PRODUCTION OF LENTIL FLOUR AND HYDROXYPROPYL METHYLCELLULOSE BASED NANOFIBER BY USING ELECTROSPINNING METHOD

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Submitted by **NİLAY TAM** in partial fulfillment of the requirements for the degree of **Master of Science in Food Engineering Department, Middle East Technical University** by,

Prof. Dr. Halil Kalıpçılar
Dean, Graduate School of **Natural and Applied Sciences** _____

Prof. Dr. Serpil Şahin
Head of Department, **Food Engineering Dept, METU** _____

Prof. Dr. S. Gülüm Şumnu
Supervisor, **Food Engineering Dept, METU** _____

Prof. Dr. Serpil Şahin
Co-Supervisor, **Food Engineering Dept, METU** _____

Examining Committee Members:

Prof. Dr. Göknur Bayram
Chemical Engineering Dept., METU _____

Prof Dr. Gülüm Şumnu
Food Engineering Dept., METU _____

Prof. Dr. Serpil Şahin
Food Engineering Dept., METU _____

Assoc. Prof. Dr. Mecit Öztop
Food Engineering Dept., METU _____

Asst. Prof. Dr. Elif Yolaçaner
Food Engineering Dept., Hacettepe University _____

Date: 31.05.2018

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name: NİLAY TAM

Signature:

ABSTRACT

PRODUCTION OF LENTIL FLOUR AND HYDROXYPROPYL METHYLCELLULOSE BASED NANOFIBER BY USING ELECTROSPINNING METHOD

Tam, Nilay

M. S. Department of Food Engineering

Supervisor: Prof. Dr. Gülüm Şumnu

Co-Supervisor: Prof. Dr. Serpil Şahin

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Nanofibers have recently become very popular in food industry for their utilization as highly functional ingredients, high-performance packaging materials, processing aids and food quality and safety sensors. The objective of this study was to obtain homogeneous nanofibers from lentil flour (LF) and hydroxypropyl methylcellulose (HPMC) blend by using electrospinning method. The effects of pH (7, 10 and 12), LF concentration (1% and 2% (w/v)), HPMC concentration (0.25%, 0.5% and 1% (w/v)) and microfluidization (3 and 5 pass) on solution properties and fiber morphology were investigated. When the pH was increased, the viscosity of both 1% and 2% LF containing solutions decreased while the electrical conductivity increased. At pH value of 7, homogeneous nanofibers couldn't be obtained whereas fibers were perfectly homogeneous at alkaline pH values. Nanofiber diameter decreased with increase in pH when 2% LF was used. On the other hand, diameter of fibers didn't show any significant change with pH for 1% lentil flour. When the LF concentration was increased, viscosity and fiber diameter increased at pH 10. When HPMC

concentration was increased, both viscosity and fiber diameter increased. Average fiber diameters ranged between 198±4 and 254±5 nm. Microfluidization did not have a positive effect on obtaining homogeneous nanofibers and resulted in fibers with beads. For the solutions containing 2% lentil flour, increasing applied voltage increased nanofiber diameter whereas increasing flow rate decreased nanofiber diameter. When LF concentration was increased, water vapor permeability of electrospun nanofibers showed a significant increase. No significant change was observed in water vapor permeability when HPMC was added into the electrospinning solutions. Neither LF concentration nor HPMC addition did not have a significant effect on color parameters.

Keywords: Electrospinning, lentil flour, hydroxypropyl methylcellulose (HPMC), nanofiber

ÖZ

ELEKTROEĞİRME METODU İLE MERCİMEK UNU VE HİDROKSİPROPİL METİLSELÜLOZ BAZLI NANOLİF ÜRETİMİ

Tam, Nilay

Yüksek Lisans, Gıda Mühendisliği Bölümü

Tez Yöneticisi: Prof. Dr. Gülüm Şumnu

Ortak Tez Yöneticisi: Prof. Dr. Serpil Şahin

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Nanolifler fonksiyonel olma, paketleme malzemesi olarak kullanılma, işleme yardımcı olma ve gıda kalite ve güvenliği alanında sensör olarak kullanılabilme özellikleriyle son yıllarda gıda endüstrisinde popüler olmaya başlamışlardır. Bu çalışmanın ana amacı, mercimek unu ve hidroksipropil metilselüloz (HPMC) kullanarak elektroeğirme metoduyla homojen nanolifler elde etmektir. Çözelti pH'sı (7, 10 ve 12), mercimek unu konsantrasyonu (%1 ve %2), hidroksipropil metilselüloz konsantrasyonu (%0,25, %0,5 ve %1) ve mikroakışkanlaştırma yönteminin (3 ve 5 döngü) çözeltilerin ve nanoliflerin özellikleri üzerindeki etkileri araştırılmıştır. pH'nın arttırılması hem %1 hem de %2 mercimek unu içeren çözeltilerin viskozitelerinde azalmaya sebep olmuştur. Bazik koşullarda tamamen homojen nanolifler elde edilebilmişken, pH 7 değerinde homojen nanolif elde edilememiştir. %2 mercimek unu içeren çözeltilerden elde edilen nanoliflerin çapları, pH'nın yükselmesiyle azalma göstermiştir. Diğer yandan %1 mercimek unu içeren çözeltilerden elde edilen nanoliflerin çaplarında önemli bir değişim gözlenmemiştir.

pH 10 değerinde mercimek unu konsantrasyonu arttırıldığında hem viskozite hem de nanoliflerin çaplarında artma olmuştur. HPMC konsantrasyonundaki artış çözeltilerin viskozitelerini ve nanoliflerin çaplarını arttırmıştır. Farklı mercimek ve HPMC konsantrasyonları kullanılarak hazırlanan çözeltilerin ortalama çapları 198±4 nm ve 254±5 nm arasında değişmektedir. Mikroakışkanlaştırma yönteminin nanolifler üzerinde olumlu bir etkisi görülmemiştir ve boncuk oluşumuna sebep olmuştur. %2 mercimek unu içeren çözeltilerde voltajın arttırılması nanolif çapında artmaya, akış hızının arttırılması ise nanolif çapında azalmaya sebep olmuştur. Mercimek unundaki artış nanoliflerin su buharı geçirgenliğinde artışa neden olmuştur. HPMC konsantrasyonun nanoliflerin su buharı geçirgenliğine etkisi görülmemiştir. Ne mercimek unu konsantrasyonunun ne de HPMC eklenmesinin renk parametreleri üzerinde önemli bir etkisi olmamıştır.

Anahtar kelimeler: Elektroeğirme, mercimek unu, hidroksipropil metilselüloz (HPMC), nanolif

To myself...

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FTIR	Fourier-transform infrared spectroscopy
HPMC	Hydroxypropyl methylcellulose
LF	Lentil flour
MW	Molecular weight
n	Flow behavior index.
PEO	Polyethylene oxide
PVA	Poly (vinyl alcohol)
SEM	Scanning electron microscopy
SPI	Soy protein isolate
WPI	Whey protein isolate
WVP	Water vapor permeability
K	Consistency index
τ	Shear stress
$\dot{\gamma}$	Shear rate

CHAPTER 1

INTRODUCTION

1.1. Electrospinning

Electrospinning is used to produce fibers with a range of submicron to nanometer. It comes to the forefront with its simple mechanism, cheap construction and short processing time among the other methods (Kriegel, Arrechi, Kit, McClements, & Weiss, 2008). Electrospinning process has shown the most promising results for fiber manufacturing (Haghi, 2009). Researches related to electrospinning method is getting more popular (Coles & Woolridge, 2015). There are various studies in the literature related to electrospinning process, parameters that affect the process and characterization of the fibers produced by this method.

1.1.1. Equipment for electrospinning

Electrospinning system is highly controllable due to its versatility. Almost every piece of the system can be altered according to the necessities of the study. In the electrospinning system, there are three main components, which are high voltage supplier, a syringe with metal tip containing the solution and a collector. There is a simple demonstration of the electrospinning system shown in Figure 1. Electrospinning solution (a) is ejected by a simple pump (b). Both electrospinning solution and the collector (c) are electrically charged by a high voltage supplier (d). Anode is attached to the metal tip of the needle (e) whereas cathode is attached to the collector. Electrically charged electrospinning solution elongates and creates a Taylor

cone (f), which is clarified in section 1.1.2. Finally, fibers are collected on the surface of the collector.

Orientation of the system can be altered. Figure 1 presents a horizontal electrospinning system. It can also be built vertically. If a vertical system is used, gravitational forces on the electrospinning solution can be used as initiator. In this orientation, more uniform fibers can be obtained (Haghi, 2009). The same gravitational force prevents a perfect flow in a horizontal system. However, in the vertical orientation there is a droplet risk. The needle is right above the collector; therefore, all of the droplets will fall on to the collector. In the horizontal orientation, with the right electrospinning conditions, droplet formation can be avoided.

The pump in the system can be excluded. Sometimes application of the high voltage to the electrospinning solution is enough to start the flow. As it is mentioned above, a vertical orientation can be used for the same purpose. However, applied voltage or gravitation may not be enough for solutions with high viscosities. In addition, without a pump, the control on the flow rate will not be sufficient (Coles & Woolridge, 2015). In some of the cases using a pump might be unnecessary but it definitely depends on the study that is conducted.

The type of the collector can also be changed. Haghi (2009) stated that using more complex shaped collectors was possible but it should be handled very carefully. For instance, using a rotating cylindrical collector instead of a stationary collector could result in more aligned fibers (Pham, Sharma, & Mikos, 2006). The effects of the collectors on fiber morphology can be seen in Figure 2. The drawback of rotating cylinder system is that the speed of the rotation will be another parameter that should be controlled (Haghi, 2009).

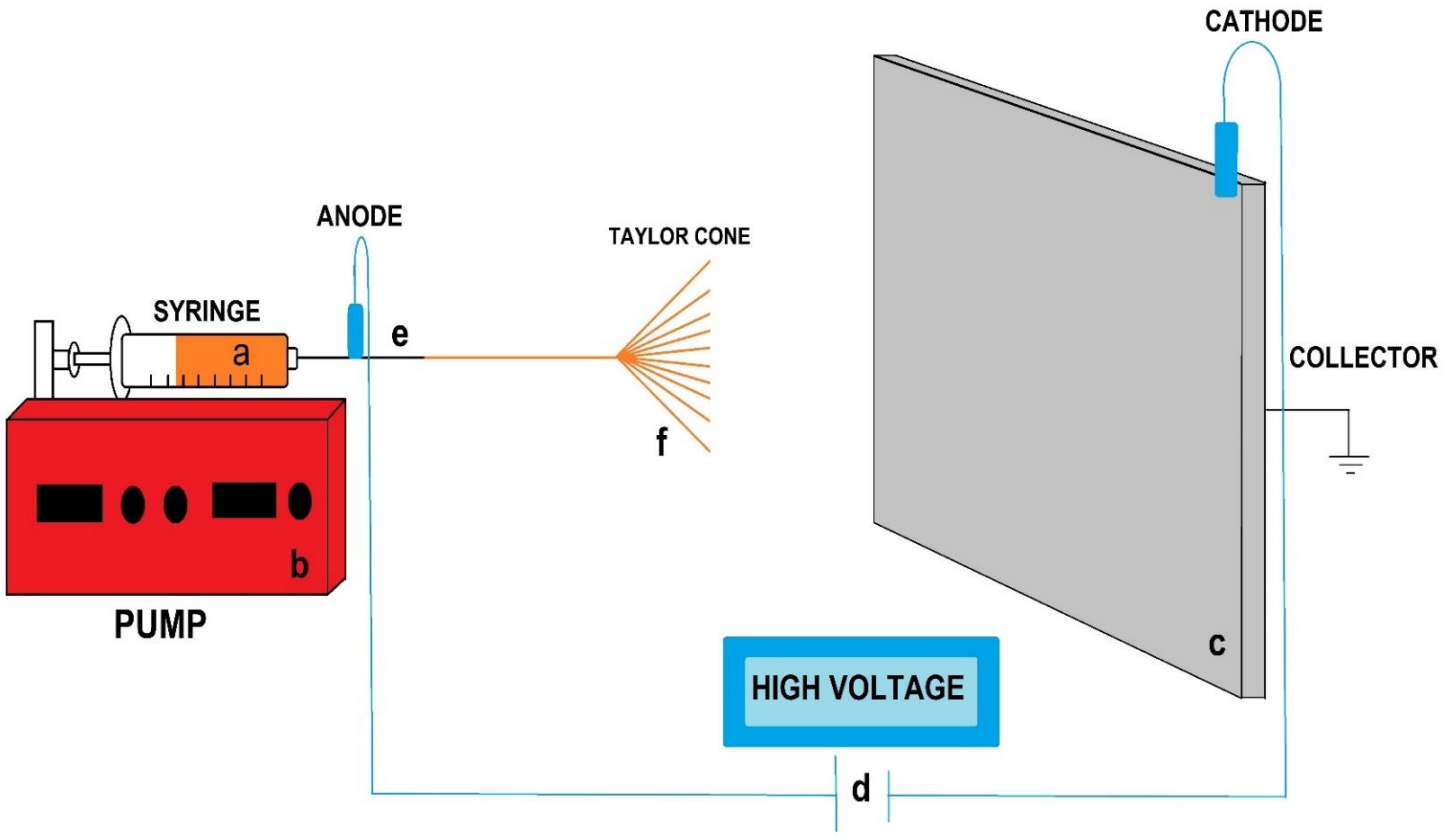
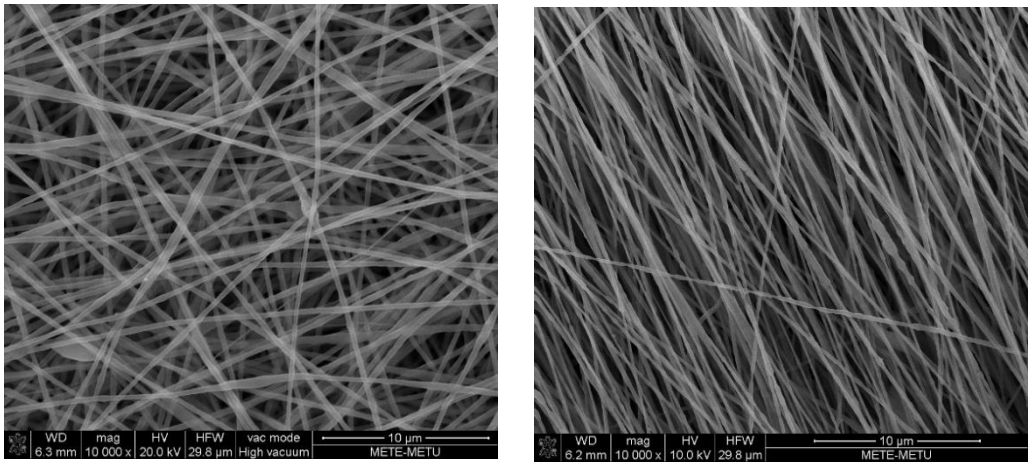


Figure 1. Demonstration of the electrospinning system



A **B**

Figure 2. Effects of the type of the collector ((A) stationary and (B) rotating cylinder) on fiber morphology

1.1.2. Principles of electrospinning

The basic principle of electrospinning is that an electrical charge is induced through the high voltage supplier to the polymer solution inside the syringe. The electrostatic forces start spinning process which distort the hemispherical surfaces of the droplets. Taylor studied the influence of this electrostatic force on liquid droplets and developed a theoretical understanding (Taylor, 1969). In electrospinning, electrically charged particles accumulates on this droplet when an electric field is applied (Coles and Woolridge, 2015). When these charged particles overcome the surface tension due to the charge repulsion, droplet elongates. This elongation creates a conical shape at the tip the needle, which is called as Taylor cone. Then, charged liquid jets are derived from the tip of this Taylor cone. During the movement of these jets to the collector, evaporation occurs and jets elongates; consequently, fibers are collected on the surface of the collector in a solid form (Anu Bhushani & Anandharamakrishnan, 2014; Huang, Zhang, Kotaki, & Ramakrishna, 2003; Schiffman & Schauer, 2008).

1.1.3. Solution characteristics

Characteristics of the electrospinning solution is very important. In the literature, there are many studies that investigate the effects of these properties on the electrospinning process. There are basically four solution characteristics, which are viscosity, conductivity, surface tension and volatility of solvent (Cadafalch Gazquez et al., 2017; Paul, 2005). It was mentioned in previous section that in order to initiate the electrospinning process, charged particles had to overcome the surface tension of the solution. This is why surface tension is an important parameter for electrospinning solution. Yang et al. (2004) stated that different solvents affected surface tension of the solutions differently. It is also known that high surface tension causes bead formation (Vega-Lugo & Lim, 2009). Surfactants are mostly used in order to reduce surface tension (Aceituno-Medina, Mendoza, Lagaron, & López-Rubio, 2013; Perez-Masia, Lagaron, & Lopez-Rubio, 2014; Pérez-Masiá, Lagaron, & López-Rubio, 2014). Solvent volatility is important because jets can elongate adequately only if solvent evaporation is adequate. Otherwise, bead formation occurs and homogeneous fibers cannot be obtained (Anu Bhushani & Anandharamakrishnan, 2014; Huang et al., 2003; Schiffman & Schauer, 2008). The effects of viscosity and electrical conductivity on electrospinning process are explained detailed in sections 1.1.3.1 and 1.1.3.2.

1.1.3.1. Solution concentration

Solution concentration is directly related to the viscosity of the electrospinning solution (Cheong, Heng, & Wong, 1992; Kriegel et al., 2008; Lim, Gwon, Jeun, & Nho, 2010; Vega-Lugo & Lim, 2012). Since changing concentration is quite easy, it is one of the most studied parameters in electrospinning studies (Coles & Woolridge, 2015). Many studies showed that obtaining homogeneous nanofibers at very low and very high viscosities was not possible (Frenot, Henriksson, & Walkenström, 2007;

Larrondo & St. John Manley, 1981; Sukigara, Gandhi, Ayutsede, Micklus, & Ko, 2003).

Coles and Woolridge (2015) explained the effect of the changing viscosity on electrospinning in detail. Increasing the viscosity from low to the critical value leads to obtaining homogeneous fibers. After critical point, diameter of fiber starts to increase. When the viscosity of the solution was not in the range proper for electrospinning process, bead formation occurs and homogenous fibers cannot be obtained again. Many studies in the literature reported an increase in diameter of fibers with an increase in solution concentration. This phenomenon was explained with the increase in viscosity of the electrospinning solution (Beachley & Wen, 2009; Cho, Nnadi, Netravali, & Joo, 2010; Ramji & Shah, 2014; Uyar & Besenbacher, 2009).

1.1.3.2. Electrical conductivity

Charged ions in the electrospinning solution influences the jet formation. As it is explained in section 1.1.2, in order to initiate the electrospinning process, charged particles must overcome the surface tension due to the charge repulsion (Paul, 2005). Therefore, electrical conductivity of the solution is another important parameter in order to initiate electrospinning process. At low electrical conductivity values, the electrospinning solution cannot be electrically charged. In this situation, formation of Taylor cone cannot take place (Bhardwaj & Kundu, 2010; Lu, Zhu, Guo, Hu, & Yu, 2006).

It is also important to obtain homogeneous nanofibers with thinner fiber diameter. Raghavan et al, (2012) stated that an increase in electrical conductivity led to an increase in charge carrying capacity of the jets. When the electric field is applied, the tension become higher and fibers can be collected more aligned. Similarly, Beachley & Wen (2009) stated that increasing the electrical charge of the solution to a critical

value allowed the electrospinning to occur. Also, the diameter of the nanofibers decreased with increasing electrical conductivity.

Methods like addition of ionic salts, using organic acids as the solvent or changing the pH can be used to increase the electrical conductivity of the electrospinning solutions (Fong, Chun, & Reneker, 1999; Vega-Lugo & Lim, 2012; Zong et al., 2002).

1.1.4. Electrospinning conditions

As well as the solution characteristics, process parameters are also important factors for electrospinning (Haider, Haider, & Kang, 2015; Nezarati, Eifert, & Cosgriff-Hernandez, 2013). Flow rate, voltage and distance between syringe and the collector are three main process parameters for electrospinning. There are many studies in literature about the effects of electrospinning parameters on the production and the morphology of the fibers. Deitzel, Kleinmeyer, Harris, & Beck Tan (2001) showed that feed rate and voltage had a strong influence on the fiber morphology. In fact, it was stated that production of homogeneous fiber cannot be succeeded without using the unique optimum conditions for the polymer used in electrospinning process.

1.1.4.1. Voltage

Applied voltage is a very important parameter because without reaching a critical voltage value, electrospinning process cannot be started. To initiate this process, an electric field must be created and voltage is the parameter that creates this electric field. Şener, Altay, & Altay (2011) stated that applied voltage determines the strength of the electric field. As it is explained in section 1.1.2, when the charged particles overcome the surface tension, droplet elongates and charged jets derived from the tip of the Taylor cone (Coles & Woolridge, 2015). In order to create Taylor cone, applied voltage must be higher than a specific threshold value (Li & Wang, 2013).

The effects of the applied voltage on the fiber morphology and fiber diameter is a controversial issue. In the literature there are some studies which showed that using high voltage values increased bead formation (Buchko, Chen, Shen, & Martin, 1999; Deitzel et al., 2001; Demir, Yilgor, Yilgor, & Erman, 2002). Paul (2005) also stated that increasing the applied voltage increased bead density due to the instability of the charged jets. Higher applied voltage values may also lead to smaller fiber diameters. Due to the stronger electric field created by applied voltage, charged jets will stretch more (Buchko et al., 1999; Lee et al., 2004; Megelski, Stephens, Bruce Chase, & Rabolt, 2002; Şener et al., 2011).

Paul (2005) stated that flight time of the charged jets also affects fiber diameter. Fibers can elongate and volatile solvent can evaporate more when the flight time is longer. Creating a weaker electric field by using low voltage values decreases the acceleration of the charged jets. Therefore, the flight time becomes longer, which can create smaller fibers (Zhao, Wu, Wang, & Huang, 2004).

On the other hand, many studies reported no significant difference of fiber morphology or fiber diameter with the change of applied voltage (Andrady, 2008; Fong et al., 1999; Pham et al., 2006). Due to these confounding observations, the effects of the voltage should not be separated from other electrospinning parameters, especially the feed rate and distance (Şener, Altay, & Altay, 2011b)

1.1.4.2. Flow rate

Flow rate determines the availability of the electrospinning solution for the process. The electrospinning system need to be fed by the electrospinning solution in order to create a stable droplet at the tip of the needle (Coles & Woolridge, 2015). Gravitational force or a pump can be used to start the electrospinning process. Even though gravity is enough to initiate the process, replication cannot be performed consistently (Coles & Woolridge, 2015). Controlling the system pressure or using a pump give higher control on the system and help producing more consistent fibers.

For a specific voltage value, there is a specific flow rate range. The relation between flow rate and applied voltage must be balanced to create a stable Taylor cone (Paul, 2005). Beachley and Wen (2009) stated that increasing flow rate too much causes an accumulation at the tip of the needle due to excess amount of solution. Using low flow rates is more favorable to give enough time to the volatile solvent of electrospinning solution for evaporation (Beachley & Wen, 2009b; Paul, 2005). According to Yuan, Zhang, Dong, & Sheng (2004), if the time is not enough for evaporation, fibers may create webs by fusing each other.

In some studies, an increase in the diameter of fibers was observed with an increase in flow rate (Hohmanmichael et al., 2001; Zong et al., 2002). Similarly, Beachley and Wen (2009), reported that at high flow rates, obtaining smooth fibers with small fiber diameter was hard due to lack of time for evaporation. Thick fibers and bead formation occur since stretching forces are not enough. Hohmanmichael et al. (2001) stated that increasing the fiber dimeter would stop at some point. In order to keep process stable, there should be an increase in voltage with the increase in flow rate.

1.1.4.3. Distance

As it was discussed in section 1.1.4.1 and 1.1.4.2, both the strength of the electric field and the flight time of the charged jets were important parameters for electrospinning process. The distance between the tip of the needle and the collector has influence on both of these parameters. A decrease in distance reduces the flight time while it increases the electric field strength (Paul, 2005). Huang et al. (2003) stated that fibers may stick to the surface of the collector and also each other if the distance between the tip of the needle and collector is too short. The reason is explained as at short distance, volatile solvent cannot evaporate adequately. High field strength also causes bead formation due to instable jets (Deitzel et al., 2001; Zong et al., 2002). Similarly, Li & Wang (2013) stated that as fibers could not solidify at short distance whereas bead formation occured at long distance. Some studies showed when the distance was increased, diameter of fibers decreased (Ayutsede et

al., 2005; Yuan et al., 2004). Reneker, Yarin, Fong, & Koombhongse (2000) explained that increasing the distance gave more flight time, which increased stretching of the jets before reaching the collector. However, literature reviews showed that there were exact opposite cases as well. Costolo, Lennhoff, Pawle, Rietman, & Stevens (2008) stated that there was a direct relation between the diameter of fiber and distance. Moreover, Zhao et al. (2004) reported that none of the fibers could reach on the surface of the collector at too long distance. The reason is that the field strength decreases with increase in the distance, which leads to a decrease in fiber stretching (Faridi-Majidi, Ziyadi, Naderi, & Amani, 2012). In addition to that there are studies which reported no significant effect of the distance on the fiber morphology (Zhang, Yuan, Wu, Han, & Sheng, 2005). Paul (2005) suggested that there should be an optimum distance which created the optimum field strength. Overall, unique electrospinning conditions must be succeeded in order to obtain smooth electrospun fibers.

1.1.5. Environmental conditions

As it was explained in sections 1.1.3 and 1.1.4, morphology of the nanofibers depends on many variables. While solution characteristics and electrospinning conditions can be altered easily, changing environmental conditions is a little bit more challenging (Haghi, 2009). Especially ambient parameters temperature and humidity have strong effect on electrospinning process (Coles & Woolridge, 2015; Haider et al., 2015; Paul, 2005).

The temperature of the environment where the electrospinning process is taking place must be considered. Thompson, Chase, Yarin, & Reneker (2007) stated that the effects of the temperature on the electrospinning process should not be considered alone. Haider et al. (2015) explained that there were two main effects of the temperature on the electrospinning. The first one was the temperature effect on the solvent evaporation rate. The second one was the effect of temperature on viscosity

of the electrospinning solution. Coles and Woolridge (2015) reported when temperature was increased, the viscosity of the electrospinning solution decreased, which affected both fiber morphology and fiber diameter. Mit-uppatham, Nithitanakul, & Supaphol (2004) proved that an increase in temperature reduced the diameter of the fibers due to the decrease in the solution viscosity.

There are many researches showing that there is a clear influence of the humidity of the environment on the electrospinning. The change in humidity affects both nanofiber morphology and nanofiber diameter due to its effects on solidification of the electrically charged jets during the electrospinning process (Haider et al., 2015). Li & Wang (2013) stated when the humidity is high, diameter of the fibers become thicker due to the small stretching forces on the jets. Compared to normal atmosphere, water vapor molecules have a tendency to condense on the fiber collected on the collector at high humidity (Paul, 2005). In addition to that, the size and the frequency of the pores on the fiber increase at high humidity (Coles & Woolridge, 2015; Paul, 2005). At lower humidity, especially in dry conditions, evaporation of the volatile solvent increases; therefore, smoother fibers can be obtained (Li & Wang, 2013; Paul, 2005).

1.2. Nanofibers

Nanofibers which have 1 to 100 nm diameter range show different mechanical, electrical and optical properties due to the higher surface area/volume ratio than macrofibers (Neethirajan & Jayas, 2011). Usage of nanofibers in many different areas like textile, biomedical, cosmetic and pharmaceutical industries have become popular in recent years. Paul (2005) stated that almost in all areas, there is a demand in developing novel materials. Nanofibers obtained by electrospinning can be examples for these new materials. However, there is not enough study about the use of nanofibers in food industry applications. One of the most important reason is that the

solvents used for electrospinning are not food safe. Selecting a proper solvent is detailed in the next section.

1.2.1. Use of solvents in electrospinning

Selecting a proper solvent is an important parameter for electrospinning solution. There is a limitation for choosing a proper solvent for a specific electrospinning system. Since some of the solvents have potential hazards on human health, any of the residual of these solvents can restrict the applications of the electrospinning process. The solvents commonly used for electrospinning process are 1,1,1,3,3,3-hexafluoro-2-propanol (HFP), trifluoroacetic acid, and 2,2,2-trifluoroethanol (TFE) ethyl acetate, tetrahydrofuran (THF) dimethylformamide (DMF), methyl ethyl ketone, and 1,2-dichloroethane (Haider et al., 2015). However, they are toxic and prohibited from food-related applications (Vega-Lugo & Lim, 2012).

Electrospinning of the biopolymers by using water as solvent is a challenging topic. Yet, there are many studies which could obtain homogeneous nanofibers by dissolving of polymer in water. Son, Youk, Lee, & Park (2004) and Deitzel, Kleinmeyer, Hirvonen, & Tan (2001) obtained nanofibers from the most common polymer used in electrospinning process, polyethylene oxide (PEO), by dissolving it in the water. Zhang et al. (2005) dissolved poly (vinyl alcohol) (PVA) in water and also obtained nanofibers. Besides polymer-based nanofibers, it is possible to obtain protein-based nanofibers by electrospinning. Sullivan, Tang, Kennedy, Talwar, & Khan (2014) and Vega-Lugo & Lim (2012) obtained nanofiber by dissolving whey protein isolate (WPI) and PEO in water. Cho et al. (2010) reported homogeneous nanofiber production from PVA and soy protein isolate (SPI) blend in the water. In many studies conducted with carbohydrates, nanofibers could be obtained using water as a solvent. Şener et al. (2011b) used water as the solvent of the sodium alginate and PVA blend. Kayaci, Sen, Durgun, & Uyar (2014) dissolved geraniol/cyclodextrin inclusion complexes in the water and obtained bead-free and uniform nanofibers.

Selecting a proper solvent is also important to obtain bead free homogeneous nanofibers because selected solvent directly affects the surface tension of the electrospinning solution. Yang et al. (2004) stated that different solvents affect surface tension of the solutions differently. When the concentration of the solution is kept constant, reduction of the surface tension reduces bead formation and then homogeneous fibers can be obtained. Fong et al. (1999) suggested the usage of ethanol as a solvent because its surface tension is low, which helps obtaining smooth fibers.

1.2.2. Use of polymers in electrospinning

Due to their high availability and low cost, synthetic polymers are more commonly used in electrospinning process (Paul, 2005). However, with the increase in the environmental awareness, biodegradable polymers started to be preferred as compared to synthetic polymers (Cho, Netravali, & Joo, 2012). Many of these polymers have been electrospun successfully (Paul, 2005).

1.2.2.1. Protein and carbohydrate

Pulses, which are the seeds of legumes, are known as high nutritional value foods. According to Food and Agricultural Organization of United Nation's the global production of pulses increased by 57.4% from 1981 to 2011 (Ariyawardana, Govindasamy, & Lisle, 2015). However, Previtali et al. (2014) stated that with the change of eating habits, legume consumption decreased. Consequently, scientists started to search for brand-new areas to use pulses. Thus, pulses have been used in pharmaceutical formulations and in biodegradable materials, such as plastics, inks and dyes (Graham & Vance, 2014).

As it is mentioned in section 1.2.1, there are many studies in which homogeneous nanofibers are obtained from protein and carbohydrates. Whey protein isolate, soy protein isolate, sodium alginate and cyclodextrin can be given as examples (Cho et al., 2010; Kayaci et al., 2014; Şener et al., 2011b; Sullivan et al., 2014; Vega-Lugo & Lim, 2012). In addition to that nanofibers could be obtained from collagen, gelatin, fibrinogen, silk, cellulose and so on (Paul, 2005).

Lentil, which is the second biggest traded pulse crop in developing countries, is a rich protein, vitamin and mineral source (Ariyawardana et al., 2015). Therefore, it takes an important part of the diets of people. Lentil is used especially in flour form in various food applications such as soups, snacks, baked products and so on (Ahmed, Taher, Mulla, Al-Hazza, & Luciano, 2016). In addition to that, scientists started to use lentil flour for production of functional foods such as to increase the nutritional value of the bread, as pre-gelatinized starch, to improve soil health and to remove hazardous dye as a novel absorbent (Çelekli, Tanriverdi, & Bozkurt, 2012; Pathiratne, Shand, Pickard, & Wanasundara, 2015; Previtali et al., 2014; Sharma & Banik, 2015). The usage of lentil flour in electrospinning process has not been studied so far.

1.2.2.2. Polyethylene oxide

Polyethylene oxide (PEO) is chosen as a carrier polymer matrix due to its non-toxic, bio-soluble and chemical resistant and water-soluble nature (Safi, Morshed, Hosseini Ravandi, & Ghiaci, 2007). PEO has been used in many studies in order to increase the spinnability of the solutions. PEO was used to increase the spinnability of cellulose and chitosan (Pakravan, Heuzey, & Ajji, 2011; Samad, Asghar, & Hashaikeh, 2013). Uyar & Besenbacher (2009) added PEO into their solutions, which was composed of cyclodextrins (CD), for same reason. PEO was added into protein isolate solutions as well. To increase the spinnability of the soy protein isolate (SPI) solutions and whey protein isolate (WPI) solutions, PEO was used as carrier polymer

matrix in many studies (Ramji & Shah, 2014; Shankar, Seyam, & Hudson, 2013; Sullivan et al., 2014; Vega-Lugo & Lim, 2012; Xu, Jiang, Zhou, Wu, & Wang, 2012).

1.2.2.3. Hydroxypropyl methylcellulose

Hydroxypropyl methylcellulose (HPMC) is a cellulose derivative. It is used in food industry in many areas. Xuan et al. (2017) studied the effects of HPMC on frozen storage of wheat gluten and recently stated that HPMC could stabilize gluten network. Tanti, Barbut, & Marangoni (2016) showed that HPMC could be used as a shortening in sandwich cookie creams. Mariotti, Pagani, & Lucisano (2013) reported that the presence of HPMC could make the crumb of the gluten free bread softer and slow down the staling process. HPMC has been used for edible film production in many studies as well (Akhtar et al., 2013; Bilbao-Sáinz, Avena-Bustillos, Wood, Williams, & Mchugh, 2010; Brindle & Krochta, 2008; Perone, Torrieri, Cavella, & Masi, 2014). In addition to these, there are many electrospinning studies have been conducted based on HPMC. Frenot et al. (2007) showed that it was possible to obtain homogeneous nanofibers from HPMC in dimethyl acetamide solution.

1.3. Objective of the study

Nanofibers have recently become very popular in food industry for their utilization as highly functional ingredients, high-performance packaging materials, processing aids and food quality and safety sensors. Electrospinning is a method which is used to produce nanofibers. Due to its simple mechanism, cheap construction and short processing time among the other methods, electrospinning has come to the forefront.

Studies related to lentil have increased due to it is a rich protein, vitamin and mineral source. The flour form of lentil is used in many food applications. Recently, the

interest in production of biopolymer-based nanofibers has increased. However, there is lack of research on the usage of lentil flour in electrospinning process.

The aim of the study is to produce homogeneous nanofibers suitable for food industry from a solution containing lentil flour and HPMC by using electrospinning method. The effects of pH, lentil flour concentration, HPMC concentration and microfluidization on solution characteristics and fiber morphology are studied. In addition to that the effects of electrospinning conditions on fiber morphology are investigated. Finally, the effects of lentil flour concentration and HPMC concentration on water vapor permeability and color of nanofibers are highlighted.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Lentil flour (LF) containing 22.2 % protein, 1.7 % fat, 8.9 % moisture and 3 % ash was obtained from Smart Chemical Trading Co. Inc. (Turkey). Polyethylene oxide, PEO (molecular weight = 900,000 Da) and hydroxyl propyl methyl cellulose (HPMC) were bought from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). The emulsifier, Tween 80 was supplied by Merck (Darmstadt, Germany) (Fig. 3).

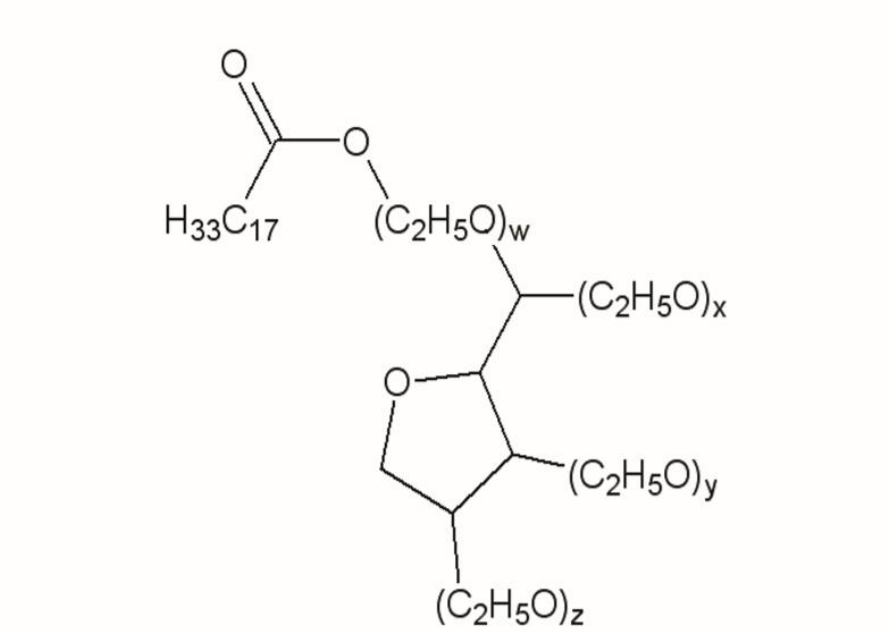


Figure 3. Tween 80 molecular structure. x, y, z, and w were selected as 5 (Karjiban, Basri, Rahman, & Salleh, 2012)

2.2. Solution preparation and characteristics

PEO solution of 3.5% (w/v) was prepared and dissolved at 1,000 rpm for overnight at room temperature by using a magnetic stirrer (Daihan Scientific Co, KR). Distilled water was used as the solvent. Lentil flour was added into the 3.5% (w/v) PEO solution at two different concentrations (1% and 2 % (w/v)). Solutions were homogenized with a high-speed homogenizer at 12,000 rpm for 3 min (IKA T25 Digital Ultra-Turrax; IKA®-Werke GmbH & CO. KG, Staufen, Germany). After that, pH of the solutions was adjusted to 7, 10 and 12 by the addition of 2M NaOH solution. Then, solutions were heated to 80°C in a water bath and mixed with a magnetic stirrer at 1,000 rpm and at 80°C for 2h. After 2 hours, solutions were left to cool down until they reach the room temperature. Tween 80 (2% (w/v)) and HPMC (0.25%, 0.5% and 1% (w/v)) were added into the solutions and homogenized with a high-speed homogenizer at 10,000 rpm for 5 min. Solutions were stirred at 750 rpm for overnight at room temperature by using a magnetic stirrer. These solutions were used for determination of effects of lentil flour concentration, HPMC concentration and pH on rheological behavior, electrical conductivity and fiber morphology (Fig. 4).

For water vapor permeability, color and FTIR experiments, four different solutions were prepared. 2% (w/v) and 5.25% (w/v) lentil flour were added into 3.5% (w/v) PEO solution. Solutions were divided into two. 2% (w/v) Tween 80 was added into all of the solutions and 0.5% (w/v) HPMC was added into half of them (Fig. 4).

PEO solution

↓ 1,000 rpm for overnight

Lentil Flour

↓ High-speed homogenizer at 12,000 rpm for 3 min

Adjusting pH (with 2M NaOH solution)

↓ Heated to 80°C in a water bath

Mixed (1,000 rpm and at 80°C for 2h)

↓ Cooled down to room temperature

Tween 80 and HPMC

↓ High-speed homogenizer at 10,000 rpm for 5 min

Stirred at 750 rpm for overnight

Figure 4. Solution preparation for determination of effects of lentil flour concentration, HPMC concentration and pH on solution characteristics and fiber morphology and characterization of fibers

For microfluidization part, first, lentil flour solutions of 5.25% (w/v) and 7.5% (w/v) were prepared. Solutions were homogenized with a high-speed homogenizer at 12,000 rpm for 3 min (IKA T25 Digital Ultra-Turrax; IKA®-Werke GmbH & CO. KG, Staufen, Germany). After that, pH of the solutions was adjusted to 10 by the addition of 2M NaOH solution. Then, solutions were heated to 80°C in a water bath and mixed with a magnetic stirrer at 1,000 rpm and at 80°C for 2h. Tween 80 (2% (w/v)) and HPMC (0.5% (w/v)) were added into the solutions, which were left to cool down until they reach the room temperature, and homogenized with a high-speed homogenizer at 10,000 rpm for 5 min. Then 3.5% (w/v) and 2.5% (w/v) PEO was added into the solutions containing 5.25% (w/v) and 7.5% (w/v) lentil flour,

respectively. After that, solutions were homogenized with a high-speed homogenizer at 10,000 rpm for 5 min again. Final solutions were stirred at 750 rpm for overnight at room temperature by using a magnetic stirrer (Fig. 5).

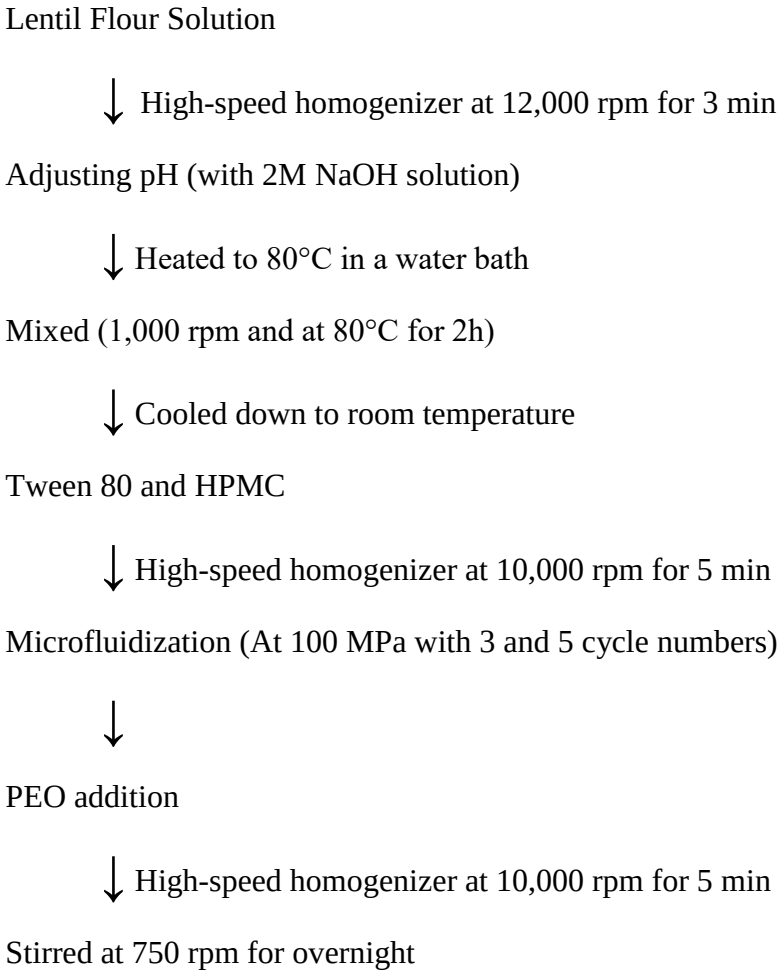


Figure 5. Solution preparation for determination of effects of microfluidization on solution characteristics and characterization of fiber morphology

2.2.1. Rheological measurements

Rheological behavior of the solutions was measured by using a controlled strain cone & plate rheometer (Kinexus, Malvern Instruments, UK). The cone angle was 4° and the plate had 40 mm diameter. Shear rate varied from 1 to 100 s^{-1}. Temperature was set to 25°C. The shear stress (τ) and shear rate ($\dot{\gamma}$) data were collected. Experiments were replicated three times.

2.2.2. Electrical conductivity measurements

Electrical conductivity of the solutions was investigated at 25°C by using a conductivity meter (Inolab® 7110, Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Experiments were replicated three times.

2.3. Microfluidization

Four different solutions, 3.5% (w/v) PEO solution containing 5.25% (w/v) lentil flour and 2.5% (w/v) PEO solution containing 7.5% (w/v) lentil flour with and without 0.5% HPMC, were processed with microfluidizer (Suflux, ILSHIN AUTOCLAVE) at 100 MPa pressure and different cycle numbers (3 and 5) to see the effect of microfluidization on electrospinning.

2.4. Electrospinning

Solution prepared for spinning was placed in 5 mL syringes, which had 11.58 mm inner diameter. The needle was positioned horizontally on the syringe pump and connected to the positively charged electrode, which had high voltage supplier. The stationary collector, which was connected to the negatively charged part, was covered

with aluminum and fixed 30 cm away from the tip of the needle. For solutions used for water permeability and color measurements, flow rate was adjusted to 0.8 mL/h and the voltage was varied between 8-15 kV. Flow rate and applied voltage in electrospinning device (NanoWeb 103, Mersin, Turkey) were varied between 0.6-1.5 mL/h and 8-20 kV for solutions prepared with microfluidization, respectively. Flow rate of 0.6 mL/h and voltage of 11 kV were used for rest of the nanofibers obtained for investigating the effects of pH, lentil flour concentration and HPMC concentration. The electrospinning process was performed at around 40% relative humidity and 20°C. Process time was fixed to 3 hours for the nanofibers obtained for investigating the effects of pH, lentil flour concentration and HPMC concentration. The time was varied between 50-90 hours for nanofibers used for water vapor permeability, color and FTIR analyses.

2.5. Characterization of fibers

2.5.1. Scanning electron microscopy (SEM)

For the morphological investigation, the nanofiber samples were collected on the stationary collector. Before SEM analysis, samples were coated with Au/Pd. After that, images were taken by using Field Emission Scanning Electron Microscopy (FESEM) (JEOL, Japan) at magnification 10,000× at Metallurgical and Materials Engineering Department, Middle East Technical University. The diameter of the nanofiber was measured by using the Image J 1.50i analysis software. For each image, diameters of 100 fibers were measured. These measurements were used to determine range of diameter and to calculate the average diameter for each sample.

2.5.2. Water vapor permeability analysis

Water vapor permeability of the nanofibers was measured with ASTM, E96 method (Busolo et.al. 2009). When nanofibers were collected enough, they were kept in a desiccator with aluminum foil for 24 hours. After that, obtained nanofibers were peeled off from the aluminum foil. The thickness of nanofibers was measured with calipers by taking 10 measurements for each sample. The surface area of the nanofibers was calculated as the base area of the plastic container. Specially designed plastic containers with 4 cm diameter were filled with water, covered up by the peeled nanofiber and kept at controlled environment. The weight of the containers was measured until the steady-state weight loss was obtained. Water vapor permeability (WVP) was calculated with equation 1.

$$WVP = \frac{W\,x}{t\,A\;P_{satı n}(R_1 - R_2)} \quad (1)$$

W/t = Slope of the weight loss and time graph (g/s)

x: Average nanofiber thickness (mm)

A: Surface area of the nanofiber (m^2)

$P_{satı n}$: Saturated vapor pressure (kPa)

R_1= Relative humidity of the environment (%)

R_2= Relative humidity of the container (%)

Experiments were replicated two times.

2.5.3. Color analysis

Color of the samples was measured using a color reader (Minolta, CR10, Osaka, Japan). White light was used and the angle was 90°. The color values were expressed by CIE coordinates, L* a* and b*, where L* indicates whiteness/darkness, a* indicates redness/greenness, and b* indicates blueness/yellowness values. Two color data were taken from different locations for each sample. For ΔE calculations, 93.2, -1.4 and 0.12 reference values were used, which were L_0*, a_0* and b_0* values of $BaSO_4$.

2.5.4. Fourier-transform infrared spectroscopy (FTIR)

FTIR analyses of PEO powder, HPMC powder and nanofibers obtained from electrospinning were conducted by using a FTIR spectrophotometer (IR-Affinity1, Shimadzu, Kyoto, Japan). The analysis was performed in attenuated total reflectance (ATR) mode using a diamond ATR crystal. The infrared regions analysis was recorded with 16 scans. FTIR spectra were collected over the wave number range 600–4000 cm^{-1}.

2.6. Statistical analysis

Analysis of variance (ANOVA) was performed to determine whether there was significant difference between the factors ($p \leq 0.05$). Tukey Single Range test was used to compare variable means by using MINITAB statistics programme (MINITAB for Windows, Version 16, Minitab Inc., State College, Pa., USA) (Appendix A).

CHAPTER 3

RESULT AND DISCUSSION

When the solutions composed of only lentil flour and HPMC homogeneous nanofibers could not be obtained. Therefore, to increase the spinnability, polyethylene oxide (PEO) was added into the solution. PEO was chosen as a carrier polymer matrix due to its non-toxic, bio-soluble and chemical resistant nature as well as its solubility in water (Safi et al., 2007). PEO has been used in many other studies to increase the spinnability of the solutions. It was shown to increase the spinnability of cellulose and chitosan (Pakravan et al., 2011; Samad et al., 2013). For the same reason, Uyar & Besenbacher (2009) also added PEO into their solutions, which composed of cyclodextrins. To increase the spinnability of the soy protein isolate solutions and whey protein isolate solutions, PEO was used as carrier polymer matrix in many studies (Ramji & Shah, 2014; Sullivan et al., 2014).

In this study, a non-ionic surfactant 2% Tween 80 was added into the solution to decrease the surface tension, which increased the chance of obtaining more homogeneous nanofibers. Vega-Lugo & Lim (2009) stated that high surface tension could cause bead formation. Yang et al. (2004) stated that different solvents affected surface tension of the solutions differently. When the total polymer concentration of the solution was kept constant, reduction of the surface tension reduced bead formation and as a consequence homogeneous fiber could be obtained (Aceituno-Medina et al. (2013) used Tween 80 as surfactant in their study. In an electro-spraying study, Perez-Masia et al. (2014) added various surfactants (Tween20, Span20 and lecithin) into solutions composed of two different low molecular weight carbohydrates (maltodextrin and commercial resistant starch). It was shown that all of the surfactants decreased the surface tension of the solution. When the solutions, which did not contain any of the surfactants were used, extensive dropping was

observed due to unstable electro-spraying. Solution properties (polymer concentration and electrical conductivity) and electrospinning conditions (voltage and flow rate) are important for electrospinning process. Therefore, in this study, the effects of pH, lentil flour and HPMC concentrations on the solution characteristics and fiber morphology were discussed. In addition, the effects of electrospinning conditions on fiber morphology was examined. Lastly, the effects of lentil flour concentration on water vapor permeability and color of nanofibers were analyzed.

3.1. Obtaining nanofibers with high lentil flour and low PEO concentrations

In order to obtain homogeneous nanofibers, solutions with different lentil flour, PEO and HPMC concentrations were prepared. In Table 1, morphology of fibers obtained from different solutions are given.

All solutions were prepared at pH 10. Effect of the pH will be discussed later. Lu et al. (2006) stated that electrospinning of natural biopolymers was challenging. In most of the studies, bead formation was observed or nanofiber could not be obtained at all. With the addition of nontoxic, biocompatible carrier polymers like PEO, spinnability of the solutions could be improved. Below 2.5% PEO concentration, no matter what the protein concentration was, homogeneous nanofibers could not be obtained (Table 1). Thus, it can be concluded that PEO concentration less than 2.5% was not enough to obtain a solution with sufficient spinnability.

Table 1. Nanofiber morphology of electrospun solutions with different lentil flour, PEO and HPMC concentrations

Concentration (%)			Nanofiber Morphology
PEO	**Lentil Flour**	**HPMC**	
3.5	5.25	0	HNF*
3.5	5.25	0.5	HNF
3.5	3.5	0	HNF
3.5	3.5	0.5	HNF
3.5	2	0	BF**
3.5	2	0.5	HNF
3.5	1.5	0	NF***
3.5	1.5	0.5	HNF
3.5	1	0	BF
3.5	1	0.5	HNF
3	6	0	HNF
3	6	0.5	BF
3	3	0	HNF
3	3	0.5	HNF
2.5	7.5	0	HNF
2.5	7.5	0.5	HNF
2.5	5	0	HNF

Table 1. (Continued)

Concentration (%)			Nanofiber Morphology
PEO	**Lentil Flour**	**HPMC**	
2.5	5	0.5	BF
2.5	2.5	0	HNF
2.5	2.5	0.5	BF
2	5	0	BF
2	5	0.5	BF
2	4	0	BF
2	4	0.5	BF
2	2	0	NF
2	2	0.5	BF
1.5	3	0	NF
1.5	3	0.5	BF
1.5	2.25	0	BF
1.5	2.25	0.5	BF
1	5	0	NF
1	5	0.5	BF
1	4	0	NF
1	4	0.5	BF
1	3	0	NF

Table 1. (Continued)

Concentration (%)			Nanofiber Morphology
PEO	**Lentil Flour**	**HPMC**	
1	3	0.5	BF
0.5	2.5	0	NF
0.5	2.5	0.5	BF
0.5	1.5	0	NF
0.5	1.5	0.5	BF

*HNF means homogenous nanofiber

**BF means bead formation

***NF means homogenous nanofibers couldn't be obtained

All of the solutions were prepared at pH 10 and contained 2% Tween80

One of the purposes of this study was to obtain nanofibers with high biopolymer concentration. Lentil flour is mainly composed of proteins and carbohydrates. Therefore, it was tried to use higher amount of lentil flour. However, concentration could not be increased more than 5.25% for solutions prepared with 3.5% PEO. Increasing lentil flour concentration more than 1.5-fold for solutions containing 3.5% PEO affected spinnability negatively and homogeneous nanofibers could not be obtained. This proportion was 2-fold and 3-fold for solutions with 3% and 2.5% PEO concentrations, respectively. Similarly, Ramji & Shah (2010) observed bead formation when solutions were prepared with 7% soy protein and 5% PEO whereas homogeneous nanofibers could be obtained when concentrations were changed to 12% soy protein and 10% PEO.

3.2. Effect of pH on solution characteristics and fiber morphology

The shear stress (τ) versus shear rate ($\dot{\gamma}$) data obtained from rheological experiments were fitted well to Power Law model (Eq. (2)) with high coefficient of determination values (r^2=0.965–0.997).

$$\tau = K\,(\dot{\gamma})^{n} \qquad (2)$$

where, τ is the shear stress (Pa), γ is the shear rate (s^{-1}), *K* is the consistency index (Pa s^n) and n is flow behavior index.

The power law parameters of spinning solutions at different pH values (7, 10 and 12) are shown in Table 2. Since *n* values ranged between 0.879 and 0.944, which were smaller than 1, it could be inferred that the solutions showed pseudoplastic (shear-thinning) behavior. As it can be seen in Figure 6, apparent viscosities of spinning solutions decreased as the shear rate increased.

Table 2. Effects of the lentil flour concentration and pH on solution characteristics and fiber morphology

Lentil flour concentration (%)	pH	*K*(Pa s^n)	*n*	R^2	Electrical conductivity (mS/cm)	Nanofiber diameter (nm)	Nanofiber Morphology	Apparent Viscosity**** (Pa.s)
1	7	$1.859\pm0.035^{c*}$	0.916^{b}	0.997	0.369 ± 0.027^{d}	-	**BF	1.235^{c}
1	10	1.294 ± 0.075^{d}	0.944^{a}	0.997	0.541 ± 0.014^{cd}	210 ± 4^{c}	***HNF	0.980^{d}
1	12	1.428 ± 0.032^{d}	0.939^{a}	0.998	1.042 ± 0.22^{ab}	223 ± 3^{bc}	***HNF	1.056^{d}
2	7	2.937 ± 0.036^{a}	0.879^{e}	0.997	0.572 ± 0.012^{cd}	-	**BF	1.680^{a}
2	10	2.358 ± 0.010^{b}	0.895^{d}	0.997	0.834 ± 0.077^{bc}	254 ± 5^{a}	***HNF	1.431^{b}
2	12	2.016 ± 0.047^{c}	0.906^{c}	0.997	1.255 ± 0.005^{a}	231 ± 3^{b}	***HNF	1.285^{bc}

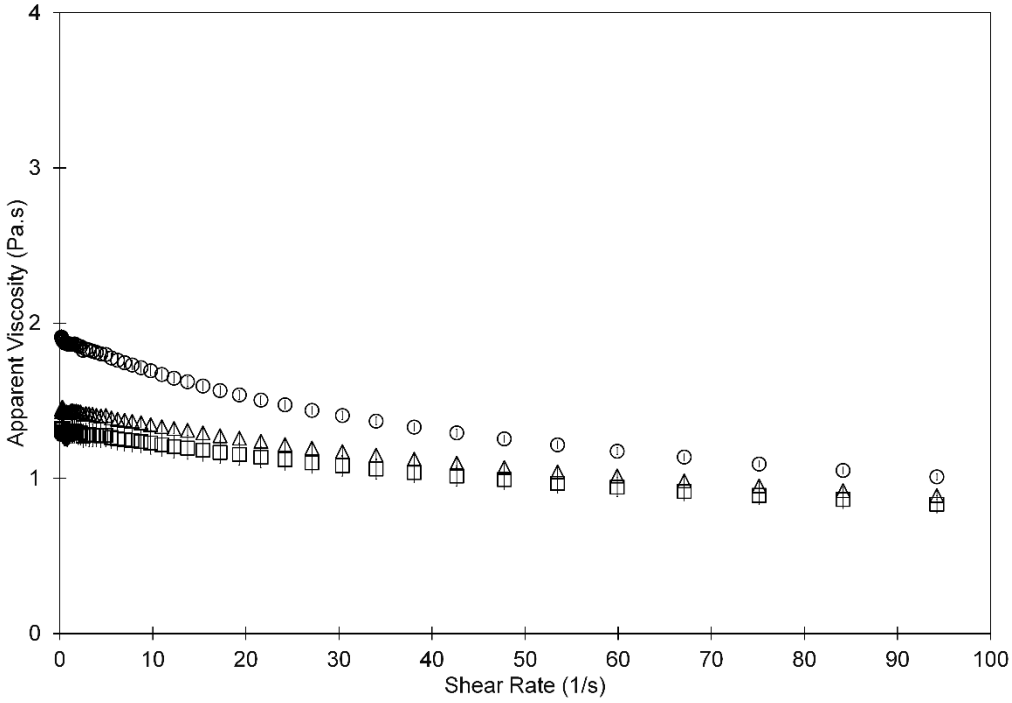
All of the solutions were prepared by using %3.5 PEO, %0.5 HPMC and 2%Tween80 at the same electrospinning conditions

*Columns with different letters differ statistically ($p\leq0.05$)

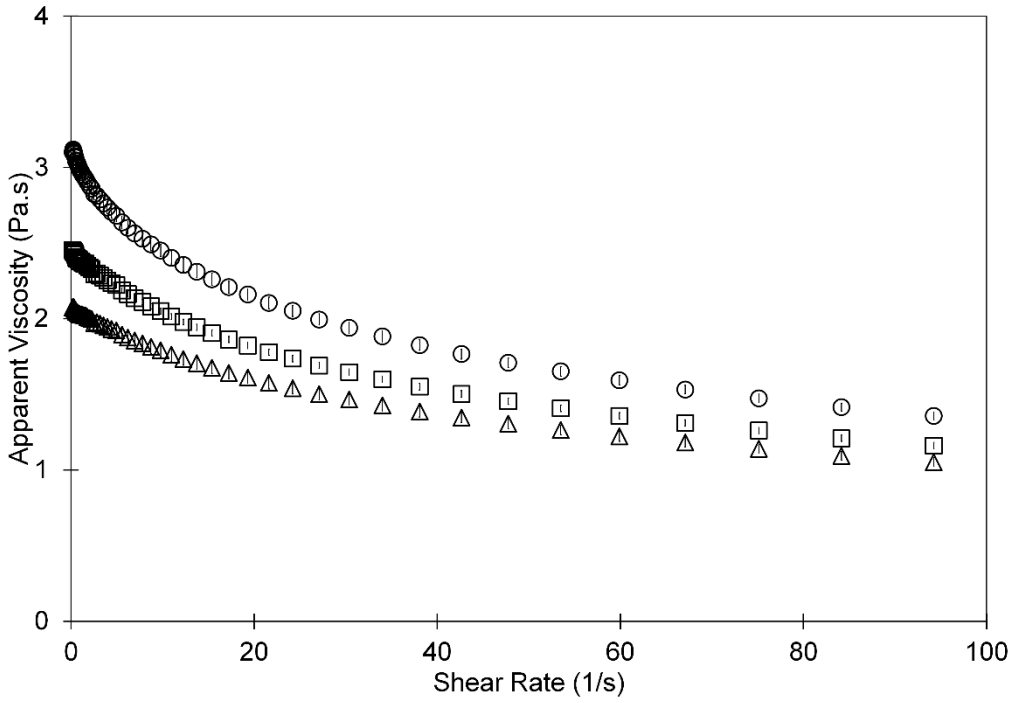
**BF means bead formation

***HNF means homogeneous nanofiber

****Apparent viscosity values are measured at 50 s^{-1} shear rate



A



B

Figure 6. The effects of different pH on apparent viscosity of different spinning solution (○: pH 7, □: pH 10, Δ: pH 12) PEO:LF ratio of (A) 3.5:1 and (B) 3.5:2

Polymer concentration is very important in electrospinning due to its effects on viscosity of the solution. Obtaining homogeneous nanofibers at very low and very high viscosities was not possible (Larrondo & St. John Manley, 1981; Sukigara et al., 2003).

pH was also effective on rheological properties and as a consequence on electrospinning. Table 2 shows the effect of pH on rheological characteristics of solutions composed of 1% and 2% lentil flour. As the pH values increased, the consistency index of solutions decreased significantly ($p \leq 0.05$) for both 1% and 2% lentil flour containing solutions (Table A1). Demetriades, Coupland, & McClements (1997) prepared corn oil-in-water emulsions stabilized by 2 wt% whey protein isolates with a pH range of (3–7). In their study, the lowest viscosity values could be obtained at only the pH values far away from the isoelectric point of the whey protein. It was also reported that to obtain the highest viscosity values, the pH of the solution should be near the isoelectric point. In another research, Cho et al. (2010) showed that the viscosity values of soy protein solutions decreased when pH level was increased. The reason of this was explained by the fact that at high pH values protein molecules were unfolded due to the reduction of entanglements. Dissanayake, Ramchandran, & Vasiljevic (2013) reported that increasing pH values of the whey protein solutions decreased viscosity. The first reason was explained by the charged nature of the whey protein which might affect the rheological behavior of the solution. When pH moved away from the isoelectric point, net charge on protein molecules became greater. Thus, water molecules had higher affinity and hydration degree of protein molecules could increase. Secondly, when intermolecular repulsion between whey proteins predominated at lower shear rates, weak interactions between protein aggregates could be easily disrupted with the increase in shear. Similar to the studies in literature, in the case of lentil flour, when the pH values were moved away from the isoelectric point of the lentil protein, which was ~pH 4.5 (Bamdad, Dokhani, & Keramat, 2009), the viscosity of the solution decreased.

Electrical conductivity of the solution is an important parameter to obtain homogeneous nanofibers since charged ions in the solution influenced jet formation. With increasing electrical conductivity, charge carrying capacity of the jets increased; consequently, the tension became higher in the presence of electric field and fibers could be collected more aligned (Raghavan et al., 2012). Moreover, fibers with smaller diameters could be obtained (Li & Wang, 2013). In order to increase the electrical conductivity of the electrospinning solutions, different methods can be used such as addition of ionic salts, using organic acids as the solvent or changing the pH. In a study conducted with various salts (KH_2PO_4, NaH_2PO_4, and NaCl), it was reported that the addition of salts increased electrical conductivity; as a result of that, more homogeneous and bead free fibers with smaller diameters was obtained (Zong et al., 2002). Similarly, Fong et al. (1999) added NaCl into PEO in order to increase the carried charge. Vega-Lugo & Lim (2012) showed that the increase in pH values of soy protein isolate solutions from 1 to 12 increased electrical conductivity of the solutions almost twentyfold.

Lentil flour contains 22.2% protein and has an isoelectric point of ~pH 4.5 (Bamdad et al., 2009). When the pH of the solution was increased, it was expected that they would be negatively charged in alkali conditions. As it was shown in Table 2, when the pH was increased from 7 to 12 or 10 to 12 by the addition of NaOH solution, the electrical conductivity increased as expected for solutions containing both 1% and 2% lentil flour. This result is similar to the results found in literature. Vega-Lugo & Lim (2012) reported that electrical conductivity values were 0.49, 1.30 and 9.64 (mS/cm) when the pH of the solution was 1, 7 and 12, respectively. That is, electrical conductivity values increased with increase in pH from 1 to 12. This alteration was because of the isoelectric points of the proteins, which was the point when the net charge on the protein molecules was equal to zero (Singh, Kaur, & Sandhu, 2005). When pH was altered, the distance to isoelectric point of the protein changed; consequently, the amount of the charged particles inside the solution changed. Therefore, the electrical conductivity of the solution was expected to change. In

another study, when the pH of the soy protein solution was increased, the conductivity increased as well (Vega-Lugo & Lim, 2008).

In this study, homogeneous nanofibers could not be obtained from the solutions prepared at pH 7 (Fig. 7A, 7B) whereas perfectly homogeneous ones were obtained at pH 10 and pH 12 (Fig. 7C, 7D, 7E, 7F). In many other studies conducted with proteins, researchers could not obtain homogeneous nanofibers at neutral pH either. Cho et al. (2010) studied with soy protein isolates and poly (vinyl alcohol) (PVA) blends and bead formation occurred for solutions prepared at pH 7. When the pH was increased to 9 and 12, bead formation decreased and eventually homogeneous nanofibers could be obtained. Vega-Lugo & Lim (2008) reported bead formation for WPI: PEO (10%:0.4%) solutions prepared at neutral conditions as well. At alkaline conditions (pH 12) bead formation problem decreased while at acidic conditions (pH 1) totally homogeneous nanofibers were obtained. Colín-Orozco, Zapata-Torres, Rodríguez-Gattorno, & Pedroza-Islas (2015) also observed bead formation for PEO:WPI solutions prepared with 30:70 and 20:80 proportions and at pH values of 7.24 and 7.16, respectively. For the solution prepared with 30:70 (PEO: WPI) ratio, above pH value of 7.28 homogeneous nanofibers were observed. Sullivan et al. (2014) prepared WPI: PEO solutions at four different pH values, which were 2.0, 4.0, 5.2 and 7.5. Uniform fibers were obtained at pH 2.0 and 7.5, whereas fibers contained beads at pH 4.0 and 5.2. Monahan, German, & Kinsella (1995) stated that, the solubility of the proteins increased as the distance from the isoelectric point increased. Malik & Saini (2017) also reported the same result. Isoelectric point of lentil protein was the same with soy protein and whey protein, which were around pH 4.5 (Bamdad et al., 2009; Elizalde, Bartholomai, & Pilosof, 1996; Pelegrine & Gasparetto, 2005). Therefore, an increase in solubility of the proteins by moving away from pH 4.5 was an expected result. When the results of the researches were examined, homogeneous nanofibers were obtained when the pH value was far away from the isoelectric points of the proteins.

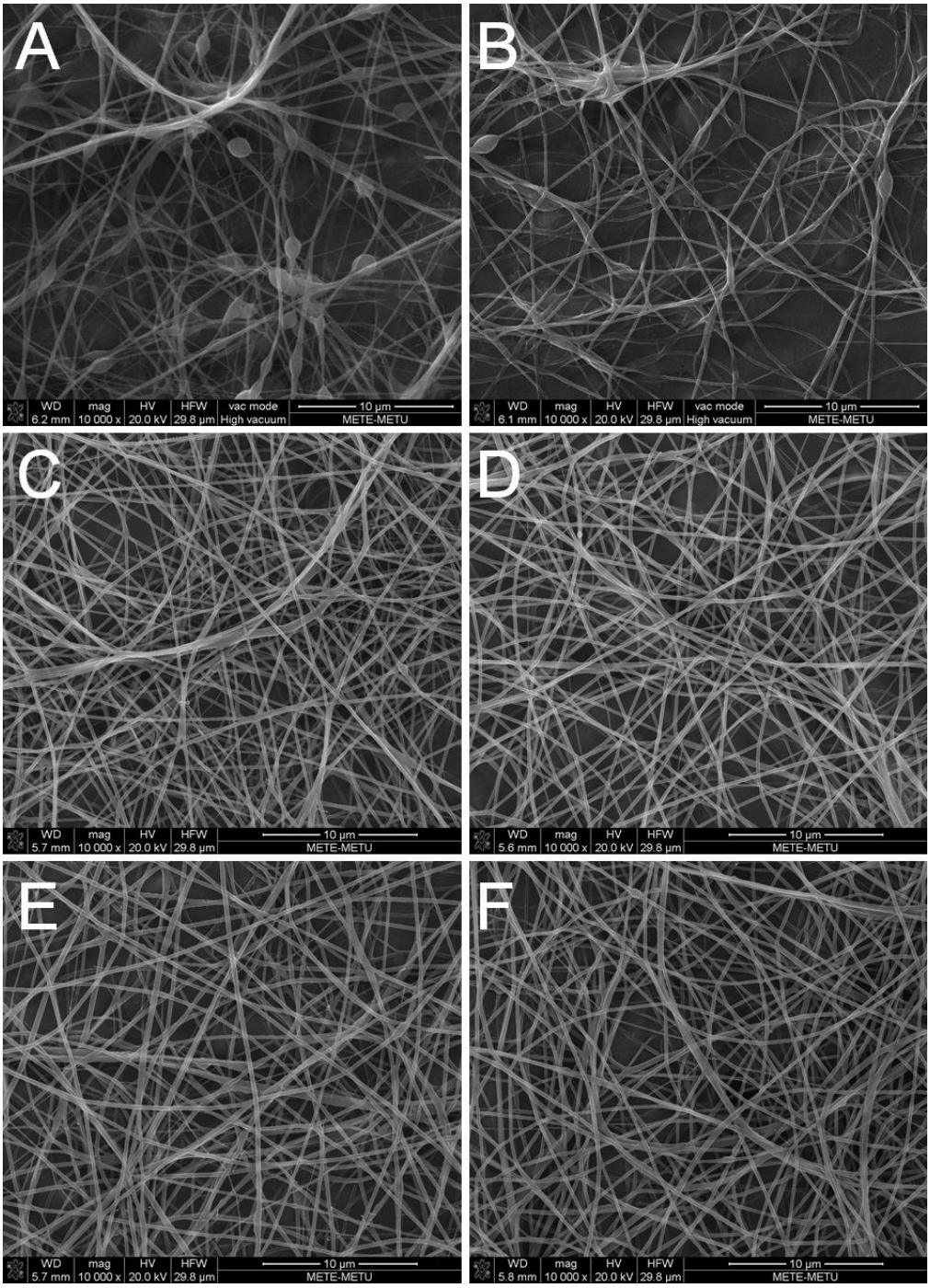
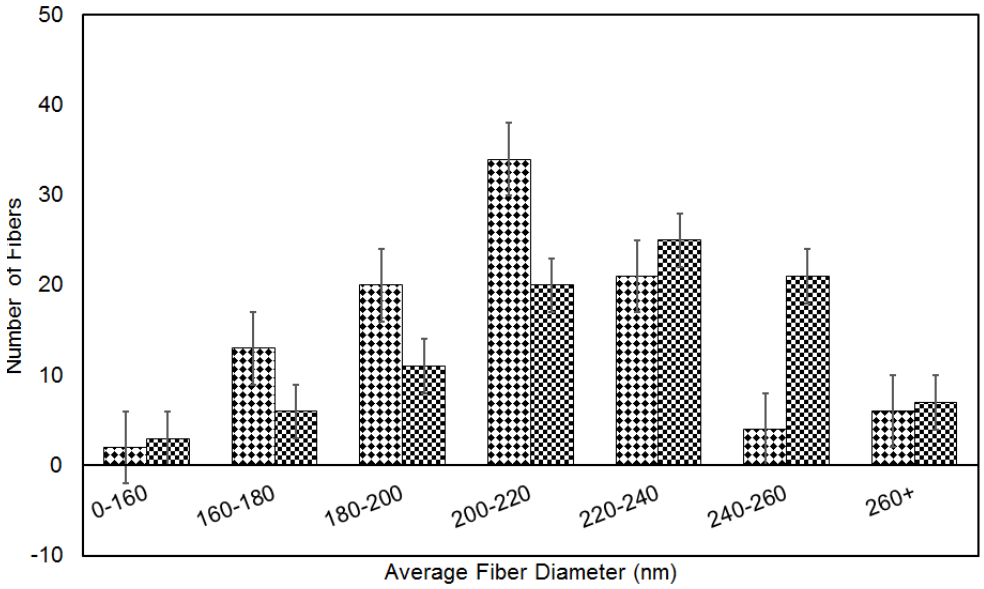


Figure 7. SEM images of different nanofibers at different pH values and with different formulations (A) PEO: LF ratio of 3.5:1 at pH 7, (B) PEO: LF ratio of 3.5:2 at pH 7, (C) PEO: LF ratio of 3.5:1 at pH 10, (D) PEO: LF ratio of 3.5:2 at pH 10, (E) PEO: LF ratio of 3.5:1 at pH 12, and (F) PEO: LF ratio of 3.5:2 at pH 12

As discussed before, moving away from the isoelectric point of the solution not only affected the solubility but also the electrical conductivity and rheological properties of the solutions. This change in the electrical conductivity and viscosity of the solution had also significant effects on the diameter of the nanofibers. Bhardwaj & Kundu (2010) reported that solutions having low electrical conductivity could not be electrically charged. Thus, Taylor cone formation and electrospinning could not take place. It can be see seen from Table 2 that low electrical conductivity was an obstacle for the production of homogeneous nanofibers (Fig. 7A, 7B). Similar to our results, Beachley & Wen (2009) stated that increasing the electrical charge of the solution to a critical value allowed the electrospinning process to occur and also reduced the diameters of the nanofibers. In another research, the production of homogeneous nanofibers by using proteins could not be achieved due to the low electrical conductivity of the solution (Lu et al., 2006). Vega-Lugo & Lim (2012) mentioned that high electrical conductivity was desirable in electrospinning process because it increased the repulsion on the charged particles which induced the critical parameters for fiber formation like the bending instability and stretching. On the other hand, it was emphasized that even though the electrical conductivity of the solution was really small at pH 1 as compared to that at pH 12, homogeneous nanofibers could still be obtained, which indicated that conductivity was not the main contributor of fiber formation. Our experimental results were in agreement with results of Vega-Lugo & Lim (2012). For the solution containing 1% lentil flour, the change in pH did not have a significant effect on fiber diameter even though the electrical conductivity increased significantly (Table A3 and A4). However, the fiber diameter decreased from 254±5 nm to 231±3 nm significantly for the solution containing 2% lentil flour while the electrical conductivity increased significantly. The reason could be explained by the viscosity change. The viscosity of the solution with 1% lentil flour concentration did not change significantly with the increase in the pH value from 10 to 12 whereas the viscosity of the solution with 2% lentil flour concentration decreased significantly (Table 2). Similarly, when the pH value was increased from 10 to 12, fiber diameter of the solutions decreased for the solution containing 2% lentil flour but it did not

change significantly for the solution containing 1% lentil flour. Shahreen & Chase (2015) explained this phenomenon with the combined effects of the viscosity and electrical conductivity. When a decrease in viscosity was combined with an increase in the electrical conductivity, viscoelastic force and charge density reduced; consequently, fiber diameter decreased. It was stated that very low and very high viscosities were considered as an obstacle for fiber production. It was suggested that increasing the solution viscosity at ideal range would increase the diameter of the fibers. Diameter distribution was varied for different lentil flour concentrations. In other words, fibers were not uniform in size. For solutions containing 1% lentil flour, both at pH 10 and pH 12 narrow diameter distributions were observed (Fig. 8A). Diameters were concentrated around 200-220 nm at pH 10 whereas they were concentrated around 220-240 nm at pH 12. When the diameter distributions were examined, a narrower distribution was obtained for the one with pH 12 as compared to the one with pH 10 for 2% lentil flour containing solution (Fig. 8B). Most of the diameter results were concentrated around 220-240 nm at pH 12 for the solution containing 2% lentil flour. After all, many studies showed that fiber diameter did not depend on only one factor, but on a combination of many factors like electrical conductivity, pH, viscosity and polymer concentration. For 2% lentil flour concentration, the average fiber diameter decreased significantly with pH with the combined effect of viscosity and electrical conductivity (Table 2).



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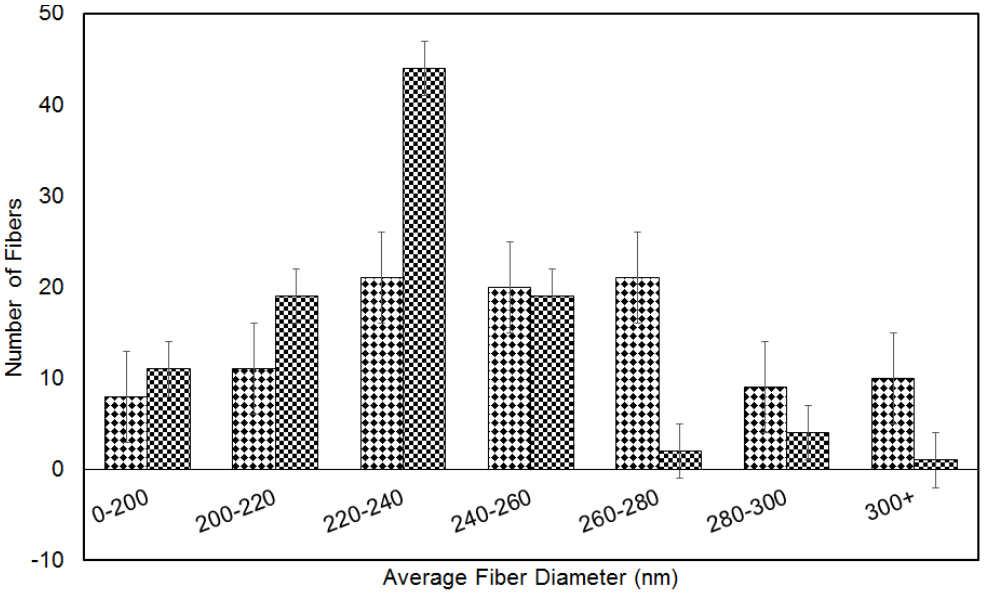

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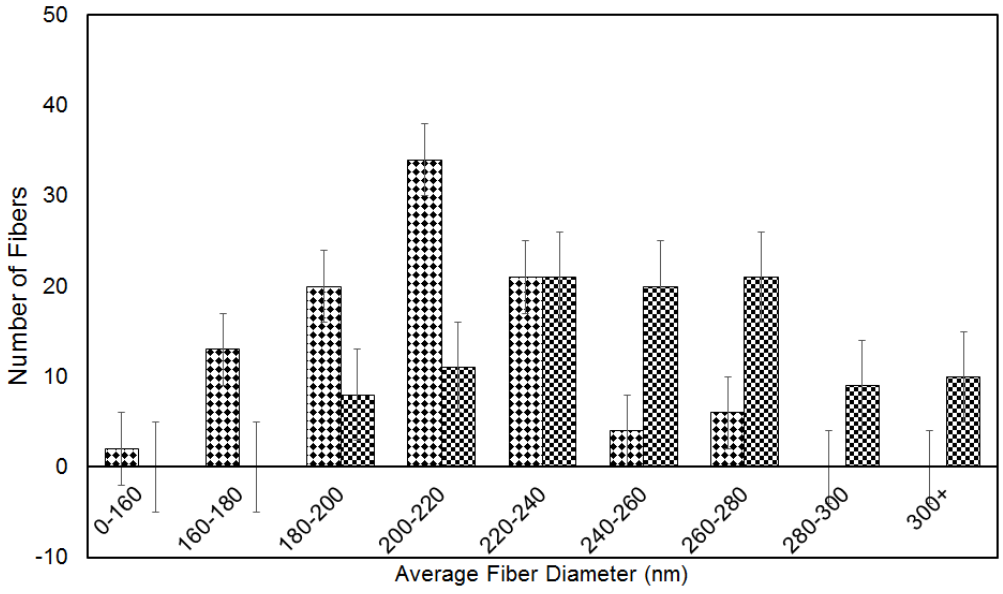
Figure 8. The effects of (⊠) : pH 10 and (▩) : pH 12 on diameter distribution of nanofibers formulation with PEO: LF of (A) 3.5:1 and (B) 3.5:2

3.3. Effect of the lentil flour concentration on solution characteristics and fiber morphology

Shear thinning behavior was seen for both 1% and 2% lentil flour containing solutions at different pH values since n values were smaller than 1. It was observed that *K* values increased significantly with the increase in lentil flour concentration at the same pH value (Table 2). In other words, it was clearly seen that when the lentil flour concentration increased from 1% to 2% at the same pH level, the viscosity of the solution increased. Kriegel et al. (2008) stated that most of the polymers had a tendency to make hydrogen bonding. Therefore, when the polymer concentration increased, the number of hydrogen bonds was expected to increase. Especially carbohydrates, had significant effect on viscosity of the solutions due to their high bonding capacities. Lentil flour is mainly composed of natural polymers which are proteins and carbohydrates. In fact, the amount of carbohydrates are almost three times more than the amount of protein in lentil flour. Thus, increasing in solution viscosity when the lentil flour concentration increased was expected due to increase in the total polymer concentration of the solution. According to the literature, polymer concentration and the viscosity of the solution were found to be directly correlated (Kriegel et al., 2008; Vega-Lugo & Lim, 2012). Fong et al. (1999) showed that viscosity of solution increased as PEO (MW 900kDa) concentration increased from 1% to 4.5%. The lowest viscosity value was 0.013 Pa.s whereas the highest one was 1.84 Pa.s. Uyar & Besenbacher (2009) incorporated three different types of cyclodextrins (α, β, and γ) in PEO. Viscosity was measured for different PEO concentrations combined with different concentrations and types of cyclodextrins. Viscosities ranged between 0.523 and 1.180 Pa.s. Addition of proteins into the electrospinning solutions was more challenging than carbohydrates; consequently, the number of studies increased in recent years. In a study, PEO (MW 600 kDa) and WPI were combined by using water as solvent (Sullivan et al., 2014). Viscosity values were increased with increase in WPI concentration and ranged between 0.34 and 2.33 Pa.s. Colín-Orozco et al. (2015) studied the effects of WPI and PEO (MW 300kDa) on viscosity as well. The highest viscosity was obtained as 9.233 Pa.s for

PEO: WPI ratio of 100:0 whereas the lowest one was obtained as 0.076 Pa.s for PEO: WPI ratio of 0:100. In this study, viscosity values measured at 50 s^{-1} shear rate were ranged between 0.980 and 1.680 Pa.s for different lentil flour concentrations and pH values (Table 2). The viscosity results of this study were in a reasonable range when it was compared to other studies. When the studies in the literature were compared, different viscosity ranges were determined in each study. One of the reason of these different ranges might be using PEO with different molecular weight. It can be concluded that the addition of both proteins and carbohydrates in solution increase viscosity of the solution. Also, increasing the amount of the biopolymers, which can be either protein or carbohydrate, causes an increase in the viscosity as well. Also, the type of the biopolymer is important as well. For instance, addition of carbohydrates increases viscosity of the solutions more than proteins due to their higher water binding capacities.

The increase in lentil flour concentration did not change electrical conductivity of solution significantly (Table 2, Table A3). The reason of that could be explained by the decrease in mobility of ions due to increase in viscosity with increase in lentil flour concentration (Sekhon, 2003). Colín-Orozco et al. (2015) and Sullivan et al. (2014) reported electrical conductivity ranges between 0.078-0.248 (mS/cm) and 0.1-3.4 (mS/cm) for PEO and WPI blends, respectively. For lentil flour, electrical conductivity of the solutions ranged between 0.369 and 1.255 (mS/cm) which was in accordance with other studies. In the literature, several studies reported that the increase in polymer concentration did not cause an increase in electrical conductivity. Tort & Acartürk (2016) reported that when PEO concentration was increased, electrical conductivity of the solutions did not change significantly. In a similar study, Vega-Lugo & Lim (2012) also reported no significant change of conductivity with increase in PEO concentration.



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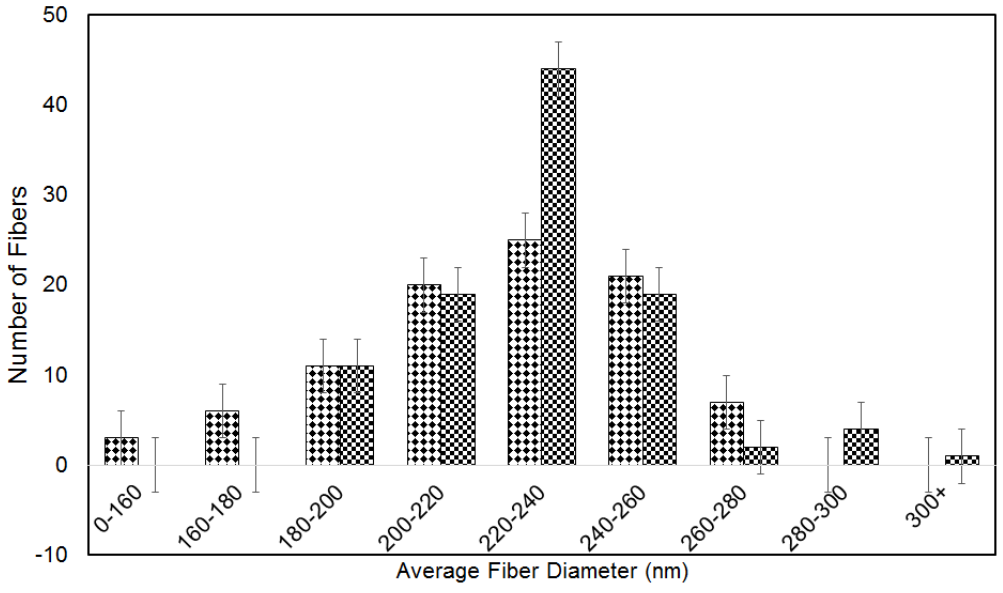

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Figure 9. The effects of lentil flour (▚: 3.5:1 (PEO: LF) and ▚: 3.5:2 (PEO: LF)) on diameter distribution of nanofibers formulation at (A) pH 10 and (B) pH 12

The results of fiber diameter showed that with the increase in lentil flour concentration, the average diameter of the nanofibers increased from 210±4 to 254±5 nm at pH 10 (Table 2, Fig. 7C, 7D). On the other hand, no significant difference was observed at pH 12 in terms of lentil flour concentration (Fig. 7E, 7F). When the diameter distributions were examined, a wider distribution was obtained for the one with 2% lentil flour concentration as compared to the one with 1% lentil flour concentration at pH 10 (Fig. 9A). The opposite trend was obtained for pH 12. In other words, most of the diameter results were concentrated around 220-240 nm, which was the average diameter, for the solution containing 2% lentil flour while a wider distribution was observed for solution containing 1% lentil flour at pH 12 (Fig. 9B). There are many studies showing that increasing the protein content in the solution resulted an increase in fiber diameter. Cho et al. (2010) prepared SPI and PVA blends with 9, 11 and 13 wt% concentrations. When the SPI concentration was increased, the average nanofiber diameters increased from 0.6±0.2 to 4.5±1.5 μm, which was explained by increasing the viscosity of the solutions. In another research, Ramji & Shah (2014) reported similar results as well. Four different solutions were prepared with 5, 7, 10 and 12% SPI concentrations and 5% PEO. The average diameter increased from 30 to 90nm when the SPI concentration increased. Increasing the carbohydrate concentrations in the solution also led to an increase in the fiber diameter. In another research with the increase in the cyclodextrin concentrations from 25% to 50% in the solutions fiber diameters increased (Uyar & Besenbacher, 2009). The ranges of the diameters increased from 95-255, 110-200 and 110-203 nm to 140-180, 105-210 and 120-240 nm for α, β and γ-cyclodextrins, respectively. Fiber diameter varied from one study to another, since different types of proteins and carbohydrates were used in each study. Similar to the most of the researches, the diameter of the nanofibers increased with increasing lentil flour concentration at pH 10 (Table 2). The reason could be explained by the significant increase in the viscosity. However, at pH 12, the fiber diameter did not show significant difference with increasing lentil flour concentration even though viscosity of the solution increased. However, conductivity values were almost twofold for pH value of 12 as

compared to pH value of 10. It was stated before that high electrical conductivity could cause a decrease in the fiber diameter. Thus, while the diameters of the fibers increased with increase in viscosity, they decreased with increase in conductivity. This might be the most probable reason for the nonsignificant diameter change observed at pH 12.

3.4. Effect of the HPMC concentration on solution characteristics and fiber morphology

Table 3 and Figure 10 show rheological properties (*K* and n) of spinning solutions for different HPMC concentrations. Apparent viscosity of the solutions measured at 50 s^{-1} shear rate, significantly increased with increase in HPMC concentration. The highest viscosity values, which were 1.671 and 2.085 Pa.s, were obtained at the highest HPMC concentration (1%) for both 1% and 2% lentil flour concentrations, respectively. This was due to the fact that HPMC was a cellulose based molecule which had many hydroxyl groups on it. These hydroxyl groups increased water binding capacity of the HPMC which caused an increase in the viscosity of the solution (Lim et al., 2010). Frenot et al. (2007) reported that solution containing 2.86% HPMC could not be spinned due to its high viscosity. When the HPMC concentration was decreased to 2.14%, a spinnable solution with lower viscosity was obtained. These results were in agreement with the results of Cheong et al. (1992), where they reported an increase in viscosity for higher HPMC concentrations. Lim et al. (2010) also showed that the solution viscosity increased from 1.284 to 8.614 (Pa.s) when the HPMC concentration was increased from 0.5% to 1.0%, respectively.

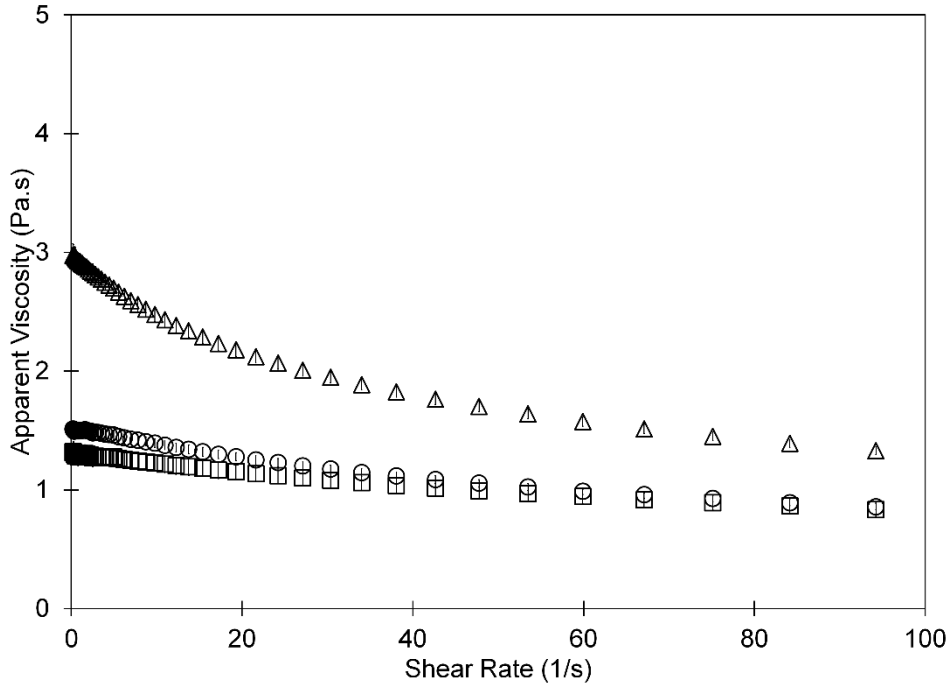
Table 3. Effects of the HPMC concentration on solution characteristics and fiber diameter

Lentil flour concentration (%)	**HPMC concentration (%)**	***K*(Pa s^n)**	***n***	**R^2**	**Electrical conductivity (mS/cm)**	**Nanofiber diameter (nm)**	**Apparent Viscosity** (Pa.s)**
1	0.25	1.494±0.036^{de*}	0.937^{b}	0.997	0.595±0.005^{bc}	198±4^{c}	1.040^{d}
1	0.50	1.294±0.075^{e}	0.950^{a}	0.997	0.541±0.014^{bc}	210±4^{bc}	0.980^{d}
1	1.00	2.879±0.067^{b}	0.899^{d}	0.996	0.505±0.001^{c}	220±3^{b}	1.671^{b}
2	0.25	1.836±0.101^{d}	0.913^{c}	0.997	0.851±0.036^{a}	203±4^{c}	1.148^{d}
2	0.50	2.358±0.010^{c}	0.907^{c}	0.997	0.834±0.077^{a}	254±5^{a}	1.431^{c}
2	1.00	3.844±0.111^{a}	0.882^{e}	0.996	0.738±0.081^{ab}	242±4^{a}	2.085^{a}

All of the solutions were prepared at pH 10 by using %3.5 PEO and 2%Tween80 at the same electrospinning conditions

* Columns with different letters differ statistically ($p \leq 0.05$)

**Apparent viscosity values are measured at 50 s^{-1} shear rate



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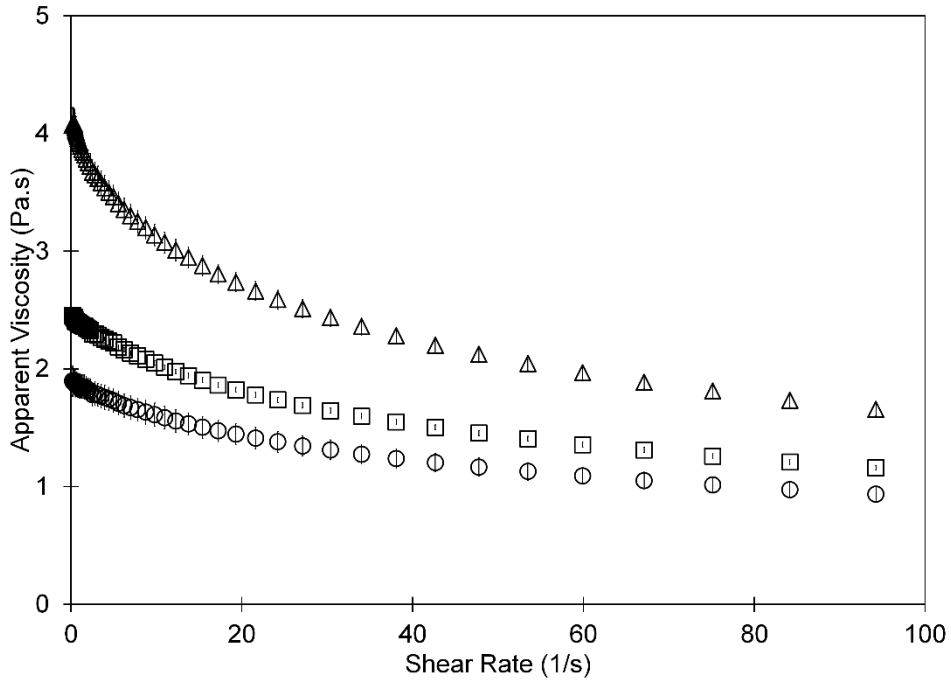


B

Figure 10. The effects of different HPMC concentrations (○: 0.25% HPMC, □: 0.5% HPMC, Δ: 1.0% HPMC) on apparent viscosity of spinning solutions PEO: LF ratio of (A) 3.5:1 and (B) 3.5:2

The electrical conductivity ranged between 0.505 and 0.595 mS/cm for solutions containing 1% lentil flour whereas the range was between 0.738 and 0.851 mS/cm for solutions prepared with 2% lentil flour (Table 3). Since HPMC is a cellulose derivative, it cannot be electrically charged by changing the pH of the solution. Therefore, increasing HMPC concentration of the solutions did not create a significant change in electrical conductivities for both solutions containing 1% and 2% lentil flour. Solutions with 2% lentil flour concentration had higher electrical conductivity values than solutions with 1% lentil flour concentration regardless of HPMC concentration. Having higher amount of charged particles (charged protein molecules) was the reason.

Bead-free nanofibers were obtained from all of the solutions with different HPMC concentrations (Fig. 11A, 11B, 7C, 7D, 11C, 11D). Increasing the HPMC concentration affected the fiber diameters and diameter distributions. HPMC, which is a derivative of the cellulose, is a polymer itself. Therefore, when the concentration of HPMC was increased, viscosity also increased. Thus, it was not surprising to observe a larger fiber diameter with increase in HPMC concentration whatever the lentil flour concentration was (Table 3). Solutions with 1% lentil flour resulted in wider diameter distributions for each HPMC concentrations. For the solutions contain 2% lentil flour with 0.25% HPMC concentration, the frequency of the fibers was the highest for the mean diameter around 200-220 nm. With increasing HPMC concentration, the variation of diameter became higher (Fig. 12A, 12B). These results were in agreement with Beachley & Wen (2009) when polycaprolactone (PCL) was used as a polymer in their electrospinning study. The diameter of nanofiber was reported to be between 350 nm to 1 μm. When the polymer concentration was increased from 8% to 20%, fiber diameters showed significant variation. Increasing the diameter was explained by increasing the viscosity of the solution similar to our study. Frenot et al. (2007) studied two different types of HPMC, which had different methoxy contents. Average diameters were measured as 128 and 127 nm for HPMC types used in the study. Since HPMC was mixed with both lentil flour and PEO, larger diameters were obtained in our study.

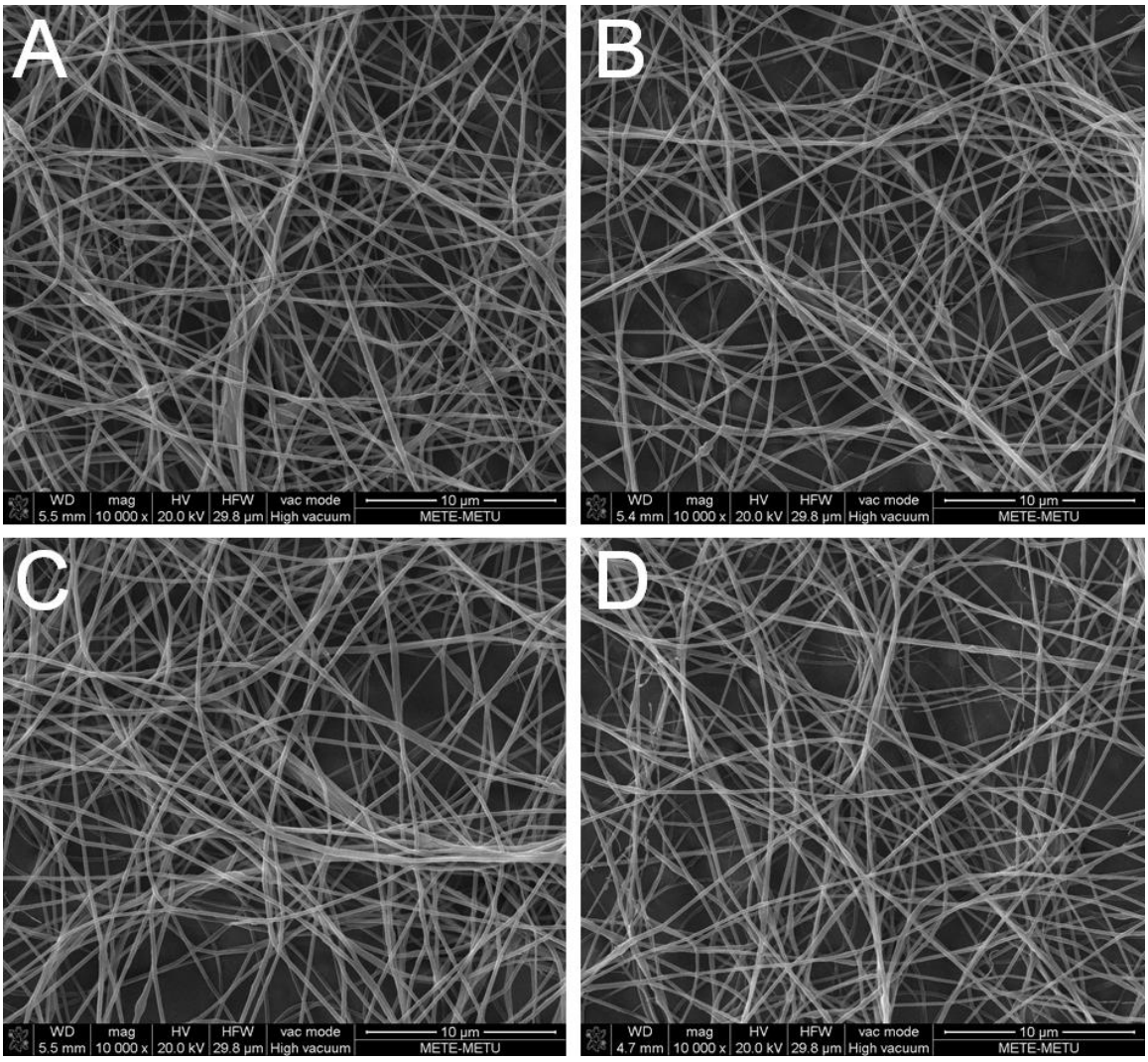
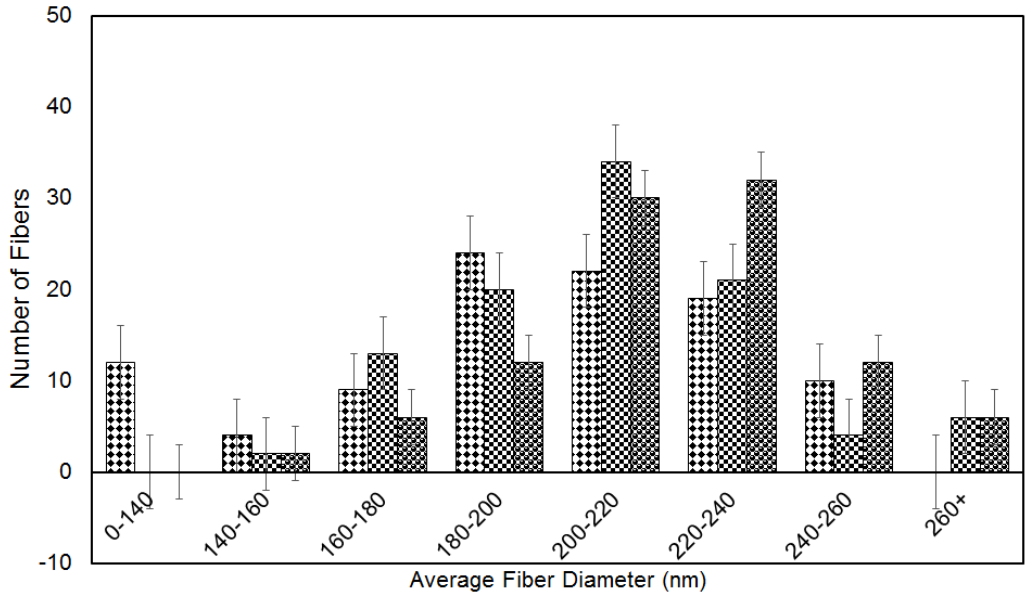


Figure 11. SEM images of different nanofiber formulations (A) PEO: LF ratio of 3.5:1 and 0.25% HPMC, (B) PEO: LF ratio of 3.5:2 and 0.25% HPMC, (C) PEO: LF ratio of 3.5:1 and 1% HPMC, and (D) PEO: LF ratio of 3.5:2 and 1% HPMC



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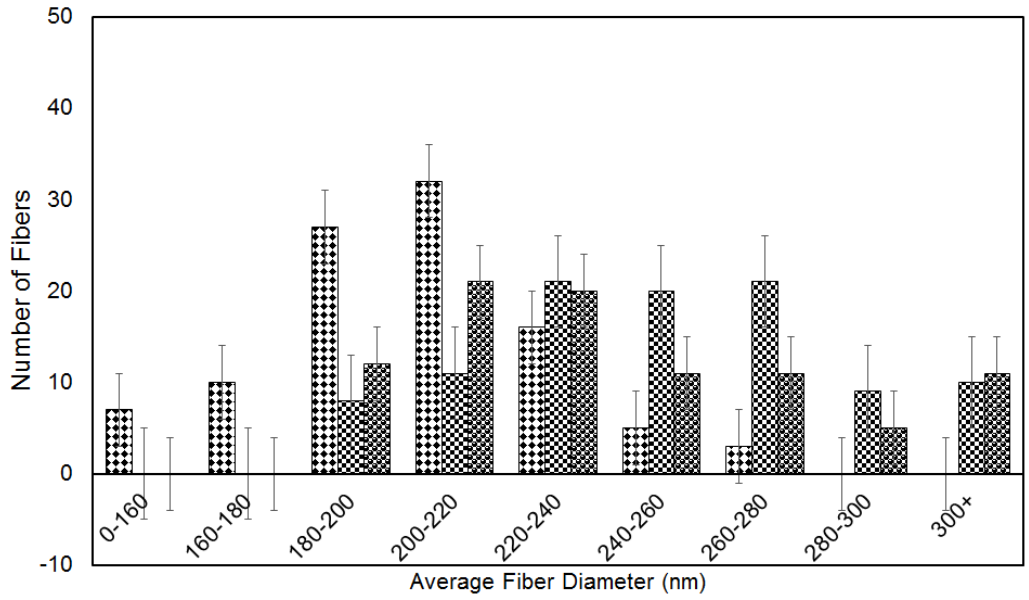

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Figure 12. The effects of HPMC (▩: 0.25% HPMC, ▩: 0.5% HPMC, and ▩: 1% HPMC) on diameter distribution of nanofibers formulation with PEO: LF of (A) 3.5:1 and (B) 3.5:2

3.5. Effects of microfluidization on solution properties and nanofiber characteristics

Preparation of a homogeneous solution is very important factor for electrospinning process. Therefore, a homogenization technique, microfluidization, was applied to the electrospinning solutions in order to increase the solubility. Microfluidization was performed at 100 MPa with 3 and 5 pass numbers. Two different solutions were prepared for this process. The first one was 3.5% PEO and 5.25% lentil flour containing solution and the second one was 2.5% PEO and 7.5% lentil flour containing one. Solutions containing the highest lentil flour concentration were chosen. The reason of that could be explained as microfluidization was an intermediary step. It was applied to the solutions contained lentil flour, HPMC and Tween 80. PEO concentration could not be fixed because each solution had unique proportions in order to be suitable for electrospinning process. Table 4-7 showed the effect of microfluidization on rheological characteristics and electrical conductivity of solutions and the morphology of fibers.

Table 4. Effect of microfluidization on the morphology of fibers and rheological characteristics and electrical conductivity of solutions prepared by using 3.5% PEO, 5.25% Lentil Flour, 0.5% HPMC and 2% Tween80

Pass Number	**Electrical Conductivity (µS/cm)**	***K* (Pa s^n)**	**n**	**Nanofiber Morphology**
0	1614^{a***}	3.40^b	0.83^a	HNF*
3	1083^b	10.06^a	0.61^b	BF**
5	1230^b	9.41^a	0.64^b	BF

*HNF means homogenous nanofiber

**BF means bead formation

***Columns with different letters differ statistically ($p \leq 0.05$)

Microfluidization was performed at 100 MPa

Electrospinning conditions were 30 cm distance, 0.6 mL/h flow rate and 10-15 kV voltage

Table 5. Effect of microfluidization on the morphology of fibers and rheological characteristics and electrical conductivity of solutions prepared by using 2.5% PEO, 7.5% Lentil Flour, 0.5% HPMC and 2% Tween80

Pass Number	**Electrical Conductivity (µS/cm)**	***K* (Pa s^n)**	**n**	**Nanofiber Morphology**
0	2390^{a***}	8.37^b	0.71^a	HNF*
3	1763^b	12.29^a	0.64^c	BF**
5	1722^c	12.50^a	0.65^b	BF

*HNF means homogenous nanofiber

**BF means bead formation

***Columns with different letters differ statistically ($p \leq 0.05$)

Microfluidization was performed at 100 MPa

Electrospinning conditions were 23-30 cm distance, 0.8-1.5 ml/h flow rate and 8-20 kV voltage

As it was shown in the Tables 4-7, when microfluidization process was applied to the solutions, the consistency indices of solutions increased significantly. Vega Lugo and Lim (2012) stated that globular structure of proteins decreased the interaction between polymeric substances and proteins in the electrospinning solutions. Lentil flour used in electrospinning solutions contains 22.2% protein. Floury, Desrumaux, & Legrand (2002) explained that during microfluidization process, strong mechanical forces were applied to the electrospinning solution. Because of these strong mechanical forces, temperature of the solutions increased which led to an increase in the protein denaturation rate. When denaturation rate increased, protein molecules in the electrospinning solution increased. Therefore, PEO molecules and protein molecules started to make more bonds with each other. Similar to Vega Lugo and Lim (2012), viscosity of the electrospinning solutions increased due to increase in these bonds between PEO and protein molecules. This result was similar to the results found in literature. Kie, Kruk, Czerniewicz, Warmi*ƒ*ska, & Haponiuk (2003) studied milk homogenization. Viscosity of the control sample was reported as 1.80 mPa.s whereas viscosity of the samples, which were homogenized in the range of 20 MPa to 140 MPa, varied from 1.86 to 1.96 mPa.s. In other words, the viscosity of milk increased when homogenization process was applied. Moreover, there were many studies related to tomato processing, which showed that the viscosity of tomato products increased with homogenization (Bayod & Tornberg, 2011; Den Ouden, van VLIET, Sciences, & Box, 2002; Lopez-Sanchez et al., 2011).

Table 6. Effect of microfluidization on the morphology of fibers and rheological characteristics and electrical conductivity of solutions prepared by using 3.5% PEO, 5.25% Lentil Flour and 2% Tween80

Pass Number	Electrical Conductivity (µS/cm)	*K* (Pa s^n)	n	Nanofiber Morphology
0	1672^{a***}	0.75^{b}	0.88^{a}	HNF^{*}
3	1697^{a}	5.40^{a}	0.64^{b}	BF^{**}
5	1711^{a}	5.04^{a}	0.65^{b}	BF

* HNF means homogenous nanofiber

**BF means bead formation

***Columns with different letters differ statistically ($p \leq 0.05$)

Microfluidization was performed at 100 MPa

Electrospinning conditions were 30 cm distance, 0.6 mL/h flow rate and 10-15 kV voltage

Table 7. Effect of microfluidization on the morphology of fibers and rheological characteristics and electrical conductivity of solutions prepared by using 2.5% PEO, 7.5% Lentil Flour and 2% Tween80

Pass Number	Electrical Conductivity (µS/cm)	*K* (Pa s^n)	n	Nanofiber Morphology
0	1973^{c***}	2.93^{c}	0.88^{a}	HNF^{*}
3	2080^{a}	8.71^{b}	0.70^{b}	BF^{**}
5	2060^{b}	12.25^{a}	0.67^{b}	BF

*HNF means homogenous nanofiber

**BF means bead formation

***Columns with different letters differ statistically ($p \leq 0.05$)

Microfluidization was performed at 100 MPa

Electrospinning conditions were 23-30 cm distance, 0.8-1.5 mL/h flow rate and 8-20 kV voltage

Electrical conductivities of solutions showed different trends for different solutions and different microfluidization pass numbers. As it was stated before, strong mechanical forces and increasing the solution temperature could cause an increase in protein denaturation (Floury et al., 2002). It is known that proteins are charged particle. When the protein denaturation increased, electrical conductivity was expected to increase as well. This case was observed only in< Table 7 for pass number 3. In other cases, either no significant change or a decrease in electrical conductivity of solutions were observed. The reason of that can be explained in previous parts. Sekhon (2003) stated that increasing of the viscosity caused decreasing of the mobility of ions in the electrospinning solution.

Morphology of nanofibers showed that microfluidization did not have a positive effect on obtaining homogeneous nanofibers from lentil flour and HPMC based electrospinning solutions (Fig. 13-16). Microfluidization resulted in fibers with beads. Many studies showed that electrospinning solutions must be in an optimum viscosity and electrical conductivity ranges in order to obtain homogeneous nanofibers. As it was discussed in previous parts, at very high or low viscosity and electrical conductivity values, obtaining homogeneous nanofibers was not possible. When microfluidization process was applied, viscosity and electrical conductivity values must have been shifted out their optimum ranges. Their combined effects on electrospinning process also affected formation of homogeneous nanofibers.

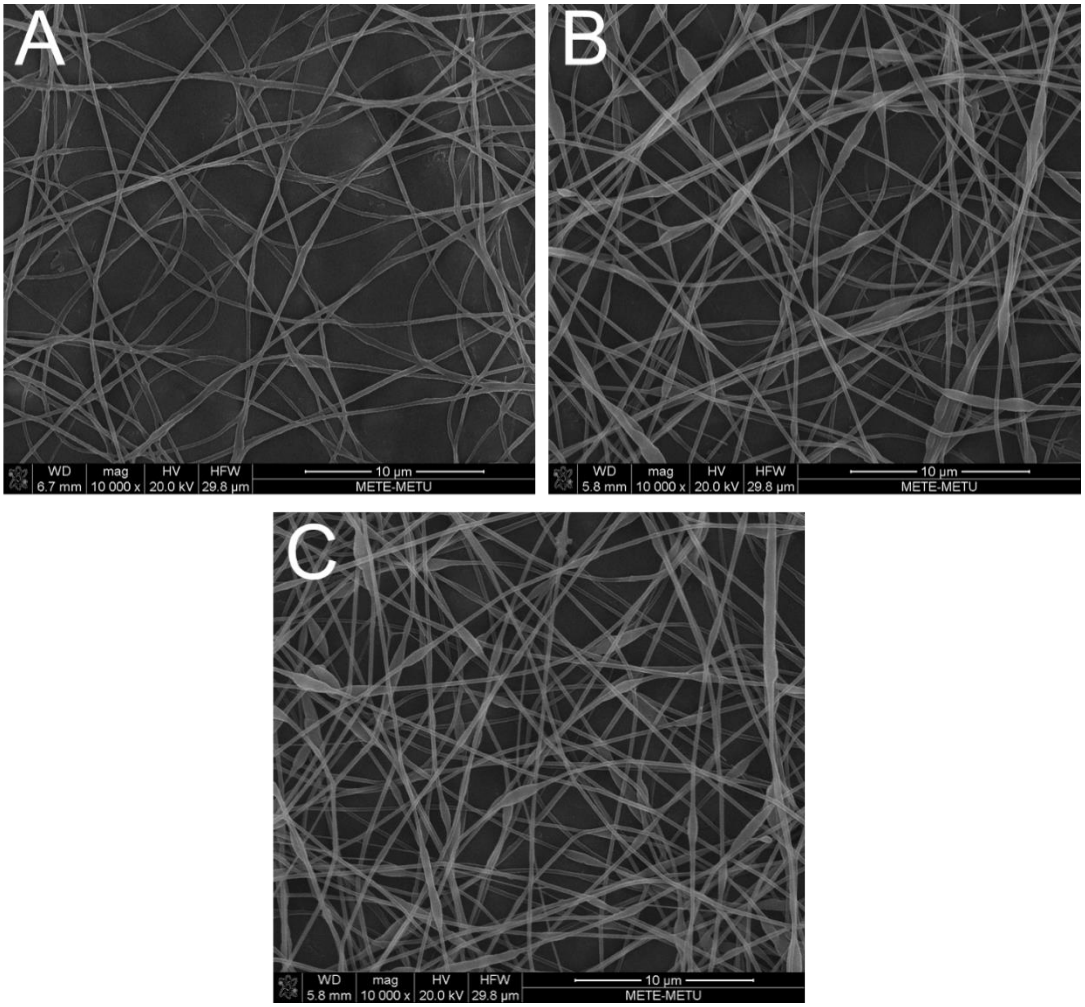

Figure 13. SEM images of nanofibers prepared with PEO: LF ratio of 3.5:5.25 and 0.5% HPMC at different microfluidization pass numbers (A) 0 pass, (B) 3 pass and (C) 5 pass

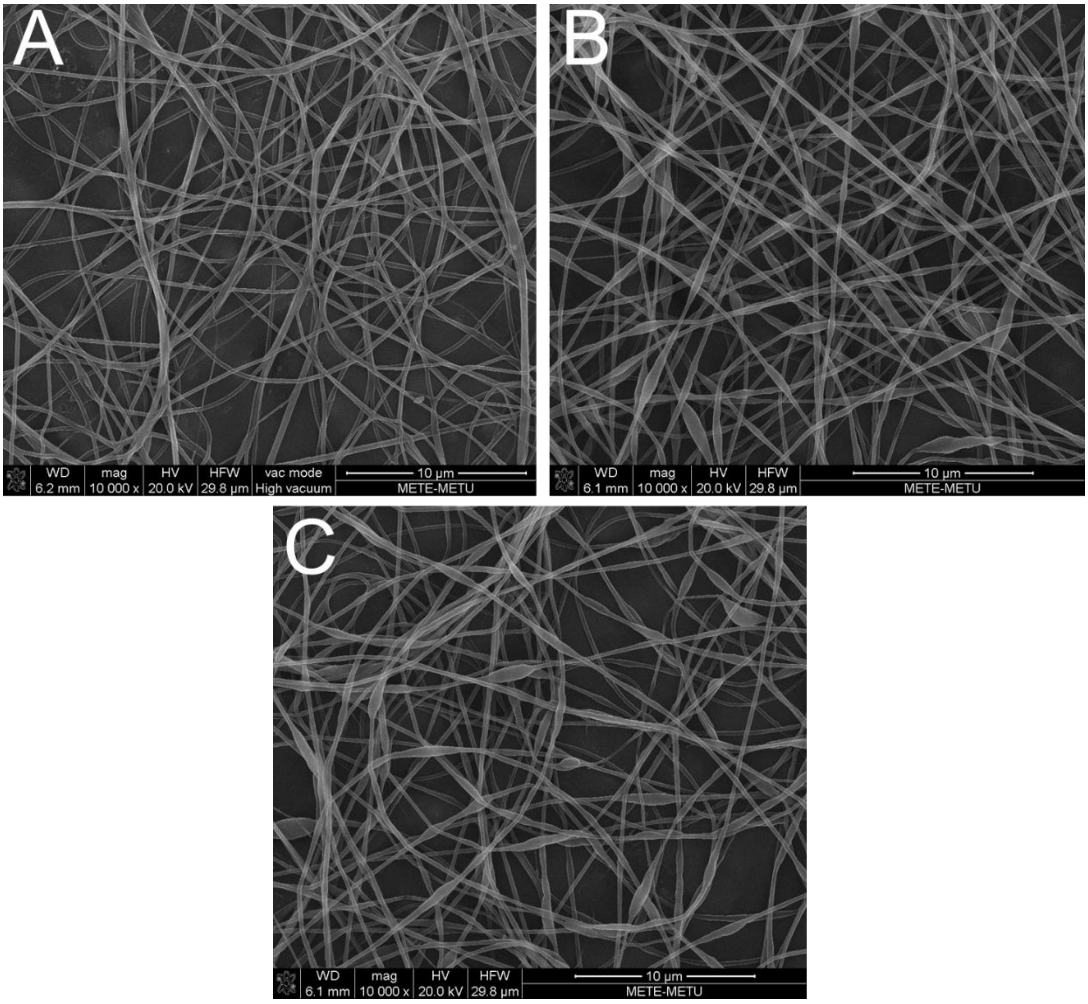

Figure 14. SEM images of nanofibers prepared with PEO: LF ratio of 2.5:7.5 and 0.5% HPMC at different microfluidization pass numbers (A) 0 pass, (B) 3 pass and (C) 5 pass

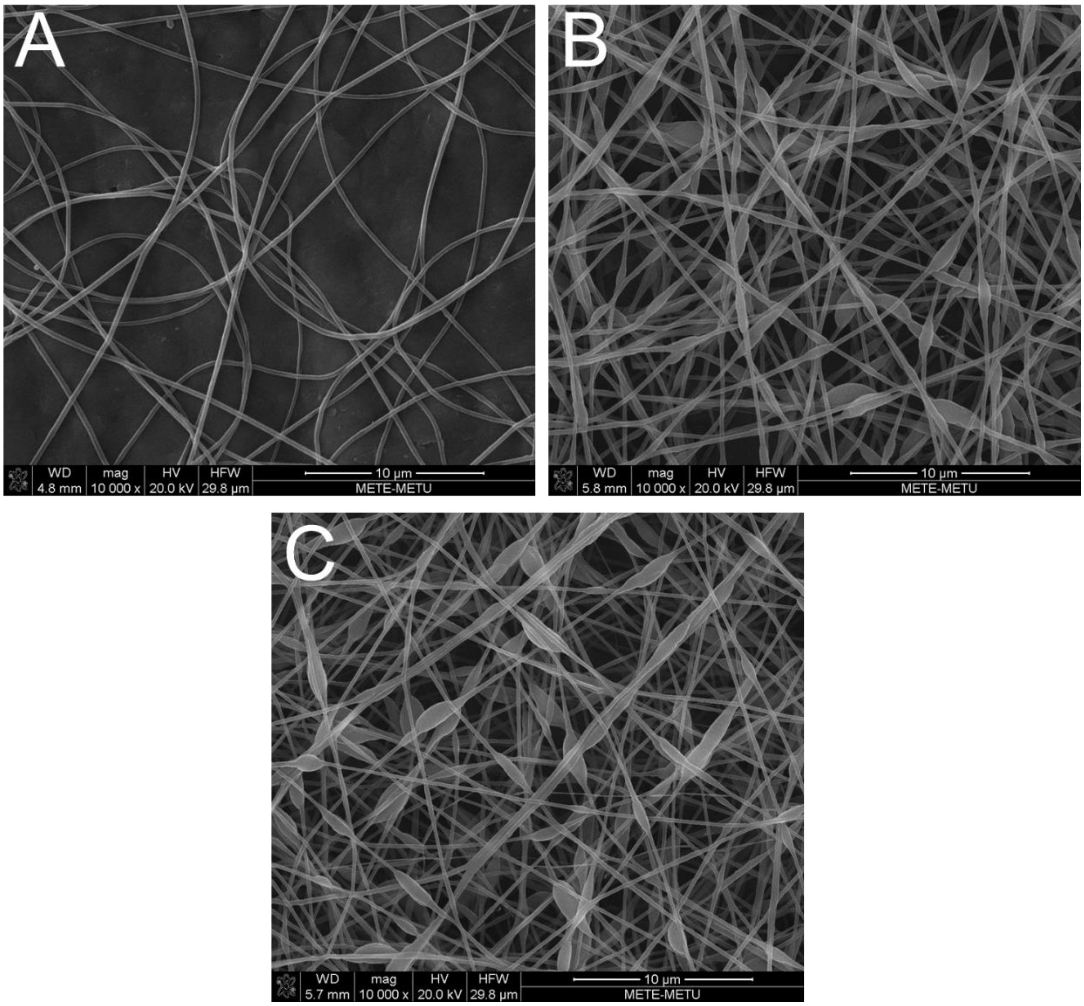

Figure 15. SEM images of nanofibers prepared with PEO: LF ratio of 3.5:5.25 at different microfluidization pass numbers (A) 0 pass, (B) 3 pass and (C) 5 pass

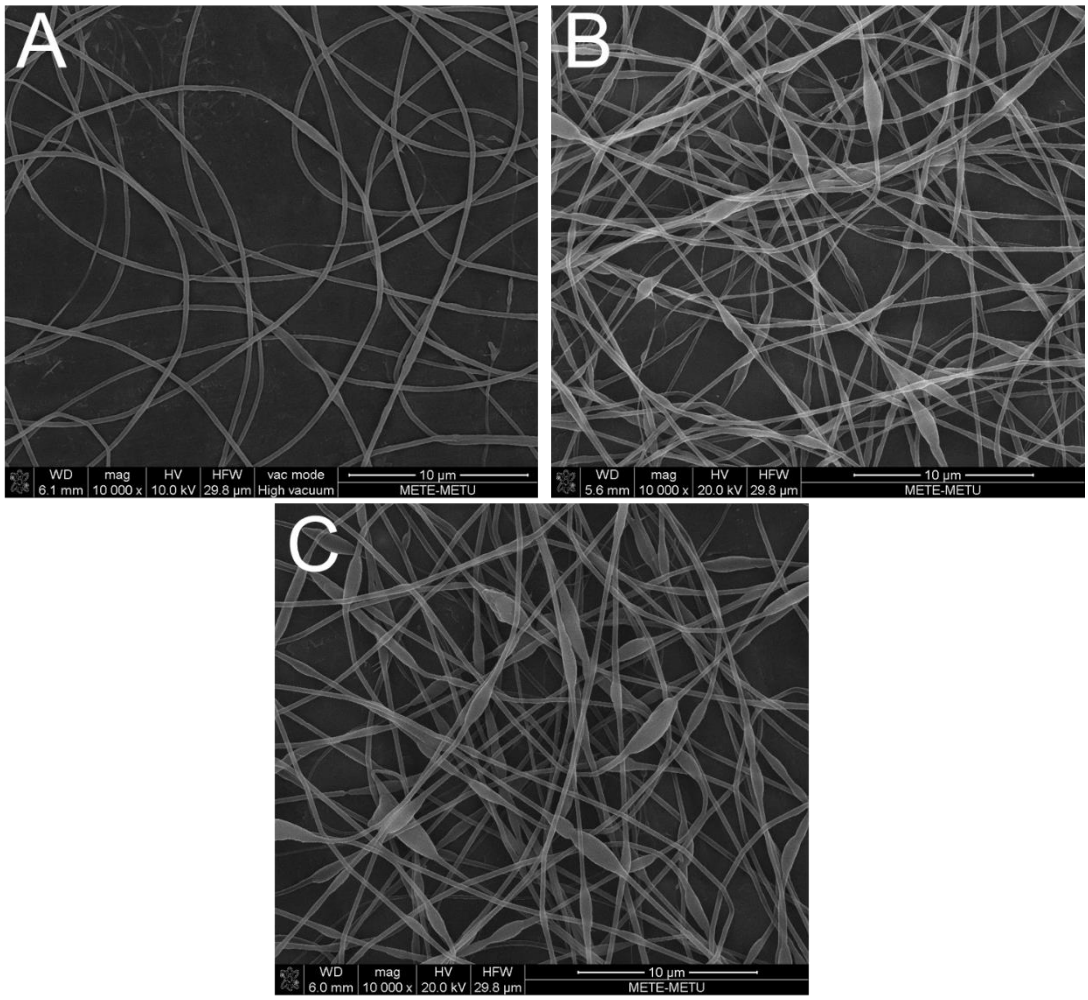

Figure 16. SEM images of nanofibers prepared with PEO: LF ratio of 2.5:7.5 at different microfluidization pass numbers (A) 0 pass, (B) 3 pass and (C) 5 pass

3.6. Effects of electrospinning conditions on fiber morphology

In the literature, there were many studies related to the effects of electrospinning conditions, which were applied voltage, flow rate and distance between the collector and the tip of the needle. Li and Wang (2013) stated that electrospinning conditions were very important for obtaining homogeneous nanofibers. In order to obtain nanofibers with the desired morphology and diameter, electrospinning conditions must be controlled. In this study, distance between collector and tip of the needle were fixed to 30 cm while the effects of voltage and flow rate were examined. Two different voltage and flow rate values were selected as 7 and 10 kV and 0.6 and 1.0 mL/h, respectively.

3.6.1. Effect of voltage on fiber morphology

Reneker et al. (2000) stated that in order to start the flow of the charged solution, sufficient voltage must be applied. The reason of that was explained as surface tension of the solution must be overcome in order to initiate the flow and create a Taylor cone.

For each concentration (1.5% and 2%) and voltages (7 kV and 10 kV), homogeneous nanofibers were obtained (Fig. 17). As it can be seen in Table 8, increasing the voltage did not have a significant effect on the diameter of nanofibers for solutions containing 1.5% lentil flour. Similarly, Reneker & Chun (1996) studied on PEO based nanofibers. It was reported that there was no significant effect of the applied voltage on nanofiber diameter. For the solutions containing 2% lentil flour, nanofiber diameter increased with increasing applied voltage. Similar to that Zhang et al. (2005) obtained poly (vinyl alcohol) PVA and water-based nanofibers and reported an increase in the nanofiber diameter with the increasing voltage. In addition to that high voltage reduced the time that charged jets needed to reach the collector, which caused an increase in diameter of nanofibers (De Schoenmaker, Van Der Schueren, Ceylan, & De Clerck, 2012).

Table 8. Effects of voltage on diameter of nanofibers

Lentil Flour Concentration (%)	Voltage (kV)	Diameter (nm)
1.5	7	220 ± 4^{b}
1.5	10	$230 \pm 3^{b*}$
2.0	7	203 ± 3^{c}
2.0	10	254 ± 5^{a}

* Columns with different letters differ statistically ($p \leq 0.05$)

All of the solutions were prepared at pH 10 by using 3.5% PEO, 0.5% HPMC and 2% Tween80

Electrospinning conditions were 30 cm distance and 0.6 mL/h flow rate

There were many studies with different outcomes in the literature. Many researchers were agreed that the effect of applied voltage was unpredictable. All the other parameters must be taken into consideration as well (Li & Wang, 2013; Pham et al., 2006).

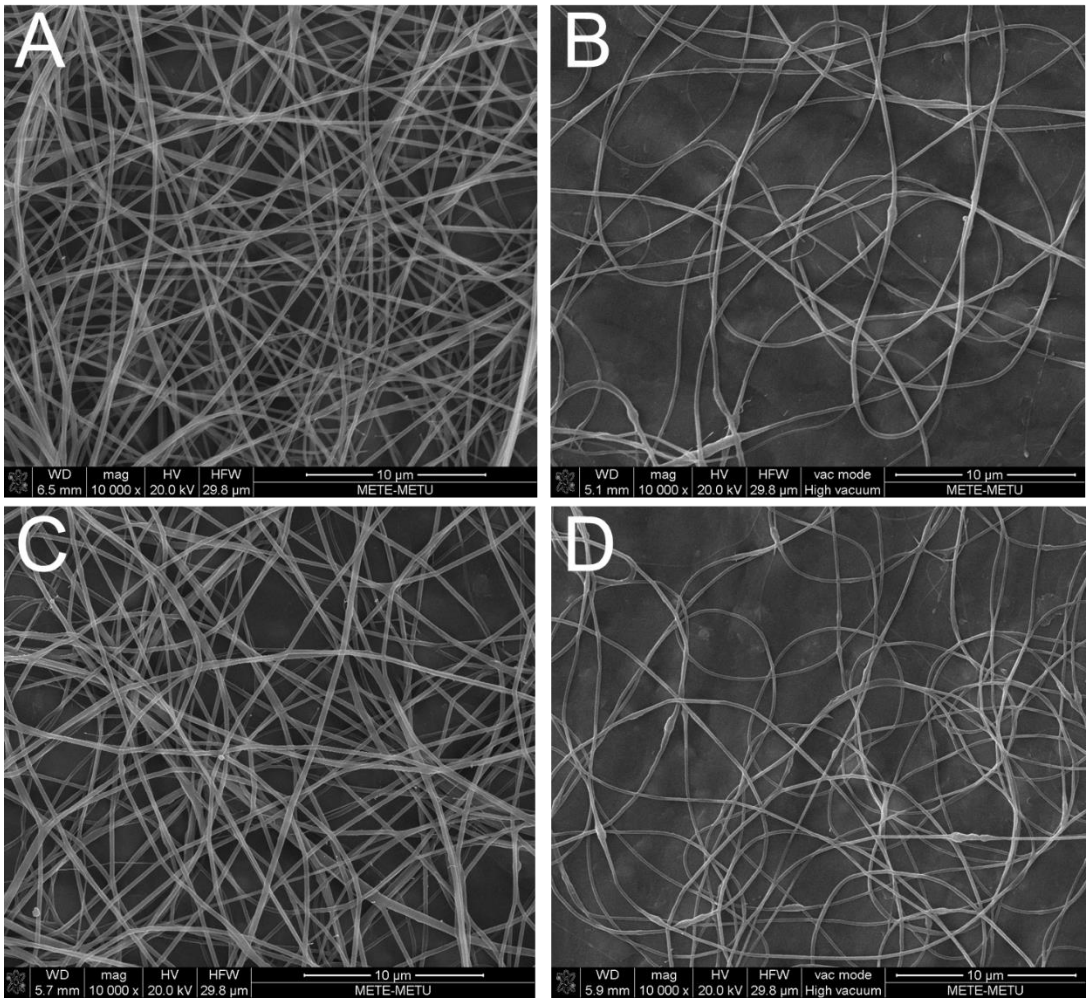

Figure 17. SEM images of nanofiber prepared at same flow rates and different applied voltages (A) 1.5% LF at 10 kV, (B) 1.5% LF at 7 kV, (C) 2% LF at 10 kV, and (D) 2% LF at 7 kV

3.6.2. Effect of flow rate on fiber morphology

Haider et al. (2015) stated that homogeneous nanofibers can be obtained at a critical flow rate value. This critical value depends on the composition of the electrospinning solution. Using lower flow rates was generally recommended. Yuan et al. (2004) explained that evaporation during the electrospinning process would be much more

efficient, which could prevent bead formation. Li and Wang (2013) also stated that obtaining bead free homogeneous nanofibers was easier at lower flow rates.

In this study, when flow rate was changed, homogeneous nanofibers could be obtained (Fig. 18). As it can be seen in Table 9, increasing flow rate did not have a significant effect on diameter of nanofibers for solutions containing 1.5% lentil flour.

De Schoenmaker et al. (2012) also studied the effects of flow rate. They reported that diameter of nanofibers first increased then decreased with increase in the flow rate. It was explained by the fact that higher amount of solution volume at the tip of the needle caused an increase in nanofiber diameter. After that with the increase in flow rate, amount of charges increased, which limited the increase in the diameter of nanofibers. Overall, it was reported that flow rate did not have significant effect on diameter of nanofiber.

Table 9. Effects of flow rate on diameter of nanofibers

Lentil Flour Concentration (%)	**Flow Rate (mL/h)**	**Diameter (nm)**
1.5	0.6	$230 \pm 3^{b*}$
1.5	1.0	221 ± 4^{b}
2.0	0.6	254 ± 5^{a}
2.0	1.0	203 ± 5^{c}

***** Columns with different letters differ statistically ($p \leq 0.05$)

All of the solutions were prepared at pH 10 by using 3.5% PEO, 0.5% HPMC and 2% Tween80

Electrospinning conditions were 30 cm distance and 10 kV voltage

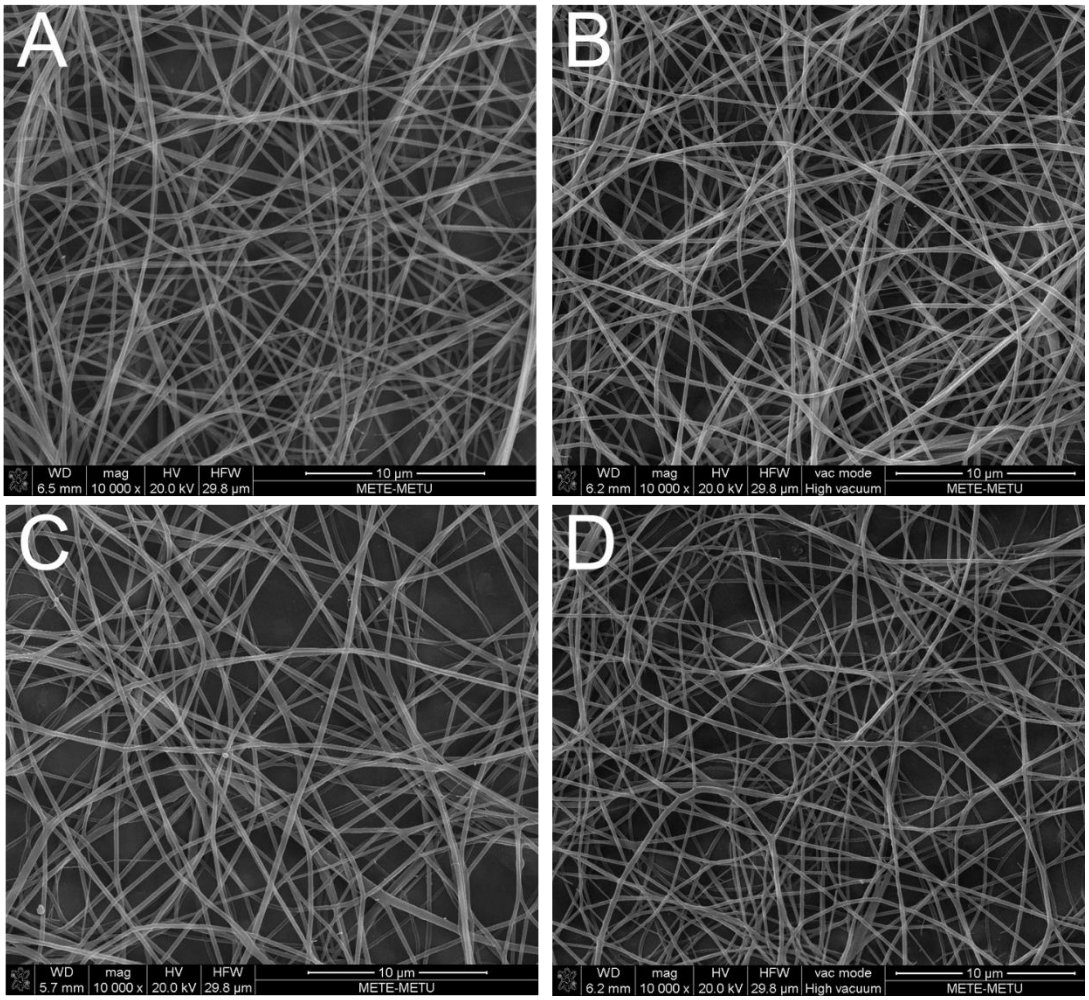

Figure 18. SEM images of nanofiber prepared at same applied voltage and different flow rates (A) 1.5% LF at 0.6 mL/h, (B) 1.5% LF at 1.0 mL/h, (C) 2% LF at 0.6 mL/h, and (D) 2% LF at 1.0 mL/h

When flow rate was increased, diameter of nanofibers showed a decrease for solutions containing 2% lentil flour (Table 9). Adabi, Saber, Faridi-Majidi, & Faridbod (2015) studied with polyacrylonitrile-based carbon nanofibers and reported a decrease in nanofiber diameter with increase in flow rate. Faridi-Majidi et al. (2012) explained that electrospinning solution on the tip of the needle evaporated less when flow rate increased. In this way, solution concentration decreased on the tip of the needle, which caused a decrease in diameter of nanofibers.

3.7. Effect of lentil flour and HPMC concentrations on characteristics of nanofibers

Due to their high mechanical properties, nanofibers obtained from electrospinning process can be used as food packaging material in the future studies. Thus, permeability properties of these nanofibers become an important parameter. Appearance of this material is also important when consumer preferences are taken into consideration. Therefore, color of the nanofibers obtained from electrospinning was measured.

In this study, solutions with 2% and 5.25% lentil flour were chosen in order to measure water vapor permeability and color of electrospun nanofibers. PEO concentration was selected as 3.5%. The efficiency of nanofiber collection on aluminum foil and proper separation of these nanofibers from the surface of the aluminum were considered while these concentrations were chosen.

3.7.1. Effect of lentil flour and HPMC concentrations on water vapor permeability of nanofibers

As it can be seen in Table 10, water permeability values of electrospun nanofibers ranged between 11.35×10^{-13} and 15.61×10^{-13} g/m.s.Pa. Water vapor permeability results of edible films obtained from lentil flour was reported as 3.1×10^{-10} g/m.s.Pa (Bamdad, Goli, & Kadivar, 2006). In another similar study, water vapor permeability range of HPMC and PEO based nanofibers was reported as 7.4×10^{-11} - 12×10^{-11} g/m.s.Pa (Aydogdu, Sumnu, & Sahin, 2018).

When lentil flour concentration was increased from 2% to 5.25%, water vapor permeability of electrospun nanofibers showed a significant increase (Table 10). According to the literature, it is known that increasing the hydrophilic compounds generally increases water vapor permeability (McHugh & Krochta, 1994). In many studies, it was stated that proteins increased water vapor permeability due to its

hydrophilic nature (Atarés, Bonilla, & Chiralt, 2010; McHugh, 2000). Fabra, Lopez-Rubio, & Lagaron, (2013) also stated that proteins have high water uptake capacity which may cause an increase in the water vapor permeability of films.

Table 10. Effect of lentil flour and HPMC concentrations on water vapor permeability of nanofibers

Concentration (%)		**Water Vapor Permeability**
Lentil Flour	**HPMC**	**(g/m.s.Pa)*10^{13}**
2.00	0	$11.35 \pm 0.2^{b*}$
2.00	0.5	11.63 ± 0.2^{b}
5.25	0	15.61 ± 1.0^{a}
5.25	0.5	13.98 ± 0.2^{ab}

* Columns with different letters differ statistically ($p \leq 0.05$)

All of the solutions were prepared at pH 10 by using 3.5% PEO and 2% Tween80

Electrospinning conditions were 20-30 cm distance, 8-15 kV voltage and 0.8 mL/h flow rate

There was no significant change in water vapor permeability results when HPMC was added into the electrospinning solutions (Table 10). It is already known that HPMC is a cellulose derivative. Möller, Grelier, Pardon, & Coma (2004) reported that cellulose derivatives swell when there is an interaction with water as the nature of polysaccharides. Swelling with water leads to poor vapor barrier property. De Moura, Avena-Bustillos, McHugh, Krochta, & Mattoso (2008) also stated a similar fact. Water vapor barrier properties of cellulose based films are not good due to their hydrophilic nature. Therefore, when HPMC was added, the water vapor permeability of electrospun nanofibers could not be improved.

3.7.2. Effect of lentil flour and HPMC concentrations on color of nanofibers

Color of the nanofibers obtained from electrospinning process was analyzed in terms of CIE L^*, a^* and b^* which are whiteness/darkness, redness/greenness, blueness/yellowness, respectively. Neither lentil flour concentration nor HPMC addition did not have a significant effect on color parameters (Table 11).

Sobral, Dos Santos, & García (2005) studied edible films formed by using proteins. Similarly, they reported no significant effect of protein concentration on color of films. Since color is a very important parameter in food industry, there are many studies related to color in the literature. However, in order to identify the relation between physical properties and polymer structural chemistry, more researches are needed.

Table 11. Effect of lentil flour and HPMC concentrations on color of nanofibers

Lentil Flour Concentration (%)	**HPMC Concentration (%)**	**L***	**a***	**b***	**ΔE***
2.00	0	68.850±4.59[a**]	1.095±0.09[a]	3.435±0.60[a]	24.707±4.59[a]
2.00	0.5	77.390±3.71[a]	1.180±0.06[a]	2.695±0.59[a]	16.239±3.70[a]
5.25	0	74.920±0.30[a]	1.405±0.03[a]	2.965±0.06[a]	18.712±0.31[a]
5.25	0.5	76.295±1.68[a]	1.040±0.07[a]	3.475±0.51[a]	17.410±1.71[a]

** Columns with different letters differ statistically ($p \leq 0.05$)

All of the solutions were prepared at pH 10 by using 3.5% PEO and 2% Tween80

Electrospinning conditions were 20-30 cm distance, 8-15 kV voltage and 0.8 mL/h flow rate

3.8. Fourier-transform infrared (FTIR) analysis of nanofibers

Interactions between the components in the nanofibers obtained by electrospinning could be examined by FTIR analysis. FTIR analysis gives the information related to the functional groups in the samples (Aydoğdu et al., 2018). FTIR spectra of nanofibers containing different compositions of lentil flour, PEO and HPMC and pure components was shown in Figure 19. The concentrations of the samples were given in Table 12.

Table 12. Compositions of samples used in FTIR measurement

Sample	Concentration (%)		
	PEO	**Lentil Flour**	**HPMC**
L_1*	3.5	2	0
L_2*	3.5	5.25	0.5
LH_1*	3.5	2	0
LH_2*	3.5	5.25	0.5
PEO**	100	0	0
Lentil Flour**	0	100	0
HPMC**	0	0	100

* All of the solutions were prepared at pH 10 by using 2% Tween80 and electrospinning conditions were 20-30 cm distance, 8-15 kV voltage and 0.8 mL/h flow rate

** Components were analyzed in pure powder form

Between the wavenumbers of 750 cm^{-1} and 1500 cm^{-1}, each substance showed a pattern which was special to that specific substance. This range is called as fingerprint region (Aydogdu et al., 2018). When the FTIR spectrum of lentil flour powder was examined intense bands were observed at 842, 962, 1097, 1340, 1471 and 2900 cm^{-1}. In the literature, wavenumbers 1520 cm^{-1} and 1660 cm^{-1} represent amide II (N-H bending) and amide I (C=O stretching), respectively (Carbonaro, Maselli, Dore, &

Nucara, 2008). The reason of the difference between our results and the literature might be the variety of lentil from which flour was obtained. Moreover, the amount of carbohydrate in the sample might vary. Ahmed, Varshney, & Ramaswamy (2009) analyzed lentil flour by FTIR and wavenumbers of the peaks were reported as 1163, 1408, 1550 and 1658 cm^{-1}. Similar reasons were provided as the reason of the difference from the literature.

When pure PEO powder was analyzed intense peaks were observed at 840, 960, 1058, 1095, 1145, 1244, 1282, 1340 and 1467 cm^{-1} wavenumbers. Similarly, Vega-Lugo and Lim (2012) stated that PEO had peaks at 1058, 1095 and 1145 cm^{-1} in the fingerprint region. Pielichowski & Flejtuch (2005) reported CH_2 twisting, wagging and scissoring in the fingerprint region of PEO at 1280, 1340 and 1467 cm^{-1}, respectively. In another electrospinning study, very similar results were found. 860, 1058, 1095, 1100, 1145, 1280, 1340, 1467 and 2900 cm^{-1} wavenumbers were reported as the intense peaks of the pure PEO (Aydoğdu et al., 2018).

Pure HPMC powder showed intense peaks at 945, 1053, 2900 and 3400 cm^{-1} (Fig. 19). Ding, Zhang, & Li (2015) reported five peaks for HPMC at 1066 cm^{-1} (C-O stretching vibrations), 1119 cm^{-1} (C-O-C asymmetric stretching vibration), 1456 cm^{-1} (CH_3 asymmetric bending vibration), 2931 cm^{-1} (C-H stretching) and 3461 cm^{-1} (O-H stretching vibrations). In another similar study, 1060, 2900 and 3400 cm^{-1} wavenumbers were stated as intense peaks (Aydoğdu et al., 2018).

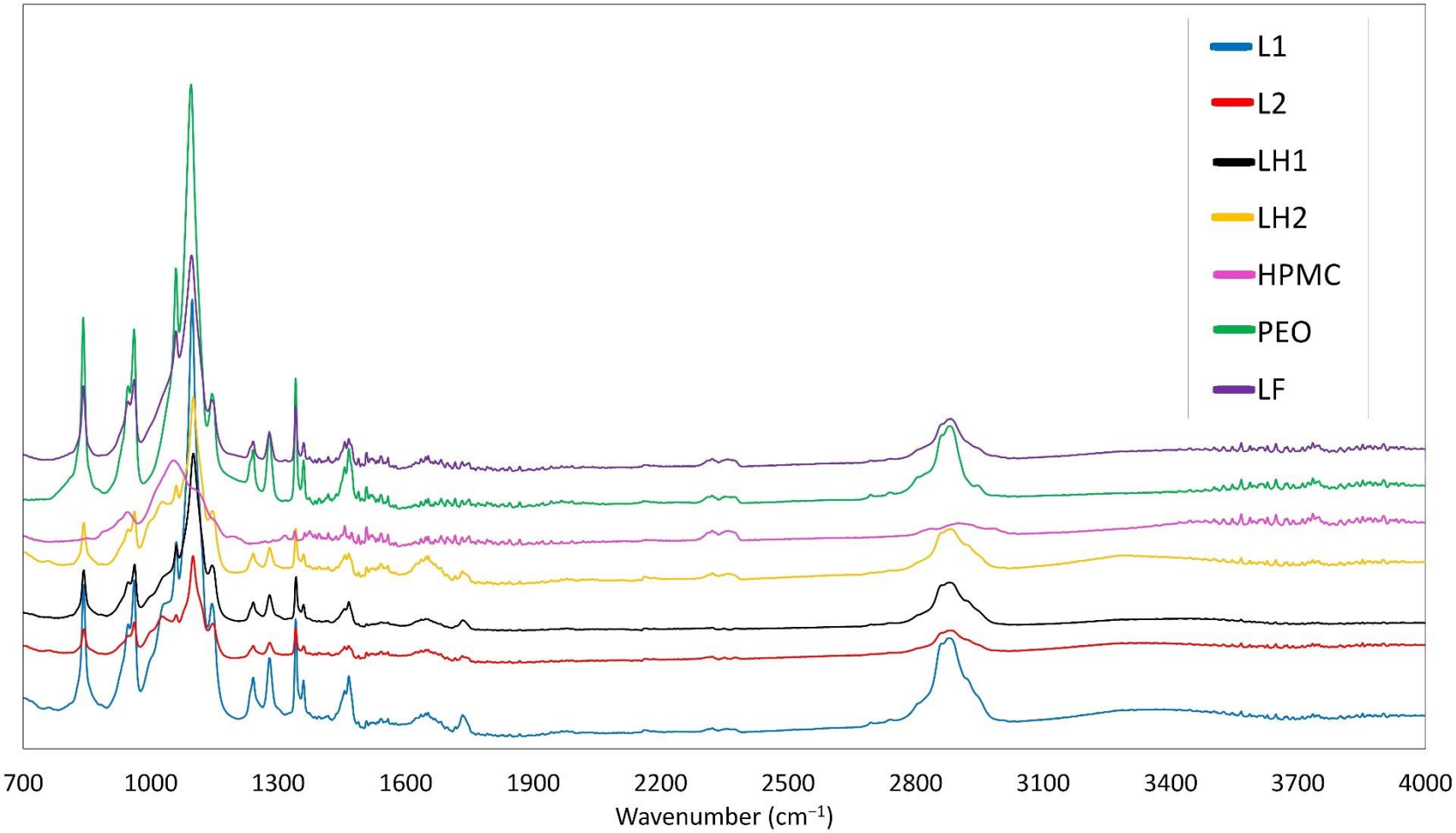

Figure 19. FTIR spectra of nanofibers containing different compositions of lentil flour, PEO and HPMC

Lee et al. (2007) stated that the peak at 2900 cm^{-1} represents CH_2 stretching in other words methylene stretching. At this wavenumber, the peak obtained from pure PEO was much higher than pure HPMC. Therefore, when L_1-L_2 and LH_1-LH_2 were examined, the ones with higher PEO, content L_2 and LH_2, showed higher peaks compared to L_1 and LH_1, respectively. Kriegel et al. (2008) reported that CH_2 stretching increased at 2885 cm^{-1} for nanofibers obtained from chitosan and PEO blend. Similarly, LH_1 and LH_2 showed smaller peak compare to L_1 and L_2, respectively. The reason of that was the addition of HPMC into the electrospinning solutions.

FTIR results of nanofibers showed more intense peaks around 842, 962, 1060, 1100, 1652, 1734 and 2900 cm^{-1}. The reason of that was probably the addition of Tween 80 in the electrospinning solutions. Liu et al. (2015) stated the peaks of pure Tween 80 at 855 & 946 cm^{-1} (-H_2C-O-CH_2-), 1110 cm^{-1} (-CO-O-CH_2-), 1648 cm^{-1} (-HC=CH-), 1732 (C=O) and 2855 & 2900 cm^{-1} (CH_3-CH_2-). The peak at 1060 cm^{-1} was C-O stretching vibration coming from HPMC. Even though there were small shifts, all the other peaks of nanofibers matched with the peaks of Tween 80.

As a general fact, not compatible polymers show their own peaks in the FTIR analysis. Distinct peaks can be shown in FTIR spectra. When polymers are compatible, chemical interactions can occur. Because of that some peaks may shift. In this study, the reason of obtaining different FTIR spectra for nanofibers was the possible interactions between lentil flour, PEO, HPMC and Tween 80.

CHAPTER 4

CONCLUSION & RECOMMENDATIONS

Homogeneous nanofibers could be obtained from solutions containing PEO, lentil flour and HPMC at different combinations and at different pH values by using electrospinning technique. pH was found to be a significant factor in affecting nanofiber production. Usage of solutions at neutral pH resulted in bead formation. On the other hand, homogeneous nanofibers could be obtained from all solutions at alkaline pH values. Fiber diameter increased with increase in lentil flour and HPMC concentration. Homogeneous nanofibers could not be obtained from solutions treated by microfluidization. Significant effects of electrospinning conditions on nanofiber diameter were observed. Increasing applied voltage increased nanofiber diameter whereas increasing flow rate decreased nanofiber diameter.

Nanofiber obtained from electrospinning could be considered to be used as packaging material. Thus, two of the important parameters for packaging materials, water vapor permeability and color values of nanofibers, were determined. With an increase in lentil flour concentration, water vapor permeability of nanofibers increased. Color of the nanofibers was not affected by neither lentil flour nor HPMC concentrations. The intensity of FTIR peaks shifted, which showed that the good miscibility of polymer compounds used in electrospinning solutions. Therefore, using PEO, lentil flour and HPMC blend as the electrospinning solution was a good choice for production of nanofibers. Nanofiber obtained from solutions containing lentil flour, 0.25% HPMC and at pH value of 10 had the smallest diameter. Thus, this composition can be considered as good candidate for packaging material because it gives uniform homogenous nanofibers with the desired smallest diameter.

In future studies, bioactive compounds can be added to these nanofibers to be used as active package materials.

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APPENDIX A

STATISTICAL ANALYSES

Table A.1. Two way ANOVA and Tukey's comparison test for *K* values of electrospinning solutions prepared by using 3.5% PEO, 0.5% HPMC and 2% Tween 80 at the same electrospinning conditions

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Factor   Type   Levels  Values
Protein  fixed       2  1; 2
pH       fixed       3  7; 10; 12


Analysis of Variance for k, using Adjusted SS for Tests

Source      DF  Seq SS  Adj SS  Adj MS       F      P
Protein      1  3,7246  3,7246  3,7246  656,94  0,000
pH           2  1,5911  1,5911  0,7956  140,32  0,000
Protein*pH   2  0,2332  0,2332  0,1166   20,57  0,000
Error       12  0,0680  0,0680  0,0057
Total       17  5,6170


S = 0,0752972   R-Sq = 98,79%   R-Sq(adj) = 98,28%


Unusual Observations for k

Obs        k      Fit   SE Fit  Residual  St Resid
  5  1,14591  1,29395  0,04347  -0,14804     -2,41 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein  N  Mean  Grouping
2        9   2,4  A
1        9   1,5    B

Means that do not share a letter are significantly different.
```

Table A.1. (Continued)

```
Grouping Information Using Tukey Method and 95,0% Confidence

pH  N  Mean  Grouping
 7  6   2,4  A
10  6   1,8    B
12  6   1,7    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein  pH  N  Mean  Grouping
2         7  3   2,9  A
2        10  3   2,4    B
2        12  3   2,0      C
1         7  3   1,9      C
1        12  3   1,4        D
1        10  3   1,3        D

Means that do not share a letter are significantly different.
```

Table A.2. Two way ANOVA and Tukey's comparison test for n values of electrospinning solutions prepared by using 3.5% PEO, 0.5% HPMC and 2% Tween 80 at the same electrospinning conditions

```
Factor   Type   Levels  Values
Protein  fixed       2  1; 2
pH       fixed       3  7; 10; 12


Analysis of Variance for n, using Adjusted SS for Tests

Source       DF     Seq SS     Adj SS     Adj MS       F      P
Protein       1  0,0071481  0,0071481  0,0071481  989,74  0,000
pH            2  0,0022303  0,0022303  0,0011152  154,41  0,000
Protein*pH    2  0,0002091  0,0002091  0,0001045   14,47  0,001
Error        12  0,0000867  0,0000867  0,0000072
Total        17  0,0096742


S = 0,00268742   R-Sq = 99,10%   R-Sq(adj) = 98,73%


Unusual Observations for n

Obs         n       Fit    SE Fit   Residual  St Resid
 14  0,888700  0,894933  0,001552  -0,006233     -2,84 R

R denotes an observation with a large standardized residual.
```

Table A.2. (Continued)

```
Grouping Information Using Tukey Method and 95,0% Confidence

Protein  N  Mean  Grouping
1        9   0,9  A
2        9   0,9    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

pH  N  Mean  Grouping
12  6   0,9  A
10  6   0,9  A
 7  6   0,9    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein  pH  N  Mean  Grouping
1        10  3   0,9  A
1        12  3   0,9  A
1         7  3   0,9    B
2        12  3   0,9      C
2        10  3   0,9        D
2         7  3   0,9          E

Means that do not share a letter are significantly different.
```

Table A.3. Two way ANOVA and Tukey's comparison test for electrical conductivity values of electrospinning solutions prepared by using 3.5% PEO, 0.5% HPMC and 2% Tween 80 at the same electrospinning conditions

```
Factor   Type   Levels  Values
Protein  fixed       2  1; 2
pH       fixed       3  7; 10; 12


Analysis of Variance for Conductivity, using Adjusted SS for Tests

Source      DF   Seq SS   Adj SS  Adj MS      F      P
Protein      1   288156   201356  201356  13,24  0,005
pH           2  1030923  1031579  515789  33,92  0,000
Protein*pH   2     6539     6539    3269   0,22  0,811
Error        9   136838   136838   15204
Total       14  1462456


S = 123,305   R-Sq = 90,64%   R-Sq(adj) = 85,45%
```

Table A.3. (Continued)

Unusual Observations for Conductivity

Obs	Conductivity	Fit	SE Fit	Residual	St Resid
7	823,00	1041,50	87,19	-218,50	-2,51 R
8	1260,00	1041,50	87,19	218,50	2,51 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein	N	Mean	Grouping
2	7	887,1	A
1	8	650,6	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

pH	N	Mean	Grouping
12	4	1148,2	A
10	6	687,8	B
7	5	470,5	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein	pH	N	Mean	Grouping
2	12	2	1255,0	A
1	12	2	1041,5	A B
2	10	3	834,3	B C
2	7	2	572,0	C D
1	10	3	541,3	C D
1	7	3	369,0	D

Means that do not share a letter are significantly different.

Table A.4. Two way ANOVA and Tukey's comparison test for diameter values of nanofibers obtained from electrospinning solutions prepared by using 3.5% PEO, 0.5% HPMC and 2% Tween 80 at the same electrospinning conditions

```
Factor   Type   Levels  Values
Protein  fixed       2  1; 2
pH       fixed       2  10; 12


Analysis of Variance for Diameter, using Adjusted SS for Tests

Source       DF    Seq SS    Adj SS    Adj MS      F      P
Protein       1  0,066510  0,065328  0,065328  48,80  0,000
pH            1  0,003524  0,003143  0,003143   2,35  0,126
Protein*pH    1  0,031771  0,031771  0,031771  23,73  0,000
Error       389  0,520765  0,520765  0,001339
Total       392  0,622570


S = 0,0365886   R-Sq = 16,35%   R-Sq(adj) = 15,71%


Unusual Observations for Diameter

Obs  Diameter       Fit    SE Fit   Residual  St Resid
 16  0,098000  0,210420  0,003659  -0,112420     -3,09 R
 61  0,297000  0,210420  0,003659   0,086580      2,38 R
 62  0,345000  0,210420  0,003659   0,134580      3,70 R
 75  0,381000  0,210420  0,003659   0,170580      4,69 R
 96  0,292000  0,210420  0,003659   0,081580      2,24 R
187  0,135000  0,222753  0,003794  -0,087753     -2,41 R
190  0,144000  0,222753  0,003794  -0,078753     -2,16 R
209  0,121000  0,254210  0,003659  -0,133210     -3,66 R
251  0,400000  0,254210  0,003659   0,145790      4,00 R
254  0,180000  0,254210  0,003659  -0,074210     -2,04 R
263  0,159000  0,254210  0,003659  -0,095210     -2,62 R
265  0,376000  0,254210  0,003659   0,121790      3,35 R
269  0,511000  0,254210  0,003659   0,256790      7,05 R
278  0,349000  0,254210  0,003659   0,094790      2,60 R
289  0,346000  0,254210  0,003659   0,091790      2,52 R
321  0,389000  0,230560  0,003659   0,158440      4,35 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein    N  Mean  Grouping
2        200   0,2  A
1        193   0,2    B

Means that do not share a letter are significantly different.
```

Table A.4. (Continued)

```
Grouping Information Using Tukey Method and 95,0% Confidence

pH    N  Mean  Grouping
10  200   0,2  A
12  193   0,2  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein  pH    N  Mean  Grouping
2        10  100   0,3  A
2        12  100   0,2    B
1        12   93   0,2    B C
1        10  100   0,2      C

Means that do not share a letter are significantly different
```

Table A.5. Two way ANOVA and Tukey's comparison test for apparent viscosity of electrospinning solutions prepared by using 3.5% PEO, 0.5% HPMC and 2% Tween 80 at the same electrospinning conditions

```
Factor   Type   Levels  Values
Protein  fixed       2  1; 2
pH       fixed       3  7; 10; 12


Analysis of Variance for Apparent Viscosity, using Adjusted SS for Tests

Source      DF   Seq SS   Adj SS   Adj MS       F      P
Protein      1  0,61361  0,61361  0,61361  156,49  0,000
pH           2  0,28758  0,28758  0,14379   36,67  0,000
Protein*pH   2  0,04445  0,04445  0,02222    5,67  0,018
Error       12  0,04705  0,04705  0,00392
Total       17  0,99269


S = 0,0626194   R-Sq = 95,26%   R-Sq(adj) = 93,28%


Unusual Observations for Apparent Viscosity

      Apparent
Obs  Viscosity      Fit   SE Fit  Residual  St Resid
  5    0,86910  0,99757  0,03615  -0,12847     -2,51 R
  6    1,10510  0,99757  0,03615   0,10753      2,10 R

R denotes an observation with a large standardized residual.
```

Table A.5. (Continued)

```
Grouping Information Using Tukey Method and 95,0% Confidence

Protein  N  Mean  Grouping
2        9   1,5  A
1        9   1,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

pH  N  Mean  Grouping
 7  6   1,5  A
10  6   1,2    B
12  6   1,2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein  pH  N  Mean  Grouping
2         7  3   1,7  A
2        10  3   1,4    B
2        12  3   1,3    B C
1         7  3   1,2      C
1        12  3   1,1        D
1        10  3   1,0        D

Means that do not share a letter are significantly different.
```

Table A.6. Two way ANOVA and Tukey's comparison test for *K* values of electrospinning solutions prepared at pH 10 by using 3.5% PEO and 2% Tween 80 at the same electrospinning conditions

```
Factor     Type   Levels  Values
Protein_1  fixed       2  1; 2
HPMC       fixed       3  0,25; 0,50; 1,00


Analysis of Variance for k_1, using Adjusted SS for Tests

Source          DF   Seq SS   Adj SS  Adj MS       F      P
Protein_1        1   2,8122   2,8122  2,8122  165,79  0,000
HPMC             2  10,5211  10,5211  5,2605  310,13  0,000
Protein_1*HPMC   2   0,4587   0,4587  0,2294   13,52  0,001
Error           12   0,2035   0,2035  0,0170
Total           17  13,9955


S = 0,130239   R-Sq = 98,55%   R-Sq(adj) = 97,94%
```

Table A.6. (Continued)

```
Unusual Observations for k_1

Obs      k_1      Fit   SE Fit  Residual  St Resid
 18  4,05682  3,84376  0,07519   0,21306      2,00 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein_1  N  Mean  Grouping
2          9   2,7  A
1          9   1,9    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

HPMC  N  Mean  Grouping
1,00  6   3,4  A
0,50  6   1,8    B
0,25  6   1,7    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein_1  HPMC  N  Mean  Grouping
2          1,00  3   3,8  A
1          1,00  3   2,9    B
2          0,50  3   2,4      C
2          0,25  3   1,8        D
1          0,25  3   1,5        D E
1          0,50  3   1,3          E

Means that do not share a letter are significantly different.
```

Table A.7. Two way ANOVA and Tukey's comparison test for n values of electrospinning solutions prepared at pH 10 by using 3.5% PEO and 2% Tween 80 at the same electrospinning conditions

```
Factor     Type   Levels  Values
Protein_1  fixed       2  1; 2
HPMC       fixed       3  0,25; 0,50; 1,00
```

Table A.7. (Continued)

Analysis of Variance for n_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Protein_1	1	0,0044998	0,0044998	0,0044998	677,80	0,000
HPMC	2	0,0065583	0,0065583	0,0032791	493,93	0,000
Protein_1*HPMC	2	0,0007363	0,0007363	0,0003681	55,45	0,000
Error	12	0,0000797	0,0000797	0,0000066		
Total	17	0,0118741				

S = 0,00257660 R-Sq = 99,33% R-Sq(adj) = 99,05%

Unusual Observations for n_1

Obs	n_1	Fit	SE Fit	Residual	St Resid
14	0,888700	0,894933	0,001488	-0,006233	-2,96 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein_1	N	Mean	Grouping
1	9	0,9	A
2	9	0,9	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

HPMC	N	Mean	Grouping
0,50	6	0,9	A
0,25	6	0,9	B
1,00	6	0,9	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein_1	HPMC	N	Mean	Grouping
1	0,50	3	0,9	A
1	0,25	3	0,9	B
2	0,25	3	0,9	C
2	0,50	3	0,9	C
1	1,00	3	0,9	D
2	1,00	3	0,9	E

Means that do not share a letter are significantly different.

Table A.8. Two way ANOVA and Tukey's comparison test for electrical conductivity values of electrospinning solutions prepared at pH 10 by using 3.5% PEO and 2% Tween 80 at the same electrospinning conditions

```
Factor   Type   Levels  Values
Protein  fixed       2  1; 2
HPMC     fixed       3  0,25; 0,50; 1,00

Analysis of Variance for Conductivity, using Adjusted SS for Tests

Source        DF  Seq SS  Adj SS  Adj MS      F      P
Protein        1  306675  306675  306675  43,73  0,000
HPMC           2   31843   31843   15922   2,27  0,146
Protein*HPMC   2    2676    2676    1338   0,19  0,829
Error         12   84149   84149    7012

Total         17  425343

S = 83,7403   R-Sq = 80,22%   R-Sq(adj) = 71,97%

Unusual Observations for Conductivity

Obs  Conductivity      Fit  SE Fit  Residual  St Resid
 14       688,000  834,333  48,347  -146,333     -2,14 R
 16       901,000  738,333  48,347   162,667      2,38 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein  N   Mean  Grouping
2        9  807,9  A
1        9  546,8    B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

HPMC  N   Mean  Grouping
0,25  6  722,8  A
0,50  6  687,8  A
1,00  6  621,4  A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein  HPMC  N   Mean  Grouping
2        0,25  3  851,0  A
2        0,50  3  834,3  A
2        1,00  3  738,3  A B
1        0,25  3  594,7    B C
1        0,50  3  541,3    B C
1        1,00  3  504,5      C

Means that do not share a letter are significantly different.
```

Table A.9. Two way ANOVA and Tukey's comparison test for diameter values of nanofibers obtained from electrospinning solutions prepared at pH 10 by using 3.5% PEO and 2% Tween 80 at the same electrospinning conditions

Factor	Type	Levels	Values
Protein	fixed	2	1; 2
HPMC	fixed	3	0,25; 0,50; 1,00

Analysis of Variance for Diameter, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Protein	1	0,079311	0,081187	0,081187	53,26	0,000
HPMC	2	0,130296	0,130147	0,065073	42,69	0,000
Protein*HPMC	2	0,038837	0,038837	0,019418	12,74	0,000
Error	585	0,891751	0,891751	0,001524		
Total	590	1,140195				

S = 0,0390431 R-Sq = 21,79% R-Sq(adj) = 21,12%

Unusual Observations for Diameter

Obs	Diameter	Fit	SE Fit	Residual	St Resid	
16	0,098000	0,210420	0,003904	-0,112420	-2,89	R
61	0,297000	0,210420	0,003904	0,086580	2,23	R
62	0,345000	0,210420	0,003904	0,134580	3,46	R
75	0,381000	0,210420	0,003904	0,170580	4,39	R
96	0,292000	0,210420	0,003904	0,081580	2,10	R
135	0,105000	0,198160	0,003904	-0,093160	-2,40	R
140	0,111000	0,198160	0,003904	-0,087160	-2,24	R
160	0,276000	0,198160	0,003904	0,077840	2,00	R
178	0,119000	0,198160	0,003904	-0,079160	-2,04	R
186	0,102000	0,198160	0,003904	-0,096160	-2,48	R
187	0,363000	0,198160	0,003904	0,164840	4,24	R
193	0,117000	0,198160	0,003904	-0,081160	-2,09	R
223	0,128000	0,220050	0,003904	-0,092050	-2,37	R
236	0,317000	0,220050	0,003904	0,096950	2,50	R
256	0,327000	0,220050	0,003904	0,106950	2,75	R
288	0,340000	0,220050	0,003904	0,119950	3,09	R
316	0,121000	0,254210	0,003904	-0,133210	-3,43	R
358	0,400000	0,254210	0,003904	0,145790	3,75	R
370	0,159000	0,254210	0,003904	-0,095210	-2,45	R
372	0,376000	0,254210	0,003904	0,121790	3,14	R
376	0,511000	0,254210	0,003904	0,256790	6,61	R
385	0,349000	0,254210	0,003904	0,094790	2,44	R
396	0,346000	0,254210	0,003904	0,091790	2,36	R
408	0,340000	0,202600	0,003904	0,137400	3,54	R
442	0,313000	0,202600	0,003904	0,110400	2,84	R
489	0,069000	0,202600	0,003904	-0,133600	-3,44	R
491	0,092000	0,202600	0,003904	-0,110600	-2,85	R
588	0,325000	0,242187	0,004093	0,082813	2,13	R
589	0,337000	0,242187	0,004093	0,094813	2,44	R
590	0,339000	0,242187	0,004093	0,096813	2,49	R
591	0,351000	0,242187	0,004093	0,108813	2,80	R

Table A.9. (Continued)

```
R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein    N  Mean  Grouping
2        291   0,2  A
1        300   0,2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

HPMC    N  Mean  Grouping
0,50  200   0,2  A
1,00  191   0,2  A
0,25  200   0,2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein  HPMC    N  Mean  Grouping
2        0,50  100   0,3  A
2        1,00   91   0,2  A
1        1,00  100   0,2    B
1        0,50  100   0,2    B C
2        0,25  100   0,2      C
1        0,25  100   0,2      C

Means that do not share a letter are significantly different.
```

Table A.10. Two way ANOVA and Tukey's comparison test for apparent viscosity of electrospinning solutions prepared at pH 10 by using 3.5% PEO and 2% Tween 80 at the same electrospinning conditions

```
Factor   Type   Levels  Values
Protein  fixed       2  1; 2
HPMC     fixed       3  0,25; 0,50; 1,00


Analysis of Variance for Apparent Viscosity, using Adjusted SS for Tests

Source        DF   Seq SS   Adj SS   Adj MS       F      P
Protein        1  0,47343  0,47343  0,47343   76,35  0,000
HPMC           2  2,15801  2,15801  1,07901  174,02  0,000
Protein*HPMC   2  0,10690  0,10690  0,05345    8,62  0,005
Error         12  0,07441  0,07441  0,00620
Total         17  2,81275


S = 0,0787440   R-Sq = 97,35%   R-Sq(adj) = 96,25%
```

Table A.10. (Continued)

```
Grouping Information Using Tukey Method and 95,0% Confidence

Protein  N  Mean  Grouping
2        9   1,6  A
1        9   1,2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

HPMC  N  Mean  Grouping
1,00  6   1,9  A
0,50  6   1,2    B
0,25  6   1,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein  HPMC  N  Mean  Grouping
2        1,00  3   2,1  A
1        1,00  3   1,7    B
2        0,50  3   1,4      C
2        0,25  3   1,1        D
1        0,25  3   1,0        D
1        0,50  3   1,0        D

Means that do not share a letter are significantly different.
```

Table A.11. One way ANOVA and Tukey's comparison test for electrical conductivity values of electrospinning solutions prepared by using 3.5% PEO, 5.25% Lentil Flour, 0.5% HPMC and 2% Tween80 and microfluidized by different pass numbers

```
Factor  Type   Levels  Values
Pass    fixed       3  0; 3; 5


Analysis of Variance for Conductivity, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS      F      P
Pass     2  300684  300684  150342  36,13  0,008
Error    3   12482   12482    4161
Total    5  313166


S = 64,5032   R-Sq = 96,01%   R-Sq(adj) = 93,36%
```

Table A.11. (Continued)

```
Grouping Information Using Tukey Method and 95,0% Confidence

Pass  N    Mean  Grouping
0     2  1614,0  A
5     2  1230,0    B
3     2  1083,0    B

Means that do not share a letter are significantly different.
```

Table A.12. One way ANOVA and Tukey's comparison test for *K* values of electrospinning solutions prepared by using 3.5% PEO, 5.25% Lentil Flour, 0.5% HPMC and 2% Tween80 and microfluidized by different pass numbers

```
Factor  Type   Levels  Values
Pass    fixed       3  0; 3; 5


Analysis of Variance for k, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
Pass     2  69,141  69,141  34,570  375,29  0,000
Error    4   0,368   0,368   0,092
Total    6  69,509


S = 0,303506   R-Sq = 99,47%   R-Sq(adj) = 99,20%


Grouping Information Using Tukey Method and 95,0% Confidence

Pass  N  Mean  Grouping
3     2  10,1  A
5     2   9,4  A
0     3   3,4    B

Means that do not share a letter are significantly different.
```

Table A.13. One way ANOVA and Tukey's comparison test for n values of electrospinning solutions prepared by using 3.5% PEO, 5.25% Lentil Flour, 0.5% HPMC and 2% Tween80 and microfluidized by different pass numbers

```
Factor  Type   Levels  Values
Pass    fixed       3  0; 3; 5


Analysis of Variance for n, using Adjusted SS for Tests

Source  DF    Seq SS    Adj SS    Adj MS       F      P
Pass     2  0,068862  0,068862  0,034431  545,10  0,000
Error    4  0,000253  0,000253  0,000063
Total    6  0,069115
```

Table A.13. (Continued)

```
S = 0,00794759   R-Sq = 99,63%   R-Sq(adj) = 99,45%

Grouping Information Using Tukey Method and 95,0% Confidence

Pass  N  Mean  Grouping
0     3   0,8  A
5     2   0,6    B
3     2   0,6    B

Means that do not share a letter are significantly different.
```

Table A.14. One way ANOVA and Tukey's comparison test for electrical conductivity values of electrospinning solutions prepared by using 2.5% PEO, 7.5% Lentil Flour, 0.5% HPMC and 2% Tween80 and microfluidized by different pass numbers

```
Factor  Type   Levels  Values
Pass    fixed       3  0; 3; 5

Analysis of Variance for Conductivity, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS           F      P
Pass     2  561080  561080  280540  1683241,00  0,000
Error    3       1       1       0
Total    5  561081


S = 0,408248   R-Sq = 100,00%   R-Sq(adj) = 100,00%


Grouping Information Using Tukey Method and 95,0% Confidence


Pass  N    Mean  Grouping
0     2  2390,0  A
3     2  1762,5    B
5     2  1722,0      C

Means that do not share a letter are significantly different.
```

Table A.15. One way ANOVA and Tukey's comparison test for *K* values of electrospinning solutions prepared by using 2.5% PEO, 7.5% Lentil Flour, 0.5% HPMC and 2% Tween80 and microfluidized by different pass numbers

```
Factor  Type   Levels  Values
Pass    fixed       3  0; 3; 5
```

Table A.15. (Continued)

```
Analysis of Variance for k, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS      F      P
Pass     2  32,519  32,519  16,260  63,73  0,000
Error    6   1,531   1,531   0,255
Total    8  34,050


S = 0,505100   R-Sq = 95,50%   R-Sq(adj) = 94,01%


Unusual Observations for k

Obs        k      Fit  SE Fit  Residual  St Resid
  6  11,4300  12,2933  0,2916   -0,8633     -2,09 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pass  N  Mean  Grouping
5     3  12,5  A
3     3  12,3  A
0     3   8,4    B

Means that do not share a letter are significantly different.
```

Table A.16. One way ANOVA and Tukey's comparison test for n values of electrospinning solutions prepared by using 2.5% PEO, 7.5% Lentil Flour, 0.5% HPMC and 2% Tween80 and microfluidized by different pass numbers

```
Factor  Type   Levels  Values
Pass    fixed       3  0; 3; 5


Analysis of Variance for n, using Adjusted SS for Tests

Source  DF     Seq SS     Adj SS     Adj MS       F      P
Pass     2  0,0083193  0,0083193  0,0041596  196,59  0,000
Error    6  0,0001270  0,0001270  0,0000212
Total    8  0,0084462


S = 0,00459988   R-Sq = 98,50%   R-Sq(adj) = 98,00%

Grouping Information Using Tukey Method and 95,0% Confidence

Pass  N  Mean  Grouping
0     3   0,7  A
5     3   0,7    B
3     3   0,6      C

Means that do not share a letter are significantly different.
```

Table A.17. One way ANOVA and Tukey's comparison test for electrical conductivity values of electrospinning solutions prepared by using 3.5% PEO, 5.25% Lentil Flour and 2% Tween80 and microfluidized by different pass numbers

```
Factor  Type   Levels  Values
Pass    fixed       3  0; 3; 5


Analysis of Variance for Conductivity, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS     F      P
Pass     2    1519    1519     760  0,19  0,833
Error    3   11704   11704    3901
Total    5   13223


S = 62,4620   R-Sq = 11,49%   R-Sq(adj) = 0,00%


Grouping Information Using Tukey Method and 95,0% Confidence

Pass  N    Mean  Grouping
5     2  1711,0  A
3     2  1697,0  A
0     2  1672,5  A

Means that do not share a letter are significantly different.
```

Table A.18. One way ANOVA and Tukey's comparison test for *K* values of electrospinning solutions prepared by using 3.5% PEO, 5.25% Lentil Flour and 2% Tween80 and microfluidized by different pass numbers

```
Factor  Type   Levels  Values
Pass    fixed       3  0; 3; 5


Analysis of Variance for k, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
Pass     2  40,045  40,045  20,023  546,52  0,000
Error    6   0,220   0,220   0,037
Total    8  40,265


S = 0,191407   R-Sq = 99,45%   R-Sq(adj) = 99,27%


Unusual Observations for k

Obs        k      Fit   SE Fit  Residual  St Resid
  5  5,72400  5,39567  0,11051   0,32833      2,10 R

R denotes an observation with a large standardized residual.
```

Table A.18. (Continued)

```
Grouping Information Using Tukey Method and 95,0% Confidence

Pass  N  Mean  Grouping
3     3   5,4  A
5     3   5,0  A
0     3   0,8    B

Means that do not share a letter are significantly different.
```

Table A.19. One way ANOVA and Tukey's comparison test for n values of electrospinning solutions prepared by using 3.5% PEO, 5.25% Lentil Flour and 2% Tween80 and microfluidized by different pass numbers

```
Factor  Type   Levels  Values
Pass    fixed       3  0; 3; 5


Analysis of Variance for n, using Adjusted SS for Tests

Source  DF    Seq SS    Adj SS    Adj MS       F      P
Pass     2  0,115799  0,115799  0,057900  714,31  0,000
Error    6  0,000486  0,000486  0,000081
Total    8  0,116286


S = 0,00900315   R-Sq = 99,58%   R-Sq(adj) = 99,44%


Unusual Observations for n

Obs         n       Fit    SE Fit   Residual  St Resid
  2  0,866700  0,884067  0,005198  -0,017367     -2,36 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pass  N  Mean  Grouping
0     3   0,9  A
5     3   0,6    B
3     3   0,6    B

Means that do not share a letter are significantly different.
```

Table A.20. One way ANOVA and Tukey's comparison test for electrical conductivity values of electrospinning solutions prepared by using 2.5% PEO, 7.5% Lentil Flour and 2% Tween80 and microfluidized by different pass numbers

```
Factor  Type   Levels  Values
Pass    fixed       3  0; 3; 5


Analysis of Variance for Conductivity, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS  Adj MS         F      P
Pass     2  12990,3  12990,3  6495,2  38971,00  0,000
Error    3      0,5      0,5     0,2
Total    5  12990,8


S = 0,408248   R-Sq = 100,00%   R-Sq(adj) = 99,99%


Grouping Information Using Tukey Method and 95,0% Confidence

Pass  N    Mean  Grouping
3     2  2080,0  A
5     2  2060,5    B
0     2  1973,0      C

Means that do not share a letter are significantly different.
```

Table A.21. One way ANOVA and Tukey's comparison test for *K* values of electrospinning solutions prepared by using 2.5% PEO, 7.5% Lentil Flour and 2% Tween80 and microfluidized by different pass numbers

```
Factor  Type   Levels  Values
Pass    fixed       3  0; 3; 5


Analysis of Variance for k, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS  Adj MS       F      P
Pass     2  132,576  132,576  66,288  150,53  0,000
Error    6    2,642    2,642   0,440
Total    8  135,218


S = 0,663592   R-Sq = 98,05%   R-Sq(adj) = 97,39%


Unusual Observations for k

Obs        k      Fit  SE Fit  Residual  St Resid
  7  13,4600  12,2467  0,3831    1,2133      2,24 R

R denotes an observation with a large standardized residual.
```

Table A.21. (Continued)

```
Grouping Information Using Tukey Method and 95,0% Confidence

Pass  N  Mean  Grouping
5     3  12,2  A
3     3   8,7    B
0     3   2,9      C

Means that do not share a letter are significantly different.
```

Table A.22. One way ANOVA and Tukey's comparison test for n values of electrospinning solutions prepared by using 2.5% PEO, 7.5% Lentil Flour and 2% Tween80 and microfluidized by different pass numbers

```
Factor  Type   Levels  Values
Pass    fixed       4  0; 3; 5; 10


Analysis of Variance for n, using Adjusted SS for Tests

Source  DF    Seq SS    Adj SS    Adj MS       F      P
Pass     3  0,077277  0,077277  0,025759  139,59  0,000
Error    8  0,001476  0,001476  0,000185
Total   11  0,078753


S = 0,0135845   R-Sq = 98,13%   R-Sq(adj) = 97,42%


Unusual Observations for n

Obs         n       Fit    SE Fit   Residual  St Resid
  7  0,647900  0,672900  0,007843  -0,025000     -2,25 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pass  N  Mean  Grouping
 0    3   0,9  A
10    3   0,7    B
 3    3   0,7      C
 5    3   0,7      C

Means that do not share a letter are significantly different.
```

Table A.23. Two way ANOVA and Tukey's comparison test for diameter values of nanofibers obtained from electrospinning solutions prepared at pH 10 by using 3.5% PEO, 0.5% HPMC and 2% Tween 80 by using different voltages

Factor	Type	Levels	Values
Protein	fixed	2	1,5; 2,0
Voltage	fixed	2	7; 10

Analysis of Variance for Diameter, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Protein	1	1552	1552	1552	0,99	0,320
Voltage	1	93086	93086	93086	59,35	0,000
Protein*Voltage	1	41250	41250	41250	26,30	0,000
Error	396	621052	621052	1568		
Total	399	756940				

S = 39,6019 R-Sq = 17,95% R-Sq(adj) = 17,33%

Unusual Observations for Diameter

Obs	Diameter	Fit	SE Fit	Residual	St Resid
1	86,000	219,760	3,960	-133,760	-3,39 R
2	96,000	219,760	3,960	-123,760	-3,14 R
3	118,000	219,760	3,960	-101,760	-2,58 R
100	310,000	219,760	3,960	90,240	2,29 R
101	312,000	203,390	3,960	108,610	2,76 R
200	74,000	203,390	3,960	-129,390	-3,28 R
201	96,000	229,960	3,960	-133,960	-3,40 R
301	121,000	254,210	3,960	-133,210	-3,38 R
302	159,000	254,210	3,960	-95,210	-2,42 R
396	346,000	254,210	3,960	91,790	2,33 R
397	349,000	254,210	3,960	94,790	2,41 R
398	376,000	254,210	3,960	121,790	3,09 R
399	400,000	254,210	3,960	145,790	3,70 R
400	511,000	254,210	3,960	256,790	6,52 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein	N	Mean	Grouping
2,0	200	228,8	A
1,5	200	224,9	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Voltage	N	Mean	Grouping
10	200	242,1	A
7	200	211,6	B

Table A.23. (Continued)

```
Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein  Voltage    N   Mean  Grouping
2,0      10       100  254,2  A
1,5      10       100  230,0    B
1,5       7       100  219,8    B
2,0       7       100  203,4      C

Means that do not share a letter are significantly different.
```

Table A.24. Two way ANOVA and Tukey's comparison test for diameter values of nanofibers obtained from electrospinning solutions prepared at pH 10 by using 3.5% PEO, 0.5% HPMC and 2% Tween 80 by using different flow rates

```
Factor     Type   Levels  Values
Protein    fixed       2  1,5; 2,0
Flow Rate  fixed       2  0,6; 1,0


Analysis of Variance for Diameter, using Adjusted SS for Tests

Source              DF  Seq SS  Adj SS  Adj MS      F      P
Protein              1    1099    1099    1099   0,58  0,446
Flow Rate            1   93300   93300   93300  49,44  0,000
Protein*Flow Rate    1   43827   43827   43827  23,23  0,000
Error              396  747281  747281    1887
Total              399  885507


S = 43,4405   R-Sq = 15,61%   R-Sq(adj) = 14,97%


Unusual Observations for Diameter

Obs  Diameter      Fit  SE Fit  Residual  St Resid
  1    96,000  229,960   4,344  -133,960     -3,10 R
101   121,000  254,210   4,344  -133,210     -3,08 R
102   159,000  254,210   4,344   -95,210     -2,20 R
196   346,000  254,210   4,344    91,790      2,12 R
197   349,000  254,210   4,344    94,790      2,19 R
198   376,000  254,210   4,344   121,790      2,82 R
199   400,000  254,210   4,344   145,790      3,37 R
200   511,000  254,210   4,344   256,790      5,94 R
201    99,000  220,350   4,344  -121,350     -2,81 R
202   111,000  220,350   4,344  -109,350     -2,53 R
299   321,000  220,350   4,344   100,650      2,33 R
300   395,000  220,350   4,344   174,650      4,04 R
301    94,000  202,730   4,344  -108,730     -2,52 R
302    96,000  202,730   4,344  -106,730     -2,47 R
303   104,000  202,730   4,344   -98,730     -2,28 R
```

Table A.24. (Continued)

```
304   107,000  202,730   4,344   -95,730     -2,21 R
305   114,000  202,730   4,344   -88,730     -2,05 R
306   114,000  202,730   4,344   -88,730     -2,05 R
395   294,000  202,730   4,344    91,270      2,11 R
396   330,000  202,730   4,344   127,270      2,94 R
397   331,000  202,730   4,344   128,270      2,97 R
398   338,000  202,730   4,344   135,270      3,13 R
399   338,000  202,730   4,344   135,270      3,13 R
400   344,000  202,730   4,344   141,270      3,27 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein    N   Mean  Grouping
2,0      200  228,5  A
1,5      200  225,2  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flow
Rate    N   Mean  Grouping
0,6   200  242,1  A
1,0   200  211,5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

         Flow
Protein  Rate    N   Mean  Grouping
2,0      0,6   100  254,2  A
1,5      0,6   100  230,0    B
1,5      1,0   100  220,3    B
2,0      1,0   100  202,7      C

Means that do not share a letter are significantly different.
```

Table A.25. Two way ANOVA and Tukey's comparison test for water vapor permeability values of nanofibers obtained from electrospinning solutions prepared at pH 10 by using 3.5% PEO and 2% Tween 80 by using different lentil flour and HPMC concentrations

```
Factor                        Type   Levels  Values
Lentil Flour Concentration %  fixed       2  2,00; 5,25
HPMC Concentration %          fixed       2  0,0; 0,5
```

Table A.25. (Continued)

Analysis of Variance for Water Vapor Permeability, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
Lentil Flour Concentration %	1	2,18594E+17	2,18594E+17	2,18594E+17	39,48
HPMC Concentration %	1	9,05786E+15	9,05786E+15	9,05786E+15	1,64
Lentil Flour Concentration %* HPMC Concentration %	1	1,82196E+16	1,82196E+16	1,82196E+16	3,29
Error	4	2,21485E+16	2,21485E+16	5,53712E+15	
Total	7	2,68020E+17			

Source	P
Lentil Flour Concentration %	0,003
HPMC Concentration %	0,270
Lentil Flour Concentration %* HPMC Concentration %	0,144
Error	
Total	

S = 74411814 R-Sq = 91,74% R-Sq(adj) = 85,54%

Grouping Information Using Tukey Method and 95,0% Confidence

Lentil Flour Concentration %	N	Mean	Grouping
5,25	4	1,47920E+09	A
2,00	4	1,14860E+09	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

HPMC Concentration %	N	Mean	Grouping
0,0	4	1,34755E+09	A
0,5	4	1,28025E+09	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Lentil Flour Concentration %	HPMC Concentration %	N	Mean	Grouping
5,25	0,0	2	1,56057E+09	A
5,25	0,5	2	1,39783E+09	A B
2,00	0,5	2	1,16268E+09	B
2,00	0,0	2	1,13453E+09	B

Means that do not share a letter are significantly different.

Table A.26. Two way ANOVA and Tukey's comparison test for color parameter L^* values of nanofibers obtained from electrospinning solutions prepared at pH 10 by using 3.5% PEO and 2% Tween 80 by using different lentil flour and HPMC concentrations

Factor	Type	Levels	Values
Lentil Flour Concentration %	fixed	2	2,00; 5,25
HPMC Concentration %	fixed	2	0,0; 0,5

Analysis of Variance for L, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Lentil Flour Concentration %	1	12,38	12,38	12,38	0,66	0,463
HPMC Concentration %	1	49,15	49,15	49,15	2,61	0,182
Lentil Flour Concentration %* HPMC Concentration %	1	25,67	25,67	25,67	1,36	0,308
Error	4	75,46	75,46	18,86		
Total	7	162,65				

S = 4,34326 R-Sq = 53,61% R-Sq(adj) = 18,82%

Grouping Information Using Tukey Method and 95,0% Confidence

Lentil Flour Concentration %	N	Mean	Grouping
5,25	4	75,6	A
2,00	4	73,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

HPMC Concentration %	N	Mean	Grouping
0,5	4	76,8	A
0,0	4	71,9	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Lentil Flour Concentration %	HPMC Concentration %	N	Mean	Grouping
2,00	0,5	2	77,4	A
5,25	0,5	2	76,3	A
5,25	0,0	2	74,9	A
2,00	0,0	2	68,8	A

Means that do not share a letter are significantly different.

Table A.27. Two way ANOVA and Tukey's comparison test for color parameter a* values of nanofibers obtained from electrospinning solutions prepared at pH 10 by using 3.5% PEO and 2% Tween 80 by using different lentil flour and HPMC concentrations

```
Factor                          Type   Levels  Values
Lentil Flour Concentration %  fixed       2  2,00; 5,25
HPMC Concentration %          fixed       2  0,0; 0,5


Analysis of Variance for a, using Adjusted SS for Tests

Source                          DF    Seq SS    Adj SS    Adj MS      F
P
Lentil Flour Concentration %     1  0,014450  0,014450  0,014450   1,77
0,254
HPMC Concentration %             1  0,039200  0,039200  0,039200   4,80
0,094
Lentil Flour Concentration %*    1  0,101250  0,101250  0,101250  12,39
0,024
  HPMC Concentration %
Error                            4  0,032700  0,032700  0,008175
Total                            7  0,187600


S = 0,0904157   R-Sq = 82,57%   R-Sq(adj) = 69,50%


Grouping Information Using Tukey Method and 95,0% Confidence

Lentil Flour
Concentration %  N  Mean  Grouping
5,25             4   1,2  A
2,00             4   1,1  A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

HPMC
Concentration %  N  Mean  Grouping
0,0              4   1,2  A
0,5              4   1,1  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Lentil Flour     HPMC
Concentration %  Concentration %  N  Mean  Grouping
5,25             0,0             2   1,4  A
2,00             0,5             2   1,2  A
2,00             0,0             2   1,1  A
5,25             0,5             2   1,0  A

Means that do not share a letter are significantly different.
```

Table A.28. Two way ANOVA and Tukey's comparison test for color parameter b^* values of nanofibers obtained from electrospinning solutions prepared at pH 10 by using 3.5% PEO and 2% Tween 80 by using different lentil flour and HPMC concentrations

Factor	Type	Levels	Values
Lentil Flour Concentration %	fixed	2	2,00; 5,25
HPMC Concentration %	fixed	2	0,0; 0,5

Analysis of Variance for b, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Lentil Flour Concentration %	1	0,0481	0,0480	0,0480	0,10	0,767
HPMC Concentration %	1	0,0264	0,0265	0,0265	0,06	0,826
Lentil Flour Concentration %* HPMC Concentration %	1	0,7813	0,7813	0,7813	1,64	0,270
Error	4	1,9110	1,9110	0,4778		
Total	7	2,7668				

S = 0,691195 R-Sq = 30,93% R-Sq(adj) = 0,00%

Grouping Information Using Tukey Method and 95,0% Confidence

Lentil Flour Concentration %	N	Mean	Grouping
5,25	4	3,2	A
2,00	4	3,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

HPMC Concentration %	N	Mean	Grouping
0,0	4	3,2	A
0,5	4	3,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Lentil Flour Concentration %	HPMC Concentration %	N	Mean	Grouping
5,25	0,5	2	3,5	A
2,00	0,0	2	3,4	A
5,25	0,0	2	3,0	A
2,00	0,5	2	2,7	A

Means that do not share a letter are significantly different.

Table A.29. Two way ANOVA and Tukey's comparison test for color parameter ΔE^* values of nanofibers obtained from electrospinning solutions prepared at pH 10 by using 3.5% PEO and 2% Tween 80 by using different lentil flour and HPMC concentrations

Factor	Type	Levels	Values
Lentil Flour Concentration %	fixed	2	2,00; 5,25
HPMC Concentration %	fixed	2	0,0; 0,5

Analysis of Variance for ΔE, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Lentil Flour Concentration %	1	11,64	11,64	11,64	0,62	0,477
HPMC Concentration %	1	47,72	47,72	47,72	2,53	0,187
Lentil Flour Concentration %* HPMC Concentration %	1	25,69	25,69	25,69	1,36	0,308
Error	4	75,60	75,60	18,90		
Total	7	160,65				

S = 4,34737 R-Sq = 52,94% R-Sq(adj) = 17,65%

Grouping Information Using Tukey Method and 95,0% Confidence

Lentil Flour Concentration %	N	Mean	Grouping
2,00	4	20,5	A
5,25	4	18,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

HPMC Concentration %	N	Mean	Grouping
0,0	4	21,7	A
0,5	4	16,8	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Lentil Flour Concentration %	HPMC Concentration %	N	Mean	Grouping
2,00	0,0	2	24,7	A
5,25	0,0	2	18,7	A
5,25	0,5	2	17,4	A
2,00	0,5	2	16,2	A

Means that do not share a letter are significantly different.