

CHARACTERIZATION OF PEA FLOUR BASED
NANOFIBERS PRODUCED BY ELECTROSPINNING METHOD

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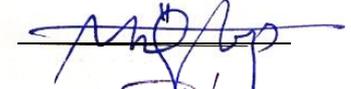


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ABSTRACT

CHARACTERIZATION OF PEA FLOUR BASED NANOFIBERS PRODUCED BY ELECTROSPINNING METHOD

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Electrospinning is a process that produces continuous nanofibers through the action of an electric field imposed on a polymer solution. In this thesis, it was aimed to produce pea flour and hydroxypropyl methylcellulose (HPMC) based homogenous nanofibers by electrospinning. The effects of pH, pea flour and HPMC concentration, and microfluidization on apparent viscosity, electrical conductivity of solutions and nanofiber characteristics were studied. In addition, the effects of voltage and flow rate were analyzed. Solutions were prepared at different pH values (7, 10, 12), with different pea flour concentrations (1%, 2% (w/v)) and HPMC concentrations (0.25%, 0.5%, 1.0% (w/v)). For all pea flour concentrations, increase in pH cause a significantly rise in consistency coefficient (k). Increase in pea flour concentration also increased consistency coefficient for alkali conditions. Electrical conductivities were not affected by pH, pea flour and HPMC concentration significantly. It was possible to obtain homogenous fibers when solutions were prepared at basic pH. On the other hand, neutral solutions produced

fibers with beads. The increase in pH, flour and HPMC concentration increased nanofiber diameter. Nanofibers obtained from solution containing 1% pea flour and 0.25% or 0.5% HPMC at pH value of 10 had the smallest diameter ranging from 177 to 179 nm. Microfluidization cause an increase in consistency coefficient and a deformation on the fiber morphology. Nanofibers were characterized by water vapor permeability (WVP) and color. Flour concentration increased WVP but did not affect color. The composition of solutions was suitable for electrospinning process which was confirmed by Fourier-transform infrared (FTIR) analysis.

Keywords: Nanofiber, pea flour, pH, hydroxypropyl methylcellulose (HPMC), electrospinning

ÖZ

ELEKTROEĞİRME METODUYLA BEZELYE UNUNDAN ÜRETİLEN NANOLİFLERİN KARAKTERİZASYONU

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Elektroeğirme, elektrik alanının bir polimer çözeltisi üzerine uygulanarak çözülden homojen nanolif üretme işlemidir. Bu tezde, bezelye unu ve hidrokispropil metilselüloz (HPMC) polimerinin karıştırılmasıyla hazırlanan çözülden elektroeğirme metoduyla homojen nanolif üretimi amaçlanmıştır. pH, bezelye unu ve HPMC konsantrasyonunun ve mikroakışkanlaştırmanın çözümlerin görünür viskozitesi, elektriksel iletkenliği, nanolif morfolojisi ve nanolif çapı üzerindeki etkisi çalışılmıştır. Ayrıca, voltaj ve akış hızının nanolif çapına olan etkisi analiz edilmiştir. Çözümler farklı pH değerlerinde (7, 10, 12), farklı bezelye unu konsantrasyonlarında (%1 ve %2 (g/ml)) ve farklı HPMC konsantrasyonlarında (%0.25, %0.5, %1.0 (g/ml)) hazırlanmıştır. Her iki un konsantrasyonu için kıvam katsayısı (k) pH artışı ile önemli oranda artmıştır. Bezelye unu konsantrasyonunun artması da alkali çözümlerde kıvam katsayısında önemli oranda artışa sebep olmuştur. Elektrik iletkenlikleri pH, un konsantrasyonu ve HPMC konsantrasyonu değişiminden etkilenmemiştir. Çözümler alkali pH değerlerinde hazırlandığında homojen nanolif üretilebilmiştir. Diğer yandan, nötr çözümlerden elde edilen

nanoliflerde boncuk oluşumu gözlenmiştir. PH, bezelye unu ve HPMC konsantrasyonunun artması nanolif çapında artışa sebep olmuştur. Hem %0.25 hem de %0.5 HPMC konsantrasyonuna sahip ve pH değeri 10'da hazırlanan, %1 bezelye unu içeren çözeltilerden elde edilen nanolifler, sırasıyla 177 ve 179 nm çapında olup, en düşük çapa sahiplerdir. Mikroakışkanlaştırma ise çözeltilerin kıvam katsayısında artışa sebep olmuştur; ama bu homojenleştirme tekniğinin nanolif morfolojisinde negatif etkisi görülmüştür. Nanoliflerin su buharı geçirgenliği ve renk analizleri yapılmıştır. Un konsantrasyonu, su buharı geçirgenliğinde artışa sebep olurken; nanolif rengine etki etmemiştir. Fourier dönüşümlü kızılötesi (FTIR) analizi ile çözeltilerin polimer içeriği elektroegirme işlemi için uygun bulunmuştur.

Anahtar kelimeler: Nanolif, bezelye unu, pH, hidroksipropil metilselüloz (HPMC), elektroegirme

To my beloved family, without whom I could have never come so far...

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CHAPTER 1

INTRODUCTION

1.1 Nanotechnology

Nanotechnology is introduced as the design, characterization, application and production of materials, devices and systems by controlling the size and shape of the nanoscale (Neethirajan & Jayas, 2011). Nanotechnology and nanoscience cover a wide range of fields from biology, physics and chemistry to engineering, medicine and electronics. Application fields of nanotechnology are categorized into four groups as: nanomaterials, nanomedicine, nanometrology, electronics, optoelectronics, and information and communication technology (Dowling et al., 2004). The nanoscale is considered to involve the range from 1 to 100 nm (Ramsden, 2011). Nanomaterials have the unique advantages such as high surface area, flexibility in surface functionality, and highly porous membranes with perfect interconnectivity. These properties have created enhancements in a wide range of fields such as biomedical, electronics, healthcare, cosmetics, protective clothing, environmental protection, and filtration. The most investigated forms of nanomaterials are nanofibers, nanotubes, nanoparticles, and nanowires (Afshari, 2017). Nanotechnology has gained importance in food technology. The driving force for this development stems from the enormous potential benefits of nanotechnology in specific areas of food science and technology, both in food processing and safety. Most progress has been made so far in food quality monitoring, food packaging and encapsulation and delivery of nutraceuticals. Recent reviews have been published highlighting the latest developments and future directions for the use of nanotechnology in the food area (Lopes Da Silva, 2012).

1.2 Electrospinning

Nanofibers have gained lots of interest from both industry and academia. There are several methods consist of electrospinning, drawing, melt or solution blowing, template synthesis, self-assembly, and phase separation in order to produce fibers from a few to several tens of nanometers when they are necessary for specific applications (Afshari, 2017; Huan et al., 2015). A comparison of various capabilities related to some methods for obtaining nanofiber is given in Table 1.1. Apart from other methods, electrospinning can be seen as the most widely studied technique because it is easy to handle, convenient to process, cost effective, simple and reproducible in fiber processing. Moreover, it requires minimum consumption of solution, and it has controllable fiber diameter, (Ramakrishna, Fujihara, Teo, Lim, & Ma, 2005; Thenmozhi, Dharmaraj, Kadirvelu, & Kim, 2017). In addition, these unique capabilities allow this method to obtain nanofibers from different materials such as polymers and ceramics with different morphologies, functionalities and patterns (Afshari, 2017). The electrospun nanofibers offer several remarkable features which are high aspect ratio, large surface area, flexible surface functionality, controllable surface morphologies and excellent mechanical performance(Sun et al., 2014).

Table 1.1 Comparison of processing methods to produce nanofibers (Ramakrishna et al., 2005)

Process	Advances	Process can be scaled.	Repeatability	Convenient process	Control on fiber size
Drawing	Laboratory	X	✓	✓	X
Template Synthesis	Laboratory	X	✓	✓	✓
Self-Assembly	Laboratory	X	✓	X	X
Phase-Separation	Laboratory	X	✓	✓	X
Electrospinning	Laboratory	✓	✓	✓	✓

It is well-understood that electrospinning is already used for nanotechnology by producing nanofibers. Apart from food industry, there were several applications of electrospun nanofibers including filtration, catalyst, tissue engineering scaffolds, affinity membranes and recovery of metal ions, wound healing, drug release, and energy storage. In food science, it is used in enzyme immobilization, enzymatic membrane reactors, sensors, and encapsulation of food bioactive compounds and cells (Lopes Da Silva, 2012). Electrospinning is a novel technology for creating nanofibers from a synthetic or natural polymer solution by using an electric field (Shanette, 2006).

Figure 1.1 shows schematic illustration of electrospinning device. For this device, the typical setup composes of a high voltage source (1-30 kV), a blunt ended stainless steel needle, a syringe pump and a grounded collector flat plate which are four essential components (Anu Bhushani & Anandharamakrishnan, 2014). At the beginning of working principle of electrospinning, the polymer solution in the needle is brought to the needle tip to form a droplet as a result of the pump pressure.

When the sufficient high voltage (10-19 kV) is applied between the needle and a grounded collecting plate, the droplets are intensively charged. Then, the droplet is influenced by two types of electrostatic forces. The first one is the electric field force created between the needle and the collector. The second one is the repulsive forces between same charges distributed on the surface of droplet (Dabirian, Hosseini Ravandi, Pischevar, & Abuzade, 2011). For a fixed quantity of fluid, the Coulomb repulsion between the ions, which carry charges, helps the formation of shapes such as a jet, while the surface tension caused sphere-like shapes with smaller surface area/unit mass (Reneker & Yarin, 2008). Once a spherical liquid droplet is exposed to an electrical field for the first time, the spherical shape can not continue to be stable beyond a threshold of electric potential difference. The electrical potential of the needle increases until the electrical forces overcome the surface tension forces of the liquid droplet. The ensuing imbalance in the forces causes a jet to launch from the liquid droplet. After, Taylor Cone-jet is obtained as a structure for a range of applied voltage corresponding to a stable conical liquid meniscus at the needle exit with a tiny jet at the end. At the same time, the solvent in the liquid jet evaporates as long as the jet moves towards a grounded plate. Therefore, the jet dries to become a solid fiber (Morad, Rajabi, Razavi, & Pejman Sereshkeh, 2016; Shahreen & Chase, 2015).

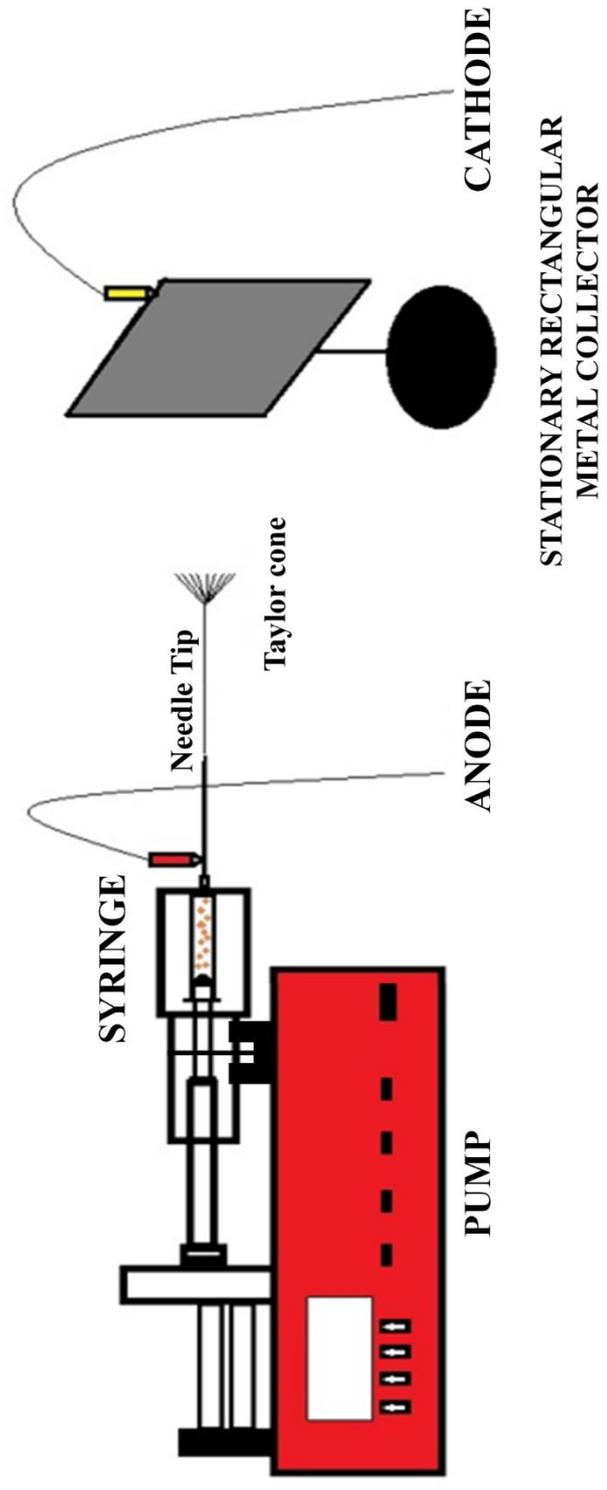


Figure 1.1 Diagram of electrospinning apparatus

There are a wide variety of parameters that play significant roles in producing the desired homogeneous nanofiber diameter which are namely properties of electrospinning solutions (viscosity, solution conductivity, surface tension), process parameters (applied voltage, feed rate, distance between needle tip and collector), and ambient parameters (temperature and humidity) (Ghelich, Rad, & Youzbashi, 2015; Rijal, Adhikari, & Bhattarai, 2017).

Viscosity of the polymer solution used in electrospinning is a key property which affects spinnability. It is controlled by both the polymer concentration and the used solvent type. Viscosity of solution is critical for the creation of a stable jet of polymer solution. Among the affecting parameters, the viscosity of precursor solution has uncertain impacts on bead formation and diameter of fibers (Villarreal-Gómez, Cornejo-Bravo, Vera-Graziano, & Grande, 2016). If the viscosity is too low, the polymer molecules will not be entangled. Then, the electrospinning process may cause bead or droplet formation. On the other hand, an extremely high viscosity will result in obstructing the flow of polymer solution through the capillary. Then, it leads to localized gel formation, which will prevent fiber formation (Liu, Gu, Hong, Cheng, & Li, 2017). In other words, an optimum viscosity, which means not too low or too high, is required to prepare bead-free fibers.

Electrical conductivity determines the ability of electrical charge to move to the surface of the pendant droplet, directly affecting the creation of an electrostatic repulsion force that is critical to initiate jetting (Vega-Lugo & Lim, 2012). In other words, electrospinning process requires the transfer of electric charges from the electrode to the spinning droplet. Since a certain electrical conductivity is essential for nanofiber formation, solutions with zero conductivity cannot be electrospun into nanofibers. Solution conductivity is basically determined by the polymer type, polymer concentration, solvent used, temperature and the presence of ionizable salts. In case the polymer in solution has ionic functionalities, the conductivity depends on the polymer concentration (Bhardwaj & Kundu, 2010; Okutan, Terzi,

& Altay, 2014). Usage of salts could increase electrical conductivity, which could increase the uniformity of fibers and decrease bead generation (Bhardwaj & Kundu, 2010; Li & Wang, 2013; Thenmozhi et al., 2017). The high conductivity is desirable in electrospinning since it increases the repulsive charges on the suspended droplet at the needle tip. However, the higher conductivity leads greater surface charge to move through the spinning jet. Therefore, greater electrostatic repulsion force is responsible for the bending instability and stretching that is critical to create submicron fibers (Vega-Lugo & Lim, 2012). In literature, optimum electrical conductivity is reported for solutions to produce homogenous nanofibers.

Natural polymers show higher surface tension which results in poor nanofiber formation as opposed to the synthetic polymers. Surface tension should be overcome by repulsive force so that jet erupts from the tip of needle at a critical voltage. In literature, it was stated that surface tension increased bead formation. Therefore, decreased surface tension is beneficial for the formation of fibers without beads. Surface tension depends on the used polymers and solvent (Fong, Chun, & Reneker, 1999).

A high voltage source is the main part of electrospinning device since it determines electric field between tip of needle and collector (Figure 1.1). Nanofiber morphology is influenced by applied voltage. The alteration in nanofiber morphology with voltage is correlated to alterations in the droplet shape. At low voltage values, the droplet remains pendant at the tip of the needle. As voltage increases, the volume of the externally visible droplet decreases (Deitzel, Kleinmeyer, Harris, & Beck Tan, 2001). Increase in applied voltage results in an enhancement in fiber size since solution moves faster from needle tip to collector. This caused polymer jet to be stretched in less time and jet length increases, resulting in bigger nanofiber size (Adabi, Saber, Faridi-Majidi, & Faridbod, 2015; Baumgarten, 1971).

Flow rate, which is known as feed rate, influences nanofiber morphology. Adabi and coauthors (2015) found that the effect of flow rate on the nanofiber size is contradictory. They recognized that enhancement in flow rate of polymer solution led to increase in nanofiber size which was not in accordance with literature. However, according to another research, flow rate was shown to have a minor effect on fiber size (De Schoenmaker, Van Der Schueren, Ceylan, & De Clerck, 2012).

The distance between the needle tip and the collecting plate may also affect the fiber diameter and morphologies. In short, if the distance is too short, there will be not enough time for the solution to solidify until reaching the grounded plate, while if the distance is too long, fibers with bead may be produced. An optimum distance is recommended because of the significant physical feature of the electrospun nanofiber that is the dryness from solvent meaning evaporation of solvent in liquid jet (Li & Wang, 2013). In literature, no significant difference in uniformity of fibers was reported with increase in distance. Besides, fiber diameter was shown to decrease with increase in distance from tip of needle to collector (Yuan, Zhang, Dong, & Sheng, 2004).

1.3 Nanofibers

Generally, polymers in electrospinning are divided into groups which are synthetic polymers and natural polymers. Traditionally, electrospun nanofibers have been produced from single polymer sources. However, more recently, polymer mixtures have been used to obtain so-called polyblend nanofibers.

1.3.1 Synthetic nanofibers

Synthetic polymers are found to be dissolved in an organic solvent easily; therefore, they are often electrospun from organic solutions. For electrospinning, volatility, toxicity and effectivity in dissolving the polymer are the properties that belong to not all organic solvents available for a given polymer (Lu, Zhu, Guo, Hu, & Yu, 2006; Salas, 2017). Generally, synthetic polymers are chosen for the production of nanofibers since they have high availability, low cost and a wide variety of well-defined functional and molecular characteristics (Kriegel, Arrechi, Kit, McClements, & Weiss, 2008). Synthetic polymers have high flexibility in modification and synthesis; however, they have low hydrophilicity. For drug delivery, synthetic ones which are especially biodegradable polymers, gained private attention in electrospinning by eliminating a second surgery to remove the implanted carrier. For tissue engineering and drug delivery applications, polymers such as poly(ϵ -caprolactone) (PCL), poly (lactic acid) (PLA), and copolymers, such as poly(lactide-co-caprolactone) (PCLA) and poly (lactic-co-glycolic acid) (PLGA), have been comprehensively studied to produce fibers with desired features. Since synthetic polymers are water-soluble such as PEO (poly(ethylene oxide) and poly (vinyl alcohol)(PVA), they can also be electrospun from aqueous solutions (Liu et al., 2017). Non-biodegradable synthetic polymers were used to obtain biodegradable copolymer by blending with biodegradable polymers (Cohn, Stern, Gonza, & Epstein, 2001). Several (bio) polymers, most of which are non-degradable, include PVA, PEO, poly (glycolic acid) (PGA), poly (lactic-co-glycolic acid) (PLGA), poly(ϵ -caprolactone) (PCL). They have been successfully fabricated into ultrafine (nano/micro) fibers by using electrospinning (Villarreal-Gómez et al., 2016).

1.3.2 Natural nanofibers

Compared to synthetic polymers, natural biopolymers which are carbohydrates and proteins derived from animal or plant, are biodegradable and biocompatible (Hu et al., 2014; Liu et al., 2017). If a polymer is degraded by the biological activity of microorganisms or enzymes, it is biodegradable polymer (Villarreal-Gómez et al., 2016). Recently, there has been an increase in production of biopolymer-based nanofibers rather than synthetic materials.

In literature, there are a few studies about natural biopolymer based nanofibers by electrospinning method. According to researches on electrospinning of biopolymer based solutions, biopolymers that are used are comprised of two groups which are proteins and polysaccharides. In protein group, there are gelatin, collagen, elastin, silk, soy zein, hordein, casein, amaranth, whey and wheat while there are chitosan, starch, alginate, cellulose, cellulose derivatives, pullulan, dextran and cyclodextrins in polysaccharide group (Mendes, Stephansen, & Chronakis, 2016). Instead of cellulose, soluble derivatives of cellulose that are the cellulose acetate, hydroxy ethyl cellulose, ethyl cellulose and hydroxypropyl methylcellulose (HPMC) are most commonly used as nanofiber scaffolds (Esentürk, Erdal, & Güngör, 2016).

Natural polymers are generally polyelectrolytes, where the charge carrying ability of the polymer jet are increased by the ions, exposing to higher tension under the electrical potential. This results in the poor fiber formation differed from the synthetic counterpart (Bhardwaj & Kundu, 2010; Li & Wang, 2013; Mendes et al., 2016; Thenmozhi et al., 2017). Also, there were limited researches of natural biopolymers electrospun from organic solutions because an organic solvent may not be easily found for biopolymers. In other words, the reason is that many biopolymers have difficulty with dissolving in water and most organic solvents. In case that they dissolve, it is needed to use a coadjutant polymer such as synthetic one whose usage was favorable to adjust the viscosity and improve mechanical properties of the electrospun nanofibers (Salas, 2017).

1.3.3 Polyblend nanofibers

Polyblend nanofibers are obtained from mixtures of synthetically and naturally derived polymers to display unique combinations of structural, mechanical and biochemical properties. This flexibility has been an advantage for the application of polyblend nanofibers in drug delivery and tissue engineering systems. For biomedical applications, nanofibers have been produced from synthetic or naturally derived polymers by established techniques, one of which is electrospinning. Nanofibers from multiple or premixed polymer solutions have lots of advantages in contrast to single-component systems. Firstly, they have the combination of the characteristic attributes of several unique polymers rather than demonstrating the singular properties of an individual polymer. Even though any particular polymer can be lack of the structural or chemical dimensions of native tissue, mixture of polymers can draw advantage from the varying strengths and bioactivities of each polymers (Gunn & Zhang, 2010). Secondly, some natural polymers are not compatible to electrospinning due to their solubility or molecular weight. Otherwise, biopolymers including chitosan, collagen, silk and soluble eggshell membrane proteins are generally dissoluble in aqueous solutions; however, it seems difficult to electrospin them alone from aqueous solutions (Lu et al., 2006). They can be successfully electrospun by using a non-toxic, non-ionic and biocompatible synthetic polymer PVA or PEO since the electrospinnable carrier polymers which enhance the processability of natural polymers while keeping their biocompatibility (Salas, 2017; Tort & Acarturk, 2016). Hydrophilic polymers such as PVA or PEO are widely used as coadjuvant polymers to obtain biopolymer scaffolds with electrospinning method (Salas, 2017). In recent literature, there were some examples on the electrospinning of solution from polymer blends. Lu and coauthors (2006) worked with alginate and PEO to obtain homogenous nanofiber that could take the benefit from hydrogen bonds between the polyelectrolytic natural polymer and the non-ionogenic synthetic polymer, PEO. The effects of electrospinning on mechanical properties of chitosan/PVA nanofibers were studied (Koosha & Mirzadeh, 2015). It can be explained that the repulsion force between polycationic

chitosan molecules is decreased to a certain degree, which favored electrospinning by combining with PVA (Li & Hsieh, 2006). Huang and coauthors (2001) had an investigation on engineered collagen-PEO nanofibers and fabrics. Moreover, Cho et al. (2010) produced nanofibers from a solution containing soy protein isolate and PVA.

1.4 Possibility of nanofiber formation from PEO, pea flour and HPMC blending solution by electrospinning

In literature, polymers used in nanofiber formation were also used in edible film formation. For example, chitosan, which can be used for nanofiber formation by using electrospinning method (Koosha & Mirzadeh, 2015), was also used in edible film formation (Pal Singh, Kumar Chatli, & Sahoo, 2014). Similarly, soy protein has been used in nanofiber formation (Cho et al., 2010) and edible film formation (Soliman, Tawfik, El-Sayed, & Moharram, 2007).

In nanofiber formation, PEO has already been used alone or blended with other polymers as mentioned in section 1.3.3. PEO can be electrospun from aqueous solutions (Liu et al., 2017). In addition, PEO is water soluble, biocompatible and non-biodegradable (Villarreal-Gómez et al., 2016).

Legumes have been known as a valuable and low cost source of high quality protein products such as flour, isolates and concentrates (M. B. Barać, Pešić, Stanojević, Kostić, & Čabrilo, 2015). Peas (*Pisum sativum L.*) are becoming a significant source of proteins in Europe. The increased usage of pea proteins is due to their enhanced functional attributes in food area, high nutritional value, high availability, and relatively low cost (M. Barać et al., 2010). Whole peas, split peas and pea ingredients are generally used in feed and food industries. Pea flour, which is produced from whole pea or split pea, is becoming major optional components by

presenting possibilities to add value for the food industry due to production maintenance, allergen-free, low carbon footprint, non-GMO, clean label and single-ingredient trends in the global market. Furthermore, the major properties of pea flour are oil binding, water-binding, gelation, emulsification, foaming and texturizing (Tulbek, Lam, Wang, Asavajaru, & Lam, 2017). Since pea flour is introduced as a polysaccharide, protein and mineral compounds rich raw material, it has good emulsifying and foaming capacity (Kohajdová, Karovičová, & Magala, 2013). In literature, production of nanofibers containing pea protein or derivative has not been reported yet. However, there were some works on production of edible films from pea starch, denatured pea protein concentrate (PPC) solution (Choi & Han, 2001; Saberi, Thakur, Bhuyan, et al., 2016; Saberi, Thakur, Vuong, et al., 2016; Sun, Sun, & Xiong, 2013; Zhang & Han, 2006).

HPMC backbone is composed of linkage in glucose units by β -1,4-glycosidic bonds. The hydrophobic (methyl) and hydrophilic (hydropropyl) parts are distributed throughout the cellulose backbone (Lim, Gwon, Jeun, & Nho, 2010). There is no problem with HPMC usage because hydrophilic non-ionic derivatives of cellulose, HPMC, are physiologically safe and are preferred to be used in foodstuffs and pharmaceutical industry (Sovilj & Petrovic, 2006). In the food industry, HPMC, which is a modified chemically polysaccharide, is used to control the texture and rheological attributes of dispersions. It is used as an emulsifier for its water binding capacity (Perez, Sanchez, Pílosof, & Rodríguez Patino, 2008). HPMC was also used with Aloe-vera for water retention capacity (Uslu, Keskin, Gül, Karabulut, & Aksu, 2010). HPMC is a viscoelastic polymer and the most commonly used polymers for hydrophilic matrices (Phadtare, Phadtare, & Asawat, 2014). When adsorbed at fluid interface, it can reduce the surface tension. OH⁻ groups of HPMC molecule participate in intermolecular hydrogen bonds that allows the cellulose chains to create fibrils or bundles (Lim et al., 2010). HPMC is a hydrophilic cellulose ether hydrocolloid with enhanced film forming properties. Bilbao-Sáinz and coauthors (2010) studied about reinforcement of HPMC based edible films. Osorio et al. (2011) also investigated the characteristics of HPMC

based edible film developed for blueberry coatings. Nanofibers from HPMC can be a candidate for packaging material.

Surfactants are generally added in the electrospinning solution which are Tween® 80, Pluronic F68, Sodium dodecyl sulfate (SDS) and Vitamin E-TPGS. Electrospinning researches showed that the availability of various surfactants did not adversely influence formation or characteristics fiber (Ignatious, Sun, Lee, & Baldoni, 2010). The presence of the non-ionic surfactant Tween80 appears to have a synergistic effect with the polymers present in the formulations (Nazari et al., 2017).

For packaging development being one of food application areas, natural polymers can be an alternative source due to their palatability and biodegradability. There has been an attention in producing edible film from renewable and natural polymer such as polysaccharide and protein. They may be used for versatile food goods to lessen moisture loss, to limit oxygen absorption, to reduce lipid migration, to improve mechanical handling attributes, to offer physical protection and to give an alternative to the commercial packaging materials (Bourtoom, 2009). Generally, there are some advantages and disadvantages about protein and carbohydrate based edible films. Polysaccharide based films display superior gas permeability attributes because of the make up of the polymer chains, leading to desirable modified atmospheres which improve the shelf life of the product without forming anaerobic conditions. In addition, polysaccharide based films and coatings can be used for increase in the shelf-life of foods by preventing oxidative rancidity, dehydration, and browning. However, their hydrophilic nature causes them to be poor barriers for water vapor (Dhanapal et al., 2012). The mechanical and gas barrier attributes of protein-based edible films are much better than those of polysaccharide and lipid based films since proteins have a specific structure (based on 20 different monomers) that gives a wider range of functional attributes, one of which is the high intermolecular binding potential. Nevertheless, protein based films have poor water vapor resistance and lower mechanical strength in contrast to

synthetic polymers restrict their application in food packaging. Thus, because protein and carbohydrate have advantages separately, making film from them can combine their advantages. For packaging, making nanofiber is also more advantageous as similar as making film. HPMC as a polysaccharide and pea flour, containing protein and polysaccharide, can be good candidates to be used as packaging material due to their mechanical and barrier properties. So far, no nanofiber was produced from and also from blending solution of pea flour and HPMC.

1.5 The objectives of the study

Electrospinning method has gained more interest in recent years due to its simplicity and potential in various applications. Electrospun nanofibers have been broadly investigated for its use in various applications. In food application, researchers have recently become more interested in studying nanofibers. Researches on pea flour have increased since it is rich in protein, polysaccharide, and mineral compounds. Production sustainability is one of its valuable attributes in the food applications. Other attributes are being allergen-free, low carbon footprint, clean label and single-ingredient trends in the marketplace. Recently, there has been an increase in obtaining biopolymer-based nanofibers. In literature, two groups of biopolymers which are protein and carbohydrates were studied. However, studies about natural biopolymer based nanofibers by using electrospinning method were limited. There is a literature gap on production of nanofibers from pea flour by electrospinning method.

The main aim of this study is to obtain nanofibers from solution containing pea flour and HPMC by using electrospinning method. After preliminary literature research, this study was undertaken to evaluate the effects of pH, flour concentration, and HPMC concentration on solution properties and nanofiber

characteristics. In addition, the effects of electrospinning parameters which are voltage and flow rate were also analyzed. The effect of microfluidization process, which is one of the best homogenization technique, on nanofiber morphology was also evaluated. In this study, obtained nanofibers were characterized in terms of diameter, water vapor permeability (WVP) and color.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Pea flour containing $22\pm 2\%$ protein, $55\pm 5\%$ carbohydrate, $12\pm 2\%$ dietary fiber, $2\pm 2\%$ fat, 7-10% moisture, and $3\pm 1\%$ ash was purchased from Molar Chemical Materials Trading Co. Inc. (Turkey). PEO (Average MW = 900 kDa) and HPMC were obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Tween80 was provided from Merck (Darmstadt, Germany).

2.2 Solution preparations

Figure 2.1 showed the basic solution preparation method. First, PEO was added into deionized water. The solution was stirred at 1,000 rpm for 24 h by a magnetic stirrer (Daihan Scientific Co, KR) to ensure complete dissolution. After 24 h, pea flour was added to the mixture at different concentrations. The solutions were homogenized with high speed homogenizer (IKA T25 Digital Ultra-Turrax; IKA®-Werke GmbH & CO. KG, Staufen, Germany) at 12,000 rpm for 4 min to provide complete dissolution. Then, pH of solutions was adjusted to 7, 10 and 12 by using 2 M NaOH solution with a pH Portable Meter (SG2 SevenGo™, Mettler, Toledo, USA). Later, they were sealed to avoid water evaporation and were heated to 80°C in a water bath. After that, they were stirred at 1,000 rpm at 80°C for 2 h. At the end of 2 h, they were cooled down to reach room temperature. Then, Tween80, which is a surfactant, was added into the prepared solutions at 2% (w/v). HPMC

was added at three different concentrations as 0.25%, 0.5% and 1% (w/v). Then, solutions were mixed with high speed homogenizer 10,000 rpm for 5 min and stirred at 750 rpm at room temperature for overnight by using a magnetic stirrer in order to remove the air bubbles. Figure 2.1 also showed the parameters, which influence solution properties and nanofiber morphology.

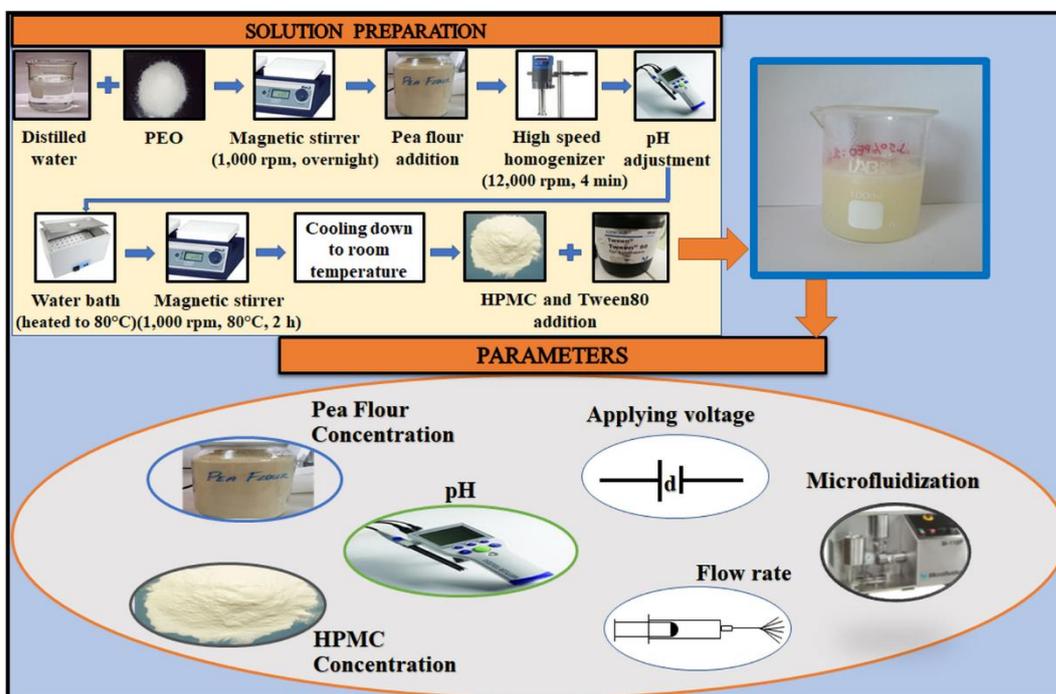


Figure 2.1 Demonstration of solution preparation.

2.3 Electrospinning

Electrospinning apparatus (Nanoweb 103, Mersin, Turkey) was used for solution electrospinning. It consists of high voltage source (1-30 kV), a needle, a syringe pump and a grounded rectangular metal collector. The prepared solution was loaded into a 5-mL plastic syringe (11.58 mm inner diameter) with a 21 gauge steel needle that was connected to a positive charge of a direct current (DC) high-voltage power supply. Collector was covered with aluminium foil and fibers were collected on it.

2.4 Usage of PEO and pea flour at different concentrations to obtain homogenous nanofiber

Solutions were prepared by changing both PEO and pea flour concentration according to efficiency of nanofiber formation by providing that pea flour concentration was higher than PEO concentration. pH of solutions was adjusted to 10, which was the optimum alkali pH value to obtain protein based nanofiber according to the literature (Raikos, Neacsu, Russell, & Duthie, 2014). It was aimed to observe whether smooth beadless nanofibers from PEO and pea flour blending solution were formed or not. PEO concentration varied as 3.5%, 3%, 2.5%, 2%, 1.5%, 1.0% and 0.5%. Accordingly, pea flour concentration varied as 1%, 1.5%, 2%, 2.25%, 2.5%, 3%, 4.5%, 5%, 5.25%, 7.5%. Each solution for this analysis was prepared with 0.5% HPMC concentration.

2.5 Effects of PEO, pea flour, pH, HPMC concentrations, voltage and flow rate on nanofiber characteristics

Firstly, nanofibers from pea flour and HPMC blending solution with minimum PEO concentration were obtained with different electrospinning conditions as shown in Table 2.1. For the observation of the effects of pH, HPMC and pea flour concentration, the parameters which are voltage, flow rate, distance and spinning time were kept constant at 11 kV, 0.4 mLh⁻¹, 0.3 m and 3 h, respectively. When voltage and flow rate values changed, the experiments were conducted with the same distance of 0.3 m and spinning time of 3 h. The solutions were electrospun with two different voltage values of 7 and 11 kV and with flow rate values of 0.4 and 0.8 mL/h.

Table 2.1 Electrospinning conditions for the observations of the effects of pH, PEO, pea flour, HPMC concentration, voltage and flow rate

PEO	Concentrations (%)		pH	Voltage (kV)	Flow rate (mL/h)	
	Pea Flour	HPMC				
3.5	1	0.5	7	11	0.4	
			10	11	0.4	
			12	11	0.4	
	2	0.5	7	11	0.4	
			10	11	0.4	
			12	11	0.4	
3.5	1	0.25		11	0.4	
		0.5	10	11	0.4	
		1.0		11	0.4	
	2	0.5	0.25		11	0.4
			10	11	0.4	
			1.0		11	0.4
3.5	1.5	0.5	10	11	0.8	
				7	0.4	
	2	0.5	10	11	0.8	
				7	0.4	
3.5	1.5	0.5	10	11	0.4	
				7		
	2	0.5	10	11	0.4	
				7		

Table 2.1 (Continued)

PEO	Concentrations (%)		pH	Voltage (kV)	Flow rate (mL/h)
	Pea Flour	HPMC			
3.5	1	0.5	10	11	0.4
	1.5	0.5	10	11	0.4
	2	0.5	10	11	0.6
	5.25	0.5	10	12	0.8
3	4.5	0.5	10	8	0.8
2.5	2.5	0.5	10	15	1
	5	0.5	10	15	1
	7.5	0.5	10	12	0.8
2	2	0.5	10	15	1
	4	0.5	10	12	1
	5	0.5	10	12	1
1.5	2.25	0.5	10	15	1
	3	0.5	10	15	0.8
	6	0.5	10	10	1
1	3	0.5	10	15	1
	4	0.5	10	20	1
	5	0.5	10	12	1
0.5	2	0.5	10	15	1
	2.5	0.5	10	15	1
	5	0.5	10	15	1

2.6 Determination of the effect of microfluidization (MF)

In order to see the effect of microfluidization (MF), the solutions with 3.5% and 2.5% PEO concentration were prepared with 5.25% and 7.5% pea flour concentration, respectively. Order of solution preparation given in Figure 2.1 was changed due to inconvenience structure of PEO for microfluidization. Firstly, pea flour was added to solutions. pH was adjusted to 10. After stirring at 1,000 rpm at 80°C for 2 h, solutions were cooled to room temperature. Tween80 of 2% was added to solutions. Also, solutions were prepared with 0.5% HPMC or without HPMC. Then, solutions without PEO were fed to the inlet chamber of ISA-N-10M Nano Disperser equipment (Ilshin Autoclave, South Korea). MF pressure was adjusted to 100 MPa. Each solution was pressurized by 10 pass through the 75 μm diameter nozzle of the interaction chamber. The device was installed with a cooling unit supplying temperature control kept at the range of 20-25°C, depending on the applied pressure. After that, PEO was added to each solution and solutions were stirred at 1,000 rpm for 24 h to ensure complete dissolution. Electrospinning conditions of the solutions were given in Table 2.2.

Table 2.2 Electrospinning conditions for the observation of the effect of MF

Concentrations (%)			Pass Number	Voltage (kV)	Flow rate (mL/h)	Distance (m)	Time (min)
PEO	Pea Flour	HPMC					
3.5	5.25	0	0	12	0.8	0.2	45
			10	10	0.6	0.3	45
2.5	7.5	0	0	15	0.8	0.25	45
			10	20	0.6	0.3	45
		0.5	0	12	1.0	0.23	45
			10	20	0.6	0.3	45

2.7 Measurement of solution properties

2.7.1 Rheological properties

Rheological properties were measured by using a controlled strain rheometer (Kinexus Pro+ Rheometer, Malvern) equipped with titanium cone-plate geometry (0.04 m diameter, 4° cone, 0.1425 mm gap) at a shear rate that was varied in the range of 0.1-100 s⁻¹. During measurements, temperature was kept at 25±1°C. Shear stress was recorded as a function of shear rate. Measurement was repeated three times.

2.7.2 Electrical conductivity

Electrical conductivity was measured at $25\pm 1^\circ\text{C}$ using conductivity meter (InoLab[®] Cond 7110, Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Conductivity results were expressed as siemens per m and experiments were replicated twice.

2.8 Nanofiber analysis

2.8.1 Morphology of nanofibers

Samples were accumulated on the surface of aluminium covered rectangular metal collector. They were coated with gold (10 nm) using a sputter coater (Au/Pd). Fiber morphology was examined using a Field Emission Scanning Electron Microscopy (FESEM) (JEOL, Japan) at magnification of $10,000\times$. Fiber diameters were measured from FESEM images using ImageJ-1.50i analysis software. After measurement, average diameters and standard errors were calculated from 100 nanofibers.

2.8.2 Fourier-transform infrared (FTIR) analysis of nanofibers

FTIR analyses of nanofibers and powders were done by using a FTIR spectrophotometer (IR-Affinity1, Shimadzu, Kyoto, Japan) in attenuated total reflectance (ATR) mode using a diamond ATR crystal. The infrared regions analysis was recorded with 16 scans over wavenumber range of $400\text{-}6000\text{ cm}^{-1}$.

FTIR analysis were performed for the powder samples of pea flour, HPMC, and PEO and nanofibers from blending solutions of pea flour and PEO with HPMC or

without HPMC. Solutions were prepared by blending 3.5% of PEO solutions with pea flour at concentrations of 2% and 5.25%. pH was adjusted to 10. Each solution for this analysis was prepared with 0.5% HPMC or without HPMC.

2.8.3 Water vapor permeability (WVP) of nanofibers

WVP of fibers was determined according to a modified ASTM method E96 as described by Sothornvit and Krochta (2000). Fibers were mounted in the permeation cups with a diameter of 0.04 m filled with water. They were stored in the desiccator containing silica gel. Samples were weighed at two hour intervals until steady-state was reached. Each experiments were replicated twice. The water vapor transmission rate of nanofibers was easily determined from the slope of weight loss versus time plot following equation (1).

$$WVP = \frac{(WVTR) \times (\Delta x)}{S \times (R_1 - R_2)} \quad (1)$$

where WVTR is the water vapor transmission rate ($\text{kgm}^{-2}\text{s}^{-1}$) and R_1 is the relative humidity inside the cup, R_2 is the relative humidity in desiccator outside the cup (Pa) and Δx is the thickness of the fiber (m). Relative humidity was determined by using multichannel data logger (EBI 20-TH1, WTW Wissenschaftlich-Technische Werkstätten GmbH, Ingolstadt). Fiber thickness was determined by using digital micrometer (LYK 5202, Loyka, Ankara, Turkey).

WVP analysis was performed for 3.5% of PEO solutions containing pea flour at concentrations of 2% and 5.25%. pH was adjusted to 10. Each solution for this analysis was prepared with 0.5% HPMC concentration. Two blending solutions were electrospun and nanofibers were analyzed. Electrospinning conditions were given in Table 2.3.

Table 2.3 Electrospinning conditions for the observation of variation in WVP and color of nanofiber.

Concentrations (%)		Voltage (kV)	Flow rate (mL/h)	Distance (m)	Time (h)
PEO	Pea Flour HPMC				
2	0.5	8	0.8	0.2	36 h + 20 min
3.5	5.25	12	0.8	0.3	56 h + 15 min

2.8.4 Color of nanofibers

Color of fibers was determined as color coordinates in CIELAB color space (L^* , a^* , b^*) and total color change (ΔE^*) by using a Konica Minolta Chroma Meter (Minolta, CR10, Osaka, Japan). Two replications were used in each measurement. The value of L^* , a^* and b^* of $BaSO_4$ was taken as L_0 , a_0 , b_0 in equation (2).

$$\Delta E^* = [(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2]^{1/2} \quad (2)$$

Color analysis were performed for 3.5% of PEO solutions containing pea flour at concentrations of 2% and 5.25%. pH was adjusted to 10. Each solution for this analysis was prepared with 0.5% HPMC concentration. Two blending solutions were electrospun and nanofibers were analyzed. Electrospinning conditions were given in Table 2.3.

2.9 Statistical analysis

Analysis of variance (ANOVA) was used to determine whether there were significant differences or not between conductivities, viscosities and diameter values. Statistical analysis was performed using MINITAB 16.1.1 software (Minitab Inc., State College, PA, USA). Tukey Single Range test was used for comparisons ($p \leq 0.05$).

CHAPTER 3

RESULTS AND DISCUSSION

One of the objective of this study was to obtain nanofiber from pea flour and HPMC blending solution with minimum PEO concentration. As can be seen in Table 3.1, homogenous nanofiber formation (HNF) was mostly obtained from the solutions with PEO concentrations equal or greater than 2.5%. The Scanning Electron Microscope (SEM) images of these nanofibers were given in Figure 3.1. Uniform beadless nanofiber could not be obtained from the solutions with PEO concentration lower than 2.5% (Figure 3.2). PEO was so important for electrospinning of the blending solutions. For example, soybean proteins, gelatin, and casein can be successfully electrospun when they are blended with PEO that enhances the processability of natural polymers by maintaining their biocompatibility (Salas, 2017). In a study related to collagen based nanofiber production, fibers could not be formed from 1–2 wt% pure collagen solution, but fibers were obtained after the addition of PEO (Huang et al., 2001). Tort and Acarturk (2016) showed that combining of HPMC, sodium alginate and κ -carrageenan with PEO made electrospinning successful. Although natural biopolymers were difficult to be electrospun alone, PEO polymer not only made them electrospun but also increased their electrospinnability. PEO, which is nontoxic and biocompatible carrier polymer, enhances entanglement of polymer chain by preventing division of polymer jet into droplets so prevents beads observed in nanofibers (Lu et al., 2006; Ramji & Shah, 2014). The reason of bead observation in nanofibers from solutions with 0.5%, 1%, 1.5%, and 2% PEO concentration may be that PEO concentration was not sufficient to improve electrospinning ability of blending solution prepared from natural biopolymers. As shown in SEM images, fibers become smoother as PEO concentration increased (Figure 3.1). Below certain concentration, chain entanglements were not sufficient for jet stabilization and the reduction in the

diameter of the jet driven by surface tension. Thus, beads were formed. At higher concentration, viscoelastic force which prevented the rapid changes in fiber shape resulted in production of uniform fiber (Lim et al., 2010). Improvement in nanofiber morphology was achieved in the solution with 2.5% PEO concentration at different flour concentrations. Splash, which is circular droplet on the nanofiber morphology, could be eliminated by increasing flour concentration from 2.5% to 5% and to 7.5% (Figure 3.1 (g, h, i)). This achievement was expected. As pea flour (containing 22% protein) concentration increased, protein concentration increased. Then, more protein molecules were destructed during heating of solution. In addition, alkali pH value (10) caused high charge density leading to irreversible unfolding structure of protein molecules. In other words, protein was stable against thermal aggregation and the refolding of the biopolymer was restrained even at room temperature. Because of more binding ability of unfolded protein chains with PEO polymer, smooth nanofiber formation was easier during electrospinning process and nanofibers with beads were converted to beadless nanofiber (Ramji & Shah, 2014). However, only increasing protein concentration was not sufficient to obtain beadless nanofibers when PEO concentration was lower than 2.5% which can be seen in Figure 3.2. It can be concluded that the effect of flour concentration on electrospinnability and nanofiber morphology was related to PEO polymer. A synergistic factor of unfolded protein chains and entanglement of PEO polymers were also observed by Ramji and Shah (2014) who worked with soy protein and PEO blending solutions. They also did not find specific relation between protein and PEO polymer. Similarly, they concluded also that fiber morphology was affected by both protein and PEO concentration and beaded nanofibers were obtained from lower PEO and protein concentrations.

Table 3.1 Nanofiber morphology of nanofibers from pea flour and HPMC blending solution at pH value of 10 with minimum PEO concentration

Component Concentrations (%)			Nanofiber Morphology
PEO	Pea Flour	HPMC	
0.5	2	0.5	BF*
	2.5	0.5	BF
	5	0.5	BF
1.0	3	0.5	BF
	4	0.5	BF
	5	0.5	BF
1.5	2.25	0.5	BF
	3	0.5	BF
	6	0.5	BF
2.0	2	0.5	BF
	4	0.5	BF
	5	0.5	BF
2.5	2.5	0.5	HNF**
	5	0.5	HNF
	7.5	0.5	HNF
3	4.5	0.5	HNF
3.5	1	0.5	HNF
	1.5	0.5	HNF
	2	0.5	HNF
	5.25	0.5	HNF

* BF: Bead Formation;
** HNF: Homogeneous nanofiber formation

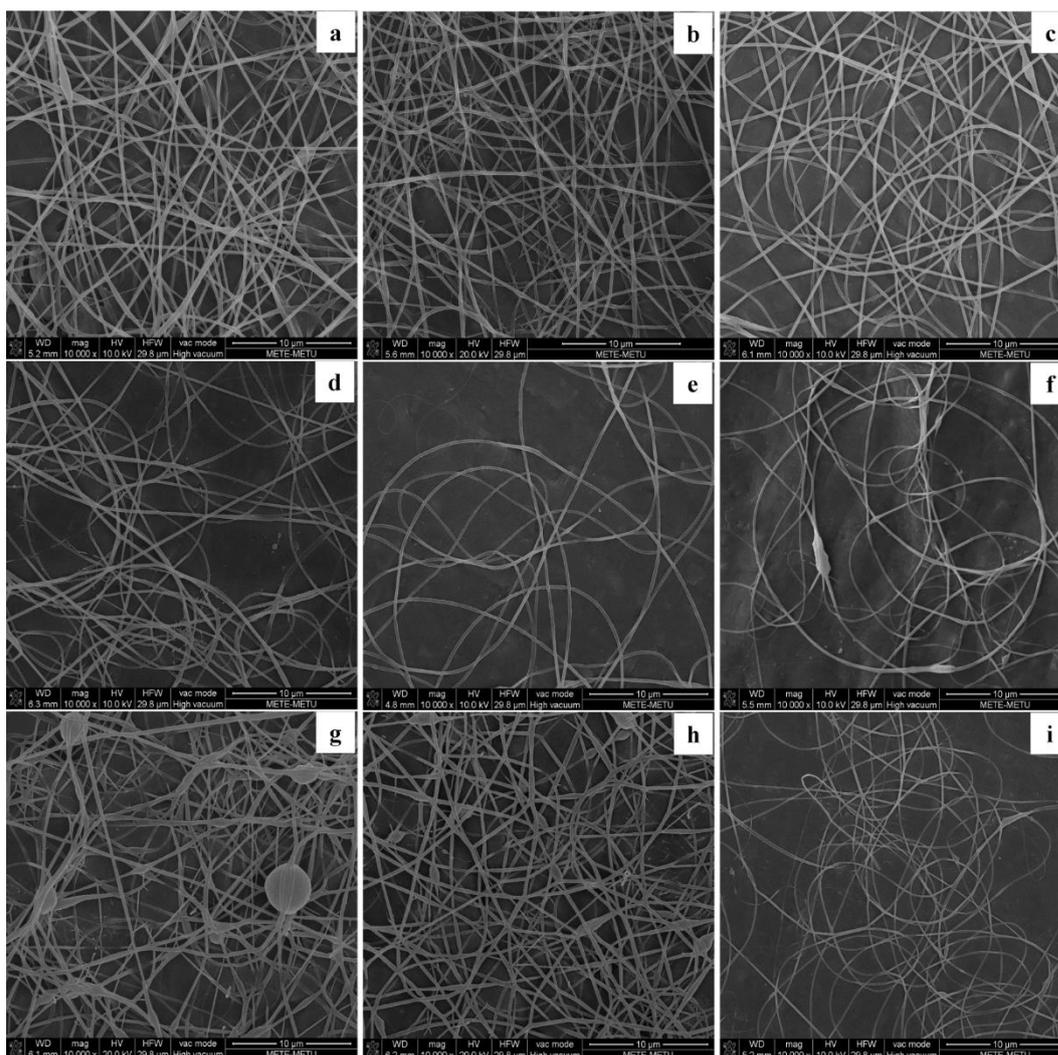


Figure 3.1 SEM images ($10,000\times$) of the nanofibers, obtained from solution with PEO:Pea flour concentrations at (a) 3.5:1, (b) 3.5:1.5, (c) 3.5:2, (d) 3.5:5.25, (e) 3:4.5, (f) 2.5:1.5, (g) 2.5:2.5, (h) 2.5:5, (i) 2.5:7.5.

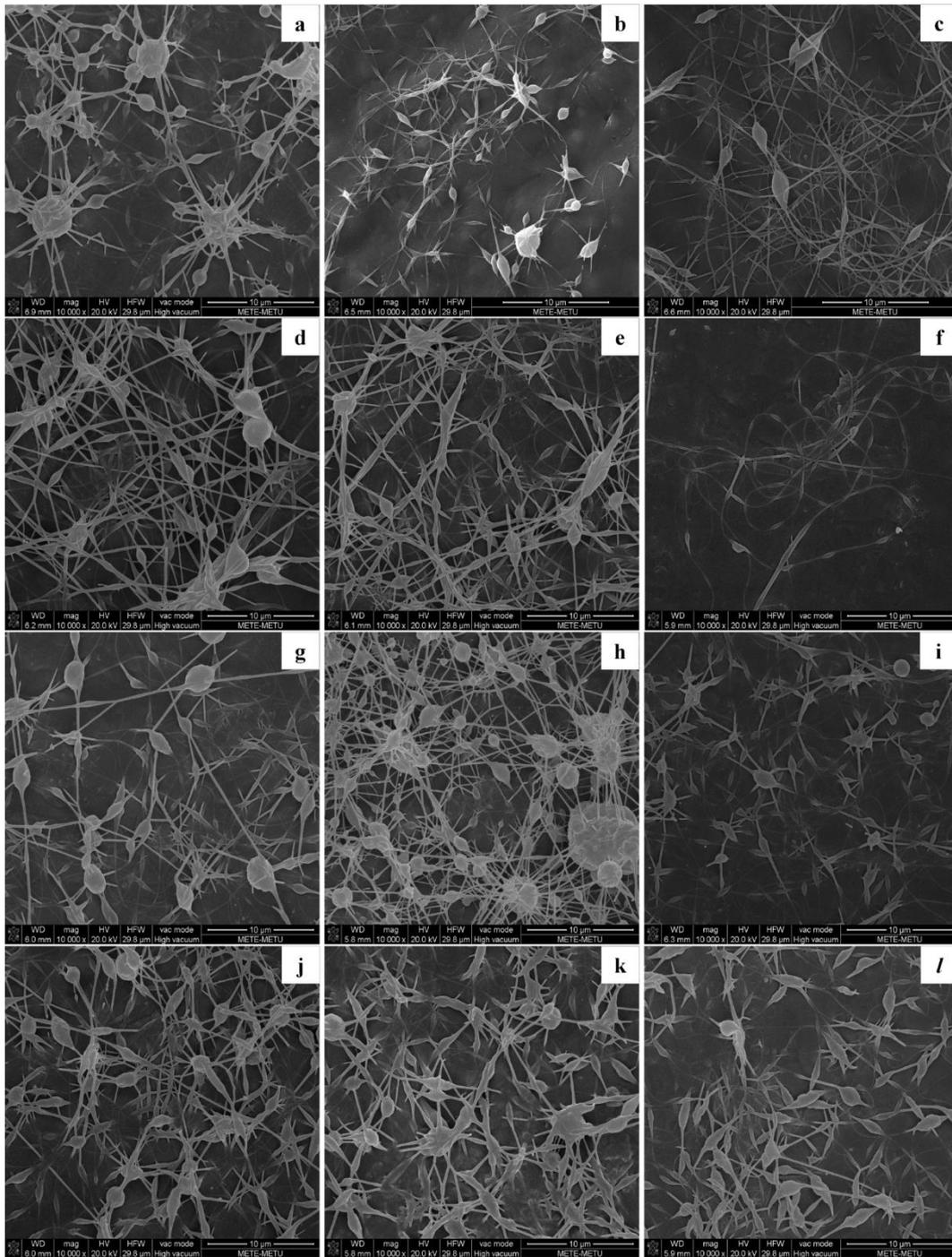


Figure 3.2 SEM images (10 000 ×) of the nanofibers, obtained from solution with PEO:Pea flour concentrations at (a) 2:2 , (b) 2:4, (c) 2:5, (d) 1.5:2.25, (e) 1.5:3, (f)1.5:6, (g) 1:3, (h) 1:4, (i) 1:5, (j) 0.5:2, (k) 0.5:2.5, (l) 0.5:5.

After preliminary experiments, it was confirmed that solution properties, which was related to solution composition, affected nanofiber morphology. In this study, alkali pH value was selected by considering solubility of pea flour (Ahmed, Ahmed, Eltayeb, Ahmed, & Babiker, 2011; Periago et al., 1998). After these observations, the effects of pH, pea flour and HPMC concentration on solution properties of the selected solutions were evaluated. Nanofiber morphology and size were affected by solution properties (viscosity, conductivity), process parameters (applied voltage, feed rate, distance between capillary and collector), and ambient parameters (humidity and temperature) (Ghelich et al., 2015; Okutan et al., 2014). In this study, process parameters were kept constant to observe the effects of solution properties on electrospinning.

3.1 Apparent viscosity

As mentioned in Section 1.2, viscosity was a key parameter affecting electrospinnability. An optimum viscosity was needed to prepare homogenous nanofiber.

Flow properties of resulting solutions obeyed “Power Law Model”;

$$\tau = k\dot{\gamma}^n$$

where τ is shear stress (Pa), $\dot{\gamma}$ is shear rate (s^{-1}), k is consistency coefficient ($Pa \cdot s^n$), n is flow behavior index.

Apparent viscosity ($\eta = k\dot{\gamma}^{n-1}$) is a function of consistency coefficient (Sahin & Sumnu, 2006). All prepared solutions were shear-thinning fluids (pseudoplastic) since $n < 1$ (Table 3.2 and Table 3.3). The apparent viscosity versus shear rate graphs can be seen in Figure 3.3 and 3.4.

Table 3.2 Effects of pH and pea flour concentration on solution properties and nanofiber morphology when 0.5% HPMC and 3.5% PEO were used.

Pea flour concentration (%)	pH	Electrical conductivity (S/m)	Consistency coefficient, k (Pa.s ⁿ)	Flow behavior index, n	R ²	Average diameter (nm)	Nanofiber morphology
1	7	0.046±0.006 ^{b*}	1.138±0.03 ^e	0.942±0.003 ^a	0.998	-	BF**
1	10	0.066±0.004 ^{ab}	1.746±0.02 ^{cd}	0.933±0.001 ^{ab}	0.998	179±3 ^c	HNF***
1	12	0.099±0.0002 ^{ab}	1.851±0.04 ^c	0.908±0.002 ^c	0.998	233±3 ^b	HNF
2	7	0.065±0.008 ^{ab}	1.511±0.11 ^d	0.926±0.003 ^b	0.998	-	BF
2	10	0.107±0.028 ^{ab}	2.536±0.02 ^b	0.894±0.001 ^d	0.997	238±4 ^b	HNF
2	12	0.136±0.011 ^a	2.803±0.07 ^a	0.888±0.002 ^d	0.997	250±3 ^a	HNF

*Columns with different letters were different statistically (p≤0.05).

BF: Bead Formation; *HNF: Homogeneous Nanofiber Formation

Table 3.3 Effect of HPMC concentration in solutions containing 3.5% PEO at pH 10 on solution properties and nanofiber diameter

Pea flour concentration (%)	HPMC concentration (%)	Electrical conductivity (S/m)	Consistency coefficient, k (Pas ⁿ)	Flow behavior index, n	R ²	Average diameter, (nm)
1	0.25	0.060±0.002 ^{a*}	1.349±0.087 ^d	0.923±0.005 ^a	0.998	177±4 ^c
1	0.50	0.066±0.004 ^a	1.746±0.020 ^d	0.933±0.001 ^a	0.998	179±3 ^c
1	1.00	0.053±0.002 ^a	3.336±0.210 ^a	0.871±0.010 ^d	0.996	239±4 ^a
2	0.25	0.085±0.014 ^a	2.039±0.025 ^{cd}	0.913±0.001 ^{ab}	0.997	217±3 ^b
2	0.50	0.107±0.028 ^a	2.536±0.020 ^{bc}	0.894±0.001 ^{bc}	0.997	238±4 ^a
2	1.00	0.084±0.026 ^a	2.903±0.300 ^{ab}	0.884±0.001 ^{cd}	0.997	242±3 ^a

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

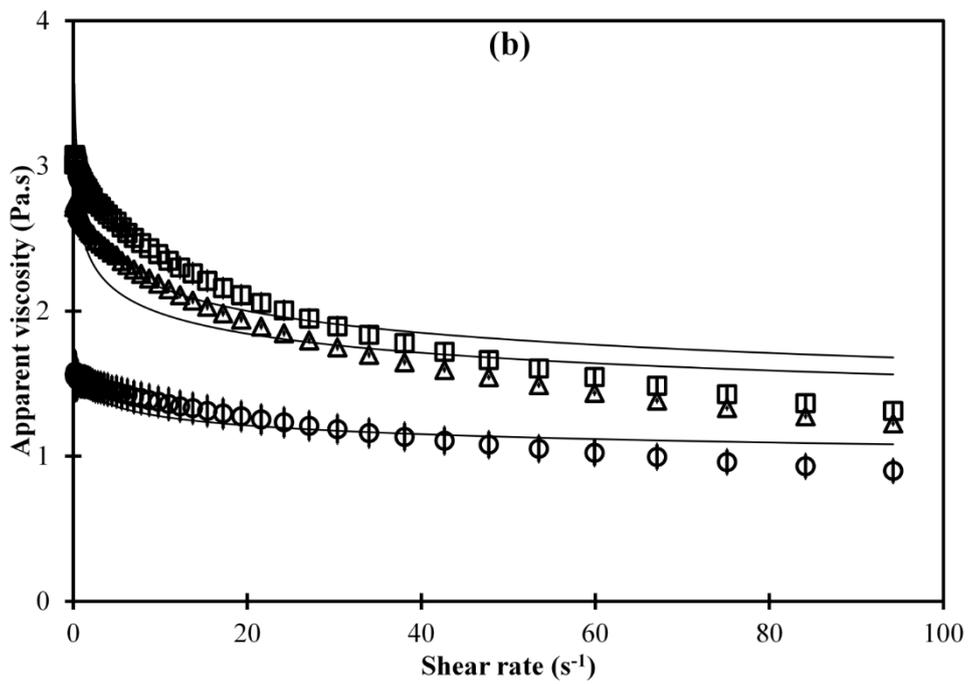
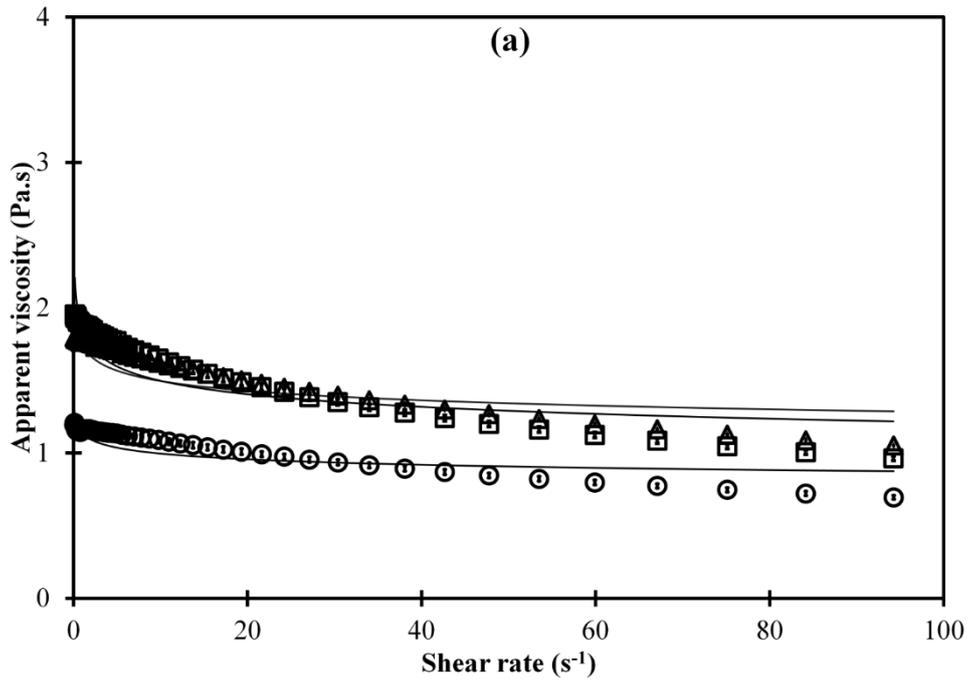


Figure 3.3 Effects of different pH values on apparent viscosity of solutions containing (a) 1% pea flour, (b) 2% pea flour at (●): pH 7, (▲): pH 10, (■): pH 12.

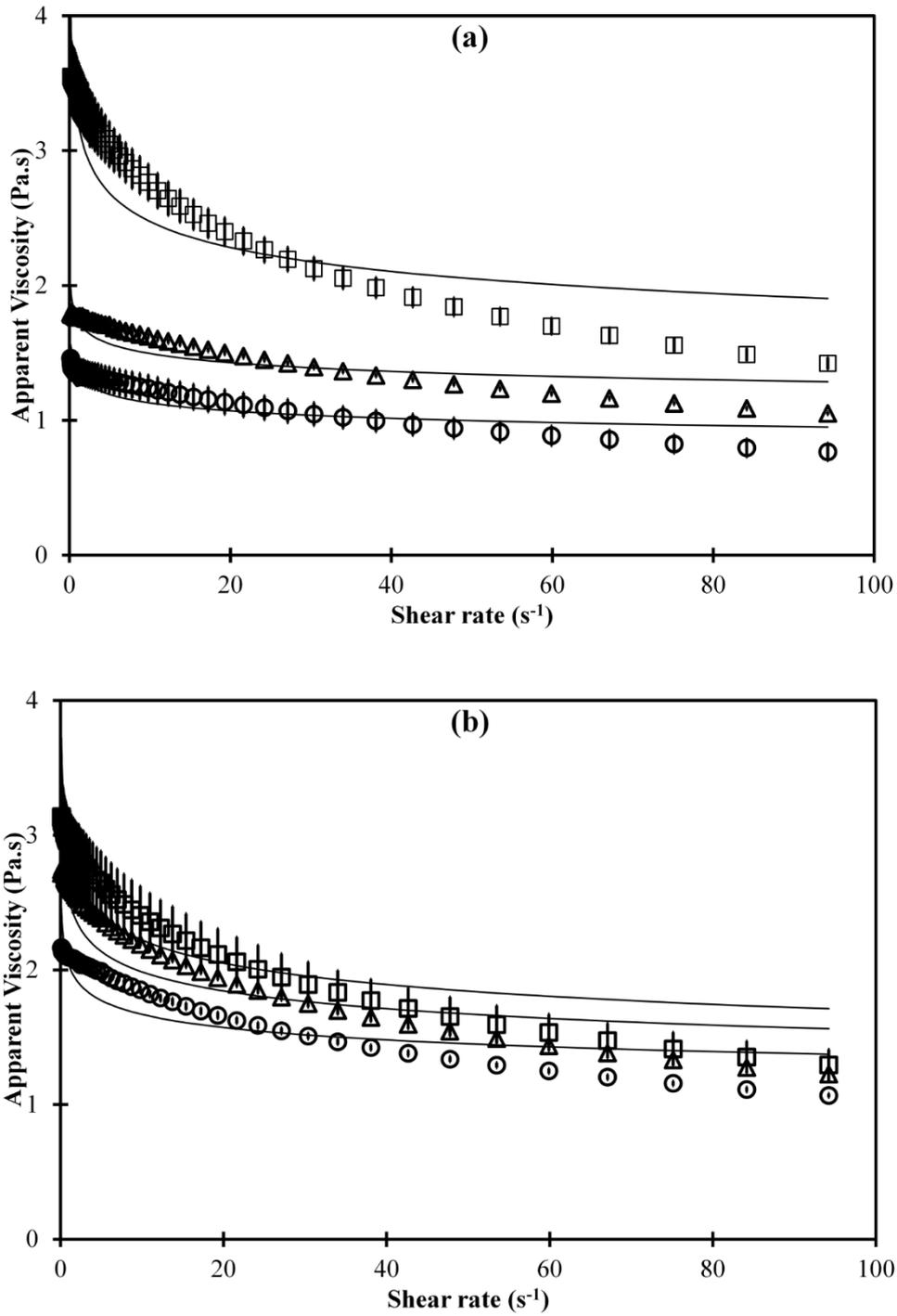


Figure 3.4 Effect of different HPMC concentrations on apparent viscosity of solutions at pH 10 containing (a) 1% pea flour, (b) 2% pea flour. (○): 0.25% HPMC, (△): 0.5% HPMC, (□): 1% HPMC.

3.1.1 Effect of pH

As can be seen in Table 3.2 as pH increased, consistency coefficient (k) of solutions increased significantly ($p \leq 0.05$) which can be seen in Table A.1. For both pea flour concentrations, n values decreased with increasing pH, indicating more shear thinning behaviors. Similarly, Xu, Carson and Kim (2015) who worked with solutions of 25% wheat protein isolate observed the same trend of decrease in n values from 0.8 to 0.5 and increase in k values from 0.46 to 1.08 Pa.sⁿ as pH values increased from 4 to 7.

Since apparent viscosity is a function of consistency coefficient (k), it can be said that apparent viscosity increased as pH increased as shown in Figure 3.3. It was due to the increase in solubility of pea protein. The relationship between solubility and viscosity were shown in many studies before. Briscoe and coauthors (2000) found that increase in solubility of solute in solvent increased viscosity of solution. The increase in solubility of solute was explained by the increase in the degree of hydrogen bonding between the solute chains and water molecules. An increase in viscosity was anticipated by the increase in the interaction between solute and solvent which was increased by solubility. In other words, strong intermolecular hydrogen bonds formed could in turn contribute to the polymerization process and to the formation of more stable and viscous structures.

Pea flour contained $22 \pm 2\%$ protein which was high enough to be affected by pH in electrospinning. Hydrophilic and hydrophobic status of proteins could be different at different pH levels so that the network and chain-chain interactions of molecules might behave differently (Xu et al., 2015). In addition, protein molecules are zwitterions which contain free amino and carboxyl groups, and the molecular net charge depends on the solution pH. For every protein, there is a specific pH value, named as the isoelectric point pI, at which the molecular net charge approaches to zero (Ulaganathan et al., 2015). The isoelectric point of pea proteins was determined to be 4.5 (M. B. Barać et al., 2015). At this pI level, there will be a

tendency for the protein to aggregate. It was also found that pea protein molecules showed minimum viscosity at their pI values where minimum solubility was observed, due to the minimum hydration (Lam & Nickerson, 2013; Lin, Breene, & Sargent, 1990). For the present study, protein molecule above pI value carried a net negative charge. Pea flour also contains carbohydrates (55%). Thus, the accumulation of negative charge that was brought about by the attachment of polysaccharides to protein molecules might cause electrostatic repulsion and might inhibit further interactions (Lin et al., 1990; Tang, Ozcam, Stout, & Khan, 2012). Increase in pH above isoelectric point affected both solubility and viscosity. The dependence of viscosity to pH was also clearly stated by Franco and coauthors (2000) in a way that the increase in pH above pI led to smaller droplet sizes which increased interaction of interdroplet and improved stability against aggregation. As a result, viscosity increased.

As stated before, increase in apparent viscosity was related to the increase in solubility of pea protein because high alkaline concentration caused breaking down of the hydrogen bonds and dissociation of hydrogen from carboxylic and sulphate groups. Then, the increased net charge of protein molecules at alkaline conditions could lead to the unfolding of the flour protein, therefore, an enhanced solubility in water (Nnadozie, Kelechi, & Deborah, 2015; Shen, Wang, Wang, Wu, & Chen, 2008). The unfolding of the flour protein was reported as greater at alkaline than at lower pH values (Ahmed et al., 2011). In literature, solubility of whey protein, unhydrolyzed pea protein, and cowpea with 26.8% protein displayed an increase at alkaline pH values (Pelegri & Gasparetto, 2005; Periago et al., 1998; Ragab, Babiker, & Eltinay, 2004). Pea protein adopted a globular conformation, limiting its physical interaction with the PEO macromolecules. After being unfolded in alkaline environments, chain entanglements between pea protein and PEO could occur. This might explain why apparent viscosity values were significantly higher in the alkaline solutions when compared with those in the neutral solution ($p \leq 0.05$) (Figure 3.3). As shown in Table 3.2, solutions had the lowest consistency coefficient values of 1.138 and 1.511 Pa.sⁿ at neutral pH (7) in comparison to those

values between 1.746 and 2.803 Pa.sⁿ at pH values of 10 and 12. Similarly, Vega Lugo and Lim (2012) figured out the same behavior for whey protein isolate solution.

Consistency coefficient of solution with 1% pea flour concentration did not change significantly as pH increased from 10 to 12. On the other hand, consistency coefficient increased significantly as pH increased from 7 to 10 or 7 to 12 (Table 3.2). The increase in pH from 10 to 12 affected the viscosity of solution containing high flour concentration (2%). Since it is difficult to homogenize more viscous solutions with 2% pea flour as compared to 1% pea flour solution, the effect of pH change (10 to 12) on viscosity and solubility of this solution became more significant. Similarly, Prinyawiwatkul et al. (1997) figured out that the increase in pH from 8.0 to 10.0 did not change solubility of cowpea proteins while increasing pH from 4.0 to 8.0 considerably increased protein solubility of flours.

3.1.2 Effect of pea flour concentration

As can be seen in Table 3.2, consistency coefficient values increased significantly ($p \leq 0.05$) as pea flour concentration increased from 1% to 2% (w/v) (Table A.1). In Figure 3.4, it can also be seen that apparent viscosity over the entire range of shear rates significantly increased with increasing pea flour concentration from 1% to 2% (w/v). According to Kriegel et al. (2008), this was caused by binding tendency of biopolymers with more hydrogens. Similarly, in literature, it was found that consistency coefficient and apparent viscosity increased as wheat protein isolate concentration increased (Xu et al., 2015). Table 3.2 also showed that n value decreased significantly as pea flour concentration increased. This meant that higher pea flour concentration enhanced shear thinning behavior ($n < 1$). Similarly, Xu, Carson and Kim (2015) found a decrease in n values from 0.8 to 0.5 as concentrations of wheat protein isolate increased from 25% to 35%.

Pea flour consists of $22 \pm 2\%$ protein and $55 \pm 5\%$ carbohydrate. From this study, it was inferred that apparent viscosity increased with increasing pea flour concentration which could be related to an increase in carbohydrate concentration. In literature, there were similar studies. In a study related to chitosan based electrospun nanofiber, the increase in the ratio of chitosan to total polymer in solution increased viscosity (Triyana et al., 2015). Santos and coauthors (2014) also showed that viscosity of solution increased with increasing the polysaccharide concentration.

Concentration was stated to be a primary factor that determined the viscosity of solution (Lu et al., 2006; Yang et al., 2004). This can be explained by the fact that viscosity was proportional to the energy required to align the molecules and at high concentrations, the disturbance around one molecule overlapped or interacted with disturbance around another molecule. Thus, this caused an increase in the viscosity (Lin et al., 1990).

3.1.3 Effect of HPMC concentration

As HPMC content increased from 0.25% to 1.0%, consistency coefficients (k) increased significantly ($p \leq 0.05$) (Table 3.3 and Table A.5). On the other hand, HPMC had a decreasing effect on flow behavior index values (Table A.6). HPMC is a well-known mucoadhesive modified chemically polymer and it shows quickly swelling in contact with water (Tort and Acarturk 2016). It is also a water-soluble hydrophilic non-ionic cellulose ether which has polymeric gel layer formed when contacted with aqueous media (Balogh et al., 2016). As can be seen in Figure 3.4, apparent viscosity over the entire range of shear rates significantly increased with increasing HPMC concentration.

The increase in k values, which refers to viscosity increase, with increase in HPMC was in accordance with the literature. It is known that the viscosity of solution increases as polymer concentration increases. HPMC molecule contains many OH groups to be part of intermolecular hydrogen bonds, allowing to creation of bundles or fibrils (Zhao et al., 2009). Lim et al. (2010) showed that viscosity of solution increased from 1.284 to 8.614 Pa.s as HPMC concentration increased from 0.5 to 1.0 wt%. In addition to polysaccharide based nanofiber researches, in a study related to chitosan based electrospun nanofiber, the increase in chitosan to total polymer ratio from 1:10 to 3:10 resulted in an increase in viscosity from 2 to 15.5 Pa.s, respectively (Triyana et al., 2015).

In the study, for both solutions, consistency coefficient did not change significantly ($p>0.05$) as concentration of HPMC was increased from 0.25% to 0.5% (w/v). The increase in HPMC content from 0.5% to 1.0% was enough to change viscosity values significantly for 1.0% (w/v) pea flour but the same change could not be observed for greater amount of pea flour. It can be concluded that the necessary amount of HPMC increase for 2% pea flour containing solution was greater than 0.5% (w/v) in order to increase viscosity.

3.1.4 Effect of microfluidization (MF)

In this study, microfluidization (MF) technique was applied to solutions with high concentration to obtain homogenous nanofiber. Solutions in which flour concentration was higher than PEO concentration were chosen (Table 3.1). In the experiments, there was difficulty in MF with the usage of HPMC. As can be seen in Table 3.4 and Table 3.5, k value increased and n value decreased significantly after MF technique for both concentrations (Table A.9, Table A.10, Table A.12 and A.13). The reason of applying MF technique is to make solutions more homogenous by reducing particle size and decreasing viscosity of solution

(Bonilla, Atarés, Vargas, & Chiralt, 2012). However, in this study, opposite behavior was observed. The reason could be caused by preparation method of solution. PEO was added to solutions after passing through MF device and unfolded biopolymers in microfluidized solutions could bind more with PEO leading to increase in k value. Similarly, Vega Lugo and Lim (2012) found that globular structure of protein limited binding interaction of protein with PEO and then unfolding of protein increased this interaction which caused increase in viscosity. Another reason of increase in viscosity could be related to high pressure applied by microfluidization process. Pea flour solution contains pea protein and pea starch. In a study conducted by Kasemwong et al. (2011), gelatinization degree was zero for non-microfluidized starch while it increased to 46.2% and 57.9% after microfluidization of 90 MPa and 120 MPa, respectively. In this current study, there could be gelatinization of pea starches under the microfluidization pressure which is 100 MPa. Then, gelatinization induced the increase in consistency coefficient.

Table 3.4 Effect of MF technique applied on solutions at pH 10 with 3.5% PEO and 5.25% pea flour concentration without HPMC on solution properties and nanofiber morphology

Pass Number	Electrical conductivity (S/m)	Consistency coefficient, k (Pas ⁿ)	Flow behavior index, n	R ²	Nanofiber Morphology
0	0.139 ^{a***}	2.74 ^b	0.87 ^a	0.998	HNF*
10	0.088 ^b	13.24 ^a	0.56 ^b	0.992	BF

*HNF: Homogenous Nanofiber Formation
**BF: Bead formation
***Columns with different letters were different statistically ($p \leq 0.05$).

Table 3.5 Effect of MF technique applied on solutions at pH 10 with 2.5% PEO and 7.5% pea flour concentration on solution properties and nanofiber morphology

HPMC Content (%)	Pass Number	Electrical conductivity (S/m)	Consistency coefficient, k (Pas ⁿ)	Flow behavior index, n	R ²	Nanofiber Morphology
0	0	0.198 ^{b***}	0.43 ^c	0.93 ^a	0.999	BF**
0.5	0	0.223 ^a	13.05 ^b	0.51 ^b	0.992	HNF*
0	10	0.189 ^c	14.57 ^b	0.43 ^c	0.991	BF
0.5	10	0.143 ^d	32.31 ^a	0.39 ^c	0.999	BF

*HNF: Homogenous Nanofiber Formation,
**BF: Bead formation, *HNF: Homogenous Nanofiber Formation,
***Columns with different letters were different statistically (p≤0.05).

3.2 Solution electrical conductivity

As mentioned in section 1.2, it was stated that there was an optimum electrical conductivity for solutions to produce homogenous nanofibers. In addition, if the solution has zero conductivity, it can not be electrospun into nanofibers because a certain electrical conductivity is required for nanofiber formation. In this study, conductivities of solutions were not zero due to solution components except HPMC (Sovilj & Petrovic, 2006).

3.2.1 Effect of pH

As can be seen in Table 3.2, electrical conductivities of solutions were not influenced by pH change ($p > 0.05$). It was expected that net charges carried by flour protein changed as the pH changed (Fagbemi, Oshodi, & Ipinmoroti, 2006). In this study, no significant change in conductivity could be explained by an increase in viscosity when pH was increased. Increase in viscosity reduced mobility of ions (Sekhon, 2003). As a result, conductivity did not change.

In literature, electrical conductivity of pea flour solution at pH 6.42 containing $20.78 \pm 0.35\%$ protein in dry basis was determined as 0.135 ± 0.001 S/m (Soria-Hernández, Serna-Saldívar, & Chuck-Hernández, 2015). In the current study, pea flour contains $22 \pm 2\%$ protein in wet basis and electrical conductivities were 0.046 and 0.065 S/m for 1% and 2% pea flour containing solutions at pH 7, respectively. The differences in results between literature and this present study could be owing to the availability of different materials and measurement conditions in both studies.

3.2.2 Effect of pea flour concentration

Table 3.2 showed that electrical conductivity was not affected significantly by increasing pea flour concentration at the same pH and HPMC concentration, respectively ($p > 0.05$). The reason could be explained that increase in viscosity decreased mobility of ions (Sekhon, 2003). Similar results were found in literature. It was shown that the increase in Aloe vera concentration from 1% to 3% led to an insignificant increase in conductivity of the solution from 0.0908 S/m to 0.0925 S/m (Uslu et al., 2010). In literature, there were also works in which the effects of polymer addition on conductivity were studied. For example, Vega Lugo and Lim (2012) showed that the addition of PEO to whey protein solution did not change the conductivity value significantly for all the pH conditions tested. In the study, PEO addition, meaning total polymer increase, changed conductivity from 1.092 ± 0.015 to 0.964 ± 0.009 S/m at pH 12 and from 0.144 ± 0.002 to 0.130 ± 0.001 S/m at pH 7. Similarly, in another study, it was shown that as polymer concentration increased from 6% to 7%, conductivity increased from 0.089 to 0.098 S/m which was not significant (Tort & Acarturk, 2016). In other words, the increase in polymer content did not affect electrical conductivity, significantly.

3.2.3 Effect of HPMC concentration

As can be seen in Table 3.3, there were no significant differences in conductivity values ($p > 0.05$) as HPMC concentration was changed. It was known that HPMC was a natural polymer similar to pea flour. The study conducted by Vega-Lugo and Lim (2012) might be also given as an example for this situation because they added polymer to whey protein isolate solutions and observed no significant change in electrical conductivity. Similarly, the effects of concentration of PEO, when mixed with different types of HPMC on conductivity was not significant. (Tort & Acarturk, 2016). Okutan et al. (2014) stated that solution conductivity was

concentration independent when the polymer was non-ionic. HPMC is a non-ionic hydrophilic polymer (Majumder, Biswas, & Majee, 2016; Sovilj & Petrovic, 2006). Therefore, the independence of conductivity on HPMC concentration was an expected result.

3.2.4 Effect of microfluidization (MF)

Table 3.4 and 3.5 showed that there was a significant decrease in electrical conductivity after MF process (Table A.11 and Table A.14). In this study, PEO was added to solutions after MF process and unfolded biopolymers in microfluidized solutions could bind more with PEO leading to increase in viscosity as discussed in Section 3.1.4. The reason of decrease in conductivity could be that the increase in viscosity decreased mobility of ions (Sekhon, 2003).

3.3 Nanofiber Characterization

In the preliminary experiments of this study, it was tried to electrospin solutions prepared from only pea flour and HPMC; however, it was not possible to produce nanofibers. Then, solutions were prepared by mixing pea flour and HPMC with PEO. Viscosity, conductivity and surface tension of the solutions play a decisive role in the electrospinning process. These parameters mostly define the morphology and size of the fibers (Colin-Orozco, Zapata-Torres, Rodriguez-Gattorno, & Pedroza-Islas, 2014). In this study, two of them were mainly discussed.

3.3.1 Effect of pH

In the experiments, bead formation (BF) occurred at pH 7 for both pea flour concentrations (Table 3.2). By increasing pH from 7 to alkaline values, homogenous nanofiber formation (HNF) could be achieved for both flour concentrations. This could be seen in Figure 3.5 where SEM images of nanofibers at different pH values were given. The images showed that the increase in pH favored the electrospinnability of pea flour solutions. This was consistent with the literature. The conversion of spherical beads to elongated spindles as the pH of the solutions increased from 7 to alkaline values, could be due to the increased polymer chain entanglement between pea protein and PEO that stabilized the polymer jet. Similarly, Vega Lugo and Lim (2012) observed the same transformation for whey protein isolate as pH increased from 7 to 12. In another study, it was shown that nanofibers displayed the same behavior for solutions of soy protein isolate and poly vinyl alcohol. At pH 7, nanofibers with beads were observed and with increasing pH to 9, beads became smaller. At pH 12, beads disappeared and homogenous nanofiber was obtained (Cho, Netravali, & Joo, 2012). Zhu and coauthors (2007) also showed that regenerated silk fibroin aqueous solutions with a pH value of 6.9 could not be electrospun.

As shown in Table 3.2, between pH 7 and alkaline pH values, the significant differences were observed in only consistency coefficient values; but minimal difference was observed in conductivity values. A significant correlation between k values and nanofiber diameter ($r = 0.812$, $p = 0.014$) was observed while no significant correlation was found between conductivity and nanofiber diameter ($r = 0.601$, $p = 0.115$). It was already known that viscosity was an important factor in affecting electrospinnability (Okutan et al., 2014). Formation of smooth fibers was shown to be dependent mainly on the viscosity in the presence of pH change. Similarly, Fong and coauthors (1999) showed that higher viscosity favored formation of fibers without beads. They observed uniform nanofiber without beads at viscosity values of 0.527-1.835 Pa.s while nanofibers with beads at lower

viscosity values of 0.013-0.160 Pa.s. Higher viscosity beyond a critical value enabled the charged jet to totally withstanding the columbic stretching force. Thus, uniform and smooth fibers were observed (Santos et al., 2014).

According to literature, pH could favor charge density and consequently conductivity; therefore, electrospinnability increased by increasing charge density. It was found that the increase in the electrical conductivity caused a significant reduction in bead formation (Fong et al., 1999; Jun, Hou, Schaper, Wendorff, & Greiner, 2003). In the present study, conductivity did not increase significantly as pH was increased from 7 to 12 (Table 3.2).

As can be seen in Table 3.2, nanofiber diameter increased as pH increased from 10 to 12 for both concentrations (Table A.4). Since there was no significant difference in conductivity, the increase in fiber diameter might be explained by the increase in viscosity values only. Lu et al. (2006) implemented a study about electrospinning of sodium alginate blending with PEO and showed that the increase in viscosity generally increased the fiber size whereas the increase in conductivity showed the opposite effect. In that case, the results demonstrated that the effect of viscosity on fiber diameter was more significant than that of conductivity leading to an increase in fiber size (Lu et al., 2006). This finding was similar to our results. As can be seen in Figure 3.6, for solution of 2% pea flour concentration, the diameter range was from 180 nm to 200 nm at pH value of 10, while at pH value of 12, the range was from 200 nm to 220 nm for 2% pea flour solution. For 1% pea flour solution, diameter range was between 120-140 nm at pH 10, while it was changed between 140-160 nm at pH 12. For both concentrations, unimodal distribution was seen.

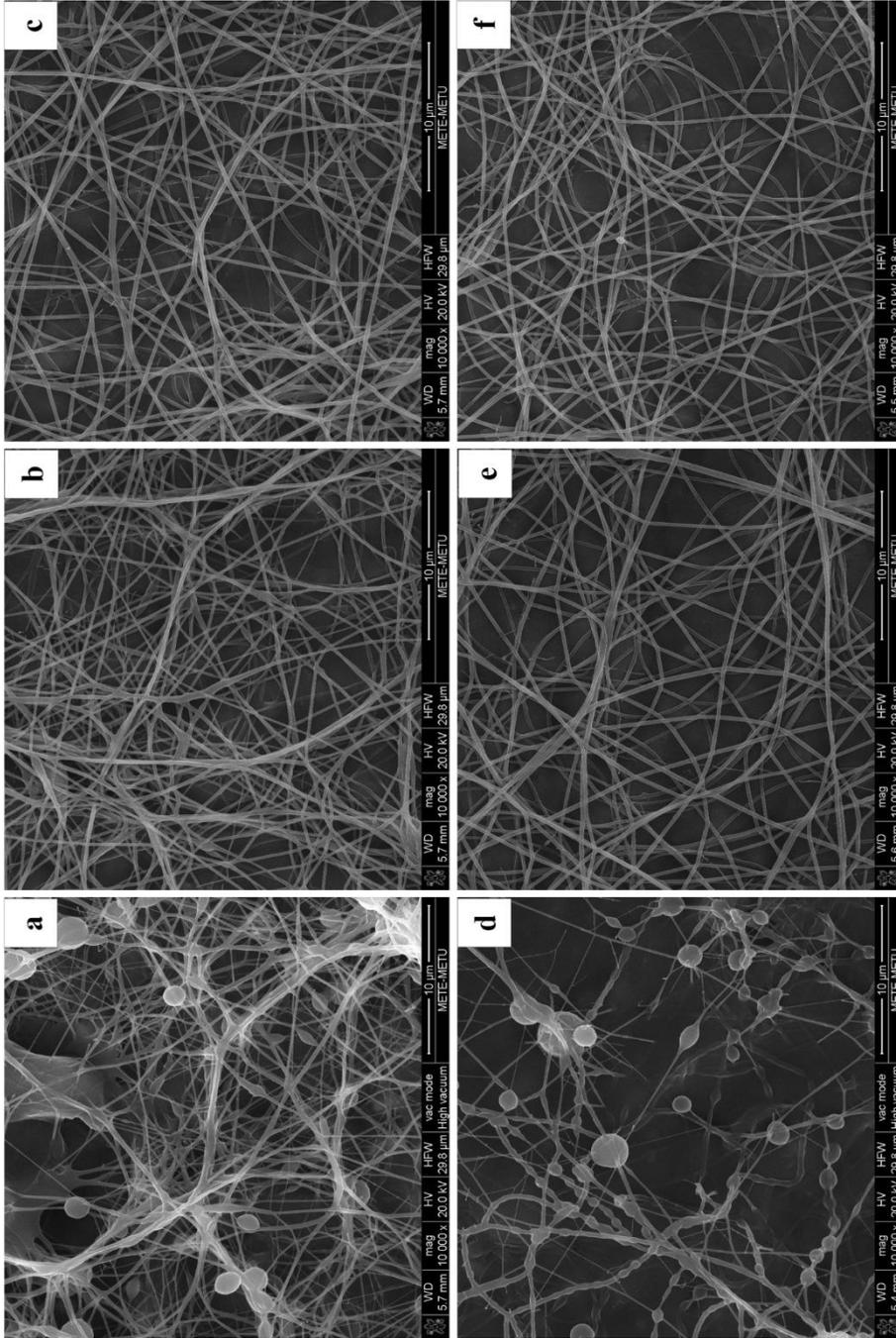


Figure 3.5 SEM images ($10,000\times$) of the nanofibers, obtained from solution with 1% pea flour at (a) pH 7, (b) pH 10, (c) pH 12; solution with 2% pea flour at (d) pH 7, (e) pH 10, (f) pH 12.

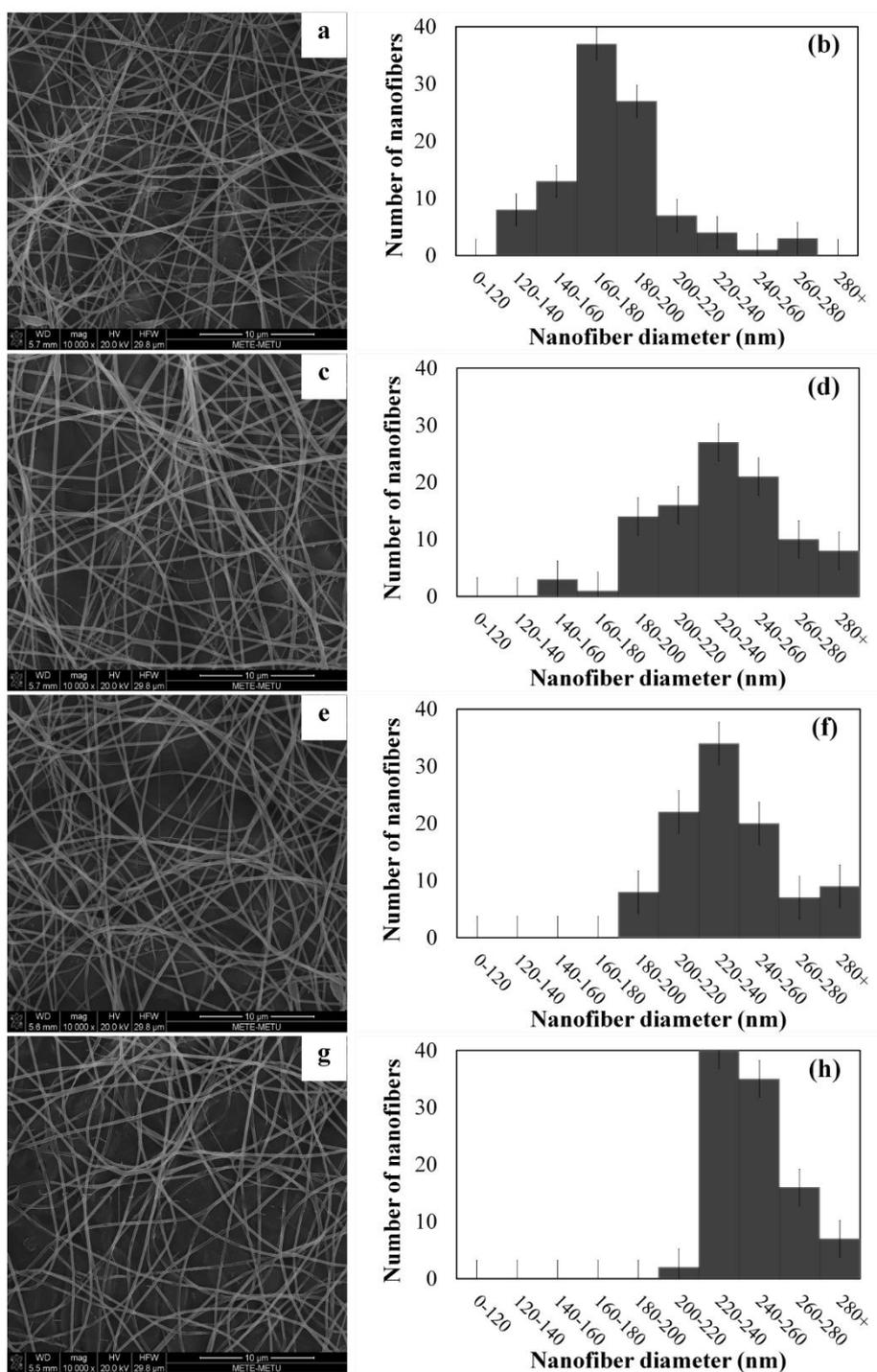


Figure 3.6 SEM images (10,000 \times) and diameter histogram of the nanofibers, obtained from solution with 1% PF (a and b) at pH 10, (c and d) at pH 12; with 2% PF (e and f) at pH 10, (g and h) at pH 12. Each solution contains also 3.5% PEO, 0.5% HPMC, and 2% Tween80.

3.3.2 Effect of pea flour concentration

Table 3.2 showed that bead formation was observed for both pea flour concentrations at pH value of 7. At pH 7, the apparent viscosity of solution with 1% pea flour was too low among the other solutions which can be seen in Figure 3.4. Similarly, Zhu and coauthors (2007) observed that aqueous solutions of regenerated silk fibroin at pH value of 6.9 could not be electrospun because the viscosity was too low to electrospin. Generally, the formation of beads has been attributed to jet instability, which was reduced upon increasing viscosity of solution with the addition of polymer (Huang et al., 2001). In this study, increasing pea flour concentration from 1% to 2% increased consistency coefficient from 1.138 to 1.151 Pa.sⁿ (Table 3.2). However, the increase in flour concentration was not sufficient to obtain smooth fibers at pH value of 7. Actually, viscosity rather than concentration was more useful parameter in electrospinning methodology (Nezarati, Eifert, & Cosgriff-Hernandez, 2013). With this information, homogenous nanofibers could be obtained when consistency coefficient was between 1.746 and 2.803 Pa.sⁿ which was proportional to apparent viscosity (Table 3.2).

Nanofiber diameter increased significantly as pea flour concentration in solution increased from 1% to 2% (w/v) at both pH values (Table A.4). Bhardwaj and Kundu (2010) have attempted to find a relation between gelatin solution concentration and fiber diameter and they found that as the concentration of solution increased, the fiber diameter increased. Generally in literature, increasing the concentration of solution could cause an increase in fiber diameter (Guo, Zhou, & Lv, 2013; Li & Wang, 2013; Sullivan, Tang, Kennedy, Talwar, & Khan, 2014).

Figure 3.6 showed a histogram of the measured diameters of solutions given in Table 3.2. It was seen that there was unimodal distribution of diameter of nanofibers prepared from solutions at different pH values. The reason of unimodal distribution was low pea flour concentration (1% and 2%). Unimodal distribution could be explained by no branching of nanofibers. Fibers prepared from solutions with high

solute concentration displayed branching when compared to ones containing low solute. The branching was attributed to uneven distribution of the charge carried by the jets, which was caused by jet elongation and solvent evaporation. The jets could reduce their local surface charge density by splitting into multiple smaller jets. When improved charge distribution and solution homogeneity were achieved, the fibers were more uniform, continuous and smooth. Xu et al. (2012) observed the transition from bimodal to unimodal distribution with decreasing branching. In addition, in a study conducted by Sullivan and coauthors, diameter range increased as ratio of whey protein isolate to PEO increased (Sullivan et al., 2014).

3.3.3 Effect of HPMC concentration

As shown in Table 3.3, there were significant changes in diameter of nanofibers with HPMC addition from 0.25% to 1.0% (w/v) for both 1% and 2% pea flour concentrations (Table A.8). For 1% (w/v) pea flour concentration, the mean diameter significantly increased from 177 ± 3 nm to 239 ± 4 nm. For 2% pea flour containing solution, the mean diameter of nanofibers significantly increased from 217 ± 3 nm to 242 ± 3 nm. It was known that polymer concentration played a major role in the electrospinning process. Similarly, Yang and coauthors (2004) showed that the mean fiber diameters increased as polymer concentration increased leading to increase in viscosity. Also, it was found by Lu and coauthors (2006) that the fiber diameter increased with increasing solution concentration of sodium alginate from 46 nm (1%) to 228 nm (3%). It was known that polymer concentration plays a major role in the electrospinning process. Under the same electrospinning conditions, increasing the polymer concentration will increase viscosity and then, the diameter of the electrospun fibers (Yener, Jirsak, & Gemci, 2014). In this study, the increase in fiber diameter could be explained by the significant increase in viscosity which was the dominant factor in influencing nanofiber diameters.

For 1% pea flour containing solution, there was insignificant increase in fiber diameter as HPMC content increased from 0.25 to 0.5%. In addition, for 2% pea flour solution, fiber diameter did not increase significantly as HPMC concentration increased from 0.5% to 1%. That is the change in viscosity with increase in HPMC concentration did not change fiber diameter significantly (Table 3.3).

There was unimodal distribution of measured diameter of solutions prepared from 0.25%, 0.5% and 1% HPMC (Figure 3.7). As discussed in Section 3.3.2, the distribution can be caused by no branching which was observed in the presence of low solute concentration. The distribution became narrower as HPMC concentration increased for both pea flour concentrations. As shown in Figure 3.7, the peak diameter observed shifted to the right as HPMC concentration increased from 0.25% to 1.0% (w/v). Similar distribution was also observed by Nazari et al. (2017).

3.3.4 Effect of applied voltage and flow rate

As mentioned in previous sections, nanofiber morphology and size were affected from not only solution properties but also process parameters which were applied voltage, feed rate, and distance between capillary and collector (Ghelich et al., 2015; Haider, Haider, & Kang, 2015). Two different solutions with 1.5% and 2% pea flour concentration, from which homogeneous nanofibers were obtained (Figure 3.1), were chosen (Table 3.1) in order to observe the effect of voltage and flow rate clearly.

3.3.4.1 The effect of applied voltage

Voltage values of 7 and 11 kV were chosen to observe the effect of voltage value on nanofiber morphology and diameter. Figure 3.8 showed that for both concentrations, homogeneous nanofibers were collected on the plate obtained by changing voltage values. However, voltage value of 7 kV might not be enough for solution charge and was found less efficient than the higher voltage. Therefore, less nanofibers were collected on the plate. This was caused by low applied voltage inducing the ejection of less fluid in the jet (Garg & Bowlin, 2011). According to Rijal et al. (2017), the high voltage value range between 10 and 19 kV is important to cause the polymer solution to be charged enough, and the charge is distributed equally through the body of the polymer droplet which is at the needle tip.

As can be seen in Figure 3.8, for 1.5% pea flour containing solutions, the peak observed in the particle size histograms moved to right meaning higher diameters as voltage value increased. For 2% pea flour concentration, nanofibers gathered around higher diameter ranges as voltage increased. As stated in previous sections, unimodal distribution was observed because of low concentration (Xu et al., 2012). Table 3.6 illustrated change of nanofiber diameter with increasing voltage value. As can be seen, the increase in voltage from 7 to 11 kV led to a significant increase

in nanofiber diameter (Table A.15). The applied voltage is the most significant one among the process parameters because it displays the degree of electrostatic interaction forces that cause the ejection of a jet (Kriegel et al., 2008). Garg and Bowlin (Garg & Bowlin, 2011) stated that more fluid ejection caused by higher voltage led to thicker fibers. In literature, there were similar researches showing that nanofiber diameter increased with increasing voltage value. Zhang et al. (2005) observed in their study that nanofiber size increased as voltage value increased from 5 kV to 10 kV. This was caused by increase in length and speed of jet and decrease in Taylor-cone diameter. High voltage increased velocity of jet and reduced the reach time of jet to collector; then, nanofiber diameter increased (Adabi et al., 2015; De Schoenmaker et al., 2012; Deitzel et al., 2001). As a result of this analysis, it was stated that voltage value should not be lower than 11 kV for efficient collection of nanofibers on grounded plate.

Table 3.6 Effect of voltage value on diameter of nanofibers obtained from 3.5% PEO solutions with 0.5% HPMC and 2% Tween80 concentration

Pea Flour Concentration (%)	Voltage (kV)	Nanofiber Diameter (nm)
1.5	7	183 ± 4 ^{d*}
1.5	11	225 ± 3 ^b
2	7	208 ± 3 ^c
2	11	238 ± 4 ^a

*Columns with different letters were different statistically (p≤0.05).

3.3.4.2 The effect of flow rate

Two different flow rates, which were 0.4 and 0.8 mL/h were chosen to observe its effects on nanofiber morphology and diameter. Figure 3.8 showed that nanofibers had bead formation as flow rate increased. As can be seen in SEM images, there was no efficiency problem in collecting nanofibers with increasing flow rate unlike decreasing voltage effect. Similarly, in a study conducted by Rodoplu and Mutlu (2012), the increase in bead size was observed with increasing flow rate value. Yuan and coauthors (2004) recommended that lower flow rates were useful to obtain homogenous nanofibers. From the morphology of nanofibers, flow rate value of 0.4 mL/h, which was used in analyzing the effect of pH, pea flour, and HPMC concentration, could be considered as optimum.

Table 3.7 showed that for both concentrations, there was significant decrease in nanofiber diameter with increasing flow rate (Table A.16). The reason could be that solvent on needle tip was evaporated at higher injection degree than lower flow rate and this led to an increase in amount of repulsive charges and then decrease in nanofiber size (De Schoenmaker et al., 2012). In literature, it was found that nanofiber size decreased significantly with increasing flow rate from 0.5 to 1.0 mL/h (Adabi et al., 2015). Similarly, Rodoplu and Mutlu (2012) observed the same behavior by increasing flow rate value from 1.1 to 1.6 mL/h and keeping other parameters constant.

Table 3.7 Effect of flow rate value on diameter of nanofibers obtained from 3.5% PEO solutions with 0.5% HPMC and 2% Tween80 concentration

Pea Flour Concentration (%)	Flow Rate (mL/h)	Nanofiber Diameter (nm)
1.5	0.4	225 ± 3 ^{b*}
1.5	0.8	166 ± 3 ^c
2	0.4	238 ± 4 ^a
2	0.8	173 ± 3 ^c

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

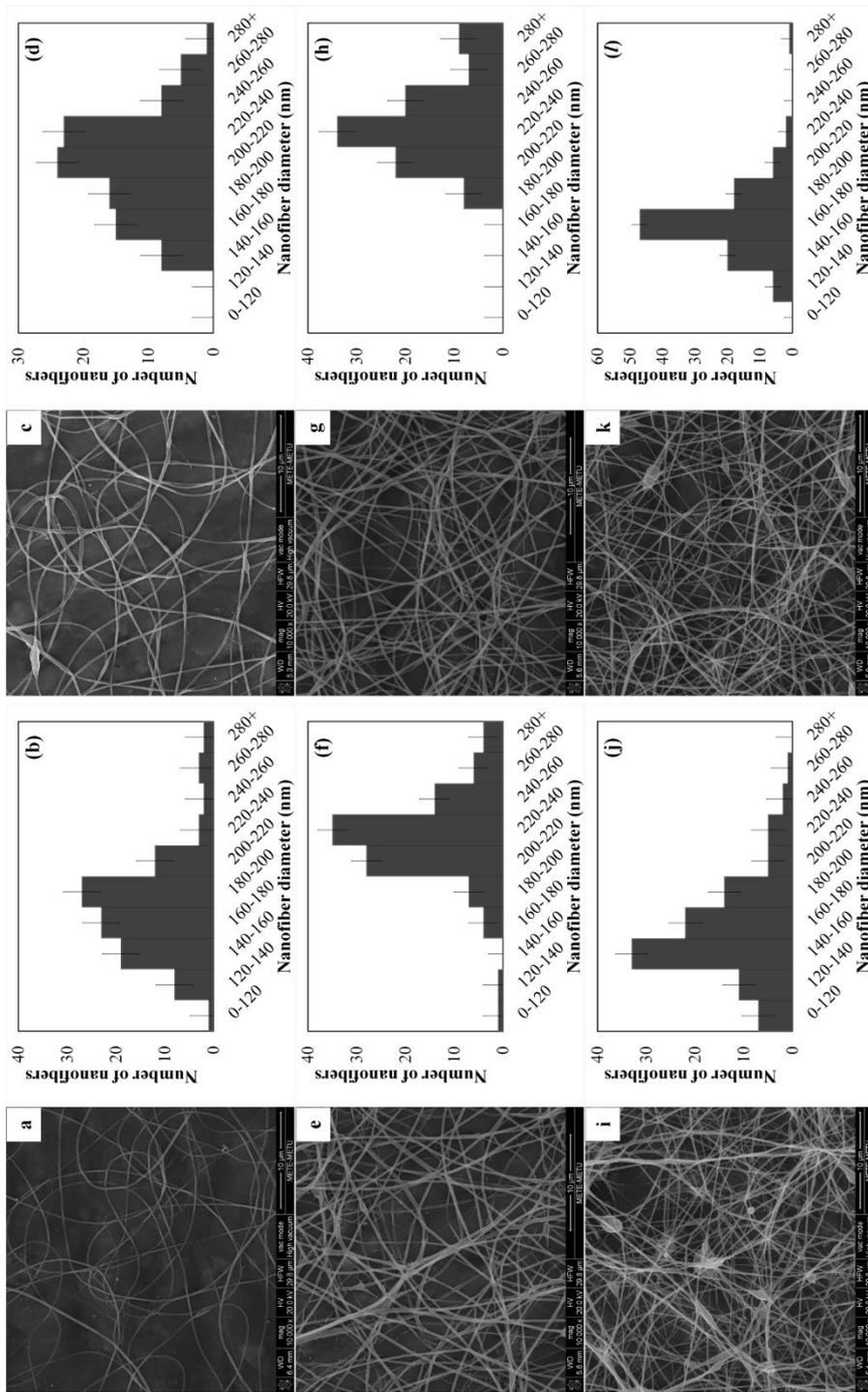


Figure 3.8 SEM images (10,000 ×) and diameter histogram of the nanofibers, obtained from solution of 1.5% pea flour operated with voltage:flow rate values of (a and b) 7 kV:0.4 mL/h, (e and f) 11 kV:0.4 mL/h, (i and j) 11 kV:0.8 mL/h; solution of 2% pea flour with (c and d) 7 kV:0.4 mL/h, (g and h) 11 kV:0.4 mL/h, (k and l) 11 kV:0.8 mL/h.

3.3.5 Effect of microfluidization (MF)

Table 3.4 and 3.5 showed that MF process had negative effect on nanofiber morphology. Nanofibers obtained from the solution with 3.5% PEO and 5.25% pea flour concentration had bead formation (BF) after MF process while homogeneous nanofibers could be obtained when MF was not applied (Figure 3.9). Similar to this result, nanofibers from solution with 2.5% PEO, 7.5% pea flour, and 0.5% HPMC concentration had the same defects after MF process which can be seen in Figure 3.10. Unlike these results, there was an improvement in nanofiber that bead size and number of beads decreased after applying MF process to solution with 2.5% PEO and 7.5% pea flour concentration. To sum up, MF process did not improve the morphology of nanofibers and therefore, it was not suggested to be used for electrospinning.

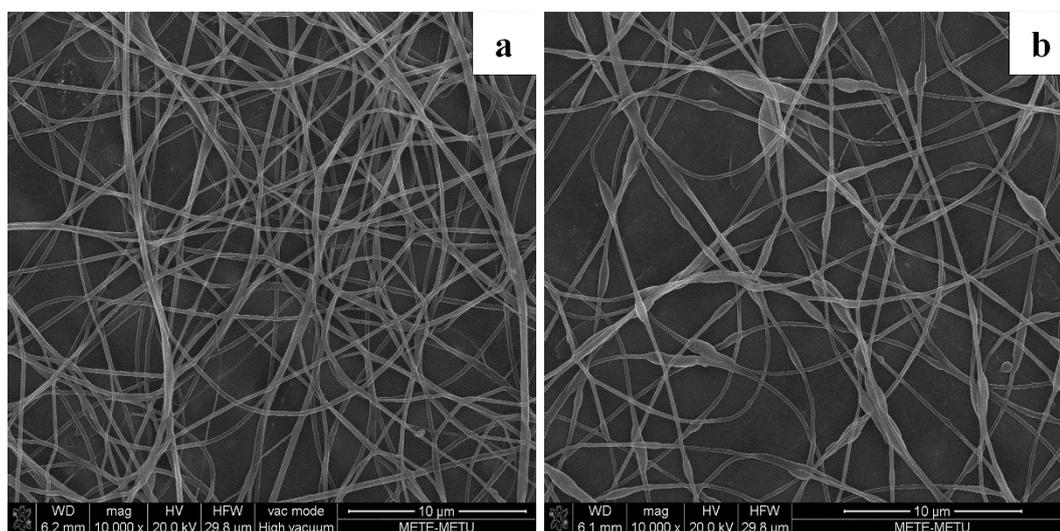


Figure 3.9 SEM images (10,000 ×) of the nanofibers, obtained from solution of 3.5% PEO and 5.25% pea flour concentration after (a) no microfluidization and (b) applying microfluidization with 10 pass.

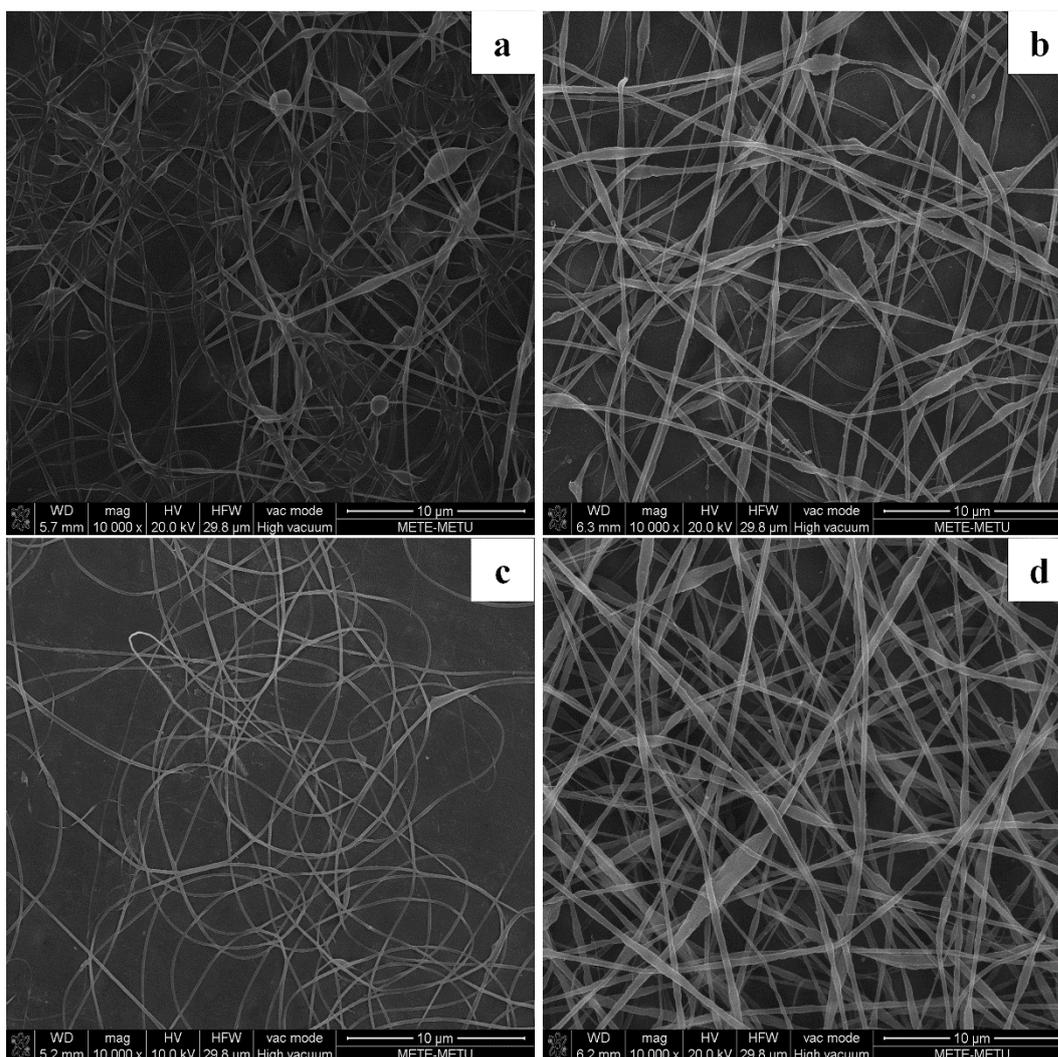


Figure 3.10 SEM images (10,000 ×) of the nanofibers, obtained from solution of 2.5% PEO and 7.5% pea flour concentration after (a) no microfluidization and (b) applying microfluidization with 10 pass; from solution of 2.5% PEO, 7.5% pea flour and 0.5% HPMC concentration after (c) no microfluidization and (d) applying microfluidization with 10 pass.

3.4 Water vapor permeability (WVP) and color analysis

Water vapor permeability (WVP) of nanofibers was obtained from solutions containing 3.5% PEO and 2% or 5.25% pea flour. Table 3.8 showed that the diameter of the fibers increased with increase in pea flour concentration. Solution with 5.25% pea flour concentration had k value of 6.672 Pa.sⁿ which was found to be significantly higher than the one observed with 2% pea flour containing solution having a k value of 2.140 Pa.sⁿ. The increase in k value with increasing pea flour concentration was due to more hydrogen binding of biopolymers as explained in Section 3.1.2. In addition, WVP value increased as pea flour concentration increased (Table A.17). This result was expected because pea flour contained 55±5% carbohydrate and 22±2% protein. In literature, WVP of edible films from alginate (Rangel-Marrón, Montalvo-Paquini, Palou, & López-Malo, 2013) and gelatin (Jongjareonrak, Benjakul, Visessanguan, Prodpran, & Tanaka, 2006) increased as polymer concentration increased because of the hydrophilic nature of some polysaccharides and proteins films being ineffective as moisture barrier. As can be seen in Table 3.8, WVP values also depended on fiber diameter. The reason may be that the increase in fiber diameter led to increase in pore size (Ryu, Kim, Lee, Park, & Lee, 2003). In this current study, WVP values of fibers varied in 12.18-17.54 × 10⁻¹⁶ kg.m⁻¹s⁻¹Pa⁻¹ which was lower compared to edible films in literature. Edible films of pea protein isolate and pea starch had WVP values of 8.4 × 10⁻¹⁴ kg.m⁻¹s⁻¹Pa⁻¹ (Kowalczyk & Baraniak, 2011) and 7.3 × 10⁻¹³ kg.m⁻¹s⁻¹Pa⁻¹ (Choi, Patel, & Han, 2016), respectively. This showed that combining pea flour with HPMC and PEO might be an advantage in food packaging area in terms of reduction of WVP values.

Table 3.8 showed that pea flour concentration did not affect nanofiber color significantly. Similar to this research, Nwachukwu (2010) also showed that fibers did not show any color change visually with the increase in polymer concentration.

Table 3.8 Effect of pea flour concentration in solutions containing 3.5% PEO, 0.5% HPMC at pH 10 on water vapor permeability and color of nanofiber

Pea flour concentration	Average diameter (nm)	WVP ($\text{kgm}^{-1}\text{s}^{-1}\text{Pa}^{-1}$) * 10^{16}	L*	a*	b*	ΔE^*
2	238±4 ^{b*}	12.18 ± 0.1 ^b	79.05 ± 0.8 ^a	1.135 ± 0.03 ^a	3.700 ± 0.04 ^a	14.81 ± 0,8 ^a
5.25	252±3 ^a	17.54 ± 1.0 ^a	73.01 ± 2.6 ^a	1.420 ± 0.06 ^a	3.315 ± 0.02 ^b	20.64 ± 2.6 ^a

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

3.5 Fourier-transform infrared (FTIR) analysis

Fourier-transform infrared (FTIR) spectrum supplies information of the functional groups in the sample, which is required to analyze the interaction between the components in the electrospun nanofibers. Table 3.9 showed the composition of samples analyzed by FTIR. Figure 3.11 showed the FTIR spectra of electrospun nanofibers with different compositions, pure pea flour, HPMC and PEO.

Table 3.9 The composition of samples analyzed in FTIR device

PEO concentration (%)	Pea Flour concentration (%)	HPMC concentration (%)	Sample letter
0	100	0	A*
100	0	0	B
0	0	100	C
3.5	5.25	0.5	D**
3.5	2	0.5	E**
3.5	5.25	0	F**
3.5	2	0	G**

*The samples were in powder form.

**The samples were solutions electrospun to nanofibers.

The region between 1500 cm^{-1} to 750 cm^{-1} was denoted by Aydogdu et al. (2018) as fingerprint region which consisted of bending vibrations within the molecule. The specific peaks of each component were produced in this region. Also, all samples had at least one peak. In the spectra of HPMC (C) and pea flour powder (A), the largest peaks were at 1053 and 1016 cm^{-1} , respectively. The peak at 1053

cm^{-1} was caused by C-O stretching vibrations (Ding, Zhang, & Li, 2015) and 1016 cm^{-1} attributed to C-O out of plane bending (Lee et al., 2007). When the region in spectrum of PEO powder (B) was examined, there was a triplet absorbance at 961, 1059, and 1144 cm^{-1} . Maximum absorbance displayed at 1096 cm^{-1} corresponding to crystallinity of PEO. Vega Lugo and Lim (2012) denoted maximum absorbance at 1100 cm^{-1} as ether peak caused by stretching vibrations of ether bond and C-O-C complex. Except pea flour and HPMC powders, the samples had similar structural features detected by FTIR spectra and similar absorbance bands but with some bands shifted from their original positions. The FTIR spectra of nanofibers obtained from solutions with different compositions showed intense absorbance at 843, 962, 1061, 1099, 1144, 1242, 1279, 1341, 1466, 2882 cm^{-1} . The small peaks at 1242 cm^{-1} corresponding to C-N stretching (Ding et al., 2015) which were not observed in HPMC spectrum. Most of the peaks in nanofiber spectra similar to peaks displayed in PEO spectrum, whose wavelength data were given in Figure 3.11. They were difference in wavelength and intensity. From the characteristic peaks observed in PEO spectrum, ones at 1144, 1279, 1341, and 1466 cm^{-1} were caused by C-O stretching, CH_2 twisting, CH_2 wagging, CH_2 scissoring, respectively. From the PEO spectrum, maximum peak at 2882 cm^{-1} which was close to the absorbance datum (2894 cm^{-1}) obtained from PEO by Safi and coauthors (2007). This peak at 2882 cm^{-1} was displayed in spectra of samples except A and C. Peak at around 2900 cm^{-1} corresponded to CH_2 (methylene) stretching (Aydogdu, Sumnu, & Sahin, 2018). Thus, absorbance intensity of CH_2 stretching of B and G spectra was the largest and similar each other. Generally, characteristic peaks in PEO spectrum were not masked when blending with other polymers to obtain nanofibers (D, E, F, G).

As a result, if the polymers are miscible because of the possible chemical interactions between polymers, there could be movements in the wavelength which is confirmed by Aydogdu et al. (2018). In this analysis, the FTIR spectra of blending nanofibers differed from that of the pure powder forms and miscibility of pea flour, HPMC and PEO blends was proved by FTIR analysis.

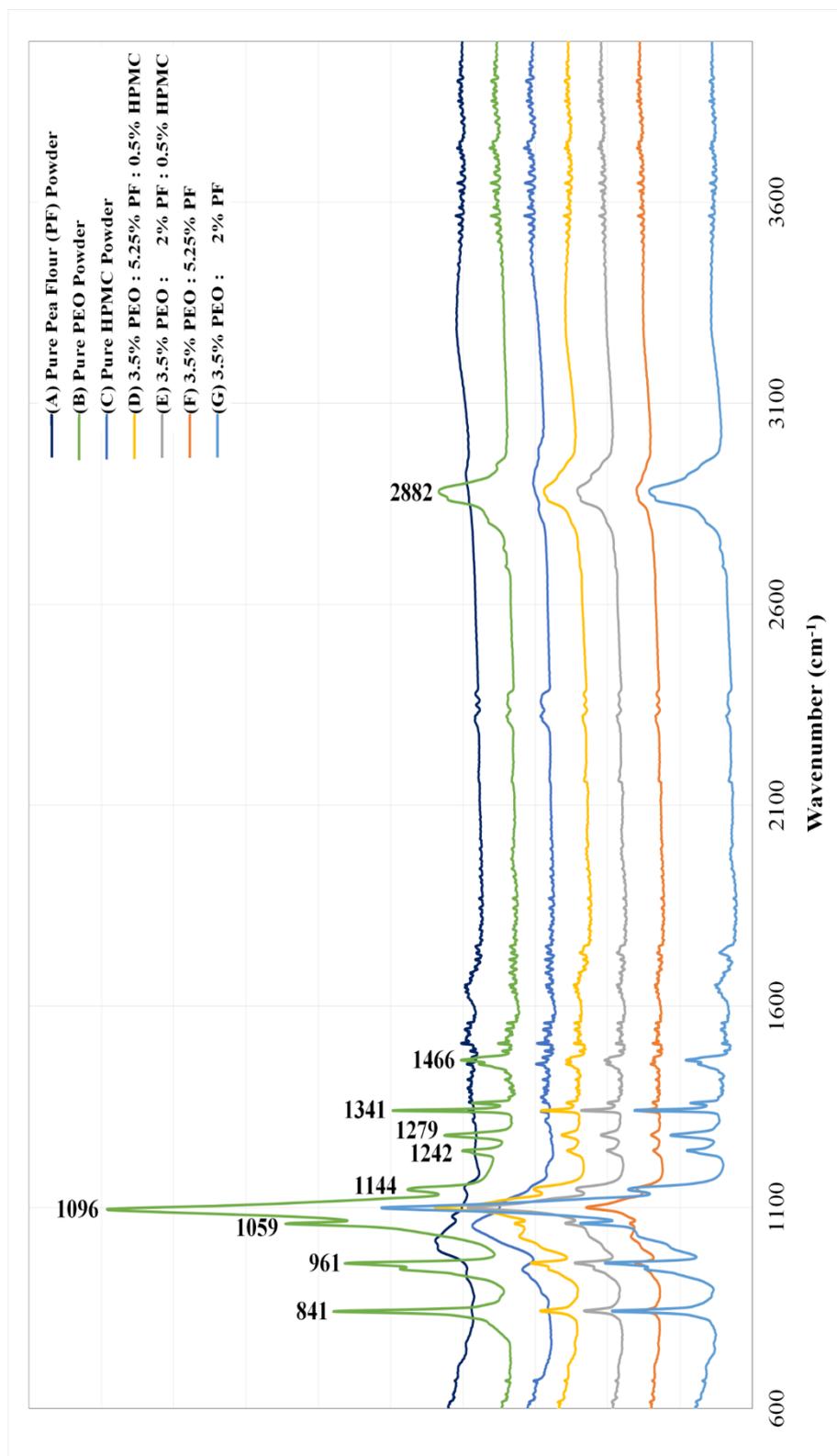


Figure 3.11 FTIR Spectra of powder forms of components and nanofibers whose denotations which are given in graph.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In this research, nanofibers from pea flour and HPMC were produced by using electrospinning. It was found that pH was a significant factor to obtain beadless nanofibers. The increase in pH from neutral value to alkaline value had positive effect on nanofiber morphology for both pea flour concentrations. Homogeneous nanofibers were obtained from solutions at alkaline conditions. On the other hand, no significant change was observed in conductivity within the range of studied variables. The increase in pH and pea flour concentration increased apparent viscosity of solution significantly which led to an increase in fiber diameter. Addition of HPMC increased nanofiber diameter. Microfluidization affected electrospinning adversely. The effect of electrospinning conditions on fiber diameter was also important. As voltage value increased, diameter of fibers increased while flow rate had an opposite effect on fiber diameter.

This study showed that electrospinning was a promising method for the production of biopolymer based nanofibers to be used in packaging industry. For this reason, water vapor permeability (WVP) and color analysis were performed. As increasing pea flour concentration from 2% to 5.25%, diameter of nanofibers increased leading to increase in WVP of nanofibers. The effect of pea flour concentration on color of nanofibers was not significant. FTIR analysis showed that good miscibility of polymers was achieved because there were movements in wavelengths at which characteristic peaks were observed. Thus, using PEO, pea flour and HPMC blending solution for electrospinning was a good alternative to produce nanofibers. It can be suggested that solutions with 1% pea flour at pH 10 containing either 0.5% or 0.25% HPMC can be good candidates for the preparation of packaging material

because they provided nanofibers with the smallest diameter and possibly lower WVP.

In future studies, active components can be included into electrospinning solutions in order to fabricate nanofibers to be used for active package materials. These components will give the packaging materials antioxidant effect. Therefore, the packages can increase shelf life of the product by reducing oxidation.

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APPENDICES

A. ANOVA TABLES

Table A. 1 Two way Analysis of Variance (ANOVA) and Tukey's comparison test for the effects of pH and pea flour concentration on consistency coefficient (k) of solution when 3.5% PEO, 0.5% HPMC and 2% Tween80 were used.

General Linear Model: k versus Pea Flour Concentration (%); pH

Factor	Type	Levels	Values
Pea Flour Concentration (%)	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
Pea Flour Concentration (%)	1	2,2360	2,2360	2,2360	237,32	0,000
pH	2	3,4131	3,4131	1,7066	181,13	0,000
Pea Flour Concentration (%) *pH	2	0,2683	0,2683	0,1341	14,24	0,001
Error	12	0,1131	0,1131	0,0094		
Total	17	6,0305				

S = 0,0970671 R-Sq = 98,13% R-Sq(adj) = 97,34%

Unusual Observations for k

Obs	k	Fit	SE Fit	Residual	St Resid
5	1,67113	1,51060	0,05604	0,16053	2,03 R
6	1,30813	1,51060	0,05604	-0,20247	-2,55 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Pea Flour Concentration (%)			
(%)	N	Mean	Grouping
2	9	2,3	A
1	9	1,6	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
12	6	2,3	A
10	6	2,1	B
7	6	1,3	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pea Flour Concentration

(%)	pH	N	Mean	Grouping
2	12	3	2,8	A
2	10	3	2,5	B
1	12	3	1,9	C
1	10	3	1,7	C D
2	7	3	1,5	D
1	7	3	1,1	E

Means that do not share a letter are significantly different.

Table A. 2 Two way Analysis of Variance (ANOVA) and Tukey’s comparison test for the effects of pH and pea flour concentration on flow behavior index (n) of solution when 3.5% PEO, 0.5% HPMC and 2% Tween80 were used

General Linear Model: n versus Pea Flour Concentration (%); pH

Factor	Type	Levels	Values
Pea Flour Concentration (%)	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				
Pea Flour Concentration (%)	1	0,0028376	0,0028376	0,0028376
224,92				
pH	2	0,0040332	0,0040332	0,0020166
159,84				
Pea Flour Concentration (%) * pH	2	0,0004819	0,0004819	0,0002410
19,10				
Error	12	0,0001514	0,0001514	0,0000126
Total	17	0,0075041		

Source	P
Pea Flour Concentration (%)	0,000
pH	0,000
Pea Flour Concentration (%) * pH	0,000
Error	
Total	

Table A. 2 (Continued)

S = 0,00355192 R-Sq = 97,98% R-Sq(adj) = 97,14%

Grouping Information Using Tukey Method and 95,0% Confidence

Pea Flour
Concentration

(%)	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
7	0,9	A
10	0,9	B
12	0,9	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pea Flour
Concentration

(%)	pH	N	Mean	Grouping
1	7	3	0,9	A
1	10	3	0,9	A B
2	7	3	0,9	B
1	12	3	0,9	C
2	10	3	0,9	D
2	12	3	0,9	D

Means that do not share a letter are significantly different.

Table A. 3 Two way Analysis of Variance (ANOVA) and Tukey's comparison test for the effects of pH and pea flour concentration on electrical conductivity of solution when 3.5% PEO, 0.5% HPMC and 2% Tween80 were used

General Linear Model: Electrical conductivity versus Pea flour (%); pH

Factor	Type	Levels	Values
PEa flour (%)	fixed	2	1; 2
pH	fixed	3	7; 10; 12

Analysis of Variance for Electrical conductivity, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
PEa flour (%)	1	0,31137	0,31137	0,31137	8,85	0,025
pH	2	0,76142	0,76142	0,38071	10,82	0,010
PEa flour (%) * pH	2	0,02872	0,02872	0,01436	0,41	0,682
Error	6	0,21109	0,21109	0,03518		
Total	11	1,31259				

S = 0,187568 R-Sq = 83,92% R-Sq(adj) = 70,52%

Unusual Observations for Electrical conductivity

Obs	Electrical conductivity	Fit	SE Fit	Residual	St Resid
9	1,35500	1,07150	0,13263	0,28350	2,14 R
10	0,78800	1,07150	0,13263	-0,28350	-2,14 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

PEa flour (%)	N	Mean	Grouping
2	6	1,0	A
1	6	0,7	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	1,2	A
10	4	0,9	A B
7	4	0,6	B

Means that do not share a letter are significantly different.

Table A. 3 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

PEa flour (%)					
	pH	N	Mean	Grouping	
2	12	2	1,4	A	
2	10	2	1,1	A B	
1	12	2	1,0	A B	
1	10	2	0,7	A B	
2	7	2	0,6	A B	
1	7	2	0,5	B	

Means that do not share a letter are significantly different.

Table A. 4 Two way Analysis of Variance (ANOVA) and Tukey's comparison test for the effects of pH and pea flour concentration on nanofiber diameter when 3.5% PEO, 0.5% HPMC and 2% Tween80 were used and nanofibers were obtained at the same electrospinning conditions

General Linear Model: Diameter (nm) versus Pea flour (%); pH

Factor	Type	Levels	Values
Pea flour (%)	fixed	2	1; 2
pH	fixed	2	10; 12

Analysis of Variance for Diameter (nm), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pea flour (%)	1	149692	149692	149692	141,21	0,000
pH	1	110623	110623	110623	104,36	0,000
Pea flour (%) * pH	1	43098	43098	43098	40,66	0,000
Error	396	419781	419781	1060		
Total	399	723193				

S = 32,5585 R-Sq = 41,95% R-Sq(adj) = 41,51%

Unusual Observations for Diameter (nm)

Obs	Diameter (nm)	Fit	SE Fit	Residual	St Resid
26	271,000	178,600	3,256	92,400	2,85 R
33	244,000	178,600	3,256	65,400	2,02 R
49	262,000	178,600	3,256	83,400	2,57 R
53	265,000	178,600	3,256	86,400	2,67 R
106	308,000	238,050	3,256	69,950	2,16 R
112	342,000	238,050	3,256	103,950	3,21 R
115	421,000	238,050	3,256	182,950	5,65 R
144	381,000	238,050	3,256	142,950	4,41 R
149	342,000	238,050	3,256	103,950	3,21 R

Table A. 4 (Continued)

229	146,000	232,620	3,256	-86,620	-2,67	R
261	152,000	232,620	3,256	-80,620	-2,49	R
262	298,000	232,620	3,256	65,380	2,02	R
278	327,000	232,620	3,256	94,380	2,91	R
293	153,000	232,620	3,256	-79,620	-2,46	R
314	354,000	250,550	3,256	103,450	3,19	R
315	342,000	250,550	3,256	91,450	2,82	R
335	360,000	250,550	3,256	109,450	3,38	R
366	372,000	250,550	3,256	121,450	3,75	R
376	409,000	250,550	3,256	158,450	4,89	R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Pea flour (%)			
	N	Mean	Grouping
2	200	244,3	A
1	200	205,6	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	200	241,6	A
10	200	208,3	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pea flour (%)				
	pH	N	Mean	Grouping
2	12	100	250,6	A
2	10	100	238,1	B
1	12	100	232,6	B
1	10	100	178,6	C

Means that do not share a letter are significantly different.

Table A. 5 Two way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of HPMC concentration in solutions at pH 10 on consistency coefficient (k) of solution when 3.5% PEO and 2% Tween80 were used

General Linear Model: k versus Pea flour (%); HPMC (%)

Factor	Type	Levels	Values
Pea flour (%)	fixed	2	1; 2
HPMC (%)	fixed	3	0,25; 0,50; 1,00

Analysis of Variance for k, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pea flour (%)	1	0,5499	0,5499	0,5499	7,98	0,015
HPMC (%)	2	6,3714	6,3714	3,1857	46,24	0,000
Pea flour (%)*HPMC (%)	2	1,3855	1,3855	0,6928	10,06	0,003
Error	12	0,8267	0,8267	0,0689		
Total	17	9,1334				

S = 0,262466 R-Sq = 90,95% R-Sq(adj) = 87,18%

Unusual Observations for k

Obs	k	Fit	SE Fit	Residual	St Resid
17	2,33731	2,90250	0,15153	-0,56520	-2,64 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Pea flour (%)	N	Mean	Grouping
2	9	2,5	A
1	9	2,1	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	3,1	A
0,50	6	2,1	B
0,25	6	1,7	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Table A. 5 (Continued)

Pea flour (%)	HPMC (%)	N	Mean	Grouping
1	1,00	3	3,3	A
2	1,00	3	2,9	A B
2	0,50	3	2,5	B C
2	0,25	3	2,0	C D
1	0,50	3	1,7	D
1	0,25	3	1,3	D

Means that do not share a letter are significantly different.

Table A. 6 Two way Analysis of Variance (ANOVA) and Tukey’s comparison test for the effect of HPMC concentration in solutions at pH 10 on flow behavior index (n) of solution when 3.5% PEO and 2% Tween80 were used

General Linear Model: n versus Pea flour (%); HPMC (%)

Factor	Type	Levels	Values
Pea flour (%)	fixed	2	1; 2
HPMC (%)	fixed	3	0,25; 0,50; 1,00

Analysis of Variance for n, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pea flour (%)	1	0,0006564	0,0006564	0,0006564	12,26	0,004
HPMC (%)	2	0,0059480	0,0059480	0,0029740	55,55	0,000
Pea flour (%)*HPMC (%)	2	0,0020811	0,0020811	0,0010406	19,44	0,000
Error	12	0,0006424	0,0006424	0,0000535		
Total	17	0,0093280				

S = 0,00731680 R-Sq = 93,11% R-Sq(adj) = 90,24%

Unusual Observations for n

Obs	n	Fit	SE Fit	Residual	St Resid
12	0,852900	0,870767	0,004224	-0,017867	-2,99 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Pea flour (%)	N	Mean	Grouping
1	9	0,9	A
2	9	0,9	B

Means that do not share a letter are significantly different.

Table A. 6 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

HPMC (%)	N	Mean	Grouping
0,25	6	0,9	A
0,50	6	0,9	A
1,00	6	0,9	B

Means that do not share a letter are significantly different.
Grouping Information Using Tukey Method and 95,0% Confidence

Pea flour

(%)	HPMC (%)	N	Mean	Grouping
1	0,50	3	0,9	A
1	0,25	3	0,9	A
2	0,25	3	0,9	A B
2	0,50	3	0,9	B C
2	1,00	3	0,9	C D
1	1,00	3	0,9	D

Means that do not share a letter are significantly different.

Table A. 7 Two way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of HPMC concentration in solutions at pH 10 on electrical conductivity of solution when 3.5% PEO and 2% Tween80 were used

General Linear Model: Electrical conductivity versus Pea flour (%); HPMC (%)

Factor	Type	Levels	Values
Pea flour (%)	fixed	2	1; 2
HPMC (%)	fixed	3	0,25; 0,50; 1,00

Analysis of Variance for Electrical conductivity, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pea flour (%)	1	0,31883	0,31883	0,31883	5,62	0,055
HPMC (%)	2	0,07373	0,07373	0,03687	0,65	0,555
Pea flour (%)*HPMC (%)	2	0,01338	0,01338	0,00669	0,12	0,891
Error	6	0,34040	0,34040	0,05673		
Total	11	0,74635				

S = 0,238189 R-Sq = 54,39% R-Sq(adj) = 16,38%

Grouping Information Using Tukey Method and 95,0% Confidence

Table A. 7 (Continued)

Pea flour			
(%)	N	Mean	Grouping
2	6	0,9	A
1	6	0,6	A

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	4	0,9	A
0,25	4	0,7	A
1,00	4	0,7	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pea flour				
(%)	HPMC (%)	N	Mean	Grouping
2	0,50	2	1,1	A
2	0,25	2	0,8	A
2	1,00	2	0,8	A
1	0,50	2	0,7	A
1	0,25	2	0,6	A
1	1,00	2	0,5	A

Means that do not share a letter are significantly different.

Table A. 8 Two way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of HPMC concentration in solutions at pH 10 on nanofiber diameter when 3.5% PEO and 2% Tween80 were used and nanofibers were obtained at the same electrospinning conditions

General Linear Model: Diameter (nm) versus Pea flour (%); HPMC (%)

Factor	Type	Levels	Values
Pea flour (%)	fixed	2	1; 2
HPMC (%)	fixed	3	0,25; 0,50; 1,00

Analysis of Variance for Diameter (nm), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pea flour (%)	1	178055	178055	178055	133,83	0,000
HPMC (%)	2	206191	206191	103096	77,49	0,000
Pea flour (%)*HPMC (%)	2	79896	79896	39948	30,02	0,000
Error	594	790313	790313	1330		
Total	599	1254455				

S = 36,4759 R-Sq = 37,00% R-Sq(adj) = 36,47%

Unusual Observations for Diameter (nm)

Obs	Diameter (nm)	Fit	SE Fit	Residual	St Resid
8	281,000	176,530	3,648	104,470	2,88 R
10	271,000	176,530	3,648	94,470	2,60 R
14	284,000	176,530	3,648	107,470	2,96 R
16	250,000	176,530	3,648	73,470	2,02 R
19	262,000	176,530	3,648	85,470	2,35 R
21	258,000	176,530	3,648	81,470	2,24 R
24	254,000	176,530	3,648	77,470	2,13 R
30	90,000	176,530	3,648	-86,530	-2,38 R
33	100,000	176,530	3,648	-76,530	-2,11 R
63	94,000	176,530	3,648	-82,530	-2,27 R
65	87,000	176,530	3,648	-89,530	-2,47 R
93	102,000	176,530	3,648	-74,530	-2,05 R
96	90,000	176,530	3,648	-86,530	-2,38 R
134	119,000	216,660	3,648	-97,660	-2,69 R
148	322,000	216,660	3,648	105,340	2,90 R
181	296,000	216,660	3,648	79,340	2,19 R
226	271,000	178,600	3,648	92,400	2,55 R
249	262,000	178,600	3,648	83,400	2,30 R
253	265,000	178,600	3,648	86,400	2,38 R
312	342,000	238,050	3,648	103,950	2,86 R
315	421,000	238,050	3,648	182,950	5,04 R
344	381,000	238,050	3,648	142,950	3,94 R
349	342,000	238,050	3,648	103,950	2,86 R
410	118,000	238,560	3,648	-120,560	-3,32 R
427	315,000	238,560	3,648	76,440	2,11 R
438	155,000	238,560	3,648	-83,560	-2,30 R
445	338,000	238,560	3,648	99,440	2,74 R
463	89,000	238,560	3,648	-149,560	-4,12 R

Table A. 8 (Continued)

464	155,000	238,560	3,648	-83,560	-2,30	R
469	92,000	238,560	3,648	-146,560	-4,04	R
481	341,000	238,560	3,648	102,440	2,82	R
489	138,000	238,560	3,648	-100,560	-2,77	R
507	373,000	242,340	3,648	130,660	3,60	R
554	364,000	242,340	3,648	121,660	3,35	R
555	330,000	242,340	3,648	87,660	2,42	R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Pea
flour

(%)	N	Mean	Grouping
2	300	232,3	A
1	300	197,9	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	200	240,4	A
0,50	200	208,3	B
0,25	200	196,6	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pea
flour

(%)	HPMC (%)	N	Mean	Grouping
2	1,00	100	242,3	A
1	1,00	100	238,6	A
2	0,50	100	238,0	A
2	0,25	100	216,7	B
1	0,50	100	178,6	C
1	0,25	100	176,5	C

Means that do not share a letter are significantly different.

Table A. 9 One way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of MF technique operating with 10 passes applied on solutions at pH 10 on consistency coefficient (k) of solution when 3.5% PEO, 5.25% pea flour and 2% Tween80 concentrations were used

General Linear Model: k value versus Pass Number

Factor	Type	Levels	Values
Pass Number	fixed	2	0; 10

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

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pass Number	1	186,95	186,95	186,95	320,26	0,000
Error	4	2,34	2,34	0,58		
Total	5	189,29				

S = 0,764039 R-Sq = 98,77% R-Sq(adj) = 98,46%

Grouping Information Using Tukey Method and 95,0% Confidence

Pass Number	N	Mean	Grouping
10	3	13,2	A
0	3	2,1	B

Means that do not share a letter are significantly different.

Table A. 10 One way Analysis of Variance (ANOVA) and Tukey’s comparison test for the effect of MF technique operating with 10 passes applied on solutions at pH 10 on flow behavior index (n) of solution when 3.5% PEO, 5.25% pea flour and 2% Tween80 concentrations were used

General Linear Model: n value versus Pass Number

Factor	Type	Levels	Values
Pass Number	fixed	2	0; 10

Analysis of Variance for n value, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pass Number	1	0,12936	0,12936	0,12936	335,42	0,000
Error	4	0,00154	0,00154	0,00039		
Total	5	0,13090				

S = 0,0196384 R-Sq = 98,82% R-Sq(adj) = 98,53%

Grouping Information Using Tukey Method and 95,0% Confidence

Pass Number	N	Mean	Grouping
0	3	0,9	A
10	3	0,6	B

Means that do not share a letter are significantly different.

Table A. 11 One way Analysis of Variance (ANOVA) and Tukey’s comparison test for the effect of MF technique operating with 10 passes applied on solutions at pH 10 on electrical conductivity of solution when 3.5% PEO, 5.25% pea flour and 2% Tween80 concentration were used.

General Linear Model: Electrical conductivity versus Pass Number

Factor	Type	Levels	Values
Pass Number	fixed	2	0; 10

Analysis of Variance for Electrical conductivity, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pass Number	1	0,0026010	0,0026010	0,0026010	30,99	0,031
Error	2	0,0001678	0,0001678	0,0000839		
Total	3	0,0027688				

Table A.11 (Continued)

S = 0,00916079 R-Sq = 93,94% R-Sq(adj) = 90,91%

Grouping Information Using Tukey Method and 95,0% Confidence

Pass	Number	N	Mean	Grouping
	0	2	0,1	A
	10	2	0,1	B

Means that do not share a letter are significantly different.

Table A. 12 Two way Analysis of Variance (ANOVA) and Tukey’s comparison test for the effect of MF technique operating with 10 passes applied on solutions at pH 10 on consistency coefficient (k) of solution when 2.5% PEO and 7.5% pea flour concentration were used with HPMC or without HPMC

General Linear Model: k value versus HPMC %; Pass Number

Factor	Type	Levels	Values
HPMC %	fixed	2	0,0; 0,5
Pass Number	fixed	2	0; 10

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

Source	DF	Seq SS	Adj SS	Adj MS	F	P
HPMC %	1	688,16	688,16	688,16	76,51	0,000
Pass Number	1	840,70	840,70	840,70	93,47	0,000
HPMC %*Pass Number	1	20,27	20,27	20,27	2,25	0,172
Error	8	71,96	71,96	8,99		
Total	11	1621,08				

S = 2,99907 R-Sq = 95,56% R-Sq(adj) = 93,90%

Unusual Observations for k value

Obs	k value	Fit	SE Fit	Residual	St Resid
12	25,7160	32,3143	1,7315	-6,5983	-2,69 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

HPMC %	N	Mean	Grouping
0,5	6	22,6	A
0,0	6	7,5	B

Means that do not share a letter are significantly different.

Table A. 12 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

Pass			
Number	N	Mean	Grouping
10	6	23,4	A
0	6	6,7	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pass				
HPMC %	Number	N	Mean	Grouping
0,5	10	3	32,3	A
0,0	10	3	14,6	B
0,5	0	3	13,0	B
0,0	0	3	0,4	C

Means that do not share a letter are significantly different.

Table A. 13 Two way Analysis of Variance (ANOVA) and Tukey’s comparison test for the effect of MF technique operating with 10 passes applied on solutions at pH 10 on flow behavior index (n) of solution when 2.5% PEO and 7.5% pea flour concentration were used with HPMC or without HPMC.

General Linear Model: n value versus HPMC %; Pass Number

Factor	Type	Levels	Values
HPMC %	fixed	2	0,0; 0,5
Pass Number	fixed	2	0; 10

Analysis of Variance for n value, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
HPMC %	1	0,13380	0,13380	0,13380	185,55	0,000
Pass Number	1	0,26829	0,26829	0,26829	372,08	0,000
HPMC %*Pass Number	1	0,09019	0,09019	0,09019	125,07	0,000
Error	8	0,00577	0,00577	0,00072		
Total	11	0,49804				

S = 0,0268525 R-Sq = 98,84% R-Sq(adj) = 98,41%

Unusual Observations for n value

Obs	n value	Fit	SE Fit	Residual	St Resid
1	0,857900	0,907000	0,015503	-0,049100	-2,24 R

R denotes an observation with a large standardized residual.

Table A. 13 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

HPMC %	N	Mean	Grouping
0,0	6	0,7	A
0,5	6	0,5	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pass	Number	N	Mean	Grouping
	0	6	0,7	A
	10	6	0,4	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

HPMC %	Pass	Number	N	Mean	Grouping
0,0		0	3	0,9	A
0,5		0	3	0,5	B
0,0		10	3	0,4	C
0,5		10	3	0,4	C

Means that do not share a letter are significantly different.

Table A. 14 Two way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of MF technique operating with 10 passes applied on solutions at pH 10 on electrical conductivity of solution when 2.5% PEO and 7.5% pea flour concentration were used with HPMC or without HPMC.

General Linear Model: Electrical conductivity versus HPMC %; Pass Number

Factor	Type	Levels	Values
HPMC %	fixed	2	0,0; 0,5
Pass Number	fixed	2	0; 10

Analysis of Variance for Electrical conductivity, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
HPMC %	1	0,0002112	0,0002112	0,0002112	168921,00	0,000
Pass Number	1	0,0039383	0,0039383	0,0039383	3150625,00	0,000
HPMC %*Pass Number	1	0,0024816	0,0024816	0,0024816	1985281,00	0,000
Error	4	0,0000000	0,0000000	0,0000000		
Total	7	0,0066310				

Table A. 14 (Continued)

S = 0,0000353553 R-Sq = 100,00% R-Sq(adj) = 100,00%

Unusual Observations for Electrical conductivity

Obs	Electrical conductivity	Fit	SE Fit	Residual	St Resid
2	0,198100	0,198050	0,000025	0,000050	2,00 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

HPMC %	N	Mean	Grouping
0,0	4	0,2	A
0,5	4	0,2	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pass	Number	N	Mean	Grouping
0	4	0,2	A	
10	4	0,2	B	

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

HPMC %	Pass	Number	N	Mean	Grouping
0,5	0	2	0,2	A	
0,0	0	2	0,2	B	
0,0	10	2	0,2	C	
0,5	10	2	0,1	D	

Means that do not share a letter are significantly different.

Table A. 15 Two way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of voltage values on diameter of nanofibers obtained from 3.5% PEO solutions with 0.5% HPMC and 2% Tween80 concentration.

General Linear Model: Diameter (nm) versus Pea Flour (%); Voltage

Factor	Type	Levels	Values
Pea Flour (%)	fixed	2	1,5; 2,0
Voltage	fixed	2	7; 11

Analysis of Variance for Diameter (nm), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pea Flour (%)	1	36176	36176	36176	30,39	0,000
Voltage	1	131189	131189	131189	110,21	0,000
Pea Flour (%)*Voltage	1	3856	3856	3856	3,24	0,073
Error	396	471364	471364	1190		
Total	399	642586				

S = 34,5009 R-Sq = 26,65% R-Sq(adj) = 26,09%

Unusual Observations for Diameter (nm)

Obs	Diameter (nm)	Fit	SE Fit	Residual	St Resid
6	308,000	238,050	3,450	69,950	2,04 R
12	342,000	238,050	3,450	103,950	3,03 R
15	421,000	238,050	3,450	182,950	5,33 R
44	381,000	238,050	3,450	142,950	4,16 R
49	342,000	238,050	3,450	103,950	3,03 R
199	301,000	208,040	3,450	92,960	2,71 R
207	296,000	225,240	3,450	70,760	2,06 R
228	300,000	225,240	3,450	74,760	2,18 R
230	135,000	225,240	3,450	-90,240	-2,63 R
275	304,000	225,240	3,450	78,760	2,29 R
282	106,000	225,240	3,450	-119,240	-3,47 R
302	259,000	182,810	3,450	76,190	2,22 R
321	321,000	182,810	3,450	138,190	4,03 R
343	314,000	182,810	3,450	131,190	3,82 R
379	277,000	182,810	3,450	94,190	2,74 R
384	270,000	182,810	3,450	87,190	2,54 R
385	270,000	182,810	3,450	87,190	2,54 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Pea Flour (%)	N	Mean	Grouping
2,0	200	223,0	A
1,5	200	204,0	B

Table A. 15 (Continued)

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Voltage	N	Mean	Grouping
11	200	231,6	A
7	200	195,4	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pea Flour (%)	Voltage	N	Mean	Grouping
2,0	11	100	238,1	A
1,5	11	100	225,2	B
2,0	7	100	208,0	C
1,5	7	100	182,8	D

Means that do not share a letter are significantly different.

Table A. 16 Two way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of flow rate values on diameter of nanofibers obtained from 3.5% PEO solutions with 0.5% HPMC and 2% Tween80 concentration.

General Linear Model: Diameter (nm) versus Pea Flour (%); Flow rate

Factor	Type	Levels	Values
Pea Flour (%)	fixed	2	1,5; 2,0
Flow rate	fixed	2	0,4; 0,8

Analysis of Variance for Diameter (nm), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pea Flour (%)	1	9516	9516	9516	9,55	0,002
Flow rate	1	389438	389438	389438	390,94	0,000
Pea Flour (%) * Flow rate	1	933	933	933	0,94	0,334
Error	396	394479	394479	996		
Total	399	794367				

S = 31,5620 R-Sq = 50,34% R-Sq(adj) = 49,96%

Unusual Observations for Diameter (nm)

Obs	Diameter (nm)	Fit	SE Fit	Residual	St Resid
13	232,000	165,890	3,156	66,110	2,11 R
32	264,000	165,890	3,156	98,110	3,12 R
42	94,000	165,890	3,156	-71,890	-2,29 R
57	99,000	165,890	3,156	-66,890	-2,13 R
67	241,000	165,890	3,156	75,110	2,39 R
73	252,000	165,890	3,156	86,110	2,74 R
74	231,000	165,890	3,156	65,110	2,07 R

Table A. 16 (Continued)

83	99,000	165,890	3,156	-66,890	-2,13	R
91	90,000	165,890	3,156	-75,890	-2,42	R
100	235,000	165,890	3,156	69,110	2,20	R
125	293,000	172,590	3,156	120,410	3,83	R
128	238,000	172,590	3,156	65,410	2,08	R
206	308,000	238,050	3,156	69,950	2,23	R
212	342,000	238,050	3,156	103,950	3,31	R
215	421,000	238,050	3,156	182,950	5,83	R
244	381,000	238,050	3,156	142,950	4,55	R
249	342,000	238,050	3,156	103,950	3,31	R
307	296,000	225,240	3,156	70,760	2,25	R
328	300,000	225,240	3,156	74,760	2,38	R
330	135,000	225,240	3,156	-90,240	-2,87	R
375	304,000	225,240	3,156	78,760	2,51	R
382	106,000	225,240	3,156	-119,240	-3,80	R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Pea			
Flour			
(%)	N	Mean	Grouping
2,0	200	205,3	A
1,5	200	195,6	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Flow			
rate	N	Mean	Grouping
0,4	200	231,6	A
0,8	200	169,2	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pea				
Flour				
(%)	Flow rate	N	Mean	Grouping
2,0	0,4	100	238,1	A
1,5	0,4	100	225,2	B
2,0	0,8	100	172,6	C
1,5	0,8	100	165,9	C

Means that do not share a letter are significantly different.

Table A. 17 One way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of pea flour concentration in solutions containing 3.5% PEO, 0.5% HPMC at pH 10 on water vapor permeability (WVP).

General Linear Model: WVP versus Pea Flour Concentration %

Factor	Type	Levels	Values
Pea Flour Concentration %	fixed	2	2,00; 5,25

Analysis of Variance for WVP, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS
F				
Pea Flour Concentration %	1	2,92391E+17	2,92391E+17	2,92391E+17
29,66				
Error	2	1,97187E+16	1,97187E+16	9,85934E+15
Total	3	3,12110E+17		

Source	P
Pea Flour Concentration %	0,032
Error	
Total	

S = 99294200 R-Sq = 93,68% R-Sq(adj) = 90,52%

Grouping Information Using Tukey Method and 95,0% Confidence

Pea Flour Concentration %	N	Mean	Grouping
5,25	2	1,75348E+09	A
2,00	2	1,21274E+09	B

Means that do not share a letter are significantly different.

Table A. 18 One way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of pea flour concentration in solutions containing 3.5% PEO, 0.5% HPMC at pH 10 on L value of nanofibers.

General Linear Model: L versus Pea Flour Concentration %

Factor	Type	Levels	Values
Pea Flour Concentration %	fixed	2	2,00; 5,25

Analysis of Variance for L, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pea Flour Concentration %	1	36,482	36,482	36,482	4,89	0,158
Error	2	14,931	14,931	7,466		
Total	3	51,413				

S = 2,73233 R-Sq = 70,96% R-Sq(adj) = 56,44%

Grouping Information Using Tukey Method and 95,0% Confidence

Pea Flour Concentration %	N	Mean	Grouping
2,00	2	79,0	A
5,25	2	73,0	A

Means that do not share a letter are significantly different.

Table A. 19 One way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of pea flour concentration in solutions containing 3.5% PEO, 0.5% HPMC at pH 10 on a value of nanofibers.

General Linear Model: a versus Pea Flour Concentration %

Factor	Type	Levels	Values
Pea Flour Concentration %	fixed	2	2,00; 5,25

Analysis of Variance for a, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pea Flour Concentration %	1	0,081225	0,081225	0,081225	16,83	0,055
Error	2	0,009650	0,009650	0,004825		
Total	3	0,090875				

S = 0,0694622 R-Sq = 89,38% R-Sq(adj) = 84,07%

Grouping Information Using Tukey Method and 95,0% Confidence

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

Means that do not share a letter are significantly different.

Table A. 20 One way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of pea flour concentration in solutions containing 3.5% PEO, 0.5% HPMC at pH 10 on b value of nanofibers

General Linear Model: b versus Pea Flour Concentration %

Factor	Type	Levels	Values
Pea Flour Concentration %	fixed	2	2,00; 5,25

Analysis of Variance for b, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pea Flour Concentration %	1	0,14823	0,14823	0,14823	66,62	0,015
Error	2	0,00445	0,00445	0,00222		
Total	3	0,15268				

S = 0,0471699 R-Sq = 97,09% R-Sq(adj) = 95,63%

Grouping Information Using Tukey Method and 95,0% Confidence

Pea Flour Concentration %	N	Mean	Grouping
2,00	2	3,7	A
5,25	2	3,3	B

Means that do not share a letter are significantly different.

Table A. 21 One way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of pea flour concentration in solutions containing 3.5% PEO, 0.5% HPMC at pH 10 on ΔE value of nanofibers.

General Linear Model: ΔE versus Pea Flour Concentration %

Factor	Type	Levels	Values
Pea Flour Concentration %	fixed	2	2,00; 5,25

Analysis of Variance for ΔE , using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pea Flour Concentration %	1	33,929	33,929	33,929	4,71	0,162
Error	2	14,397	14,397	7,199		
Total	3	48,326				

S = 2,68303 R-Sq = 70,21% R-Sq(adj) = 55,31%

Grouping Information Using Tukey Method and 95,0% Confidence

Pea Flour Concentration %	N	Mean	Grouping
5,25	2	20,6	A
2,00	2	14,8	A

Means that do not share a letter are significantly different.