ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL

ANALYTICAL STUDIES ON BIOACTIVE COMPONENTS OF NATURAL MATERIALS

Ph.D. THESIS

Veselina ADIMCILAR

Department of Chemistry

Chemistry Programme

FEBRUARY 2024

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL

ANALYTICAL STUDIES ON BIOACTIVE COMPONENTS OF NATURAL MATERIALS

Ph.D. THESIS

Veselina ADIMCILAR
(509172014)

Department of Chemistry

Chemistry Programme

Thesis Advisor: Prof. Dr. F. Bedia ERİM BERKER

FEBRUARY 2024

ISTANBUL TEKNİK ÜNİVERSİTESİ ★ LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

DOĞAL MALZEMELERİN BİYOAKTİF BİLEŞENLERİ ÜZERİNE ANALİTİK ÇALIŞMALAR

DOKTORA TEZİ

Veselina ADIMCILAR
(509172014)

Kimya Anabilim Dalı

Kimya Programı

Tez Danışmanı: Prof. Dr. F. Bedia ERİM BERKER

ŞUBAT 2024

Veselina ADIMCILAR, a Ph.D. student of ITU Graduate School 509172014, successfully defended the thesis/dissertation entitled "ANALYTICAL STUDIES ON BIOACTIVE COMPONENTS OF NATURAL MATERIALS", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor : **Prof. Dr. F. Bedia ERİM BERKER** ....
Istanbul Technical University

Jury Members : **Prof. Dr. Ayşegül GÖLCÜ** ....
Istanbul Technical University

Prof. Dr. Duri Şehvar ÜNAL ....
Istanbul University

Assoc. Prof. Dr. Nevin ÖZTEKİN ....
Istanbul Technical University

Prof. Dr. Sezgin BAKIRDERE ....
Yıldız Technical University

Date of Submission : 26 December 2023
Date of Defense : 12 February 2024

To my family,

FOREWORD

I would like to express my profound and sincere gratitude to my supervisor, Prof. Dr. F. Bedia ERİM BERER, for her guidance and support in every aspect of my academic journey spanning over a decade. I am also deeply grateful for her providing me the valuable opportunity to work in her group, which I am honored to be a part of.

I also would like to thank my thesis committee members, Prof. Dr. Ayşegül GÖLCÜ and Prof. Dr. Duri Şehvar ÜNAL for their valuable evaluation of my work. Furthermore, I would like to thank Prof. Dr. Gülşen AKIN EVİNGÜR and Prof. Dr. Emrah TORLAK for their scientific support.

I would like to thank my coworkers with whom I shared the laboratory during my studies, Dr. Zeynep KALAYCIOĞLU for her support and particularly Assoc. Prof. Dr. Nevin ÖZTEKİN for her help and support. Additionally, I would thank to Research Foundation of Istanbul Technical University for financial support through the BAP project Grant ID: 42445.

I would like to express my thanks to my dearest friends, Kristina TSVETANOVA WINTER, Dr. Nazlıcan ÇİVİLİBAL TANG, Yasemin VAROL, Gözde ABDULKERİMOĞLU, Öykü GÜNGÖR, and Gizem KELEŞ KOÇ for their unconditional support. Also, I would like to thank Selin GÜMRÜKÇÜ, Dr. Nazlı FARAJZADEH ÖZTÜRK and former labmate Dr. Parya HASHEMİMYANDOAB and my many other friends, whose names I cannot mention, made the days pleasant at ITU with their friendship. I am also grateful to my students who continously supported me.

Finally, I would also like to express my deepest and most heartfelt gratitude to my mother, father, and uncle, who made everything easier for me with their unwavering support. They are making every challenge conquerable for me. I consider myself fortunate to have them in my life, I am grateful for their infinite understanding and love.

December 2023 Veselina ADIMCILAR
(M.Sc. Chemist)

TABLE OF CONTENTS

Page

ABBREVIATIONS

ANOVA	: Analysis of variance
BGE	: Background electrolyte
CA	: Carnosic acid
CE	: Capillary electrophoresis
CMC	: Critical micelle concentration
CFU	: Colony forming unit
CZE	: Capillary zone electrophoresis
DAD	: Diode array detector
DPPH	: 2,2-Diphenyl-1-picrylhydrazyl
E	: Young's modulus
EOF	: Electroosmotic flow
FRAP	: Ferric-reducing antioxidant power
FTIR	: Fourier transform infrared
GAE	: Gallic acid equivalent
GC	: Gas chromatography
HPLC	: High-performance liquid chromatography
IC_{50}	: Half maximal inhibitory concentration
LOD	: Limit of detection
LOQ	: Limit of quantification
MEKC	: Micellar electrokinetic chromatography
RA	: Rosmarinic acid
RSD	: Relative standard deviation
SDS	: Sodium dodecyl sulfate
SEM	: Scanning electron microscope
TPC	: Total phenolic content
TPTZ	: 2,4,6-tripyridyl-s-triazine
UV-Vis	: Ultraviolet-Visible
WHO	: The World Health Organization
WVP	: Water vapor permeability
WVTR	: Water vapor transmission rate

SYMBOLS

ν	: Velocity
t	: Time
ζ	: Zeta potential
μ	: Electrophoretic mobility
Ɛ	: Molar absorption coefficient
l	: Path length
$\boldsymbol{\varepsilon_r}$	: Dielectric constant
E	: Applied electric field
V	: Voltage
°C	: Degree celcius
λ	: Wavelength
$\mathbf{cm^{-1}}$	: Wavenumber
η	: Viscosity
ρ	: Density
θ	: X-ray diffraction angle
d	: Thickness
L*	: Lightness
C	: Chroma
h	: Hue
a*	: Redness in color
b*	: Yellowness in color
WI	: Whiteness index
ΔE	: Difference between two colors in the Lab color space

LIST OF TABLES

Page

LIST OF FIGURES

Page

ANALYTICAL STUDIES ON BIOACTIVE COMPONENTS OF NATURAL MATERIALS

SUMMARY

Since historical eras, plants have been considered the most valuable resource in nature due to their high nutritional value as food and their healing properties. Many plants are classified as herbal medicines as a result of their use as traditional remedies. The various healing properties of plants have been discovered and documented over time, and these medicinal plants have been used for the treatment of heart diseases and skin problems, lowering high cholesterol and sugar levels in the blood, and also curing cold-like symptoms. Today, the demand for medicinal plants persists. These plants not only grow in the geography where they originated, but they have propagated across many parts of the world.

Plants are rich in phenolic compounds, and carotenoids, as well as vitamins and minerals, and they mostly have antioxidant and anticancer effects. A diet rich in plants is crucial for maintaining health, but identification and isolation of the bioactive components are also necessary for the production of new products and drug formulations, as encouraged by the World Health Organization (WHO). Türkiye has different climates and ecological conditions in different regions, which leads to a rich plant flora. Thus, the determination of the bioactive constituents in local medicinal and aromatic plants is especially significant and contributes to their commercial and export value.

Determination and separation of the active ingredients and components in medicinal plants, herbs, and spices have been studied by using many analytical techniques. A relatively new but also very powerful technique, capillary electrophoresis (CE), which was introduced in the 1980s, facilitates studies in this field. Diminution of the sample and solvent consumption, very short analysis time, and high resolution are highlighted as the advantages of CE. The separation principle depends on the formation of a bulk flow in capillaries under high voltage during separation, where all analytes separate within the column according to their relative motion.

Moreover, traditional medicinal plants and some plants with therapeutic effects attract great attention as a good source of additives in food products and the development of some food packaging materials. Large polymeric molecules derived from living organisms or from their products are called biopolymers, which are extensively used for this purpose due to their non-toxic nature and biodegradability. Nowadays, the development of novel materials with desirable properties is achieved by using biopolymer blends, and enhancement of the protective properties of the materials with the addition of natural extracts and products has become a highly investigated research area. The studies are focused on limiting the use of plastics and diminishing waste accumulation; however, replacing them with safer alternatives and increasing food protection with natural additives also meet consumers' demands.

In the first part of the thesis study, two important bioactive components khellin and visnagin amounts were determined in 5 different plant parts of *Ammi visnaga* L. and *Ammi majus* L., two plants used as traditional herbal medicine. A micellar electrokinetic chromatography (MEKC) method was successfully applied and completed in 8.5 minutes. Separation conditions were optimized, 20 mM borate, 20 mM SDS and 5% (v/v), and pH 9.6. Detection of the species was carried out with a UV detector at 245 nm wavelength. There are a limited number of studies on the determination of khellin and visnagin from *A. visnaga* and *A. majus* flowers. However, this study is the first report on the determination and comparison of the khellin and visnagin contents of these two plants grown in Türkiye in their five different parts, namely: flowers, leaves, roots, seeds and stems, as well as the determination of the antioxidant properties applied to the plant extracts. Linear calibration curves were constructed and regression coefficients were calculated as 0.997 and 0.998 for khellin and visnagin, respectively. The applied method was successfully validated, and the amounts of khellin and visnagin in the samples were calculated in the range of 1.60 to 22.60 mg g^{-1} dry weight of the plant. The limit of detection (LOD) of the method was calculated as 0.83 mg L^{-1} for khellin and 0.99 mg L^{-1} for visnagin. Visnagin was found only in *A. visnaga* and its amount was found to be between 5.80 and 18.50 mg g^{-1} dry weight of the plant. The antioxidant power of the extracts was determined by the DPPH method, which measured the ability to react with the 2,2-diphenyl-1-picrylhydrazyl radical, and the highest effect was found in the flowers of both species, followed by the seeds and leaves.

In the second part of the thesis, a capillary electrophoresis method was developed to determine the amounts of rosmarinic and carnosic acids, in 14 *Salvia* species obtained from various regions of Anatolia. There are numerous literature studies on the determination of *Salvia* ingredients grown in various regions and countries. However, this study covers 14 *Salvia* species growing in Anatolia were examined and the correlation between the amount of bioactive species and antioxidant properties was reported. Optimized separation conditions were 20 mM borate and pH 9.6. A silica capillary column with an effective length of 59 cm was used for separations, and the detection of the analytes was carried out with UV detection at a wavelength of 210 nm. The migration time for carnosic acid was 4.1 minutes, while for rosmarinic acid was 5.8 minutes. Linear calibration curves were constructed for both analytes and the regression coefficients were calculated as 0.998 for rosmarinic and carnosic acids. The detection limit of the method was calculated as 1.86 mg mL^{-1} for rosmarinic acid and 1.72 mg mL^{-1} for carnosic acid. Recovery studies were carried out for the validation of the method, and the values were found to be between 94.8% and 101% for rosmarinic acid and 90.2% and 96.7% for carnosic acid. While rosmarinic acid contents in *Salvia* species were found to be between 1.08 and 18.70 mg g^{-1} dry plant, carnosic acid was found to be 11.00 mg g^{-1} dry plant in only one *Salvia* specie. The antioxidant power of the extracts was determined using the iron (III) ion-reducing antioxidant power method (FRAP) and the DPPH method, in which the 2,2-diphenyl-1-picrylhydrazyl radical is used and the ability of the studied substance to undergo radical reactions is measured. The Follin-Ciocalteu method was used for total phenolic substance determination. The correlation between rosmarinic acid content and DPPH method results is 0.77, and the correlation between rosmarinic acid content and FRAP method results was found to be 0.82.

In the third part of the thesis, vinegars which were obtained by fermentation of various fruits were used as safe and non-toxic solvents to produce chitosan-based, non-toxic, biodegradable materials with improved properties. Since chitosan is a natural biopolymer and obtained by the deacetylation of chitin, which is abundant in the shells of crustaceans, and dissolves in dilute solutions of organic acids, films were prepared by dissolving 1% (w/v) chitosan in vinegar and water ratio of 1:1 (v/v). Chitosan was also preferred due to its antimicrobial and non-toxic properties, and its physical and mechanical properties are compatible with the desired coating materials. In this study, kinds of vinegar produced from pomegranate, apple, grape and hawthorn fruits were used. The spectroscopic, physical and mechanical properties of these films were compared with each other. It was observed that vinegar films had the ability of blocking ultraviolet rays in the range of 200 to 350 nm, and especially the increase in the elasticity of the film prepared with pomegranate vinegar was obtained. An 11-fold increase in the antioxidant power of the film prepared with pomegranate vinegar, determined by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method, was observed compared to the control film *i.e*. chitosan film prepared in commercial acetic acid. It was also determined that prepared films showed a higher antimicrobial effect against gram-positive and gram-negative bacteria, compared to the control film. Materials prepared with four vinegar varieties showed improved antimicrobial, antioxidant, optical and elastic properties, and they are promising in the application of these materials as potential and economical food packaging materials.

In the fourth and last part of this thesis, a potential biopolymer-based food packaging material was produced by using the extract of the purple basil (*Ocimumbasilicumpurpurascen*). Aromatic basil leaves, known for their antioxidant, antiviral and antibacterial properties, are rich in phenolic compounds, minerals, vitamins, and anthocyanins, which are the pigments responsible for the strong purple color of the plant. The chemical structures of the anthocyanins change with pH variation, and as a result, color changes are observed. Thus, the prepared material is non-toxic and biodegradable, and it can be used as a natural indicator for the determination of food freshness. In order to provide the desired physical properties of the material, pectin and alginate biopolymers were used together. Films were prepared by mixing 1% (w/v) pectin and 0.5% (w/v) alginate with purple basil extract in 1:1 (v/v) water in a total volume of 30 mL for 5 hours. The polymer mass was formed. It was prepared by adding 1:1 glycerol by mass and dissolving it, then pouring it into petri dishes and drying it. In order to prevent the obtained biopolymeric films from dissolving in water, the films were cross-linked with $CaCl_2$ solution. The optical, spectroscopic and physical properties of the obtained material (swelling, water solubility, water vapor permeability, moisture content analysis), antioxidant and antimicrobial properties, and the release of anthocyanins over time were evaluated. In addition, it was tested with a real food sample to see if this material could detect food spoilage as expected. The resulting material showed high antioxidant properties and antimicrobial power against both gram-positive and gram-negative bacteria and was observed to block ultraviolet light. It has been proven that its physical properties are close to the values stated in the literature for these materials and that it is a potential food packaging material with a potential natural indicator feature, with the color change that accompanies food degradation, which is also accompanied by pH change.

DOĞAL MALZEMELERİN BİYOAKTİF BİLEŞENLERİ ÜZERİNE ANALİTİK ÇALIŞMALAR

ÖZET

Doğanın en değerli kaynaklarından olan bitkiler, tarihi çağlardan itibaren yalnızca yiyecek olarak tüketilmemiş, aynı zamanda iyileştirici güçlerinden yararlanılmış ve tedavi edici ürünler olarak da öne çıkmıştır. Bazı bitkilerin sahip olduğu farklı iyileştirici özellikleri zamanla ve insanoğlunun artan tecrübesiyle keşfedilmiş ve kaydedilmiştir. Bu tıbbi bitkiler kalp rahatsızlıklarından cilt problemlerinin tedavisine, yüksek kolesterol ve şekerin düşürülmesine, soğuk algınlığının iyileştirilmesine kadar pek çok durumda kullanılmış ve halen de talep görmektedir. Bu bitkiler yalnızca ortaya çıktıkları coğrafyada kullanılmakla kalmamış; Dünya'nın pek çok yerine de yayılmıştır.

Bitkiler; vitamin, mineral içeriklerinin yanı sıra fenolik bileşikler, flavanoidler ve karotenoidler içerdiğinden antioksidan etkilerinin yüksek olması, antikanser etkiye sahip olmaları, besleyiciliğinin yüksek olmasından dolayı sağlıklı yaşam açısından önem kazanmıştır. Sağlıklı ve doğal ürünler olan bu bitkilerin gıda olarak tüketilmesi kadar bu ürünlerin katkısıyla yeni ürünlerin geliştirilmesi son yıllarda önem kazanmıştır. Ayrıca doğal kaynaklardan elde edilen bileşenler ile hazırlanan ilaçların Dünya Sağlık Örgütü (DSÖ) tarafından da teşvik edilmesi ve her geçen gün bu ilaç etken maddelerinin sayısının artışı ile bu kaynakların içeriklerinin aydınlatılmasına dair yapılan araştırmalar da ön plana çıkmıştır.

Türkiye; farklı iklim ve ekolojik koşulları barındırdığından, zengin bir bitki florasına sahiptir. Özellikle ülkemizde yetişen bazı yerel bitkisel kaynakların çeşitli bölümlerindeki biyoaktif bileşen içeriklerinin belirlenmesi ve bu bileşen miktarları ile antioksidan etki gibi biyoaktif özelliklerin belirlenmesi ve arasındaki ilişkinin incelenmesi oldukça önemli bir bilgi kaynağıdır. Yetiştiği coğrafyaya özgü olan bu bitkilerdeki önemli bileşen miktarların bilinmesi, bu kaynakların ticari ürünlerin geliştirilmesinde ya da ilaç etken maddesi eldesinde kullanılmalarının önünü açmaktadır. Bir başka deyişle, bu bitkilerin ülke çapındaki ticari değeri ve ihraç değerinin artması sağlanmaktadır. Bu ürünler hakkındaki bilgilerin artması ile gıda katkısı olarak kullanılmaları ile yeni gıda ile ilişkili ürün geliştirmeleri adına yapılan çalışmalar artmaktadır.

Bu iyileştirici etkilere sahip tıbbi ve aromatik bitki, bitkisel doğal ürünler ve baharatların, biyoaktif bileşen içerikleri yüksek performanslı sıvı kromatografisi gibi sıklıkla kullanılan analitik yöntemlerle belirlenmeye çalışılmaktadır. Bu yöntemlerden biri, görece daha yeni ve oldukça güçlü bir yöntem olan ve basit bir ayırma ve analiz prensibini kullanan kapiler elektroforezdir. Yüksek voltaj altında, ince kapiler kolonlara doldurulan tampon çözelti içerisinde meydana gelen yığın akışı sonucunda küçük iyonlardan büyük moleküllere kadar her bir türün ayrılmasını sağlayan bu yöntem 1980'li yıllarda bugünkü haline kavuşmuştur. Kapiler elektroforez, yüksek ayrım gücünün yanı sıra, kısa analiz süresi ve az çözelti ve örnek sarfiyatı sebebiyle çevresel sebeplerle de tercih edilmektedir.

Ayrıca son yıllarda gittikçe artan ve pek çoğu yenilebilir olan yenilikçi ve güvenli gıda ambalajı adaylarının üretilmesi ile bu alana olan ilgi de artmıştır. Gıdaların özellikle uzun süreler, bozulmadan korunması, gıdanın gözle görülebilmesi, koruyucu malzemenin antimikrobiyal özellikleri olması sıklıkla tercih edilen özellikler olduğundan bu gibi malzemelerin hazırlanması için biyopolimerler tercih edilmektedir. Biyopolimerler, biyolojik yapıda olan büyük moleküllerdir, doğada bozunur olmaları da bu amaçla plastiklerin yerine tercih edilme sebepleri arasındadır. Bu yenilikçi malzemelerin hazırlanması sırasında doğal bitkisel kaynaklar özellikle bu malzemelerin koruyucu özelliklerinin arttırılması için eklenmektedir. Farklı biyopolimerle ve farklı doğal ürünler ya da ekstraktlarıyla hazırlanan malzemelerin incelenmesi ve potansiyellerinin değerlendirilmesi üzerine çalışmalar yapılmaktadır.

Bu tez çalışmasının ilk aşamasında, tıbbi özellikleri ile pek çok rahatsızlığın tedavisinde kullanılan ve ticari formları da bulunan *Ammi* türüne ait Hatay ve Balıkesir illerinden toplanmış iki bitki olan, *Ammi visnaga* L. ve *Ammi majus* L. bitkilerinin 5 farklı bölgesinde bulunan iki önemli bileşen olan kelin ve visnagin miktarları misel elektrokinetkik kromatografi (MEKC) yöntemiyle 8.5 dakika içerisinde yüksek hassasiyetle belirlenmiştir. Kelin ve visnagin yüksüz moleküller olduğundan, bu iki bileşenin birbirinden ayrılması amacıyla kapiler kolon içerisine, kritik misel konsantrasyonu üzerinde yüzey aktif madde ilave edilerek misel yapıları oluşturulmuş ve polarite farklılarına göre ayrım gerçekleşmiştir. Ayırma şartları 20 mM borat, 20 mM SDS ve %5 (h/h) ve pH 9,6 olarak optimize edilmiştir. Çalışmada, 50 µm iç çaplı, etkin uzunluğu 50 cm olan silika kapiler kullanılmıştır. Türlerin dedeksiyonu UV dedektör ile 245 nm dalga boyunda gerçekleştirilmiştir. İnjeksiyonlar hidrodinamik yöntemle olup 50 mbar basınçta 6 saniye süreyle yapılmış olup analiz sırasında uygulanan voltaj 25 kV'tur.

A. visnaga ve *A. majus* çiçeklerinde bulunan kelin ve visnagin miktarı tayinine ait çalışmalar literatürde sınırlı sayıda mevcuttur; ancak bu çalışmanın özgün yanı Türkiye'de yetişen bu iki bitkinin kelin ve visnaginin içeriğinin bitkilerin çiçek, yaprak, kök, tohum ve saplarında belirlenmesi ve karşılaştırılması ile bitki ekstraklarına uygulananan antioksidan özelliklerin de belirlenmesi olmuştur. Lineer kalibrasyon doğruları oluşturulmuş ardından regresyon katsayıları kelin ve visnagin için sırasıyla 0,997 ve 0,998 olarak hesaplanmıştır. Uygulanan yöntem başarı ile valide edilmiş ve örnekler içerisindeki kelin ve visnagin miktarları kelin için 1,60 ile 22,60 mg g^{-1} kuru bitki aralığında, visnagin içinse 5,80 ile 18,50 mg g^{-1} kuru bitki aralığında bulunmuştur. Visnagine ise yalnızca *A. visnaga* bitkisinin çiçekleri, yaprakları ve tohumlarında rastlanmıştır. Yöntemin gözlenebilme sınırı (LOD) kelin için 0,83 mg L^{-1} visnagin için ise 0,99 mg L^{-1} olarak hesaplanmıştır. Metod validasyonu gün içi ve günler arası tekrarlanırlıklar hesaplanmış ve gerçek örnekler kullanılarak geri kazanım çalışmaları gerçekleştirilmiştir. Kelin ve visnagin için geri kazanımlar %89,7 ile %100,6 arasında bulunmuştur. Ekstraktların antioksidan gücü, 2,2-difenil-1-pikrilhidrazil radikali ile reaksiyona girme kabiliyetinin ölçüldüğü (DPPH) yöntemi ile belirlenmiş ve en yüksek etki her iki türe ait çiçeklerde bulunmuş bunu tohumlar ve yapraklar takip etmiştir.

Tez çalışmasının ikinci aşamasında, Anadolu coğrafyasının çeşitli bölgelerinden temin edilmiş *Salvia* (Adaçayı) türlerinde bulunan ve bitkinin önemli iyileştirici özellikleri ve antioksidan gücünden sorumlu iki organik asit yapısındaki rozmarinik ve karnosik asit miktarlarının tayin edilmesi için kapiler elektroforez yöntemi geliştirilmiştir. Çeşitli bölge ve ülkelerde yetişen *Salvia* içeriklerinin belirlenmesi ile ilgili çok sayıda ve ilgi gören literatür çalışmaları mevcuttur; ancak bu çalışmanın özgün yanı Anadolu coğrafyasında yetişen 14 *Salvia* türünün incelenmiş ve raporlanmış olmasıdır. Uygun ayırma şartları, 20 mM borat ve pH 9,6 olarak seçilmiştir. Ayırmalarda etkin uzunluğu 59 cm, iç çapı 50 µm silika kapiler kolon kullanılmış, analitlerin dedeksiyonu 210 nm dalga boyunda UV dedeksiyonla gerçekleştirilmiştir. İnjeksiyonlar hidrodinamik yöntemle gerçekleştirilmiş ve 50 mbar basınçta 6 sn süreyle yapılmış olup ayırma voltajı 28 kV'tur. Analiz sırasında, karnosik asit için göç süresi 4,1 dakika iken rozmarinik asit için bu süre 5,8 dakikadır. Lineer kalibrasyon doğruları, oluşturulmuş ve regresyon katsayıları rozmarinik ve karnosik asit için 0,998 olarak hesaplanmıştır. Yöntemin gözlenebilme sınırı rozmarinik asit için 1,86 mg mL^{-1} ve karnosik asit için 1,72 mg mL^{-1} olarak hesaplanmıştır. Metodun validasyonu için geri kazanım çalışmaları uygulanmış olup, değerler rozmarinik asit için %94,8 ile %101 ve karnosik asit için ise %90,2 ile %96,7 arasında bulunmuştur. *Salvia* türlerindeki rozmarinik asit içerikleri 1,08 ile 18,70 mg g^{-1} kuru bitki arasında bulunmuşken karnosik asite yalnızca bir *Salvia* türünde rastlanmış ve miktarı 11,00 mg g^{-1} kuru bitki olarak bulunmuştur. Ekstraktların antioksidan gücü 2,2-difenil-1-pikrilhidrazil radikalinin kullanıldığı, çalışılan maddenin radikalik reaksiyona girme kabiliyetinin ölçüldüğü (DPPH) yöntemi ile demir (III) iyonu indirgeyici antioksidan güç yöntemi (FRAP) kullanılarak belirlenmiştir. Toplam fenolik madde tayini içinse Follin-Ciocalteu metodu kullanılmıştır. Rozmarinik asit içeriği ile DPPH yöntemi sonuçları arasındaki korelasyon 0,77; rozmarinik asit içeriği ile FRAP yöntemi sonuçları arasındaki korelasyon ise 0,82 olarak bulunmuştur.

Tez çalışmasının üçüncü bölümünde ise, şeker içeriği yüksek olan özellikle çeşitli meyvelerin fermentasyonu ile elde edilen ve eski çağlardan beri gıdaların korunmasında ve tatlandırılmasında ilk akla gelen ürün olan sirkeler güvenli ve toksik olmayan çözücüler olarak kullanılarak kitosan bazlı toksik olmayan, biyo bozunur ve çeşitli özellikleri iyileştirilmiş malzemeler hazırlanmıştır. Doğal bir biyopolimer olan ve kabuklu deniz canlılarının kabuklarında çokça bulunan kitinin deasetillenmesi ile meydana gelen kitosan organik asitlerin seyreltik çözeltilerinde çözündüğünden asetik asit kaynağı olan sirkeler ve su içerisinde 1:1 (h/h), %1 (a/h) oranında çözülerek filmler hazırlanmıştır. Kitosan ayrıca, antimikrobiyal özelliğe sahip olması, toksik olmaması ile fiziksel ve mekanik özelliklerinin arzu edilen malzemelere göre uygunluğu ile de tercih edilmiştir. Bu çalışmada nar, elma, üzüm ve alıç meyvelerinden üretilmiş sirkeler kullanılmıştır. Bu filmlerin spektroskopik, fiziksel ve mekanik özellikleri birbirleri ile karşılaştırmalı olarak incelenmiştir. Sirke filmlerinin 200 ile 350 nm aralığında ultraviyole ışınları bloke etme güçleri olduğu gözlenmiş ve özellikle nar sirkesi ile hazırlanan filmin elastikiyetinde artış kaydedilmiştir. Yine nar sirkesi ile hazırlanan filmin, asetik asitle hazırlanan kitosan kontrol filmine kıyasla DPPH yöntemiyle belirlenen antioksidan gücünde 11 kat artış gözlenmiştir. Antimikrobiyal etkiye sahip kontrol filmine oranla nar ve alıç sirkesi ile hazırlanan filmlerin gram pozitif ve gram negatif bakterilere karşın antimikrobiyal etkisinin arttığı belirlenmiştir. Bu malzemelerin gıdaların paketlenmesi, korunması ve saklanmaları sırasında kullanılabileceği sonucuna ulaşılmıştır.

Bu tez çalışmasının dördüncü ve son bölümünde ise, Türkiye sınırları içerisinde de yetiştirilen mor reyhan (*Ocimumbasilicumpurpurascen*) bitkisinin ekstraktı kullanılarak, biyopolimer bazlı potansiyel bir gıda ambalaj malzemesi üretilmiştir. Antioksidan, antiviral ve antibakteriyal özellikleri bilinen aromatik reyhan yapraklarının, flavanoidler ile fenolik bileşenlerin yanı sıra vitaminler ve mineraller bakımından zengin olduğu bildirilmiştir; ancak bu çalışmada tercih edilmesinin başlıca sebebi mor reyhanın, güçlü mor renginden sorumlu pigmentler olan antosiyaninlerin pH'a karşı yüksek duyarlılığa sahip olmasıdır. Antosiyaninlerin, değişen pH'a karşı kimyasal yapıları değişmekte ve bunun sonucunda ise renk değişimi gözlenmektedir. Bu özellikleri sebebiyle hazırlanan malzemenin, toksik olmayan, biyo bozunur ve koruyucu olmasının yanı sıra, reyhanın renk değiştirmesi ile gıda tazeliğinin tespit edilmesinde doğal bir indikatör olarak kullanılması amaçlanmıştır. Bu amaçla hazırlanan malzemenin istenen fiziksel özelliklerinin sağlanabilmesi için, pektin ve aljinat biyopolimerleri bir arada kullanılmıştır. Pektin, bitkilerin hücre duvarından elde edilen bir karbonhidrat olup, jelleşme kabiliyeti sebebiyle gıda endüstrisinde çokça tercih edilmektedir. Kullanılan bir diğer biyopolimer sodyum aljinat ise, kahverengi alglerden elde edilir, anyonik yapıdadır ve gıda endüstrisinde sıklıkla ve benzer sebeplerle tercih edilmektedir.

Bu amaçla hazırlanan, %1 (a/h) pektin ve %0,5 (a/h) aljinatın toplam 30 mL hacimde 1:1 (h/h) suda ekstrakte edilen mor reyhan ekstraktı ve su içerisinde 5 saat karıştırılması, polimer kütlesine oranla kütlece 1:1 gliserol ilavesi ve çözülmesi ardından petri kaplarına dökülüp, kurutulmasıyla hazırlanmıştır. Elde edilen biyopolimerik filmlerin suda çözünmelerinin önüne geçilmesi için, filmler $CaCl_2$ çözeltisi ile çapraz bağlanma işlemine tabi tutulmuştur. Elde edilen malzemenin optik, spektroskopik, fiziksel özellikleri ile antioksidan ve antimikrobiyal özellikleri ile zamanla gerçekleşen antosiyaninlerin salım miktarı değerlendirilmiştir. Ek olarak bu malzemenin beklenildiği gibi, gıda bozunmasını tespit edip edemediği gerçek bir gıda örneği paketlemesi ile denenmiştir. Elde edilen malzeme oldukça yüksek antioksidan özellik göstermiş, hem gram pozitif hem de gram negatif bakteriler üzerinde antimikrobiyal gücü olduğu kanıtlanmış, ultraviyole ışığı bloke ettiği gözlenmiş; fiziksel özelliklerinin bu malzemeler için literatürde belrtilen değerlere yakın olduğu ve aynı zamanda pH değişminin de beraberinde gerçekleştiği gıda bozunmasına eşlik eden renk değişimi ile potansiyel bir doğal indikatör özelliğe sahip potansiyel bir gıda ambalaj malzemesi olduğu kanıtlanmıştır.

1. INTRODUCTION

Plants are one of the most valuable resources nature provides to humanity and have been utilized for a variety of purposes since ancient times. Being one of the best sources of nutrition, plants are also widely used for their health-promoting properties and are referred to as medicinal plants. These herbal medicines are still considered alternative treatments and support treatment all over the world (Teke & Kuete, 2014). The molecules naturally found in medicinal herbs responsible from their positive physiologic and immunologic properties are bioactive constituents (Hamzalıoğlu & Gökmen, 2024).

The plants are rich in minerals and primary and secondary metabolites, which are known as bioactive components, namely polyphenols, dietary fiber, carotenoids, organic acids, nucleotides, and vitamins (Kamiloglu et al, 2021; Samtiya et al, 2021). Bioactive constituents obtained from plants are reported to have mainly antioxidant properties, an anticancer effect on various types of cancer, an anti-diabetic effect, prevent cardiovascular diseases, maintain brain health, regulate the blood lipid profile, and also have an antiviral and anti-inflammatory effect (Samtiya et al, 2021; Dincheva et al, 2023). Especially, chemical compounds in phenolic structures are considered primary antioxidants or free radical inhibitors due to their ability to donate hydrogen ions or electrons, resulting in many other beneficial aspects of human health (Singh et al, 2016; Kim et al, 2016).

The traditional uses of medicinal herbs as remedies finally led to the development of novel drug molecules after intensive clinical research on those natural sources (Dias et al, 2012). Since the process of drug discovery is laborious, time-consuming and highly expensive and also has a huge risk of failure, during drug development studies, the compounds isolated from natural sources and their chemically synthesized forms have become promising sources. Morphine a drug molecule obtained from opium and aspirin, a commonly known anti-inflammatory agent, a great example of a compound derived from salicin obtained from *Salix alba* L. white willow bark are drug molecules in the market (Dias et al, 2012; Gurnani et al, 2014).

Following the success of the natural bioactive compounds and their derivatives as novel drugs many new drug molecules were introduced (Gurnani et al, 2014). It was reported that since 1994, almost 50% of the approved novel drugs were prepared from natural products (Katiyar et al, 2012). Lately, natural sources are especially under study for their potential in treatments of cancer (Butler, 2008; Gurnani et al, 2014).

The isolation and quantification of the compounds in plant extracts and study on their pharmacological properties have been studied, especially after the realization of their importance as potential drug candidates. Studies include several analytical techniques such as capillary electrophoresis (CE), which is an effective technique frequently employed for pharmaceutical analysis nowadays. CE offers rapid, highly sensitive and simple analysis of a wide range of analytes from small ions to large molecules.

Besides the medicinal importance and traditional uses of the natural sources and they are also good alternatives to use as a food additive to increase the nutritive value of food. In addition, novel materials such as non-toxic, and biodegradable food packaging prepared with the addition of plant extracts and natural sources with known bioactivities are getting a considerable amount of attention from consumers. Intelligent and safer materials from edible and biodegradable sources are under study (Sun et al, 2017 ; Rambabu et al, 2019 ; Sani et al 2021).

The main purpose of this thesis study was to determine the amount of bioactive substances in plants grown in our country which was used in traditional medicine and to determine the antioxidant properties of the extracts with several methods. Antioxidant tests were used to establish a correlation between the amounts of active substances in the plants and their bioactivities. In this way, the bioactive substances and bioactivities of different plant families and different species of the same family were elucidated. Additionally, due to the ascending interest in using renewable sources instead of large plastic consumption and waste accumulation, biodegradable and non-toxic food packaging materials from biopolymers with the addition of some extracts and natural products were prepared and their physical and bioactive properties were evaluated comparatively.

In the first part of this thesis, two main bioactive components in two plant species from *Apiaceae* family were investigated with the CE method. The amounts of khellin and visnagin in five different organs of *Ammi visnaga* L. and *Ammi majus* L. plants were studied and the amounts were compared for the first time in our study. The plant parts were also compared for their antioxidative abilities. The plants are both well-known for their health benefits and have traditionally been used for a long time and their trade values were revealed.

In the second part of the thesis, a successful CE method was developed for the determination and quantification of the rosmarinic and carnosic amounts in 14 different *Salvia* species collected from Anatolia including endemic species. The on capillary UV detection was performed and the method was validated. The important bioactive components in each plant species were reported and also the correlation between their amounts in the species and the antioxidant properties and total phenolic contents were investigated and evaluated. There are several studies including rosmarinic acid quantification (Jordán et al, 2012 ; Koşar et al, 2011 ; Li et al, 2019), however, this study covered the investigation of a large number of Anatolian *Salvia* species and their bioactivities.

The third part of the thesis was focussed on the use of five different vinegar varieties and the preparation of safe and non-toxic biopolymeric material with enhanced properties for potential food packaging. Natural sources usually employed for the preparation of that kind of materials. The developed materials were comparatively investigated and their different tensile, optical, spectroscopical, and physical properties and bioactivities namely antioxidant and antimicrobial activities were comparatively evaluated.

The fourth and last part of the thesis was aimed at preparing an intelligent biopolymeric material using a natural purple basil (*Ocimumbasilicumpurpurascen*) extract in order to monitor real-time food freshness via the color change in the material due to the strong color sensitivity of the anthocyanins present in purple basil. In the literature, pectin and sodium alginate biopolymer blends were not frequently studied. Alginate-pectin composite films with purple basil extract were developed and the indicator property of the material was proved for the first time.

The increased antioxidant and antibacterial properties of the film with the addition of natural plant extract were evaluated. Also, the spectroscopic and physical properties of the prepared film were comperatively investigated with the film prepared without extract.

2. GENERAL SCIENTIFIC INFORMATION

2.1 Capillary Electrophoresis

Capillary electrophoresis (CE) is a relatively new analytical technique that was introduced around the 1980s and is used for both the separation and quantification of a wide range of analytes. CE offers separation of analytes of different sizes, from very small ions to large molecules such as DNA, as well as charged or uncharged, chiral or achiral compounds. CE analysis is performed in small capillaries under high voltage, which offers fast analysis, diminution of sample and solvent volume consumption, and high resolution and sensitivity. The advantages of using thin columns made it easier to analyze many analytes and CE has been widely used in food analysis, clinical, pharmaceutical and environmental studies (Whatley, 2001).

2.1.1 Historical background of capillary electrophoresis

Electrophoresis, the basis of CE, is simply defined as the separation of charged particles as a result of their differential movement under the influence of an electrical field. The 1948 Nobel Prize winner Swedish biochemist Arne Tiselius first introduced the separation technique in 1937. He studied serum proteins and observed that the proteins in the sample moved in a certain direction and speed under the electric field resulting from the direct current applied to the sample mixture added into a tube filled with buffer solution. The electrophoresis technique has been used for many years to analyze proteins and similar molecules. (Li, 1992).

In 1967, Hijerten successfully separated a group of analytes in a 3 mm wide glass tube filled with gel. The widths of the tubes used here are in the millimeter range; however, over time, this width has been expressed in μm. In 1979, Mikkers and colleagues performed electrophoresis in polymeric capillary tubes. The biggest disadvantage of the applied method was the determination of analytes at low concentrations, and this problem paved the way for the development of modern capillary electrophoresis.

The first and highly approved introduction of capillary electrophoresis was performed by Jorgenson and Lukacs in 1981. In the method, meter-long fused silica capillaries with an inner diameter of 75 μm were used and fluorescent amino acids and peptides were separated. They also clarify the relationship between the power of separation, that is, the resolution, and the conditions of the system; and also explain the theory of the capillary electrophoresis method. Then, the commercial CE instrument became available not until the late 1980s and afterwards rapidly gained popularity and today it is still used in routine analytical procedures (Li, 1992).

2.1.2 Advantages of capillary electrophoresis over electrophoresis

One of the two different methods used in electrophoretic separation is gel electrophoresis and the other is CE. Although gel electrophoresis has been the classical method used for the separation of high molecular weight molecules for years, it is still preferred today with different support media. CE, has gained popularity in recent years and is an instrumental version of electrophoresis, has replaced gel electrophoresis in most cases with many advantages which is listed in Table 2.1.

Table 2.1 Comparison of gel electrophoresis and capillary electrophoresis.

Gel Electrophoresis	Capillary Electrophoresis
Low voltage applicability	High voltage applicability
Longer analysis time	Shorter analysis time
A large amount of sample and solvent consumption	Diminution of the sample and solvent volume consumption
Manual procedure	Instrumentation
A limited number of analytes	A wide range of analytes
Variable conditions	Optimization of the analysis conditions

2.1.3 Theory of capillary electrophoresis

2.1.3.1 Electrophoretic mobility

The heart of the separation in electrophoresis depends on the movement of the sample ions at different speeds under an electrical field as a result of an applied potential. Ionic species move from one electrode to the other, placed at both ends migrate at their own speeds. There are two factors that affect the migration speed of a charged particle. The first of these is the intensity of the electric field, E (Vcm^{-1}), and the other is the electrophoretic mobility, also known as mobility, μ_e ($cm^2V^{-1}s^{-1}$). The product of these two terms gives the migration velocity of the relevant ion (Li, 1992 ; Dziubakiewicz and Buszewski, 2013).

$$\nu = \mu_e E \tag{2.1}$$

$$\nu = \mu_e \frac{V}{L} \tag{2.2}$$

Where ν is the migration velocity, μ_e is the electrophoretic mobility, E is the electrical field and the, V is the running voltage, and L represents the length of the separation capillary. As expressed in the equation, the intensity of the electric field is directly proportional to the applied potential and inversely proportional to the length of the column. The migration velocity of ions increases with voltage.

The electrophoretic mobility of a charged species is constant under specified conditions and is a characteristic feature of ions (Dziubakiewicz and Buszewski, 2013). The electrophoretic mobility of all ionic species is proportional to the electrical force acting on them and the friction force in the environment, and when these two forces balance each other, the electrophoretic mobility can be calculated.

$$\mu_e \approx \frac{\text{Electrical Force } (F_e)}{\text{Frictional Force } (F_f)} \tag{2.3}$$

Electrical Force,

$$F_e = qE \tag{2.4}$$

Frictional force of an ion according to the Stokes' law for a spherical particle represent as,

$$F_f = -6\Pi\eta r\nu \tag{2.5}$$

Where, q is the ionic charge, η is the viscosity of the solution, r is the ionic radius, and ν is the migration velocity.

During electrophoresis, these two forces are equal and opposite to each other.

$$F_e = -F_f \tag{2.6}$$

$$qE = 6\Pi\eta r\nu \tag{2.7}$$

If equation 2.1 substituted and solved instead of velocity in this equation, electrophoretic mobility is obtained.

$$\mu_e = \frac{q}{6\Pi\eta r} \tag{2.8}$$

According to the equation, the electrophoretic mobility of a particle is directly proportional to its charge and inversely proportional to its radius. While highly charged particles with a small radius have higher mobility and move faster, particles with a larger radius and lower charges move slowly due to their low mobility.

2.1.3.2 Electroosmotic flow (EOF)

The basis of analysis and separation performed by capillary electrophoresis depends on electroosmosis. The bulk flow of the electrolyte solution along the capillary is called electroosmotic flow. This flow occurs as a result of high voltage applied to the electrolyte solution and causes all ionic species in the column to move in the same direction.

The ionization of the silanol groups on the inner surface of the fused silica capillary column causes an excess negative charge at the interphase with the aqueous electrolytic solution (Marina et al, 2005). The ionization of the silanol groups depends on the pH value of the background electrolyte (BGE) solution filled in capillary and stated as following reaction equation (Dziubakiewicz and Buszewski, 2013). When the pH value of the BGE solution is 3 or above, the inner surface of the capillary becomes negatively charged due to the ionization.

$$SiOH(s) \rightarrow SiO^{-}(s) + H^{+}(aq)$$

Owing to the negatively charged surface of the capillary wall, the ions with opposite charges are attracted to the wall forming an electrical double layer by a stationary coat known as the Stern layer and a weakly bounded mobile layer called Gouy-Chapman (Dziubakiewicz and Buszewski, 2013 ; Marina et al, 2005). Under the influence of the electrical field caused by the applied high voltage to the column, positive charges that are solvated move rapidly towards the cathode, dragging the solvent with them and creating strong bulk flow. The creation of the electrical double layer and the electroosmotic flow are represented in Figure 2.1.

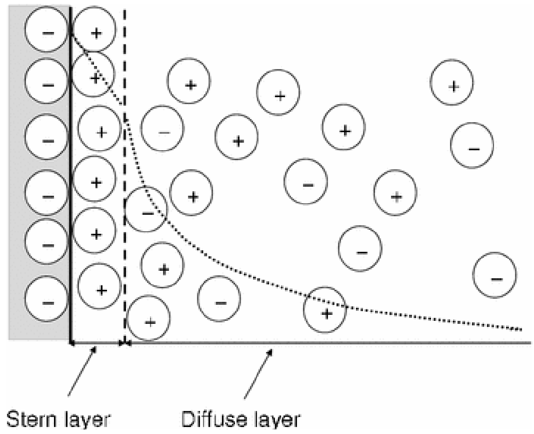

Figure 2.1 : Electrical double layer formed in capillary column (Dziubakiewicz and Buszewski, 2013).

EOF mobility and velocity can be calculated by using some equations, including using the potential occurring at the boundary of two layers, defined as zeta potential (ζ). ζ depends on the pH of the electrolyte solution which directly affects the charge density on the inner wall of the column.

$$\zeta = \frac{4\Pi\eta\mu_{eof}}{\varepsilon_r} \tag{2.9}$$

Here, η represents the viscosity of the solution, μ_{eof} is electroosmotic flow mobility and ε_r is the dielectric constant of the solution respectively. The velocity of EOF (ν_{eof}) can be calculated as follows:

$$\nu_{eof} = \frac{\varepsilon_r \zeta E}{4\Pi\eta} \tag{2.10}$$

Where E represents the strength of the electric field during analysis. Another value for describing electroosmotic flow is μ_{eof} which is expressed as:

$$\mu_{eof} = \frac{\nu_{eof}}{\varepsilon_r} \tag{2.11}$$

Following the substitution of equation 2.10 in equation 2.11 the μ_{eof} express as:

$$\mu_{eof} = \frac{\varepsilon_r \zeta}{4\Pi\eta} \tag{2.12}$$

The main difference between the pressure driven separations, as in liquid chromatography and CE is flow profiles. The pressure driven systems, the flow has a parabolic profile throughout the column, where the flow rate is higher in the middle parts of the column, it drops to almost zero where it approaches the stationary phase, causing the pressure to drop significantly throughout the column. On the other hand, in CE since an electric field is used instead of pressure, the flow progresses regularly throughout the column. It does not show any change along the column as there is no pressure change. Therefore, in CE the separation efficiency is high and the peaks obtained are quite sharp. The flow profiles are provided in Figure 2.2.

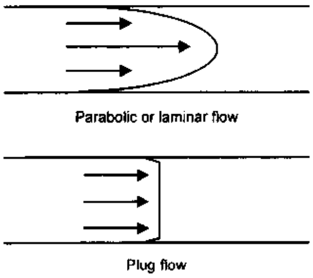

Figure 2.2 : Difference between electroosmotic and laminar flow profiles (Whatley, 2001).

2.1.3.3 Factors affecting electroosmotic flow

a) pH value of the electrolyte solution

As explained in the previous section, the ionization degree of the silanol groups at the inner side of the capillary wall strongly depends on the pH of the buffer solution. As the silanol groups are completely ionized at pH values from 3 to 9, the charge density on the inner wall of the capillary increases, resulting in a high zeta potential and greater electrical double layer. Therefore, electroosmotic flow mobility increases and EOF accelerates, and the time for analytes to reach the detector shortens. On the other hand, pH values below 2 will not be able to ionize the capillary wall, leaving the silica surface neutral. (Marina et al, 2005).

b) Concentration of the electrolyte solution

The increase in buffer solution concentration, or other words, ionic strength, causes the EOF to slow down and thus, reach the detector late. The increase in ionic strength in the capillaries creates a phenomenon called Joule heating in the capillary column. As a result, the system tries to reduce the temperature and causes the flow to slow down (Landers, 2007).

c) Temperature

Temperature is one of the key parameters in CE analysis, especially the repeatability of the analysis. An increase in the temperature decreases the viscosity of the water

(Jorgenson and Lukacs, 1985 ; Lechnou, 2008). As a result of the decrease in the viscosity of the electrolyte solution, the electroosmotic flow rate increases and the analysis time decreases, but may decrease the efficiency. Furthermore, an uncontrolled increase in the temperature produces Joule heating can cause the central zone in the column to migrate faster than the zones near the wall, creating a variation in the migration rates (Dziubakiewicz and Buszewski, 2013). Thus, Joule heating causes more broadened peak shapes. Temperature must be controlled and optimized during operation.

d) Electric field strength

Any change in voltage primarily affects the electric field and therefore the EOF. As the voltage increases, the electric field will increase, so the EOF rate also will increase. Therefore, the time it takes for analytes to reach the detector will be shortened and the resolution will be improved (Landers, 2007). However, the voltage should be increased in some extent in order to prevent the formation of Joule heating (Landers, 2007). In order to prevent the changes in EOF rate during analysis, the voltage is required to remain constant.

e) Addition of an organic solvent

The addition of organic solvents to the buffer solution affects mainly viscosity and zeta potential. It generally causes a decrease in the current in the capillary column, causing a slowdown in the EOF rate. It is known that with the addition of acetonitrile or methanol, viscosity increases and EOF decreases.

f) Addition of surfactants

Surfactant addition in buffer composition is to increase, decrease or completely reverse EOF. With the use of anionic surfactants, the EOF rate increases as the negative charge density on the inner wall of the capillary increases, whereas with the addition of cationic surfactants, the EOF rate can be reduced by reducing the amount of negative charge on the wall. Using high concentrations of cationic surfactants, the wall can be made positively charged with ionic and hydrophobic interactions and the EOF direction can be switched from cathode to anode.

g) Addition of polymeric substances

The rate of EOF can be modified by adding hydrophobic or hydrophilic polymeric substances to the buffer. It can be reduced or increased by using charged polymers, and the EOF rate can be reduced and reversed with cationic polymers. Neutral polymers, on the other hand, can significantly slow down or even stop the EOF. These substances are used for coating the inner wall of the capillary for changing its nature; such as polyethyleneimine (PEI) and polyvinyl alcohol (PVA) (Dziubakiewicz and Buszewski, 2013).

2.1.4 Separation modes of capillary electrophoresis

CE is not only used for the separation of charged species and includes the analysis of many different compounds. Different modes, differing in their separation mechanisms, have been developed. For instance, while micellar electrokinetic chromatography is used to separate neutral molecules from each other, in gel electrophoresis, molecules are separated according to size differences by passing through pores. Table 2.2 below shows the applied methods and separation mechanisms in CE analysis.

Table 2.2: Modes and separation mechanisms in CE (Whatley, 2001).

CE Mode	Mechanism of Separation
Capillary Zone Electrophoresis (CZE)	Separation of charged particles according to charge/mass in solution.
Micellar Electrokinetic Chromatography (MEKC)	Difference in hydrophobic/hydrophilic interactions with micelles formed by surfactants added above the critical micelle concentration.
Capillary Gel Electrophoresis (CGE)	Separation of macromolecules in a gel environment according to size and shape differences.
Capillary Isotachophoresis (CITP)	Separation of analytes according to their charge/size ratio between two buffer solutions with different conductivities.
Capillary Isoelectric Focussing (CIEF)	Separation of proteins according to their isoelectric points by gradual pH change in the capillary.
Capillary Electrochromatography (CEC)	Distribution of non-ionic species between the stationary phase placed in the capillary column and the stationary and mobile phase.

2.1.4.1 Capillary zone electrophoresis (CZE)

CZE is the simplest mode in CE analysis where the charged particles are separated into specific zones, in a capillary column filled with a buffer solution, under the influence of high voltage. All the species are separated from each other according to their charge/mass ratios. CZE is frequently used, due to its simplicity, ease of adaptability, and efficiency during analysis (Marina et al, 2005). The separation principle of CZE completely reflects the principle of CE. Buffer solution composition and separation conditions are the same throughout the capillary column; separation of analytes from each other is achieved by changing parameters such as pH and buffer concentration and can be carried out in an aqueous or non-aqueous buffer solution environment.

In the mode of CZE, the charged analytes and the analytes that become charged can be separated, while neutral analytes cannot be separated. CZE is frequently preferred especially in pharmaceutical and food analysis; because most components are mostly polar due to the amino, carboxyl or hydroxyl groups present in their structure. The basic CZE mode is represented in Figure 2.3.

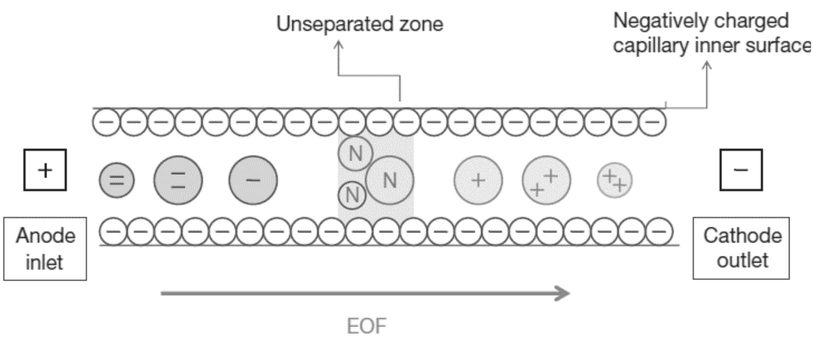

Figure 2.3: Separation of charged species in CZE (Shallan et al, 2013).

2.1.4.2 Micellar electrokinetic chromatography (MEKC)

Following the simplest mode, CZE, MEKC is also frequently used in CE separations. Since nonionic species cannot be separated by CZE only using electrolytic buffer solution filled capillary, MEKC developed. MEKC was first introduced in 1984 by Terabe and colleagues (Garcia et al, 2005). Unlike the CZE in MEKC, the buffer solution contains surfactant molecules. Surfactants are molecules both containing hydrophilic and hydrophobic parts. In order to separate nonionic molecules or increase selectivity between ionic species, surfactants form spherical structures called micelles as a result of the aggregation in the buffer. In MEKC, different kinds of surfactant molecules above their critical micelle concentrations (CMC) form micelles which work as an additional phase in the column like chromatographic techniques (Garcia et al, 2005 ; Shallan et al, 2013 ; Kalaycıoğlu ans Erim, 2022). Mostly anionic surfactants are used especially, sodium dodecyl sulfate (SDS); but also, different ionic or neutral surfactants or charged additives can be used (Altira, 1996). Analytes interact with the micelles depending on their differences in hydrophilicity and differentiate in migration times and separate from each other. The separation principle is simple, like reverse phase HPLC, micelles turn the capillary column to a chromatographic column (Altira, 1996). With MEKC mode, a wide range of analytes can be separated such as aromatic compounds, nucleic acids, vitamins, and pharmaceutical compounds which proved to be separated faster than the HPLC technique (Garcia et al, 2005). The separation principle in MEKC mode is given in Figure 2.4.

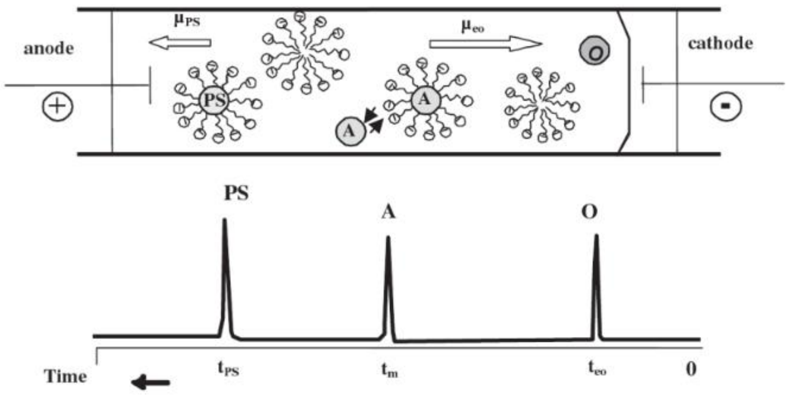

Figure 2.4 : Separation of species in MEKC (Garcia et al, 2005). PS: Pseudo micelle phase, A: neutral hydrophobic species and O: neutral small molecule.

2.1.5 Electrophoretic mobilities and migration times of analytes

In CE analysis, absence of stationary phase in the column, resulting no retention of the analytes. Thus, arrival times of the analytes from injection end to the detector at the detection end stated as migration time (t_m). Under the influence of electroosmotic flow, all ionic species injected from the anodic end moves towards the cathodic end where detector is placed and detected in a certain order. EOF is a bulk flow and because of it all particles move to the same direction in the column; positive, neutral and negative particles have different migration times, due to the difference in their velocities (Shallan et al, 2013). The observed (apparent) mobilities (μ_{obs}) of the species are their net mobilities in the presence of EOF (Shallan et al, 2013).

Since the direction of movement of cations is towards the cathode, their own mobility contributes to electroosmotic mobility. For this reason, cations become the fastest moving species and are detected first. Since neutral species do not have their own electroosmotic mobility, they move with the same mobility as the electroosmotic flow and reach the detector at the same time. Since negatively charged ions will want to move towards the positive anode instead of the cathode direction, their electrophoretic mobility is oppoite to the EOF mobility accordingly. For this reason, anions become the slowest moving species and the last to leave the column. Below are the relationships between visible mobility and speed of species and figure 2.5 represents the movements of the ions in the column.

$$\mu_{obs} = \mu_e \pm \mu_{eof} \tag{2.13}$$

$$v_{+} = (\mu_{eof} + \mu_{e\,+})E \tag{2.14}$$

$$v_0 = (\mu_{eof})E \tag{2.15}$$

$$v_{-} = (\mu_{eof} - \mu_{e\,-})E \tag{2.16}$$

where, μ_{obs} is the observed mobility, ν is the velocity of the species and μ_e represents the electrophoretic mobilities of the species.

The order of the species with the same charge is in accordance with Equation 2.8 . The charge/size ratio affects the magnitude of the electrophoretic mobility of ions. A cation with a small diameter with high electrophoretic mobility will move faster, whereas in the opposite case, a negatively charged ion is willing to move in the opposite direction, causing its apparent mobility to be low.

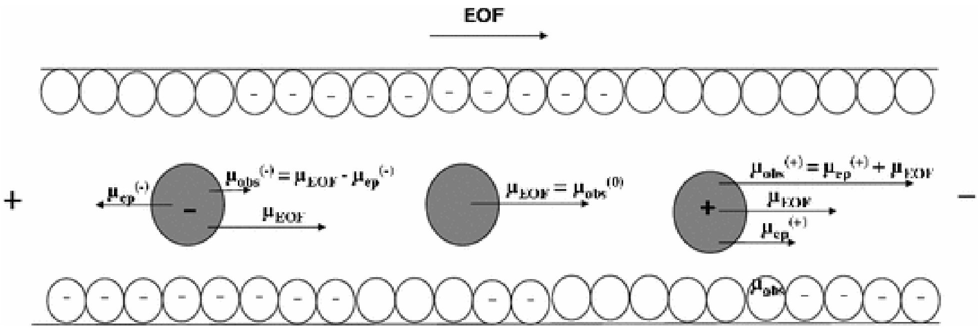

Figure 2.5 : Order of the separated species in capillary (Dziubakiewicz and Buszewski, 2013).

In order to calculate the tm and μ_{obs} of the analytes, some experimental parameters can be used. Migration time is simply related to the distance and the velocity as follows.

$$t_m = \frac{L_d}{v} \tag{2.17}$$

where, L_d is the length of the column from injection to the detector which is also called effective length. Thus, μ_{obs} can be calculated:

$$\mu_{obs} = \frac{L_d}{t_m E} = \frac{L_d L_{tot}}{t_m V} \tag{2.18}$$

E, is the electrical field strength, Ltot is the total length of the capillary and V is the running voltage.

2.1.6 Instrumentation

Modern CE instruments are simply constructed systems available in the market (Lele et al, 2001). CE instrument consists of some main components including anode and cathode electrodes, sample and inlet and outlet buffer vials, a capillary column where separation occurs, a high voltage power supply and a detection system. Construction of a CE instrument does not require high levels of expertise; however, the control over temperature must be handled in order to develop methods and perform separations (Lele et al, 2001). The sample introduction is usually performed via anodic side hydrodynamically or electrokinetically and analytes are separated in the column under the influence of an electrical field and detected from a detector placed on the cathodic side. The modern CE instrument is given in Figure 2.6.

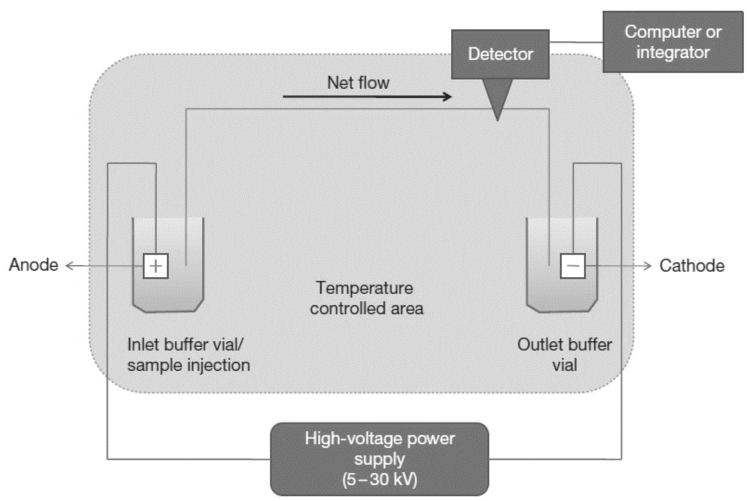

Figure 2.6 : A scheme of a modern CE instrument (Shallan et al, 2013).

2.1.6.1 High voltage power supply

The necessary electric field within the capillary is generated by a power supply. A high-voltage power supply can generate voltages of 5 to 30 kV which creates up to 60 kV/m electric field strength (Kuhn and Hoffstetter-Kuhn, 2013 ; Fekete and Schmitt-Kopplin, 2007). Since high voltages are used during analysis, it is important that the power supply has the necessary safety mechanisms and offers programming in case of the need for switching polarities (Marina et al, 2005). Also, the power source must be very stable and the voltage must remain constant throughout the analysis for the repeatability of migration times. Apart from voltage, it is also possible to work with constant current or constant power, and especially working with constant current can be preferred in cases where there is no thermostat since solution viscosity depends on temperature.

2.1.6.2 Capillary column

As the capillary column is crucial in CE separation, various types of capillaries were employed. Several materials such as fused silica, glass and Teflon were used for the construction of columns. Among these materials, fused silica was the most preferred material due to its many advantages, such as ease of production, low impurities and costs and optical transparency to UV radiation (Marina et al, 2005 ; Shallan et al, 2013).

Fused silica columns, with polyimide coating which makes them easy to handle and more flexible, are widely in use. The capillary column between 50 to 100 cm in length with inner diameters of 25 μm, 50 μm and 75 μm are frequently preferred in cylindrical shapes (Marina et al, 2005). Even, smaller diameters provide shorter analysis time and increase efficiency, however sensitivity decreases. Also, the shorter capillaries decrease analysis time; but the increase in field strength should be controlled. Prior to the first use of the capillary columns, activation of the silanol groups and formation of EOF ensures with the washing of the capillary for 30 minutes with 1 M NaOH, water and buffer respectively. As long as necessary, the capillary column should be regenerated with washings between runs, with 0.1 M NaOH, water and buffer respectively.

2.1.6.3 Sample injection systems

The sample was introduced into the capillary column in a narrow band. One of the advantages of CE which provides high resolution due to the very small amount of sample injection. Sample injection into the column is achieved in two main different ways.

a) Electrokinetic Injection

A sample may be introduced into the capillary column by using the same and simple principle as electrophoresis (Breadmore, 2009). Applying potential to the sample-containing solution for few a seconds after exchanging the buffer reservoir with the sample reservoir is the method of application of electrokinetic injection (Camilleri 1997 ; Shallan et al, 2013). The applied voltage is usually lower than the separation voltage and applied only for shorter periods. Thus, the electric field strength is low as the time of applied voltage ensures that the amount of sample injected is quite small.

In this injection method, analytes are introduced to the capillary in a narrow zone, however the amount of the analytes in the sample enters in capillary according to EOF, and also their mobilities. If the mobilities are different between the analytes, a non-homogeneous sample mixture containing more of the ions with higher mobilities and fewer amount of the ions with low mobilities, will be introduced into the column. In this case, it is necessary to use internal standards for more accurate results. Since the composition of the sample in the sample container may change after each injection, the solution must be prepared again before being injected into the device. The repeatability of this method is lower than hydrodynamic injection. The amount of sample given by electrokinetic injection depends on the applied voltage, time and capillary length as in the formula below (Altira, 1996 ; Breadmore, 2009).

$$Q = \frac{(\mu_e + \mu_{eof}) V \Pi r^2 C t}{L} \tag{2.19}$$

where, Q is the amount of sample introduced, μe and μ_{eof} are the mobilities of the ionic specie and EOF respectively, V is the applied voltage, C is the concentration of the sample, t is the time of voltage applied, r is the capillary radius and L, is the length of the capillary.

b) Hydrodynamic injection

In hydrodynamic injection, the sample introduction is achieved by applying pressure or vacuum-as negative pressure to the end of the capillary to obtain a difference in pressure between the vials in which capillary column placed (Altira, 1996). However, another simple technique was also practiced for sample introduction. Elevating the inlet vial above from the outlet and creating siphoning of the sample was also practiced; but this technique allows the introduction only a small amount of sample which is not suitable for viscous electrolytes (Altira, 1996).

Hydrodynamic injection with applying pressure is widely used for sample introduction in commercial instruments. The amount of sample introduced to the column is generally around 1 to 3% volume of the capillary for preventing the broadening of the peaks (Altira, 1996). The volume of the sample introduced (*Vinj*) via the hydrodynamic approach can be calculated as the following equation:

$$V_{inj} = \frac{\Delta p \Pi {r_d}^4 t}{8 \eta L} \qquad (2.20)$$

where, Δp is the pressure difference, rd is the inner diameter of the column, t corresponds to the injection time, L is the total length of the capillary column and η is the viscosity of the buffer solution. Representation of different injection methods are given in Figure 2.7.

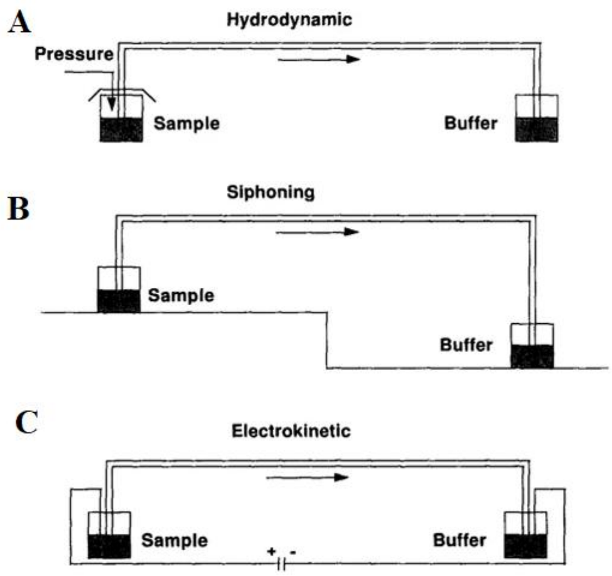

Figure 2.7 : Injection methods used in CE instruments (Altira, 1996).

2.1.6.4 Detector

Highly sensitive detectors are crucial in CE, due to the small volumes of sample injected to the columns which have very small inner diameters. Detection of the analytes can be performed either on-capillary when analytes are still in the column or end-capillary by collecting the samples from the column into the detector. On-column detections such as spectrophotometric and conductivity detection do not reduce the resolution and do not cause any distortion in peak shapes unlike end-capillary methods (Shallan et al, 2013). The widely accepted detection methods, advantages and disadvantages and also detection limits range are summarized in Table 2.3.

Table 2.3 : Most common detection methods in CE (Shallan et al, 2013).

Method	Detection Limit (M)	Advantages/Disadvantages
UV-Vis Absorption	10^{-5} to 10^{-8}	Universal, cheap, DAD offers spectral data
Laser-induced fluorescence	10^{-14} to 10^{-16}	Sensitive/Expensive and needs derivatization of the analytes
Amperometry	10^{-10} to 10^{-11}	Sensitive and selective/Only useful for electroactive species, need special requirements
Conductivity	10^{-7} to 10^{-8}	Universal and simple
Mass spectrometry	10^{-8} to 10^{-9}	Sensitive and gives information about structure
Indirect UV, fluorescence, amperometry	-	Universal and detection limits are lower than direct methods

UV-Visible absorption spectroscopy detection

Spectroscopic detection in CE depends on the investigation of the interaction between the analyte and the light. Detection is performed while analytes migrate in the column to the detector side. A window is usually created by removal of the polymeric coating of the column. The sensitivity of the detection strictly depends on the path length, which is related to the capillary diameter (Shallan et al, 2013). The widely used detection system is UV-Vis absorption detection since it is universal and applicable to many analytes. Recent instruments are mostly equipped with a diode array detector (DAD) which gives spectral information by measuring more than one wavelength (Shallan et al, 2013). Low costs and no need for any derivatization of analytes make UV-Vis detection one of the simplest detection methods.

2.2 Biopolymers

In the last decades, there has been a huge growing attention on environmental issues related to the negative impacts of the accumulation of non-degradable industrial waste and the excessive use of non-renewable sources. Synthetic polymers are becoming the main problem since they are used in great amounts worldwide, nearly 140 million tonnes in each year (Rao et al, 2014). Their extreme resistance to biodegradation and toxicity are the main reasons behind the efforts to limit in use of conventional plastics (Rao et al, 2014 ; Niaounakis, 2015). To encourage to reduction of the use of petroleum-based plastics, biopolymers become a highly promising alternative (Balart et al, 2021). Biopolymers are replacing traditional polymeric materials since they are environmentally friendly and safer and have similar properties as plastics.

The specific term of biopolymer or bioplastic, even though many other terms are wrongly confused, such as biodegradable, bio-based or compostable, is used depending on two features; biodegradability and the origin of the raw material (Niaounakis, 2015). Three different classifications can be summarized depending on the source and degradability. The first group is biopolymers produced from renewable and biodegradable materials, the second group is also produced from renewable materials, but not biodegradable, and the third and last class includes biopolymers formed from fossil fuels and biodegradable (Niaounakis, 2015). To sum up; biopolymers are polymeric structures produced from renewable sources and also biodegradable polymers obtained from biological or fossil-fuel sources. Table 2.4 represents the classification and examples of some biopolymers.

Table 2.4: Classification and examples of biopolymers (Niaounakis, 2015).

Biopolymers				
Biodegradable				**Non-biodegradable**
Biological Source			**Fossil Based**	**Biological Source**
Plants	**Animals**	**Microorganisms**		
Polysaccharides (Cellulose, Alginate, Pectin)	Polysaccharides (Chitin/Chitosan, Hyaluronan)	PHAs	Poly(alkylene dicarboxylates)	PE, PP and PVC
Monosaccharides (Starch)	Proteins (Whey, Casein, Collagen, Albumin, Keratin, Leather)	PHF	PGA	PET and PPT
Triglycerides (Lipids)		Bacterial Cellulose	PCL	PU
Proteins (Wheat, corn etc.)		Polysaccharides (Hyaluronan, Xanthan, Curdlan, Pullulan)	Poly(vinyl alcohol)	PC
Lignin		Protein (Silk)	Poly(ortho ester)	Poly(ether-esters)
Gums			Polyanhydrides	Polyamides, Polyester amides, Unsaturated polyesters
Carrageenan			PPHOS	Epoxy
PLA				Phenolic resins

2.2.1 Applications of biopolymers

Biopolymers have been using in vast number of applications since their properties are adjustable with blending different biopolymers together. Desired mechanical properties, transparencies, resistance to extrinsic factors, barrier properties could be obtained for specific purposes. Thus, they are highly in use for many different applications. Biopolymers largely studied for use in medical applications. So far, health related applications including wound dressings for closure and healing ability, implants, scaffolds in tissue engineering studies, biosensing devices working *in vivo*, some materials such as clips, staples and sutures and drug delivery approaches were reported (Van de Velde and Kiekens, 2002 ; Niaounakis, 2015). These materials are using due to their ability to degrade into non-toxic products in body over longer periods of time (Niaounakis, 2015).

Another area where biopolymers are highly in use is packaging applications. Plastics are generally used for protection of a product from environmental factors, even longer or shorter periods of time use or for single use or use for more than once (Niaounakis, 2015). The waste accumulation due to plastics, sources of raw materials and their disposal problems are promoting the design and use of materials prepared from renewable sources with biodegradable behavior. The film forming ability of some biopolymers, especially increasing the number of studies on the area. From different kinds of films to bags or nets like materials were reported so far (Van de Velde and Kiekens, 2002 ; Niaounakis, 2015). Food packaging applications are also highly under studied since safer packaging materials in contact with food are favored by consumers in last years.

Biopolymers are also using in many other fields including electronics, cosmetics, agricultural purposes, fishery and related products, manufacturing of toys and other households and sports related items, construction industry includes making cement, and lime. Biopolymers are frequently using in food industry effectively for enhancing the properties of product such as stability and emulsification (McClements, 2009).

2.2.2 Biopolymers in polysaccharide structure

Polysaccharides are very common types of biopolymers present in nature and they are consisting of long carbohydrate chains as repeating units (Gupta et al, 2022). Monosaccharides are connecting with glycosidic bonds to each other. Chitin, chitosan, pectin, alginate, cellulose, dextran, xanthan gum are the most known examples of the polysaccharides.

2.2.2.1 Chitosan

Chitosan is a well-known biopolymer derivative of chitin, which is known as the second most abundant natural polymer in nature (Honarkar and Barikani, 2009 ; Gonçalves et al, 2021). Chitin presents in exoskeletons of shrimps and other crustaceans also insects and even found in bacteria, fungi and mushrooms (Gonçalves et al, 2021 ; Kou et al, 2021). Chitosan is a chitin derivative, obtained from deacetylation of chitin under alkaline conditions with several methods. The repeating units of chitosan are D-glucosamine and N-acetyl-D-glucosamine with β-1–4 links and the chemical structure of chitin and chitosan are represented in Figure 2.8. Chitin and then chitosan in recent years, are mostly obtaining from biowastes of sea creatures (Kim, 2010). The natural source, deacetylation degree (DD) and molecular weight are the main features of the chitosan.

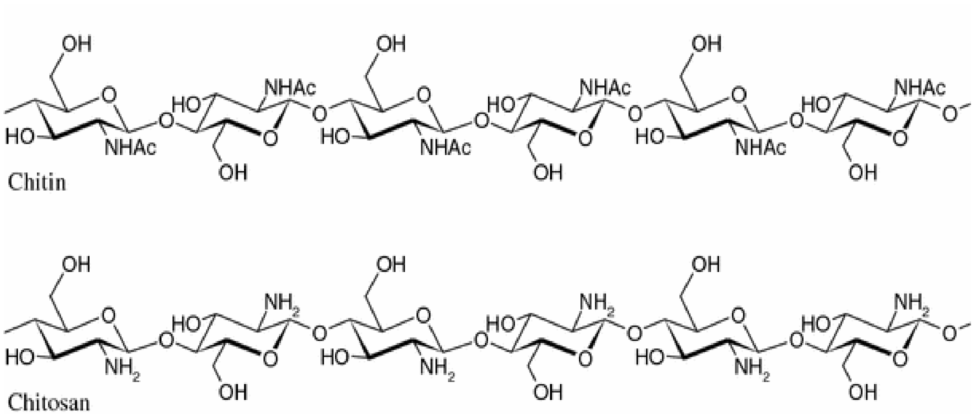

Figure 2.8 : Structures of chitin and chitosan (Honarkar and Barikani, 2009).

Chitosan is in the form of a pale yellowish colored powder. As a result of being a cationic biopolymer, it dissolves in dilute solutions of weak organic acids by protonation of the -NH_2 units (Kumar, 2020). It forms a slightly yellow viscous solution. Chitosan is usually prepared in acetic acid solution of 1% (v/v); the protonated amine groups of chitosan using acetic acid increase solubility as a result of the repulsion of charged groups (Giraldo and Rivas, 2021). The commercial chitosan differs in molecular weight, and ranges from between 50-2000 kDa and DD ranging from 50 -100% (Gonçalves et al, 2021). The molecular weight and solubility are dependent. The low molecular weight chitosan has higher solubility in acidic solutions and is mostly used (Aider, 2010). Another parameter is considered as DD, which has an effect on physical properties. The water solubility increases in high DD chitosan, which also shows greater biological actives (Kou et al, 2021).

Chitosan is highly known and worldwide popular polysaccharide type biopolymer due to its properties such as low-toxicity, biodegradability and also biocompatibility and antimicrobial and antioxidant ability (Pillai et al, 2009). Also, possibility of modification of the properties and even enhance with using other biopolymers or compounds makes the chitosan a good candidate to use in scientific and industrial purposes. Different forms of the materials can be prepared with chitosan such as membranes, films, hydrogels.

Chitosan is used as a food additive, as a preservative and gelling agent. Chitosan is often used in the preparation of edible biofilms with casting method for many food related applications, including antimicrobial food packaging materials and their properties were investigated and improved (Honarkar and Barikani, 2009 ; Rahman and Goswami, 2021 ; Zhu, 2021). Moreover, chitosan is used in the production of coatings, surface conditioners and also in biosensors (Honarkar and Barikani, 2009). Chitosan products are also used in biomedical applications, including drug delivery and preparation of materials for treatment in the form of multicarbon nanotubes, nanofibers, nanosheets, scaffolds, nanoparticles, sponge-like structures (Honarkar and Barikani, 2009 ; Kumar, 2020). Apart from medical studies, chitosan is in use for agricultural purposes as a pesticide, in the textile industry, for paper production and water treatment purposes.

2.2.2.2 Pectin

Pectin has a complicated polysaccharide structure and it is an important part of plants cell walls (Rolin, 1993 ; Thakur et al, 1997). Pectin is a structural component in plants also responsible for hydration of the plant and grows also in softer parts (Thakur et al, 1997). Many fruits contain pectin in different amounts (Thakur et al, 1997). Even though many plants have pectin in their structure, commercially available pectin is usually obtaining from apple pomace or citrus peels. The physical properties of the pectin such as gelation ability, solubility depend on the source. Pectin has been using in food and cosmetic industry due to its gelling ability as well as pharmaceutical purposes. Pectin is an edible polysaccharide and has a part in human diet; but not nutritive and mainly using in jams, ice creams and some dietary products. Pectin also has emulsifying, stabilizing and thickening abilities (Ropartz and Ralet, 2020).

The complexity of pectin is due to its different branching units and molecular weight. The main part of the commercial pectin molecule consists of linear galacturonic acid units linked with covalent bonds. Pectin and pectic biopolymers are heteropolysaccharides that have 70% or more galacturonic acid units linked at the O-1 and the O-4 positions and some of the carboxylic groups are esterified with methyl units (Rollin 1993 ; Mohnen, 2008 ; Ropartz and Ralet, 2020). The structure of the pectin molecule is given in Figure 2.9. In the figure, the arrow showing the group at O-2 position which is seldom presented in commercial pectin (Rolin, 1993).

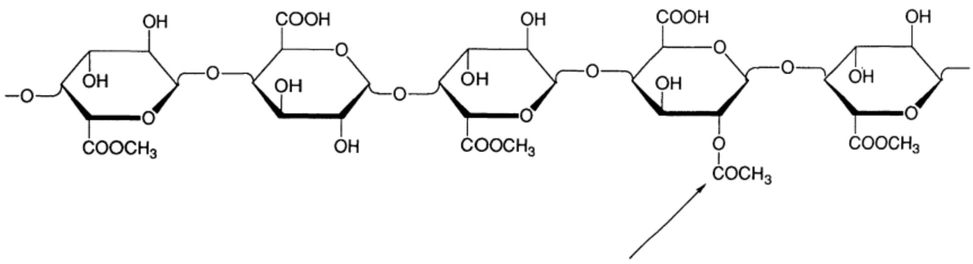

Figure 2.9 : The main structure of methyl esterified pectin molecule (Rolin, 1993).

Degree of the methoxyl esterified groups in pectin molecule, plays a role on the solubility and gel formation. Pectins can classified into two groups depend on the number of those groups. High methoxyl (HM) pectin contains 20-50% and low methoxyl (LM) pectin contains 50-80% methoxyl groups (Thakur et al, 1997). It can be said that solubility increases with the increase in the esterified groups and decrease in the molecular weight (Thakur et al, 1997). HM pectin are forms gels in the presence of sugars at low pH values, on the other hand LM pectin forms gels in the presence of Ca^{2+} ions. The gel formation in LM pectin rely on the formation of bridge structure with Ca^{2+} ions between carboxylic acid groups (Thakur et al, 1997). The egg box model structure formed with Ca^{2+} ions is represented in Figure 2.10. Recent reports suggest that also HM pectin forms gels with Ca^{2+} (Yang et al, 2013 ; Cao et al, 2020). The intrinsic and extrinsic factors affecting the gelling ability of pectin were also explained (Cao et al, 2020).

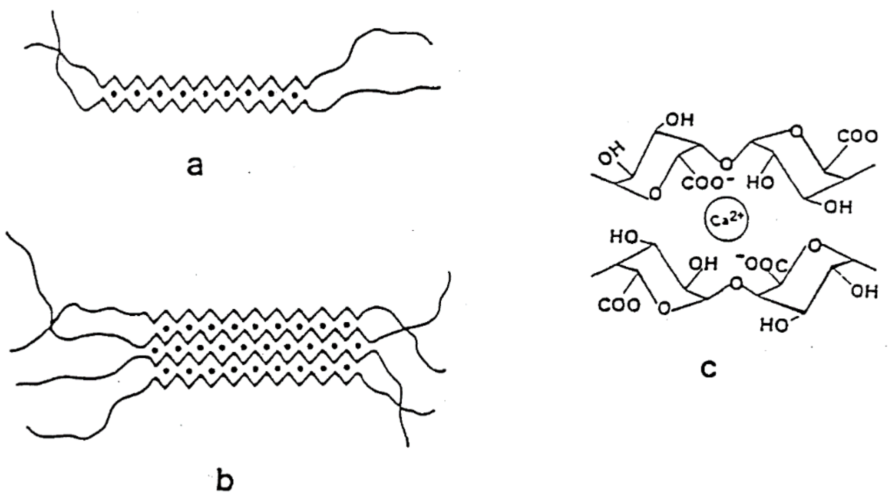

Figure 2.10 : Egg-box model representation of pectin molecule. a) dimer structure, b) dimer aggregates, c) ionic bridge (Thakur et al, 1997).

Pectin has also some positive effects reported on health including decreasing levels of cholesterol and glucose, immune response stimulation and causing apoptosis of cancer cells (Inngjerdingen et al, 2007 ; Jackson et al, 2007 ; Mohnen, 2008). The possibility of modification of the properties, pectin has been using in many other purposes and preparation of materials and devices such as implants, drug delivery agents, edible films, adhesives, foams which are some of the examples (Mohnen, 2008).

2.2.2.3 Alginate

Alginate is another well-known biopolymer in polysaccaharide structure. Alginate is biocompatible, biodegradable, low toxic, low cost and has good gelling ability (Lee and Mooney, 2012 ; Hurtado et al, 2022). It is an anionic, linear copolymer of homopolymeric units of (1–4) linked α-L-guluronic acid (G) and β-D-mannuronic acid (M) in alternating order. The physical properties were depended on the distribution of these units and the ratio of G and M units which is related with its source.

Alginate is obtained from brown seaweeds (algae) and also present in few bacteria (Raus et al, 2021). The extraction from algae usually perform with alkaline solutions and then precipitated into the salts. Molecular weights of commercially available alginates are in between 32,000 and 400,000 g/mol (Lee and Mooney, 2012). It has white to pale yellow colored appearance. Alginate is non-toxic and water soluble. The solubility strongly depends on the molecular weight and pH. Increase in the molecular weight also increases the viscosity of the solution. Similarly to pectin alginate also forms gels in the presence of divalent or trivalent cations such as Ca^{2+}. The structure of the alginate structure and the egg-box model gelation are represented in Figure 2.11. Cations are trapping between the chains during gel formation.

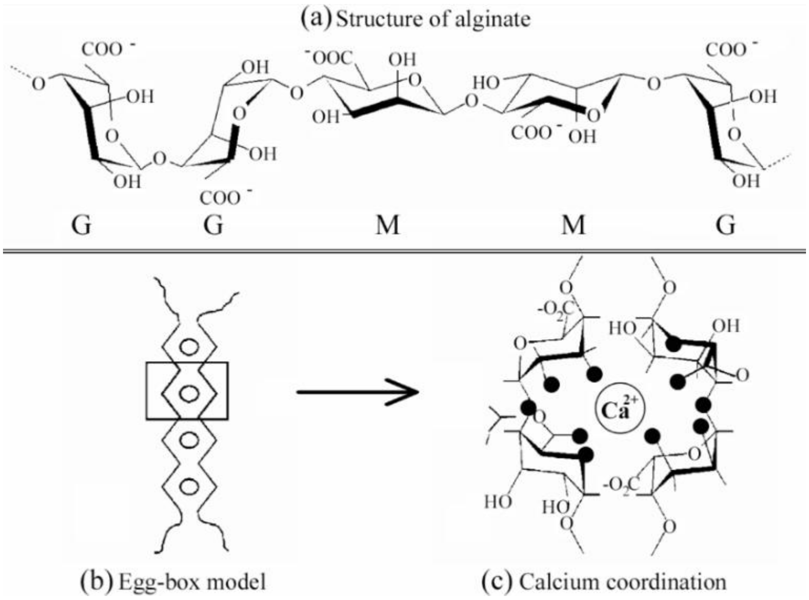

Figure 2.11 : a)The chemical structure of alginate G: Gluronic acid units, M: Mannuronic acid units, b) egg-box model, c) Ca^{2+} crosslinking (Hurtado et al, 2022).

Alginates are safe and approved for the use in food production and also biomedical, biotechnological and pharmaceutical applications. In food industry, due to their good gel forming abilities they are using in ice cream production as well as beer (Hurtado et al, 2022). Owing to their abilities, alginates are using for drug delivery, tissue engineering, production dressings for wound healing, dental molding and also as supplements (Mandal et al, 2009 ; Kaygusuz et al, 2015 ; Hurtado et al, 2022 ; Kostenko et al, 2022). Alginates are also using in many other industries such as textile, paper, construction, cosmetics and art & crafts for production of some materials such as adhesives, cleaning compounds, insulation materials and toys.

2.3 Bioactive Compounds and Their Plant Sources

2.3.1 *Ammi visnaga* L. and *Ammi majus* L.

The species known as *Ammi* comes from the family of *Apiaceae (Umbelliferae)* commonly known as the parsley family (Hashim et al, 2014). The Parsley family includes carrot, parsnip, celery as vegetables and some herbs such as cumin, anise, coriander and have about 455 genera and over 3000 species (Hashim et al, 2014). They are cultivated largely in many different areas in Europe, Asia and Africa and lately in America. *Ammi* species are herbal plants and have great value as well as their natural products. These are rich in bioactive components especially in coumarins and flavonoids. *Ammi visnaga L. (A. visnaga)* and *Ammi majus L. (A.*

majus) are two highly investigated *Ammi* species due to their rich chemical constituents and known for their positive health effects. In Figure 2.12 *A. visnaga* and *A. majus* were shown.

Figure 2.12 : *A.visnaga* (left) and *A. majus* (right) plants (Hashim et al, 2014 ; Hossain and Touby 2020).

A. visnaga is also known by some other names: bisnaga, Toothpick weed, Bishop's weed in English and khella in Arabic, disotu, hiltan in Turkish (Günaydın and Beyazıt, 2004 ; Khalil et al, 2020). *A. visnaga* is a known medicinal plant that grows about 1-meter in height (Khalil et al, 2020). It originated in the Mediterranean region, including the Aegean region, the South and West parts of Anatolia in Türkiye (Günaydın and Beyazıt, 2004). *A. visnaga* traditionally were used for the treatment of several medical conditions. Flowers of the plant was found to be useful for supporting the treatment of respiratory related conditions asthma and bronchitis (Günaydın and Beyazıt, 2004 ; Al-Snafi, 2013 ; Hashim et al, 2014). Also, extraction of the plant was used to treat skin conditions vitiligo and psoriasis. It was also employed as diuretic substance and used to treat vertigo, diabetes and kidney stones. Different parts including seeds were also used for treatment of aches and pains. More recently, it has been employed due to its antispasmodic effect coming from its fruits which have vasodilator activity (Günaydın and Beyazıt, 2004). The extract of the plant had also showed antimicrobial, antioxidant, antidiabetic, and anti-inflammatory activity (Hashim et al, 2014 ; Ahmed et al, 2021). Furthermore, it was reported that *A. visnaga* showed cytotoxic activity towards several cancer lines namely: cervical and breast cancer (Pakfetrat et al, 2015 ; Khalil et al, 2020). The diverse chemical constituents are present in different parts of the plant in different amounts were listed (Khalil et al, 2020 ; Ahmed et al, 2020). The region of the plant harvested, conditions while growing has an influence on the amounts of those bioactive compounds (Sellami et al, 2013). Main groups of those compounds are: γ-pyrones, coumarins, flavonoids and as minor constituents include essential oils (Al-Snafi, 2013). γ-pyrones are furanochromone derivatives and their amounts are about 4% and two major compounds known as khellin and visnagin (Al-Snafi, 2013). Some other γ-pyrones are khellinol, visammiol, khellol. The second major group called coumarins and their amount are in reported in between 0.2 to 0.5% (Al-Snafi, 2013). Primarily, γ-pyrone constituents of *A. visnaga* are responsible for the positive health effects. Two main components visnagin, and khellin are isolated and studied.

A. majus is from the same family as *A. visnaga* and is also used as herbal medicine. *A. majus* locally known as Khella shaytani, Greater Ammi or Bishop's weed Al-Snafi, 2013). Plant was first discovered in Egypt and then distributed all around the world. It grows about 1 to 2-meter height (Hossain and Touby 2020). From the ancient times different parts and forms of the plant was used as cure for some medical conditions. Aerial part was used for skin problems such as vitiligo while different forms of fruits was used for kidney and gallbladder stones, for curing infections. Also, fruits used by Egyptians after extraction for treatment of hypertension, depression and allergic symptoms and rashes also as diuretic and for leukoderma (Hossain and Touby 2020 ; Otman et al, 2023). *A. majus* is also known with beneficial effects on coronary and chronic asthma and also for its antispasmodic and antiseptic activities. The extract of plant with several solvents also reported to have some properties such as cytotoxic effects, antihyperlipidemic, antioxidant, antimicrobial, antidiabetic, anti-inflammatory, analgesic, antipyretic and wound healing activities (Koriem et al, 2012 ; Hossain and Touby 2020). The chemical composition of *A. majus* mainly consists furanocoumarins followed with imperatorin total amount of 1.9% (Al-Snafi, 2013). Aerials and flowers and fruits were also investigated in specific regions.

2.3.1.1 Khellin and visnagin

Khellin and visnagin are bioactive chemical compounds in the furanochromone structure. They are both insoluble in water, and soluble in alcohol, chloroform and acetone. Khellin and visnagin does not have any color or odor and they are stable in the solid state. The chemical structures of both compounds are given in Figure 2.13.

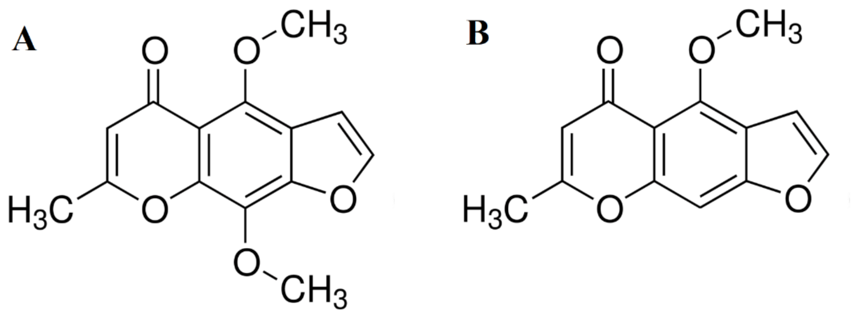

Figure 2.13 : Chemical structures of A) Khellin and B) Visnagin.

The both bioactive constituents are responsible from the reported health benefits of the *Ammi* species explained in previous section, since they are the major components in *A. visnaga* and *A. majus*. Specifically, khellin and visnagin reported to have calcium channel blocking ability, vasolidator activity to use in treatment of heart related diseases, and antispasmodic activity (Rauwald et al, 1994 ; Hashim et al, 2014 ; Duarte et al, 2000). Visnagin reported to have an effect of reducing vascular resistance and khellin increases the HDL-cholesterol (Schindler, 1953 ; Harvengt and Desager, 1983). Also, it was prooved that khellin and visnagin can prevent renal epithelial cell damage (Vanachayangkul et al, 2010). Cytotoxic activities of the khellin and visnagin were also showed against various cell lines (Beltagy and Beltagy, 2015).

2.3.2 *Salvia Officinalis* L.

Salvia Officinalis L. is a plant commonly known as sage has a great reputation and importance since early times. Sage is a medicinal and also highly aromatic plant have been using both for medical and culinary purposes. It belongs to the Lamiaceae family and originated in Mediterranean parts of the world. Sage has around 1000 different species and distributed to all over the Europe, Southeastern part of Asia and America (Jakovljević et al, 2019). *Salvia* species have wood like stems, green leaves and purple colored flowers (Miraj and Kiani, 2016). The appearance of plant *Salvia* is shown in Figure 2.14.

Figure 2.14 : The plant of *Salvia Officinalis L.*

Highly aromatic *Salvia* species are important in the kitchen; However, over time, its pharmacological positive effects have become well known. Traditional uses of *Salvia* species include treating dizziness, inflammation, seizures, tremors, paralysis, ulcers, and hyperglycemia. The widespread use of *Salvia* species as medicine has led to the investigation of the biological activities of the plant, and it has been reported that the species has anticancer, anti-inflammatory, antioxidant, antimicrobial, antimutagenic, antidementia, hypoglycemic and also hypolipidemic effects (Hernández-Saavedra et al, 2016 ; Ghorbani and Esmaeilizadeh, 2017).

Active chemical constituents in *Salvia* species were identified and reported. Salvia species are rich in phenolic compounds, alkoloids, carbohydrates, steroids, terpenes and terpene derivatives (Ghorbani and Esmaeilizadeh, 2017). Flavonoid-type compounds, including rosmarinic acid, uteolin-7-glucoside and some phenolic acids like caffeic acid, were identified in the alcoholic extracts of the species and two main bioactice components were identified as rosmarinic acid and ellagic acid as the most abundant compounds in *Salvia* species and rutin, chlorogenic acid, and quercetin were found in high amounts and biological activities of bioactive compounds have been under investigation.

2.3.2.1 Rosmarinic and carnosic acid

Rosmarinic acid (RA) and some other active ingredients are formerly known as Labiatengerbstoffe, from family of Lamiaceae. However, RA was purely isolated from and named after *Rosmarinus officinalis* (rosemary) by two chemists (Petersen and Simmonds, 2003 ; Petersen, 2013). It is an ester molecule of caffeic and 3,4-

dihydroxyphenyllactic acid (Petersen and Simmonds, 2003). Many RA derivatives and natural compounds were then identified. RA can be found in many plants and herbs using for their medicinal properties. RA can be found in subfamily of Lamiaceae called Nepetoideae, including rosemary, spearmint and lemon balm and also in some other plants from different families especially from Boraginaceae (Petersen, 2013 ; Amoah et al, 2016). The are several extraction and isolation methods for RA and they were summarized by Amoah et al. (2016).

RA and derivatives were largely investigated since the plants with RA were showed great properties. The biological and also pharmacological activities of RA and similar compounds were proved that RA have antioxidant, anti-inflammatory, anticancer, anti-allergic, antimutagen, antibacterial, antiviral and antifungal activities and were summarized in reviews (Petersen and Simmonds, 2003 ; Petersen, 2013 ; Nadeem et al, 2019). The RA was also showed that it has very low toxicity and the amount in bloodstream decreases rapidly (Petersen and Simmonds, 2003). In the light of these studies, RA have been using in cosmetic industry due to its high antioxidant ability and *Rosmarinus officinalis* and *Sanicula europaea* are using as plant sources (D'Amelio Sr, 1998).

Carnosic acid (CA) is a phenolic compound in diterpene structure. The main natural sources of CA are known as sage and rosemary. The chemical structures of RA and CA were both represented in Figure 2.15. CA is a secondary metabolite in plants and firstly discovered as carnasol as a result of the oxidation of CA, and then as CA from *Rosmarinus officinalis* L (Birtić et al, 2015 ; Bahri et al, 2016). The compound has a protective role in plants and can be transform into several derivatives in the case of excess exposure to solar UV radiation and enormous lack of water. CA works as protector against free radicals and improves cell integrity. The biologic activities of CA also under study and so far, antioxidative, antimicrobial and anti-inflammatory abilities were reported (Birtić et al, 2015). Furthermore, CA showed the ability to reduce the fat gain on mice by inhibition of triglyceride accumulation (Ninomiya et al, 2004). CA, is also reported to be unstable and transform into carnasol and rosmanol type compounds however, it was proved that it increases the stability of food and approved as additives as an antioxidant by European Commission (Birtić et al, 2015). Since both RA and CA has protective abilities, their sources are become significant products in use for food and cosmetic industry. Thus, there are several studies on determination of their amounts in plant species of *Salvia* and *Rosmarinus officinalis* L with chromatographic techniques (Hidalgo et al, 1998 ; Bonoli et al, 2003 ; Başkan et al, 2007 ; Jordán et al, 2012 ; Cao et al, 2014 ; Li et al, 2019).

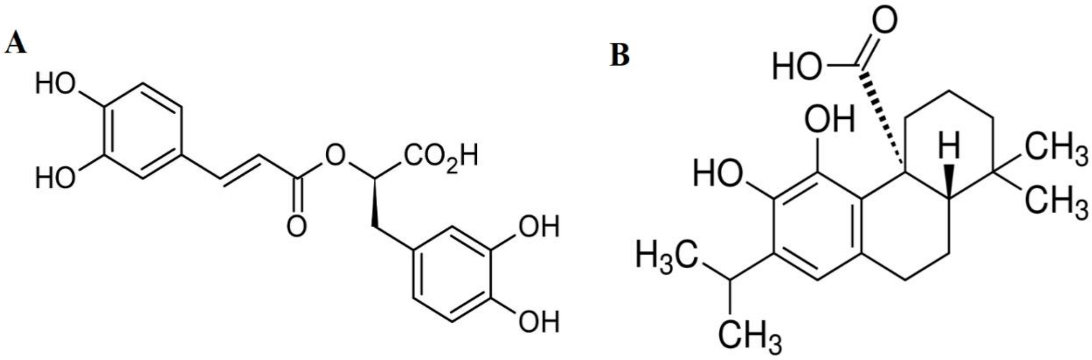

Figure 2.15 : Chemical structures of A) Rosmarinic and B) Carnosic acid.

2.3.3 Vinegar

Vinegar is a well-known fermented food product with a characteristic pungent and sour taste. The name vinegar is coming from French word 'vin aigre', translate as sour wine (Conner and Allgeier, 1976). Vinegar have been using for centuries and first records about vinegar was found about 5,000 years ago (Johnston and Gaas, 2006). In culinary, vinegar is mainly using for preservation of foods and for seasoning purposes. It is widely in use for preparation of various kinds of pickles and sauces. Following to the realization of its many positive properties today vinegar is using for cleaning and medicinal approaches.

Any natural raw material including fermentable sugars and starches can be used for vinegar production such as fruits, fruit juices, molasses, beets and malts. Vinegar produced by process known as fermentation in two steps including formation of ethanol by yeasts and then followed by oxidation of ethanol to acetic acid. Oxidation of ethanol is carried out by 2 main type of bacteria *Acetobacter* and *Gluconobacter* belonging to the *Acetobacteraceae* family (Budak et al, 2014). Thus, acetic acid is the most significant compound present in the vinegar. There are 2 main methods used in production of commercial vinegar varieties namely: surface and submerged culture fermentation (Budak et al, 2014). In surface culture technique, the oxygen presents at the surface used for the bacteria to grow, meanwhile submerged culture in order to shorten fermentation oxygen provides during the fermentation process (Garcia-Parilla et al, 1997 ; Fan et al, 2021). A study reported that the latter method causing loss of many bioactive compounds of source material compared to the surface culture method (Molelekoa et al, 2018). Furthermore, the fermentation process during vinegar production can either be slow or rapid (Fan et al, 2021). The slow process can take even years using for traditional vinegar production whereas the fast fermentation occurs within hours or days by addition of acetic acid bacteria (Fan et al, 2021). Following to the fermentation, filtration, clarification and also distillation and pasteurization steps are necessary to produce bottled vinegar (Fan et al, 2021). The general reaction mechanisms of the fermentation steps were shown in Figure 2.16.

$$C_6H_{12}O_6 \text{ (Glucose)} \xrightarrow{\text{Yeast}} 2C_2H_5OH \text{ (Ethyl Alcohol)} + 2CO_2 + \text{Energy (ATP)}$$

$$2C_2H_5OH \xrightarrow[\text{Oxidation}]{\text{Acetic Acid Bacteria}} 2CH_3CHO \text{ (Acetaldehyde)} + 2H_2$$

$$2CH_3CHO + O_2 \xrightarrow[\text{Oxidation}]{\text{Acetic Acid Bacteria}} 2CH_3COOH \text{ (Acetic Acid)} + 2H_2O$$

Figure 2.16 : The reaction mechanisms of fermentation reactions.

The acetic acid amount in vinegar varieties varies in between 5-7% (Johnston and Gaas, 2006). After reaching the desired amounts of acetic acid fermentation process is terminated. Besides acetic acid, there are many different types of organic acids such as citric, tartaric, formic and lactic present in different amounts due to the acetic acid bacteria. The table about the organic acid contents in different vinegars was given in a review (Budak et al, 2014). The ingredients and their amounts in the vinegar depends on both the choice of raw material and the process of fermentation and effects the taste (Johnston and Gaas, 2006). The selection of the raw materials is usually depending on the climate of the region and the economic concerns (Conner and Allgeier, 1976). There are many different types of vinegars such as; wine, rice, apple cider, balsamic, potato, tarragon and also vinegars prepared with different fruits including pomegranate, hawthorn, grape and apple. Thus, vinegars contain several organic acids, polyphenols, vitamins, and minerals. Local products are in use for production of specific well-known vinegar types such as balsamic vinegar producing from sugary grapes originated to Modena, Italy (Hailu et al, 2012).

Over the years, vinegars have been using for medicinal purposes. The first records on vinegar using as medicine was goes back to ancient times. The antioxidative ability of vinegars is high due to their high polyphenolic content and also the presence of melanoidins (negatively charged macromolecules) by the mechanism of free radical scavenging, chelating of the metal ions and reducing the oxidants (Chen et al, 2016 ; Kelebek et al, 2017 ; Ousaaid et al, 2021). Vinegar varieties and especially fruit vinegars fights with inflammation by decreasing the levels of cytokins (Ousaaid et al, 2021). Vinegar has also some positive effects on cardiovascular diseases. The blood pressures of the rats with hypertension were reduced with diet including vinegar regularly (Na et al, 2016). Beside antihypertensive effect, vinegar also have effects on lowering the cholesterol levels due to their polyphenolic content. (Laranjinha et al 1994 ; Budak et al, 2011). Vinegar has also ability to decrease the blood glucose levels when digested working as antidiabetic and also has antiobesity effect (Ebihara and Nakajima 1988 ; Brighenti et al, 1995 ; Budak et al, 2014). It has also been proven that vinegar has anticancer properties on leukemia cancer cells and that it inhibits proliferation of cancer cells (Samad et al, 2016). Vinegar has antimicrobial activity and especially using for protection of food and food products safely (Entani et al, 1998 ; Rhee et al, 2003). This feature also has been using as natural remedy for hair lice, nail fungus and some infections (Rutala et al, 2000).

2.3.4 Purple basil *(Ocimumbasilicumpurpurascen)*

The economical and pharmacologically valuable plant *Ocimum basilicum* L. known as basil in English, basilic in French, and reihan in Persian language (Shahrajabian et al, 2020). Basil plant is a member of the Lamiaceae family. The genus known as *Ocimum* includes 30 different species (Shahrajabian et al, 2020). Basil species differentiates in the color of the flowers, morphologic structures, and chemical compositions. Purple basil (*Ocimumbasilicumpurpurascens*) has a very unique purple colored leaves and also very strong odor.

Figure 2.17 : The leaves of *Ocimum basilicum* L. species.

Widespread distribution and highly aromatic nature of the basil plays an important role in its use for culinary purposes. Thus, basil has a great reputation in culinary use since historical times and called as “God of spices” (Dhama et al, 2023). Basil is a herb with high nutritional value, rich in vitamins including C, E, K, A, β-carotene, thiamine (B_1), riboflavin (B_2), niacin (B_3), and minerals including Ca, Fe, Mg, and Zn and also contains great amounts of essential oils, phenolic compounds, flavanoids, anthocyanins, tannins (Filip, 2017 ; Dhama et al, 2023).

Basil species are traditionally used for treatments of some disorders and after the further research on their pharmacological properties, it was proved that the species have anticancer, antifungal, antiviral, antibacterial, and antispasmodic activity (Shahrajabian et al, 2020 ; Dhama et al, 2023). Moreover, basil extracts were showed analgesic and anti-inflammatory effect by inhibiting the mediators of inflammation (Ch et al, 2015 ; Dhama et al, 2023). Alcholic, chloroform and carbon tetrachloride extracts of basil species are also showed antioxidant activity, while the water extracts of basil proved to decrease the cholesterol levels in blood and both aqueous and alcholic extracts showed immunomodulatory and antidiabetic effects (Mousavi et al, 2018 ; Shahrajabian et al, 2020 ; Dhama et al, 2023).

Basil is a medicinal herb, rich in essential oils, polyphenols, flavonoids, phenolic acids, and anthocyanins. Purple basil has a unique property due to its high anthocyanin content, which extracts of the plant undergoes visible color change in different pH values. Thus, the plant has a great color sensitivity to detect pH cahnge, working as a natural pH indicator. In the light of the studies revealing the positive aspects of the basil, it become a great candidate as food additive to increase the nutritive value and aslo protective properties. Furthermore, a natural polysaccharide from basil seeds known as basil seed gum (BSG). BSG has many features including emulsifying, thickening, stabilizing, texurizing effects and a good natural source for food related application especially enhancing the quality of the frozen food products (Khazaei et al, 2014 ; Shahrajabian et al, 2020).

3. MATERIALS AND INSTRUMENTATION

3.1 Chemicals and Reagents

3.1.1 Chemicals used for capillary electrophoretic separations

Sodium tetraborate decahydrate ($Na_2B_4O_7.10H_2O$), sodium hydroxide (NaOH), sodium dodecyl sulfate (SDS) and methanol were obtained from Merck (Darmstadt, Germany). Rosmarinic and carnosic acid were obtained from Sigma Aldrich (St. Louis, MO, USA). Khellin and visnagin from *A. visnaga* was obtained and used as reference compounds for analysis from Associate Professor Neslihan Beyazıt after isolation. The compounds purity were confirmed with the following information.

Khellin. Crystallized from MeOH-water, m.p. 154–155 °C, UV λ_{max} nm: 275, 322. 1H-NMR (200MHz; $CDCl_3$): δ, ppm 7.65 (d, J = 2 Hz, H-2) 7.03 (d, J = 2 Hz, H-3) 6.06 (s, H-6) 4.19 (s,OCH_3) 4.06 (s,OCH_3) 2.40 (s, CH3).

Visnagin. Crystallized from MeOH–water, m.p. 142–143 °C, UV λ_{max} nm: 270, 320. 1H-NMR (200 MHz; CDCl3): δ, ppm 7.59 (d, J = 2 Hz, H-2) 7.03 (d, J = 2 Hz, H-3) 7.20 (s, H-9)6.02 (s, H-6) 4.18 (s, OCH_3) 2.32 (s, CH_3)

3.1.2 Chemicals used for antioxidant and antimicrobial tests

Folin–Ciocalteu reagent, gallic acid, 2,2 diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), and $FeCl_3.6H_2O$ were purchased from Sigma Aldrich (St. Louis, MO, USA). Methanol (CH_3OH), sodium hydroxide (NaOH), hydrochloric acid (HCl), acetic acid (CH_3COOH) (glacial) 100%, and ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) were obtained from Merck (Darmstadt, Germany).

Lyophilized cultures of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 6538), Brain Heart Infusion Broth, Nutrient Agar, and Nutrient Broth were supplied from Liofilchem (RosetoDegli Abruzzi, Italy) and from Lab M (Burry, UK). All reagents were analytical grade and were used as received.

3.1.3 Chemicals used for biopolymeric film preparation

Low molecular weight chitosan (50,000-190,000 Da) with viscosity of 20-300cP, 1wt % in 1% acetic acid, 25 °C and 75-85 % degree of deacetylation, and acetic acid (CH_3COOH) (glacial) 100%, were purchased from Merck (Darmstadt, Germany). Pectin Citrus (yellow to pale brown powder, galacturonic acid units ≥74.0% and methoxy groups ≥6.7%) was purchased from Alfa-Aesar (Haverhill, Massachusetts, USA). Alginic acid sodium salt (medium viscosity) was purchased from Sigma Aldrich (St. Louis, MO, USA). All other reagents were analytical grade and were used as received. The commercial pomegranate, hawthorn, grape, and apple vinegar were obtained from local markets. Also, commercial package of dried leaves of purple basil was purchased from a well-known local herb market known as Arifoğlu.

3.2 Plant Materials

3.2.1 *Ammi visnaga* L. and *Ammi majus* L.

The plant *A. Visnaga* L. was obtained from Salam Farm located in Hatay city of Türkiye. *A. Majus* L. was obtained from Balıkesir city of Türkiye by collecting from 9 km Northen region of Ayvalık. The both plants were obtained from Professor Keriman Günaydın. The botanist Professor Emine Akalın Uruşak from, Istanbul University, Faculty of Pharmacy was authenticated the plants. Both plants have voucher specimens, Number 83915 for *A. majus* L. and Number 83916 for *A. visnaga* L. kept at Herbarium of Faculty of Pharmacy of Istanbul University.

3.2.2 *Salvia Officinalis* L. species

Anatolian *Salvia* species which were collected from 7 different locations were used in the studies explained in Chapter 5. The 14 samples included three endemic species which are *Salvia potentillifolia*, *Salvia pisidica*, and *Salvia euphratica var. leocalycina*. The table 3.1 below indicates the detail about the species such as collection dates, coordinates, and Taxon numbers. The identification of the species was performed by Ahmet Kahraman and Tuncay Dirmenci. Their voucher specimens were kept in the Balıkesir and Uşak University.

Table 3.1 : Taxon numbers, collection dates and localities, height, coordinate, and endemism information of the *Salvia* species studied.

Salvia Species Name	Taxon Number	Collection dates and localities	Height (m)	Coordinate
S. pisidica	AK-2481	June 2016, Antalya : Elmalı, Cedar Research Forest	1285	N36 58 840
*S. euphratica var. leocalycina**	TD-4569	June 2016, Sivas: between Divriği - İliç¸ 47. km		
S. palaestina	TD-4574	June 2016, Sivas: between Divriği - Kemaliye		
*S. potentillifolia**	AK-2478	June 2016, Antalya : Elmalı, Cedar Research Forest	1200	
S. candidisima subsp. candidissima	TD-4578	June 2016, Kayseri: roadsides	1040	
*S. chionantha**	AK-2505	June 2016, Antalya : Elmalı to Korkuteli, roadsides	1300	N36 85 903 E30 02 339
S. hedgeana	TD-4570	June 2016, Sivas: between Karayol - Divriği	1428	
S. hypargeia	TD-4576	June 2016, Kayseri : between Pınarbaşı - Sarız	1690	
S. nutans	AK-2417	May 2016, İstanbul: Ağva to Şile, Mahmutdere	Sea level	N41 15 428 E29 73 032
S. aramiensis	AK-2348	April 2016, Hatay: Arsuz, Hacıahmetli-Karagöz road, above Karagöz village	608	N38 16 341 E37 17 460
S. caespitosa	AK-2155	June 2015, K.Maras¸ : Elbistan, Şar Mountain, rocky and stony slope	1619	N38 16 341 E37 17 460
S. cerino-pruinosa	TD-4568	June 2016, Sivas: between Divriği - İliç¸ 47. km		
S. blephorocleana	TD-4575	June 2016, Kayseri : between Pınarbaşı - Sarız 8. km	1640	
S. verbenaca	AK-2321	April 2016, Burdur: Isparta-Antalya road, Çamlık village to Kremna, roadsides	397	N37 48 627 E30 73 544

*: Endemic *Salvia* species, N: Nort, E: East, AK: Ahmet Kahraman, TD: Tuncay Dirmenci.

3.3 Instrumentation

3.3.1 CE system

An Agilent 1600 capillary electrophoresis system (Waldbronn, Germany) with a bare capillary column with a 50 µm inner diameter obtained from Polymicro Technology (Phoenix, AZ, USA) was used in separation. The CE system was coupled with online DAD system. The detection window was created at the detection side by removing the polymer coating of the column. The data analysis was performed by using Agilent ChemStation software. In the first use, the fused silica capillary column was conditioned by flushing with 1 mol L^{-1} NaOH for 30 min and then deionized water for 15 min before use.

3.3.2 Laboratory apparatus

During preparation standard and sample solutions IKA KS 260 (Staufen, Germany) orbital shaker, and vortex, Bandelin SONOREX (Berlin, Germany) ultrasonic bath, and IKA (Staufen, Germany) magnetic stirrers were used. pH adjustments were performed by Thermo Scientific Orion Dual Star pH meter (Beverly, MA, USA). MColorpHast™ Merck (Darmstadt, Germany) pH indicator strips were used for pH testing. Electrical balance from Mettler Toledo (tare range: 0–100 g; sensitivity: 0.00001, Ohio, USA) was used. A hand-held Digital Caliper (0-150 mm) was employed.

The prepared film samples were stored in test cabinet of Nüve TK 120, Ankara, Türkiye. Shimadzu UV-1800 model UV-VIS spectrophotometer (Kyoto, Japan) was used for spectroscopic analysis and transmittance studies. Optical measurements were performed by using Agilent Cary 630 Fourier transform infrared (FTIR) Spectrometer (CA, USA), X-ray diffractometer (XRD RIGAKU, SmartLab 9kW, Tokyo, Japan), Scanning electron microscope (SEM) from METU Central Laboratory with QUANTA 400F Field Emission and Polaron Sputtering system. White Lenata cards were used for determination of color properties.

Mechanical properties were determined with Instron 3345 testing machine (Norwood, MA, USA) attached to a 10 N force transducer. Shimadzu MOC63U

UniBloc Moisture Analyzer (Kyoto, Japan), an oven (Nüve EV 018, Ankara, Türkiye), a Nüve ST-402 shaking water bath (Ankara, Türkiye) were also used during investigation of physical properties.

All solutions were prepared in pure water obtained by Elga Purelab Option-7–15 model system with 18.2 Ω resistivity.

3.4 Statistical Analysis

The mean values of the obtained data from analysis of bioactive components in different plant samples and data from physical properties of the film samples were statistically evaluated. One-way ANOVA for multiple comparisons and Tukey's post-hoc ($p < 0.05$) test was used to reveal the significant differences between the groups in pairs. IBM® SPSS® 24 (NY, USA) was used for statistical analyses. Different letters indicate significant differences among the groups.

4. DETERMINATION OF KHELLIN AND VISNAGIN AMOUNTS IN FIVE DIFFERENT PARTS OF AMMI VISNAGA AND AMMI MAJUS AND THEIR FREE RADICAL SCAVENGING ABILITIES

4.1 Preparation of the Plant Materials and Standard Solutions of Khellin and Visnagin

4.1.1 Plant materials

The five different parts of both *A. Visnaga* and *A. Majus* were finely grained, homogenized and then 100 mg of each part was weighed. Mehanol was used for the extraction solvent as methanol was selected as the optimum solvent in a previous study reported by our group (Günaydın and Erim, 2002) .10 mL methanol was added in plant parts and the mixtures was vortexed about 5 min. Afterwards, the mixtures were ultrasonicated about an hour. The extracts were filtrated through a Whatmann No:1 filter paper and the volumes were adjusted to 10 mL with methanol. The filtrates were again filtered from 0.45 µm pore sized cellulose acetate microfilters before introducing into the CE column.

4.1.2 Preparation of khellin and visnagin standard solutions

Bioactive compounds of khellin and visnagin were dissolved in methanol at a concentration of 800 mg L^{-1} were prepared. The diluted standard solutions from each stock solution were prepared freshly with methanol as needed. The stock solutions were kept at 4 °C in a refrigerator.

4.1.3 Operation conditions

Bare fused silica capillary column was used for separations with 50 µm inner diameter, total length of 65 cm and effective length of 50 cm. The sample was introduced to the capillary column hydrodynamically at 50 mbar for 6 seconds from the anodic side. Detection was carried out online with UV detection, which was performed at 245 nm. Separation voltage was 25 kV and was set constant during analysis. All analysis and sample injections were carried out at 25 °C.

The regeneration of the capillary column for daily use was performed. Capillary column was flushed by 1 M NaOH solution for 15 minutes, water and buffer solutions for 10 minutes respectively. Between each run during analysis, capillary was washed with 0.1 M NaOH solution, water, and buffer for 5 minutes each respectively.

4.1.4 Evaluation of free radical scavenging activity

Free radical scavenging activity of each part of the plant extracts was tested with the method reported by Blois (1958) with some minor changes. A hundred μL of plant extracts were mixed with the methanolic solution of 20 mg L^{-1} DPPH volume of 1900 μL. Following to the incubation at dark for 30 minutes, the absorbance of the solutions was measured at 517 nm against the blank solution of methanol. The results were calculated as percentage inhibition % according to the equation given below.

$$Inhibition\% = \frac{\text{A control} - \text{A sample}}{\text{A control}} \tag{4.1}$$

where A sample is the absorbance of the samples and A control is the absorbance of the control solution prepared with addition of methanol as sample.

4.2 Results and Discussions

4.2.1 Optimization of the MEKC method

Khellin and visnagin are both nonionic compounds and their structures were given in Figure 2.13. They both are not soluble in water. The successful capillary electrophoretic separation of the compounds can be performed by using MEKC method. The separation principle in this method depending on the polarity differences between molecules as in chromatographic separations. In order to perform the separation, a surfactant was used in buffer solution above its critical micelle concentration (CMC). Surfactants, forms micelle structures within the columns where the partition of analytes occurs between the micelles and aqueous phase. Phosphate and borate as BGE solution were studied and borate was provided stable baseline signal. Borate concentrations between 5 and 30 mM were tested and the best resolution for the separation in real plant samples were obtained with 20 mM of borate. SDS was used at a concentration of 20 mM where micelles formed. The pH was used as it is. Methanol and acetonitrile addition to the buffer solution were studied in order to enhance the sensitivity and resolution. The optimum separation electrolyte composition was selected as 20 mM borate solution and 20 mM SDS at pH of 9.6 with 5% (v/v) methanol. All analysis was performed under optimized conditions under constant voltage of 25 kV and the conditions given 4.1.3. The standard electropherogram of khellin and visnagin obtained under optimized separation conditions was represented in Figure 4.1.

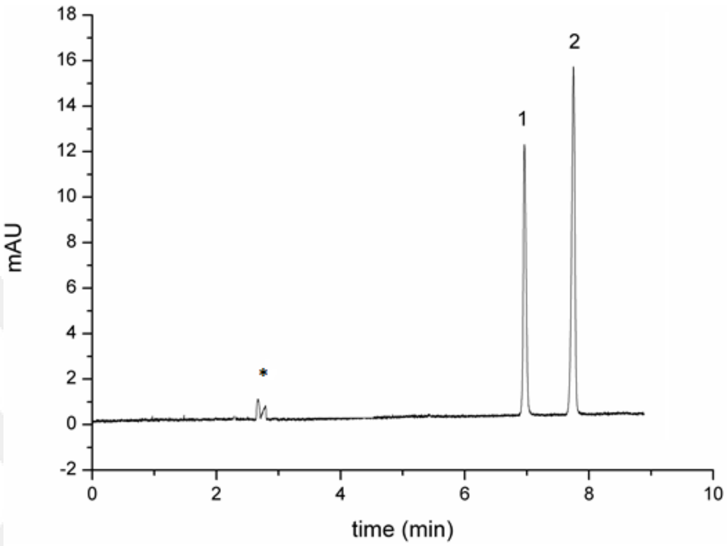

Figure 4.1 : Standard electropherogram of 20 mg L^{-1} khellin and visnagin under optimized separation conditions. 1: Khellin, 2: Visnagin, *: Electroosmotic flow.

4.2.2 Method validation

The linear calibration curves were plotted with using khellin and visnagin standard solutions concentrations against corrected peak areas (peak area/migration time) in the concentration levels of 3, 6, 10, 25, 50 and 100 mg L^{-1} and 4, 6, 10, 25, 50 and 100 mg L^{-1} for khellin and visnagin respectively. The regression equations were given in Table 4.1. The regression coefficients were found to be 0.997 and 0.998 respectively for khellin and visnagin. Limit of detection (LOD) and limit of quantification (LOQ) values were calculated as three and ten times the average signal to noise ratio, respectively. The LOD and LOQ values for khellin and visnagin were given in Table 4.1. The repeatability of the method was tested for migration times and corrected peak areas for intra-day and inter-day measurements and results were listed in Table 4.1. The precision results were calculated for seven injections for the same day and seven injections for three consecutive days. Relative standard deviations (RSD)% of migration times were $\leq$ 0.31 for intra-day and $\leq$ 1.92 for inter-day measurements. The precision of the corrected peak areas in terms of RSD% were ≤ 3.12 and ≤ 4.89 for intra-day and inter-day measurements respectively.

Table 4.1 : MEKC method validation parameters.

Method Validation Parameters	Khellin	Visnagin
Regression Equation	y= 0.00314x + 0.00126	y= 0.00373x + 0.00129
Correlation Coefficient (R^2)	0.997	0.998
LOD (mg L^{-1})	0.83	0.99
LOQ (mg L^{-1})	2.77	3.30
Intra-day Precision (RSD%)		
Migration Time (t) (n=7)	0.29	0.31
Corrected Peak Area (A/t) (n=7)	1.91	3.12
Concentration (n=7)	2.47	2.82
Inter-day Presicion (RSD%)		
Migration Time (t) (n=21)	1.42	1.92
Corrected Peak Area (A/t) (n=21)	3.83	4.89
Concentration (n=21)	4.74	5.41

RSD%: Relative Standard Deviation.

Method validation was also performed by applying recovery experiments. Standard khellin and visnagin solutions were added to *A. visnaga* and *A. majus* plant extract samples at three different concentration levels (50%, 100% and 200% of the amount determined in sample). The good recovery results were calculated between 89.7 and 100.6% and results are given in Table 4.2.

Table 4.2 : Recovery results.

Analyte	Amount Determined	Amount Added	Amount Found	Recovery % ± SD
Khellin		11.8	31.7	89.7 ± 0.7
*A.**Visnaga***	23.5	23.5	42.5	90.3 ± 0.9
		46.1	69.5	100.6 ± 0.9
Visnagin		9.4	26.3	93.4 ± 0.3
*A.**Visnaga***	18.8	18.8	35.2	93.7 ± 0.7
		37.5	52.1	92.4 ± 0.4
Khellin		7.9	22.9	96.8 ± 1.1
*A.**Majus***	15.8	15.8	28.9	91.6 ± 0.7
		31.5	43.0	91.0 ± 0.9

n=2, ± SD: Standard Deviations.

4.2.3 Analysis of plant samples

The amounts of bioactive components in plants parts were calculated from the equations of calibration curves constructed with standard substances. The extracts were sufficiently diluted with methanol and directly injected to the column after filtration without any further pretreatment. The analysis for each part of the plants was duplicated. The electropherogram of a plant extract is shown in Figure 4.2. The khellin amounts were found in between 6.80 and 22.60 mg g^{-1} dry weight of *A. Visnaga* plant parts. Visnagin only found in some parts of *A. visnaga* in between 5.80 and 18.50 mg g^{-1} dry weight of plant part and did not detected in any parts of *A. majus*. The respective khellin and visnagin amounts in different parts of *Ammi* species were represented in Table 4.3.

Table 4.3 : Amounts of khellin and visnagin in different parts of *Ammi* species.

Plant Sample	Khellin (mg g^{-1} dry weight ± SD)	Visnagin (mg g^{-1} dry weight ± SD)
A. Visnaga Flower	22.60 ± 0.06	18.50 ± 0.06
A. Visnaga Leaf	6.80 ± 0.14	13.20 ± 0.27
A. Visnaga Seed	11.00 ± 0.03	5.80 ± 0.01
A. Visnaga Stem	n.d	n.d
A. Visnaga Root	n.d	n.d
A. Majus Flower	2.40 ± 0.01	n.d
A. Majus Leaf	n.d	n.d
A. Majus Seed	1.60 ± 0.01	n.d
A. Majus Stem	n.d	n.d
A. Majus Root	n.d	n.d

n=2, n.d: not detected, ±SD: Standard Deviations.

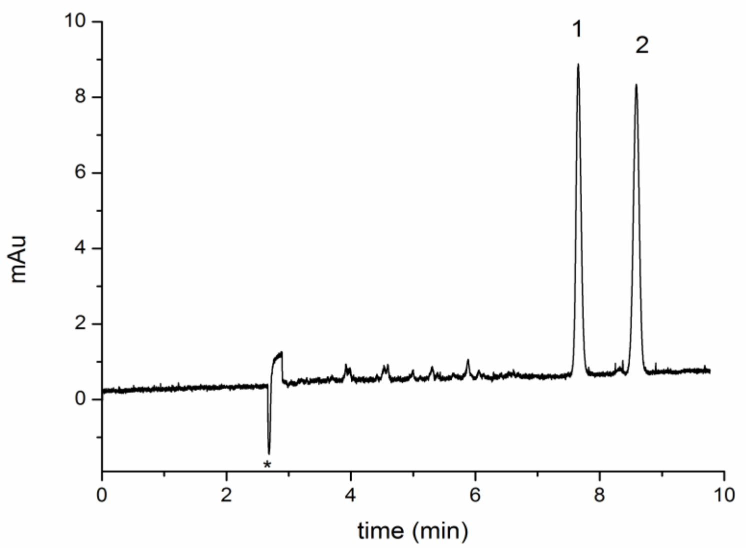

Figure 4.2 : 10 times diluted *A. visnaga* flower extract electropherogram under optimized separation conditions. 1: Khellin, 2: Visnagin, *: Electroosmotic flow.

4.2.4 Free radical scavenging activity of the plant extracts

Free radicals or reactive oxygen species (ROS) are compounds formed as a result of oxidative stress, causing serious damage to the biological systems including aging, immune related disorders and even cancer (Baliyan et al, 2022). Antioxidant compounds are working as inhibtors by reacting with those reactive species and eliminate their undsired and harmful chain reactions. Especially, secondary metabolites in the plants posses antioxidant activity by blocking the activity of ROS. Thus, the natural sources of antioxidant compounds are getting considerable attention since those antioxidants are safer alternatives due to their low toxicity. In order to evaluate the antioxidant power, DPPH method is usually applied. DPPH is a rapid, simple and easily accessible well-known technique used for determination of the antioxidantive power of plant extracts. DPPH is a stable radical molecule with characteristic purple color, in DPPH method the molecule mixed with a solution containing compound capable of hydrogen transfer, as a result the color changes from the purple to yellow and absorbance was measured.

The results of the antioxidant power of five different parts of both *Ammi* species as DPPH inhibition % are represented in Figure 4.3. The flowers of *A. majus* and followed with the flowers of *A. visnaga* were showed higher DPPH % inhibition activity 76.68% and 63.21% respectively where stems and roots of both species were showed lower DPPH % inhibition.

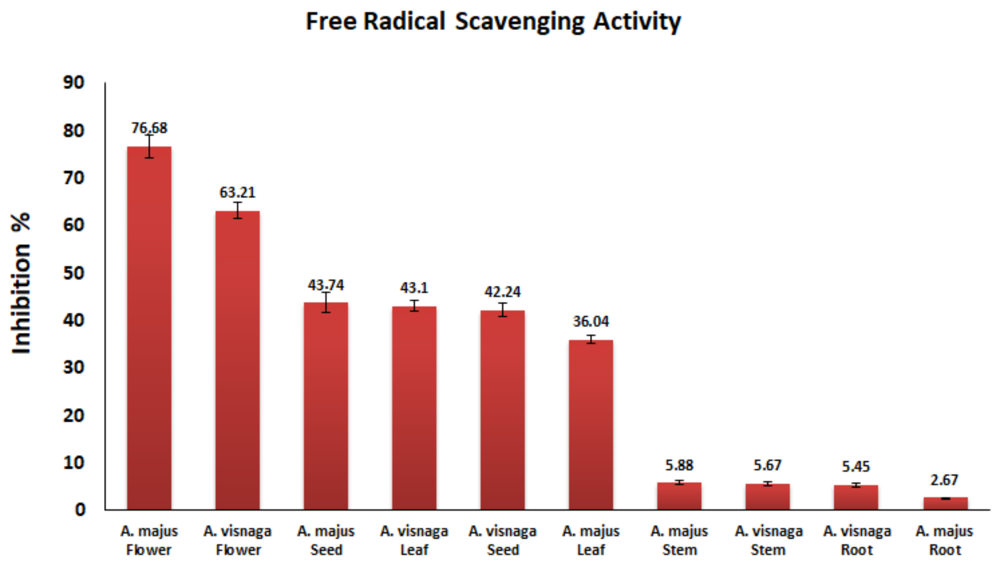

Figure 4.3 : DPPH inhibition % values of different parts of *Ammi* species.

4.2.5 Discussions

In this study, khellin and visnagin amounts in five different organs of two plants were investigated together first time with using a sensitive, simple and rapid MEKC analysis method with UV detection in about 8.5 minutes. *A. visnaga* was found to be a good source of khellin and visnagin and these bioactive components were found in flowers, seeds and leaves. Visnagin did not found in any plant part of *A. majus*, however khellin were detected in flowers and seeds of *A. majus*. Moreover, the antioxidative power of the plant parts were evaluated and the flowers of each species were found to be good sources of antioxidants. The previous studies were reported the khellin and visnagin amounts especially in *A. visnaga* fruits. Günaydın and Erim (2002) reported khellin amounts as 5.8 mg g^{-1} dry weight and visnagin amounts as 1.6 mg g^{-1} only in fruits. The developed method offers simple, sensitive and especially rapid analysis compared to the previous reports including spectroscopic and chromatographic techniques such as HPLC and GC technique (Martelli et al, 1984 ; El-Domiaty, 1992 ; Zgorka et al, 1998 ; Kursinszki et al, 2000 ; Günaydın and Beyazıt, 2004 ; Badr et al, 2014 ; Alqasoumi et al, 2014).

5. DETERMINATION OF ROSMARINIC AND CARNOSIC ACID AMOUNTS IN ANATOLIAN *SALVIA* SPECIES AND INVESTIGATION OF THEIR TOTAL PHENOLIC CONTENTS AND ANTIOXIDANT ACTIVITIES

5.1 Preparation of the *Salvia* Extracts and Standard Solutions

5.1.1 *Salvia* plants

Leaves of 14 *Salvia* species collected from different regions of Anatolia were dried and homogeneously grounded before weighing. 0.1 g of leaves were extracted with 10 mL methanol. The mixtures were vortexed for a minute and then ultrasonicated for 30 minutes. Before the analysis, the extracts were filtered from a Whatmann No:1 filter paper and completed to a final volume of 10 mL. The second filtration was performed through cellulose acetate microfilters with a pore size of 0.22 μm before injection to the CE system. After the necessary dilutions with methanol, the solutions were also used for antioxidant activity tests.

5.1.2 Preparation of rosmarinic and carnosic acid standard solutions

Standard solutions of rosmarinic and carnosic acids at a concentration of 1000 mg L^{-1} were prepared by dissolving the necessary amount of the standard substance in methanol. The stock solutions were kept in a refrigerator set at −20 °C. Each day of the study, the working standard solutions were freshly prepared by dilution with methanol.

5.1.3 Operation conditions

Uncoated silica capillary with a total length of 67 cm and effective length of 57 cm with an inner diameter of 50 μm was used for separations. Prior to its first use capillary was conditioned by washing with 1 M of NaOH solution for 30 minutes then 10 minutes with water. Each working day the regeneration was also performed by flushing the capillary with 1 M NaOH for 15 minutes and 10 minutes with water and the separation electrolyte consecutively. The capillary column was washed with

0.1 M NaOH solution for 2 minutes, water for 2 minutes, and buffer for 5 minutes after each injection. All the analysis procedures were performed at 25 ℃. CE analysis was performed at constant voltage set at 28 kV and the sample injections were performed hydrodynamically 50 mbar for 6 seconds from anodic side and detection was carried out with UV detection performed at 210 nm.

5.1.4 Evaluation of free radical scavenging activity

Antioxidative power of the *Salvia* extracts was evaluated with using a common radical scavenging method. The molecules present in the extract with antioxidant power reacts with DPPH radical. The method was performed with slight modifications as explained in Section 4.1.4. The 1980 µL methanolic 20 mg L^{-1} solution of DPPH was mixed with twenty µLof extract. The mixtures were vortexed and than incubated for 30 minutes at dark and absorbances were measured at 517 nm. The resulting DPPH inhibiton % of the each *Salvia* extract was determined by using the equation 4.1.

5.1.5 Determination of total phenolic contents (TPC)

Total phenolic contents of the *Salvia* species were evaluated with the method known as Folin-Ciocalteu proposed by Singleton and Rossi (1965) with some slight modifications. The prepared extracts (0.3 mL) were mixed with 1:10 diluted aqueous Folin-Ciocalteu reagent (1.5 mL) and aqueous sodium carbonate solution (1.2 mL) at a concentration 7.5% (w/v) in a small glass vial. The mixture incubated at room temperature for 15 minutes and absorbance was measured at 760 nm against water as blank. A calibration curve was constructed with the same method by using gallic acid (GA) as standard substance. The results were calculated as gallic acid equivalents (GAE) and expressed as mg GAE /g dry weight.

5.1.6 Evaluation of ferric reducing antioxidant power (FRAP)

The FRAP evaluation of 14 *Salvia* species extracts were performed accordingly a proposed method by Benzie and Strain (1996). The FRAP reagent was prepared by mixing 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution dissolved in 40 mM aqueous HCl, 20 mM $FeCl_3$, and 0.3 mM acetate buffer at pH 3.6 in 1:1:10 ratio. The reagent was incubated for 10 minutes at 37 ℃. *Salvia* extract (0.1 mL), FRAP reagent (1.8 mL) and deionized water (0.1 mL) was mixed and again incubated for

10 minutes at 37 °C. Following to the cooling down to room temperature the absorbance was measured at 593 nm against deionized water as blank. The FRAP results calculated as mg Fe(II) in g dry weight of *Salvia* species.

5.2 Results and Discussions

5.2.1 Optimization of the CZE method

The separation of two components in acidic structures where given in Figure 2.15, can be achieved by CZE method with using buffer solutions with increased pH values. According to the findings of a previous study conducted in our laboratory, borate buffer was selected as the separation electrolyte (Başkan et al, 2007). Concentrations in the range 10-40 mM were examined and the best resolution with the lowest noise was obtained at a borate concentration of 20 mM. The pH was set at 9.6. All analysis was performed under optimized conditions explained in 5.1.3 under constant voltage of 28 kV at 25 °C. Under the optimized conditions migration times of carnosic acid and rosmarinic acid were 4.1 and 5.8 minutes respectively. The standard electropherogram of carnosic and rosmarinic was given in Figure 5.1. The whole analysis procedure was completed in a total of one hour in order to prevent the decomposition of carnosic acid into carnosol under the influence of heat and light. The standard solutions were prepared daily.

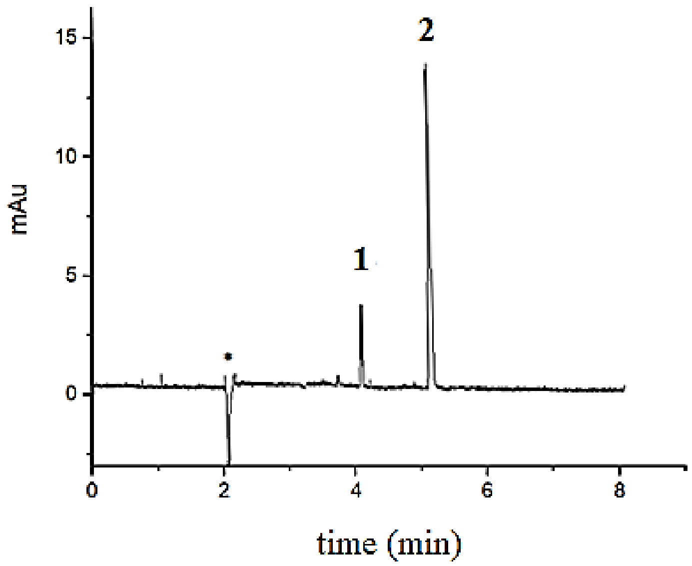

Figure 5.1 : Standard electropherogram of 20 mg L^{-1} CA and RA under optimized separation conditions. 1: CA, 2: RA, *: Electroosmotic flow.

5.2.2 Method validation

Method linearity was shown by the construction of six-point linear calibration curves for carnosic and rosmarinic acid from the standard solution concentration levels of 6, 10, 20, 50, 100, 200 mg L^{-1} and 7, 10, 20, 50, 100 and 200 mg L^{-1} respectively against corrected peak areas (peak area/migration time). The regression equations and correlation coefficients of both rosmarinic and carnosic acid and other method validation parameters were given Table 5.1. The calibration equations for carnosic and rosmarinic acid were y=0.2371x-0.3335 and y=0.1053x-0.1754 respectively. LOD values (S/N=3) were calculated 1.72 g mL^{-1} and 1.86 g mL^{-1} for carnosic and rosmarinic acid respectively where LOQ values (S/N=10) were 5.72 g mL^{-1} for carnosic acid and 6.20 g mL^{-1} for rosmarinic acid. The reproducibility of the method was tested in terms of intra and inter-day precisions of carnosic and rosmarinic acid. The precision results were represented as RSD% values listed in Table 5.1 for migration times and corrected peak areas. Seven injections were made on the same day, and seven injections were performed on each three consecutive days.

Table 5.1 : CZE method validation parameters.

Method Validation Parameters	Rosmarinic Acid	Carnosic Acid
Correlation Coefficient (R^2)	0.998	0.998
LOD (mg L^{-1})	1.86	1.72
LOQ (mg L^{-1})	6.20	5.72
Intra-day Precision (RSD%)		
Migration Time (t) (n=7)	1.16	1.39
Corrected Peak Area (A/t) (n=7)	1.77	1.90
Concentration (n=7)	2.12	2.34
Inter-day Precision (RSD%)		
Migration Time (t) (n=21)	1.98	1.64
Corrected Peak Area (A/t) (n=21)	5.15	4.85
Concentration (n=21)	5.31	5.10

RSD%: Relative Standard Deviation.

The recovery studies were applied to *Salvia* species extract by addition of standard carnosic and rosmarinic acid in 50%, 100% and 200% levels of the amount found for each acid in extract. Satisfactory recovery values were obtained in between 94.8 ± 2.8 and 101.0 ± 5.0 for rosmarinic acid and 90.2 ± 2.9 and 96.7 ± 1.4 for carnosic acid.

5.2.3 Method application to *Salvia* samples

According to the results of the CE analysis, rosmarinic acid was found in all 14 *Salvia* species in a range of 1.08 and 18.70 mg g^{-1} dry weight. The highest amount of rosmarinic acid was found in *S. caespitosa*, followed by *S. hypargeia* at 14.40, *S. nutans* at 13.60 and *S. aramiensis* at 10.10 mg g^{-1} dry weight. Among the endemic species, *S. chionantha* contains the lowest amount of rosmarinic acid. Furthermore, *S. caespitosa* is the only *Salvia* species that contains carnosic acid. The amounts of rosmarinic and carnosic acids with standard deviations and as average values were summarized in Table 5.2 with indicating the significant difference within the groups by different letters according to the test of Tukey's post hoc comparisons ($p < 0.05$). The electropherogram of *Salvia* extracts under optimized conditions, *S. potentillifolia* (a) and *S. caespitosa* (b) are represented in Figure 5.2.

Table 5.2 : Rosmarinic and carnosic acid amounts in *Salvia* species.

***Salvia* Species Name**	**Rosmarinic Acid (mg g^{-1} dry weight ± SD)**	**Carnosic Acid (mg g^{-1} dry weight ± SD)**
S. pisidica	8.64 ± 0.13^{cde}	n.d
S. euphratica var. leocalycina	9.81 ± 0.23^{bcd}	n.d
S. palaestina	2.65 ± 0.01^{gh}	n.d
S. potentillifolia	7.96 ± 0.89^{def}	n.d
S. candidisima subsp. candidissima	2.96 ± 0.10^{fg}	n.d
S. chionantha	1.08 ± 0.01^{h}	n.d
S. hedgeana	3.88 ± 0.13^{efg}	n.d
S. hypargeia	14.40 ± 1.20^{ab}	n.d
S. nutans	13.60 ± 2.40^{abc}	n.d
S. aramiensis	10.10 ± 0.82^{bcd}	n.d
S. caespitosa	18.70 ± 1.97^{a}	11.00 ± 0.20
S. cerino-pruinosa	6.35 ± 0.15^{defg}	n.d
S. blephorocleana	1.76 ± 0.07^{h}	n.d
S. verbenaca	3.35 ± 0.45^{efg}	n.d

n=2, n.d: not detected, ± SD: Standard Deviations. Mean values with the different letter are significantly different from each other according to Tukey's test ($p < 0.05$).

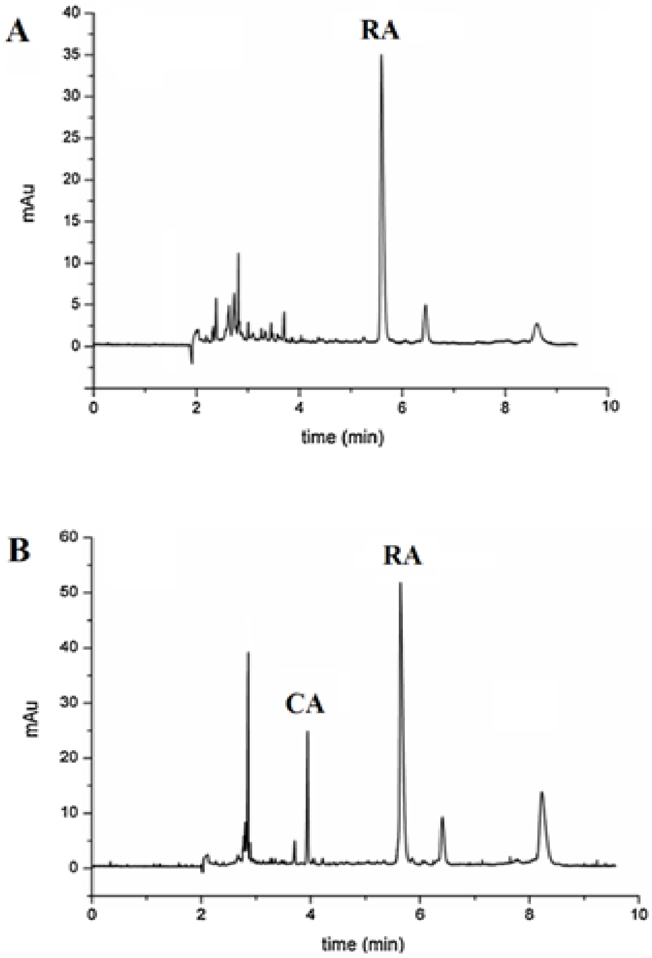

Figure 5.2 : Electropherograms of A) *Salvia potentillifolia* B) *Salvia caespitosa* extract under optimized separation conditions. CA: Carnosic acid, RA: Rosmarinic acid.

5.2.4 TPC, DPPH activity and FRAP results of *Salvia* samples

The DPPH method was used for the radical scavenging activity evaluation of the species. The results are listed in Table 5.3. Among the *Salvia* species, the higher DPPH inhibition percentages were calculated for *S. aramiensis* and *S. caespitosa* at 93.2% and 92.3%, respectively. The values for DPPH inhibition activities varied between 3.76% and 93.2%.

The total phenolics of the *Salvia* species were determined to be between 6.5 and 260.0 mg GAE g^{-1} dry weight of the plants. *S. aramiensis* showed the highest TPC, followed by *S. caespitosa* where both species showed a higher DPPH% as well. The results are summarized in Table 5.3.

The FRAP test depends on the measurement of a biological system or an extract's defense ability to resist oxidative damage (Benzie and Strain, 1996). In the method, the formed Fe(III)-TPTZ complex reduces Fe(II) form as a result of the ability of the sample, and the results were expressed as mg Fe(II) g^{-1} dry weight of the plant. The FRAP results were calculated between 10.9 and 138.0 mg Fe(II) g^{-1} dry weight. *S. caespitosa* showed the highest antioxidant power, while *S. palaestina* showed the lowest antioxidant power. The results are presented in Table 5.3.

Table 5.3 : TPC, DPPH and FRAP assay results of *Salvia* species.

***Salvia* Species Name**	**TPC (mg GAE g^{-1} dry weight ± SD)**	**DPPH Activity (% inhibition ± SD)**	**FRAP (mg Fe(II) g^{-1} ± SD)**
S. pisidica	27.0 ± 1.5^{h}	42.7 ± 0.1^{e}	115.0 ± 7.1^{abc}
S. euphratica var. leocalycina	97.3 ± 0.2^{d}	36.7 ± 1.5^{ef}	85.8 ± 8.4^{cd}
S. palaestina	51.4 ± 0.1^{f}	12.4 ± 0.9^{hi}	10.9 ± 0.1^{g}
S. potentillifolia	86.1 ± 0.5^{e}	66.2 ± 2.8^{d}	132.0 ± 5.6^{ab}
S. candidisima subsp. candidissima	94.5 ± 0.1^{d}	22.2 ± 0.6^{gh}	44.1 ± 4.2^{ef}
S. chionantha	6.5 ± 0.1^{k}	3.76 ± 0.1^{i}	15.6 ± 0.1^{fg}
S. hedgeana	12.1 ± 0.9^{jk}	30.3 ± 0.3^{fg}	66.4 ± 5.5^{de}
S. hypargeia	41.3 ± 0.1^{g}	46.1 ± 1.4^{e}	114.0 ± 8.5^{bc}
S. nutans	105.0 ± 0.1^{c}	82.1 ± 3.5^{bc}	146.0 ± 0.1^{a}
S. aramiensis	260.0 ± 1.0^{a}	93.2 ± 4.5^{a}	133.0 ± 10.0^{ab}
S. caespitosa	221.0 ± 0.2^{b}	92.3 ± 1.9^{ab}	138.0 ± 6.0^{ab}
S. cerino-pruinosa	85.4 ± 0.1^{e}	73.3 ± 1.0^{cd}	122.0 ± 1.1^{ab}
S. blephorocleana	15.3 ± 0.1^{ij}	42.3 ± 0.9^{e}	77.2 ± 5.0^{d}
S. verbenaca	12.5 ± 1.4^{jk}	36.5 ± 0.3^{ef}	64.9 ± 5.6^{de}

n=2, ±SD: Standard Deviations. Mean values with the different letter are significantly different from each other according to Tukey's test ($p < 0.05$).

5.2.5 Discussions

The RA amounts of various *Salvia* species have been reported so far. The amounts were differentiated between the species. A study reported the RA amounts of 3 Chinese *Salvia* species by Cao and colleagues (2014), in the range of 1.30 and 21.4 mg g^{-1} where no CA were reported. The RA amounts were reported in the range of 13.7 to 18.4 mg g^{-1} for *Salvia* samples from Tunusia (Ben Farhat et al, 2013). The RA amounts in samples of *Salvia halophila* from Türkiye were reported in the range of 5.98 - 48.90 mg g^{-1} (Koşar et al, 2011). Another study was reported the RA levels in eight different *Salvia* samples from Türkiye in the range of 2.3 - 114 mg g^{-1}

(Tosun et al, 2011). Another study was conducted by Tepe and coworkers (2007), and the RA levels reported 28.7 and 24.1 mg g^{-1} for two *Salvia* species *S. verticillata subsp. verticillata* and *S. verticillata subsp. amasiaca* respectively and it was stated that there is a strong correlation between the antioxidant activity and RA amounts. Tepe (2008) reported similar values for RA amounts found in this study as well as Zengin and coworkers (2018) reported for different *Salvia* species. The commercial sage tea samples were reported and RA amounts were between 2.50 - 4.01 mg g^{-1} (Başkan et al, 2007).

The antioxidant abilities of the *Salvia* samples were compared with the previous reports. FRAP results of another group of *Salvia* species from Anatolia were reported between 5.8 - 93.7 mg $Fe^{2+}g^{-1}$ dry weight (Gezek et al, 2019). DPPH inhibition % values were also reported between 1.90% and 24.5% (Gezek et al, 2019). The antioxidant abilities of the species in this study found to be higher. A study was reported DPPH scavenging values for Anatolian *Salvia* samples between 0.74 - 89.23 µg mL^{-1} (Şenol et al, 2010). DPPH scavenging values for another group of samples were reported between 18.3 - 88.2% (Tosun et al, 2009).

TPC contents of the *Salvia* species in this study was found to be higher as in antioxidant abilities. Tosun et al. (2010), reported TPC values of *Salvia* species between 50.3-167.1 mg g^{-1} dry weight, while another study was reported TPC values of 4 *Salvia* species in the range of 67.67 and 161.37 mg g^{-1} dry weight (Ben Farhat et al, 2013).

The correlations between the DPPH inhibition % and the significant bioactive component RA, and FRAP and RA were investigated and found to be 0.77 and 0.82 respectively. In this work, large number of *Salvia* species were evaluated and information about their bioctive components and bioactivities were revealed.

6. PREPARATION AND INVESTIGATION OF THE PROPERTIES OF THE CHITOSAN BASED BIOPOLYMERIC FILMS PREPARED IN BIOACTIVE FRUIT VINEGARS

6.1 Preparation of the Chitosan Films in Vinegar Varieties

Chitosan films at a final chitosan concentration of 1% (w/v) were prepared by dissolving in a mixture of 50% (v/v) vinegar: water. Four different fruit vinegars were used as bioactive and safer solvent namely: pomegranate, grape, apple and hawthorn. Each film solution at a total volume of 30 mL were allowed to stir on a magnetic stirrer at a rate of 200 rpm for 24 hours at room temperature. Below 30 mL the films were not easily processed. The beakers were sealed during stirring with parafilm. A control film without containing vinegar varieties was prepared by dissolving the same amount of chitosan in 1% (v/v) aqueous acetic acid solution at the same conditions. Only apple vinegar containing film was not obtained smooth enough and was fragile as a proposed food packaging material a small amount of glycerol (0.2% (v/v)) was added to the film formulation.

Following to the 24 hours of stirring the all film solutions were poured into 9 cm plastic petri dishes after quick filtration through the Whatman No:1 filter paper. The films were dried for 48 h at 25 ℃ at dark in controlled environment without increasing temperature. All the film samples were stored in a preconditioned test cabinet until further studies (Nüve TK 120) at 25 ℃ ± 0.1 and 50 ± 5% relative humidity (RH). The physical appearances of the films are represented in Figure 6.1

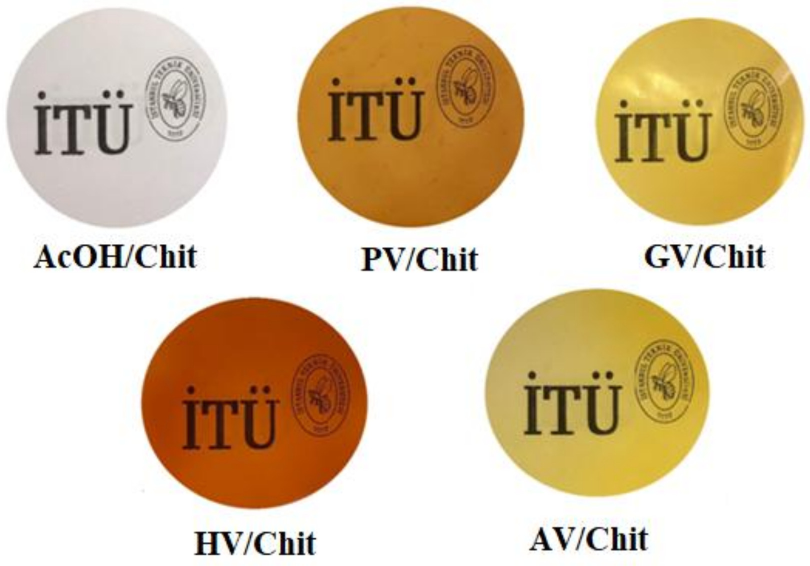

Figure 6.1 : Vinegar/Chitosan film samples. AcOH: Acetic acid, Chit: Chitosan, GV: Grape vinegar, AV: Apple vinegar, HV: Hawthorn vinegar, PV: Pomegranate vinegar.

6.2 Investigation of Optical, Spectroscopic and Morphological Properties of the Prepared Films

The film thickness of each film was measured with using a hand-held digital caliper from five different random positions of the films. The average values were used in further calculations comparatively.

The morphological structures of the films were investigated comparatively for both the film surfaces and the cross-sections by using scanning electron microscope (SEM -QUANTA 400F Field Emission) analysis conducted at 20 kV. All the films were coated 3 mm in thickness with Au-Pd particles in order to provide electrical conductivity. The morphological data were obtained in different and suitable magnifications.

The films are transparent enough to provide visual examination of the food as a potent food packaging, also their transmittance spectra were recorded by using a Shimadzu UV-1800 UV-Vis spectrophotometer and their UV-light blocking abilities were also investigated and compared with sole chitosan film. The opacity values (mm^{-1}) were calculated at 600 nm according to the following formula 6.1 as used in the literature to ease the comparison of the results. The opacity reveals the ligth scattering ability of the material, as used in turbidity determination in microbiologic studies for detection of cell concentration (Myers et al, 2013).

$$Opacity_{600} = \frac{Abs_{600}}{d} \tag{6.1}$$

d is the average thickness value of the films in mm.

Each vinegar variety has different and unique color depending on their source raw material thus, the films prepared by dissolving in vinegars are also different in their colors. The color properties of the films, according to the CIE-L*a*b* system introduced by International Optical Committee as color space coordinates were determined by using white Leneta card and measurements were performed in two different positions. Color of the films represents with several indices. According to the system, L* stands for lightness ranging from 0 (complete darkness) to 100, a* stands for greenness to redness in the range of -128 to +127, b* represents the color blueness to yellowness again ranging from -128 to +127, and ΔE reveals the color difference between different color points in the coordinate system (Martin, 2015). Also, WI represents the whiteness index, C is the chroma, which is the saturation of the color, and h is the hue, which means the distinct value for each color.

The FTIR analysis was performed in order to investigate the interactions between the compounds in the vinegar varieties and chitosan. The transmittance spectra of the 5 films were obtained in the range of 400-4000 cm^{-1} with a resolution of 4 cm^{-1} and 64 scans for both background (air) and samples.

The X-ray diffraction patterns of the film samples were also investigated with an X-ray diffractometer. Direction angles were scanned (2θ) from 5° to 40° with an increment of 0.01° per second.

6.3 Determination Physical and Mechanical Properties of the Prepared Films

The physical properties of the films were also investigated. The surface pH values of the films were tested in order to evaluate the acidity level of the films. The pH test strips were used for this evaluation. The film surfaces were wetted with a little amount of water, and then the test was performed with strips, and the corresponding color changes were noted.

The moisture content of the films during storage was comparatively evaluated by using a Shimadzu MOC63U UniBloc Moisture Analyzer gravimetrically. The moisture content of each sample was measured twice, and average values were calculated. The small pieces of the films in 2 × 2 cm^2 dimensions were taken and then placed on the balance. The pieces were subjected to heating up to 105 °C, which was the maximum suitable heat for food and food-related materials, with a constant rate of 1 °C increments every 30 seconds. Moisture contents were recorded gravimetrically when the change in moisture content in the sample reached 0.03%.

The properties related to the water uptake such as swelling was also investigated. The swelling properties of the stored films were measured in distilled water at room temperature. The films were cut into 2 × 2 cm^2 pieces and then placed into Nüve EV 018 oven for completely drying at 40 °C for 24 h as reaches the constant weight. The completely dried film pieces were weighed immediately after cooling down and immersed in a 50 mL distilled water containing beakers. The beakers were placed in a Nüve ST-402 shaking water bath and they were shaken at 50 rpm constant rate. All film pieces were taken out and wiped gently with a filter paper for removing of the excess water and then weighed at 1, 5, 15, 30, 45, and 60 minutes. The results were expressed as average values for each films piece and the swelling percentages (Swelling %) were calculated. The following equation was used calculations.

$$Swelling\ \% = \frac{(\mathrm{M_{final}} - \mathrm{M_{initial}})}{\mathrm{M_{initial}}} \times 100 \tag{6.2}$$

where Minitial and Mfinal are masses of films before and after swelling.

ASTM E96–95M (1994) “Water Method” is a common method for gravimetric determination of the barrier properties of the materials proposed for use in especially food related applications since it is affecting the both quality and the shelf-life of the product. In this part of the study, two samples for each type of film were mounted on the glass vials. The vials filled with distilled water below 2 cm from the top and then tightly covered with the films. The initial weighing of the vials was taken with a Mettler Toledo electrical balance and then the vials were placed in a test cabinet conditioned at 37 °C ±0.1 and 20% ±5 room humidity. The changes in their weights were recorded for several time at predetermined time intervals. The curve was constructed according to the weight loss over time and linear regression was used for the calculation of the slope. The water vapor transmission rate (WVTR) for each film was calculated with the following equation 6.3 as given below.

$$WVTR = \frac{\mathrm{m}}{A} \tag{6.3}$$

Where m, represents slope and A represents the area of the films subjected to the water vapor transmission.

$$Permeance = \frac{\mathrm{WVTR}}{\Delta \mathrm{P}} \tag{6.4}$$

Permeance can be derived from the equation 6.4 above by using the pressure difference between the sides of the film and then the water vapor permeance (WVP) can be calculated by taken consideration of the film thickness as expressed in equation 6.5.

$$WVP = Permeance\ \times Thickness\ (mm) \tag{6.5}$$

The mechanical endurance of the packaging materials designed for the protection of food has to be evaluated in order to evaluate their potential and processability. The mechanical properties of the films were determined comparatively by employing Instron 3345 testing machine (USA) attached to a 10 N force transducer. For the analysis, the films were cut into pieces of 5 × 40 mm^2 and placed between the 15 mm distanced jaws and the measurements were performed at a speed of 0.05 mm/s at room temperature. Stress-strain curves were constructed and from the curves Young's modulus (E), Elongation at break percentage % (EB %), and Ultimate strengths were calculated.

6.4 Investigation of the Antioxidant and Antimicrobial Properties of the Prepared Films

Antioxidant properties of the films were determined and also the antioxidant power of each vinegar type was evaluated. In order to enhance the protection ability of a food packaging material usually an antioxidant compound is used in formulation. The common method known as DPPH as explained in previous sections was employed for this evaluation. The measurement of radical scavenging activity was performed with using 0.05 mM DPPH solution which was prepared in methanol. Vinegar samples were firstly filtered from 0.45 μm pore-sized microfilters, and then 40 μL of each vinegar solution was mixed with 1960 μL DPPH solution. The mixtures were vortexed for 30 seconds at 1000 rpm and they were incubated at dark and room temperature for 30 minutes. The absorbance measurements were performed at 517 nm against blank solution of methanol. In order to measure the antioxidant abilities of the films, the conditions were optimized and according to the conditions, 50 mg of each dried film sample was mixed with 4 mL of distilled water and vortexed for a minute at 1000 rpm and ultrasonicated for 30 minutes. The 250 μL portion of film solutions were mixed with 1750 μL of the DPPH solution and followed the same procedure as vinegar samples. After 30 minutes, the absorbance values were measured at 517 nm against blank and the inhibition % were calculated according to the equation 4.1. All the measurements were duplicated.

The antimicrobial activities of the five different film were also measured and compared according to the ISO 22196 standard (2007). Two different bacteria Escherichia coli (ATCC 25922) as Gram-negative and Staphylococcus aureus (ATCC 6538) as Gram-positive were used. Film samples (50 mm x 50 mm) were UV sterilized and placed on glass plates and plates were placed into Petri dishes. A 200 μL of test inoculums which were prepared in 1/500 nutrient broth (Lab M, Burry, UK) at a density of approximately 6 log colony-forming unit (CFU)/mL were placed onto the film samples. The film samples were covered with plain sterile polypropylene (PP) film (40 mm x 40 mm) and incubated for about 4 h at 35 °C under relative humidity above 90%. Antimicrobial activity was determined after incubation by the number of viable microorganisms on the film samples was determined and the results were expressed as a logarithmic reduction in the number of viable microorganisms.

6.5 Results and Discussions

6.5.1 Optical, spectroscopic and morphological properties of the prepared films

The slight increase in the film thickness were observed in all films prepared by dissolving chitosan in bioactive vinegar species, compared to the film prepared dissolving chitosan in 1% acetic acid solution however, higher increase in the film thickness was observed for the film prepared with the pomegranate vinegar. The average film thickness for each film are listed in Table 6.1. The differences between the thickness values were depend on the type of vinegar used. The vinegars produced from different natural fruits also differ in color, pH, and taste. The thickness of the material increases with the higher amount of solid incorporated to the structure with natural extracts also making compact structure (Rambabu et al, 2019 ; Wu et al, 2022). Moreover, the film prepared with the pomegranate vinegar showed the higher increase in thickness may due interaction of chitosan with the large phenolic compound called punicalagin which is also responsible for the high antioxidant power of pomegranate juices compared to the other fruit juices (Kalaycıoğlu and Erim, 2017).

The differences in the color parameters measured as L*, a*, b*, C and h values were also compatible with the distinctive and unique colors of the films. The redness (+a*) values were significantly increase for the films which have more intense in color such as hawthorn and pomegranate as showed in digital images in Figure 6.1. The color parameters of the films are listed in Table 6.2.

The transmittance spectra of the films were also measured between 200-800 nm, and the results proved that the all films prepared with the vinegar varieties have UV-light blocking ability between 200 nm and 350 nm, which is strongly desirable for the potential food packaging material, as also reported for the films enriched with extracts (Kalaycıoğlu et al, 2017 ; Rambabu et al, 2019 ; Zeng et al, 2021 ; Wu et al, 2022). The transmittance spectra of the films were represented in Figure 6.2 comparatively. The control film prepared in a 1% (v/v) acetic acid solution did not have any UV-light blocking property. Furthermore, the opacity values were calculated and are also provided in Table 6.1. The grape and hawthorn vinegar included films showed increased opacity, and other films have closer opacity values to the control film. All films are transparent enough to fulfill the consumer’s demand for examining the food product.

Table 6.1 : Thickness, and opacity values of the vinegar/chitosan film samples.

Film Sample	Thickness** (mm) ± SD	Opacity (mm^{-1}) ± SD
AcOH/Chit	0.040 ± 0.005	1.05 ± 0.04
GV/Chit	0.060 ± 0.012	1.99 ± 0.13
AV/Chit	0.070 ± 0.017	1.08 ± 0.01
HV/Chit	0.090 ± 0.009	2.20 ± 0.16
PV/Chit	0.150 ± 0.017	1.01 ± 0.01

n=2, ** n=5, ± SD: Standard Deviations, AcOH: Acetic acid, Chit: Chitosan, GV: Grape vinegar, AV: Apple vinegar, HV: Hawthorn vinegar, PV: Pomegranate vinegar.

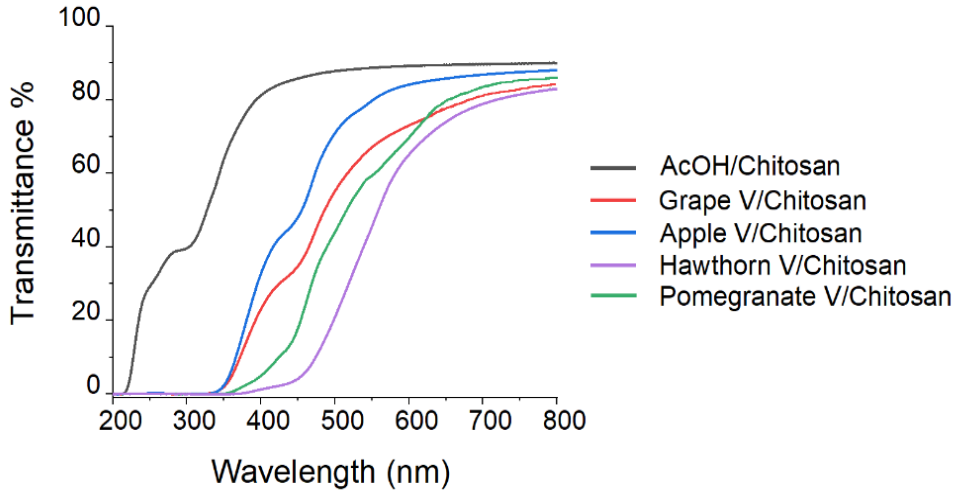

Figure 6.2 : Transmittance spectra of the vinegar/chitosan film samples between 200 and 800 nm.

Table 6.2 : Color properties of the vinegar/chitosan film samples.

Film Sample	L* ± SD	a * ± SD	b* ± SD	C ± SD	h ± SD
AcOH/ Chit	92.15 ± 0.12	-1.73 ± 0.03	5.90 ± 0.15	6.14 ± 0.14	106.4 ± 0.2
GV/ Chit	74.49 ± 2.92	6.32 ± 3.27	58.54 ± 5.59	58.91 ± 5.91	83.97 ± 2.60
AV/ Chit	82.00 ± 0.63	1.41 ± 0.79	68.54 ± 1.56	68.55 ± 1.58	88.83 ± 0.64
HV/ Chit	60.81 ± 5.81	28.29 ± 5.58	57.03 ± 7.29	63.92 ± 4.04	63.39 ± 7.40
PV/ Chit	57.81 ± 4.76	26.16 ± 3.71	53.25 ± 3.39	59.43 ± 1.41	63.79 ± 4.66

n=2, ± SD: Standard Deviations, AcOH: Acetic acid, Chit: Chitosan, GV: Grape vinegar, AV: Apple vinegar, HV: Hawthorn vinegar, PV: Pomegranate vinegar. L*: Ligthness, a*: Redness, b*: Yellowness, C: Chroma, h: Hue.

The SEM images of the films were revealed the surface morphologies and cross-sectional area of the films. The SEM images of the films are given in Figure 6.3. The images showed that the films have uniform surfaces due to the smooth distribution of the vinegar species beside the grape vinegar. The SEM image of the film prepared with grape vinegar showed a slightly rough structure compared to the other vinegar films and sole chitosan film. The reports on SEM images were similar for the films prepared with natural extracts (Rambabu et al, 2019 ; Brás et al, 2020).

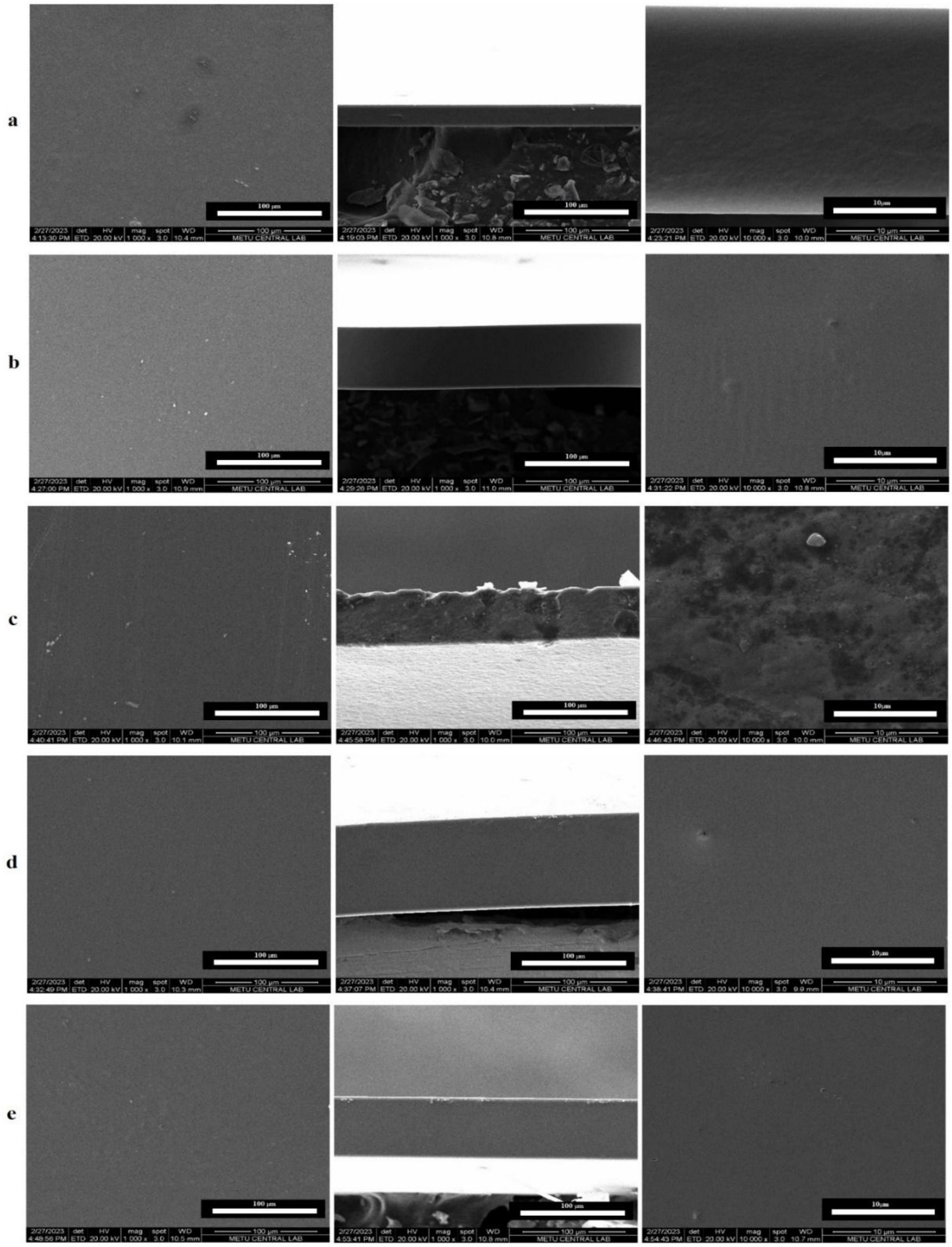

Figure 6.3 : SEM images of the surface (left) and cross-section (middle) and higher magnification of cross-section (right) of each vinegar/chitosan film sample. a) AcOH/Chitosan film b) Pomegranate V/Chitosan film c) Grape V/Chitosan film d) Hawthorn V/Chitosan film e) Apple V/Chitosan film.

The FTIR spectra of the films were measured and investigated. The spectra of the vinegar containing films did not showed distinct differences from the spectrum of the chitosan film prepared only in acetic acid solution as can be seen in the Figure 6.4. However, the intensities were increased and slight shifts were observed as expected (Brás et al, 2020). The slight shifts of the characteristic peaks and changes in the intensities indicating the absence of the covalent bond formation between the chitosan and the vinegar (Brás et al, 2020).

N-H stretching vibrations overlapped with the O-H stretchings were observed at 3265 cm^{-1} as characteristic broad peaks, and also the peak at 2916 cm^{-1} corresponded to the C-H stretching vibrations (Anicuta et al, 2010 ; Kalaycıoğlu et al, 2017). The characteristic amide I and amide II vibrations were assigned at 1636 cm^{-1} and 1543 cm^{-1} for amide I and amide II structures respectively (Brás et al, 2020). At 1013 cm^{-1} C-O stretching vibration peak was observed, and asymmetric C–O–C and C–N stretches were observed at 1151 cm^{-1}, and also -CH_3 symmetrical vibrations at 1377 cm^{-1} and the -CH_2 bending vibrations at 1403 cm^{-1} were assigned (Anicuta et al, 2010 ; Melro et al, 2020).

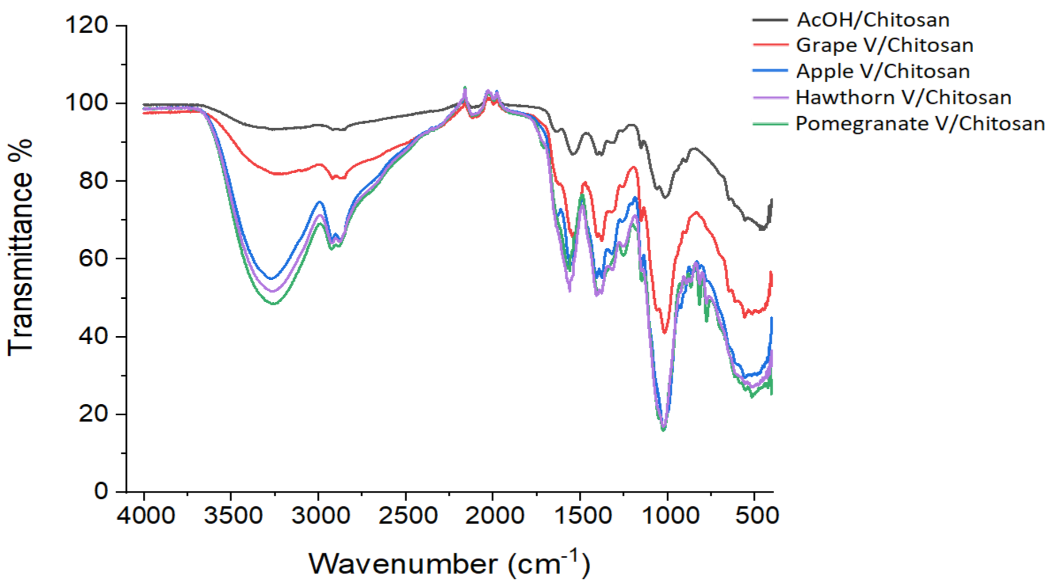

Figure 6.4 : The FTIR spectra of the vinegar/chitosan film samples.

The XRD patterns of the films prepared with vinegars and sole chitosan are represented in Figure 6.5. As in presented in figure peaks around 2θ of 8°, 11°, and 20° were observed for the film prepared with sole chitosan. The peak at 20° (2θ) indicating the amorphous structure of the chitosan (Zhang et al, 2020). The peaks both observed at 8° and 11° were not shown in the images of apple, hawthorn and pomegranate films. On the other hand, the film prepared with grape vinegar showed a weak diffraction peak around 11° might indicating semi-crystallinity. The intensities of the peak around 20° showed slightly increase in pomegranate vinegar added film may be attributed to the thickness of the film while the decrease was observed for grape vinegar added film. The other vinegar added films were not shown difference in intensities with sole chitosan film. The difference in the SEM images of the grape vinegar film is compatible with the results in XRD and also FTIR analysis.

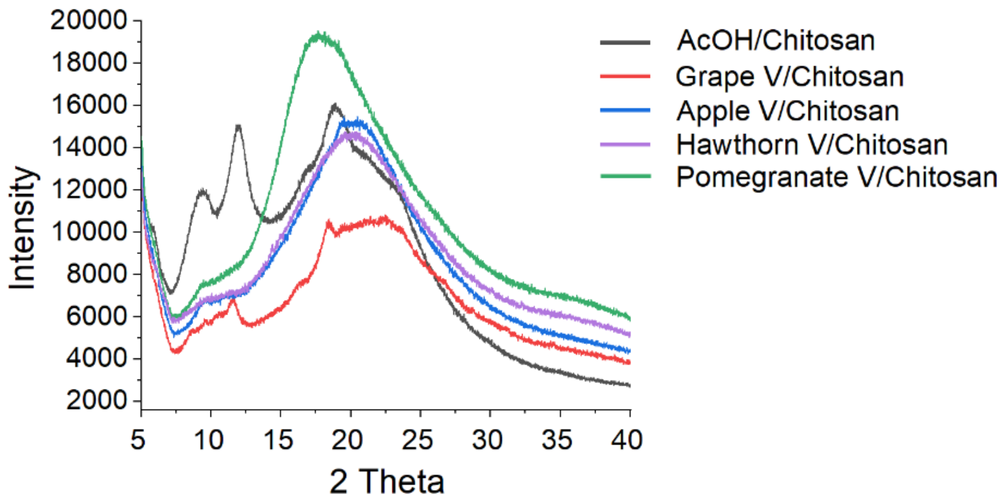

Figure 6.5 : XRD patterns of the vinegar/chitosan film samples.

6.5.2 Physical and mechanical properties of the prepared films

The surface pH values of the films were determined between 4 and 5, showing slightly acidic nature, higher than the pH values of the vinegar varieties itself. The pH values of the studied vinegars were between 2.8 and 3.3. It was reported that, enhancement of protection ability of chitosan achieved by moderate acidic nature of the films (Dutta et al, 2009).

The moisture contents of the films are listed in Table 6.2. The slight increase of the moisture contents of the films prepared with grape and apple vinegars were obtained compared to the control film prepared with sole chitosan. The decrease in the moisture contents were observed for the films prepared with pomegranate and hawthorn vinegar which have higher thickness values. The results may indicate the strong indication of the vinegar components and the functional groups of the chitosan such as hydroxyl and amino groups and limitation of the hydrogen bond formation between biopolymer and the water (Wang et al, 2019).

Swelling % of the films were determined and compared to the sole chitosan film and the results are showed in Table 6.2 and indicating that all films were swelled. The highest swelling % was observed for the sole chitosan film after an hour and this might be related with the lack of crosslinking and also due to the hydrophilic units in chitosan structure (Rohindra et al, 2004 ; Cadogan et al, 2014). Grape vinegar film was swelled about 200 times greater than its initial weight and the swelling ratios were differentiated between the vinegar films probably depending on their raw material and its unique organic acid and phenolic contents. The organic compounds in vinegar may provide crosslinking like interactions thus decreasing the swelling % of the films from sole chitosan film. In Figure 6.6, the swelling % trends of the films are represented.

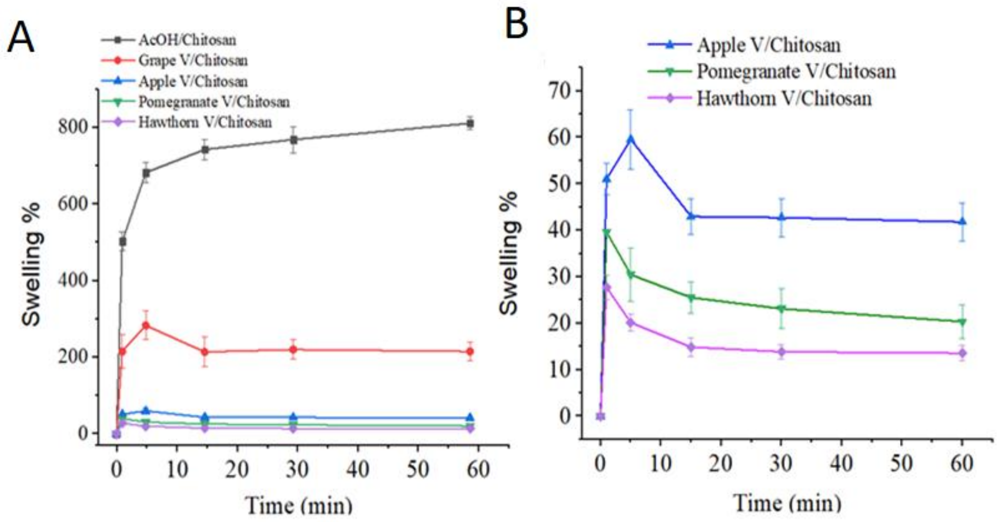

Figure 6.6 : The swelling % of the vinegar/chitosan film samples.

During the shelf life of the product the amount of moisture transfer is one of the crucial parameters for food quality. The moisture exchange should be prevented in order to maintain the freshness and the quality of the packaged food (Adjouman et al, 2017). As a result of the lack of crosslinking, sole chitosan film was showed the highest WVTR due to the less porous structure of the film and was found to be closer in the previous reports (Kalaycıoğlu et al, 2017). The rest of the films prepared with vinegar were showed slightly decrease. In the case of WVP, a slight increase was observed for the films prepared with vinegar varieties especially hawthorn and pomegranate vinegar films. The result may indicate the interaction of the components in vinegar and chitosan limiting the amount of moisture absorbed within the structure where permeance increased as in accordance with the swelling properties of the films. The results are presented in Table 6.3.

Table 6.3 : Physical properties of the vinegar/ chitosan film samples.

Film Sample	Moisture Content % ± SD	Swelling % (1 h) ± SD	WVTR (g/day.m^2) ± SD	WVP (g.mm/day.m^2.mm Hg) ± SD
AcOH/Chit	20.90 ± 3.06^b	811.00± 17.00^c	1132 ± 18^b	0.96 ± 0.02^a
GV/Chit	28.84 ± 2.72^b	215.00 ± 25.00^b	1011 ± 6^a	1.29 ± 0.01^b
AV/Chit	29.43 ± 1.22^b	41.79 ± 4.11^a	1017 ± 11^a	1.52 ± 0.02^c
HV/Chit	7.56 ± 1.10^a	13.56 ± 1.64^a	1121 ± 32^b	2.14 ± 0.06^d
PV/Chit	11.76 ± 2.48^a	20.32 ± 3.66^a	1027 ± 5^a	3.28 ± 0.01^e

n=2, ± SD: Standard Deviations. Different letters within a column indicate significant differences among film samples ($p < 0.05$). AcOH: Acetic acid, Chit: Chitosan, GV: Grape vinegar, AV: Apple vinegar, HV: Hawthorn vinegar, PV: Pomegranate vinegar.

The tensile properties of the films were also investigated. The elasticity of a potential food packaging material is significant due to its processability. Young's modulus is a measure of the strength and elasticity of a solid substance. The higher Young's modulus indicates lower flexibility. Among the films, the smaller Young's modulus was calculated for the film prepared with pomegranate vinegar, which is also the most flexible film. The rest of the vinegar films also showed a lower Young's modulus than the sole chitosan film. The significantly low Young's modulus of the film prepared with pomegranate vinegar is quite similar to rubber-like elastomers. The films prepared with especially pomegranate and hawthorn vinegars are flexible and highly suitable for the use as potential food packaging. The ultimate strength values of the films were decreased for the films prepared with vinegars which is in accordance with the Young's modulus. Furthermore, elongation at break % values were increased for the films prepared with vinegar varieties. This increase shows the materials higher ductility. The vinegar films can easily deform without breaking compared to the sole chitosan film. Pomegranate vinegar film showed higher elongation at break % may be related with the interaction of the biopolymer chains with the large phenolic compounds as discussed in 6.5.2. The results according to the tensile test are given in Table 6.4. The addition of glycerol into the apple vinegar film did not caused a significant flexibility such as pomegranate vinegar film since the added glycerol amount is limited. The physical properties of the films were mainly affected by the type of the vinegar. The report on the chitosan films suggested that the acid type used during film preparation such as acetic, malic, lactic or citric, changes the physical properties of the obtained material (Park et al, 2002).

Table 6.4 : Mechanical properties of the vinegar/chitosan film samples.

Film Sample	**Young's Modulus (MPa)**	**Ultimate Strength (MPa)**	**Elongation at Break %**
AcOH/Chit	1927	23.10	4.28
GV/Chit	1034	9.927	10.1
AV/Chit	660.4	11.43	9.44
HV/Chit	79.12	4.258	8.31
PV/Chit	0.663	0.151	22.2

AcOH: Acetic acid, Chit: Chitosan, GV: Grape vinegar, AV: Apple vinegar, HV: Hawthorn vinegar, PV: Pomegranate vinegar.

6.5.3 Antioxidant and antimicrobial properties of the prepared films

Food deterioration due to the reactive free radicals are usually overcome by using antioxidant substances as additives to the food itself or to the packaging material. As discussed in Section 2.3.3 vinegars are known for their antioxidant properties. The films prepared with vinegars were expected to have higher antioxidant power than the film prepared with dissolving chitosan in acetic acid. Pomegranate is an antioxidant rich fruit, due to its phenolic content (Bakır et al, 2017 ; Kalaycıoğlu and Erim, 2017). Also, the antioxidant power of the adulterated fruit juices of pomegranate with apple juice was showed significant decrease (Tezcan et al, 2009). Pomegranate peels were also employed for increasing antioxidant properties of the films (Zeng et al, 2021). Thus, the first part of the study the vinegar varieties were tested and as expected pomegranate vinegar showed higher antioxidant activity, followed by hawthorn, grape, and apple vinegar. The quite similar trend was observed from the analysis of the film samples and the pomegranate vinegar film was increased the antioxidant property of the sole chitosan film 11 times. Dissolving chitosan in vinegar varieties has enhanced the protective ability of the materials prepared for food packaging. The inhibition % results are provided in Figure 6.7 comparatively for film samples and fruit vinegars.

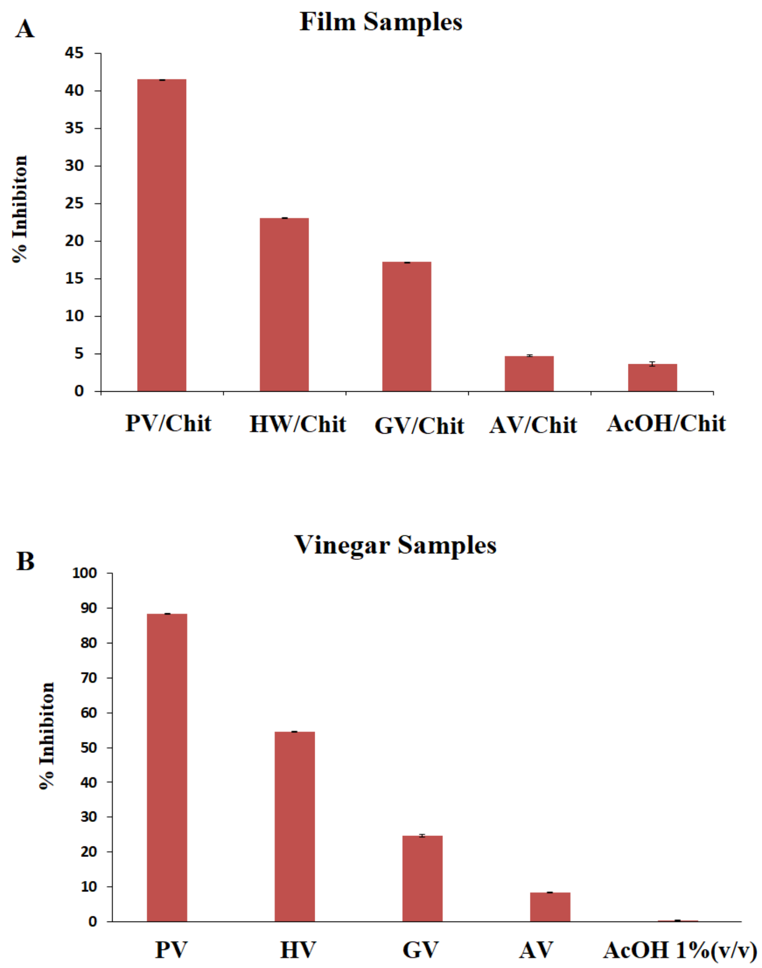

Figure 6.7 : DPPH inhibition % values of the A) Film samples B) Vinegar varieties. AcOH: Acetic acid, Chit: Chitosan, GV: Grape vinegar, AV: Apple vinegar, HV: Hawthorn vinegar, PV: Pomegranate vinegar.

The antimicrobial activity of a food packaging material is also enhancing the protection ability of the materials. The antimicrobial activity of chitosan towards gram-positive and gram-negative bacteria also some yeast and fungi were reported so far (Li and Zhuang, 2020) and also vinegar varieties known for their antimicrobial properties as discussed in Section 2.3.3. The antimicrobial activity of the films prepared in vinegar were tested against *Staphylococcus aureus* (*S. Aureus*) and *Escherichia coli* (*E. coli*). The findings proved that the vinegar addition enhanced the antimicrobial activity of the sole chitosan film for both bacteria type. The film prepared with pomegranate vinegar showed the highest protection ability towards *S. aureus*, followed by the film prepared with hawthorn vinegar and the results are shown in Figure 6.8 A. On the other hand, the films prepared with hawthorn vinegar, followed by pomegranate vinegar showed higher protection for *E. coli* and also the results are represented in Figure 6.8 B. The antimicrobial activities of the film samples in terms of logarithmic reduction of the viable microorganisms. The different enhancement in antimicrobial activity of the films depend on again the content of the vinegar varieties since different acids showed different antimicrobial activity (Cherrington et al, 1991).

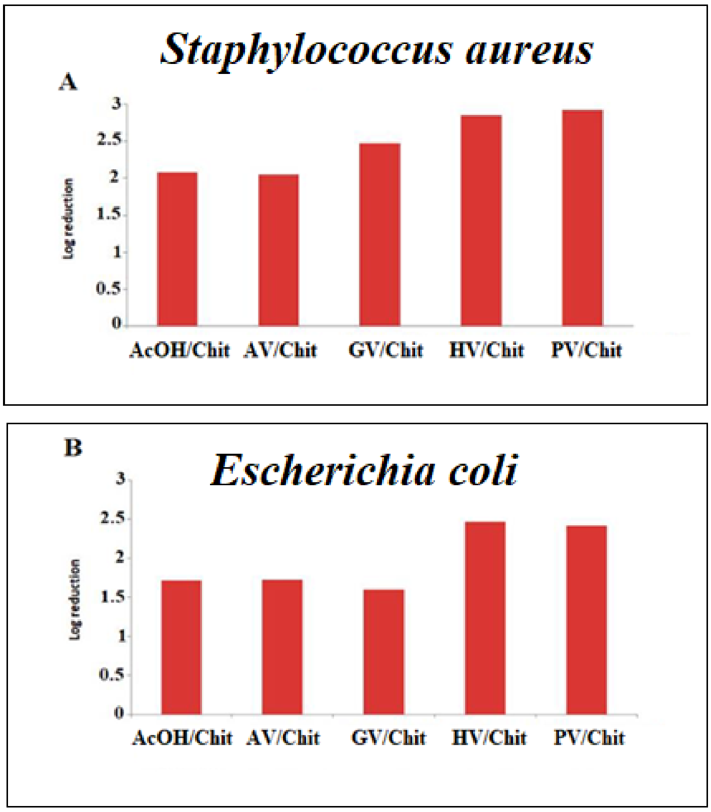

Figure 6.8 : Antimicrobial activities of the vinegar/chitosan film samples against A) *Staphylococcus aureus* B) *Escherichia coli*.

7. PREPARATION AND INVESTIGATION OF INTELLIGENT BIOPOLYMERIC FILM WITH pH SENSING ABILITY FOR FOOD FRESHNESS DETECTION BY USING PURPLE BASIL EXTRACT

7.1 Preparation of the Purple Basil Extract and Biopolymeric Films

Finely grounded and homogenized dried purple basil leaves were used and ten grams of the leaves were extracted with freshly boiled deionized water. Following to the incubation for 5 minutes in water bath the filtration was performed from Whatman No:41 filter paper. Freshly prepared extract was used for film preparations and the extract was protected from day light and heat and kept in the laboratory refrigerator wrapped with aluminum foil until use.

Citrus pectin and sodium alginate were both used to produce biopolymer films with optimized physical properties. For all film-forming solutions citrus pectin 1% (w/v) was dissolved in 1:1 (v/v) aqueous purple basil and water. Each film solution was allowed constant stirring at a rate of 300 rpm on a magnetic stirrer for 2 hours at room temperature. Then, an amount of sodium alginate corresponding to 0.5% (w/v) was weighed and added to the mixture and allowed to stir for 2 h more. Glycerol was added to the film-forming solution mixture (350 µL) and allowed to stir for an hour. During agitation of the film-forming solutions including purple basil extract, beakers were wrapped with aluminum foil in order to prevent anthocyanins from degradation.

As a control film sample for comparison of the enhancement of the purple basil film, a film was prepared by dissolving 1% (w/v) citrus pectin and 0.5% (w/v) sodium alginate in deionized water and after glycerol addition mixture was allowed to stir for a total of 5 hours at room temperature. All film forming solutions were poured into plastic 9 cm diameter Petri dishes and left to dry homogeneously in an Nüve EV 018 oven (Ankara, Türkiye) for 48 h at 40 °C.

The dried films after casting were not physically stable when placed in water. The films were disintegrating and solubilized, proving that they are not suitable for use in food-related applications. For this purpose, the films were subjected to crosslinking to produce films with water resistance. 3% (w/v) $CaCl_2$ solution was prepared and the dried films were immersed in 30 mL of this solution and left for 15 minutes. Following to the crosslinking, films were washed with deionized water to remove excess $CaCl_2$ and left to dry in dark and at room temperature for about 12 hours and then used for further analysis. The prepared films are shown in Figure 7.1.

Figure 7.1 : Digital photographs of purple basil and control films.

7.2 Investigation of Optical and Spectroscopic Properties of the Purple Basil Extract and Films

Color changes with respective pH of the purple basil extract were observed in the pH range of 2.0 to 12.0. pH levels of the extract adjusted with adding HCl or NaOH solutions and digital images were recorded. The UV-visible spectra of the extracts were measured by using a Shimadzu UV-1800 model UV-VIS spectrophotometer in the range of 400 and 800 nm. Ultra-purified water was the extraction solvent and thus used as the blank solution.

The film thickness of both films was measured with using a digital, hand-held caliper from five different random positions of the films. The average values were calculated used in further calculations.

The light transparency of the films was measured with Shimadzu UV-1800 model UV-VIS spectrophotometer. An apparatus was used for standard sample cell and the films were cut into 2×2.5 cm^2 dimensions. The transmittance spectrum of each film was obtained at the wavelength range of 200-800 nm against air was used as reference. The opacity values were calculated at 600 nm with the equation 6.1.

The Fourier transform infrared (FTIR) spectroscopic analysis of both films was performed by using an Agilent Cary 630 FTIR Spectrometer. The transmittance spectra of the films were recorded for both air as background and samples in the wavenumber range of 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1} and 64 scans.

7.3 Determination Physical Properties of the Prepared Films

Surface acidity of the biopolymeric films were assessed by using MColorpHast™ Merck (Darmstadt, Germany) pH indicator strips. The pH strips were placed on the wetted surfaces of each film until the strip was wet enough and the color change was evaluated according to the color change.

The amount of water present in the films, moisture contents (MC) were determined in according to the method explained in Section 6.3. 2 × 2 cm^2 pieces of each film was placed on the balance of the moisture analyzer and heated up to 105 ℃ with a constant rate of 1°C increment of 30 seconds. When the difference between masses reached 0.03%, moisture content was recorded. Measurements were triplicated.

The swelling properties of each film were determined according to the method explained in Chapter 6.3 with few changes. Films were cut into 2 × 2 cm^2 pieces and completely dried in an oven (Nüve EV 018) at 70 ℃ for 24 h. Then the initial weighing of the films was recorded, and films were rapidly immersed in 50 mL distilled water containing beakers shaken at 50 rpm at 25 ℃ in Nüve ST-402 shaking water bath (Ankara, Türkiye). At predetermined time intervals (1, 5, 15, 30, 45, and 60 mins to 2, 3, 24 hours) films were taken from the water, and excess surface water on the films was dried gently and carefully weighed. All measurements were conducted with three samples for each film. The swelling percentages (Swelling %) were calculated according to equation 6.2.

The water vapor permeabilities (WVP) and the water vapor transmission rate (WVTR) of the control and the purple basil films were determined gravimetrically as explained in Chapter 6.3 with minor changes. Both types of films were cut into circular shapes and then tightly mounted on the glass bottles containing distilled water 2 cm below the top. Each bottle was delicately weighed before and then placed in a desiccator at 25 °C preconditioned to 75% humidity with using saturated NaCl solution. The bottles were weighed with an electrical balance for definite time intervals. (Mettler Toledo, tare range: 0–100 g; sensitivity: 0.00001). The WVP and WVTR values were calculated according to the equations 6.3, 6.4, and 6.5 given at Chapter 6.3.

Also, physical integrity of the films was assessed as calculating the total water solubility (TWS) of each film. The prepared films were cut into 2 × 2 cm^2 dimensions and completely dried in an oven at 70 °C for 24 hours (Nüve EV 018). Then the initial weighing of the films was recorded, and films were immersed in 50 mL distilled water containing beakers at 25 °C placed in Nüve ST-402 shaking water bath and shaken at a constant rate of 50 rpm for 24 h. Then the pieces were carefully taken and dried in the oven at 70 °C for another 24 h to allow completely dry. Then the films were re-weighed. The difference between the masses was calculated as following equation 7.1 and the total water solubility % values were recorded. The experiment was triplicated for each film.

$$TWS\% = (\frac{M2-M1}{M1}) \times 100 \tag{7.1}$$

where M_1 and M_2 are masses of the films, where M_1 dried film before immersing in water, M_2 dried film following the immersing in water.

7.4 Determination Bioactivities of the Purple Basil Extract and the Films

The antioxidative power of the purple basil extract was determined with a well-known technique based on the measurement of radical scavenging activity by using DPPH solution. 0.05 M methanolic DPPH solution was prepared. The extract of dried leaves was diluted with water to five different concentrations and 40 μL of each solution was mixed with 1960 μL of DPPH solution and vortexed for 30 seconds. Then the solutions were incubated in the dark for 30 minutes at room temperature. The absorbance values of the solutions were measured at 517 nm using methanol as a blank solution with a Shimadzu UV-1800 model UV-VIS spectrophotometer. The antioxidant activity was reported as inhibition % and was calculated with equation given as 4.1. Then a calibration curve was constructed with % inhibition against mg/L of extract. Ascorbic acid was used as a standard substance and the same procedure was applied. The regression equations for basil extract and the standard ascorbic acid were found y = 24505x + 11.576, R^2 = 0.9708 and y = 2.252x + 0.0985, R^2 = 0.9927, respectively. The concentrations where 50% inhibition was achieved were reported as IC_{50} values.

The radical scavenging activities of the films were also determined by DPPH method. Between 2 mg to 25 mg film samples were mixed with 5 mL of distilled water. The mixture was vortexed for a minute and then ultrasonicated for 20 minutes. 50 μL from the solutions were mixed with 1950 μL of the DPPH solution, vortexed, and kept dark for 30 minutes, and again the absorbance values of the solutions were measured at 517 nm against methanol. The antioxidant activity reports as IC_{50} values where 50% inhibition was achieved. The equation of the calibration curve is y = 4.699x + 6.362, R^2 = 0.9996. All experiments were duplicated.

The total phenolic contents of the film solutions were evaluated based on the Folin-Ciocalteu method explained in Chapter 5.1.5 with some minor modifications. Three hundred μL of film extract was mixed with 1:10 diluted Folin-Ciocalteu's reagent (1.5 mL) and 7.5% (w/v) aqueous sodium carbonate solution (1.2 mL). The mixture was allowed to stand for 15 min at room temperature and absorbance was measured at 760 nm against water as blank. The total phenolic content was calculated from the calibration curve constructed with standard gallic acid solutions. The 5-point calibration equation is y = 0.0953x + 0.0014 with a regression coefficient of 0.9995. The results were expressed as mg gallic acid equivalents (GAE) in the g film sample. For the extraction of the film sample, 10 mg of film samples were extracted in 5 mL of deionized water. The mixture was vortexed for a minute and then ultrasonicated for 20 minutes. All experiments were duplicated.

As one of the major constituents of the basil extracts, the total anthocyanin content was determined for purple basil extract using the pH differential method. Anthocyanin pigments change their structures with pH reversibly, thus absorbance spectrums are also changing. The color differences at pH 1.0 and pH 4.5 were recorded using UV-Vis spectroscopy. The absorbance spectrums were obtained for the extract with different dilution ratios, to obtain absorbance values within the range of 0.4-0.9 at 516 nm. After the appropriate dilution ratio was determined, 10 mL from the diluted extract was taken and pH values were adjusted to pH 1.0 and 4.5 using 1 M and 0.1 M HCl solutions. Afterward, each solution was diluted to 50 mL using appropriate buffer solutions, potassium chloride buffer for pH 1.0, and sodium acetate buffer for pH 4.5. After incubating the solutions in the dark and in containers wrapped with aluminum foil in the refrigerator for 2 hours, absorbance (A) values were measured. The absorbances were measured against a blank cell filled with distilled water at 516 and 700 nm. To facilitate the comparison of the results with the literature they were expressed as cyanidin-3-glucoside (Cy-3-glc) equivalents by using equation 7.3

$$A = (A_{516} - A_{700})\, pH_{1.0} - (A_{516} - A_{700})\, pH_{4.5} \tag{7.2}$$

$$\text{Total anthocyanin amount (TAA) (mg/L)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \tag{7.3}$$

where MW is the molecular weight, DF is the total dilution factor, ε is the molar absorption coefficient, l is the path length. TAA calculated as cyanidin-3-glucoside as common anthocyanin, MW = 449.2 g/mol and ε= 26900 (L/mol.cm)

The released total anthocyanin amount (TAA) with time was calculated. 30 mg film samples were placed in a 50 mL distilled water-containing beaker wrapped with an aluminum foil and shaken at a constant rate of 150 rpm with IKA KS 260 (Staufen, Germany) orbital shaker at room temperature. The calculation of the TAA amount in the solution was determined by using the pH differential method. The same volume of 4 mL (2 mL used for each pH) was removed from the release medium and fresh distilled water was added each time. The released TAA from the purple basil film was calculated using the following equation. The experiment was duplicated.

$$\text{TAA Release } \% = \frac{M_t}{M_i} \times 100 \tag{7.4}$$

where M_t is the calculated TAA released at predetermined times (5 min, 15 min, 30 min, 1 h, 2 h, 4 h, and 6 h), M_i is the TAA amount present in the films after the crosslinking procedure.

Antibacterial activities of films were determined by quantifying the survival of bacteria held in intimate contact with film samples according to ISO 22196 (2007) standard. Two microorganism species representing Gram negatives and positives were used in the antibacterial assay of the films. Stock cultures of microorganisms were stored in Brain Heart Infusion Broth (Liofilchem) supplemented with 20% glycerol at −18 °C. Working cultures were grown on Nutrient Agar (Liofilchem) slants. The overnight slant cultures were transferred in 1/500 Nutrient Broth (Liofilchem) and the cell density of suspensions was adjusted to the 0.5 McFarland turbidity standard, which represents approximately 1.5 × 108 colony forming units (CFU)/mL. These suspensions were diluted in 1/500 Nutrient Broth to obtain a working concentration of about 1.0 × 106 CFU/mL. Test film samples of 50 x 50 mm square dimensions, sterilized under UV light, were placed in separate sterile Petri dishes and 200 µL of test inoculum of microorganisms was pipetted onto the film samples. Then, the inoculated film samples were covered with sterilized plain polypropylene (PP) film pieces of 40 x 40 mm to spread the inoculum on the test films. Sterile plain PP film pieces of the same size as the film samples were used as controls. Petri dishes containing the inoculated test and control films for 6 h at 35 °C under a relative humidity of above 90%.

The numbers of viable microorganisms on the test and control films were determined by plate count technique immediately after inoculation and after 6 h of contact time. The microorganisms on the films were recovered in D/E Neutralizing Broth (Acumedia, Lansing, USA). Then, D/E Neutralizing Broth and its decimal dilutions prepared with phosphate-buffered physiological saline were plated on Plate Count Agar (Liofilchem), and inoculated plates were incubated at 35°C for 48 hours. The resultant colonies were counted, and the number of viable microorganisms were calculated as log CFU/sample.

7.5 Application to a Real Food Sample and Determination of Changes in Color Properties

The developed potential intelligent film was tested for its applicability as an indicator of food freshness. Firstly, prepared films were placed in several buffer solutions with different pH values (2.0, 4.0, 6.0, 8.0, 10.0, and 12.0) where distinct and strong color changes were observed. Then, as a protein-rich food, fresh chicken breast meat was used for testing purposes. A 4 x 4 cm^2 square piece of film containing purple basil extract was placed inside the package, without touching the chicken breast, and the mouth of the petri dish was closed so as not to come into contact with air. The visual observations for chicken breast meat and the film sample indicating the freshness of the product were conducted in two different conditions of 4 ℃ and 25 ℃. After the change in the color of the film with the deterioration of the food, color properties were measured by using white Lenata cards from three different positions of each film pieces, and average values were calculated and total color change of the films were also calculated by the equation 7.5.

$$\Delta E = \sqrt{(L^*{}_2 - L^*{}_1)^2 + (a^*{}_2 - a^*{}_1)^2 + (b^*{}_2 - b^*{}_1)^2} \tag{7.5}$$

Where, L*1, a*1 and b*1 reference color parameters and L*2, a*2 and b*2 for changed color gives the ΔE color change.

7.6 Results and Discussions

7.6.1 Optical and spectroscopic properties of the purple basil extract and films

Anthocyanins are plant pigments in color and undergo structural changes with varying pH and the color change also accompanies the pH change due to the high color sensitivity (Prinsi et al, 2019). The characteristic purple color of the basil extract represents the intact color of the anthocyanins. A drastic decrease of pH at 2.0, the strong reddish color observed due to the flavylium cation, while the increase in the pH, around 4.0 - 5.0, hydration of flavylium decreases the strong red color and turns colorless to a grey-colored carbinol pseudobase form (Sani et al, 2021). Violet to bluish color is observed with an elevated pH (6.0 - 8.0) due to the quinonoidal base formation and further increases in pH to strong alkaline conditions, chalcone forms, and a yellowish color is seen (Sani et al, 2021 ; Hoffmann et al, 2022). The changes in the structures of anthocyanins are shown in Figure 7.2.

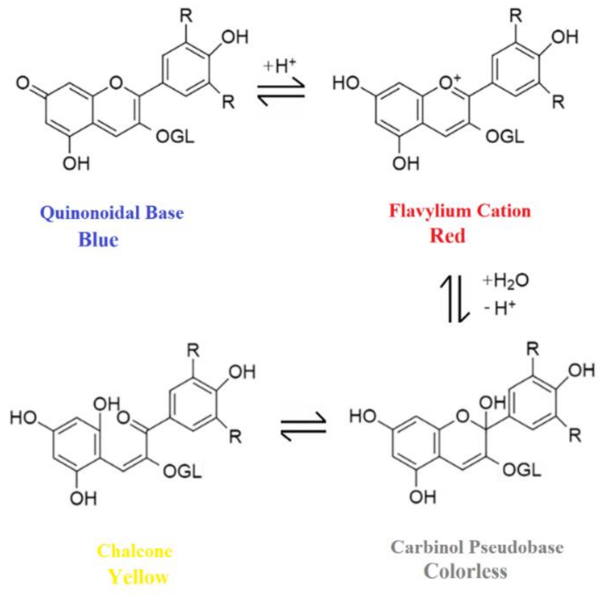

Figure 7.2 : The structural changes and respective colors of the anthocyanins at different pH values.

The color changes of basil extract regarding the pH changes between 2.0 and 12.0 were recorded, and a digital image of the color changes is represented in Figure 7.3.

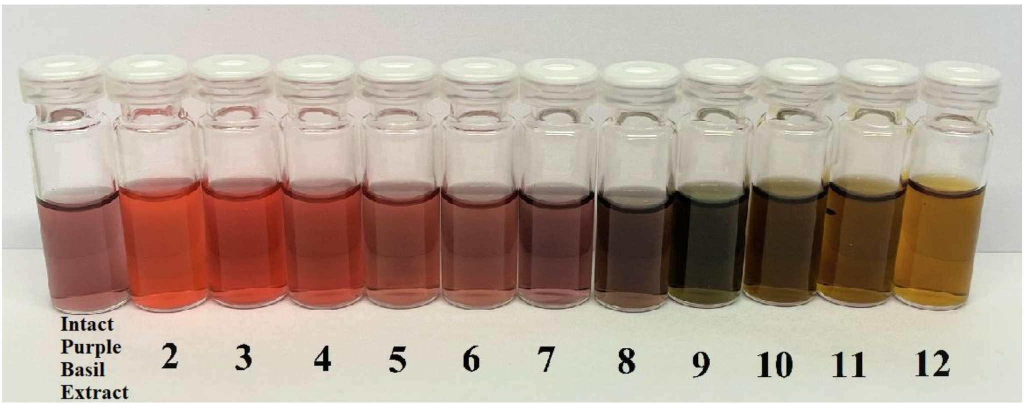

Figure 7.3 : The color changes of the purple basil extract at different pH values between 2.0 and 12.0.

The UV-Vis spectra of the extract were also recorded and the color changes proved with the changing maximum wavelengths. At lower pH values the maximum wavelength was 520 nm whereas with the increase in the pH, the maximum wavelengths were shifted to higher values around 595 nm and the peak intensities were decreased. In Figure 7.4 the UV-Visible spectra of the extract at different pH values are provided.

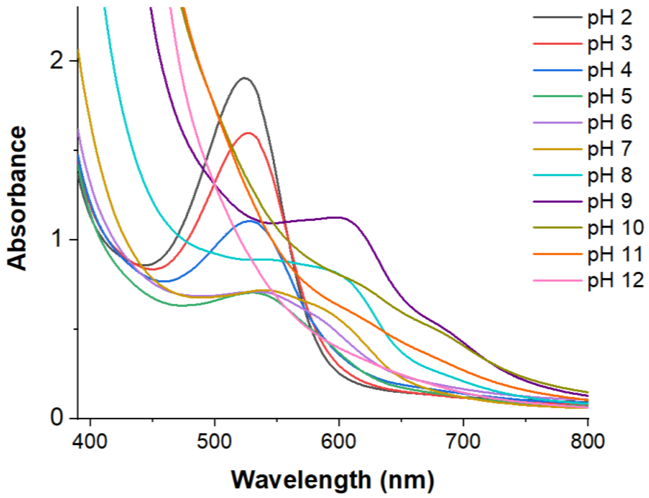

Figure 7.4 : UV-Visible spectra between the wavelength range of 400 and 800 nm of the purple basil extract at different pH values from 2.0 to 12.0.

The film thickness values were determined, and a slight decrease in thickness was observed for the purple basil film. The results are given in Table 7.1. The average thickness value of the films was used for the calculation of opacity values at 600 nm.

The opacity of the films was comparatively calculated. The opacity value of the film prepared with the extract was increased significantly and can be seen from Table 7.1, as a result of the natural and strong purple color. The light transmittance of the film was decreased as a result and contributed to the protection ability of the film. Higher opacity is desirable for a packaging material especially since the food could easily spoil by light (Oluwasina et al, 2019 ; Sudheesh et al, 2023). The UV-Vis light transmittance of the films was recorded and the film with basil was showed better light-blocking ability in the range of 200 and 400 nm, however still enough transparent to give a chance to visual examination of the product. The transmittance spectra of both films in between 200 and 800 nm are represented in Figure 7.5.

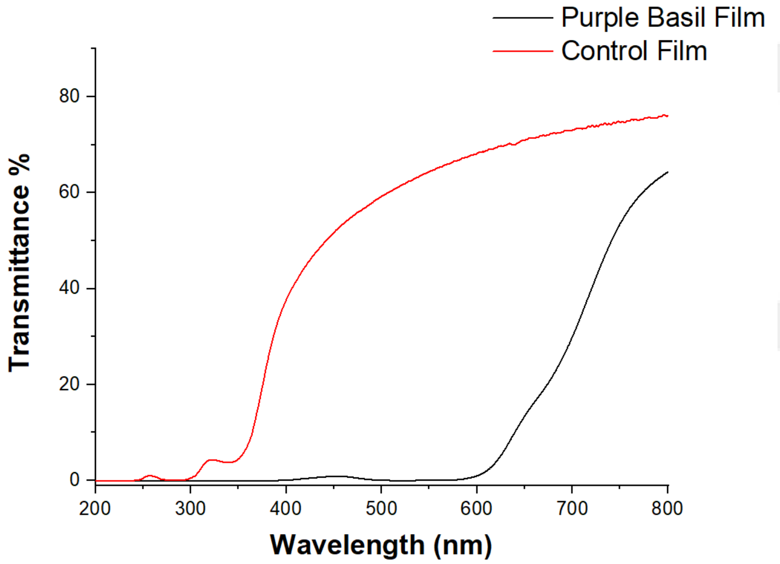

Figure 7.5 : The UV-Vis transmittance spectra of the purple basil and the control film prepared without extract.

The FTIR spectra of the both films were compared. Characteristic O-H stretching vibration of the sole pectin-alginate film was obtained at the frequency of 3267 cm^{-1}, asymmetric -COO^- stretching was obtained at 1595 $cm^{-1,}$ and symmetric -COO^- stretching was observed at 1418 cm^{-1}. Also, the -C=O vibrations of the -$COOCH_3$ group were observed at 1735 cm^{-1} (Makaremi et al, 2019). The minor shifts and the absence of a new peak indicates the lack of covalent interactions between the polymer blend and the extract. The spectra of the film obtained with the purple basil extract was similar, however the peak intensities were increased and slight shifts were obtained due to the interactions. O-H stretching and asymmetric -COO^- stretching vibrations were shifted to shorter wavenumbers, 3246 cm^{-1} and 1593 cm^{-1} respectively. The FTIR spectra of the both films are provided in Figure 7.6.

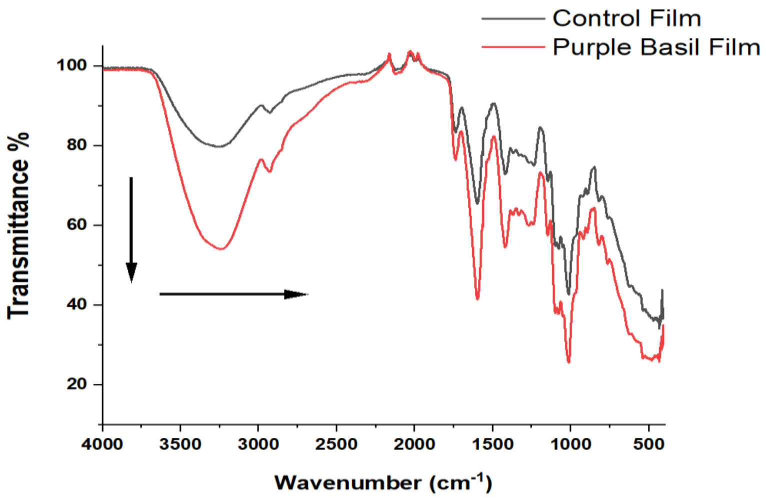

Figure 7.6 : The FTIR spectra of the purple basil film and the film prepared without extract.

7.6.2 Physical properties of the prepared films

The surface pH level of the film with the extract was found to be around 3. Purple basil extract pH was also measured as pH 5. The slightly acidic nature of the extract was decreased when incorporated in the pectin including structure. It was reported that acidic character of the films increases the protection ability of the material (Lund et al, 2020).

The moisture content of the films was evaluated and summarized in Table 7.1. The amount of water present at the room temperature and storage conditions were revealed that the film with the purple basil extract has lower moisture content. The result suggested that the interaction between the moisture and the material is limited due to the interaction between the plant extract and the polymer backbones of pectin and alginic acid. It is consistent with the previous study explained in Chapter 6 and also reported for the materials enriched with natural hibiscus extract (Peralta et al, 2019).

The swelling abilities of the films were investigated. The water uptake of the film with purple basil extract was found to be 1.5 times higher than the film prepared without extract. The swelling % of the films after 24 hours are presented in Table 7.1. Purple basil extract as many natural plants extract rich in hydrophilic constituents like polyphenols and makes water molecules contact easier with the biopolymeric film. The results are in accordance with the studies using natural extracts (Sun et al, 2017). The swelling trends of the films are given in Figure 7.7.

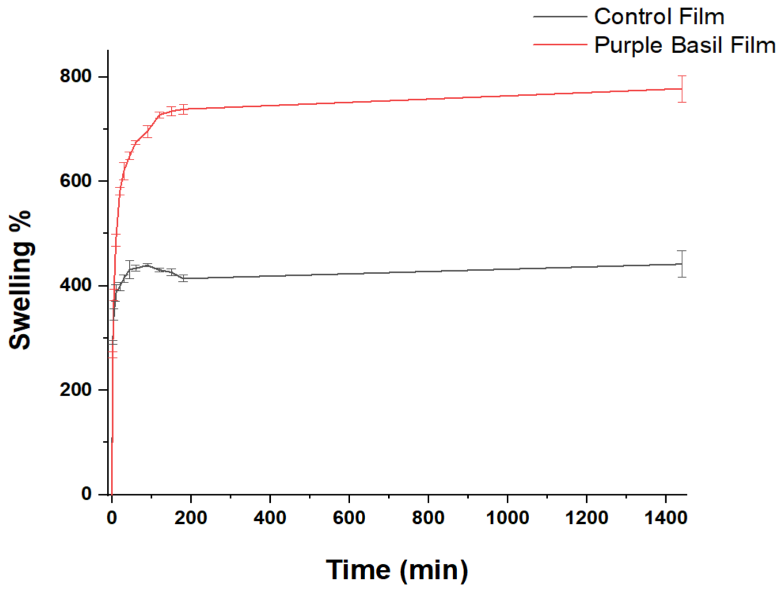

Figure 7.7 : The swelling % of the purple basil film and the film prepared without extract.

The water solubility % of the both films were assessed and the results are shown in Table 7.1 and proved that the films did not disintegrates in the water completely the water solubility amounts were consistent with the similar studies (Prietto et al, 2017 ; Wang et al, 2019 ; Zhang et al, 2020 ; Hoffmann et al, 2022). The water solubility of the purple basil included film was slightly increased due to the hydrophilic constituents which has affinity of water.

Barrier properties as WVP and WVTR of the films were also comparatively investigated. The WVTR of the film with the extract was slightly increased. The crosslinking like interactions between purple basil components and biopolymer blend increased the porous structure and thus WVTR. The WVP of the films were found to be similar and slightly decreased for the film with the extract, since it is related to the film thickness. The results are given in Table 7.1.

Table 7.1 : Physical and optical properties of the prepared biopolymeric film samples.

Physical Properties	Control Film	Purple Basil Film
Thickness (mm) * ± SD	0.124 ± 0.024	0.074 ± 0.011
Opacity (mm^{-1}) ± SD	0.77 ± 0.01	11.31 ± 0.04
Moisture Content % ± SD	26.96 ± 1.46	15.24 ± 1.93
Total Water Solubility % ± SD	21.08 ± 2.07	32.38 ± 0.82
Swelling % ± SD	441 ± 26	777 ± 25
WVTR (g/day.m^2) ± SD	12.92 ± 0.02	20.79 ± 0.01
WVP (g.mm/day.m^2.mmHg) ± SD	0.067 ± 0.001	0.065 ± 0.001

n=3, *n=5, ± SD: Standard Deviations.

7.6.3 Bioactive properties of the purple basil extract and the films

The antioxidant power of the purple basil extract and the film were evaluated by using the DPPH method and the IC_{50} values calculated. The extract was diluted for different ratios and then the IC_{50} value was found to be 1.57 ± 0.06 mg/mL. The IC_{50} value of the ascorbic acid standard substance was also tested and found to be 22.16 ± 3.87 mg/mL. The antioxidative power of the purple basil extract was found to be higher, it is a good natural product to use as an additive for food packaging applications.

The control film prepared without purple basil extract did not show any antioxidant activity while the antioxidant ability of the film containing basil extract as IC_{50} value was found to be 1.86 ± 0.7 mg film /mL. Comparatively high antioxidant activity increases the protection ability of a material which is strongly desired for the materials designed for use in food-related fields. Similar results were reported in the increased antioxidant activities of the films prepared using several extracts such as green tea, murta leaves, and borage added to chitosan and gelatin (Wang et al, 2012).

The total phenolic content of the purple basil film extract was evaluated. The control film showed no TPC, however the basil film showed a TPC value of 24.1 ± 1.66 mg GAE g^{-1} film sample. The high amounts of phenolic compounds increase the potential protection ability of the film. Purple basil is well known for its beneficial properties and the result supported the literature reports (Dhama et al, 2021).

The total anthocyanin amount of the basil extract was determined by the pH differential method. The method is based on the color change of the anthocyanins regarding the changes in their structures. The total monomeric anthocyanin content of the purple basil extract was found to be 4.87 ± 0.49 mg/g as cyanidin-3-glucoside (Cy-3-glc) equivalents. The results were found to be in the range of the literature values given for purple basil extracts (Gülhan et al, 2022).

The release of the anthocyanins from the biopolymeric materials enriched with a natural extract was investigated. Food freshness indicator property and potential food preservation ability of the prepared material depend on the anthocyanin content in the film. Thus, the release behavior of these compounds from the film into distilled water used as a water-containing food simulant was studied. After 2 hours total release % reached the limit of 27.06%. It was understood that a significant amount of the anthocyanins was still in the film structure. Therefore, it can be assumed that the decrease in the contents of anthocyanins did not reduce the indicator properties of the film. The limited release of these compounds may also prove the interaction between the purple basil extract and the biopolymers via hydrogen bonding (Wang et al, 2023). Similar release trends were also reported for biopolymeric films prepared with different natural extracts (Wu et al, 2021 ; Nguyen et al, 2022 ; Wang et al, 2023).

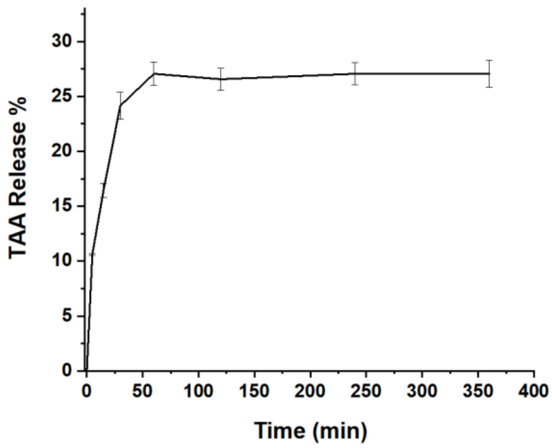

Figure 7.8 : Total anthocyanin release % of the film prepared with purple basil extract.

In the light of the previous studies which proved that the purple basil extracts and their essential oils have a great antimicrobial effect towards various bacteria (Stanojevic et al, 2017 ; Adıgüzel et al, 2005). In our study, the film enriched with the extract had great antimicrobial effect after 6 h of contact time for both gram-positive and gram-negative bacteria. The control film prepared without extract had very limited antimicrobial effect as expected. The improvement of the antimicrobial ability of the purple basil film was due to the natural extract addition. The results are represented comparatively in Figure 7.9.

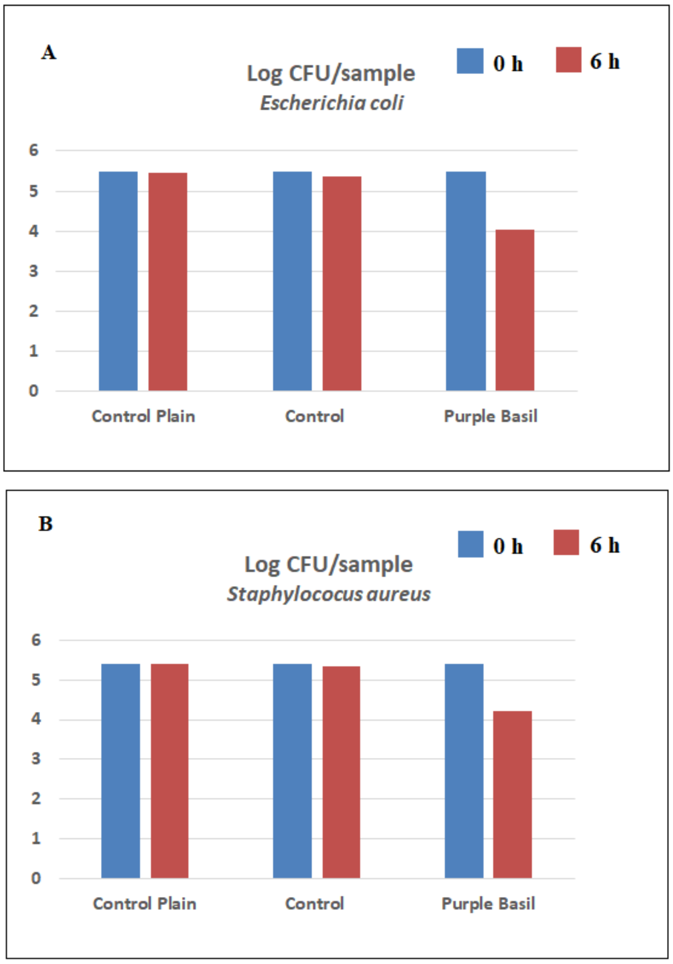

Figure 7.9 : Antimicrobial activities of the film prepared with and without purple basil extract against bacterial species A) *Escherichia coli* and B*) Staphylococcus aureus.* Control Plain: Sterile control polypropylene film and Control: Film prepared without purple basil extract.

7.6.4 Application to a real food sample and color changes of the films

As mentioned in Section 7.6.1 pH sensitive property of the purple basil extract, was tested by using several buffer solutions. The films were cut into rectangular-shaped pieces and were immersed in buffer solutions and the changing colors were recorded and given in Figure 7.10. The intact original purple color of the film was turned into strong pink at lower pH values and got paler around pH 4 and then get darker at high pH values and at pH 12 color was turned green. The prepared material proved to have color sensitivity to use as indicator of a pH change occurs during food spoilage.

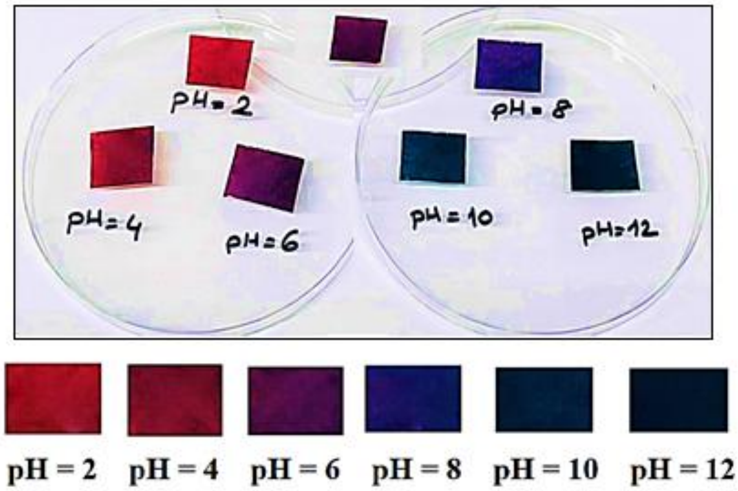

Figure 7.10 : Digital images of the color changes of the pH sensitive film in various buffer solutions.

Food spoilage is the term used for foods that are no longer safe to consume regarding to some chemical, microbial, or physical changes (Petruzzi et al, 2017). Both manufacturers and consumers share food safety concerns and promote the necessity of intelligent packaging materials. The film prepared with purple basil is a promising material for prolonging the shelf life of the product due to its antioxidant and antibacterial activity, but also color-changing property of the anthocyanins in the film enables the film as a natural indicator of food freshness. All species of animal meat and meat products are good sources of protein and are consumed in daily life and they all deteriorate even in chilled storage conditions (Triki et al, 2018). They all undergo spoilage depending on several factors such as pH, contents and structure of food and also storage conditions, and packaging (Comi, 2017). During deterioration, microbial growth and proteolysis occur (Triki et al, 2018). The peptides and free amino acids formed by proteolysis are used by microorganisms, and then the products such as biogenic amines form (Triki et al, 2017). As a result, the pH value of the chicken meat was found to increase with the storage time proportionally with the spoilage (Yang and Chen, 1993 ; Allen et al, 1997). The applicability of the prepared material as an intelligent packaging to serve as an indicator for food spoilage was tested by using chicken breast meat stored in two different conditions: at room temperature of 25 ℃ and in a refrigerator at 4 ℃. After 3 days the color change was started for the film placed inner surface of the package of the chicken breast stored at room temperature. The film placed on the inner surface of the package of the refrigerated chicken meat changed color after 12 days. The visual changes in both the color of the film and the meat are represented in Figure 7.11. The changes in color properties of the films are represented in Table 7.2. Redness (a) values were significantly decreased as expected due to the color change from purple to green. Yellowness (b) values were also slight, especially for the film stored with chicken breast meat at 25 °C. ΔE, the color differences were calculated and represented indicating the pH-sensitive color-changing ability of the prepared material.

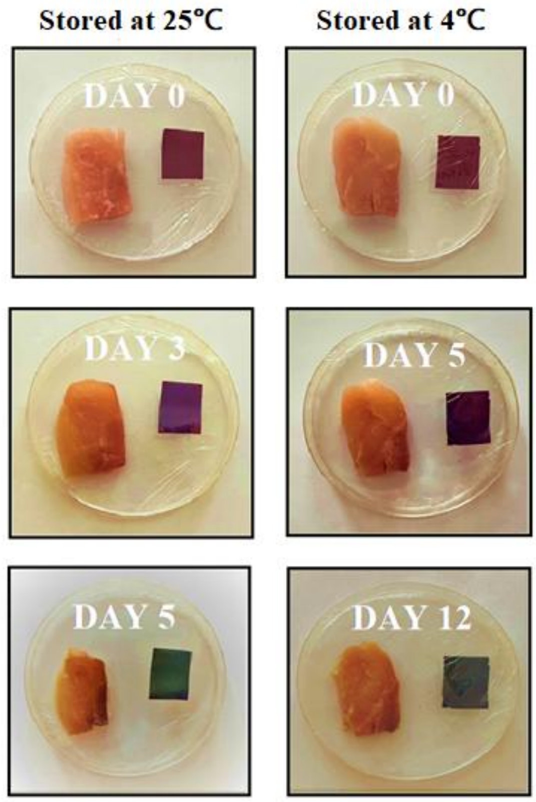

Figure 7.11 : The color change of the film prepared with the purple basil extract during 2 different storage conditions of chicken breast meat.

Table 7.2 : Changes in color properties of the films.

Sample	L* ± SD	a* ± SD	b* ± SD	C ± SD	WI ± SD	ΔE ± SD
Original Film	28.37 ± 0.32	10.07 ± 0.40	-2.18 ± 0.66	10.32 ± 0.51	29.67 ± 7.00	-
Stored at 25 °C	30.41 ± 1.48	4.52 ± 0.23	0.23 ± 0.11	4.53 ± 0.22	4.32 ± 0.70	43.16 ± 2.65
Stored at 4 °C	28.71 ± 0.80	2.27 ± 0.04	-1.19 ± 0.86	2.63 ± 0.40	19.14 ± 9.60	63.21 ± 6.82

n=3, ± SD: Standard Deviations. L*: Ligthness, a*: Redness, b*: Yellowness, C: Chroma, WI: Whiteness index, ΔE: Color difference.

8. CONCLUSIONS AND FUTURE ASPECTS

In the scope of the first part of the thesis study, the amounts of khellin and visnagin in five different organs: leaves, flowers, stems, roots and seeds of two significant medicinal *Ammi* species, *Ammi visnaga* and *Ammi majus* collected from Türkiye were investigated for the first time. Khellin and visnagin, which are the two main bioactive compounds responsible for the positive pharmacological properties of these species, were quantified by using a simple, rapid, low-cost and highly sensitive MEKC method. There are few studies on those herbs, which are significant traditional medicinal herbs. Furthermore, the free radical scavenging abilities of the plant parts were evaluated and compared.

In the second part of the thesis, the study covered the investigation of the rosmarinic and carnosic acid contents of *Salvia* species from Anatolia and also correlated antioxidant abilities and total phenolic contents. A simple and sensitive CE method was developed and successfully applied to numerous species. *Salvia* is an aromatic plant and traditional herbal medicine, and the results of the study contribute to the scientific literature on *Salvia* species. Rosmarinic acid is the most common bioactive component of *Salvia* species and is responsible for their important pharmacological properties. The correlation between the antioxidant power of plants and rosmarinic acid contents was also provided.

The results of CE analysis and bioactivity studies provide significant and reliable information for the scientists working on developing pharmaceutical formulations, including these medicinal herbs, which are highly rated topics of investigation. The results will also reveal the values of Anatolian Ammi and Salvia species in terms of trade and export.

In the third part of the study, bioactive vinegar species were used as both solvents and as an additive for the preparation of chitosan films. The mechanical properties of chitosan films were enhanced by using economical alternative natural sources as solvents. The films prepared with pomegranate, hawthorn, grape and apple kinds of vinegar showed enhanced antioxidant and antimicrobial activities. The obtained films have, UV-light blocking ability; but they are still transparent, also they are elastic enough to process, to conclude materials are promising, environmentally friendly, non-toxic and alternative active food packaging, instead of food packaging made from petroleum-based plastics.

In the last part of the study another natural source, purple basil extract was used for the preparation of an intelligent packaging material with alginate and pectin, to monitor the food freshness and quality in real-time. Additionally, the antioxidant and antimicrobial ability of the material was highly increased. The anthocyanin structures present in the extract provided a color change of the biopolymer matrix respective to the pH changes during food spoilage. The successful results were obtained and the physical and optical properties were also tested and the film proved to have a UV-light barrier ability. As a result, prepared material is a good alternative for use in food-related applications as a safe, edible, and the promising results from especially standard testing of antimicrobial activity and barrier properties. This alternative active and smart material for use as food packaging may lead to developing industrial scale production of these materials with further engineering and testing.

REFERENCES

Adigüzel, A., Güllüce, M., Şengül, M., Öğütcü, H., Şahin, F., & Karaman, I. (2005). Antimicrobial effects of Ocimum basilicum (Labiatae) extract, *Turkish Journal of Biology, 29*(3), 155-160.

Adjouman, Y. D., Nindjin, C., Tetchi, F. A., Dalcq, A. C., Amani, N. G., & Sindic, M. (2017). Water vapor permeability of edible films based on improved Cassava (Manihot esculenta Crantz) native starches, *Journal of Food Processing and Technology, 8*.

Ahmed, S. S. T., Fahim, J., & Abdelmohsen, U. R. (2021). Chemical and biological potential of Ammi visnaga (L.) Lam. and Apium graveolens L.: a review (1963-2020), *Journal of Advanced Biomedical and Pharmaceutical Sciences, 4*(3), 160-176.

Aider, M. (2010). Chitosan application for active bio-based films production and potential in the food industry, *LWT-Food Science and Technology, 43*(6), 837-842.

Allen, C. D., Russell, S. M., & Fletcher, D. L. (1997). The relationship of broiler breast meat color and pH to shelf-life and odor development, *Poultry Science, 76*(7), 1042-1046.

Alqasoumi, S. I., Alam, P., Anwer, M. K., & Abdel-Kader, M. S. (2014). Qualitative and quantitative analysis of khellin in Ammi visnaga fruits and pharmaceutical preparations using HPTLC and HPLC. *Journal of Liquid Chromatography & Related Technologies, 37*(1), 61-72.

Al-Snafi, A. E. (2013). Chemical constituents and pharmacological activities of Ammi majus and Ammi visnaga. A review, *International Journal of Pharmacy and Industrial Research, 3*(3), 257-265.

Altria, K. D. (1996). Fundamentals of capillary electrophoresis theory. Capillary Electrophoresis Guidebook: Principles, Operation, and Applications, 3-13.

Altria, K. D. (1996). Overview of capillary electrophoresis and capillary electrochromatography, *Journal of Chromatography A, 856*(1-2), 443-463.

Amoah, S. K., Sandjo, L. P., Kratz, J. M., & Biavatti, M. W. (2016). Rosmarinic acid–pharmaceutical and clinical aspects, *Planta Medica, 82*(05), 388-406.

Anicuta, S. G., Dobre, L., Stroescu, M., & Jipa, I. (2010). Fourier transform infrared (FTIR) spectroscopy for characterization of antimicrobial films containing chitosan. Analele Universită Ńii din Oradea Fascicula: Ecotoxicologie, Zootehnie şi Tehnologii de Industrie Alimentară, 1234-1240.

ASTM E96-95, (1994). Standard test methods for water vapor transmission of materials.. In *Annual Book of ASTM Standards*. Philadelphia, PA, USA: American Society for Testing Materials.

Badr, J. M., Hadad, G. M., Nahriry, K., & Hassanean, H. A. (2015). Validated HPLC method for simultaneous estimation of khellol glucoside, khellin and visnagin in Ammi visnaga L. fruits and pharmaceutical preparations. *Natural Product Research, 29*(7), 593-601.

Bahri, S., Jameleddine, S., & Shlyonsky, V. (2016). Relevance of carnosic acid to the treatment of several health disorders: Molecular targets and mechanisms, *Biomedicine & Pharmacotherapy, 84*, 569-582.

Bakir, S., Devecioglu, D., Kayacan, S., Toydemir, G., Karbancioglu-Guler, F., & Capanoglu, E. (2017). Investigating the antioxidant and antimicrobial activities of different vinegars, *European Food Research and Technology, 243,* 2083-2094.

Balart, R., Garcia-Garcia, D., Fombuena, V., Quiles-Carrillo, L., & Arrieta, M. P. (2021). Biopolymers from natural resources, *Polymers, 13*(15), 2532.

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa, *Molecules, 27*(4), 1326.

Başkan, S., Öztekin, N., & Erim, F. B. (2007). Determination of carnosic acid and rosmarinic acid in sage by capillary electrophoresis. *Food Chemistry, 101*(4), 1748-1752.

Beltagy, A. M., & Beltagy, D. M. (2015). Chemical composition of Ammi visnaga L. and new cytotoxic activity of its constituents khellin and visnagin. *Journal of Pharmaceutical Sciences and Research, 7*(6), 285.

Benzie, I. F. F. & Strain, J. J. (1996). Ferric reducing ability of plasma (FRAP) as a measure ofantioxidant power: the FRAP assay, *Analytical Biochemistry, 239*,70–76.

Birtić, S., Dussort, P., Pierre, F. X., Bily, A. C., Roller, M. (2015). Carnosic acid, *Phytochemistry, 115*, 9-19.

Blois, M. S. (1958) Antioxidant determinations by the use of a stable free radical, Nature, *181*, 1199–1200.

Bonoli, M., Pelillo, M., & Lercker, G. (2003). Fast separation and determination of carnosic acid and rosmarinic acid in different rosemary (Rosmarinus officinalis) extracts by capillary zone electrophoresis with ultra violet-diode array detection, *Chromatographia, 57*, 505-512.

Brás, T., Rosa, D., Gonçalves, A. C., Gomes, A. C., Alves, V. D., Crespo, J. G., ... & Neves, L. A. (2020). Development of bioactive films based on chitosan and Cynara cardunculus leaves extracts for wound dressings, *International Journal of Biological Macromolecules, 163*, 1707-1718.

Breadmore, M. C. (2009). Electrokinetic and hydrodynamic injection: making the right choice for capillary electrophoresis. *Bioanalysis, 1*(5), 889-894.

Brighenti, F., Castellani, G., Benini, L., Leopardi, E., Crovetti, R., Testolin, G. (1995). Effect of neutralized and native vinegar on blood glucose and acetate responses to a mixed meal in healthy subjects, *European Journal of Clinical Nutrition, 49*, 242–7.

Budak, H. N., Kumbul Doguc, D., Savas, C. M., Seydim, A. C., Kök Tas, T., Ciris, I. M., Güzel-Seydim, Z. B. (2011). Effects of apple cider vinegars produced with different techniques on blood lipids in high-cholesterol-fed rats, *Journal of Agricultural and Food Chemistry, 59*, 6638–44.

Budak, N. H., Aykin, E., Seydim, A. C., Greene, A. K., & Guzel-Seydim, Z. B. (2014). Functional properties of vinegar. *Journal of Food Science, 79*(5), R757-R764.

Butler, M.S. (2008). Natural products to drug: Natural product-derived compounds in clinical trials. *Natural Product Reports, 25*, 475-516.

Cadogan, E. I., Lee, C. H., Popuri, S. R., & Lin, H. Y. (2014). Effect of solvent on Physico-chemical properties and antibacterial activity of chitosan membranes, *International Journal of Polymeric Materials and Polymeric Biomaterials, 63*(14), 708-715.

Camilleri, P. (Ed.). (1997). Capillary electrophoresis: theory and practice. CRC press.

Cao, J., Wei, J., Tian, K., Su, H., Wan, J., Li, P. (2014). Simultaneous determination of seven phenolic acids in three Salvia species by capillary zone electrophoresis with β-cyclodextrin as modifier, *Journal of Separation Science, 37*, 3738-3744.

Cao, L., Lu, W., Mata, A., Nishinari, K., & Fang, Y. (2020). Egg-box model-based gelation of alginate and pectin: A review, *Carbohydrate Polymers, 242***,** 116389.

Ch, M., Naz, S., Sharif, A., Akram, M., & Saeed, M. (2015). Biological and pharmacological properties of the sweet basil (Ocimum basilicum), *British Journal of Pharmaceutical Research, 7*(5), 330-339.

Chen, H., Chen, T., Giudici, P., & Chen, F. (2016). Vinegar functions on health: Constituents, sources, and formation mechanisms, *Comprehensive Reviews in Food Science and Food Safety, 15*(6), 1124-1138.

Cherrington, C. A., Hinton, M., Mead, G. C., & Chopra, I. (1991). Organic acids: chemistry, antibacterial activity and practical applications. Advances in microbial physiology, 32, 87-108.

Comi, G. (2017). Spoilage of meat and fish. In The microbiological quality of food (pp. 179-210). Woodhead Publishing.

Conner, H. A., & Allgeier, R. J. (1976). Vinegar: its history and development. In Advances in Applied Microbiology (Vol. 20, pp. 81-133). Academic Press.

D'Amelio Sr, F. S. (1998). Botanicals: a phytocosmetic desk reference. CRC Press.

Dhama, K., Sharun, K., Gugjoo, M. B., Tiwari, R., Alagawany, M., Iqbal Yatoo, M., ... & Farag, M. R. (2023). A comprehensive review on chemical profile and pharmacological activities of Ocimum basilicum. *Food Reviews International, 39*(1), 119-147.

Dias, D. A., Urban, S., & Roessner, U. (2012). A historical overview of natural products in drug discovery. *Metabolites*, *2*(2), 303-336.

Dincheva, I., Badjakov, I., & Galunska, B. (2023). New insights into the research of bioactive compounds from plant origins with nutraceutical and pharmaceutical potential. *Plants*, *12*(2), 258.

Duarte, J., Torres, A. I., & Zarzuelo, A. (2000). Cardiovascular effects of visnagin on rats. *Planta Medica*, 66(01), 35-39.

Dutta, P. K., Tripathi, S., Mehrotra, G. K., & Dutta, J. (2009). Perspectives for chitosan based antimicrobial films in food applications, *Food Chemistry*, *114*(4), 1173-1182.

Dziubakiewicz, E., Buszewski, B. (2013). Principles of Electromigration Techniques, In Electromigration Techniques, B. Buszewski (Ed.), Springer, Berlin, Heidelberg.

Ebihara, K., & Nakajima, A. (1988). Effect of acetic acid and vinegar on blood glucose and insulin responses to orally administered sucrose and starch, *Agricultural and Biological Chemistry*, *52*(5), 1311-1312.

El-Domiaty, M. M. (1992). Improved high-performance liquid chromatographic determination of khellin and visnagin in Ammi visnaga fruits and pharmaceutical formulations. *Journal of Pharmaceutical Sciences, 81*(5), 475-478.

Entani, E., Asai, M., Tsujihata, S., Tsukamoto, Y., Ohta, M. (1998). Antibacterial action of vinegar against food-borne pathogenic bacteria including Escherichia coli O157:H7, *Journal of Food Protection, 61*(8), 953-959.

Fan, H. Y., Shya, L. J., Abdul Hamid, M., Mamat, H., Akanda, J. H., Amin, M., & Faridah, S. (2021). A Short Review on the Potential of Fruits for the Production of Vinegar with Functional Properties. *Transactions on Science and Technology, 8*(3-2), 159-163.

Farhat, M. B., Landoulsi, A., Chaouch-Hamada, R., Sotomayor, J. A., & Jordán, M. J. (2013). Characterization and quantification of phenolic compounds and antioxidant properties of Salvia species growing in different habitats. *Industrial Crops and Products*, *49*, 904-914.

Fekete, A., Schmitt-Kopplin, P. (2007). Chapter-15 Capillary Electrophoresis, Food Toxicants Analysis, Techniques, Strategies and Developments, 561-597.

Filip, S. (2017). Basil (Ocimum basilicum L.) a source of valuable phytonutrients. Int. *Journal of Clinical Nutrition and Dietetics, 3*, 118.

Garcia M. A., Marina, M. L., Ríos, A., Valcárcel, M. (2005). Separation modes in capillary electrophoresis Marina, M. L., Ríos, A., & Valcárcel, M. (Eds.). In Analysis and detection by capillary electrophoresis. Elsevier.

Garcia-Parilla, M. C., Gonzalez, G. A., Heredia, F. J., Troncoso, A. M. (1997). Differentiation of wine vinegars based on phenolic composition, *Journal of Agricultural and Food Chemistry, 45*, 3487–92.

Gezek, G., Hashemi, P., Kalaycıoğlu, Z., Kaygusuz, H., Sarıoğlu, G., Döker, S., ... & Bedia Erim, F. (2019). Evaluation of some Turkish Salvia species by principal component analysis based on their vitamin B2, mineral composition, and antioxidant properties, *LWT,100*, 287–293.

Ghorbani, A., & Esmaeilizadeh, M. (2017). Pharmacological properties of Salvia officinalis and its components, *Journal of Traditional and Complementary Medicine*, *7*(4), 433-440.

Giraldo, J. D., & Rivas, B. L. (2021). Direct ionization and solubility of chitosan in aqueous solutions with acetic acid, *Polymer Bulletin, 78*, 1465-1488.

Gonçalves, C., Ferreira, N., & Lourenço, L. (2021). Production of low molecular weight chitosan and chitooligosaccharides (COS): A review, *Polymers*, *13*(15), 2466.

Gupta, I., Cherwoo, L., Bhatia, R., & Setia, H. (2022). Biopolymers: Implications and application in the food industry. *Biocatalysis and Agricultural Biotechnology*, 102534.

Gurnani, N., Mehta, D., Gupta, M., & Mehta, B. K. (2014). Natural products: source of potential drugs. *African Journal of Basic & Applied Science, 6*(6), 171-186.

Gülhan, A., Coklar, H., & Akbulut, M. (2022). Influence of drying method and infusion time on purple basil leaves tea, *International Journal of Secondary Metabolite*, *9*(4), 457-466.

Günaydın, K. and Erim F. B. (2002). Determination of khellin and visnagin in Ammi visnaga fruits by capillary electrophoresis. *Journal of Chromatography A. 954* (1-2), 291–294.

Günaydin, K., & Beyazit, N. (2004). The chemical investigations on the ripe fruits of Ammi visnaga (Lam.) Lamarck growing in Turkey. *Natural Product Research*, *18*(2), 169-175.

Hailu, S., Admassu, S., & Jha, K. (2012). Vinegar production technology—An overview, *Beverage Food World, 2*, 29-32.

Halliwell, B. (2011). Free radicals and antioxidants–quo vadis?, *Trends in Pharmacological Sciences*, *32*(3), 125-130.

Hamzalıoğlu, A. and Gökmen, V. (2024). Interaction between bioactive carbonyl compounds and asparagine and impact on acrylamide. In Acrylamide in Food, 433-455. Academic Press.

Harvengt, C., & Desager, J. P. (1983). HDL-cholesterol increase in normolipaemic subjects on khellin: a pilot study, *International Journal of Clinical Pharmacology Research, 3*(5), 363-366.

Hashim, S., Jan, A. S. A. D., Marwat, K. B., & Khan, M. A. (2014). Phytochemistry and medicinal properties of Ammi visnaga (Apiacae), *Pakistan Journal of Botany, 46*(3), 861-867.

Hernández-Saavedra, D., Pérez-Ramírez, I. F., Ramos-Gómez, M., Mendoza-Díaz, S., Loarca-Pina, G., & Reynoso-Camacho, R. (2016). Phytochemical characterization and effect of Calendula officinalis, Hypericum perforatum, and Salvia officinalis infusions on obesity-associated cardiovascular risk, *Medicinal Chemistry Research, 25*, 163-172.

Hidalgo, P. J., Ubera, J. L., Tena, M. T., & Valcárcel, M. (1998). Determination of the carnosic acid content in wild and cultivated Rosmarinus officinalis, *Journal of Agricultural and Food Chemistry, 46*(7), 2624-2627.

Hoffmann, T. G., Angioletti, B. L., Bertoli, S. L., & de Souza, C. K. (2022). Intelligent pH-sensing film based on jaboticaba peels extract incorporated on a biopolymeric matrix, *Journal of Food Science and Technology, 59(*3), 1001-1010.

Honarkar, H., & Barikani, M. (2009). Applications of biopolymers I: chitosan, *Monatshefte für Chemie-Chemical Monthly, 140*, 1403-1420.

Hossain, M. A., & Al Touby, S. (2020). Ammi majus an endemic medicinal plant: A review of the medicinal uses, pharmacological and phytochemicals, *Annual Toxicology, 2*, 9-14.

Hurtado, A., Aljabali, A. A., Mishra, V., Tambuwala, M. M., & Serrano-Aroca, Á. (2022). Alginate: Enhancement strategies for advanced applications, *International Journal of Molecular Sciences, 23*(9), 4486.

Inngjerdingen, K. T., Patel, T. R., Chen, X., Kenne, L., Allen, S., Morris, G. A., ... & Paulsen, B. S. (2007). Immunological and structural properties of a pectic polymer from Glinus oppositifolius, *Glycobiology, 17*(12), 1299-1310.

ISO 22196 (2007). Plastics—Measurement of Antibacterial Activity on Plastics Surfaces.

Jackson, C. L., Dreaden, T. M., Theobald, L. K., Tran, N. M., Beal, T. L., Eid, M., ... & Mohnen, D. (2007). Pectin induces apoptosis in human prostate cancer cells: correlation of apoptotic function with pectin structure, *Glycobiology, 17*(8), 805-819.

Jakovljević, M., Jokić, S., Molnar, M., Jašić, M., Babić, J., Jukić, H., & Banjari, I. (2019). Bioactive profile of various Salvia officinalis L. preparations, *Plants, 8*(3), 55.

Johnston, C. S., & Gaas, C. A. (2006). Vinegar: medicinal uses and antiglycemic effect, *Medscape General Medicine, 8*(2), 61.

Jordán, M. J., Lax, V., Rota, M. C., Lorán, S., & Sotomayor, J. A. (2012). Relevance of carnosic acid, carnosol, and rosmarinic acid concentrations in the in vitro antioxidant and antimicrobial activities of Rosmarinus officinalis (L.) methanolic extracts, *Journal of Agricultural and Food Chemistry, 60*(38), 9603-9608.

Jorgenson, J. W., & Lukacs, K. D. (1985). Capillary Zone Electrophoresis. In Journal of Chromatography Library (Vol. 30, pp. 121-131). Elsevier.

Kalaycıoğlu, Z., & Erim, F. B. (2017). Total phenolic contents, antioxidant activities, and bioactive ingredients of juices from pomegranate cultivars worldwide, *Food Chemistry, 221*, 496-507.

Kalaycıoğlu, Z., Torlak, E., Akın-Evingür, G., Özen, İ., & Erim, F. B. (2017). Antimicrobial and physical properties of chitosan films incorporated with turmeric extract, *International Journal of Biological Macromolecules, 101*, 882-888.

Kalaycıoğlu, Z., & Erim, F. B. (2022). Capillary electrophoresis: basic principles. Capillary Electrophoresis in Food Analysis, 1. Castro-Puyana, M., Herrero, M., & Marina, M. L. (Eds.). Bentham Sicence Publishers.

Kamiloglu, S., Tomas, M., Ozdal, T., Yolci-Omeroglu, P., & Capanoglu, E. (2021). Bioactive component analysis. In Innovative food analysis, 41-65. Academic Press.

Katiyar, C., Gupta, A., Kanjilal, S., & Katiyar, S. (2012). Drug discovery from plant sources: An integrated approach. *Ayu, 33*(1), 10.

Kaygusuz, H., Uysal, M., Adımcılar, V., & Erim, F. B. (2015). Natural alginate biopolymer montmorillonite clay composites for vitamin B2 delivery, *Journal of Bioactive and Compatible Polymers, 30*(1), 48-56.

Kelebek, H., Kadiroğlu, P., Demircan, N. B., & Selli, S. (2017). Screening of bioactive components in grape and apple vinegars: Antioxidant and antimicrobial potential, *Journal of the Institute of Brewing, 123*(3), 407-416.

Khalil, N., Bishr, M., Desouky, S., & Salama, O. (2020). Ammi visnaga L., a potential medicinal plant: A review, *Molecules, 25*(2), 301.

Khazaei, N., Esmaiili, M., Djomeh, Z. E., Ghasemlou, M., & Jouki, M. (2014). Characterization of new biodegradable edible film made from basil seed (Ocimum basilicum L.) gum, *Carbohydrate Polymers, 102*, 199-206.

Kim, M. J., Moon, Y., Tou, J. C., Mou, B., & Waterland, N. L. (2016). Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.). *Journal of Food Composition and Analysis, 49*, 19-34.

Kim, S. K. (Ed.). (2010). Chitin, chitosan, oligosaccharides and their derivatives: biological activities and applications. CRC Press.

Koriem, K. M., Asaad, G. F., Megahed, H. A., Zahran, H., & Arbid, M. S. (2012). Evaluation of the antihyperlipidemic, anti-inflammatory, analgesic, and antipyretic activities of ethanolic extract of Ammi majus seeds in albino rats and mice, *International Journal of Toxicology, 31*(3), 294-300.

Kostenko, A., Swioklo, S., Connon, C. J. (2022) Alginate in corneal tissue engineering, *Biomedical Materials, 17*, 022004.

Koşar, M., Göger, F., & Başer, K. H. C. (2011). In vitro antioxidant properties and phenolic composition of Salvia halophila Hedge from Turkey. *Food Chemistry, 129*(2), 374-379.

Kou, S. G., Peters, L. M., & Mucalo, M. R. (2021). Chitosan: A review of sources and preparation methods, *International Journal of Biological Macromolecules, 169*, 85-94.

Kuhn, R., & Hoffstetter-Kuhn, S. (2013). Capillary electrophoresis: principles and practice. Springer Science & Business Media.

Kumar, M. N. R. (2000). A review of chitin and chitosan applications, *Reactive and Functional Polymers, 46*(1), 1-27.

Kursinszki, L., Troilina, J., & Szoke, E. (2000). HPLC-determination of visnagin in genetically transformed root cultures of Ammi visnaga growing in liquid medium. *Chemia Analityczna, 45*(2), 229-236.

Landers, J. P. (Ed.). (2007). Handbook of capillary and microchip electrophoresis and associated microtechniques. CRC press.

Laranjinha, J. A., Almeida, L. M., Madeira, V. M. (1994). Reactivity of dietary phenolic acids with peroxyl radicals: antioxidant activity upon low density lipoprotein peroxidation, *Biochemical Pharmacology, 48*, 487-494.

Lechnou, Christopher N. (2008) Capillary Electrophoresis Used As An Alternative to HPLC for Pharmaceutical Analysis of Antifungal Agents. Retrieved from 1. https://jdc.jefferson.edu/diss_masters/1

Lee, K. Y., & Mooney, D. J. (2012). Alginate: properties and biomedical applications, *Progress in polymer science, 37*(1), 106-126.

Lele, M., Lele, S. M., Petersen, J. R., & Mohammad, A. (2001). Capillary electrophoresis: General overview and applications in the clinical laboratory. *Clinical and Forensic Applications of Capillary Electrophoresis*, 3-19.

Li, J., & Zhuang, S. (2020). Antibacterial activity of chitosan and its derivatives and their interaction mechanism with bacteria: Current state and perspectives, *European Polymer Journal, 138*, 109984.

Li, P., Liu, A., Li, Y., Yuan, B., Xiao, W., Liu, Z., ... & Lin, H. (2019). Development and validation of an analytical method based on HPLC-ELSD for the simultaneous determination of rosmarinic acid, carnosol, carnosic acid, oleanolic acid and ursolic acid in rosemary, *Molecules, 24*(2), 323.

Li, S. F. Y. (1992). Capillary electrophoresis: principles, practice and applications. Elsevier.

Lund, P. A., De Biase, D., Liran, O., Scheler, O., Mira, N. P., Cetecioglu, Z., ... & O'Byrne, C. (2020). Understanding how microorganisms respond to acid pH is central to their control and successful exploitation, *Frontiers in Microbiology, 11*, 556140.

Makaremi, M., Yousefi, H., Cavallaro, G., Lazzara, G., Goh, C. B. S., Lee, S. M., ... & Pasbakhsh, P. (2019). Safely dissolvable and healable active packaging films based on alginate and pectin, *Polymers, 11*(10), 1594.

Mandal, S., Basu, S.K., Sa, B. (2009). Sustained release of a water-soluble drug from alginate matrix tablets prepared by wet granulation method, *AAPS PharmSciTech, 10,* 1348-1356.

Marina, M. L., Ríos, A., & Valcárcel, M. (2005). Fundamentals of capillary electrophoresis**,** Marina, M. L., Ríos, A., & Valcárcel, M. (Eds.). Analysis and detection by capillary electrophoresis. Elsevier.

Martelli, P., Bovalini, L., Ferri, S., & Franchi, G. G. (1984). Rapid separation and quantitative determination of khellin and visnagin in Ammi visnaga (L.) Lam. fruits by high-performance liquid chromatography. *Journal of Chromatography A. 301*, 297-302.

Martin, A. (2015). 4.4 Lab Colour Space and Delta E Measurements. Graphic design and print production fundamentals, 95.

McClements, D. J. (2009). Biopolymers in food emulsions. In Modern biopolymer science,129-166. Academic press.

Melro, E., Antunes, F. E., da Silva, G. J., Cruz, I., Ramos, P. E., Carvalho, F., & Alves, L. (2020). Chitosan films in food applications. Tuning film properties by changing acidic dissolution conditions, *Polymers*, *13*(1), 1.

Miraj, S., & Kiani, S. (2016). A review study of therapeutic effects of Salvia officinalis L, *Der Pharmacia Lettre*, *8*(6), 299-303.

Mohnen, D. (2008). Pectin structure and biosynthesis, *Current Opinion in Plant Biology*, *11*(3), 266-277.

Molelekoa, T. B., Regnier, T., da Silva, L. S., & Augustyn, W. A. (2018). Potential of marula (Sclerocarya birrea subsp. caffra) waste for the production of vinegar through surface and submerged fermentation, *South African Journal of Science*, *114*(11-12), 1-6.

Myers, J. A., Curtis, B. S., & Curtis, W. R. (2013). Improving accuracy of cell and chromophore concentration measurements using optical density. *BMC Biophysics*, *6*(1), 1-16.

Mousavi, L., Salleh, R. M., & Murugaiyah, V. (2018). Phytochemical and bioactive compounds identification of Ocimum tenuiflorum leaves of methanol extract and its fraction with an anti-diabetic potential, *International Journal of Food Properties, 21*(1), 2390-2399.

Na, L., Chu, X., Jiang, S., Li, C., Li, G., He, Y., ... & Sun, C. (2016). Vinegar decreases blood pressure by down-regulating AT1R expression via the AMPK/PGC-1α/PPARγ pathway in spontaneously hypertensive rats, *European Journal of Nutrition, 55*, 1245-1253.

Nadeem, M., Imran, M., Aslam Gondal, T., Imran, A., Shahbaz, M., Muhammad Amir, R., ... & Martins, N. (2019). Therapeutic potential of rosmarinic acid: A comprehensive review, *Applied Sciences, 9*(15), 3139.

Nguyen, T. T., Pham, B. T. T., Le, H. N., Bach, L. G., & Thuc, C. H. (2022). Comparative characterization and release study of edible films of chitosan and natural extracts, *Food Packaging and Shelf Life, 32*, 100830.

Niaounakis, M. (2015). Biopolymers: applications and trends. William Andrew.

Ninomiya, K., Matsuda, H., Shimoda, H., Nishida, N., Kasajima, N., Yoshino, T., ... & Yoshikawa, M. (2004). Carnosic acid, a new class of lipid absorption inhibitor from sage, *Bioorganic & Medicinal Chemistry Letters*, *14*(8), 1943-1946.

Oluwasina, O. O., Olaleye, F. K., Olusegun, S. J., Oluwasina, O. O., & Mohallem, N. D. (2019). Influence of oxidized starch on physicomechanical, thermal properties, and atomic force micrographs of cassava starch bioplastic film, *International Journal of Biological Macromolecules*, *135*, 282-293.

Otman, E. G., Drioua, S., Ameggouz, M., Salhi, N., Sayah, K., Zengin, G., ... & Benzeid, H. (2023). Antioxidant activity, analgesic activity, and phytochemical analysis of Ammi majus (L.) extracts, *International Journal of Secondary Metabolite, 10*(1), 23-37.

Ousaaid, D., Mechchate, H., Laaroussi, H., Hano, C., Bakour, M., El Ghouizi, A., ... & El Arabi, I. (2021). Fruits vinegar: Quality characteristics, phytochemistry, and functionality, *Molecules*, *27*(1), 222.

Pakfetrat, H., Nemati, N., Shiravi, A. (2015). Cytotoxicity effects of Ammi visnaga extract on Hela and MCF7 cancer cell lines, *Journal of Animal Biology*, *7*(2), 25-33.

Park, S. Y., Marsh, K. S., & Rhim, J. W. (2002). Characteristics of different molecular weight chitosan films affected by the type of organic solvents, *Journal of Food Science*, *67*(1), 194-197.

Peralta, J., Bitencourt-Cervi, C. M., Maciel, V. B., Yoshida, C. M., & Carvalho, R. A. (2019). Aqueous hibiscus extract as a potential natural pH indicator incorporated in natural polymeric films, *Food Packaging and Shelf Life*, *19*, 47-55.

Petersen, M. (2013). Rosmarinic acid: new aspects, *Phytochemistry Reviews*, *12*, 207-227.

Petersen, M., & Simmonds, M. S. (2003). Rosmarinic acid, *Phytochemistry*, *62*(2), 121-125.

Petruzzi, L., Corbo, M. R., Sinigaglia, M., & Bevilacqua, A. (2017). Microbial spoilage of foods: Fundamentals. In The microbiological quality of food 1-21. Woodhead Publishing.

Pillai, C. K., Paul, W., & Sharma, C. P. (2009). Chitin and chitosan polymers: Chemistry, solubility and fiber formation, *Progress in Polymer Science*, *34*(7), 641-678.

Prietto, L., Mirapalhete, T. C., Pinto, V. Z., Hoffmann, J. F., Vanier, N. L., Lim, L. T., ... & da Rosa Zavareze, E. (2017). pH-sensitive films containing anthocyanins extracted from black bean seed coat and red cabbage, *LWT*, *80*, 492-500.

Prinsi, B., Morgutti, S., Negrini, N., Faoro, F., & Espen, L. (2019). Insight into composition of bioactive phenolic compounds in leaves and flowers of green and purple basil, *Plants*, *9*(1), 22.

Rahman, L., & Goswami, J. (2021). Recent development on physical and biological properties of chitosan-based composite films with natural extracts: A review, *Journal of Bioactive and Compatible Polymers, 36*(3), 225-236.

Rambabu, K., Bharath, G., Banat, F., Show, P. L., & Cocoletzi, H. H. (2019). Mango leaf extract incorporated chitosan antioxidant film for active food packaging, *International Journal of Biological Macromolecules, 126*, 1234-1243.

Rao, M. G., Bharathi, P., & Akila, R. M. (2014). A comprehensive review on biopolymers, *Scientific Reviews & Chemical Communications, 4*(2), 61-68.

Raus, R. A., Nawawi, W. M. F. W., & Nasaruddin, R. R. (2021). Alginate and alginate composites for biomedical applications, *Asian Journal of Pharmaceutical Sciences, 16*(3), 280-306.

Rauwald, H. W., Brehm, O., & Odenthal, K. P. (1994). The Involvement of a Ca^{2+} Channel Blocking Mode of Action in the Pharmacology of Ammi visnaga Fruits1, *Planta Medica*, *60*(02), 101-105.

Rhee, M. S., Lee, S. Y., Dougherty, R. H., Kang, D. H. (2003). Antimicrobial effects of mustard flour and acetic acid against Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella enterica Serovar typhimurium, *Applied and Environmental Microbiology, 69*, 2959–2963.

Rohindra, D. R., Nand, A. V., & Khurma, J. R. (2004). Swelling properties of chitosan hydrogels, *The South Pacific Journal of Natural and Applied Sciences, 22*(1), 32-35.

Rolin, C. (1993). Pectin. In Industrial gums, 257-293. Academic Press.

Ropartz, D., & Ralet, M. C. (2020). Pectin structure. Pectin: Technological and physiological properties, 17-36.

Rutala, W. A., Barbee, S. L., Agular, N. C., Sobsey, M. D., Weber, D. J. (2000). Antimicrobial activity of home disinfectants and natural products against potential human pathogens, *Infection Control & Hospital Epidemiology, 21*, 33-38.

Samad, A., Azlan, A., & Ismail, A. (2016). Therapeutic effects of vinegar: a review, *Current Opinion in Food Science, 8,* 56-61.

Samtiya, M., Aluko, R. E., Dhewa, T., & Moreno-Rojas, J. M. (2021). Potential health benefits of plant food-derived bioactive components: An overview. *Foods, 10*(4), 839.

Sani, M. A., Tavassoli, M., Hamishehkar, H., & McClements, D. J. (2021). Carbohydrate-based films containing pH-sensitive red barberry anthocyanins: Application as biodegradable smart food packaging materials, *Carbohydrate Polymers, 255*, 117488.

Schindler, H. (1953). The genuine ammei, Ammi visnaga (L.) LAM, a Mediterranean spasmolytic drug with khellin content. *Die Pharmazie, 8*(2), 176-179.

Sellami, H. K., Napolitano, A., Masullo, M., Smiti, S., Piacente, S., & Pizza, C. (2013). Influence of growing conditions on metabolite profile of Ammi visnaga umbels with special reference to bioactive furanochromones and pyranocoumarins, *Phytochemistry, 95*, 197-206.

Shahrajabian, M. H., Sun, W., & Cheng, Q. (2020). Chemical components and pharmacological benefits of Basil (Ocimum basilicum): A review. *International Journal of Food Properties, 23*(1), 1961-1970.

Shallan, A., Guijt, R., & Breadmore, M. (2013). Capillary electrophoresis: basic principles, Siegel, J. A., Saukko, P. J., Houck, M. M. (Eds.) In Encyclopedia of Forensic Sciences (Second Ed.), 549-559. Academic Press.

Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2016). Bioactive compounds in banana and their associated health benefits–A review. *Food Chemistry, 206*, 1-11.

Singleton, V. L. &. Rossi, J. A. (1965) Colorimetry of total phenolics withphosphomolybdic- phosphotungstic acid reagents, *American Journal of Enology and Viticulture, 16*, 144-158.

Stanojevic, L. P., Marjanovic-Balaban, Z. R., Kalaba, V. D., Stanojevic, J. S., Cvetkovic, D. J., & Cakic, M. D. (2017). Chemical composition, antioxidant and antimicrobial activity of basil (Ocimum basilicum L.) essential oil, *Journal of Essential Oil Bearing Plants, 20*(6), 1557-1569.

Sudheesh, C., Sunooj, K. V., Aaliya, B., Navaf, M., Akhila, P. P., Mir, S. A., ... & George, J. (2023). Effect of energetic neutrals on the kithul starch retrogradation; Potential utilization for improving mechanical and barrier properties of films, *Food Chemistry, 398*, 133881.

Sun, L., Sun, J., Chen, L., Niu, P., Yang, X., & Guo, Y. (2017). Preparation and characterization of chitosan film incorporated with thinned young apple polyphenols as an active packaging material, *Carbohydrate Polymers, 163*, 81-91.

Şenol, F. S., Orhan, I., Celep, F., Kahraman, A., Doğan, M., Yilmaz, G., & Şener, B. (2010). Survey of 55 Turkish Salvia taxa for their acetylcholinesterase inhibitory and antioxidant activities, *Food Chemistry, 120*(1), 34-43.

Teke, G. N. and Kuete, V. (2014). Acute and subacute toxicities of African medicinal plants, *Toxicological survey of African Medicinal Plants*, 63-98, Elsevier.

Tepe, B., Eminagaoglu, O., Akpulat, H. A., & Aydin, E. (2007). Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of Salvia verticillata (L.) subsp. verticillata and S. verticillata (L.) subsp. amasiaca (Freyn & Bornm.) Bornm, *Food Chemistry, 100*(3), 985-989.

Tepe, B. (2008). Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of Salvia virgata (Jacq), Salvia staminea (Montbret & Aucher ex Bentham) and Salvia verbenaca (L.) from Turkey. *Bioresource Technology, 99*(6), 1584-1588.

Tezcan, F., Gültekin-Özgüven, M., Diken, T., Özçelik, B., & Erim, F. B. (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices, *Food Chemistry, 115*(3), 873-877.

Thakur, B. R., Singh, R. K., Handa, A. K., & Rao, M. A. (1997). Chemistry and uses of pectin—A review, *Critical Reviews in Food Science & Nutrition*, ***37***(1), 47-73.

Tosun, M., Ercisli, S., Sengul, M., Ozer, H., Polat, T., & Ozturk, E. (2009). Antioxidant properties and total phenolic content of eight Salvia species from Turkey. *Biological Research, 42*(2), 175-181.

Triki, M., Herrero, A. M., Jiménez-Colmenero, F., & Ruiz-Capillas, C. (2018). Quality assessment of fresh meat from several species based on free amino acid and biogenic amine contents during chilled storage, *Foods, 7*(9), 132.

Van de Velde, K., & Kiekens, P. (2002). Biopolymers: overview of several properties and consequences on their applications, *Polymer Testing, 21*(4), 433-442.

Vanachayangkul, P., Byer, K., Khan, S., & Butterweck, V. (2010). An aqueous extract of Ammi visnaga fruits and its constituents khellin and visnagin prevent cell damage caused by oxalate in renal epithelial cells. *Phytomedicine, 17*(8-9), 653-658.

Wang, F., Xie, C., Tang, H., Li, H., Hou, J., Zhang, R., ... & Jiang, L. (2023). Intelligent packaging based on chitosan/fucoidan incorporated with coleus grass (Plectranthus scutellarioides) leaves anthocyanins and its application in monitoring the spoilage of salmon (Salmo salar L.), *International Journal of Biological Macromolecules, 252*, 126423.

Wang, L., Guo, H., Wang, J., Jiang, G., Du, F., & Liu, X. (2019). Effects of Herba Lophatheri extract on the physicochemical properties and biological activities of the chitosan film, *International Journal of Biological Macromolecules, 133*, 51-57.

Wang, S., Marcone, M. F., Barbut, S., & Lim, L. T. (2012). Fortification of dietary biopolymers-based packaging material with bioactive plant extracts, *Food Research International, 49*(1), 80-91.

Wang, X., Yong, H., Gao, L., Li, L., Jin, M., & Liu, J. (2019). Preparation and characterization of antioxidant and pH-sensitive films based on chitosan and black soybean seed coat extract, *Food Hydrocolloids, 89*, 56-66.

Whatley, H. (2001). Basic principles and modes of capillary electrophoresis. In J.R. Petersen, A.A. Mohammad, (Eds.), *Clinical and Forensic Applications of Capillary Electrophoresis* (pp.21-58). New Jersey: Humana Press Inc.

Wu, L. T., Tsai, I. L., Ho, Y. C., Hang, Y. H., Lin, C., Tsai, M. L., & Mi, F. L. (2021). Active and intelligent gellan gum-based packaging films for controlling anthocyanins release and monitoring food freshness, *Carbohydrate Polymers, 254*, 117410.

Wu, S., Li, G., Li, B., & Duan, H. (2022). Chitosan-based antioxidant films incorporated with root extract of Aralia continentalis Kitagawa for active food packaging applications, *E-Polymers*, *22*(1), 125-135.

Yang, C. C., & Chen, T. C. (1993). Effects of refrigerated storage, pH adjustment, and marinade on color of raw and microwave cooked chicken meat, *Poultry Science*, *72*(2), 355-362.

Yang, Y., Zhang, G., Hong, Y., Gu, Z., & Fang, F. (2013). Calcium cation triggers and accelerates the gelation of high methoxy pectin, *Food Hydrocolloids*, *32*(2), 228-234.

Zeng, J., Ren, X., Zhu, S., & Gao, Y. (2021). Fabrication and characterization of an economical active packaging film based on chitosan incorporated with pomegranate peel, *International Journal of Biological Macromolecules*, *192*, 1160-1168.

Zengin, G., Llorent-Martínez, E. J., Fernández-de Córdova, M. L., Bahadori, M. B., Mocan, A., Locatelli, M., & Aktumsek, A. (2018). Chemical composition and biological activities of extracts from three Salvia species: S. blepharochlaena, S. euphratica var. leiocalycina, and S. verticillata subsp. amasiaca. *Industrial Crops and Products*, *111*, 11-21.

Zhang, K., Huang, T. S., Yan, H., Hu, X., & Ren, T. (2020). Novel pH-sensitive films based on starch/polyvinyl alcohol and food anthocyanins as a visual indicator of shrimp deterioration, *International Journal of Biological Macromolecules, 145*, 768-776.

Zhang, X., Lian, H., Shi, J., Meng, W., & Peng, Y. (2020). Plant extracts such as pine nut shell, peanut shell and jujube leaf improved the antioxidant ability and gas permeability of chitosan films. *International Journal of Biological Macromolecules, 148*, 1242-1250.

Zhu, F. (2021). Polysaccharide based films and coatings for food packaging: effect of added polyphenols, *Food Chemistry, 359,* 129871.

CURRICULUM VITAE

Name Surname : Veselina ADIMCILAR

EDUCATION :

- **B.Sc.** : 2014, Istanbul Technical University, Science and Letters Faculty, Department of Chemistry
- **M.Sc.** : 2016, Istanbul Technical University, Graduate School of Science and Engineering, Chemistry

PROFESSIONAL EXPERIENCE AND REWARDS:

- 2017- present Research Assistant at Istanbul Technical University, Department of Chemistry
- 2014 - Graduation at 1st rank from Istanbul Technical University, Department of Chemistry
- 2017-2019-Researcher-ITU-BAP-GAP- TGA-2017-40917- Kemoterapötik İlaç Adayları Geliştirme Çalışmaları
- 2017-2020-Researcher-ITU-BAP-GAP- TGA-2017-40695- Seryum İyonu ve Nano Seryum Oksit İçerikli Yeni Yara Kapatıcı Çitosan Bazlı Hidrojel Filmlerin Hazırlanması: Antibakteriyel ve Fiziksel Özelliklerinin İncelenmesi

PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:

- **Adımcılar, V.,** Kalaycıoğlu, Z., Akın-Evingür, G., Torlak, E., & Erim, F. B. (2023). Comparative physical, antioxidant, and antimicrobial properties of films prepared by dissolving chitosan in bioactive vinegar varieties. International Journal of Biological Macromolecules, 242, 124735. (Scopus Q1)

- **Adımcılar, V.,** Beyazit, N., & Erim, F. B. (2023). Khellin and visnagin in different organs of Ammi visnaga and Ammi majus. Natural Product Research, 37(1), 164-166. (Scopus Q1)

- **Adımcılar, V.,** Kalaycıoğlu, Z., Aydoğdu, N., Dirmenci, T., Kahraman, A., & Erim, F. B. (2019). Rosmarinic and carnosic acid contents and correlated antioxidant and antidiabetic activities of 14 Salvia species from Anatolia. Journal of pharmaceutical and biomedical analysis, 175, 112763. (Scopus Q1)

- **Adımcılar, V.,** Torlak, E., Erim, F.B. Preparation and Characterization of Intelligent Pectin and Alginate Based Biopolymeric Films Enriched with Purple Basil Extract for Potential Detection of Food Freshness with pH Sensing Ability. *Submitted for publication-Under Review.*

- **Adımcılar, V.,** Erim, F.B. Preparation of pH Sensitive Purple Basil Extract Enriched Biopolymeric Film for Potential Food Preservation and Monitoring of Food Freshness, 12th Aegean Analytical Chemistry Days (AACD2023), Oral Presentation, İstanbul, Türkiye, 19-22 September 2023.
- **Adımcılar, V.,** Akın-Evingür, G., Torlak, E., Erim, F.B. Preparation and Characterization of Novel Chitosan Based Biopolymeric Films: A Promising Food Packaging Material, 25th JCF Spring Symposium 2023, Oral Presentation, Giessen, Germany, 21-24 March 2023
- **Adımcılar, V.,** Günaydın K., Erim, F. B. Determination of Khellin and Visnagin Contents of Ammi Visnaga and Ammi Majus by Capillary Electrophoresis with UV Detection, 8th Black Sea Basin Conference on Analytical Chemistry, Oral Presentation, Şile, Türkiye, 09-11 May 2018

OTHER PUBLICATIONS, PRESENTATIONS AND PATENTS:

- **Adımcılar, V.,** Saygılı, M. T., Cansever, M. Ş., & Öztekin, N. (2023). Highly Sensitive Rapid Determination of Orotic Acid in Urine Samples Using a Field-Amplified Sample Stacking Approach in Capillary Electrophoresis Coupled with Contactless Conductivity Detection. Journal of Pharmaceutical and Biomedical Analysis, 115826.
- Demir, S., **Adımcılar, V.,** Cini, N., & Gölcü, A. (2022). In-vitro release study of Pt (II) and Fe (III) metallocefotaxime drug candidates in pH dependent releasing mediums mimicking human biological fluids. Journal of Drug Delivery Science and Technology, 71, 103328.
- **Adımcılar, V.,** Çeşme, M., Şenel, P., Danış, İ., Ünal, D., & Gölcü, A. (2021). Comparative study of cytotoxic activities, DNA binding and molecular docking interactions of anticancer agent epirubicin and its novel copper complex. Journal of Molecular Structure, 1232, 130072**.**
- Kalaycıoğlu, Z., Kahya, N., **Adımcılar, V.,** Kaygusuz, H., Torlak, E., Akın-Evingür, G., & Erim, F. B. (2020). Antibacterial nano cerium oxide/chitosan/cellulose acetate composite films as potential wound dressing. European Polymer Journal, 133, 109777.
- Yılmaz, Z. T., Odabaşoğlu, H. Y., Şenel, P., **Adımcılar, V.,** Erdoğan, T., Özdemir, A. D., ... & Büyükgüngör, O. (2020). Investigations on crystal structure of a novel 3-((4, 6-dimethylpyrimidin-2-yl) amino) isobenzofuran-1 (3H)-one, and related theoretical studies. Arabian Journal of Chemistry, 13(6), 5564-5580.
- Yılmaz, Z. T., Odabaşoğlu, H. Y., Şenel, P., **Adımcılar, V.,** Erdoğan, T. A. N. E. R., Özdemir, A. D., ... & Odabaşoğlu, M. (2020). A novel 3-((5-methylpyridin-2-yl) amino) isobenzofuran-1 (3H)-one: Molecular structure describe, X-ray diffractions and DFT calculations, antioxidant activity, DNA binding and molecular docking studies. Journal of Molecular Structure, 1205, 127585.
- **Adımcılar, V.,** & Öztekin, N. (2019). A fast and convenient analysis method for the determination of cations in pomegranate juices by capillary electrophoresis with contactless conductivity detection. Turkish Journal of Chemistry, 43(2), 547-554.

- **Adımcılar, V.,** Öztekin, N., & Erim, F. B. (2018). A direct and sensitive analysis method for biogenic amines in dairy products by capillary electrophoresis coupled with contactless conductivity detection. Food analytical methods, 11, 1374-1379.
- Kaygusuz, H., Uysal, M., **Adımcılar, V.,** & Erim, F. B. (2015). Natural alginate biopolymer montmorillonite clay composites for vitamin B2 delivery. Journal of Bioactive and Compatible Polymers, 30(1), 48-56.
- Cansever, M. Ş., Öztekin, N., **Adımcılar, V.,** Zübarioğlu, T., Kıykım, E., & Aktuğlu, A. (2020). İdrarda Orotik Asit'in Temassız İletkenlik Dedektörü Kullanılarak Kapiler Elektroforez Yöntemi İle Hızlı Tayini. Süleyman Demirel University Faculty of Arts and Science Journal of Science, 15(2), 194-202.
- **Adımcılar, V.,** Taşer-Saygılı, M., Cansever, M. Ş., Öztekin, N. Fast and Sensitive Determination of Orotic Acid in Urine Samples with Capillary ElectrophoresisCoupled with Contactless Conductivity Detection, PBA 2023 33rd International Symposium on Pharmaceutical and Biomedical Analysis, Oral Presentation, Ankara, Türkiye, 02- 06 July 2023.
- **Adımcılar, V.,** Altay, F., Bütün, V., Gölcü, A. Preparation and comparison of electrospun matrice and hydrogel based controlled release systems for chemotherapeutic agent epirubicin and metal complexes, Euroanalysis XX Conference, Oral Presentation, İstanbul, Türkiye, 01- 05 September 2019.
- **Adımcılar, V.,** Bütün, V., Gölcü, A. In Vitro Controlled Release of an Anticancer Drug Epirubicin from pH Sensitive Hydrogel Systems**,** 1st International Congress on Analytical and Bioanalytical Chemistry, Oral Presentation, Antalya, Türkiye, 27 - 30 March 2019.
- **Adımcılar, V.,** Kahya, N., Akın-Evingür, G., Torlak, E., Erim, F.B. Physical properties of chitosan/cellulose acetate composites, 22nd Liquid State Symposium, Oral Presentation, İstanbul, Türkiye, 7 December 2018.