trials which means that the small portion of polyphenols were flavonoids for the extraction with 40 s/s ratio. TFC and DPPH reached the significantly ($p > 0.05$) the highest values at 20 s/s ratio (176.45 mg QE/ g sample and 82.44 %inhibition, respectively) so, most of the DPPH-responded phenolic compounds were flavonoids.

According to Pearson correlation analysis, there was a negative correlation between TPC and TFC ($r = -0.318$, $p > 0.05$), and also it was observed that while TPC increased, TFC decreased (Figure 3.6). At 10 and at 40 s/s ratio, TPC was higher than others while TFC had the lowest values. TPC and TFC values of the blueberry pomace increased by increasing solvent/solid ratio from 10 to 40 and results indicated that high solvent/solid ratio increase the contact surface between the material and solvent and increased the solubility by enhancing mass transfer [Bamba et al., 2018], [Zhou et al., 2019].

DPPH and ABTS had negative correlation ($r = -0.064$, $p > 0.05$) with each other that indicates the difference in scavenging activity mechanisms of DPPH and ABTS. This is because of the restrictive characteristic of DPPH when evaluating hydrophilic

antioxidants and the ability of ABTS to measure the hydrophilic and lipophilic nature of phenolic compounds in the extract samples.

The effect of ultrasound power on the TPC, DPPH, ABTS and TFC of ultrasound-assisted extraction of galangal was studied at the extraction temperature of 25 °C, extraction time of 20 min, ethanol/water ratio of 50%, solvent/solid ratio of 10 and ultrasound cycle of 5 (Figure 3.7).

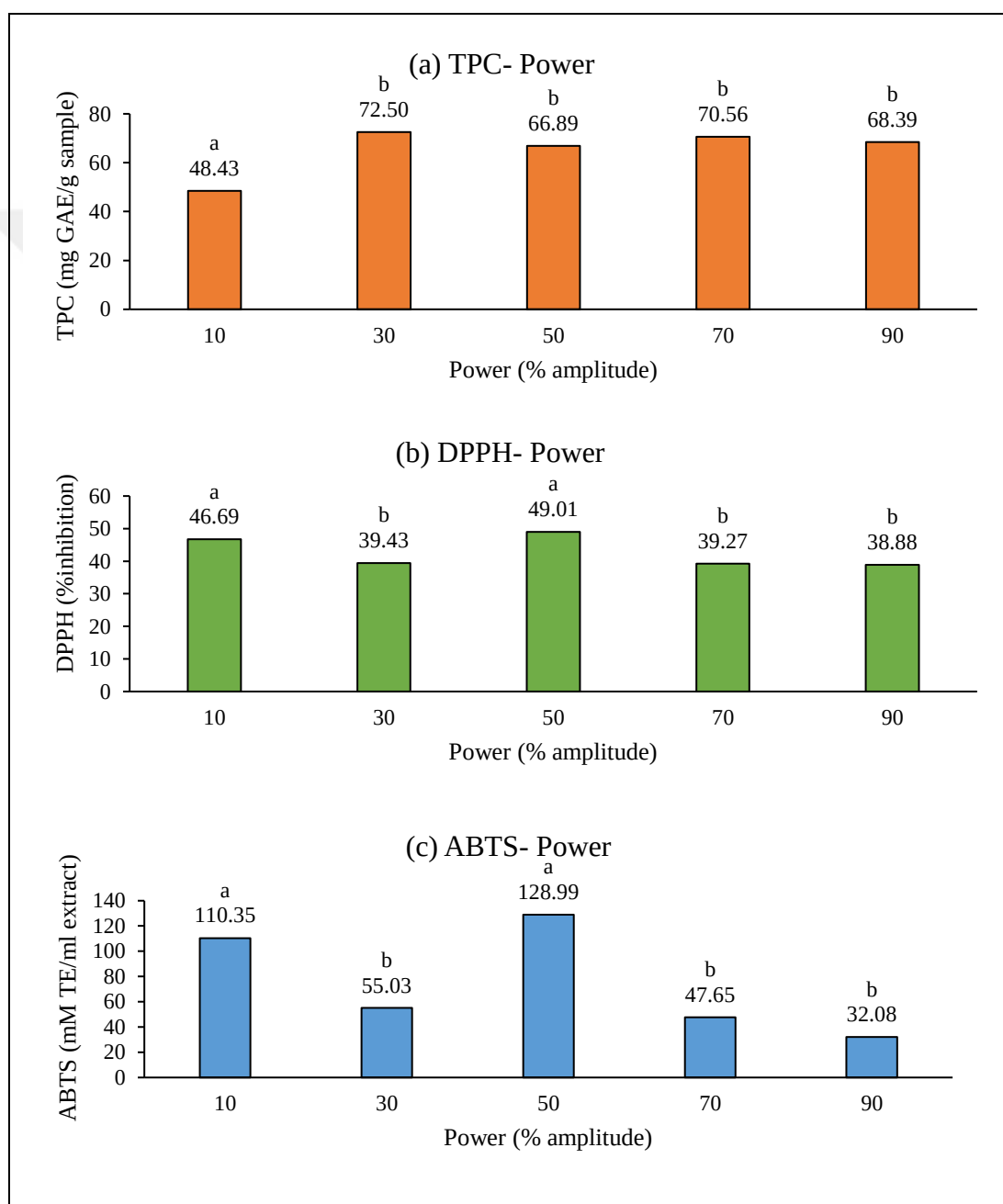


Figure 3.7: Effect of ultrasound power on (a) TPC, (b) DPPH, (c) ABTS and (d) TFC of galangal at the extraction temperature of 25 °C, extraction time of 20 min, ethanol/water ratio of 50%, solvent/solid ratio of 10 and ultrasound cycle of 5.

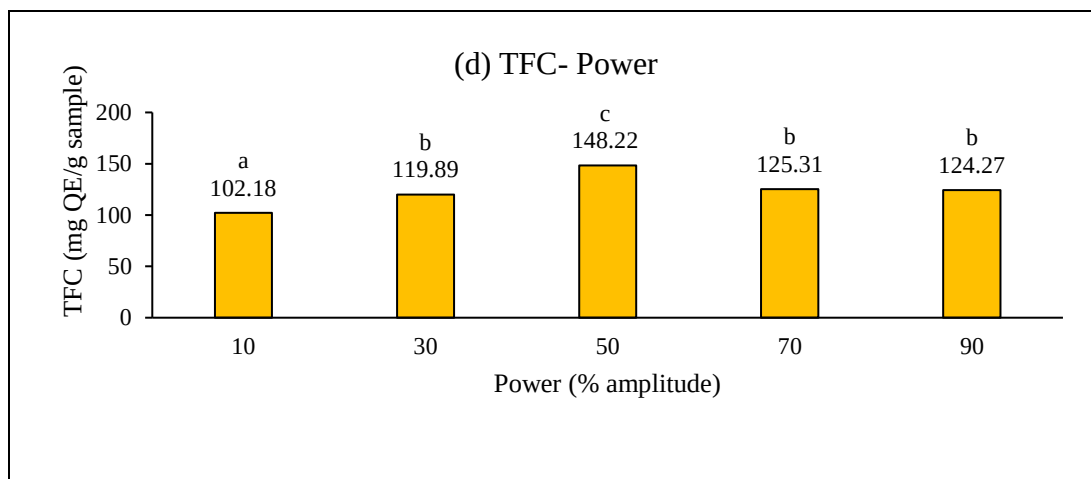


Figure 3.7: Continued.

Increased ultrasound power attributed to increased cavitation effect caused enhancement of TPC (Figure 3.7a) with a significant difference between 10% and 30% ($p \leq 0.05$) (48.43 and 72.50 mg GAE/g sample, respectively) while after 30% there wasn't a significant difference ($p > 0.05$) among the power levels. Although statistically no difference was observed among TPC values, high power of ultrasound destroys extracted phenolic compounds and thereby reducing the antioxidant activity [Li et al., 2021], [Xu et al., 2022]. DPPH (Figure 3.7b) and ABTS (Figure 3.7c) reached at maximum value at 50% power of ultrasound (49.01 %inhibition and 128.99 mM TE/ml extract) and started to decrease by increasing ultrasound power. In a study about the ABTS value of *Melastoma sanguineum*, the reported results support the phenomenon that antioxidant activity increases with increasing ultrasound power but decreases when the ultrasound power exceeds a certain level, possibly due to degradation of some antioxidant compounds.

TFC reached the highest value significantly at 50% power level of 148.22 mg QE/g sample (Figure 3.7d). There weren't significant differences between 70% and 90% values of TPC, TFC, DPPH and ABTS ($p > 0.05$).

TPC was correlated with TFC ($r = 0.603$ $p > 0.05$) which means that the considerable amount of soluble phenolic compounds were flavonoids. TPC showed negative correlation with DPPH ($r = -0.592$, $p > 0.05$) and ABTS ($r = -0.567$, $p > 0.05$). This result can be attributed of the fact that the antioxidant activity ability does not depend only phenolic compounds but also the other compounds such as vitamin E or C which can found in galangal [Rusea et al., 2014], [Rachkeeree et al., 2018]. Also the

negative correlation of DPPH and ABTS antioxidant activities with may indicate that the antioxidant activities of the phenolic compounds in the extract were not very strong. On the side of the TFC there was positive correlation between DPPH ($r = 0.233$, $p > 0.05$) and ABTS ($r = 0.205$, $p > 0.05$) which means that the considerable amount of soluble phenolic compounds were flavonoids and that contribute to antioxidant activity [More and Arya, 2021], [Vo et al., 2022]. Conversely to TPC, both DPPH and ABTS showed positive correlation with TFC which is interpreted as a significant part of the phenolic compounds that affected the radical scavenging activities consisting of flavonoids.

DPPH and ABTS were strongly correlated ($r = 0.988$, $p \leq 0.01$) with each other. Similar results obtained by Osman et al., (2021) about the antioxidant activity of *Artemia salina* with a strong correlation between DPPH and ABTS ($r = 0.790$) [Osman et al., 2021]. The result indicates that these two antioxidant activity methods support each other under these conditions.

The effect of ultrasound cycle on the TPC, DPPH, ABTS and TFC of ultrasound-assisted extraction of galangal was studied at the extraction temperature of 25 °C, extraction time of 20 min, ethanol/water ratio of 50%, solvent/solid ratio of 10 and ultrasound power of 50% (Figure 3.8).

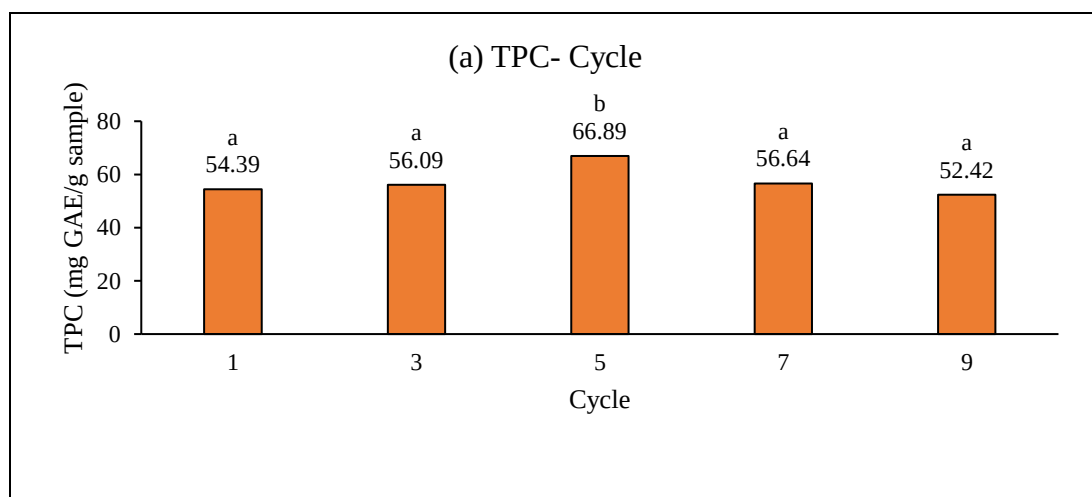


Figure 3.8: Effect of ultrasound cycle on (a) TPC, (b) DPPH, (c) ABTS and (d) TFC of galangal at the extraction temperature of 25 °C, extraction time of 20 min, ethanol/water ratio of 50%, solvent/solid ratio of 10 and ultrasound power of 50%.

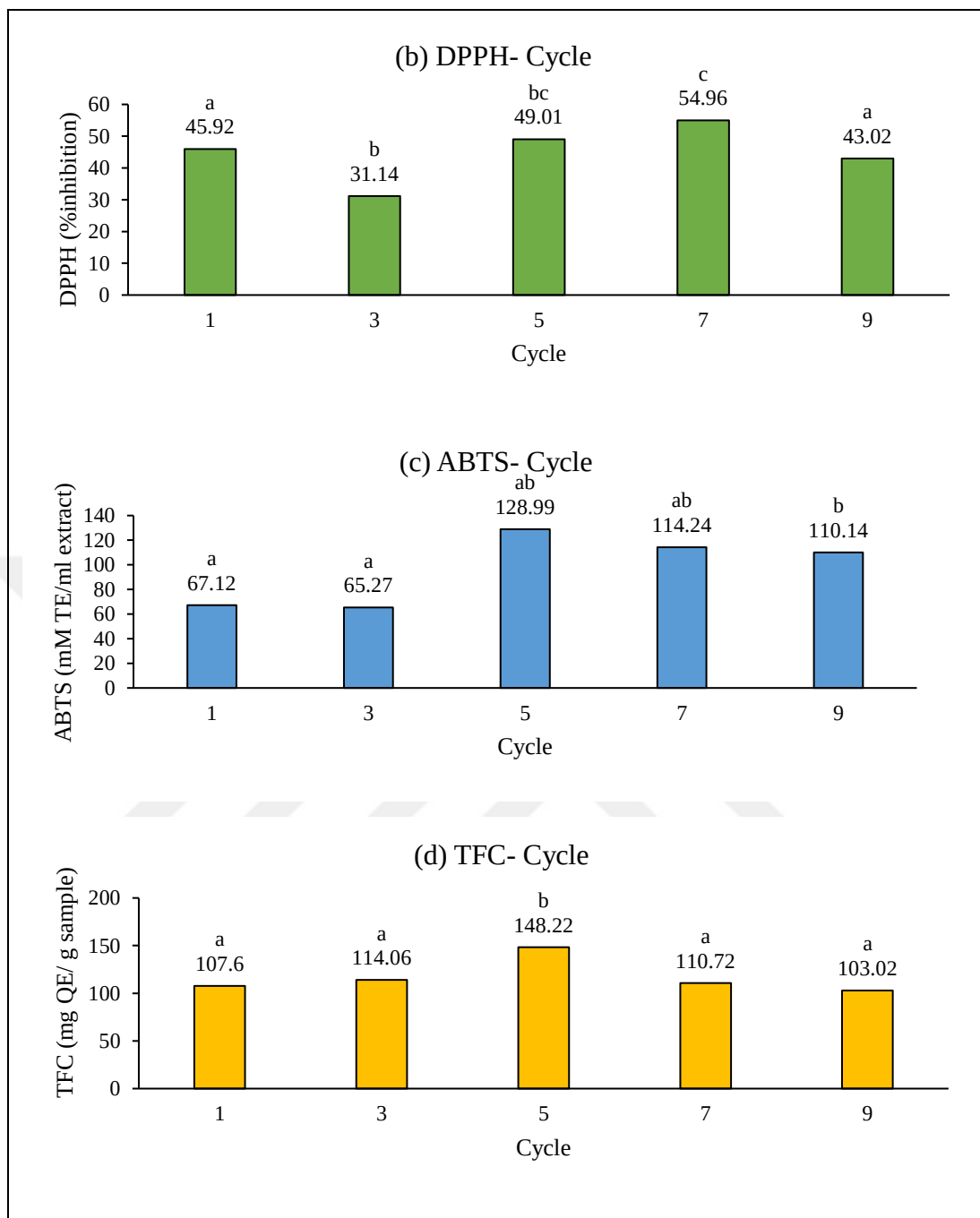


Figure 3.8: Continued.

The highest TPC (Figure 3.8a) and TFC (Figure 3.8d) values were obtained at 5 cycle (66.89 mg GAE/g sample and 148.22 mg QE/g sample, respectively) and there wasn't a significant difference ($p > 0.05$) among other cycle levels. The generation of higher cycle of ultrasound (7 and 9) had no significant ($p > 0.05$) effect on TPC, similar to the study on high frequency ultrasound waves in the study about the extraction of phenolic compounds from olive oil done by [Juliano et al., 2017]. Pearson correlation

of TPC and TFC was very strong ($r = 0.992$, $p \leq 0.05$) that shows most of the phenolic compound were flavonoids at specified cycles of ultrasound.

High cycle of ultrasound generates a large number of free radicals that degrade the polyphenols and reduce their antioxidant activity, as shown in DPPH and ABTS at high frequency levels (Figures 3.8b and 3.8c). DPPH was at the highest value of 54.96 %inhibition at 7 cycle while ABTS was at the highest value of 128.99 mM TE/ml extract at 5 cycle. According to Pearson correlation analysis, DPPH and ABTS were moderately correlated ($r = 0.667$, $p > 0.05$) that shows these two methods were appropriate to determine antioxidant capacity under these conditions. The TPC, TFC, DPPH and ABTS of *Lobelia nicotianifolia* leaves increased up by increasing ultrasound frequency to some point and decreased by increasing frequency [Zimare et al., 2021].

3.3. Optimization of Ultrasound-Assisted Extraction of Polyphenols

Screening experiments were done to determine the effect of temperature, time, ethanol/water ratio, solvent/solid ratio, power of ultrasound, and cycle of ultrasound on the four responses (TPC, DPPH, ABTS and TFC). Ethanol/water ratio of 50%, ultrasound power of 50% and ultrasound cycle of 5 were chosen as constant. Independent variables for the optimization study were extraction temperature (20-60 °C), extraction time (10-60 min) and solvent/solid ratio (10-30 ml/g).

Experimental design is used to understand the effect of independent parameters on the extraction process, decrease experiment number, and save time and resources. Additionally, experimental errors are minimized, and statistical approaches measure the effect of changes and their interaction on the process [Maran et al., 2015]. In this study, effect of three independent factors (temperature, time, and solvent/solid ratio) optimized and investigated the impact on TPC, DPPH, ABTS and TFC. Table 3.3 showed the experimental data of ultrasound-assisted extraction conditions according to Box- Behnken design (BBD) and experimental data were fitted second-order polynomial Equation 3.4.

Model adequacy was examined by four high degree polynomial models (linear, quadratic, cubic, and interactive (2FI). Quadratic model was found to be the most suitable model to express the ultrasound-assisted extraction process.

Regression of the fitted models was developed for all the responses, and experimental data were fitted, and analysis of variance (ANOVA) (Table 3.5). The statistical significances were evaluated by probability value (p-value) for all responses for developed models are statistically significant $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$ for all responses, and not significant $p > 0.05$ for lack of fit of the models adequately describes the relationship between responses and factors. This showed that the developed models satisfactorily represented the real relationship between independent variables and responses. The R^2 and adjusted R^2 values were higher than 0.90 and 0.78, respectively, indicating that the experimental data and the quadratic models fit satisfactorily, and there was a good correlation between the experimental and predicted values (Table 3.8). The regression coefficients of the quadratic surface models were given in Table 3.7. In the model equation, positively signed terms tend to increase response values, while negative values tend to decrease response values. The three-dimensional 3D response surface plots were generated by keeping one independent variable at the middle and for three independent variables shown in Figure 3.9-3.13.

Table 3.5 represents the summary of regression coefficients from the variance analysis (ANOVA) according to the second-degree polynomial equations (Table 3.7) determination coefficients and significance (p-value) of the models. Quadratic equations were obtained by significant responses ($p \leq 0.001$ for TPC, ABTS and TFC; $p \leq 0.01$ for DPPH). Analyzing of determination of coefficients R^2 values showed high model accuracy and acceptable R^2 values (0.9659 for TPC; 0.9078 for DPPH; 0.9838 for ABTS and 0.9587 for TFC). The closer the R^2 value to 1.000, the empirical model fits with real data better [Hishmath et al., 2011]. Adjusted R^2 (0.9220 for TPC; 0.9629 for ABTS and 0.9057 for TFC) were also very high and suggested high significant model. DPPH models show a lower adjusted R^2 value than other responses (0.7893) because of non-significant terms that the DPPH model has included. However, it indicates that the reliability of the developed model was low but still sufficient for a model with good desirability.

ANOVA variance analysis results showed that the lack of fit p-values of responses was not significant ($p > 0.05$) (0.8713 for TPC; 0.8710 for DPPH; 0.5861 for ABTS and 0.2810 for TFC), which are validated of the suitability of the model. CV% (coefficient of variance) values were low (2.64 for TPC; 5.39 for DPPH; 5.79 for ABTS and 5.49 for TFC) and the CV lower than 10% suggests significantly high accuracy and reliability models [Zhang and Lee, 2021]. Low CV % values clearly

indicate the high degree of precision and good reliability of the experimental values. High R^2 values with low CV % values indicate that the developed model can make a fairly good prediction of the system's response in the studied range [Maran et al., 2015].

Based on ANOVA results (Table 3.5), for TPC, linear X_1 , X_2 , and X_3 ; quadratic X_1^2 , X_3^2 , and interaction values X_1X_2 , X_1X_3 and X_2X_3 were significant ($p \leq 0.05$) parameters, while quadratic X_2^2 was not significant ($p > 0.05$). For DPPH, the linear X_3 value was significant ($p \leq 0.05$), while the other linear X_1 , X_2 ; quadratic X_1^2 , X_2^2 , X_3^2 , and interaction values of X_1X_2 , X_1X_3 , and X_2X_3 were not significant ($p > 0.05$). For ABTS, linear X_1 , X_3 ; quadratic X_2^2 , X_1^2 , and interaction values of X_1X_2 , X_1X_3 , and X_2X_3 were significant ($p \leq 0.05$), while linear X_2 and quadratic X_1^2 values were not significant ($p > 0.05$). For TFC, linear X_1 , X_2 ; quadratic X_1^2 , X_3^2 values, and interaction values of X_1X_2 were significant ($p \leq 0.05$) but linear X_3 , quadratic X_2^2 values, and interaction values of X_1X_3 and X_2X_3 were not significant ($p > 0.05$).

Regression coefficients of the second-degree polynomials for response parameters were given in Table 3.6. Negative values of quadratic terms of X_1^2 , X_2^2 , X_3^2 for TPC; linear and quadratic terms of X_2 , X_2^2 , X_3^2 for DPPH; X_2 , X_1^2 , X_2^2 for ABTS and X_3 , X_1^2 , X_3^2 for TFC indicate the negative effect of these terms on responses of process. All other terms were highly dependent on temperature value (X_1), as it was shown by equation coefficients. High temperature led to increased solubility and diffusion of bioactive compounds better [Adil et al., 2008].

Increasing extraction time (X_2) impresses TPC and TFC positively. On the other hand, time had a negative effect on the ANOVA analysis (-0.52 for DPPH and -4.7887 for ABTS) for both antioxidant activity determination methods, indicating that the bioactive compounds in extracts begin to lose their antioxidant activity with prolonged time and the effect of applied ultrasound [Li et al., 2019].

The increased s/s ratio (X_3) caused a positive effect and improvement in all responses except TFC. The decrease of TFC may depend on the low concentration gradient of flavonoids in increasing the solvent/solid ratio. Similarly, some studies have shown that some subgroup phenolic compounds (anthocyanins, isoflavones) were extracted more effectively at a lower solvent/solid ratio [Cacace and Mazza, 2003] [Rostagno et al., 2004].

Table 3.3: Experimental values for extracted polyphenols from galangal by Box-Behnken design.

Run No	X ₁ : Temperature (°C)	X ₂ : Time (min)	X ₃ : Solvent/solid ratio (ml/g)	Responses			
				TPC (mg GAE/g sample)	DPPH (%inhibition)	ABTS (mM TE/ml extract)	TFC (mg QE/g sample)
1	40	60	30	68.10 ± 0.73	46.52 ± 1.62	144.93 ± 38.85	140.15 ± 3.38
2	40	35	20	63.73 ± 1.08	47.82 ± 0.74	115.8 ± 6.38	136.87 ± 6.25
3	20	35	10	47.19 ± 1.84	40.03 ± 2.32	87.40 ± 2.45	78.38 ± 5.08
4	40	35	20	62.32 ± 0.47	42.60 ± 1.63	131.45 ± 10.45	123.85 ± 4.73
5	20	10	20	56.05 ± 1.83	40.45 ± 0.85	66.81 ± 6.64	103.02 ± 3.55
6	40	35	20	66.70 ± 0.76	40.99 ± 1.10	118.26 ± 17.85	137.6 ± 2.00
7	40	10	30	65.02 ± 0.77	49.52 ± 2.28	178.43 ± 19.82	103.90 ± 5.49
8	60	35	10	61.77 ± 0.96	36.61 ± 0.71	92.36 ± 7.10	140.15 ± 6.52
9	60	10	20	60.62 ± 0.54	45.63 ± 1.24	147.84 ± 14.04	117.6 ± 1.30
10	40	10	10	50.58 ± 0.78	34.14 ± 0.49	90.72 ± 7.84	111.71 ± 1.36
11	20	35	30	66.73 ± 1.34	46.27 ± 2.74	122.81 ± 10.02	82.96 ± 5.21
12	40	35	20	66.82 ± 0.65	43.42 ± 0.46	124.28 ± 14.63	134.27 ± 3.55
13	20	60	20	62.67 ± 1.56	41.71 ± 0.90	98.06 ± 14.31	113.85 ± 2.60
14	40	35	20	63.82 ± 0.11	46.57 ± 0.67	112.81 ± 23.40	136.97 ± 7.81
15	40	60	10	61.61 ± 0.29	34.48 ± 0.61	93.18 ± 3.54	140.15 ± 1.65
16	60	35	30	59.16 ± 1.10	52.35 ± 3.73	215.62 ± 10.68	118.9 ± 3.01
17	60	60	20	67.45 ± 1.06	42.87 ± 0.66	109.32 ± 8.70	161.14 ± 4.85

For all measurements, data are expressed as mean values with standard deviations. Mean value is the average of three replications.

The maximum TPC value was obtained in experiment #1 at the conditions of 40 °C, 60 min and 30 solvent/solid ratio (68.10 mg GAE/g sample). The minimum TPC value was obtained in the experiment #3 at the conditions of 20 °C, 35 min and 10 solvent/solid ratio (47.19 mg GAE/g sample).

The maximum DPPH value was obtained in experiment #16 at the conditions of 60 °C, 35 min and 30 solvent/solid ratio (52.35 %inhibition). The minimum DPPH value was obtained in the experiment #10 at the conditions of 40 °C, 10 min and 10 solvent/solid ratio (34.14 %inhibition).

The maximum ABTS value was obtained in experiment #16 at the conditions of 60 °C, 35 min and 30 solvent/solid ratio (215.62 mM TE/ml extract). The minimum ABTS value was obtained in the experiment #5 at the conditions of 20 °C, 10 min and 20 solvent/solid ratio (87.40 mM TE/ml extract).

The maximum TFC value was obtained in experiment #17 at the conditions of 60 °C, 60 min and 20 solvent/solid ratio (161.14 mg QE/g sample). The minimum TFC value was obtained in the experiment #3 at the conditions of 20 °C, 35 min and 10 solvent/solid ratio (78.38 mg QE/g sample).

3.3.1. Pearson Correlation Analysis of Optimization

Table 3.4: Pearson correlation coefficient of optimization responses.

		TPC	DPPH	ABTS	TFC
TPC	Pearson	1.000	0.418	0.327	0.561
	Correlation				
DPPH	Significance		0.095	0.200	0.019*
	Pearson	0.418	1.000	0.793	-0.68
ABTS	Correlation				
	Significance	0.095		0.000*	0.795
TFC	Pearson	0.327	0.793	1.000	0.019
	Correlation				
	Significance	0.200	0.000*		0.942
	Pearson	0.561	-0.068	0.019	1.000
	Correlation				
	Significance	0.019*	0.795	0.942	

Pearson correlation analysis results of the seventeen experimental runs were given in Table 3.4. According to Taylor (1990) Pearson correlation coefficient of less than 0.35 shows weak correlation, between 0.35 and 0.68 shows moderate correlation, and between 0.68 and 1.00 indicates a strong correlation [Taylor, 1990]. The different approaches were observed of independent parameters on DPPH and ABTS because of the different mechanisms of these two methods. Still, there was also a strong correlation between DPPH and ABTS ($r = 0.793$, $p \leq 0.05$) for the samples which shows that similar scavenging antioxidant activity was observed between runs for these two methods. TPC showed moderate correlation with DPPH ($r = 0.418$, $p > 0.05$) and TFC ($r = 0.561$, $p \leq 0.05$) this confirms that DPPH radical scavenging activity is related with higher TPC and TFC content. DPPH and affect each other with a moderate correlation ($r = 0.500$, $p \leq 0.05$). Pearson correlation analysis results showed that there is a negative correlation between TFC and DPPH values according to Pearson coefficient ($r = -0.68$, $p \leq 0.05$)

Table 3.5: Analysis of variance (ANOVA) for all the responses for galangal extract samples.

Source	df	TPC		DPPH		ABTS		TFC	
		Sum squares	of p-value	Sum squares	of p-value	Sum squares	of p-value	Sum squares	of p-value
Model	9	526.52	0.0002***	371.43	0.0068**	20664.55	≤0.0001***	7358.67	0.0005***
Linear									
X ₁	1	33.46	0.0094**	10.13	0.2128	4515.35	≤0.0001***	3183.22	≤0.0001***
X ₂	1	94.94	0.0006***	2.16	0.5465	183.46	0.0934	1771.91	0.0004***
X ₃	1	179.17	≤0.0001***	305.05	0.0001***	11110.19	≤0.0001***	74.91	0.2391
Quadratic									
X ₁ ²	1	32.96	0.0097**	1.13	0.6612	158.74	0.1140	878.53	0.0031**
X ₂ ²	1	0.1406	0.8247	19.15	0.1014	331.46	0.0350*	82.83	0.2182
X ₃ ²	1	42.25	0.0053**	4.06	0.4139	968.64	0.0029**	869.43	0.0032**
Interaction									
X ₁ X ₁	1	0.0110	0.0003***	4.04	0.4153	1216.96	0.0016**	267.49	0.0453*
X ₁ X ₃	1	122.66	0.0449*	22.56	0.0800	1929.41	0.0004***	166.80	0.0963
X ₂ X ₃	1	15.80	0.0097**	2.79	0.4952	323.28	0.0367*	15.25	0.5498
Residual	7	18.61				340.94		316.76	
Lack of fit	3	2.74	0.8713	5.56	0.8710	120.51	0.5861	183.69	0.2801
Pure error	4	15.87		32.16		220.43		133.07	
R ²		0.9659		0.9078		0.9838		0.9587	
Adj. R ²		0.9220		0.7893		0.9629		0.9057	
CV (%)		2.64		5.39		5.79		5.49	

Significance level: ***p ≤ 0.001. **p ≤ 0.01 and *p ≤ 0.05.

Table 3.6: Regression coefficient of the second-degree polynomials for response parameters for UAE extracted polyphenols from galangal under different extraction conditions of temperature, time and solvent/sample ratio.

Coefficients		Responses								
			TPC		DPPH		ABTS		TFC	
			p-values		p-values		p-values		p-values	
Intercept		β_0	64.678		44.28		120.52		133.912	
Linear	X_1	β_1	2.045	0.0094**	1.125	0.2128	23.7575	≤ 0.0001 ***	19.9475	≤ 0.0001 ***
	X_2	β_2	3.445	0.0006***	-0.52	0.5465	-4.78875	0.0934	14.8825	0.0004***
	X_3	β_3	4.7325	≤ 0.0001 ***	6.175	0.0001***	37.2663	≤ 0.0001 ***	-3.06	0.2391
Quadratic	X_1^2	β_{11}	-2.79775	0.0097**	0.5175	0.6612	-6.14	0.1140	-14.4448	0.0031**
	X_2^2	β_{22}	-0.18275	0.8247	-2.1325	0.1014	-8.8725	0.0350*	4.43525	0.2182
	X_3^2	β_{33}	-3.16775	0.0053**	-0.9825	0.4139	15.1675	0.0029**	-14.3687	0.0032**
Interaction	X_1X_2	β_{12}	0.0525	0.9505	-1.005	0.4153	-17.4425	0.0016**	8.1775	0.0453*
	X_1X_3	β_{13}	-5.5375	0.0003***	2.375	0.0800	21.9625	0.0004***	-6.4575	0.0963
	X_2X_3	β_{23}	-1.9875	0.0449*	-0.835	0.4952	-8.99	0.0367*	1.9525	0.5798

Significance level: *** $p \leq 0.001$. ** $p \leq 0.01$ and * $p \leq 0.05$.

Table 3.7: Equations of the second-degree polynomials for response parameters.

Reponses	Equations
TPC	$Y = 64.48 + 2.05X_1 + 3.45X_2 + 4.73X_3 - 2.80X_1^2 - 0.1828X_1^2 - 3.17 X_3^2 + 0.0525X_1X_2 - 5.54X_1X_3 - 1.99X_2X_3$
DPPH	$Y = 44.28 + 1.13X_1 - 0.52X_2 + 6.18X_3 + 0.517X_1^2 - 2.13X_1^2 - 0.9825X_3^2 - 1.01X_1X_2 + 2.38X_1X_3 - 0.8350X_2X_3$
ABTS	$Y = 120.52 + 23.56X_1 - 4.79X_2 + 37.27X_3 - 6.14X_1^2 - 8.87X_1^2 + 15.17X_3^2 - 17.44X_1X_2 + 21.96X_1X_3 - 8.99X_2X_3$
TFC	$Y = 133.91 + 19.95X_1 + 14.88X_2 - 3.06X_3 - 14.44X_1^2 + 4.44X_1^2 - 14.37X_3^2 + 8.18X_1X_2 - 6.46X_1X_3 + 1.95X_2X_3$

The experimental results obtained using the Box-Behnken experimental design model and quadratic polynomial equations with interaction terms between the independent variables were given in Table 3.7 with the coded values. Both significant and non-significant values were taken into account. X_1 was coded for temperature, X_2 for time, and X_3 for solvent/solid ratio.

3.3.2. Effect of Ultrasound-Assisted Extraction Conditions on Total Phenolic Contents (TPC)

The total phenolic content (TPC) of galangal extracts ranged from 47.19 to 68.10 mg GAE/g sample depending on the ultrasound-assisted extraction conditions (Table 3.3). The maximum total phenolic content was consistent with (%50 ethanol/water ratio, 50% ultrasound power level and 5 level of cycle) 40 °C, 60 min and 30 solvent/solid ratio conditions. The terms with effect on TPC were the linear terms of s/s ratio, time and temperature; quadratic terms of s/s ratio, temperature and time and interaction terms of temperature with s/s ratio ($p \leq 0.001$) in descending order. The most effective term was s/s ratio on TPC.

To understand the interaction of different experimental factors on the effects of responses better, 3D response surface plots were determined. These plots showed the impact of two variables on the response while the third independent factor was constant as its middle level. Figure 3.9 shows the three-dimensional response surface plots showing the interaction effects of independent variables on TPC. The

investigated design spaces were depicted in Figure 3.9a as a function of time (min), and temperature (°C) in Figure 3.9b as a function of solvent/solid ratio (ml/g) and temperature (°C), and Figure 3.9c as a function of solvent/solid ratio (ml/g) and time (min).

According to the regression coefficient table, the interaction of time (X_2), and temperature (X_1) wasn't significant ($p > 0.05$). Figure 3.9a displayed the effect of time and temperature, when the solvent/solid ratio was constant at 20. TPC changed when the extraction time or temperature increased and reached the maximum at 60 °C and 60 min of 67.45 mg GAE/g sample while minimum at 20 °C and 10 min of 56.05 mg GAE/g sample when the s/s ratio was 20. When the temperature increases, effect of time increased more on the TPC. The concave characteristics in 3D response surface due to temperature change and TPC increased until 40 °C then decreased by increasing temperature. Increasing temperature in long time extraction more effective than short time extraction. When the temperature was constant, TPC increased by increasing time. Maximum TPC was observed when the temperature and time TPC was maximum.

According to regression coefficient table, interaction of solvent/solid ratio (X_3), and temperature (X_1) was significant ($p \leq 0.001$). Figure 3.9b displayed the effect of solvent/solid ratio and temperature and temperature when the time was constant at 45 min. TPC changed when the extraction time or temperature increased and reached the maximum at 30 s/s ratio and 20 °C of 66.73 while minimum at 10 s/s ratio and 20 °C of 47.19 mg GAE/g sample when the time was 35 min. TPC increased by increasing temperature and effect of temperature was considerable because of its effects on diffusivity, mass transfer, solvent tension, viscosity and etc. Concave characteristic of the graph due to s/s ratio. Increasing s/s ratio the TPC increased considerably and then decreased sharply. High s/s ratio with low temperature conditions gave better results of TPC. When the s/s ratio and temperature were minimum, TPC value was the lowest as 47.19 mg GAE/g sample. According to 3D graph 1.9b, it can be said that the s/s ratio is effective on TPC and the moderate conditions gave better results.

According to regression coefficient table, interaction of solvent/solid ratio (X_3), and temperature (X_2) was significant ($p \leq 0.05$). Figure 3.9c displayed the effect of solvent/solid ratio and time when the temperature was constant at 40 °C. TPC changed when the extraction time or temperature increased and reached the maximum at 30 s/s ratio and 60 min of 68.10 mg GAE/g sample while minimum at 10 s/s ratio and 10 min

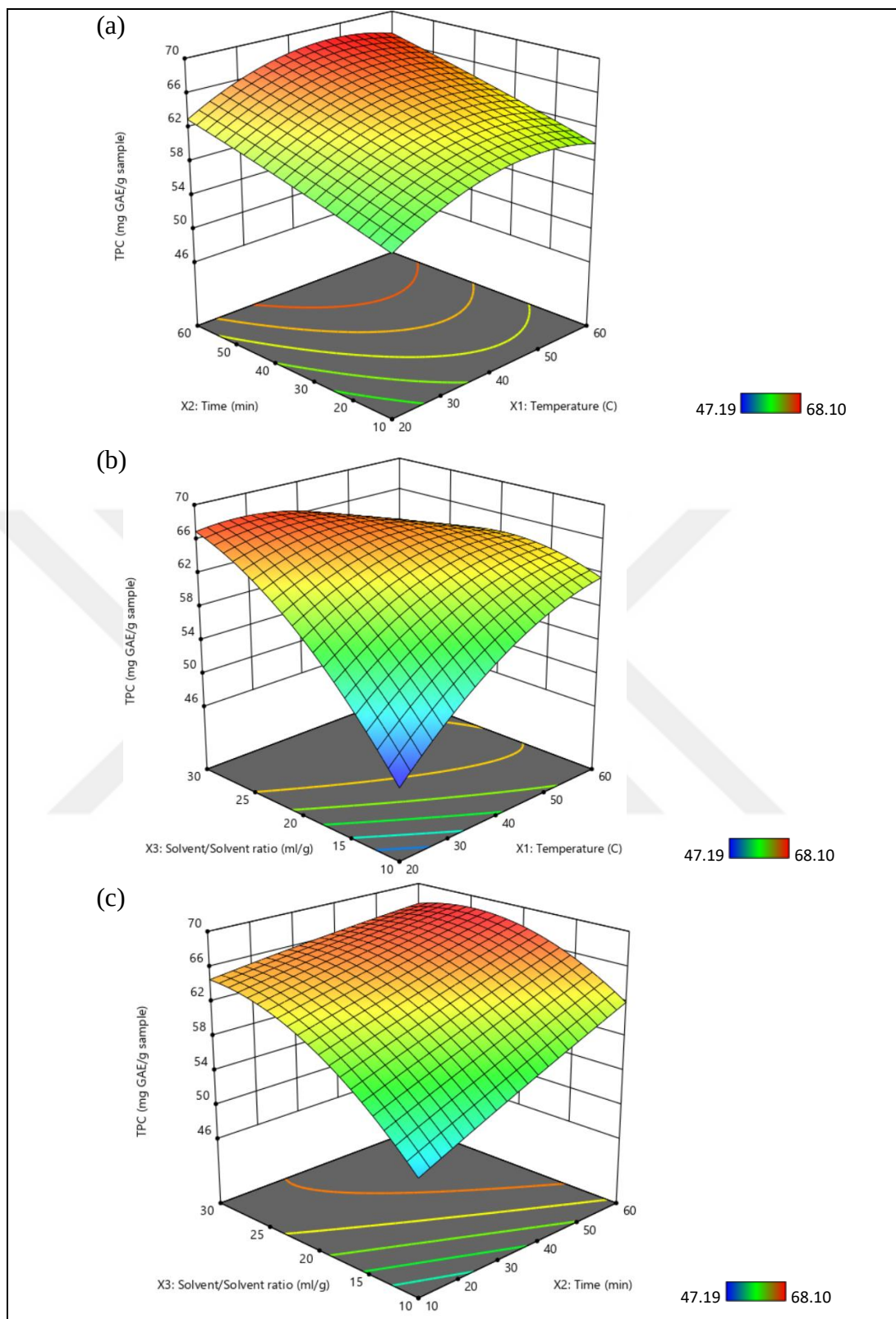


Figure 3.9: Three dimensional surface plots showing interaction effects of independent variables on the total phenolic content (TPC) a) temperature-time effect on extraction efficiency at s/s ratio of 20 ml/g b) s/s ratio-temperature effect on extraction efficiency at 35 min, c) s/s ratio-time effect on extraction efficiency at 40 °C.

of 50.58 mg GAE/g sample when the temperature was 40 °C. Increasing s/s ratio increased TPC and then slightly decreased. The reason for the increase in TPC depending on the increasing s/s ratio is that phenolic compounds can pass into the solvent more easily with the increase in solvent-material interaction [Wang et al, 2013]. The results are consistent with the results provided by the optimization study of the *Sparganii rhizome* extraction by Wang et al. (2013) [Wang et al, 2013]. Extraction time has a strong effect on TPC when the s/s ratio was kept at higher levels. Increasing time increased TPC greatly and the highest value was obtained at the maximum time and maximum s/s ratio as similar with the optimization study of *Rhamnus alaternus* leaves [Nekkaa et al., 2021]. Concave characteristic of the graph due to s/s ratio. The best results were obtained at high s/s ratio and high temperature values while low s/s ratio and low extraction time negatively affected the TPC.

3.3.3. Effect of Ultrasound-Assisted Extraction Conditions on Antioxidant Activity

Antioxidant activity of samples determined by DPPH and ABTS radical scavenging activity methods. The action mechanisms of DPPH and ABTS methods are to capture radical or transfer hydrogen atoms [Prior et al., 2005]. The difference between DPPH and ABTS is explained by the effecting mechanisms of these two methods. It is known that the ABTS method is practicable for both hydrophilic and hydrophobic systems. However, DPPH is more efficient in hydrophobic systems [Arend et al., 2017].

3.3.3.1. DPPH Method

The DPPH antioxidant activity of galangal extract ranged from 34.14 to 52.35 %inhibition, depending on the ultrasound-assisted extraction conditions (Table 3.3). The maximum DPPH antioxidant activity was consistent with (%50 ethanol/water ratio, 50% ultrasound power level, and 5 level of cycle) 60 °C for 35 min and 30 solvent/solid ratio conditions. The terms with the highest effect on the DPPH were the linear terms of s/s ratio, temperature and time the quadratic terms of time, s/s ratio and temperature in descending order. The most effective term on DPPH antioxidant activity was the s/s ratio ($p \leq 0.001$).

To understand the interaction of different experimental factors on the effects of responses better, 3D response surface plots were determined. These plots showed the impact of two variables on the response while the third independent factor was constant as its middle level. Figure 3.10 shows the three-dimensional response surface plots showing the interaction effects of independent variables on the antioxidant activity (DPPH). The investigated design spaces were depicted in Figure 3.10a as a function of time (min), and temperature (°C) in Figure 3.10b as a function of solvent/solid ratio (ml/g) and temperature (°C) and Figure 3.10c as a function of solvent/solid ratio (ml/g) and time (min).

According to the regression coefficient table, the interaction of time (X_2), and temperature (X_1) was not significant ($p > 0.05$). Figure 3.10a displayed the effect of time and temperature when the solvent/solid ratio was constant at 20. DPPH changed when the extraction time or temperature increased and reached the maximum at 35 min and 40 °C of 47.82% inhibition while minimum at 20 °C and 10 min of 40.15% inhibition when the s/s ratio was 20. DPPH slightly increased by increasing time and then slightly decreased. The concave characteristic of the graph due to time. Increasing temperature increased the DPPH better at low extraction time. DPPH increased by increasing temperature and high temperatures with short time conditions give better results.

According to regression coefficient table, interaction of solvent/solid ratio (X_3), and temperature (X_1) was not significant ($p > 0.05$). Figure 3.10b displays the effect of solvent/solid ratio and temperature when the time was constant at 45 min. DPPH changed when the extraction time or temperature increased and reached the maximum at 30 s/s ratio and 60 °C of 52.35% inhibition while minimum at 10 s/s ratio and 60 °C of 36.61% inhibition when time was 35 min. At low temperatures, increasing s/s ratio was more negatively effective than high temperatures. Similarly, at low s/s ratio, increasing temperature was more negatively effective than high s/s ratio. Because of the negatively effect of the conditions the DPPH was observed better at low s/s and low temperature conditions.

According to regression coefficient table, interaction of solvent/solid ratio (X_3), and temperature (X_2) was not significant ($p > 0.05$). Figure 3.10c displays the effect of the solvent/solid ratio and the time when the temperature was constant at 40 °C.

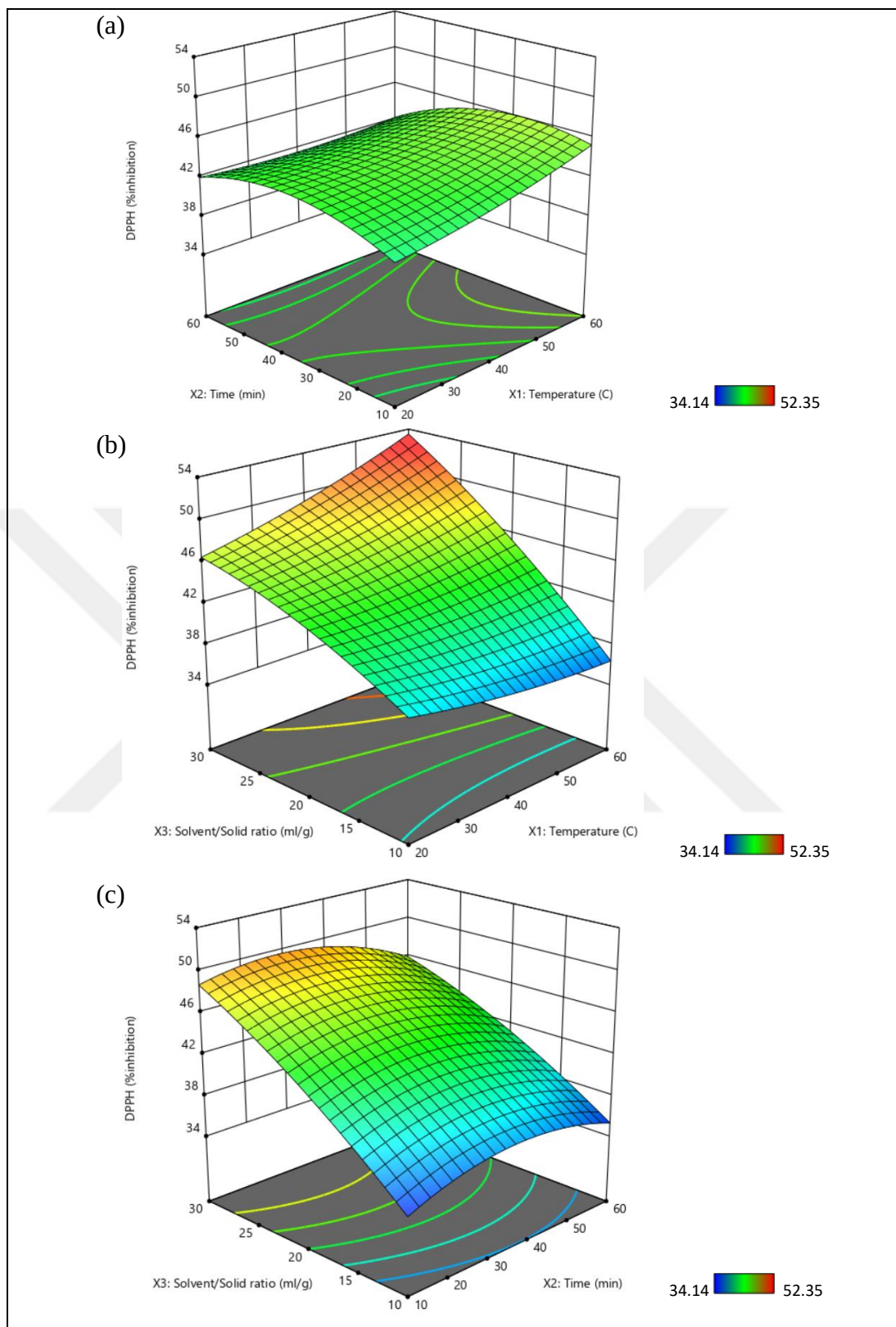


Figure 3.10: Three dimensional surface plots showing interaction effects of independent variables on the DPPH antioxidant activity a) temperature-time effect on extraction efficiency at s/s ratio of 20 ml/g b) s/s ratio-temperature effect on extraction efficiency at 35 min, c) s/s ratio-time effect on extraction efficiency at 40 °C.

DPPH changed when the extraction time or temperature increased and reached the maximum at 30 s/s ratio and 10 min of 49.52% inhibition while minimum at 10 s/s ratio and 10 min 34.14% inhibition when the temperature was 40 °C. Increased s/s ratio increased the DPPH linearly at constant time conditions. The concave characteristic of the graph due to time and increasing time increased the DPPH slightly and then decreased slightly. At low extraction time, increasing s/s ratio increased better the DPPH than high extraction time. Highest s/s ratio with moderate extraction time gave the best results.

3.3.3.2. ABTS Method

The ABTS antioxidant activity of galangal extract ranged from 66.81 to 215.32 mM TE/ml extract depending on the ultrasound-assisted extraction conditions (Table 3.3). The maximum ABTS antioxidant activity was consistent with (%50 ethanol/water ratio, 50% ultrasound power level, and 5 level of cycle) 60 °C for 35 min and 30 solvent/solid ratio conditions. The terms with the highest effect on the TPC were the linear terms of s/s ratio, temperature and time, the quadratic terms of s/s ratio, time and temperature in descending order. The most effective term was s/s ratio on ABTS ($p \leq 0.001$).

To understand the interaction of different experimental factors on the effects of responses better, 3D response surface plots were determined. These plots showed the impact of two variables on the response while the third independent factor was constant as its middle level. Figure 3.11 shows the three-dimensional response surface plots showing the interaction effects of independent variables on the antioxidant activity (ABTS). The investigated design spaces were depicted in Figure 3.11a as a function of time (min), and temperature (°C) in Figure 3.11b as a function of solvent/solid ratio (ml/g) and temperature (°C) and Figure 3.11c as a function of solvent/solid ratio (ml/g) and time (min).

According to the regression coefficient table, the interaction of time (X_2) and temperature (X_1) was significant ($p \leq 0.01$). Figure 3.11a displayed the effect of time and temperature, and temperature when the solvent/solid ratio was constant at 20. ABTS changed when the extraction time or temperature increased and reached the maximum at 10 min and 60 °C of 147.84 mg TE/ml extract and minimum at 20 °C 10 min of 66.81 mg TE/ml extract when the s/s ratio was 20. Increasing time slightly

effect the ABTS at low temperatures while hardly effect at high temperatures. At high temperatures ABTS decreased by increasing time. Temperature affected the ABTS by increasing rate. The best results were obtained at high temperatures and moderate time conditions. Short-time extraction at low temperature did not give good results.

According to the regression coefficient table, the interaction of solvent/solid ratio (X_3), and temperature (X_1) was significant ($p \leq 0.001$). Figure 3.11b displayed the effect of solvent/solid ratio and temperature and temperature when the time was constant at 35 min. ABTS changed when the extraction time or temperature increased and reached the maximum at 30 s/s ratio and 60 °C of 215.62 mg TE/ml extract and minimum at 10 s/s ratio and 20 °C of 87.4 mg TE/ml extract when the time was 35 min. Increasing s/s ratio and temperature negatively affect the ABTS and inner concave characteristic of the graph due to temperature. Extreme conditions degraded the antioxidant activity of the bioactive compound and low temperature and low s/s ratio ensured better ABTS results. Similar ABTS trend was observed by Silva et al., (2022) on the optimization study of kiwiberry leaves and the short extraction time with low s/s ratio was found better. [Silva et al., 2022]

According to the regression coefficient table, the interaction of solvent/solid ratio (X_3), and temperature (X_1) was significant ($p \leq 0.05$). Figure 3.11c displayed the effect of the solvent/solid ratio and time when the temperature was constant at 40 °C. ABTS changed when the extraction time or temperature increased and reached the maximum at 30 s/s ratio and 10 min of 178.43 mg TE/ml extract and minimum at 10 s/s ratio and 10 min of 90.72 mg TE/ml extract when the temperature was 40 °C. ABTS increased by increasing s/s ratio better at low temperatures than high temperatures. Depending on the increasing time, not much change was observed in ABTS at low s/s ratio while at high s/s ratio ABTS decreased by increasing time. The best results were obtained at high s/s ratio and low temperature values.

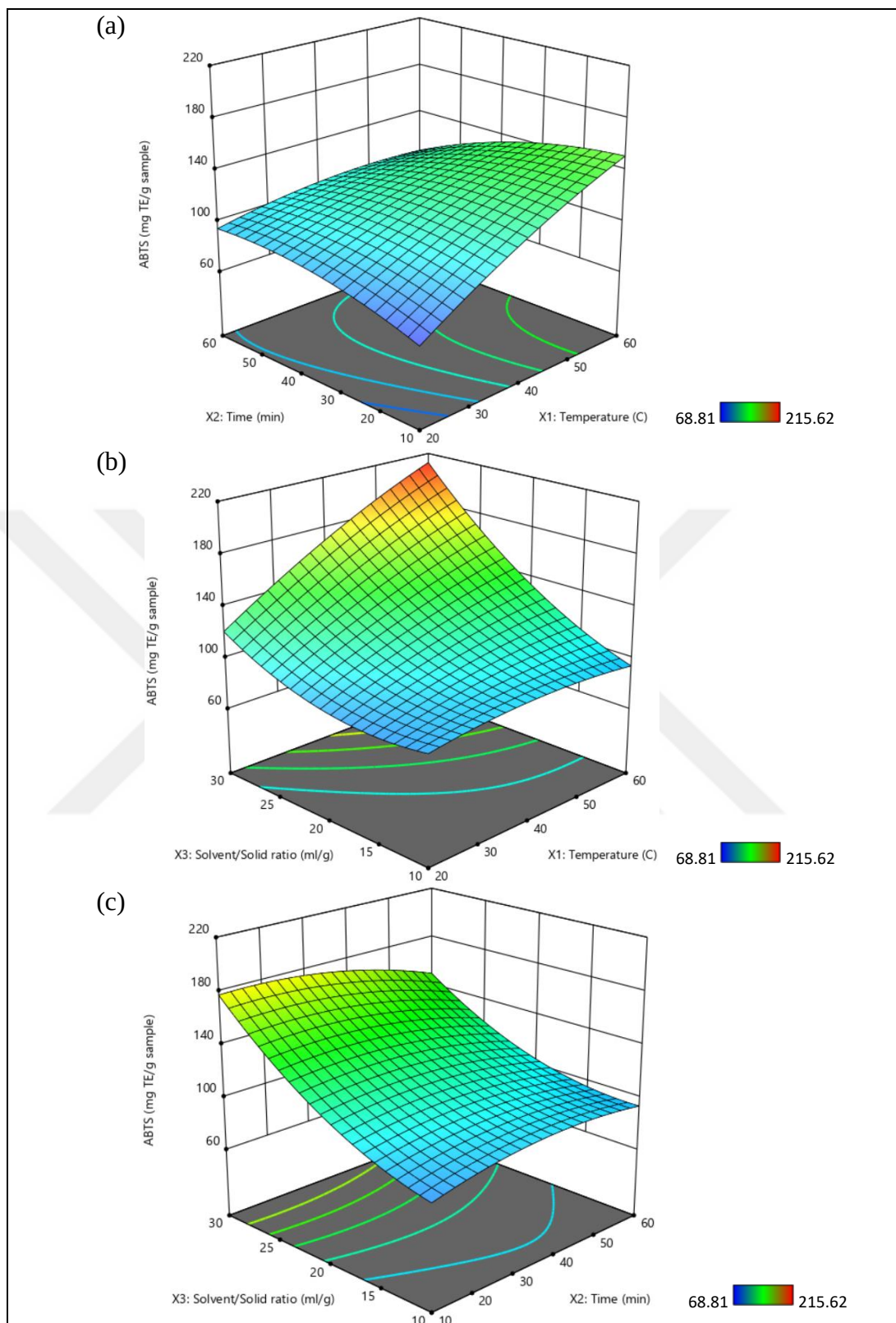


Figure 3.11: Three dimensional surface plots showing interaction effects of independent variables on the ABTS antioxidant activity a) temperature-time effect on extraction efficiency at s/s ratio of 20 ml/g b) s/s ratio-temperature effect on extraction efficiency at 35 min, c) s/s ratio-time effect on extraction efficiency at 40 °C.

3.3.4. Effect of Ultrasound-Assisted Extraction Conditions on Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of galangal extract ranged from 78.38 to 161.14 mg QE/g depending on the ultrasound-assisted extraction conditions (Table 3.3). The maximum TFC was consistent with (%50 ethanol/water ratio, 50% ultrasound power level, and 5 level of cycle) 60 °C for 60 min and 20 solvent/solid ratio conditions. The terms with the highest effect on the TFC were the linear terms of temperature, time and s/s ratio, the quadratic terms of temperature, time and s/s ratio in descending order. The most effective term on TFC was temperature ($p \leq 0.001$).

To understand the interaction of different experimental factors on the effects of responses better, 3D response surface plots were determined. These plots showed the impact of two variables on the response while the third independent factor was constant as its middle level. Figure 3.12 shows the three-dimensional response surface plots showing the interaction effects of independent variables on the total flavonoid content (TFC). The investigated design spaces were depicted in Figure 3.12a as a function of time (min), and temperature (°C) in Figure 3.12b as a function of solvent/solid ratio (ml/g) and temperature (°C) and Figure 3.12c as a function of solvent/solid ratio (ml/g) and time (min).

According to the regression coefficient table, the interaction of time (X_2) and temperature (X_1) was significant ($p \leq 0.05$). Figure 3.12a displayed the effect of time and temperature and temperature when the solvent/solid ratio was constant at 20. TFC changed when the extraction time or temperature increased and reached the maximum at 60 min and 60 °C of 161.14 mg QE/g sample and minimum at 10 min and 20 °C of 103.02 when the s/s ratio was 20. At low temperatures, increasing time not much effected on TFC while at high temperatures TFC increased by increasing time. The concave characteristic of the graph due to temperature. Increasing temperature increased the TFC and maximum TFC values were obtained by simultaneous increase of time and temperature. The maximum TFC of apple skins was observed at high temperature and time conditions when the other extraction parameters were constant [Casazza et al., 2020]. Therefore, it can be said that the flavonoid compounds are better extracted at excessive conditions.

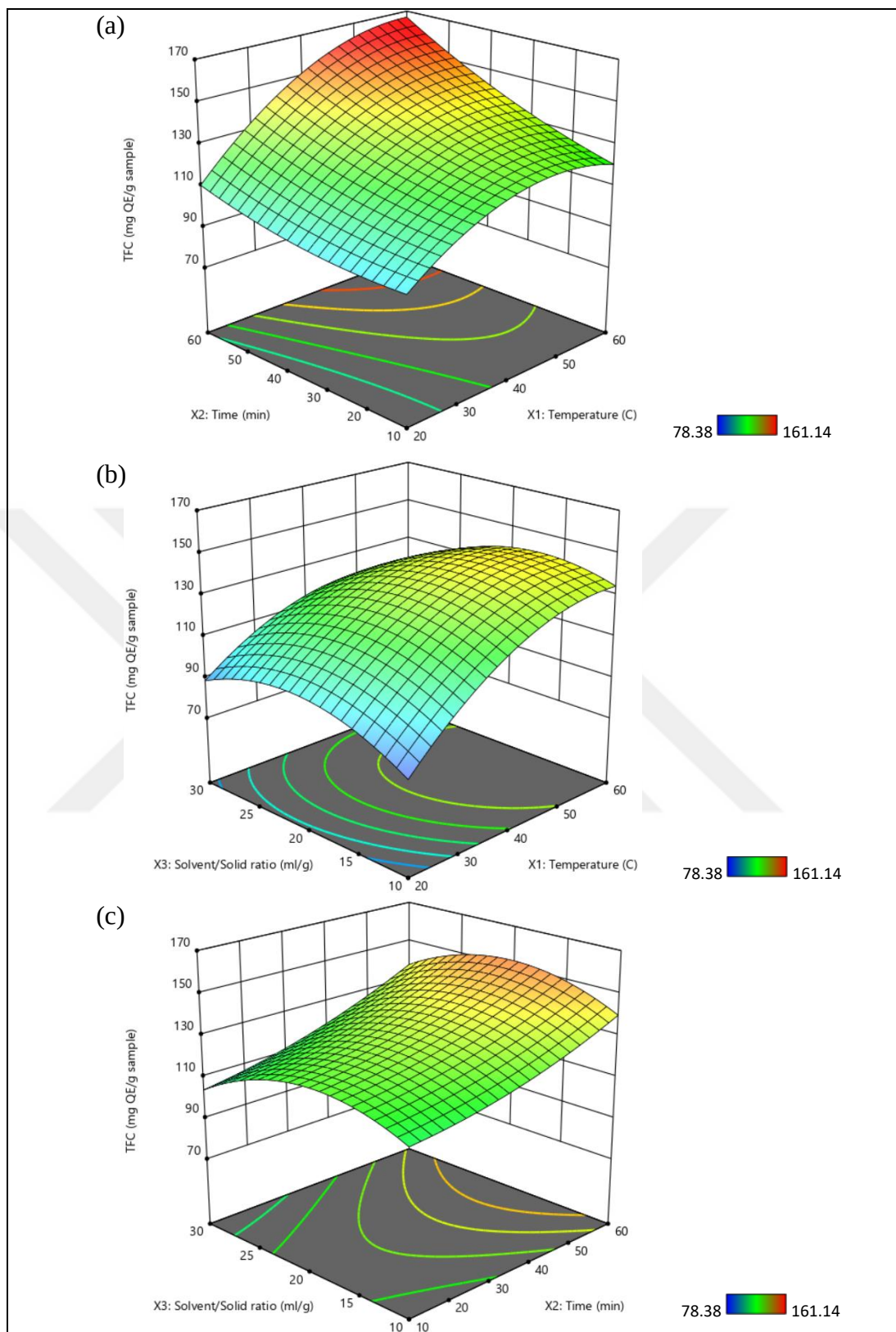


Figure 3.12: Three dimensional surface plots showing interaction effects of independent variables on the total flavonoid content (TFC) a) temperature-time effect on extraction efficiency at s/s ratio of 20 ml/g b) s/s ratio-temperature effect on extraction efficiency at 35 min, c) s/s ratio-time effect on extraction efficiency at 40 °C.

According to the regression coefficient table, the interaction of solvent/solid ratio (X_3) and temperature (X_1) was not significant ($p > 0.05$). Figure 3.12b displayed the effect of solvent/solid ratio and temperature and temperature when the time was constant at 45 min. TFC changed when the extraction time or temperature increased and reached the maximum at 60 °C and 10 s/s ratio of 140.15 QE/g sample and minimum at 20 °C and 10 s/s ratio of 78.38 mg QE/g sample when the time was 35 min. The concave characteristic of the graph due to both s/s ratio and temperature. By increasing s/s ratio and temperature, TFC increased but then slightly decreased when the temperature and s/s ratio reached maximum. While increasing temperature TFC increased at low s/s ratio, temperature caused a decrease in TFC as s/s increased. TFC decreased with increasing s/s ratio at high temperature. The effect of temperature was more effective at low s/s values ($p > 0.05$) of TFC and it has been observed that the TFC value the highest at the highest temperature and lowest s/s value.

According to the regression coefficient table, the interaction of solvent/solid ratio (X_3), and temperature (X_2) was not significant ($p > 0.05$). Figure 3.12c displayed the effect of the solvent/solid ratio and the time when the temperature was constant at 40 °C. TFC changed when the extraction time or temperature increased and reached the maximum at 60 min 20 s/s ratio of 161.14 QE/g sample and minimum at 10 min and 30 s/s ratio of 103.9 mg QE/g sample when the temperature was 40 °C. Increasing s/s increased the TFC than decreased slightly. Moderate s/s values were more convenient. S/s ratio was more effective at long extraction time than short extraction time to increase TFC. Maximum TFC were obtained at the long-time extraction with moderate s/s ratio.

3.4 Optimization of Ultrasound-Assisted Extraction and Validation

The multi-response optimization was applied to determine optimum conditions required to achieve high TPC, DPPH, ABTS and TFC. The optimum conditions were temperature of 47.51 °C, time of 52.66 min, and s/s ratio of 30 ml/g with the desirability of 0.77. The validation experiments were performed triplicate under optimized conditions to verify their reliability models. The predictive and experimental results were given in Table 3.8. There wasn't a significant ($p \leq 0.05$) difference between predicted and experimental values except for TFC.

Table 3.8: Predicted and experimental values at optimum conditions.

Optimum point			
Responses	Predicted	Experimental	p-value
TPC (mg GAE/g sample)	65.49	64.74 ± 1.63	0.742
DPPH (%inhibition)	48.56	48.49 ± 2.32	0.935
ABTS (mM TE/ ml extract)	170.42	167.98 ± 6.97	0.259
TFC (mg QE/g sample)	135.81	132.54 ± 6.72	0.037

For all measurements, data are expressed as mean values with standard deviations. Mean value is the average of three replications.

3.5. Comparison of Ultrasound-Assisted Extraction and Maceration

Optimized ultrasound-assisted extraction conditions (47.51 °C, 52.6 min, and 30 solvent/solid ratio with a constant 50% ethanol/water ratio, 50% ultrasound power, and 5 ultrasound cycle) were applied to galangal powder. Under the same conditions, maceration was applied by silencing the ultrasound parameters, and TPC, DPPH, ABTS and TFC were analyzed.

Table 3.9 represents the comparison of maceration and ultrasound-assisted extraction. TPC (12.68%), DPPH (12.65%), ABTS (83.24%) and TFC (6.5%) increased significantly ($p \leq 0.001$) by ultrasound-assisted extraction at the same conditions. Figure 3.13 represents the scanning electron microscopy (SEM) micrographs of galangal powder at 2000X before and after ultrasound-assisted extraction at the optimum point. When two micrographs are compared, it can be seen that the ultrasound treatment causes damage and fragmentation to the tissues.

Table 3.9: Comparison of extracted polyphenols from galangal by ultrasound-assisted extraction and maceration (without ultrasound) under optimum conditions.

Optimum point			
Responses	Maceration	Ultrasound-assisted extraction	p-value
TPC (mg GAE/g sample)	57.45 ± 0.46	64.74 ± 1.63	≤ 0.001
DPPH (%inhibition)	38.33 ± 0.66	48.49 ± 2.23	≤ 0.001
ABTS (mM TE/ ml extract)	91.67 ± 6.45	167.98 ± 6.59	≤ 0.001
TFC (mg QE/g sample)	124.45 ± 1.69	132.54 ± 6.72	≤ 0.001

For all measurements, data are expressed as mean values with standard deviations. Mean value is the average of three replications.

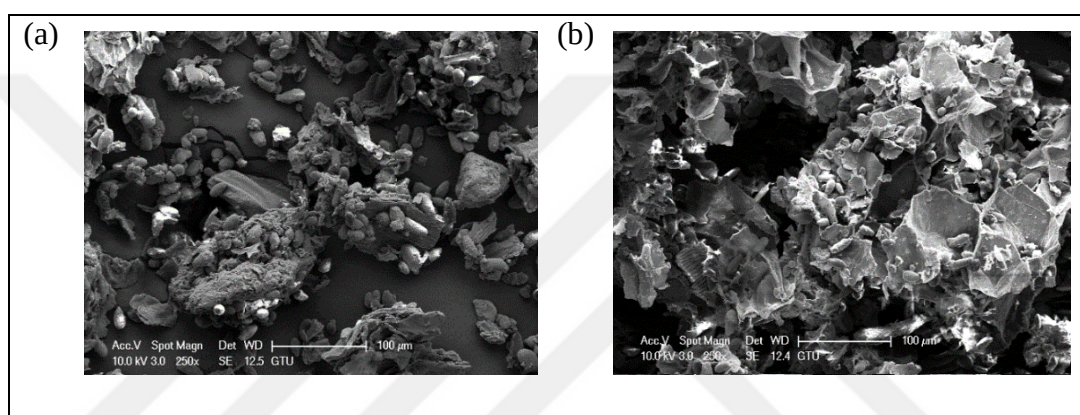


Figure 3.13: Scanning electron microscopy (SEM) micrographs of galangal powder at 2000X (a) before and (b) after ultrasound-assisted extraction at optimum point.

4. CONCENTRATION of POLYPHENOLS from GALANGAL by MEMBRANE PROCESSES

4.1. Materials and Methods for Concentration of Polyphenols from Galangal

Membrane processes offer sustainable solutions as they can operate prominent features are that they do not use chemicals or solvents, work with a closed system that prevents product from contamination, and work at room temperature with low energy consumption. In this way, it ensures the protection of the biological activity of the target compounds. [Conidi et al., 2020]. Membrane processes are based on the principle of selective permeability of semipermeable membranes, using mechanical pressure as a driving force. Major pressure-driven membrane processes are microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). Figure 4.1 presents schematically the primary sieving mechanism of the MF, UF and NF membranes. MF is used to separation fine particles, removing suspended high molecular weight particles and pass the water, high added-value compounds, and some micro-macro molecules. UF is used for separation of macromolecules and rejection of the micro-macro molecules, and NF membranes reject high molecular weight solutes and multivalent ions. And NF and RO have been successfully used for separation and concentration of polyphenols and other bioactive compounds with high antioxidant activity from natural products. Water and low molecular weight compound can pass through the NF permeates [Conidi et al., 2017]. Particles in all MF, UF and NF processes rely on a separation mechanism based on the sieving effect according to their size, and some other factors such as charge, shape and the interactions of the membrane with filtered particles [Bruggen et al., 2003]. Membranes can be used individually in separation and concentration processes or the combination of at least two membranes to achieve a better separation (UF/NF, UF/RO, MF/UF, and MF/UF/NF) [Nyamien et al., 2017].

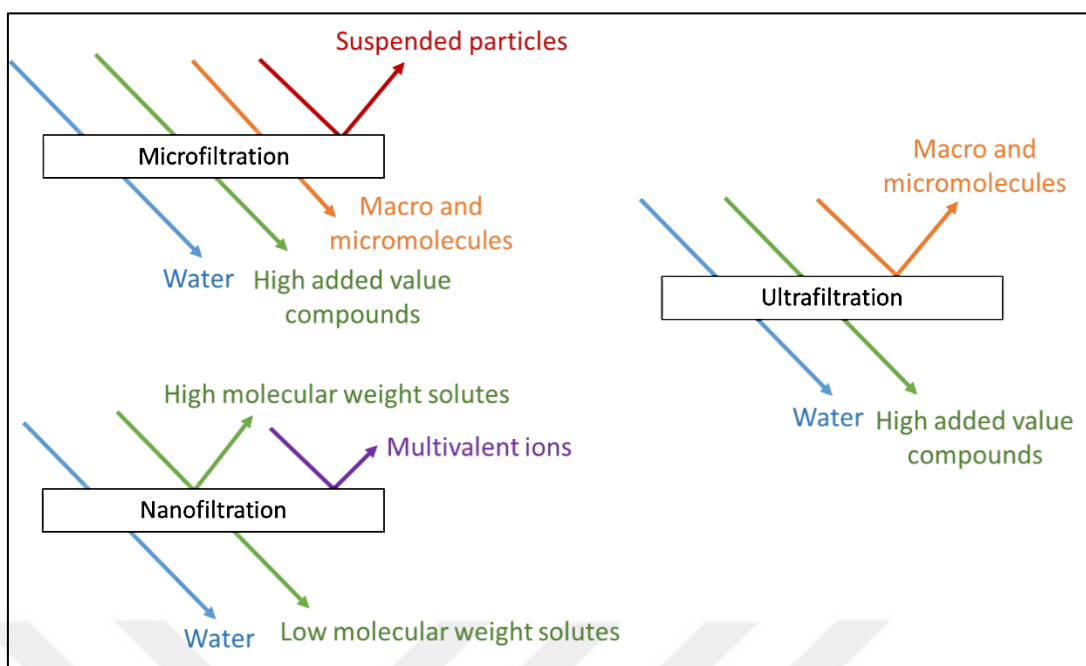


Figure 4.1: Sieving mechanism of microfiltration, ultrafiltration and nanofiltration.

UF and NF membranes which are the most commonly used, are classified according to MWCO (molecular weight cut-off value), described as the upper molecular weight limits of less than 10% of the compounds transported from the membranes [Lopez-Munoz et al., 2009]. Most UF membranes have MWCO values between 1000 and 100.000 Da and pore sizes between 2 and 100 nm [Conidi et al., 2020]. The UF membranes have been used to retain colloidal substances, proteins, and macromolecular pigments in the feed while allowing the recovery of larger molecular weight polyphenols in the extract [Khemakhem et al., 2017]. On the other hand, NF membranes have been successfully used to separate micromolecules of 0.5 to 2 nm and molecular weights of 150 to 1000 Da [Conidi et al., 2020].

There are several factors that effects on separation mechanisms of membranes in addition to MWCO. Specific interactions between solute and membrane, material and structure of the membrane, operating parameters (stirring speed, applied pressure) and membrane fouling during filtration affect the membrane performance individually or combined.

Commercially, the most commonly used membranes are polymeric membranes such as cellulose acetate membranes (CA), polysulphone membranes (PS), polyamide membranes (PA), etc. The material and structure of the membrane affect the separation mechanism. For example, polysulphone membranes consist of aromatic and aliphatic

units that prevent the rejection of water and hydrophilic compounds, while polyethersulphone membranes, which are the sub-group of polysulphone, are less hydrophobic and can bind water molecules [Cassano et al., 2017].

UF and NF membranes are often preferred for the separation and concentration of bioactive compounds. Polymeric membranes are suitable for separating polyphenols with amphipathic and hydrophobic aromatic rings and hydrophilic hydroxyl groups. The molecular weights of the phenolic compounds are smaller than the average pore size of the membranes. Still, an entirely successful separation is not always possible because of the other separation mechanisms [Vernhet and Moutounet, 2002]. Membranes reject phenolic compounds depending on their molecular weight as well as their hydrophobic properties and whether they contain aromatic rings. [Arsuaga et al., 2010]. The membrane structural features such as hydrophobicity cause an increase in phenolic compounds' adsorption and facilitate their diffusion transport, thus decreasing membrane retention [Cassano et al., 2017].

Higher retention is attributed to the presence of additional hydroxyl groups and solute-membrane steric interactions of ions and molecules with membranes that affect activity and performance. The position of substituted functional groups and the aromatic ring of polyphenols also affects membranes' retention by increasing order para, meta, orto [Braeken et al., 2005]. The membrane adsorbs part of target compounds while some target molecules pass through. Adsorption of polyphenols during filtration causes a decrease in the pH of the solution and reduced electrostatic repulsion between membrane surface and phenolic compounds and affects the membrane's separation ability during the filtration period. With increasing exposure to phenolic compounds over time, the hydrophilic compounds on the membrane surface increase, and PES membranes become more hydrophilic; therefore, the contact angle, which is a measure of hydrophobicity of the membranes, decreases [Cassano et al., 2017].

UF membranes generally work with 2-5 bar, while NF membranes with 8-20 bar pressure [Singh and Hankins, 2016]. Applied pressure is a main factor in separating polyphenols, and high pressures cause pore size restrictions and change the results [Cisse et al., 2011]. Roselle (*Hibiscus sabdarbifia* L.) extract anthocyanins were concentrated under the pressure of 5-30 bar by 5 kDa and 150 kDa UF membranes about 97% and 24%, respectively. The molecular weight of anthocyanins were 600 g/mol on average so direct MWCO values cannot explain the high rejection of 5 kDa

membrane. The higher MWCO membranes are more sensitive to pressure changes. High pressure leads to polyphenols accumulating on the membrane and by clogging the pores, decreased the membrane performance [Khemakhem et al., 2017].

The accumulation of substances on the membrane surface or inside the membrane pores and thus effect of membrane performance is called membrane fouling [Nady et al., 2011]. The accumulation on the membrane surface causes the concentration polarization and flux to decline over time [Miyamoto et al., 2015]. A highly concentrated layer creates a membrane surface that resists the mass transfer of permeability. The membrane characteristics such as pore size, electrical charge, hydrophobicity, etc., critically affect the membrane fouling. Hydrophobic membranes are more prone to being fouled by organic materials than hydrophilic membranes because of the adsorption of non-polar compounds more in hydrophobic membranes [Marel et al., 2009],[Vieira et al., 2018]. Fouling decreases permeate flux and efficiency while increasing the consumption of energy.

In general, membrane processes are carried out with a feed of water and water-soluble/insoluble compounds. Pequi fruit (*Caryocar brasiliense* Camb.) aqueous extract was concentrated by various membrane processes, and each separation method yielded varying rates of successful separation, with about 100% recovery [Magalhaes et al., 2019]. Aqueous extract of goji (*Lycium Barbarum* L.) leaves were purified and concentrated by UF and NF membranes, and 73-80% retention of total polyphenols and antioxidant activity were achieved [Conidi et al., 2020]. The factors affecting membrane performance related not only to the membrane structure and the operating conditions but also to the feed composition [Zhang et al., 2015]. A study about grape seed polyphenols rejection done by Nawaz et al. (2006) has shown that smaller pore size membranes ensure better results because of the efficient removal of undesirable compounds while theoretically, the opposite was expected [Nawaz et al., 2006]. Similarly, the separation performances of UF membranes (MWCO of 1 and 10 kDa) were determined by Giacobbo et al.(2017) and the membrane's rejection performance with a smaller MWCO value is more excellent than other (about 60% and 50%) [Giacobbo et al., 2017]. Studies showed that using organic solvents or an aqueous mixture as an extraction solvent is more effective than using only aqueous for polyphenol extraction. [Alara et al., 2021]. The presence of a non-water solute such as ethanol can lead to unexpected consequences from attractive interactions between non-polar surfaces and polar solutes. These consequences may prevent the separation

of the target substance or contribute to membrane performance. For example, polar interactions positively affect the retention of polyphenols by PES membranes [Ulbricht et al., 2009]. However, using organic solvents instead of aqueous has considerable adverse effects on the transport mechanism of organic compounds because of the membrane-solvent interactions [Tylkowski et al., 2011]. In addition, an organic solvent may cause some structural changes in the membrane and affect its selectivity [Vieira et al., 2018]. Pequi (*Caryocar brasiliense* Comb) aqueous and ethanolic extracts were concentrated by NF90 membrane. Retention of polyphenols and carotenoids in aqueous extracts (97% and 100%, respectively) was higher than in alcoholic extracts (15% and 10%, respectively) depending on the hydrophobicity phenomenon of membranes against solute [Machado et al., 2013]. While separation processes of aqueous extracts by membrane processes are carried out successfully, applying membrane processes using organic solvents (ethanol, acetone) is still being investigated [Moraes et al., 2018].

Higher molecular weight polysaccharides such as cellulose and pectin and their subgroups, proteins, and other unwanted compounds also exist with phenolic compounds in the extract during the extraction of phenolic compounds, affect the membrane performance [Balyan and Sarkar, 2016]. Pre-treatments such as vacuum filtration, precipitation, adding absorbent, and centrifugation after extraction are not effective enough to remove these macromolecules. However, reduce the membrane's load, and contribute to increasing the flux [Zhang et al., 2015].

Separation with membrane processes aims to remove impurities and maximize phenolic and flavonoid compounds in the extract. The membrane processes can remove total solids; however, excess solids plug the pores of membranes and decrease the flux and the filtration performance. Controlling fouling and increasing permeate fluxes is possible by applying a pre-treatment to feed, selecting and modifying the membrane, optimizing operating parameters, etc. Pre-treatments such as precipitation, coagulation, centrifugation, pre-filtration, or adding absorbents can be applied. Total solids and total phenolic content retention were the highest with the nanofiltration membrane of treating phenolic wastewater successfully combining UF with NF and RO processes. [Sun et al., 2015]. Destani et al. (2013) recovery of blood orange juice phenolic compounds in a total solid content study was made by UF and osmotic distillation [Destani et al., 2013]. Ultrafiltration permeates with a soluble solid content of 10.5 Brix to 61.4 Brix by osmotic distillation.

UF and NF membranes achieved the rejection of polyphenols individually or combined in several studies. Recovery of phenolic compounds from the extract of pequi fruit by UF and NF was studied by Martins Sa et al. (2019), comparing direct and sequential processes. Almost 100% of the phenolic compound were recovered by direct NF process [Martins Sa et al., 2019]. Another study about aqueous and alcoholic extraction of pequi polyphenols and carotenoids was concentrated by nanofiltration with high efficiency of around 97% [Machado et al., 2013]. Rosella extracts UF and NF concentrated anthocyanins, and UF membranes succeeded above 80%, and nanofiltration membranes had more than 95% concentration [Cisse et al., 2011]. UF and NF membranes were used to separate and purify phenolic compounds from pomegranate juice, with a yield of 84.8% for polyphenols and 90.7% for anthocyanins [Conidi et al., 2017]. Hydro-alcoholic extracts of graviola pruning waste bioactive compounds were concentrated by UF and NF combined process providing 77.6% and 76.3% phenolic compounds retention coefficients for 50% ethanol and 70% ethanol extracts, respectively [Moraes et al., 2018]. Oleuropein concentration from olive leaf extracts was studied by combining MF, UF, and NF processes by 95.07% retention for total phenolic compounds, 97.86% retention for total flavonoid content, and 84.62% retention for antioxidant activity [Khemakhem et al., 2017]. Rabelo et al. (2016) concentrated hydro-alcoholic extract of artichoke solid waste about 95% of the chlorogenic acid using NF membranes [Rabelo et al., 2016]. 70% ethanolic extract of propolis concentrated by NF with a rejection of more than 95% for each kind of analyzed compound [Tylkowski et al., 2011]. A similar study about propolis aqueous and the ethanolic extract was made by Mello et al. (2010) regarding the concentration of flavonoids and phenolic compounds [Mello et al., 2010]. The retention coefficient was better with ethanolic extract, 90%, and 94% with aqueous extracts.

Increasing interest in the recovery of phenolic compounds from plant sources and their use as raw materials in cosmetics, pharmaceuticals, and nutraceuticals makes it essential to develop environmentally-friendly extraction and separation, concentration-purification methods to preserve the stability of phenolic compounds [Cassano et al., 2017]. The second part of this study aims to i) evaluate different membrane performances of concentration of phenolic compounds with high antioxidant capacity by directly and combining processes with an optimized ultrasound-assisted extract from *Alpinia officinarum*.

4.1.1. Materials

The materials step was performed as given in Section 3.1.1.

Ultrafiltration membranes UP005, UP020, and UV150 were supplied from Sterlitech Corporation (Auburn, USA) and NF90 from DOW Filmtech Corporation (Minnesota, USA).

4.1.2. Sample Preparation

The extract preparation step was performed as given in Section 3.1.2

4.1.2.1. Centrifugation

After extraction and vacuum filtration, centrifugation experiments were performed with a centrifuge device (OHAUS FC5714, Germany). The extracts were centrifuged at 4300 rpm for 15 min to ensure the separation of total solids that cannot be filtered in vacuum filtration to remove the turbidity and reduce the membrane load used in solids separation. The supernatant was collected for the membrane filtration experiments.

It has also been examined whether the centrifuge effect TPC, DPPH, ABTS, TFC, total carbohydrates, color (CEILAB), turbidity, color (spectrophotometrically), clarity and TC at the optimum point.

4.1.2.2. Membrane Filtration

Membrane filtration experiments were applied to concentrate bioactive compounds in optimized galangal extracts. Filtrations were performed on High Pressure Stirred Cell (Sterlitech HP4750, USA) stainless steel vertical cross-flow membrane module with a feed volume capacity of 300 ml. The cell was 19.9 cm in height and 14.6 cm in width (with high-pressure couplings), and the cell diameter was 5.1 cm. Membrane surface area was 0.00146 m^2 . To provide the desired pressure and control the gas transfer, high purity (%99.998) nitrogen gas (Linde Gaz, Kocaeli, Turkey) tube with pressure regulator assembly was directly connected with a high-pressure hose to the module. There was a pressure on/off valve in the system and a

pressure regulating valve connected to the transmission and discharge section to provide safe operation. Experiments were executed at 25 °C. The stirring rate was kept at 375 rpm to minimize concentration polarization [Tylkowski et al., 2010], controlled with a magnetic stirrer and PTFE-coated stir bar inside the module. The schematic diagram of the system was given in Figure 4.2.

The ultrafiltration (UF) and nanofiltration (NF) experiments were carried out under operating pressure of 5 and 12 bar, respectively. The permeate was discharged with a permeate tube to a glass beaker covered with parafilm to prevent loss of volatile compounds and ethanol in permeate during filtration. The permeate weight was recorded at regular time intervals with a digital scale transferred to the computer through an automation system as the RsKey ComCOMS (A&D Weighing, South Australia) software. Volumetric permeate flux (J_v) measured in $L/(m^2h)$ which is defined as the amount of permeate passed through the membrane area per unit of time. UF and NF processes were carried out for about 2.5 hours until steady-state permeate flux was obtained. Permeate flux was expressed by using Equation 4.1.

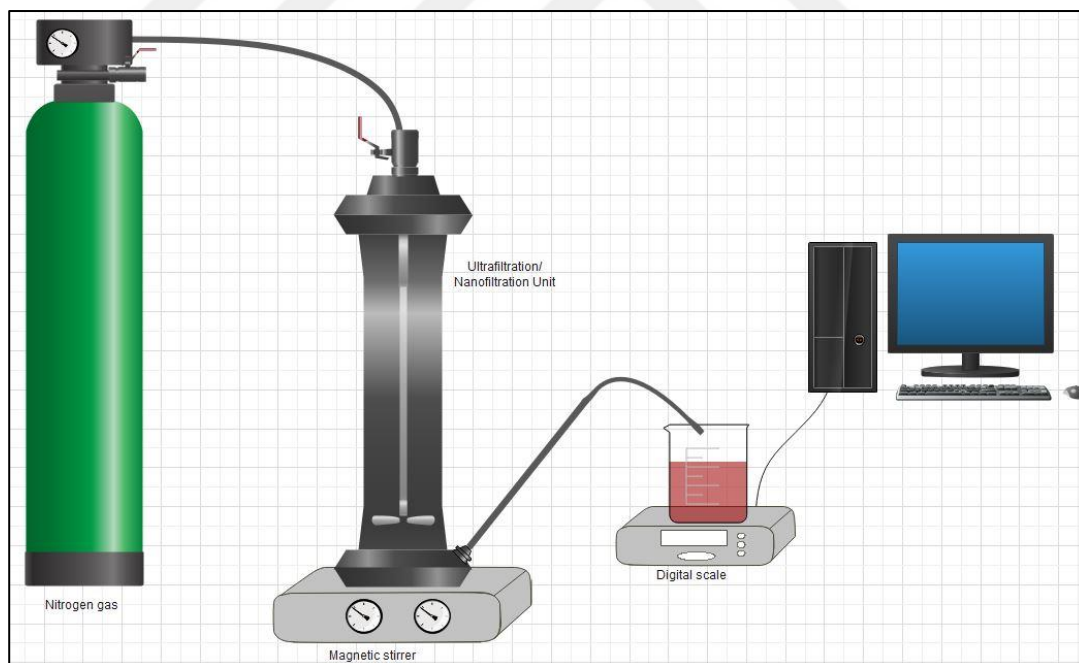


Figure 4.2: Schematic representation of the membrane filtration system.

$$J_v = \frac{1}{A_m} * \frac{dV}{dt} \quad (4.1)$$

where, J_v is volumetric permeate flux in L/m^2h , A_m is the effective membrane area in m^2 V is total volume of permeate in m^3 and t is the filtration time in min.

Ultrafiltration and nanofiltration processes indicate higher initial permeate fluxes at the beginning of the filtration. Permeate flux decreased gradually over time and inclined to be constant. The flux decrease may be due to the thickness of the gel layer formed on the membrane surface increasing over time, thus creating resistance to the solvent flow, which causes a decrease in the permeate flow [Khemakhem et al., 2017]. Percentile flux decrease was calculated by using Equation 4.2.

$$D_f = \left(\frac{J_i - J_s}{J_i} \right) * 100 \quad (4.2)$$

where, J_i is initial permeate flux in L/m^2h and J_s is steady-state permeate flux in L/m^2h .

The volume reduction factor (VRF) determination is essential in membrane applications. It related to a concentration degree and was calculated by Equation 4.3.

$$VRF = \frac{V_f}{V_r} \quad (4.3)$$

where, V_f is feed volume in ml and V_r is retentate volume in ml.

Membranes were pre-conditioned by immersing them in deionized water for 24 hours at room temperature. A new membrane was used in each trial to ensure the same initial conditions. This pre-treatment improves the permeability flow, facilitates the membrane's permeability, and aims to provide that the membrane is thoroughly wetted and free of contamination before using the specifications. The used membranes were given in Table 4.1. In the first stage, UF and NF processes were carried out individually (direct processes), then a combined membrane treatment using UF and NF to concentrate phenolic compounds from galangal extract was evaluated. In combined processes, the membrane with the largest pore size of UF was performed to reduce

suspended solids and reject polyphenols and the filtrate from the UF was used as feed for the NF treatment, intended to concentrate the compounds.

Feeds, retentates, and permeates were analyzed for total phenolic content (TPC), total flavonoid content (TFC). DPPH% inhibition and ABTS radical scavenging activities, total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), total carbohydrates (TC), color removal, turbidity, and clarity.

Retention coefficients (R_f) were expressed in total phenolic content (TPC), total flavonoid content (TFC), DPPH% inhibition and ABTS radical scavenging activities, total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), total carbohydrates (TC) and color removal were determined according to Equation 4.4 [Vieira et al., 2018].

$$R_f = \left(1 - \frac{W_p}{W_r}\right) * 100 \quad (4.4)$$

where, W_p is concentration of compounds in permeate and W_r is concentration of compounds in retentate.

4.1.3. Extract Analysis

4.1.3.1. Determination of Total Phenolic Content (TPC)

The TPC step was performed as given in Section 3.1.3. The TPC of extracts was quantified by using the gallic acid standard curve ($R^2=0.9968$) and expressed as mg gallic acid equivalent (GAE) per ml of extract.

4.1.3.2. Determination of Total Flavonoid Content (TFC)

The TFC step was performed as given in Section 3.1.4. The TFC of extracts was quantified by using the standard curve of quercetin ($R^2=0.9833$) and expressed as mg quercetin equivalent (QE) per ml of extract.

Table 4.1: Characteristics of the used membranes in ultrafiltration and nanofiltration experiments.

Membrane type	Manufacturer	Designation	Material	Nominal MWCO (Da)	Water permeability ($\text{kg h}^{-1} \text{m}^{-2} \text{bar}^{-1}$)	Maximum Operating Pressure (bar)	Maximum Operating temperature ($^{\circ}\text{C}$)
UF	Microdyn-Nadir	UP005	Polyethersulphone	5.000	5.2	14	50
		UP020	Polyethersulphone	20.000	23.2	14	
		UV150	Polyvinylidene Fluoride	150.000	285	14	95
NF	Dow-Filmtech	NF270	Polyamide thin film composite	300	8.9	45	45
		NF90	Polyamide thin film composite	200	3.8	35	35

4.1.3.3. Determination of Antioxidant Activity

The antioxidant activity step was performed as given in Section 3.1.5. The DPPH free radical scavenging activity of extracts was expressed as percent (%) inhibition. The ABTS antioxidant capacity of extracts was quantified by using the standard curve of Trolox ($R^2=0.9886$) and expressed as mM Trolox equivalent per ml extract.

4.1.3.4. Determination of Color (CEILAB)

Color measurements of ultrasound-assisted extract samples of galangal were performed by using Minolta CR-400 (Konica Minolta, Sensing Inc., Osaka, Japan) using the CIELAB scale (L^* , a^* and b^*) color space. The luminosity L^* ($L^* = 0$ black and $L^* = 100$ white), a^* (green (-) to red (+)) and b^* (yellow (-) to blue (+)) were obtained using equipment D_{65} . The chroma (C^*) and hue angle (H^*) parameters were calculated using Equation 4.5 and 4.6, respectively [Vieira et al., 2018].

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (4.5)$$

$$H^* = \arctan\left(\frac{b^*}{a^*}\right) \quad (4.6)$$

Calibration was performed with the calibration tile in white color ($Y = 86.6$, $x = 0.3188$, $y = 0.3364$) prior to every color measurement. Extract samples were placed in a quartz cell and 3 random readings of each sample were taken. Results were given as L^* , a^* , b^* , C^* and H^* . The average values of the readings were reported.

4.1.3.5. Determination of Total Solids (TS), Total Suspended Solids (TSS), Total Dissolved Solids (TDS)

The total solids (TS) step was performed as given in Section 3.1.6. The results were expressed in mg of total solids/ml of extract.

Removal of TS of the extracts was expressed as % TS removal by Equation 4.7. All determinations were performed in triplicate, and average values of the measurements were reported.

$$\%TS \text{ removal} = 1 - \left(\frac{TS_p}{TS_r} \right) * 100 \quad (4.7)$$

where, TS_p is total solids in permeate in mg/ml and TS_r is total solids in retentate in mg/ml.

Total suspended solids (TSS) were determined using a centrifuge device (OHAUS FC5714, Germany). 1 ml of pre-weight samples were centrifuged at 4000 rpm for 20 min. The subsided solids were weighed after removing the supernatant [Conidi et al., 2020]. The removal of TSS was expressed as %. TSS removal was calculated by Equation 4.8. All determinations were performed in triplicate, and average values of the measurements were reported.

$$\%TSS \text{ removal} = 1 - \left(\frac{TSS_p}{TSS_r} \right) * 100 \quad (4.8)$$

where, TSS_p is total suspended solids in permeate in mg/ml and TSS_r is total suspended solids in retentate in mg/ml.

Total dissolved solids (TDS) were determined using a portable conductivity and TDS meter (HACH, HQ40D Conductivity and TDS meter, Germany). The results were reported as and results were reported as a unit of mg/ml. The removal of TDS was expressed as %. TDS removal was calculated by Equation 4.9.

$$\%TDS \text{ removal} = 1 - \left(\frac{TDS_p}{TDS_r} \right) * 100 \quad (4.9)$$

where, TDS_p is total dissolved solids in permeate in mg/ml and TDS_r is total dissolved solids in retentate in mg/ml. Three separate measurements were done, and results were given as the average of three separate measurements.

4.1.3.6. Determination of Total Carbohydrates

Total carbohydrates (TC) of the samples were evaluated using the phenol-sulfuric acid method [Dubois et al., 1956]. 0.5 ml of diluted sample was mixed with 0.5 ml of 5% phenol reagent, and 2.5 ml of concentrated sulfuric acid. The mixture

was vortexed (VWR Vortex Mixer, VWR International, West Chester, PA, USA) and left to stand for 10 min in the dark. Then samples were placed in a water bath set at 25 °C for 20 min. Absorbance values of solutions were measured at 490 nm with a UV-VIS spectrophotometer (Perkin Elmer Lambda 35, Shelton, CT, USA). The total carbohydrate value of galangal was quantified by using the standard curve of glucose ($R^2 = 0.9748$) and expressed as mg glucose equivalent per ml extract (mg GE/ml extract). Total carbohydrate removal of the extracts was expressed as % TC value as absorbance by Equation 4.10.

$$\%TC = 1 - \left(\frac{TC_s}{TC_r} \right) * 100 \quad (4.10)$$

where, TC_p is total carbohydrates in permeate in mg GE/ml extract and TC_r is total carbohydrates in retentate in mg GE/ml extract. Three separate measurements were done, and results were given as the average of three separate measurements.

4.1.3.7. Determination of Turbidity

The turbidity of the extracts was determined using a portable Turbidimeter (HACH 2100Q Portable Turbidimeter. Germany). The results were reported as Nephelometric Turbidity Units (NTU) of the extract [Abdullah et al., 2007]. Turbidity removal % was expressed by given Equation 4.11.

$$\%Turbidity\ removal = 1 - \left(\frac{T_p}{T_r} \right) * 100 \quad (4.11)$$

where, T_p is turbidity of permeate in NTU and T_r is turbidity of retentate in NTU. Three separate measurements were done against deionized water, and results were given as the average of three separate measurements.

4.1.3.8. Determination of Clarity

Clarity was determined by a standard method [Aponso et al., 2017]. The absorbance of all samples was measured at 660 nm using the UV-Vis Spectrophotometer (Perkin Elmer Lambda 35, Shelton, CT, USA) using deionized water as reference [Brujin et al., 2003]. Clarity of the extracts was expressed as % clarity value as absorbance by given Equation 4.12.

$$\%C = 1 - \left(\frac{A_s}{A_r} \right) * 100 \quad (4.12)$$

where A_s is absorbance of sample and A_r is absorbance of retentate. Three separate measurements were done, and results were given as the average of three separate measurements.

4.1.3.9. Determination of Color (Spectrophotometrically)

The color change was measured using the UV-Vis Spectrophotometer (Perkin Elmer Lambda 35, Shelton, CT, USA) at 720 nm [Diaz-Montes et al., 2020]. The rate of color removal was calculated using Equation 4.13. Absorbance readings were made triplicate against deionized water. Color removal and were expressed using Equation 4.10.

$$\%CR = 1 - \left(\frac{A_s}{A_r} \right) * 100 \quad (4.13)$$

where A_s is absorbance of sample and A_r is absorbance of retentate. Three separate measurements were done, and results were given as the average of three separate measurements.

4.1.4. Membranes Analysis

4.1.4.1. SEM Analysis

SEM analyses were performed as given in section 3.1.11. Images of the treated and dried membranes (UP005, UP020, UV150, NF27, NF90, UF+NF270, UF+NF90) were analyzed.

4.1.4.2. Contact Angle Analysis

All treated and untreated membranes were analyzed for their affinity to water with Meniscus method by Attension Theta Lite Optical Contact Angle Tensiometer (Terralab, Turkey) equipped with a high-resolution camera at room temperature [Vieira et al., 2018]. Membrane samples were set on an acrylic plate that was fixed to have a smooth surface by double side adhesive tape. 5 µl of deionized water dropped on the surface. Contact angle values were controlled with TetaLite OneAttension software by left, right, and average at checkpoints (1-10 s).

4.1.5. Statistical Analysis

All physical and chemical analyzes were statistically analyzed using SPSS Statistics 22 software (SPSS Inc., Chicago, IL, USA) according to Tukey test with a significance level of $p \leq 0.05$.

4.2. Results and Discussion for Concentration of Polyphenols by Membrane Processes

4.2.1. Centrifugation of Optimized Extracts

Before membrane filtrations, the optimized extract was pre-treated by centrifugation. The aim was to obtain a clear extract by reducing the total solids content and the load on the membrane processes [Martins Sa et al., 2019]. Centrifugation is an essential pre-treatment before membrane processes, affecting membrane fouling and filtration kinetics [Zhu et al., 2016]. Total solids (TS), total

dissolved solids (TDS), total suspended solids (TSS), turbidity, color, and clarity values were measured in the supernatant obtained after centrifugation. Besides these, TPC, DPPH, ABTS and TFC were determined to observe the effect of centrifugation. Results were expressed in Table 4.2.

TPC and DPPH were observed at a higher value after centrifugation at 1.96% and 4.62%, respectively. There wasn't a significant difference ($p > 0.05$) between values. Therefore, it can be said that centrifugation does not cause significant removal of phenolics and DPPH antioxidant activity. A study about bioactive compounds recovery from purple sweet potatoes demonstrated that centrifugation as pre-treatment enhances the recovery of polyphenols from 23% to 29% [Zhu et al., 2016]. ABTS and TFC of galangal extracts were not significantly ($p > 0.05$) changed by the centrifuge effect.

As expected, total solids (TS) and total dissolved solids (TDS) were reduced after centrifugation by 6.90% and 28.65%, respectively. There wasn't a significant difference between before and after centrifugation in TS ($p \leq 0.05$) while TDS was significantly low after centrifugation ($p \leq 0.05$). Total suspended solids (TSS) were higher after centrifugation; it can be said that the centrifuge does not remove the TSS; on the contrary, it ensures that they are found more. A comparison study about the effect of pre-treatment methods on permeate flux showed that most of the larger suspended solids were separated by centrifugation [Rai et al., 2007]. Also, total carbohydrates were higher in the sample after centrifugation, but there wasn't a significant difference between the results ($p > 0.05$).

The turbidity value of extract was 940 ± 2.00 NTU before centrifugation. After centrifugation, turbidity value reduced to 474 ± 3.05 NTU. The turbidity was removed as 49.57% ($p \leq 0.05$). Spectrophotometric color value which is the redness intense of sample was reduced 54.19% ($p \leq 0.05$) and clarity value was reduced 44.85% ($p \leq 0.05$) of the non-centrifuged extract. It was applied as a pre-treatment because centrifugation did not significantly affect the removal of bioactive substances but decreased the amount of some solids, increasing the clarity and removing the turbidity, and reducing the load of the membrane processes.

Table 4.2: Comparison of the determined values for the optimized extract of galangal before and after centrifugation.

	Before Centrifugation	After Centrifugation	p-value
TPC (mg GAE/ml sample)	69.05 ± 4.80	70.4 ± 0.47	0.356
DPPH (%inhibition)	49.38 ± 5.03	51.66 ± 3.15	0.170
ABTS (mg TE/ml extract)	161.26 ± 18.19	133.56 ± 21.7	0.079
TFC (mg QE/ml extract)	12.7 ± 6.88	12.61 ± 0.54	0.751
TS (mg/ml)	0.029 ± 0.01	0.027 ± 0.01	0.819
TDS (ml/ml)	61.40 ± 1.48	43.81 ± 0.79	0.000
TSS (g/ml)	0.0150 ± 0.001	0.0220 ± 0.001	0.001
TC (mg GE/ml extract)	6.62 ± 0.14	7.30 ± 0.36	0.036
Color (720 nm)	0.919 ± 0.04	0.421 ± 0.02	0.000
Turbidity (NTU)	940 ± 2.00	474 ± 3.05	0.005
Clarity (660 nm)	1.01 ± 0.02	0.557 ± 0.03	0.000

For all measurements, data are expressed as mean values with standard deviations. Mean value is the average of three replications.

4.2.2. Extract Analysis

4.2.1.1. Color Determination of Filtration Experiments (CEILAB)

Optimized ultrasound-assisted extracts with three-level, three factors Box-Behnken experimental design of RSM (Response Surface Methodology) were filtrated through different ultrafiltration and nanofiltration membranes by individually and combined processes. Colors of extracts according to CEILAB system were determined. Result were given in Table 4.3. Color is the most important characteristic feature of food products. Anthocyanins and some of the flavonoid compounds, which are sub-groups of phenolic compounds, are responsible for the reddish color of the food products [Arend et al., 2017]. Color intensities increased by increasing the total phenolic content of the fluids. According to visual observation, permeates showed significantly lower color intensity than feed and retentates. L*, a*, b* color spaces indicate that the L* is the lightness of the samples black (0) and white (100); a* is for red/green value and b* for blue/yellow value. C* is the Chroma color is the distance of the point from L*. H* is the hue angle which implies the red (0°) to cyan blue (360°) [Web 2, 2020]. Table 4.3 represents the feed, retentates, and permeates UF and NF direct and combined processes L* a* b* values and C* and H* calculated data.

L*, which is the brightness index, was 21.37 in feed extract. After membrane processes, brightness increased for all permeates ($p \leq 0.05$). L* values of the permeates were 24.12 for UP005, 23.70 for UP020, 21.76 for UV150, 23.91 for NF270, 24.03 for NF90, 23.47 for UF+NF270, 23.55 for UF+NF90. Differences can be explained by removing colloidal suspensions from the extract by filtrating and retention of phenolic compounds by UF and NF membranes, causing colorless permeates with higher luminosity. There wasn't a significant difference ($p > 0.05$) between the feed and the L* value of retentates for all samples. Similar results were reported by Vieira et al. (2018), the study about anthocyanin concentration using nanofiltration membranes from jussara (*Euterpe edulis*) [Vieira et al., 2018]. L* values of the galangal extracts were 24.01 for UP005, 23.70 for UP020, 22.40 for UV150, 23.91 for NF270 and 24.03 for NF90 in direct processes. Maximum brightness was achieved with UP005 retentate. After pre-treatment with UV150, there wasn't a significant difference between ($p > 0.05$) NF270 and NF90 permeates and retentates for the combined process.

a* is redness index for red/green color was 3.66 in feed extract. All permeates (1.51 for UP020, 2.30 for UV150, 1.48 for NF270, 1.74 for NF90) except UP005 (4.35) gave lower a* results in direct processes. a* value of UP005 permeate increased after filtration process. The direct process showed similar results for permeates of NF membranes (1.60 for NF270 and 1.88 for NF90). NF270 membrane gave the lowest value of a* index for both direct and indirect processes. There were significant differences ($p \leq 0.05$) between the values of permeates. b* is yellowness index or blue/yellow color was 2.17 in feed extracts. In the permeates of UP005 (1.37), NF270 (0.74) and NF90 (0.70) b* value decreased while in the permeates of UP020 (2.23) and UV150 (3.73) b* value increased in direct processes. The lowest value was obtained in NF90 permeate. In contrast, in combined processes, NF270 (0.59) showed lower value NF90 (1.66) of b* there was significant difference ($p \leq 0.05$).

C* which is the chroma color as the distance of the point from L* was 9.06 in feed extract. In the permeates of UP005 (10.42) and UV150 (9.57) C* value increased, while in the permeates of UP020 (3.61), NF270 (1.36), and NF90 (3.13) C* value decreased in direct processes. The lowest value was obtained in NF270 permeate.

Table 4.3: Color determination of optimized extract of galangal for UF and NF experiments.

Membrane		Fluid	L*	a*	b*	C*	H*
Direct Processes	UP005	Feed	21.37 ± 0.47 ^{ab}	3.66 ± 0.29 ^{ab}	2.17 ± 0.36 ^{ad}	9.06 ± 1.72 ^{ad}	0.53 ± 0.05 ^a
		Retentate	21.14 ± 0.02 ^{ab}	3.47 ± 0.08 ^{ac}	1.83 ± 0.03 ^{ab}	7.68 ± 0.24 ^{ab}	0.48 ± 0.02 ^b
		Permeate	24.01 ± 0.02 ^c	4.35 ± 0.07 ^c	1.37 ± 0.06 ^c	10.42 ± 0.29 ^c	0.30 ± 0.02 ^c
	UP020	Feed	21.37 ± 0.47 ^{ab}	3.66 ± 0.29 ^{ab}	2.17 ± 0.36 ^{ad}	9.06 ± 1.72 ^{ad}	0.53 ± 0.05 ^a
		Retentate	20.86 ± 0.02 ^a	3.41 ± 0.04 ^c	1.81 ± 0.08 ^{ab}	7.44 ± 0.10 ^{ab}	0.48 ± 0.02 ^b
		Permeate	23.70 ± 0.35 ^c	1.51 ± 0.02 ^d	2.23 ± 0.01 ^{de}	3.61 ± 0.05 ^c	0.97 ± 0.01 ^e
	UV150	Feed	21.37 ± 0.47 ^{ab}	3.66 ± 0.29 ^{ab}	2.17 ± 0.36 ^{ad}	9.06 ± 1.72 ^{ad}	0.53 ± 0.05 ^a
		Retentate	21.76 ± 0.01 ^{abd}	3.74 ± 0.10 ^b	2.37 ± 0.04 ^e	9.81 ± 0.32 ^{df}	0.56 ± 0.02 ^{cf}
		Permeate	22.40 ± 0.68 ^d	2.30 ± 0.04 ^e	3.73 ± 0.10 ^f	9.57 ± 0.36 ^e	1.01 ± 0.02 ^{af}
	NF270	Feed	21.37 ± 0.47 ^{ab}	3.66 ± 0.29 ^{ab}	2.17 ± 0.36 ^{ad}	9.06 ± 1.72 ^{ad}	0.53 ± 0.05 ^a
		Retentate	20.97 ± 0.12 ^a	3.39 ± 0.06 ^c	1.94 ± 0.05 ^{abd}	7.63 ± 0.10 ^{ad}	0.51 ± 0.02 ^b
		Permeate	23.91 ± 0.01 ^c	1.48 ± 0.03 ^d	0.74 ± 0.02 ^g	1.36 ± 0.05 ^b	0.46 ± 0.01 ^g
	NF90	Feed	21.37 ± 0.47 ^{ab}	3.66 ± 0.29 ^{ab}	2.17 ± 0.36 ^{ad}	9.06 ± 1.72 ^{ad}	0.53 ± 0.05 ^a
		Retentate	22.06 ± 0.01 ^{bd}	4.01 ± 0.04 ^f	2.79 ± 0.01 ^h	11.90 ± 0.14 ^f	0.60 ± 0.004 ^h
		Permeate	24.03 ± 0.04 ^c	1.74 ± 0.01 ^{gh}	0.70 ± 0.03 ^g	1.75 ± 0.04 ^g	0.38 ± 0.01 ^g
Combined Processes	UV150 and NF270	Feed	22.40 ± 0.32 ^d	2.30 ± 0.46 ^e	3.73 ± 0.41 ^f	9.67 ± 0.45 ^e	1.02 ± 0.14 ^{af}
		Retentate	21.54 ± 0.38 ^{abd}	3.10 ± 0.06 ⁱ	3.50 ± 0.02 ^f	10.93 ± 0.14 ^h	0.84 ± 0.01 ⁱ
		Permeate	23.47 ± 0.23 ^c	1.60 ± 0.12 ^d	0.59 ± 0.01 ^g	1.45 ± 0.19 ^g	0.35 ± 0.03 ^g
	UV150 and NF90	Feed	22.40 ± 0.32 ^d	2.30 ± 0.46 ^e	3.73 ± 0.41 ^f	9.67 ± 0.45 ^e	1.02 ± 0.14 ^{af}
		Retentate	21.68 ± 0.10 ^{abd}	3.07 ± 0.14 ⁱ	3.71 ± 0.08 ^f	11.59 ± 0.44 ^h	0.87 ± 0.03 ^h
		Permeate	23.55 ± 0.03 ^c	1.88 ± 0.06 ^h	1.66 ± 0.01 ^c	3.13 ± 0.10 ⁱ	0.72 ± 0.02 ^e

For all measurements, data are expressed as mean values with standard deviations. Mean value is the average of three replications.

Similarly, NF270 (1.45) in combined processes is lower than NF90 (3.13) of C* value. There was significant difference ($p \leq 0.05$) between the NF processes.

H* hue angle, which implies the red (0°) to cyan blue (360 °) was 0.53 in feed extract. According to the definition of H*, a low hue angle shows the tendency to red color, and high hue angle values show the tendency to yellow color [Aponso et al., 2017]. Concentrating phenolic compounds reduced the H* value in the permeate compared with feed in the permeates of UP005 (0.30) and NF270 (0.46) and NF90 (0.38) H* value, and there wasn't a significant difference between the direct NF processes. In the permeates of UP020 (0.97), UV150 (1.01) H* value increased in direct processes. The lowest value was obtained in UP005 permeate ($p \leq 0.05$). In contrast, in combined processes, NF270 (0.35) showed a lower value of NF90 (1.72) of b* There was a significant difference between them ($p \leq 0.05$). According to the two Preucil-style conversions of RGB, hue angles of 0-15 represent colors from red to vermilion (orange-red). In addition, the luminous value of these colors is in the range of 0-30, which is confirmed in the measurements made [Defining and Communicating Color: The CIELAB System, 2013].

4.2.1.2. Total Phenolic Content (TPC), Antioxidant Activity (DPPH, ABTS) and Total Flavonoid Content (TFC) Determination of Filtration Experiments

Ultrasound-assisted extracts optimized with three-level, three factors Box-Behnken experimental design of RSM (Response Surface Methodology) were filtrated through different ultrafiltration and nanofiltration membranes by individually and combined processes. Table 4.4 shows the TPC, DPPH, ABTS and TFC values.

In direct processes, all membranes yielded extracts with higher TPC values than feed from 7.04 to 7.44-9.03 mg GAE/ml extract. Some amount of the phenolic compounds passes into permeate for each membrane. When permeates were examined the highest recovery of phenolic compounds was with the UV150 membrane as 9.03 mg GAE/ml extract. The larger pore size of the UV150 membrane compared to other UF and NF membranes caused greater diffusion of phenolic compounds. Besides, the extract with the most reddish filtrate was observed in UV150, as can be seen in Figure 4.9. This may indicate that phenolic substances in the class of anthocyanins, called colorants, remain in retention. The difference was that the VRF ratio changes while

keeping the filtration time constant for each membrane [Murakami et al., 2011]. There wasn't significant difference ($p > 0.05$) between retentates and permeates of UP005 (7.44 and 1.48 mg GAE/ml extract, respectively) and UP020 (7.57 and 1.87 mg GAE/ml extract, respectively).

DPPH antioxidant activity showed similar results with TPC as % inhibition. DPPH value of the feed extract was 51.55%. DPPH was increased in UP020 and UV150 membranes by increasing membrane pore size from 51.66% to 57.49- 60.11% inhibition (Table 4.4). UP005 membrane lower DPPH value in retentate from feed. The reduction may be because of the losses during the process or the phenolic compounds that respond to DPPH radical scavenging activity more passed into permeate [Mello et al., 2010], [Conidi et al., 2017]. There wasn't a significant difference ($p > 0.05$) between retentates and permeates in DPPH values of UP020 (57.59 and 15.56% inhibition, respectively) and UV150 (60.11 and 114.53% inhibition, respectively).

ABTS antioxidant activity was 133.56 mM TE/ml extract was in feed extract. The highest ABTS was in the retentate of UP005 (139.40 mM TE/ml extract). There wasn't significant difference ($p > 0.05$) among the ABTS-responsive phenolic compounds found in permeates of UP005, UP020 and UV150 membranes (0.138, 0.134, and 0.78 TE/ml extract, respectively). Similarly, TFC values in retentates of each UF membranes were significantly the same ($p > 0.05$) in UP005 and UP020 (13.73 and 13.52 mg QE/ml extract, respectively). In contrast, the UV150 (17.67 mg QE/ml extract) membrane did not let the flavonoid compounds pass into permeate. Difference may be due to the different structure material of UV150 membrane (polyvinylidene) from the other two UF membranes (polyethersulfone) (Table 4.1). There wasn't a significant difference in TFC of the permeates among UF membranes (1.31, 0.95, 1.82 mg QE/ml extract, respectively).

NF270 and NF90 membranes showed close results to each other for all analyses. There wasn't a significant difference between TPC values of the permeates of NF membranes (0.59 and 0.55, respectively). Even if there wasn't a significant difference in TFC values of UF and NF membranes ($p > 0.05$), NF membranes were more successful in retaining total flavonoids (14.61 and 14.16 mg QE/ml extract, respectively). Both DPPH (50.48 and 46.85% inhibition, respectively) and ABTS (109.90 and 105.18 mM TE/ml extract, respectively) antioxidant activities were lower at retentates than feed (51.66% inhibition and 133.56 mM TE/ml extract, respectively).

Table 4.4: Determination of the TPC, DPPH, ABTS and TFC of optimized extract of galangal for UF and NF experiments.

Membrane		Pressure (bar)	Fluid	TPC (mg GAE/ ml extract)	DPPH (%inhibition)	ABTS (mM TE/ml extract)	TFC (mg QE/ml extract)
Direct Processes	UP005	5	Feed	7.04 ± 0.47 ^a	51.66 ± 3.15 ^{ag}	133.56 ± 21.7 ^{ab}	12.61 ± 0.54 ^a
			Retentate	7.44 ± 0.18 ^{ac}	47.30 ± 0.49 ^{ab}	139.40 ± 15.17 ^b	13.73 ± 0.40 ^a
			Permeate	1.48 ± 0.02 ^b	8.84 ± 0.09 ^{cj}	0.138 ± 0.01 ^c	1.31 ± 0.16 ^b
	UP020	5	Feed	7.04 ± 0.47 ^a	51.66 ± 3.15 ^{ag}	133.56 ± 21.7 ^{ab}	12.61 ± 0.54 ^a
			Retentate	7.57 ± 0.34 ^c	57.49 ± 0.63 ^{df}	97.91 ± 1.06 ^a	13.52 ± 1.31 ^a
			Permeate	1.87 ± 0.06 ^{bd}	15.56 ± 0.07 ^e	0.134 ± 0.06 ^{cd}	0.95 ± 0.05 ^b
	UV150	5	Feed	7.04 ± 0.47 ^a	51.66 ± 3.15 ^{ag}	133.56 ± 21.7 ^{ab}	12.61 ± 0.54 ^a
			Retentate	9.03 ± 0.10 ^e	60.11 ± 1.55 ^f	135.26 ± 12.03 ^{ab}	17.67 ± 0.46 ^c
			Permeate	2.81 ± 0.02 ^f	14.53 ± 1.52 ^e	0.78 ± 0.03 ^{cd}	1.82 ± 0.19 ^b
	NF270	12	Feed	7.04 ± 0.47 ^a	51.66 ± 3.15 ^{ag}	133.56 ± 21.7 ^{ab}	12.61 ± 0.54 ^a
			Retentate	7.67 ± 0.15 ^c	50.48 ± 4.99 ^{dg}	109.90 ± 23.01 ^a	14.61 ± 4.71 ^c
			Permeate	0.59 ± 0.02 ^g	3.06 ± 0.72 ^h	3.58 ± 1.07 ^c	0.37 ± 0.03 ^b
	NF90	12	Feed	7.04 ± 0.47 ^a	51.66 ± 3.15 ^{ag}	133.56 ± 21.7 ^{ab}	12.61 ± 0.54 ^a
			Retentate	7.96 ± 0.12 ^c	46.85 ± 0.13 ^b	105.18 ± 16.11 ^a	14.16 ± 0.23 ^{ac}
			Permeate	0.55 ± 0.01 ^g	3.52 ± 0.02 ^h	0.030 ± 0.01 ^c	0.42 ± 0.04 ^b
Combined Processes	NF270	12	Feed	2.21 ± 0.11 ^d	26.90 ± 4.54 ⁱ	4.90 ± 6.77 ^c	1.94 ± 0.72 ^b
			Retentate	4.31 ± 0.02 ^h	25.44 ± 0.33 ⁱ	126.49 ± 26.45 ^{ab}	2.64 ± 0.11 ^b
			Permeate	0.49 ± 0.01 ^g	2.23 ± 0.02 ^h	0.037 ± 0.01 ^c	0.14 ± 0.02 ^b
	NF90	12	Feed	2.21 ± 0.11 ^d	26.90 ± 4.54 ⁱ	4.90 ± 6.77 ^c	1.94 ± 0.72 ^b
			Retentate	3.58 ± 0.32 ⁱ	12.42 ± 0.56 ^{ej}	49.51 ± 9.41 ^d	1.58 ± 0.23 ^b
			Permeate	0.56 ± 0.02 ^g	5.93 ± 0.12 ^{ch}	5.09 ± 0.07 ^c	0.50 ± 0.02 ^b

For all measurements, data are expressed as mean values with standard deviations. Mean value is the average of three replications.

The result may be due to the loss of phenolics that respond to antioxidant activity during filtration or, more likely, their retention on NF membranes [Mello et al., 2010]. For the two antioxidant activity methods, there wasn't a significant difference ($p > 0.05$) between the permeates on NF membranes. Total phenolic content was better in NF90 than NF270 (7.96 and 7.67 mg GAE/ml sample). However, statistically no difference between their extractions yields ($p > 0.05$).

In combined processes, optimized extracts were filtrated through UV150 membranes, and then the permeates were given to NF270 and NF90 membranes as feed. Considering that the TPC of feeds were the same (2.21 mg GAE/ml extract), retention of phenolic compounds was higher in NF270 membrane (4.31 mg GAE/ml extract) than NF90 membrane (3.58 mg GAE/ml extract) ($p > 0.05$) in contrast to that observed in direct processes ($p > 0.05$). Nevertheless, TPC values of permeates in direct and combined processes were not significantly different from each other ($p > 0.05$) (0.59, 0.15, 0.49, 0.56 mg GAE/ml extract, respectively). DPPH value of the NF feed (26.90% inhibition) and the retentates of the NF270 combined process were not significantly different ($p > 0.05$). Considerable decrease of DPPH (from 26.90 to 12.42% inhibition) but in NF90 membrane ($p > 0.05$). For ABTS, there was a pretty good rate of increase (from 4.90 to 126.49 and 49.51 mM TE/ml extract) for both membranes. Although phenolic compounds affecting ABTS lost their antioxidant capacity in direct processes, when UV150 was applied as a pretreatment, it was observed that ABTS value increased quite well in both membranes. The difference may be due to better performance in the NF step due to the decomposition of phenolic compounds that do not affect ABTS. There wasn't a significant difference ($p > 0.05$) between ABTS values of permeates in combined processes (4.90 and 5.09 mM TE/ml extract). Still, the NF270 membrane performed better than the NF90 membrane for the ABTS value in both direct and combined processes.

There wasn't a significant difference ($p > 0.05$) between the feed, retentates, and permeates of combined NF processes. The TFC (1.94 mg QE/ml extract in feed) was higher at NF270 (2.64 mg QE/ml extract) than NF90 (1.58 mg QE/ml extract) in the final product, which may be confirmation that the NF270 membrane is more suitable for flavonoids. When compared with direct NF processes, it was seen that there was no statistical difference ($p > 0.05$) between the amounts of TFC in the permeates of all processes. The retention of some phenolic compounds by the UV150 membrane and the low residual phenolic compounds in the liquid fed to the NF membranes indicate

that the combined processes are unsuccessful in the final product so, was not found to be good because it is a two-stage process and causes more energy and material consumption in total phenolic and other evaluation parameters [Magalhaes et al., 2019].

4.2.1.3. Turbidity, Clarity and Color (Spectrophotometrically) Determination of Filtration Experiments

Before membrane filtrations, optimized extracts were centrifuged, and some cloudy and colloidal suspensions that would cause turbidity were partially removed (Table 4.2). Changes in turbidity during direct UF, NF, and combined processes given in Table 4.5. The pre-treated feed extract still has a high turbidity value of 460 NTU. All UF and NF membranes retained the molecules, and the turbidity value decreased to acceptable NTU ($p > 0.05$) in direct processes. Considering that turbidity molecules were retained on the membrane based on the particle size phenomenon, the results were in line with the increase in pore sizes of UF membranes (1.41 NTU for UP005, 3.93 NTU for UP020, and 5.17 NTU for UV150). There wasn't a significant difference between the permeates of direct processes and combined processes ($p > 0.05$). In combined processes by feeding the filtrate from UV150 pre-filtration to NF270 and NF90 membranes, the turbidity of feed was 5.17 NTU, which was considerably low, decreased to 1.44 NTU for NF270 1.04 NTU/ml for NF90 membranes ($p > 0.05$). In direct processes, large molecular weight solids that cause turbidity clog the membrane after a while, causing the membrane's filtering performance to decrease. Since the turbidity molecules removed in the pre-UF process do not pass through in the combined NF process, easier filtering and successful turbidity removal were achieved. Magalhaes et al. (2019) studied direct and sequential membrane processes from pequi (*Caryodarbraziliense* Camb.) extract. They found direct processes reduced turbidity to the same extent as sequential processes [Magalhaes et al., 2019]. Brujin et al. (2003) reported that, although turbidity removal depends on the particle size and structure of the turbid molecules, the applied pressure and other filtration parameters are also effective [Brujin et al., 2003].

Clarity is an index of the quality of transparency or purity of extract. Although it is not aimed to obtain a clear and pure end product in this study, the measurement of clarity is also involved in evaluating the quality of the permeate and, therefore the

membrane performance. The absorbances of the samples were measured at UV-VIS at 660 nm, and the results were given in Table 4.5. The clarity of the feed extract was 0.575 abs. UF membranes (0.005 abs for UP005 and 0.011 abs for UV150) and NF membranes gave the approximately the same result in clarity of permeate (0.001 abs for both NF270 and NF90), and there wasn't a significant difference between the results except for UP020 (0.041 abs), and clarity was almost completely provided. Higher clarity values were observed in NF270 (0.004 abs) and NF90 (0.003 abs) membrane filtrations performed after UV150 compared to direct processes. The results of combined processes were not significantly different ($p > 0.05$) from direct processes. Still, these extracts yielded acceptably clear filtrates. Brujin et al. (2003) determined the clarity of apple juice in different pore sizes of UF membranes, and there wasn't a significant difference between the results [Brujin et al., 2003].

The spectrophotometric color value which is the equivalent of the red color in the visible region, and it was made to verify how much the reddish color of the extract was removed by membrane filtrations. The absorbances of the samples were measured at UV-VIS at 720 nm, and the results were given in Table 4.5. The retention of color-providing phenolic compounds by membrane processes and the removal of color in permeate were also evaluated. Results differ with turbidity and clarity, as the interactions of color-producing phenolic compounds with membranes differed from colloidal suspensions that cause turbidity and affect clarity. The filtration dynamics of these bioactive compounds were related to membrane pore sizes and membrane-bioactive compound interactions [Balyan and Sarkar, 2016], [Cassano et al., 2017]. In direct processes, removal of color was observed the best in the NF90 membrane (0.001 abs), NF270 (0.002 abs), and UP005 (0.003 abs) membranes following NF90, respectively. UV150 (0.011 abs) showed better performance than UP020 (0.021) membrane. There wasn't a significant difference ($p \leq 0.05$) between direct and combined processes. Most of the color was removed by the UV150 membrane in combined processes. The NF270 and NF90 membranes decolorize no better than direct processes. Considering the removal of the color, since the maximum result can be achieved with the direct process, two-stage process was not needed for color removal. According to the results of color in L^* , a^* , b^* and color (spectrophotometrically) determinations, all permeate values were low because of the removal of solids and color compounds. As data to support this, the clarity (660 nm) measurements and a^* values (red color intensity) are approach 0 as the filtering activity increases in

permeates. Similar results were obtained by Arend et al. (2017), a nanofiltration study on strawberry juice [Arend et al., 2017].

Table 4.5: Determination of turbidity, clarity, and color of optimized extract of galangal for UF and NF experiments.

Membrane	Fluid	Turbidity (NTU)	Clarity (660 nm)	Color (720 nm)
Direct Processes	UP005	Feed	460 ± 2.00 ^a	0.575 ± 0.020 ^a
		Retentate	288.33 ± 0.57 ^b	0.521 ± 0.010 ^{bf}
		Permeate	1.41 ± 0.015 ^c	0.003 ± 0.001 ^c
	UP020	Feed	460 ± 2.00 ^a	0.575 ± 0.020 ^a
		Retentate	638.66 ± 0.57 ^d	0.497 ± 0.002 ^b
		Permeate	3.93 ± 0.03 ^c	0.041 ± 0.001 ^d
	UV150	Feed	460 ± 2.00 ^a	0.575 ± 0.020 ^a
		Retentate	716 ± 3.6 ^e	0.625 ± 0.003 ^e
		Permeate	5.17 ± 0.06 ^c	0.011 ± 0.001 ^c
	NF270	Feed	460 ± 2.00 ^a	0.575 ± 0.020 ^a
		Retentate	641.3 ± 3.21 ^e	0.543 ± 0.001 ^g
		Permeate	3.93 ± 0.03 ^c	0.001 ± 0.000 ^c
Combined Processes	NF90	Feed	460 ± 2.00 ^a	0.575 ± 0.020 ^a
		Retentate	296.6 ± 2.08 ^f	0.526 ± 0.002 ^{fg}
		Permeate	2.22 ± 0.02 ^c	0.001 ± 0.001 ^c
	UV150 and NF270	Feed	5.17 ± 0.06 ^c	0.010 ± 0.001 ^f
		Retentate	11.37 ± 0.54 ^g	0.036 ± 0.001 ^d
		Permeate	1.44 ± 0.01	0.004 ± 0.001 ^c
	UV150 and NF90	Feed	5.17 ± 0.06 ^c	0.010 ± 0.001 ^f
		Retentate	16.08 ± 0.01 ^h	0.043 ± 0.001 ^d
		Permeate	1.04 ± 0.03 ^c	0.003 ± 0.001 ^c

For all measurements, data are expressed as mean values with standard deviations. Mean value is the average of three replications.

4.2.1.4. Total Solids (TS), Total Dissolved Solids (TSS), Total Suspended Solids (TSS) and Total Carbohydrate Analysis of Filtration Experiments

Centrifugation pre-treatment aims to remove a certain part of the solids of the optimized extract and reduce the membranes' load. Despite all the pre-treatments, there was still some suspended, dissolved solid matter in the galangal extract. These solids were retained in various proportions by the membranes, and the total solids (TS), total dissolved solids (TDS), total suspended solids (TSS) and total carbohydrate (TC) evaluations were given in Table 4.6. Separation of solids by the membrane process is based on the particle size-pore size phenomenon. The particles holding the membrane clog the pores over time, reducing performance and restricting filtration [Balyan and Sarkar, 2016]

Feed extract included 0.0270 mg/ml total solids (TS). UF membranes retained the most amount of total solids. In the retentates of UF filtration total solids were 0.0240, 0.0270, 0.0189 mg/ml, (UP020, UV150, UP005 respectively) and there wasn't significant difference ($p > 0.05$) between feed and the retentates. The UV150 membrane with the largest pore size did not give the best results. Around 43% retention was achieved due to the application of ultrasound; only the particles) were broken into very small pieces and some of these particles were broken into small particles were removed by centrifugation. In addition, during the filtration, the solids come together and precipitate and form a cake layer, making it difficult to pass into permeate. NF270 and NF90 membranes retained the solids more successfully due to the small pore sizes [Lopez-Munoz et al., 2009]. There wasn't a significant difference between the retentates (0.0300 and 0.0302 mg/ml) and permeates (0.0060 for both) of direct NF membranes. The difference was probably because some of the solids were retained on the membranes at different rates and could not pass through to permeate or stay in the retentate. Combined processes consist of two stages: pre-filtration by UV150 and then NF270 and NF90 filtration. Although the UV150 membrane could not retain most of the solids when applied alone; as a pre-treatment, it increased the filtration capacity as it reduced the load of the feed entering the NF membranes and provided permeates with the lowest total solid content of 0.0030 mg/ml to NF270 and NF90 permeates ($p \leq 0.05$).

Total dissolved solids (TDS) express a measure of dissolved organic and inorganic matter substances in the extracts. These are minerals and inorganic salts such as potassium, calcium, magnesium, bicarbonates, etc. [Chen et al., 2021]. Although advanced filtration systems such as reverse osmosis are more useful due to their solubility and small molecular size, a certain decrease was also achieved in the TDS content of the extracts with UF and NF [Kim et al., 2018]. Feed extract included 43.81 mg/ml of total dissolved solids. In the permeates of UF filtration, total dissolved solids were 4.67, 8.42, and 13.22 (UP020, UP005, UV150, respectively). These results were significantly different and feed ($p > 0.05$). UV150 showed the lowest retention rate as well as the total solids. The result is directly related to the pore size phenomena [Sahai, 2000]. In contrast to total solid, UP005 better retained total dissolved solids. Although NF270 (6.74 mg/ml) and NF90 (5.31 mg/ml) ($p > 0.05$) are not as successful as the UP005 membrane, they provided low TDS content in permeate in direct processes. In the combined processes, as with the total solids, a part of the total dissolved solids was

filtered in UV150, which is a pre-treatment, and the load of the NF membranes decreased, and they showed good performance. The fact that there was no statistical difference ($p > 0.05$) between the performances of both NF membranes when viewed as a percentage (Table 4.7) may be due to the difference in the VRF ratio [Murakami et al., 2011]. However, the UP005 membrane direct process was found to be the most successful in TDS retention.

Total suspended solids are small particles found in the form of non-dissolved suspensions in fluid [Moran et al., 1980]. Suspended solids cause turbidity in the liquid and must be removed. Feed extract included 0.022 mg/ml of total suspended solids. All UF membranes (UP005, UP020, and UV150) and NF270 membrane showed the same results of total suspended solids in permeates (0.010 mg/ml) ($p > 0.05$). However, NF90 permeate has a lower total suspended solid content (0.008 g/ml) but was still not significantly different from others ($p > 0.05$). When retentates were examined, it was noticed that the TSS retained amount was different. UP005 and UV150 membranes retained the 0.014 g/ml of TSS while UP020 retained 0.010 g/ml, NF270 retained 0.018 g/ml and NF90 retained 0.016 g/ml. There wasn't a significant difference ($p > 0.05$) between the results of direct and combined process retentates. Conidi et al.(2020) filtrated goji (*Lycium Barbarum* L.) leaves extract through ultrafiltration, and it was observed an increase in total suspended solid content in retentate (from 5.2 to 6.2 mg/ml) and in the permeate total suspended solids were not detected [Conidi et al., 2020]. In combined processes, total suspended solids, some of which were retained by UV150 and could not be adsorbed, were also more filtered in NF270 and NF90 membranes. The inconsistency between retentate and permeate is that some of the molecules were retained on the membrane in two-stage filtration.

Total carbohydrate contents of fluids were determined by the phenol-sulfuric acid method. The main principle of this method is the reaction of sulfuric acid and phenol with furfural derivatives of carbohydrates [Quero-Jimenez et al., 2019]. Sulfuric acid breaks the polysaccharides, oligosaccharides, and disaccharides into monosaccharides and the compounds react with phenol to generate color [Nielsen, 2010]. The presence of the carbohydrates was similar in UP020, and UV150 permeates as 5.41 mg GE/ml extract. UP005 permeate filtrated less than other UF membranes as 5.14 mg GE/ml extract in the permeate. Significantly, there wasn't any difference ($p > 0.05$) among the permeates of UF membranes.

Table 4.6: Determination of total solids, total dissolved solids, total suspended solids and total carbohydrates of optimized extract of galangal for UF and NF experiments.

Membrane		Fluid	Total Solids (TS) (mg/ml)	Total Dissolved Solids (TDS) (mg/ml)	Total Suspended Solids (TSS) (g/ml)	Total Carbohydrates (TC) (mg GE/ml extract)
Direct Processes	UP005	Feed	0.0270 ± 0.002 ^{ab}	43.81 ± 0.790 ^a	0.022 ± 0.008 ^a	7.30 ± 0.36 ^a
		Retentate	0.0240 ± 0.001 ^{ab}	39.13 ± 0.020 ^b	0.014 ± 0.001 ^b	8.60 ± 0.19 ^{be}
		Permeate	0.0143 ± 0.001 ^c	8.72 ± 0.010 ^c	0.010 ± 0.001 ^b	5.14 ± 0.08 ^c
	UP020	Feed	0.0270 ± 0.002 ^{ab}	43.81 ± 0.790 ^a	0.022 ± 0.008 ^a	7.30 ± 0.36 ^a
		Retentate	0.0270 ± 0.001 ^{ab}	41.21 ± 0.020 ^d	0.010 ± 0.048 ^b	7.89 ± 0.27 ^d
		Permeate	0.009 ± 0.001 ^d	4.67 ± 0.005 ^e	0.010 ± 0.001 ^b	5.41 ± 0.08 ^c
	UV150	Feed	0.0270 ± 0.002 ^{ab}	43.81 ± 0.790 ^a	0.022 ± 0.008 ^a	7.30 ± 0.36 ^a
		Retentate	0.0189 ± 0.001 ^{ab}	35.73 ± 0.025 ^f	0.014 ± 0.001 ^b	8.23 ± 0.19 ^{bd}
		Permeate	0.0135 ± 0.001 ^c	13.22 ± 0.080 ^g	0.010 ± 0.001 ^b	5.41 ± 0.08 ^c
	NF270	Feed	0.0270 ± 0.002 ^{ab}	43.81 ± 0.790 ^a	0.022 ± 0.008 ^a	7.30 ± 0.36 ^a
		Retentate	0.0300 ± 0.001 ^b	38.82 ± 0.020 ^b	0.018 ± 0.002 ^b	9.10 ± 0.23 ^{eg}
		Permeate	0.0060 ± 0.001 ^d	6.74 ± 0.012 ^h	0.010 ± 0.001 ^b	2.19 ± 0.09 ^f
	NF90	Feed	0.0270 ± 0.002 ^{ab}	43.81 ± 0.790 ^a	0.022 ± 0.008 ^a	7.30 ± 0.36 ^a
		Retentate	0.0302 ± 0.010 ^b	35.73 ± 0.010 ^f	0.016 ± 0.001 ^b	9.33 ± 0.20 ^g
		Permeate	0.0060 ± 0.001 ^d	5.31 ± 0.004 ^e	0.008 ± 0.001 ^b	1.56 ± 0.11 ^h
Combined Processes	UV150 and NF270	Feed	0.0150 ± 0.001 ^c	15.59 ± 2.390 ^k	0.010 ± 0.001 ^b	5.41 ± 0.08 ^c
		Retentate	0.0208 ± 0.001 ^c	14.64 ± 0.002 ^k	0.009 ± 0.001 ^b	9.10 ± 0.23 ^{ai}
	UV150 and NF90	Permeate	0.0030 ± 0.001 ^d	7.49 ± 0.010 ^{ch}	0.015 ± 0.001 ^b	1.51 ± 0.05 ^h
		Feed	0.0150 ± 0.001 ^c	15.59 ± 2.390 ^k	0.010 ± 0.001 ^b	5.41 ± 0.08 ^c
		Retentate	0.0150 ± 0.001 ^c	11.92 ± 0.006 ⁱ	0.017 ± 0.001 ^b	6.69 ± 0.07 ⁱ
		Permeate	0.0030 ± 0.001 ^d	1.75 ± 0.003 ^j	0.015 ± 0.001 ^b	1.39 ± 0.05 ^h

For all measurements, data are expressed as mean values with standard deviations. Mean value is the average of three replications.

In all UF membranes, retentate contains more total carbohydrates than feed. (8.60, 7.89, 7.30 mg GE/ml extract for UP005, UP020 and UV150 membranes, respectively). NF membranes concentrated better than UF membranes; the total carbohydrate content of the feed was 9.10 and 9.33 mg GE/ml extract for NF270 and NF90 ($p > 0.05$). Also, in permeates, total carbohydrate contents were 2.19 and 1.56, respectively. Due to the results, NF90 was the most effective membrane for retaining total carbohydrates in direct processes. There was a significant difference ($p \leq 0.05$) between the results. Similarly, Diaz-Montes et al. (2020) fractionated the *Stevia rebusiana* extract through ultrafiltration for both membranes tested; there was a significant increase in TC of retentate and a decrease in TC of permeate [Diaz-Montes et al., 2020]. These decreases in the total carbohydrate content in permeates are explained by the interaction of suspended solids and carbohydrates [Conidi et al., 2020]. In combined processes in the fluid given as feed, UV150 has reduced the total carbohydrate content, NF270 (1.51 mg GE/ml extract) and NF90 (1.39 mg GE/ml extract) ($p > 0.05$) membranes showed better activity than direct processes ($p > 0.05$).

4.2.1.5. Retention Coefficients of Analysis of Filtration Experiments

Retention coefficients of TPC, DPPH, ABTS, TFC, TS, TDS, TSS, turbidity, clarity, color (spectrophotometrically), and TC evaluation for all membranes were shown in Table 4.7. VRF of the membranes processes were between 1.06 and 2.00, which were similar to a study done by Cisse et al. (2011) about the concentration of anthocyanins from rosella extract VRF of 1.70-2.00 [Cisse et al., 2011].

UF (UP005, UP020, and UV150) membranes retention of TPC 80.12%, 75.75%, and 68.88% respectively for 5 bar transmembrane pressure ($p \leq 0.05$). Unexpectedly high retention coefficients of TPC (above 75.25) were observed for UP020 and UP005 membranes. High retention probably depends on the membrane and the solute interactions and retained macromolecules [Cisse et al., 2011]. Similar observations were found in a study done by Moraes et al. (2018) about the polyphenols' UF and NF retention performances from the hydro-alcoholic extract of graviola [Moraes et al., 2018]. Phenolic compounds are known as a molecular weight between 100 and 700 g/mol and this is smaller than the molecular weight cut-off (MWCO) of UF membranes (5.000 for UP005 and 20.000 for UP020). Therefore, UF membranes are expected to be unable to retain phenolic compounds. High retention coefficients of the

UF membranes can be explained by the fact that phenolic compounds, which provide antioxidant capacity, are bound to macromolecules in the matrix, especially complex carbohydrates, with strong bonds. Thus the phenolic compound-macromolecule complex reached sizes that cannot pass through the membrane pores and their transition to the permeate prevented. The macromolecules attached to the pores during filtration also reduce the pore size, causing the molecules that can pass through the pores under normal conditions not to be filtered.

Retention of TPC was the highest value of 93.13% for NF90 and 92.27% for NF270 membrane when all of the direct and combined processes were evaluated. Similarly, Koop et al. (2021) found NF270 and NF90 membranes to concentrate phenolic compounds in jambolan (*Syzygium cumini* L.) extract better [Koop et al., 2021]. The low final efficiency in combined processes was the retention of some of the phenolic compounds in the UV150 membrane, the pre-treatment for total solids retention and the NF270 membrane was more successful ($p \leq 0.05$) than the NF90 membrane of total phenolic retention when UV150 was applied as a pre-treatment.

Evaluation of retention coefficients of flavonoids, which are the sub-groups of phenolic compounds, similar results with TPC were observed. Retention of TFC varied from 89.68%- 93.01% for UF membranes ($p \leq 0.05$) and 97.45%, 96.01% for NF membranes, respectively with highest Similarly, flavonoid concentration by NF90 membrane from propolis 99% of flavonoids of in aqueous extract and 90% of flavonoids of ethanolic extract was retained [Mello et al., 2010]. The presence of ethanol in the extract, might cause membrane swelling and dilation and effects the diffusion of flavonoids. TFC from *Sideritis* ssp L. ethanolic extract was concentrated by different NF membranes, and about 98% retention was succeeded [Tylkowski et al., 2011].

NF270 showed higher retention than NF90 of 94.76% when applied as a combined process after UV150, while NF90 (67.46%) has the lowest retention among all membrane filtrations. In order to understand the losses, mass balances before and after filtration were compared, and the cake formation during filtration caused flux decline and some of the low solubility bioactive compounds retained in this cake layer on the membranes. Combined processes double the cake formation phenomenon, which will cause a decrease in productivity because it is a two-stage filtration system.

Phenolic compounds responsible for antioxidant activity were determined by DPPH and ABTS radical scavenging methods. The highest retention of DPPH was

observed in NF membranes as 93.93% and 92.48% for NF270 and NF90 membranes, respectively. There wasn't a significant difference ($p > 0.05$) between direct NF membranes. UF membranes, as with TPC, showed unexpectedly high retention. ($> 72.93\%$) this was due to the presence of phenolic compounds in concentrate, which are responsible for antioxidant activity. UP005 showed the highest retention (81.32%) of DPPH among the UF membrane. Combined processes of NF270 gave as successful results as the direct NF process, despite the absence of phenolic compounds remaining in the concentration of the UV150 membrane (75.82%) with the pre-treatment. DPPH in the UV150 permeate as the feed of the N270 membrane ensured the retention of 91.24% the phenolic compounds responsible for the antioxidant activity.

ABTS is an alternative antioxidant capacity method and DPPH is soluble only in organic solvents (especially in alcoholic solvents). Still, ABTS is also soluble in aqueous media, allowing to measurement the hydrophilic and lipophilic nature of the phenolic compounds in the extracted sample [Andres et al., 2020]. Additionally, ABTS includes reactive toward not only phenolics but also vitamin C, which is a main antioxidant molecule in foods [Walker and Everette, 2009]. The retention coefficient values of all direct membrane processes higher than 99%. NF membranes showed higher retention coefficients than UF membranes. There wasn't a significant difference ($p \leq 0.05$) between UF membranes and NF membranes. This indicates that almost all of the phenolic compounds that affect ABTS were retained by the membrane and cannot pass through to the permeate. Similarly, graviola (*Annona muricata* L.) leaves 50% hydro-alcoholic extracts were concentrated by UF and NF membranes, and the retention coefficient of direct processes was between 86.8 – 97.1% for ABTS analysis [Moraes et al., 2018]. Combined processes had the also high value of ABTS value (99.97% and 99.90%, respectively).

Low retention coefficient of total solids with UV150 membranes (28.75%), NF270 and NF90 membranes showed better results (85.64% and 80% respectively) ($p \leq 0.05$) with similar to direct processes (80.10% and 80.16% respectively) because of the retention of some of solids by UV150 and it facilitated the passage through NF membranes and only retained smaller molecular weight that UV150 did not retain. In direct processes, total solids retentions were the highest with NF membranes, and the lowest retention was with UV150 membrane. Magalhaes et al. (2019) investigated total solid content of direct NF/UF and sequential UF/NF processes [Magalhaes et al.,

2019]. Similarly, they reported that the direct NF process has better total solids retention, and 5 kDa UF membrane gives the lowest total solids retention.

Evaluation of total dissolved solids (TDS) advanced in a similar trend with TS in UF membranes. The highest retention of TDS was in the UP020 membrane (88.68%) following by UP005 (77.72%) and UV150 (62.99%). Retention of TDS with direct NF membranes was significantly different ($p \leq 0.05$) from each other (82.62% and 85.15%, respectively). In combined processes, NF90 (80%) showed a significantly better result ($p > 0.05$) than NF270 (48.85%) TDS retention performance after the pretreatment of UV150. TSS retention of UP020, UV150 and NF270 was exactly the same (54.54%) while NF90 (63.63%) showed better performance ($p \leq 0.05$). UP005 and NF90 were able to retain 42.10% and 50.00% of the TSS in the feed. Combined NF270 and NF90 processes showed low retention performances (33.33%, 11.76%, respectively) but were not as good as direct processes.

Turbidity was defined as the quality and clarity of a liquid. Removal of turbidity leads to increased clarity. Turbidity was removed by UP005 (94.04%) with the highest value. In direct processes, all membranes had removed turbidity considerably (83.89 – 94.54%). Although UV150 membrane removed the 83.89% of turbid materials so in combined processed, the membranes showed good performances (88.89% and 85.33%, respectively). In combined processes NF270 was significantly better than NF90 ($p \leq 0.05$)

Clarity values in direct processes were obtained between 91.75%- 99.82%. The best results were with NF membranes (99.82%) and UF005 (99.04%), which have the smallest pore size of UF membranes. In combined processes, the fact that most of the turbidity was removed and clarity was ensured caused the percentages to below in the following NF processes (42.86% and 78.38%, respectively).

The spectrophotometric color value, which is a measure of the removal of phenolic compounds that provide the color of the extract physical clarification and the removal of color, shows parallelism with clarity. Highest retention coefficients were observed with NF270, NF90, and UF005 membranes (99.49%, 99.75%, and 98.20% respectively) and also UP020 and UV150 membranes showed considerably high color removal values (94.70% and 98.14%). The decrease in color and increase in clarity, which is one of the physical properties of the galangal feed extract, during the filtration processes indicate that the highest percentage of pigmented compounds were retained [Diaz-Montes et al., 2020].

Table 4.7: Retention coefficient of TPC, DPPH, ABTS, TFC, TS, TDS, TSS, turbidity, clarity, color and TC of optimized extract of galangal for UF and NF experiments.

Membranes		Retention coefficient										
		TPC (%)	DPPH (%)	ABTS (%)	TFC (%)	TS (%)	TDS (%)	TSS (%)	Turbidity (%)	Clarity (%)	Color (%)	TC (%)
Direct Processes	UP005	80.12 ±0.53 ^a	81.32 ± 0.03 ^a	99.90 ± 0.01 ^a	90.48 ±0.14 ^a	40.32± 0.46 ^a	77.72 ±0.03 ^a	42.10 ±1.60 ^a	94.54 ±0.01 ^a	99.04 ±0.25 ^a	99.20 ±0.35 ^a	31.40 ±6.04 ^a
	UP020	75.25 ±0.38 ^b	72.93 ±1.94 ^b	99.86 ±0.01 ^a	93.01 ±0.33 ^b	66.82 ±3.01 ^b	88.68 ±0.02 ^b	22.20 ±9.09 ^b	86.27 ±0.42 ^b	91.75 ±0.18 ^b	94.7 ±0.25 ^b	25.84 ±4.07 ^b
	UV150	68.88 ±2.1 ^c	75.82 ±3.1 ^b	99.43 ±0.05 ^{ab}	89.68 ±0.69 ^c	28.75 ±0.17 ^c	62.99 ±0.18 ^c	28.57 ±9.09 ^c	83.89 ±0.01 ^a	98.24 ±0.17 ^a	98.14 ±0.23 ^a	34.20 ±4.07 ^b
	NF270	92.27 ±0.30 ^d	93.93 ±0.16 ^c	99.96 ±0.01 ^a	97.45 ±0.32 ^d	80.10 ±0.44 ^d	82.62 ±0.03 ^d	15.09 ±6.09 ^d	86.27 ±0.01 ^b	99.82 ±0.00 ^a	99.49 ±0.00 ^a	75.93 ±0.21 ^d
	NF90	93.13 ±0.30 ^d	92.48 ±0.14 ^c	99.97 ±0.01 ^a	97.01 ±0.31 ^d	80.16 ±0.30 ^d	85.15 ±0.01 ^e	50.00 ±4.54 ^e	83.63 ±0.01 ^c	99.82 ±0.00 ^a	99.75 ±0.00 ^a	83.29 ±2.25 ^e
	UV150 and NF270	88.55 ±0.44 ^e	91.24 ±0.06 ^c	99.97 ±1.51 ^a	94.76 ±1.54 ^e	85.64 ±0.27 ^e	48.85 ±0.05 ^f	33.33 ±12.0 ^f	88.89 ±0.15 ^d	42.86 ±3.33 ^c	99.81 ±2.31 ^c	77.80 ±0.30 ^d
Combined Processes	UV150 and NF90	84.22 ±0.93 ^f	52.27 ±0.21 ^c	99.90 ±1.26 ^a	68.46 ±0.69 ^f	80.00 ± 0.69 ^d	80.00 ±0.02 ^d	11.76 ±12.0 ^g	85.33 ±1.21 ^b	78.73 ±3.33 ^d	89.66 ±2.5 ^d	79.25 ±0.99 ^d

For all measurements, data are expressed as mean values with standard deviations. Mean value is the average of three replications

Total carbohydrates (TC) is a method for understanding the number of carbohydrates (mono-, di-, oligo- and polysaccharides) by breaking them down into monosaccharides [Nielsen, 2010]. NF270 (75.93%) and NF90 (83.29%) showed better performance ($p \leq 0.05$) to retain carbohydrates than UF membranes (31.40%, 25.84% and 34.20%). Combined processes retained more TC (77.80% and 79.25%, respectively) ($p > 0.05$). Total carbohydrates from Goji leaves were fractionated by UF membrane from 6.33 g glucose/L to 9.65 g glucose/L and 15% retention [Conidi et al., 2020].

4.2.1.6. Determination of Permeate Fluxes

Table 4.8 presents the pure water fluxes, initial and steady-state fluxes for all proposed membranes, and calculated flux decline (%) values. The steady-state permeate fluxes were ranged from 1.64 to 8.63 L/m²h.

The initial permeate fluxes of direct UP005 and UP020 were 5.25 and 4.11 L/m²h, respectively. However, when they reached to steady-state (2.05 and 1.64 L/m²h, respectively) the flux declines were statistically same ($p \leq 0.05$) (60.95% and 60.09%, respectively). The membranes became clogged over time, and their steady-state flux decreased related to the structural and adhesive properties, hydrophobicity of the membrane, and the applied pressure [Balyan and Sarkar, 2016]. UV150 (24.66 L/m²h) had the highest initial flux than other membranes. The decrease in flux (75.02%) was statistically ($p \leq 0.05$) higher than other UF membranes. Although the direct NF membranes had higher initial fluxes (6.58 and 8.22 /m²h , respectively) than direct UF membranes, the flux declines (62.46% and 64.90%) were similar to UP005 and UP020. Also, the steady-state fluxes of NF270 (2.47 L/m²h) and NF90 (2.88 L/m²h) not statistically different ($p > 0.05$). The evaluation of the combined process differed from direct processes. NF270 membrane showed the lowest flux decline (4.53%) by initial flux of 9.04 L/m²h and steady-state flux of 8.63 /m²h which was also the highest ($p \leq 0.05$) steady-state flux of direct and combined processes. Considering the steady-state fluxes, the NF90 (4.52 L/m²h) membrane performed only half as well as the NF270 (8.57 L/m²h) membrane despite the low flux decline (21.39%), under the same conditions. The initial flux of the NF90 (5.75 L/m²h) membrane was much lower although UV150 pre-filtration step.

The reported fluxes are similar to the literature for the membrane filtration of pequi fruit extract [Martins Sa et al., 2019]. In a study about recovery of phenolic compounds from pequi fruit extracts, final fluxes of UF and NF experiments were ranged from 1.17-5.67 L/m²h [Magalhaes et al., 2019]. Conidi et al. (2017) found 3-17 L/m²h steady-state permeate fluxes by UF and NF membranes of pomegranate juice [Conidi et al., 2017]. Destani et al. (2013) studied the recovery of phenolic compounds in blood orange juice with UF and the initial permeate flux was 14.02 L/m²h and decreased by time to 2.7 L/m²h. [Destani et al., 2013]. NF membranes were used to concentrate biologically active compounds from propolis with 2.66-4.05 L/m²h permeate fluxes [Tylkowski et al., 2011]. Stevia extracts were fractionated by two-step UF with steady-state permeate fluxes of 0.65-3.3 L/m²h [Diaz-Montes et al., 2020]. The concentration study of phenolic compounds from strawberry juice, and the average fluxes were 4.0 L/m²h of direct NF membrane and for combined MF+NF membrane 3 L/m²h [Arend et al., 2017]. Bioactive compounds were concentrated by NF from watermelon juice with an average flux of 2.3 L/m²h [Santos et al., 2014]. These results were in accordance with the results observed in the present study.

According to analysis, untreated UV150 has the highest water flux and treated UV150 has the highest flux decline. Therefore, higher fouling occurred and the high contact angle (59.56%) of it shows the hydrophobicity of the membrane (Table 4.10).

Table 4.8: Permeate fluxes of optimized extract of galangal for ultrafiltration and nanofiltration membranes.

Membranes		Permeate fluxes			
		Water flux (L/m ² h)	Initial flux (L/m ² h)	Steady-state flux (L/m ² h)	Flux decline (%)
Direct Processes	UP005	26.00	5.25	2.05	60.95
	UP020	116.00	4.11	1.64	60.09
	UV150	1425.00	24.66	6.16	75.02
	NF270	106.80	6.58	2.47	62.46
	NF90	45.60	8.22	2.88	64.90
Combined Processes	UV150 and NF270	106.80	9.04	8.63	4.53
	UV150 and NF90	45.6	5.75	4.52	21.39

The changes against time within the fluxes were taken per minute of direct processes of UP005, UP020, UV150, NF270 and NF90 combined processes of NF270 and NF90 were given in Figure 4.3. While the initial fluxes were higher in all trials (4.11 - 24.66 L/m²h) they decreased over time and fixed after a while. The flux decreases in direct processes while the combined NF270 and NF90 fluxes are the same/close to the initial flux at certain intervals, and therefore a, more diffuse graphs were obtained.

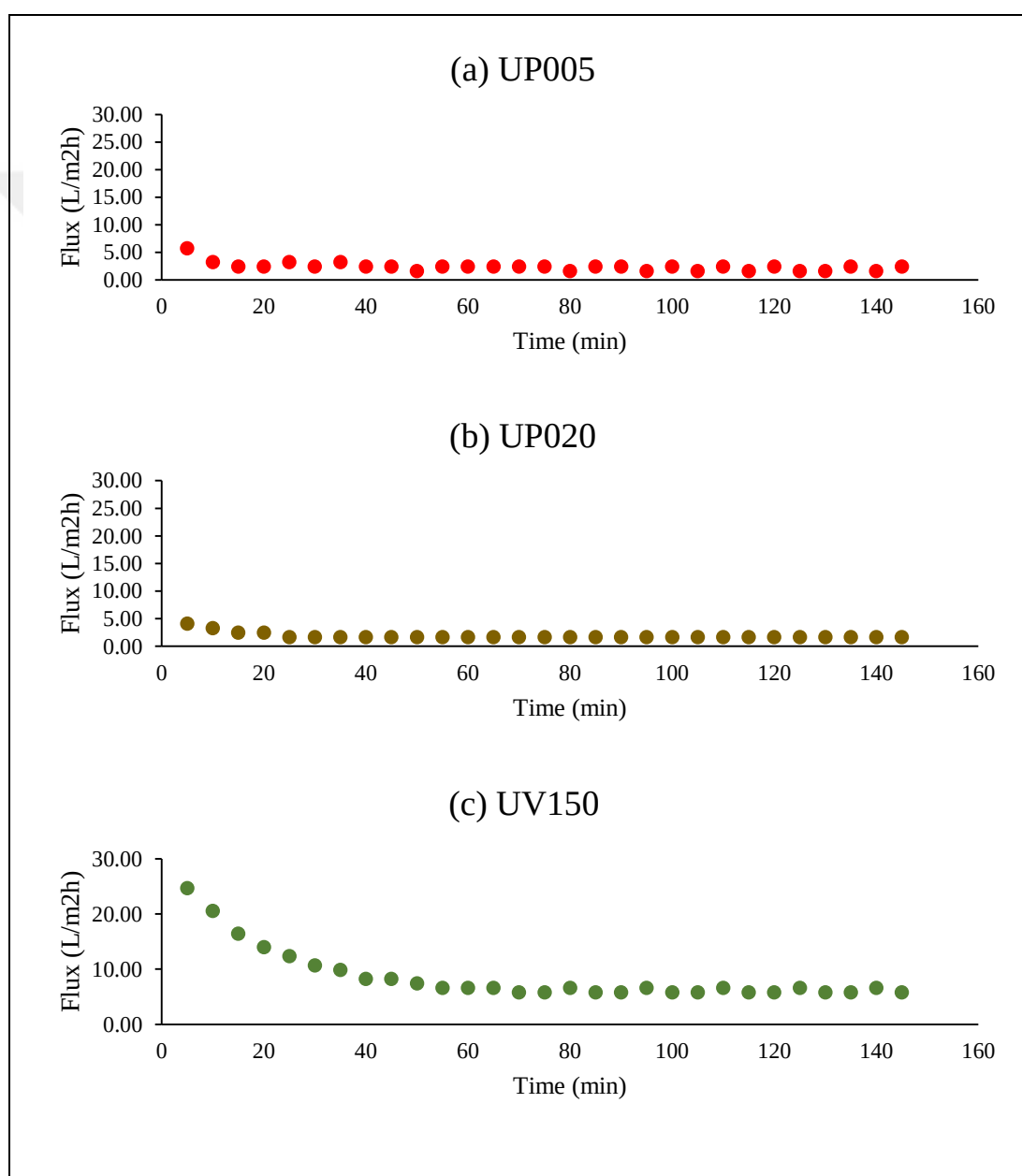


Figure 4.3: Permeate flux graphs of optimized extract of galangal for ultrafiltration (a) UP005, (b) UP020, (c) UV150, nanofiltration (d) NF270, (e) NF90 and combined (f) UF + NF270 and (g) UF+ NF90 experiments.

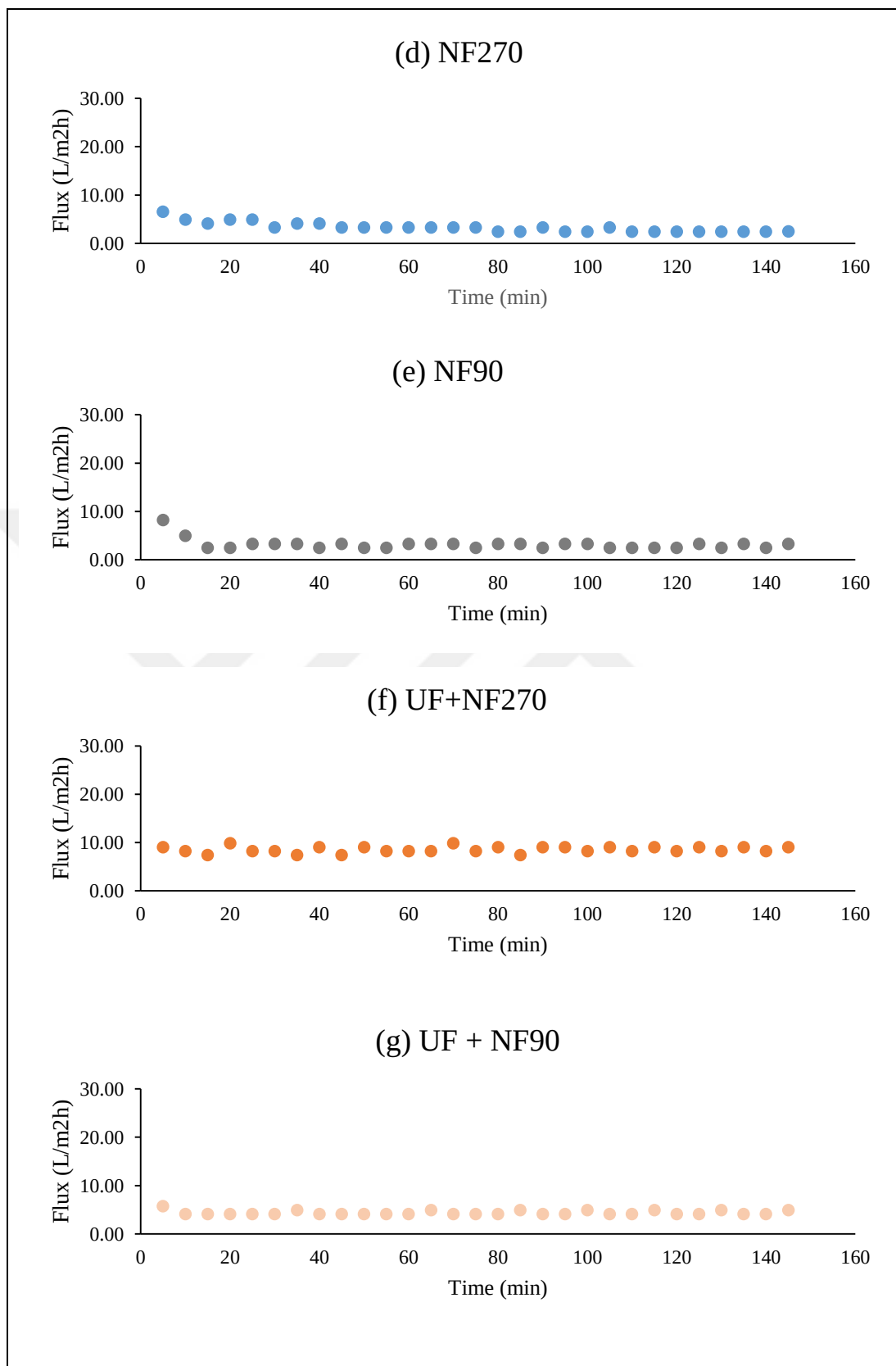


Figure 4.3: Continued.

4.2.1.7. Summary of Membrane Filtration Experiments

Figure 4.4 summarizes the optimized ultrasound-assisted extracted galangal pre-treatments with hydro-alcoholic solvent and membrane filtration experiments as direct processes of UF (UP005, UP020, UV150) and NF (NF270 and NF90) and combined processes as UV150+NF270 and UV150+NF90. Images of hydro-alcoholic feed extract, retentates and permeates. Table 4.9 compares images of optimized extract of galangal as feed, retentate, permeate, retentate and permeate.



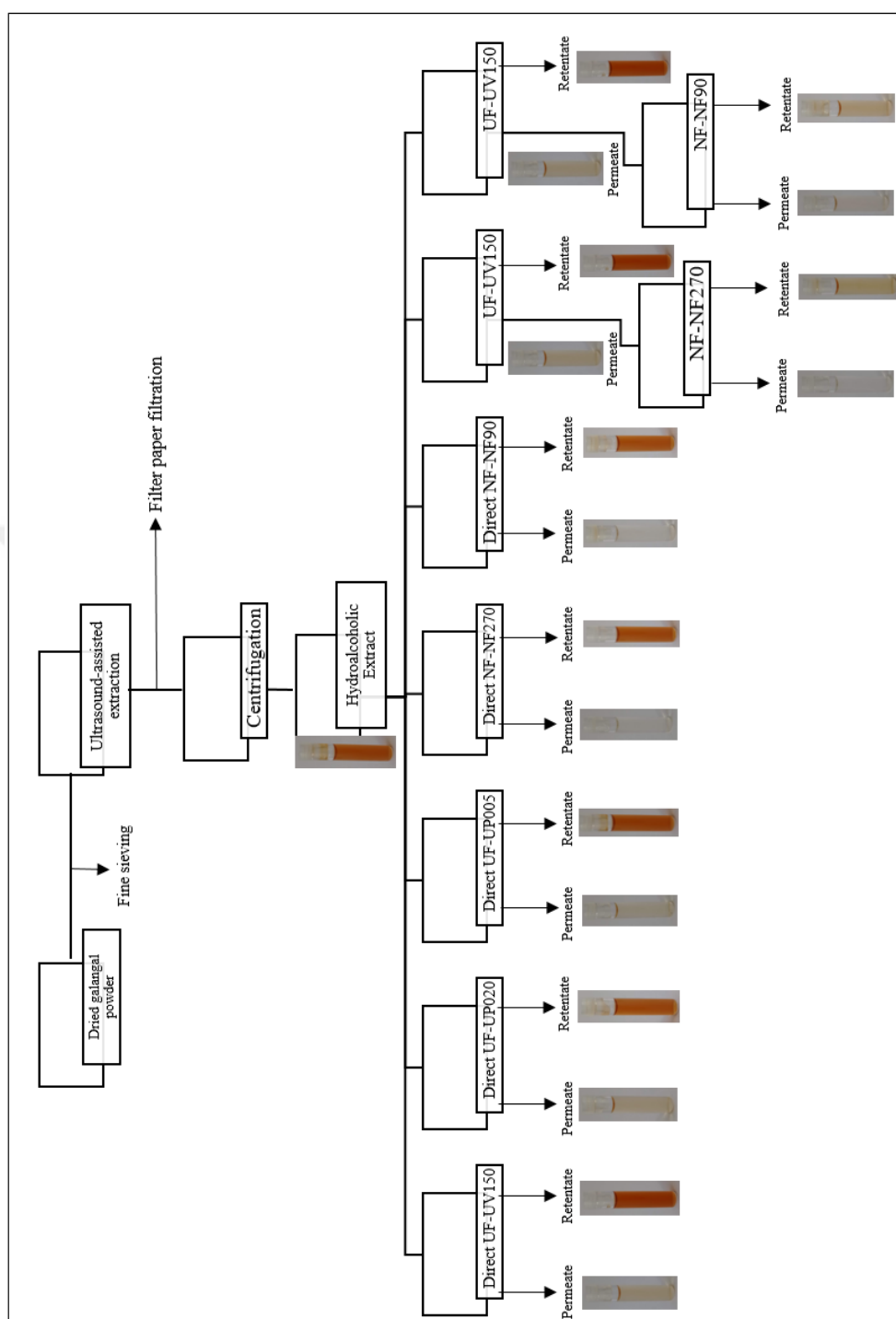
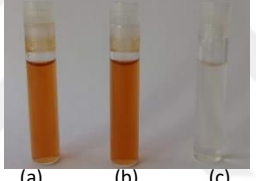
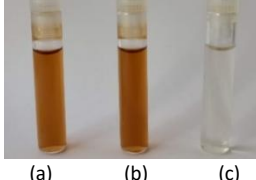


Figure 4.4: Schematic summary of the membrane process.

Table 4.9: Comparison of images of optimized extract of galangal as (a) feed, (b) retentate, (c) permeate, (d) retentate, (e) permeate.

Membrane		
Direct Processes	UP005	
		(a) (b) (c)
	UP020	
		(a) (b) (c)
	UV150	
		(a) (b) (c)
NF270		
		(a) (b) (c)
	NF90	
		(a) (b) (c)
Combined Processes	UV150 and NF270	
		(a) (b) (c) (d) (e)
UV150 and NF90		
		(a) (b) (c) (d) (e)

4.2.3. Membrane Analysis

4.2.3.1. Contact Angle Analysis

Contact angle (θ) measurements were made to evaluate the hydrophobicity of the membranes. Lower contact angle value indicates a more hydrophilic character of the membrane. Hydrophobicity depends on polar and non-polar groups on the membranes surface [Rabelo et al., 2016]. It is crucial to evaluate the cake layer formed on the membranes due to the more excellent absorption of non-polar compounds on hydrophobic surfaces [Vieira et al., 2018]. The contact angles of the all treated and untreated membranes were given in Table 4.10.

In direct processes, the most hydrophobic membrane was UP005 (53.60°) followed by UV150 (35.86°) and UP020 (35.59°), respectively. The same order was observed for the untreated membranes for UF (62.70° , 59.56° , 45.94°). Untreated NF membranes (27.08° for NF270 and 29.71° for NF90) were more hydrophilic than UF membranes. The structural material of NF270 and NF90 are polyamide thin-film composite that contains polar amide groups in their backbone. These amide groups make hydrogen bonds with polar solvents, so, they have lower contact angle values [Vieira et al., 2018]. Filtration enhanced the hydrophobicity of the NF membranes (53.42° for NF270 and 58.12° for NF90) while decreasing UF membranes. Similarly, Rabelo et al. (2016) and Vieira et al. (2018) measured the contact angles of NF membranes before and after filtration, and they reported that the contact angles were higher in treated membranes than in untreated ones [Rabelo et al., 2016] [Vieira et al., 2018]. Hydrophobicity was lower in NF270 membrane than NF90 in treated and untreated membranes. The reduction in permeability flux was higher in the NF90 (74.77%) membrane than in the NF270 (72.83%) membrane (Table 4.8), which was confirmed by the contact angles of the NF270 and NF90 membranes. (53.42° and 53.46° , respectively), indicating greater fouling [Yammine et al., 2019].

In combined processes, compared to the untreated membrane (27.08° for NF270 and 29.71° for NF90). The increase in the untreated membrane (28.04° for NF270 and 27.92° for NF90) showed a lower increase in contact angle than the direct process (53.46° for NF270 and 58.12° for NF90). The highest contact angle was the untreated UP005 (62.70°) membrane which indicated the most hydrophobic membrane of all. According to Marel et al. (2009), hydrophobic membranes are more prone to being

fouled by organic materials due to hydrophobic adsorption between the contaminants and the membrane [Marel et al., 2009]. An increase in the contact angle indicates increased hydrophobicity, and more hydrophobic molecules were retained on the membrane. The decrease in the UV150 membrane from 59.56° to 35.86° means that hydrophobic molecules were absorbed; therefore, the presence of a more hydrophilic membrane in the NF step of the combined process can be mentioned.

Table 4.10: Contact angle measurements of treated and untreated membranes.

Membrane	Untreated membrane	Avr.	Treated membranes	Avr.
Direct Processes				
UP005		62.70		53.60
UP020		45.94		35.59
UV150		59.56		35.86
NF270		27.08		53.46
NF90		29.71		58.12
Combined Processes; (UV150 and)				
NF270		27.08		28.04°
NF90		29.71		27.92

4.2.3.2. SEM Analysis

Table 4.11 represents the scanning electron microscopy (SEM) micrographs of the treated ultrafiltration UP005, UP020, UV150, nanofiltration NF270, NF90 and combined UF + NF270 and 111 UF+ NF90 experiments. In direct processes, it has been seen from the SEM images that all the membranes were blocked after the filtration experiments. Combined membranes were less blocked, and uncontaminated parts of the membranes were also visible. This was because bioactive molecules and other contaminants were retained by UV150 pre-treatment, and a clearer extract was fed to the NF membranes.

Visual differences were observed between the UF membranes and NF membranes. Among UF membranes, cake layer formations were observed in direct proportion of pore sizes. Similar results were observed by Saha et al., (2007) in the ultrafiltration study of sugarcane juice [Saha et al., 2007]. The results were usual while considering the pore sizes and membrane-compound interactions. There was more accumulation on the NF membranes, and the results of the various analyzes and the retention coefficient calculations also support this.

Table 4.11: Scanning electron microscopy (SEM) micrographs of the treated ultrafiltration (a) UP005, (b) UP020, (c) UV150, nanofiltration (d) NF270, (e) NF90 and combined (f) UF + NF270 and (g) UF+ NF90 experiments.

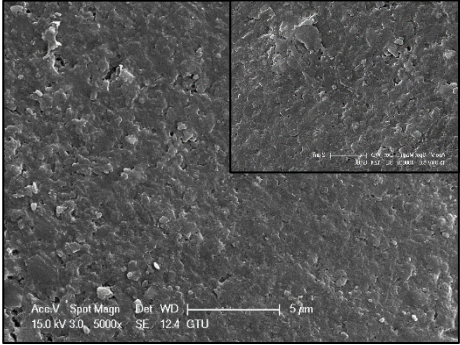
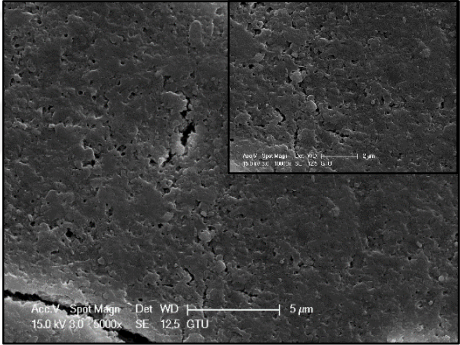
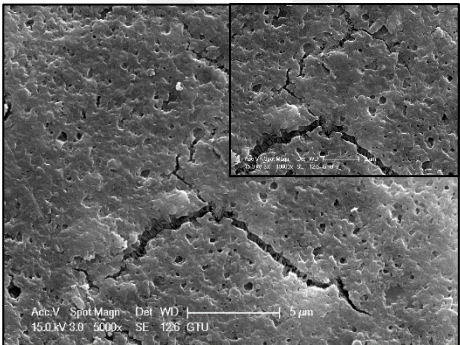
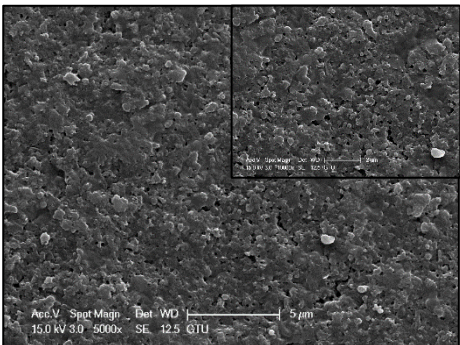
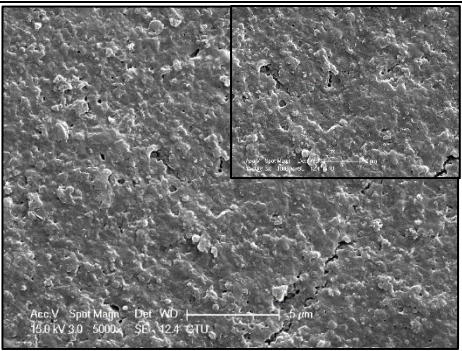
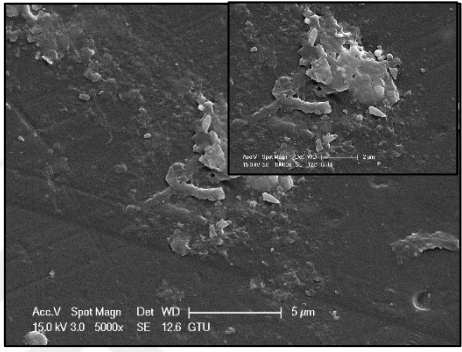
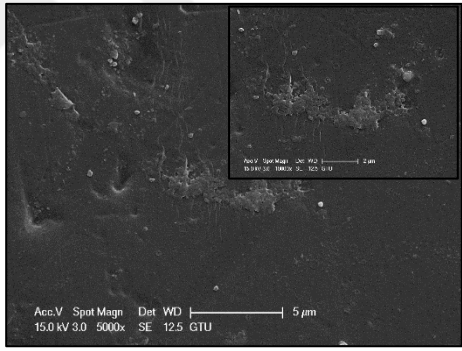
Membrane	SEM
Direct Processes	
UP005	
UP020	
UV150	
NF270	

Table 4.11: Continued.

Combined Processes; UV150 and	NF90	
	NF270	
	NF90	

5. CONCLUSIONS

The extraction temperature, extraction time, solvent/solid ratio, ethanol/water ratio, ultrasound power, and ultrasound cycle are the factors that affect TPC, TFC, and antioxidant activity (DPPH and ABTS) of ultrasound-assisted extracted galangal. 50% ethanol/water was found to be the best solvent for TPC compared to four protic, four aprotic and two mixture solvents. Moderate temperature, time and s/s ratio ranges; constant values of ethanol/water ratio, ultrasound power, and ultrasound cycle were determined using screening studies.

Predictive models were described using Response Surface Methodology by the functions of independent factors of temperature, time, and s/s ratio. The approach of the desirability function determined optimum conditions. The optimum conditions were temperature of 47.51 °C, time of 52.66 min, and s/s ratio of 30 ml/g with the maximum desirability function of 0.77. The optimum conditions results of ultrasound-assisted extracted galangal were TPC of 64.74 mg GAE/g sample, DPPH of 48.49 % inhibition, ABTS of 167.98 mg TE/ml extract and TFC of 132.54 mg QE/g sample. Experimental results were found very close to predicted values. The temperature, time, and s/s ratio were the critical factors on TPC, DPPH, ABTS and TFC. The most effective factors were the s/s ratio for TPC, DPPH, and ABTS while temperature for TFC.

Filtration experiments were maintained by UF membranes of UP005, UP020, and UV150; NF membranes of NF270 and NF90, directly and combined. TPC, DPPH, ABTS, TFC, color (CEILAB), TS, TDS, TSS, TC, turbidity, color and clarity (spectrophotometrically) values were determined in feed, retentate and permeate solutions. Retention performances, steady-state flux performances, contact angles, and SEM images of the membranes were evaluated. Direct NF membranes were found more successful in the recovery of the compounds according to the retention coefficient values. The performances of the membranes were better in direct NF270 for DPPH, ABTS, and TFC and direct NF90 for TPC, ABTS UP020 was better at the retention of TDS. UP005 and UP020 had the best removed turbidity. TS and color were recovered better with combined processes of UF + NF270, while clarity and TC were better with combined processes of UF + NF90.

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BIOGRAPHY

Veranur GÜNGÖR received her B. Sc. degree from Department of Food Engineering, İstanbul Sabahattin Zaim University in 2019 and Department of Nutrition and Dietetics, İstanbul Sabahattin Zaim University by double-major program. In 2020, she was qualified as “Research assistant” and she has been pursuing her M. Sc. under the supervision of Prof. Dr. Murat ÖZDEMİR at the Department of Chemical Engineering, Institute of Natural and Applied Sciences, Gebze Technical University since 2020.



APPENDICES

Appendix A: Conference Proceeding

Güngör V., Özdemir M., (2021), “Solvent extraction of polyphenols from galangal”, 5. GTU Lisansüstü Araştırmalar Sempozyumu, Gebze-Kocaeli, 10-11 June.

