ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

CURCUMINOIDS: ANALYTICAL CHEMISTRY, BIOCHEMISTRY, AND POTENTIAL APPLICATIONS

Ph.D. THESIS

Zeynep KALAYCIOĞLU

Department of Chemistry

Chemistry Programme

JUNE 2020

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

CURCUMINOIDS: ANALYTICAL CHEMISTRY, BIOCHEMISTRY, AND POTENTIAL APPLICATIONS

Ph.D. THESIS

Zeynep KALAYCIOĞLU
(509142022)

Department of Chemistry

Chemistry Programme

Thesis Advisor: Prof. Dr. Fatma Bedia BERKER

JUNE 2020

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

KURKUMİNOİDLER: ANALİTİK KİMYA, BİYOKİMYA VE POTANSİYEL UYGULAMALARI

DOKTORA TEZİ

Zeynep KALAYCIOĞLU
(509142022)

Kimya Anabilim Dalı

Kimya Programı

Tez Danışmanı: Prof. Dr. Fatma Bedia BERKER

HAZİRAN 2020

Zeynep KALAYCIOĞLU, a Ph.D. student of ITU, Graduate School of Science Engineering and Technology, student ID 509142022, successfully defended the thesis entitled "CURCUMINOIDS: ANALYTICAL CHEMISTRY, BIOCHEMISTRY, AND POTENTIAL APPLICATIONS", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor :	**Prof. Dr. Fatma Bedia BERKER** Istanbul Technical University	.....
Jury Members :	**Prof. Dr. Ayşegül GÖLCÜ** Istanbul Technical University	.....
	Assoc. Prof. Dr. Nevin ÖZTEKİN Istanbul Technical University	.....
	Prof. Dr. Ayşen YARAT Marmara University	.....
	Prof. Dr. Sezgin BAKIRDERE Yıldız Technical University	.....

Date of Submission : 12 May 2020
Date of Defense : 15 June 2020

To my family,

FOREWORD

First and foremost I wish to thank my advisor, Prof. Dr. Fatma Bedia BERKER. It has been an honor to be her Ph.D. student. She has supported me not only by providing academically but also emotionally over almost six years. The joy and enthusiasm she has was motivational for me during tough times. I am also thankful for the excellent example she has provided as a successful woman.

I would like to thank to the members of the thesis committe; Prof. Dr. Ayşen YARAT and Assoc. Prof. Dr. Nevin ÖZTEKİN for their help and allowing time to my thesis. I would like to acknowledge Prof. Dr. Keriman GÜNAYDIN. She has guided me through the isolation of curcuminoids experiments. I very much appreciated her willingness to teach. I also want to thank to Assoc. Prof. Dr. Işıl GAZİOĞLU, with whom we have worked together on bioactivity experiments. In regards to the some parts film experiments, I thank to Prof. Dr. Emrah TORLAK, Prof. Dr. İlhan ÖZEN, and Assoc. Prof. Dr. Gülşen AKIN-EVİNGÜR.

I would like to thank to Dr. Parya HASHEMI who I have had the pleasure to work with on capillary electrophoresis. I would like to thank to Asst. Prof. Dr. Hakan KAYGUSUZ for his support. I also would like to thank the past and present group members of Berker Research Group, Dr. Filiz TEZCAN, Dr. Sesil ERKAN, Nilay KAHYA, Veselina ADIMCILAR, and the numerous students who come through the lab. I am also thankful to Fatih ŞİMŞİT for his help in bureaucratic processes. I gratefully acknowledge the Research Foundation of ITU (TDK-2018-41174).

My time at ITU was made enjoyable due to the many friends which became a part of my life. I am grateful for time spent with them. We shared the same office with Esra SU for 5 years. Thank you my lovely friend for unforgettable memories and trips. And thank to Hande TINAS, with whom my flatmate since 2018. I am grateful for her delicious foods and amazing Turkish coffee sessions. They both are the true friends of mine. Lastly, I wish to acknowlegde a friendship that began six years ago and became ripe throughout years: thank you to Sirun ÖZÇELİK. She unconditionally supported me in many ways. I hope this friendship continue for decades!

Last but not least, I would like to express my deepest gratitude to my family. This dissertation would not have been possible without their warm love, continued patience, and endless support. I need to thank them in Turkish now: Bana her daim sevgilerini ve desteklerini veren dedeme, anneanneme, rahmetli babaanneme, canım teyzelerime ve kuzenlerime teşekkür ederim. Son olarak çocukluğumdan beri bana bilginin peşinden gitmeyi, üretmeyi ve sevmeyi öğreten annem ile babama şükranlarımı sunarım. Hayat sizlerle güzel!

May 2020

Zeynep KALAYCIOĞLU
(M.Sc. Chemist)

TABLE OF CONTENTS

Page

ABBREVIATIONS

2-HP-β-CD : 2-hydroxypropyl-beta-cyclodextrin
ACh : Acetylcholine
AChE : Acetylcholinesterase
AcI : Acetylcholine iodide
AD : Alzheimer's disease
ATCC : American type culture collection
BChE : Butrylcholine esterase
BDMC : Bisdemethoxycurcumin
BHA : Butylated hydroxyanisole
BHT : Butylated hydroxytoluene
CE : Capillary electrophoresis
CFU : Colony forming unit
CZE : Capillary zone electrophoresis
DMC : Demethoxycurcumin
DPPH : 2,2-Diphenyl-1-picrylhydrazyl
DTG : Derivate thermo gravimetric
DTNB : 5,5′-dithiobis (2-nitrobenzoic) acid
E : Young's modulus
EC : Enzyme code
EL : Ductility
FRAP : Ferric-reducing antioxidant power
FTIR : Fourier transform infrared
I : Inhibition
IC_{50} : The half maximal inhibitory concentration
ID : Internal diameter
LIF : Laser induced fluoresence
LOD : Limit of detection
LOQ : Limit of quantification
MEEKC : Micro emulsion electrokinetic chromatography
MEKC : Micellar electrokinetic chromatography
NACE : Non-aqueous capillary electrophoresis
NMR : Nuclear magnetic resonance
***p*-NPG** : *p*-Nitrophenyl-*α*-D-glucopyranoside
PP : Polypropylene
R_f : Retention factor
RP : Reversed phase
RSD : Relative standard deviation
TEE : Turmeric ethanol extract
TLC : Thin layer chromatography
TPTZ : 2,4,6-tripyridyl-s-triazine
TG : Thermo gravimetric
TS : Tensile strength

UV/VIS **:** Ultraviolet/visible
WVP **:** Water vapor permeability
WVTR **:** Water vapor transmission rate

LIST OF TABLES

LIST OF FIGURES

Page

CURCUMINOIDS: ANALYTICAL CHEMISTRY, BIOCHEMISTRY, AND POTENTIAL APPLICATIONS

SUMMARY

Natural plant products have been utilized for various purposes throughout human history. Many of plants have taken the initial form of crude medicine as being tinctures, teas, and powders. An oral history had been created for certain plants used and their application methods for specific ailments. After all, a cultural knowledge base has been recorded regarding medicinal plants. In more recent history, natural plant products have opened a new research area in modern medicine. Bioactive constituents isolated and characterized have been focused for pharmaceutical drug discovery and drug design.

Turmeric (*Curcuma longa* Linn., family Zingiberaceae), is one of the most popular herb used as spice due to its fragrance and yellowish colour worldwide. Except the uses as a spice, it has been used as traditional medicine due to its beneficial properties on gastrointestinal diseases, diabetic wounds, inflammation, cough, and rheumatism. Modern researches have authenticated turmeric as antioxidant, antimicrobial, anticancer, antidiabetic, anti-inflammatory, anti-HIV etc. Wide spectrum of pharmacological activities made turmeric a highly considerable spice as a universal panacea. One of the essential component of turmeric is curcumin that provides the color and bioactivity to this plant.

Extensive researches have revealed that commercial turmeric powder is combined of naturally occuring curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC). Curcuminoid is a collective name, covering these three compounds that only vary in methoxy substitution on the aromatic ring. These structural differences provide different pharmaceutical properties. Individual curcuminoids should be taken into account for the accurate and repetable clinical studies based on the contents of bioactive components.

Despite recent progresses in analytical techniques, curcuminoids suffer from poor separation and broad peaks. Therefore, there is a necessity to develop a reliable separation and determination technique to express the individual quantities of curcuminoids in real samples. Comparisons of the individual curcuminoid bioactivities are promising approaches for development into a modern drug. In addition, curcuminoids are also known as natural antibacterial agents. This potency of turmeric can be converted into an application such as food packaging.

This thesis aims a series of investigations on curcuminoids to contribute to the large research pool on this subject. To achieve this aim, an isolation method has been developed for turmeric plant. Three curcuminoids were isolated purely by column chromatography and subsequent thin layer chromatography, the structures were determined by nuclear magnetic resonance (NMR) spectroscopy. A capillary electrophoresis-laser induced fluorescence technique was developed for the separation

and determination of individual curcuminoids. Both the isolation and analysis method were used for curcuminoids to be used at all levels of the scope of thesis in order to determine of their quantity and purity.

In the first part, the amount of three curcuminoids in turmeric powder and herbal supplements was determined by capillary electrophoresis technique. Capillary electrophoresis (CE) is a separation and analysis technique which is well-known for its short analysis time, high sensitivity, and low sample and solvent consumption. The developed CE technique was combined with laser induced fluorescent detector (LIF) taking advantage of floresence nature of curcuminoids. LIF detection presents high sensitivity due to the great intensity of the incidental light. Borate buffer (pH: 9.6) was chosen as suitable separation medium. Hydrolytic degradation of curcuminoids in the basic medium is prevented by the addition of 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD) to the borate buffer. Presence of 2-HP-β-CD in the separation medium provided also the increasement of fluoresence intensities of curcuminoids. Three curcuminoids of turmeric were completely separated and determined quantitatively in 4.5 min. The method was well-validated and applied to powdered and rhizome turmeric samples and a turmeric herbal supplement in order to find the curcuminoid amounts. Being the first part of the thesis, it was published on a peer-reviewed SCI indexed journal (Electrophoresis 36 (2015) 2516-2521).

In the second part, antioxidant, anticholinesterase, and antidiabetic activities of the curcuminoids were determined and compared. The antioxidant potential of curcuminoids were evaluated using 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging activity and ferric-reducing antioxidant power (FRAP) assays. Curcumin showed the highest antioxidant activity with both assays. Antioxidant activities of DMC and BDMC came after those of curcumin, respectively. In order to test the anti-Alzheimer activity, acetylcholinesterase (AChE) and butyrlcholinesterase (BChE) inhibition powers of curcuminoids were evaluated. Since AChE and BChE ezymes have been proven to be the most viable therapeutic target for symptomatic improvement in Alzheimer disease (AD), inhibition of these enzymes are considered to be the most accepted approach for the treatment of AD. BDMC showed substantial inhibitory activity, even better than the activity of galantamine that is a medication used to treat symptoms of Alzheimer's disease. The activity of DMC came after BDMC, whereas curcumin showed very little acetylcholinesterase inhibition activity. BDMC showed inhibitory activity against butrylcholinesterase enzyme, while curcumin and DMC did not show activity. Curcumin and DMC showed no inhibitory activity against butrylcholinesterase enzyme. However, BDMC was active. Antidiabetic activities were determined via their α-glucosidase inhibitory activities. The method of inhibition of α-glucosidase enzyme is one of the most useful way for the control of hyperglycemia in patient with type 2. All curcuminoids showed α-glucosidase inhibitory activities with decreasing order as BDMC > curcumin > DMC. In this context, a manuscript was published on a peer-reviewed SCI indexed journal (Natural Product Research 36 (2017) 2914-2917).

In the third part, mechanically stable and antibacterial turmeric incorporated chitosan food packaging films were prepared using solvent-casting method and cross-linked with sodium sulfate. Mechanical, optical, thermal properties, water vapor permeabilities and antimicrobial activities of the films were measured. Tensile strength and the ultraviolet-visible (UV) light barrier of the films were improved with the addition of turmeric extract. Furthermore, turmeric incorporated chitosan film showed a significant antimicrobial effect on two common food pathogens; *Salmonella* and

Staphylococcus aureus. As a result, turmeric extract incorporation into chitosan film improve the functional properties. Thereby, it can be suggested as a strong candidate to be used in food packaging industry. This study was published on a peer-reviewed SCI indexed journal (International Journal of Biological Macromolecules 101 (2017) 882-888).

Taking into consideration all of the studies conducted in the present thesis, we report here a systematic study describing chemical and physical properties of turmeric based on curcuminoids which could in turn help develop innovative therapeutic strategies. The developed CE technique is important for the rapid and fully separation and determination of individual curcuminoids in the quality control of herbal tablets and turmeric samples. Each curcuminoid has different biochemical potentials. Inspired by this phenomenon, the differences of antioxidant, anti-Alzheimer, and antidiabetic activities of individiual curcuminoids were revealed and compared. The results may contribute the literature survey dealing with an area of current resarch interest in designing new drugs. All of these properties make turmeric not only as a part of clinical studies but also an important candidate in the ranges of application areas. Food packaging technology has gained a great development since the packaging has an important issue on the conservation and marketing of foodstuffs. There is an increasing interest on films produced by biopolymers. Besides having excellent biodegradability and biocompatibility, biopolymers serve as carriers of many functional ingredients. At this point, utilizing turmeric as natural additive enables the development of film properties.

KURKUMİNOİDLER: ANALİTİK KİMYA, BİYOKİMYA VE POTANSİYEL UYGULAMALARI

ÖZET

Tarih boyunca insanlar bitkisel ürünleri çeşitli amaçlarla kullanmıştır. Bitkilerin çoğundan ilaç olarak tentür, çay ve toz halinde yararlanılmıştır. Belirli hastalıklar için kullanılan bitkiler ve bitkilerin uygulama metodları nesilden nesile aktarılmıştır. Sonuç olarak şifalı bitkiler için kültürel bir veritabanı kaydedilmiştir. Yakın tarihte doğal bitki ürünleri modern tıpta yeni bir araştırma alanı açmıştır. Bu konudaki çalışmalar, farmasötik ilaç keşfi ve ilaç tasarımı için tıbbi bitkilerden aktif bileşenlerin izolasyonu ve karakterizasyonuna odaklanmıştır.

Zerdeçal, zencefil familyasından lifli bir bitki olan *Kurkuma longa* bitkisinin kökünden elde edilmektedir. Kokusu, aroması ve sarı rengi nedeniyle dünya çapında baharat olarak kullanılan en popüler bitkilerden biridir. Baharat olarak kullanılması dışında, gastrointestinal hastalıklar, diyabetik yaralar, iltihaplanma, öksürük ve romatizma üzerindeki faydalı etkileri nedeniyle geleneksel ilaç olarak kullanılmıştır. Modern araştırmalar, zerdeçalın antioksidan, antimikrobiyal, antikanser, antidiyabetik, antienflamatuar, anti-HIV özelliklere sahip olduğunu ortaya çıkarmıştır. Geniş spektrumdaki farmakolojik aktiviteleri, zerdeçalı evrensel her derde deva olan önemli bir baharat haline getirmiştir. Zerdeçala kendine özgü sarı rengini veren madde kurkumindir. Kurkumin, zerdeçalın temel biyoaktif bileşiği olarak bilinir.

Kapsamlı araştırmalar zerdeçalın doğal olarak kurkumin, demetoksikurkumin (DMC) ve bisdemetoksikurkumin (BDMC)'nin bir karışımı olduğunu ortaya koymuştur. Aromatik halka üzerinde sadece metoksi sübstitüsyonu farklılık gösteren bu üç bileşik toplu olarak kurkuminoidler olarak adlandırılır. Çok yakın bir zamana kadar bunlardan sadece kurkumin yüksek fiyatlarda ticari olarak saf olarak temin edilebilmekte, diğer iki kurkuminoidin ticari satışı bulunmamaktaydı. Günümüzde ise çok düşük miktarlarına yüksek fiyatlarla ulaşılabilmektedir. Literatürdeki çalışmalar kurkuminin farmakolojik özellikleri, antioksidan, antiseptik, antimikrobiyal, antienflamatuvar ve antikanserojen etkileri üzerinde olumlu sonuçlar alınan tıbbi çalışmalar ve bu bileşiğin ilaç olarak kullanımı için çözünürlüğünü ve vücut tarafından kullanılabilme etkinliğini arttırıcı araştırmalar üzerinde yoğulaşmaktadır. Ancak, yeni ilaç tasarımının yolunu açacak bilgi birikiminin sağlanması için yapısal değişikliklerin neden olduğu biyoaktivite farklılıklarının incelenmesi göz ardı edilmemelidir. Ayrıca, biyoaktif bileşenlerin içeriğine dayanan klinik çalışmaların doğru ve tekrarlanabilir bir şekilde yürütülebilmesi için her bir kurkuminoidin tek tek ele alınması gerekir.

Analitik yöntemlerdeki son gelişmelere rağmen, kurkuminoidlerin ayırım etkinliği düşük olabilmektedir. Bu nedenle, gerçek numunelerde kurkuminoidlerin ayrı ayrı miktarlarını belirlemek için güvenilir bir ayırma ve analiz metodunun geliştirilmesi gerekmektedir. Ayrıca, kurkuminoidlerin biyoaktivitelerinin karşılaştırmalı olarak değerlendirilmesi de modern ilaç tasarımı konusunda umut verici bir yaklaşımdır.

Kurkuminoidler doğal antibakteriyel ajanlar olarak bilinir. Zerdeçal ekstraktının bu özelliğinden gıda ambalaj maddesi katkısı olarak yararlanılabilir. Böyle bir çalışmanın uygulamaya geçirilmesi halinde, ucuz ve kolay ulaşılabilir olan zerdeçalın, gıda ambalaj endüstrisine katılması sağlanabilecektir.

Bu tez çalışması, bu konudaki büyük araştırma havuzuna katkıda bulunmak için kurkuminoidler üzerine bir dizi araştırmayı amaçlamaktadır. Bu amaç doğrultusunda, zerdeçal bitkisi için bir izolasyon yöntemi uygulanmıştır. Kolon kromatografisi ve takip eden ince tabaka kromatografisi ile 3 kurkuminoid (kurkumin, DMC ve BDMC) saf olarak izole edilmiş, yapıları nükleer manyetik rezonans (NMR) spektroskopisi ile belirlenmiştir. Kurkuminoidlerin ayrım ve analizi için bir kapiler elektroforez-lazer indüklenmiş floresans yöntemi geliştirilmiştir. Bu tez kapsamının her kademesinde, saf kurkuminoidlerin izolasyonu, miktarlarının ve saflıklarının tayini için bu izolasyon ve analiz yöntemi kullanılmıştır.

Birinci bölümde üç kurkuminoidin zerdeçal tozu ve bitkisel takviyelerdeki miktarının belirlenmesi lazer indüklenmiş floresans (LIF) dedektör ile birleştirilmiş kapiler elektroforez tekniği ile gerçekleştirilmiştir. Kapiler elektroforez yüksek bir elektrik alan altında, silika kapiler kolon içinde analitlerin ayrımlarının gerçekleştiği bir ayırma ve analiz metodudur. Bu yöntemin başlıca avantajları kısa analiz süresi, güçlü ayırma etkinliği ve az miktarda solvent ve örnek tüketimidir. Kapiler elektroforez sistemine LIF dedektörün bağlanması floresans özellik gösteren maddelerin eser miktar tayinini mümkün kılmaktadır. Kurkuminoidlerin doğal floresans özelliğinden yararlanarak, kapiler elektroforezle ayrılan kurkuminoidler lazer indüklenmiş floresans dedektör ile dedekte edilmiştir. Borat tamponu (pH:9.6) uygun ayırma olarak belirlenmiş, ayırma ortamına 2-hidroksipropil-β-siklodekstrin (2-HP-β-CD) ilave edilmiştir. Kurkuminoidlerin bazik ortamdaki hidrolitik ayrışması, 2-HP-β-CD ilavesi ile önlenmiştir. Aynı zamanda bu sayede kurkuminoidlerin floresans şiddetleri artırılmıştır. Üç kurkuminoid 4.5 dakikadan daha kısa bir sürede birbirinden tamamen ayrılmıştır. Yöntem kesinlik ve doğruluk parametreleri açısından valide edilmiştir. Ticari toz ve kök zerdeçal örnekler ile takviye edici kurkumin bitkisel kapsüle uygulanmıştır. Bu çalışma bir SCI dergisinde yayınlanmıştır (Electrophoresis 36 (2015) 2516-2521).

İkinci bölümde, zerdeçaldan izole edilen üç kurkuminoidin antioksidan, anti-Alzheimer ve antidiyabetik aktiviteleri çeşitli *in vitro* yöntemlerle incelenmiştir. Antioksidan aktiviteleri; 2,2-difenil-1-pikrilhidrazil (DPPH) radikal yakalama kapasitesi yöntemi ve demir (II) indirgeyici antioksidan kapasite yöntemi (FRAP) ile tayin edilmiştir. Her iki yöntemle de kurkuminin, antioksidan aktivitesi en yüksek olan kurkuminoid olduğu tespit edilmiştir. Kurkumini sırasıyla DMC ve BDMC takip etmiştir. Alzheimer hastalığının tedavisi için asetilkolin ve butirilkolin hidrolizinden sorumlu olan asetilkolinesteraz (AChE) ve butirilkolinesteraz (BChE) enziminin inhibisyonu yöntemi en çok kabul gören yaklaşımdır. Bu yaklaşım göz önünde bulundurularak antikolinesteraz aktivite testleri asetilkolinesteraz ve butirilkolinesteraz enzimlerinin inhibisyonu odaklı gerçekleştirilmiştir ve sonuçlar günümüzde Alzhemier tedavisinde kullanılan galantaminin aktivitesi ile karşılaştırılmıştır. BDMC galantaminin AChE inhibisyon aktivitesinden bile daha iyi bir inhibisyon aktivitesi göstermiştir. BDMC'yi sırasıyla DMC ve kurkumin takip etmiştir. BChE inhibisyon aktiviteleri karşılaştırıldığında kurkumin ve DMC'nin aktiflik göstermediği, BDMC'nin ise düşük de olsa aktivite gösterdiği görülmüştür.

Antidiyabetik aktiviteleri ise kurkuminoidlerin α-glukozidaz enzimini inhibe etmeleri esasına göre değerlendirilmiş, pozitif kontrol olarak genistein kullanılmıştır.

α-Glukozidaz enziminin inhibisyonu yöntemi, tip 2 diyabet hastalarda hipergliseminin kontrolünde en yararlı yoldur. Tüm kurkuminoidler, BDMC > kurkumin > DMC olarak azalan sırada α-glukozidaz inhibitör aktiviteleri göstermiştir. DPPH radikal yakalama kapasitesi yöntemi ile AChE, BChE ve α-glukozidaz enzim inhibisyonu çalışmaları 96 kuyucuklu mikroplakalarda spektroskopik yöntemle gerçekleştirilmiştir. FRAP metodu için ultraviyole-görünür bölge (UV-GB) spektrofotometre kullanılmıştır. Bu çalışma bir SCI dergisinde yayınlanmıştır (Natural Product Research 36 (2017) 2914-2917).

Üçüncü ve son bölümde, zerdeçal ekstraktı içeren mekanik olarak kararlı ve antibakteriyel özellikte kitosan temelli gıda ambalaj filmi literatürde ilk kez hazırlanmıştır. Kitosan, toksik olmayan, biyobozunur özelliği yanında, doğal anti-bakteriyel özelliği nedeniyle literatürde sıkça araştırılan gıda ambalaj filmleri olarak ön plana çıkmaktadır. Kitosanın içine doğal antibakteriyel ve antioksidan ilavelerle aktif gıda ambalaj maddelerinin ortaya konması güncel bir araştırma konusudur. Kurkuminoidlerin zerdeçal ekstraktlarından izolasyonu çalışmaları esnasında, ekstraktın antibakteriyel özelliğinin olabileceği düşünülmüştür. Bu amaçla ekstrakt, kitosan filmlere katılmış ve zerdeçal ekstraktı ilavesinin kitosan filmlerin fiziksel ve antibakteriyel özellikleri üzerindeki etkisi araştırılmıştır. Mukayese amacıyla zerdeçal ekstraktı içermeyen bir kitosan film de hazırlanmıştır. Filmler, çözücü dökme yöntemi ile hazırlanmış ve sodyum sülfat ile çapraz bağlama yapılmıştır. Filmlerin mekanik, optik, termal özellikleri, su buharı geçirgenlikleri ve antibakteriyel aktiviteleri ölçülmüştür. Filmin gerilme mukavemeti ve UV-GB ışık bariyeri, zerdeçal ekstraktı ilavesiyle iyileştirilmiştir. Ayrıca, zerdeçal katkılı kitosan filmi, iki yaygın gıda patojeni olan *Salmonella* ve *Staphylococcus aureus* üzerinde önemli bir antibakteriyel etki göstermiştir. Sonuç olarak, kitosan filmlere zerdeçal ekstraktının dahil edilmesi fonksiyonel özelliklerini geliştirmiştir. Zerdeçal gıda ambalaj endüstrisinde kullanılmak üzere güçlü bir aday olarak önerilebilir. Bu çalışma bir SCI dergisinde yayınlanmıştır (International Journal of Biological Macromolecules 101 (2017) 882-888).

Bu tezde yapılan tüm çalışmalar göz önünde bulundurulduğunda, zerdeçalın kurkuminoidlere dayanan kimyasal ve fiziksel özelliklerini tanımlayan sistematik bir çalışma rapor edilmektedir. Bu yaklaşım ve tezin çıktıları yenilikçi terapötik stratejilerin geliştirilmesine yardımcı olabilir. Geliştirilen CE tekniği kurkuminoidlerin hızlı ve etkin bir şekilde ayrım ve analizini sağlaması açısından önemlidir. Çünkü moleküllerdeki yapısal farklılıklar onların biyoaktifliklerinin de farklı olmasına sebep olur. Bu olgudan esinlenerek kurkuminoidlerin antioksidan, anti-Alzheimer ve antidiyabetik aktivitelerinin farklılıkları ortaya çıkarılmış ve karşılaştırılmıştır. Sonuçlar, yeni ilaç tasarımı gibi güncel bir araştırma alanına katkıda bulunabilir. Bu özelliklerin tümü, zerdeçalı sadece klinik çalışmaların bir parçası olarak değil, aynı zamanda uygulama alanları arasında önemli bir aday haline getirir. Gıda ambalaj teknolojisi, gıda maddelerinin korunması ve pazarlanmasında önemli bir konu haline gelip büyük bir gelişme kaydetmiştir. Son yıllarda biyopolimerler temelli gıda ambalaj filmlerine artan bir ilgi vardır. Mükemmel biyobozunurluğa ve biyouyumluluğa sahip olmanın yanı sıra, biyopolimerler birçok fonksiyonel bileşenin taşıyıcısı olarak işlev görür. Bu noktada, zerdeçalın doğal katkı maddesi olarak kullanılması film özelliklerinin gelişmesini sağlamıştır.

1. INTRODUCTION

Plants have not only been utilized as food or culinary purposes but also as medicines for many years. Mother Nature has always been generous for humankind in this respect. The abundance and availability of natural products in large quantities have supplied population groups for centuries. Plants have been consumed as teas, powders, tinctures in the prevention and/or treatment of a variety of fatal or non-fatal diseases. Eventually, a resource regarding medicinal plants was accumulated.

When we come closer to recent history, the utilization of plants have been moved to research areas related with isolation of active compounds. The active compounds of medicinal plants are important sources of new drugs. Despite the recent interest in synthetic chemical methods and molecular modeling by pharmaceutical companies, isolation of natural sources from plants still keeps its importance. After the plant materials are extracted, bioactive compounds are isolated and characterized by bioassay-guided fractionation are completed. The standardization of herbal medicine is necessary to respect the Intellectual Property Rights. Moreover consumers are needed to reach prospectus of herbal medicines. Because of all these, analytical and biological marker compounds should be taken more rigourusly.

Turmeric plant (also known as Indian saffron and yellow beetroot) is used as a spice worldwide. In the scientific community, turmeric is called as *Curcuma longa*. *Curcuma longa* is a member of the ginger (Zingerberaceae) family which is widely cultivated in tropical Thailand, India, and Indonesia. Turmeric has been used in traditional medicine for many years. The science has taken turmeric from the kitchen shelves and moved into the clinical researches.

Turmeric contains various significant constituents. Curcumin, which gives the turmeric its typical yellowish color, known as the most important and the main bioactive compound. It was firstly isolated in 1815, its solid form was obtained in 1870 and identified as 1,7-bis(4-hydroxy-3-methoxy-phenyl)hepta-1,6-diene-3,5-dione) or diferuloylmethane [1, 2]. The first scientific paper of curcumin goes back to precisely

1871 and from that date, curcumin writes it own history. Up to 21600 publications have been reported in connection with curcumin until the year of 2020. In the last 10 years (2010-2020), 17331 curcumin papers in the field of analytical chemistry, biochemistry, food chemistry, medicinal chemistry and pharmacology are also appeared according to Web of Science database search. Curcumin has been proved as having antibacterial, anticancer, antiviral and antioxidant activities by both *in-vivo* and *in-vitro* techniques on these researches. Some clinical studies have also shown that curcumin has a potential against several diseases such as diabetes, arthritis, and Alzhemier's diease.

Besides including curcumin, turmeric also contains structurally related compounds of curcumin which are collectively named as curcuminoids. Demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) are the main natural analogues of curcumin. The main constituent of curcuminoids in commercial turmeric is curcumin with roughly 75 percent. DMC (~20%) and BDMC (~5%) follow curcumin. The only structural difference between these three compounds is the methoxy substitution on the aromatic ring. Curcumin has two symmetric *o*-methoxy phenols connected with α, β-unsaturated β-diketone component. BDMC, which is also symmetric, lacks of two *o*-methoxy phenol substitutions. Unlike curcumin and BDMC, DMC shows an asymmetric structure with *o*-methoxy phenol substitution on the one of phenyl rings.

It is known that even the slight differences in chemical structures of analogues affect the functional properties of these compounds. Since the curcuminoids vary structurally, it is possible that the chemical and pharmacological properties will vary among the curcuminoids. Pure standards of DMC and BDMC were not commercially avaliable until very recently. Thus, techniques that determine individual curcuminoids were very narrow.

Today, despite pure DMC and BDMC are commercially available, they are both scarce and expensive. Therefore, it is important to obtain pure curcuminoids for the individual determination. The development of isolation techniques is currently a topic of intense research in phytochemical researches. Isolated compounds are determined with improved analytical methods. However curcuminoids suffer from poor resolution. Therefore, there is a need to improve the separation efficiency of curcuminoids.

1.1 Purpose of Thesis

The main aim of this thesis is to contribute the clinical and experimental science regarding three curcuminoids; curcumin, DMC, and BDMC. Studies were grouped into three different strategies following the main aim: 1) evaluation of individual curcuminoids in a manner of qualitative and quantitative determination in real samples, 2) individual comparisons of curcuminoids in the *in-vitro* biochemical activities, 3) formation of a food coating used as curcuminoids ingredient carriers. These three major parts are organized in such that each part has its own introduction, experimental, results and discussion, and conclusion.

In the first part, curcumin, DMC, and BDMC were separated by capillary electrophoresis (CE) and detected by laser induced fluorescence dedector (LIF). Curcuminoids are not practically soluble in water at neutral and acidic pH. An alkali medium is necessary in order to convert these compounds into their ionic forms. Thus, a good separation can be obtained by capillary zone electrophoresis (CZE). However, it is known that the curcuminoids decomposed very fast in alkali solution. In this context, cyclodextrins (CDs) presents great advantages as complexing agents. According to the previous experiments of our research group, complexation with cyclodextrin protects the molecules from decomposition and improve the aqueous solubility of molecules. As will be shown below, 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD) including borate buffer (pH:9.6) provided an excellent separation of curcuminoids in a short time without any decomposition. The advantage of the detection method should also be mentioned. Curcuminoids are naturally florescence which makes possible its dedection via LIF detector. LIF provides very low detection limits. 2-HP-β-CD presence in the separation buffer considerably improved the sensitivity of curcuminoids. The developed method was applied in the determination of curcumin, DMC, and BDMC in turmeric samples and a curcumin herbal supplement. The relationship between diet and health is the curiosity of many consumer. Nutrient content information from food and herbal supplement labels create product safety and quality.

In the second part, the *in-vitro* bioactivities of curcuminoids were comparatively tested. The rich knowledge base in medicinal plants has attracted the attention of pharmaceutical companies. The efficacy of synthetic drugs is limited and these drugs

show dose-dependent side-effects. Many research and development programmes utilize active principles of medicinal plants in the pursuit of discovering novel drugs. This part mainly aims to lighten curcumin, DMC, and BDMC invidually in terms of antioxidant, anti-Alzheimer, and antidiabetic activities. The structural differences of curcuminoids may affect the bioactivities. This comparative approach can provide an oppurtunity to focus future researches on target species that show different pharmacological activities. Antioxidant activities were tested by two different assay methods: DPPH and FRAP. Certain levels of antioxidant activities are critical if the curcuminoids are to become a competitive phytonutrient. Anti-Alzhemier activities were determined based on the inhibition of two enzymes (acetlycholinesterase and butrylcholinesterase) in the cholinergic nervous system. For the antidiabetic activities of curcuminoids, α-glucosidase enzyme inhibition powers were evaluated. α-Glucosidase enzyme inhibitors delay the carbohydrate absorption from the small intestine and reduce the postprandial blood glucose and insulin levels. Thus, α-glucosidase inhibitors are largely applied in the curing of type 2 diabetes.

In the third part, we report, for the first time to our knowledge, turmeric extract incorporated chitosan-based film. Both physical properties and antibacterial activities of the film were improved due to the turmeric extract incorporation. The antibacterial properties of turmeric extract is drawing increasing scientific interest particularly due to curcuminoids contained. Curcumin as a natural additive for food packaging was reported in the literature. The original contribution of our study is the first use of turmeric extract in biopolymer for food packaging. Since turmeric is abundant and economic comparing with its standard active agents, our study is more applicable for industry.

2. THE SENSITIVE CAPILLARY ELECTROPHORETIC-LIF METHOD FOR SIMULTANEOUS DETERMINATION OF CURCUMINOIDS IN TURMERIC BY ENHANCING FLUORESCENCE INTENSITIES OF MOLECULES UPON INCLUSION INTO 2-(HYDROXYPROPYL)-β-CYCLODEXTRIN[1]

Turmeric, a member of the Zingiberaceae family cultivated generally in India and Southeast Asia, has received great attention in the last years through the scientific communications due to its incredible properties. For centuries powdered rhizomes of turmeric has been extensively used as a spice for its fragrance and yellowish color. Moreover, in Asian medicine turmeric has been very popular in treatment of coryza, hepatic disorders and rheumatism [3]. Major bioactive components of turmeric are curcumin, demethoxycurcumin (DMC) and, bisdemethoxycurcumin (BDMC) which responsible for its yellow color and pharmacological properties [4].

Recent investigations on medicinal properties of curcuminoids in turmeric has proved the anti-inflammatory, antioxidant, anti-atherogenic, antipsoriatic, antidiabetic, antibacterial, immune-stimulatory, and anticancer effects of the molecules [5-7].

The increasing pharmaceutical popularity of turmeric rhizomes has led to various column chromatographic reports on the separation of its curcuminoids for the quality control of this plant [8-10]. Liquid chromatographic methods suffer from the disadvantages of high cost, time consumption in sample preparation to prevent the retention of hydrophobic compounds in column, and production of large quantities of organic waste. Capillary electrophoresis techniques could be a viable alternative to compensate these deficiencies. Low cost, high resolution, separation speed, low

[1]This chapter is based on the paper 'Kalaycıoğlu, Z., Hashemi, P., Günaydın, K., & Erim, F.B. The sensitive capillary electrophoretic-LIF method for simultaneous determination of curcuminoids in turmeric by enhancing fluorescence intensities of molecules upon inclusion into 2-(hydroxypropyl)-β-cyclodextrin, *Electrophoresis*, *36* (20), 2516-2521 (October 2015) DOI: 10.1002/elps.201500253'.

sample consumption, and environmental friendly feature of capillary electrophoresis makes it one of the most important candidates for food analysis [11]. There have been several reports on separation and determination of curcuminoids by capillary electrophoretic methods like capillary zone electrophoresis (CZE) [12-15], micro emulsion electrokinetic chromatography (MEEKC) [16, 17], micellar electrokinetic chromatography (MEKC) [18, 19], and non-aqueous capillary electrophoresis (NACE) [20]. In all of these methods, curcuminoids were detected by diode-array detectors, except one amperometric detection only for curcumin [12], after capillary electrophoretic separations.

Recently, a huge increase has been observed in the number of scientific studies on the activities of curcuminoids against various cancer species and Alzheimer's disease and significant and distinctive pharmaceutical contributions of curcuminoids were reported [21-23]. Sensitive detection of curcuminoids became important in plasma and tumor tissue, besides in plants. In recent years, with the combination of capillary electrophoresis systems with laser induced fluorescent (LIF) detectors, very low detection limits have been reached for many molecules that are fluorescent or that are derivatized with fluorescent dyes [24-26]. Based on the naturally fluorescent features of curcumin, DMC, and BDMC, this is the first CE-LIF report in the literature for the simultaneous analyses of curcuminoids. Moreover, in this study the fluorescence intensities of curcuminoids were further enhanced by adjusting the composition of surrounding microenvironment of analytes using a buffer additive based on our previous experience [27].

2.1 Experimental Part

2.1.1 Materials

Curcumin analytical standard (≥ 98%) and 2-HP-β-CD (average M_w~1460 and 0.8 molar substitution) were purchased from Sigma-Aldrich (Steinheim, Germany). Di-sodium tetraborate decahydrate, hexane, methanol, chloroform, and sodium hydroxide were obtained from Merck (Darmstadt, Germany). All commercial samples of powdered turmeric, rhizomes, and medicinal turmeric capsule were purchased from a local market in Istanbul, Turkey.

2.1.2 Isolation of curcuminoids

All curcuminoids were isolated as described in literatures with a few modifications [28, 29]. The rhizomes were powdered in a pestle and 100 g of fine powder were extracted into acetone by a Soxhlet for 2 days. The volatile oils were removed with hexane (500 mL) and then extract was filtered and concentrated to dryness using a rotary vacuum evaporator at 40^{o}C. This crude curcuminoids mixture (8 g) was dissolved in methanol and subjected to seven TLC Merck silica gel 60 F 254 preparative plates (20x20 cm layers; 0.5 mm thickness) using chloroform:methanol (95:5 *v*/*v*) as developing solvent. The isolation of the curcuminoids was performed by scraping off the three separated bands with decreasing R_f values from BMDC to curcumin as 0.27, 0.55, and 0.75, respectively. Elution of the components from silica gel was performed with methanol. Traces of soluble silica gel were removed using LiChrolut RP-18E columns (Merck). The solvent was evaporated and yielded the pure compounds of curcumin (15 mg), DMC (8 mg), and BDMC (5 mg). The perfect agreement between R_f values and capillary electrophoretic migration times of isolated curcumin and commercial curcumin was obtained. The identification of other curcuminoids was done by comparison of the TLC orders and R_f values of curcuminoids with literature values [28, 29]. The purity of each individual curcuminoids was determined by capillary electrophoresis.

2.1.3 Standard solutions

The stock standard solutions of curcumin, DMC, and BDMC were prepared in methanol separately at concentration of 1000 µg/mL and protected from light in amber glasses at deep freeze. Working standard solutions were freshly prepared by further dilution of stock standards with methanol.

2.1.4 Sample preparation

Rhizomes of turmeric samples were grounded in a pestle and then 100 mg of fine powdered turmeric, rhizomes, and medicinal turmeric capsule were accurately weighed. Samples were extracted into 10 mL of methanol by vortexing for 5 min at 2500 rpm and sonicating for 30 min. The mixture was then centrifuged at 2500 rpm for 5 min. Appropriate amount of the extract was diluted with methanol and filtered through 0.45 µm pore sized micro filters before injections.

2.1.5 Instrumentation and separation conditions

All experiments were carried out with an Agilent 1600 capillary electrophoresis system (Waldbronn, Germany) equipped with a ZETALIF 2000 laser-induced fluorescence detector (Picometrics, Montlaur, France). The excitation was performed at 488 nm by Ar ion laser. Data processing was performed using Agilent Chemstation software. The bare fused silica capillary with total length of 65 cm (effective length of 50 cm to detector) and 75 μm I.D. (Polymicro Technologies, AZ) was used throughout the analysis. The new fused silica capillary was conditioned prior to use by rinsing with 1 M NaOH for 30 min and then water for 10 min. The capillary was flushed with 1 M NaOH for 15 minutes, water for 2 minutes, and buffer for 5 minutes at the beginning of each working day. Between runs, the capillary was flushed with 0.1 M NaOH and water for 2 min, and the running buffer for 5 min. Separations were performed at 25 kV and injections were made at 50 mbar and 6 s. The temperature was set at 25 ^{o}C.

2.2 Results and Discussion

2.2.1 Separation of curcuminoids

The chemical structures of curcuminoids are given in Figure A.1.

Curcuminoids are insoluble in water at acidic or neutral pH values. These compounds are soluble in alkali medium, so that basic buffers come as first candidates for converting curcuminoids to ionic targets and achieve good separation results in CZE.

However curcuminoids undergo a rapid hydrolytic degradation within minutes in alkali solution [30]. Tonnesen et al. reported that the formation of inclusion complexes between cyclodextrins and curcumin improve the water solubility and stability of compound under alkaline conditions [31]. On the other hand, Baglole et al. reported the significant enhancement of the curcumin fluorescence as a result of binding by different cyclodextrins, but mostly with 2-HP-β-CD [32]. Thereby, we added 2-HP-β-CD to our basic separation electrolyte to prevent their hydrolytic degradation and increase their fluorescence intensities.

In the preliminary experiments, phosphate, carbonate, and borate buffer at different concentrations were tried as the separation medium. According to peak migration time and peak heights, the borate buffer was chosen to perform separations. The borate

concentration was fixed at 25 mM and the effects of 2-HP-β-CD concentration, methanol, and buffer pH were studied to obtain optimal migration conditions for the curcuminoids in term of migration time, peak symmetry, and peak height. The concentration of 2-HP-β-CD was changed in the buffer between 10 mM to 40 mM at pH 9.6. When we increased the 2-HP-β-CD concentration, good resolution of all curcuminoids was observed. Finally, 35 mM 2-HP-β-CD was chosen for the best separation. We have checked the effect of buffer pH between pH 9 and 10. At pH 9, the resolution of curcuminoids could not be achieved. Eventually, pH 9.6 was chosen to perform rapid separations with excellent resolution of three curcuminoids. In further experiments, we examined the presence of methanol in the selected buffer system. But the migration times were retarded by gradual addition to 20% methanol into the buffer and no visible improvement was observed in resolution and detection. Consequently, 25 mM borate, 35 mM 2-HP-β-CD at pH 9.6 was selected as an optimized background electrolyte.

Even if the maximum excitation wavelengths of the molecules (around 426 nm) do not exactly match the wavelength of the commercial argon laser, the molecule gives a strong emission under Ar laser excitation at 488 nm, which occurs at the tail of excitation wavelength of the molecules.

Figure 2.1B shows the electropherogram of curcuminoids standard solution in borate buffer and 2-HP-β-CD containing borate buffer (Figure 2.1A). According to the comparison of the detection limits of the molecules in two buffer systems, 30, 40, and 54 fold enhancements in sensitivity were obtained by the use of 2-HP-β-CD in the buffer for curcumin, DMC, and BDMC, respectively.

2.2.2 Validation of method

System suitability of the method was evaluated by analyzing the resolution between the BDMC, DMC, and curcumin peak, analyzing the symmetry and plate numbers of each curcuminoids peak. Table 2.1 shows the data obtained. The validation of the proposed method was performed according to the Eurachem guidelines [33]. The calibration curves were obtained in the range of 0.5-70 μg/mL for each individual curcuminoids with correlation coefficients of 0.999, 0.999, and 0.993 for BDMC, DMC, and curcumin, respectively. The precision of the method was performed in terms of intra-day and inter-day repeatability of corrected peak areas and migration

times. Intra-day analysis was determined by injecting the curcuminoids mixture seven times in the same day. Inter-day repeatability was calculated by injection of standard solutions on three different days, seven injections in each day.

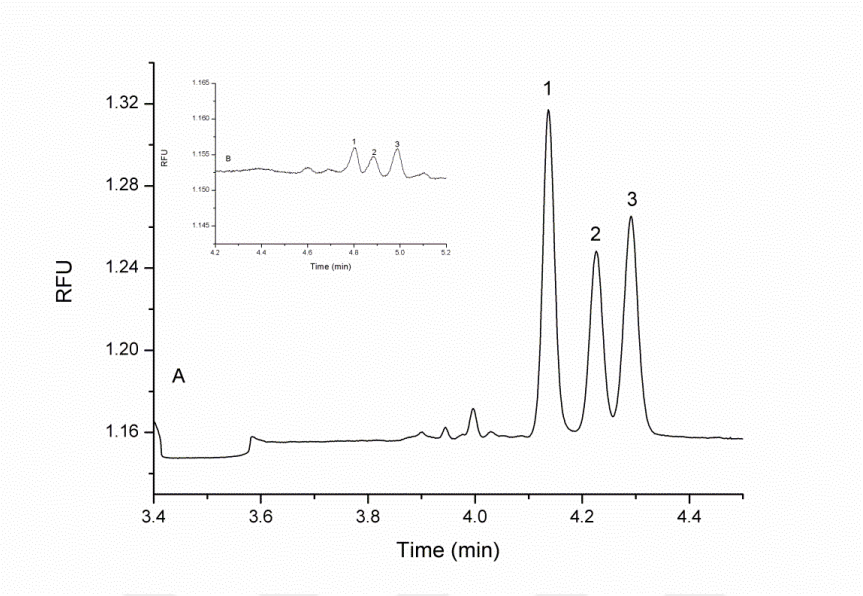


Figure 2.1 : (A) Buffer: 25 mM borate, 35 mM 2-HP-β-CD; (B) Buffer: 25 mM borate. 1: BDMC; 2: DMC; 3: Curcumin.

Table 2.1 : System suitability of the proposed method.

Parameter	BDMC	DMC	Curcumin	BDMC:DMC	DMC:Curcumin
Resolution (Rs)*				2.79	2.07
Symmetry**	1.01	1.04	0.96		
Plate number**	90363	99330	100979		

* $Rs=2(t_n-t_{n-1})/(W_n+W_{n-1})$, where t is the migration time (min) and w is the baseline peak width
** n=7

The precision of the corrected peak areas and migration times (RSD %) were between 1.82-2.26 and 0.73-0.85 in the same day and 1.95-2.55 and 1.29-1.88 in three days, respectively. The limit of detection (LOD) was calculated as three times the average noise taken for three different baseline areas. LOD values for curcumin, DMC, and BDMC were 0.081, 0.039, and 0.005 μg/mL, respectively. From comparing the previously reports in the literature using various techniques of capillary electrophoresis, our results show a considerable lower detection limits for curcuminoids [14-19]. Only in one CE study which reports curcumin analysis with amperometric detection, LOD of curcumin is comparable with our result [12]. We obtained a very low detection limit for BDMC which exists in the smallest amount in the turmeric. The limit of quantification (LOQ) was calculated as ten times the average noise taken for three different baseline areas. LOQ values of the method for curcumin,

DMC, and BDMC were 0.270, 0.130, and 0.016 μg/mL, respectively. Analytical method validation values are given in Table 2.2. Recovery experiments were established in three different levels. Satisfactory recoveries were between 90–104% and are summarized in Table 2.3.

Table 2.2 : Analytical performance of the proposed method.

Parameter	BDMC	DMC	Curcumin
Intra-day Precision (n=7)			
Corrected peak area (RSD, %)	1.94	2.26	1.82
Migration time (RSD, %)	0.76	0.73	0.85
Inter-day Precision (n=3x7)			
Corrected peak area (RSD, %)	2.03	2.55	1.95
Migration time (RSD, %)	1.46	1.29	1.88
Linearity			
Linear range (μg/mL)	0.5-70	0.5-70	0.5-70
Slope	0.0016	0.0002	0.0002
Intercept	-0.00074	0.000006	0.00038
Coefficient of regression	0.999	0.999	0.993
Standard errors of slope	0.001185	0.000177	0.000771
Standard errors of intercept	0.000033	0.000005	0.000024
LOD (μg/mL)	0.005	0.039	0.081
LOQ (μg/mL)	0.016	0.130	0.270

2.2.3 Real sample analyses

The developed method for determination was applied for the determination of curcuminoids in three turmeric powders (P1, P2, P3), three different turmeric rhizomes (R1, R2, R3), and herbal supplement tablet (T). The turmeric extract was prepared as explained before. Figure 2.3 is a representative electropherogram of a powdered turmeric sample. The curcuminoids content of turmeric samples are given in Table 2.4. As seen from the table, herbal supplement tablet contains considerable amount of curcuminoids. The labeled amount of tablet is 475 mg curcuminoids per tablet. We found 435 mg total curcuminoids equivalent per tablet. The label on the product declares that at least 90% of the stated amount should occur in the tablet.

Table 2.3 : Recovery values of curcuminoids for turmeric samples at three concentration level.

Samples		Sample Content(μg/mL)	Curcuminoid added (μg/mL)	Recovery (RSD) (%)	Bias (%)
Turmeric powder	BDMC	5.31	2.65	90.0 (1.73)	3.33
			5.31	90.5 (0.91)	4.75
			10.60	98.5 (1.29)	1.00
	DMC	8.57	4.28	99.3 (1.74)	0.23
			8.57	101.0 (2.04)	0.50
			17.10	104.0 (1.49)	2.67
	Curcumin	8.69	4.35	97.0 (0.80)	1.00
			8.69	102.0 (2.75)	1.00
			17.38	102.0 (3.43)	1.33
Turmeric rhizome	BDMC	5.99	3.00	95.0 (1.92)	1.67
			6.00	95.2 (1.78)	2.40
			12.00	101.0 (1.54)	0.67
	DMC	9.75	4.87	95.8 (2.95)	1.40
			9.75	92.3 (3.53)	3.85
			19.50	99.5 (1.59)	0.33
	Curcumin	13.0	6.51	101.0 (1.91)	0.52
			13.02	98.6 (2.69)	0.68
			26.00	103.0 (3.16)	2.00

2.3 Conclusions

In this study, a very rapid, simple, and highly sensitive capillary electrophoresis method for the simultaneous determination of three curcuminoids was developed. Curcuminoids were extracted with a short and simple extraction method, followed by a CZE separation coupled with LIF detector using borate buffer and 2-HP-β-CD as background electrolyte. The use of 2-HP-β-CD as buffer additive has further enhanced the sensitivity of LIF detection. This method was successfully applied for determination of curcuminoids content of turmeric samples and medicinal turmeric tablet.

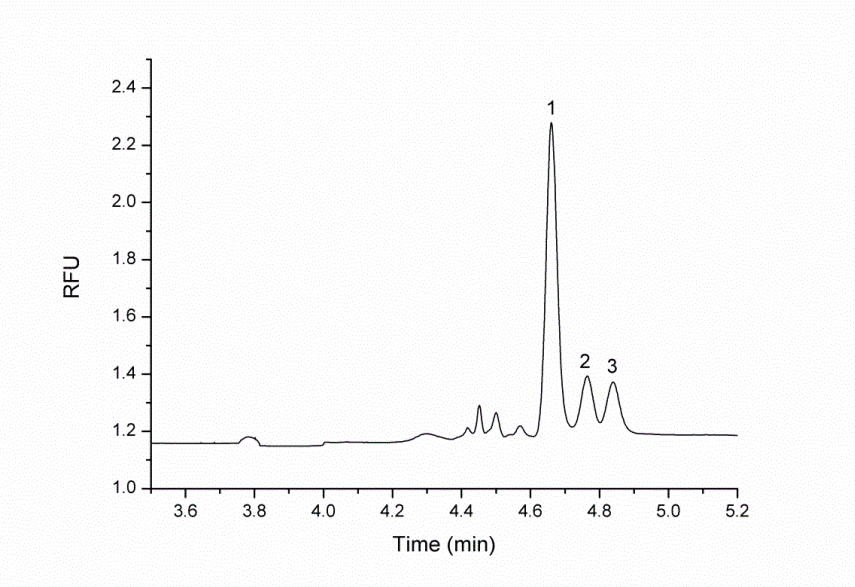


Figure 2.2 : Electropherogram of a powdered turmeric extract.

Table 2.4 : Curcuminoids contents of turmeric samples.

	Mean (g/100g) (RSD%) (n=2)		
Sample	**BDMC**	**DMC**	**Curcumin**
(a) Powdered Turmeric Sample			
P1	0.61 (1.64)	0.92 (2.17)	1.60 (2.50)
P2	0.43 (9.30)	0.51 (1.96)	0.90 (1.11)
P3	0.69 (4.35)	0.87 (4.60)	1.46 (1.37)
(b) Rhizome Turmeric Sample			
R1 (Fresh Rhizome)	1.17 (6.84)	0.75 (1.33)	1.17 (1.71)
R2 (Dried Rhizome)	0.89 (6.74)	1.03 (2.91)	1.73 (3.47)
R3 (Dried Rhizome)	0.48 (2.08)	0.60 (3.33)	1.10 (1.82)
(c) Herbal Turmeric Tablet			
T	6.47 (0.46)	24.9 (5.18)	55.7 (3.64)

3. COMPARISON OF ANTIOXIDANT, ANTICHOLINESTERASE, AND ANTIDIABETIC ACTIVITIES OF THREE CURCUMINOIDS ISOLATED FROM *CURCUMA LONGA* L.[2]

Many plants have medicinal values due to their bioactive ingredients. Medicinal plants may be useful in decreasing of blood pressure, prevention of cardiovascular diseases, and reducing the risk of cancer, mainly due to their antioxidant activities [34]. Although synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have been widely used as antioxidants in the food industry, they may cause liver damage and be carcinogenesis. Therefore, it is strongly recommended to intake the plant materials as effective antioxidant sources [35, 36].

Alzheimer's disease (AD) is a progressive neurodegenerative illness mostly affecting elderly people. AD is characterized by the decrease in the level of a brain neurotransmitter, namely acetylcholine (ACh). Acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) are enzymes which are both present in the brain and catalyze the breakdown of acetylcholine. The first approach to medicate AD was to use drugs restoring the levels of acetylcholine through the inhibition of AChE and BChE enzymes. However synthetic drugs approved for this purpose have undesirable side effects. Therefore, there is an increasing demand to discover new products having AChE and BChE inhibitory activites. Plant ingredients are candidates of this aim. Many medicinal plants have been reviewed for this purpose [37, 38].

Diabetes Mellitus is a chronic metabolic disorder characterized by increase in blood glucose levels. Non-insulin dependent diabetes (Type 2 diabetes) is the most common form of the disease worldwide. Drugs used in the treatment of non-insulin-dependent

[2]This chapter is based on the paper 'Kalaycıoğlu, Z., Gazioğlu, I., & Erim, F.B. Comparison of antioxidant, anticholinesterase, and antidiabetic activities of three curcuminoids isolated from *Curcuma Longa* L. *Nat. Prod. Res., 31* (24), 2914-2917 (March 2017) DOI: 10.1080/14786419.2017.1299727'.

Diabetes Mellitus delay production or absorption of glucose by inhibiting carbohydrate hydrolyzing enzymes such as *α*-glucosidase (EC 3.2.1.20). Current synthetic agents used for this purpose have prominent adverse effects. Alternative approach is isolation of new potent antidiabetic phytochemicals from medicinal plants. There have been several reviews on antidiabetic plants or antidiabetic ingredients isolated from plants [39, 40].

Curcuma longa is a member of Zingiberaceae family and grown generally in Asian countries. *Curcuma longa* or with a common name turmeric is extensively used worldwide as a spice, food preservative, and coloring material. This plant has been used in traditional medicine for the treatment of numerous disorders. Recently, extensive studies have been done with turmeric to reveal the biological activity of this spice and interest is mainly focus on curcumin as the important bioactive component of turmeric [41-44]. However recent chromatographic assays revealed that three curcuminoids, namely curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC), always exist together in turmeric samples [45-47]. On the other hand, there exist only a very few reports on the biological activities of other curcuminoids of turmeric [48-51].

Traditionally used medicinal plants offer great potential for the discovery of new drugs. Especially comparison of bioactive powers of similar structures or chemical isomers helps to understand the structure related to the effect of activities. In our previous study, we developed a sensitive analysis method to quantify three curcuminoids in turmeric [45]. Following this work, in the present study, antioxidant, anticholinesterase, and antidiabetic activities of the isolated curcuminoids from the same turmeric source were found and compared using *in-vitro* models.

3.1 Experimental Part

3.1.1 Materials

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), 5,5′-dithiobis (2-nitrobenzoic) acid (DTNB), acetylthiocholine iodide (AcI) and butyrylthiocholine chloride (BuCl), galantamine, 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), *p*-nitrophenyl-*α*-D-glucopyranoside (*p*-NPG), genistein, 2,4,6-tripyridyl-s-triazine (TPTZ) and $FeCl_3.6H_2O$ were obtained from Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany). Acetic acid, methanol,

dimethyl sulfoxide (DMSO), sodium acetate trihydrate, hydrochloric acid, ascorbic acid and $FeSO_4.7H_2O$ were purchased from Merck (Darmstadt, Germany).

3.1.2 Spectral measurements

NMR analyses were performed onan Agilent VNMRS 500 MHz NMR spectrometer (Waldbronn, Germany). The identification of each compound was confirmed by the complete ^1H and ^{13}C NMR spectra in DMSO-d_6 with 500 MHz. The chemical structures of curcuminoids are given in Figure A.1. ^1H and ^{13}C NMR spectral data of the isolated curcuminoids are given in Tables A.1 and A.2, respectively. Chemical shifts of the curcuminoids were compared and found to be in agreement with those in the literature [28].

Bioactivity measurements were carried out on a 96-well microplate reader, BioTek Power Wave XS (USA). The measurements and calculations of the activity results were evaluated by using Gen5 Data Analysis software.

3.1.3 DPPH free radical scavenging assay

The free radical-scavenging activity of each curcuminoid was determined by the DPPH assay described by Blois [53] with slight modification. In its radical form, the absorption wavelength of DPPH is at 517 nm. Under reduction by an antioxidant or a radical species, its absorption decreases. Briefly, 90 μL methanolic 0.1 mM DPPH solution was added to 10 μL sample solutions in methanol at different concentrations. Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Inhibition of free radical DPPH in percent (I%) was calculated by using the equation 3.1 where A_{sample} is the absorbance of the compounds/references and $A_{control}$ is the absorbance of the control. The concentration of each curcuminoid causing 50% inhibition (IC_{50}) of DPPH radical was estimated using standard calibration curve.

$$I\% = \frac{A_{control} - A_{sample}}{A_{control}} x100 \qquad (3.1)$$

3.1.4 FRAP assay

The ferric-reducing antioxidant power (FRAP) of each curcuminoid was determined, following the method of Benzie and Strain [54]. The FRAP reagent was prepared containing 1:1:10 ratio of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, 20 mM $FeCl_3.6H_2O$, and 0.3 M acetate buffer at pH 3.6, and incubated at 37 °C for 10 min before use. 200 µL of each curcuminoid was allowed to react with 1.8 mL of the FRAP reagent for 10 min at 37 °C in the dark condition. The absorbance of the coloured product (ferrous tripyridyltriazine complex) was measured by a Shimadzu UV-1800 spectrophotometer at 593 nm. Results were expressed as µM Fe(II)/g analyte and compared with that of ascorbic acid. The calibration curve of $FeSO_4.7H_2O$ was constructed in the range of 6.25-40.0 µM with the correlation coefficient of 0.999. The calibration equation was 'y = 0.0216x – 0.009'.

3.1.5 Anticholinesterase activity

Acetyl- and butyryl-cholinesterase inhibitory activities were measured by slightly modifying the spectrophotometric method developed by Ellman et al [55]. Acetylthiocholine iodide and butyrylthiocholine iodide were used as substrates of the reaction and DTNB were used for the measurement of the anticholinesterase activity. Each curcuminoid was dissolved in methanol to prepare their stock solutions. One hundred-fifty microliter of 100 mM sodium phosphate buffer (pH 8.0), 10 µg/mL of sample solution and 20 µL AChE (or BChE) enzyme solution were mixed and incubatedfor 15 min at 25 °C, and then 10 µg/mL of DTNB is added. The reaction was then initiated by the addition of 10 µg/mL acetylthiocholine iodide (or butyrylthiocholine iodide). Final concentrations of the curcuminoids in solutions were 0.04, 7.5, 15 and 30 µg/mL. The hydrolysis of these substrates was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine iodide, at a wavelength of 412 nm. Methanol was used as a solvent to dissolve the samples and control

3.1.6 α-Glucosidase enzyme inhibitory activity

The isolated curcuminoids were assessed for the inhibition of *α*-glucosidase from *Saccharomyces cerevisiae* according to the slightly modified method of Tsuji et al [56]. Briefly, a 40 µL solution of *α*-glucosidase (3.0 U/mL, dissolved in phosphate buffer, pH 6.8) was pre-incubated in the presence of 10 µL of each curcuminoid in DMSO at 37 °C for 30 min. The enzymatic reaction was initiated by the addition of 100 µL of *p*-nitrophenyl-*α*-D-glucopyranoside (*p*-NPG, final concentration 0.5 mM), and then the mixture was incubated for another 30 min. The *α*-glucosidase activity was determined by monitoring the *p*-nitrophenol released from *p*-NPG at 405 nm. Genistein was used as the positive control.

3.1.7 Statistical analysis

All data, for antioxidant, anticholinesterase, and antidiabetic activity tests, are the average of triplicate analyses. The data were reported as mean ± standard deviation. The results were analyzed using the IBM SPSS 23.0 statistical software program for windows. To compare the significant differences of the mean values at $p<0.05$, one way analysis (ANOVA) and the Duncan's new multiple range test were applied to the result.

3.2 Result and Discussion

3.2.1 Antioxidant activity

Strong antioxidant activity of curcumin has been known [57]. However antioxidant activities of its isomers have been hardly investigated. In the present study, antioxidant activity of curcuminoids was carried out in the two common test models, namely DPPH free-radical scavenging and FRAP assays. The results were compared with that of the reference compound BHA (butylated hydroxyanisole, a synthetic antioxidant). As seen from Table 3.1, all individual curcuminoids possess antioxidant activities according to the two methods investigated. Lower IC_{50} value indicates a higher DPPH free radical scavenging activity, higher Fe(II) value indicates higher ferric-reducing antioxidant powers. The antioxidant capacities of curcuminoids decrease in the order: curcumin > demethoxycurcumin > bisdemethoxycurcumin. The decreasing order of antioxidant activities of curcuminoids correlates well between the results of DPPH and

FRAP assays. The inhibition of curcumin was found to be close to that of standard antioxidant, BHA, as seen from Table 3.1. Only two reports exist in the literature giving antioxidant power of three curcuminoids from turmeric. Ramsewak et al. [50] reported antioxidant capacities of three curcuminoids with iron-catalyzed liposome model and % inhibition of liposome peroxidation of curcuminoids were reported. Jayaprakasha et al. [49] reported phosphomolybdenum and linoleic acid method for antioxidant activities of curcuminoids. The correlations between the antioxidant activity orders of curcuminoids with our results and results from two studies are in agreements. Our study is the first report on the simultaneous application of the most commonly used antioxidant assays, i.e. DPPH and FRAP, to three curcuminoids.

Table 3.1 : *In vitro* inhibition and FRAP of the compounds for antioxidant activity.

Compounds	**DPPH assay (IC_{50}, μM)**	**FRAP assay (μM Fe(II)/g)**
Curcumin	0.72 ± 0.01	1884 ± 63
DMC	6.62 ± 0.03	1432 ± 43
BDMC	80.2 ± 0.1	667 ± 19
BHA*	1.18 ± 0.03	
Ascorbic acid*		10846 ± 126

Data represents the means ± S.D of three paralel measurements (p<0.05).
*Standard compounds.

3.2.2 Anticholinesterase activity

The results of AChE and BChE inhibitory activities of tested curcuminoids were expressed as IC_{50} (μM) in Table 3.2. Decreasing values show higher activities. As seen from Table 3, BDMC shows significant AChE inhibition activity (2.14 ± 0.78 μM), which is even better than the AChE inhibition value (2.41 ± 0.12 μM) of galantamine using as standard. Galantamine is a drug used to treat symptoms of Alzheimer's disease. Between three curcuminoids curcumin shows much less activity as acetylcholinesterase inhibitor (51.8 ± 0.59 μM). When compared for BChE activities in Table 3.2, curcumin and DMC show no inhibitory activity against BChE, whereas BDMC shows enzyme activity inhibition power (67.2 ± 0.62 μM). There is only one study reporting AChE inhibitory activities of curcumin, DMC and BDMC by both in-vitro and ex-vivo models [51]. In the in-vitro AChE assay, the individual components of the curcuminoids showed AChE inhibitory activity in the decreasing order as BDMC (16.84 μM), DMC (33.14 μM) and curcumin (67.69 μM) as in agreement with

the order of this study. The present study examined the BChE activity of three curcuminoids for the first time. According to both AChE and BChE assays, BDMC displayed pronounced high inhibitory activity against cholinesterase enzymes.

Table 3.2 : *In vitro* inhibition of the compounds for anticholinesterase activity.

Compounds	**AChE assay (IC_{50}, µM)**	**BChE assay (IC_{50}, µM)**
Curcumin	51.8 ± 0.6	Non-active
DMC	19.7 ± 0.2	Non-active
BDMC	2.14 ± 0.78	67.2 ± 0.6
Galantamine*	2.41 ±0.12	17.4 ± 0.6

Data represents the means ± S.D of three paralel measurements (p<0.05).

*Standard compounds.

3.2.3 Antidiabetic activity

The result of present study is given in Table3.3 as the inhibitory activities of three curcuminoids against α-glucosidase. The concentrations of curcuminoids for 50% inhibition of the α-glucosidase enzymatic activity decrease in the order: bisdemethoxycurcumin (12.5 µM) > curcumin (21.1 µM) > demethoxycurcumin (26.4 µM). BDMC was significantly the most potent compared to the others. The activities of curcumin and DMC are almost similar. The beneficial role of curcumin on diabetes has been reported before [44]. There is only one literature report on the potential of BDMC as inhibitor of human pancreatic α-amylases, but inhibitory potential of other curcuminoids were not exist in the same study [52].

Table 3.3 : *In vitro* inhibition of the compounds for antidiabetic activity.

Compounds	**α-Glucosidase inhibitory assay (IC_{50}, µM)**
Curcumin	21.1 ± 0.3
DMC	26.4 ± 0.2
BDMC	12.5 ± 0.2
Genistein*	2.50 ± 0.02

Data represents the means ± S.D of three paralel measurements (p<0.05).
*Standard compounds.

3.3 Conclusion

Antioxidant activities, acetylcholinesterase (AChE) and butrylcholinesterase (BChE) inhibitory activities, and α-glucosidase inhibitory activities of three curcuminoids

isolated from *Curcuma longa* L. were simultaneously investigated in the present study. Curcumin was found as the strongest antioxidant compound amongst others. Bisdemethoxycurcumin showed significantly higher inhibitory effect against acetylcholinesterase enzyme, followed by demethoxycurcumin. Curcumin has very little acetylcholinesterase inhibitory activity. Whereas curcumin and demethoxycurcumin are not active as butrylcholinesterase inhibitor, bisdemethoxycurcumin shows activity. Consequently, bisdemethoxycurcumin may be a candidate drug for Alzheimer's disease. All three curcuminoids showed potent antidiabetic activity of decreasing order from bisdemethoxycurcumin to demethoxycurcumin.

All these studies suggested that small differences in the chemical structures of isomers strongly affect biological activities of individual curcuminoids. The findings in this research might be useful in designing new drugs for Alzheimer’s and Diabetes Mellitus.

4. ANTIMICROBIAL AND PHYSICAL PROPERTIES OF CHITOSAN FILMS INCORPORATED WITH TURMERIC EXTRACT[3]

Food packages extend the shelf life of the food product protecting them from external factors such as micro-organisms, moisture, and ultraviolet light. Recently, food packages based on biopolymers have gained a significant interest concerning the environmental issues. Moreover, incorporation of antimicrobial agents into biodegradable food packaging systems in order to prevent microbial growth on the food surface has been an area of research for the last decades [58].

Chitosan is a polysaccharide composed of β-(1–4)-D-glucosamine and β-(1–4)-N-acetyl-D-glucosamine, produced by alkaline deacetylation of chitin. Chitin, a natural biopolymer, is derived from exoskeletons of crustaceans. Chitosan has been widely used in food industry due to its biocompatible, biodegradable, antimicrobial, and eco-friendly nature [59]. The excellent film forming property and high mechanical resistance of chitosan resulted in a significant interest on chitosan based packaging materials [60]. The improvement of film properties by the incorporation of several natural additives such as essential oils [61–64], fruit extracts [65–67] have been demonstrated in the recent years.

Turmeric is a spice cultivated from the rhizomes of *Curcuma longa* which is a member of Zingiberaceae family. It is very popular worldwide as a spice, food preservative, and coloring material. The chemical constituents of turmeric and its biological activities were reviewed by Gupta, Patchva, Koh, and Aggarwal [68] and Niranjan and Prakash [14]. Turmeric has been used in traditional medicine and now many scientific studies have been proven its many bioactivities like anti-inflammatory, anti-HIV,

[3]This chapter is based on the paper 'Kalaycıoğlu, Z., Torlak, E., Akın-Evingür, G., Özen, İ & Erim, F.B. (2017). Antimicrobial and physical properties of chitosan films incorporated with turmeric extract. *Int. J. Biol. Macromol.*, *101*, 2914-2917 (August 2017) DOI: 10.1016/j.ij.biomac.2017.03.174.'

antibacterial, antioxidant, antidiabetes, and anticarcinogenic activities. The most studied component of turmeric is curcumin or curcuminoids. Many bioactivities of turmeric are accepted as related to curcumin [42, 68, 69]. However, curcumin constitutes 2–5% of turmeric and many other important ingredients such as polyphenols, sesquiterpenes, diterpenes, triterpenoids, sterols have been isolated from turmeric [68]. High doses of the spice (0.5–1.5 g/day/person) can be tolerated without any toxic effects [69].

Recently, Gul and Bakht [70] showed the antimicrobial potentials of turmeric extracts. This property together with its commercial availability and cost effective features makes turmeric extracts as a good candidate for additive to chitosan films aimed as food packaging.

The objective of this study is to prepare chitosan films by incorporation of turmeric extracts. As far as we know, this is the first report on the incorporation of turmeric extract to chitosan films. The thermal, mechanical, and optical properties, antimicrobial activities and water vapor permeability of the crosslinked chitosan films with and without turmeric extract were evaluated. Turmeric extract is a cost effective additive compared to standard bioactive compounds used as additive. These properties may promote the film to be used in food packaging industry.

4.1 Experimental Part

4.1.1 Materials

Low molecular weight and 75-85% degree of deacetylation chitosan was purchased from Sigma-Aldrich (Steinheim, Germany). Ethanol, sodium sulfate, and acetic acid was obtained from Merck (Darmstadt,Germany).

Antimicrobial tests were done with a Gram-positive foodborne pathogenic bacteria (*Staphylococcus aureus* ATCC 25923) and a Gram-negative foodborne pathogenic bacteria (*Salmonella typhimurium* ATCC 14028). Lyophilized cultures of microorganisms were purchased from Microbiologics Inc. (Saint Cloud, USA).

Commercial sample of powdered turmeric with a known trade mark was from a local market in Istanbul, Turkey.

4.1.2 Preparation of stock turmeric extract solution

500 mg of fine powdered turmeric sample was accurately weighed and extracted into 25 mL of ethanol. After vortexing for 5 min at 2500 rpm, it was sonicated for 30 min with a frequency of 35 kHz, and a power of 160 W. Centrifugation was done at 2500 rpm for 5min. The extract was filtered through a Whatman 41 filter paper and diluted to 25 mL with ethanol.

4.1.3 Preparation of inoculums

Microorganisms were stored in Nutrient Broth (Merck) containing 20% glycerol at −18 °C. Working cultures were grown on Nutrient Agar (Merck) slants and kept at 4 °C. The inoculums were prepared and adjusted to cell density of approximately 5×10^6 colony-forming unit (CFU)/mL in 1/500 nutrient broth (Merck).

4.1.4 Analyses of curcumionid in stock turmeric extract solution

The curcuminoids content of turmeric-ethanol extract (TEE) was determined by capillary electrophoresis using an Agilent 1600 capillary electrophoresis system (Waldbronn, Germany) equipped with a ZETALIF 2000 laser-induced fluorescence detector (Picometrics, Montlaur, France) [45]. The excitation was performed at 488 nm by Ar ion laser. The results were calculated as mg curcuminoid per mL of extract using calibration curves constructed in the range of 0.5-70 µg/mL for each individual curcuminoids with correlation coefficients of 0.999, 0.999, and 0.993 for bisdemethoxycurcumin (BDMC), demethoxycurcumin (DMC), and curcumin, respectively. Curcuminoids content of the film was calculated according to the added stock extract solution and expressed as mg curcuminoid per g of film. Duplicate measurements were done.

4.1.5 Film formation and characterization

4.1.5.1 Film formation

A 2% (*w/v*) chitosan solution was prepared in a 1% (*v/v*) acetic acid aqueous solution. The solution was magnetically stirred for 6 hours and then degassed by an ultrasonicator for 1 hour.

Film-forming solutions were prepared by performing 1:2 (*v/v*) dilution of this solution with ethanol and TEE. These solutions were magnetically stirred for 30 min and then

degassed for 30 min. Films were obtained by casting the solutions onto petri dishes which were dried for 72 h at room temperature with 25% humidity. The films show twistible feature which is suitable for packing.

The dried films were also soaked in water for 3 hours to check the water uptake capability of the films. However negligible increases in film masses were observed.

4.1.5.2 Cross-linking process

Chitosan is soluble in dilute organic and inorganic acid solutions. Since chitosan solution was prepared in acetic acid, after the evaporation of water, the acid will remain in the film. These films may dissolve and degrade when they contact with food products. In order to obtain water insoluble films, chitosan films were crosslinked with Na_2SO_4 as non toxic agent. The cross-linking formation was achieved by the ionic interaction between the positively charged functional groups of chitosan and the sulfate anions [71]. For crosslinking procedure, Na_2SO_4 aqueous solution (20%) was prepared. The dried films were peeled from the petri dishes, soaked into the Na_2SO_4 solution (20%) and crosslinked for 15 min. The films were removed from the counter-ion solution, washed with distilled water, and dried at room temperature and at 25% relative humidity.

4.1.5.3 Thickness measurement and mechanical properties

The film thickness measurement was achieved by a digital micrometer (Mitutoyo Manufacturing Co. Ltd., Tokyo, Japan) having a sensitivity of 0.001 mm. Ten thickness values were taken randomly at different positions on both films.

The mechanical properties of the films were evaluated by stress-strain curves (Young's modulus, strain at break, stress at break, and toughness) using a INSTRON 3345 model, set at cross head speed of 0.1 cm/min, probe size of 10 cm and load cell of 500 N sensibility. According to the standard method ASTM D882-10 [72], the tensile strength (TS) and ductility (percent elongation EL%) of the films were evaluated. The films were cut into 9 mm x 36 mm [72]. The measurements were done at room temperature. Tensile strength (TS) and ductility (percent elongation EL%) of the films were calculated using the equation 4.1 and 4.2, respectively:

$$TS = \frac{load\ at\ break}{original\ \ width}\ x\ original\ \ thickness \tag{4.1}$$

$$EL\% = \frac{elongation \quad rupture}{initial \quad gage \quad length} \, x \, 100 \tag{4.2}$$

4.1.5.4 Thermal properties

Calorimetric analysis was carried out on by using a simultaneous thermal gravimetric analyzer (Seiko Exstar 6200). Sample weights examined were 12.86 and 16.22 mg for pure chitosan film and turmeric incorporated chitosan film, respectively. Each film was heated up from 30 to 800 °C at a heating rate of 10 °C/min. under nitrogen flow of 150 mL/min. The thermo gravimetric (TG) and differential thermo gravimetric (DTG) analyses were performed on pure chitosan and TEE incorporated chitosan film.

4.1.5.5 Water vapor permeability

The water vapor transmission rate measurements of the films were performed according to the wet cup method described by ASTM E96-95 [73]. In this method, the test film covered a Petri dish filled with distilled water. The water mass lost from the dish was monitored as a function of time, and the water vapor transmission rate (WVTR) was calculated from the steady-state region by using a Labthink TSY-T3 water vapor permeability tester. The measurements were carried out at 38 °C with 90% relative humidity. The water vapor pressure at the outer side of the film is 44.73 mm-Hg. Molecular sieve was used as adsorbent inside the dish to adsorb all water molecules passing through the film and to maintain partial water vapor pressure. The water vapor permeabilities (g.mm/h.m^2.Pa) of the films were calculated by equation 4.3 where d is the average film thickness (mm) and ΔP is the partial water vapor pressure gradient.

$$WVP = \frac{WVTR \; x \; d}{\Delta P} \tag{4.3}$$

4.1.5.6 Optical properties

The ultraviolet and visible light barrier properties weredetermined by a Shimadzu UV-1800 spectrophotometer. The analyses were performed in triplicate. Film samples (5 cm in length and 3 cm in width) were fixed in place of the cuvette so the light beam could pass over the film surfaces. Transmittance measurements were taken at wavelengths between 200 and 800 nm. Film transparency was determined at 600 nm,

which is a typical wavelength used for film transparency [74]. The percentage transparency was calculated as follows:

$$Transparen\ cy\ value = \log \frac{T_{600}}{t} \quad (4.4)$$

where T_{600} is the transparency at 600 nm and t is film thickness (mm).

4.1.5.7 FTIR analysis

Fourier-transform infrared spectroscopy (FTIR) analyses were performed by using a Perkin Elmer spectroscopy (Perkin Elmer, USA). The spectra were determined in a spectral range from 600 cm^{-1} to 3600 cm^{-1} with a resolution of 4 cm^{-1}. The data were analyzed by FTIR Spectrum software (Perkin Elmer).

4.1.5.8 Antibacterial activities

Antibacterial activities of chitosan films were determined according to ISO 22196 standard [75]. A polypropylene (PP) film was used as control. Film samples (50 mm×50 mm) were sterilized by UV treatment and then placed into sterile petri dishes. Test inoculums (200 µL) were pipetted onto film samples and films were covered with a piece of UV sterilized PP films (40 mm×40 mm). After intimate contact for 1, 2 and 3 h at 35 °C under relative humidity of above 90%, survival bacteria colonies on plates were counted and results were reported as log CFU/g sample.

4.1.6 Statistical analysis

All data, for mechanical and antimicrobial tests, are the average of triplicate analyses. The data were reported as mean±standard deviation. Analysis of variance (ANOVA) procedure was performed at a level of significance set at $p < 0.05$ using SPSS 23.0 software. Differences were accepted as significant when p-values < 0.05.

4.2 Results and Discussion

Transparent yellow chitosan films were obtained by the incorporation of TEE as seen Figure 4.1. Film properties were studied and compared with and without TEE.

4.2.1 Curcuminoids contents of chitosan film

The contents of three curcuminoids in TEE were found as 0.32 ± 0.01 mg/mL, 0.18 ± 0.02 mg/mL, and 0.12 ± 0.01 mg/mL, for curcumin, DMC, and BDMC, respectively. After casting and drying, turmeric incorporated films contain 197 mg curcumin, 113 mg DMC, and 75 mg BDMC per g chitosan film.

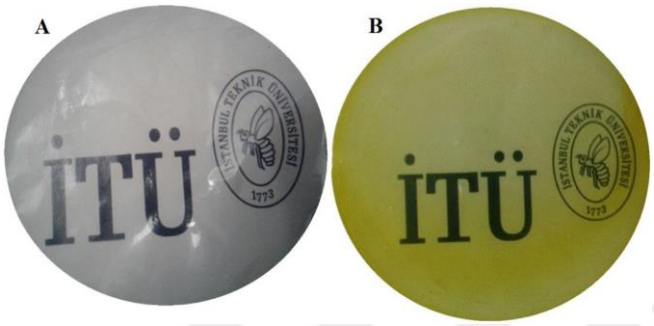


Figure 4.1 : (A) Pure chitosan film and (B) TEE incorporated chitosan film.

4.2.2 Film characterization

4.2.2.1 Thickness and mechanical properties

The thickness of the films are shown in Table 4.1. The incorporation of TEE did not make any change in thickness. This value was used for the tensile strength (TS), ductility (percent elongation EL%), and water vapor permeability (WVP), and transparency calculations.

The mechanical properties of films were characterized by evaluating the tensile strength, ductility, Young's modulus, fracture strength, fracture strain, toughness, and the results were summarized in Table 4.1. Figure 4.2 shows the comparison of the stress-strain graphs for both films with and without turmeric extract.

The incorporation of TEE caused a significant increase ($p < 0.05$) in TS from 32.21 to 63.50 MPa. This improvement of TS may be due to the interaction between -OH group of curcuminoids and other phenolics and -NH_2 groups in chitosan. In the literature, the trends in the change of TS values of chitosan films with the incorporation of different additives are not the same as one another. With some additives TS increases, with others it decreases. Generally, an increase is attributed to a strong interaction between additives and chitosan chains which causes an increase in film rigidity. Contrarily, a decrease in TS is due to a decrease in intermolecular interactions between chitosan chains in the presence of additives.

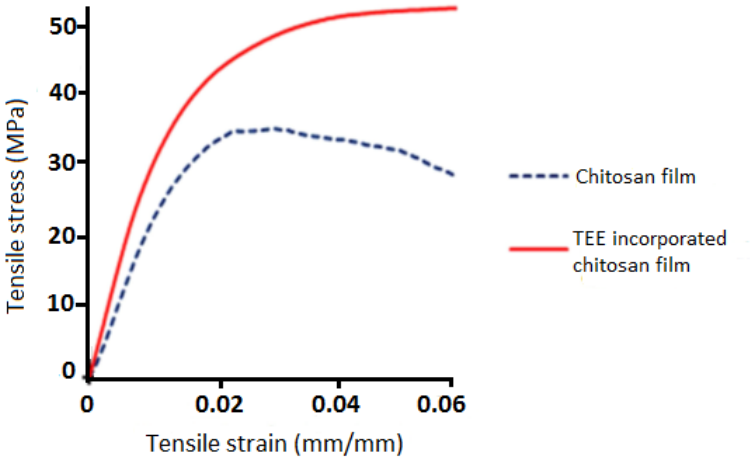


Figure 4.2 : Tensile stress-strain curves of chitosan-based films.

In this study, the addition of turmeric extracts to the chitosan matrix increases film rigidity, significantly. Incorporation of turmeric extracts increases TS value of film as 1.97-fold of TS of pure chitosan films. Similar results have been obtained with chitosan films incorporated with standard curcumin addition and the increase in TS was reported as 2-fold [76]. This improvement was interpreted as the result of strong interaction between curcumin and chitosan backbone. Curcumin is one of the important ingredients of turmeric however curcumin is not the one constituent in turmeric, two important curcuminoids exist together with curcumin, *i.e.* DMC and BDMC, which likely contribute the interaction with chitosan and this explains higher increase in TS. Similarly, TS increase in gelatin films was reported with the addition of curcumin probably due to the interaction between gelatin peptides and curcumin [77].

Ductility (percent elongation EL%) values were almost same for turmeric incorporated chitosan and the pure chitosan films (Table 4.1). Turmeric incorporation leads to significant changes ($p < 0.05$) in the chitosan films, which have impact on the physical characteristics such as fracture strength, fracture strain, and toughness. The results also show that the turmeric incorporated film exhibits a significant increase ($p < 0.05$) in Young's modulus compared to pure chitosan film. In other words, chitosan film established that the modulus of elasticity and the energy needed to break the film increase with the addition of turmeric within the film.

Tensile strength, Young's modulus, fracture strength, and toughness of turmeric incorporated chitosan films increase compared to these values of pure chitosan (Table

4.1). This may be an indication of an interaction between chitosan molecules and probably curcumin which is dominant bioactive component of turmeric. Chitosan and curcumin interaction may give an extra crosslink effect to incorporated film.

Table 4.1 : Physico-chemical properties of chitosan-based films.

Parameter	Pure chitosan film	Turmeric incorporated chitosan film
Thickness, (mm)	$0.04 \pm 2x10^{-4}$	$0.04 \pm 3x\ 10^{-4}$
Tensile strength*, (MPa)	32.2 ± 0.9	47.9 ± 2.6
Ductility (EL), (%)	6.35 ± 0.01	6.20 ± 0.01
Young's Modulus (E), (MPa)	2064 ± 155	2786 ± 125
Fracture strength*, (MPa)	26.4 ± 0.5	47.9 ± 2.6
Fracture strain (mm/mm)	0.06 ± 0.00	0.06 ± 0.00
Toughness*, (kJ/m^3)	1376 ± 110	2378 ± 100
WVTR*, ($g/m^2.day$)	1443 ± 53	1972 ± 113
WVP, ($g.mm/h.m^2.Pa$)	$4.03 \times 10^{-4} \pm 1.52 \times 10^{-5}$	$5.51 \times 10^{-4} \pm 3.18 \times 10^{-5}$

Results were expressed as mean ± standard deviations (n = 3).
Measurements were done 10 times for thickness.
WVTR: Water Vapor Transmission Rate. WVP: Water Vapor Permeability.
*Significant difference between two groups (p<0.05).

4.2.2.2 Thermal behaviour

Figure 4.3 shows the TG and DTG curves of films. As seen from the TG curves, there are two significant weight loss stages: The first small weight loss was from 50 to 150 °C. It may be due to the moisture vaporization. The second weight loss from 250 to 400 °C is caused by the thermal degradations of both films. In each weight loss stage, the loss rates were almost the same. The weight loss stages could be observed better in the DTG curves. The decomposition temperatures of both films are close to each other as around 285 °C.

4.2.2.3 Water vapor permeability

Water vapor is particularly important in food packaging and preservation. Sorption and diffusion of water vapor through polymeric films may also affect the barrier and mechanical properties of the polymer [78]. Hence this property can affect the shelf life of the food product. The results showed that the water vapor permeability (WVP) of chitosan film was increased slightly ($p > 0.05$) with the addition of TEE as shown in Table 4.1. Comparable WVP results for chitosan-based films were obtained [79, 80].

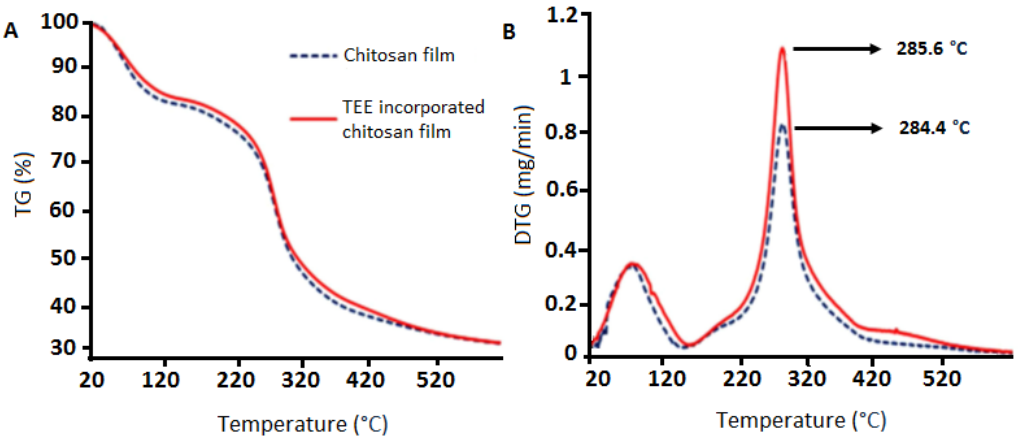


Figure 4.3 : (A) TGA and (B) DTG thermograms of chitosan-based films.

4.2.2.4 Light transmission and transparency

The light transmission of films in a wavelength range from 200 to 800 nm was 0.01–55.8% in the range of visible wavelength (350–800 nm). The incorporation of TEE into the chitosan film significantly ($p < 0.05$) decreased the transmittance values compared to the transmittance of chitosan film (Figure 4.4).

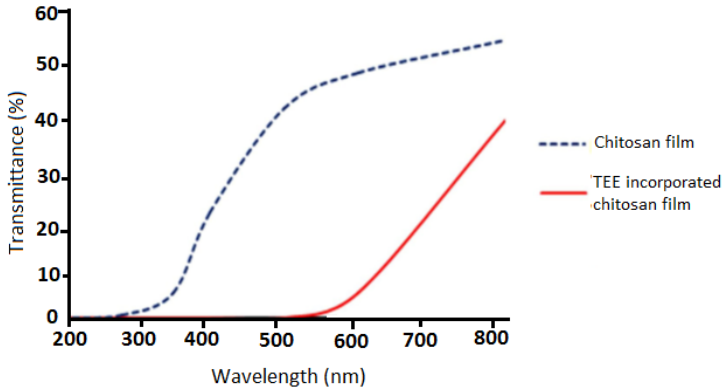


Figure 4.4 : UV/VIS light barrier properties (wavelenghts in the 200-800 nm range as a function of transmittance) of chitosan-based films.

The transmittance value of the film containing TEE was 0.01% in the wavelength range of 200-500 nm. This indicates that TEE incorporated chitosan films have a good barrier property against UV/VIS light transmission toward the food product. These results can be attributed to aromatic groups of the curcuminoids in the turmeric, which are responsible for the absorption of UV/VIS radiation. These groups were also detected at 1280 cm^{-1} in FTIR spectra (Figure 4.5).

The transparency of a film is an important optical parameter for food packaging industry, as well. See-through packaging materials are generally demanded by consumers. The transparency values of the pure chitosan and TEE-incorporated chitosan film at 600 nm were 3.09 and 2.10, respectively. The reduction in transparency of TEE incorporated films is related to yellow color of turmeric. However, turmeric incorporated film still allows product visibility to satisfy consumers demand.

4.2.2.5 FTIR analysis

The infrared spectra of the pure chitosan and TEE incorporated chitosan films are shown in Figure 4.5. The peaks of the TEE incorporated film show the characteristic of pure chitosan film. A broad absorption band at the range of 3200 and 3550 cm^{-1} due to overlapping of hydroxyl group and amino group stretching vibration was observed in both films. The peaks at 2920 and 2872 cm^{-1} were due to the $-CH_2-$ stretching. In both spectrums, it can be said that the absorption band at 1634 cm^{-1} belongs to amide I. Peaks at 1535 and 1554 cm^{-1} represent the N-H (amide II band) of chitosan carbon chains.

The peak at 1280 cm^{-1} in TEE incorporated films was assigned to the -C-O- stretching vibration of benzene ring, which was regarded as a characteristic peak belonging to curcuminoids [81]. As seen from the spectra, typical peaks have blue shifted in TEE incorporated film. This result can be due to the hydrogen bonds formed between chitosan and curcuminoids in turmeric. Liu, Cai, Jiang, Wu, and Le [76] had also similar observations with the addition of curcumin into the chitosan film.

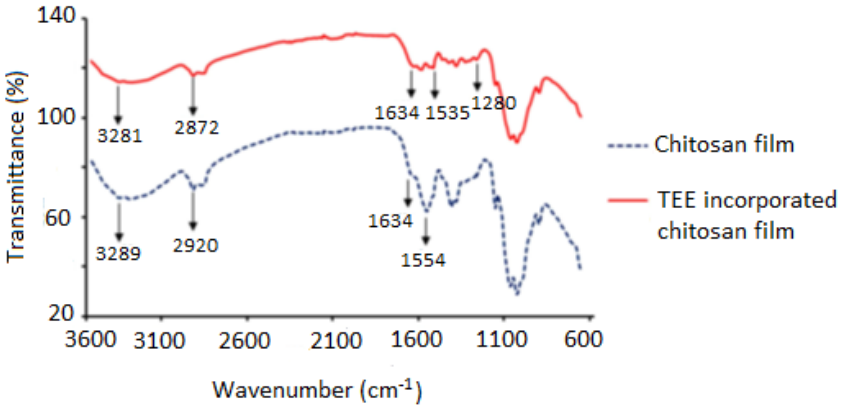


Figure 4.5 : FTIR spectra of chitosan-based films.

4.2.2.6 Antimicrobial activity

Figure 4.6 shows the inhibitory effect of polypropylene (PP) control film, pure chitosan film, and TEE incorporated chitosan film against *Salmonella* and *S. aureus* which are common food poisoning bacteria.

Chitosan is already known as having strong antimicrobial activities [82]. As expected, chitosan films showed a significant reduction ($p < 0.05$) in the counts of all microorganisms tested compared to PP control film during the 3h exposure period (Figure 4.6). Results have also shown that inhibitory efficiency of each chitosan film against *S. aureus*, a Gram-positive bacteria was higher compared to those against *Salmonella*, a Gram-negative bacteria. Similarly, there are some reports indicating that chitosan was more effective on Gram-positive bacteria than Gram-negative [63, 83]. Fernandez-Saiz, Lagaron, and Ocio [84] suggested that antimicrobial activity of chitosan against Gram-positive bacteria is due to the electrostatic interactions occuring between teichoic acid backbone in their cell wall and chitosan molecules.

As expected, antimicrobial activity was improved with the addition of TEE into chitosan film. TEE incorporated chitosan film reduced the counts of *Staphylococcus aureus* and *Salmonella* from significantly ($p < 0.05$), compared to pure chitosan film during the 3 h exposure period (Figure 4.6). It was not observed any significant positive effect of ethanol ($p > 0.05$) to bacterial activity, when chitosan film was compared with chitosan film containing ethanol as seen in Figure. 4.6.

The antibacterial properties of chitosan edible films incorporated with TEE could be related with the presence of mainly curcuminoids and also terpenoids. Gul and Bakht [70] investigated that turmeric itself exhibited antimicrobial activity against *S. aureus* in addition to *Candida albicans*, *Salmonella typhi*, and *Escherichia coli*.

The enhanced antibacterial properties of films are very important for application in food packaging industry to delay or prevent the growth of microorganisms on the product's surface.

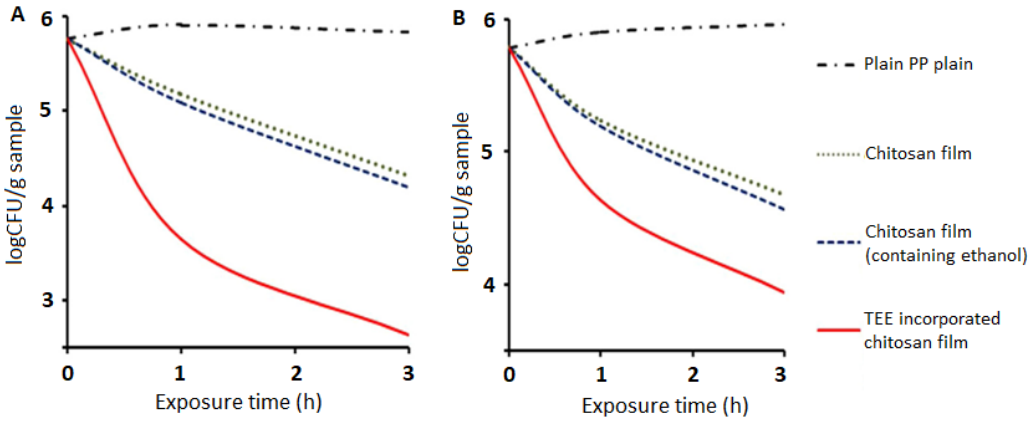


Figure 4.6 : Effects of chitosan-based films on the growth of pathogens during 3 h exposure period (A) *S. aureus* and (B) *Salmonella*.

4.3 Conclusions

Turmeric extract incorporation increased the physical and antibacterial film properties of pure chitosan. Tensile properties were increased considerably by the incorporation of turmeric extract, the film being stiffer than pure chitosan film. Although turmeric incorporation did not modify the water vapor barrier property and thermal behavior of the films, significantly. Films have also good light barrier properties but transparent enough to fulfill consumer demands. Moreover, turmeric incorporated chitosan films exhibit the antibacterial activity. Thereby, turmeric extract incorporated chitosan films can be suggested as potent coating agents with improved film properties and antimicrobial activities against food pathogens.

5. CONCLUSION

Curcuminoids are currently a topic of intense research. Nevertheless, until now most analytical and biochemical approaches to curcuminoids have been limited with only curcumin. Due to the fact that the other two curcuminoids DMC and BDMC were not commercially available until very recently. The pure curcuminoids can be purchased today, but they are both expensive and insufficient. Therefore, it is challenging to maintain the experiments which is relevant to DMC and BDMC. As presented here, those curcuminoids were isolated from the turmeric plant, first. We were able to separate and determine individual curcuminoids, evaluate their bioactivities, and generate antibacterial films using turmeric extract which is a cheap curcuminoid source. This unified approach combined the existing and potential applications related curcuminoids.

In the first part of the thesis, a CE-LIF method for the separation and determination of curcumin, DMC, and BDMC has been successfully developed. The separation medium was optimized as 30 mM borate buffer (pH: 9.6) containing 35 mM of 2-HP-β-CD. Considerable enhancement of the curcumin (30-fold), DMC (40-fold), and BDMC (54-fold) fluorescence occured as a result of 2-HP-β-CD presence in the buffer. The method was used for the analysis of three individual curcuminoids in turmeric powder, turmeric root, and a curcumin herbal supplement. Curcumin, DMC, and BDMC were determined as between 0.90-1.60, 0.51-0.92, and 0.43-0.69 g/100 g in turmeric powder samples, respectively. In turmeric root, curcumin, DMC, and BDMC were reported as between 1.10-1.73, 0.60-1.03, and 0.48-1.17 g/100 g, respectively. As for the curcumin herbal supplement, curcumin, DMC, and BDMC were found as 55.7, 24.9, 6.47 g/100 g, respectively. The method can be used for the identification of these curcuminoids in natural products, herbal supplements, and any foodstuff. The quality control of any product containing turmeric is also possible. Moreover, the analysis of individual curcuminoids in these products are very important not only for quality control issues but also for confirming the efficiency of these compounds as active constituents in functional foods and pharmaceutical forms. Besides, satisfying baseline separation and

good values of resolution factors obtained can make the method applicable at the preparative free-flow electrophoresis scale.

In the second part of the thesis, comparative studies on the antioxidant, anti-Alzheimer, and antidiabetic potential of curcumin, DMC, and BDMC were reported. Curcumin appeared to be more active than both DMC and BDMC in DPPH free radical scavenging assay and FRAP assay related antioxidant activities. The methoxy substituent on the aryl rings seem to increase the antioxidant activity. An opposite trend to antioxidant activity for the curcuminoids were observed in the case of AcHE inhibition power corresponding to anti-Alzheimer activity. As for BcHE inhibition, BDMC was the most active curcuminoid whereas DMC and curcumin was non-active. A similar conclusion in terms of BDMC was obtained for antidiabetic activity which has been detected by α-glucosidase inhibition. Curcumin and DMC followed BDMC, respectively. The results of each assay were compared with those of standard active compounds. Curcuminoids showing better or almost the same activity can replace these synthetic compounds in the pharmaceutical preparations.

In the third part of this thesis, mechanically stable and antibacterial turmeric extract incorporated chitosan film was produced. The physical and mechanic film characteristics fulfills its use in food coating. It is simple and quick to fabricate for many commercial applications. By incorporating turmeric extract within chitosan, an enhancement in antibacterial activity was achieved due to the curcuminoids presence. Turmeric as an antibacterial film additive has advantages economically compared its standard active constituents.

REFERENCES

[1] **Aggarwal, B. B., Surh, Y. J. & Shishodia, S.**(2007). The molecular targets and therapeutic uses of curcumin in health and disease. *Adv. Exp. Med. Biol., 595,* Springer publication.

[2] **Daybe, F. V. (1870).** Uber Curcumin. *Den Farbstoff der Curcumawurzzel Ber 3,* 609.

[3] **Ammon, H. P. T. & Wahl, M. A.** (1991). Pharmacology of Curcuma Longa. *Planta Med., 57,* 1–7.

[4] **Govindarajan, V. S.** (1980). Turmeric—chemistry, technology and quality. *Crit. Rev. Food Sci. Nutr., 12,* 199-301.

[5] **Epstein, J., Sanderson, I. R. & MacDonald, T. T.** (2010). Curcumin as a therapeutic agent: the evidence from in vitro, animal and human studies. *Brit. J. Nutr., 103,* 1545-1557.

[6] **Noorafshan, A. & Ashkani-Esfahani, S.** (2013). A review of therapeutic effects of curcumin. *Curr.Pharm. Des., 19,* 2032-2046.

[7] **Vallianou, N. G., Evangelopoulos, A., Schizas, N. & Kazazis, C.** (2015). Potential anticancer properties and mechanisms of action of curcumin. *Anticancer Res., 35,* 645-651.

[8] **Zhang, J., Jinnai, S., Ikeda, R., Wada, M.,Hayashida, S. & Nakashima, K.** (2009). A simple HPLC-fluorescence method for quantitation of curcuminoids and its application to turmeric products. *Anal. Sci., 25,* 385-388.

[9] **Shen, Y., Han, C., Chen, X., Hou, X. & Long, Z.** (2013). Simultaneous determination of three Curcuminoids in *Curcuma wenyujin* Y. H. chen et C. Ling. by liquid chromatography-tandem mass spectrometry combined with pressurized liquid extraction. *J. Pharm. Biomed. Anal., 81-82,* 146-150.

[10] **Imran, A., Ashanul, H. & Kishwar, S.** (2014). Separation and identification of curcuminoids in turmeric powder by HPLC using phenyl column. *Anal. Methods, 6,* 2526-2536.

[11] **Garcia-Canas, V., Simo, C., Castro-Puyana, M. & Cifuentes, A.** (2014). Recent advances in the application of capillary electromigration methods for food analysis and Foodomics. *Electrophoresis, 35,*147-169.

[12] **Sun, X., Gao, C., Cao, W., Yang, X. & Wang, E.** (2002). Capillary electrophoresis with amperometric detection of curcumin in Chinese herbal medicine pretreated by solid-phase extraction. *J. Chromatogr. A., 962,* 117-125.

[13] **Lechtenberg, M., Quandt, B. & Nahrstedt, A.** (2004). Quantitative determination of curcuminoids in Curcuma rhizomes and rapid differentiation of Curcuma domestica Val. and Curcuma xanthorrhiza Roxb. by capillary electrophoresis. *Phytochem. Anal*., *15,* 152-158.

[14] **Yuan, K., Weng, Q., Zhang, H.,Xiong, J. & Xu, G.** (2005). Application of capillary zone electrophoresis in the separation and determination of curcuminoids in urine. *J. Pharm. Biomed. Anal*., *38,* 133–138.

[15] **Maráková, K., Mikuš, P., Piešťanský, J. & Havránek, E.** (2011). Determination of curcuminoids in substances and dosage forms by cyclodextrin-mediated capillary electrophoresis with diode array detection. *Chem. Pap*., *65*, 398–405.

[16] **Nhujak, T., Saisuwan, W., Srisa-art, M. & Petsom, A.** (2006). Microemulsion electrokinetic chromatography for separation and analysis of curcuminoids in turmeric samples. *J. Sep. Sci*., *29*, 666–676.

[17] **Li, F., Liu, R., Yang, F., Xiao, W., Chen, C. & Xia, Z.** (2014). Determination of three curcumin in Curcuma longa by microemulsion electrokinetic chromatography with protective effects on the analytes. *Anal. Methods*, *6*, 2566–2571.

[18] **Watanabe, T., Mazumder, T. K., Yamamoto, A., Nagai, S. & Terabe, S.** (2000). Separation and determination of curcuminoids in turmeric samples by micellar electrokinetic chromatography with a high molecular mass surfactant. *J. Jpn. Soc. Food Sci*. *Technol*., *47*, 780–786.

[19] **Lin, X. L., Xue, L., Zhang, H. Y. & Zhu, C.F.** (2006). Determination of curcumins in turmeric by micellar electrokinetic capillary chromatography. *Can. J. Anal. Sci. Spect*., *51*, 35–42.

[20] **Anubala, S., Sekar, R. & Nagaiah, K.** (2014). Development and validation of an analytical method for the separation and determination of major bioactive curcuminoids in *Curcuma longa* rhizomes and herbal products using non-aqueous capillary electrophoresis. *Talanta*, *123*, 10–17.

[21] **Yodkeeree, S., Chaiwangyen, W., Garbisa, S. & Limtrakul, P.** (2009). Curcumin, demethoxycurcumin and bisdemethoxycurcumin differentially inhibit cancer cell invasion through the down-regulation of MMPs and uPA. *J. Nutr. Biochem*., *20*, 87–95.

[22] **Ni, X., Zhang, A., Zhao, Z., Shen, Y., & Wang, S.** (2012). Demethoxycurcumin inhibits cell proliferation, migration and invasion in prostate cancer cells. *Oncol. Rep*., *28*, 85–90.

[23] **Ahmed, T. & Gilani, A. H.** (2014). Therapeutic potential of turmeric in Alzheimer's disease: Curcumin or curcuminoids?. *Phytother. Res*., *28*, 517–525.

[24] **Başkan, S., Tezcan, F, Köse, S., Öztekin, N. & Erim, F. B.** (2010). Non-ionic micellar electrokinetic chromatography with laser-induced fluorescence: A new method tested with biogenic amines in brined and dry-salted fish. *Electrophoresis*, *35*, 2174–2179.

[25] **Uzaşcı, S., Başkan, S. & Erim, F. B.** (2012). Biogenic amines in wines and pomegranate molasses – a non-ionic micellar electrokinetic chromatography assay with laser-induced fluorescence detection. *Food Anal. Methods, 5*, 104–108.

[26] **Tezcan, F., Uzaşcı, S., Uyar, G., Öztekin, N. & Erim, F. B.** (2013). Determination of amino acids in pomegranate juices and fingerprint for adulteration with apple juices. *Food Chem., 141*, 1187–1191.

[27] **Uzaşcı, S. & Erim, F. B.** (2014). Enhancement of native fluorescence intensity of berberine by (2-hydroxypropy1)-beta-cyclodextrin in capillary electrophoresis coupled by laser-induced fluorescence detection: Application to quality control of medicinal plants. *J. Chromatogr. A., 1338*, 184–187.

[28] **Péret-Almeida, L., Cherubino, A. P. F., Alves, R. J., Dufossé, L. & Glória,M. B. A.** (2005). Separation and determination of the physico-chemical characteristics of curcumin, demethoxycurcumin and bisdemethoxy curcumin. *Food Res. Int., 38*, 1039–1044.

[29] **Revathy, S., Elumalai, S., Benny, M., & Antony, B.** (2012). Isolation, purification and identification of curcuminoids from turmeric (Curcuma longa L.) by column chromatography. *J. Exp. Sci., 2*, 21–25.

[30] **Tonnesen, H. H. & Karlsen, J.** (1985). Studies of curcumin and curcuminoids: VI. Kinetics of curcumin degradation in aqueous solutions. *Z. Lebensm. Unters. Forsch., 18*, 132–134.

[31] **Tonnesen, H. H., Másson, M. & Loftsson, T.** (2002). Cyclodextrin complexation: solubility, chemical and photochemical stability. *Int. J. Pharm., 24*, 127–137.

[32] **Baglole, K. N., Boland, P. G. & Wagner, B. D.** (2005). Fluorescence enhancement of curcumin upon inclusion into parent and modified cyclodextrins. *J. Photoch. Photobio. A., 173*, 230–237.

[33] **EURACHEM**, The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics, 1st ed., 1998.

[34] **Škrovánková, S., Mišurcová, L. & Machů, L.** (2012). Antioxidant activity and protecting health effects of common medicinal plants. *Adv. Food Nutr. Res., 67*, 75–139.

[35] **Krishnaiah, D., Sarbatly, R. & Nithyanandam, R.** (2011). A review of the antioxidant potential of medicinal plant species. *Food Bioprod. Process., 89*, 217–233.

[36] **Barlow, S. & Schlatter, J.** (2010). Risk assessment of carcinogens in food. *Toxicol. Appl. Pharm., 243*, 180–190.

[37] **Adewusi, E. A., Moodley, N. & Steenkamp, V.** (2010). Medicinal plants with cholinesterase inhibitory activity: a review. *Afr. J. Biotechnol., 9*, 8257–8276.

[38] **Mukherjee, P. K., Kumar, V., Mal, M. & Houghton, P. J.** (2007). Acetylcholinesterase inhibitors from plants. *Phytomedicine, 14*, 289–300.

[39] **Marles, R. J. & Farnsworth, N. R.** (1995). Antidiabetic plants and their active constituents. *Phytomedicine, 2*, 137–189.

[40] **Elekofehinti, O. O.** (2015). Saponins: anti-diabetic principles from medicinal plants – a review. *Pathophysiology, 22*, 95–103.

[41] **Hatcher, H., Planalp, R., Cho, J., Torti, F. M. & Torti, S. V.** (2008). Curcumin: from ancient medicine to current clinical trials. *Cell. Mol. Life Sci., 65*, 1631–1652.

[42] **Chattopadhyay, I., Biswas, K., Bandyopadhyay, U. & Banerjee, R. K.** (2004). Turmeric and curcumin: biological actions and medicinal applications. *Curr. Sci., 87*, 44–53.

[43] **Hamaguchi, T., Ono, K. & Yamada, M.** (2010). Review: curcumin and alzheimer's disease.*CNS Neurosci. Ther., 16*, 285–297.

[44] **Fazel Nabavi, S., Thiagarajan, R., Rastrelli, L., Daglia, M., Sobarzo-Sánchez, E., Alinezhad, H. & Mohammad Nabavi, S.** (2015). Curcumin: a natural product for diabetes and its complications. *Curr. Top. Med. Chem. 15*, 2445–2455.

[45] **Kalaycıoğlu, Z., Hashemi, P., Günaydın, K. & Erim, F. B.** (2015). The sensitive capillary electrophoretic–LIF method for simultaneous determination of curcuminoids in turmeric by enhancing fluorescence intensities of molecules upon inclusion into (2-hydroxypropyl)-β-cyclodextrin. *Electrophoresis, 36*, 2516–2521.

[46] **Wichitnithad, W., Jongaroonngamsang, N., Pummangura, S. & Rojsitthisak, P.** (2009). A simple isocratic HPLC method for the simultaneous determination of curcuminoids in commercial turmeric extracts, *Phytochem. Analysis, 20*, 314-319.

[47] **Song, W., Qiao, X., Liang, W. F., Ji, S., Yang, L., Wang, Y., Xu, Y. W., Yang, Y., Guo, D. A. & Ye, M.** (2015). Efficient separation of curcumin, demethoxycurcumin, and bisdemethoxycurcumin from turmeric using super critical fluid chromatography: from analytical to preparative scale. *J. Sep. Sci., 38*, 3450–3453.

[48] **Sivasothy, Y., Sulaiman, S. F., Ooi, K. L., Ibrahim, H. & Awang, K.** (2013). Antioxidant and antibacterial activities of flavonoids and curcuminoids from *Zingiber spectabile* Griff. *Food Control, 30*, 714–720.

[49] **Jayaprakasha, G. K., Jaganmohan Rao, L. & Sakariah, K. K.** (2006). Antioxidant activities of curcumin, demethoxycurcumin and bisdemethoxycurcumin. *Food Chem., 98*, 720–724.

[50] **Ramsewak, R. S., DeWitt, D. L. & Nair, M. G.** (2000). Cytotoxicity, antioxidant and anti-inflammatory activities of curcumins I-III from *Curcuma longa*. *Phytomedicine, 7*, 303-308.

[51] **Ahmed, T. & Gilani, A. H.** (2009). Inhibitory effect of curcuminoids on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia may explain medicinal use of turmeric in alzheimer's disease. *Pharmacol., Biochem. Behav., 91*, 554–559.

[52] **Ponnusamy, S., Zinjarde, S., Bhargava, S., Rajamohanan, P. R. & RaviKumar, A.** (2012). Discovering bisdemethoxycurcumin from *Curcuma longa* rhizome as a potent small molecule inhibitor of human pancreatic *α*-amylase, a target for type-2 diabetes. *Food Chem.*, *135*, 2638–2642.

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

[54] **Benzie, I. F. F. & Strain, J. J.** (1996). The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Anal. Biochem.*, *239*, 70–76.

[55] **Ellman, G. L., Courtney, K. D., Andres Jr., V. & Featherstone, R. M.** (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*,*7*, 88–95.

[56] **Tsujii, E., Muroi, M., Shiragami, N. & Takatsuki, A.** (1996). Nectrisine is a potent inhibitor of alpha-glucosidases, demonstrating activities similarly at enzyme and cellular levels. *Biochem. Biophys. Res. Commun.*, *220*, 459–466.

[57] **Ak, T. & Gülçin, İ.** (2008). Antioxidant and radical scavenging properties of curcumin. *Chem. Biol. Interact.*, *174*, 27–37.

[58] **Malhotra, B., Keshwani, A. & Kharkwal, H.** (2015). Antimicrobial food packaging: potential and pitfalls. *Front. Microbiol.*, *6*, 1–9.

[59] **Shahidi, F., Arachchi, J. K. V. & Jeon, Y. J.** (1999). Food applications of chitin and chitosans. *Trends Food Sci. Technol.*, *10*, 37–51.

[60] **Van den Broek, L. A. M., Knoop, R. J. I., Kappen, F. H. J. & Boeriu, C. G.** (2015). Chitosan films andblends for packaging material. *Carbohydr. Polym.*, *116*, 237–242.

[61] **Hafsa, J., Smach, M. A., Khedher, M. R. B., Charfeddine, B., Limem, K., Majdoub, H. & Rouatbi, S.** (2016). Physical, antioxidant and antimicrobial properties of chitosan films containing Eucalyptus globulus essential oil. *LWT— Food Sci. Technol. 68*, 356–364.

[62] **Sánchez-González, L., González-Martínez, C., Chiralt, A. & Cháfer, M.** (2010). Physical and antimicrobial properties of chitosan–tea tree essential oil composite films. *J. Food Eng.*, *98*, 443–452.

[63] **Torlak, E. & Nizamlıoğlu, M.** (2011). Antimicrobial effectiveness of chitosan-essential oil coated plastic films against foodborne pathogens. *J. Plast. Film Sheeting*, *27*, 235–248.

[64] **Yuan, G., Chen, X. & Li, D.** (2016). Chitosan films and coatings containing essential oils: the antioxidant and antimicrobial activity and application in food systems. *Food Rest. Int.*, *89*, 117–128.

[65] **Fan, J., Zhang, Z. H., Qin, Y. Y., Zhao, T. R. & Cheng, C. S.** (2013). Characterization of antioxidant chitosan film incorporated with pomegranate peel extract. *Adv. Mater. Res.*, *706*, 24–27.

[66] **Genskowsky, E., Puente, L. A., Pérez-Álvarez, J. A., Fernandez-Lopez, J., Muñoz, L. A. & Viuda-Martos, M.** (2015). Assessment of

antibacterial and antioxidant properties of chitosan edible films incorporated with maqui berry (Aristotelia chilensis). *LWT—Food Sci. Technol., 64,* 1057–1062.

[67] **Tan, Y. M., Lim, S. H., Tay, B. Y., Lee, M. W. & Thian, E. S.** (2015). Functional chitosan-based grape fruit seed extract composite films for applications in food packaging technology. *Mater. Res. Bull., 69,* 142–146.

[68] **Gupta, S. C., Patchva, S., Koh, W. & Aggarwal, B. B.** (2012). Discovery of curcumin, a component of golden spice, and its miraculous biological activities. *Clin. Exp. Pharmacol. Physiol., 39,* 283–299.

[69] **Niranjan, A. & Prakash, D.** (2008). Chemical constituents and biological activities of turmeric (Curcuma longa L.)—a review. *J. Food Sci. Technol., 45,* 109–116.

[70] **Gul, P. & Bakht, J.** (2015). Antimicrobial activity of turmeric extract and its potential usein food industry. *J. Food Sci. Technol., 52,* 2272–2279.

[71] **Al-Remawi, M. M. A.** (2012). Properties of chitosan nanoparticles formed using sulfateanions as crosslinking bridges. *Am. J. Appl. Sci., 9,* 1091–1100.

[72] **ASTM** (2010). Standard Test Method for Tensile Properties of Thin Plastic Sheeting D882-10 Annual Book of American Standard Testing Methods, ASTM, Philadelphia.

[73] **ASTM E96-95** (1994). Standard test methods for water vapor transmission of materials, in: In: Annual Book of ASTM Standards, No. E96-95, American Society forTesting Materials, Philadelphia, PA, USA, pp. E95–E96.

[74] **Han, J. H. & Floros, J. D.** (1997). Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. *J. Plast. Film Sheeting, 13,* 287–298.

[75] **ISO 22196** (2007). Plastics—Measurement of antibacterial activity on plastics surfaces.

[76] **Liu, Y., Cai, Y., Jiang, X., Wu, J. & Le, X.** (2016). Molecular interactions, characterization and antimicrobial activity of curcumin-chitosan blend films. *Food Hydrocolloids, 52,* 564–572.

[77] **Bitencourt, C. M., Fávaro-Trindade, C. S., Sobral, P. J. A. & Carvalho, R. A.** (2014). Gelatin-based films additivated with curcuma ethanol extract: antioxidant activity and physical properties of films. *Food Hydrocolloids, 40,* 145–152.

[78] **Rubino, M., Tung, M. A., Yada, S. & Britt, I. J.** (2001). Permeation of oxygen, water vapor, and limonene through printed and unprinted biaxially oriented polypropylene films. *J. Agric. Food Chem., 49,* 3041–3045.

[79] **Bourbon, A. I., Pinheiro, A. C., Cerqueira, M. A., Rocha, C. M. R., Avides, M. C., Quintas, M. A. C. & Vicente, A. A.** (2011). Physicochemical characterization of chitosan-based edible films incorporating bioactive compounds of different molecular weight. *J.Food Eng., 106,* 111–118.

[80] **Silva, M. D. F., Lopes, P. S., Da Silva, C. F. & Yoshida, C. M. P.** (2016). Active packaging material based on buriti oil—Mauritia flexuosa L.f. (Arecaceae) incorporated into chitosan films. *J. Appl. Polym. Sci.*, *133*, 1–9.

[81] **Feng, F., Liu, Y., Zhao, B. & Hu, K.** (2012). Characterization of half N acetylated chitosan powders and films. *Procedia Eng.*, *27*, 718–732.

[82] **Hosseinnejad, M. & Jafari, S. M.** (2016). Evaluation of different factors affecting antimicrobial properties of chitosan. *Int. J. Biol. Macromol.*, *85*, 467–475.

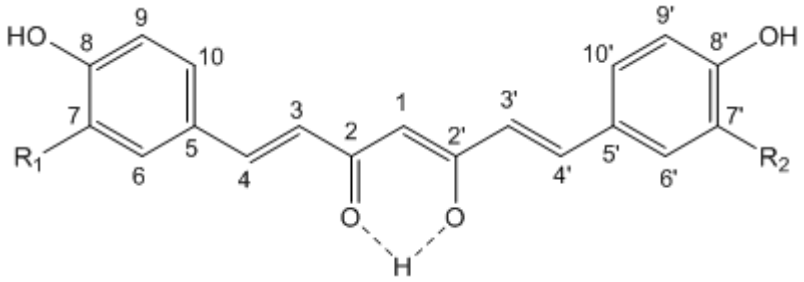
[83] **No, H. K., Park, N. Y., Lee, S. H. & Meyers, S. P.** (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *Int. J. Food Microbiol.*, *74*, 65–72.

[84] **Fernandez-Saiz, P., Lagaron, J. M. & Ocio, M. J.** (2009). Optimization of the biocide properties of chitosan for its application in the design of active films of interest in thefood area. *Food Hydrocolloids*, *23*, 913–921.

APPENDICES

APPENDIX A: NMR spectral data

APPENDIX A



Compound	R_1	R_2
Curcumin	$-OCH_3$	$-OCH_3$
DMC	-H	$-OCH_3$
BDMC	-H	-H

Figure A.1 : The chemical structures of three main curcuminoids.

Table A.1 : ^{1}H NMR spectral data of the isolated curcuminoids.

^{1}H	Curcumin	DMC	BDMC
1	6.04 (2H, s)	6.02 (2H, s)	5.99 (2H, s)
2,2'	-	-	-
3,3'	6.75 (1H, d J=15.7 Hz)	6.68 (2H, d J=15.8 Hz)	6.69 (1H, d J=15.7 Hz)
4,4'	7.54 (1H, d J=15.7 Hz)	7.55 (2H, d J=15.8 Hz)	7.55 (1H, m)
5,5'	-	-	-
6,6'	7.30 (1H br. s)	7.32 (1H br. s)	7.55 (1H, m)
7,7'	-	7.53 (1H, d J=8 Hz)	7.55 (1H, m)
8,8'-OH	-	-	-
9,9'	6.82 (1H, d J=7.8 Hz)	6.81 (1H, d J=7.8 Hz)	6.81 (1H, d J=7.8 Hz)
10,10'	7.14 (1H, dd, J=7.8 and 1.8 Hz)	7.14 (1H, dd, J=7.8 and 1.8 Hz)	7.55 (1H, m)
O-CH_3	3.82 (s, 6H)	3.82 (s, 3H)	-

s:singlets; d:doublets; br. s: broad singlet; m: multiple
Conditions of NMR: ^{1}H 500 MHz, DMSO-d_6

Table A.2 : ^{13}C NMR spectral data of the isolated curcuminoids.

^{13}C	**Curcumin**	DMC	BDMC
1	101.0	101.0	101.0
2,2'	188.6/188.0	188.6/188.0	188.6/188.0
3,3'	123.4/119.6	123.4/119.6	123.4/119.6
4,4'	152.7/131.2	152.7/131.2	152.7/131.2
5,5'	120.1	127.8/120.1	127.8
6,6'	116.8	115.8/116.8	115.8
7,7'	144.9	157.7/144.9	157.7
8,8'	151.3	115.8/151.3	115.8
9,9'	112.0	127.8/112.0	127.8
10,10'	128.8	127.8/128.8	127.8
O-CH_3	56.2/56.2	56.2	

Conditions of NMR: ^{13}C 500 MHz, DMSO-d_6

CURRICULUM VITAE

Name Surname **:** Zeynep Kalaycıoğlu

Place and Date of Birth: Üsküdar–1989

E-Mail **:** kalayciogluz@itu.edu.tr

EDUCATION:

B.Sc. **:**Yıldız Technical University – Faculty of Arts and Sciences/ Chemistry Department (2007–2013)

M.Sc. **:**Istanbul Technical University – Institute of Engineering and Science / Chemistry Program (2013–2015)

PROFESSIONAL EXPERIENCE:

Research & Teaching Assistant, Chemistry Department, Istanbul Technical University (Jan. 2015–Feb. 2020)

Instructor, Chemistry Department, Istanbul Technical University (Feb. 2020–...)

PUBLICATIONS ON THE PhD THESIS:

- **Kalaycıoğlu, Z.**, Hashemi, P., Günaydın, K., and Erim, F.B. (2015). The sensitive capillary electrophoretic–LIF method for simultaneous determination of curcuminoids in turmeric by enhancing fluorescence intensities of molecules upon inclusion into 2-(hydroxypropyl)-β-cyclodextrin. *Electrophoresis*, 36, 2516–2521.

- **Kalaycıoğlu, Z.**, Gazioğlu, I., and Erim, F.B. (2017). Comparison of antioxidant, anticholinesterase, and antidiabetic activities of three curcuminoids isolated from *Curcuma Longa* L. *Nat. Prod. Res.* 31, 2914–2917.

- **Kalaycıoğlu, Z.**, Torlak, E., Akın-Evingür, G., Özen, I., and Erim, F.B. (2017) Antimicrobial and physical properties of chitosan films incorporated with turmeric extract. *Int. J. Biol. Macromol.* 101, 882–888.

PUBLICATIONS FROM THE MSc THESIS:

- **Kalaycıoğlu, Z.** and Erim, F.B. (2016). Simultaneous determination of nitrate and nitrite in fish products with improved sensitivity by sample stacking-capillary electrophoresis. *Food Anal. Methods*, 9, 706–711.

OTHER PUBLICATIONS:

- **Kalaycıoğlu, Z.** and Erim, F.B. (2019). Nitrate and nitrites in foods: worldwide regional distribution in view of their risks and benefits. *J. Agric. Food Chem.* 67, 7205–7222.

- 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. *J. Pharmaceut. Biomed. 175*.

- **Kalaycıoğlu, Z.**, Karadaş, N., Çınar, M. E., Ayşil Ozkan, S., Ozer Unal, D., Gölcü, A., Erim, F.B. (2019). Electrochemical oxidation of curcuminoids: an experimental and computational investigation. *Turk J Chem.* 43, 834–840.

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

- Akkaya, N. E., İşçi, S., Ertem, A. F., **Kalaycıoğlu, Z.**, Giz, H. (2019). Increasing the water solubility of curcumin by alginate in ultrasound medium. *Sigma Journal of Engineering and Natural Sciences.* 37, 85–92.

- **Kalaycıoğlu, Z.**, Uzaşçı, S., Dirmenci, T. Erim, F.B. (2018). Glucosidase enzyme inhibitory effects and ursolic and oleanolic acid contents of fourteen Anatolian *Salvia* species. *J. Pharmaceut. Biomed.* 155,284–287.

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

- **Kalaycıoğlu, Z.**, Kaygusuz, H., Döker, S., Kolaylı, S., Erim, F.B. (2017). Characterization of Turkish honeybee pollens by principal component analysis based on their individual organic acids, sugars, minerals, and antioxidant activities. *LWT - Food Sci. Technol.* 84, 402–408.

- Özgür, M., **Kalaycıoğlu, Z.**, Dülger, Ö. (2017). Simultaneous spectrophotometric determination of phenolic antioxidant (BHA and BHT) concentrations in pharmaceutical preparations and chewing gums using the H-point standard addition method. *Maced J Chem En.* 36, 211–222.

- Çatalgil Giz, H.,İşçi, S., Bahadori, F., **Kalaycıoğlu, Z.**, Akkurt, B. (2017). Curcumin, increasing its water solubility by ultrasound and PEG. *Sigma Journal of Engineering and Natural Sciences.* 35, 47–58.

- Tezcan, F., Uzaşcı, S., Uyar, G., **Kalaycıoğlu, Z.**, Erim, F.B. (2017). Comparison of proline and arginine contents of pomegranate and grape juices in Turkey for the detection of juice adulteration. *Hacettepe J. Biol. Chem.* 45, 557–562.