Encapsulation of Carotenoids Extracted from Tomato Peels into Nanofibers by Electrospinning Method to Develop their Efficiency and Stability

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Tuğba İNANÇ HORUZ

ABSTRACT

ENCAPSULATION OF CAROTENOIDS EXTRACTED FROM TOMATO PEELS INTO NANOFIBERS BY ELECTROSPINNING METHOD TO DEVELOP THEIR EFFICIENCY AND STABILITY

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In this study, it was aimed to encapsulate the carotenoids, which had been extracted from tomato peels, into zein and gelatin polymers by electrospinning technique. Firstly, the process parameters of electrospinning were optimized for each polymer to obtain the thinnest and homogeneous distributed fibers by electrospinning process. Secondly, the tomato peel extract (TPE) was encapsulated into gelatin and zein fibers by using these optimum conditions. According to DSC results, thermal stability of the extract was improved by nanoencapsulation. FTIR spectra showed that the TPE could be compatibly entrapped into the electrospun fibers. Compared to non-encapsulated extract, the encapsulated one had better retention of lycopene and antioxidant activity during 14-days storage. Moreover, antioxidant activity of the extract increased about 10-fold by nanoencapsulation. More interestingly, the solubility of TPE in water was highly enhanced when it was encapsulated into gelatin nanofibers. Thirdly, the encapsulated TPE within gelatin and zein nanofibers applied in yogurt as a model food to investigate their effect on the antioxidant and physicochemical properties of yogurt during storage at 4°C. The yogurt samples enriched with TPE had closer characteristics to control sample in terms of pH, acidity, syneresis, and viscosity parameters. The incorporation of TPE-loaded fibers resulted in increasing of the free radical scavenging activity of yogurt by 40-60%. The yogurts enriched with TPE-loaded fibers were redder, darker, and more yellow than control yogurt. Overall, it was realized that nanoencapsulation by electrospinning is an effective way to stabilize carotenoids and improve their functional properties and it is promising to use them in food processing. However, future toxicological studies are needed to establish the potential adverse effects of nanotechnology on human health and environment.

Key words: electrospinning, nanoencapsulation, carotenoids, tomato peel, gelatin, zein

ÖZET

DOMATES KABUKLARINDAN ELDE EDİLEN KAROTENOİDLERİN ELEKTROSPİN YÖNTEMİYLE NANOLİFLERE ENKAPSÜLE EDİLEREK ETKİNLİK VE STABİLİTELERİNİN GELİŞTİRİLMESİ

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Bu çalışmada domates kabuklarından elde edilen karotenoidlerin jelatin ve zein polimerlerine elektrospin yöntemiyle enkapsüle edilmesi amaçlanmıştır. İlk olarak, elektrospin işleminde kullanılan faktörler, en düzgün, homojen dağılımlı ve küçük çaptaki lifler elde edilecek şekilde zein ve jelatin için ayrı ayrı optimize edilmiştir. İkinci olarak, domates kabuğundan elde edilen karotenoid ekstraktları optimum koşullar kullanılarak elektrospin yöntemiyle jelatin ve zein liflerine enkapsüle edilmiştir. DSC sonuçlarına göre, nanoenkapsülasyonla ekstraktın termal stabilitesi arttırılmıştır. FTIR spektralar domates kabuğu ekstraktının (DKE) liflere uyumlu bir şekilde hapsedildiğini göstermiştir. Enkapsüle edilmemiş ekstraktla kıyaslandığında, 14 günlük depolama süresince enkapsüle edilmiş ekstrakt daha iyi antioksidan aktivitesi ve likopen alıkonulması özellikleri göstermiştir. Dahası, nanoenkapsülasyon ile ekstraktın antioksidan aktivitesi 10 kat artmıştır. Daha ilginç olarak, DKE jelatin nanoliflerine enkapsüle edildiğinde suda çözününürlüğü hayli iyileşmiştir. Üçüncü olarak, nanoliflere enkapsüle edilmiş DKE 4°C de depolama süresince antioksidan ve fiziko-kimyasal etkilerini araştırmak amacıyla model gıda olarak yoğurda uygulanmıştır. DKE yüklü liflerce zenginleşmiş yoğurt kontrol örneklerle yakın pH, asitlik, sineresis ve viskozite özellikleri göstermiştir. DKE yüklü lifler yoğurdun serbest radikal süpürücü aktivitesini %40-60 civarında arttırmıştır. Ekstrakt yüklü liflerce zengin yoğurtta kontrol örneğine kıyasla daha fazla kırmızılık, koyuluk ve sarılık görülmüştür. Sonuç olarak, electrospin yöntemiyle nanoenkapsülayonun karotenoidlerin stabilizasyonu ve fonksiyonel özelliklerinin iyileştirilmesi hususunda etkili bir yol olduğu anlaşılmıştır ve bunların gıdalarda kullanımı umut vaadedicidir. Bununla beraber, nanoteknolijinin insan sağlığı ve çevre üzerindeki potansiyel etkilerinin ortaya konması için geleceğe yönelik toksikolojik çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: elektrospin, nanoenkapsülasyon, karotenoidler, domates kabuğu, jelatin, zein

To my love and son

Erhan and Kerem Tahir

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NOMENCLATURE

AA	Antioxidant Activity
ATR-FTIR	Attenuated Total Reflectance-Fourier Transform Infrared
DPPH	1,1-Diphenyl-2-Picrylhydrazil
DSC	Differential Scanning Calorimetry
EE	Encapsulation Efficiency
EtOH	Ethanol
HAc	Acetic Acid
HPLC	High Performance Liquid Chromatography
LC	Loading Capacity
MTBE	Methyl Tert-Butyl Ether
RSA	Radical Scavenging Activity
SEM	Scanning Electron Microscope
S/N	Signal-to-Noise
T_g	Glass Transition Temperature
TPE	Tomato Peel Extract
TPE-Ge	TPE-Loaded Gelatin Nanofibers
TPE-Ze	TPE-Loaded Zein Nanofibers

CHAPTER 1: LITERATURE REVIEW

1.1 Nanofibers and electrospinning

A fiber having a diameter below 100 nm may be defined as a nanofiber. The diameter of a single human hair is about 100,000 nm (Figure 1.1), a typical atom is 1/5 nm in diameter and viruses vary from 25 nm to 300 nm. Nanofibers have special properties such as very large surface-to-volume ratios, and small pore size with high porosity (Huang et al., 2003). These properties make nanofibers ideal candidates for many innovative applications such as drug delivery, filter media, tissue engineering, and wound dressing. Although there are various techniques to fabricate nanofibers, electrospinning is the most common technique because of its advantages. The important advantages of the electrospinning technique are that it is relatively simple, versatile and not expensive to produce nanofibers from a wide variety of polymers (Ramakrishna et al., 2006). Other advantages are the ability to control the fiber diameters, high aspect ratio, and pore size as non-woven fabrics.

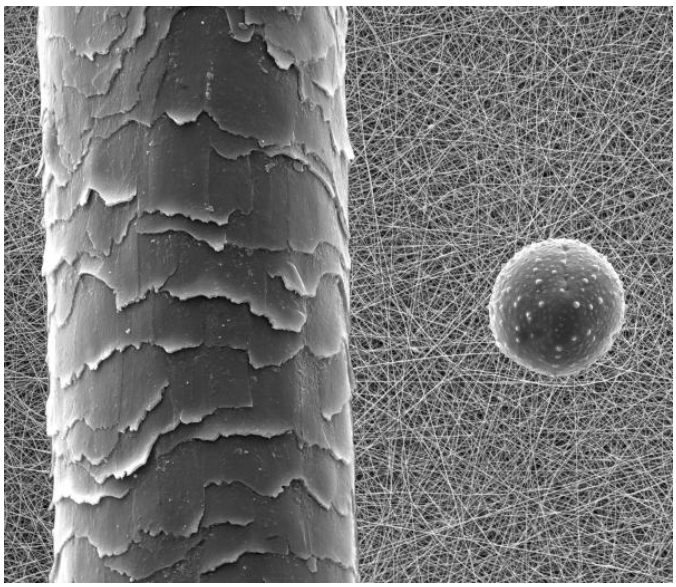


Figure 1.1 Comparison of the scale of human hair, pollen, and nanofibers

1.2 Electrospinning mechanism

Electrospinning has been considered as a significant breakthrough and a promising technology that produces continuous polymer fibers with nanometer sizes. The fundamental idea of this process dates back to more than 80 years ago; however, the first 'electrospinning' term was introduced in 1994. It was Formhals (1934) who first published the patent related to the set-up needed to produce polymeric filaments by means of electrostatic forces in 1934 and then published a number of patents related to this field. Since 1980s there has been an increase in the attention paid by researchers to this method due to increase in interest in nanotechnology.

For a basic electrospinning is shown in Figure 1.2. There are three main components to conduct the process: a syringe with a metal spinneret of small diameters, a high voltage supplier, and a collector. The spinneret is usually attached to the positive electrode, while the counter electrode (usually grounded) is attached to the collector. During the electrospinning process, a high voltage power supply created an electrically charged jet of polymer fluids. By increasing voltage applied the shape of droplet at the end of the tip of the needle changes from hemispherical shape into a conical shape, which is known as the Taylor cone. The charge of the polymer droplet overcomes its surface tension by applying a critical voltage to the spinneret and causes the polymer solution to eject from the spinneret to the collector (Figure 1.3). The electrical forces stretch and thin the jets by very large ratios. The solvent finally evaporates as the polymer jet travels in the air towards the collector and results in the solidification of jet, resulting in a fibrous mat on the collector (Doshi and Reneker, 1995; Reneker and Chun, 1996; Supaphol et al., 2005; Carroll, and Joo, 2006).

1.3 Factors affecting electrospinning process

The electrospinning process can be operated and altered by a number of variables. The parameters are generally classified as polymer solution properties, controlled process parameters, and ambient parameters. Solution properties include the conductivity, viscosity, surface tension, concentration, and polymer molecular weight. Change in one parameter may results in the change in the other solution properties, therefore it is difficult to isolate them. Controlled process variables include the applied voltage, flow

rate, collector, distance between tip and collector, and spinneret geometry. Ambient parameters include humidity, temperature, and air velocity.

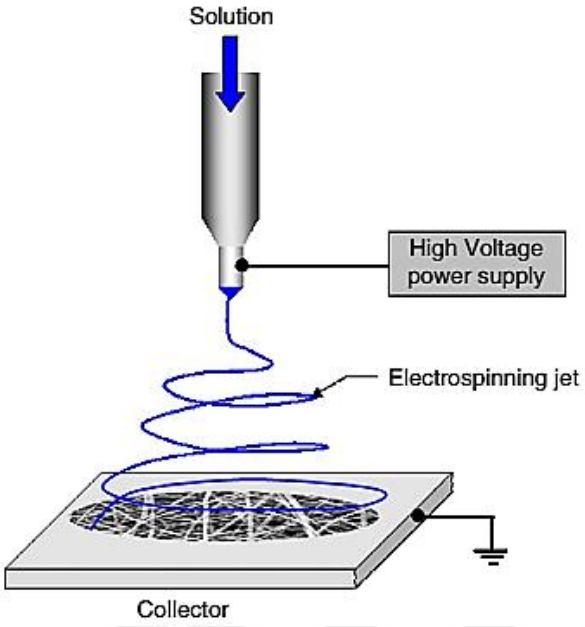


Figure 1.2 A typical electrospinning set-up using a grounded static collector (Teo and Ramakrishna, 2006)

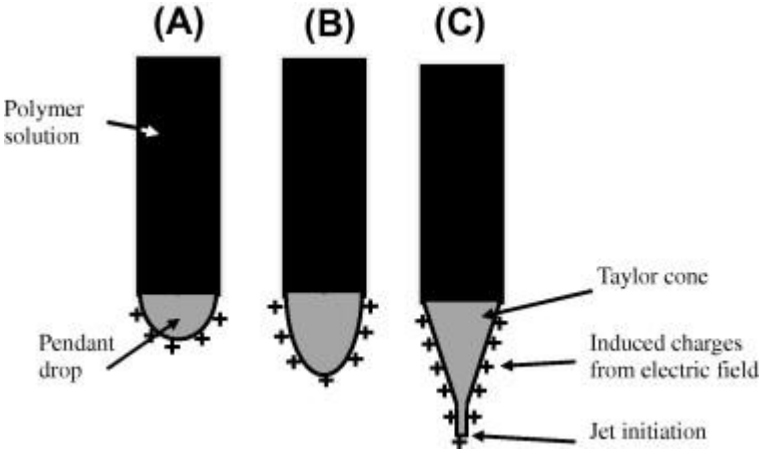


Figure 1.3 Schematic illustration of the Taylor cone formation under increasing voltage (from left to right): (A) Induction of surface charges in the polymer solution due to the electric field. (B) Elongation of the pendant drop. (C) Deformation of the pendant drop to the form the Taylor cone due to the charge-charge repulsion. A fine jet initiates from the cone (Baji et al., 2010)

Defects, such as beads, occur among the resultant fibers, or even as the major product sometimes depending on the operating conditions and the material properties. Beads are the main problem of electrospun fibers. The presence of beads along the fibers not only reduces the fiber uniformity, but also leads to reduced fiber surface area and poor mechanical strength. The main factors influencing the occurrence of beads are the applied voltage, surface tension, viscosity and conductivity of the solution (Lin and Wang, 2009).

1.3.1 Viscosity

Solution viscosity plays an important role in determining the fiber size and morphology produced by electrospinning (Fong et al., 1999) because the viscous forces prevent fiber stretching by resisting the electrical forces during electrospinning process. It has been found that viscous solutions tend to produce continuous fiber in the optimal range of viscosity for a polymer solution. The cohesiveness of the solution prevents fiber formation at higher viscosities. On the contrary, lower viscosities are more dilute and have a tendency to produce fibers embedded with beads (Huang et al., 2003).

It is known that the viscosity of the polymeric solution strongly influences the fiber diameter, and both the molecular weight and the concentration of the polymer can affect the viscosity of the polymer solution. Generally, when a high molecular weight polymer is solved by a solvent, the viscosity of the solution is higher than the solution prepared from the low molecular weight of same polymer. It has been found that at very low concentration of polymer solution, continuous fiber cannot be formed and causes the production of droplets instead of fibers (Beachley and Wen, 2009; Ramakrishna et al., 2005). It was proven that higher viscosity solutions resulted in fibers with larger diameters (Supaphol et al., 2005).

1.3.2 Conductivity

The other solution parameters that is effective in controlling the electrospinning process is the conductivity of polymer solutions. The potential of a solution to carry an electrical current is known as conductivity. Thus, it affects the rate which shows the amount of charge moving through a solution. Solution conductivity usually

depends on the type of polymer, and solvent used and the availability of ionisable salts (Bhardwaj and Kundu, 2010). It has been proven that solutions having higher conductivity resulted in thinner electrospun fibers (Tan et al., 2005; Lee et al., 2005). The smaller diameters were attributed to higher charge density and increasing stretching, and fiber elongation.

1.3.3 Surface tension

The phenomenon caused by the cohesive forces between liquid molecules is known as surface tension. Generally, atoms on the surface of a solution are at the lowest energy level, so they configure themselves to minimize surface area, thereby lowering the number of available bonding sites on the surface. Molecules on the surface have an attractive force towards the interior molecules, this is called surface tension. In electrospinning, the solution droplet at the tip of the needle is held by the surface tension until the electric voltage is applied enough to overcome the surface tension (Yang et al., 2004).

Surface tension is the most critical parameter since it determines the upper and lower limits of the electrospinning process if other parameters are kept constant. High surface tension is generally known to inhibit the electrospinning process, thus resulting in the formation of drops instead of spinning (Hohman et al., 2001). Electrospinning can occur at a lower electric field by using a polymer solution with a lower surface tension. However, it does not mean that not a lower surface tension of a solution will always be more suitable for electrospinning (Haghi and Akbari, 2007).

1.3.4 Flow rate

The flow rate of the polymer solution during electrospinning is an important process parameter. Generally, it is more recommended to use lower flow rate during electrospinning because it provides with enough time for polarization of the polymer solution. If the flow rate is very high, the produced fibers will be thick with many beads. However, the smooth fibers with small diameters can be produced when the flow rate is lower, probably due to the short drying time prior to reaching the collector and low stretching forces (Li and Wang, 2013).

1.3.5 Applied voltage

The applied voltage to the solution is a crucial element in the electrospinning process. Only after attainment of threshold voltage, fiber formation occurs; this induces the required charges on the solution along with electric field and initiates the electrospinning process. Researchers have suggested that there is more polymer ejection with higher voltages applied, and this facilitates the formation of thicker fibers (Zhang et al., 2005). It was reported that an increase in the applied voltage resulted in increase the electrostatic repulsive force on the jet fluid. This ultimately favors the formation of thinner fibers (Larrondo and Manley, 1981). Generally, a higher voltage leads to greater stretching of the solution because of the greater columbic forces in the jet as well as a stronger electric field. These effects cause reducing fiber diameter, leading to evaporation of solvent from the fibers in a shorter time. Therefore, voltage affects diameter of fiber, but the level of effect changes with the concentration of polymer solution and the distance between the collector and tip of the needle (Yördem et al., 2008).

1.3.6 Collectors

During the spinning process, types of collectors can also affect morphology of the fibers. Generally aluminum foil is used as a collector but other collectors such as conductive paper, wire mesh, and liquid non-solvent coagulation bath are also used (Bhardwaj and Kundu, 2010; Wang et al., 2005) Movement of the collector can also affect the electrospinning process. It is possible to produce aligned fiber by using a rotating drum type collector. Rotating collectors also provide the fast solvent evaporation (Bhardwaj and Kundu, 2010; Ramakrishna et al., 2005).

1.3.7 Distance between collector and tip of the syringe

The distance between the collector and the tip of the needle can also be an effective parameter on the diameters and morphology of the fibers. Simply, when the distance is too short, the fiber does not have sufficient time to solidify before being accumulated on the collector, whereas if the distance is too long, fibers with bead structures may be obtained. One of the important physical criteria related to electrospun fibers is the

dryness from the solvent, thus an optimum distance is recommended (Li and Wang, 2013).

1.3.8 Ambient parameters

Ambient parameters, including humidity and temperature may also influence the diameters and morphology of fibers. The increase in temperature results in thinner fibers, which is attributed to the decrease in the viscosity of the solution at increased temperatures (Mit-Uppatham et al., 2004). As for the humidity, low humidity may dry the solvent may be dried totally by low humidity and this leads to fast solvent evaporation. On the contrary, high humidity results in the thick fibers because the charges on the jet may be neutralized, leading to the smaller stretching forces. Recently, Casper et al. (2004) demonstrated that the change in humidity can also influence the surface morphologies of electrospun polystyrene fibers.

1.4 Application of nanofibers in foods

Nanofibers produced by electrospinning process have been used in a number of applications such as separation membranes, wound dressing materials, artificial organs, and protective clothing (Chen et al., 2006; Frenot et al., 2007; Huang et al., 2003; Li and Xia, 2004; Sill and Von Recum, 2008). However, the application of these versatile materials in food area is relatively new. In food area, electrospun fibers have a strong potential to be used mainly in packaging, enzyme immobilization, filtration, and encapsulation of food ingredients.

1.4.1 Food packaging

Electrospinning is a hopeful technique for production of active packaging materials and production of nanostructured layers for food packaging. It is advantageous to use electrospun fibers as active packaging materials because of its large surface to volume ratio, submicron to nano-scale diameter, high resistance to changes in surrounding atmosphere and high encapsulation ability of active compounds (Vega-Lugo and Lim, 2009).

Vega-Lugo and Lim (2009) used electrospun nanofiber made of soy protein or PLA fibers with allyl isothiocyanate as an active packaging and found that the electrospun fibers can release the active compound though humidity triggering. Similarly, zein/chitosan nanofibers with biocide was studied by Torres-Giner et al. (2009). Additionally, Su et al., (2012) found that the nanosized tea powder loaded PVA electrospun fibers showed antibacterial activity against Escherichia coli. The common point of these studies is to investigate the use of bioactive components in active packaging on the basis of the activation mechanism for controlled release. Recently, Wen et al. (2016) produced electrospun fibers within cinnamon oil and applied as active packaging material for fresh strawberry to prolong shelf-life of it.

Electrospun nanofibers can also been used as a smart packaging which has great significance in the food industry. Smart packaging is useful to monitor the quality changing during storage. Van der Schueren et al. (2010) designed a fiber mat within pH-sensitive dye to monitor real-time pH change in food system. Similarly, Teng et al. (2012) added multiple pH dyes inside of fibers to detect pH from a large range.

Additionally, electrospun fibers can be used as multilayered structures. The electrospun fibers could be used as composite packaging material with oxygen barrier to reduce food oxidation during storage (Busolo et al., 2009; Fabra et al., 2013).

1.4.2 Enzyme immobilization

Enzymes are natural catalysts and they have numerous industrial applications with high efficiency and specificity. However, the usage of enzymes for commercial purposes is limited because of their high cost, temperature sensitivity, difficulty of recovery from a chemical reaction, pH limitations and the possibility of inactivation by a variety of agents (Keyes and Saraswathi, 1985). Enzyme immobilization technique is used in order to overcome these difficulties. Enzyme immobilization is defined as the process of restricting enzymes either on a physical support or encapsulating within solid matrices for retention of their catalytic activities (Brena and Batista-Viera, 2006). Immobilization gives the enzyme enhanced stability against environmental changes (temperature or pH) and also serves as a medium for controlled release (Lopez-Rubio et al., 2006). Due to large surface area provided by electrospun

fiber, it considered a potential martial substitution for food industry. Despite from encapsulation, enzymes can be bond on nanofibers through physically adsorbed or covalently attached. Here are some examples of electrospun nanofibers with enzyme immobilization. Wu et al. (2005) proved that the use of electrospun nanofibers could increase cellulase catalyzing ability because of higher loading enzymes on the surface of fibers. El- Aassar et al. (2012) immobilized β-galactosidase on the surface of poly (acylonitrile-co-methyl methacrylate) fibers by crosslinking using glutaraldehyde. They found that immobilized enzyme showed greater pH, temperature, and storage stabilities compared to free enzyme. Wang et al. (2012) produced electrospun fibers for immobilizing lipase by physical adsorption. No matter which polymers had been used, lipase immobilized on nanofibers showed enhanced storage stability.

Additionally, immobilization of glucose oxidase (GOD) have been carried out by various studies. In one study, Ren et al. (2006) immobilized GOD into PVA fibers and used these fibers to produce enzymatic electrode for biosensor application. In other study, it was shown that chitosan and PVA biocomposite fibers improved the stability of GOD and it was used in designing glucose biosensor (Wu and Yin, 2013).

1.4.3 Filtration

Due to the high surface to volume ratio, porous surface and interwined fibrous structure of electrospun fibers, they are good candidates for filtering material, gas and liquid filtration. The electrospun nanofibers can be applied in air filtration on commercial systems due to their small pore size and large surface area. In food industry, nanofibrous membranes are able to filter liquids through pressure-driven liquid separation systems.

Polyvinylidene fluoride (PVF) nanofibers were used in liquid separation for clarification of water (Feng et al., 2008). Similarly, Rajesh and Natarajan (2009) investigated the feasibility of using sulfonated poly (ether ketone) nanofibers for pretreatment of water. Additionally, a few studies have reported the feasible use of electrospun nanofibers in the filtration of beverages. Veleirinho and Lopes-da-Silva (2009) used poly (ethylene terephthalate) nanofibers for the clarification of apple juice and found that the highest filtration rate was obtained with nanofibrous membrane

compared to traditional methods. Moreover, it is required to use lower pressure during filtration for nanofibrous membrane, compared to the ultra-filtration method. Also, Fuenmayor et al. (2014) used nylon nanofibers for apple juice filtration. This membrane demonstrated suitable mechanical properties that are required in filtration process. It is advantageous to use nylon nanofibers because they have affinity with selective adsorption of the polyphenols. This gives the juice a bitter taste. In another study, Lemma et al. (2015) filtered bacteria and yeast in the beer by using nylon nanofibers. It was seen that beer lost more than 95% of its turbidity at the end of filtration. This result also indicated the removal of microorganisms as was confirmed by microorganism assay methods.

1.4.4 Encapsulation of bioactive components

Food encapsulation is the most important application area of electrospun nanofibers in food industry. Generally, the main purposes of the encapsulation of food materials are to protect food materials against light, moisture, oxidation, etc. Additionally, controlled release of food ingredients, immobilization of cells and enzymes, masking unpleasant odor or taste of nutrients, enhancing the probiotics viability and facilitating their handling, and making functional foods can be provided with electrospun fibers.
In a study, α-Tocopherol (α-TOC) was encapsulated into 3 different hydrocolloid matrixes (soybean protein isolate (SPI), whey protein isolate (WPI), and zein) by electrospinning technique (Fabra et al., 2016). Higher encapsulation efficiency of α-TOC (100%) was obtained for the zein fibers compared to others. In another study, Moomand and Lim (2014) encapsulated fish oil into zein fibers by electrospinning with higher encapsulation efficiency, compared to conventional methods. They also observed that the oxidative stability of fish oil during storage was improved compared to non-encapsulated fish oil. In a recent study, electrospun fibers loaded with fish oil were produced with coaxial electrospinning by Yang and coworkers (2017). They showed that coaxial electrospinning significantly enhanced the oxidative stability and shelf life of encapsulated fish oil, compared to single electrospinning (Yang et al., 2017a).

Water-soluble vitamins are also encapsulated into fibers by electrospinning. Alborzi et al. (2013) demonstrated that almost 100% retention of the encapsulated folic acid

within the electrospun fibers was seen after 41 days of storage in the dark at pH 3, while the retention of non-encapsulated folic acid after 1 day of storage at pH 3 was 8% and 0% in the absence and the presence of light, respectively.

Encapsulation of bioactive components into nanofibers is also useful for control releasing of the encapsulant. For example, homogeneous and uniform hybrid chitosan/phospholipid nanofibers were produced to deliver curcumin (Mendes et al., 2016). According to the release profile it was proven that the sustained release behavior of curcumin from fibers for over 7 days (around 75%) without significant burst effect. In another study, curcumin was encapsulated within amaranth protein isolate and pullulan nanofibers and similar release profile had been observed (Blanco-Padilla et al., 2015).

Several studies showed that nanofibers loaded with food active components could be used as antimicrobial and antioxidant. For example, Bui et al. (2014) reported that the zein nanofibers containing 1.6 wt % curcumin can efficiently inhibit growth of Staphylococcus aureus suggesting potential application in antibacterial nonwoven mat. Similarly, Wang et al. (2017) showed good antimicrobial activity and antioxidant capacity of curcumin loaded zein electrospunfibers. Gallic acid, which is a natural antioxidant, was successfully encapsulated into zein nanofibers by Neo et al. (2013). By this way, they made functional edible nanofibers having antioxidant properties.

Numerous studies have showed that electrospinning is a very effective technique to improve stability and bioactivity of essential oil. Yao et al. (2016) used coaxial electrospinning for encapsulation of rose hip seed oil into a zein matrix and they showed the significant effect of the fibers on prolonging shelf-life of bananas and cumquats. In another study, Wen and coworkers (2016) successfully encapsulated the cinnamon essential oil into polyvinyl alcohol (PVA) and β-cyclodextrin matrix using electrospinning. The resulting nanofilm had better antimicrobial activity compared to the casting film, and it significantly enhanced the strawberry shelf-life. In a research study Kayaci et al. (2013), encapsulated the eugenol into PVA nanofibers and showed that the thermal stability of eugenol was improved by encapsulating it.

Electrospinning has been also considered as a promising encapsulation technique for probiotics because this technique overcomes the difficulties, such as the use of organic solvents or high temperature related to the other techniques including extrusion, spray drying, etc. López-Rubio and coworkers (2009) showed that the electrospinning technique is feasible for encapsulating Bifidobacterium strains in food hydrocolloids and it can be considered for functional food applications. The viability of the cells was not affected by the encapsulation process and the viability of encapsulated bacteria was found to be significantly higher than that of non-encapsulated bacteria after 40 days of storage at 20 °C and 130 days at 4 °C (López-Rubio et al., 2009). In another work, Fung et al. (2011) demonstrated good survivability of Lactobacillus acidophilus (78.6–90%) after electrospinning and protected its viability for over 21 days at 4 °C.

1.5 Limitations and future needs

In the past two decades, electrospinning technique has been significantly progressed in the laboratory scale. However, there are still problems that hamper the application of electrospinning technique in the food industry. Low throughput is the main limitation of electrospinning and it limits its large-scale industrial production. Thus, to overcome this constraint by modifying the structure of electrospinning setup (e.g., multi-needle arrangement) is an essential research area. Additionally, to determine the general parameters for controlled release and successful encapsulation of bioactive compounds is a big challenge because of the myriad of experimental conditions, solvents, polymers, and compounds to choose from. Moreover, the possible applications of electrospun fibers have to be efficiently translated to their use in food systems. In other words, the number of studies should be increased to prove the practicability of the obtained products as active/smart food packaging materials without changing the chemical and physical properties of food. Finally, with respect to food safety, one reason of that nanofibers has not been widely used in the food area can be attributed to the predominance of studies on synthetic polymers rather than on natural ones. Additionally, there are still questions on consuming nano-scale food materials and thus, further studies are required to determine their effects on human health and the environment. In order to provide with the safe of food materials with nano-size, it is also required to arrange legislation by government agencies on the application of nanomaterials in the food industry.

Electrospinning technique has great potential to be used to develop innovative products. However, the application of electrospinning in food area is still new and needs to be developed. Antioxidants, antimicrobials, and bioactive compounds can be easily encapsulated into nanofibers by electrospinning technique. Thus, one of the main areas where electrospun nanofibers can be developed by further studies are novel food packaging materials and functional foods. Additionally, the use and modifications of coaxial electrospinning in order to produce nanofibers having a core-shell structure may be required to obtain sustained release or construct a suitable delivery device for the encapsulation of fragile compounds, which are not suitable for blend electrospinning. Moreover, in order to broaden the possibilities for new products with improved properties electrospinning may be combined with other technologies, such as nanoparticles, liposomes, from the micro/nano encapsulation field. Currently, the production of electrospun fibers is feasible at commercial scale, however, advanced researches are still required to provide the industrial application in food area, as well as the collaborations between researchers and manufacturers (Wen et al., 2017).

1.6 Biopolymers for electrospinning

Encapsulation of bioactive compounds into fibers is generally achieved by mixing the bioactive compounds with polymer solution followed by electrospinning. Electrospinning can be applied to both natural and synthetic polymers. However, natural polymers have attracted more attention of researchers in the field of electrospun nanofibers because of their better biocompatibility, biodegradability and non-toxicity. Generally, proteins and carbohydrates are used as the natural biopolymers for encapsulation studies. Generally, proteins are preferred over synthetic polymers (more importantly carbohydrate polymers) because they are the major component of the human body, and are often in themselves, a functional food enhancer and valuable dietary supplements (Nieuwland et al., 2013). However, it is difficult to electrospin proteins, because of their complex secondary and tertiary structures. To overcome this, the proteins should be changed into a random coil conformation by well dissolving them. Globular proteins have too little internal interaction to entangle during the spinning process (Nieuwland et al., 2013). Egg albumen, casein, collagen, whey

protein isolate, whey protein concentrate, gelatin, soy protein isolate, and zein are common protein-based encapsulating materials used in electrospinning process.

1.6.1 Zein

Zein, which is a class of prolamine protein found in corn, has been used particularly, in food and pharmaceutical industry as a coating material because of its hydrophobicity and good elasticity (Lawton, 2002). Zein is particularly rich in glutamic acid (21–26%), leucine (20%), proline (10%) and alanine (10%). Zein exhibits various characteristics such as flexibility, glossiness, toughness, compressibility, antioxidant activity, and resistance to microbial attack. It also possesses the benefits of being renewable and biodegradable (Shukla and Cheryan, 2001). Zein nanofibers have the potential to be used for controlling release of active components, and as an active packaging material, etc. (Zhong et al., 2009; Alkan et al., 2011). There are also various studies about electrospinning of zein, especially by incorporating of various functional compounds inside of them (Fernandez et al., 2009; Neo et al., 2013; Moomand and Lim, 2014; Yang et al, 2017b). Moreover, composite zein fibers were produced by the blending of zein with several polymers in order to improve its electrospinnability (Torres-Giner et al., 2009; Kayacı and Uyar, 2012).

1.6.2 Gelatin

Gelatin, which is a natural polymer, is derived from collagens. It is an aqueous polymer, i.e. dissolvable in water. It is known that aqueous systems are generally unsuitable to produce electrospun fibers from natural biopolymers, because of the low volatility or high surface tension of the aqueous solution. Therefore, gelatin and other natural polymers generally require toxic and aggressive solvents for successful production of electrospun nanofibers, which causes significant problems in utilization of them, particularly in food and biomedical science, cosmetics, pharmaceuticals, and other related fields (Huang et al., 2004; Ki et al., 2005). Fortunately, the number of studies investigating the identification of non-toxic and less aggressive solvents to be used in production of electrospun nanofibers from gelatin has increased, recently. Li et al. (2006) and Zhang et al. (2009) showed that it is possible to spin gelatin from water by heating the solution above melting point if the electrospinning device includes a temperature control system. Song et al. produced electrospun nanofibers

from gelatin in different combinations of water, acetic acid and ethyl acetate (Song et al., 2008). In another study, Okutan et al. (2014) successfully produced gelatin nanofibers by using 20% acetic acid solution as a solvent. Erencia et al. (2015) also showed the viability to obtain electrospun gelatin nanofibers at 25% acetic acid concentration in their study.

1.7 Tomato waste

Tomatoes are one of the most widely used and cultivated fruit crops. They are commonly consumed fresh and in the form of processed products such as tomato juice, canned tomato, ketchup, paste, and so on. The processing of tomatoes generates significant amounts of tomato waste (seeds, skins, and pulp) which is usually used for animal feeds or similar areas having low economic value. However, tomato waste, especially skin, is a rich source of antioxidant compounds including carotenoids mainly in the form of lycopene (80-90% of total carotenoids) (Knoblich et al., 2005; Riggi et al., 2008). Moreover, Sharma and Maguer (1996) proved that the tomato peel could contain about 5 times more lycopene than tomato pulp. The recovery of this carotenoid could represent an alternative for the valorisation of the by-products of the tomato industry (Sharma and Maguer, 1996). Waste materials and by-products from the food processing industry can be a money spinner if they are appropriately utilized using newer knowledge and technologies (Potty, 2009).

1.8 Tomato carotenoids

Carotenoids are synthesized in the flowers, leaves, and fruits in tomatoes. In the leaf tissues, carotenoids act as photoprotectors, being lutein the main carotenoid present, confer the characteristic yellow coloration to flowers (Standfuss et al., 2005). However, lycopene is the main carotenoid in ripe tomato fruits, and it causes its red coloration.

The carotenoids contents are highly affected by the environmental conditions and genotype of the tomato as well as other chemoprotective substances. Taking this variability into account, the concentrations of lycopene in standard tomato cultivars range from 7.8 to 18.1 mg in 100 g of fresh tomato. The second main carotenoid in tomato is β-carotene, and it is responsible for orangey colors. Its concentration is

generally max 1.2 mg in 100 g of fresh tomato. Apart from these major carotenoids, lesser amounts of lutein, α-carotene, γ-carotene, δ-carotene, neurosporene, and other carotenoids can also be present in tomatoes (Marti et al., 2016).

Tomato carotenoids are generally intended for use as natural food colorants and antioxidant in a wide variety of food areas including dairy products, baked goods, beverages, breakfast cereals, candy, soups, salad dressings and many others (Choksi and Joshi, 2007). They can be also used as food supplement providing health benefits such as modulation of the immune system, vitamin A precursor, prevention of the risk of cancer and cardiovascular diseases (Arab and Steck, 2000; Palozza et al., 2011).

However, it is difficult to incorporate the carotenoids into different food formulations because they are insoluble in water and partially soluble in oils at room temperature. Additionally, they are relatively unstable in food systems because they are susceptible to oxygen, light, and heat (Xianquan et al., 2005). Fortunately, encapsulation of the carotenoids can be an efficient way in order to improve their solubility and preserve them from deterioration during processing.

Among the many different encapsulation techniques, considerable attention is currently being centered on electrospinning technique due to their versatility, simplicity and cost effectiveness. Additionally, nanofibers produced by electrospinning have many structural and functional advantages, such as a large surface-to-volume ratio due to high porosity, high encapsulation efficiency without heat application and enhanced stability of encapsulated compounds (Bhardwaj and Kundu, 2010).

1.9 Aims and significance of the study

In this PhD thesis study, it was aimed to increase thermal and storage stability of carotenoids, extracted from tomato peel, and to give them functional features (water solubility and enhanced antioxidant activities) by encapsulating them into nanofibers composed of zein and gelatin biopolymers by using electrospinning technique.

This thesis consisted of three main parts. In the first part, electrospinning process parameters were optimized by using an experimental design: Taguchi's orthogonal arrays. In the second part, the carotenoids were extracted from tomato peel and encapsulated into nanofibers by electrospinning using the optimized conditions. In the third part, the encapsulated tomato carotenoids within the nanofibers were applied on yogurt as a model food to investigate their effect on the antioxidant and physicochemical properties of yogurt during storage at 4°C.

With this study, production of high added-value and stable carotenoids within nanofibers, which may be an alternative recovery way of treatment of waste tomato peel, could be provided by encapsulating carotenoids obtaining from wastes to nanofibers. Thus, by-products which are food waste material both will redound to economy as value added products and environmental impact of these wastes will be decreased. Moreover, it will be contributed to popularise nanofibers produced by electrospinning method of which usage is new in food systems.

CHAPTER 2: OPTIMIZATION STUDY FOR ELECTROSPINNING OF ZEIN AND GELATIN

2.1 Introduction

Basically, electrospun nanofibers are formed by spinning of a polymeric solution in an electric field into fibrils whose diameters are ranging from 10 nm to several hundred nanometers. The solution and process parameters have to meet a number of requirements for a successful electrospinning process. For example, the polymer solution must have adequate concentration, viscosity, surface tension and conductivity. In addition to this, several process parameters such as voltage applied, flow rate of solution and distance between the collector and tip of the needle must be in a suitable range of values. Controlling the factors above, uniform, bead-free and homogeneously distributed electrospun nanofibers with small diameter and desired morphology can be produced. However, all of these parameters must be adjusted specifically depending on the types of polymer-solvent systems. Therefore, optimization of the conditions for each polymer-solvent system becomes a challenging task in order to get the best results.

One of the aim of this part of the study was to understand how the morphology and diameter of produced electrospun fibers from gelatin and zein was influenced by solvent type and concentration, and process parameters. Another was optimization of the electrospinning process parameters to get thinner fibers and narrower diameter size range indicating less jet instabilities. Up to now, a few numbers of optimization studies on electrospinning of gelatin and zein, which investigated the effects of limited solution parameters with one or two process parameters neglecting the others, have been carried out (Huang et al., 2004; Selling et al., 2007; Torres-Giner et al., 2008; Okutan et al., 2014). However, this optimization study differs from others in the way of taking the effects of multiple variables acting simultaneously into account by using Taguchi's orthogonal design for the production of nanofibers by electropinning. The

final aim of the study was to further determine the best polymer concentration to achieve thin, bead-free, smooth and homogeneously distributed fibers by conducting several experiments at determined optimum conditions.

2.2 Materials and Methods

2.2.1 Materials

Food grade, beef-hide gelatin (200 bloom) was supplied from a local market and zein (Z 3625), glacial acetic acid (HAc) (>99.85%), and ethanol (EtOH) was purchased from Sigma Aldrich (St. Louis, Mo., U.S.A).

2.2.2 Experimental design

Taguchi' s orthogonal design, which is based on statistical design of experiments, was conducted by using Minitab 17 (Minitab Inc., USA) with two important aims. The first aim was to investigate the influences of three levels of four factors (voltage supplied, flow rate of solution, distance between collector and needle, and solvent concentration of solution) on the diameter of electrospun fibers and dispersion of these sizes of diameters on the mat. The second aim was to optimize these parameters to get thinner fibers and narrower diameter size range indicating less jet instabilities. The experimental designs with levels of factors for gelatin and zein were shown in Table 2.1 and Table 2.2, respectively. A combination of factors with different levels were designed according to the orthogonal array of L_9 type shown in Table 2.3 and Table 2.4. A "smaller the better" characteristic formula has been used to determine the optimum combination of factors to minimize both the diameters of fibers and their variation in production of electrospun gelatin nanofibers as indicated in the equation below (Ranjit, 1990; Ross, 1996; Ghani et al., 2004):

$$\frac{S}{N} = -10log(\frac{1}{n}\sum_{i=1}^{n} y_i^2)$$

where S/N is the signal-to-noise ratio, n is the number of observations, and y is the diameter of gelatin nanofibers measured.

Table 2.1 Selected factors and their levels used in Taguchi's design for gelatin nanofibers

Factor	Level		
	1	**2**	**3**
A, Voltage (kV)	15	18	21
B, Flow rate (μL/min)	5	10	15
C, Distance (cm)	10	12.5	15
D, HAc (%)	20	50	80

Table 2.2 Selected factors and their levels used in Taguchi's design for zein nanofibers

Factor	Level		
	1	2	3
A, Voltage (kV)	12	16	20
B, Flow rate (μL/min)	10	15	20
C, Distance (cm)	10	12.5	15
D, Solvent	HAc	EtOH	HAc/EtOH

Table 2.3 L_9 Orthogonal design for selected factors with their levels for gelatin nanofibers

Run	Factors			
	Voltage, kV	Flow rate, μL/min	Distance, cm	HAc,%
1	15	5	10	20
2	15	10	12.5	50
3	15	15	15	80
4	18	5	12.5	80
5	18	10	15	20
6	18	15	10	50
7	21	5	15	50
8	21	10	10	80
9	21	15	12.5	20

Table 2.4 L_9 Orthogonal design for selected factors with their levels for zein nanofibers

Run	Factors			
	Voltage, kV	Flow rate, µL/min	Distance, cm	Solvent
1	12	10	10.0	HAc
2	12	15	12.5	EtOH
3	12	20	15.0	HAc/EtOH
4	16	10	12.5	HAc/EtOH
5	16	15	15.0	HAc
6	16	20	10.0	EtOH
7	20	10	15.0	EtOH
8	20	15	10.0	HAc/EtOH
9	20	20	12.5	HAc

2.2.3 Preparation of solutions

For optimization study, gelatin solutions were prepared at 25% (w/w) concentration by using three different concentrations (20, 50, 80%, v/v) of aqueous acetic acid (HAc) solution. Similarly, 25% zein solutions were prepared by using pure acetic acid (HAc), 80% (v/v) aqueous ethanol solution (EtOH), and the mixture of acetic acid and 80% (v/v) aqueous ethanol solution, HAc/EtOH (1:1) as three different solvents. After optimization study, five different concentrations (16, 20, 24, 28, and 32%, w/w) of gelatin and zein solutions were prepared by using the determined concentration of acetic acid solution and solvent type, respectively. The gelatin solutions were stirred for about 2 hours using a magnetic stirrer at 40 ˚C for complete dissolving of gelatin, whereas the zein solutions were obtained after constant stirring using a magnetic stirrer at room temperature for 1 hour.

2.2.4 Characterization of solutions

Viscosities of the prepared polymer solutions were determined at 24°C by using Haake Rheostress RS1 (Karslruhe, Germany) equipped with a plate-plate sensor (dia 35 mm, gap 1 mm). The shear rate ranged from 0 to 200 s^{-1} in 60 s during viscosity measurement.

Surface tension and electrical conductivity values of the solutions were determined by using a tensiometer (Krüss K6, Germany) and a conductivity meter (Orion 115A, Thermo, USA), respectively, at room temperature.

2.2.5 Electrospinning

Electrospinning process was carried out by using the Nanotel (NTEE 80-1, Turkey) electrospinning device consisting of a syringe pump, a high voltage power supply and a collector plate covered in non-stick aluminum foil for easy removal of mats. The solutions were placed in 5 ml syringes fixed horizontally on the syringe pump delivering the solution to the tip of a metal needle where the voltage was applied. Fibers were then collected on the aluminum foil. Production of nanofibers was firstly made according to the process conditions shown in Table 2.3 and Table 2.4. Secondly, nanofibers were produced from different concentrations of polymer solutions at the further determined optimum conditions of parameters.

2.2.6 Morphological characterization

The morphology and diameters of the produced nanofibers were examined by using a scanning electron microscope (SEM) (JEOL, JSM-6390LV, Japan) with accelerating voltage of 20 kV. Fiber diameter analysis was carried out by randomly counting 50 fibers per experiment using a software (ImageJ, 1.48V). The mean diameter and standard deviation were calculated as the responses.

2.2.7 Statistical analysis and optimization

The diameter values of the nanofibers obtained were statistically analyzed by using the Minitab-17 program (Minitab Inc., USA) and optimum conditions were determined. For the confirmation, one more experiment was conducted at the determined conditions.

2.3 Results and Discussion

2.3.1 Results related to production of gelatin nanofibers

2.3.1.1 Morphologies of gelatin nanofibers

According to the images obtained by scanning electron microscopy (SEM) in Figure 2.1, electrospun gelatin nanofibers showed bead-free, smooth, and homogeneously distributed morphology. They had also small diameters ranging from 76 to 288 nm with not very high deviation (Table 2.5). At first glance to the SEM images, it can be easily seen that gelatin nanofibers obtained in Run-3 and Run-8 had larger diameters than others'. The common parameter used in these runs was acetic acid (HAc) concentration with the highest level. However, bead-free and smooth nanofibers with smaller diameter were produced by electrospinning of the gelatin solutions at lower HAc concentrations.

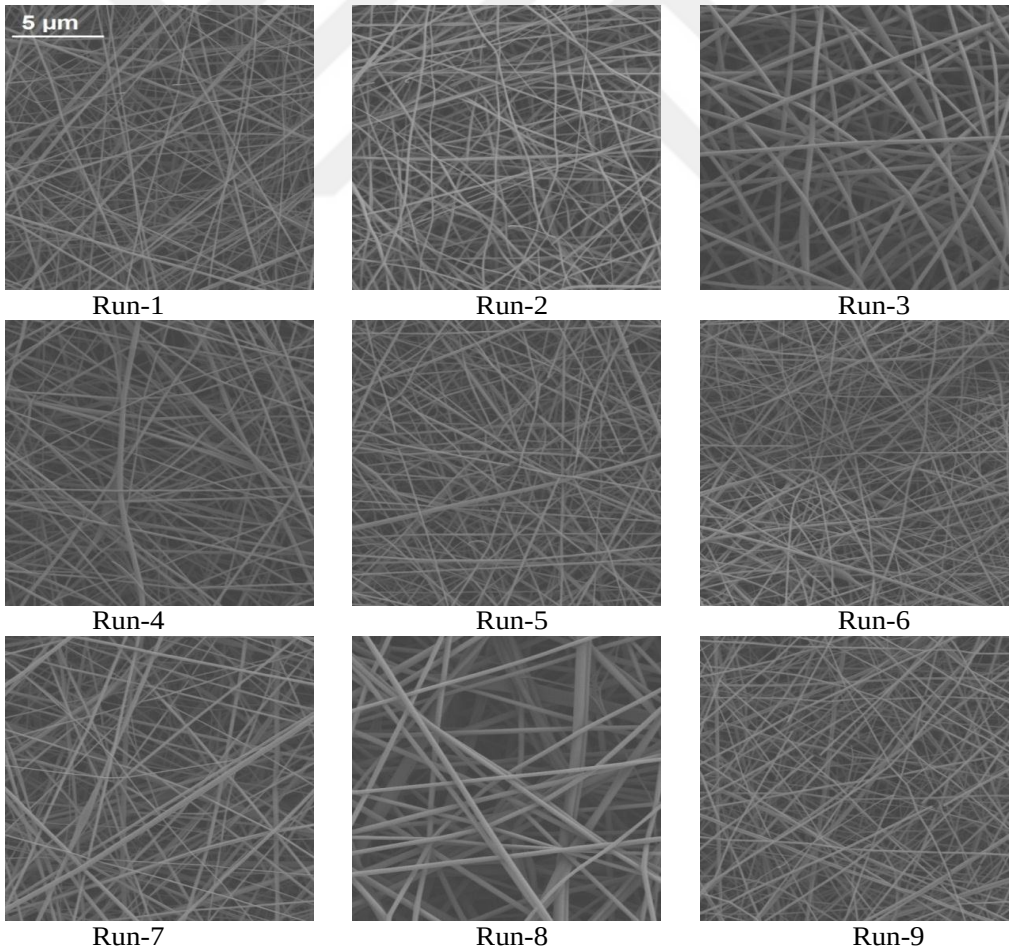


Figure 2.1 SEM images of electrospun gelatin nanofibers produced according to the experimental design shown in Table 2.3

Erencia et al. (2015) also produced bead-free nanofibers from both solution combinations of low acetic acid concentration (25%) with higher concentrations of gelatin (>25%) and high acetic acid concentrations (>50%) with low gelatin concentrations (<25%). However, these fibers had larger diameters (>200 nm) compared to our fibers produced. In another study, Gu et al. (2009) obtained nanofibers from a solution combination of low gelatin concentration (10%) with high acetic acid concentration (>80%). Although these produced nanofibers had thin structures with small diameters (80-100 nm), many bead formations were observed on them.

Table 2.5 Gelatin fiber diameter with standard deviation and their corresponding signal-to-noise (S/N) ratios based on ‘‘smaller the better’’

Run	Designation	Average fiber diameter, nm	S/N values
1	A1B1C1D1	77 ± 28	-37.73
2	A1B2C2D2	98 ± 34	-39.82
3	A1B3C3D3	212 ± 49	-46.53
4	A2B1C2D3	109 ± 25	-40.75
5	A2B2C3D1	96 ± 26	-39.64
6	A2B3C1D2	76 ± 21	-37.62
7	A3B1C3D2	107 ± 47	-40.59
8	A3B2C1D3	288 ± 55	-49.19
9	A3B3C2D1	97 ± 30	-39.74

2.3.1.2 Characteristics of gelatin solutions

In the current study, the change in acetic acid concentration also affected conductivity, surface tension, and viscosity values as shown in Table 2.6. It is well-known that the morphology of electrospun nanofibers depends on not only operating conditions, but also these solution characteristics (Fong et al., 1999). The most critical parameter among them is surface tension since it determines the upper and lower limits of the electrospinning process if other parameters are kept constant. High surface tension is generally known to inhibit the electrospinning process, thus resulting in the formation of drops instead of spinning (Hohman et al., 2001). The decrease in surface tension caused by increasing concentration of acetic acid in the solution is due to lower surface tension value of acetic acid (28.8 mN/m) than water (72 mN/m) at room temperature

(Song et al., 2008; Gu et al., 2009). In the present study, surface tension values also showed a decreasing trend with increasing the acetic acid concentration as shown in Table 2.6. It can be seen in Figure 2.1 that bead-free and smooth nanofibers could be obtained from all of the runs from 1 to 9 thanks to suitable surface tension values of the solutions.

Table 2.6 Characteristics of prepared gelatin solutions

Run	Solution	Viscosity (Pa.s)	Conductivity (μS/cm)	Surface Tension (mN/m)
1,5,9	Gelatin+20%HAc	0.2592±0.00	2053±5.0	40.8±0.05
2,6,7	Gelatin+50%HAc	0.2920±0.00	1939±2.5	40.0±0.10
3,4,8	Gelatin+80%HAc	0.4364±0.00	1079±2.0	36.4±0.20

The viscosity values of the solutions given in Table 2.6 were to be increased with increasing the acetic acid concentration probably due to increased aggregation of molecules at acidic pH levels (Davanço et al., 2007; Salles et al., 2015). Increased viscosity caused the formation of the larger diameter of fibers as shown in Figure 2.1 and Table 2.5. When the concentration of acetic acid in the gelatin solution was 80% in Run-3, Run-4, and Run-8, the fibers with the largest diameters (respectively 212, 109, and 288 nm) were produced. It is consisted with several studies showing that higher viscosity in the feed polymer solution gives rise to the formation of uniform fibers with larger diameters due to polymer chain entanglements, which makes jet stretching more difficult (Erencia et al., 2015; Salles et al., 2015; Ramakrishna et al., 2005; Kulkarni et al., 2010).

It was shown in Table 2.6 that the conductivity values of solutions decreased with increasing acetic acid concentration. This caused a significant increase in the diameter of the electrospun fibers. This phenomenon can be explained by the fact that increasing of conductivity also increases the charge density on the surface of the drop in the needle, which gives rise to increases in elongation forces exerted on the polymer jet providing a smaller fiber (Zong et al., 2002).

2.3.1.3 Analyzing the results by Taguchi's Method

The Taguchi method, in which orthogonal arrays are used to organize the factors and the levels affecting the process, offers the opportunity to be able to evaluate all parameters together or independently with the minimum number of experiments (Ross, 1996). Taguchi used signal-to-noise (S/N) ratio, which measures the deviation of quality characteristics from the desired values in order to assess the quality. In this study, S/N ratio values calculated by rearranging the results by 'Minitab-17' statistical software according to "smaller the better" characteristic formula and were given in Table 2.5. As depicted in Table 2.5, Run-8, which contained the highest levels of voltage applied and acetic acid concentration, yielded the highest diameter of gelatin nanofiber with the highest range of variation in diameter (288 ± 55 nm) and the lowest value of S/N ratio. In contrast, the thinnest nanofibers (76-77 nm) with the highest value of S/N ratio values were obtained in Run-1 and Run-6 containing lower levels of voltage applied and acetic acid concentration.

In order to realize the effects of each factor and level on the response, the average fiber diameter and S/N ratio value for different levels of each parameter were calculated as shown in Table 2.7. According to range analysis in this Table, the differences between maximum and minimum values of the three levels were calculated and the degree of importance of each factor on the fiber diameter was determined in a way that the factor with larger range had a more significant effect on the fiber diameter.

Our results indicate that the concentration of acetic acid in the solution is the most influential factor on the diameter of electrospun gelatin fiber. The effect of acetic acid concentration is quite clear as mentioned above; increasing of the acetic acid concentration gave rise to increases in the viscosity, whereas decreases in the conductivity and surface tension values of the solutions causing larger fiber diameters. As depicted in Figure 2.1, the electrospun nanofibers obtained from the gelatin solution containing 20% HAc and 50% HAc showed similar morphology because of their close characteristic values in Table 2.6 and mean S/N ratio values in Table 2.7.

The applied voltage was found as the second influential factor on the gelatin fiber diameter. The fiber diameters were seen as smaller when the applied voltage was 18

kV. However, the experimental runs, in which the voltage levels of 15 and 21 kV were applied, caused increases in the fiber diameters. In literature, the effect of voltage on the fiber diameter is also crucial and still controversial. Several researchers suggested that very high levels of voltage applied accelerates jet extending and causes a greater volume of polymer solution to be drawn from the needle, resulting in the formation of the larger diameter of fibers (Demir et al., 2002; Zhang et al., 2005). On the other hand, several researchers suggested that a strong electrical charge provides with a greater stretch of the polymer solution leading to usually decrease the diameter of the fibers (Yuan et al., 2004; Bhardwaj and Kundu, 2010). In addition to this, Sill and Recum (2008) showed that with an increase in the applied voltage beyond a critical value, the fiber diameter decreases initially because of a higher degree of jet stretching and then increases after a definite point. In another study, Yördem et al. (2008) indicated that the effect of applied voltage on fiber diameter may vary by type of polymer-solvent system and the distance between the collector and the tip of the needle.

It is inferred that the flow rate and the distance between the collector and the tip were found to have fewer effects on the fiber diameter compared to other factors (Table 2.7). Generally, increasing the flow rate increases the fiber diameter because of more fluid ejection in a jet (Zuo et al., 2005). Although the distance between the collector and the tip is generally thought as the least important factor, the effect of it is almost the same as for increasing voltage (Frenot and Chronakis, 2003; Ramakrishna et al., 2005; Homayoni et al., 2009). In the current study, the effects of the flow rate and the distance on the fiber diameter showed both increasing and decreasing trends. The explanation for this fact is that the effects of these two parameters also vary with other parameters.

2.3.1.4 Optimum combinations of factors and confirmation experiment

Figure 2.2 shows the mean effects of each factor on the mean fiber diameter. According to Table 2.7 and Figure 2.2, optimum levels of the factors were determined as; voltage 18 kV, flow rate 5 μL/min, distance 12.5 cm, and HAc concentration 20% in order to obtain the thinnest nanofiber. Figure 2.3 shows the SEM images with distribution of fiber diameters of electrospun nanofibers obtained with optimized

conditions. According to the images and bar graphs, bead-free, smooth and homogenous gelatin nanofibers with small diameters could be obtained. The confirmation tests were conducted for all of the three levels of flow rate because 5 µL/min was thought as not so high level to further electrospin the solutions with high gelatin concentration. It might cause accumulation of the polymer solution in the needle preventing fiber formation. Therefore, it was decided to use 15 µL/min as the optimum flow rate because no significant differences were observed among the morphology of the fibers obtained using different flow rates.

Table 2.7 Response table for means of fiber diameters and S/N values

Level	**Means of fiber diameters (nm)**				**Means of S/N values**			
	A	B	C	D	A	B	C	D
1	129.00	97.67	147.00	90.00	-41.36	-39.69	-41.51	-39.04
2	93.67	160.67	101.33	93.67	-39.34	-42.88	-40.10	-39.34
3	164.00	128.33	138.33	203.00	-43.17	-41.30	-42.25	-45.49
Range	70.33	63.00	45.67	113.00	3.83	3.19	2.15	6.45
Importance	2	3	4	1	2	3	4	1
Best level	18 kV	5µL/min	12.5cm	20%				

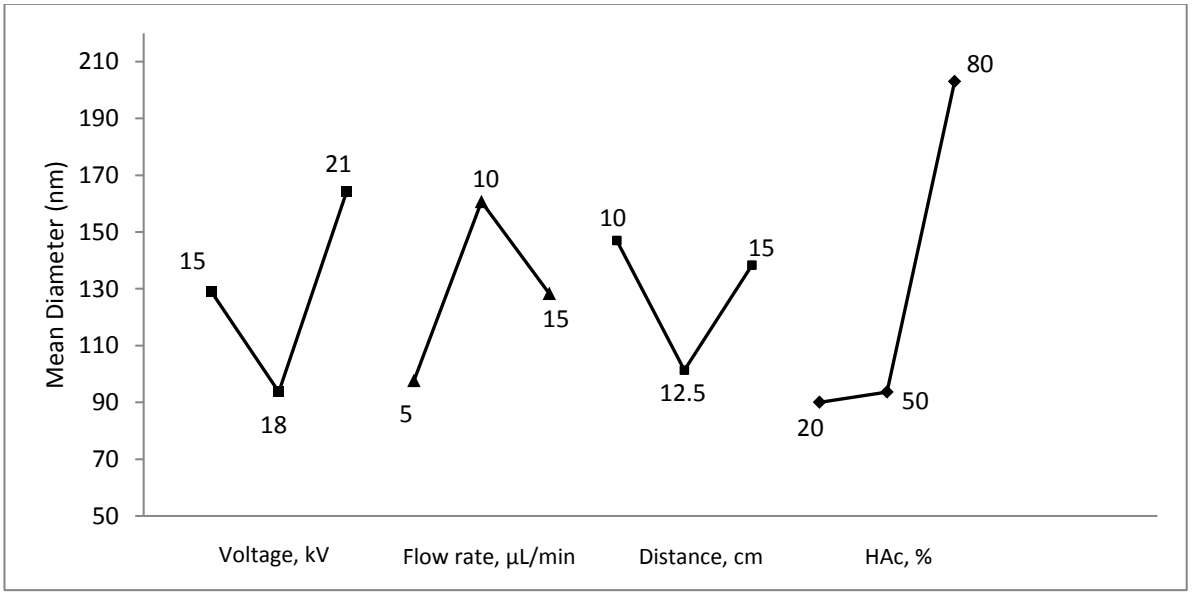


Figure 2.2 Main effect plots of each parameter on means of nanofiber diameter

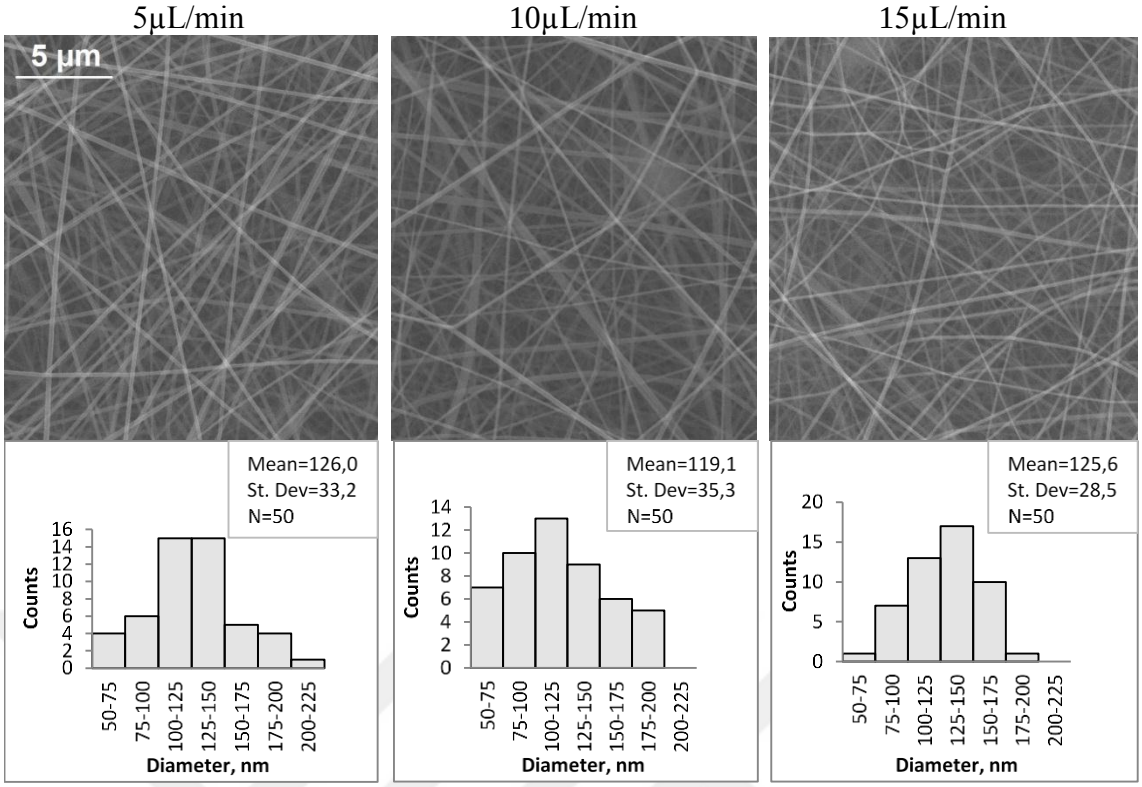


Figure 2.3 SEM images and bar graphs of diameter ranges of electrospun gelatin nanofibers obtained at optimized conditions (18 kV; 12.5 cm; 20% HAc) with different flow rates

2.3.1.5 Effect of gelatin concentration

The morphologies of the electrospun gelatin nanofibers at various concentrations obtained by using the optimum conditions were illustrated in Figure 2.4. Even though the most homogenous nanofibers with the smallest diameter (about 50 nm) were obtained from the gelatin solution at 16% (w/w), many beads were observed along the fibers probably due the low viscosity resulting in spraying of the solution (Ki et al., 2005). However, fewer beads were formed when the gelatin concentration increased to 20% (w/w), contributing to increase in the viscosity (Table 2.8). With further increase of concentration, no bead formation was observed along the electrospun nanofibers, but the diameters of the fibers markedly increased because of the high viscosity (Deitzel et al., 2001).

Table 2.8 Characteristics of the gelatin solutions at different concentrations

Gelatin (%,w/w)	Viscosity (Pa.s)	Conductivity (mS/cm)	Surface Tension (mN/m)
16	0.0448±0.00	2049±5.0	37,8±0.25
20	0.1226±0.00	2030±2.5	41,2±0.25
24	0.2499±0.00	2011±0.5	42,5±0.00
28	0.8244±0.05	1931±2.0	46,5±0.50
32	3.0130±1.30	1773±1.0	48,5±0.50

In the current study, both viscosity and surface tension values of the solutions increased with increasing gelatin concentration. It is probably attributed to that the increase in solution concentration could increase the cohesiveness among the water molecules at the liquid/air interface leading to the observed increase in surface tension of the solutions (Choktaweesap et al., 2007). However, many studies observed that surface tension decreased, while viscosity increased with gelatin concentration (Regismond et al., 1999; Choktaweesap et al., 2007; Okutan et al., 2014).

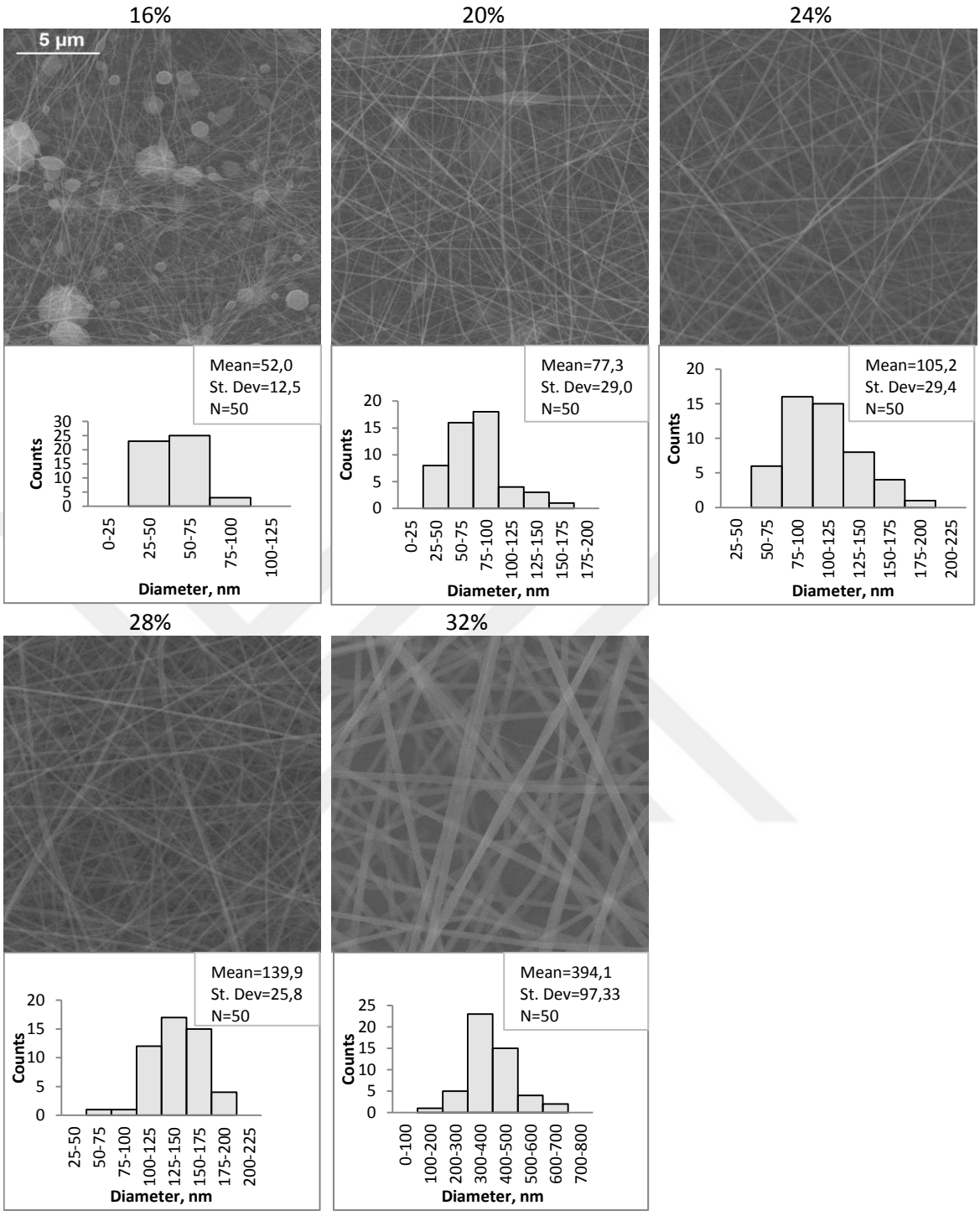


Figure 2.4 SEM images and bar graphs of diameter ranges of electrospun gelatin nanofibers obtained from different concentrations of gelatin solutions at optimized conditions (18 kV; 15 µL/min; 12.5 cm; 20% HAc)

2.3.2 Results related to production of zein nanofibers

2.3.2.1 Morphologies of zein nanofibers

At first glance to the SEM images in Figure 2.5, it can be easily seen that zein nanofibers obtained in Run-1, Run-5, and Run-9, in which HAc was used as a solvent, showed bead-free, smooth, and homogeneously distributed morphology. This better morphology compared to the morphologies of fibers produced from EtOH solutions was a result of that the spinning continuity of the HAc solution was much better than that obtained when using EtOH solutions. This might be because of the lower vapor pressure of the HAc which would reduce the amount of solvent that was evaporating at the needle tip (Selling et al. 2007). The mean diameters of these fibers range from 106 to 264 nm with not very high deviation (Table 2.9). However, Selling et al. (2007) and Miri et al. (2016) produced nanofibers with larger diameters (above 400 nm) from the zein solution in HAc, probably due to the combined effect of higher voltage (above 20 kv) and higher zein concentrations used in their study.

Table 2.9 Experimental results of zein fiber diameters with their corresponding standard deviations and signal-to-noise (S/N) ratios based on ‘‘smaller the better’’

Run	Designation	Mean fiber diameter, nm	S/N values
1	A1B1C1D1	106 ± 29	-40.51
2	A1B2C2D2	220 ± 74	-46.84
3	A1B3C3D3	80 ± 18	-38.06
4	A2B1C2D3	136 ± 35	-42.67
5	A2B2C3D1	181 ± 43	-45.15
6	A2B3C1D2	207 ± 59	-46.32
7	A3B1C3D2	308 ± 102	-49.77
8	A3B2C1D3	127 ± 28	-42.08
9	A3B3C2D1	264 ± 80	-48.43

The fibers with larger diameters, ranging from 207 to 308 nm, were obtained by electrospinning of zein solution in EtOH compared to that in HAc or HAc/EtOH. The diameters of these fibers (Run-2, Run-6, and Run-7) were also distributed in a wide range of values with very high standard deviations (Table 2.9). Neo et al. (2013) also obtained the similar results in their study, in which 25% zein solution in EtOH was electrospun using similar process conditions in the present study. These EtOH-based

zein fibers are also different from others in their morphology. They have ribbon-like fiber morphology resulted from rapid skin formation and collapsing of fibers due to the high volatility of EtOH (Miyoshi et al., 2005; Torres-Giner et al., 2008). The high volatility of EtOH also caused clogging of the polymer at the tip of the needle, which is a general problem occur during electrospinning (Kanjanapongkul et al., 2010; Augustine et al., 2015). This made the electrospinning process quite laborious and caused the formation of non-smooth irregular shaped fibers (Figure 2.5).

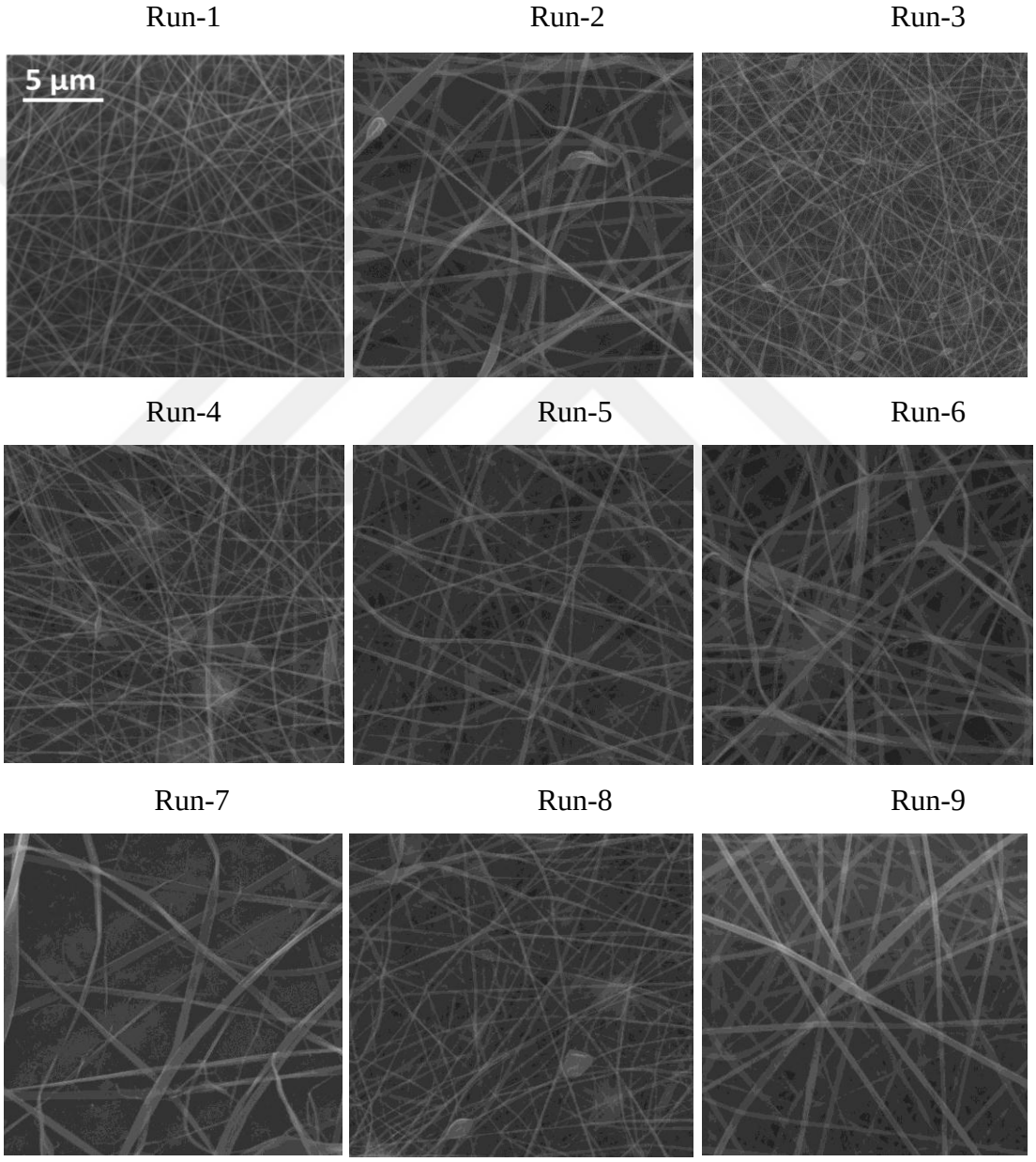


Figure 2.5 SEM images of electrospun zein nanofibers produced according to the experimental design shown in Table 2.4

The mean diameter of electrospun fibers and standard deviation values remarkably decreased when HAc/EtOH was used as a solvent (Table 2.9). However, a number of beads were observed along the electrospun fibers (Figure 2.5), probably due to the combined effect of low viscosity and high surface tension. More beads were formed in the fiber structures obtained in Run-3 and Run-8 compared to Run-4. This might be explained by the higher flow rate values used in these two runs. According to Li and Wang (2013), high flow rates cause formation of beaded fibers, which is attributed to the larger droplet at the end of the capillary. This results in incomplete drying because of non-evaporation of the solvent and low stretching of the solution in the flight between the needle and the collector.

2.3.2.2 Characteristics of zein solutions

In the current study, the change of solvent type caused also the changes in conductivity, surface tension, and viscosity values as shown in Table 2.10. It is well-known that the morphology of electrospun nanofibers depends on not only operating conditions, but also these solution characteristics (Fong et al., 1999). The most critical parameter among them is surface tension since it determines the upper and lower limits of the electrospinning process if other parameters are kept constant. High surface tension is generally known to inhibit the electrospinning process, thus resulting in the formation of drops instead of spinning (Hohman et al., 2001). However, in this study surface tension values of the solutions were not so high that it mostly did not hinder the spinning process. Despite having the highest surface tension value (30 mN/m) as shown in Table 2.10, smooth and homogenous nanofibers were produced via continuous spinning from HAc-based zein solution because of its moderate viscosity value. However, as shown in Figure 2.5 beaded fibers were produced by spinning of EtOH/HAc-based zein solution because of the combined effect of low viscosity and high surface tension. It is important to be aware of that for a solution of low viscosity, surface tension is the dominant factor and beaded fiber may form (Li and Wang, 2013).

The viscosity values of the solutions were seen in Table 2.10 to be higher in the presence of the HAc probably due to increased aggregation of molecules at acidic pH levels (Davanço et al., 2007; Salles et al., 2015). Generally, increased viscosity causes the formation of the larger diameter of fibers. However, in Run-2, Run-6, and Run-7

the thickest zein fibers (respectively 220, 207, and 308 nm) were formed from the EtOH-based solution, in spite of its lower viscosity than others (Figure 2.5 and Table 2.10). This observation might be attributed to the fact that EtOH is more volatile and thus evaporated faster. This results in a greater increase of viscosity of polymer solution at the tip of the needle during spinning, leading to formation of thicker fibers (Fong et al. 1999).

Table 2.10 Characteristics of prepared zein solutions

Run	Solution	Viscosity (Pa.s)	Conductivity (μS/cm)	Surface Tension (mN/m)
1,5,9	Zein in HAc	0.730±0.015	37.1±0.2	30.0±0.0
2,6,7	Zein in EtOH	0.173±0.002	762.7±1.7	27.7±0.2
3,4,8	Zein in HAc/EtOH	0.259±0.005	455.7±0.5	29.8±0.5

When HAc-based solution was used in Run-1, Run-5, and Run-9, the fibers with the larger diameters (respectively 106, 181, and 264 nm) were produced compared to that of fibers produced from HAc/EtOH-based solution. It is consisted with several studies showing that higher viscosity in the feed polymer solution gives rise to the formation of uniform fibers with larger diameters due to polymer chain entanglements, which makes jet stretching more difficult (Ramakrishna et al., 2005; Salles et al., 2015; Erencia et al., 2015).

It was shown in Table 2.10 that the conductivity values of solutions significantly increased when EtOH used. This caused a significant decrease in the diameter of the electrospun fibers in Run-3, Run-4, and Run-8 (respectively 80, 136, and 127 nm). This phenomenon can be explained by the fact that increasing of conductivity also increases the charge density on the surface of the drop in the needle, which gives rise to increases in elongation forces exerted on the polymer jet providing a smaller fiber (Li and Wang, 2013). However, when EtOH was used as a single solvent, the fibers with larger diameters were obtained, despite its very high conductivity value. This might be due to the fact that very high volatility of EtOH resulted in greater viscosity as mentioned above and might create a dominant effect over the conductivity effect.

2.3.2.3 Analyzing the results by Taguchi's Method

The Taguchi method, in which orthogonal arrays are used to organize the factors and the levels affecting the process, offers the opportunity to be able to evaluate all parameters together or independently with the minimum number of experiments (Ross, 1996). Taguchi used signal-to-noise (S/N) ratio, which measures the deviation of quality characteristics from the desired values in order to assess the quality. In this study, S/N ratio values calculated by rearranging the results by 'Minitab-17' statistical software according to "smaller the better" characteristic formula and were given in Table 2.9. As depicted in Table 2.9, Run-7, where EtOH-based solution was electrospun by using the highest level of voltage applied, yielded the highest diameters of zein nanofibers with the highest range of variation in diameter (308±102 nm) and the lowest value of S/N ratio (-49.77). In contrast, the thinnest nanofibers (80±18 nm) with the highest value of S/N (-38.06) ratio value were obtained in Run-3, where HAc/EtOH-based solution was electrospun by using the lowest level of voltage applied.

In order to realize the effects of each factor and level on the response, the average fiber diameter and S/N ratio value for different levels of each parameter were calculated as shown in Table 2.11. According to range analysis in this table, the differences between maximum and minimum values of the three levels were calculated and the degree of importance of each factor on the fiber diameter was determined in a way that the factor with larger range had a more significant effect on the fiber diameter.

From these results, it is clearly understood that the type of solvent in the solution is the most influential factor on the diameter of electrospun zein fiber. When EtOH was used only solvent in the zein solution, the diameters of electrospun fibers were higher; when it was used with HAc, thinner but beaded fibers were produced. However, as depicted in Figure 2.5, smooth, bead-free, and homogeneously distributed fibers could be obtained by using HAc as a single solvent, but the diameters of these fibers not less than that of fibers produced from HAc/EtOH-based solution.

The applied voltage was found as the second influential factor on the zein fiber diameter. The fiber diameters were seen as smaller when the applied voltage was 12

kV and they increased with increasing voltage. In literature, the effect of voltage on the fiber diameter is also crucial and still controversial. Several researchers suggested that very high levels of voltage applied accelerates jet extending and causes a greater volume of polymer solution to be drawn from the needle, resulting in the formation of the larger diameter of fibers (Demir et al., 2002; Zhang et al., 2005). On the other hand, several researchers suggested that a strong electrical charge provides with a greater stretch of the polymer solution leading to usually decrease the diameter of the fibers (Yuan et al., 2004; Bhardwaj and Kundu, 2010). In addition to this, Sill and Recum (2008) showed that with an increase in the applied voltage beyond a critical value, the fiber diameter decreases initially because of a higher degree of jet stretching and then increases after a definite point. In another study, Yördem et al. (2008) indicated that the effect of applied voltage on fiber diameter may vary by type of polymer-solvent system and the distance between the collector and the tip of the needle.

Table 2.11 Response table for means of fiber diameters and S/N values

Level	**Means of fiber diameters (nm)**				**Means of S/N values**			
	A	B	C	D	A	B	C	D
1	135.3	183.3	146.7	183.7	-41.81	-44.32	-42.97	-44.70
2	174.7	176.0	206.7	245.0	-44.71	-44.69	-45.98	-47.65
3	233.0	183.7	189.7	114.3	-46.76	-44.27	-44.33	-40.94
Range	97.7	7.7	60.0	130.7	4.95	0.42	3.02	6.71
Importance	2	4	3	1	2	4	3	1
Best level	12 kV	15µL/min	10 cm	HAc/EtOH				

It is inferred from Table 2.11, the flow rate and the distance between the collector and the tip were found to have fewer effects on the fiber diameter compared to other factors. Generally, increasing the flow rate increases the fiber diameter because of more fluid ejection in a jet (Zuo et al., 2005). Although the distance between the collector and the tip is generally thought as the least important factor, the effect of it is almost the same as for increasing voltage (Frenot and Chronakis, 2003; Ramakrishna et al., 2005, Homayoni et al., 2009). In the current study, the effects of the flow rate and the distance showed both increasing and decreasing trends. The explanation for

this observation is that the effects of these two parameters also vary with other parameters.

2.3.2.4 Optimum combinations of factors and confirmation experiment

Figure 2.6 shows the mean effects of each factor on the mean fiber diameter. According to Table 2.11 and Figure 2.6, optimum levels of the factors were determined as following conditions: 12 kV of applied voltage, 15 µL/min of solution flow rate, 10 cm of distance between needle tip to collector, and HAc/EtOH as solvent in order to obtain the thinnest nanofiber. However, it was decided to use HAc instead of HAc/EtOH because of the bead formation on the fibers produced from HAc/EtOH-based solution and its poor spinning due to the high volatility of EtOH.

Figure 2.7 shows the SEM images with distribution of fiber diameters of electrospun zein nanofibers obtained on optimized conditions. According to the images and bar graphs, bead-free, smooth and homogenous zein nanofibers with small diameters were able to obtain.

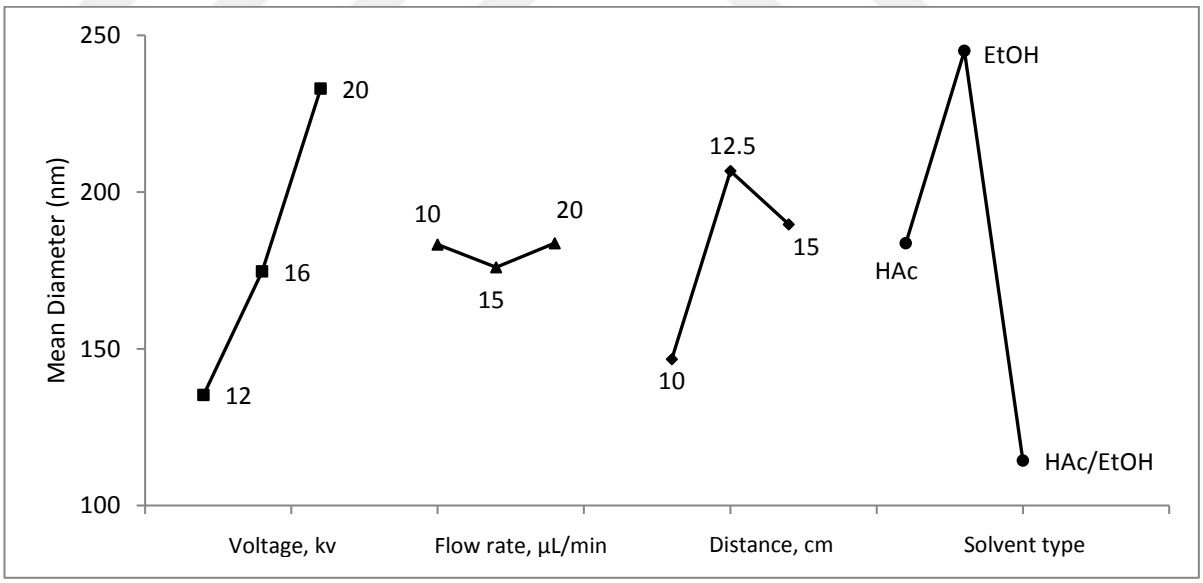


Figure 2.6 Main effect plots of each parameter on means of nanofiber diameter

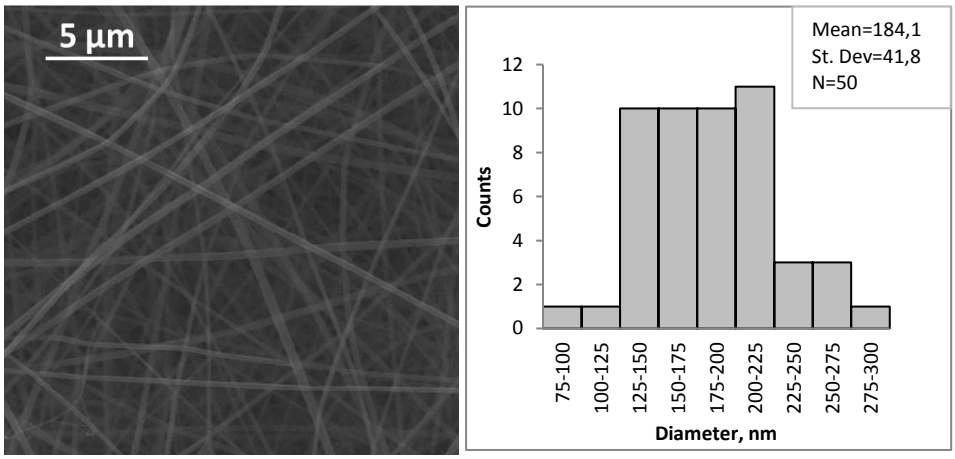


Figure 2.7 SEM image and bar graph of diameter ranges of electrospun zein nanofibers obtained with the optimized conditions (12 kV; 15 µL/min; 10 cm; HAc)

2.3.2.5 Effect of zein concentration

The morphologies of the electrospun zein nanofibers at various concentrations obtained by using the optimum conditions were illustrated in Figure 2.8. Even though the most homogenous nanofibers with the smallest diameter (about 66 nm) were obtained from the zein solution at 16% (w/w), many beads were observed along the fibers probably due the low viscosity resulting in spraying of the solution (Ramakrishna et al., 2005). However, fewer beads were formed when the zein concentration increased to 20% (w/w), attributing to increase in the viscosity (Table 2.12). With further increase of concentration, no bead formation was observed along the electrospun nanofibers, but the mean diameters of the fibers increased to 141.5 nm because of the high viscosity (Ramakrishna et al., 2005). When the zein concentration was 28% (w/w), the mean diameters of the fibers markedly increased to 368 nm. There was no fiber production from the zein solution at 32% (w/w) due to hindering spinning because of very high viscosity combined with very high surface tension as shown in Table 2.12. In the current study, both viscosity and surface tension values of the solutions increased with increasing zein concentration. It is probably attributed to that the increase in solution concentration could increase the cohesiveness among the water molecules at the liquid/air interface leading to the observed increase in surface tension of the solutions (Choktaweesap et al., 2007).

Table 2.12 Characteristics of the zein solutions prepared with HAc at different concentrations

Zein (%,w/w)	Viscosity (Pa.s)	Conductivity (mS/cm)	Surface Tension (mN/m)
16	0.080±0.011	22.6±0.2	28.8±0.2
20	0.212±0.009	28.5±0.6	29.5±0.4
24	0.549±0.005	36.4±1.2	29.8±0.2
28	1.328±0.009	38.9±0.2	30.5±0.0
32	3.185±0.181	39.6±0.0	30.8±0.2

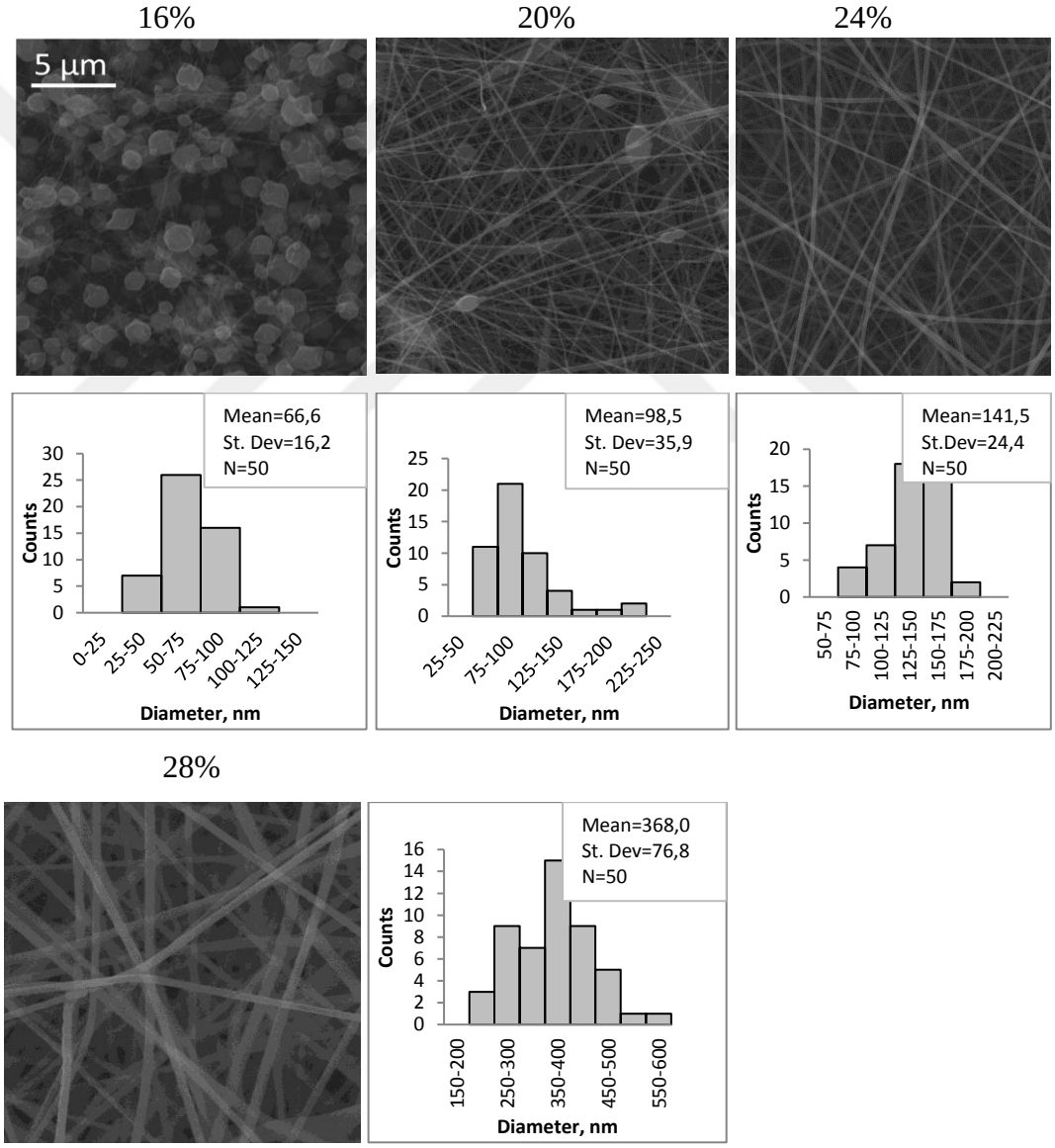


Figure 2.8 SEM images and bar graphs of diameter ranges of electrospun zein nanofibers obtained from different concentrations of zein solutions at the optimized conditions (12 kV; 15 µL/min; 10 cm; HAc)

2.4 Conclusion

In this part of the study, electrospinning of gelatin and zein was separately optimized by employing an L9 orthogonal array along with S/N ratios of Taguchi's Method, in which effects of four factors with three different levels on the morphologies of electrospun nanofibers were investigated. The acetic acid concentration was found as the most influential factor on the diameter of electrospun gelatin fibers. The smooth and bead-free nanofibers with smaller diameters were produced by electrospinning of the gelatin solutions at lower HAc concentrations. The solvent type was found as the most influential factor on the diameter of zein fibers. The smooth and bead-free nanofibers with smaller diameters were produced by electrospinning of the HAc-based zein solutions. The applied voltage was the second most influential factor on both the gelatin and zein fiber diameter. However, the flow rate and the distance between the collector and the tip were found to have fewer effects on the fiber diameter compared to other factors. The optimum combination of factors was determined an applied voltage of 18 kV, a flow rate of 15 μL/min, a distance of 12.5 cm, and a HAc concentration of 20% for the production of gelatin nanofibers, whereas an applied voltage of 12 kV, a flow rate of 15 μL/min, a distance of 10 cm, and a solvent type of HAc for the production of zein nanofiber. According to the electrospun fibers with different polymer concentrations which were produced by using these optimum conditions, the thinnest fibers were produced at the polymer concentrations of 16 and 20%, but many bead structures were observed along these fibers. When the polymer concentration of the solutions increased, the bead-free and smooth fibers could be produced, but the diameters of these fibers increased. However, the thinnest bead-free fibers were produced by electrospinning of the polymer solutions at the concentration of 24%.

CHAPTER 3: ENCAPSULATION OF TOMATO PEEL EXTRACT INTO NANOFIBERS BY ELECTROSPINNING

3.1 Introduction

Up to now, the stabilization of carotenoids by electrospinning technique has been less investigated with only a few studies on encapsulation of β-carotene into nanofibers (Fernandez et al., 2009; Zômpero et al., 2015; Peinado et al., 2016; Reksamunandar et al., 2017). These studies focused on encapsulation of only β-carotene, not any other carotenoid. Additionally, no study related to encapsulation of lycopene by electrospinning technique to improve its stability and water solubility has been reported in literature. However, the present study explored the use of electrospinning for encapsulation of total carotenoids extracted from tomato peel (composed of mainly lycopene and moderately β-carotene) into gelatin and zein nanofibers to improve its thermal and storage stability, antioxidant activity, and water solubility. The morphology, structure, encapsulation efficiency of produced composite fibers and the compability of components within the fibers were also explored.

3.2 Materials and Methods

3.2.1 Materials

Type B gelatin powder from bovine skin, zein (Z 3625), lycopene standard, HPLC grade solvents; glacial acetic acid (HAc), hexane, acetone, methanol, methyl tert-butyl ether (MTBE), were purchased from Sigma Aldrich (St. Louis, Mo., U.S.A). Tomato was purchased from a local market.

3.2.2 Drying of tomato peels and extraction of carotenoids from dried tomato peels

The moisture content of the fresh peels of tomatoes was determined by 105 °C oven method (AOAC, 1995). The fresh peels of tomatoes were dried by using a dehydrator at 50 °C until they reached to 6-7% moisture. After that, dried peels were ground into about 1 mm size. The dried and ground peels were stored in glass jars covered with aluminum foil at -70 °C until extraction process.

Carotenoids were extracted from tomato peels by the modified method described by Strati and Oreopoulou (2011) and Albanese et al. (2014). Homogenized dry tomato waste was placed in the extraction vessel and homogenized with acetone and hexane (50:50) as the extraction solvent in a ratio of 1:10 (w/v). The mixture was stirred for 30 min at room temperature. At the end of the first extraction step, the mixture was filtered through filter paper. The residue collected was put again in the vessel and re-extracted with fresh extraction solvent. The whole procedure was repeated three times to complete the successive extraction steps. The obtained extract-solvent mixture was concentrated to about 10% of mixture by using a rotary vacuum evaporator at 35-40 °C. The tomato peel extract (TPE) was then stored in amber colored bottles at – 70 °C for further use in the encapsulation process.

3.2.3 Preparation and characterization of polymer solutions

Three different concentrations (5, 10, and 20 wt%- based on the weight of polymer) of the dried extract under N_2 and powder gelatin (24% wt/wt) were dissolved in aqueous acetic acid (20%, v/v). These solutions were designated as Ge–5, Ge–10 and Ge–20, respectively. Similarly, three different concentrations (5, 10, and 20 wt% - based on the weight of zein) of the dried extract under N_2 and powder zein (24% wt/wt) were dissolved in pure acetic acid. These prepared solutions were designated as Ze–5, Ze–10 and Ze–20, respectively. For comparison, a gelatin solution and a zein solution without extract were also prepared for the production of neat fibers. Each gelatin solution was stirred by using a magnetic stirrer at 40 °C for about 2 hours and each zein solution was stirred by using a magnetic stirrer at room temperature for about an hour.

The viscosities of the prepared polymer solutions were determined at 24°C by using Haake Rheostress RS1 (Karslruhe, Germany) equipped with a plate-plate sensor (dia 35 mm, gap 1 mm). The shear rate ranged from 0 to 200 s-1 in 60 s during viscosity measurement.

The surface tension and electrical conductivity values of the solutions were determined by using a tensiometer (Krüss K6, Germany) and a conductivity meter (Orion 115A, Thermo, USA) at room temperature.

3.2.4 Electrospinning

Electrospinning process was carried out at room temperature (24 ± 2 °C) by using the Nanotel (NTEE 80-1) electrospinning device consisting of a syringe pump, a high voltage power supply and a collector plate covered in non-stick aluminum foil for easy removal of mats. The solutions were placed in 10 ml syringes fixed horizontally on the syringe pump delivering the solution to the tip of a metal needle where the voltage was applied. Fibers were then collected on a stainless-steel collector plate covered with aluminum foil. The electrospinning process for gelation and zein solutions was carried out by using the following conditions decided in the previous optimization studies: 18 kV of applied voltage, 15 µL/min of solution flow rate, 12.5 cm of distance between the needle tip to the collector and 12 kV of applied voltage, 15 µL/min of solution flow rate, 10 cm of distance between the needle tip to the collector, respectively. The fiber mats produced were kept at -70 °C until being used for characterization analysis.

3.2.5 Characterization of nanofibers

The morphology and diameters of the produced nanofibers were examined by using a scanning electron microscope (SEM) (JEOL, JSM-6390LV, Japan) with accelerating voltage of 20 kV. Fiber diameter analysis was carried out by randomly counting 50 fibers per experiment using a software (ImageJ, 1.48V). The mean diameter and standard deviation were calculated as the responses.

The thermal behavior of the fiber samples and the extract was determined by a differential scanning calorimetry (DSC) (DSC-6, Perkin Elmer, Netherlands). A

sample (about 5 mg) was heated from 20 to 300 °C at a heating rate of 10 °C/min under a constant flow rate of nitrogen gas of 50 mL/min.

Functional group analysis of the samples was examined by using Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy device (Perkin Elmer Spectrum 100, Netherlands) by scanning the samples from 600 to 4000 cm^{-1}. Each measurement was an average of 32 scans at 4 cm^{-1} resolution.

3.2.6 Encapsulation efficiency and loading capacity

Encapsulation efficiency (EE) and loading capacity (LC) were determined according to the method established by Moomand and Lim (2014), which is based on the measurement of the amount of non-encapsulated extract. The electrospun nanofiber was submerged into hexane:acetone (1:1) mixture for 30 s to remove the free extract. The absorbance of the solution was read at 450 nm by using UV-Vis Spectrophotometer (SP-3000nano, OPTIMA, Japan). The amount of extract present in hexane:acetone (1:1) was determined by using a calibration curve (R=0.99) prepared by measuring the absorbance of a series of solutions with different concentrations of TPE dissolved in hexane:acetone (1:1). The percentage EE and LC values were calculated as:

$$EE = (A-B)/A *100$$

$$LC = (A-B)/C *100$$

where A was the total theoretical amount of extract, B was the free amount of extract in the solution, and C was the weight of fibers

3.2.7 Storage stability of encapsulated tomato peel extract

Storage studies of the encapsulated extract in the electrospun fibers (with the highest concentration of extract in fiber, 20%) and the non-encapsulated extract (dried under N_2) as a control sample were carried out under both light and dark environment at three different temperatures (-20, 4, 25 °C) for 14 days. Some tubes were wrapped in the aluminum foil to protect the content from light in order to test the effect of light. The change in lycopene retention and the antioxidant activity of the samples were examined after 3, 7, and 14 days of storage. The fiber samples were magnetically

stirred in hexane and in hexane:acetone (1:1) mixture with a ratio of 2:1 (w/v) for 2 hours at room temperature prior to respectively, HPLC and antioxidant activity analysis. Additionally, an equal concentration of non-encapsulated dry extract was prepared by the same way.

3.2.7.1 HPLC analysis

All sample solutions were filtered through a 0.45 μm membrane filter to remove particulate residues before injection. The HPLC (High Performance Liquid Chromatography) system was equipped with a YMC (Tokyo, Japan) C30 column (250x4.6mm I.D.). 20 μL of sample was injected for HPLC analysis. A mobile phase consisting of (A) methanol/MTBE/water (81/15/4), and (B) methanol/MTBE/water (7/90/3) was used with a following gradient system: 0-100 %B (0-90 min). The flow rate was maintained at 1 ml/min, and the detection was carried out at 450 nm.

Lycopene within the extract-loaded fibers and the non-encapsulated extract was identified by comparing its retention time with the corresponding peak obtained with the standard solution. Quantification of lycopene in the electrospun fibers was carried out by using the calibration curve, which was prepared by using a calibration curve (R=0.99) prepared by measuring the peak areas of standard lycopene solutions with different concentrations.

3.2.7.2 Determination of antioxidant activity

The antioxidant activity of each sample was evaluated using 1,1-diphenyl-2-picrylhydrazil (DPPH) assay method (Neo et al., 2013; Albanese et al., 2014). One hundred micro liter of the sample solutions was allowed to react with 3ml of DPPH (0.1mM solution in methanol) for 30 min in dark. Then, the absorbance was measured at 517 nm and the antioxidant activity (AA %) was calculated using the following equation:

$$AA\% = [(A_0 - A_1)/A_0] \times 100\%$$

where A_0 is the absorbance of the control reaction, and A_1 is the absorbance of the sample

3.2.8 Carotenoid extract solubility in water

An accurately weighed amount of 5 mg carotenoids extracted from tomato peel or 25 mg of gelatin fibers loaded with 20% carotenoid extract (containing 5 mg carotenoid extract) was dissolved in 10 mL of distilled water. The mixture was vortexed for 2 min prior to centrifugation at 3000 rpm for 10 min. The absorbance of the clear solution was measured at 450 nm with UV-Vis Spectrophotometer (SP-3000nano, OPTIMA, Japan). The percentage of extract dissolved in water was calculated from the previously prepared standard calibration curve.

3.2.9 Statistical analysis

All the experiments were performed in triplicate and results were expressed as the mean values ± standard deviations. Significant differences among samples were evaluated with analysis of variance (One-way ANOVA with Duncan test) at 5% significance level by using the statistical software SPSS (IBM SPSS Statistics, Version 22, New York, USA)

3.3 Results and Discussion

3.3.1 Results related to encapsulation of tomato peel extract into gelatin nanofibers

3.3.1.1 Fabrication of composite gelatin nanofibers

The SEM images with fiber diameter distributions of gelatin nanofibers with and without the carotenoid extract from tomato peel are given in Fig 3.1. The extract-loaded fibers did not show different morphology from the neat gelatin fibers. All nanofibers were almost bead-free, smooth, and homogeneously distributed. However, the diameters of nanofibers significantly increased by the addition of tomato peel extract (TPE), which could be explained by the characteristics of gelatin solutions shown in Table 3.1. By the addition of TPE into the gelatin solutions, the surface tension and conductivity values decreased, whereas the viscosity of the solutions increased. The change in the surface tension values was not found to be significant to hinder the spinning process causing bead formation. Generally, increased viscosity causes increasing fiber diameter. The tomato peel extract significantly ($P<0.05$)

increased the viscosities of gelatin solutions from 256 to 397 cP probably due to more entanglements of gelatin molecules caused by more solutes in the solution (Sriyanti et al., 2017). In addition to this, the significant decrease in the conductivity values (from 2.51 to 2.23 mS/cm) caused a significant ($P<0.05$) increase in the fiber diameters. Static charges come into prominence on jet surface by a reduction in conductivity values, leading to an increase in capacity of polymer solutions on the tip of the needle, which in turn increased the fiber diameter (Wang et al., 2013).

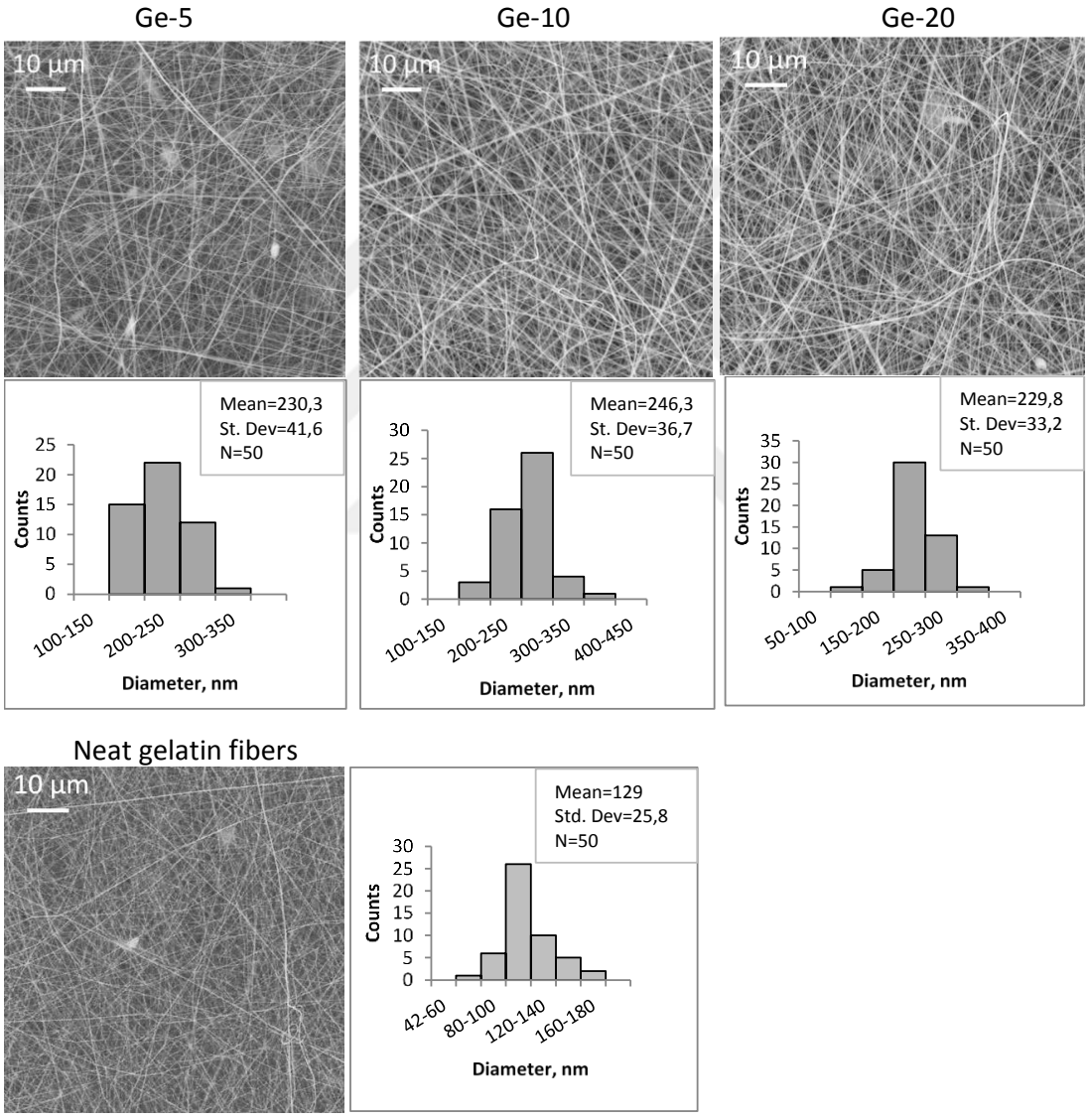


Figure 3.1 SEM images with histogram graphs of diameters of TPE-loaded and neat gelatin fibers

Table 3.1 Characteristics of prepared gelatin solutions and fiber diameters

TPE concentration, %	Viscosity (cP)	Conductivity (mS/cm)	Surface Tension (mN/m)	Diameter, nm
0	256.2±4.2[a]	2.51±0.4[a]	40.0±0.2[a]	129±25.8[a]
5	321.1±3.4[b]	2.39±0.2[b]	38.0±0.4[b]	230.3±41.6[b]
10	371.4±1.8[c]	2.38±0.2[b]	37.9±0.2[b]	246.3±36.7[b]
20	397.1±2.9[c]	2.23±0.2[c]	37.6±0.0[b]	229.8±33.2[b]

Data with the same superscript letter in the same column indicate that they are not statistically different ($p>0.05$).

3.3.1.2 Characterization of electrospun gelatin nanofibers

The differential scanning calorimetry (DSC) thermograms of the carotenoid extract from tomato peel, neat gelatin fibers and the extract-loaded gelatin fibers are represented in Figure 3.2. The carotenoid extract exhibited a sharp endothermic peak corresponding to the melting point of 175.8 °C, and an exothermic peak at 242.0 °C, indicating its degradation. The carotenoid extract thermogram was consistent with the studies reported in the literature (Faisal et al., 2013; Pu and Tang, 2017). The thermograms of carotenoid extract-loaded gelatin fibers did not show any characteristic melting peak of carotenoid extract, indicating that the crystalline structure of carotenoids had turned to the amorphous state in gelatin fiber by electrospinning. It can be concluded that the electrospinning process prevents the crystallization and remains the amorphous solid solution state of carotenoids which is well incorporated into the gelatin nanofibers.

Only a broad endotherm appeared at about 200 °C in the thermograms of electrospun gelatin fibers rather than a longer endothermic peak at about 230 °C, which was reported as the decomposition temperature of pure gelatin in the literature (Cortesi et al., 1998). These changes could be attributed to an increase in the amorphous structure of random coil conformations of the gelatin, that is, a decrease in its crystallinity (Ki et al., 2005; Erencia et al., 2015).

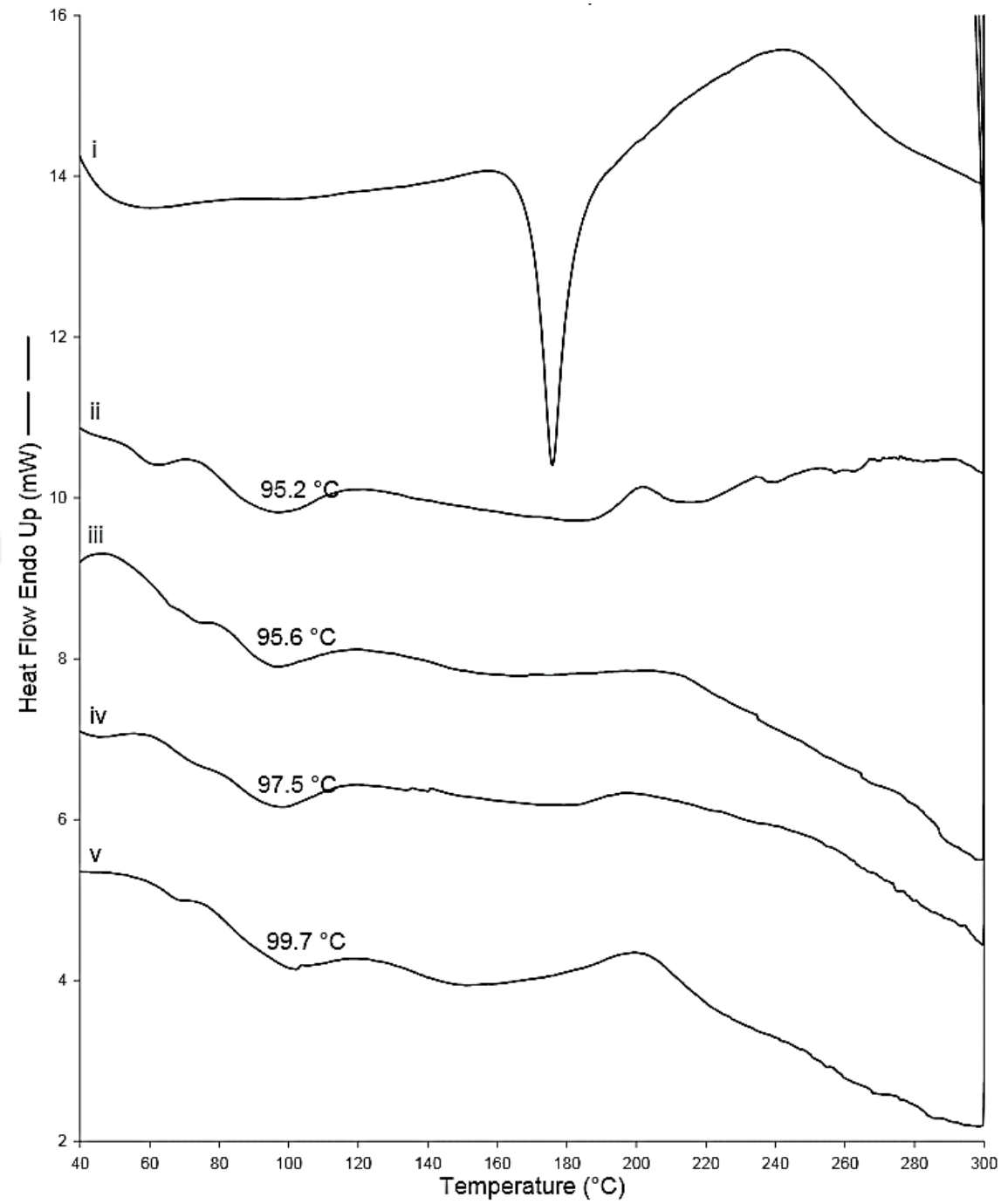


Figure 3.2 DSC thermograms of different samples: (i) carotenoid extract; (ii) neat gelatin fiber; (iii) Ge-5; (iv) Ge-10; (v) Ge-20 fibers with the extract concentrations of 5%, 10% and 20% (w/w), respectively

The melting temperature (Tm) of pure gelatin fiber was at 95.2°C and agreed with the reported value. Addition of carotenoid extract into the gelatin fibers resulted in a shift in Tm (to 99.7°C) and no phase segregation or secondary peaks are visible, suggesting that the resulting fibers are homogenous (Li et al., 2006).

The compability among the components and the second-order interactions within the materials has been frequently examined by using Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy method. The compability among the structures are crucial for high-quality and stable products. This compability and stability could be attributed by second-order interactions such as hydrogen bonding, electrostatic, and hydrophobic interactions (Li et al., 2009; Yang, et al., 2013). The ATR-FTIR spectra of tomato peel extract, neat gelatin fiber, and extract-loaded gelatin fibers; Ge-5, Ge-10, and Ge-20 are presented in Figure 3.3. The characteristic gelatin peaks at 1642 cm^{-1} (-C=O streching vibration), and 1540 cm^{-1} (C-N stretch). Gelatin has also exhibited peaks at 3291 cm^{-1} (N–H stretching vibrations), and 2956 cm^{-1} (C–H stretching vibrations of aliphatic groups), meaning that it could act as both potential proton donors and receptors for hydrogen bonding (Erencia et al., 2015). This indicates the presence of hydrogen-bonding interactions within the extract-loaded gelatin fibers. Compared to neat gelatin fiber, almost no changes in the spectra of extract-loaded gelatin fibers were observed, showing that the tomato peel extract could be compatibly entrapped into the gelatin fibers. The characteristic spectrum of tomato peel extract within the spectrum of gelatin fibers demonstrates that molecular structure of the extract is maintained during electrospinning. Moreover, the encapsulated extract within the biopolymer wall was realized to be physical in nature rather than a chemical interaction of any sort (Hani et al., 2017). A complete disappearance of few signature sharp peaks of solvents within the extract was observed in the spectra of extract-loaded gelatin fibers because of solvent evaporation during the spinning process.

3.3.1.3 Encapsulation efficiency and loading capacity

The values of encapsulation efficiency (EE) and loading capacity (LC) are essential to give an idea about the quality of encapsulation. At the theoretical concentrations of 5, 10, 20%, the extract LC values of the gelatin fibers were 4.52±0.07, 9.43±0.14, and 19.25±0.25, respectively, and the EE values were 90.47±1.33, 94.28±1.36, and 96.26±1.23, respectively. These results indicated that there was almost no loss of tomato peel extract during the electrospinning process and above 90% of the extract could be encapsulated into gelatin fibers, meaning that the carotenoid extract from tomato peel can be efficiently encapsulated into gelatin by electrospinning technique.

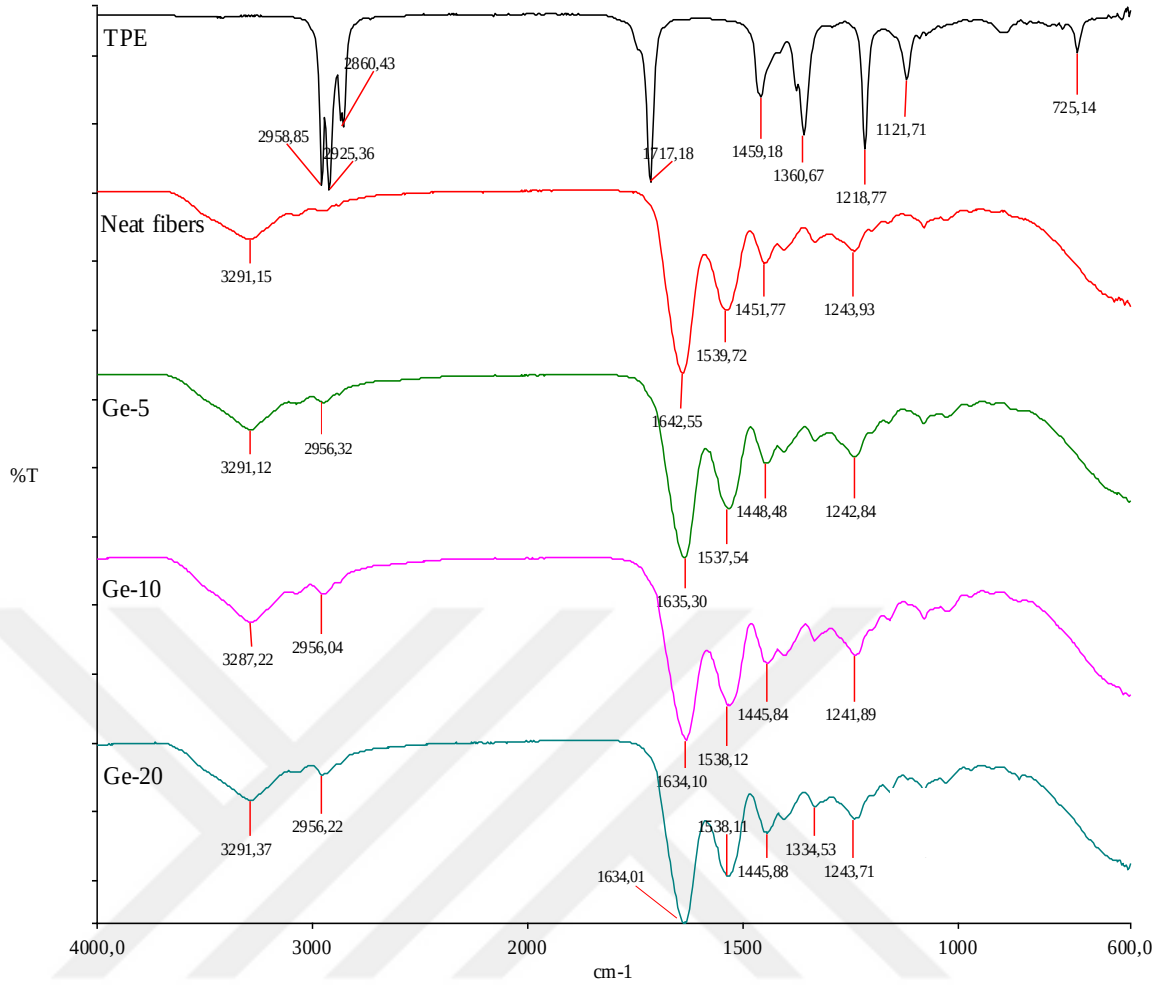


Figure 3.3 FTIR spectra of TPE, neat gelatin nanofibers, the Ge-5, Ge-10, and Ge-20 composite fibers

3.3.1.4 Change in lycopene content during storage

The changes in the retention of lycopene, which is the major carotenoid of tomato peel, were analyzed throughout 14-day storage of encapsulated and non-encapsulated tomato peel extract at different temperatures (-20, 25, 4 °C) and in dark or lighted conditions. At first glance to Fig 3.4, it can be clearly seen that the non-encapsulated extract showed the highest lycopene degradation (above 90%) under all tested conditions during the first 3 days. However, the encapsulated extract into gelatin fibers showed better retention of lycopene because of the preserving effect of nano-encapsulation by electrospinning (Moomand and Lim, 2014; Fernandez et al., 2009).

The higher lycopene degradation was seen in the encapsulated extract samples stored at 25 °C under light conditions compared to 4 °C in the dark. The detrimental effect of

light and temperature on carotenoids has been introduced number of times in the literature (Boon et al., 2010; Britton, 1995). The encapsulated extract in the gelatin fibers stored at -20 °C showed the highest retention of lycopene with almost no lycopene degradation.

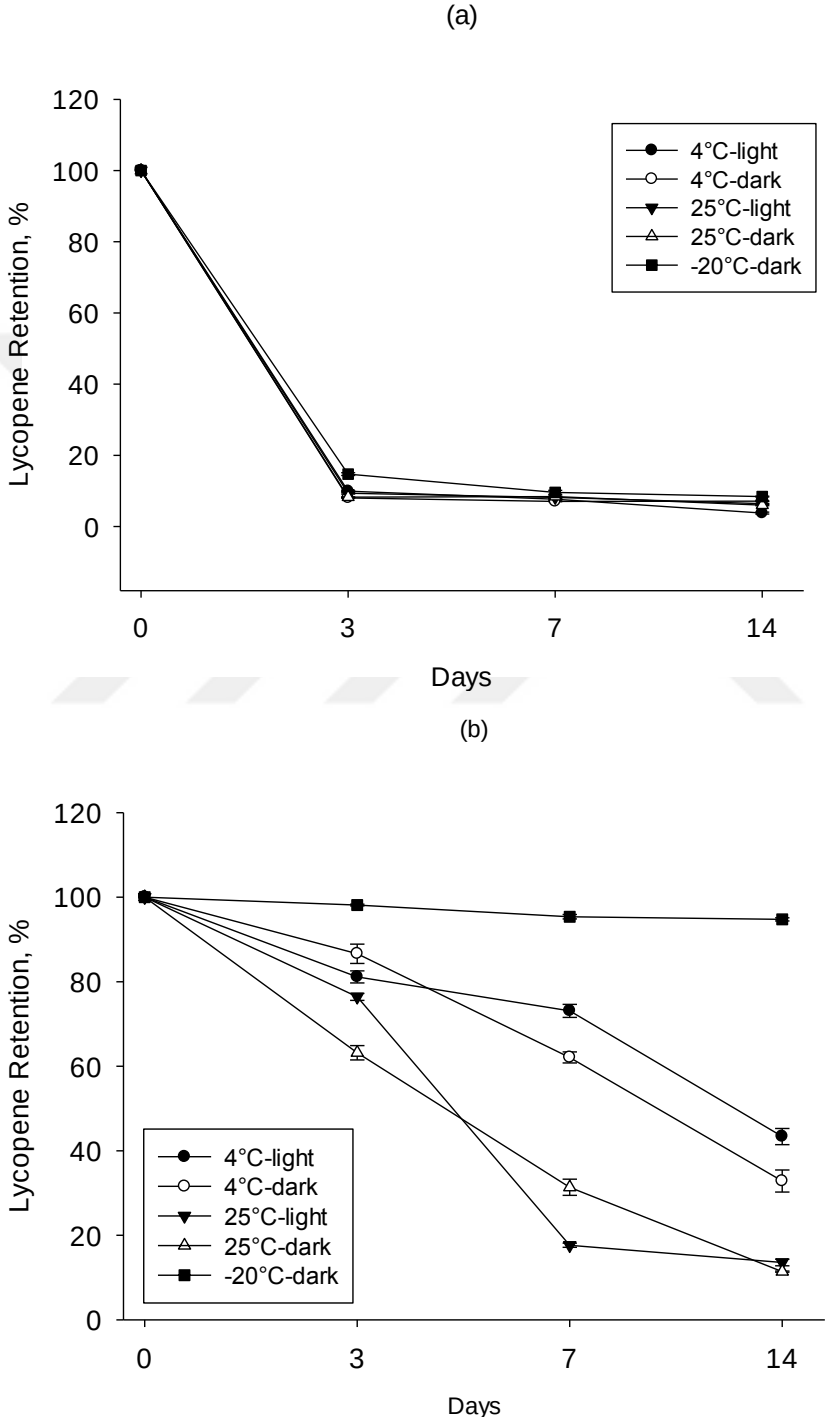


Figure 3.4 Change in lycopene retention (a) non-encapsulated tomato peel extract; (b) extract-loaded electrospun gelatin fibers during 14-day storage

3.3.1.5 Change in antioxidant activity during storage

Since tomato carotenoids are already known to have antioxidant properties due to the presence of different phytochemicals (Kalogeropoulos et al., 2012), the effectiveness of the antioxidant properties of the carotenoids extract from tomato peels after being encapsulated into the gelatin nanofibers was determined by measuring antioxidant activiy (AA) using the DPPH assay method. The change in the antioxidant activity (AA) of the fiber samples loaded with tomato peel extract and the non-encapsulated extract was evaluated during 14-day storage under different conditions. It is inferred from Table 3.2 that the values of AA mainly showed a decreasing trend during 14 days. However, it changed significantly among all samples except the ones at the temperature of -20 °C. The decrease in AA of the non-encapsulated extract samples after 14 days was higher than 35 %, whereas it was less than 27% for the encapsulated ones into the gelatin fibers. This clearly confirms the protective effect of nanoencapsulation on the AA of the tomato peel extract. It can be explained that nanoencapsulation by electrospinning protects the encapsulant material from the light and oxygen and consequently prevents its degradation by virtue of its nano-scale dimensions and very large surface area. Moreover, the nanoencapsulation of the extract into nanofibers significantly ($p<0.05$) increased the antioxidant activity of the extract about 10 times, probably due to the fact that nanoencapsulation helps in enhancing the antioxidant potential of encapsulant by reducing the size of its molecules into the nano-metric size (Külkamp et al., 2011; Shah et al., 2016; Hussein et al., 2017).

3.3.1.6 Solubility of carotenoid extract in water

Carotenoids are usually not soluble in water and the attempts have been going on with different techniques in order to improve their solubility. In this study, the effect of encapsulation by electrospinning on the solubility of carotenoids extracted from tomato peel in water was investigated. The percentage of extract dissolved in water for non-encapsulated extract and encapsulated one within the gelatin fibers were 0.89±0.017 and 99.08±0.20%, respectively. In other words, almost all of the encapsulated carotenoid extract was dissolved in water, whereas only less than 1 % of the non-encapsulated one was dissolved. This astonishing result is presumably due to the combined effect of owing to the high solubility of gelatin in water and reducing the size of the carotenoid molecules into the nano-metric size by electrospinning.

Additionally, the loss of crystalline structure of the carotenoid extract and the intimate contact with gelatin might be another reason to highly improve its water solubility (Gómez-Estaca et al., 2015).

Table 3.2 Change in antioxidant activity (%) of fiber samples loaded with TPE and non-encapsulated extract during 14-day storage

Sample	Condition	Days				Change
		0	3	7	14	(%)
TPE	4°C-light	1.24 ± 0.02^{Xx}	1.17 ± 0.02^{Xx}	1.18 ± 0.03^{Xx}	0.79 ± 0.05^{Xy}	36.3
	4°C-dark	1.24 ± 0.02^{Xx}	1.19 ± 0.08^{Xx}	1.20 ± 0.02^{Xx}	0.81 ± 0.03^{Xy}	34.7
	25°C-light	1.24 ± 0.02^{Xx}	0.84 ± 0.01^{Yy}	0.85 ± 0.00^{Yy}	0.75 ± 0.04^{Xz}	39.5
	25°C-dark	1.24 ± 0.02^{Xx}	1.08 ± 0.02^{Zy}	0.85 ± 0.02^{Yz}	0.77 ± 0.03^{Xd}	37.9
	- 20°C	1.24 ± 0.02^{Xx}	1.23 ± 0.03^{Xx}	1.16 ± 0.02^{Xy}	1.12 ± 0.03^{Yy}	9.7
Ge-20 fibers	4°C-light	12.03 ± 0.33^{Aa}	9.42 ± 0.49^{Ab}	8.86 ± 0.56^{ACb}	9.85 ± 1.09^{Ab}	18.1
	4°C-dark	12.03 ± 1.00^{Aa}	9.47 ± 0.23^{Ab}	10.93 ± 0.79^{Bc}	9.44 ± 0.40^{Ab}	21.5
	25°C-light	12.03 ± 0.79^{Aa}	9.01 ± 0.44^{Ab}	8.30 ± 1.20^{Ac}	8.83 ± 1.23^{Abc}	26.6
	25°C-dark	12.03 ± 0.24^{Aa}	8.73 ± 0.52^{Ab}	9.47 ± 0.93^{Cb}	8.91 ± 0.87^{Ab}	25.9
	- 20°C	12.03 ± 0.23^{Aab}	11.89 ± 1.15^{Bab}	12.43 ± 0.79^{Db}	11.37 ± 0.57^{Ba}	5.5

Means with different letters and superscripts in each column (X, Y, and Z for TPE; A, B, C, and D for Ge-20 fibers) and row (x, y, and z for TPE; a, b, and c for Ge-20 fibers) differ significantly ($P < 0.05$) from each other. Data are presented as means ± SD (n = 3)

3.3.2 Results related to encapsulation of tomato peel extract into zein nanofibers

3.3.2.1 Fabrication of composite zein nanofibers

The SEM images with fiber diameter distributions of zein nanofibers with and without the carotenoid extract from tomato peel are given in Fig 3.5. The extract-loaded fibers did not show different morphology from the neat zein fibers. All nanofibers were bead-free, smooth, and homogeneously distributed. However, it was found that the diameters of nanofibers significantly increased by the addition of tomato peel extract (TPE), which could be explained by the characteristics of zein solutions shown in Table 3.3. The addition of TPE into the zein solutions decreased the surface tension and conductivity values, whereas increased the viscosity of the solutions. The slight decrease in the surface tension values was not found to have significant effects on the fibers morphologies. Generally, increased viscosity causes increasing fiber diameter.

The addition of TPE significantly (P<0.05) increased the viscosities of zein solutions from 425 to 520 cP probably due to more entanglements of zein molecules caused by more solutes in the solution (Sriyanti et al., 2017). In addition to this, the significant decrease in the conductivity values (from 35 to 25 µS/cm) caused a significant (P<0.05) increase in the fiber diameters. Static charges come into prominence on the jet surface by a reduction in conductivity values, leading to an increase in capacity of polymer solutions on the tip of the needle, which in turn increased the fiber diameter (Vega-Lugo and Lim, 2008; Wang et al., 2013).

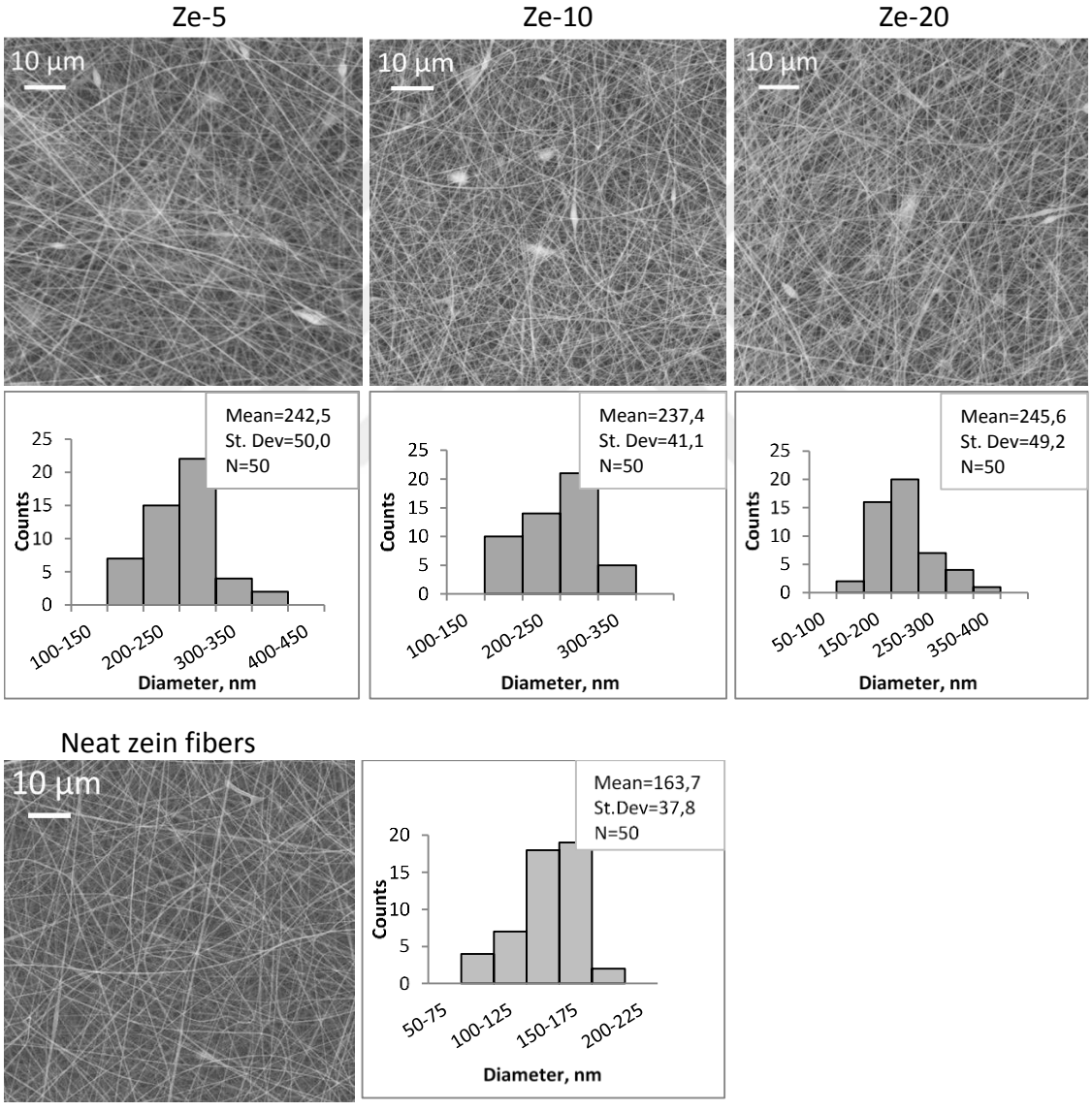


Figure 3.5 SEM images with histogram graphs of diameters of TPE-loaded and neat zein fibers

Table 3.3 Characteristics of prepared zein solutions

TPE concentration (%)	Viscosity (cP)	Conductivity (μS/cm)	Surface Tension (mN/m)	Diameter (nm)
0	425.0±3.1[a]	35.1±0.4[a]	30.0±0.0[a]	163.7±37.8[a]
5	474.4±2.8[b]	29.9±0.2[b]	28.0±0.2[b]	242.5±50.0[b]
10	497.8±1.4[b]	26.9±0.2[b]	27.8±0.2[b]	237.4±41.1[b]
20	520.7±2.9[c]	25.0±0.2[b]	27.5±0.4[b]	245.6±49.2[b]

Data with the same superscript letter in the same column indicate that they are not statistically different ($p>0.05$).

3.3.2.2 Characterization of electrospun nanofibers

The differential scanning calorimetry (DSC) thermograms of the carotenoid extract from tomato peel, neat zein fibers and the extract-loaded zein fibers are displayed in Figure 3.6. The carotenoid extract exhibited a sharp endothermic peak corresponding to the melting point of 175.8 °C, and an exothermic peak at 242.0 °C, indicating its degradation. The carotenoid extract thermogram was consistent with the studies reported in the literature (Faisal et al., 2013; Pu and Tang, 2017). On the other hand, zein fibers showed a broad endotherm with a peak at around 100 °C. These characteristic endotherms of zein fibers were due to the evaporation of bound water from the molecules (Luo et al., 2011; Muller et al., 2011; Neo et al., 2013).

The thermograms of extract-loaded zein fibers did not show any characteristic melting peak of carotenoid extract, indicating that the crystalline structure of carotenoids had turned to the amorphous state in zein fiber by electrospinning. The glass transition temperature (T_g) of zein fiber was 154.3±2.6 °C as shown in Table 3.4, which was similar to that reported in the literature (Torres-Giner et al., 2008; Torres-Giner et al., 2009). However, the extract-loaded fibers exhibited lower T_g values than neat zein fibers, in the range of 135-142 °C. The drop of T_g values could be a result of that carotenoids had created a plasticizing effect and increased the mobility of zein molecular chains. Additionally, thermal degradation of the extract-loaded fibers had shifted to significantly higher temperatures than neat zein fibers.

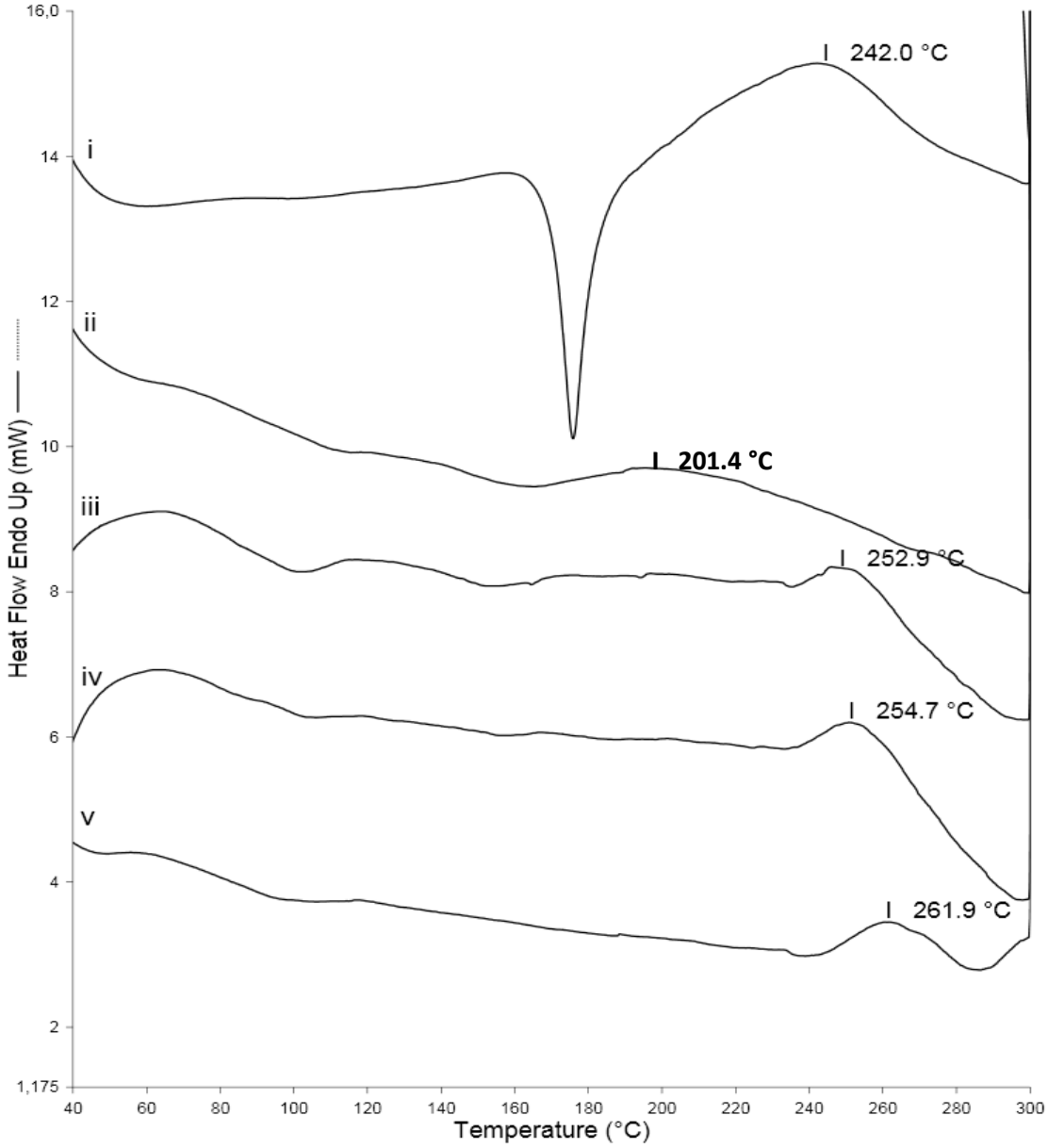


Figure 3.6 DSC thermograms of different samples: (i) carotenoid extract; (ii) neat zein fiber; (iii) Ze-5; (iv) Ze-10; (v) Ze-20 fibers with the extract concentrations of 5%, 10% and 20% (w/w), respectively

Moreover, comparing the thermogram of the extract with extract-loaded zein nanofibers, it was observed that the electrospinning process for encapsulation of the carotenoid improved its thermal stability performance by retarding its degradation temperature from 242.0 °C to 261.9 °C. The degradation temperature slightly

increased with the increase of carotenoid concentration. This might be attributed to that the increase in concentration of carotenoids created more antioxidant effect, leading to retarding of its degradation temperature.

Table 3.4 Glass transition temperatures, loading capacity (LC) and encapsulation efficiency (EE) values of zein fibers

Sample	T_g (°C)	LC (%)	EE (%)
Neat zein fibers	154.3±2.6[a]	-	-
Ze-5 fibers	141.7±3.3[b]	4.62±0.08[a]	92.49±1.67[a]
Ze-10 fibers	138.4±1.2[b]	9.41±0.10[b]	94.12±0.99[a]
Ze-20 fibers	134.4±1.9[b]	19.39±0.20[c]	96.95±0.99[a]

Data with the same superscript letter in the same column indicate that they are not statistically different ($p>0.05$).

The compability among the components and the second-order interactions within the materials has been frequently examined by using Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy method. The compability among the structures are crucial for high-quality and stable products. This compability and stability could be attributed by second-order interactions such as hydrogen bonding, electrostatic, and hydrophobic interactions (Li et al., 2009; Yang et al., 2013). The ATR-FTIR spectra of tomato peel extract, neat zein fiber, and extract-loaded zein fibers; Ze-5, Ze-10, and Ze-20 are presented in Figure 3.7. Zein has exhibited characteristic peaks at 3297 cm-1 (N–H stretching vibrations), 2958 cm-1 (C–H stretching vibrations of aliphatic groups), 1652 cm-1 (-C=O streching vibrations), meaning that it could act as both potential proton donors and receptors for hydrogen bonding. This indicates the presence of hydrogen-bonding interactions within the extract-loaded zein fibers.

Compared to neat zein fiber, no significant changes in the spectra of extract-loaded zein fibers was observed, showing that the tomato peel extract could be compatibly entrapped into the zein fibers. Moreover, the encapsulated extract within the biopolymer wall was realized to be physical in nature rather than a chemical interaction of any sort (Hani et al., 2017). In other words, no new peaks appeared which indicates that no new bonds were formed during encapsulation process and only physical

interactions occured between zein and extract. A complete disappearance of few signature sharp peaks of solvents within the extract was observed in the spectra of extract-loaded zein fibers because of solvent evaporation during spinning process.

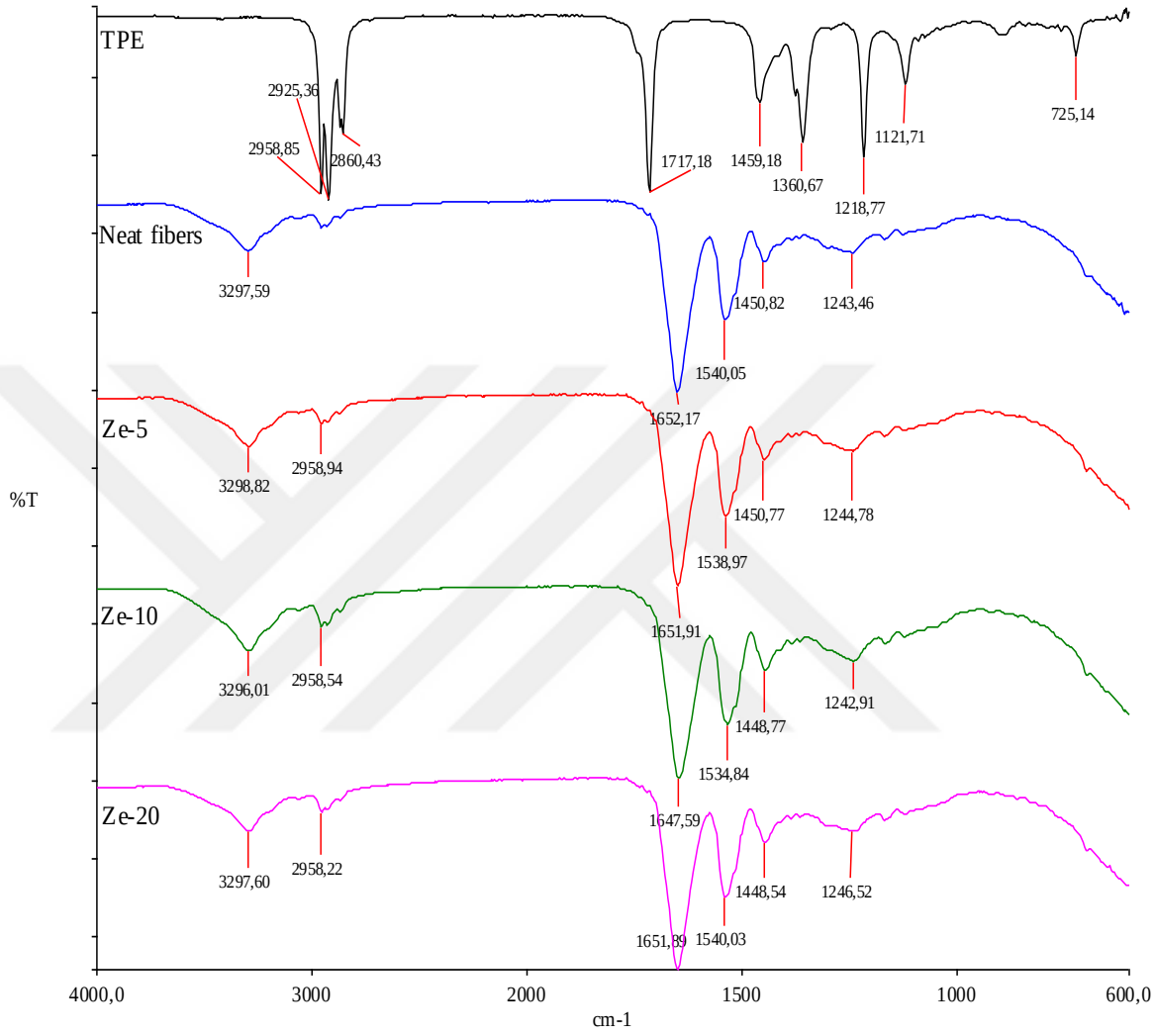


Figure 3.7 FTIR spectra of TPE, neat zein nanofibers, the Ze-5, Ze-10, and Ze-20 composite fibers

3.3.2.3 Encapsulation efficiency and loading capacity

The values of encapsulation efficiency (EE) and loading capacity (LC) are essential to give an idea about the quality of encapsulation. At the theoretical concentrations of 5, 10, 20%, the extract LC values of the zein fibers were 4.62±0.08, 9.41±0.10, and 19.39±0.20, respectively, and the EE values were 92.49±1.67, 94.12±0.99, and 96.95±0.99, respectively as tabulated in Table 3.4. These results indicated that there

was almost no loss of tomato peel extract during the electrospinning process and above 90% of the extract could be encapsulated into zein fibers, meaning that the carotenoid extract from tomato peel can be efficiently encapsulated by electrospinning technique.

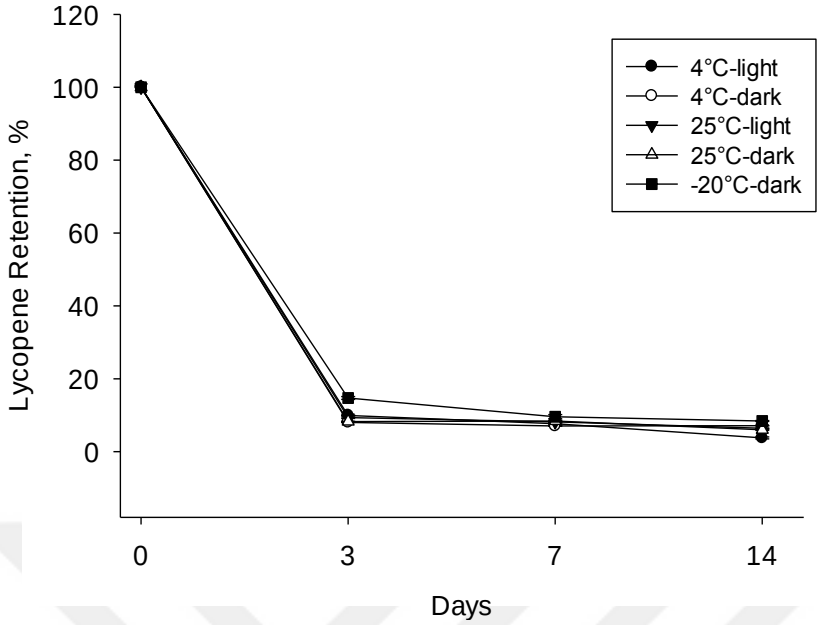
Compared to the other encapsulation techniques, the higher encapsulation efficiency of electrospinning has been reported for many times (Drosou et al., 2017). For example, Nougeira et al. (2017) encapsulated the carotenoids by lyophilization technique with an encapsulation efficiency of around 65%. Similarly, β-carotene was encapsulated by spray drying with very low efficiencies (20.9-38.8%) in another study reported by Deng et al. (2014).

3.3.2.4 Change in lycopene content during storage

The changes in the retention of lycopene, which is the major carotenoid of tomato peel, were analyzed throughout 14-day storage of encapsulated and non-encapsulated tomato peel extract at different temperatures (-20, 25, 4 °C) and in dark or lighted conditions. At first glance to Fig 3.8, it can be clearly seen that the non-encapsulated extract showed the highest lycopene degradation (above 90%) under all tested conditions during the first 3 days. However, the encapsulated extract into zein fibers showed better retention of lycopene because of the preserving effect of nano-encapsulation by electrospinning (Moomand and Lim, 2014; Fernandez et al., 2009).

The higher lycopene degradation was seen in the encapsulated extract samples stored at 25 °C under light compared to 4 °C in the dark. The detrimental effect of light and temperature on carotenoids has been introduced number of times in the literature (Boon et al., 2010; Britton, 1995). The encapsulated extract in the zein fibers stored at -20 °C showed the highest retention of lycopene with almost no lycopene degradation.

(a)



(b)

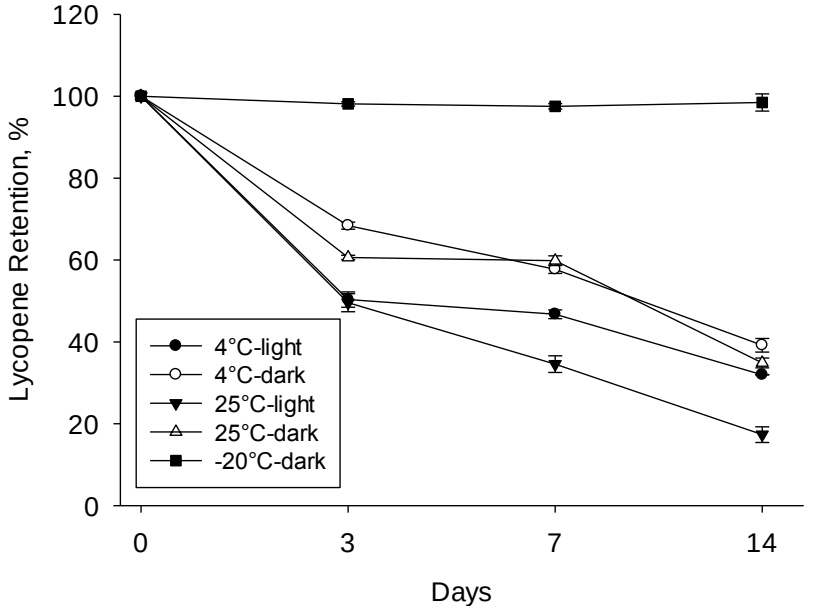


Figure 3.8 Change in lycopene retention (a) non-encapsulated TPE; (b) TPE-loaded electrospun zein fibers during 14-day storage

3.3.2.5 Change in antioxidant activity during storage

Since tomato carotenoids are already known to have antioxidant properties due to the presence of different phytochemicals (Kalogeropoulos et al., 2012), the effectiveness of the antioxidant properties of the carotenoids extract from tomato peels after being

encapsulated into the zein nanofibers was determined. The change in the antioxidant activity (AA) of the fiber samples loaded with tomato peel extract and the non-encapsulated extract was evaluated during 14-day storage under different conditions. It is inferred from Table 3.5 that the values of antioxidant activity decreased during 14 days. DPPH radical scavenging activity changed considerably among all samples except the ones at the temperature of -20 °C. The decrease in AA of the non-encapsulated extract samples after 14 days was higher than 35 %, whereas it was less than 20% for the encapsulated ones into the zein fibers. This clearly confirms the protective effect of the nanoencapsulation by electrospinning technique on the AA of the tomato peel extract. Moreover, AA of the encapsulated extract was about 11 times higher compared to non-encapsulated one, probably due to the fact that nanoencapsulation helps in enhancing the antioxidant potential of encapsulant by reducing the size of its molecules into the nano-metric size (Külkamp et al., 2011; Shah et al., 2016; Hussein et al., 2017).

Table 3.5 Change in antioxidant activity (%) of fiber samples loaded with TPE and non-encapsulated extract during 14-day storage

Samples	Conditions	Days				Change (%)
		0	3	7	14	
TPE	4°C-light	1.24 ± 0.02^{Xx}	1.17 ± 0.02^{Xx}	1.18 ± 0.03^{Xx}	0.79 ± 0.05^{Xy}	36.3
	4°C-dark	1.24 ± 0.02^{Xx}	1.19 ± 0.08^{Xx}	1.20 ± 0.02^{Xx}	0.81 ± 0.03^{Xy}	34.7
	25°C-light	1.24 ± 0.02^{Xx}	0.84 ± 0.01^{Yy}	0.85 ± 0.00^{Yy}	0.75 ± 0.04^{Xz}	39.5
	25°C-dark	1.24 ± 0.02^{Xx}	1.08 ± 0.02^{Zy}	0.85 ± 0.02^{Yz}	0.77 ± 0.03^{Xd}	37.9
	- 20°C	1.24 ± 0.02^{Xx}	1.23 ± 0.03^{Xx}	1.16 ± 0.02^{Xy}	1.12 ± 0.03^{Yy}	9.7
Ze-20 fibers	4°C-light	14.36 ± 0.30^{Aa}	12.30 ± 0.17^{Ab}	11.57 ± 0.3^{Ac}	11.72 ± 0.17^{Ac}	18.4
	4°C-dark	14.36 ± 0.30^{Aa}	13.26 ± 0.37^{Bb}	12.18 ± 0.21^{Ac}	11.97 ± 0.10^{Ac}	16.6
	25°C-light	14.36 ± 0.30^{Aa}	11.59 ± 0.23^{Ab}	11.66 ± 0.23^{Ab}	11.48 ± 0.29^{Ab}	20.0
	25°C-dark	14.36 ± 0.30^{Aab}	11.96 ± 0.08^{Ab}	12.01 ± 0.37^{Ab}	11.74 ± 0.20^{Ab}	18.2
	- 20°C	14.36 ± 0.30^{Aa}	14.18 ± 0.18^{Ca}	14.01 ± 0.24^{Bb}	14.03 ± 0.12^{Bb}	2.3

Means with different letters and superscripts in each column (X, Y, and Z for TPE; A, B, C, and D for Ge-20 fibers) and row (x, y, and z for TPE; a, b, and c for Ge-20 fibers) differ significantly ($P < 0.05$) from each other. Data are presented as means ± SD (n = 3)

3.4 Conclusion

The study showed that the carotenoids extract from tomato peel was succesfully encapsulated into the gelatin and zein nanofibers by electrospinning with high encapsulation efficiency. The obtained extract-loaded fibers did not show different morphology from the neat fibers with a bead-free, smooth, and homogeneously-distributed morphology. However, increasing the concentration of extract resulted in larger fibers. Thermal stability performance of the extract was improved by nanoencapsulation via electrospinning process. Additionally, thermal degradation of the extract-loaded fibers had shifted to significantly higher temperatures than neat zein fibers. FTIR spectra revealed that the tomato peel extract could be compatibly entrapped into the gelatin and zein fibers with a physical process rather than chemical interaction between the extract and polymer molecules. Compared to non-encapsulated extract, the encapsulated one inside fibers showed better retention of lycopene and antioxidant activity under all tested conditions during 14-days storage. Moreover, antioxidant activity of the extract increased about 10-fold by nanoencapsulation because of reduction in its molecular size into the nano-metric size. It is also remarkable to point out that the solubility of carotenoid extract in water was highly enhanced when it was encapsulated into gelatin nanofibers. These results indicated that nanoencapsulation by electrospinning is an effective way to stabilize carotenoids and improve their antioxidant activity and water solubility, therefore it is promising to use them in food processing, particularly including aqueous food matrix.

CHAPTER 4: APPLICATION OF TOMATO PEEL EXTRACT LOADED FIBERS IN A MODEL FOOD

4.1 Introduction

There is growing interest in the valorization of by-products from the food industry. When tomatoes are processed, 3-7% of their weight becomes waste consisting of mainly fruit peels. The peel of tomato is a rich source of carotenoids (mainly in the form of lycopene). However, it is currently disposed as a solid waste or used for animal feeding. Tomato carotenoids have potential to be used as colorant, antioxidant, and food supplement because of their protective action by providing health benefits such as modulation of the immune system, vitamin A precursor, prevention of the risk of cancer and cardiovascular diseases. However, it is difficult to incorporate the carotenoids into different food formulations because they are insoluble in water and partially soluble in oils at room temperature. Additionally, they are relatively unstable in food systems because they are susceptible to oxygen, light, and heat (Xianquan et al., 2005).

In this part of the study, the carotenoids extracted from tomato peel were encapsulated by electrospinning and applied them in a model food. Although the incorporation of carotenoids into nanofiber material by electrospinning had been demonstrated (Fernandez et al., 2009; Zômpero et al., 2015; Peinado et al., 2016; Reksamunandar et al., 2017), there is no report about the application of encapsulated carotenoids within nanofibers for preparing any food material by directly addition of it. In the current study, the produced fibers were then used in yogurt preparation as a model food and their effect on its antioxidant and physicochemical properties during storage was assessed. By this way, it also contributed to the production of water-soluble carotenoids by nanoencapsulation via electrospinning and use them in aqueous food matrix.

4.2 Materials and Methods

4.2.1 Materials

Zein (Z 3625), Type B gelatin powder from bovine skin, glacial acetic acid (HAc), hexane, and acetone were purchased from Sigma Aldrich (St. Louis, Mo., U.S.A). Tomato and raw milk were purchased from a local market.

4.2.2 Drying of tomato peels and extraction of carotenoids from dried tomato peels

The moisture content of the fresh peels of tomatoes was determined by 105 °C oven method (AOAC, 1995). The fresh peels of tomatoes were dried by using a dehydrator at 50 °C until they reached to 6-7% moisture. After that, dried peels were ground into about 1 mm size. The dried and ground peels were stored in glass jars covered with aluminum foil at -70 °C until extraction process.

Carotenoids were extracted from tomato peels by the modified method described by Strati and Oreopoulou (2011) and Albanese et al. (2014). Homogenized dry tomato waste was placed in the extraction vessel and homogenized with acetone and hexane (50:50) as the extraction solvent in a ratio of 1:10 (w/v). The mixture was stirred for 30 min at room temperature. At the end of the first extraction step, the mixture was filtered through filter paper. The residue collected was put again in the vessel and re-extracted with fresh extraction solvent. The whole procedure was repeated three times to complete the successive extraction steps. The obtained extract-solvent mixture was concentrated to about 10% of mixture by using a rotary vacuum evaporator at 35-40 °C. The tomato peel extract (TPE) was then stored in amber colored bottles at – 70 °C for further use in the encapsulation process.

4.2.3 Preparation of electrospinning solutions

The gelatin-based solution was prepared by dissolving the dried extract (30 wt% - based on the weight of gelatin) under N_2 and powder gelatin (24% wt/wt) were in aqueous acetic acid (20%, v/v) under constant stirring at 40 °C for about 2 hours. The zein-based solution was prepared by dissolving the dried extract (30 wt% - based on

the weight of zein) under N_2 and powder zein (24% wt/wt) were in pure acetic acid under constant stirring at room temperature for about an hour.

4.2.4 Electrospinning process

Electrospinning process was carried out at room temperature (24 ± 2 °C) by using the Nanotel (NTEE 80-1) electrospinning device consisting of a syringe pump, a high voltage power supply and a collector plate covered in non-stick aluminum foil for easy removal of mats. The solutions were placed in 10 ml syringes fixed horizontally on the syringe pump delivering the solution to the tip of a metal needle where the voltage was applied. Fibers were then collected on a stainless-steel collector plate covered with aluminum foil. The electrospinning process was carried out by using the following conditions decided in the previous optimization studies: 18 kV of applied voltage, 15 μL/min of solution flow rate, 12.5 cm of distance between the needle tip to the collector and 12 kV of applied voltage, 15 μL/min of solution flow rate, 10 cm of distance between the needle tip to the collector for gelatin and zein fibers production, respectively. The fiber mats produced were kept at the temperature of -70 °C until being used for characterization analysis.

4.2.5 Characterization of nanofibers

The morphology and diameters of the produced nanofibers were examined by using a scanning electron microscope (SEM) (JEOL, JSM-6390LV, Japan) with accelerating voltage of 20 kV. Fiber diameter analysis was carried out by randomly counting 50 fibers per experiment using a software (ImageJ, 1.48V). The mean diameter and standard deviation were calculated as the responses.

4.2.6 Application in the food system

Yogurt was chosen as the model system for the produced nanofibers application. The following yogurts were prepared: (A) with TPE-loaded gelatin nanofibers (TPE-Ge); (B) with TPE-loaded zein nanofibers (TPE-Ze) and (C) a standard yogurt without the addition of nanofibers (Control). Pasteurized and homogenized milk was used for yogurt preparation. Fibers were added into the milk at the concentrations of 0.1% and mixed using Ultra Turrax (IKA, T18) at 12000 rpm for 5 min before milk heat

treatment. The samples were heated to 45°C and 0.02% freeze dried yogurt starter culture, containing two types of *Lactobacillus* strains (*Lactobacillus delbrueckki* ssp. *bulgarius*, and *Streptococcus thermophilus*) was added. Inoculated milk samples were incubated at 45°C in an oven for 5 hours until coagulation occurred. The samples were then cooled and stored at 4°C for 15 days to monitor their properties.

4.2.7 Yogurt analysis

4.2.7.1 pH and acidity

pH measurement was conducted using a pH meter (Hanna, HI 8314, Romania, Europe). Titratable acidity was evaluated by mixing 10 g of yogurt sample with 10 mL hot distilled water and titrating with 0.1 N NaOH using 0.5% phenolphthalein indicator. The titratable acid value was calculated by converting it to lactic acid percentage (AOAC, 1995).

4.2.7.2 Syneresis

Syneresis properties of yogurt samples were analyzed by centrifuging of 20 g of yogurt at 5000 rpm for 5 min. Syneresis percentage was calculated using the following equation (Achanta et al., 2007):

Syneresis = 100 x (Total weight of separated liquid (g) / Total weight of yogurt (g))

4.2.7.3 Free radical scavenging activity by DPPH

Radical scavenging activity (RSA) was measured according to the Brand-Williams (1995) method with slight modifications. 250 μL of sample was mixed with 3 mL of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol solution and vortexed for 30 s. The mixture was covered with aluminum foil and kept in the dark at room temperature for 30 min. Reaction was carried out in the dark for 30 min. Then, the samples were centrifuged at 1400 rpm for 5 min and absorbances were measured at 517 nm. Free radical scavenging activity as expressed as following:

$$RSA\ (\%) = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control reaction, and A_1 is the absorbance of the sample

4.2.7.4 Color properties

HunterLab Colorflex (A-60-1010-615, HunterLab, Reston, VA) was used to measure the color change produced by the addition of TPE-loaded nanofibers to the yoghurt samples during storage period of 15 days. Before the test, the instrument was standardized with standard black glass and white tile as specified by the manufacturer. The color values were expressed as L* (whiteness or brightness/darkness), a* (redness/greenness) and b* (yellowness /blueness) values according to the CIELAB system. Total color change, ΔE was calculated using the following formula:

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$$

where, L_0^*, a_0^*, b_0^* were values of the yogurt samples at 1st day of storage; L*, a*, b* were values of the oil samples at various storage days.

4.2.7.5 Rheological measurements

The rheological behavior of the yogurt samples was determined in the shear rate range of 0-300 s^{-1} during 120 s at 20℃ by using Haake Rheostress RS1 (Karlsruhe, Germany) equipped with TCP/P Peltier temperature controller unit. A plate-plate sensor (diameter 3.5 cm; gap 1 mm) was used throughout the measurements. The shear rate versus shear stress data were analysed with a Herschel-Bulkey model (RheoWin 3, Haake, Karlsruhe, Germany) according to the following equations:

$$\tau = \tau_0 + K\dot{\gamma}^n \qquad \eta = \frac{\tau_0}{\dot{\gamma}} + K\dot{\gamma}^{n-1}$$

where τ is the shear stress (Pa), τ_0 is the yield strength, $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency index ($Pa.s^n$), n is the flow behavior index, and η is the viscosity (Pa.s). Apparent viscosity (η_{app}) was calculated at 10 s^{-1} of shear rate.

4.2.7.6 Statistical analysis

All the experiments were performed in triplicate and results were expressed as the mean values ± standard deviations. Significant differences among samples were evaluated with analysis of variance (One-way ANOVA with Duncan test) at 5% significance level by using the statistical software SPSS (IBM SPSS Statistics, Version 22, New York, USA)

4.3 Results and Discussion

4.3.1 Morphologies of produced nanofibers

In this study, encapsulation of tomato peel extract (TPE) into gelatin and zein nanofibers was achieved successfully by via electrospinning. Figure 4.1 shows SEM images of TPE-loaded nanofibers produced by electrospinning. The SEM images did not contain beads, tomato peel extract crystals, or other types of aggregates over the surface of the fibers, implying that TPE was perfectly incorporated within the nanofibers. The mean fiber diameters were found to be less than 250 nm.

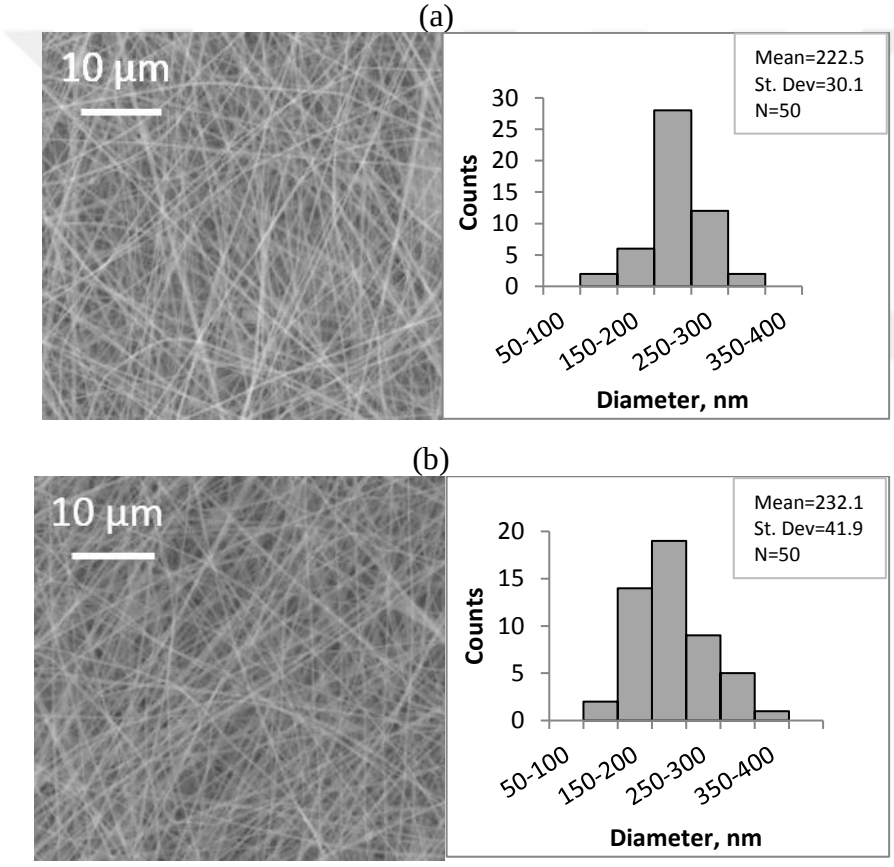


Figure 4.1 SEM images with histogram graphs of diameters of TPE-loaded (a) gelatin and (b) zein fibers

4.3.2 Application of the TPE-loaded nanofibers in model food

The TPE-loaded gelatin and zein fibers were applied in yogurt in order to evaluate their effect on quality and physico-chemical characteristics of the yogurt during storage.

4.3.2.1 Change in pH and acidity of yogurt samples during storage

The acidity and pH values of yogurt samples were given in Table 4.1. The yogurt samples enriched with TPE-loaded fibers had relatively lower pH values compared to the control sample. Additionally, the samples with TPE-loaded zein fibers had the lowest pH values and they were found to be as statistically different from both control and samples with TPE-loaded gelatin fibers throughout the storage. Moreover, the pH of each sample significantly decreased during storage.

Table 4.1 Changes in acidity, pH, radical scavenging activity (RSA), and apparent viscosity values of yogurt samples during storage 4°C

Parameter	Days	Control	TPE-Ge	TPE-Ze
Acidity, %	1	0.643 ± 0.006^{Aa}	0.675 ± 0.003^{Aa}	0.707 ± 0.041^{Aa}
	5	0.708 ± 0.007^{Ab}	0.713 ± 0.004^{Ab}	0.755 ± 0.009^{Bb}
	10	0.722 ± 0.001^{Abc}	0.741 ± 0.006^{Bc}	0.785 ± 0.007^{Cc}
	15	0.744 ± 0.010^{Ac}	0.745 ± 0.005^{Ac}	0.780 ± 0.001^{Bc}
pH	1	5.115 ± 0.011^{Aa}	5.038 ± 0.013^{Ba}	5.020 ± 0.007^{Ba}
	5	4.973 ± 0.004^{Ab}	4.918 ± 0.004^{Bb}	4.860 ± 0.000^{Cb}
	10	4.938 ± 0.004^{Ac}	4.908 ± 0.004^{Bb}	4.825 ± 0.005^{Cc}
	15	4.915 ± 0.005^{Ac}	4.865 ± 0.005^{Bc}	4.800 ± 0.000^{Cd}
RSA,%	1	44.91 ± 0.11^{Aa}	64.48 ± 0.17^{Ba}	64.79 ± 0.28^{Ba}
	5	35.20 ± 0.80^{Aab}	43.57 ± 0.46^{Bb}	49.16 ± 1.00^{Bb}
	10	30.85 ± 0.93^{Ab}	44.36 ± 0.70^{Bb}	46.24 ± 0.78^{Bb}
	15	28.61 ± 0.49^{Ab}	44.62 ± 0.46^{Bb}	45.83 ± 0.39^{Bb}
$\eta_{app,}$ Pa.s	1	$0,89\pm0,02^{Aa}$	$1,13\pm0,05^{Ba}$	$0,82\pm0,25^{Aa}$
	5	$0,45\pm0,05^{Ab}$	$0,41\pm0,00^{Ab}$	$0,27\pm0,01^{Bb}$
	10	$0,31\pm0,00^{Ac}$	$0,23\pm0,01^{Bc}$	$0,24\pm0,01^{Bb}$
	15	$0,28\pm0,04^{Ac}$	$0,21\pm0,04^{Ac}$	$0,24\pm0,03^{Ab}$

Means with different superscripts (A, B, C) in each row and column (a, b, c) differs significantly ($P<0.05$) from each other. Data are presented as means ± SD (n = 3)

Table 4.1 also shows that no significant difference was observed in acidity values of all samples ($p>0.05$). However, the acidity values significantly increased during storage for each sample ($p<0.05$). It was reported in literature that lactic acid bacteria cause an increase in metabolic activity and other biochemical changes at refrigerated temperature, leading to an increase in acidity during storage (Brabandere and Baerdemaeker 1999; Yadav et al., 2018). The direct incorporation of TPE-loaded zein fibers into yogurt resulted in significantly higher acidity values compared to control and samples with TPE-loaded gelatin fibers during storage ($p<0.05$). Lactic acid was higher accumulated at the yogurt enriched with TPE-loaded fibers because the accumulation of lactic acid was stimulated by TPE-loaded fibers such as protein and carotenes (Radiati et al., 2016).

4.3.2.2 Syneresis

Syneresis is a measure of the quantity of whey separated from the yogurt and is one of the most important quality characteristics of yogurt. The change in syneresis of all samples during 15 days at 4°C storage was demonstrated in Figure 4.2. Initially, no significant difference among the groups was observed ($p>0.05$). However, the percent syneresis value of yogurt enriched with TPE-loaded zein fibers was significantly higher compared to other two samples ($p<0.05$) at the end of fifth and tenth days. This could be attributed to water insolubility of zein fibers. The presence of insoluble fiber, which does not incorporate within protein network, leads to a higher level of syneresis (Miocinovic et al., 2018). An increasing trend was observed with storage time in all yogurt samples, probably due to severe casein network rearrangements promoting whey expulsion (Ramirez-Santiago et al., 2010). Lucey (2002) also stated that when the casein particles rearrange in the gel network, whey expulsion is spontaneous, as the gel shrinks without the application of some external force. It could be also directly related to the acidity and therefore inversely related to the pH values (Panesar and Shinde, 2011). There was no significant difference among all yogurt samples at the end of storage ($p>0.05$).

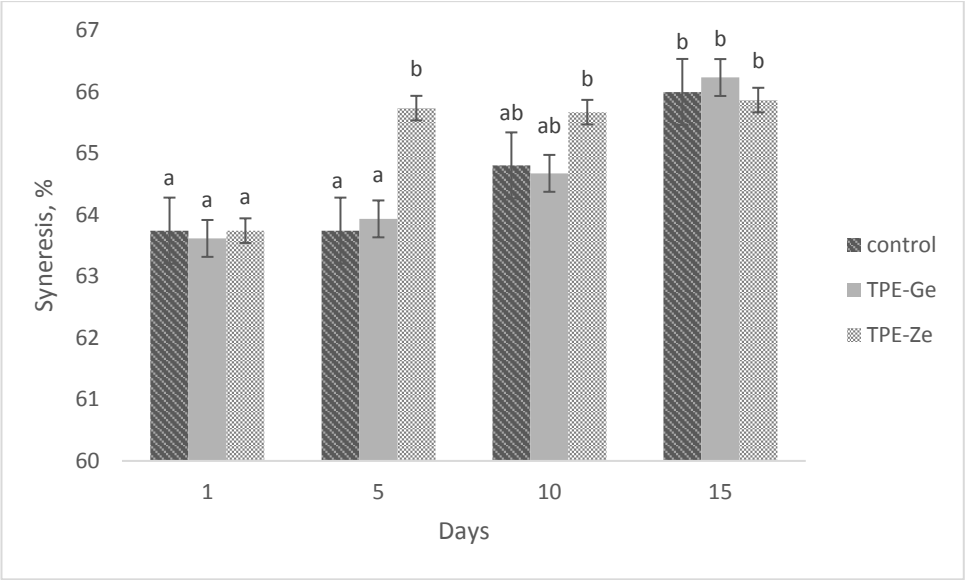


Figure 4.2 Changes in syneresis of different yogurt samples during storage 4°C (Different letters show statistically significant results ($P < 0.05$) of each sample during storage.)

4.3.2.3 Color properties

Changes in the color of samples stored at 4°C for 15 days are presented in Table 4.2. The incorporation of fiber into milk resulted in decreasing of ($P<0.05$) L* value of the yogurt samples by 2–4 units because fiber showed a darkening effect, probably because of the water absorption. The L* value of foods is associated to the amount of free water in the surface of a product more than the complete product (García-Pérez et al., 2005). It is clearly seen from Table 4.2 that L* values of the yogurt samples supplemented with TPE-Ge fibers were less compared to ones supplemented with TPE-Ze fibers because of better water absorptivity of gelatin fibers. The L* values for all the samples increased slightly, but significantly by elevating the storage period from 1 to 15 days, probably due to the slight increase in syneresis.

The control yogurt showed the lowest a* and b* values, indicating redness and yellowness, respectively, and they significantly increased with fiber addition ($p<0.05$). The a* and b* values were significantly higher for the yogurt samples supplemented with TPE-Ge fibers compared to ones supplemented with TPE-Ze fibers. This difference could be explained by the different degree of solubility of gelatin and zein in water. Overall, the results showed that the yogurts enriched with TPE-loaded fibers were redder, darker, and more yellow than control yogurt. The tomato peel extract is

dark red, and its color undoubtedly contributed to the color of yogurt by decreasing the L* value and increasing the a* and b* values.

Table 4.2 Changes in color properties of yogurt samples during storage 4°C

Parameter	Days	Control	TPE-Ge	TPE-Ze
L*	1	89.027 ± 0.031^{Aa}	85.977 ± 0.005^{Ba}	87.057 ± 0.009^{Ca}
	5	90.430 ± 0.014^{Ab}	86.020 ± 0.014^{Bb}	87.247 ± 0.005^{Cb}
	10	90.460 ± 0.014^{Ab}	86.873 ± 0.005^{Bc}	88.190 ± 0.029^{Cc}
	15	90.827 ± 0.005^{Ac}	86.703 ± 0.012^{Bc}	88.263 ± 0.005^{Cd}
a*	1	-1.263 ± 0.012^{Aa}	4.510 ± 0.022^{Ba}	1.397 ± 0.021^{Ca}
	5	-2.147 ± 0.012^{Abc}	4.873 ± 0.033^{Bb}	1.443 ± 0.009^{Ca}
	10	-2.140 ± 0.008^{Ab}	4.870 ± 0.016^{Bb}	2.027 ± 0.038^{Cb}
	15	-2.167 ± 0.005^{Ac}	4.860 ± 0.008^{Bb}	2.840 ± 0.008^{Cc}
b*	1	8.287 ± 0.017^{Aa}	18.607 ± 0.045^{Ba}	12.843 ± 0.054^{Ca}
	5	8.430 ± 0.028^{Ab}	18.050 ± 0.037^{Bb}	13.167 ± 0.026^{Cb}
	10	8.640 ± 0.022^{Ac}	19.240 ± 0.014^{Bc}	14.807 ± 0.024^{Cc}
	15	8.950 ± 0.016^{Ad}	19.220 ± 0.022^{Bc}	14.707 ± 0.021^{Cd}

Means with different superscripts (A, B, C) in each row and column (a, b, c) differs significantly ($P<0.05$) from each other. Data are presented as means ± SD (n = 3)

4.3.2.4 Free radical scavenging activity by DPPH

The free radical scavenging activity (RSA) of yogurt samples during storage for 15 days at 4°C are represented in Table 4.1. The RSA values of yogurt significantly increased by the addition of TPE-loaded fibers ($p<0.05$). Antioxidant activity of yoghurt is known in the literature and depends on the ingredients used (Şanlıdere Aloğlu and Öner, 2011). Since tomato carotenoids are already known to have antioxidant properties due to the presence of different phytochemicals (Kalogeropoulos et al., 2012), TPE-loaded fibers led to increasing antioxidant activity of yogurt. However, the RSA value of yogurt enriched with TPE-loaded gelatin fibers was not statistically different from the one of yogurt enriched with TPE-loaded zein

fibers ($p>0.05$). It was also clearly seen from the Table 4.1 that the RSA values of yogurt enriched with TPE-loaded fibers were still higher than the control yogurt at the end of the 15th day, although the antioxidant activity significantly decreased throughout the storage for all yogurt samples ($p<0.05$).

4.3.2.5 Rheological properties

The data of shear stress versus shear rate of yogurt samples after 1-day and 15-day storage was illustrated in Figure 4.3. All the yogurt samples studied in the present study showed non-Newtonian shear-thinning behavior, meaning that apparent viscosity decrease with increasing shear rate.

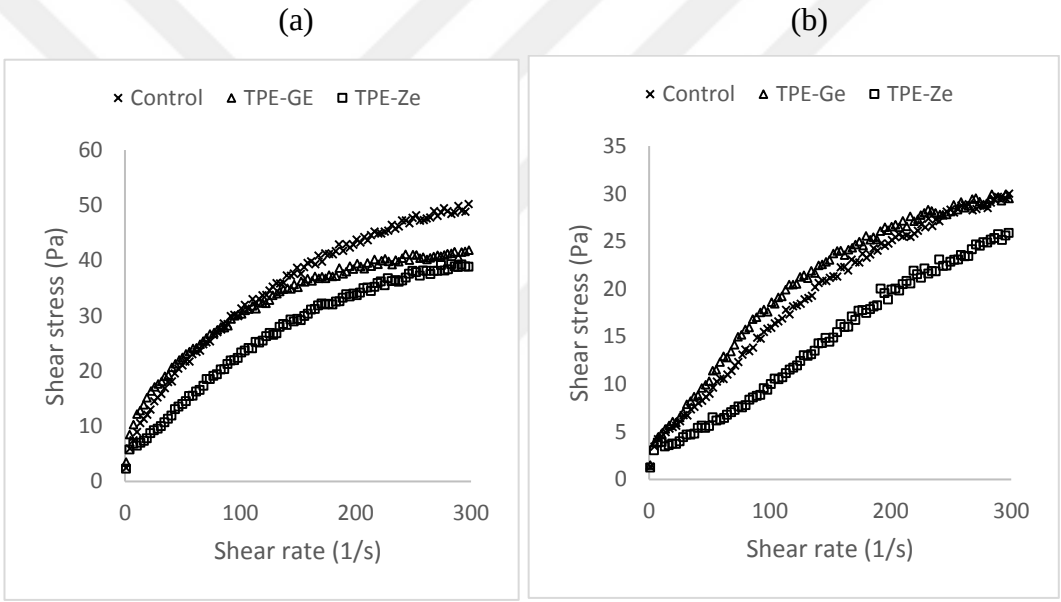


Figure 4.3 Comparison of rheological behavior of yogurt samples after (a) 1-day and (b) 15-day storage at 4°C

As it is seen in Table 4.1, the initial apparent viscosity of enriched yogurt with TPE-loaded gelatin fiber is higher than the other two groups because gelatin improves the texture of yogurt. It is attributed to the interaction of gelatin with the casein matrix of yogurt to develop a stronger three- dimensional network leading to increase in viscosity (Fiszman et al., 1999). However, the viscosities of yogurt enriched with TPE-loaded gelatin fiber and control yogurt were not statistically different after 5-day storage. The viscosities of all samples showed a sharp decrease, especially for the first

week, as if the system reached an equilibrium state and did not change significantly throughout the storage period (Toniazzo et al., 2014).

4.4 Conclusion

In this study, the nanoencapsulated of tomato peel extract (TPE) within gelatin and zein nanofibers achieved by electrospinning was successfully incorporated into yogurt. The results revealed that adding nanoencapsulated TPE into yogurt gave closer characteristics to control sample in terms of pH, acidity, syneresis, and viscosity parameters despite the significant differences among some of them. However, the addition of TPE-loaded nanofibers enhanced antioxidant potential and color properties of yogurt throughout the storage. Future toxicological studies are required because there is concern about potential adverse effects associated with the application of nanotechnology in foods.

CHAPTER 5: CONCLUSIONS

In the first part of present study, electrospinning of gelatin and zein was separately optimized by employing the Taguchi's Method. The optimum combination of factors was determined an applied voltage of 18 kV, a flow rate of 15 μL/min, a distance of 12.5 cm, and a HAc concentration of 20% for the production of gelatin nanofibers, whereas an applied voltage of 12 kV, a flow rate of 15 μL/min, a distance of 10 cm, and a solvent type of HAc for the production of zein nanofiber. According to the electrospun fibers with different polymer concentrations which were produced by using these optimum conditions, the thinnest bead-free fibers were produced by electrospinning of the polymer solutions at the concentration of 24%.

In the second part, that the carotenoids extract from tomato peel (TP) was successfully encapsulated into the gelatin and zein nanofibers by electrospinning with high encapsulation efficiency. The obtained extract-loaded fibers did not show different morphology from the neat fibers with a bead-free, smooth, and homogeneously-distributed morphology. FTIR spectra also showed that the TP extract could be compatibly entrapped into the gelatin and zein fibers. Thermal and storage stability of the extract was improved by nanoencapsulation. Moreover, antioxidant activity of the extract increased about 10-fold by nanoencapsulation. It is also remarkable to point out that the solubility of carotenoid extract in water was highly enhanced when it was encapsulated into gelatin nanofibers, therefore it is promising to use them in food processing, particularly including aqueous food matrix.

In the final part, the nanoencapsulated of tomato peel extract (TPE) within gelatin and zein nanofibers achieved by electrospinning was successfully incorporated into yogurt. adding nanoencapsulated TPE into yogurt gave closer characteristics to control sample in terms of pH, acidity, syneresis, and viscosity. However, it significantly changed the color and antioxidant properties of the yogurt.

By this study it has been proven that high added-value and stable carotenoids within nanofibers could be produced successfully by electrospinning technique and it may be considered as an alternative recovery way of treatment of waste tomato peel. However, future toxicological studies are required because there is concern about potential adverse effects associated with the application of nanotechnology in foods.

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CURRICULUM VITAE

PERSONAL INFORMATION

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FOREIGN LANGUAGE

English (YDS=81.25; March 2016)

EDUCATION

Degree	Instution	Year
Ph.D	Gaziantep University	2018
M.Sc.	Gaziantep University	2012
B.Sc.	Gaziantep University	2009

WORK EXPERIENCE

Duration	Place	Enrollment
Nov. 2009-Present	Gaziantep University	Research Assistant

PUBLICATIONS

Articles in Peer-Reviewed Journals

1- İNANÇ HORUZ, T., BELİBAĞLI, K. B. (2018). Nanoencapsulation of carotenoids extracted from tomato peels into zein fibers by electrospinning. Journal of the Science of Food and Agriculture, doi: 10.1002/jsfa.9244 (Accepted).

2- İNANÇ HORUZ, T., BELİBAĞLI, K. B. (2018). Nanoencapsulation by electrospinning to improve stability and water solubility of carotenoids extracted from tomato peels. Food Chemistry, 268, 86-93.

3- İNANÇ HORUZ, T., BELİBAĞLI, K. B. (2018). Effects of solvent type and process parameters on electrospinnability of zein through orthogonal experimental design. Materials Science (Medžiagotyra), 24, 10-17.

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5- İNANÇ HORUZ, T., MASKAN, M. (2015). Effect of cinnamaldehyde on oxidative stability of several fats and oils at elevated temperatures, Cogent Food and Agriculture.

6- İNANÇ HORUZ, T., MASKAN, M. (2015). Effect of the phytochemicals curcumin, cinnamaldehyde, thymol and carvacrol on the oxidative stability of corn and palm oils at frying temperatures. Journal of Food Science and Technology, 52:12, 8041-8049.

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8- İNANÇ, T., MASKAN, M. (2013). Testing the Antioxidant Effect of Essential Oils and BHT on Corn Oil at Frying Temperatures: a Response Surface Methodology. Journal of American Oil Chemists' Society, 90, 1845-1850.

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Presentations

1- İNANÇ HORUZ, T., BELİBAĞLI, B., Fabrication of tomato peel extract-loaded gelatin nanofibers by electrospinning, 5th International ISEKI_Food Conference, July, 2018, Stuttgart, Germany (Oral presentation).

2- İNANÇ HORUZ, T., BELİBAĞLI, B., Encapsulation of Tomato Peel Extract into Zein Nanofibers by Electrospinning, International Congress on Engineering and Life Science, April, 2018, Kastamonu, Turkey (Oral presentation)

3- İNANÇ HORUZ, T., BELİBAĞLI, B., Production and Optimization of Gelatin Nanofibers by Using Electrospinning Technique, The 5th Conference on Nanomaterials, 14-16, January, 2016, Bangkok, Thailand (Oral presentation)

4- İNANÇ HORUZ, T., MASKAN, M., Effect of cinnamaldehyde on oxidative stability of some fats and oils 2nd International Congress of Agriculture, Food and Gastronomy, April, 2015, Antalya, Turkey

5- İNANÇ, T., MASKAN, M., Effect of carvacrol on oxidative stability of corn and palm oils during frying, International Bioscience Conference, 29-30 September, 2014, Phuket, Thailand. (Oral Presentation)

6- İNANÇ, T., BELİBAĞLI, B., Quality Characteristics of Frozen Pistachio Nuts, 21-24 June 2014, IFT Annual Meeting and Food Expo, New Orleans, USA.

7- BELİBAĞLI, B., İNANÇ, T., ENGİN, S. Investigation of Some Changes during Isot Pepper Productıon, The 2nd International Symposium on Traditional Foods from Adriatic to Caucasus, 24-26 October, 2013, Struga /Macedonia.

8- İNANÇ, T., MASKAN, M., Effect of Some Essential Oils on Stability of Corn Oil at Frying Temperatures. 11th Euro Fed Lipid Congress. Antalya, Turkey, 27-30 October, 2013.

9- İNANÇ, T., MASKAN, M., Effect of Plant Based Active Components on Stability of Corn and Palm Oils at Frying Temperatures. 11th Euro Fed Lipid Congress. Antalya, Turkey, 27-30 October, 2013.

10- MASKAN, M., NACAROĞLU, S., İNANÇ, T., Effect of essential oils of Thymbra spicata on stability of palm Oil during deep-fat frying. The 2nd International Conference in Food Industries and Biotechnology. Homs, Syria, 01-03 November, 2010.

11- İNANÇ, T., MASKAN, M., Use of natural antioxidants in frying of foods: A Review. The 2nd International Conference in Food Industries and Biotechnology. Homs, Syria, 01- 03 November, 2010.

Chapter in a book

Her Yönüyle Gıda, Bölüm adı: (İşlemeye Hazır Ürünler) (2015), BELİBAĞLI KADİR BÜLENT, İNANÇ HORUZ TUĞBA, Sidas, Editör:F. Özkaya, S. Coşansu, K. Ayhan, Türkçe (Ders Kitabı)

PROJECTS AND SCHOLARSHIP

Domates kabuklarından elde edilen karotenoidlerin elektrospinning yöntemiyle nanoliflere enkapsüle edilerek etkinlik ve stabilitelerinin arttırılması, TÜBİTAK PROJESİ, Bursiyer, 2014-2017.

Tübitak 2211-C (Öncelikli alanlara yönelik yurt içi doktora burs programı)

Bazı baharatların uçucu yağlarının hızlandırılmış yöntemle oksidasyon dayanıklılığının optimizasyonu; Mısırözü ve palm yağlarında kızartma işleminde antioksidan olarak kullanılması, BAP PROJESI, Bursiyer, 2010-2012.

TRAINING AND INTERNATIONAL EXPERIENCE

- Erasmus Öğrenci Hareketliliği, Warsaw University of Life Science (SGGW), 2011.
- Vth Training School on Bioencapsulation, 09-12 April, 2013, Nantes, France.
- International Training School on Characterization of Electrospun Nanofibers: Hands-on Experience, 11-13 June, 2014, Ankara, Turkey.
- Workshop- "Composite, nanofabrication, food and pharma related application and packaging, controlled release", Novi Sad, Serbia, March, 2015.