

**REPUBLIC OF TURKEY  
YILDIZ TECHNICAL UNIVERSITY  
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES**

**FORMATION OF POLYMERIC MICRO/NANO PARTICLES AND  
FIBERS INCLUDING BITTER MELON (*Momordica charantia* L.)  
EXTRACT BY ELECTROSPINNING METHOD**

**AYŞEGÜL BEŞİR**

**MSc. THESIS  
DEPARTMENT OF FOOD ENGINEERING  
PROGRAM OF FOOD ENGINEERING**

**ADVISER  
ASSOC. PROF. DR. TALİP KAHYAOĞLU**

**İSTANBUL, 2015**

**REPUBLIC OF TURKEY**  
**YILDIZ TECHNICAL UNIVERSITY**  
**GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES**

**FORMATION OF POLYMERIC MICRO/NANO PARTICLES AND  
FIBERS INCLUDING BITTER MELON (*Momordica charantia* L.)  
EXTRACT BY ELECTROSPINNING METHOD**

A thesis submitted by Ayşegül BEŞİR in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** is approved by the committee on 26 November 2015 in Department of Food Engineering, Food Engineering Program.

**Thesis Adviser**

Assoc. Prof. Dr. Talip KAHYAOĞLU  
Yıldız Technical University

**Approved By the Examining Committee**

Assoc. Prof. Dr. Talip KAHYAOĞLU  
Yıldız Technical University

---

Assoc. Prof. Dr. Aylin ALTAN METE, Member  
Mersin University

---

Assist. Prof. Dr. M. Zeki DURAK, Member  
Yıldız Technical University

---

## ACKNOWLEDGEMENTS

---

Firstly, I would like to express my deep and sincere gratitude to my advisor Assoc. Prof. Dr. Talip KAHYAOĞLU for his suggestions, guidance, encouragement and support throughout my M.Sc. study. I thank to Assist. Prof. Dr. M. Zeki DURAK and Assoc. Prof. Dr. M. Tahsin YILMAZ for their supporting. I also would like to thank Prof. Dr. Ayfer SARAÇ for her helping about some analysis and friendly fondness.

I warmly express my special thanks to Gülsüm UÇAK, Hilal YILMAZ and all of my lab-friends with who I studied together in Yıldız Technical University Food Engineering Laboratory for their helps, patience and friendships. I want to express special thanks to Fatih BOZKURT and P. Kübra ÇİÇEK for their guidance like a co-adviser.

I warmly express my special thanks to my high school friends Dilek MAZLUM, Gözdem BAYRAKTAR and Hicret KIZILKAYA. I have always their supports with me.

I would like to extend my thanks to my family especially Hacer and Ferhat BEŞİR for their encouragement and supports in all of my life.

November, 2015

Ayşegül Beşir

## TABLE OF CONTENTS

---

	Page
LIST OF SYMBOLS.....	vii
LIST OF ABBREVIATIONS.....	viii
LIST OF FIGURES .....	ix
LIST OF TABLES.....	xi
ABSTRACT.....	xii
ÖZET .....	xiv
CHAPTER 1	
INTRODUCTION .....	1
1.1 Literature Review .....	1
1.2 Objective of the Thesis .....	2
1.3 Hypothesis .....	2
CHAPTER 2	
GENERAL INFORMATION.....	3
2.1 Bitter Melon.....	3
2.1.1 Phytochemical Composition of the Bitter Melon.....	4
2.1.2 Usage Fields of Bitter Melon .....	4
2.2 Nanotechnology .....	5
2.2.1 Properties of Nanostructures.....	6
2.2.2 Production of Nanostructures .....	7
2.2.3 Nanotechnology in the Food Area .....	7

2.2.4. Toxicology of nanotechnology .....	10
2.3 Encapsulation.....	11
2.3.1 Encapsulation Methods.....	11
2.3.2 Technological Advantages of the Encapsulation Process.....	12
2.4 Electrohydrodynamics atomization .....	13
2.4.1 Electrospray .....	17
2.4.2 Electrospinning .....	19
2.4.3 The Application of Electrohydrodynamics Atomization Technical for Encapsulation.....	19
2.4.4 Parameters Affecting Electrospinning Method.....	21
2.4.5 Polymers used in the electrospinning method .....	27
2.4.6 Use of Electrospinning Method in Food Industry as Encapsulation Technique	30
2.4.7 Characterization of produced nano-micro materials by electrospinning method .....	31
 CHAPTER 3	
MATERIALS AND METHODS.....	32
3.1 Chemicals.....	32
3.2 Methods .....	32
3.2.1 Extraction.....	32
3.2.2 Preparation of feed solution .....	32
3.2.3 Characterization of feed solutions .....	34
3.2.4 Electrospinning .....	35
3.2.5 Particle/fiber characterization .....	37
 CHAPTER 4	
RESULTS AND DISCUSSION.....	40
4.1 The Total Phenolic Content and Antioxidant Activity of Bitter Melon Extract.....	40
4.2 Characterization of Feed Solutions.....	42

4.2.1 Conductivity, pH and Surface Tension of Feed Solutions.....	42
4.2.2 Rheological Properties of Feed Solutions.....	45
4.3 Electrospinning .....	47
4.4 Characterization of Particles/Fibers.....	47
4.4.1 SEM and Optical Characterization of Nanofibers .....	48
4.4.2 Zeta Potential of Dispersions with Nanofibers/Nanoparticles.....	58
4.4.3 Total Phenolic Content and Antioxidant Content of Particles/Fibers.....	59
4.4.5 Thermal Analysis .....	60
4.4.6 FTIR Analysis.....	63
CHAPTER 5	
CONCLUSION.....	68
REFERENCES .....	70
CURRICULUM VITAE.....	77

## LIST OF SYMBOLS

---

$T_e$	End temperature (°C)
$\Delta H$	Enthalpy (J/g)
$n$	Flow behavior index
$K$	Flow consistency index (Pa.s)
$T_g$	Glass transition temperature (°C)
$T_m$	Melting temperature (°C)
$T_o$	Onset temperature (°C)
$T_p$	Peak temperature (°C)
$\tau$	Shear stress (Pa)
$\gamma$	Shear rate (s <sup>-1</sup> )
$\zeta$	Zeta potential value (mV)

## LIST OF ABBREVIATIONS

---

AFM	Atomic Force Microscopy
DSC	Differential Scanning Calorimetry
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry weight
EG	Gelatin feed solution with bitter melon extract
EM	Maltodextrin feed solution with bitter melon extract
EZ	Zein feed solution with bitter melon extract
FESEM	FieldScanning Electron Microscopy
FC	Folin Ciocalteu reagent
FTIR	Fourier Transform Infra Red
GAE	Gallic acid equivalents
h	Hour
kV	Kilo voltage
NG	Gelatin neat feed solution
NM	Maltodextrin neat feed solution
NZ	Zein neat feed solution
M	Meter
μ	Mikron
min	Minutes
nm	Nanometer
N	Newton
NMR	Nuclear Magnetic Resonance
PCL	Poly(ε-caprolactone)
PGA	Poly(glycolic acid)
PLA	Poly(lactic-acid)
PVA	Poli vinyl Alcohol
PEO	Polyethylene Oxide
SEM	Scanning Electron Microscopy
S	Siemens
TEM	Transmission Electron Microscopy
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
XRD	X-Ray Diffraction

## LIST OF FIGURES

	Page
Figure 2.1 Bitter melon plant.....	4
Figure 2.2 Nano size samples.....	6
Figure 2.3 Classification of nanostructures.....	7
Figure 2.4 Taylor cone and jet formation.....	14
Figure 2.5 Schematic representation of electrohydrodynamic systems.....	15
Figure 2.6 Electrospinning and electrospray.....	16
Figure 2.7 Advantages of electrospinning.....	17
Figure 2.8 Electrospray method.....	18
Figure 2.9 Electrospray forms.....	18
Figure 2.10 Electrospinning system setup.....	19
Figure 2.11 Schematic representation of electroencapsulation techniques.....	20
Figure 2.12 Different structures obtained by electrospinning .....	21
Figure 2.13 Co-axial nanofiber.....	26
Figure 2.14 Application areas of electrospinning method in food industry.....	31
Figure 3.1 Schematic representation of the preparation of the extract and feed solutions.....	34
Figure 3.2 Electrospinning setup .....	36
Figure 4.1 Nanofibers obtained from gelatin solution at 20% prepared with aqueous bitter melon extract .....	48
Figure 4.2 Optical images obtained from 20% NM.....	49
Figure 4.3 Optical images obtained from 30% NM.....	49
Figure 4.4 SEM images obtained from 5% NG.....	50
Figure 4.5 SEM images obtained from 20% NG.....	51
Figure 4.6 SEM images obtained from 25% NG.....	51
Figure 4.7 SEM images obtained from 30% NG.....	52
Figure 4.8 SEM images obtained from 2,5% NZ.....	53
Figure 4.9 SEM images obtained from 5% NZ.....	53
Figure 4.10 SEM images obtained from 15% NZ.....	54
Figure 4.11 SEM images obtained from 20% NZ.....	54
Figure 4.12 SEM images obtained from 25% NZ.....	55
Figure 4.13 Optical images of electrosprayed particles.....	56
Figure 4.14 SEM images of electrospun fibers.....	56
Figure 4.15 SEM images of electrospun fibers.....	57
Figure 4.16 Zeta potential values of electrospinning samples.....	58
Figure 4.17 DSC thermogram of electrospun gelatin nanofibers.....	61
Figure 4.18 DSC thermogram of electrosprayed maltodextrin particles.....	61
Figure 4.19 DSC thermogram of electrospun zein nanofibers.....	62
Figure 4.20 FTIR spectrum of aqueous bitter melon extract.....	64

Figure 4.21 FTIR spectrum of electrospun fibers obtained from gelatin.....	64
Figure 4.22 FTIR spectrum of electrospayed particles obtained from maltodextrin....	65
Figure 4.23 FTIR spectrum of alcoholic bitter melon extract.....	66
Figure 4.24 FTIR spectrum of electrospun fibers obtained from zein.....	66

## LIST OF TABLES

---

	Page
Table 2.1 Application of nanotechnology in food sector.....	8
Table 4.1 pH, electrical conductivity and surface tension value of the feed solution...43	43
Table 4.2 Viscosity values of feed solutions.....	46
Table 4.3 Electrospinning process parameters.....	47
Table 4.4 Total phenolic content and antioxidant activity of electrosprayed particles and electrospun fibers.....	59
Table 4.5 DSC parameters of electrosprayed particles and electrospun fibers.....	62
Table 4.6 Functional groups.....	67

## ABSTRACT

---

# FORMATION OF POLYMERIC MICRO/NANO PARTICLES AND FIBERS INCLUDING BITTER MELON (*Momordica charantia* L.) EXTRACT BY ELECTROSPINNING METHOD

Ayşegül BEŞİR

Department of Food Engineering

MSc. Thesis

Adviser: Assoc. Prof. Dr. Talip KAHYAOĞLU

In this study we tried to produce polymeric micro/nano particles and fibers by electrospinning method using natural polymers such as maltodextrin, gelatin and zein for to be used in food formulations and bitter melon extract was used to provide functional features to the structures. In the first part of the study we obtained the structures from neat feed solutions that prepared with water for maltodextrin, 20% diluted acetic acid for gelatin and 70% ethanol for zein. We determined appropriate electrospinning parameters (flow rate, voltage and distance) parameters for each polymer. We examined the effect of different polymer concentration on diameters that formed after electrospinning.

In the second part of the study bitter melon was extracted with water and 70% ethanol and used as a solvent instead of neat water and alcohol for preparing feed solutions. While water extract was used for maltodextrin and gelatin, alcohol extract was used for zein polymer. The total phenolic contents of water and alcohol extracts were found as 196.95 and 287.50 mg GAE/l extract, respectively. When we compared the total antioxidant activities; alcohol extracts showed higher antioxidant activity (32%) than water extracts (27%). We compared infeed solutions prepared with/without bitter melon extract and structures obtained from them after electrospinning process. We want to know how to change electrospun samples with adding extract. To determine the characterization of the feed solutions; pH, electrical conductivity, surface tension and rheology analyzes were performed. SEM images, surface zeta potential values, DSC thermogram, FTIR spectrum, total phenol and antioxidant content were obtained for characterization of electrospayed particles and electrospun fibers. According the results it was observed that electrospun samples obtained from infeed solutions prepared with bitter melon extracts had better surface morphology and smaller diameter. And among polymers, zein nanofibers

including bitter melon extract showed the best properties. Zeta potential, total phenolic content and total antioxidant capacity (DPPH) analyses results of zein fibers were 21.77 mV, 2.6 mg GAE/100 g fiber and 32.90%, respectively. The results indicated that bitter melon extracts could be used to obtain structures with better properties via electrospinning process. These particles and fibers may be used to enhance functional properties by adding as an ingredient in food formulations.

**Keywords:** Bitter melon, electrospinning, nanoencapsulation , nanofiber, nanoparticles

**KUDRET NARI (*Momordica charantia* L.) EKSTRAKTI İÇEREN  
POLİMERİK MİKRO/NANO PARTİKÜL VE LİFLERİN  
ELEKTROSPİNNİNG YÖNTEMİ İLE ELDESİ**

Ayşegül BEŞİR

Gıda Mühendisliği Anabilim Dalı

Yüksek Lisans Tezi

Tez Danışmanı: Doç. Dr. Talip KAHYAOĞLU

Gıda formülasyonlarında da kullanılabilirliği olması açısından maltodekstrin, jelatin ve zein doğal polimerleri kullanılarak elektrodöndürme yöntemiyle polimerik mikro/nano yapılar üretilmiştir ve bu yapılara fonksiyonel özellik kazandırmak için kudret narı ekstraktı kullanılmıştır. Çalışmanın ilk kısmında maltodekstrin için su, jelatin için %20 seyreltik asetik asit ve zein için %70'lik etil alkol çözücüleri kullanılarak elektrospinning yöntemi ile partikül/lif üretilmiştir. Elektrodöndürme sonucu oluşan yapılar üzerinde polimer konsantrasyonunun etkisi incelemek için farklı konsantrasyonda polimer çözeltileri kullanılmıştır. Çalışmanın ikinci kısmında ise kudret narı su ve %70'lik etil alkol ile ayrı ayrı ekstrakte edilmiş ve bu ekstraktlar elektrospinningde çözücü olarak kullanılmıştır. Yani maltodekstrin ve jelatin için su yerine kudret narının su ile hazırlanan ekstraktı, zein için ise alkol yerine %70'lik etil alkol ile hazırlanan kudret narı ekstraktı kullanılmıştır. Ekstraktların toplam fenolik madde ve antioksidan aktiviteleri sırasıyla; sulu ekstraktın 196.95 mg GAE/l ekstrakt ve %27, alkollü ekstraktın ise 287.50 mg GAE/l ekstrakt ve %32 bulunmuştur. Kudret narı ekstraktı kullanarak hazırladığımız besleme çözeltilerini ve bu çözeltilerden elde ettiğimiz yapıları ekstrakt kullanılmadan hazırlanan besleme çözeltileri ve bu çözeltilerden elde edilen yapılar ile karşılaştırdık. Ekstrakt ile hazırlamanın herhangi bir etkisi olup olmayacağını araştırdık. Besleme çözeltisi karakterizasyonu için pH, elektriksel iletkenlik, yüzey gerilimi ve reolojik analizler yapılmıştır. Elde edilen partikül/lif karakterizasyonu için ise SEM görüntüleri, yüzey zeta potansiyeli, DSC termogramları, FTIR spektrumları, toplam fenolik ve antioksidan kapasite analiz sonuçları değerlendirilmiştir. SEM görüntülerine göre kudret narı ekstraktı ile hazırlanan besleme çözeltilerinden daha homojen ve küçük boyutlu yapılar elde edilmiştir. Polimerler arasında da en iyi özellikler zein polimerinden elde edilen yapılarda gözlenmiştir. Kudret narının etanol ile hazırlanmış ekstraktı ve zein kullanılarak

elde edilen lifli yapıların zeta potansiyel, toplam fenolik ve antioksidan kapasitesi (DPPH) sırasıyla 21.77 mV, 2.6 mg GAE/100g lif ve %32.90 olarak bulunmuştur. Yapılan bu çalışma sonucunda kudret narı ekstraktı kullanılarak elektrospinning yöntemi ile daha iyi özellikte yapıların elde edilebileceği belirlenmiştir. Elde edilen partikül ve liflerin gıda bileşeni olarak fonksiyonel özelliği arttırmak amacıyla gıda formülasyonlarına ilave edilebileceği söylenebilir.

**Anahtar Kelimeler:** Elektrospinning, Kudret narı, nanoenkapsülasyon, nanolif, nanopartikül

### INTRODUCTION

#### 1.1 Literature Review

Elderly population and unhealthy living are increasing in the worldwide. People want to solve encountered health problems with consuming functional foods in addition to nutrition. The number of people over 60 years is estimated to reach 1.5 billion until 2050, that is 16% of the world population. However, it was only 5% in 1950s [1]. Since ancient times the use of plants for medicinal purposes is known [2], [3]. Plant-derived bioactive phenolic compounds are interesting additives for functional foods [4]. The researches prove the therapeutic use of plants and is ultimately approved for use in the traditional medicine of many plants. World Health Organization (WHO) reports that 80% of world population that treatment with various herbal medicines at the initial stage of ill [3], [5]. The use of plants for treatment was widespread across the world at the beginning of the 1900s, while it has become the subject of research in Turkey in the 1970s. Informations about using of medicinal plants date back to China, Egypt and Greek's history. While around 20,000 plants are used worldwide, 600 is the number of plants used for medicine in Turkey [5]. They contain high amounts of phenolic flavonoids, such as anthocyanins, phenolic compounds, which are known to display a wide range of biological activities like antioxidant, anti-inflammatory, antimicrobial or anti-carcinogenic activities, improvement of vision, induction of apoptosis and neuroprotective effects [4]. In addition, thanks to their antioxidant and antimicrobial contents, they also increasingly being used as an additive in food and cosmetics industries [2], [6]. The novel methods that enhance functionality of food by protecting bioactive compounds, demonstrating minimal effect during processing and showing a positive effect on digestion are increasingly developed [7]. One of these methods is encapsulation process. The traditional encapsulation methods (spray drying, freeze drying etc.) and in particular

electrospinning method has some advantages as producing more bioactive molecules in room temperatures [8], [9], [10].

## **1.2 Objective of the Thesis**

The aim of this study was to produce micro/nano-biomaterials that can be used in food formulations by electrospinning method using natural food-grade polymers such as maltodextrin, gelatin and zein. To produce powder form from 1:20 (solid: liquid) ratio obtained bitter melon extract via electrospinning method without subjecting the additional processing. To prepare infeed solutions using both proper solvents for each polymer and bitter melon extracts as a solvent and to compare the effect of each feed solutions properties such as surface tension and conductivity on the final product. To evaluate functional, thermal and morphology features of micro/nano structures produced electrospinning method.

## **1.3 Hypothesis**

The hypothesis is that functional micro/nano biostructures could be produced by electrospinning method. Bitter melon extract may be used to obtain the functional particles and fibers. It is expected that feed solutions (with and without extract) could demonstrate different properties from each other. Thus particle/fiber structures obtained from these feed solutions could have different features. The micro/nano fiber obtained from bitter melon extract with electrospinning might demonstrate functional properties.

### GENERAL INFORMATION

Besides nutritive values demand for healthy foods has increased substantially over the recent years due to the growth in the world population and an increased perception of unhealthy lifestyles. As the population ages, there is expected to be increasing demand for that have a potentially positive effect on health beyond basic nutrition-functional foods. There is strong motivation to progressively improve the performance of food products to provide this type of consumer benefit. One approach that could help to address this problem is the development in encapsulation of food ingredients that are rich in vitamins and antioxidants [1]. Encapsulated ingredients can be formulated to survive travel through the gastro-intestinal (GI) system to deliver their payload at a particular point, thus maximizing the beneficial effect.

#### 2.1 Bitter Melon

In studies *Momordica charantia* is referred to as more than twenty local names such as bitter melon, karela (India), balsam pear, cundeamor (South America) and bitter gourd [11], [12], [13], [14], [15]. Bitter melon, a member of the Cucurbitaceae family [16], [17], [12], [18], [19], [20],[21] is tropical and edible plant [14], [15], [22]. Besides it is widely grown and consumption in India, China, Malaysia [18], [23] East Africa, Asia [24], the Caribbean Islands, the Amazon Basin and South America [11], [13], [15], [16], [17], [25], it can be adapted the place showed a wide variation in climate [14], [20]. It is grown in Western Anatolia in Turkey [26]. It is one-year, slimline and climbing plant with long stems [14]. Its raw fruits are green. As ripe, its fruits turn orange-red colour with 10-20 cm length, indented and rough. The taste of all parts of plants is bitter therefore generally known as bitter melon [17], [14], [19], [20]. Phytochemicals in nature are shown as a source of bitter taste (alkaloids) [21]. Bitter melon plant are shown in Figure 2.1.

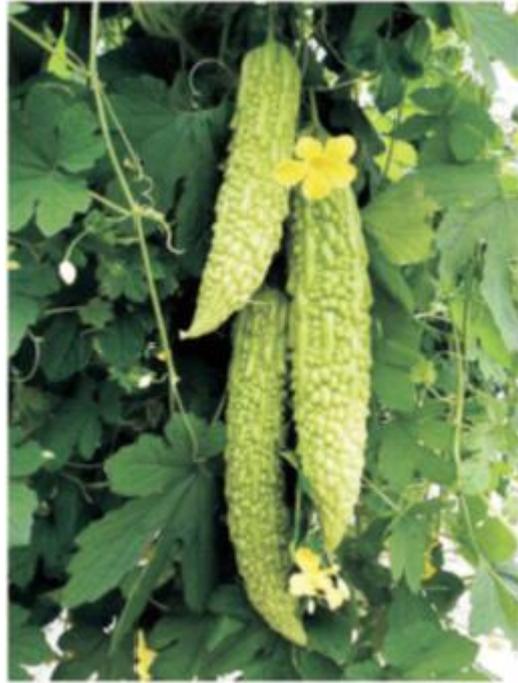


Figure 2.1 Bitter melon plant [11]

### **2.1.1 Phytochemical Composition of the Bitter Melon**

Studies have associated with bitter melon with a wide range of functional properties are linked to several bioactive compounds that are present in fruits, seeds, leaves [15], [27]. Its fruits are important part of plant for human nutrition, due to carbohydrates, proteins, vitamins, minerals and other nutrients. Some glycosides isolated from the root and fruit [17]. Research results have been reported that bitter melon contains charantin (blend of sterol glycosides), vicuna (pyrimidine nucleoside), and p-insulin (polypeptide) as active ingredients [28]. Especially pericarp tissue and seeds are rich in phenolics, catechin, epicatechin and gallic acid components [13]. It incorporates different bioactive compounds such as saponin, phenolic acids, flavonoids, carotenoids, triterpenoids and phytochemicals [21]. These components are named as secondary metabolites and investigated their phytochemical and pharmacological effects [24], [27], [29]. Thanks to the active components, it is investigated potential use as adjuvants to obtain functional foods [22].

### **2.1.2 Usage Fields of Bitter Melon**

There is possibility of use in many areas as feed and therapeutic plants. It has been studied effect on glucose tolerance and development of lower blood cholesterol about type 2 diabetes; studies on the effectiveness of chemical components such as alkaloids and

polypeptides in the composition is still ongoing [14], [24]. Its antidiabetic and hypoglycemic effect has been subject of many research studies [21], [28]. The juice taken orally decreases blood glucose level and improves the glucose tolerance in normal and diabetic humans and animals. In addition to antidiabetic effects, processed and raw form are used for tonic or emetic in Sri Lanka and India, tea form is used for cold, abdominal pain, prevent constipation in children. For medical purposes, the core, wine, fruit and leaf of bitter melon is consumed by Chinese in the treatment of gastrointestinal disease, viral infections and tumor inhibitions [15], [28]. Alternatively, bitter melon is used mostly in the western and southwestern Anatolia peptic ulcer and tumor treatment in Turkey [26]. With many epidemiological studies done in the laboratory, it is reported that bitter melon demonstrate cardiovascular [25], antiulcerogenic [26], hypoglycemic effect, anti-HIV, antitumor, anticarcinogenic, anti-inflammatory, antioxidant and antimalarial effect [16], [18], [19], [20], [21], [22], [28], [29]. Bitter melon is used in treatment of skin diseases, wounds, eczema, rheumatism [14]. It is indicated that bitter melons' fruit show antimicrobial activity against *Aspergillus niger* and *E.coli*. [18]. Bitter melon contains polyphenols that have antioxidant effect [13]. Thanks to its antioxidant activity, it is used to suppress oxidative stress, so treat some diseases such as cancer diabetes [30] Also available for Type 1 and Type 2 diabetes. In recent researches it is demonstrated that although insulin intake remains important for type 1 diabetes, insulin-like proteins that is obtained from therapeutic plants can be used alternatively to insulin. Insulin-like peptides, charantin, vicuna, fruit, seed extracts, fruit juice and powder form isolated from the bitter melon are used to treat diabetes by lowering blood glucose levels effect [21], [24], [27], [29]. Also it could be used to treat obesity because it helps in the regulation of blood sugar [23]. Especially unripe fruits have similar to the effect of insulin in lowering blood sugar levels and is effective in the treatment of diabetes. It is thought that this effect associated with charantin component, Charantin consist of two glycosides structures called as stigmasterol and sitosterol [16].

## **2.2 Nanotechnology**

Nanotechnology is a field of science and technology which is generally interested in particles that are smaller than 1-100 nm (billionth of a meter) and it is grown rapidly in recent years. It deals with molecules, atoms and these kind of structures which has nano-scale dimensions [31], [32]. All areas in which used words beginning with nano refers to

the existence of an innovative approach. Nano means dwarf in the Greek language. According to the international system, nano refers to any measure one billionth ( $10^{-9}$ ), nanometer is one billionth of a meter [31]. Nanotechnology is a new and developing field that aims to improve the properties or to give completely new physical, chemical and biological properties in atomic or molecular level [32] to materials with workings as processing, measurement, modeling and design workings performed on a nanometer scale [33]. It has focused on the production, processing and characterization of biological or non-biological structures with smaller than 100 nm [8], [32]. The most general expression "Nanoscience" is the science that studies the matter and energy in the nanometer scale [31]. In 2006, nanotechnology was used by The National Nanotechnology Initiative to identify the particles changing diameters between 1 to 100 nm, while materials that are rendered new and superior characteristics in micrometers and smaller dimensions were defined as nanomaterials by the FDA [33], [34], [35], [36]. Several examples are given for better understanding of the nanoscale in Figure 2.2.

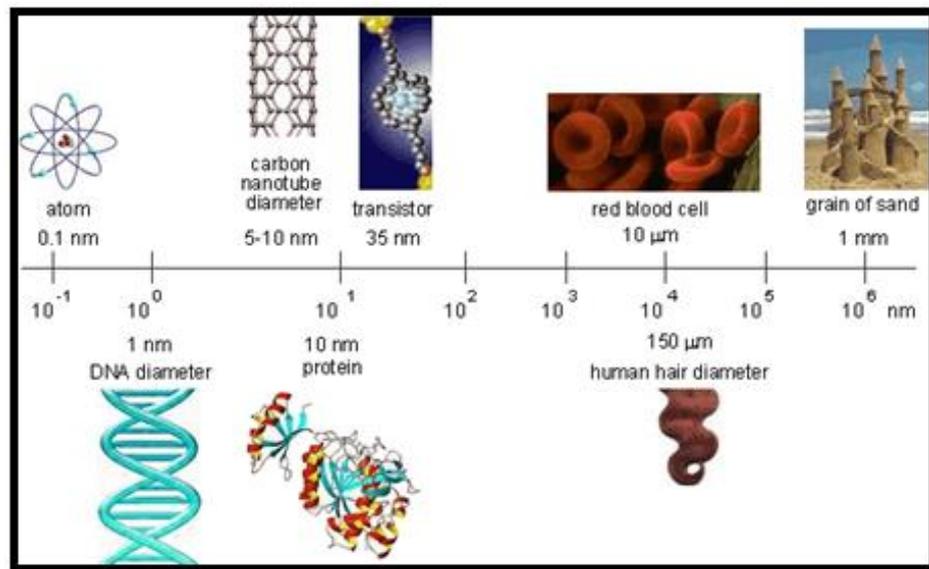


Figure 2.2 Nano-size samples [37]

### 2.2.1 Properties of Nanostructures

Nanostructures can be classified as shown in Figure 2.3.

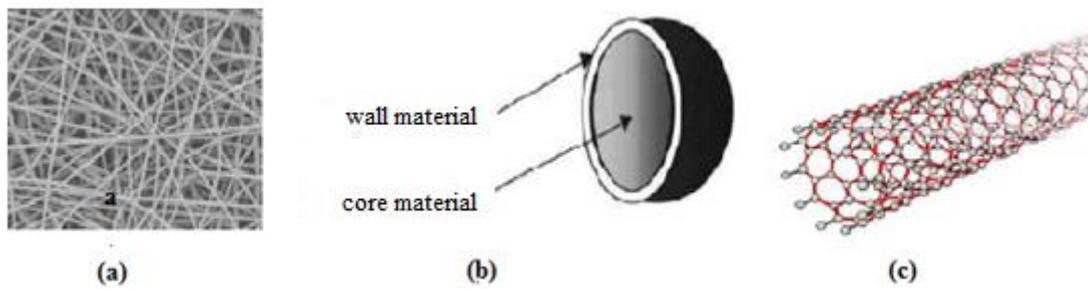


Figure 2.3 Classification of nanostructures: (a) nanofiber, (b) nanocapsules, (c) nanotubes

Dimensions are down to the nanoscale with the production of nanostructures so that the materials have completely new or enhanced size [38] improved distribution and morphology properties by quantum effect. According to the materials consisting of larger particles with same mass, nanomaterials have a larger and effective surface area and consequently more reactive and easily interact with other materials. It is obtained new products which they have differentiated properties as optical, electrical and magnetic [33], [39].

### 2.2.2 Production of Nanostructures

The production of nanomaterials is explained by called two approaches from top to bottom (top-down) and bottom up. Top-down approach is defined as the acquisition of nano-level structure from large materials as a result of crushing, grinding, mechanical, chemical and different high pressure processes [35]. Mostly, this approach is preferred. Production of nanofibers by electrospinning method are also performed with the top-down approach [33]. With these methods structures gains some improved properties. For example dry milling of wheat flour is the method implemented to increase the water holding capacity and this method is described with the top-down approach [36]. The bottom up approach for creating more complex structures refers to chemical synthesis reactions such as self-assembly [33].

### 2.2.3 Nanotechnology in the Food Area

With nanotechnology has become increasingly popular science, it is continuing to work in this field in the world's leading research centers. European Union countries, particularly the US and Japan, including China, Brazil, India allocate sizable budgets on nanotechnology researches [40]. Food systems are composed of delicate and complex

structures. Because of insufficient information about interaction between nanostructures and food systems, the use of nanotechnology in the food sector has been slow compared to other industries [32]. Today, the world's leading food companies HJ Heinz, Nestlé, Hershey, Unilever and Kraft have researched investments in nanotechnology researches and developments [36]. Kraft was created first laboratory that was founded the field of nanotechnology in the food in 1999. Leading companies in the food industry such as Kraft, Nestle, Unilever have use nanotechnology in some process. For example drink production containing nanocapsules for color and flavor entrapment- nanoparticles production for spreadable products to improve texture, chocolate, chips and ice cream that is changed their characteristics in various ways benefit from nanotechnology [32], [33]. Nanotechnology has been applied more than 150 food products and more than 400 packing material have already taken its place in the market. While nano food sector was 2.6 billion dollars in 2003, rose to 7.0 billion dollars in 2006. It is estimated that nanotechnology has been used approximately 40% of the food industry in 2015 [33]. Nanofood can be defined as a new food that is manufactured using nanotechnology in its production, processing, packaging and transport safely of food to consumers [33], [36], [38], [39]. Applications of nanotechnology in the food science and technology can be summarized as shown in Table 2.1.

Table 2.1 Application of nanotechnology in food sector [38]

<b>Chain phase</b>	<b>Application</b>	<b>Nanotechnology</b>	<b>Function</b>
Agricultural production	Nanosensors Pesticides Water purification/soil cleaning	Nanospray on food commodities Hand-held devices Filters with nanopores Nanoparticles	Binds and colors microorganisms Detection of contaminants, mycotoxins and microorganism Increased efficacy and water solubility Pathogen/contaminant removal

Table 2.1 (cont'd)

Production and processing of food	Food production  Refrigerators, storage containers, food preparation equipment	Nano-ceramic devices,  Incorporated nanosized particles, mostly silver, occasionally zinc-oxide	Large reactive surface area  Antibacterial coating
Conservation	Food products  Packaging materials	Nano-sized silver spray  Incorporated sensors  Incorporated particles  Incorporated active nanoparticles	Antibacterial action  Detection of food deterioration, monitoring storage conditions  Increasing barrier properties, strength of materials  Oxygen scavenging, prevention of growth pathogen
Functional food consumption	Supplements	Colloidal metal nanoparticles  Delivery system 'nano clusters'  Nano-sized/-clustered food/drinks(nutrients)	Claimed enhanced desirable uptake of metal  Protecting and targeted delivery of content

The use of nanotechnology arises as to be used for various purposes in food applications as nanoemulsions composed of macromolecules, biopolymer nanoparticles, nanocomposites, nanofibers, nanotubes and nanosensors [38]. Applications of nanotechnology in the food can be expressed under four main headings. The samples that are examined under these headings can be considered as a summary of studies using nanotechnology in the food area:

- 1- Food processing and developing functional products [31], [32], [33], [38],[41],[42]
- 2- The transportation of bioactive substances, nutraceuticals and their controlled release [31], [32], [33], [35], [36],[43]
- 3-Pathogen detection and provide food security [31], [32], [33], [38], [44]

4-The development of traceability and packaging system that will positively affect product quality and shelf life [31],[32], [33], [36],[42], [45].

#### **2.2.4. Toxicology of nanotechnology**

Beyond all these advantages, due to the limited knowledge about nanomaterials (specially nanoparticles and nano grinding particles) behaviour while their using on food applications, there are some doubt about their toxicity. Additionally their interaction mechanism during processing and storage is not known accurately [32]. The subject discussed and concerned in the world are toxicity of nanoparticles, routes of administration, intake with food and the risks that may occur if taken by inhalation and contact path [38]. We can get nanomaterials in our body with 3 possible ways:

1-Through the dermal absorption: Effects on the body of nanomaterials depends on the ability to access the epidermis or dermis and to penetrate the outer protective layer,

2- By respiratory: Solid materials with aerodynamic diameter less than 10 mm accumulate in the lungs by passing the nasal cavity and can lead to chronic diseases,

3- By digestion: For toxicology particle size and surface area are important material characteristics. Particles larger than 1 mm can not exceed the intestinal mucus barrier [35].

The suspicions of the toxicological effects of nanomaterials due to the following sentences:

- To be more reactive compared to the large materials,
- Enhanced and fast access ability,
- Pathological effects arise in the long term [36].

Some researched showed that people living in Europe are more cautious about the use of nanotechnology products than people in the United States [32]. According to the survey results the use of nanotechnology in the packaging have been met more positively than the use of nanomaterials in food [32], [36]. In order to investigation of the toxicological effects of manufactured nanomaterials, ADME properties are examined. These properties described as A; Absorption, D; Distribution, M; Metabolism, E; Excretion. There is no applicable international law about applications of nanotechnology in assessing the toxicological aspects. while packaging applications are organized by the US FDA in the

USA, they are organized by Food Standards Australia and New Zealand (FSANZ) in Australia [36].

### **2.3 Encapsulation**

Encapsulation is defined as simply components called as core, filling, interior, inner phase and active materials are coated by some wall, shell materials [46], [47], [48]. In other words liquid or gaseous food components, enzymes, cells [36] and microorganisms coated with coating materials based on protein or carbohydrate with encapsulation [46]. Encapsulation is used for protection of food components from heat, light, pH. Also used for stabilization and slow release during processing and storage [35], [49], [50]. It is not only used for protection of the active ingredient but also suppress the undesired taste and odor components [4], [51], [52]. Functional properties of the product is improved and the shelf life is extended by encapsulating of food components mostly fats, oils, flavor, vitamins, minerals, enzymes and color [7], [47]. According to the preparation of method, encapsulated materials are produced varying size at the range from millimeters to nanometers [53]. According to the dimensions, encapsulation can be divided into three section:

Nanoencapsulation (200 nm= less than 0.2  $\mu\text{m}$ )

Microencapsulation (0.2-5,000  $\mu\text{m}$ )

Macroencapsulation ( bigger than 5,000  $\mu\text{m}$ ) [46].

#### **2.3.1 Encapsulation Methods**

There are many encapsulation method in the present. These are; Spray drying, Freeze Drying, Air Suspension Coating Method, Rotating Suspension Separation Method, Extrusion-Emulsion Method, Removal of water by centrifugation Method, Cocrystallization, Phase Separation Method, Molecular Complex Rendering Method, Liposomes, Microemulsions of Microencapsulation, Nanoemulsions of Nanoencapsulatin [7], [32], [46]. And these days electrospinning method is thought as an encapsulation precess.

### **2.3.1.1 Spray drying**

The principle of encapsulation is based on feeding the mixture, prepared with coating material including dispersed active material, to the spray system that is set to the desired temperature. Solid particles are obtained results of evaporation of the solvent. The produced particles are range from 10 to 100  $\mu\text{m}$ . Spray dryer is a preferred method for many products in the food industry due to its low cost [2], [7].

### **2.3.1.2 Emulsification**

Emulsion is a system provided to be used in food formulations by providing the distribution of active ingredients insoluble in water or oil in both environments. If oil droplets disperse in water phase that is continuous phase, this emulsion called as 'oil in water emulsion'. However if water phase disperse in oil phase, this defined as 'water in oil emulsion'. Size of the droplets are generally in the range 0.1-100  $\mu\text{m}$  [2], [7].

### **2.3.1.3 Liposome Formation**

It is defined as the formation of colloidal particles, that involve the active phase, at the end of hydrophilic - lipophilic interactions between oil and water. It is possible to obtain particles varying size from 30 nm to micron. But it is not widely used because of its high cost [2].

### **2.3.1.4 Freeze Drying**

This method, also called lyophilization is usually applied to the water-soluble heat-sensitive components. It can be explained as the removal of water, that is frozen under high pressure, with sublimation converting from solid form to gas form [2], [7].

## **2.3.2 Technological Advantages of the Encapsulation Process**

Thanks to increasing effective surface area, bioavailability increases, desired properties are imparted by the use of a smaller amount [10], [35] right transportation to target area is ensured on time [51]. Food components, which are not fully soluble in water or oil, are converted into the appropriate form so they can be used for both the system [35]. Optical transmittance especially important for beverage is developed [35], new products modified of sensory and mechanical properties are produced [38]. Food coating material as a physical barrier protect food against environmental conditions such as heat, light, pH,

moisture. Thus storage life is extended [35], [47]. It allows the formation of more homogeneous food systems [35]. It provides ease of transport by creating the appropriate physical form for storage [10], [35]. Functional products that are easy to digest are produced by facilitating the addition of active or bioavailability components to food formulations [35], [46]. It contributes positive effects on food processing as shorten the ripening time, easy handling [46].

#### **2.4 Electrohydrodynamics atomization**

Electrohydrodynamic atomization is a process wherein the electric field is used in the formation of nano or micro materials [2]. Electrohydrodynamic atomization technique is not a new technology. For the first time in 1897, it was described by Lord Rayleigh. It is patented in 1902 by John F. Cooley. Electrohydrodynamics system have become popular again in conjunction with the increase of the interest in nanotechnology [54]. In 1934 Anton Formhals developed the method and got a new patent by gathering textiles nanofibers on a moving collector [33], [45]. System advantages of over other methods are:

- It is a single-stage system.
- Characterization of produced fiber / particle can be made.
- Many biodegradable or synthetic polymers are used or use in blend form.
- Its cost is advantageous [55].

In this technique, after dissolving the polymer in a suitable solvent is placed into a syringe. During the process the polymer solution is pumped to the feed pipe from the feed pump with a constant flow rate. The feed that comes to end of syringe is loaded electrical charges through electrodes in contact [45]. Then voltage is applied between a collector plate and the polymer solution. The positive terminal of the power supply that provide high voltage is connected to the metal tip of the syringe. Collector plate connects to ground. It has the appearance of a closed circuit system [33]. Polymer droplet that is at the end of needle tip in the feeder unit stands spherical shape up to critical voltage value due to the force of surface tension. The surface tension of the polymer solution that has sufficient viscosity shows resistance to electrical stress. As soon as the applied potential difference reaches a threshold value electrostatic forces equal to the surface tension forces. At this point the polymer drops takes the cone form changing the shape. This cone

is called a Taylor cone. After polymer drop takes Taylor cone form, jet flow occurs with a very small increase in voltage. When jet flows between metal needle and collector plate, solvent evaporates with the applied electric field effect. Nano-micro particles or fibers are collected on collector plates [2], [33], [38], [45]. Taylor cone is show in Figure 2.4 [38].

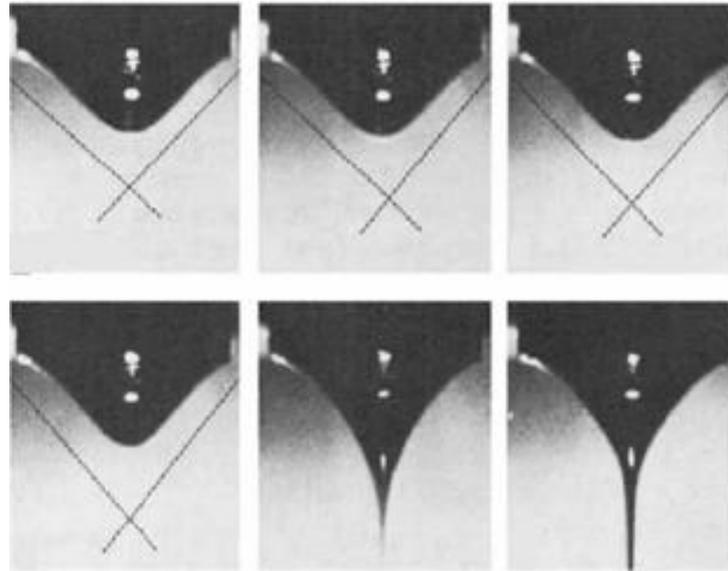


Figure 2.4 Taylor cone and jet formation

Taylor cone formation is monitored at the point where the applied electrical field strength and surface tension of the solution is equivalent. These values are different for each polymer solution and should apply higher voltage in the feed solution with high surface tension. In generally, Taylor cone structure formation is observed in the polymer solution that has a high viscosity smooth and stable [2], [45], [56], [57]. When the Taylor cone is not stable, some drops of polymer solution are ejected from the capillary tip [58], [59]. With a wide size range from nanometers to micrometers products can be obtained. Simply electrohydrodynamics system consists of voltage source, feed unit, pump and collector surface (1-30 kV) [33], [38], [45], [51], [60], [61]. Electrohydrodynamics system can be positioned horizontally or vertically with respect to the desired properties [38]. Different electrohydrodynamic systems are shown in Figure 2.5 [33], [38].

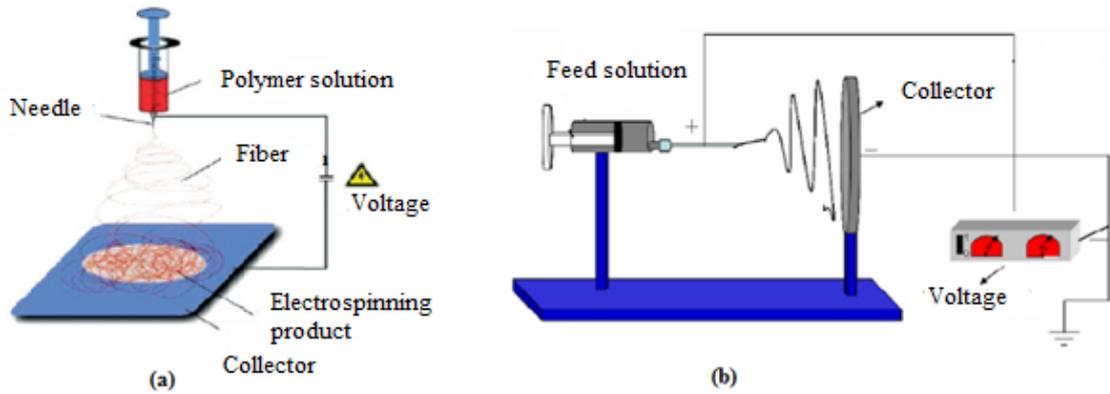


Figure 2.5 Schematic representation of the Electrohydrodynamic systems: vertical (a), horizontal (b) [33], [38]

Forces acting in electrohydrodynamic method can be listed as gravity (FG), electrostatic force (FE), the Coulomb force (FC), viscoelastic force (FV), surface tension force (PA) and friction force(FS);

**Gravitational force:** The gravitational force acts in the opposite direction to the electric field and vertical to the collector plate. It varies depending on the solution concentration [33], [38].

**Electrostatic forces:** It is the force towards from needle to collector plate. This force is determined by the applied electric field and material properties [33].

**Coulomb force:** It is the force that defines the instability and whipping movement formed on the surface of the polymer jets. The intensity of the Coulomb force varies according to the characteristics of the polymer and solvent type [33], [38].

**Viscoelastic forces:** Applied force prevents elongation of the jet in the electric field. Viscoelastic forces depends on polymer molecular weight, solvents type and added other substances [33], [38].

**Surface tension force:** It occurs against elongation of the polymer jet. The surface tension force varies with used polymer, solvents and additives [33], [38].

**Frictional force:** It emerges between jet surface and air surrounding [33].

There are two main electrohydrodynamic atomization technique. Electrohydrodynamics systems are named differently depending on the product obtained. Products can be particles or fiber. They called as electrospray and electrospinning, respectively [2], [51]. Electrospray and electrospinning are shown schematically in Figure 2.6 [51].

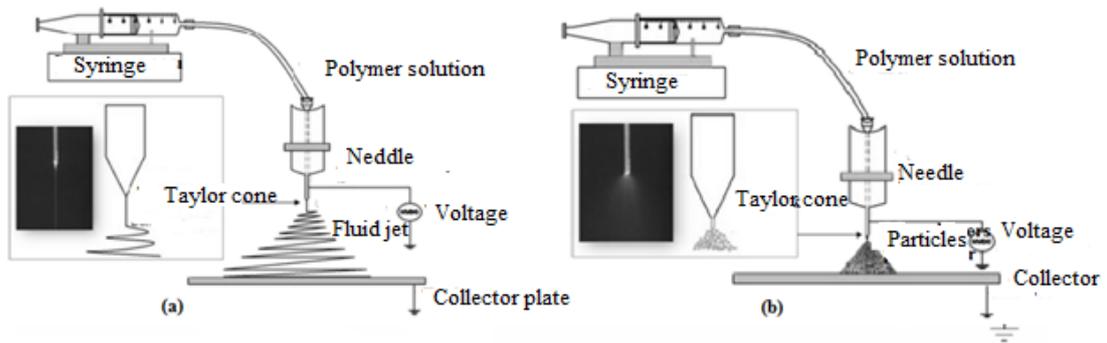


Figure 2.6 Electrospinning (a) and Electrospray (b) [51]

The difference between electrospray and electrospinning is due to the polymer concentration significantly. Samples are collected in the form of particles on the collector plate when the polymer concentration is low and irregular flow. This kind of production is called electrospray. When the polymer concentration increases, the flow becomes more regular and fiber structure is observed, this production method is also called electrospinning [51], [62].

Advantages:

- Production costs are advantageous,
- System operation principle is simple,
- The process parameters can be changed easily,
- Proper polymers are varied as compared to other methods [37]. Advantages of the electrohydrodynamics system are listed in Figure 2.7.

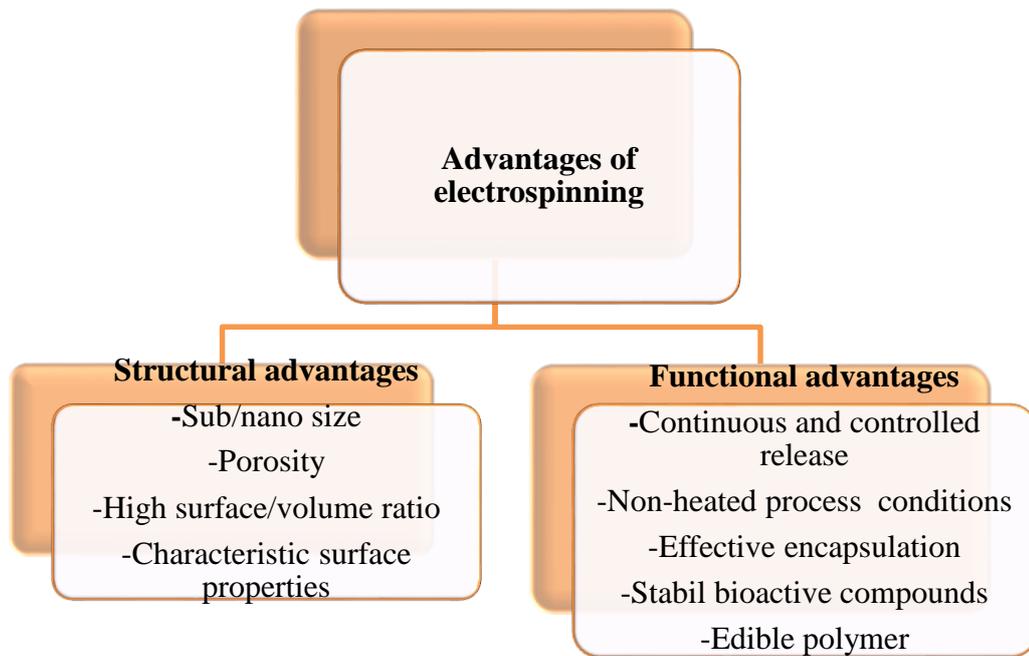


Figure 2.7 Advantages of electrospinning

#### Disadvantages

- The result of the influence of various parameters complex production system takes place.
- Fine-tuning of parameters are required in production.
- The amount of product is low.
- Some blockage occurs in the system.
- There are some inadequate mechanical properties.
- Some problems emerge like inhomogeneous distribution of diameter [37], [54].

#### 2.4.1 Electro spray

Electrospray is a method in which atomization of feed solution realizes using electrical forces. System consists of a capillary nozzle and electrode. Feed solution that flows from the nozzle is pushed by electric potential and so solvent evaporates. Solid particles collect on the collector plate [2], [62], [63]. Electro spray figure is shown in Figure 2.8.

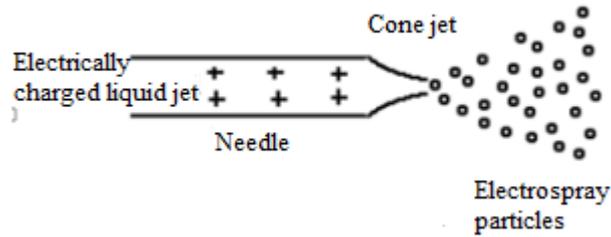


Figure 2.8 Electro-spray method [62]

Electro-spray process has many spray form depending on shape of semi-sphere, formation of droplets and type of flow behaviour. Electro-spray forms are summarized in Figure 2.9 [62].

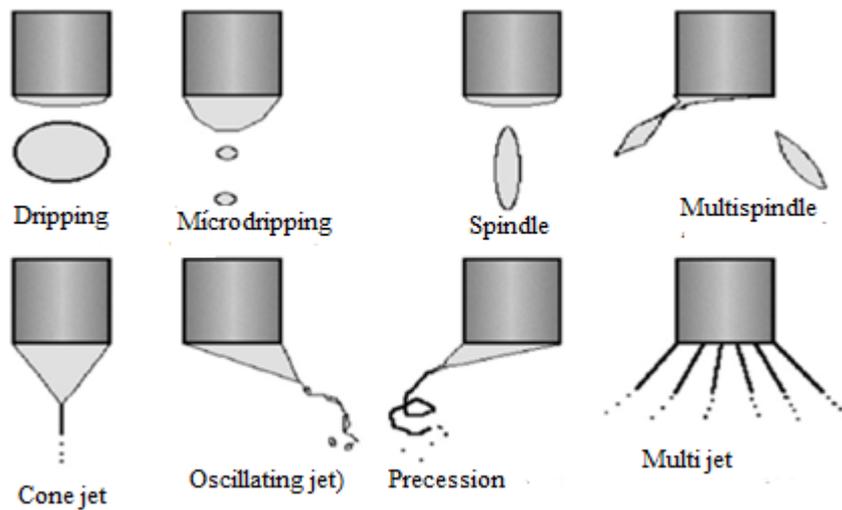


Figure 2.9 Electro-spray forms [62]

Compared with a conventional electromechanical atomizer spray it has the following advantages:

- It allows obtaining uniform powder product in different particle sizes ranging from nanometers to micrometers,
- When droplets are loaded in the electric field, they repel each other and coagulation is prevented by distributing themselves in space,
- The movement of charged droplets can be easily controlled via the electric field. Changes direction or focus can be done,
- When used for the encapsulation studies, active material loaded into the delivery system is usually carried out in one step,

- The active substance is homogeneously dispersed in the polymeric matrix. High active substance loading capacity and encapsulation efficiency is obtained,
- It is a cheap and easily applicable method [62].

### 2.4.2 Electrospinning

Electrospinning can be defined as production method of micro/nano-sized fibers [8], [55]. Schematic representation of the electrospinning process is shown in Figure 2.10 [60].

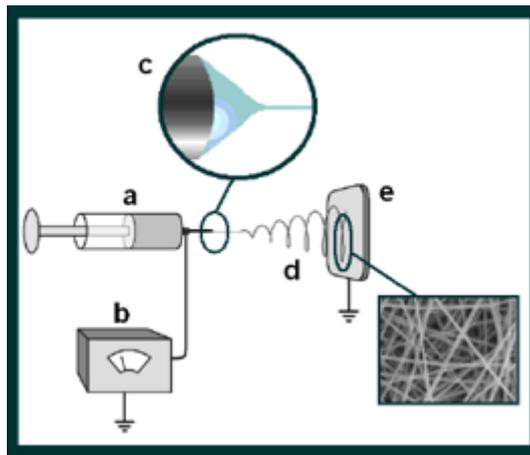


Figure 2.10 Electrospinning system set up: polymer feed solution (a), high voltage (b), cone form (c), instability area (d), micro-nano fiber (e) [60]

### 2.4.3 The Application of Electrohydrodynamics Atomization Technical for Encapsulation

Encapsulation is one of the electrospinning applications area. This encapsulation carried out with the help of the electric field is also named electroencapsulation [62], [63]. It has become one of the alternative methods used in encapsulation of bioactive components in polymer materials. Heat sensitive bioactive components can be encapsulated with this method in food and cosmetic industry because there is no need to heat. Therefore the method has been researched in many areas of the food industry [64]. There are various encapsulation techniques employed. These are:

- The interaction of two oppositely charged droplets: In this method, two oppositely charged feed solutions spray from two nozzles standing side by side. As a result of Coulomb interactions of this two feed solutions, droplets having high surface tension are trapped in the droplets have a smaller surface tension [62].

- b) Electrospray and evaporating the solvent: Active ingredient is suspended in a suitable polymer solution. When feed solution that was homogenized moves to the collector plate by the effect of the electric field, feed solutions' solvent evaporates. Shell becomes rigid. Powder product remains on the collector plate. So active ingredient takes place in the polymer encapsulation [62].
- c) Electro-spray and gelatinization process: The feed solution also incorporates suspended active ingredient sprays into a bath in which there is chemical and ionic crosslinking agent. The active ingredient is trapped in the polymer by forming a hard coating on the core material [62].
- d) Electro-coextrusion (coaxial electro-spray): It is the process that two different liquids spray from simultaneously coaxial (concentric) nozzles. While active substance flows through the nozzle that is in the center, shell material flows from the circular space between the two nozzles. This technique has the advantage of providing high loading capacity and encapsulation efficiency. Encapsulation models with the aid of electric fields are shown schematically in Figure 2.11 [62].

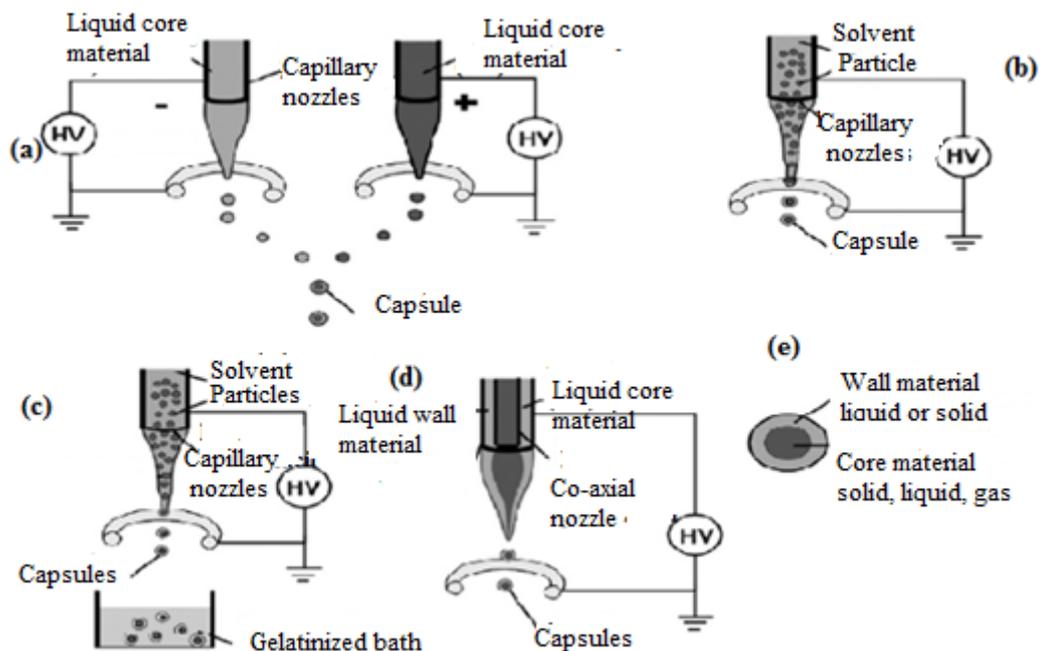


Figure 2.11 Schematic representation of electroencapsulation techniques; the interaction of two oppositely charged droplets (a), evaporation of the solvent and electrospray process (b), electrospray and gelatinization (c), electro-coextrusion (d), micro / nanocapsules structure (e) (62)

#### 2.4.4 Parameters Affecting Electrospinning Method

Many features as morphology and structure of products produced electrospinning change depending on some parameters during production. Different structures obtained from electrospinning method are shown in Figure 2.12 [65].

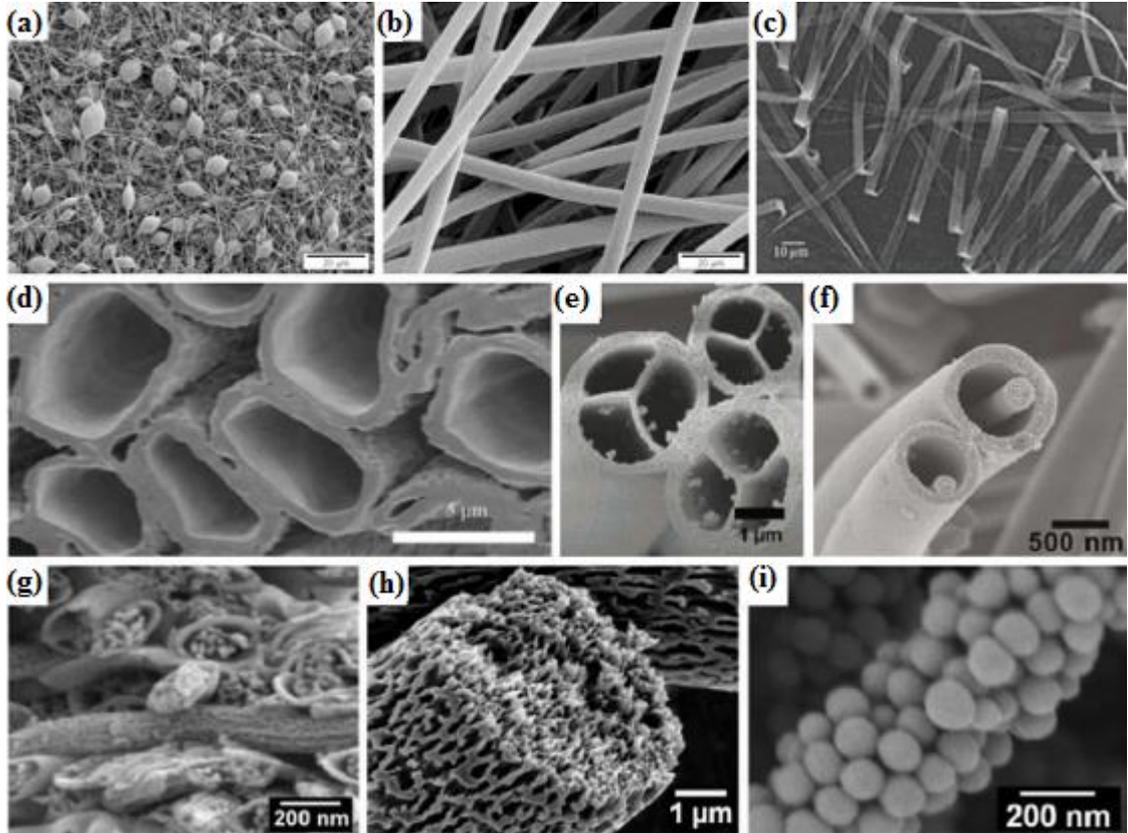


Figure 2.12 Different structures obtained by electrospinning; beaded (a), smooth (b), ribbon (c), hollow (d), multichannel tubular (e), nanowire-in-microtube (f), multi-core cable-like (g), porous (h, i) (65)

As shown in Figure 2.12 reason of the formation different structure and morphology material structures is that many parameters are effective on method [66]. Factors affecting system in electrospinning method can be classified as feed solution, electrospinning process and environmental parameters. [33], [38], [66], [67], [68], [69], [70], [71], [72].

**Solution variables:** The temperature of the solution, rheological properties, polymer concentration, molecular weight of the polymer, conductivity, surface tension [33], [38], [67].

**Process Parameters:** Applied voltage, flow rate, electrical field, collector and needle type, distance between needle and collector plate [33], [38], [67].

Environmental Variables: Relative humidity, ambient temperature [33], [38], [67].

#### **2.4.4.1 Viscosity**

Viscosity is the most effective feed solution parameter in the system affecting properties. Therefore, the viscosity must be within the proper range. Depending on the polymer used viscosity usually ranges from 1 to 215 poise [(1 poise = 1 dyne/cm<sup>2</sup>.saniye, 1/100 poise = 1 centipoise (cp)) [38]. Viscosity range required for the production of nanofibers at different feed solution varies. Generally, when the viscosity of feed solution increases, the diameter of the product produced increases [33], [67]. Viscosity is related to the concentration and molecular weight of each polymer [31]. It can be determined by whether a solution is generally suitable to electrospinning method by solution concentration or not. When other factors are kept constant, increase in concentration causes increase in fiber diameter. But in some studies concluded that polymer molecular weight is also important for the production of nanofibers. Therefore, in order to give precise information is needed to work with different polymers having different molecular weights [45], [67], [70]. If the solution concentration is low, bead structures are observed. Fibrous structures are created together with the increase of concentration [33], [38], [67].

#### **2.4.4.2 Molecular Weight**

The molecular weight of the polymer affects some electrical and rheological properties such as viscosity, surface tension, conductivity, dielectric strength. Straight chain polymers are usually worked. Larger diameters and fiber formation is observed when high molecular weight polymers are used. However beaded structure occurs as low weight polymer is used [38], [67], [70]. High molecular weight polymer indicates complexity of chain. So it is related to the viscosity. Chain complexity plays an important role in electrospinning method.

#### **2.4.4.3 Surface Tension**

Surface layer of liquid have similar properties with flexible layer. Effect that occurs result of these similarities is called surface tension [38], [45]. It refers to the energy per unit surface and its unit is mN/ m (milinewtons/m) [45]. In electrospinning process the first force applied against the electrical potential is the surface tension [33]. One of the solution properties influencing the formation of the fibers, droplets and beads is surface tension.

While other variables constant, it can be obtained smooth fiber surface by reducing surface tension [38]. Generally applied minimum voltage increases with the rising of the surface tension [33], [67]. Value of the surface tension properties change with respect to type of polymer, solvent and temperature. In general, the surface tension decreases with the increasing of polymer concentration. Addition of small amounts of surfactants to the solution as Tween 20 and Span 20 reduces the required voltage for the production of nanofibers decreasing the surface tension [71]. The low surface tension of the solution allows the formation of thinner and smooth nanofiber without dripping [45], [67], [70], [73], [74], [75]. According to the results of study made by Ali et. al. as the surface tension of the solvent increases, the particle size distribution also expands [2].

#### **2.4.4.4 Voltage**

Applied voltage provides loading with electrical charge of feed solution during the process [45]. In terms of morphology of produced material the voltage value is important. Fiber production takes place after a critical voltage is exceeded. Some studies have indicated that material diameter is reduced by increasing the applied voltage value. After that certain voltage value, the diameter begin to increase [33], [67]. Generally, high voltage applied to the solution causes high straining (flexing). Thereby the diameter of the fibers narrows and the solvent evaporates quickly. The beaded structure is more likely to be observed at high voltage [33], [38], [67]. When examining the effect of voltage on fiber diameter, the other process parameters must be considered. Increasing the voltage value also ensures faster fiber formation [69]. Change of voltaj value affects the shape of the Taylor cone [2], [45], [67].

#### **2.4.4.5 Properties of Flow Rate and Needle Type**

Needle type affects produced nanomaterials diameter. Small diameter nanofibers are obtained with small needle. But these pipe have some problems about convey of high viscosity feed solution to needle tip. Most suitable diameter value of the needle pipe is determined as 4.5 mm with the study reported in the literature. It is amount of produced nanomaterial that is obtained from feed solution [45]. It affects the reaching time of the solution to the collection plate. Low feed rate is more preferred since it provides sufficient time to evaporate the solvent. The increase in flow velocity causes the fiber diameter to be thicker and the pore diameter to be larger. Because electrical forces that will decrease

the thickness of the material reduces at the constant voltage [33], [38], [67], [1]. Flow rate must be equivalent amount with the polymer solution moving towards the plate from the feed. Otherwise, the formation of nanofibers with diameters proportional distribution and continuous cannot obtained. Taylor cone can not occur at low flow rates. However in high flow rate nanofibers and beaded structure are formed with large diameter. High flow rate reduces the electrical loading. As a result an increased diameter of the nanofibers or nanofiber formation ceases fully [45].

#### **2.4.4.6 Electrical Conductivity**

Siemens (S) is the unit of solution conductivity (1 Siemens = 1 Amper/Volt). Solvents that are used in feed solutions have lower electrical conductivity than neat water [45]. Solvent evaporates in the electric fields generated by electrical charge in the electrospinning process. Therefore, it is important minimum electrical conductivity in the feed solution for the production that have desired characteristics [33]. Electrical conductivity is defined as transmitting the electric current applied on the material to each side of material. The lower resistance a material shows against to electric current the higher electrical conductivity it has [38], [67]. Using a solution having a very low electrical conductivity is not possible to produce nanofibers with electrospinning method. The choice of the suitable solvent to the polymer is important in terms of electrical conductivity. When the polymer dissolves in the suitable solvent, electrical conductivity is generally increases because of ionic charges [38]. But this is not the case for some polymer structure that do not contain ionic compounds in its composition. Some inorganic salts such as sodium chloride (NaCl) are added to these kind of polymers to increase electrical conductivity. Adding the ionic additives provide more stable jet formation [45]. Polymer type, polymer concentration, solvent type and measurement temperature affects the electrical conductivity. When the electrical conductivity increases, a significant reduction is observed in product diameters. But as the electrical conductivity is low, bead formation occurs because of insufficient elongation that is observed in the jet created by electrical forces [33], [67], [70]. Increase to a certain level of conductivity of solution allows the formation of thinner nanofibers. Generally studies have show that nanofibre production is not possible with the feed solutions that their conductivity is above 5 mS/cm [45], [67].

#### **2.4.4.7 Dielectric Constant ( $\epsilon$ )**

Dielectric constant indicates that how electrical charge that is within its structure. The unit is F/m (Farad/metre). The dielectric constant varies according to the polymer and solvent type. The high dielectric constant allows uniform distribution of electrical charges within feed solution. So quality nanofibers production occur with high yield. In other words, increasing of the dielectric constant provides more nanofibers collection per unit in the field. Additives or different solvents can be used to change the dielectric constant. However, change in fiber morphology may not only be connected the change in dielectric constant. Changes also affects conductivity, surface tension and the distribution of polymer chains in solution [38], [45], [67].

#### **2.4.4.8 The Volatility of the Solvent**

The vapor pressure of the solvent used is an effective parameter on electrospinning method by affecting the rate of evaporation and drying time. The high volatility solvents should be preferred in the electrospinning method so as to obtain smooth homogeneous fibers in a short time [67].

#### **2.4.4.9 The Distance Between the Syringe/Needle and the Collector Plate**

One of the effective parameters in order to control the morphology and size is the distance between needle tip and collector plates. Throughout the distance solvent evaporates. Feed solution begins to solidify and fibers or particles occurs [2]. Solvent does not find an opportunity to evaporate in case the distance, if it is shorter than necessary. These conditions leads to the formation of instability Taylor cone and defective morphological structures are observed [38], [45], [67], [1]. Generally when the distance increases, it leads to reduction in diameter of nanofibers. But in some cases it has been observed vice versa. Gap distance also affect the morphology of the fiber like the fiber diameter. The distance have to be a specific value at which the solvent can evaporate from feed solution jet. Otherwise, wet nanofibers are collected on collector plate [45].

#### **2.4.4.10 Collector Type**

Collector plate is wrapped with materials such as aluminum foil, so nanofibers or nanoparticles can be collected easily. Besides aluminum foil, conductive papers, fabrics or wires are used. The plates may be used in parallel or movable type. A study carried out

show that use of wires has better way to get sample from the surface of collector plate than aluminum foil [38], [67]. Some studies were carried out with using various collector types as rotary cylinder, rotary plate, wire frames of various geometric shapes, annular electrodes or conical shaped. The geometry of the collector will determine how the nanofibers are collected. Mobile collector plate type provides accumulation of desired density nanofibers per unit area. It provides more proportional distribution of the fiber structure and more controlled porous network. This is especially advantageous in sensor applications. The speed of the moving plate also affects the morphology of the nanofiber network [45]. Co-axial or hollow (hollow) nanofibers can be obtained with different design syringe or needle [51]. Schematic presentation of co-axial nanofiber is given in Figure 2.13.

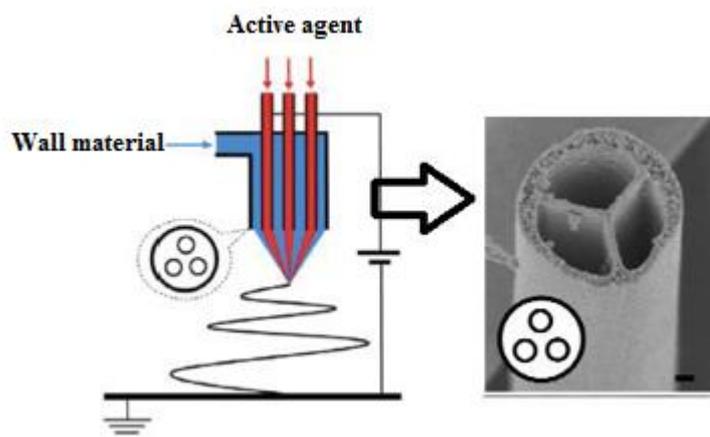


Figure 2.13 Co-axial nanofiber [37]

#### 2.4.4.11 Temperature and Humidity

The gas composition and temperature affects the evaporation time of the solvent in the solution through electrospinning process [1]. The control of these conditions is required to obtain dry fibers. Solvent evaporates more quickly with devices that have an extra heat source and circulation of heated air. But the heated air circulation rate have to be equivalent to the rate of evaporation of the solvent in nanofiber jet. Mit-Uppatham and et. all (2004) examined the effect of temperature on the production of polyamide nanofibers by electrospinning. They indicated that when temperature increased from 25°C to 60°C, diameter of the fibers reduced and it is associated with reduced viscosity. There is a contrary relationship between viscosity and temperature [38].

According to some studies made with polystyrene, increase of ambient humidity causes to formation of the beaded structure. Volatile solvent may easily evaporate from feed solution at low relative humidity conditions. As a result of the evaporation of the solvent, the needle tip is choked up by the residue so some problems occur in formation of nanofibers. It is defended that production of nanofibers by electrospinning method will be improved with the increase of humidity [33], [38]. It was examined that effect of relative humidity ranges from 10% to 70% on fiber diameter. As a result of experiments it was observed that as the relative humidity increases, the fiber diameter increases. Electric field strength, required for reduction of the fiber diameter, decreases because of relative humidity [38].

#### **2.4.5 Polymers used in the electrospinning method**

There are many synthetic and natural polymers used in electrospinning method. Synthetic polymers are generally preferred due to some features as inexpensive, accessibility and different functional properties. Poly (caprolactone) / PCL, poly (glycolic acid) / PGA, poly (D, L-lactic acid) / PLA, poly (ethylene oxide) / PEO and poly (vinyl acetate) / PVA can be given as examples of synthetic polymer widely used [8], [10], [33], [62]. The use of natural polymers is increasing day by day. Biodegradable, hydrophilic, edible, renewable and non-toxic properties are among the reasons for the increase in the use of natural polymers [8], [33]. Due to branched chain, production of nanofiber from natural polymers is not easy [70]. These branched structures are required to hydrolysis or blends with polymer that can be obtained fibers structure alone. Feed solution should be homogeneous. The viscosity should be in the appropriate range according to electrospinning method [31], [70]. The nano-micro products obtained from synthetic polymers are used in more electronic and medical fields. Natural food biopolymers are used to production of food packaging materials, create synthetic food matrix and enrichment of bacterial culture media [38]. Chitosan, alginate, dextran, cellulose and its derivatives are used as polysaccharides. Collagen, gelatin, casein, wheat protein, zein, eggshell protein, egg albumin, serum albumin are used as proteins. Additionally phospholipids, enzymes and other natural components of deoxyribonucleic acid (DNA) are used for obtaining nanofiber [33].

### **2.4.5.1 Cellulose**

Cellulose is a natural polysaccharide obtained from the cell walls of plants and most abundant in environment. Cellulose is often preferred because of cheap and having good thermal, mechanical properties. However it has extremely low solubility in aqueous solution because of the crystal and hydrogen bonds [8], [33].

### **2.4.5.2 Chitosan**

Chitosan, obtained result of partial deacetylation of chitin, is natural second polysaccharide derived from plant and animal [7], [9]. Thanks to the large hydrogen bonding and rigid crystal structure, chitosan is insoluble polymer in water but soluble in organic solvent. Chitosan consists of (1 → 4) -2-amino-2-deoxy-β-D-glucan and (1 → 4) and 2-acetamido-2-deoxy-β-D-glucan units. It is used as the antimicrobial and the chelating agent in the food industry [8]. It has high mechanical strength. It distinguishes with the similarity of proteins. Thanks to the biological properties, it is widely used in the food, biomedical, cosmetics, chemicals and pharmaceutical industry. Chitosan is often preferred due to biologically degradable and non-toxic nature. It is used in the produce of edible films with its bactericidal and fungicidal effects. [8], [33]. Chitosan is a polymer with a different structure of the solid phase and dispersed phase. Nanofibers obtained neat chitosan by electrospinning method could not been developed until 2004. Before that date, studies were performed with the aid of the polyvinyl alcohol and other solvents. After 2004 tetrahydrofolate (THF) and acetic acid has been used as solvent [38]. In studies production of nanofibers obtained from chitosan successful were carried out by using solvents such as acetic acid and TFA [33].

### **2.4.5.3 Alginate**

Alginate, has a wide range of applications thanks to versatile biopolymer structures, is obtained by alkali treatment with algae [33]. It is soluble in water but insoluble in organic solvents [8]. Alginate has linear polymers chains that are comprised of (1 → 4) α-L-gulonic acid and (1 → 4) β-D-mannuronic acid units [7]. Water-soluble alginates can form a gel even at very low concentrations [33]. It provides a strong gel structure in the presence of particular calcium ions [8].

#### **2.4.5.4 Zein**

Zein is storage protein produced from corn and can easily turn into film form [57], [61]. It is biodegradable and non-toxic, which is a by-product of the bio-ethanol industry. It constitutes 44-79% of the endosperm protein. Zein is used in some areas as production of micro-nano-particles/fibers, encapsulation of bioactive substances and biodegradable packaging [8] It can be dissolved in dimethylformamide (DMF), aqueous ethanol, methanol and acetic acid [50]. It is used in sweets and dried fruits, owing to its hydrophobic structure. Coating material in which zein is used, has properties resistant against to oil, oxygen and moisture [68].

#### **2.4.5.5 Casein**

Casein is a milk protein having a molecular weight of 19-25 kDa. It consists of amino acids more than 55% that are responsible for the formation of the polar portion of the casein micelles. It is estimated that casein is not suitable polymer for electrospinning method due to the presence of molecules intra-and intermolecular hydrogen bonds. However blend with casein and poly (ethylene oxide) (PEO) or poly (vinyl alcohol) (PVA) can be used [8].

#### **2.4.5.6 Gelatin**

Gelatin is a protein derived from by partial hydrolysis of collagen. Collagen is located in connective tissue like animal skin, bone, cartilage and tendons. Different types of gelatin are obtained as a result of treatment with the solution of collagen acid (type A gelatin) and alkali (type B gelatin) [33], [38]. The main purpose for production of gelatin is to divide into smaller pieces insoluble collagen by hydrolyzing. So it becomes water-soluble and the best gelatin that has improved gelling properties is obtained [9]. Gelatin protein content varies between 85% and 92%. The other components constituting the gelatin are remaining water after drying and mineral salts. Gelatin is a multifunction hydrocolloid [33] It has enabled to make a lot of research on gelatin due to commercially inexpensive, biocompatible, biodegradable and accessibility in various forms. Gelatine is used as foaming agent, creaminess, syneresis inhibitor, emulsion stabilizer as well as gelling agent in food industry [33], [64]. Gel strength and thermal stability of gelatin depends on gelatin molecular properties (molecular weight distribution and amino acid composition). The physical properties of gelatin affects the potential applications and the quality of the

gelatin which is associated with gel structure. It is interesting for hydrogel packaging due to interaction of multiple molecular structure that is supplied by gelatin's structure. Thanks to gelatin and collagen's high water holding capacity and swelling properties, when meat and fish products are cooked or frozen, gelatin has an active role in reducing the loss of water and preventing hydration [31].

Functional properties of gelatin and collagen are summarized as follows:

- It is easily soluble at low temperatures (at body temperature)
- It is used as gelling and water binding agent,
- It reduces the surface tension,
- It has film forming characteristics,
- It can be used as coating material in encapsulation method [31], [70], [56], [61].

Aqueous solution of gelatin transforms into gel form at room temperature. This is a disadvantage for the electrospinning method at room conditions [61].

#### **2.4.5.7 Maltodextrin**

Maltodextrin is a carbohydrate-based polymer with low molecular weight. It was not possible to obtain any electrospayed structure from the maltodextrin aqueous solution. Carbohydrate solutions presented high surface tension and low viscosity values. But we can use maltodextrin with electrospinning process thank to adding some surfactans [70], [71].

#### **2.4.6 Use of Electrospinning Method in Food Industry as Encapsulation Technique**

In the food industry electrospinning technique is used in sub mikro-, micro- and nano-encapsulation of antioxidants, probiotics and bioactive compounds [73]. Unlike traditional encapsulation methods such as spray drying, coacervation, electrospinning method is used for the encapsulation of sensitive bioactive compounds. Briefly usage areas are shown in Figure 2.14.

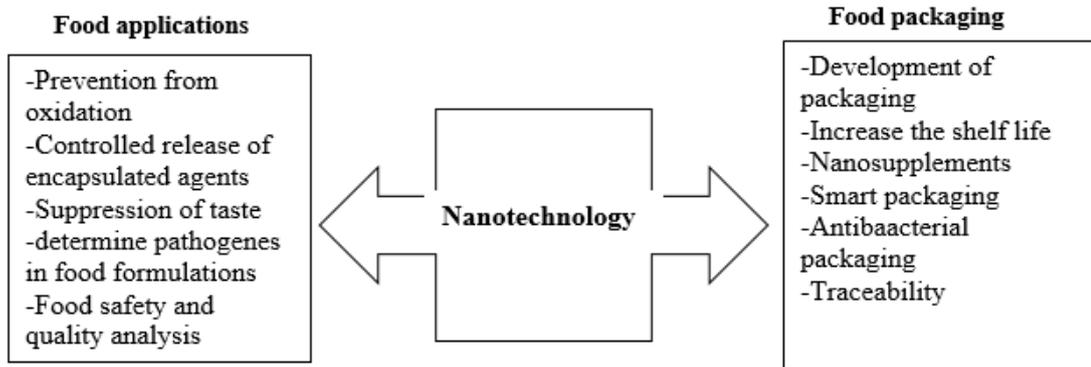


Figure 2.14 Application areas of electrospinning method in food industry [40]

#### 2.4.7 Characterization of produced nano-micro materials by electrospinning method

Various techniques can be used for the characterization of nanofibers. Physical characterization is related to the structure and morphology of the material produced; scanning electron microscopy for morphological specifications (SEM), field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM) are used for morphological properties; For chemical characterization, Fourier transform infra red (FTIR), nuclear magnetic resonance (NMR), circular dichroism (CD), differential scanning calorimetry (DSC), X-ray diffraction and X-ray scattering is used; distribution and stability in different environments of produced material may be identified with the zeta-sizer measurements. Thus it can be estimated that the suitability of the material produced by electrospinning to the food system [1], [2], [8], [34], [38],[41], [67].

### MATERIALS AND METHODS

#### 3.1 Chemicals

Maltodextrin (Smart Kimya), zein (Acros Organics), 225 g Bloom type B bovine gelatin (Sigma), Tween 80 (Merck), ethyl alcohol (Düzey Lab), 100% acetic acid (Emsure) 2N Folin-Ciocalteu (FC) reagent (Aldrich), sodium carbonate (Emsure), potassium chloride (Emsure), 2,2 - diphenyl-1-picrylhydrazyl (DPPH), nutrient broth (Sigma Aldrich) and dried Bitter melon were used.

#### 3.2 Methods

##### 3.2.1 Extraction

Dried bitter melon was pulverized by milling with grinder and passed through sieve 1 mm in diameter. Solid-liquid extraction method was used for extraction. Solid:water ratio is 1:20. Water extraction was performed with a thermo-shaking bath at 40°C speed of 150 rpm for 15 minutes. Alcohol extraction was achieved by stirring for 3 hours at 150 rpm with 70% ethyl alcohol [29]. Extracts were centrifuged at 4000 rpm for 10 min. Then, the samples were filtered through Whatman filter paper and stored at +4°C.

##### 3.2.2 Preparation of feed solution

Feed solutions in which each polymer can be dissolved, were prepared for using in the electrospinning method. Polymer solutions at different concentrations (w/v) were fed into the electrospinning device to examine the effect of polymer concentration on fiber or particle formation. The polymer feed solutions that fed to the electrospinning device prepared as described below:

**Neat maltodextrin feed solutions, NM;** 20% and 30% (w/v) maltodextrin polymer and 5% tween 80 (w/w, with respect to maltodextrin) were dissolved in water under magnetic agitation for 1 h at room temperature,

**Maltodextrin feed solution prepared with aqueous bitter melon extract, EM;** 20% (w/v) maltodextrin and 5% tween 80 (w/w, with respect to maltodextrin) were dissolved in aqueous bitter melon extract under magnetic agitation for 1 h at room temperature,

**Neat gelatin feed solutions, NG;** 5%, 20%, 25%, 30% (w/v) gelatin polymer was dissolved in 20% (v/v) diluted acetic acid solution under magnetic agitation for 4 h at 40°C temperature and cooled down to room temperature before processing,

**Gelatin feed solution prepared with aqueous bitter melon extract, EG;** 20% (w/v) gelatin is dissolved in 20% (v/v) acetic acid solution with aqueous bitter melon extract under magnetic agitation for 4 h at 40°C temperature and cooled down to room temperature before processing,

**Neat zein feed solutions, NZ;** 2.5%, 5%, 15%, 20%, 25%, 20% (w/v) zein is dissolved in 70% ethyl alcohol under magnetic agitation for 1 h at room temperature,

**Zein feed solution prepared with alcoholic bitter melon extract, EZ;** 20% (w/v) zein is dissolved in alcoholic (70% ethyl alcohol) bitter melon extract under magnetic agitation for 1 h at room temperature.

Extraction and preparation of the feed solution are summarized in the figure as shown in Figure 3.1.

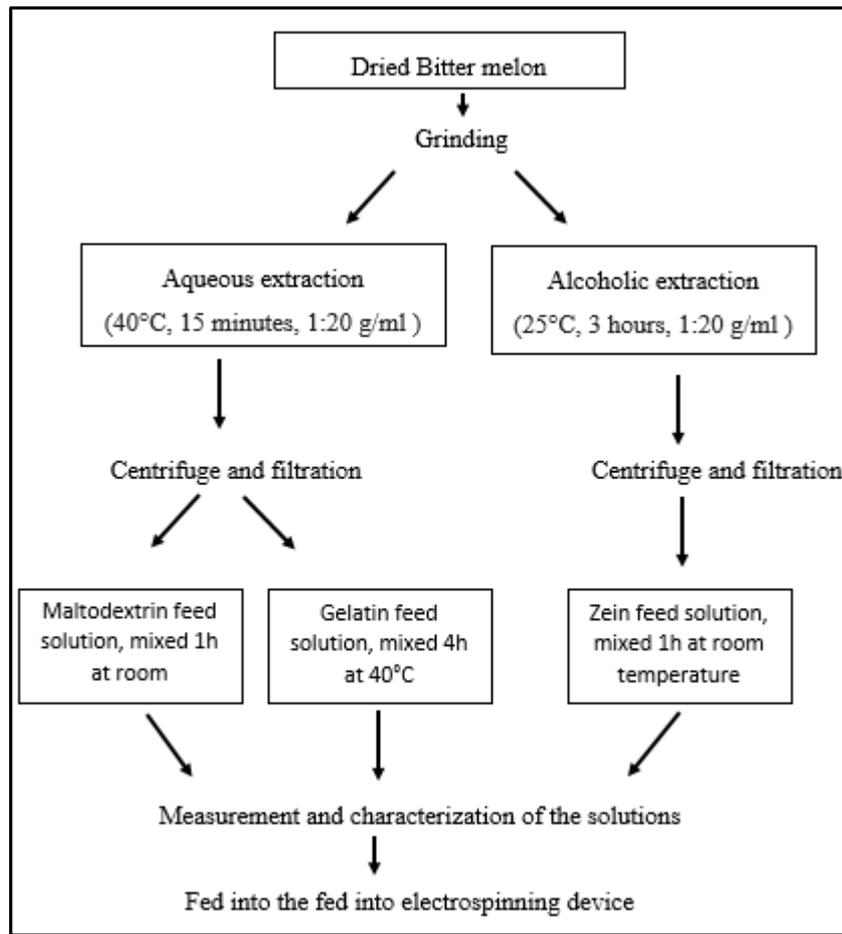


Figure 3.1 Schematic representation of the preparation of the extracts and feed solutions

### 3.2.3 Characterization of feed solutions

Some measurements were carried out to investigate the characteristics of the feed solution before the electrospinning process.

#### 3.2.3.1 Measurement of pH of solutions

The hydrogen ion concentrations of feed solutions were measured using a Hanna H 2211 pH meter room temperature. Results were presented as mean of four measurements  $\pm$  standard deviation including two replications and two parallel.

#### 3.2.3.2 Measurement of the electrical conductivity of feed solutions

The electrical conductivities of feed solutions were measured using Malvern marka Nano ZS (Worcestershire, UK) at room temperature. Results were presented as mean of four measurements  $\pm$  standard deviation including two replications and two parallel.

### **3.2.3.3 Determination of surface tension of solutions**

The surface tension of the solutions was measured using tensiometer (KSV 701 model Du Nouy tensiometer) with applying Du Nouy ring method at room temperature. Results were presented as mean of four measurements  $\pm$  standard deviation including two replications and two parallel.

### **3.2.3.4 Rheological properties of feed solutions**

Steady analysis was carried out for determining the rheological properties of feed solutions. The rheological characterization of feed solutions were determined with (Anton Paar rheometer, MCR 302) at 25°C in the shear rate range of 0 to 200 s<sup>-1</sup> and a parallel plate sensor (diameter = 35 mm, gap = 0.5 mm). We used shear rate-shear stress graph to have information about flow behaviour. The results were modeled using the software Statistica according to the power-law equation (4.1) for determining consistency index (K) and flow behavior index (n). The power-law equation was assessed Eq. (3.1):

$$\tau = K\gamma^n \quad (3.1)$$

Where  $\tau$  is the the shear stress (Pa), K is the flow consistency index (Pa.s),  $\gamma$  is the shear rate (s<sup>-1</sup>), and n is the flow behavior index (dimensionless) [31].

### **3.2.4 Electrospinning**

The electrospinning device (Nanodev, Turkey) was fed by prepared feed solution for obtaining nanoparticles or nanofibers. The process was carried out at room temperature. The electrospinning device used is shown in Figure 3.2.

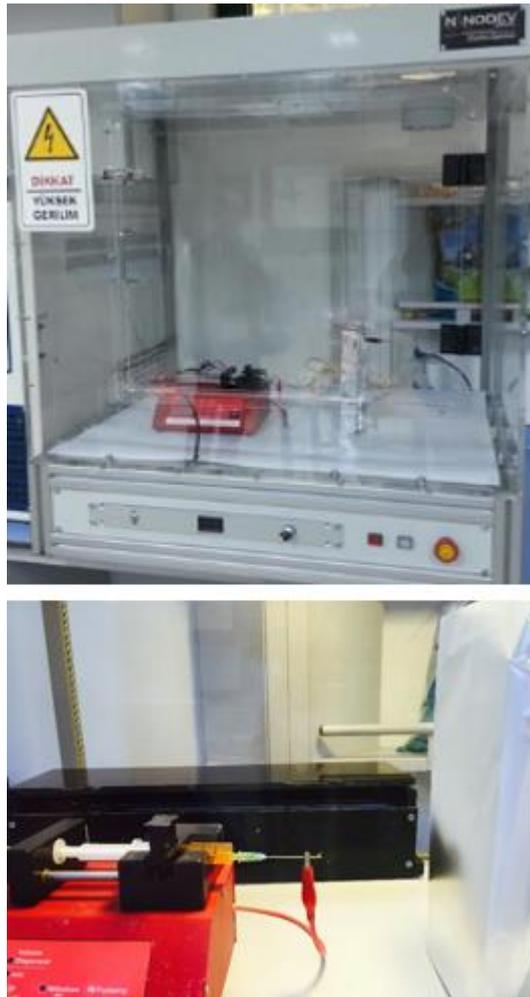


Figure 3.2 Electrospinning setup (Nanodev, Turkey)

Plastic syringe that is used for spraying feed solution to collector plate had 5 ml volume and 0,80x38 mm and 21Gx1<sup>1/2</sup>. Varied concentrations as experimental variable were tested to investigate the effect of the concentration on produced nanoparticles and nanofibers. The parameters applied to each of polymer were determined as a result of the literature studies. Different concentrations like 20-30% (w/v) maltodextrin, 5-20-25-30% (w/v) gelatin and 2.5-5-10-15-20% (w/v) zein were fed into the electrospinning device. Different voltages (10-18 kV), solution flow rates (0.1-1 ml/h), and the tip to collector plate distance (5-15 cm) were applied in electrospinning process. Conditions in which accumulation taken place without dripping and appropriate system parameters were determined by evaluating microscope images.

### **3.2.5 Particle/fiber characterization**

#### **3.2.5.1 Determination of the morphological features**

It is considered that viscosity is the most important parameters affecting on the morphology of particle and fiber forms [1]. For evaluating the effect of the concentration, the electrospinning process was performed with various concentrations. Other parameters were kept constant. Diameters and morphological properties of the obtained electrospayed nanoparticles and electrospun nanofibers were investigated by using an electron microscope (JEOL JSM-7001FTTLS LV, Kitam, Samsun). Images were captured at 5 kV with 500, 1500, and 20,000 magnification.

Electrosprayed particles obtained using maltodextrin polymer could not be analyzed for SEM because of deteriorating during transport. So images and diameter of electrospayed maltodextrin particles were obtained using optical microscope and zetasizer, respectively. For this purpose electrospayed maltodextrin particles dispersed in ethanol at 0.1%.

#### **3.2.5.2 Measurement of particle size and surface zeta potential**

The zeta potential measurements of electrospayed and electrospun samples were carried out using a dynamic light scattering instrument (Malvern Zetasizer Nano ZS, Worcestershire, UK) at 25° C. Zeta potential is defined as the potential occurring between particle surface and dispersion liquid. Zeta potential is to provide information about the stability of the colloidal system [75].

Zetasizer Nano instrument measures the Brownian movement of the particles/fibers by dynamic light scattering. Brownian motion is defined as random motion which is occurred by particles/fibers [33].

While ethanol was used as a dispersant for gelatin electrospun fibers and electrospayed maltodextrin particles, water was used for zein electrospun samples. Measurements were carried out at 0.1% (w / v) powder sample concentration. Results were presented as mean of three measurements  $\pm$  standard deviation.

#### **3.2.5.3 Determination of thermal properties of powder sample**

Differential scanning calorimetry (Q20 model DSC, USA) was performed under nitrogen atmosphere with a heating rate of 10°C/min in duplicate. Samples were sealed in

aluminum pans and heated from 20°C to 300°C for all samples. Thermograms were examined for onset temperature ( $T_o$ ), peak temperature ( $T_p$ ), end point temperature ( $T_e$ ) and enthalpy ( $\Delta H$ ) values using the DSC software program. 7 mg, 10 mg and 5 mg were amount used for DSC analysis of maltodextrin, gelatin and zein electrospinning samples, respectively. Results were presented as mean of two measurements  $\pm$  standard deviation.

#### **3.2.5.4 FTIR analysis**

All spectra were recorded by an absorption mode in the wave length range of 4000–400  $\text{cm}^{-1}$  using a Bruker (Rheinstetten, Germany) Platinum ATR equipment. The spectra were obtained by averaging 16 scans at 4  $\text{cm}^{-1}$  resolution.

#### **3.2.5.5 Determination of total phenolic content**

The content of total phenolic content (TCP) was determined by using Folin-Ciocalteu method [76]. Total phenolics content of bitter melon extracts, electrospayed and electrospun samples were analysed. 0.5 ml of extract and 2.5 ml 0.2N Folin-Ciocalteu reagent were added into a falcon tube. After 3 min at room temperature, 2 ml of 7.5 g/100 ml  $\text{Na}_2\text{CO}_3$  solution was added. Each sample was allowed to stand for 1 h at room temperature and absorbances were measured at 760 nm (UV-1800, Shimadzu, Japan).

Electrospayed and electrospun samples (10 mg) needed to be reconstituted in proper solvent for each polymer. Then analysis for total phenolic content was carried out. After adding chemicals, absorbance values were measured at 760 nm after centrifugation at 4000 rpm for 10 minutes. The linearity range for this assay was determined as 0.5–5.0 mg/L GAE ( $R^2 = 0.9990$ ). Total phenolic content was assessed using Eq. (3.2):

$$\text{TCP (mg/L)} = ((\text{Absorbance} - 0.0821) / (0.0111)) * \text{dilution factor} \quad (3.2)$$

Total phenolics were calculated on the basis of gallic acid, and expressed as  $\text{mg L}^{-1}$ .

#### **3.2.5.6 Determination of antioxidant capacity**

Antioxidant activity (%AA) was determined by the DPPH (1, 1- diphenyl-2-picrylhydrazyl) assay according to the method reported by Singh et. al [77]. 10 mg electrospayed and electrospun samples were reconstituted in proper solvent. 0.1 ml of this solution was mixed with 4.9 mL DPPH solution (0.1mM) in methanol. The mixture

was shaken vigorously and left to stand for 30 min in the dark. The absorbance was then measure at 517 nm. The free radical scavenging activity was assessed using Eq. (3.3):

$$\%AA = ((A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}})) \quad (3.3)$$

where  $A_{\text{sample}}$  is the absorbance in the presence of the extracts and  $A_{\text{control}}$  is the absorbance of the control.

### RESULTS AND DISCUSSION

Besides the use of nanotechnology in material, electronic, computer, medical, pharmaceutical, textile, environment, energy, biotechnology area, the use in food industry has also become popular [79], [80], [81], [82]. It is particularly important in the production of functional food products [39]. The demand for functional foods using plant extract is increasing day by day [61], [57], [59]. With this technology, various ingredients of the food contents could be designed at the molecular level according to desired characteristics and controlled; sensory and mechanical properties can be developed with the addition of nanostructures containing different colors [39], [83], [84], [85]. To obtain products with nanoscale via electrospinning method has enabled the examination of this method within the scope of nanotechnology science. Particle or fibrous structure can be obtained by electrospinning process depending on system and environmental parameters [61]. Structures produced by the electrospinning method are important in the food industry to support sustained and controlled release properties [1], [86], [87].

We tried to generate particles and fiber structures obtained from different polymer with bitter melon extract via electrospinning method. It is aimed to gain functional properties to food with adding these particles and fiber structures. In this chapter the results of characterization analysis performed both feed solutions and particles/fibers structure are given.

#### **4.1 The Total Phenolic Content and Antioxidant Activity of Bitter Melon Extract**

The total phenolic content of aqueous and alcoholic bitter melon extract (70% ethanol) was determined 196.95 mg GAE/l extract and 287.50 mg GAE/l extract, respectively. Antioxidant activity results were determined similar to phenolic content. It was higher in

alcoholic extract. While antioxidant activity of alcoholic extract found as 32%, aqueous extract was 27%.

IC<sub>50</sub> values (concentration of sample required to scavenge 50% free radical or to prevent lipid peroxidation by 50%) as an indicator of antioxidant activity were found, 129.94 µg/ml ve 156.78 µg/ml in water and ethanol bitter melon extract, respectively. Compared to BM-EtOH (44.0 mg/g for total flavonoids and 68.8 mg/g for total phenols), BM-H<sub>2</sub>O appeared to have a higher concentration of total flavonoids (62.0 mg/g) but a lower content in phenolic compounds (51.6 mg/g) [88].

Kubola et al. [25] evaluated the antioxidant activity of various boiled water extracts from un-ripened bitter melon fruit. Results of the DPPH assay revealed a 53.9±0.73% reduction in the DPPH radical at a concentration of 0.2 mg/g. Wu et al. [88] reported the IC<sub>50</sub> values of boiled water (129.94 mg/ml) and ethanol (156.78 mg/ml) fruit extracts.

Hee-Ock Boa et al. [89] researched phenolic and antioxidant content of various plants and antioxidant activity of bitter melon was determined as 50% (DPPH).

Horax et al. [27] reported that there was no significant difference in the antioxidant activities of the extracts among varieties ( $P = 0.2556$ ) and between drying methods ( $P = 0.1444$ ). The antioxidant activities of flesh, inner tissue, and seed ranged from 81.7% to 86.5%, 78.8% to 88.4%, and 78.5% to 85.4% inhibition, respectively. So they have concluded that bitter melon can be used in the food system as a good antioxidant source.

The amount of phenolic compounds in the extracts obtained by SWE (52.63mg gallic acid equivalents (GAE)/g dry weight (DW)) was significantly higher than those in the extracts obtained by MeOH extraction (5.00–6.00mgGAE/gDW) and soxhlet water extraction (5.00–7.00mgGAE/gDW). The IC<sub>50</sub> value of the extract obtained with SWE at 200 °C for 2 h (5.49 g/ml) was significantly lower than that of the extract obtained with solvent extraction at 65°C for 2h (17.34 g/ml) and soxhlet water extraction for 4h (15.62 g/ml). This result indicated that SWE gave the extracts with much higher antioxidant activity [90].

Phenolics were extracted from pericarp (fleshy portion) and seeds of bitter melons harvested at three maturation stages (immature, mature, and ripe) using ethanol. It was reported that when phenol content decreased, an increase EC<sub>50</sub> (efficient concentration in mg extract/mg DPPH, defined as the amount of extracts necessary to decrease the initial DPPH concentration by 50%) values was obtained [13].

Ripening degree of the fruit contents, cultivating conditions, treatments applied to the fruit prior to extraction, the extraction solvent, solid:liquid ratio are effective on phenolic and antioxidant character [21].

Some findings contradict that of the present study, where the antioxidant activity and total phenolic content of the water extracts is low. This difference may be due to the country of origin from which the fruit was obtained as well as soil type and a host of climatic factors (temperature, moisture, sunlight ) and extraction methods. Additionally our bitter melon samples were dried before extraction. Maybe this is the reason for the low antioxidant capacity according to studies.

## **4.2 Characterization of Feed Solutions**

### **4.2.1 Conductivity, pH and Surface Tension of Feed Solutions**

Polymer solution subjected to the electric field should necessarily have a certain conductivity. Otherwise it is not observed any formation of a jet. When the electrical conductivity of the solution increases, jet is charged with more load. Thus it is possible to obtain finer bead and fibers. Charged polymer solution have to overcome the surface tension forces for smooth morphology. High surface tension forces is a complicating factor for electrospinning process. Low surface tension is recommended for a homogeneous structure and small dimensions [60].

In Table 4.1 pH, electrical conductivity and surface tension measurement results of the feed solutions are given.

Table 4.1 pH, electrical conductivity and surface tension value of the feed solutions

Feed solutions	pH	Electrical conductivity (mS/cm)	Surface tension (mN/m)
NM	3.29±0.02	1.28±0.07	32.25±0.06
EM	4.82±0.02	6.59±0.09	30.88±0.39
NG	3.18±0.01	6.13±0.09	35.19±0.30
EG	3.27±0.01	7.32±0.24	34.83±0.02
NZ	5.34±0.02	0.99±0.08	24.43±0.04
EZ	5.70±0.01	2.17±0.22	21.82±0.02

\*NM: Neat maltodextrin feed solution, EM: Maltodextrin feed solution prepared with aqueous bitter melon extract, NG: Neat gelatin feed solution, EG: Gelatin feed solution prepared with aqueous bitter melon extract, NZ: Neat zein feed solution, EZ: Zein feed solution prepared with alcoholic bitter melon extract.

In contrast, with increasing the electrical conductivity the decrease in surface tension was observed. When as shown in Table 4.1 the feed solutions prepared with bitter melon extract had higher electrical conductivity and pH but lower surface tension than neat feed solutions. So when we examined the morphology of the electrospayed and electrospun samples prepared with bitter melon extract, it was observed to have more uniform morphology and smaller size in diameter (Fig. 4.13, 4.14 and 4.15).

Studies have indicated that feed solution properties affect structure and morphology of electrospinning products. Terzi et al. [33] researched to obtain edible nanofibers via electrospinning method. For this purpose they used gelatin. When they evaluated feed solutions properties they reported that as gelatin concentration from 7% to 10% electrical conductivity increased while surface tension decreased.

In generally, it can not be obtained nanofiber from gelatin feed solution using water at room temperature. This is because of the gelation. Zhang et al. [85] wanted to solve this problem. They increased the conductivity by adding sodium alginate solution to the heated gelatin solution. So they achieved to generate fibers with smooth morphology.

Eltayeb et al. [74] used the electrohydrodynamic spray technique to obtain solid lipid-based nanoparticles for food products. Reduction in produced solid fat nano particle size was observed by decreasing the surface tension.

In study made by Torres-Giner et al. [82] it had indicated that pH value is associated with the feed solution viscosity and it is thought to be effective on electrospinning zein fibers. High conductivity causes reduction in particle diameter. Coulomb repulsion forces generated in the electric field strength are in competition with viscoelastic forces of feed solution and it is easier to get rid of the polymer network. So the collection of electrospinning product on the collector plate occurs easily [71].

When blackberry extract was added to soy protein isolates solution, the conductivity of solutions were decreased. The reduction in electrical conductivity could permit an evaluation in static charges on the jet surface in the electrospinning process. This resulted in an increase in electrostatic forces which in turn increased the capacity of polymer solutions to be electrospun. So when decrease in conductivity occurs, some unstable beaded formation can observed [59].

Increase in size was obtained by decreasing conductivity that was a result of adding gallic acid to feed solution. The reason for this was shown by rising viscosity with increasing concentrations of gallic acid [91].

In addition in some studies have been mentioned about the negative effects of high conductivity on the electrospinning product. Amaranth protein was dissolved in acetic acid, formic acid and HFIP solvents and fed into electrospinning device. They observed deterioration in the fibrous structure at high conductivity. When the surface tension increases, fiber-beaded and particul structures were observed rather than fibrous structure. They infered that increased surface tension prevents the formation of a stable polymer jet by avoiding electrical forces. Low conductivity and low surface tension had shown to be effective in the formation of fibrous structure [86].

In generally when feeding solution conductivity increases, more homogeneous and uniform structure is obtained. But some studies have shown the results in opposite. In case of high conductivity it is difficult to control of electrical forces that are on the polymer jet. This affects the formation of the characteristic cone. As conductivity increases the classic cone-jet and multi-jet models can be observed.

Üstündağ and Karaca showed that low electrical conductivity, surface tension and high viscosity, pH values are more suitable for electrospinning process. Polyelectrolytes such as alginates, have fairly high conductivity values and these values are associated with solution concentrations. The resulting solution properties, the average fiber diameter and SEM images are evaluated together; as PVA ratio increases, decrease in conductivity and surface tension values; increase in viscosity and pH values was observed [60].

In a study made by Torres-Giner et al. [92] to prepare for the first time bioblends of zein/chitosan fibers by electrospinning, surface tension of the mixture increased with increasing chitosan ratio in the blend. So some beaded structures were observed.

Charernsriwilaiwat et al. [93] prepared electrospun chitosan-based nanofiber mats and to incorporate the fruit hull of *Garcinia mangostana* extracts into the mats. When the amount of *Garcinia mangostana* extracts increased, conductivity and fiber size increased but surface tension decreased.

Aqueous maltodextrin solution is not suitable polymer because of low conductivity and high surface tension. Pérez-Masiá et al. [71] could obtain particles from maltodextrin feed solution via electrospinning. They used some surfactants for increasing conductivity and decreasing surface tension.

In comparison the results of the present study have confirmed that as surface tension decreases, more homogenous and smaller size are obtained. Also a positive effect is observed on the structures when the electrical conductivity increases to a certain value.

#### **4.2.2 Rheological Properties of Feed Solutions**

The surface tension, conductivity, viscosity and polymer concentration are shown as affecting parameters on the product produced by the electrospinning method. Among these the viscosity and polymer concentration are considered as the most effective parameters [69].

Changes in viscosity value of feed solution are effective on morphology when the other parameters are constant. Polymer solution should have a suitable viscosity value suitable for the efficient electrospinning. In case of low chain complexity that is occur at low viscosity, surface tension has dominant effect on the polymer jet through electrospinning process. Therefore continuous jet form can not occur [60]. Electrospayed particles formation can be seen (Figure 4.13). When the viscosity increases, continuous jet

formation is obtained increasing the complexity of polymer chain and fiber formation was observed (Figure 4.14 and 4.15).

The  $n$  values of all feed solutions is close to 1 (data not shown). As expected the addition of the extract solution did not cause a significant change in viscosity. Rheological behaviors of all feed solutions were close to Newtonian flow properties. Similar results were reported in literature. In a study which polyphenols had been encapsulated with gelatin, made by Gómez-Mascaraque et al. [64] 5-8-10-20% concentrations of gelatin prepared with 20% acetic acid showed Newtonian flow behaviour. Aqueous solutions of gelatin show Newtonian flow behaviour even at high concentrations around the neutral pH. Another study indicated that feed solution prepared with 20% (w/w) concentration of zein shows Newtonian flow behaviour [63]. Viscosity values were determined as in Table 4.2.

Table 4.2 Viscosity values of the feed solutions

Feed solutions	$\eta$ (Pa.s)
NM	0.007±0.00
EM	0.008±0.00
NG	0.540±0.01
EG	0.650±0.02
NZ	0.083±0.01
EZ	0.086±0.01

\*NM: Neat maltodextrin feed solution, EM: Maltodextrin feed solution prepared with aqueous bitter melon extract, NG: Neat gelatin feed solution, EG: Gelatin feed solution prepared with aqueous bitter melon extract, NZ: Neat zein feed solution, EZ: Zein feed solution prepared with alcoholic bitter melon extract.

All polymers prepared at 20% concentration. So viscosity values of neat feed solutions and feed solution including extract was closed to each other. In present study zein nanofibers were observed at 0.08 viscosity value and we obtained smooth fiber structures. Gomez-Estaca et al. [63] reported that when the viscosity values of 20% zein feed solution is grater than 0.027, smooth fiber structures without beads are observed.

### 4.3 Electrospinning

Pre experiments were conducted to determine appropriate process parameters for each polymer. When the applied voltage decreased dripping was observed. As voltage increases, finer fibers formed. But some deterioration emerged in the stability of the jet. When the solution feed rate was too low, production took much time. It was seen some dripping at high flow rate. While the distance between the needle tip and the collector plate was under a certain value, there was not sufficient time to evaporate the solvent. Optimum process parameters for neat polymer solutions determined with respect to these observations are summarized in the following Table 4.3.

Table 4.3 Electrospinning process parameters

Feed solutions (w/v)	Applied voltage (kV)	Feed rate (ml/h)	Distance between tip and collector (cm)
20% Maltodextrin, (NM, EM)	18	0.1	10
20% Gelatin, (NG, EG)	18	0.25	10
20% Zein, (NZ, EZ)	14	0.2	10

\*NM: Neat maltodextrin feed solution, EM: Maltodextrin feed solution prepared with aqueous bitter melon extract, NG: Neat gelatin feed solution, EG: Gelatin feed solution prepared with aqueous bitter melon extract, NZ: Neat zein feed solution, EZ: Zein feed solution prepared with alcoholic bitter melon extract.

### 4.4 Characterization of Particles/Fibers

The samples obtained by the electrospinning method shown as an example in Figure 4.1.



Figure 4.1 Nanofibers obtained from gelatin solution at 20% prepared with aqueous bitter melon extract

#### 4.4.1 SEM and Optical Characterization of Nanofibers

System, environmental and feed solution properties parameters are effect on electrospinning process. But most important of them are the concentration and viscosity [69]. Because these parameters are effective on the size in relation to the surface tension [2]. Solution concentration is one of the most important parameters for electrospinning [68]. Therefore to examine the effect of concentration on the electrospinning method, different polymers were tested at different concentrations. Optical images for maltodextrin polymer and SEM images for gelatin and zein polymers are shown as follow. An increases in fiber diameter as the concentration of the polymer increases was reported in literature [61], [63], [74], [89].

Although using water as a solvent is a disadvantage due to its difficult volatility for electrospinning process, it is a nontoxic and can be used in many food formulations. Tween 80, Span 20, lecithin are used for some polymers having a low molecular weight like maltodextrin to be a suitable polymer in electrospinning process with using some surfactans [71].

In our study, Tween 80 was used as 5% with respect to polymer weight. Optical microscope images are provided for maltodextrin particles due to deterioration of samples during transportation to Samsun to get SEM images. Size measurement of maltodextrin particles was carried out by zeta-sizer. When images are examined, it has reached the conclusion that particles could easily be affected by environmental conditions and do not

have uniform structure. Particles obtained from 20% and 30% (w/v) concentration of neat maltodextrin feed solution are shown in Figures 4.2 and 4.3.

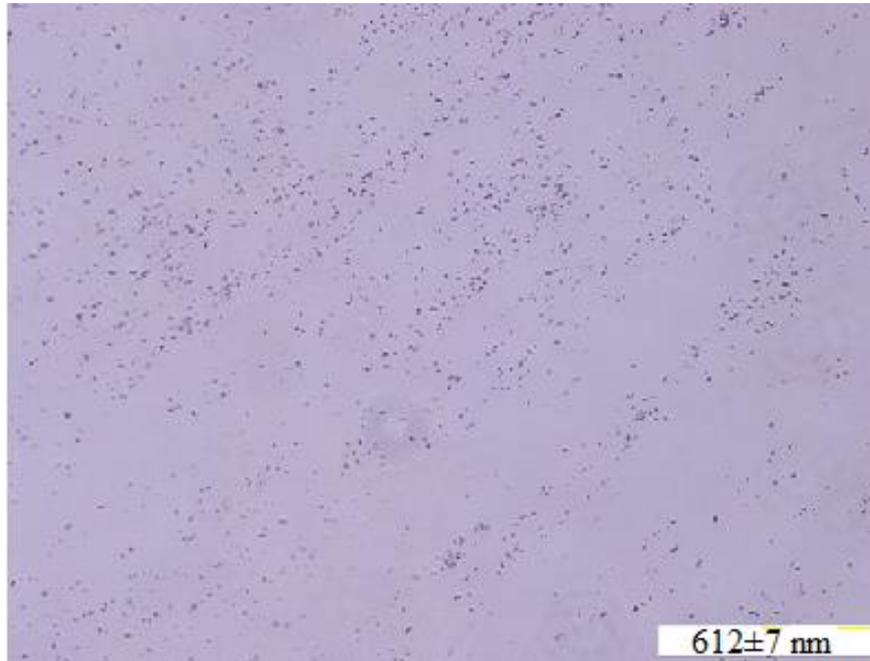


Figure 4.2 Optical image obtained from 20% NM (NM: Neat maltodextrin feed solution)

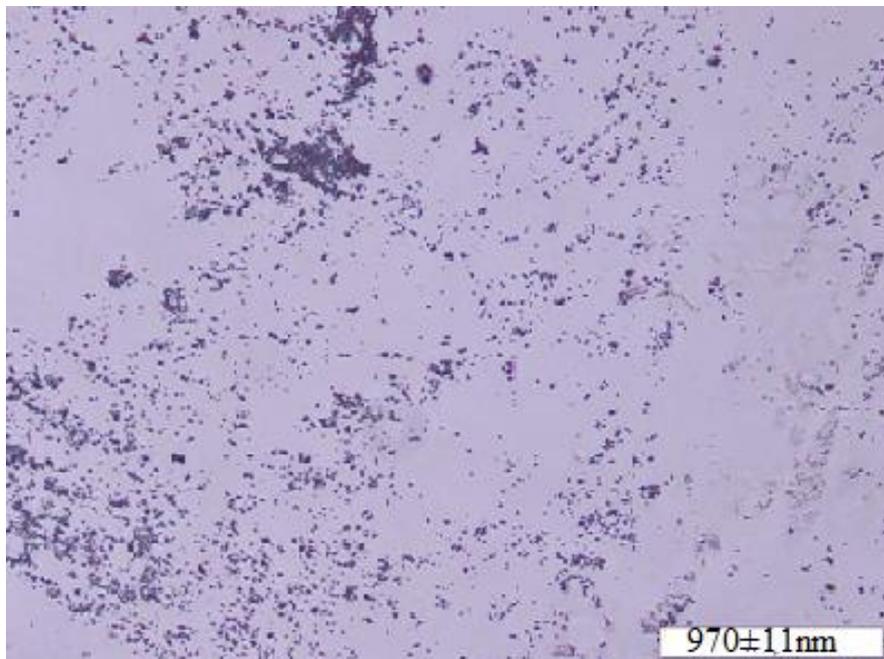


Figure 4.3 Optical image obtained from 30% NM (NM: Neat maltodextrin feed solution)

As shown in Figure 4.2 and 4.3, the diameters of particles obtained from 20% and 30% (w/v) polymer concentration are  $612 \pm 7$  nm and  $970 \pm 11$  nm, respectively. When polymer

concentration increases, increases in the particle size was observed. In a study made by Pérez-Masiá et al. [71] submicron size particles were obtained from various surfactant aided aqueous maltodextrin feed solution.

SEM image of gelatin particles obtained from 5% concentration is given in Figure 4.4. It was obtained inhomogeneous and irregular particles. Uniform and homogeneous gelatin particle structure could not be obtained from 5% (w/v) polymer feed solution prepared using 20% dilute acetic acid. Low concentration is shown as the reason. Indeed, similar results are also found in the literature [33], [45], [64], [69]. Gelatin particles obtained from 5% gelatin feed solution is shown in Figure 4.4.

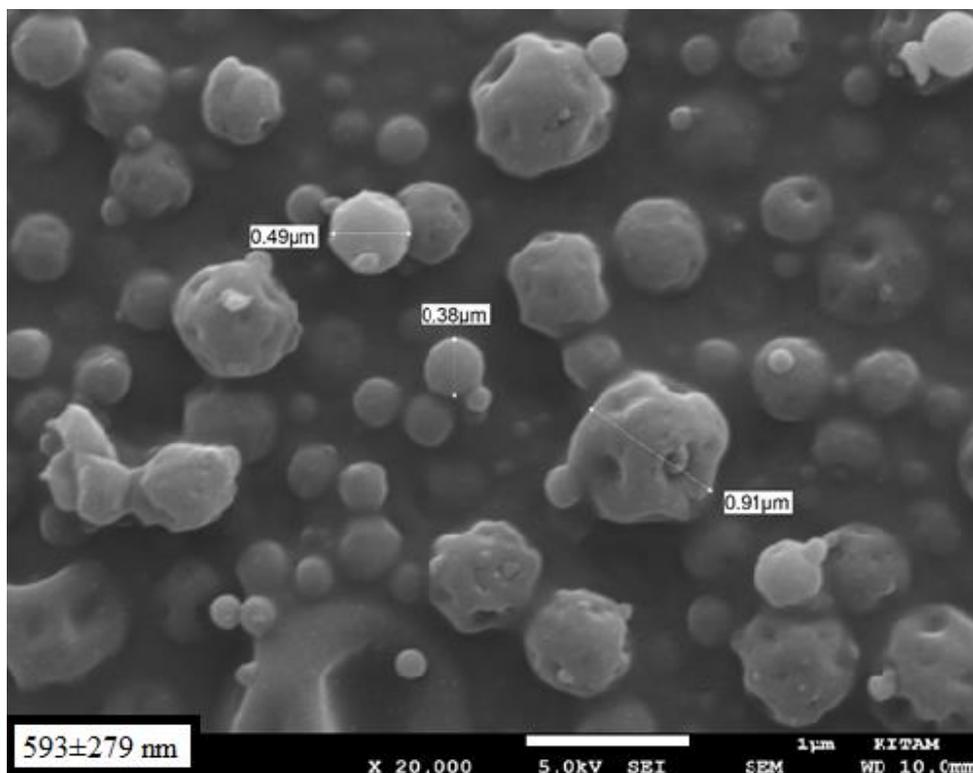


Figure 4.4 SEM image obtained from 5% NG (NG: Neat gelatin feed solution)

Ki Seok et al. could not observe uniform gelatin particles at below of 7% polymer concentrations as similar in our study. They prepared gelatin feed solution with formic acid. Low viscosity and high surface tension at low concentration were shown as the reason of unstable and irregular particle morphology [94].

When we examined morphology, fibrous structures were observed at 20% or above polymer concentration. As the polymer concentration increases, the fiber diameter increases. Fiber morphology and size that were obtained from a different gelatin polymer concentrations are demonstrated in the following Figures 4.5, 4.6 and 4.7.

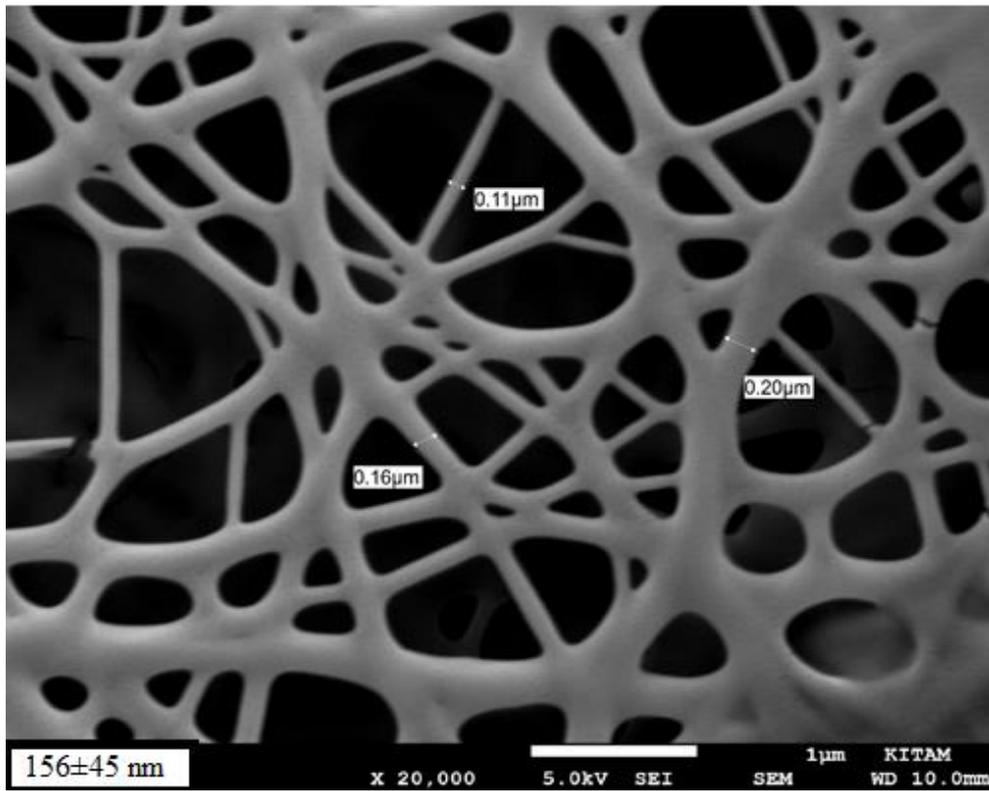


Figure 4.5 SEM image obtained from 20% NG (NG: Neat gelatin feed solution)

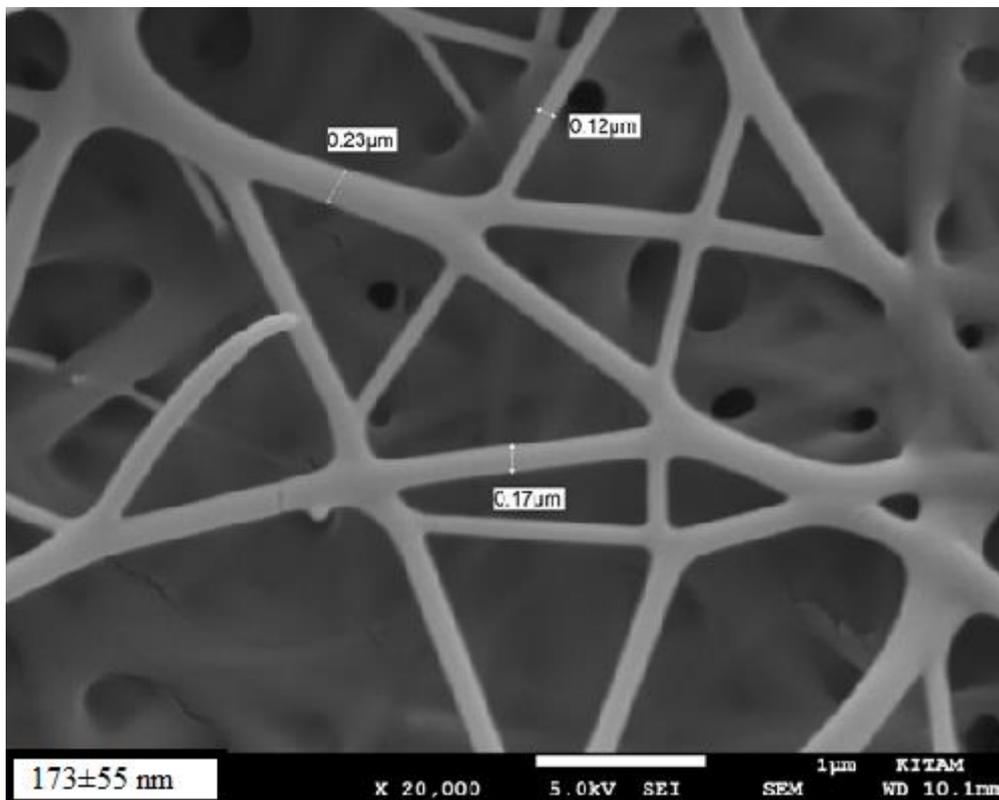


Figure 4.6 SEM image obtained from 25% NG (NG: Neat gelatin feed solution)

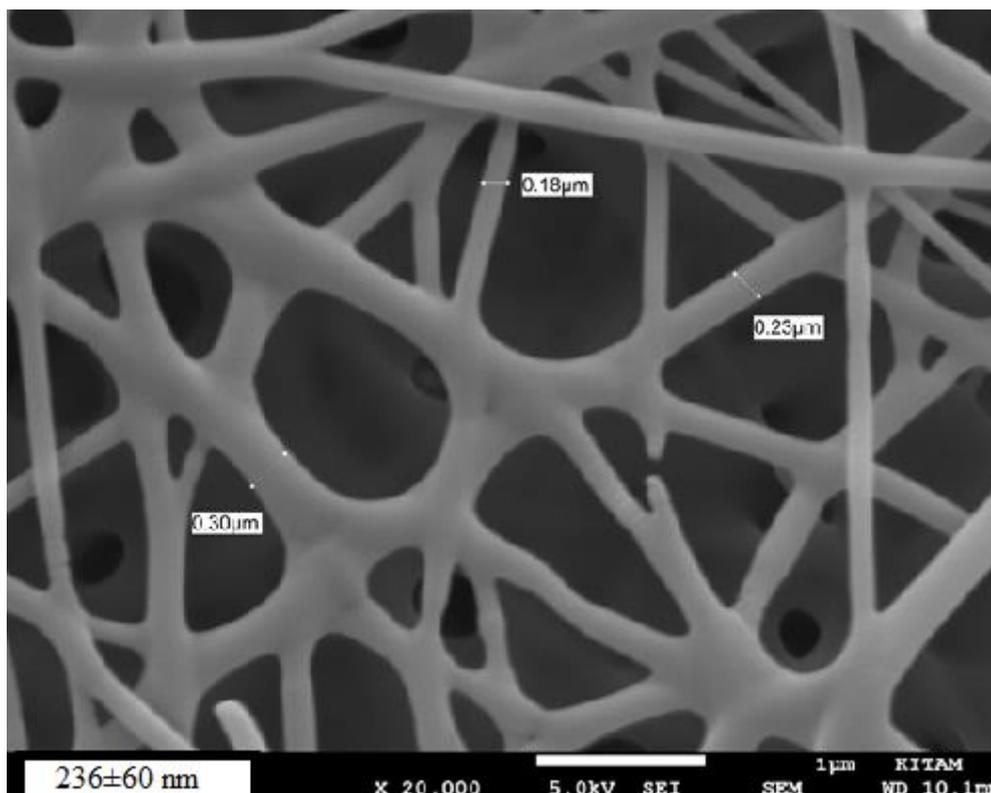


Figure 4.7 SEM image obtained from 30% NG (NG: Neat gelatin feed solution)

According to the study made by Gómez-Mascaraque et al. [64] non-uniform particles structure was obtained from gelatin solutions at 5-8-10% concentrations. The shape of the beads changed from spherical to spindle-like and finally uniform fibers with increased diameters were formed because of the higher viscosity at %20 concentration.

Homogeneous fibrous structure could not be obtained from gelatin solution at 7%, while smooth fiber morphology was observed at %20 gelatin concentration [89].

We prepared zein feed solutions at different concentrations with dissolving in 70% ethanol. SEM images of particles obtained from zein solution at 2.5 %, 5%, 15%, 20%, 25% are shown as below Figures 4.8, 4.9, 4.10, 4.11 and 4.12, respectively.

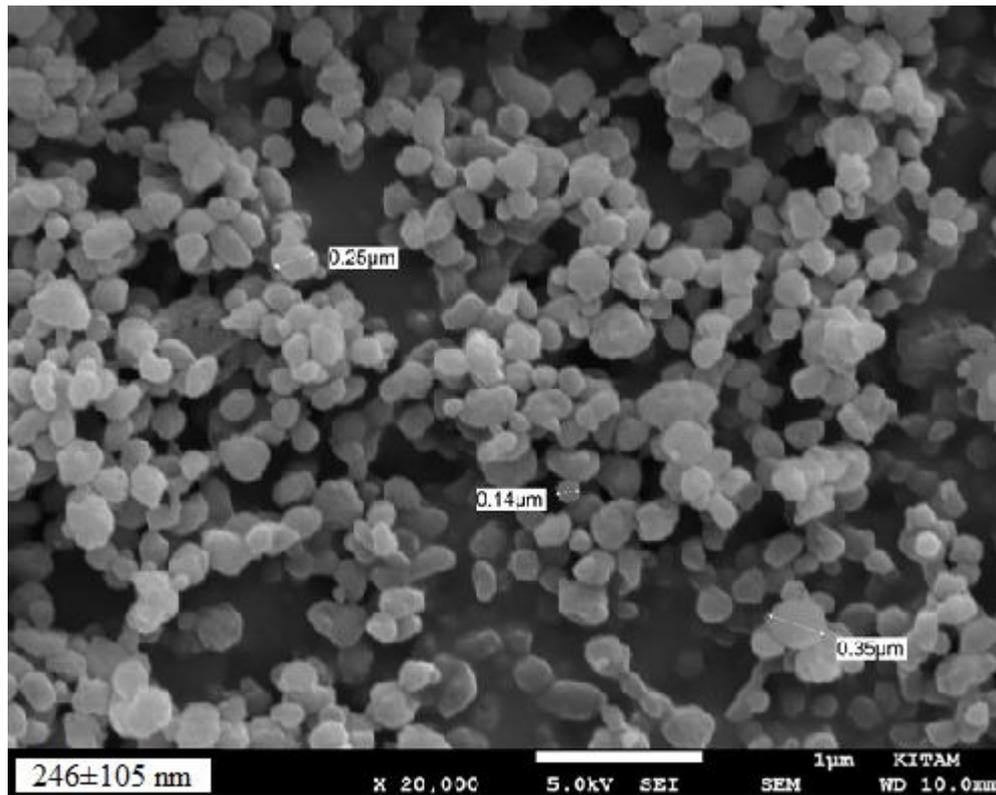


Figure 4.8 SEM image obtained from 2.5% NZ (NZ: Neat zein feed solution)

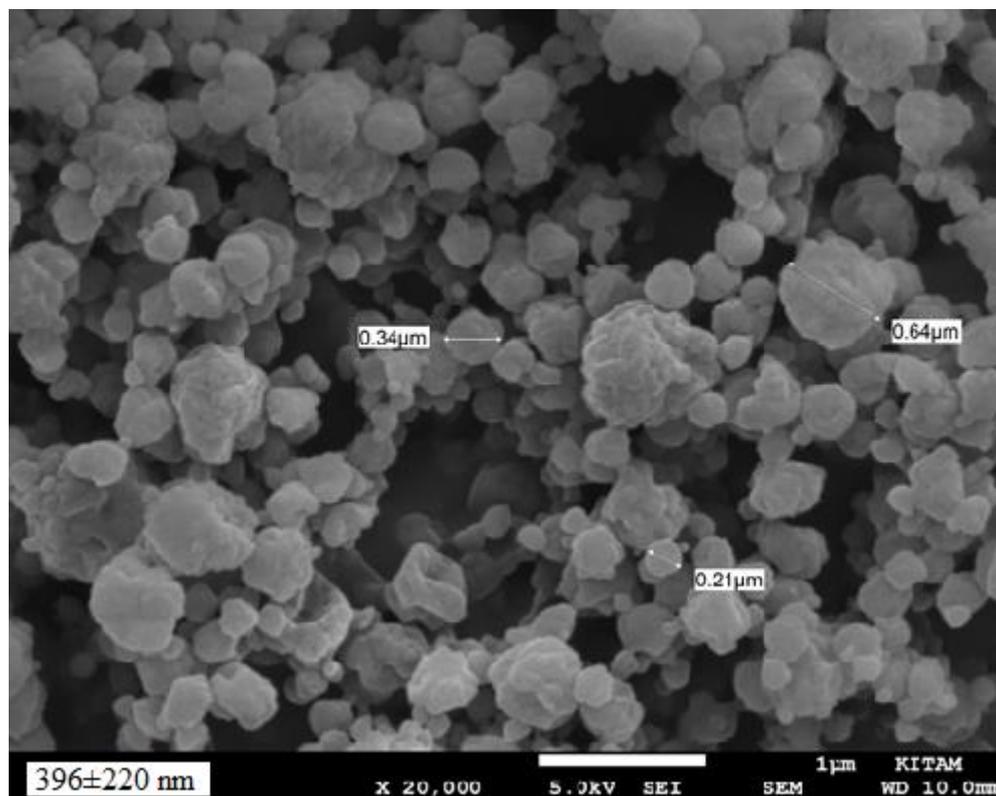


Figure 4.9 SEM image obtained from 5% NZ (NZ: Neat zein feed solution)

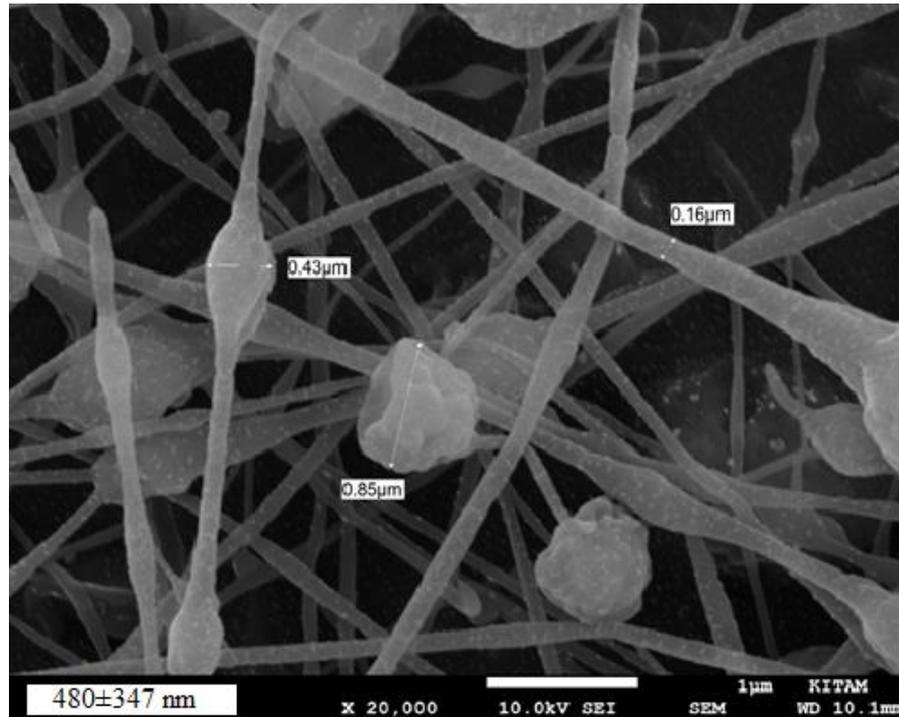


Figure 4.10 SEM image obtained from 15% NZ (NZ: Neat zein feed solution)

Zein concentration of 15% has evaluated as transition concentration from particle to fibrous structure. Miyoshi et al. [68] prepared zein solutions with 80% ethanol. 18% concentration was showed as a transition concentration from particle form to fibrous form. Also homogeneous smooth fibers were obtained from zein feed solution at 21%.

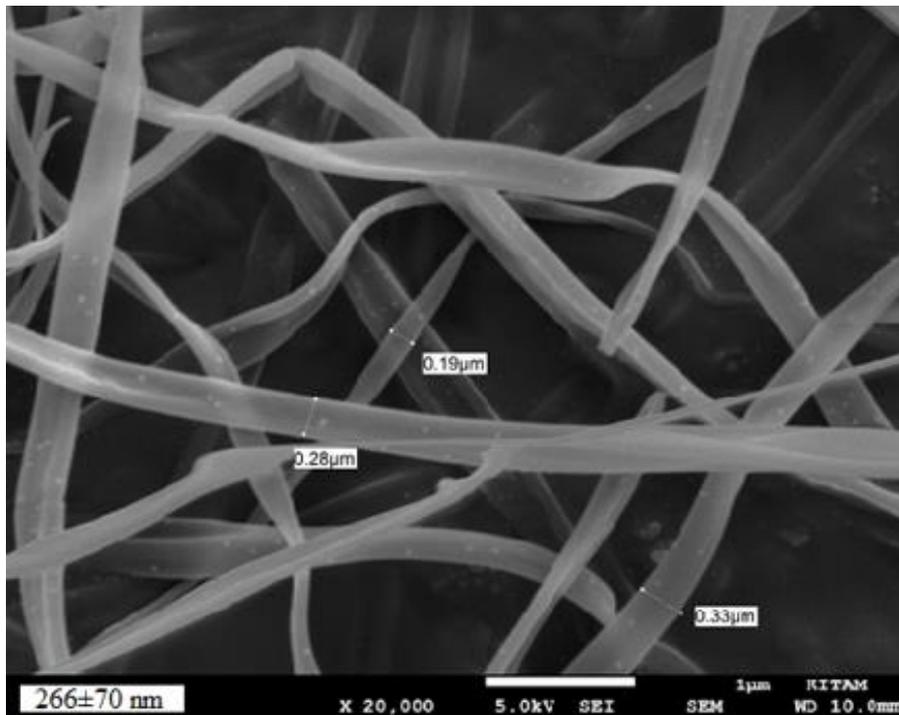


Figure 4.11 SEM image obtained from 20% NZ (NZ: Neat zein feed solution)

Fibrous structure occurred at 20% and 25% zein concentrations and diameters increased with increasing the concentration.

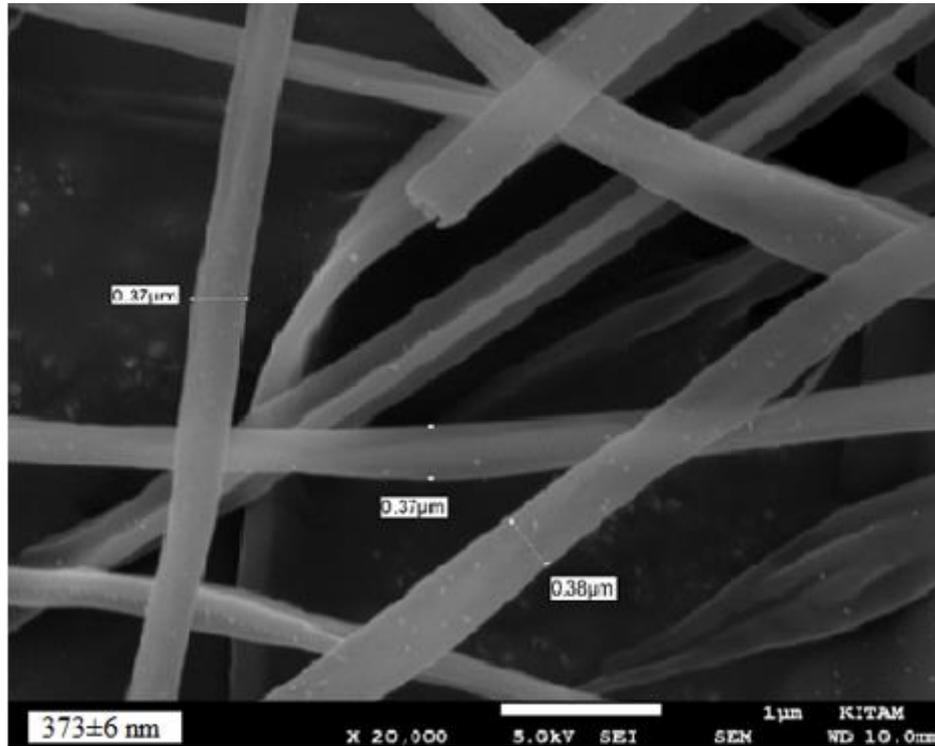


Figure 4.12 SEM image obtained from 25% NZ (NZ: Neat zein feed solution)

Zein feed solutions were prepared in concentration ranging from 1% to 15% (w/w). Particles forms were obtained from feed solutions that their concentrations within this range. Fiber formations were observed at 20% zein concentration [63], [2].

Unknown structures like dot were observed in SEM images of zein polymer. Similar unknown dots were determined in fibrous structure obtained from Hydrophobins (HP-A) in another study [34].

Miyoshi et al. [68] reported that the aspect ratio of the beads increased continuously with increasing polymer concentration, and that only fibers were formed above the critical concentration in electrospinning of polystyrene. Critical polymer concentration is more important for each polymer.

According the SEM images of neat particles/fibers, 20% polymer concentration was determined to be used in feed solutions prepared with bitter melon extract. The same process parameters ( 0.1 ml/h, 18 kV - 0.25 ml/h, 18 kV - 0.2 ml/h, 14 kV was used for maltodextrin, gelatin and zein, respectively) were used for preparing feed solutions including bitter melon extract. Images that are obtained from neat polymer solution and

feed solution prepared with bitter melon extract are given as below. Figure 4.13, 4.14 and 4.15 showed the morphology and size of maltodextrin, gelatin and zein electrospun samples, respectively.

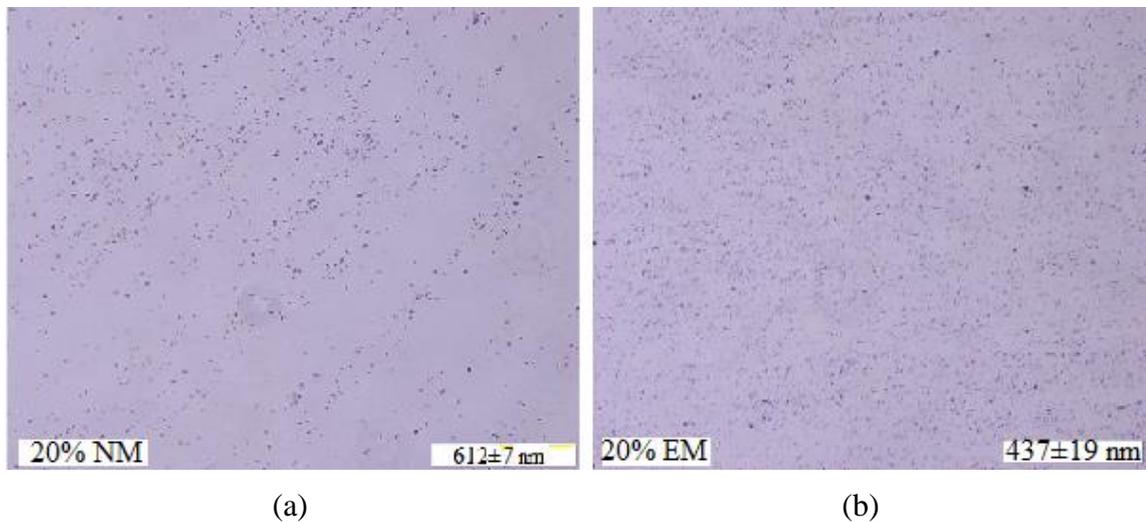


Figure 4.13 Optical images of electrospun particles: a) Electrospun particles with 612 nm obtained from 20% NM, b) Electrospun particles with 437 nm obtained from 20% EM (NM: Neat maltodextrin feed solution, EM: Maltodextrin feed solution prepared with aqueous bitter melon extract)

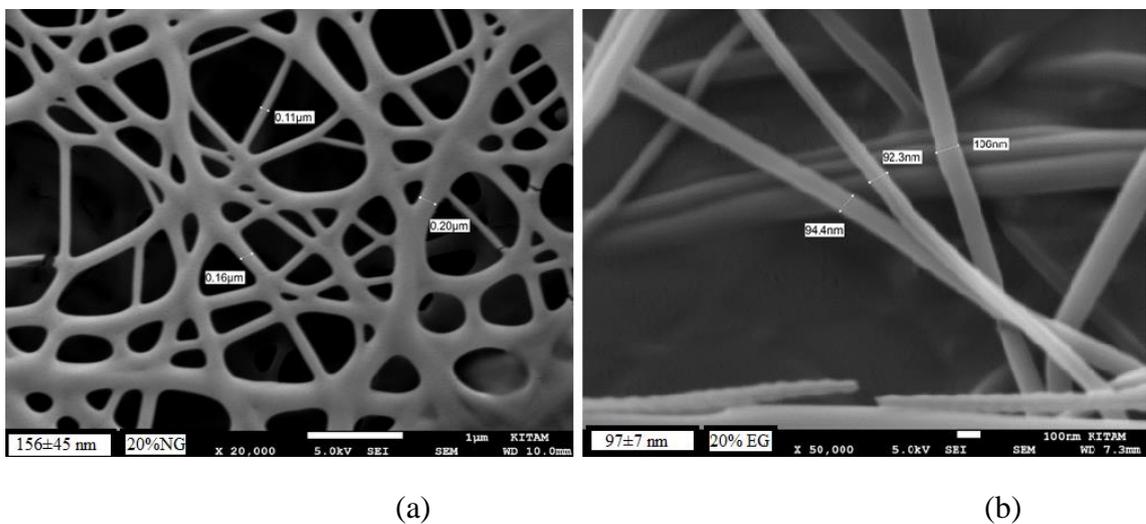
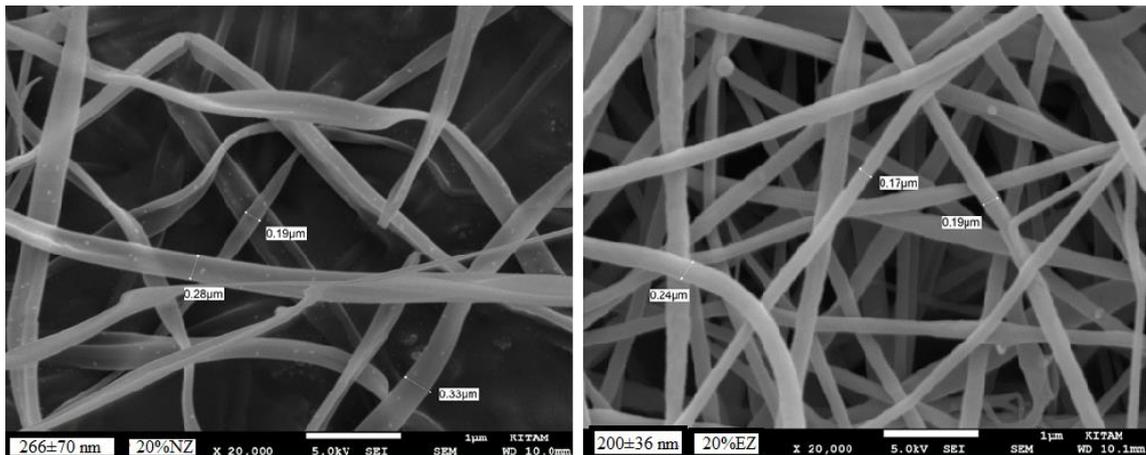


Figure 4.14 SEM images of electrospun fibers: a) Nanofibers with 156 nm size obtained from 20% NG, b) Nanofibers with 97 nm size obtained from 20% EG (NG: Neat gelatin feed solution, EG: Gelatin feed solution prepared with aqueous bitter melon extract)



(a)

(b)

Figure 4.15 SEM images of electrospun fibers: a) Electrospun fibers with 266 nm size obtained from 20% NZ, b) Electrospun fibers with 200 nm size obtained from 20% EZ (NZ: Neat zein feed solution, EZ: Zein feed solution prepared with alcoholic bitter melon extract)

Structures obtained from each polymer feed solution prepared with bitter melon extract or without are shown in figures as above. Similar results were obtained in each polymer. Nanofibers/particles diameter obtained from feed solutions prepared with bitter melon extract are smaller than diameters obtained from neat feed solutions. Low surface tension and high electrical conductivity that occurred with adding extract are shown as its reason. There are different studies and different results about electrospinning particles/fibers including bioactive components or some plant extract. Fibers diameter obtained by the addition of tannin to zein feed solution were smaller than the size of fibers obtained from only zein feed solution. Size reduction is associated with a decrease in viscosity of the solution by Mori et al. [57].

Charernsriwilaiwat et al. [93] used the mangosten fruit extract for production of chitosan nanofibers. The dimensions of the fibers increased with the increasing extract loading rate. When extract ratio was increased, viscosity and surface tension decreased but conductivity increased.

In a study nanofibers were obtained by adding *Centella asiatica* extract to gelatin solution (20% (w/v) gelatin in 70% acetic acid) in increasing concentrations (5-30% (w/w) with respect to polymer). According to the observations significant differences were not observed in the size and morphology of the fibers, depending on the ratio extracts loaded [95].

#### 4.4.2 Zeta Potential of Dispersions with Nanofibers/Nanoparticles

Investigation of electrosprayed particles and electrospun fibers surface charge is performed by calculating the zeta potential. The zeta potential is crucial in determining the stability of a suspension. [96]. Regions in where zeta potential is above +25 mV or below -25 mV value show stable regions for suspensions. Colloidal systems tend to agglomerate in case zeta potential are outside in this range [33]. If all the particles have a large negative or positive zeta potential then they will tend to repel each other, and there will be no tendency for the particles to come together, so the suspension will be stable. However, if the zeta potential is low there will be insufficient repulsion to prevent the particles coming together, so tendency for flocculation is increased [96].

Electrospinning method is thought to increase the ability of the diffusion by reducing the size until nano dimensions of the samples [45].

Zeta potential value helps us to get an idea how to behave when the samples add to aqueous food formulation [89]. In the following Figure 4.16 zeta potential of samples are provided.

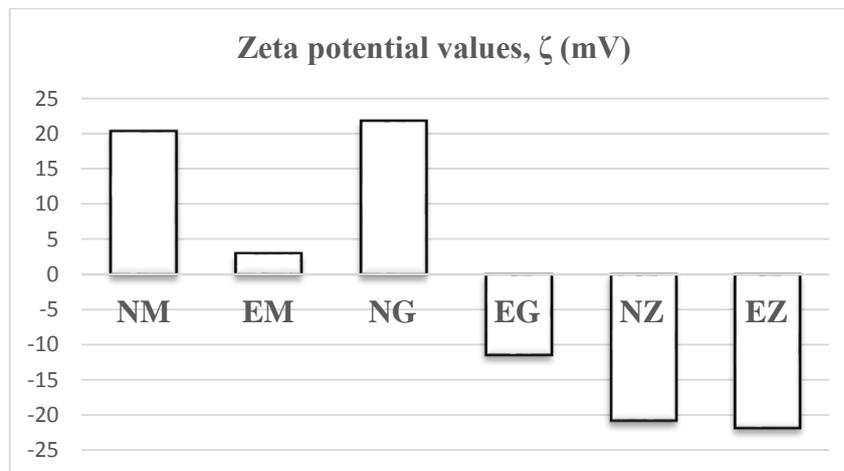


Figure 4.16 Zeta potential values of electrospinning samples

According to the Figure 4.16, the preparation of maltodextrin with bitter melon extracts caused adverse effects on stability. Indeed the degradation of maltodextrin particle within three days indicate that produced maltodextrin structures are not stable.

In our study neat gelatin fibers had positive charge but after adding bitter melon extract it turned negative electrical charge. It might be due to adding extract. Boo and al. [89] reported that while the zeta potential of the gelatin nanofibers were negative charge after electrospinning process they loaded positive. It is thought that the applied voltage is

effective on this changing. It is observed that increase in diffusion coefficient with decrease in size in this study. So the movement of the particles is easy in food formulations.

Preparation zein feed solution with extract was not a noticeable effect on the zeta potential. Accordingly, the closer value to the -25 mV belonged to the sample zein nanofibers, meaning these nanofibers may suspend in a dispersed state longer compared to the other samples.

As a result we can say that zeta potential values of the samples is outside the range of values required for stable solution described in the literature [33].

#### 4.4.3 Total Phenolic Content and Antioxidant Content of Particles/Fibers

Instead of synthetic additives the use of plant extract is becoming increasingly common while developing functional foods. Encapsulated plant extracts are used in order to produce functional foods that are positive effects on health besides contributing to nutrition [59].

Therefore phenolic and antioxidant content of micro/nano fibers and particles including bitter melon extract were examined in our study. The results obtained are shown in the following Table 4.4.

Table 4.4 Total phenolic content and antioxidant activity of electrosprayed particles and electrospun fibers

Fibers/ Particles	Total phenolic content (mg GAE/kg fiber or particles)	Total antioxidant activity (AA%)
EM	594.60±0.7	2.24±0.4
EG	8849.85±0.3	12.46±0.6
EZ	26537.54±0.9	32.90±0.5

\*NM: Neat maltodextrin feed solution, EM: Maltodextrin feed solution prepared with aqueous bitter melon extract, NG: Neat gelatin feed solution, EG: Gelatin feed solution prepared with aqueous bitter melon extract, NZ: Neat zein feed solution, EZ: Zein feed solution prepared with alcoholic bitter melon extract.

According to the Table 4.4 total phenolic content increased in direct proportion with antioxidant capacity. We can list the total phenolic content and antioxidant capacity from

high to low as EZ>EG>EM. Zein nanofibers (prepared with ethanolic bitter melon extract) had most phenolic content among the others. Because ethanol bitter melon extract had higher phenolic content than aqueous bitter melon extract or it can be thought that zein is more convenient coating material for bioactive components of bitter melon extract.

The antioxidant activity of zein nanofibers obtained from 25% polymer concentration that dissolved in 80% ethyl alcohol was determined 6% [91]. Zein nanofibers including bitter melon extract's antioxidant activity was determined as 32%.

Bitter melon extract prepared with water and ethyl alcohol were freeze-dried separately by Tan vd. powders obtained from alcoholic bitter melon extract had higher phenolic content than powders obtained from aqueous extract. The antioxidant activity were also higher in alcoholic extract similar to phenolic content [29]. Several studies have shown that bioactive compounds continue their activity after electrospinning process [97], [64].

#### **4.4.5 Thermal Analysis**

Onset temperature ( $T_o$ ), peak temperature ( $T_p$ ), end temperature ( $T_e$ ), melting temperature ( $T_m$ ), glass transition temperature ( $T_g$ ) are parameters used for DSC analysis.

In the temperature-heat flow graph, the axis point at the beginning of peak and ending of peak shows onset temperature ( $T_o$ ) and ending temperature ( $T_e$ ), respectively.  $\Delta H$  is amount of used or formed energy in the area of peaks have occurred [33]. The DSC thermograms taken in neat particles/fibers and particles/fibers with bitter melon extract.

All polymer shown endothermic peaks as reported by earlier researchers [86]. These characteristics endotherms of fibres might have often been termed as dehydration temperature ( $T_D$ ), which is due to the evaporation of bound water or volatile component from the molecules [91]. The thermogram obtained by DSC analysis are shown in Figure 4.17, 4.18 and 4.19.

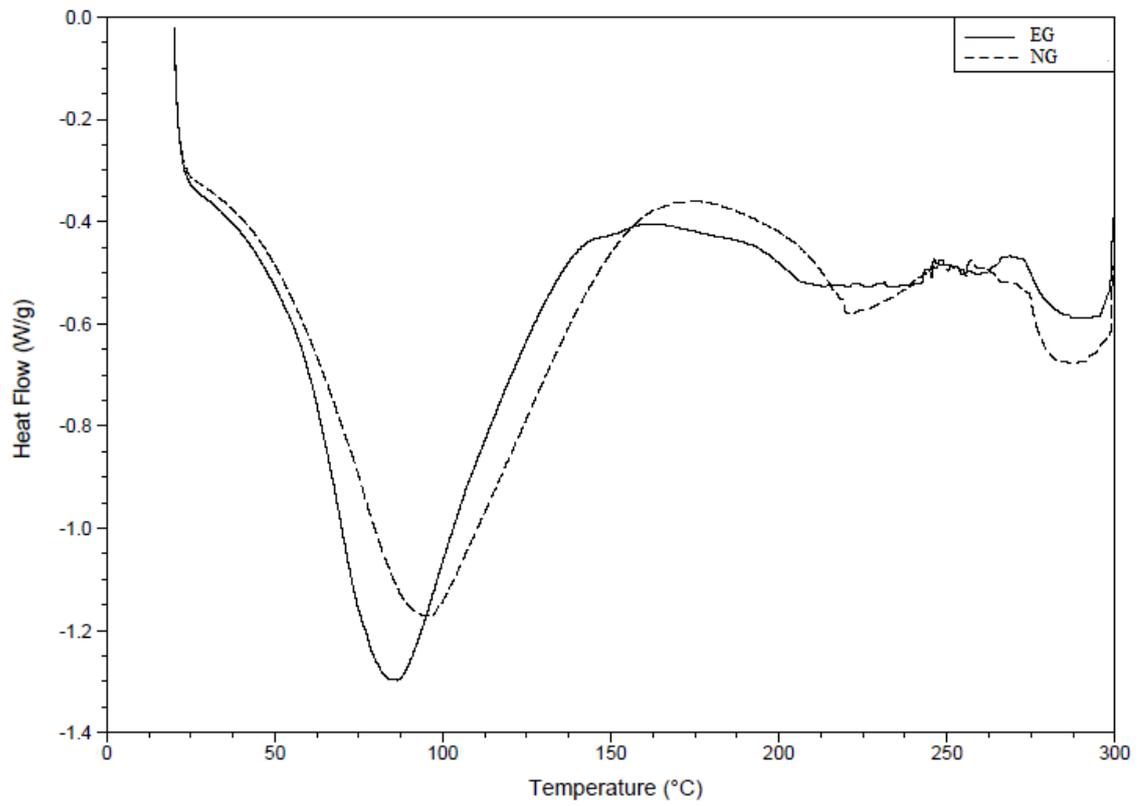


Figure 4.17 DSC thermogram of electrospun gelatin nanofibers

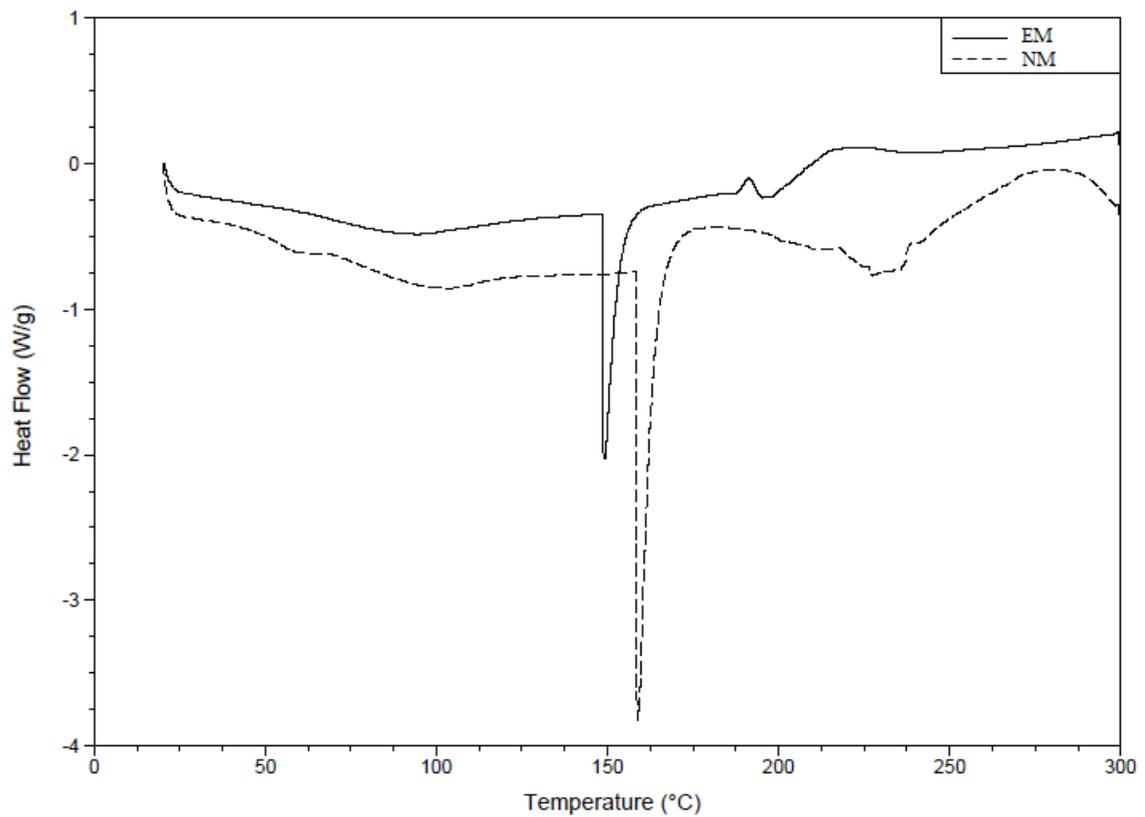


Figure 4.18 DSC thermogram of electrospayed maltodextrin particles

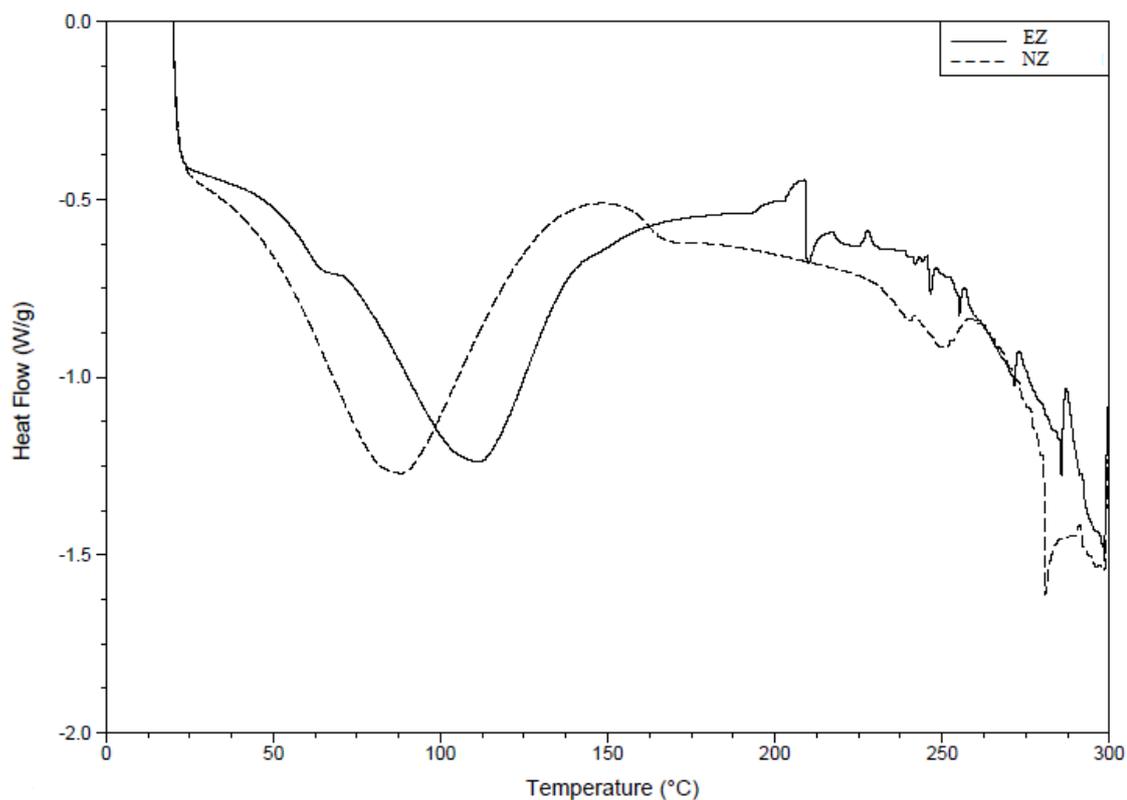


Figure 4.19 DSC thermogram of electrospun zein nanofibers

Thermal parameters obtained by DSC analysis are shown in the following Table 4.5.

Table 4.5 DSC parameters of electrospayed particles and electrospun fibers

Particles/ fibers	Thermal parameters			
	$T_0$ (°C)	$T_p$ (°C)	$T_c$ (°C)	$\Delta H$ (J/g)
NM	161.29±4.12	161.77±3.99	179.27±3.80	59.13±25.5
EM	148.48±0.0	149.13±0.0	164.37±2.02	39.08±0.9
NG	50.21±2.89	95.13±0.52	170.1±1.57	326.6±12.30
EG	46.80±5.86	84.45±0.64	143.29±10.31	237.1±66.8
NZ	41.04±0.30	86.75±0.50	144.715±1.12	249.85±15.06
EZ	56.32±1.87	109.1±0.2	167.54±3.80	278.05±36.13

When we compare the thermal stability of electrospun product, decrease in stability of maltodextrin and gelatin electrospinning products was observed with adding bitter melon extract. However the use of bitter melon extract with zein polymer increased the thermal stability. Zein has specific interesting properties in terms of water resistance, viscosity or thermal resistance as a consequence of the presence of the apolar amino acids of proline and glutamine [50]. Peak temperature of zein nanofibers increased from 86°C to 109°C with adding bitter melon extract. It could be associated with the strong interactions between the hydroxyl groups of bitter melon extract and amine groups present in zein [37].

In a study the increase in thermal stability was observed similar as the present study. Mori et al. [57] carried out generate the encapsulated tannin in zein polymer via electrospinning method. The addition of tannin increased the glass transition temperature of the nanofibers, suggesting an antiplasticizing effect of the tannin on the zein.

Gallic acid was encapsulated in zein nanofibers by Neo et al. [91]. When evaluating the thermal parameters of nanofibers, melting temperature of neat gallic acid was determined as 260 °C. A decrease in the glass transition temperature in zein fiber was observed by adding gallic acid.

Castro-Munoz et al. [98] used maltodextrin and gelatin as coating agent for encapsulation of purple cactus pear juice. The melting temperature of maltodextrin and gelatin was determined as 174.7°C and 88.74°C, respectively. According to the thermogram results the melting temperature was high due to showing harder structure. As a result of high melting point, more stable and protective fibers occurred [37], [64].

#### **4.4.6 FTIR Analysis**

In order to investigate possibility of structural change after electrospinning process, FTIR spectroscopy analysis was examined. FTIR spectra of aqueous bitter melon extract, alcoholic bitter melon extract and electrospayed particles and electrospun fibers obtained by electrospinning are shown below. FTIR spectrums of samples are shown in Figure 4.20, 4.21, 4.22, 4.23 and 4.24, respectively.

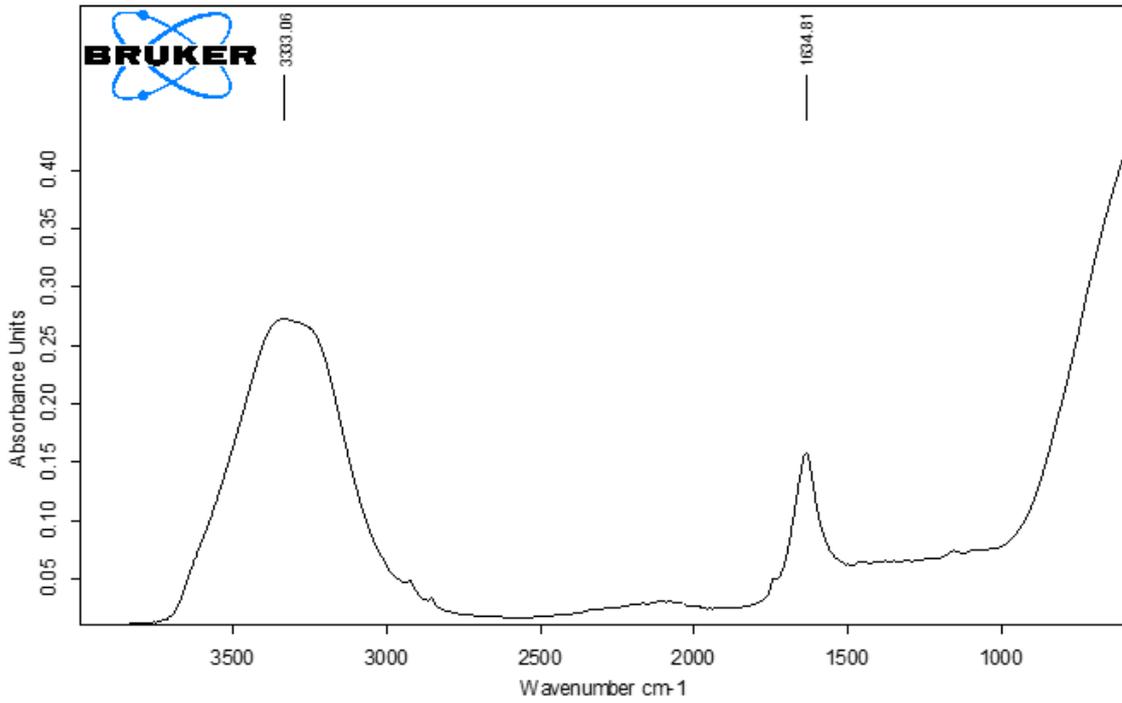


Figure 4.20 FTIR spectrum of aqueous bitter melon extract

The aqueous extract of Bitter melon showed broader band of absorption around  $3333\text{ cm}^{-1}$ , due to  $\text{-OH}$  stretching and peaks around  $1634\text{ cm}^{-1}$  were attributed to the  $\text{C=O}$  stretching vibration [99].

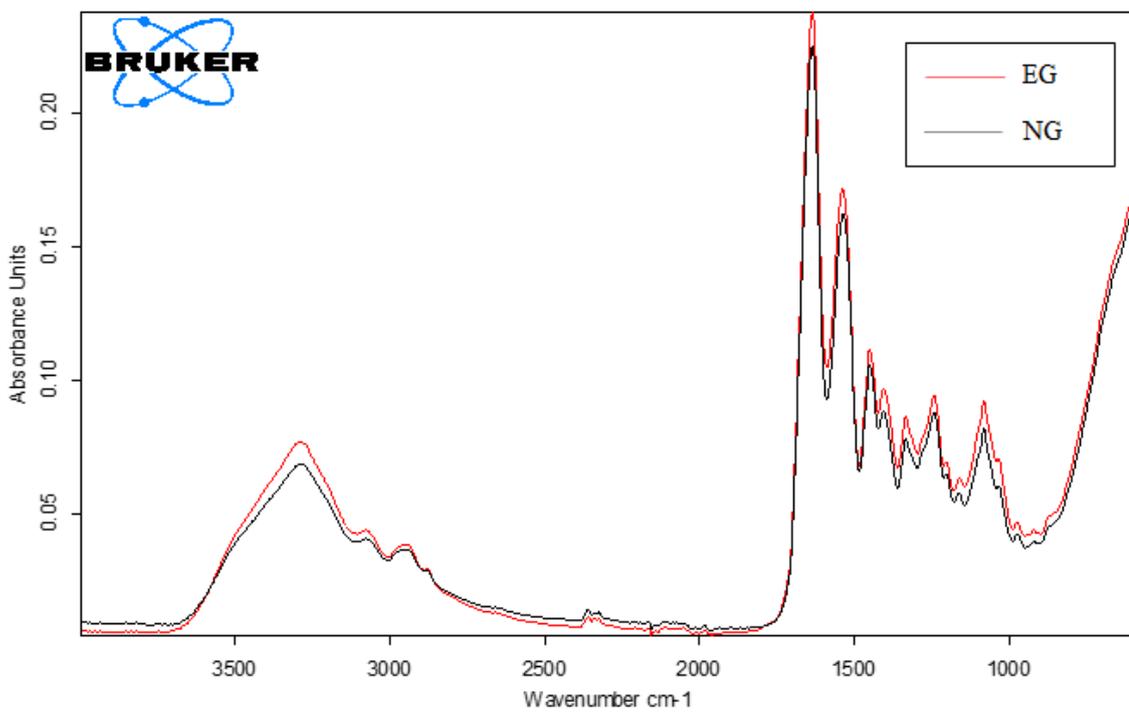


Figure 4.21 FTIR spectrum of electrospun fibers obtained from gelatin

The spectrum of gelatin nanofibers showed bands centred at  $3293\text{ cm}^{-1}$  (O-H stretching vibration),  $1635\text{ cm}^{-1}$  (C=O stretching vibration),  $1538\text{ cm}^{-1}$ , (C-N stretching vibration),  $1450\text{ cm}^{-1}$ (C-O stretching vibration) and  $1242\text{ cm}^{-1}$  (Amide III, C-N stretching), and  $1081\text{ cm}^{-1}$  (C-O-C covalent vibration ) [64].

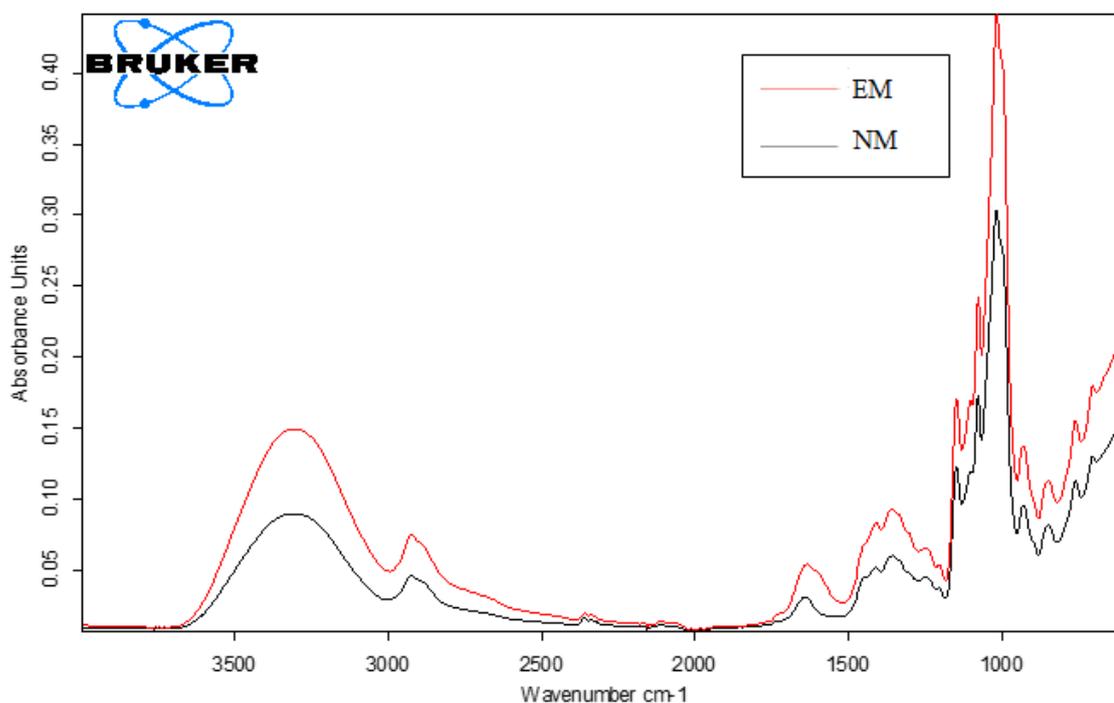


Figure 4.22 FTIR spectrum of electrospayed particles obtained from maltodextrin

The spectrum of electrospinning maltodextrin nanoparticles shows the band at  $3315$ ,  $2925$ ,  $1635$ ,  $1357$ ,  $1148$ , and  $1016\text{ cm}^{-1}$ . The characteristic peak at  $3315\text{ cm}^{-1}$  due to O-H stretching;  $2925\text{ cm}^{-1}$  due to C-H stretching vibration;  $1635$  assigned to C-O stretching and  $1357\text{ cm}^{-1}$  due to O-H bending;  $1148\text{ cm}^{-1}$  and  $1016\text{ cm}^{-1}$  due to C-O stretching [100].

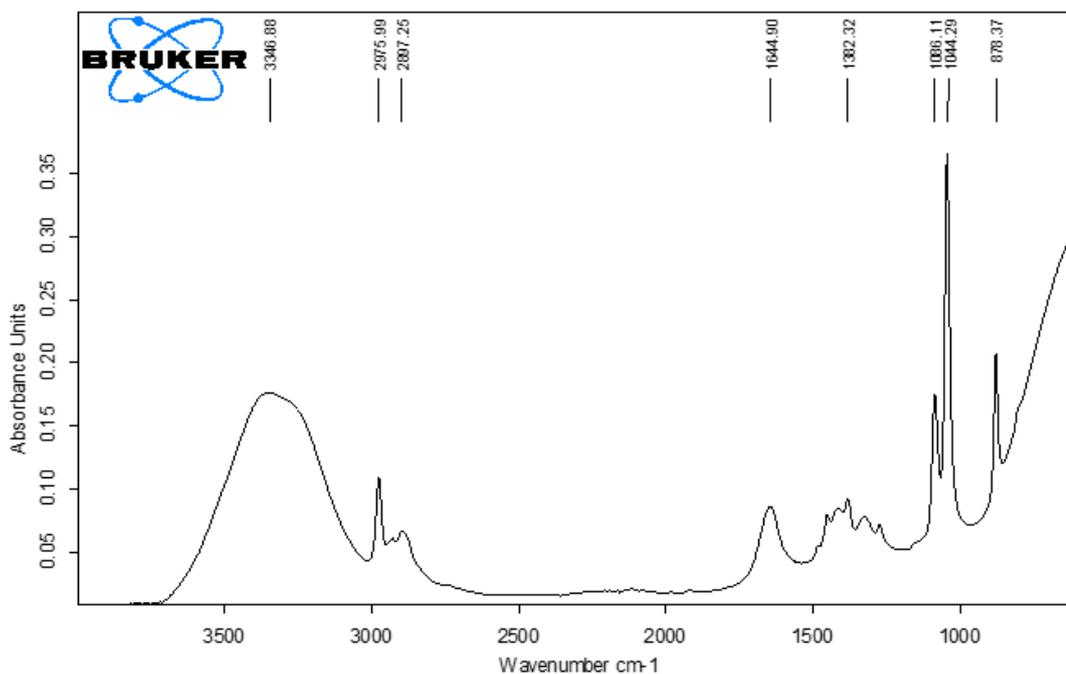


Figure 4.23 FTIR spectrum of alcoholic bitter melon extract

FTIR spectra of alcoholic extract showed the major absorption bands at 3346, 2977, 1044, 1086, 878  $\text{cm}^{-1}$ . These bands correspond to hydroxyl group (-OH) of alcohols (3346  $\text{cm}^{-1}$ ), C-H stretching vibration of alkyl groups (-CH<sub>2</sub>-) (2975  $\text{cm}^{-1}$ ), C-O-C covalent vibration of ether (-C-O-C-) groups (1044, 1086  $\text{cm}^{-1}$ ) owed to polyphenols in bitter melon extract [19], [38]. Peaks between 400-1300  $\text{cm}^{-1}$  influenced by molecular structure. All of the peaks observed in this region are specific molecules.

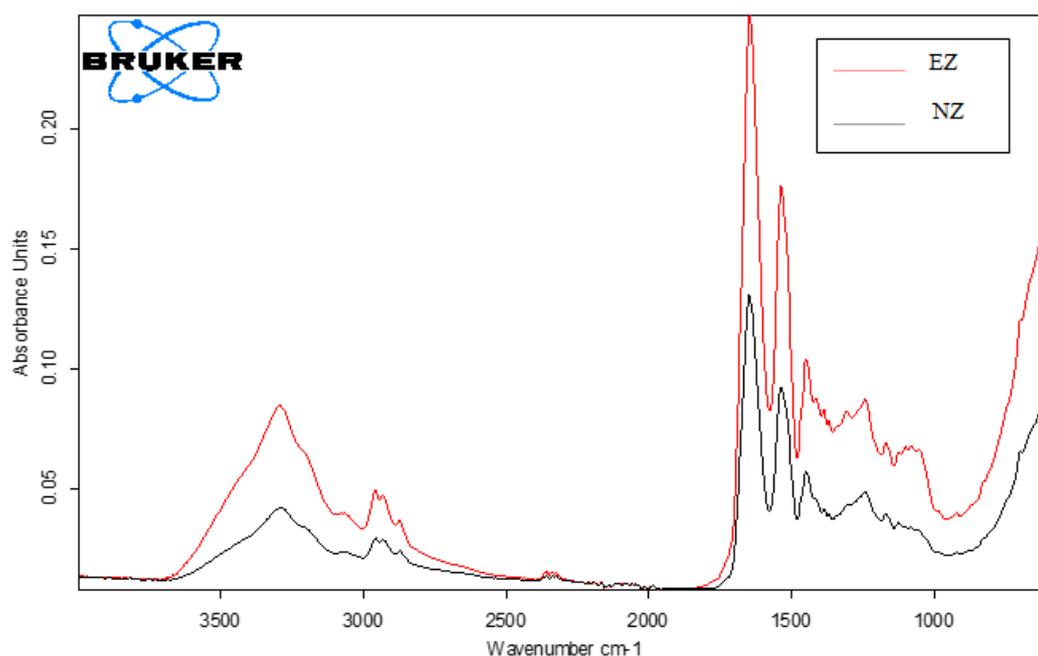


Figure 4.24 FTIR spectrum of electrospun fibers obtained from zein

Zein nanofibers has exhibited characteristic peaks at 3293  $\text{cm}^{-1}$  (N–H stretching vibrations), 2968  $\text{cm}^{-1}$ , 2873  $\text{cm}^{-1}$  and 2931  $\text{cm}^{-1}$  (C–H stretching vibrations of aliphatic groups), 1647  $\text{cm}^{-1}$  (amide I) and 1538  $\text{cm}^{-1}$  (amide II) as shown in Table 4.6. The amide I peak is due to the C=O stretch vibrations, while the amide II peak is derived from N–H bending and C–N stretching vibrations [42], [91].

It was not observed important structural change between neat polymer particles/fibers and particles/fibers prepared with bitter melon extract. Absorbance values of electrospinning samples prepared with bitter melon extract were higher. We can say that similar bonds occurred between polymers and extracts. Their intensity increased as previously reported [59], [73]. Peaks obtained from FTIR analysis and functional groups which they relate with are shown in the following Table 4.6.

Table 4.6 Functional groups

Wavelength ( $\text{cm}^{-1}$ )	Functional group	Characteristic value
3392, 3293, 3315, 3333, 3346	Hydroxyl group (-OH)	O-H stretching vibration
2924, 2925, 2957, 2958, 2975	Alkyl group (-CH <sub>2</sub> -)	C-H stretching vibration
1634, 1635, 1638, 1644, 1647, 1648	Carboxyl group (-C=O veyá -CHO)	C=O stretching vibration
1405, 1448, 1449, 1450	Carboxyl group (-COOH)	C-O stretching vibration
1334	Carboxyl group (-COOH)	C=O symmetric vibration
1241, 1242	Amide III, C-N stretching	C-N stretching
1044, 1077, 1081, 1086, 1148	Ether (-C-O-C-)	C-O-C covalent vibration
1016, 1018	Hydroxyl group (-OH)	O-H bending vibration
1535, 1538, 1539	Amid 2 (N-H)	C-N stretching vibration

Presence of glycosides, phenolics, flavonoids, alkaloids, terpenoids, carotenoids, phytochemicals in the bitter melon extract has been reported previously [13], [21], [28]. Thus, the comparison of FTIR spectra of extract and particles/fibers revealed the involvement of these functional groups in electrospinning of particles/fibers formed [19].

### CONCLUSION

Despite the use of nanotechnologies in food industry has the high potential, it is still being studied in a very limited scale. But in the last few years the world has accelerated about nanotechnology studies for the food industry. One of the potential applications of nanotechnology is also electrospinning method. With the increasing production of nano particles/fibers from biopolymers with electrospinning methods, it is likely to increase in the application in foods.

In this study micro/nano polymeric fibers and particles including bitter melon extract were obtained by electrospinning method. The features of feed solutions and micro/nano fibers and particles obtained from these feed solutions were effected in a good way adding bitter melon extract. Electrical conductivity, surface tension, rheological and thermal parameters that are effective on electrospinning method were measured. When the feed solutions prepared with bitter melon extract the electrical conductivity increased however the surface tension decreased. This was observed for all polymer types. The size of fibers and particles obtained from feed solutions including extract was in smaller and more homogeneous distribution for all polymers. The diameter of neat maltodextrin particles, neat gelatin fibers and neat zein fibers was 612 nm, 157 nm and 267 nm, respectively. With including bitter melon extract the diameter of structures decreased. The diameter of maltodextrin particles including extract, gelatin fibers including extract and zein fibers including extract was 437 nm, 97 nm and 200 nm, respectively. Among polymers nanofibers including bitter melon extract prepared with zein had the highest phenolic contents and in relation with these antioxidant capacity was determined higher. The phenolic content and antioxidant activity of zein nanofibers determined as 2.6 mg GAE/100g fiber and 32.90%, respectively. The zeta potential was measured 21.77 mV. Compared the neat zein nanofibers with adding bitter melon extract showed higher thermal stability. So zein could be used to wall material in encapsulation process. Indeed,

if nanoparticles/fibers with different functions which can be tailored by processing parameters during electrospinning will be added to foods to achieve better jobs than traditional additives or components in the food with lower amount, then it may be considered as beneficial in food industry's point of view [98]. In the future zein nanofibers including bitter melon extract could be used as additives which are very little amount compared to materials that are currently used in food industry for same functions and enhance functionality of foods. Use of bitter melon, which has restricted usage due to the bitter taste, might be increased in food formulations.

Toxicity studies of nanotechnology applications in foods need to further research. Consumers have some concerns about the use of nanotechnology applications in foods. The regulation about the nanotechnology applications in food industry should be set. At the end of these arrangements consumers will become more conscious about the use of nanomaterials in food formulations and nanotechnology applications will accelerate as predicted for food industry in the future.

## REFERENCES

---

- [1] Ghorani, B. and Tucker, N., (2015). “Fundamentals of Electrospinning as a Novel Delivery Vehicle for Bioactive Compounds in Food Nanotechnology”, *Food Hydrocolloids*, 51:227-240.
- [2] Sunguc, C., (2013). Encapsulation of *Sarcopoterim Spinosum* Extract in Zein Particle by Using Electrospray Method, Yüksek Lisans Tezi, İzmir İleri Teknoloji Enstitüsü Fen Bilimleri Enstitüsü, İzmir.
- [3] Grover, J. and Yadav, S., (2004). “Pharmacological Actions and Potential Uses of *Momordica Charantia*: a review”, *Journal of Ethnopharmacology*, 93:123–132.
- [4] Betz, M. and Kulozik, U., (2011). “Microencapsulation of Bioactive Bilberry Anthocyanins by Means of Whey Protein Gels”, *Procedia Food Science*, 1: 2047 – 2056.
- [5] Sarı, A., Oguz, B., Bilgic, A., Tort, N., Guvensen, A. and Senol, S., (2010). “Ege ve Güney Marmara Bölgelerinde Halk İlacı Olarak Kullanılan Bitkiler”, *J. of AARI*, 20(2):1-21.
- [6] Ozkan, G., Sagdic, O., Gokturk, S., Unal, O. and Albayrak, S., (2010). “Study on Cemical Composition and Biological Activities of Essential Oil and Extract from *Salvia Pisidica*”, *LWT - Food Science and Technology*, 43:186-190.
- [7] Vos, P., Faas, M., Spasojevic, M. and Sikkema, J., (2010). “Encapsulation for Preservation of Functionality and Targeted Delivery of Bioactive Food Components”, *International Dairy Journal*, 20:292-302.
- [8] Kriegel, C., Arrechi, A., Kit, K., McClements, D. and Weiss, J., (2008). “Fabrication, Functionalization, and Application of Electrospun Biopolymer Nanofibers”, *Critical Reviews in Food Science and Nutrition*, 48:775–797.
- [9] Ozkan, G. and Bilek, S., (2013). “Microencapsulation of Natural Food Colourants”, *International Journal of Nutrition and Food Sciences*, 3(3): 145-156.
- [10] Nagavarma, B V N., Hemant, K.S., Ayaz, A., Vasudha L.S., and Shivakumar, H.G.,(2012). “Different Techniques for Preparation of Polymeric Nanoparticles”, *Asian Journal of Pharmaceutical and clinical research*, 5:16-23.
- [11] Joseph, B. and Jini, D., (2013). “Antidiabetic Effects of *Momordica Charantia* (bitter melon) and its medicinal potency”, *Asian Pac J Trop Dis*, 3(2): 93-102.
- [12] Viridi, J., Sivakami, S., Shahani, S., Suthar, A., Banavalikar, M. and Biyani, M., (2003). “Antihyperglycemic Effects of Three Extracts from *Momordica Charantia*”, *Journal of Ethnopharmacology*, 88:107–111.
- [13] Horax, R., Hettiarachchy, N. and Chen, P., (2010). “Extraction, Quantification, and Antioxidant Activities of Phenolics from Pericarp and Seeds of Bitter Melons

- (*Momordica charantia*) Harvested at Three Maturity Stages (Immature, Mature, and Ripe)”, *J. Agric. Food Chemistry*, 58:4428–4433.
- [14] Akay, A. and Karaaslan, E., (2012). “Mikoriza Aşılanmış Kudret Narı (*Momordica Charantia*) Bitkisine Farklı Dozlarda Fosforlu ve Demirli Gübre Uygulamasının Yaprak Klorofil İçeriğine Etkisi”, *Iğdır Üniversite Fen Bilimleri Enstitüsü Dergisi*, 2(3):103-108.
- [15] Kenny, O., Smyth, T., Hewage, C. and Brunton, N., (2013). “Antioxidant Properties and Quantitative UPLC-MS Analysis of Phenolic Compounds from Extracts of Fenugreek (*Trigonella foenum-graecum*) Seeds and Bitter Melon (*Momordica charantia*) Fruit”, *Food Chemistry*, 141:4295–4302.
- [16] Pitipanapong, J., Chitprasert, S., Goto, M., Jiratchariyakul, W., Sasaki, M. and Shotipruk, A., (2007). “New Approach for Extraction of Charantin from *Momordica Charantia* with Pressurized Liquid Extraction”, *Separation and Purification Technology*, 52:416–422.
- [17] Leung, L., Birtwhistle, R., Kotecha, J., Hannah, S. and Cuthbertson, S., (2009). “Anti-diabetic and Hypoglycaemic Effects of *Momordica Charantia* (bitter melon): A Mini Review”, *British Journal of Nutrition*, 102:1703–1708.
- [18] Yıldız, G., Sekeroglu, N., Kulak, M. and Demirkol, G., (2015). “Antimicrobial Activity and Agricultural Properties of Bitter melon (*Momordica charantia* L.) Grown in Northern Parts of Turkey: A Case Study for Adaptation”, *Natural Product Research*, 29(6):543-545.
- [19] Ajitha, B., Reddy, Y. and Reddy, P., (2015). “Biosynthesis of Silver Nanoparticles Using *Momordica charantia* Leaf Broth: Evaluation of Their Innate Antimicrobial and Catalytic Activities”, *Journal of Photochemistry and Photobiology B: Biology*, 146:1–9.
- [20] Tuan, P., Kim, J., Park, N., Lee, S. and Park, S., (2011). “Carotenoid Content and Expression of Phytoene Synthase and Phytoene Desaturase Genes in Bitter Melon (*Momordica charantia*)”, *Food Chemistry*, 126:1686–1692.
- [21] Nagarani, G., Abirami, A. and Siddhuraju, P., (2014). “Food Prospects and Nutraceutical Attributes of *Momordica* Species: A Potential Tropical Bioresources – A review”, *Food Science and Human Wellness*, 3:117–126.
- [22] Tan, S., Parks, S., Stathopoulos, C. and Roach, P., (2013). “Greenhouse-grown Bitter Melon: Production and Quality Characteristics”, *J Sci Food Agric*, 94:1896–1903.
- [23] Lucas, E., Dumancas, G., Smith, B., Clarke, S. and Arjmandi, B., (2010). “Health Benefits of Bitter Melon (*Momordica charantia*)”, *Bioactive Foods in Promoting Health: Fruits and Vegetables*, 35:525-549.
- [24] Tan, S., Kha, T., Parks, S., Stathopoulos, C. and Roach, P., (2015). “Effects of the Spray-drying Temperatures on the Physiochemical Properties of an Encapsulated Bitter Melon Aqueous Extract Powder”, *Powder Technology*, 281:65–75.
- [25] Kubola, J. and Siriamornpun, S., (2008). “Phenolic Contents and Antioxidant Activities of Bitter Gourd (*Momordica charantia* L.) Leaf, Stem and Fruit Fraction Extracts in Vitro”, *Food Chemistry*, 110:881–890.
- [26] Semiz, A. and Sen, A., (2007). “Antioxidant and Chemoprotective Properties of *Momordica charantia* L. (bitter melon) Fruit Extract”, *African Journal of Biotechnology*, 6(3):273-277.

- [27] Horax, R., Hettiarachchy, N. and Islam, S., (2005). "Total Phenolic Contents and Phenolic Acid Constituents in 4 Varieties of Bitter Melons (*Momordica charantia*) and Antioxidant Activities of Their Extracts", *Food Chemistry and Toxicology*, 70(4):275-280.
- [28] Gürbüz, I., Akyüz, Ç., Yeşilada, E. and Şener, B., (2000). "Anti-ulcerogenic Effect of *Momordica charantia* L. Fruits on Various Ulcer Models in Rats", *Journal of Ethnopharmacology*, 71:77–82.
- [29] Tan, S., Vuong, Q., Stathopoulos, C., Parks, S. and Roach, P., (2014). "Optimized Aqueous Extraction of Saponins from Bitter Melon for Production of a Saponin-Enriched Bitter Melon Powder", *Journal of Food Science*, 79(7):1372-1381.
- [30] Choo, C., Waisundara, V. and Hoon, L., (2014). "Bittergourd (*Momordica charantia*) Scavenges Free Radicals by Enhancing the Expression of Superoxide Dismutase in Vitro Models of Diabetes and Cancer", *Journal of Food*, 12(4):378-382.
- [31] Kumru, A., (2013). Elektroüretimle Nanolif Eldesine Etki Eden Faktörlerin ve Jelatin-Pektin İçeren Nanoliflerin Model Gıdaların Reolojik Özelliklerine Etkilerinin İncelenmesi, Yüksek Lisans Tezi, İstanbul Teknik Üniversitesi, Fen Bilimleri Enstitüsü, İstanbul.
- [32] Senturk, A., Yalçın, B. and Otles, S., (2013). "Nanotechnology As A Food Perspective", *Journal of Nanomaterials & Molecular Nanotechnology*, 2:6.
- [33] Terzi, P., (2013). Elektrodöndürme Yöntemiyle Elde Edilen Jelatin Nanoliflerin Gıdalarda Kıvam Verici Olarak Kullanılması, Yüksek Lisans Tezi, İstanbul Teknik Üniversitesi, Fen Bilimleri Enstitüsü, İstanbul.
- [34] Nuansing, W., (2014). Electrosinching of Biomolecules, PhD Thesis, Univesidad del Pais Vasco, Departamento de Fisica de Materiales, Spain.
- [35] Khare, A. and Vasisht, N., (2014). Microencapsulation in the Food Industry, Chapter 14, USA.
- [36] Momin, J., Jayakumar, C., and Prajapati, J., (2013). "Potential of Nanotechnology in Functional Foods", *Food Agricultural*, 25(1):10-19.
- [37] Safak, S., (2012), Tekstilde nanolifler, Kullanım Alanları ve Nanolif Üretim Yöntemleri, [tekstil.uludag.edu.tr/seminer/semprog1213guz/nanotekno.pptx](http://tekstil.uludag.edu.tr/seminer/semprog1213guz/nanotekno.pptx), 30 Mayıs 2015.
- [38] Sener, A., (2011). Elektro Üretim Yöntemiyle Sodyum Aljinat ve Mısır Nişastası İçeren Nanolif Eldesine Etki Eden Faktörlerin İncelenmesi, Yüksek Lisans Tezi, İstanbul Teknik Üniversitesi, Fen Bilimleri Enstitüsü, İstanbul.
- [39] Tarhan, Ö., Gökmen, V. and Harsa, Ş., (2010). "Nanoteknolojinin Gıda Bilim ve Teknolojisi Alanındaki Uygulamaları", *Gıda Dergisi*, 35(3):219-225.
- [40] Dursun, S., Erkan, N. and Yeşiltaş, M., (2010). "Doğal Biyopolimer Bazlı (Biyobozonur) Nanokompozit Filmler ve Su Ürünlerindeki Uygulamaları", 4(1):50—77.
- [41] Yıkar, E. and Bölgen, N., (2014). "Elektrosinching Yöntemi ile Jelatin/kitosan Nanofiberlerin Üretimi ve Biyomimetrik Hidroksiapatit ile Kaplanması", 11. Ulusal Kimya Mühendisliği Kongresi, 2-5 Eylül, Eskişehir.
- [42] Frenot, A. and Chronakis, I., (2003). "Polymer Nanofibers Assembled by Electrosinching", *Current Opinion in Colloid and Interface Science*, 48:64-75.

- [43] Chen, L., Bai, G., Yang, R., Zang, J., Zhou, T. and Zhao, G., (2014). “Encapsulation of  $\beta$ -carotene within Ferritin Nanocages Greatly Increases Its Water-Solubility and Thermal Stability”, *Food Chemistry* 149:307–312.
- [44] Altinkut, A., Talas, T., Yavuz, H., Denizli, A. and Memon, A., (2008). “Bitkilere Gen Aktarımı”, *Tübitak Dergisi*, Ocak:12-15.
- [45] Okutan, N., (2013). Elektrodöndürme Yöntemiyle Elde Edilen Jelatin Nanoliflerinin Emülsiyonlarda Stabilize Edici Olarak Kullanılması, Yüksek Lisans Tezi, İstanbul Teknik Üniversitesi, Fen Bilimleri Enstitüsü, İstanbul.
- [46] Gokmen, S., Palamutoğlu, R. And Sarıçoban, C., (2012). “Gıda Endüstrisinde Enkapsülasyon Uygulamaları”, *Gıda Teknolojileri Elektronik Dergisi*, 7:36-50.
- [47] Koc, M., Sakin, M. and Kymak, F., (2010). “Mikroenkapsülasyon ve Gıda Teknolojisinde Kullanımı”, *Pamukkale Üniversitesi Mühendislik Bilimleri Dergisi*, 16(1):77-86.
- [48] Nedovic, V., Kalusevic, A., Manojlovic, A., Levic, S. and Bugarski, B., (2011). “An Overview of Encapsulation Technologies for Food Applications”, *Procedia Food Science* 1:1806 – 1815.
- [49] Zaidel, D., Sahat, N., Jusoh, Y. and Muhamad, I., (2014). “Encapsulation of Anthocyanin from Roselle and Red Cabbage for Stabilization of Water-in-Oil Emulsion”, *Agriculture and Agricultural Science Procedia*, 2:82-89.
- [50] Fernandez, A., Torres-Giner, S. and Lagaron, J., (2009). “Novel Route to Stabilization of Bioactive Antioxidants by Encapsulation in Electrospun Fibers of Zein Prolamine”, *Food Hydrocolloids*, 23:1427–1432
- [51] Anu Bhushani, J. and Anandharamakrishnan, C., (2014). “Electrospinning and Electrospinning Techniques: Potential Food Based Applications”, *Trends in Food Science & Technology* 38:21-33.
- [52] Coronel-Aguilera, C. and Martín-González, M., (2014). “Encapsulation of Spray Dried  $\beta$ -carotene Emulsion by Fluidized Bed Coating Technology”, *LWT-Food Science and Technology*, 62:187-193.
- [53] Derman, S., Kızılbey, K. and Akdeste, Z., (2013). “Polimerik Nanopartiküller”, *Mühendislik ve Fen Bilimleri Dergisi*, 31:109-122.
- [54] Ramakrishna, S., Fujihara, K., Teo, T., Yong, T., Ma, Z. and Ramaseshan, R., (2006). “Electrospun Nanofibers: Solving Global Issues”, *Materials Today*, 3(9): 40-50.
- [55] Kong, L. and Ziegler, G., (2014). “Fabrication of Neat Starch Fibers by Electrospinning”, *Food Hydrocolloids* 36:20-25.
- [56] Balaban, G., Nanoliflerin Elektrosppining Yöntemiyle Üretiminde Bazı Uygulamalar İçin Özel Tekniklerin Ve Polimer Karışımların İncelenmesi, [www.gso.org.tr/userfiles/file/7%20Nanoliflerin%20Elektrosppining%20Y](http://www.gso.org.tr/userfiles/file/7%20Nanoliflerin%20Elektrosppining%20Y), 03.05.2015
- [57] Mori, C., Passos, N., Oliveira, J., Mattoso, L., Mori, F., Carvalho, A., Fonseca, A., and Tonoli, G., (2014). “Electrospinning of Zein/tannin Bio-nanofibers”, *Industrial Crops and Products*, 52:298-304.
- [58] Fantini, D. and Costa, L., (2008). “Dye, Fluorophores and Pigment Coloration of Nanofibers Produced by Electrospinning”, *Polymer Advanced Technology*, 20:111-121.

- [59] Wang, S., Marcone, M., Barbut, S. and Lim, L., (2013). "Electrospun Soy Protein Isolate-Based Fiber Fortified with Anthocyanin-Rich Red Raspberry (*Rubus strigosus*) Extracts", *Food Research International*, 52:467-472.
- [60] Üstündağ, G. and Karaca, E., (2009). "Poli (Vinil Alkol)/Sodyum Aljinat Karışımlarından Elektroçekim Yöntemi ile Elde Edilen Nanolifli Yüzeylerin İncelenmesi", *Uludağ Üniversitesi Mühendislik-Mimarlık Fakültesi Dergisi*, 14(1):159-172.
- [61] Nieuwland, N., Geerdink, P., Brier, P., Eijnden, P., Henket, J., Langelaan, M., Stroeks, N., Deventer, H. and Martin, A., (2014). "Reprint of Food-Grade Electrospinning of Proteins", *Innovative Food Science and Emerging Technologies* 24:138–144.
- [62] Badıllı, U. and Tarımcı, N.,(2009). "Elektropüskürtme Yöntemi ve Nanoteknolojideki Uygulamaları", *Ankara Eczacılık Fakültesi Dergisi*, 38(2):117-135.
- [63] Gomez-Estaca, J., Balaguer, M., Gavara, R. and Hernandez-Munoz, P., (2012). "Formation of Zein Nanoparticles by Electrohydrodynamic Atomization: Effect of the Main Processing Variables and Suitability for Encapsulating the Food Coloring and Active Ingredient Curcumin", *Food Hydrocolloids*, 28:82-91.
- [64] Gómez-Mascaraque, L., Lagarón, J. and López-Rubio, A., (2015). "Electrosprayed Gelatin Submicroparticles as Edible Carriers for the Encapsulation of Polyphenols of Interest in Functional Foods", *Food Hydrocolloids*, 49:42-52.
- [65] Ding, B., Wang, M., Wang, X., Yu, J. and Sun, G., (2010). "Electrospun Nanomaterials for Ultrasensitive Sensors, *Materials Today*, 13(11):16-27.
- [66] López-Rubio, A. and Lagaron, J., (2012). "Whey Protein Capsules Obtained Through Electrospinning for the Encapsulation of Bioactives", *Innovative Food Science and Emerging Technologies*, 13:200–206.
- [67] Kulkarni, A., Bambole, V. and Mahanwar, P., (2010). "Electrospinning of Polymers, Their Modeling and Applications", *Polymer-Plastics Technology and Engineering*, 49(5):427-441.
- [68] Miyoshi, T., Toyohara, K. and Minematsu, H., (2005). "Preparation of Ultrafine Fibrous Zein Membranes via Electrospinning", *Polymer International*, 54:1187–1190.
- [69] Erencia, M., Cano, F., Tornero, J., Macanás, J. and Carrillo, F., (2014). "Resolving the Electrospinnability Zones and Diameter Prediction for the Electrospinning of the Gelatin/Water/Acetic Acid System", *Langmuir*, 30:7198–7205.
- [70] Stijnman, A., Bodnar, I. and Tromp, R., (2011). "Electrospinning of Food-grade Polysaccharides", *Food Hydrocolloids*, 25:1393-1398.
- [71] Pérez-Masiá, R., Lagaron, J. and López-Rubio, A., (2014). "Surfactant-aided Electrospinning of Low Molecular Weight Carbohydrate Polymers from Aqueous Solutions", *Carbohydrate Polymers*, 101:249– 255.
- [72] Reneker, D. and Yarin, A., (2008). "Electrospinning Jets and Polymer Nanofibers", *Polymer*, 49:2387-2425.
- [73] López-Rubio, A., Sanchez, E., Wilkanowicz, S., Sanz, Y. and Lagaron, C., (2012). "Electrospinning as a Useful Technique for the Encapsulation of Living Bifidobacteria in Food Hydrocolloids", *Food Hydrocolloids*, 28:159-167.

- [74] Eltayeb, M., Bakhshi, P., Stride, E. and Edirisinghe, M., (2013). "Preparation of Solid Lipid Nanoparticles Containing Active Compound by Electrohydrodynamic Spraying", *Food Research International*, 53:88–95.
- [75] Wojciechowski, K. and Klodzinska, E., (2015). "Zeta Potential Study of Biodegradable Antimicrobial Polymers", *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 483:204-208.
- [76] Singleton, L. and Rossi, J., (1965). "Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents", *American Journal of Enology and Viticulture*, 3:144-158.
- [77] Singh, R., Murthy, K.N. and Jayabrahkasha, G., (2002). "Studies on the Antioxidant Activity of Pomegranate (*Punica granatum*) Peel and Seed Extracts Using in Vitro Models", *Journal of Agricultural and Food Chemistry*, 50:81–86.
- [78] Lian, Y., Zhan, J., Zhang, K. and Mo, X., (2014). "Fabrication and Characterization of Curcumin-loaded Silk Fibroin/P(LLA-CL) Nanofibrous Scaffold", *Frontiers of Materials Science*, 4: 354–362.
- [79] Huang, Z., Zhang, Y., Ramakrishna, S. and Lim, C., (2004). "Electrospinning and Mechanical Characterization of Gelatin Nanofibers", *Polymer*, 45:5361–5368.
- [80] Kayaci, F. and Uyar, T., (2012). "Electrospun Zein Nanofibers Incorporating Cyclodextrins", *Carbohydrate Polymers*, 90:558– 568.
- [81] Hu, D., Lin, C., Liu, L., Li, S. and Zhao, Y., (2012). "Preparation, Characterization, and in Vitro Release Investigation of Lutein/zein Nanoparticles via Solution Enhanced Dispersion by Supercritical Fluids", *Journal of Food Engineering*, 109:545-552.
- [82] Torres-Giner, S., Gimeez, E. and Lagaron, J.M., (2008). "Characterization of the Morphology and Thermal Properties of Zein Prolamine Nanostructures Obtained by Electrospinning", *Food Hydrocolloids*, 22:601-614.
- [83] Lu, W., Ma, M., Xu, H., Zhang, B., Cao, X. and Guo, Y., (2015). "Gelatin nanofibers prepared by spiral-electrospinning and cross-linked by vapor and liquid phase glutaraldehyde", *Materials Letters*, 140:1-4.
- [84] Moon, S. and Farris, R.,(2009). "Electrospinning of Heated Gelatin-Sodium Alginate-Water Solutions", *Polymer Engineering and Science*, 49:1616-1620.
- [85] Zhang, Y., Venugopal, J., Huang, Z., Lim, C., ve Ramakrishna, S., (2006). "Crosslinking of the Electrospun Gelatin Nanofibers", *Polymer*, 47:2911–2917.
- [86] Aceituno-Medina, M., Lopez-Rubio, A., Mendoza, S. and Lagaron, J., (2013). "Development of Novel Ultrathin Structures Based in Amaranth (*Amaranthus hypochondriacus*) Protein Isolate Through Electrospinning", *Food Hydrocolloids*, 31:289-298.
- [87] Okutan, N., Terzi, P. and Altay, F., (2014). "Affecting Parameters on Electrospinning Process and Characterization of Electrospun Gelatin Nanofibers", *Food Hydrocolloids* 39:19-26.
- [88] Wu, S. and Ng, L., (2008). "Antioxidant and Free Radical Scavenging Activities of Wild Bitter Melon (*Momordica charantia* Linn. var. *abbreviata* Ser.) in Taiwan", *LWT-Food Science and Technology*, 41:323–330.

- [89] Boo, H., Hwang, S., Bae, C., Park, S., Heo, B. and Gorinstein, S., (2012). “Extraction and Characterization of Some Natural Plant Pigments”, *Industrial Crops and Products*, 40:129– 135.
- [90] Budrat, P. and Shotipruk, A., (2009). “Enhanced Recovery of Phenolic Compounds from Bitter Melon (*Momordica charantia*) by Subcritical Water Extraction”, *Separation and Purification Technology*, 66:125–129.
- [91] Neo, Y., Ray, S., Jin, J., Nikolaidis, M., Nieuwoudt, M., Liu, D. and Quek, S., (2013). “Encapsulation of Food Grade Antioxidant in Natural Biopolymer by Electrospinning Technique: A Physicochemical Study Based on Zein–Gallic Acid System”, *Food Chemistry*, 136:1013-1021.
- [92] Torres-Giner, S., Ocio, M. and Lagaron, J., (2009). “Novel Antimicrobial Ultrathin Structures of Zein/chitosan Blends Obtained by Electrospinning”, *Carbohydrate Polymers*, 77:261-266.
- [93] Charernsriwilaiwat, N., Rojanarata, T., Ngawhirunpat, T., Sukma, M. and Opanasopit, P., (2013). “Electrospun Chitosan-based Nanofiber Mats Loaded with *Garcinia Mangostana* Extracts Natthan Charernsriwilaiwat”, *International Journal of Pharmaceutics*, 452:333-343.
- [94] Ki, C., Baek, D., Gang, K., Lee, K., UM, I. and Park, Y., (2005). “Characterization of Gelatin Nanofiber Prepared from Gelatin–Formic Acid Solution”, *Polymer*, 46:5094-5102.
- [95] Sikareepaisan, P., Suksamrarn, A., and Supaphol, P., (2008). “Electrospun Gelatin Fiber Mats Containing a Herbal—*Centella Asiatica*—Extract and Release Characteristic of asiaticoside”, *Nanotechnology*, 19:1-10.
- [96] Hendessi, S., (2014). Utilization of Electrospun Nanofibers Containing Gelatin or Gelatin-Cellulose Acetate for Preventing Syneresis in Tomato Ketchup, M.Sc. Thesis, Istanbul Technical University, Science Institute, Istanbul.
- [97] G´omez-Estaca, J., Gavara, R. and Hern´andez-Mu˜noz, P., (2015). “Encapsulation of Curcumin in Electrospayed Gelatin Microspheres Enhances its Bioaccessibility and Widens its Uses in Food Applications”, *Innovative Food Science and Emerging Technologies*, 1288:1-29.
- [98] Castro-Munoz, R., Barragan-Huerta, B., and Yanez-Fernandez, J., (2014). “Use of Gelatin-Maltodextrin Composite as an Encapsulation Support for Clarified Juice From Purple Cactus Pear (*Opuntia stricta*)”, *LWT - Food Science and Technology*, 62:242-248.
- [99] Li, J., Wang, Y., Huang, J., Xu, X. and Xiang, C., (2010). “Characterization of Antioxidant Polysaccharides in Bitter Gourd (*Momordica charantia* L.) Cultivars”, *Journal of Food, Agriculture & Environment*, 8:117-120.
- [100] Wu, Y., Zou, L., Mao, J., Huang, J., and Liu., S., (2014). “Stability and Encapsulation Efficiency of Sulforaphane Microencapsulated by Spray Drying”, *Carbohydrate Polymers*, 102:497-503.

## CURRICULUM VITAE

---

### PERSONAL INFORMATION

**Name Surname** : Ayşegül BEŞİR  
**Date of birth and place** :05.05.1990 - Trabzon  
**Foreign Languages** : English  
**E-mail** : aysegulbesir@gmail.com

### EDUCATION

<b>Degree</b>	<b>Department</b>	<b>University</b>	<b>Date of Graduation</b>
Undergraduate	Food Engineering	Ondokuz Mayıs University	2013
High School	Mathematics	Kanuni Anatolian High School	2008

### WORK EXPERIENCE

<b>Year</b>	<b>Corporation/Institute</b>	<b>Enrollment</b>
2015-Continuing	Ondokuz Mayıs University	Research asistant

## **PUBLISHERMENTS**

### **Conference Papers**

1-Beşir, A., Toker, O., Durak, M.Z. and Kahyaoğlu, T., (2015). “Nano- and Microencapsulation of Honey by Electrospinning Technique”, 1. Uluslararası Kovandan Sofraya Arı Ürünleri Kongresi, 21-22 Mayıs 2015, İstanbul.

2-Beşir, A., Özçelik, G., Bozkurt, F. and Yılmaz, M.T., (2015). “Dynamic Rheological Properties of Kuymak”, The 3<sup>rd</sup> International Symposium on ‘Traditional Foods from Adriatic to Caucasus’ October 1-4, Sarajevo.

3-Beşir, A. ve Kahyaoğlu, T., (2016). “Formation of Functional Particles-Fibers Using Bitter Melon Extract via Electrospinning Method”, 2nd Food Structure and Functionality Forum Symposium - From Molecules to Functionality, 28 February – 2 March, 2016, Singex, Singapore. (Poster accepted).

### **Projects**

1. 5130023 Production of Natural Food Dyes From Tulip Petals (Tubitak Scholarship, 2014-2015).