ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

THE UNIAXIAL AND COAXIAL ELECTROSPUN NANOFIBER ENCAPSULATION OF SOUR CHERRY (*PRUNUS CERASUS* L.) ANTHOCYANINS AND THEIR *IN VITRO* BIOACCESSIBILITY

M.Sc. Thesis

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Department of Food Engineering

Food Engineering Programme

JUNE 2016

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

VİŞNE ANTOSİYANİNLERİNİN TEK EKSENLİ VE ÇİFT EKSENLİ ELEKTROEĞİRME YÖNTEMİYLE ENKAPSÜLASYONU VE BİYOYARARLILIĞININ İNCELENMESİ

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Date of Submission: 2 May 2016
Date of Defense: 10 June 2016

To my beloved family,

FOREWORD

First of all, I would like to express my special thanks and gratitude to my dear supervisor, Assist. Prof. Dr. Filiz ALTAY LOKUMCU for her valuable guidance, encouragement and support along my study.

Another important person either in this study or whole my education is Esra ÇAPANOĞLU GÜVEN whom I like to give my special gratitude for giving me an opportunity to share her excellent knowledge, encouragement, support and patience.

I would like to give special thanks to Prof Dr. Beraat Özçelik for her indulgence and encourangement during this process.

I would like to thank to Konfrut Fruit Juice Company for provision of sour cherry concentrate used in this study.

I represent my gratitude and acknowledge to Nagihan OKUTAN for her help during my experimental study.

Finally, I would like to dedicate this study to my dear parents Safiye IŞIK and Ali Ziya IŞIK. I want to thank to them becauce of their endless support, love, patience and help in my whole life.

June 2016

Beyza Şükran IŞIK

Food Engineering

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ABBREVIATIONS

ABTS: 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid
C3GE: Cyanidin-3-glucoside equivalent
CE: Catechin equivalent
DPPH: 2,2-diphenyl-1-picrylhydrazyl
G: Gelatin solution
GAE: Gallic Acid Equivalent
GI: Gastrointestinal
GL: Gelatin and lactalbumin solution
GN: Gelatin nanofiber
GLN: Gelatin and lactalbumin nanofiber
HPLC: High Performance Liquid Chromotography
IN: Intestine
PG: Post Gastric
S: Sour cherry concentrate
SG: Sour cherry and gelatin solution
SGCN: Sour cherry nanofiber coated with gelatin by coaxial electrospinning
SGL: Sour cherry, gelatin and lactalbumin solution
SGLN: Sour cherry nanofiber coated with mixture of gelatin and lactalbumin by uniaxial electrospinning
SGN: Sour cherry nanofiber coated with gelatin by uniaxial electrospinning
TAC: Total Anthocyanin Content
TEAC: Trolox Equivalent Antioxidant Activity
TFC: Total Flavonoid Content
TPC: Total Phenolic Content
Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

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THE UNIAXIAL AND COAXIAL ELECTROSPUN NANOFIBER ENCAPSULATION OF SOUR CHERRY (*PRUNUS CERASUS* L.) ANTHOCYANINS AND THEIR *IN VITRO* BIOACCESSIBILITY

SUMMARY

Sour cherry (*Prunus Cerasus* L.) is consumed worldwide and its bioactive compounds protect human body against diseases.

Although sour cherry is a good source of polyphenols, protection of polyphenols from unwanted environmental condition and improvement of bioaccessibility are very crucial. In literature, several encapsulation techniques applied to polyphenols, however encapsulation with electrospinning method is not studied yet. In addition, information about *in vitro* digestion and bioaccessibility of the encapsulated bioactive compounds is highly limited.

The aim of this study is encapsulation of sour cherry anthocyanins and other phenolic compounds coated with gelatin and mixture of gelatin and lactalbumin by electrospinning process and determination of bioaccessibility of the nanofibers. While investigating encapsulation of sour cherry polyphenols, solution properties, characterization of nanofibers, total antioxidant content and capacity by spectrophotometry, phenolic profile by chromatography and *in vitro* digestion were analyzed.

Electrical conductivity, surface tension, viscosity and dielectric constant were measured 2.25±0.01 mS/cm, 31.16±0.03 mN/m, 0.21 ± 0.01 Pa.s and 39.52± 0.33 for sour cherry concentrate with gelatin solution and 2.23±0.01 mS/cm, 30.52±0.03 mN/cm, 0.24 ± 0.01 Pa.s, 65.14± 1.96 for sour cherry with mixture of gelatin and lactalbumin mixture, respectively. Both gelatin and mixture of gelatin and lactalbumin were suitable coating materials for electrospinning of sour cherry polyphenols.

Encapsulation efficiency of nanofibers were determined by HPLC. The results were 61.74, 79.17 and 50.33 % for nanofiber coated with gelatin (SGN), mixture of gelatin and lactalbumin by uniaxial electrospinning (SGLN) and gelatin by coaxial electrospinning (SGCN), respectively.

Zeta potential and contact angle were evaluated to characterize nanofibers. All zeta potentials were negative charged. The lowest zeta potential was found for SGCN (-9.38 ± 0.73 mV). SGN (-8 ± 1.23 mV) and SGLN (-3.19 ± 0.15 mV) followed. All nanofibers showed hydrophilic properties because all contact angles were lower than 90º. However, SGLN was the most hydrophobic (84.58 ± 5.7) than others.

Total phenolic contents of sour cherry concentrate and nanofibers were measured by spectrophotometry and the highest value were found for SGCN (16.45 ± 0.83 mg GAE/g nanofiber) and the lowest total phenolic content were investigated for sour cherry concentrate (12.89 ± 0.83 mg GAE/g sample). Total flavonoid contents of

sour cherry concentrate and nanofibers were found as follows 8.58 ± 1.45, 11.19 ± 0.51, 12.01 ± 2.08, 10.88 ± 1.94 mg CE/g sample or nanofiber for sour cherry concentrate, SGN, SGLN and SGCN, respectively. Total anthocyanin contents of sour cherry concentrate and nanofibers were ranged between 0.80 ± 0.04 and 1.63 ± 0.12 mg C3GE/g sample or nanofiber.

Total antioxidant capacity was determined by DPPH and CUPRAC analyses. DPPH results were between 3.07 ± 0.20 and 6.49 ± 3.39 mg TEAC/g sample or nanofiber. CUPRAC results were found between 11.26 ± 1.36 and 39.19 ± 3.98 mg TEAC/g sample or nanofiber. In both assays, SGCN showed the lowest antioxidant capacity.

Phenolic profile of sour cherry and nanofibers were determined by HPLC. Gallic acid, cholorogenic acid, p-coumaric acid as phenolic acids and kaempherol derivatives, epicatechin and cyanidin as flavonoids were detected in sour cherry concentrate. Bioaccessibility of the sour cherry and nanofibers were determined and recovery of polyphenols were calculated according to HPLC results. Results showed that SGLN showed the highest recovery than SGCN and SGN. Another important result was that though SGCN had the lowest encapsulation efficiency, recovery of polyphenols was improved by coaxial electrospinning.

VİŞNE ANTOSİYANİNLERİNİN TEK EKSENLİ VE ÇİFT EKSENLİ ELEKTROEĞİRME YÖNTEMİYLE ENKAPSÜLASYONU VE BİYOYARARLILIĞININ İNCELENMESİ

ÖZET

Vişne (*Prunus cerasus* L.), dünya çapında tüketilen ve içerdiği biyoaktif bileşikler insan vücudunu hastalıklara karşı korur.

Vişne polifenoller bakımından iyi bir kaynak olmasına rağmen, polifenollerin zorlu çevresel şartlara karşı korunması ve biyoerişilebilirliğinin iyileştirilmesi çok önemlidir. Literatürde, polifenollere korunması amacıyla çok sayıda enkapsülasyon teknikleri uygulanmıştır ancak elektroeğirme yöntemi ile enkapsülasyon çalışılmamıştır. Buna ek olarak, enkapsülasyonu yapılmış biyoaktif bileşenlerin *in vitro* sindirim ve biyoerişilebilirliği ile bilgiler oldukça sınırlıdır.

Bu çalışmanın amacı, vişnenin antosiyaninleri ve diğer fenolik bileşenlerinin elektroeğirme yöntemiyle jelatin ve jelatin ve laktalbumin karışımı ile enkapsülasyonunu yapmak ve biyoerişilebilirliğini incelemektir. Vişne polifenollerinin elektroeğirme yöntemiyle enkapsülasyonu incelenirken, çözelti özellikleri, nanolif karakterizasyonu, spektrofotometrik ve kromatografik yöntemlerle toplam antioksidan ve kapasite ölçümleri yapıldı ve nanoliflerin biyoerişilebilirliği analiz edildi.

Sırasıyla elektriksel iletkenlik, yüzey gerilimi, viskozite ve dielektrik sabiti vişne konsantresi ve jelatin çözeltisi için 2,25±0,01 mS/cm; 31,16±0,03 mN/m; 0,21±0,01 Pa.s ve 39,52±0,33 ve vişne, jelatin ve laktalbümin karışımı çözeltisi için 2,23±0,01 mS/cm; 30,52±0,03 mN/m; 0,24±0,01 Pa.s ve 65,14±1,96 olarak ölçülmüştür. Hem jelatin hem de jelatin ve laktalbumin karışımının vişne polifenollerinin elektroeğirme yöntemiyle enkapsülasyonu için uygun kaplama polimerleri olduğu belirlenmiştir.

Enkapsülasyon verimliliği HPLC ile tespit edilmiştir. Sonuçlar; vişne polifenollerinin jelatinle tek eksenli elektoeğirme yöntemiyle elde edilen nanolif, vişne polifenollerinin jelatin ve laktalbüminle tek eksenli elektroeğirme yöntemiyle elde edilen nanolif ve vişne polifenollerinin jelatinle eş eksenli elektoeğirme yöntemiyle elde edilen nanoliflerin enkapsülasyon verimliliği için sırasıyla %61,74; 79,17 ve 50,33 olarak tespit edilmiştir. Eş eksenli elektroeğirme işleminin düşük enkapsülasyon verimliliği, işlem sırasında mekanizmadan kaynaklı sızdırma sonucu vişne konsantresinin efektif bir şekilde pompalanamamasından kaynaklandığı düşünülmektedir.

Nanolifler karakterizasyonu için zeta potansiyeli ve temas açısı değerleri ölçüldü. Nanoliflerin tümü için zeta potansiyeller negatif olarak tespit edildi. En düşük zeta potansiyeli SGCN (-9,38±0,73 mV) için bulunmuştur. SGN (-8±1,23 mV) ve SGLN (-3,19±0,15 mV) için zeta potansiyelleri daha düşük ölçülmüştür. Tüm nanolifler hidrofilik özellikler göstermiştir çünkü nanoliflerin temas açıları 90º'den daha düşük olarak bulumuştur. Bununla birlikte, SGLN diğer nanoliflere göre (84,58±5,7) en hidrofobik özellik gösteren nanoliftir.

Vişne konsantresi ve nanoliflerin toplam fenolik madde miktarı spektrofotometre ile ölçüldü ve en yüksek değer SGCN (16,45±0,83 mg GAE/g nanolif) ve en düşük toplam fenolik madde miktarı vişne konsantresi (12,89±0,83 mg GAE/g örnek) için bulunmuştur. Sonuçlara göre; jelatin ve jelatin ve laktalbumin karışımından elde edilen nanoliflerin toplam fenolik madde aktivitesi gösterdiği gözlenmiştir. Ancak bu durum jelatin ve laktalbuminin içerdiği aminoasitlerle alakalıdır. Zira aminoasitler de fenolik bileşenler gibi Folin reaktifiyle reaksiyona girerek belli bir absorbans değeri vermektedir. HPLC sonuçlarında jelatin ve laktalbumin nanoliflerinde herhangi bir polifenol tespit edilmemesi bu görüşü desteklemektedir.

Toplam flavonoid miktarı sırasıyla vişne konsantresi, SGN, SGLN ve SGCN için sırasıyla 8,58±1,45; 11,19±0,51; 12,01±2,08 ve 10,88 ± 1,94 mg CE/g örnek veya nanolif olarak tespit edilmiştir. En yüksek değer SGLN için tespit edilirken en düşük değer vişne konsantresi için ölçülmüştür. Toplam fenolik madde miktarları toplam flavonoid madde miktarlarından fazla bulunmuştur. Flavonoidler polifenollerin alt grubu olduğu bilindiğinden sonuçlar makuldür.

Toplam antosiyanin içeriği vişne konsantresi ve nanolifler için 0.80 ± 0.04 ve 1.63 ± 0.12 mg C3GE/g örnek veya nanolif arasında değişmektedir. En yüksek toplam antosiyanin miktarı vişne için bulunurken en düşük miktar SGCN için tespit edilmiştir. SGLN ve SGN örneklerinin miktarları birbirine çok yakın bulunmuştur. Antosiyaninler de flavonoid grubunun alt grubu olduğundan toplam antosiyanin miktarlarının toplam flavonoid miktarlarından düşük çıkması makuldür.

Toplam antioksidan kapasite DPPH ve CUPRAC ile analiz edilmiştir. DPPH sonuçları 3,07±0,20 ve 6,49±3,39 mg TEAC/g örnek veya nanolif arasında tespit edilmiştir. CUPRAC sonuçları 11,26±1,36 ve 39,19±3,98 mg TEAC / g örnek veya nanolif arasında bulunmuştur. Analizlerde, SGCN en düşük antioksidan kapasite gösteren örnek ve nanolif olmuştur. DPPH ve CUPRAC analizlerinden elde edilen sonuçların birbirinden farklı olması analiz tekniklerinin etki mekanizmasının farklı olmasından kaynaklanmaktadır. CUPRAC analizinde reaktifler hem lipofilik hem de hidrofilik polifenollerle etkileşime girebilmektedir ve bu nedenle CUPRAC değerleri DPPH değerlerine göre daha yüksek bulunmuştur.

Aynı zamanda, vişne konsantresi ve nanoliflerin in vitro sindirim aşamalarından mide ve bağırsak sonrası numunelerine toplam fenolik, flavonoid ve antosiyanin analizlerinin yanında toplam antioksidan kapasiteleri DPPH ve CUPRAC ile analiz edilmiştir. Vişne konsantresindeki biyoaktif bileşenler sindirim sonrası bozulup parçalanması sonucu madde miktarları ve aktiviteleri azalmıştır. Buna karşılık nanoliflerin miktarları sindirim sonrası toplam antosiyanin analizi dışında tüm analizlerde arttığı gözlenmiştir.

Vişne konsantresi ve nanoliflerin fenolik bileşen profili HPLC ile belirlenmiştir. Vişne konsantresinde fenolik asit olarak gallik asit, klorojenik asit ve p-kumarik asit olarak saptanırken vişne konsantresindeki flavonoidler kaempherol türevleri, epikateşin ve siyanidin olarak belirlenmiştir. Vişne konsantresi ve nanoliflerin biyoerişilebilirlikleri belirlendi ve polifenollerin geri kazanımı HPLC sonuçlarına göre hesaplanmıştır. Sonuçlar göstermektedir ki SGLN, SGCN ve SGN göre daha yüksek geri kazanım göstermiştir. Bir diğer önemli sonuç ise SGCN en düşük enkapsülasyon verimliliği göstermesine rağmen, eş eksenli elektroeğirme yöntemi polifenollerin geri kazanımında önemli bir etkiye sahip olduğu gözlenmiştir.

Sonuçlar göstermektedir ki, elektroeğirme yöntemi, özellikle antosiyanin gibi olumsuz çevre koşullarına ve bağırsak yüksek pH'ına dayanıksız bileşenlerin bile korunmasında ve biyoyararlılığının arttırılmasında etkin bir yöntemdir. Laktalbüminin elektroeğirme çözeltisine eklenmesi; nanoliflerin enkapsülasyon etkinliğini ve biyoerişelebilirliği olumlu etkilediği gözlenmiştir. Aynı zamanda eş eksenli elektroeğirme yönteminin biyoerişilebilirlik açısından etkin bir enkapsülasyon yöntemi olduğu belirlenmiştir.

1. INTRODUCTION

Consumption of natural and healthy food is preferred by consumers in recent years. Sour cherry (*Prunus cerasus* L.) is an attractive fruit due to both unique taste and rich source of polyphenol content especially anthocyanins. It is known that polyphenols are effective agents against free radicals which influence human health negatively and cause several diseases such as cancer, cardiovascular diseases, atherosclerosis, stroke, neurological, renal and liver disorders, hypertension, rheumatoid arthritis, adult respiratory distress syndrome, auto-immune deficiency diseases, inflammation, degenerative disorders related with aging (Lobo et al., 2010; Lü, 2010; Rahman, 2007; Singh et al., 2010). Human body is under attack by on average 10^5 oxidatives every day and consumption of polyphenols become important to avoid illnesses (Valko et al., 2004). Sour cherry phenolics can be used to protection against allergenicity of protein β-lactoglobulin, cancer, cardiovascular diseases, muscle pains and aches (Kang, 2003; Tantoush et al., 2011; Wang, 1999).

Bioaccessibility is the remaining fraction of nutrient after digestion which is suitable for absorption in the gut (Hedrén et al., 2002). *In vitro* bioaccessibility is measured by digestion stimulation in a laboratory. Stimulation of mouth, stomach and intestinal conditions in laboratory which is known as gastrointestinal models is a good alternative to *in vivo* assays. Since *in vitro* analyses are rapid, safe, has no ethical restrictions, the *in vitro* gastrointestinal models are frequently utilized (Parada and Aguilera, 2007).

Although polyphenols contribute to prevent several diseases, they are vulnerable compounds against temperature, pH, light, oxygen etc. Moreover, bioaccessibility of polyphenols is very low due to low and high pH in gastrointestinal track.

Encapsulation can be a solution to prevent degradation of polyphenols and improve bioaccessibility. Encapsulated compounds for functional foods are becoming important due to consumer's demands on high quality, convenient and healthy food (Fang and Bhandari, 2010; Garti and McClements, 2012). In 2013, functional food market was worth 29.12 billion dollars and it is expected that the number will

increase to 55 billion dollars in 2017 (2014). The market of the nanotechnology products in the food industry approaches to one billion dollars and is predicted to rise to 20 billion dollars in the next decade (Chau et al., 2007).

In literature, several techniques such as spray drying, freeze drying, extrusion etc. were applied to encapsulation of polyphenols (Belščak-Cvitanović et al., 2011; Robert et al., 2010; Sanchez et al., 2013). However, degradation of polyphenols due to high temperature, expensive, low encapsulation efficiency and bioaccessibility lead to find a new method for encapsulation of polyphenols. Electrospinning is simple, versatile and top-down process to produce nanofibers. High encapsulation efficiency, sustained release of encapsulated material, suitable encapsulation method for heat-labile compounds, improved protection from detrimental conditions, enhanced stability and functionality of encapsulated material are the main advantages of electrospinning procedure (Bhushani and Anandharamakrishnan, 2014).

The aim of this study is to investigate encapsulation of anthocyanin and other phenolic compounds of the sour cherry coated with gelatin and mixture of gelatin and lactalbumin by uniaxial and coaxial electrospinning. Moreover, changes of bioaccessibility of the sour cherry, uniaxial and coaxial nanofibers is determined.

2. LITERATURE REVIEW

2.1 Sour cherry

Sour cherry (*Prunus cerasus* L.) which is also called tart cherry belongs to *Rosaceae* family (Serradilla et al., 2015; Tokusoglu, 2011). Both fresh and processed sour cherries are consumed predominantly worldwide. They are used in beverages, bakery product, salad and dishes (Tokusoglu, 2011). Turkey is the leader producer of the sour cherry while Ukraine, Russia and Poland are the other important producers (FAOSTAT, 2010).

Sour cherry trees are deciduous trees. They prefer low winter temperatures and summer drought. Sudden freezes in winter, spring frosts, summer winds, hail and lightning influence the production of sour cherry. After a certain amount of winter chilling the growth starts and the heat is needed for full bloom. Soil properties are also important for production. Light, well-drained soils at pH of 5.5-7.5 provide better production. Although; silt loam is the best soil type for sour cherry, soil classes ranging from sandy loam to clay loam are also suitable for production (Serradilla et al.,2015).

Cultivar, maturation stage, agricultural practices and environmental conditions are significant for chemical composition (Crisosto et al., 2002; Gonçalves et al., 2006). The major chemical compound in sour cherry is carbohydrate and the amount of it is 12.8 %. In addition, 1.6% of chemical compound is dietary fiber (Canada, 2010). There is no starch in sour cherry and the available sugar content is 8.48 %. The predominant sugar is glucose with contents of 4.18-10 g/ 100 g of fresh weight (FW) in Ferprime, Burlat, Van, Bing, Lapins and Sweetheart cultivars. However, Beyrudi, Kısa ap and Uzun Turkish cultivars contain 15.1-21.5 g/100 g of FW (Gündoğdu and Bılge, 2012). The sugar composition is dependent on cultivar, climatic conditions and cultivation system (Hrotkó, 2005). Fructose is the second carbohydrate in sour cherry and the amount is 3.51 g/100 g FW. The protein content is 1 g/ 100 g FW. Sour cherry is low- fat food and the amount of total fat is 0.3 g/ 100 g FW. Potassium

is the most abundant mineral in sour cherry with levels of 173 mg/ 100 g FW and calcium (16 mg/ 100 g FW), phosphorus (15 mg/ 100 g FW), magnesium (9 mg/ 100 g FW) and sodium (3 mg/ 100 g FW) are the other minerals. Also iron, zinc, copper and manganese are found at lower amounts in sour cherry (Canada, 2010).

Sour cherry is mildly acidic fruits and their pH values are ranged from 3.1 to 3.6 (Milošević and Milošević, 2011; Papp et al., 2010). The abundant organic acid in cherries is malic acid which is ranged from 600-900 mg/ 100 g FW. However, above 1100 mg/ 100 g FW is found for other cultivars. Citric and succinic acid are other organic acids which are present in sour cherry with levels of 170-300 mg/ 100 g FW and 4-38 mg/ 100 g FW (Girard and Kopp, 1998; Gündoğdu and Bılge, 2012; Serrano et al., 2005). Sour cherry has specific and racy bitter taste. Its unique bitterness and astringency are attributed to flavonol content, pectin residues and degree of polymerization which is number of flavan-3-ol and ranged from 2.6 to 6.6 in sour cherry (Vidal et al., 2004).

2.2 Antioxidants

Free radicals occur when atoms, molecules and ions loose their electrons. Unpaired electrons are unstable and predispose to chemical reactions with other molecules. Free radicals are derived from oxygen, nitrogen and sulfur which are transformed into reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulfur species (RSS) respectively (Carocho and Ferreira, 2013). Although xanthine oxidase, peroxisomes, inflammation processes, phagocytosis, arachidonate pathways, ischemia and physical exercises are normal part of the metabolism, they are responsible for generation of free radicals. Moreover; like smoking, environmental pollutants, radiation, drugs, pesticides, industrial solvents and ozone are the external factors that can cause production of free radicals (Lobo et al.,2010). It is known that human body is under attack by on average 10^5 oxidative every day (Valko et al., 2004). These attacks by ROS, RNS and RSS cause several diseases such as cancer, cardiovascular diseases, atherosclerosis, stroke, neurological disorders, renal disorders, liver disorders, hypertension, rheumatoid arthritis, adult respiratory distress syndrome, auto-immune deficiency diseases, inflammation, degenerative disorders related with aging (Lobo et al., 2010; Lü, 2010; Rahman, 2007; Singh et al., 2010).

Antioxidants are defined as compounds which delay, remove or inhibit oxidation reactions and directly scavenge ROS, RNS and RSS (Halliwell, 2007; Halliwell and Gutteridge, 1995; Khlebnikov et al., 2007). The initiation of peroxidation by scavenging species, preventing formation of reactive species or destruction of peroxides by chelating metal ions, quenching ROS to eliminate peroxide production, inhibition of auto-oxidative chain reaction and reduction of localized oxygen concentrations are the antioxidant mechanisms for elimination of free radicals (Asimi et al., 2013).

Several studies showed that antioxidants are effective agents against inflammation, cancer, atherosclerosis, coronary diseases and gut health (Bartoszek and Polak, 2012; Boffetta et al., 2010; Cardona et al., 2013; Díaz et al., 2012; Fernández-Marín et al., 2012; Fu et al., 2011; Hamrouni-Sellami et al., 2013; Li and Beta, 2011; Machmudah et al., 2012; Ratnasooriya and Rupasinghe, 2012; Strati and Oreopoulou, 2011; Toro-Funes et al., 2012). Antioxidants are utilized in food industry widely not only their health promoting effects but also their slowing down of oxidation reaction properties which provide to improve food quality and shelf life of the food (Akoh and Min, 2008). Antioxidants can be classified into vitamins, carotenoids and polyphenols (Oroian and Escriche, 2015). The classification of antioxidants was demonstrated in Figure 2.1.

2.2.1 Vitamins

2.2.1.1 Vitamin C

Vitamin C (Figure 2.2a) which is also known as L-ascorbic acid is termed the most important hydrophilic antioxidant due to its scavenging property against superoxide radical anions, hydroxyl radicals, hydrogen peroxide, reactive nitrogen species and singlet oxygen (Lykkesfeldt, 2000). The amount of vitamin C is very crucial. Even though over 1000 mg/kg vitamin C scavenges free radicals, lower than 100 mg/kg can promote oxidation reactions (Ahn et al., 2007). Vitamin C prevents gout, chronic and degenerative diseases (Roddy and Choi, 2014; Wojcik et al., 2010). The European Food Safety and Authority (EFSA) is reported that consumption of vitamin C between 25 and 45 mg/day depends on age is beneficial for health (Tetens, 2013).

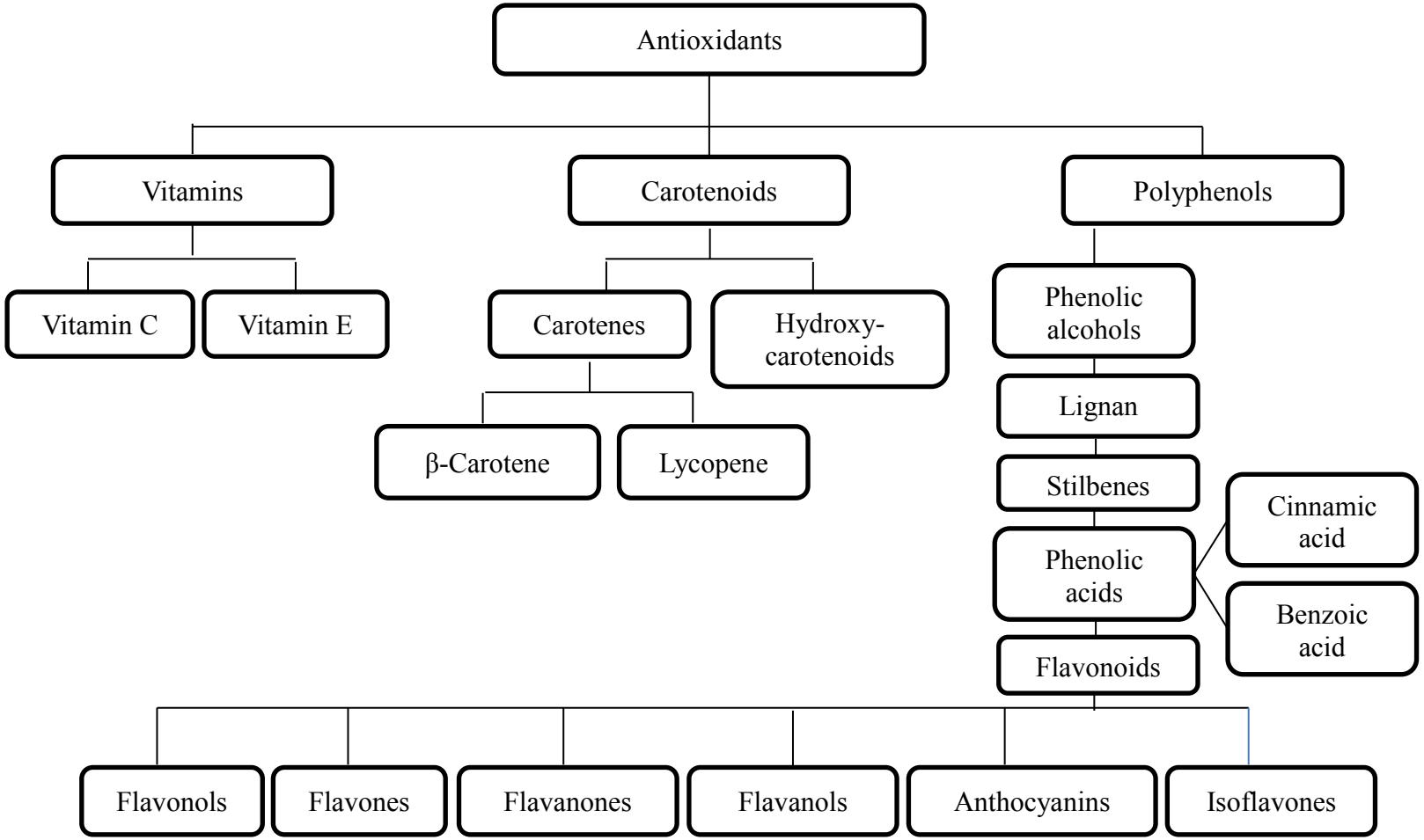

Figure 2.1. Classification of antioxidants.

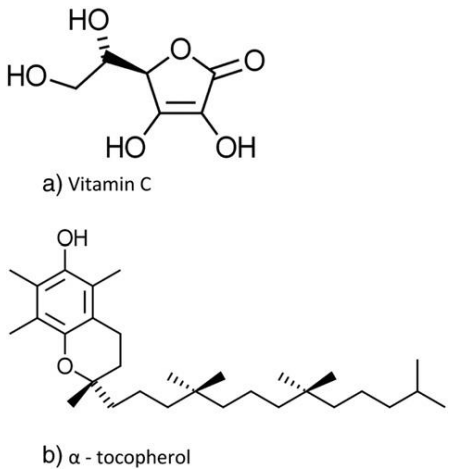

Figure 2.2. a. Vitamin C **b.** α-tocopherol.

2.2.1.2 Vitamin E

Vitamin E (Figure 2.2.b) includes tocopherols and tocotrienols. It is one of the most important lipid soluble antioxidant in the human body. Both chain breaking electron donor and acceptor are the main mechanisms of the vitamin E (Oroian and Escriche, 2015). The European Food Safety and Authority (EFSA) is reported that consumption of vitamin E between 8 and 25 mg/day depends on age is beneficial for health (Tetens, 2010).

2.2.2 Carotenoids

It is known that at least 60 carotenoids exist in the vegetable and fruits (Oroian and Escriche, 2015). Carotenoids are valuable compounds not only their provitamin A activity but also their singlet oxygen quencher and free radical scavenger properties (Böhm et al., 2012; Ramel et al., 2012). Studies showed that some cancer types, metabolic diseases, cardiovascular diseases and obesity can be prevented by carotenoids (Jomova and Valko, 2013; Kim et al., 2012). Carotenoids are classified into carotenes and hydroxy-carotenoids.

2.2.2.1 Carotenes

α-, β-carotene (Figure 2.3.a) and lycopene (Figure 2.3.b) are the most common carotenes in the foods. β- carotene which is lipid soluble provitamin is very effective agent to quench singlet oxygen and prevent lipid oxidation (Sánchez-Patán et al., 2012; Weber and Grune, 2012). Lycopene is known as the most powerful singlet

oxygen quencher in the carotenoid family (Perretti et al., 2013). Its power is associated with many conjugated double bonds of lycopene (de Cortes Sanchez-Mata, 2013).

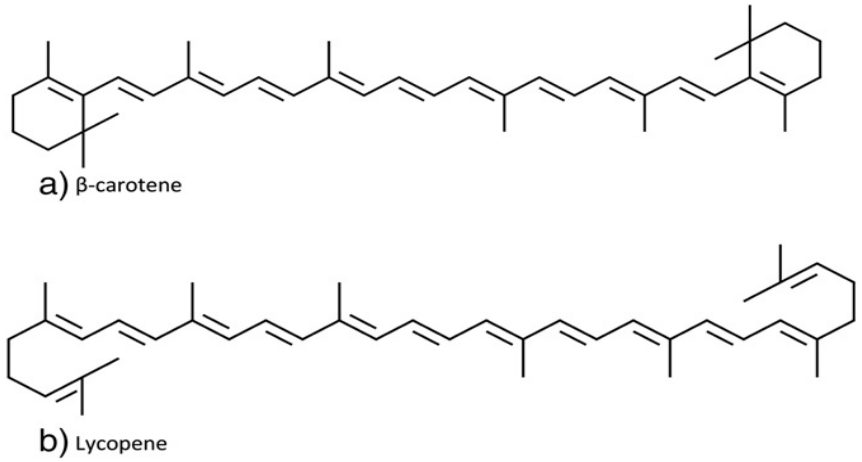

Figure 2.3. a. β-carotene **b.** Lycopene.

2.2.2.2 Hydroxy-carotenoids (xanthophylls)

Hydroxy-carotenoids which are also known as xanthophylls are obtained by oxygenation of carotenoids in the plastids. They are yellow pigments and generally found in leaves. Lutein and zeaxanthin are the most common hydroxy-carotenoids in the nature (Pasaporte et al., 2014).

2.2.3 Polyphenols

Polyphenols are prevalent compounds in all plants. It is known that more than 8000 phenolic compounds exist in the wide range of plants (Crozier et al., 2009). They are second metabolites which are synthesized plant tissue through photosynthesis. They are responsible for taste, appearance, flavor and health-promoting properties (Tomás-Barberán and Espin, 2001). Their antioxidant, free radical scavenging, anti-inflammation, modulation of signal transduction, anti-microbial and anti-proliferation activities have given rise to increase in importance since 20th century (Ahmed et al., 2015; Velderrain-Rodríguez et al., 2014). Moreover, endogenous antioxidant enzymes in the human body are protected by polyphenols therefore; they help maintain the human health indirectly (Pradeep and Sreerama, 2015; Zhang et al., 2015a; Zhang et al., 2015b). Polyphenols are classified into phenolic alcohols, lignans, stilbenes, phenolic acids and flavonoids.

2.2.3.1 Phenolic alcohols

Phenolic alcohols in the plants are 2-phenylethanol, 3,4-dihydrozyphenylethanol which is also called hydroxytyrosol, p-hydroxyphenylethanol which is also known as tyrosol and 3,4-dihydrophenylethanol glucodise (Oroian and Escriche, 2015). Tyrosol (Figure 2.4.a) and hydroxytyrosol (Figure 2.4.b) are the main o-diphenols in olives and olive oil (Franco et al., 2014). They prevent low-density lipoprotein (LDL) oxidation and their low concentration maintains human erythrocytes and DNA against oxidatives (Ilavarasi et al., 2011).

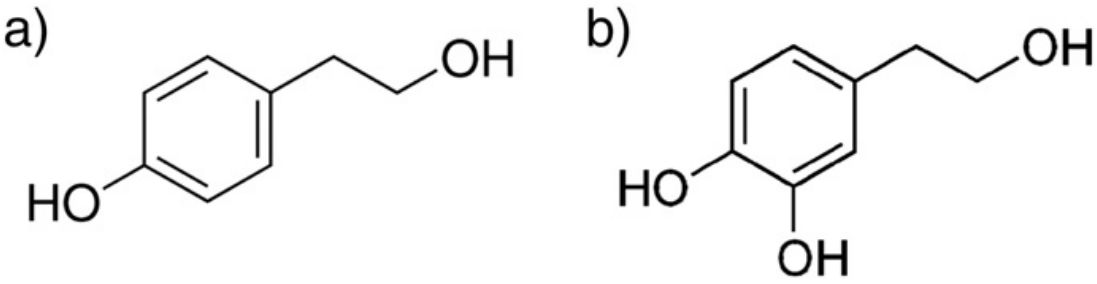

Figure 2.4.a. Tyrosol **b**. Hydroxytyrosol.

2.2.3.2 Lignans

Lignans (Figure 2.5) are found in flaxseed and other seeds, roots, leaves, fruits, woody part of the plants and grains (Ekiert et al., 2013; Gerstenmeyer et al., 2013; Landete, 2012). They are important for regulating normal colon functioning and prevent or delay mammary cancer (Landete, 2012).

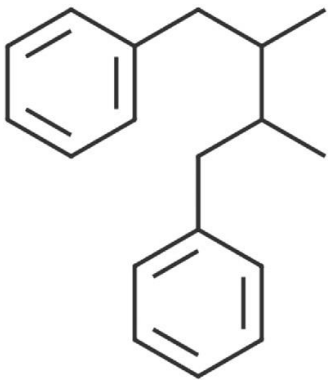

Figure 2.5. Lignans.

2.2.3.3 Stilbenes

Stilbenes are significant phenolic compounds even though they are found in low concentration. They are efficient agents against cardiovascular diseases, atherosclerosis and cancer, also they have anti-inflammatory and antiviral effects (Frombaum et al., 2012; Galindo et al., 2011). Resveratrol (Figure 2.6) is one of the most popular stilbene in the plants (Adrian and Jeandet, 2012).

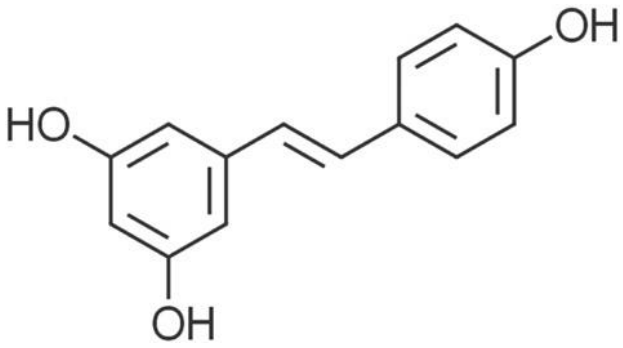

Figure 2.6. Stilbenes-Trans resveratrol.

2.2.3.4 Phenolic acids

Phenolic acids (Figure 2.7) can be classified into benzoic and cinnamic acid derivatives depends on their structure (Oroian and Escriche, 2015). They are acknowledged as powerful antioxidants and they have antibacterial, antiviral, anticarcinogenic and anti-inflammatory and vasodilatory effects (dos Santos Lima et al., 2014; Mudnic et al., 2010).

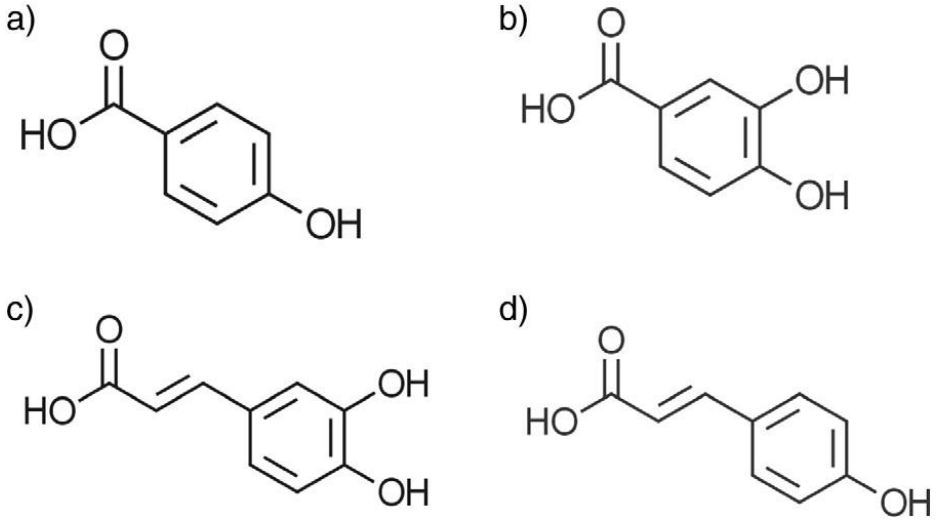

Figure 2.7. Phenolic acids a**.** p-hydroxybenzoic acid **b**. 3,4-dihydroxybenzoic acid **c.** caffeic acid **d**. p-coumaric acid.

2.2.3.5 Flavonoids

Flavonoids (Figure 2.8) are ubiquitous compounds in nature and can be grouped into flavonols, flavones, flavanones, flavanols, anthocyanins and isoflavones.

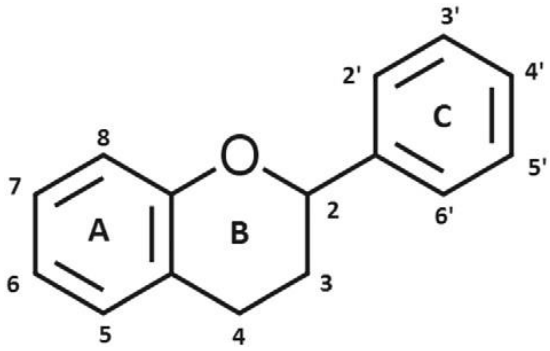

Figure 2.8. Flavonoids.

Flavonols (Figure 2.9) can be found in vegetables and fruits such as onion, pepper and grape berry skins (Alcalde-Eon et al., 2014; Kühn et al., 2014; Liang et al., 2014; Lu et al., 2011). Quercetin which is one of the most popular flavonols is efficient agent against to cancer, inflammation, viral disorders and cardiovascular diseases (Caridi et al., 2007; Santos et al., 2014). Flavanols which are also known as procyanidins are sensitive compounds to heat, food ingredients, high pH, dissolved oxygen and reactive oxygen species (Song et al., 2015). Their antioxidant, anti-thrombotic, anti-inflammatory, anti-proliferative, antibacterial and lipid metabolism regulator properties are beneficial for welfare (Monagas et al., 2010; Sánchez-Patán et al., 2012).

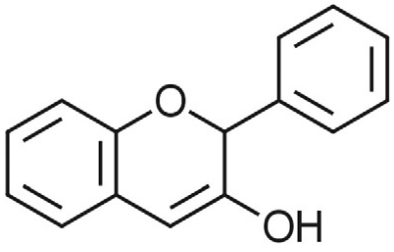

Figure 2.9. Flavonols.

Flavones (Figure 2.10) which exist in citrus peel (de Moraes Barros et al., 2012), onion(Rehman et al., 2013) and parsley(Kaiser et al., 2013) are antioxidant, anticancer, anti-inflammation, neuroprotective, anti-diabetes, anti-ulcer and

antimicrobial compounds (Hou and Kumamoto, 2010; Li et al., 2012; Sagrera et al., 2011; Singh et al., 2014; Sumbul et al., 2011; Voicescu et al., 2015; Yu et al., 2012).

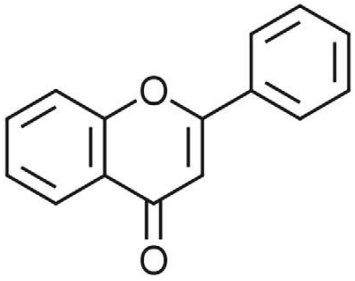

Figure 2.10. Flavones.

Flavanones (Figure 2.11) are plenty in citrus fruit and prunes (Khan and Dangles, 2014). Naringenin is one of the most important flavanones and exist in citrus fruits (Oroian and Escriche, 2015). It is responsible for anti-inflammatory, anticarcinogenic, antihypertensive and anti-atherogenic properties (Rodrigo et al., 2012).

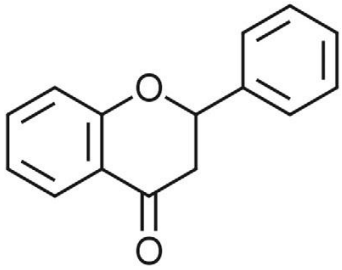

Figure 2.11. Flavanones.

Anthocyanins (Figure 2.12) are the most abundant flavonoid. The name of the anthocyanin comes from Greek words *anthos* flower and *kyanos* blue. Anthocyanins are water soluble, secondary metabolites of plants and they provide cyanic colors ranged from salmon pink through red and violet to dark blue. Although 539 different anthocyanin structures are found, the basic structure of the anthocyanins are aglycones which are also called anthocyanidins. When anthocyanidins bond one or more sugar moieties, they become anthocyanins. There are 31 identified anthocyanidins; however 90% of the anthocyanins are composed of six of them which are cyanidin, pelargonidin, delphinidin, peonidin, petunidin and malvinidin (Andersen and Markham, 2005; Nollet and Toldrá, 2012). Anthocyanins are very

vulnerable compounds against temperature, pH, light, oxygen, solvents, metallic ions, ascorbic acid, sulphite and enzymes (Castañeda-Ovando et al., 2009; Cavalcanti et al., 2011). They are reported to have antioxidant, anticarcinogenic, neuroprotective and anticancer effects (Castañeda-Ovando et al., 2009; Konczak and Zhang, 2004; Santos-Buelga et al., 2014; Tomas-Barberan and Andres-Lacueva, 2012; Tsuda, 2012). Consumption for 4 weeks provides to decrement in myopia, apoptosis, obesity and diabetes (Tsuda, 2012).

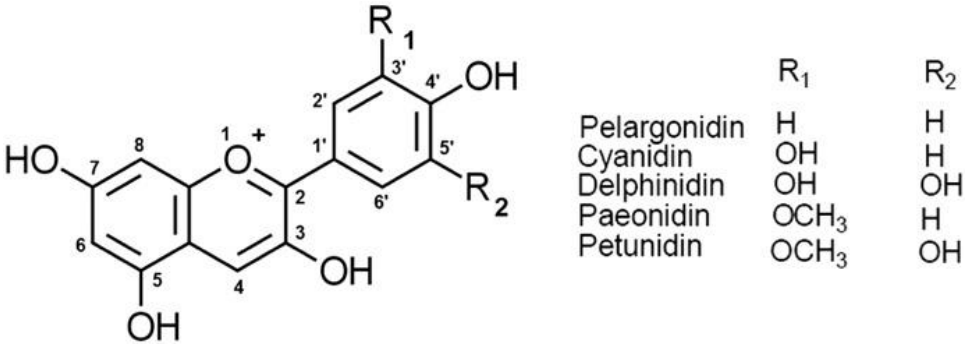

Figure 2.12. Anthocyanins.

Isoflavones are found in legumes and daidzein (Figure 2.13.a), genistein (Figure 2. 13.b) and glycitein are the most predominant compounds. They are inversely associated with cancer, cardiovascular disease and postmenopausal symptoms (He and Chen, 2013; Liu et al., 2012; Virk-Baker et al., 2014).

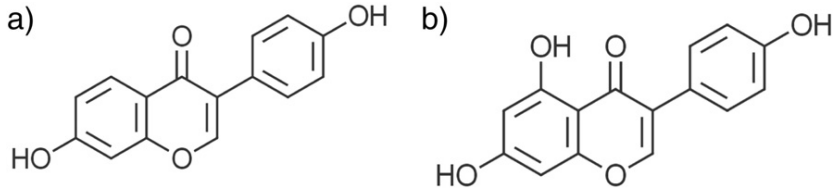

Figure 2.13. a. Daidzein **b.** Genistein.

2.3 Sour Cherry Antioxidants

Sour cherry is good source of antioxidants. Antioxidant profile of the sour cherry is composed of vitamins, anthocyanins, hydroxycinnamic acids, flavanols, flavonols and flavones (Canada, 2010; Wojdyło et al., 2014).

Sour cherry is a good source of vitamin C with 10 mg/ 100 g FW. However other water soluble vitamins are at low concentrations. The amount of vitamin E and

vitamin A which are lipid soluble vitamins are found as 0.07 mg tocopherol/ 100 g FW and 0.064 mg retinol activity equivalent/ 100 g FW (Canada, 2010).

Sour cherry is rich in anthocyanin. Wojdylo et al. (2014) determined 11 anthocyanins including 8 cyanidin, 2 peonidins and 1 pelargonidin in all 33 cultivars. In addition, 5 other antocyanins exist in some of the sour cherry cultivars. However; cyanidin-3-glucosylrutinoside was found as the most abundant anthocyanin with a mean range of 57% and the second and third anthocyanins were cyanidin-3-O-sphoroside and cyanidin-3-O-rutinoside with a mean range of 20 and 19% respectively. Other study also showed that major anthocyanins in sour cherry are cyanidin-3-glucosylrutinoside with amount of 140.3-320.9 mg/L, cyanidin-3-rutinoside with amount of 35.4-85.5 mg/L, cyanidin-3-sophoroside with amount of 2.6-21.5 and cyanidin-3-glucoside with amount of 2.0-9.9 mg/L (Damar and Ekşi, 2012).

Several studies about different sour cherry cultivars reported the total anthocyanin content between 21 and 285 mg/100 g fresh cherry (Khoo et al., 2011; Kim et al., 2005; Pedisić et al., 2007; Veres et al., 2008). Moreover, total anthocyanin content was determined by Wojdylo and her friends between 844.77 and 994 mg/ 100 g dry matter for different sour cherry cultivars (Wojdyło et al., 2014).

Sour cherry is rich in phenolic compounds and polyphenols. The content of the hydroxycinnamic acid can change due to ripening degree and the amount can fluctuate between 258.86 and 1200.90 mg/100 g dry matter from one cultivar to another. Epicatechin and catechin are the predominant flavan-3-ols in sour cherry and the amounts were found as 18.12-282.87 and 4.27-115.72 mg/100 g dry matter, respectively (Wojdyło et al., 2014). Genistein is a polyphenol compound belonging to isoflavones and major source is legumes. However, it is found that certain sour cherry cultivars are also a good source of genistein and the content of genistein in sour cherry was found between 6.2 and 16.5 mg/100 g fresh sample (Abrankó et al., 2015). Quercetin, kaempherol, isorhamnetin are other major polyphenol compounds in sour cherry (Kirakosyan et al., 2009).

Previous studies showed that total phenolic content of sour cherry were varied from 74 to 754 mg gallic acid equivalent (GAE)/100 g fresh cherry (Bonerz et al., 2007; Dragović-Uzelac et al., 2007; Khoo et al., 2011; Kim et al., 2005). Total phenolic

content of sour cherry was found between 1539.43 and 2982.51 mg/ 100 g dry weight for different cultivars (Wojdyło et al., 2014).

Antioxidant activity is related with both total polyphenol content and type of the polyphenol compounds (Bandoniene and Murkovic, 2002; Ou et al., 2002; Pantelidis et al., 2007; Rice-Evans et al., 1996; Toydemir et al., 2013) . It is determined that hydroxylation of B ring and glycosylation are responsible for antioxidant potential (Rice-Evans et al., 1996). Moreover, flavan-3-ols for example catechin, epicatechin and their oligomers have the strongest effect on the antioxidant activity (Bandoniene and Murkovic, 2002). Khoo and his friends studied with 34 different sour cherry cultivars and they reported that antioxidant capacity of sour cherries were ranged from 9 and 63 μmol trolox equivalent (TE)/ g (Khoo et al., 2011). Also, antioxidant activity was measured in another study and the results are found as 8.13-38.11, 3.72-18.40 and 1.93-12.95 mmol TE/ 100 g dry matter for ORAC, ABTS and FRAP assay, respectively (Wojdyło et al., 2014).

It is known that the concentration of pigments like anthocyanin are placed are much more in the skin than in the flesh (Chaovanalikit and Wrolstad, 2004b).Similarly, the highest amount of total phenolic compound are placed in skin followed by flesh then in pits (Chaovanalikit and Wrolstad, 2004a). Chemical properties and physical location which are closeness to membrane phospholipids, emulsion interfaces and in the aqueous phase within the food affect anti-oxidative effects of the antioxidants (Watanabe et al., 2000).

Sour cherry phenolics can be used to protection from allergenicity of protein β-lactoglobulin, cancer, cardiovascular diseases, muscle pains and aches (Kang, 2003; Tantoush et al., 2011; Wang, 1999). Based on the *in vivo* tests, dark colored cultivars of sour cherry which have highest amount of anthocyanin show the highest antiproliferative effects against human colon and gastric cancer cells (Serra et al., 2011).

2.4 Bioaccessibility and Bioavailability

Bioavailability is expressed as the rate and extent to which the active substances or therapeutic moieties contained in a drug are absorbed and become available at the site of action (Benet et al., 1996). Bioaccessibility is the remaining fraction of

nutrient after digestion which is suitable for absorption in the gut (Hedrén et al., 2002). However, bioavailability is the amount of nutrients in the blood plasma. Bioavailability and bioaccessibility measurements are very important for ascertain actual contribution of the nutrients on health and effects of food processing techniques on the food nutrient content (Parada and Aguilera, 2007).

Bioavailability and bioaccessibility are measured by *in vivo* and *in vitro* assays. *In vivo* assays carry out by living beings to obtain a result after consumption of a nutrient (Yeum and Russell, 2002). *In vivo* assays provides to measure bioavailability directly. However, individual difference, physiological state, dose and existence of other food components influence the results (Faulks and Southon, 2005). Another limiting factor is ethical restriction about existence of human and animal in the experiments (van het Hof et al., 2000a; van het Hof et al., 2000b). *In vitro* bioavailability measurement is done by stimulation of digestion and absorption and bioaccessibility is measured by only digestion stimulation in a laboratory. Stimulation of mouth, stomach and intestinal conditions in laboratory which is known as gastrointestinal models is a good alternative to *in vivo* assays. Since *in vitro* analysis are rapid, safe, has no ethical restrictions, the gastrointestinal models are frequently utilized (Parada and Aguilera, 2007). In this study, *in vitro* gastrointestinal model is selected to measure bioaccessibility.

2.5 Encapsulation

Encapsulation is an entrapment process to cover desired compounds within an appropriate material. There are two basic methods which are top-down (Figure 14.a) and bottom-up (Figure 14.b) to fabricate nanoparticles. The top-down processes scale down big particles into nanoparticles by size reduction mechanical process with energy input. On the other hand, in bottom-up processes small molecules are become together to produce nanoparticles depends on intermolecular affinity (Shimomura and Sawadaishi, 2001; Whitesides and Grzybowski, 2002).

Selection of suitable encapsulation method depends on the compound which is desired to encapsulate, physicochemical properties of final product, processing time, cost, preparation steps, amount of encapsulate, market demand and regulations (Nedovic et al., 2011). Encapsulation process provides to improve shelf life, processibility and functionality, keep the active compounds from detrimental

environmental conditions and conceal unwanted taste, flavor or smell (Luca et al., 2014; Yallapu et al., 2010). Encapsulation technology is becoming popular in food industry in last years due to protection of valuable compounds against chemical, physical and biological degradation, enhancement in handling, improvement of bioaccessibility and controlled release at a certain time and point.

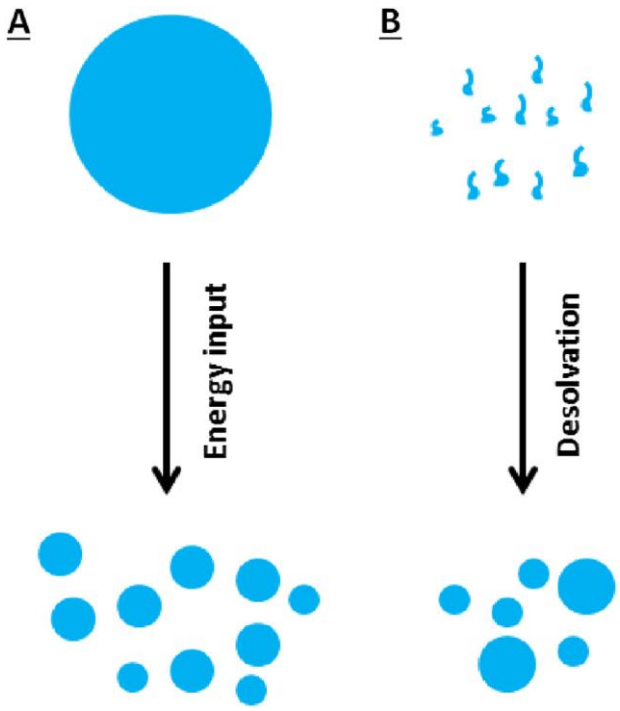

Figure 2.14.a. Top-down **b.** Bottom-up process (Li et al., 2015).

Especially bioaccessibility and controlled release are very important parameters for bioactive compounds encapsulation. The delivery of any bioactive compound to various sites within the body is directly affected by particle size. Reducing the size of the encapsulates to the nanoscale offers opportunities related to prolonged gastrointestinal retention time due to improve bioadhesiveness in the mucus covering the intestinal epithelium. Reducing the particle size may improve the bioavailability, delivery properties and solubility of the nutraceutical because of the greater surface area per unit volume and thus increased biological activity. Moreover, modulations of surface properites (e.g., coatings) can enable targeted delivery of compounds. Thus, nanoencapsulation has the potential to enhance bioavailability, improve controlled release and enable precision targeting of the bioactive compounds to a greater extent than microencapsulation (Anandharamakrishnan, 2014). According to the studies encapsulation can be accepted one of the suitable way to protect the

valuable compounds against environmental conditions, improve shelf life, functionality and bioaccessibility. Moreover, encapsulated compounds for functional foods become important due to consumer's demands on high quality, convenient and healthy food (Fang and Bhandari, 2010; Garti and McClements, 2012). In 2013, functional food market was worth 29.12 billion dollars and they expected that the number will increase to 55 billion dollars in 2017 (2014). The market of the nanotechnology products in the food industry approaches the one billion dollars and is predicted to rise to 20 billion dollars in the next decade (Chau et al., 2007). However, nanotechnology has some limitations. The main concern about the nanotechnology in food sector is the toxicity of the nanoparticles. Nucleic acids, proteins, peptides and antibody fragments act as antigens when they are in nanosized. Therefore these antigen like components cause toxicity (Desai, 2012). Moreover, surfactants are generally used in encapsulation technology. When the encapsulation efficiency is low, amount of encapsulated compounds consumption increases. Health problems can appear with consumption of excess amount of surfactants. Other limitations in encapsulation applications are unappropriare bioavailability and bioaccessibility. Gastrointestinal tract is gruelling journey for the nanoparticles. The nanoparticles can be degraded or digested before they reach the intestine and absorption. Although they can be absorbed effectively, the final structure and the characteristic of the bioactive materials in nanoparticles can change during gastrointestinal tract. Moreover, nanotechnology is an expensive implication in these days. Decrease of the cost is as important as overcoming the other limitations (Wang et al., 2014).

There are studies about encapsulation of valuable bioactive compounds. Orange oil was encapsulated with soybean proteins and it is found that encapsulation process protects orange oil from degradation of valuable compounds and oxidation (Kim et al., 1996). Phenolic compounds and anthocyanins in purple sweet potatoes were encapsulated with maltodextrin. The encapsulated valuable compounds were added in different food products and enhancement of colour, flavour, water solubility index, antioxidant content and capacity were observed (Ahmed et al., 2009). Encapsulation of anthocyanin was also studied and degradation of anthocyanins during storage was minimized thanks to encapsulation (Betz and Kulozik, 2011). Another study showed

that encapsulation of procyanidins into zein particles improves solubility and bioaccessibility (Zou et al., 2012).

In this study, electrospinning was selected for encapsulation technique.

2.5.1 Electrospinning

Electrospinning is a simple and versatile top-down process to fabricate continuous nanofibers from natural and synthetic polymers (Kulkarni et al., 2010; Yördem et al., 2008). Electrospinning was investigated by A. Formhals in 1934 (Formhals, 1934) and the technique is used in various applications for instance filtration (Agarwal et al., 2008; Veleirinho and Lopes-da-Silva, 2009), wound healing, scaffolds in tissue engineering (Agarwal et al., 2008; Li et al., 2002), drug delivery (Loh et al., 2010), enzyme immobilization, biosensors, energy generation, protective clothing, affinity membrane and cosmetics (Bhardwaj and Kundu, 2010; Shen et al., 2010). Lastly, food and agricultural applications become popular.

High voltage source, stainless steel needle, syringe pump and collector are the main components of electrospinning technique which can be seen in Figure 2.15. (Dhandayuthapani et al., 2011; Kessick et al., 2004).

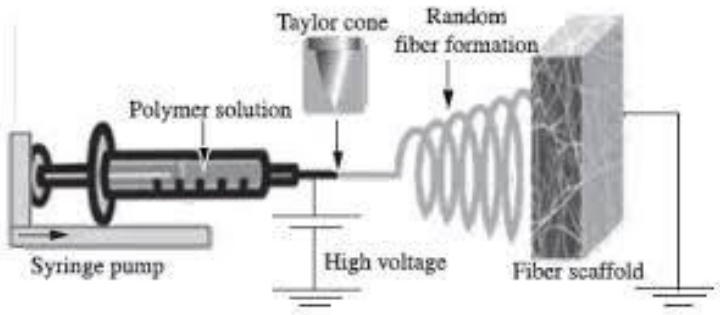

Figure 2.15. Electrospinning setup (Dhandayuthapani et al., 2011).

In electrospinning technique, a proper polymer solution is spun from needle to collector under high potential electrical field. There are two electrostatic forces at the tip of the needle which are electrostatic repulsion of like charges and Coulombic force of the electrical field. Due to both forces, the droplet on the needle changes into a conical shape which is also known as Taylor cone. While the high potential electrical field is bigger than surface tension of the droplet, a charged jet of the

polymer ejected from the Taylor cone to form continuous nanofibers on the collector (Ponhan and Maensiri, 2009; Taylor, 1969).

High encapsulation efficiency, sustained release of encapsulated material, suitable encapsulation method for heat-labile compounds, improved protection from detrimental conditions, enhanced stability and functionality of encapsulated material are the main advantages of electrospinning procedure (Bhushani and Anandharamakrishnan, 2014). However, low production amount and difficulty of find a suitable coating material are the drawbacks of the electrospinning (Noruzi, 2016). To overcome the disadvantages, it is represented several solutions. High production value can be obtained by multiple jet electrospinning or industrial needleless electrospinning equipment. In conventional electrospinning which is also called uniaxial electrospinning, it cannot be possible to work with every polymer. Synthetic polymers provide to fabricate nanofiber easily with high encapsulation efficiency however, they can be toxic and not biodegradable (López-Rubio et al., 2012). Although natural polymers are nontoxic, highly biodegradable, bioaccessible, renewable and edible, some of them such as chitosan cannot be electrospinnable because of inter and intramolecular interaction and high viscosity (Stijnman et al., 2011; Torres-Giner et al., 2016). In this respect, coaxial electrospinning method is an appropriate solution to make the electrospinning possible with the low electropinnable polymers (Pakravan et al., 2012). In coaxial electrospinning process, there are two concentric capillaries to eject polymer solution and the compound which is desired to encapsulate at the same time from different needles (Jiang et al., 2005; Pakravan et al., 2012). Coating material covers the encapsulated part and the valuable compound is placed in the core part. Though, uniaxial electrospinning process is simple than coaxial electrospinning, higher controlled release property is obtained by coaxial electrospinning procedure (López-Rubio et al., 2012).

2.5.1.1 Coating materials

In encapsulation processes, both synthetic and natural polymers are widely used. Synthetic polymers provide to fabricate nanofiber easily with high encapsulation efficiency however, they can be toxic and not biodegradable (López-Rubio et al., 2012). Natural polymers are preferred due to their high biocompatibility, hydrophilicity and nontoxicity however; handling of natural polymers are difficult

than synthetic ones (Bhattarai et al., 2006; Moon and Farris, 2009). Selecting of appropriate polymer is very crucial decision for encapsulation process.

Carbohydrates, lipids, gums, cellulose and proteins are natural polymers generally used for coating materials in encapsulation processes (Brazel, 1999). Every coating material has own advantages and disadvantages. Coating material is selected according to end product requirements, nature of the core material, encapsulation technique, legislation and economics point of view (Dabur and Kapoor, 1999). Moreover, coating material should have no reaction with core material, easy handling, good emulsion-stabilization and release properties, low viscosity at high concentration, complete elimination of solvent, and provide the highest protection against undesired environmental conditions (Trubiano and Lacourse, 1988).

Proteins have polyamide in their structure naturally therefore; they represent better physical and chemical properties than carbohydrates (Torres-Giner et al., 2016). Proteins have excellent functional properties such as solubility, viscosity, emulsification and film-forming properties due to their different chemical groups, amphiphilic properties, self-associated behavior, high molecular weight and flexible molecular chain make the proteins suitable coating materials for encapsulation process (Dalgleish, 1997; Dickinson, 2001). Moreover, proteins can interact with food components due to their functional groups. The interaction between hydrophilic or hydrophobic compounds of foods and coating materials makes the proteins more suitable carrier for encapsulation (Torres-Giner et al., 2016). In this study, gelatin and lactalbumin were selected for coating material.

Gelatin (Figure 2.16) which is obtained by hydrolysis of collagen is one of the widely used polymer and Food and Drug Administration (FDA) approved the gelatin as coating materials in encapsulation technology (Gourdel and Tronel, 2001). Gelatin is preferred in food industry due to low cost, highly biocompatible and biodegradable property (Zhang et al., 2005). In addition, gelatin is an ideal polymer because it improves elasticity, stability and consistency of food products (Giménez et al., 2005).

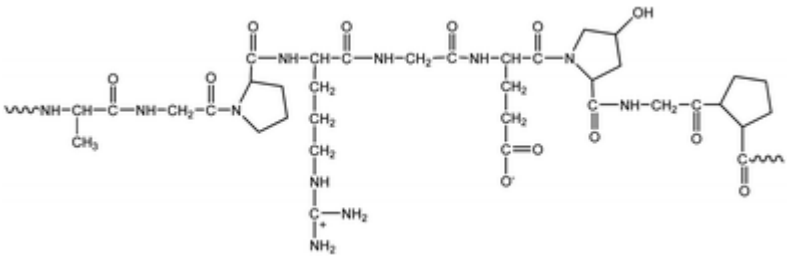

Figure 2.16. Chemical structure of gelatin.

Gelatin has been utilized to encapsulate flavor and these flavored capsules were added in foods as seasonings and other flavorings (Gourdel and Tronel, 2001). Another study showed that when gelatin is added into maltodextrin and gum arabic for coating material, gelatin provides to improve controlled release ability (Yoshii et al., 2001). Encapsulation of plant derived polyphenols and flavonoids with gelatin-pectin coating material shows greater mechanical strength and thermal stability. Also; due to less capacity expand and absorb water, encapsulated materials can be used as reduced calorie fat replacer, flavor binder and texturizer (Strauss and Gibson, 2004). Lycopene is a susceptible compound against oxidation and isomerization reactions. When lycopene is encapsulated with gelatin, lycopene is not released at pH 2-3.5 and released at basic conditions like pH 5.5-7. Therefore, encapsulated lycopene is not released in the stomach and it is released in the intestine (Chiu et al., 2007).

Lactalbumin (Figure 2.17) is the second most existent protein in milk and it is less allergic than β-lactoglobulin which is the most abundant protein in the milk (Hernández-Ledesma et al., 2007; Selo et al., 1999). Consumption of lactalbumin provides several benefits on health. Lactalbumin is highly biocompatible and digestible protein. It is good source of tryptophan which influences satiety and mood positively. Morever, lactalbumin has antimicrobial and antitumor effects.(Mehravar et al., 2009).

Lactalbumin is accepted as good stabilizer due to its low cost, good emulsifying property and highly biocompatible (Vijayaragavan et al., 2014; Yi et al., 2016). Lactalbumin is generally used for encapsulation of hydrophobic compounds. Vitamin D and retinol were encapsulated with lactalbumin and it was found that stability and binding power are improved with lactalbumin encapsulation (Delavari et al., 2015; Puyol et al., 1991).

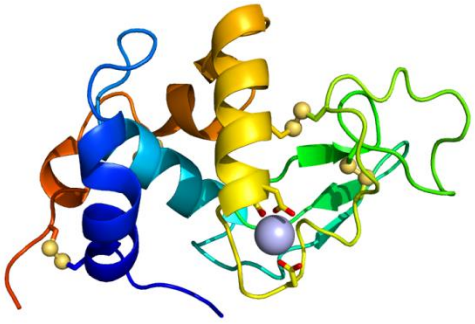

Figure 2.17. Lactalbumin structure (The Ca2+ is shown as a ball in purple, helix A in dark blue, helix B in light blue, helix C in yellow, helix D in orange, while the β-sheet is in green. The sulfur atoms in the four disulfide bonds are indicated by small yellow balls.) (Jøhnke and Petersen, 2012).

2.5.1.2 Parameters of electrospinning

Several parameters which are solution, instrumental and ambient parameters affect the electrospinning procedure (Figure 2.18) (Raghavan et al., 2012).

Solution parameters are conductivity, dielectric constant, concentration, viscosity and surface tension of the solution.

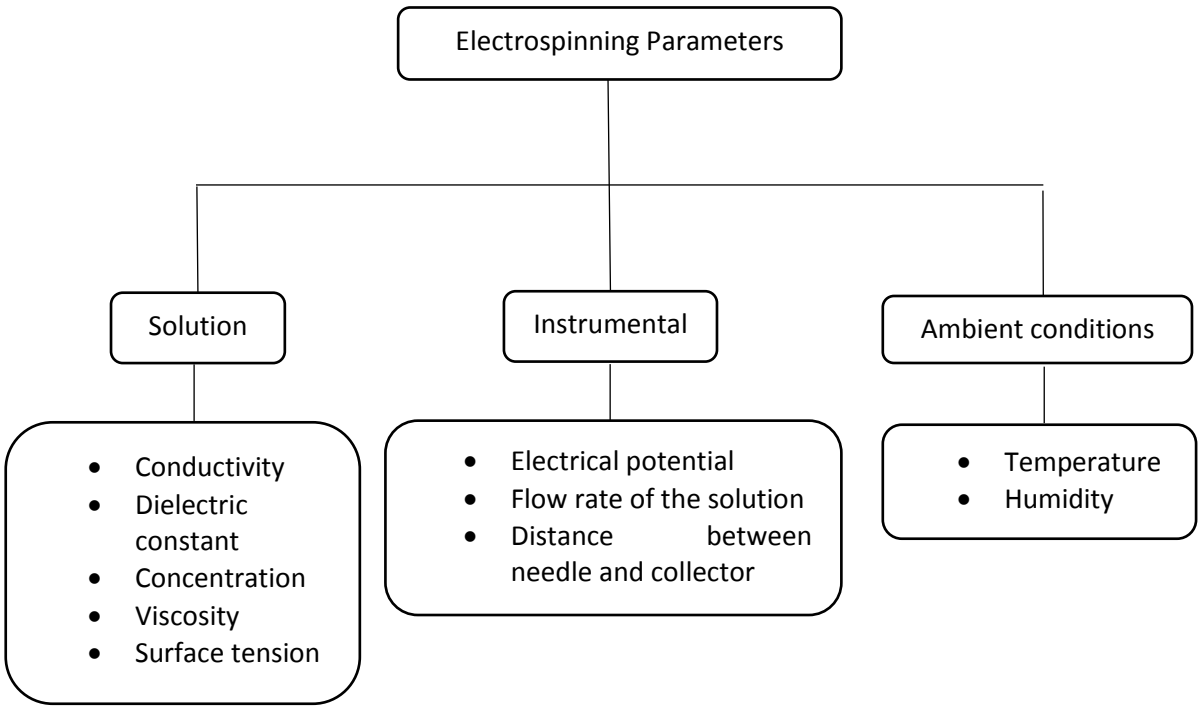

Figure 2.18. Electrospinning parameters (Raghavan et al., 2012).

Polymers are generally nonconductive however, minimal conductivity is necessary for nanofiber fabrication in electrospinning procedure. Conductivity of the polymer solution affects the formation of Taylor cone and diameter of the nanofibers. Without Taylor cone, electrospinning cannot be possible (Sun et al., 2014). Electrospinning procedure is directly related with Coulomb force between charges on the surface of the droplet and the force from the external electrical field. When charges on the surface of the droplet are not sufficient, applied electric field is not enough to form Taylor cone and start the electrospinning process (Haider et al., 2015). High conductivity provides easier nanofiber collection in aligned form and reduces diameter of the nanofibers (Zong et al., 2002). Polymer and solvent type, polymer concentration and temperature influence the electrical conductivity (Andrady, 2008).

Dielectric constant is very important parameter for electrospinning procedure and depends on solvent. Dielectric constant is measured to figure out the capability to hold electrical charge when exposed into an electrical field. High dielectric constant have stronger capability to split which happen in the high electrical field. High dielectric constant provides strong Coulomb repulsion force to overcome surface tension easily. Electrospinning process is very difficult and productivity of the nanofibers are not efficient with very low dielectric constant. Moreover, the diameter of the nanofibers is lower and neat appearance is obtained with high dielectric constant (Gu et al., 2014).

Concentration of the polymer influences the morphology of the nanofibers. When the concentration of the polymer solution is high, chain entanglement is improved. Strong chain entanglement overcomes the surface tension hence uniform and beadless nanofibers are obtained (Haider et al., 2015). Moreover, the fiber diameter is directly proportional to concentration of the solution. Powerful relationship between concentration and fiber diameter is investigated by Demir and his friends (Demir et al., 2002). The relationship can be shown as follows in Equation 2.1.;

$$Nanofiber\ diameter = (Concentration)^3 \qquad (2.1)$$

Viscosity of the solution is another important factor for nanofiber formation. The solutions with too high viscosity cannot be ejected from the needle and it is not possible to obtain nanofibers with too low viscosity (Bhardwaj and Kundu, 2010).

Moreover, higher viscosity causes larger diameter and more uniform nanofiber formation (Deitzel et al., 2001).

Surface tension is the main force against applied voltage during electrospinning procedure. Lower surface tension provides to form nanofibers without beads. Surface tension depends on concentration, chemical composition of the polymer and temperature (Andrady, 2008).

Instrumental parameters are electrical potential, flow rate of the solution, distance between needle and collector.

Electric field is necessary to deform spherical droplet to Taylor cone. Critical applied voltage is changeable from one polymer to another (Sill and von Recum, 2008). When applied electrical potential is increased, the diameter of the nanofiber decreases (Andrady, 2008). However, too high electrical potential causes larger diameter because of more polymer ejection. Also at high voltages, bead formation can happen (Demir et al., 2002).

Flow rate of the solution is another important parameter that influence the morphology of the nanofiber. To obtain complete evaporation of the solvent and solid nanofibers, low flow rate has to be selected. Flow rate and ejection rate of the solution have to be matched (Bhardwaj and Kundu, 2010). Otherwise, at very low flow rate, electrospinning cannot be achieved (Andrady, 2008) and at very high flow rate, uncompleted evaporation of solvent brings about beaded and large diameter nanofibers (Bhardwaj and Kundu, 2010).

The distance between needle and the collector is another instrumental parameter. The relationship between diameter and the distance are inversely proportional (Lin et al., 2008). Moreover, increment in the distance gives rise to longer solvent evaporation time and less bead formation (Jarusuwannapoom et al., 2005).

Ambient parameters are temperature and humidity of the chamber.

Temperature has influence on the uniformity of the nanofibers. Temperature affects both rate of the evaporation and viscosity of the polymer solution. When the temperature is increased, diameter of the nanofibers decreases (Haider et al., 2015). It is known that higher temperature provides more uniform nanofibers (Demir et al., 2002).

Effect of the humidity is vague and cannot be generalized. In a study, the diameter of nanofibers which are obtained by cellulose acetate increases at high humidity. However, the diameter of nanofibers which are obtained by poly vinylpyrrolidone decreases at high humidity. Moreover, vapor pressure and humidity influence the pore diameter and pore size distribution on the fibers (Casper et al., 2004).

2.5.1.3 Characterization of nanofibers

Zeta potential measurement gives information about surface charge of the nanofibers and it is an important parameter to determine stability of the solution.

Contact angle measurement determines the hydrophobicity and hydrophobicity of the nanofibers' surface (Ramaseshan et al., 2006). When the angle is higher than 90º, the nanofiber is hydrophobic. On the other hand, if the contact angle is lower than 90º, it indicates that the surface property of the nanofiber is hydrophilic (Herminghaus, 2000).

3. MATERIALS AND METHODS

3.1 Materials

Ethanol was purchased from AYS Ltd. Sti. (Turkey). The other chemicals and standards were purchased from Sigma-Aldrich (St. Louis, USA). Sour cherry concentrates (Brix: 65 %) were obtained by Konfrut Fruit Juice Concentrates and Purees, Turkey. 500 mg/6 ml C18 SPE cartridges were purchased from Macherey-Nagel GmbH&Co.KG, Germany.

3.2 Preparation of Feed Solutions

20% (w/v) gelatin solution was stirred with 50% (v/v) glacial acetic acid over night at 40ºC and 450 rpm. Gelatin and lactalbumin were mixed (19:1 w/w) and stirred in 50% (v/v) glacial acetic acid over night at 40ºC and 450 rpm. For uniaxial electrospinning procedure, 1 ml of sour cherry concentrate was mixed with 9 ml gelatin or gelatin, lactalbumin mixture (19:1 w/w) solution.

3.3 Determination of Solution Properties

To determine solution properties of the gelatin and mixture of gelatin lactalbumin solutions electrical conductivity, dielectric costant, surface tension and viscosity were measured.

3.3.1 Electrical conductivity

The electrical conductivity of feed solutions was measured triplicate by conductometer (WTW LF95, Germany) at 25 ºC.

3.3.2 Dielectric properties

Dielectric constant of feed solutions was measured triplicate by Agilent ENA Series Network Analyzer, USA at 25 ºC. Dielectric constants of the solutions were investigated over a wide frequency range of 30 Mhz to 3 Ghz.

3.3.3 Surface tension

The surface tension of feed solutions was measured triplicate by a tensiometer (Dataphysics DCAT 11 E, Germany) at 25 ºC.

3.3.4 Viscosity

The viscosity was measured triplicate by a rheometer (Haake Rheostress 1, Germany) using plate-plate sensor whose diameter is 35 mm and gap is 1 mm at 25 ºC. The shear rate was selected between 0.1 and 100 1/s. The results were modelled using the software which is Haake RheoWin3 Data Manager, Germany.

3.4 Electrospinning of Sour Cherry Concentrate

The electrospinning process was conducted with the equipment (Inovenso NE100, Turkey) at room temperature. The equipment has the syringe pump (New Era Pump Systems Inc., NE-300, USA), the tip of the needle, the collector plate and the high voltage power supply (Elektrosis, PW1010, Turkey). In this study, uniaxial (Figure 3.1) and coaxial electrospinning (Figure 3.2) were applied to encapsulate sour cherry concentrate with gelatine and mixture of gelatine and lactalbumin. The operation conditions were demostrated in Table 3.1. The distance between the tip of the needle and the collector plate was 10 cm and kept constant during experiments. The nanofibers were collected on an aluminum foil which was used for covering the collector plate.

Figure 3.1. Set up for uniaxial electrospinning.

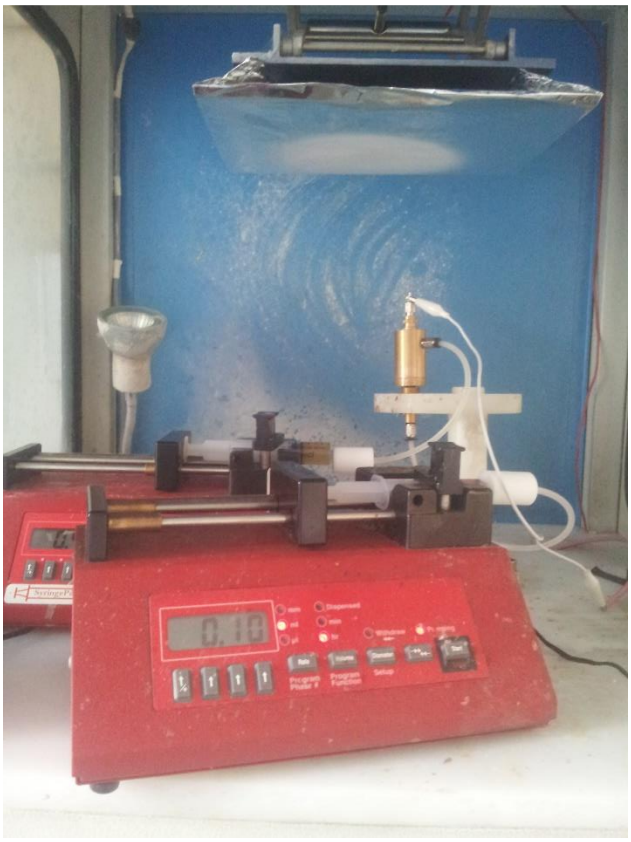

Figure 3.2. Set up for coaxial electrospinning.

Table 3.1. Electrospinning operation parameters.

Electrospinning geometry	Core composition	Shell composition	Applied voltage (kV)	Flowrate (ml/hr)
Uniaxial	-	Gelatin (20% w/v)- aqueous acetic acid solution, sour cherry concentrate 9:1	25	0.5
Uniaxial	-	Gelatin, Lactalbumin (19:1% (w/w)/v)- aqueous acetic acid solution, sour cherry concentrate 9:1	28	0.5
Coaxial	Sour cherry concentrate	Gelatin (20% w/v)- aqueous acetic acid solution	25	Sour cherry: 0.1 Gelatin: 0.4

3.5 Encapsulation Efficiency

To determine encapsulation efficiency of bioactive compounds 10 mg nanofiber were dissolved in 10 ml MQ. The amount of cyanidin was quantified by Waters 2695 HPLC system with PDA detector (Waters 2996) through interpolation of the

absorbance maximum at 520 nm for cyanidin within the calibration curves previously obtained.

3.6 Characterization of Nanofibers

To characterize the nanofibers zeta potential, contact angle measurements were performed.

3.6.1 Zeta potential

The zeta potential was measured triplicate by a dynamic light scattering instrument (Malvern Zetasizer Nano ZS, Worcestershire, UK) at 25 ºC. Ethanol was used for dispersant for all samples because gelatine cannot dissolve in ethanol. All samples were dispersed into 0.1% (w/v) in ethanol.

3.6.2 Contact angle

Contact angle of the nanofibers was measured triplicate by a tensiometer (Dataphysics DCAT 11 E, Germany).

3.7 *In vitro* Digestion

To investigate the bioaccessibility of sour cherry nanofibers invitro gastrointestinal digestion model was stimulated in laboratory conditions with slight modifications (Dinnella et al., 2007). For gastric conditions, 10 mg of nanofibers were dissolved in 5 ml of distilled water and acidified with 6M HCl to pH 2 under constant stirring. 0.6 ml of a porcine pepsin solution (160 mg/ml in 0.1 M HCl) was added and final volume of the solution was arranged to 10 ml with distilled water. The solution was placed into a water bath (Memmert WNB 7, Memmert GmbH & Co. KG, Germany) at 37ºC for 2 hours. After 2 hours, 2 ml of the solution was collected to analyze post gastric (PG) condition. After gastric digestion, the pH of the solution was increased to 5 with 0.045 M $NaHCO_3$. To stimulate the intestinal conditions, 2.4 ml of pancreatin–bile solution (pancreatin 4 mg/ml, bile 25 mg/ml in 0.1 M $NaHCO_3$) was added into the solution. pH was increased to 7.0 with 0.005 M $NaHCO_3$. The solution was placed into a water bath at 37ºC for 2 hours. After 2 hours, the intestinal condition was obtained.

3.8 Determination of Antioxidant Properties

3.8.1 Determination of total phenolic content

The total phenolic content was determined by Folin – Ciocalteu method. 100 µl extract was mixed with 750 µl Folin – Ciocalteu solution (diluted 10 times with distilled water). The mixture was stayed at room temperature for 5 minutes then 750 µl $NaHCO_3$ solution (60 g/ L) was added to the previous mixture. After 90 minutes, the absorbance was read at 725 nm against distilled water and gallic acid was used for the standard (Singleton and Rossi, 1965). The results were expressed as mg gallic acid equivalent (GAE)/ g nanofiber.

3.8.2 Determination of total flavonoid content

The total phenolic content was determined with slight modification (Dewanto et al., 2002). 100 µl extract was mixed with 500 µl distilled water and 30 µl 5% aqueous-$NaNO_2$ solution. 6 minutes later, 60 µl 10% $AlCl_3.6H_2O$ solution was added. After 5 minutes, 200 µl 1 M NaOH was mixed with the solution. Volume of the final mixture was reached to 1000 µl with distilled water. The absorbance was read at 510 nm against distilled water immediately and rutin was used for the standard. The results were expressed as mg catechin equivalent/ g nanofiber.

3.8.3 Determination of total anthocyanin content

Total anthocyanin content was determined with pH differential method (Lee et al., 2005). 100 µl was diluted with 400 µl pH 1 buffer (potassium chloride, 0.025 M) and 100 µl was diluted with 400 µl pH 4.5 buffer (sodium acetate, 0.4 M). The absorbance was measured at 517 and 700 nm. The anthocyanin content was measured as follows in Equation 3.1a and 3.1b;

$$Anthocynanin\ content\ \left(C3GE, \frac{mg}{L}\right) = \frac{A*MW*DF*10^3}{\varepsilon*l} \quad (3.1a)$$

$$\mathrm{A} = \left(\mathrm{A520nm} - \mathrm{A700nm}\right)\mathrm{pH\ 1.0} - \left(\mathrm{A520nm} - \mathrm{A700nm}\right)\mathrm{pH\ 4.5} \quad (3.1b)$$

MW: Molecular weight of cyanidin-3-glucoside which is 449.2 g/mol

DF: Dilution factor. The dilution is necessary because the absorbance has to be between 0.2 and 1.4 absorbance unit (AU) for most of the spectrophotometers. It was 5 in this study to maintain the absorbances into the interval.

ε: Extinction coefficient of cyanidin-3-glucoside which is 26900 L* mol^{-1}*cm^{-1}

l: path length in cm. It was 1 cm in this study.

The content of the anthocyanin in nanofibers were calculated with the Equation 1 and the results were expressed as mg cyanidin-3-glucoside equivalent/ g nanofiber.

3.8.4 Determination of total antioxidant capacity

To determine the total antioxidant capacity, four assays were performed.

3.8.4.1 1,1-diphenyl-2-picrylhydrazil radical scavenging activity (DPPH)

DPPH radical scavenging activity was determined (Rai et al., 2006). 100 µl extract was mixed with 2000 µl 0.1 mM DPPH solution (3.943 g DPPH was dissolved with 100 ml methanol). After 30 minutes stand in the dark, the absorbance was read at 517 nm against methanol in and trolox was used for the standard. The results were expressed as mg trolox equivalent (TEAC)/ g nanofiber.

3.8.4.2 Cupric ion reducing antioxidant capacity (CUPRAC)

CUPRAC antioxidant capacity was determined (Apak et al., 2004). 100 µl extract was mixed with 1000 µl 10^{-2}mM $CuCl_2$ solution (0.4262 g $CuCl_2.2H_2O$ was dissolved with 250 ml distilled water), 1000 µl 7.5mM neocuproine solution (0.039 g neocuproine was dissolved with 25 ml 96% aqueous-ethanol), 1000 µl NH_4Ac solution (19.27 g NH_4Ac was dissolved with 250 ml distilled water) and 1000 µl distilled water. After 30 minutes, the absorbance was read at 450 nm against distilled water and trolox was used for the standard. The results were expressed as mg trolox equivalent (TEAC)/ g nanofiber.

3.8.5 HPLC analysis of sour cherry and nanofibers phenolic profile

Before HPLC analysis of the samples, *in vitro* gastrointestinal digestion samples were purified by solid phase extraction (SPE) method (Kamiloglu et al., 2015). To condition of 500 mg/6 ml C18 SPE cartridges were rinsed by 6 ml formic acid/methanol (1:100, v/v) and 4 ml formic acid/MQ water (1:100, v/v). 1.5 ml of the

samples were acidified with 30 μL of formic acid and centrifuged (Hettich Zentrifugen Universal 32 R refrigerated centrifuge) at 16000 g for 10 minutes. The cartridges 5 ml formic acid/MQ water were washed after supernatants were added into the cartridges. The samples were eluted with formic acid/methanol (1:100, v/v) and filtered through 0.45 μm membrane filters.

Analysis were operated with HPLC system with PDA detector. The separation was performed with Luna C18 column (Phenomenex). Injection volume was 10 μL and column temperature was adjusted at 40 ºC. The gradient system consists of solvent A (MQ water with 0.1% (v/v) trifluoroacetic acid (TFA)) and solvent B (acetonitrile with 0.1% (v/v) TFA). The gradient was as follows: 0 min 5% B; 45 min 65% B; 47 min 75% B; 52 min 75% B and 55 min 5% B. The flow rate was 1 ml/ min. The detection of the eluted peaks was achieved at 280, 312, 360, and 512 nm wavelengths. Identification was performed based on the

retention times and characteristic UV spectra, and quantification was achieved by external standard curves.

4. RESULTS AND DISCUSSION

4.1 Determination of Solution Properties

To determine the solution properties, electrical conductivity, surface tension, viscosity and dielectric constant were measured. The results of the solution properties were demonstrated in Table 4.1.

Table 4.1. Solution properties of feed solutions.

Solution	Electrical conductivity (mS/cm)	Surface tension (mN/m)	Absolute viscosity at 25ºC (Pa.s)	Dielectric constant (ε′) at 30 Mhz	Dielectric constant (ε′) at 3 Ghz
S	0.68±0.01	54.81±0.03	0.12 ± 0.01	9.56± 0.33	19.26± 1.44
G	2.08±0.01	31.85±0.03	0.17 ± 0.04	30.66±1.05	27.86± 1.45
GL	2.03±0.01	31.08±0.03	0.22 ± 0.01	37.17± 1.05	30.09± 1.50
GS	2.25±0.01	31.16±0.03	0.21 ± 0.01	39.52± 0.33	24.37± 0.69
GLS	2.23±0.01	30.52±0.03	0.24 ± 0.01	65.14± 1.96	23.06± 2.50

S: Sour cheery concentrate, G: Gelatin, GL: Mixture of gelatin and lactalbumin, GS: Mixture of gelatin and sour cherry concentrate, GLS: Mixture of gelatin, lactalbumin and sour cherry concentrate.

The results showed that the electrical conductivity is very low for sour cherry concentrate (S) (0.68±0.01 mS/cm). However, coating materials which are gelatine and lactalbumin have high electrical conductivity. To initiate the electrospinning process, conductive polymer solution is necessary. Conductive polymer solution has high repulsion force to overcome surface tension of a droplet to formation of nanofiber (Wang et al., 2013). The conductivity of gelatine (G) (2.08±0.01 mS/cm) is slightly higher than mixture of gelatine and lactalbumin (GL) (2.03±0.01 mS/cm). When sour cherry and coating materials were mixed, the conductivity of the new solution was increased. The results are inconsistent with the literature. A study about quercetin encapsulation with electrospinning process showed that conductivity of the electrospinning solution increased when the coating material and quercetin were

mixed (Aceituno-Medina et al., 2015). G and GL solutions' conductivities were found appropriate for encapsulation of sour cherry concentrate with electrospinning process.

Surface tension of the S was the highest one (54.81±0.03 mN/m) and addition of coating materials provided to decrease the surface tension of the electrospinning solutions. To formation of nanofiber with electrospinning, repulsion force has to overcome the surface tension. While the surface tension is too high, drops are formed instead of nanofiber (Ki et al., 2005). The surface tension of gelatin in acetic acid is around 28.8 mN/m and the surface tension of gelatin in water is 74.2 mN/m (Okutan et al., 2014). Acetic acid was chosen as a solvent to dissolve coating materials for electrospinning procedure due to its low surface tension. The surface tension of G (31.85±0.03 mN/m) was found slightly higher than GL (31.08±0.03 mN/m). When gelatine or lactalbumin were added into sourcherry concentrate, the surface tension of the solutions decreased. Decrement of surface tension with polymers provided to electrospin the sour cherry concentrate properly.

Viscosity is another important parameter for electrospinning procedure. All solutions exhibited Newtonian behaviour. Viscosity of GL (0.22 ± 0.01 Pa.s) was slightly higher than G solution (0.17 ± 0.04 Pa.s). When the sour cherry concentrate was added into the G or GL solution, the viscosity increased. Electrospinning of the sour cherry concentrate was done properly with both of the coating materials.

Dielectric constant gives information about the polarity of the solution. When the dielectric constant is high, net charge density in the solution is high. Therefore, the solution with high dielectric constant is more polar (Son et al., 2004). The dielectric constant of G solution (30.66±1.05) was found lower than GL (37.17± 1.05). Hence, addition of lactalbumin into the solution improved polarity. The lowest dielectric constant was measured for S (9.56± 0.33). Addition of sour cherry concentrate into the G and GL solution provided to increment of dielectric constant of sour cherry solution. The most polar solution was sour cherry concentrate in GL solution and the most nonpolar solution was S. Dielectric constants were found higher at 30 Mhz than at 3 Ghz for all solutions except for sour cherry concentrate. The highest change was found for the GLS solution. The changes of dielectric constant dependent on frequency were found in accordance with the other studies (Mahendia et al., 2010; Sinha et al., 2015; Zamri et al., 2014). The sharp decrement of dielectric constant at

low frequency is explained with the polarity. At low frequencies the dipoles follow the applied voltage and the dielectric constant is measured high due to polar compound's interfacial and electrode polarizations. At high frequencies, the polar compounds do not have enough time to align with applied voltage and the the dielectric constant is found low (Sinha et al., 2015).

4.2 Encapsulation Efficiency

Encapsulation efficiency of nanofibers were demonstrated in Table 4.2.

Table 4.2. Encapsulation efficiency of nanofibers.

Nanofiber	Encapsulation Efficiency %
SGN	61.74
SGLN	79.17
SGCN	50.33

Nanofiber coated with mixture of gelatin and lactalbumin by uniaxial electrospinning had the highest encapsulation efficiency which is 79.17 % and nanofiber coated with gelatin followed. The lowest encapsulation efficiency was determined for coaxial electrospinning. The encapsulation efficiency of quercetin and ferulic acid were found 93.6 and 83.7, respectively in a study (Aceituno-Medina et al., 2015). The difference can be related with different polymer and bioactive compound. In this study, it did not used pure cyanidin compare the other study, therefore it can influence the electrospinning process and encapsulation efficiency.

4.3 Characterization of Nanofibers

Zeta potential potential and contact angle were measured to characterize the nanofibers and the results are demonstrated in Table 4.3.

All nanofibers had negative surface charges. Gelatin nanofiber (GN) (-16.6 ± 0.85 mV) was found more anionic than gelatin and lactalbumin nanofiber (GLN) (-8 ± 0.07 mV). The same behaviour was observed when the sour cherry concentrate is added into the nanofiber formation. The zeta potential of nanofiber with sour cherry concentrate and gelatin (SGN) was -8 ± 1.23 mV and for nanofiber with sour cherry concentrate, gelatin and lactalbumin (SGLN) was -3.19 ± 0.15 mV. The differences between gelatin and lactalbumin nanofibers can be explained with applied voltage.

Table 4.3. Zeta potential and contact angle of the nanofibers.

Nanofiber	Zeta potential (mV)	Contact angle (º)
SGN	-8 ± 1.23	78.45± 0.9
SGLN	-3.19 ± 0.15	84.58 ± 5.7
SGCN	-9.38 ± 0.73	n.d.
GN	-16.6 ± 0.85	89.49 ± 0.59
GLN	-8 ± 0.07	88.29± 2.02

SGN: Sour cherry nanofiber with gelatin by uniaxial electrospinning, SGLN: Sour cherry nanofiber with mixture of gelatin and lactalbumin by uniaxial electrospinning SGCN: Sour cherry nanofiber with gelatin by coaxial electrospinning, GN: Gelatin nanofiber GLN: Mixture of gelatin and lactalbumin nanofiber. n.d. Not detected.

25 and 28 kV were applied when the gelatin and gelatin and lactalbumin nanofibers were produced respectively. When the applied voltage increased, the zeta potential of the nanofiber decreased. The same decrease for zeta potential of gelatin solution were obtained when the applied voltage were increased in another study (Okutan et al., 2014). In addition, absolute value of zeta potential is the indication of stability (Koo et al., 2014). Therefore, it is possible to obtain more stable sour cherry nanofiber with gelatin than gelatin and lactalbumin mixture.

Contact angle was measured to decide the nanofiber's hydrophobicity and hydrophilicity. All nanofiber's contact angle values in water were lower than 90º. Therefore, the nanofibers showed hydrophilic properties.

4.4 Antioxidant Property

To determine antioxidant property, total phenolic (TPC), total flavonoid (TFC) and total anthocyanin content (TAC), free scavenging activity (DPPH) and reducing antioxidant capacity (CUPRAC) analyses were done.

4.4.1 Total phenolic content (TPC) analysis

Total phenolic contents of the samples were analyzed by spectrophotometric analysis and results were demonstrated in Table 4.4.

Total phenolic content of sour cherry concentrate (S) was found 12.89± 0.83 mg GAE/g sample. The total phenolic content of 32 different sour cherry cultivars were found between 0.74 and 7.54 mg GAE/ g sample (Khoo et al., 2011). The difference between the results can be due to different methods of extraction and determination processes (Wojdyło et al., 2014). Moreover, genetic background, climatic conditions

and agricultural practices have influence on nutritional content and antioxidant capacity of the fruit (Gerasopoulos and Stavroulakis, 1997).

Table 4.4. Total phenolic content (TPC) of sourcherry concentrate, nanofibers and changes after *in vitro* digestion.

Sample	Total phenolic content (TPC) mg gallic acid equivalent (GAE)/g sample or nanofiber	Post gastric (PG)	Intestine (IN)
S	12.89 ± 0.83	14.24 ± 0.08	1.94 ± 0.17
SGN	14.79 ± 0.51	33.60 ± 2.16	62.32 ± 1.51
SGLN	13.56± 1.63	34.09 ± 3.58	21.55 ± 9.81
SGCN	16.45 ± 0.83	29.60 ± 1.18	32.03 ± 2.73
GN	11.12 ± 0.57	47.09 ± 6.24	67.83 ± 4.93
GLN	13.69 ± 1.18	52.43 ± 1.65	66.42 ± 4.45

SGN: Sour cherry nanofiber with gelatin by uniaxial electrospinning, SGLN: Sour cherry nanofiber with mixture of gelatin and lactalbumin by uniaxial electrospinning SGCN: Sour cherry nanofiber with gelatin by coaxial electrospinning, GN: Gelatin nanofiber GLN: Mixture of gelatin and lactalbumin nanofiber.

According to results, gelatin and lactalbumin also showed phenolic activity. However; phenolic activity of gelatin and lactalbumin can be related with their aminoacid residues such as tyrosine, tryptophan, cysteine, histidine and asparagines. It is known that these aminoacids reduce Folin–Ciocalteu reagent like phenolic compounds (Lowry et al., 1951). When the effects of the gelatin and lactalbumin phenolic activities were subtracted from sour cherry nanofibers the results were 14.79 ± 0.51 mg GAE/g nanofiber for gelatin coated (SGN) and 13.56± 1.63 mg GAE/g nanofiber for mixture of gelatin and lactalbumin coated nanofibers (SGLN). The highest total phenolic content which is 16.45± 0.83 mg GAE/g nanofiber was determined for coaxial electrospun sour cherry concentrate (SGCN).

TPC of all samples was increased after gastric phase. Low pH and enzymatic activity can provide to hydrolysis of phenolic compounds (Rodríguez-Roque et al., 2013). Also, gastric phase can improve extractability of the phenolic compounds (Baublis et al., 2000; Liyana-Pathirana and Shahidi, 2005). TPC of all nanofibers were found higher than S. Increase of TPC of all nanofibers can be related with interaction between phenolic compounds and gelatin or lactalbumin. Similarly, Tagliazucchi et al. suggested that phenolic compounds can bound to high molecular weight molecules for instance protein and carbohydrate and the interaction improves release properties (Tagliazucchi et al., 2010).

After intestinal phase, TPC of S decrased to 1.94 ± 0.17 mg GAE/g sample. High pH during intestinal phase caused to degradation of polyphenols of the sour cherry. The decrase of TPC can be explained by transformation of polyphenols into different structural forms (Bermúdez-Soto et al., 2007). On the other hand, TPC of all nanofibers improved after *in vitro* digestion. The improvement of polyphenol content can be related with protective effect of electrospinning process against high and low pH value during digestion. When sour cherry concentrate encapsulated with gelatin or mixture of gelatin and lactalbumin, TPC increased in contrast to S. It can be said that electrospinning process provides to improve TPC efficiently.

4.4.2 Total flavonoid content (TFC) analysis

Total flavonoid contents of the samples were analyzed by spectrophotometric analysis and results were demonstrated in Table 4.5.

The total flavonoid content (TFC) of S was measured as 8.58 ± 1.45 mg CE/g sample. TFC content of sour cherry were found 1.39 mg CE/g sample and 4.41 mg CE/g sample in other studies. The different contents can be related with variation of cultivars, different extraction method and analysis method. The highest total flavonoid content was found for SGLN (12.01 ± 2.08 mg CE/ g nanofiber).

TFC of S decreased in gastric condition and continued to decrease during intestinal phase. TFC of all nanofibers decreased after gastric phase except SGN. However, after intestinal phase, TFC of all nanofibers increased and their results were higher than the initial amount. The increase of TFC after intestinal phase can be explained

Table 4.5. Total flavonoid content (TFC) of sourcherry concentrate, nanofibers and changes after *in vitro* digestion.

Sample	Total flavonoid content (TFC) mg catechin equivalent (CE)/g sample or nanofiber	Post gastric (PG)	Intestine (IN)
S	8.58 ± 1.45	5.10 ± 1.18	5.04 ± 0.84
SGN	11.19 ± 0.51	13.56 ± 3.28	27.00 ± 3.20
SGLN	12.01 ± 2.08	9.30 ± 4.68	40.52 ± 4.10
SGCN	10.88 ± 1.94	8.23 ± 1.37	32.34 ± 1.55
GN	22.95 ± 0.90	26.76 ± 1.68	74.66 ± 6.19
GLN	15.51 ± 0.81	22.70 ± 2.69	70.85 ± 4.15

SGN: Sour cherry nanofiber with gelatin by uniaxial electrospinning, SGLN: Sour cherry nanofiber with mixture of gelatin and lactalbumin by uniaxial electrospinning SGCN: Sour cherry nanofiber with gelatin by coaxial electrospinning, GN: Gelatin nanofiber GLN: Mixture of gelatin and lactalbumin nanofiber.

by longer extraction time which was additional 2 hours and intestinal digestive enzymes (Bouayed et al., 2011).

4.4.3 Total anthocyanin content (TAC) analysis

Total anthocyanin contents of the samples were analyzed by spectrophotometric analysis and results were demonstrated in Table 4.6.

Table 4.6. Total anthocyanin content (TAC) of sourcherry concentrate, nanofibers and changes after *in vitro* digestion.

Sample	Total anthocyanin content (TAC) mg cyanidin-3-glucoside equivalent(C3GE)/g sample or nanofiber	Post gastric (PG)	Intestine (IN)
S	1.63 ± 0.12	1.66± 0.47	1.57 ± 0.50
SGN	1.20± 0.32	1.83 ± 0.02	2.46± 0.17
SGLN	1.21 ± 0.28	1.71 ± 0.01	0.73 ± nd
SGCN	0.80 ± 0.04	0.39 ± 0.001	0.37± 0.17
GN	0.17 ± 0.09	6,26208E-05± 0.001	n.d
GLN	0.33 ± 0.09	8,34944E-05± 0.001	n.d

SGN: Sour cherry nanofiber with gelatin by uniaxial electrospinning, SGLN: Sour cherry nanofiber with mixture of gelatin and lactalbumin by uniaxial electrospinning SGCN: Sour cherry nanofiber with gelatin by coaxial electrospinning, GN: Gelatin nanofiber GLN: Mixture of gelatin and lactalbumin nanofiber.

Total anthocyanin contents of the samples were measured by pH method. The total anthocyanin content of the sour cherry concentrate was determined as 1.63 ± 0.12 mg C3GE/g sample. Different sour cherry cultivars' anthocyanin contents were found between 0.18 and 1.24 C3GE/ g sample. There can be several reasons to explain the difference between the results. During ripening, the anthocyanins in sour cherry do not change however, the highest amount of anthocyanin is found at the last stage of maturity. Also, growing region and ripening affect both anthocyanin content and color of the sour cherry (Pedisić et al., 2010). The concentrations of anthocyanins in different sour cherries are variable because genotype, ripening stage, year of study, agronomic and storage conditions influence the anthocyanin content (Gonçalves et al., 2007; Usenik et al., 2008; Valero and Serrano, 2010).

TAC of S increased slightly after gastric phase to 1.66 ± 0.47 mg C3GE/g sample. Also TAC of all nanofibers except SGCN increased after gastric phase. The increment of anthocyanin content can be related with low pH and pepsin digestion. At gastric condition, anthocyanins transform into some colored anthocyanin derivatives (Fazzari et al., 2008). After intestinal phase, TAC of all samples

decreased except SGN. It is known that anthocyanins are vulnerable compounds against high pH. During intestinal phase, high pH caused to degradation of anthocyanins sample and nanofibers.

4.4.4 Total antioxidant capacity analyses

4.4.4.1 1,1-diphenyl-2-picrylhydrazil radical scavenging activity (DPPH)

Free radical scavenging activity of the samples were analyzed by DPPH method spectrophotometrically and results were demonstrated in Table 4.7.

Table 4.7. DPPH of sourcherry concentrate, nanofibers and changes after *in vitro* digestion.

Sample	DPPH mg trolox equivalent (TEAC)/g sample or nanofiber	Post gastric (PG)	Intestine (IN)
S	6.49 ± 3.39	34.25 ± 4.97	nd
SGN	5.40 ± 1.31	13.82 ± 1.24	nd
SGLN	5.23 ± 3.02	34.36 ± 4.45	nd
SGCN	3.07 ± 0.20	14.85 ± 1.58	nd
GN	n.d	26.76 ± 1.68	nd
GLN	n.d	22.70 ± 2.69	nd

SGN: Sour cherry nanofiber with gelatin by uniaxial electrospinning, SGLN: Sour cherry nanofiber with mixture of gelatin and lactalbumin by uniaxial electrospinning SGCN: Sour cherry nanofiber with gelatin by coaxial electrospinning, GN: Gelatin nanofiber GLN: Mixture of gelatin and lactalbumin nanofiber. n.d. Not detected.

Free radical scavenging activity of the S was found as 6.49 ± 3.39 mg TEAC/g sample. DPPH of sour cherry in other studies were investigated as13.49 ± 2.93 mg TEAC/g sample and 0.45 ± 0.015 mg GAE/g sample (Lončarić et al.; Toydemir et al., 2013). The difference between the results can also be explained by different cultivars and applied method.

After gastric digestion, DPPH values of all samples increased. The increment can be relevant to racemization of molecules which conversion of one enantiomers into more enantiomer. The conversion of molecules influences biological reactivity. Due to low pH, racemization occurred and the molecules became more reactive than initial. Also, in intestinal phase, scavenging activity could not be measured due to low reactivity of phenolics at high pH (Jamali et al., 2008; Wootton-Beard and Ryan, 2011).

4.4.4.2 Cupric ion reducing antioxidant capacity (CUPRAC)

Cupric reducing antioxidant capacity of the samples were analyzed by CUPRAC method spectrophotometrically and results were demonstrated in Table 4.8.

Table 4.8. CUPRAC of sourcherry concentrate, nanofibers and changes after *in vitro* digestion.

Sample	CUPRAC mg trolox equivalent (TEAC)/g sample or nanofiber	Post gastric (PG)	Intestine (IN)
S	39.19 ± 3.98	30.96 ± 4.41	28.72 ± 2.29
SGN	25.17± 0.45	56.64 ± 1.04	111.77 ± 3.96
SGLN	22.36 ± 1.36	60.27 ± 6.93	115.21 ± 11.57
SGCN	11.26 ± 1.36	37.60 ± 3.83	97.28 ± 8.02
GN	130.94 ± 0.79	97.56 ± 2.26	278.27 ± 3.71
GLN	122.57 ± 6.32	98.19 ± 3.42	260.11 ± 3.41

SGN: Sour cherry nanofiber with gelatin by uniaxial electrospinning, SGLN: Sour cherry nanofiber with mixture of gelatin and lactalbumin by uniaxial electrospinning SGCN: Sour cherry nanofiber with gelatin by coaxial electrospinning, GN: Gelatin nanofiber GLN: Mixture of gelatin and lactalbumin nanofiber.

The highest antioxidant capacity was measured for sour cherry concentrate (39.19 ± 3.98 mg TEAC/ g sample). According to another study CUPRAC of the sour cherry was found as 63.67 ± 12.87 mg TEAC/ g sample (Toydemir et al., 2013). The difference between the results can be due to different sour cherry cultivars.

After gastric phase, CUPRAC of S decreased to 30.96 ± 4.41 mg TEAC/ g sample. However, for all nanofibers CUPRAC values increased after gastric digestion. Similarly, CUPRAC value of black carrot antioxidants increased after gastric phase (Tomas et al., 2015).

After intestine phase, CUPRAC value of S continued to decrease (28.72 ± 2.29 mg TEAC/g sample) and nanofibers antioxidant capacity also continued to increase. The highest CUPRAC value after in vitro gastrointestinal digestion was found for SGLN (115.21 ± 11.57 mg TEAC/g nanofiber).

4.4.5 HPLC analysis of sour cherry concentrate and nanofibers phenolic profile

Antioxidant components of sour cherry and nanofibers were analyzed by HPLC and the results were demonstrated in Table 4.9.

Gallic acid, cholorogenic acid, p-coumaric acid as phenolic acids and kaempherol derivatives, epicatechin and cyanidin as flavonoids were detected in sour cherry concentrate. The most predominant antioxidant in the S was cyanidin which is 67% of total antioxidant activity. The results are in accordance with the literature. Another study also showed that cyanidin was the most abundant antioxidant in sour cherry and its products (Toydemir et al., 2013). The phenolic compounds after gastrointestinal digestion were degraded except gallic acid and quercetin. It is known that anthocyanins are very sustainable compounds and they degrade in intestinal phase due to high pH. The lowest recovery was found for cyanidin which is 14% and total recovery of polyphenols after in vitro digestion was 34.01%.

Polyphenol content of nanofibers which were coated by gelatin or mixture of gelatin and lactalbumin also undergone degradation. However, the highest recovery was found for cyanidin for every nanofibers contrast to S. Recovery of total polyphenol content of nanofibers was 66.66, 79.70 and 79.01 % respectively. GN and GLN didn't have any polyphenol content.

The improvement of bioaccessibility with electrospinning can be related with size reduction. It is known that size is very important parameter for uptake of the compounds. It is investigated that nanoparticles with 100 nm can be uptaken 2.5 fold greater than 1μm and 6 fold higher uptake is possible with 100 nm size nanoparticle compared to 10 μm microparticles by Caco-2 cells (Desai et al., 1997).

Addition of lactalbumin into the nanofiber formation provided to improve bioaccessibility. Although, encapsulation efficiency of nanofiber by coaxial electrospinning was low, bioaccessibility of polyphenol content was found higher than nanofiber coated with gelatin by uniaxial electrospinning and slightly different from nanofiber coated with mixture of gelatin and lactalbumin by uniaxial electrospinning. In addition, Nanoparticles have greater surface area and higher soluble property than micron-sized particles. Therefore; nano-size provides to improve bioavailability, controlled release and targeting of the core material (Mozafari et al., 2008).

Table 4.9. Antioxidant components of sour cherry concentrate and nanofibers.

	Gallic acid	Chlorogenic acid	Epicatechin	p-coumaric acid	Kaempherol derivatives	Cyanidin	Quercetin	Recovery %
S	0.38±0.03	1.24±0.07	0.06±0.01	0.14±0.01	0.10±0.01	3.57±0.69	0.19±0.06	34.01
S (IN)	0.03±0.003	0.52±0.06	n.d	0.09±0.01	0.05±0.01	0.50±0.1	0.75±0.13	
SGN	n.d	0.95±0.33	0.41±0.04	0.17±0.1	0.15±0.09	3.42±0.46	n.d	66.66
SGN (IN)	n.d	0.44±0.08	n.d	n.d	n.d	2.97±0.28	n.d	
SGLN	0.97±0.01	0.99±0.11	n.d	0.13±0.01	0.10±0.02	4.39±0.13	n.d	79.70
SGLN (IN)	0.85±0.16	0.12±0.002	n.d	n.d	0.16±0.03	4.1±0.1	n.d	
SGCN	0.46±0.04	n.d	n.d	n.d	n.d	2.79±0.3	n.d	79.1
SGCN (IN)	0.34±0.04	n.d	n.d	n.d	n.d	2.23±0.04	n.d	
GN	n.d	n.d	n.d	n.d	n.d	n.d	n.d	-
GN (IN)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	-
GLN	n.d	n.d	n.d	n.d	n.d	n.d	n.d	-
GLN (IN)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	-

Amount of polyphenols were expressed as mg/g sample or nanofiber. n.d. Not detected

5. **CONCLUSION**

Sour cherry is good source of anthocyanin and phenolic compounds. Antioxidant profile of the sour cherry is composed of vitamins, anthocyanins, hydroxycinnamic acids, flavan-3-ols, flavonols and flavones. Sour cherry phenolics can be used to protection against allergenicity of protein β-lactoglobulin, cancer, cardiovascular diseases, muscle pains and aches. Antioxidants are vulnerable against undesired environmetal conditions. Especially anthocyanins which are responsible to prevent several diseases, are sustainable compounds. Encapsulation provides to protect the valuable compounds from degradation and improve bioaccessibility and controlled release. Electrospinning is cost effective, simple with high encapsulation efficiency process.

The aim of the study was to determine encapsulation of sour cherry anthocyanins and phenolic compounds coated with gelatin and mixture of gelatin and lactalbumin by uniaxial and coaxial electrospinning process. Hence, bioaccessibility of nanofibers were investigated by *in vitro* gastrointestinal digestion. For this purpose, solution properties were measured to control suitability of coating materials for electrospinning. After nanofibers were fabricated, characterization of nanofibers was analyzed by zeta potential and contact angle measurements. Total phenolic, flavonoid and anthocyanin content and total antioxidant capacity were analyzed by spectrophotometry. Moreover, phenolic profile of the sour cherry and nanofibers were investigated by chromatographic method. Finally, *in vitro* digestion was performed and bioaccessibility were calculated.

The results showed that both gelatin and mixture of gelatin and lactalbumin are suitable polymers to electrospun of sour cherry polyphenols. All nanofibers were negative charged and contact angle of all nanofibers was lower than 90º, therefore they showed hydrophilic properties. Encapsulation efficiency of nanofibers were determined according to HPLC results and the highest encapsulation efficiency were obtained by SGLN. Total phenolic, flavonoid and anthocyanin content and total antioxidant capacity were measured for samples before and after *in vitro* digestion

process by spectrophotometry. In all assays, all nanofibers had higher amount than sour cherry concentrate. Gallic acid, cholorogenic acid, p-coumaric acid, kaempherol derivatives, epicatechin and cyanidin were detected in sour cherry concentrate by HPLC. The amount of polyphenols in nanofibers after *in vitro* digestion were also found higher than sour cherry concentrate. The highest recovery was found for SGLN.

Gelatin and lactalbumin were electrospun easily and they provided high protection and improved bioaccessibility. Addition of lactalbumin into the solution provided to enhance protection of polyphenols and bioaccessibility. Moreover, the recovery results showed that coaxial electrospinning intensify bioaccessibility though encapsulation efficiency was low.

This study provides valuable data on electropinning of polyphenol compounds and bioaccessibility of nanofibers and could be a basis for the further studies. This study can be extended with the use of other natural polymers and bioactive compounds. Coaxial electrospinning is a promising method and investigation can be expanded about that field. Also, *in vivo* analysis can be performed to obtain direct results about response of living organisms to nanofibers.

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APPENDICES

APPENDIX A: Standard calibration curves

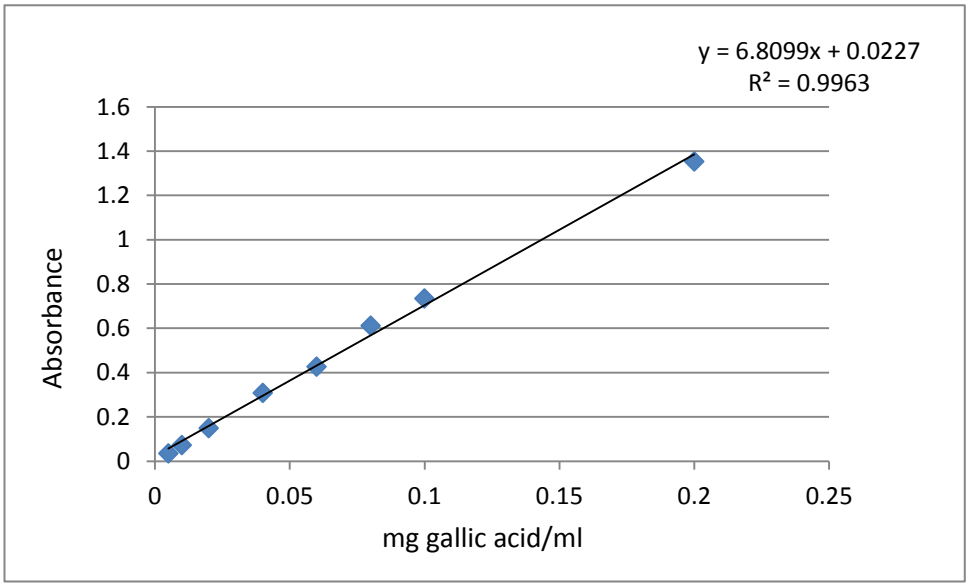

Figure A. 1: Standart calibration curve for total phenolic content analysis

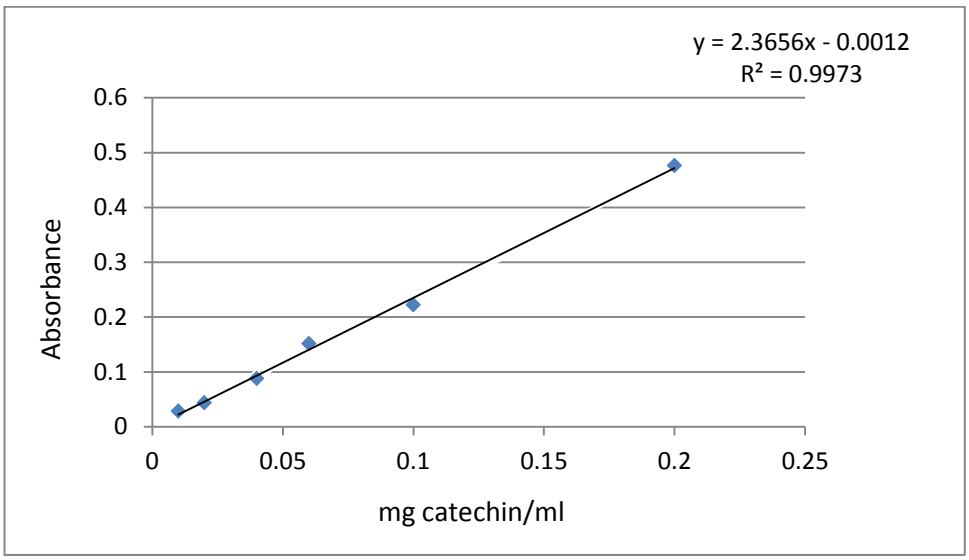

Figure A. 2: Standart calibration curve for total flavonoid content analysis

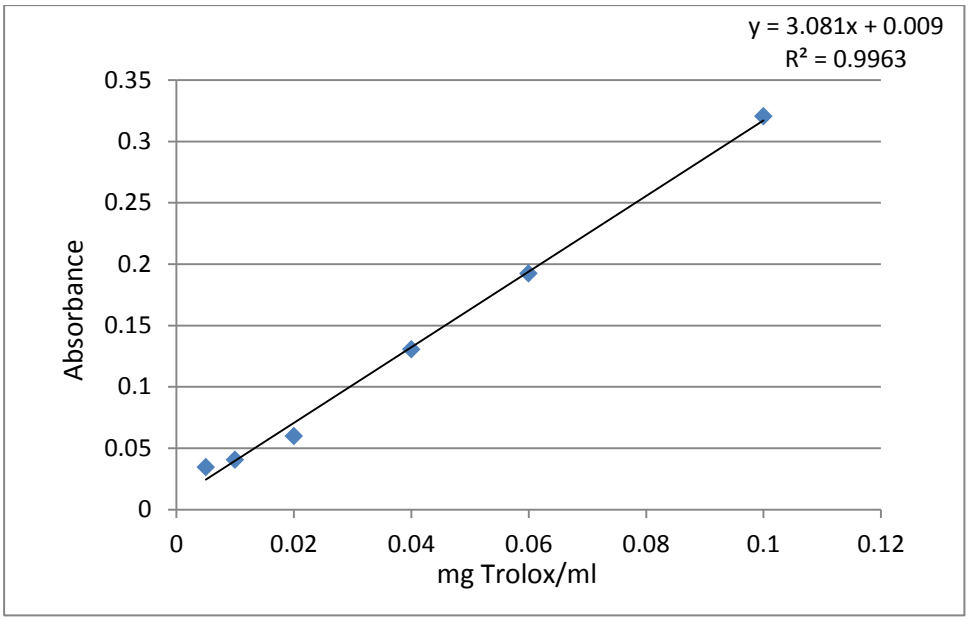

Figure A. 3: Standart calibration curve for DPPH analysis

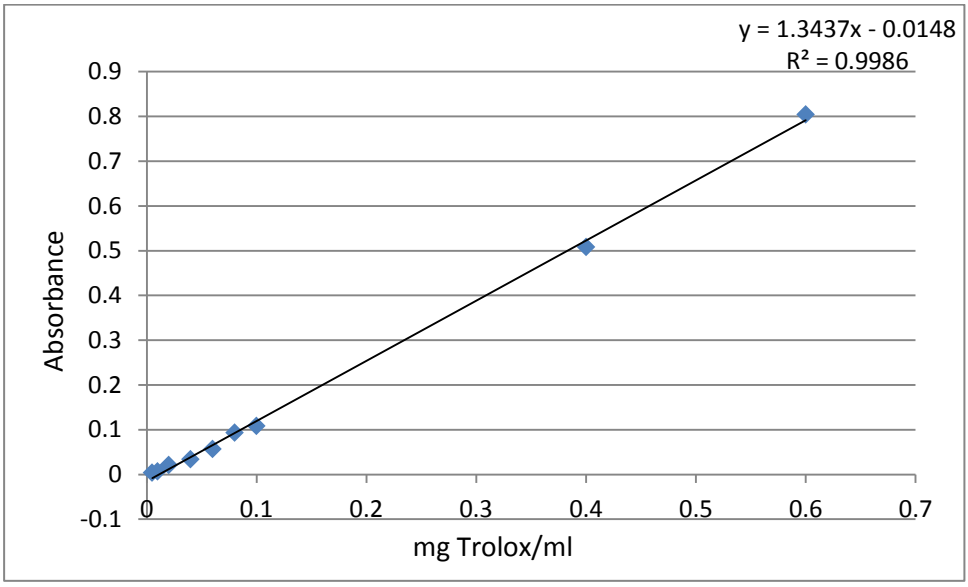

Figure A. 4: Standart calibration curve for CUPRAC analysis

APPENDIX B: HPLC Chromatograms

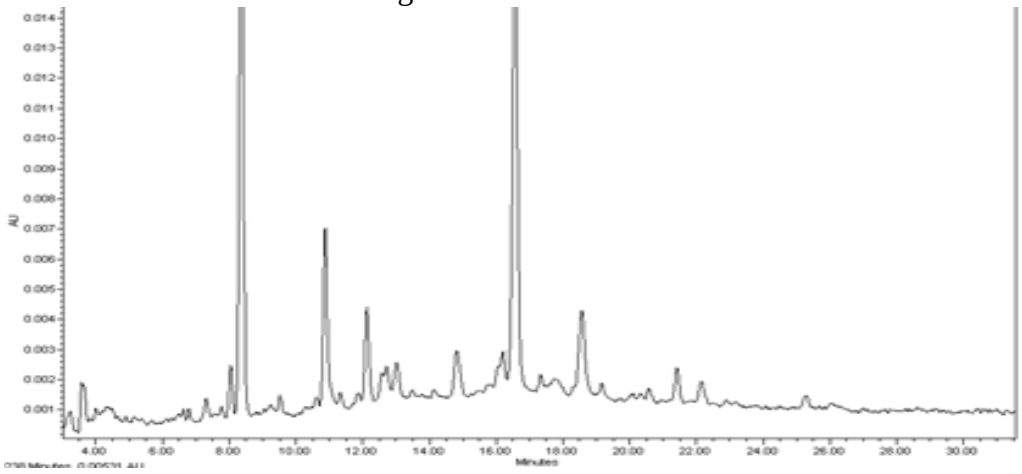

Figure B.1: Representative HPLC chromatograms of sour cherry concentrate at 280 nm.

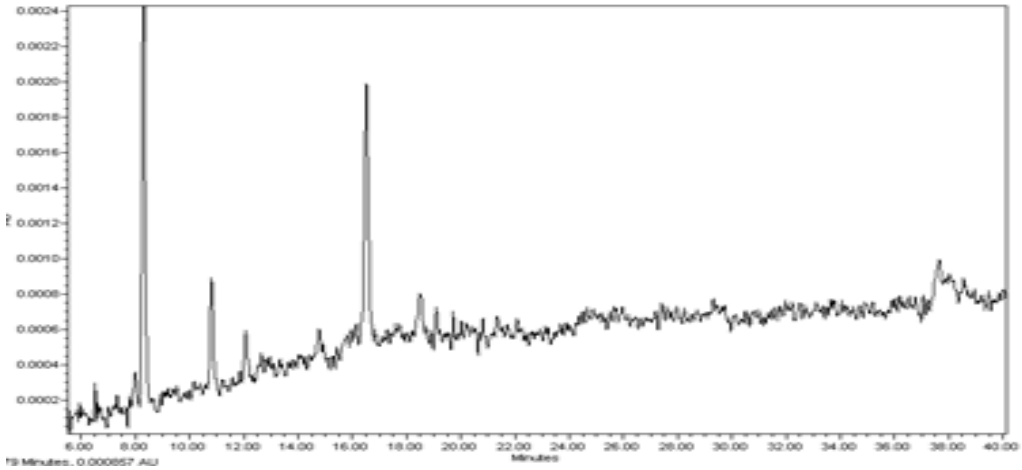

Figure B.2: Representative HPLC chromatograms of SGN at 280 nm.

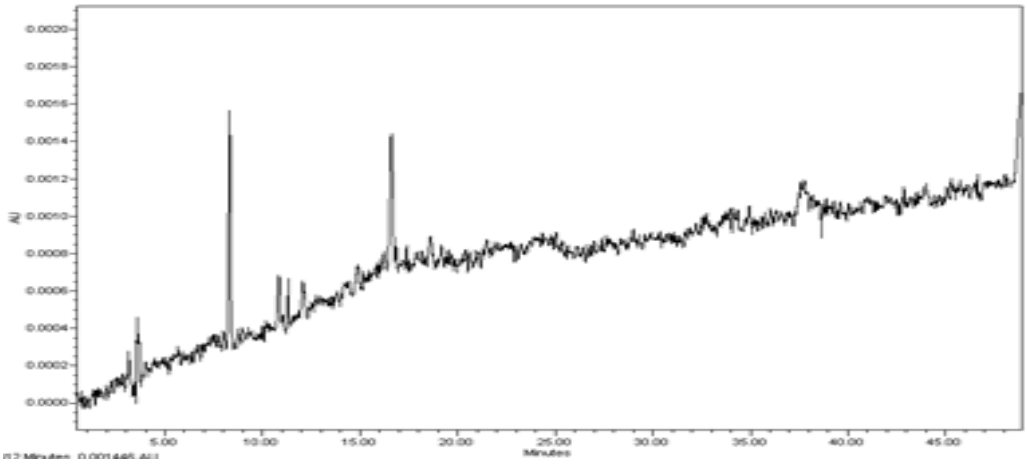

Figure B.3: Representative HPLC chromatograms of SGLN at 280 nm.

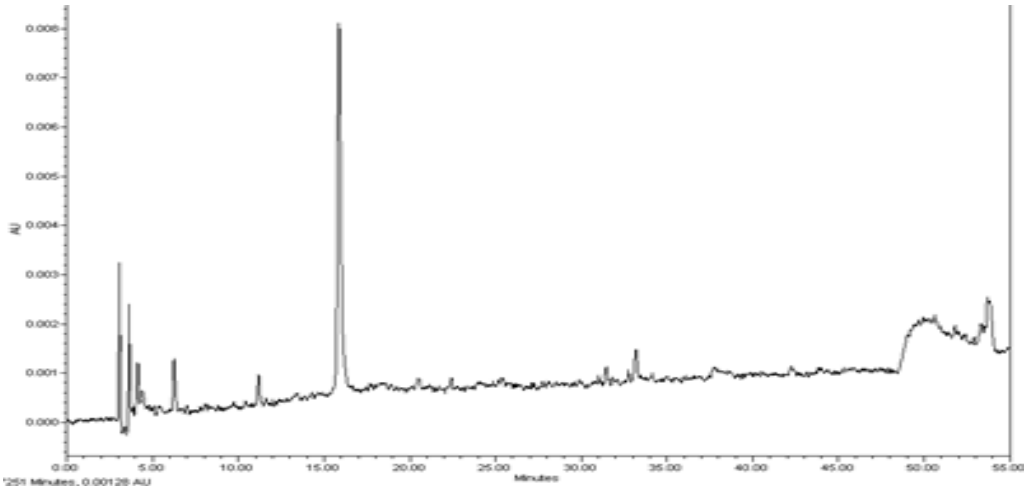

Figure B.4: Representative HPLC chromatograms of SGCN at 280 nm.

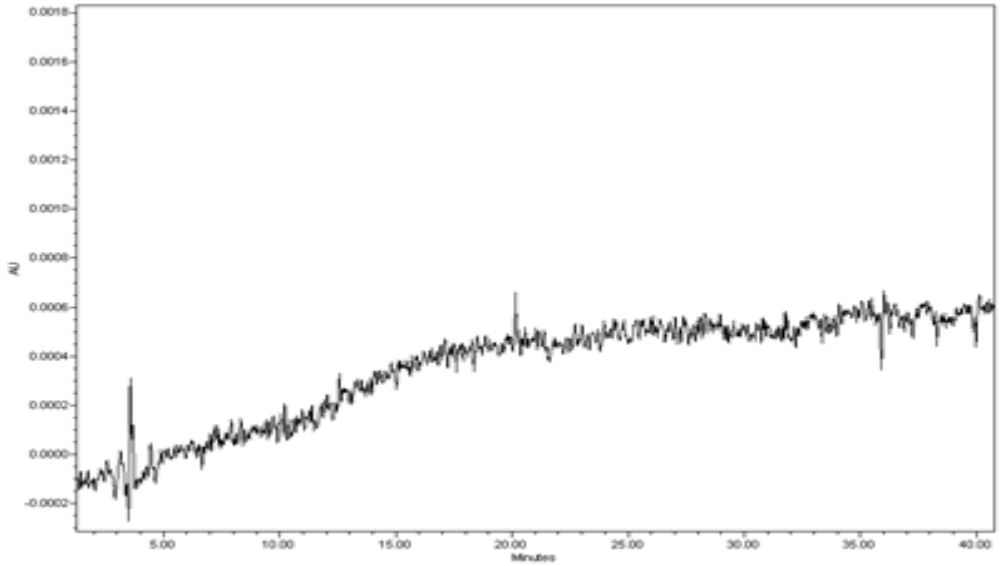

Figure B.5: Representative HPLC chromatograms of GN at 280 nm.

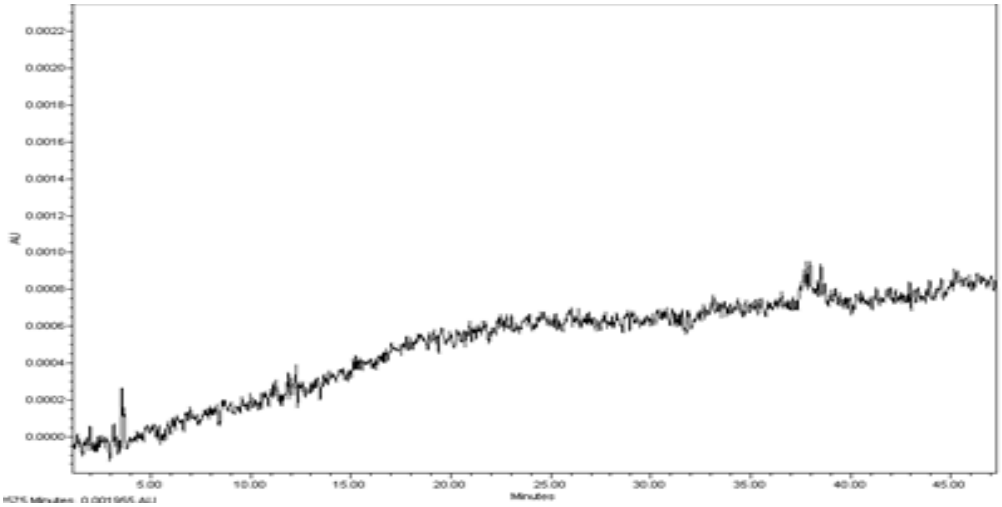

Figure B.6: Representative HPLC chromatograms of GLN at 280 nm.

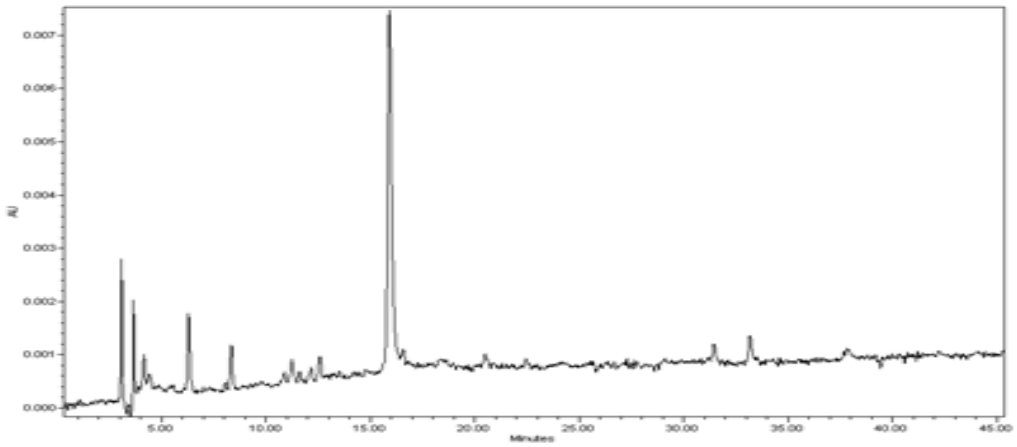

Figure B.7: Representative HPLC chromatograms of sour cherry concentrate after *in vitro* digestion at 280 nm.

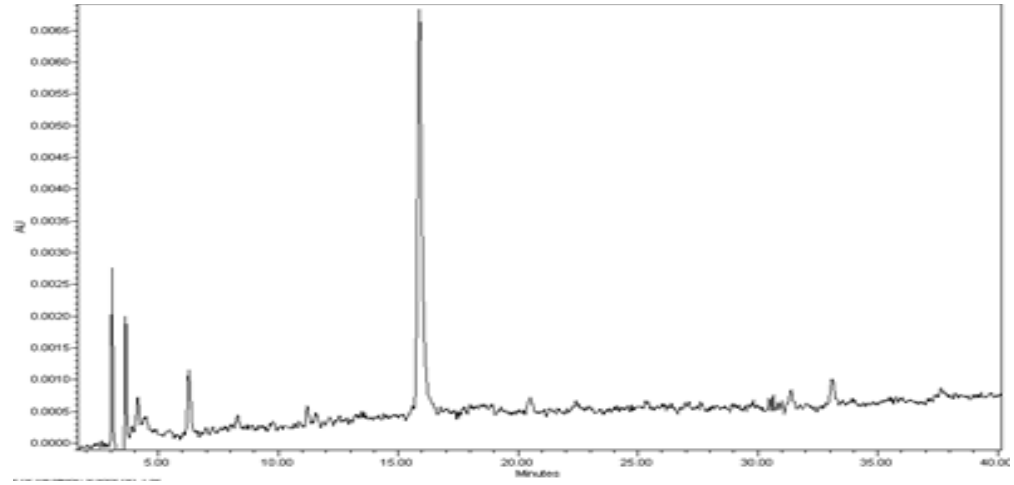

Figure B.8: Representative HPLC chromatograms of SGN after *in vitro* digestion at 280 nm.

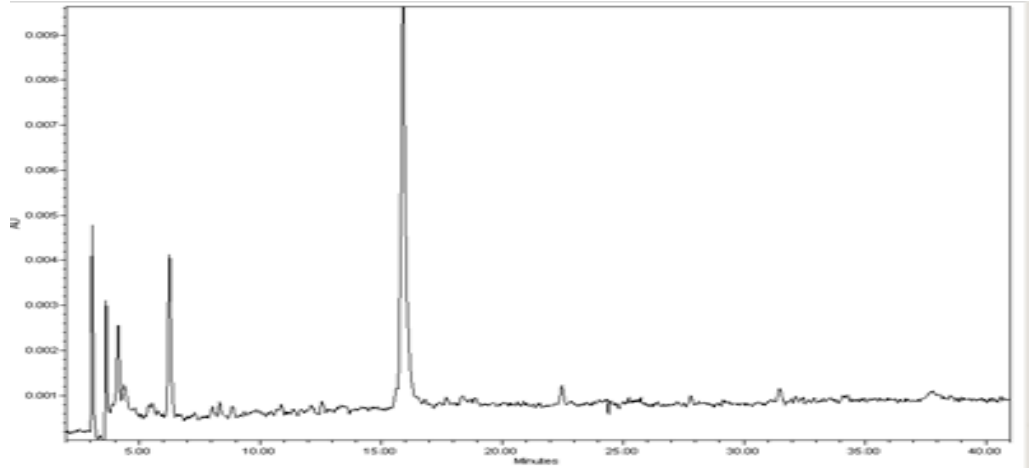

Figure B.9: Representative HPLC chromatograms of SGLN after *in vitro* digestion at 280 nm.

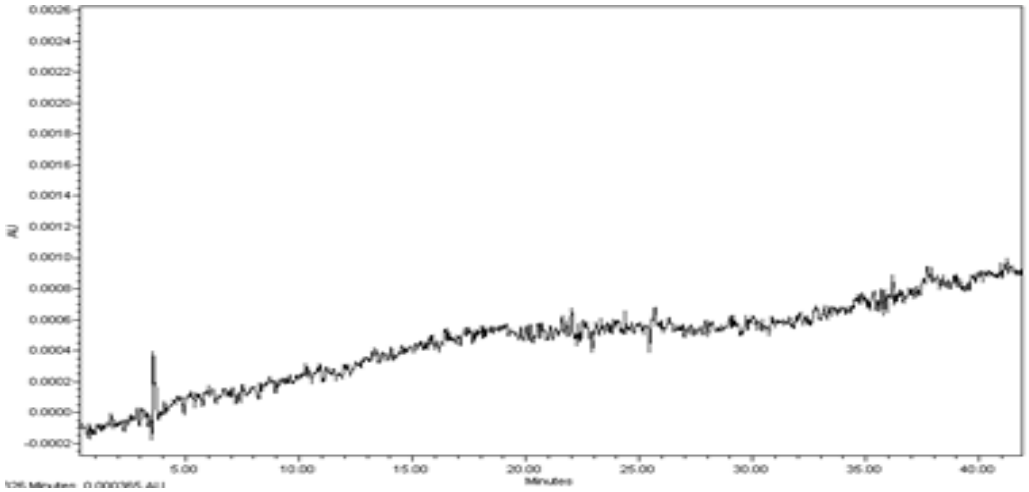

Figure B.10: Representative HPLC chromatograms of SGCN after *in vitro* digestion at 280 nm.

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