EFFECTS OF MICROWAVE HEATING ON ELECTROSPINNING OF CAROB BEAN FLOUR BASED NANOFIBERS

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EFFECTS OF MICROWAVE HEATING ON ELECTROSPINNING OF CAROB BEAN FLOUR BASED NANOFIBERS

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ABSTRACT

EFFECTS OF MICROWAVE HEATING ON ELECTROSPINNING OF CAROB BEAN FLOUR BASED NANOFIBERS

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In addition to time and energy saving advantages, microwave heating has some effects on functional properties of flours, especially starch and protein compounds. The aim of this study was to determine the effects of microwave pretreatment on physicochemical characteristics of carob flour and rice starch-based nanofibers produced by electrospinning. In addition, the effects of carob flour concentration (3%, 5% w/v) and rice starch addition (0.5% w/v) on nanofiber production were studied. Solutions were heated conventionally or by using microwave until 80°C is reached. In electrospinning process, 0.8 mL/h flow rate and 12 kV voltage were set constant. Scanning electron microscopy (SEM), water vapor permeability (WVP), X-Ray diffraction (XRD), mechanical test, differential scanning calorimeter (DSC), Fourier-transform infrared spectroscopy (FTIR) analysis were performed on the film samples. It was possible to obtain homogenous and bead free fibers when microwave heating was used. Nanofibers originated from microwave heated solution presented better characteristics in terms of water vapor permeability and mechanical properties as compared to conventionally heated one at the same concentration. While microwave heated sample had 1.68×10^{-12} g $s^{-1}m^{-1}Pa^{-1}$ water vapor permeability value, conventionally heated sample had 2.13×10^{-12} g $s^{-1}m^{-1}Pa^{-1}$ at 3% carob flour and 0.5%

rice starch concentration. In the study, solution characteristics were investigated in terms of analysis of rheological properties, electrical conductivity and free amino group determination. It was found that microwave heated solution had higher viscosity value and free amino group amount as compared to conventionally heated solution at the same concentration. This study showed that microwave heating can be considered as a promising and more advantageous method for the preparation of electrospinning solutions rather than conventional heating.

Keywords: Carob flour, microwave, electrospinning, nanofiber, starch

ÖZ

MIKRODALGA İLE ISITMANIN KEÇİBOYNUZU UNU BAZLI NANOLİFLERİN ELEKTROEĞRİLMESİ ÜZERİNE ETKİSİ

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Zaman ve enerji tasarrufu gibi avantajlarına ek olarak, mikrodalga ile ısıtmanın un bileşenlerinin özellikle nişasta ve proteinlerin fonksiyonel özellikleri üzerinde bazı etkileri vardır. Bu çalışmanın amacı, mikrodalga ile ön ısıtmanın, keçiboynuzu unu ve pirinç nişastasından üretilen nanoliflerin fizikokimyasal özellikleri üzerindeki etkilerini belirlemektir. Ayrıca, keçiboynuzu unu konsantrasyonunun (%3 ve 5%) ve pirinç nişastası ilavesinin (0.5%) nanolif üretimi üzerindeki etkileri incelenmiştir. Çözeltiler konvensiyonel olarak veya mikrodalga ile 80°C'ye gelinceye kadar ısıtılmışlardır. Elektroeğirme parametrelerinde akış hızı olarak 0.8 mL/saat ve voltaj olarak 12 kV sabit tutulmuştur. Film numuneleri üzerinde taramalı elektron mikroskobu (SEM), su buharı geçirgenliği, X-ışını kırınımı, mekanik test, diferansiyel taramalı kalorimetri (DTK) ve Fourier dönüşümlü kızıl ötesi (FDKÖ) analizleri yapılmıştır. Elde edilen sonuçlara göre, mikrodalga ile ısıtma kullanılarak hazırlanan çözeltilerden homojen ve boncuksuz lifler elde etmek mümkün olmuştur. Mikrodalga ile ısıtılan çözeltilerden üretilen nanolifler, aynı konsantrasyon kullanılarak ve geleneksel olarak ısıtılanlara kıyasla su buharı geçirgenliği ve mekanik özellikler bakımından daha iyi özellikler göstermiştir. %3 un ve %0.5 nişasta ile hazırlanan örneklerde, mikrodalga ile ısıtılan örnekten elde edilen film 1.68×10^{-12} g $s^{-1}m^{-1}Pa^{-1}$ su

buharı geçirgenliği değerini gösterirken, konvensiyonel örnek 2.13×10^{-12} g $s^{-1}m^{-1}Pa^{-1}$ değerini göstermiştir. Çalışmada çözeltilerin özellikleri; reolojik analizi, elektriksel iletkenlik ve serbest amino grubu tayini açısından incelenmiştir. Mikrodalga ile ısıtılan çözeltinin, aynı konsantrasyondaki konvensiyonel olarak ısıtılan çözeltiye kıyasla daha yüksek viskozite değerine ve serbest amino grubu miktarına sahip olduğu bulunmuştur. Bu çalışma, mikrodalga ile ısıtmanın konvensiyonel ısıtmaya kıyasla elektroeğirme çözeltilerinin hazırlanmasında daha ümit verici ve daha avantajlı bir yöntem olarak kabul edilebileceğini göstermiştir

Anahtar Kelimeler: Keçiboynuzu unu, mikrodalga, elektroeğirme, nanolif, nişasta

To my family

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ABBREVIATIONS

ΔH_m	Melting Enthalpy
DSC	Differential Scanning Calorimeter
FTIR	Fourier-transform infrared spectroscopy
OPA	The orthophthalaldehyde
PEO	Polyethylene oxide
SEM	Scanning electron microscopy
T_g	Glass transition temperature
T_m	Melting temperature
XRD	X-Ray Diffraction
WVP	Water vapor permeability

CHAPTER 1

INTRODUCTION

1.1. Nanotechnology

Nanotechnology is known as the study of extremely small structures with in the size range of 0.1 to 100 nm. The first development of this field started in 1958. Nanotechnology is based on proceeding of individual molecules, compounds or atoms into some structures to obtain different materials and devices having special properties. Additionally, nanotechnology deals with size reduction of large particles to the smallest structures. Working range of 0.1 to 100 nm is quite broad which makes creation and applications of different types of nanodevices and nanomaterials possible. Unlike large structures, nanomaterials have unique biological and physicochemical properties such as flexibility and high surface area. By nanotechnology, it is expected to observe a change in electrical conductance, chemical reactivity, magnetism, optical effects and physical strength properties of materials due to their small size (Nikalje, 2015).

It is one of the most promising technologies which can be used in a wide variety of fields such as health and medicine, energy and environment, engineering, electronics, transportation, space exploration etc. In addition to these fields, nanotechnology has become important for food science and food technology. By using nanotechnology, it is possible to set processing and packaging systems in food industry. Extension of storage life, enhancement of food security, improvement of flavor and nutrient are possible applications of nanotechnology in food systems. It is also known that many achievements have been made in various areas of food packaging by nanotechnology. Edible coating, active packaging and intelligent packaging are some examples of packaging applications (He & Hwang, 2016).

1.2. Electrospinning

It is a known fact that nanoscale fibers give promising results in many fields such as enzyme immobilization, drug delivery, textile production, biosensors and tissue engineering. Advantages of using nanofibers mainly come from increased surface to volume ratio. As particle size approaches to nanoscale, surface to volume ratio increases. This increase results in promotion of electrical, mechanical and optical properties of materials (Haider, Haider, & Kang, 2018). In this regard, promising features of nanofibers make them attractive for many applications over the years. There are many possible methods to produce nanofibers. Drawing processing, phase separation, self-assembly, solvent casting, template assisted synthesis, bicomponent and electrospinning are some of the well-known methods of nanofiber formation (Khajavi & Abbasipour, 2012). The first appearance of electrospinning dates back to early 1900s. Cooley and Morton separately had two patents of a prototype which describes a setup for electrospinning process in 1902. Between 1964 and 1969, some papers were published by Taylor which were showing mathematical representation of the process and model of change of shape of polymer solutions from spherical to conical under the effect of electrical field. In other words, formation of Taylor Cone was reported. Until 1990s, electrospinning was not a scientific research of interest (Taylor, 1964). In 1990s Reneker and Rutledge showed that various types of polymers can be transformed into nanofibers by electrospinning method. By their pioneer approach, electrospinning became a popular method to produce continuous and homogeneous fibers in nanoscale (Fong, Chun, & Reneker, 1999). After 2000s, using area of electrospinning was expanded by using different materials and formulations. As a result, production of ceramic and composite fibers, continuous lines of fibers, hollow and aligned fibers were produced. In early 2010s, by development of multiple-needle electrospinning systems and needleless electrospinning systems, industrial scale usage of electrospinning started (Xue, Wu, Dai, & Xia, 2019).

Among nanofiber formation methods, electrospinning is one of the most frequently used ones. Compared to solvent casting and phase-separation methods, nanofibers

produced by electrospinning method have higher surface area to volume ratio. It is also possible to produce well ordered, homogeneous fibers with larger number of intra-fibrous pores (D. Li & Xia, 2004). Although it is not one of the newest methods, it is quite advantageous in terms of cost and simplicity (Niu, Zeng, Mu, Nie, & Ma, 2016). Rather than these, electrospinning process can be effectively controlled and has potential for production scale up. Ability of producing nanofibers from different types of materials including natural and synthetic polymers and composites makes electrospinning attractive for many scientists. Therefore, it became a common method in various research fields such as bioengineering, environmental protection, sensors, catalysis and electronics etc. (Jiang, Carbone, Lo, & Laurencin, 2015).

Electrospinning setup mainly consists of following components as it can be seen in Figure 1; a high voltage power supply, a pump, a syringe which can be placed in the pump, a needle which is connected to the syringe and a metal collector plate.

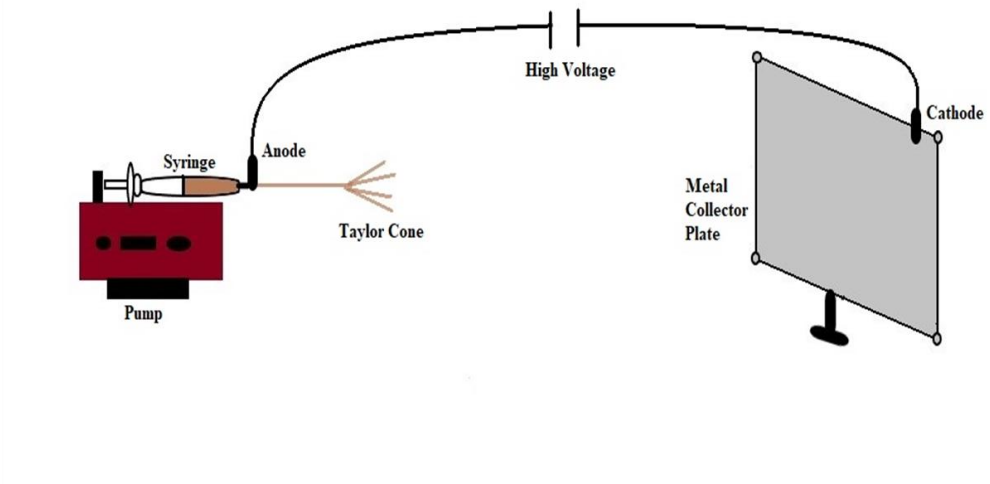

Figure 1. Demonstration of a basic electrospinning system

Working principle of electrospinning method is based on applying high electrical potential to form an electrical field between the needle and the metal collector plate. Positively charged electrode is connected to the needle, while negatively charged electrode is connected to the collector plate (Adomavičiūtė, 2007). Spinning solution should be placed in the syringe before voltage is applied. Spinning solution is pumped

at a constant flow rate and a specific voltage is applied to create an electric field between the needle tip and the collector (Kenry & Lim, 2017). At the beginning, the spinning solution of spherical droplet shape stays at the tip of the needle. To make flow to start, a certain surface tension threshold should be overcome (Bhardwaj & Kundu, 2010). High electrical potential causes instability of charged particles in the solution. At the same time, charged particles form a force which is opposite to surface tension. As a result, surface tension is overcome, and the flow of the solution starts in the same direction with electrical field. A further intensifying of electrical field leads to the deformation of spherical droplet. This deformation causes elongation of spherical droplet into a conical shape. This conical shape is named as Taylor Cone (Xue et al., 2019). After Taylor Cone is formed, solution jet is ejected towards metal collector plate. Between the tip of the needle and the collector, solution jet is exposed to electrical forces, thus elongation, solvent evaporation and change in bending instabilities are observed. The solution jet also moves in the whipped form due to electrical forces. Whipping movement of solution is the main reason of obtaining fibers in nanoscales by stretching and sliding of polymer chains. Finally, the charged jet is collected on the collector plate as randomly-oriented nanofibers (Reneker, Yarin, Fong, & Koombhongse, 2000).

Electrospinning is a complex process which can be affected by many different parameters. Providing optimum parameters is crucial in terms of having successful nanofiber formation. These parameters can be divided into three categories as environmental, processing and solution related. Environmental parameters are humidity and temperature (Kong & Ziegler, 2013). Process parameters can be categorized as flow rate, voltage, distance between tip and syringe needle, while solution related parameters are viscosity, surface tension and electrical conductivity of the solutions (Hu et al., 2014).

1.2.1. Environmental Parameters:

Relative humidity and temperature are considered as environmental parameters. The change in environmental conditions have strong impacts on electrospinning process, since it is a multi-parameter and complex procedure. Rather than the air temperature and humidity, temperature and humidity in the electrospinning chamber should be the considered. The effects of ambient parameters are directly related to fiber morphology and fiber diameter (Z. Li & Wang, 2013).

Temperature is known to have effect on solvent evaporation rate and solution viscosity. Evaporation rate of solvent decreases with decreasing temperature. As a result of lower evaporation rates, solidification of jet takes too much time and elongation cannot occur properly. Thus, solvent evaporation rate is the dominant mechanism at lower temperatures which results in smaller fiber diameters. Additionally, it is known that as temperature changes, rheological properties of solutions are affected. Rigidity of polymer chains are directly related to rheological parameters. At higher temperatures, lowering in solution viscosities can be observed since polymer chains are more mobile at relatively higher temperatures. As a result of mobility and higher stretching rate, thinner fibers are obtained (De Vrieze et al., 2009).

Similar to temperature, relative humidity also affects solvent evaporation rate. It is known that at very low relative humidity values, high solvent evaporation rates are observed. Due to high solvent evaporation rate, localized increases in polymer concentrations occur. This causes instant increases in solution viscosity. Thus, less stretching of fibers occurs and thicker fiber diameters are obtained. On the contrary, at very high relative humidity values, lower solvent evaporation rate is observed. Thus, stretching takes more time and thinner fibers are obtained (Pelipenko, Kristl, Jankovi, & Kocbek, 2013). In addition to these, it was shown that at very high relative humidity values (at above 50%) irregular fiber morphology and bead formation on fiber segments were observed (Tripatanasuwan, Zhong, & Reneker, 2007). Moderate

values of temperature and relative humidity should be selected to have optimum results.

1.2.2. Solution Parameters

1.2.2.1. Viscosity

Electrospinning is a complex procedure and can be affected by many different parameters. Viscosity is one of the solution parameters to be adjusted to provide optimum conditions since it is directly linked to solution concentration. In the literature, various studies are available about relation of viscosity and fiber morphology. In electrospinning process, since optimum conditions make possible to obtain homogeneous and bead-free fibers, too high or too low viscosity values are not preferred. In too low viscosity values, entanglement of polymers cannot be possible. Consequently, bead formation and non-homogeneity occurs. When temperature increases, a decrease in viscosity of the solutions is observed. The change in viscosity is directly related to fiber properties such as diameter and morphology. Viscosity decrease can result in a decrease in fiber diameter (Z. Li & Wang, 2013).

On the other hand, too high viscosities result in different situation. Flow throughout the syringe needle can be prevented due to extreme viscosity and local gel formations in electrospinning solutions are observed (Okutan, Terzi, & Altay, 2014).

1.2.2.2. Electrical Conductivity

Electrical conductivity is a key parameter which makes possible movements of electrically charged particles. To initiate jet movement of electrospinning solution, electrostatic repulsion force should be created. This force can be created by transferring of charges. In that regard, electrical characterization of the solution has a critical role. To make spinnability possible, spinning solutions should have a certain electrical conductivity value (Vega-Lugo & Lim, 2012). Solutions having zero electrical conductivity value cannot be spun by electrospinning method. Similarly, too low electrical conductivity values do not show successful results since these solutions

cannot be charged electrically by electrical forces. In general, it can said that diameter of the electrospun nanofibers decreases when the electrical conductivity of the solution increases (Mohammadian & Haghi, 2014). Therefore, an optimum value of electrical conductivity should be provided. Ionic salt and organic acid content, polymer concentration and type are some important factors which contribute to electrical conductivity of solutions.

1.2.2.3. Surface Tension

Surface tension is another important factor related to spinnability of solutions. It is known that electrospinning process can only initiate if repulsive forces can overcome the surface tension of the solution. Therefore, too high surface tension values make this process impossible. Polymer and solvent type are important parameters for surface tension. Too high surface tension values result in bead formation and non-homogeneous fiber structure (Vega-Lugo & Lim, 2009). To solve this problem, some surfactants can be used to decrease surface tension value of spinning solutions. Tween 80, Tween 40, Span 80 and Brij 58 are some examples of commonly used surfactants.

1.2.3. Process Parameters

Besides environmental and solution-related parameters, process parameters are quite effective ones in electrospinning process. Flow rate, voltage and distance between tip of syringe needle and the collector are process parameters. Voltage is critical in terms of creating an electrical field. Voltage is applied by electrospinning device and strength of the electrical field is determined by it. To initiate electrospinning process, charged particles should overcome surface tension. When this occurs, solution droplets start to elongate. As a result, jet movement starts from the tip of formed Taylor Cone shape. A certain amount of voltage should be applied to form Taylor Cone shape (Xue et al., 2019). At lower voltage values, droplets stay at their place at the tip of the needle. However, higher voltage application fastens ejection of droplets. Since solutions can be entangled faster, increased fiber diameter can be observed (Deitzel, Kleinmeyer, Harris, & Beck Tan, 2001).

As another parameter, flow rate is also quite effective on fiber morphology. In electrospinning process, spinning solution should be continuously fed to the system to obtain a stable process. Therefore, availability of spinning solution is crucial. As in the case of other parameters, a balanced flow rate should be selected to have proper results. It is known that too high flow rates can result in excess ejection of spinning solution. Excess amount of solution can cause difficulties such as less solvent evaporation and fiber collision. Therefore, lower flow rates are more favorable in terms of providing more time to the process to occur properly (Okutan et al., 2014). Voltage and flow rate should be in good correlation to obtain best results.

The distance between the needle tip and the collecting plate is the final parameter related to process. Most important effect of the distance is linked to evaporation rate of solvent in the electrospinning solution. As the distance shortens, volatile solvent cannot evaporate sufficiently due to the reduction in time for fluid to reach to the collector plate. If enough solvent is not evaporated, solution solidification cannot occur, thus bead formation can be observed (Z. Li & Wang, 2013).

1.2.4. Studies on Composition of Spinning Solutions

In nanofiber formation, it is possible to use synthetic, natural, and hybrid materials to prepare spinning solutions. As synthetic materials, polylactic acid (PLA), poly ε-caprolactone (PCL), polyethylene oxide (PEO) and some copolymers including poly l-lactide-co-caprolactone (PCLA) and poly lactic-co-glycolic acid (PLGA) can be listed which are widely used polymers in electrospinning method. These polymers are known to show great flexibility and thus better spinnability with high availability and low cost. Among synthetic polymers, polyethylene oxide (PEO) is biodegradable, biocompatible and water-soluble polymer which is widely used in electrospinning process. It has high polar affinity and capability of preserving organization. Also, its oxygen atoms have high electron donor power which enables them to form multiple intramolecular coordinate bonds with cations (Surov, Voronova, Afineevskii, & Zakharov, 2018).

There is an increasing demand in recent years in using environmentally friendly materials. Therefore, carbohydrates, proteins and fats are can be used as natural biopolymer sources to prepare electrospinning solutions. Being non-toxic, biocompatible and biodegradable are their most important properties. Gelatin, collagen, soy, casein, gliadin, zein and whey can be preferred as protein sources, while cellulose, chitosan, starch, pullulan, cyclodextrin and alginate were preferred as polysaccharides (Mendes, Stephansen, & Chronakis, 2017). At that point, flours can also be used as a natural source for nanofiber formation since they are good sources of carbohydrates, proteins, fats and fibers.

In recent years, many different flours such as lentil flour and pea flour are used to produce nanofibers by electrospinning. Since flours are biodegradable and are natural source of carbohydrates, proteins, fats and fibers, there is a growing interest on using them in electrospinning process. There are various studies on electrospinning of different flour types in the literature. In the study of Tam, Oguz, Aydogdu, Sumnu, & Sahin, 2017, electrospinning of lentil flour and HPMC containing solutions were studied. In their study, lentil flour was selected because of its rich content of protein, vitamin and mineral. It was found that at alkaline pH values, homogeneous nanofibers could be obtained from lentil flour and HPMC containing solutions. Additionally, it was found that fiber diameter and viscosity increased with increasing lentil flour and HPMC concentration. In this regard, lentil flour can be considered as good alternative for electrospinning. In another study of Oguz, Tam, Aydogdu, Sumnu, & Sahin, 2018, electrospinning of HMPC and pea flour containing solutions were investigated. Pea flour is a rich flour in terms of protein and polysaccharides. At alkaline pH, it was possible to obtain homogeneous and bead-free nanofibers. Increasing pea flour concentration resulted in an increase in apparent viscosity and fiber diameter. With optimum flour concentration and pH, pea flour was suggested to be a promising source for electrospinning method.

In another study, electrospinning of soy-protein and lignin containing solutions were studied (Salas, Ago, Lucia, & Rojas, 2014). Soy bean is a widely harvested crop which

is commonly used in food packaging. Soybeans are rich source of soy protein and carbohydrate. The main purpose of the study was to obtain homogeneous nanofibers from lignin and soy-protein solutions. As a result of the study, bead-free nanofibers were produced. It was concluded that unfolding of soy protein enhanced interaction with lignin macromolecules. More favorable and advantageous fiber structures were obtained due to the interaction.

Electrospinning of rice flour and PVA containing solutions was studied in another study (Woranuch, Pangon, Puagsuntia, Subjalearndee, & Intasanta, 2017). Rice is a naturally abundant crop and rice flour is a biodegradable and biocompatible material. At optimum rice flour and PVA concentrations, well defined nanofibers were obtained by electrospinning method. Uniform diameter distribution and fiber structure were achieved. The result could be attributed to interaction of a natural polymer with PVA. As a result, flours which are natural polymer sources can be considered as promising alternative sources for electrospinning method.

In the literature, there are many different studies related to starch usage in electrospinning process. Starch usage has become an emerging trend in electrospinning method. Starch types are generally used as secondary electrospinning agent, since their fiber forming properties are not enough to be used alone. Among starch types, rice starch is a commonly used and available one. In a recent study, preparation of electrospun fibers from glutinous rice starch and PVA containing solutions were investigated. To improve electrospinning properties of rice starch, PVA is added to the solution as a synthetic polymer with linear chain. At the end of the study bead-free, smooth and homogeneous fibers were obtained. Very high or very low solution concentrations resulted in bead formation and non-homogenous fiber structures. In this regard, the optimum concentrations of rice starch and PVA were selected to obtain proper nanofibers (Jaiturong et al., 2018). In another study, electrospinning of cassava starch and PLA containing solutions were studied (Sunthornvarabhas, Chatakanonda, Piyachomkwan, & Sriroth, 2011). Cassava starch was used to adjust surface properties, while PLA was used to improve chemical and

physical properties. It was found that bead-free and homogeneous fibers can be obtained. Also, starch concentration had an important effect on electrical conductivity of the solutions which were directly related to bead formation. Therefore, proper concentrations of PLA and cassava starch were chosen. In addition to these, electrospinning of high amylose maize starch and pullulan containing solutions was investigated in a former study (H. Wang & Ziegler, 2019). To improve spinnability of starch molecules, pullulan was added. At the end of the study, nanofibers were obtained by a blend of starch based natural polymer. Also, conductivity, surface tension and viscosity were regarded as important parameters to adjust solution parameters to obtain nanofibers.

1.3. Carob Flour

Carob tree is an evergreen and mostly cultivated in Mediterranean countries. Mainly mild and dry areas are preferred for cultivation. Endosperm of seeds of carob are known to have high gum content which is a water- soluble mucus, named as locust bean gum (Kamal, Youssef, El-Manfaloty, & Ali, 2013). Locust bean gum is commonly utilized in food industry. It is a polysaccharide (galactomannan) which consists of 80%–84% D-mannose and 16%–20% D-galactose. Carob is also a considerable source of vitamins C, D, E, B6, Niacin and folic acid. There are various studies which have shown that carobs can prevent some specific chronic diseases such as diarrhea symptoms, apoptotic and antiproliferative activity against cancer cells, and improve human health by its antidiabetic and antihyperlipidemic effects. By their high dietary fiber, antioxidant and polyphenol content they also have antidiabetic effects. Therefore, carob is an ideal choice for manufacture of dietetic and gluten-free products (Papaefstathiou, Agapiou, Giannopoulos, & Kokkinofta, 2018). In a recent study, carob flour was used in preparation of edible films for food packaging. The main objective of the study was to develop a completely edible packaging film for dry foods including dried vegetables and instant beverages. Carob was selected in the study for various reasons. Firstly, it has a natural flavor which can be replaced with chocolate easily. Also, carob has rich content of sugar, fiber and mineral substances which

makes carob attractive for packaging purposes. It is also considered as a source of bioactive natural compounds with low sodium and fat content. As a result, completely water-soluble and edible packaging materials were obtained (Amariei, 2019).

There are some other studies based on usage of carob flour in bread making in the literature. The effects of addition of carob flour to rice flour on quality of gluten-free doughs were studied (Tsatsaragkou, Yiannopoulos, & Kontogiorgi, 2014). Carob flour was selected in the study due to its high nutritional value and fiber content. It was found that viscoelastic properties of doughs were directly affected by carob flour content. Dough elasticity and quality increased with increasing carob flour amount. Increase in elasticity was linked to high fiber content of carob flour since fiber addition makes dough more elastic and stronger.

Among natural flours, carob flour is differentiated from others by its high content of dietary fiber (7.3%-9.61%) and total sugar content (48%-56%). It also contains protein (3%-5%). (Özcan, Arslan, & Gökçalik, 2007); (Kamal et al., 2013); (Papaefstathiou et al., 2018). High fiber content of carob flour may bring some benefits in mechanical properties of nanofiber films. Müller, Laurindo, & Yamashita, (2009) found that fiber addition to starch containing electrospinning solutions resulted in an increase in tensile strength and modulus of elasticity of the obtained films. Thereby, carob flour can be a good choice for obtaining electrospun nanofibers with better mechanical properties.

1.4. Rice Starch

Rice is one of the naturally abundant crops. It is highly available in worldwide. There are more than 240,000 varieties of rice over the world. The main differences between varieties comes from different soil characteristics and climate changes. Different rice varieties lead to differences in starch types. Textures, processing stabilities and viscosities and gelatinization temperatures of these starch types are different from each other (Tsatsaragkou et al., 2014). High population of human depending on rice as a primary source of calorie. In eastern countries, rice products are highly consumed as staple foods. Rice starch is obtained from rice flour by alkaline steeping method (Wu,

Chen, Li, & Li, 2009). It is a common biopolymer which is a rich source of amylose and amylopectin. While amylose content forms linear chain and amorphous part, amylopectin content forms branched and crystalline part of starch granules. Amylose and amylopectin are considered as attractive biomaterials which has barrier properties as packaging materials. As a result, different mechanical performances and physical structures can be achieved (Woranuch et al., 2017). To improve packaging properties of starch molecules, starches can be mixed with different types of proteins. Water vapor permeability values decreased while tensile strength values increased by combining of starch and proteins (Jagannath, Nanjappa, Das Gupta, & Bawa, 2003)

In this study, starch selection was based on their granule size. It was found that films made with starches having smaller granule size showed better mechanical properties in terms of tensile strength and elongation rate as compared to high granule size starch in polyethylene films (Lim, Jane, Rajagopalan, & Seib, 1992). Compared to potato, wheat and corn starches, rice starch has the lowest granule size (J. Y. Li & Yeh, 2001). In this study rice starch was chosen due to its smaller granule size.

1.5. Microwave Heating

In this study, to prepare electrospinning solutions, as a pre-treatment microwave heating was selected. Microwaves are electromagnetic waves with frequency ranging between 300 MHz and 300 GHz. Microwave heating is known to provide transfer of energy through the materials in a rapid rate and also with significantly reduced processing time. Principle of microwave heating is based on interaction of microwaves with charged particles and polar molecules. Heat generation occurs due to ability of these molecules to rotate in the alignment of alternating field. Water is the main component of most food products. Since it is a polar molecule, water is the major reason of interaction of food materials with microwaves (Vollmer, 2004).

Electrical properties of foods are effective on heating pattern of food products. Electrical properties are divided into two; dielectric properties and penetration depth. Dielectric properties are dielectric constant and dielectric loss factor. Dielectric

constant is known as a material's ability to store electrical energy, while dielectric loss factor is the ability of a material to dissipate electrical energy. There are some essential properties of food materials that can affect dielectric properties. Moisture content, bulk density, temperature and frequency of food materials can affect dielectric properties (Casper, Stephens, Tassi, Chase, & Rabolt, 2004). It was shown that moisture content and temperature can change dielectric properties of potato starch. When granular and gelatinized form of the starch were compared at the same temperature, it was found that granular starch showed lower dielectric loss factor than its gelatinized form (Roebuck, Goldblith, & Westphal, 1972). Also, in another study, it was shown that with increasing temperatures and increasing starch concentrations, a decrease in dielectric constant and dielectric loss factor of potato, wheat, corn and waxy corn starch solutions was observed (Ndife, Şumnu, & Bayindirli, 1998).

Penetration depth can be explained as the depth from the surface at which surface power drops to 1/e of its initial value. It is known that as frequency decreases, penetration depth increases. Too small penetration depths can result in non-uniform temperature distributions in foods. Similarly, penetration depth values lower than sample size can also cause non-uniform temperature distributions (Sumnu, 2001).

In microwave processing, as microwaves penetrates through foods, heat is generated volumetrically inside the food. Heat is transferred by convection from the surface to the center of foods which leads to a temperature difference between center and surface (Puligundla, 2014). Especially, compared to conventional heating methods microwave heating is quite advantageous with regard to be a fast process. Other advantages of microwave heating over conventional heating are precise process control, selective and volumetric heating (Sumnu, 2001). By microwave heating it is possible to preserve nutritional content of food products due to its short processing times. Moreover, by reduction in process time and energy efficiency, microwave is considered as an environmentally friendly method. Hence, using microwave in preparation of electrospinning solutions is a feasible approach as compared to classical methods by virtue of its advantages. Moreover, microwaves can change some functional properties of carbohydrates and proteins. It was observed that microwave

heating caused changes in structures and properties of potato and tapioca starches. After microwave application, potato starch shifted from Type B to Type A. Microwave heating completely disrupted integrity of starch granules while conventional heating caused only surface gelatinization. This could be related to the fact that microwave energy directly affected moisture in starch granules of crystal region which ended up easy destruction of granules (Uthumporn, Nadiah, Koh, Zaibunnisa, & Azwan, 2016). In another study, microwave heating was shown to increase in tensile strength of edible films formulated by soy protein isolate and zein up to 5%-25%. This result was explained by that the microwave might have changed the structure of the solutions. Additionally, it was also reported that the surface of the films obtained by microwave treatment was smoother with less pores. This result was linked to increasing of intramolecular forces and change in secondary structures of proteins (N. Wang et al., 2016). In another study, effects of microwave heating on properties of granules of potato starch was investigated. It was found that by 10 seconds heating of starch dispersions, microwaves caused vibration of water molecules in the crystalline region. As a result of vibration, shift in amylopectin patterns was observed even though amylopectin crystals did not reach the gelatinization temperature. By 15-20 seconds heating, an irreversible swallowing of starch granules was observed. Results were linked to complete deterioration of crystal structure of starch chains due to vibration of polar water molecules. Volumetric heating of microwave causes breaking of integrity of starch granules. Unlike microwave heating, in conventional heating, surface heating occurs, thus only surface gelatinization is observed (Xie, Yan, Yuan, Sun, & Huo, 2013). A similar study was conducted about change in mechanical properties of fibers obtained from wheat proteins. After heating of wheat protein solutions, the change in number of -SH and S-S bonds was observed. This was correlated to that microwaves changed structure of protein molecules, thus -SH bonds replaced with S-S bonds. As a result, microwave originated fibers showed higher degree of crystallinity. It was observed that higher crystallinity degree caused well-ordered structures. To break well-ordered crystal structures, higher energy levels are required. Therefore, materials having more crystal

structures show better mechanical properties (C. Liu & Ma, 2016).

Addition to these, effects of microwave heating on physico-chemical properties of different starch types including corn, wheat and waxy corn were investigated. At the end of the study, it was observed that microwave radiation caused a reduction solubility, crystallinity and swelling properties of corn and wheat starches. Moreover, gelatinization temperatures of all starch types increased to higher temperatures due to microwave treatment. It was also concluded that effects of microwave heating were in correlation with amylose content and crystal structure of starch (Lewandowicz, Jankowski, & Fornal, 2000).

1.6. Objective of the Study

Nanotechnology has recently gained importance in many different fields including food systems and food packaging. Nanofibers can be used to produce different food packaging materials. To obtain nanofibers, electrospinning is a very advantageous method in terms of cost, simplicity and short processing time. Electrospun nanofibers are known to have higher surface area to volume ratio with well-ordered and homogeneous structures.

Carob flour is known to have high amount of sugar and fiber. Although there are many studies related to the usage of carob flour in different food products in the literature, it has not been used so far to produce electrospun nanofibers.

The hypothesis of the study is that usage of microwave in preparation of electrospinning solutions can be a feasible approach as compared to conventional methods in terms of improving physical properties of electrospun nanofibers since microwave heating has some effects on changing some functional properties including gelatinization behavior, structure and solubility of starch and proteins. Microwave heating is a frequently used method due to short processing time, preservation of nutritional content and being environmentally friendly. However, to the best of our knowledge, there is no study in literature about investigation of the effects of microwave heating on production of nanofibers by electrospinning. Therefore, the aim of this study was to determine the effects of microwave heating on properties of

electrospinning solutions and electrospun nanofibers as compared to conventional heating. In addition, the effects of different carob flour concentrations and rice starch addition on production of nanofibers by electrospinning were investigated. Nanofibers were characterized by mechanical properties, differential scanning calorimeter (DSC), scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR) and water vapor permeability (WVP) analyses.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Carob flour was obtained from Havancızade Gıda (İstanbul, Turkey). Rice starch was supplied from National Starch Food Innovation (Bridgewater, NJ, USA). Polyethyleneoxide (PEO) with molecular weight of 900 kDa and sodium hydroxide pellets were bought from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Polyoxyethylene sorbitan monooleate (Tween 80) (density: 1.064 g/cm^3, viscosity: 400–620 cps at 25 °C) was obtained from Merck (Darmstadt, Germany).

2.2. Preparations of Solutions

PEO solutions of 2% (w/v) were prepared by dissolving overnight using magnetic stirrer at 400 rpm (Daihan Scientific Co, KR) at room temperature. Carob flour was added at two different concentrations which were 3% (w/v) and 5% (w/v) and rice starch was added at 0.5% (w/v) concentration into dissolved PEO solution. Solutions were homogenized by using high-speed homogenizer (IKA T25 Digital Ultra-Turrax; IKA®-Werke GmbH & CO. KG, Staufen, Germany) at 9000 rpm for 4 min. Then, pH of the solutions was adjusted 10 by adding 2M NaOH. To prepare conventionally heated solutions; solutions were heated up to 80°C in water bath (GFL, Type 1086, Germany). Then, solutions continued to be heated using magnetic stirrer at 750 rpm and 80°C for 2 h. After that, solutions were cooled down to room temperature. Then, Tween 80 was added to obtain solutions were stirred 2% (w/v) concentration further at 750 rpm for 30 min to disperse Tween 80. To prepare microwave heated solutions; solutions were heated by using microwave oven (Kenwood, New Jersey, USA) for 2.5 min at 416 W after solution temperature reaches to 80°C. Power level was determined

by IMPI 2-L test (Buffler, 1993). Then, the same procedure for further cooling and addition of Tween 80, as in the case of conventional heating method, was followed.

2.3. Characterization of Solutions

2.3.1. Rheological Characterization

Rheological behaviors of the solutions were examined by using a controlled strain rheometer with bob and cup geometry (Kinexus, Malvern Instruments, UK). Shear rate range was selected as 1 to 100 s^{-1}. Measurement temperature was 25°C. The shear stress (τ) values were recorded with respect to shear rate ($\dot{\gamma}$) values. Measurements were replicated twice.

2.3.2. Electrical Conductivity Measurement

Electrical conductivity of the solutions was measured by using conductivity meter (Inolab® 7110, Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany) at 25°C in duplicate.

2.3.3. Determination of Free Amino Groups

The orthophthalaldehyde (OPA) method was used to quantify free amino groups. The method was applied as described by Nielsen, Petersen, & Dambmann, (2001). Experiments were performed for the solutions containing 3% carob flour concentration. The solution which was not exposed to any heat treatment was considered as control. Conventionally heated and microwave heated solutions were also analyzed. Solutions were diluted with 1/20 dilution rate by using distilled water prior to analysis. Absorbance values of samples were measured by using UV/VIS Spectrophotometer at 340 nm (Optizen Pop Nano Bio, Mecasys Co. LTD, Korea). The amino groups' value was determined by taking glysine standard curve of concentration (0.004-0.0004 g/100 mL) versus absorbance at 340 nm as a reference. Free amino group concentration was expressed as g/mL in comparison with unheated solution sample.

2.3.4. Surface Tension Measurement

Surface tension of the solutions was measured by using tensiometer (Attension Theta, Biolin Scientific, China).

2.4. Electrospinning

Films were prepared by using electrospinning device (NanoWeb 103, Mavitek, Mersin, Turkey). Prepared solutions were placed into 5 mL syringe with 11.53 mm inner diameter. Needle of the syringe was placed horizontally on the pump. Positively charged electrode was connected to the needle and negatively charged electrode was connected to the collector plate. Collector plate was covered with aluminum foil and placed at 30 cm distance from the needle's tip. Flow rate was selected as 0.8 mL/h and the voltage was set as 12kV. Experiments were performed at 21±1 % relative humidity and at 20±1 °C temperature.

2.5. Characterization of the Films

The films were labeled as M-5F-0.5S, M-5F-0S, M-3F-0.5S and C-3F-0.5S with the first letter denoting heating type (microwave- M, conventional- C). Notation in the middle referred to carob flour concentration (either 5% or 3 % w/v) and the latter one represented rice starch concentration (0.5% w/v or 0) in the formulations.

2.5.1. Scanning Electron Microscopy (SEM)

Images of the films was taken by using scanning electron microscope (Nova NanoSEM 430, FEI, Oregon) for carrying out analysis of morphologies of films. Images were taken at 10000× magnification. Nanofiber diameters were measured by using Image J software to obtain diameter range and calculate average diameter of each sample. For each sample, measurements were performed using randomly selected 100 fibers.

2.5.2. Differential Scanning Calorimeter (DSC)

Thermal analysis of the films was performed by using a differential scanning calorimeter (Pyris 6 DSC, PerkinElmer, Massachusetts, USA). An empty pan was used as reference. In each measurement, around 4-5 mg sample was put in aluminum pans. Samples were firstly cooled down to -60°C and heated up to 100°C with 10°C/min heating rate. Glass transition temperatures of films were determined, and measurements were replicated twice.

2.5.3. Mechanical Test

Tensile strength, Young's modulus, and percentage elongation at break were determined by using texture analyzer (Brookfield Texture Analyzer CT310K, Middleborough, USA). Measurements were performed by using 0.1 N preload at a speed of 25 mm/min. Film samples were prepared in 25 mm width and 80 mm length. At room temperature, experiments were repeated twice.

2.5.4. Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

Fourier transform infrared (FTIR) spectroscopy analyses of film samples were performed by using a FTIR spectrometer (IR-Affinity1, Shimadzu Corporation, Kyoto, Japan) with Attenuated Total Reflectance (ATR) attachment. Analysis were carried out at 600-4000 cm^{-1} frequency range with 32 number of scans and 4 cm^{-1} resolutions.

2.5.5. Water Vapor Permeability (WVP)

Water vapor permeability of the films was determined by following ASTM, E96 method ("Stand. Test Methods Water Vap. Transm. Mater.," 1995). Firstly, thickness of nanofilms was measured with calipers and 10 measurements were taken for each film sample. Water vapor permeability cups made of plastic with 4 cm diameter were used to perform experiment. Cups were filled with 35 mL water and covered with obtained films. Relative humidity inside the cup was fixed to 100%. Cups were kept in the desiccators.

After steady state condition was reached, cups were weighed at 1.5 h intervals for 3 days. Measurements were performed duplicate. Water vapor transmission rate (WVTR) and then water vapor permeability values of the films were calculated. Water vapor transmission rate was calculated from the plot of weight loss versus time graph. Dividing surface area of the cup to the slope of weight loss versus time graph gave water vapor transmission rate. Then, using the Eq. (1) given below, water vapor permeability was calculated;

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

in which WVTR was the water vapor transmission rate (g m^{-2} s^{-1}), P_{sat} (kPa) was the saturated vapor pressure at certain temperature, R_1 (%) was the relative humidity inside the cup, R_2 (%) was the relative humidity outside the cup and Δx (m) was the average thickness of the film.

2.5.6. X-Ray Diffraction (XRD)

Crystalline property of film samples was examined by X-Ray diffractometer (Rigaku Ultima-IV, USA) using copper (Cu) irradiation with 30 mA current and 40 kV energy. Experiments were carried out between 5°-70° scanning range with 2°/min scanning rate.

2.6. Statistical Analysis

ANOVA was used to determine whether there is significant difference between samples by Minitab software (version 17, State College, PA, USA). If significant difference was found, Tukey's test was used for comparison ($p \leq 0.05$).

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Effects of Heating Method, Carob Flour Concentration and Starch Addition on Morphology of the Samples

When 3% (w/v) (Figure 2A) and 5% (w/v) carob flour containing solutions were exposed to conventional heating, homogenous fibers could not be obtained. On the other hand, homogeneous fibers could be obtained in microwave treated samples both in 3% (w/v) (Figure 2B) and 5% (w/v) carob flour concentration. Electrospinning potential of conventionally heated solutions were quite different than microwave heated ones. This could be explained by the differences in the mechanism of conventional and microwave heating. To enlighten the reason of difference in morphology of microwave and conventionally treated samples, free amino groups were determined. There was no significant difference between the solution not exposed to heating and conventionally heated one in terms of free amino groups (Table A.1). As can be seen in Table 1, sample treated by microwave heating for 2.5 mins had the highest amount of free amino group as compared to the conventionally heated samples. In microwave heating, there is internal heat generation which increases the internal pressure in the sample (Sumnu, 2001). This internal pressure gradient might have increased the release of free amino groups from the sample. Moreover, microwave heating might have a different effect on the protein molecules. Microwave heating can facilitate protein unfolding which can be linked to excitation of collective intrinsic modes in the proteins (Bohr & Bohr, 2000).

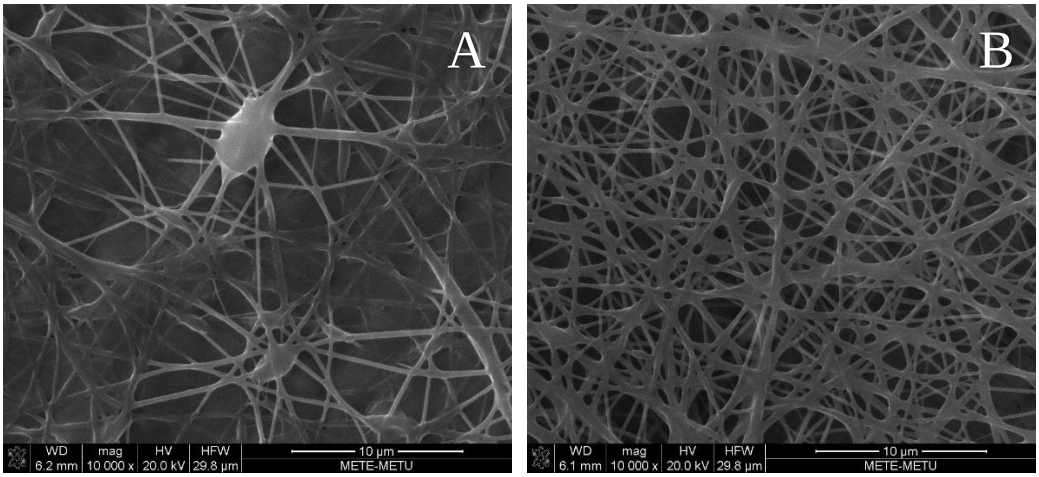

Figure 2. SEM images of electrospun nanofibers containing 3% carob flour A) treated by conventional heating B) treated by microwave heating

In this context, it was clear that microwave heating had a significant effect on obtaining bead free fibers due to the release of amino groups. It was worth to investigate how free amino groups could affect elecrospinnability. This could be related to the viscosity of the solutions. Increasing number of amino groups were shown to increase viscosity of solutions. Viscosity values of microwave treated samples were determined as 0.163 Pa.s. This value was 0.138 Pa.s for conventionally heated sample. This showed that the increase in free amino group content could be related to increase in viscosity values. The increase in viscosity enhanced elecrospinnability of solutions, thus bead free and homogeneous structures were obtained. In electrospinning process, optimum viscosity value of the solutions is considered as a key parameter to obtain homogeneous and bead free nanofibers (G. Liu, Gu, Hong, Cheng, & Li, 2017)

Table 1. Free amino group values of the samples

Sample	Free Amino Group (g/100 mL)
Control	$0.0332 \pm 0.0075^{b*}$
Microwave treated	0.0426 ± 0.0047^{a}
Conventionally treated	0.0354 ± 0.0011^{b}

*Different superscript letters show that means are significantly different ($p \leq 0.05$).

To observe the effect of starch on fiber morphology, 0.5% rice starch was added to the solutions. In both microwave heating and conventional heating, by adding 0.5% starch to 3% carob flour bead free nanofiber could be obtained. As discussed before, without addition of rice starch, bead-free and homogeneous fibers could not be achieved in conventionally heated samples (Figure 2A). The reason could be linked to the effect of amylose content on elecrospinnability. Higher amylose content is very effective on the formation of homogeneous fibers during electrospinning (Wang et al., 2018). Addition of rice starch helped to obtain homogeneous and bead free fibers in conventionally heated solutions (Figure 3). For conventional heating, 3% flour and 0.5% starch combination were the lowest possible concentration for homogeneous and bead free fiber formation. Thus, solutions containing carob flour of 3% concentration and starch of 0.5% concentration were selected in order to compare microwave and conventional heating. Viscosity and electrical conductivity of the solutions were determined, since they are important parameters for electrospinning process.

Table 2 shows the viscosity, and electrical conductivity of the solutions and average diameter values of the fibers. All the solutions showed Newtonian behavior obeying Newton's law of viscosity with high coefficient of determination value (R^2=0.99). According to the results, MW- 3F- 0.5S sample had the highest viscosity value as compared to the other samples ($p \leq 0.05$). C-3F-0.5S sample had lower viscosity value

than MW-3F-0.5S sample. This can be related to the free amino group content. As discussed before, according to OPA analysis results, microwave heating process increased the release of free amino groups in comparison with conventional heating. At that point, it was found that there was a correlation between increase in free amino group content and increase in viscosity. Viscosity increase was mainly resulted from increased resistance offered by the more structured solvent caused by the increase of moving free amino group part. Thus, solvation effects could also contribute to the movement of amino groups (Yan, Wang, & Lu, 2002); (Ali, Shahjahan, & Ansari, 2010). Thereby, viscosity difference between conventionally heated and microwave heated sample could be explained by the free amino group amount in the solutions. When the starch effect was considered, it was found that viscosity of MW-5F-0.5S and MW-5F-0S samples were not significantly different from each other (Table A.2). The reason could be that starch amount was not sufficient to cause a significant change in viscosity. In addition to these measurements, to confirm the importance of viscosity, viscosity of conventionally heated solution with 5% of carob flour concentration was measured and was found as 0.128 Pa.s which was significantly lower than those of the other samples ($p \leq 0.05$). It was already mentioned that, conventionally heated sample with 5% carob flour resulted in non-homogeneous nanofibers with beads. In this regard, it can be concluded as lower viscosity values resulted in non-homogeneous fibers with beads.

Electrical conductivity is an important factor in electrospinning process. It is known that electrical conductivity is related to movement of charges which is important to overcome surface tension forces to start spinning process (Vega-Lugo & Lim, 2012). For example, high conductivity can cause greater electrostatic repulsion force, thus better stretching properties which are responsible for inducing the bending instability and stretching (Vega-Lugo & Lim, 2012).

In Table 2, it was clear that M-3F-0.5S and C-3F-0.5S samples had significantly lower conductivity values than M-5F-0S and M-5F-0.5S samples ($p \leq 0.05$). This can be correlated to pH adjustment of the solutions. To adjust pH of the solutions to 10,

NaOH solution was added. Thus, number of charged molecules increased by the presence of Na^+ and OH^- ions. This was directly related to electrical conductivity increase. For 5% solutions, since more NaOH solution was required to adjust pH, higher conductivity values were obtained.

There was no significant effect of heating treatment difference on electrical conductivity, since M-3F-0.5S and C-3F-0.5S samples had the same electrical conductivity values (Table A.3).

Table 2. Viscosity, electrical conductivity and surface tension values of the solutions and average diameters of the fibers

Sample	Viscosity (Pa.s)	Electrical Conductivity (mS/cm)	Avarage Fiber Diameter (nm)	Surface Tension (mN/m)
M-5F-0.5S	$0.166\pm0.001^{b*}$	2.710 ± 0.042^{a}	243.68 ± 0.25^{c}	33.46 ± 0.03^{a}
M-5F-0S	0.153 ± 0.008^{bc}	2.380 ± 0.007^{b}	228.34 ± 4.26^{c}	33.25 ± 0.16^{a}
M-3F-0.5S	0.239 ± 0.005^{a}	1.504 ± 0.003^{c}	301.53 ± 0.36^{a}	33.73 ± 0.39^{a}
C-3F-0.5S	0.146 ± 0.001^{c}	1.563 ± 0.002^{c}	274.87 ± 0.79^{b}	33.96 ± 0.21^{a}

*Columns with different superscript letters are significantly different ($p\leq0.05$).

Figure 3 shows the SEM images and diameter distributions of films obtained from solutions containing different concentrations of flour and starch and different heating processes. Average fiber diameter and standard deviation results are also available. Range of average fiber diameters was between 220-300 nm. In M-3F-0.5S sample, the most uniform distribution can be seen, while M-5F-0.5S, M-5F-0S and C-3F-0.5S samples showed more uneven distributions. Table 2 shows the average fiber diameter of the films. It can be concluded that, M- 3F-0.5S sample had the largest diameter. Viscosity and electrical conductivity are considered as one of the most effective parameters that can affect fiber diameter (Hu et al., 2014). In the study of Ramji &

Shah, (2014) a correlation was found between viscosity and fiber diameter. As viscosity value of soy protein-PEO mixed solutions increased, a significant increase in fiber diameter of their films was observed. In addition, it was observed that the increase in electrical conductivity could result in the decrease in fiber diameter and less bead formation (Zhang, Yuan, Wu, Han, & Sheng, 2005). The reason could be linked to difficult elongation of jet. Low values of conductivity cause insufficient elongation, thus higher fiber diameter size (Bhardwaj & Kundu, 2010). According to the viscosity and electrical conductivity results of this study, M-3F-0.5S sample had the highest viscosity and the lowest conductivity values. For that reason, it was expected for this sample to have the highest fiber diameter.

C-3F-0.5S sample had the second biggest diameter. This could be associated with its lower viscosity value as compared to M- 3F-0.5S sample, although their electrical conductivity values were not significantly different than each other. At that point, viscosity was found to be a more effective parameter on fiber diameter rather than electrical conductivity. It was observed that 5% flour containing samples (M- 5F-0.5S, M- 5F-0S) had the lowest fiber diameters as compared to 3% flour containing ones. Again, this could be reasoned with their lower viscosity values and also their quite high electrical conductivity values. Both viscosity and electrical conductivity resulted in a decrease in fiber diameter.

Surface tension values of the samples were shown in Table 2. There was no significant difference between surface tension values of the solutions (Table A.4). All solutions had the same PEO concentration but with different biopolymer concentrations. In a former study, effects of the PEO concentration on surface tension and viscosity values of the solutions were investigated. It was found that as PEO concentration increased, surface tension values decreased while viscosity values increased (Deitzel et al., 2001). In that connection, increasing or decreasing PEO concentration could be more effective in increasing surface tension values of the solutions. It was clear that, biopolymer concentration at the selected range and also heating treatment difference had an insignificant effect on surface tension. Additionally, Tween 80 which was used

as surfactant had a lowering effect on surface tension. In all the solutions, same concentration was used which can be another reason of observing the same surface tension values.

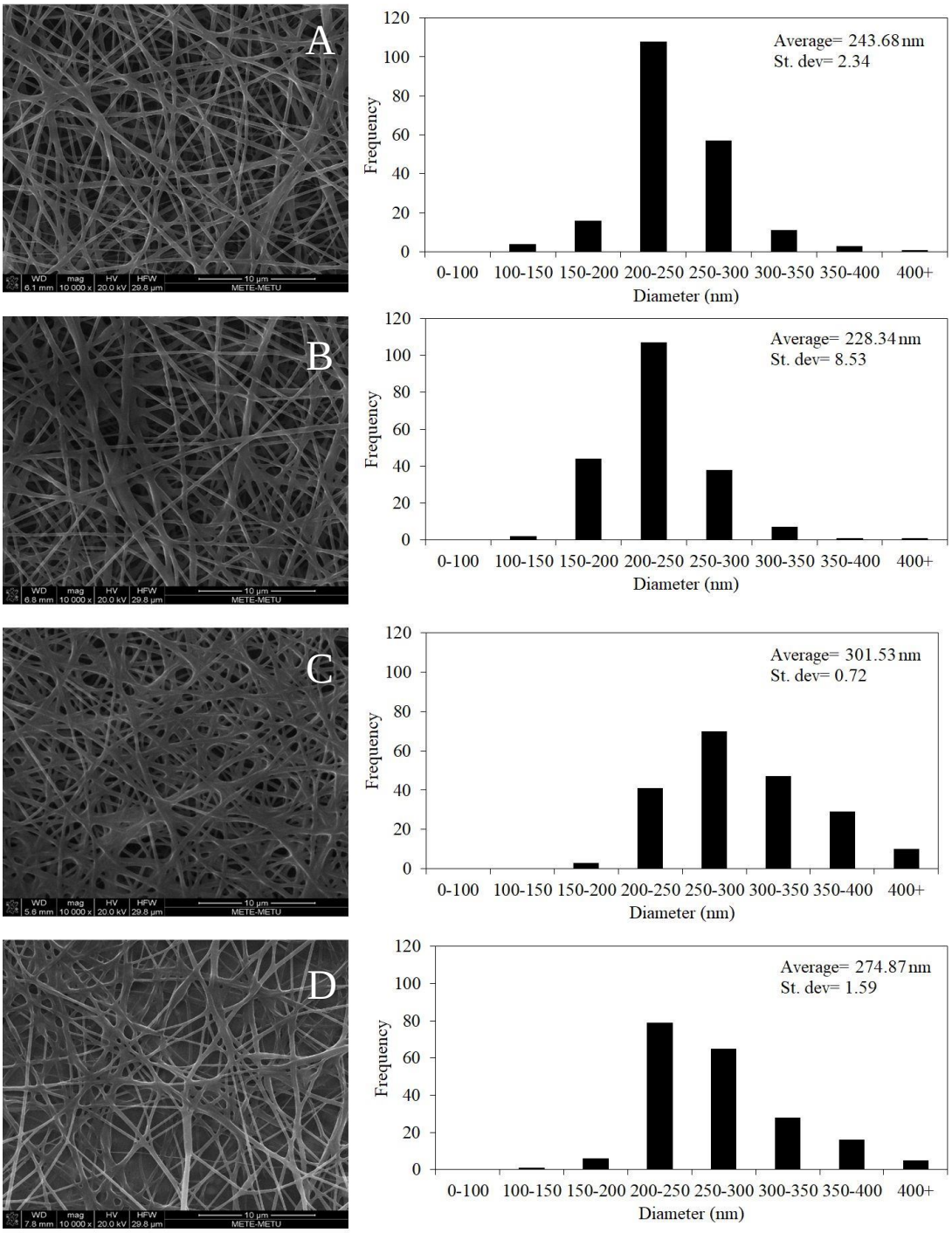

Figure 3. SEM images and diameter distributions of the films obtained from different solutions with different concentrations and heating processes (A) M- 5F-0.5S, (B)M-5F-0S, (C) M- 3F-0.5S and (D) C- 3F-0.5S

3.2. Characterization of nanofibers

Nano fibers of M- 5F-0.5S, M- 5F-0S, M- 3F-0.5S and C- 3F-0.5S were characterized by using WVP, X-ray diffraction, mechanical test, DSC and FTIR analysis.

3.2.1. Water Vapor Permeability

In fiber characterization, WVP is one of the most important criteria, due to its impact on moisture transfer through the film. Lower WVP values are more preferable in terms of packaging purposes since lower values refer to less amount of moisture transferring to the environments.

In Table 3, WVP values of films were shown. Although these values were slightly higher than that of synthetic polymers LDPE (0.36×10^{-12} g m^{-1} s^{-1} Pa^{-1}) (He & Hwang, 2016), they were lower than biodegradable PLA film (26.02×10^{-12} g m^{-1} s^{-1} Pa^{-1}) (Aydogdu, Yildiz, Ayhan, et al., 2019), cellulose-based polymer package; cellophane; (0.84×10^{-10} g m^{-1} s^{-1} Pa^{-1}) (Mali, Grossmann, Garcia, Martino, & Zaritzky, 2002) and rice flour - cellulose fiber films (0.313×10^{-10} g m^{-1} s^{-1} Pa^{-1}) (Dias, Müller, Larotonda, & Laurindo, 2011). In the literature, there are a couple of ways to lower WVP of hydrophilic films, such as the addition of lipids (Acosta, Jiménez, Cháfer, González-Martínez, & Chiralt, 2015), crossing-linking agents and different methods of film processing (Choi, Lee, Chang, Lacroix, & Han, 2018). Therefore, comparing with the literature results, although film materials had similar characteristics, electrospun nanofiber films had lower values as compared to films made by the casting method. This was one of the advantages of electrospinning. Electrospun nanofilm was formed by the deposition of nanofibers on each other. In this study, the thickness of films was recorded as approximately 0.04 mm ± 0.001mm and fiber diameter was nearly 262 ± 16 nm. Therefore, more than 150 nanofibers layered out each other. This structure formed a torturous path and made it difficult for transfer of water vapor. In this way, the permeability of nanofilms decreased.

Table 3. Water vapor permeability values and mechanical properties of films

Sample	WVP×10^{-12} (g $s^{-1}m^{-1}Pa^{-1}$)	Tensile Strength(N)	Young Modulus (N/m)	Elongation at Break (%)
M- 5F-0.5S	$2.32\pm0.03^{b*}$	$0.955\pm0.032^{c*}$	$29.55\pm1.57^{b*}$	$41.36\pm0.76^{b*}$
M- 5F-0S	2.73 ± 0.06^{a}	0.625 ± 0.053^{c}	22.27 ± 1.47^{b}	34.99 ± 0.67^{b}
M- 3F-0.5S	1.68 ± 0.02^{c}	3.875 ± 0.088^{a}	79.91 ± 2.48^{a}	61.25 ± 3.21^{a}
C- 3F-0.5S	2.13 ± 0.01^{b}	2.305 ± 0.004^{b}	69.784 ± 0.42^{a}	41.69 ± 0.76^{b}

*Columns with different superscript letters are significantly different ($p\leq0.05$)

A similar finding was also observed for PEO, HPMC and lentil flour electrospun nanofibers. In this study, depending on pea flour concentration of the film WVP values were 1.218×10^{-12} g m^{-1} s^{-1} Pa^{-1} and 1.754×10^{-12} g m^{-1} s^{-1} Pa^{-1} (Oguz et al., 2018). Furthermore, it is worth to notice that the reason of small variation between carob flour and pea flour nanofilms might be due to the variation of polar amino acid content, and amylose and amylopectin content of the flours.

Microwave treatment is another factor affecting WVP. Although films had the same formulations (M-3F-0.5S and C-3F-0.5S), microwave treated samples had significantly lower WVP than conventionally treated ones. In fact, the aim of addition of NaOH to solutions was to adjust pH 10 and to denature the proteins to make it more spinnable. However, it was recorded that alkali treatment was also used to modify the gelatinization process of starches. Disruption of intra and inter hydrogen bonding between amylose and amylopectin molecules led to decreasing of gelatinization temperature (Marques, Pérégo, Le Meins, Borsali, & Soldi, 2006). Due to the internal heat generation principle of the microwave, starch granules became more damaged and disordered (Zhao et al., 2019). This working principle also resulted in puffing of starch which might accelerate the chemical reaction. Furthermore, electromagnetic waves caused polarization and alignment of macromolecules under the field which might cause breakage of hydrogen bonds (Shi, Yin, & Jiao, 2014). To adjust pH to 10,

more NaOH solution was added to M-5F-0.5S as compared to C-3F-0.5S. The addition of more alkali solution and microwave treatment might accelerate bond formation between PEO, carob flour, and starch molecules. By the way, more linkage and the more homogenous solution might cause the formation of less permeable nanofilm. Thus, different results between M-3F-0.5S and C-3F-0.5S could be explained by the microwave effect.

The permeability difference between M-3F-0.5S and C-3F-0.5S might also be explained by difference in fiber diameter. M-3F-0.5S sample had larger fiber diameter compared to C-3F-0.5S sample (Table 2). It was shown in a previous study that the increase in fiber diameter had a decreasing effect on the porosity of films; thereby, as fiber diameter increased, the porosity of films decreased (Juklíčková et al., 2012). The decrease in porosity was more desirable in terms of reducing water vapor transfer. The same observation was also valid for M-5F-0.5S and M-3F-0.5S samples. Although both samples were treated with microwave and M-5F-0.5S had higher carob flour, the higher fiber diameter of M-3F-0.5S strongly decreased WVP.

3.2.2. X-Ray Diffraction (XRD)

Crystallinity might be another factor that strongly affected permeability characteristics of films. Higher intensity of peaks was interpreted as higher crystallinity. Figure 4 shows XRD results of the films from different solutions, concentrations and heating processes. M-3F-0.5S sample had the highest crystallinity and the lowest permeability. On the other hand, M-5F-0S had the lowest crystallinity and highest permeability. XRD pattern of other samples also showed similar correlation with WVP. In crystal regions, molecules had higher density, more compact characteristic, molecule alignments were more regular but in amorphous phases, they were less dense. Therefore, due to crystal structure and regular molecule orientation, water molecule passage through crystal parts was relatively slow.

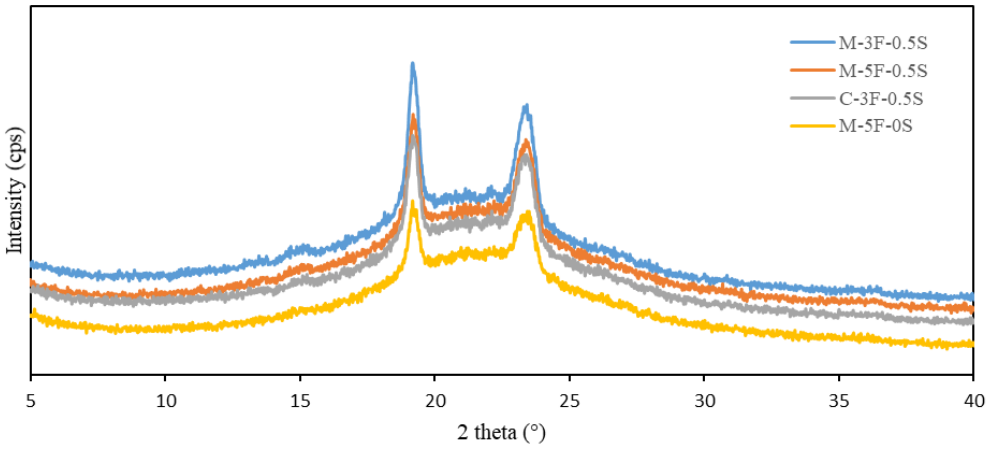

Figure 4. XRD results of electrospun nanofilms

In the literature, it was recorded that higher crystallinity improved barrier property of film since crystal structure was an inert and impermeable character. Therefore, diffusion of molecules could take place mainly in amorphous regions (Fabra, Lopez-Rubio, & Lagaron, 2013). Since PEO is a semi-crystalline polymer, it has diffraction peaks at 2θ = 19° and 23° (Uyar & Besenbacher, 2009). As can be seen in Figure 4, XRD patterns of nanofilms were very similar to that of PEO. No peak was observed rather than PEO peaks. These structures showed that both carob flour and rice starch were homogenously distributed through the carrier polymer. They did not show any unique crystal formation. This also showed how materials interacted strongly with each other.

3.2.3. Mechanical Properties

Mechanical properties of nanofibers depend on some parameters such as composition, processing condition, fiber diameter, distribution and interaction between components (Sengupta et al., 2007). Tensile strength, Young's modulus and elongation at break values of films were given in Table 3.

XRD results and mechanical properties of the films complemented each other. Fibers with high peak intensity were regarded as more crystalline structure with a numerous hydrogen bonding, and more molecular orientation. It should be noticed that tensile

strength of the films was aligned in an order of peak intensity values. Therefore, increasing crystallinity promoted tensile strength of the films.

Films with relatively high crystallinity not only had higher tensile strength but also higher Young's modulus because of the same reasons, high hydrogen bonding and more compact, rigid structure. These results were also supported by other studies. It was recorded that films with a compact structure and higher degree in crystallinity had higher elastic modulus and tensile strength. Although crystal structure was less in quantity than the amorphous phase, relative increase in crystallinity showed an increasing trend on strength of film (Fabra et al., 2013).

Fiber diameter of the film seemed to be correlated with mechanical properties. Increasing fiber diameter increased the tensile strength of the films. For example, M-3F-0.5S sample had the highest fiber diameter and tensile strength. M-5F-0.5S and M-5F-0S sample had smaller diameter with a low tensile strength.

Films containing 5% carob flour had lower tensile strength values. In the literature, hydrophobic molecules present in the film formed clusters in the matrix and decreased tensile strength by weakening bond interactions (Sivarooban, Hettiarachchy, & Johnson, 2008). Increasing carob flour concentration also increased hydrophobic protein or amino acids amount in the film forming solution. Therefore, these molecules might form aggregates and act as an impurity that decreased mechanical properties.

It was also recorded that tea polyphenol added polylactic acid (PLA) fibers had lower elongation at break and tensile strength. Presence of tea polyphenols reduced stretching ability of PLA chains by restricting PLA molecule bonding (Y. Liu, Liang, Wang, Qin, & Zhang, 2018). Hydrophobic molecules might have the same effect for higher carob flour containing samples. In addition to that, these aggregates might disturb equal force distribution on fibers.

3.2.4. DSC Analysis

In Table 4, glass transition temperatures (T_g), melting temperatures (T_m) and melting enthalpies (ΔH_m) of the films are shown. In addition to these measurements, to understand the thermal behavior of PEO, pure PEO was tested. As a result, melting point of pure PEO was found as 71.5 °C. Films have lower melting point which is around 58 °C. In X-ray diffraction results, PEO is the main reason of crystalline pattern of the films. Since all crystallinity comes from PEO itself, crystalline structure of it might be depressed by interaction with biopolymers of the films. In similar studies, it was found that biopolymers such as chitosan had a depressing effect on crystalline structure of PEO, due to the interactions between them (Kriegel, Kit, McClements, & Weiss, 2009). Enthalpy of melting of 5% flour containing samples was lower than that of 3% flour containing ones. As biopolymer concentration increased, ΔH_m value decreased. Similar to melting point results, the reason could be linked to depression of crystalline structure of PEO due to interactions with biopolymer content. In glass transition temperature results, M-5F-0S and M-5F-0.5S samples had higher Tg values as compared to M-3F-0.5S and C-3F-0.5S samples. It can be clearly said that higher amount of biopolymer concentrations caused increase in Tg values of the films. For nanofibers having both Tm and Tg value, it was concluded that they owned crystal and amorphous regions. Glass transition temperature is linked to transition between glassy state to rubbery state (Saba et al., 2017). In this regard, properties of films are mostly dominated by amorphous character. In the study of Guirguis and Moselhey as hydroxypropyl cellulose concentration increased, a decrease in Tg values of the samples was observed (Guirguis & Moselhey, 2012). Similar to this case, increase in biopolymer concentration can result in chain mobility increase, thus an increase in Tg can be observed.

Table 4. Thermal properties of the samples

Sample	Tg (°C)	Tm (°C)	ΔH_m (J/g)
M-5F-0.5S	-13.58±0.11[a]	58.66±0.11[a]	29.41±0.35[b]
M-5F-0S	-12.83±0.04[a]	58.17±0.03[a]	30.09±1.02[b]
M-3F-0.5S	-14.45±0.02[b]	58.57±0.10[a]	38.80±0.65[a]
C-3F-0.5S	-14.73±0.18[b]	58.25±0.02[a]	38.39±0.98[a]

Columns with different superscript letters are significantly different ($p \leq 0.05$). Tg: glass transition temperature, Tm: melting temperature, ΔH_m: melting enthalpy

3.2.5. FTIR Analysis

FTIR spectrum of nanofibers, PEO, carob flour and rice starch were shown in Figure 5. Generally, in FTIR spectrum, three different regions were described as the fingerprint region (800-1600 cm^{-1}), CH stretch region (2800 and 3000 cm^{-1}) and OH stretch region (3000 and 3600 cm^{-1}) (Aydogdu, Yildiz, Aydogdu, et al., 2019). Samples showed characteristic peak of pure components which was supporting the presence of these three constituents in the nanofibers, and well interaction between each other. Nano fibers and PEO showed similar peak at position 840 cm^{-1} which was due to C-O stretching, C-C stretching, CH_2 rocking (Pielichowski & Flejtuch, 2005). Peaks located at 1058, 1095, and 1145 cm^{-1} coming from characteristic peak of pure PEO which was linked stretching vibrations of the ether bond or C-O-C complex (Figure 5-A, B). The other peaks which are located around 1280, 1340 and 1467 cm^{-1} were related with PEO which were characteristics of CH_2 twisting, CH_2 wagging and CH_2 scissoring, respectively (Aydogdu, Sumnu, & Sahin, 2018). Actually, absorptions peaks at 1145 and 1058 cm^{-1} were related to crystallinity of PEO (Sim, Gan, Chan, & Yahya, 2010). After thermal and chemical treatments and electrospinning process, it was expected a reduction in intensity. Although starch and carob flour had also crystal

starch components, after heat treatment they might lose mostly their original structure. Therefore, although PEO crystallinity remains unchanged, increasing concentration of the amorphous part caused such a spectrum.

At fingerprint region, overlapping and complex spectra made difficult attribution of exact band assignment. In this part, spectra were based on vibrational form of monomer glucose units of starch, cellulose, amylose and amylopectin. Peaks positioned between 960 cm^{-1} in spectra of nanofibers was attributed to vibrations arising from C-O-C of α-1,4 glycosidic linkages (Kizil, Irudayaraj, & Seetharaman, 2002). The presence of peak at only nanofibers spectrum with a high peak intensity proved strong interaction between components, and new bond formation. The peaks at 1242 cm^{-1} corresponded to CH_2OH related mode besides the C−O−H deformation mode (Kizil et al., 2002). Small peaks located at 1600–1700 cm^{-1} and 1531 cm^{-1} regions were observed in both carob flour, starch and nanofibers spectra which are related to proteins and peptides and characteristic peaks of Amide-I and Amide-II (Mamone et al., 2019). Carob flour was composed of both starch and protein and trace amount of protein present in starch might lead to such a spectra. However, the presence of peaks in nanofibers might be interpreted as more heat stable proteins. For example, proteins in the form of β-sheet had more thermally stable structure than α-helix, β-turn and unordered conformations (Mamone et al., 2019). Therefore, applied heat treatment might not be enough for disrupting secondary the structure of some proteins. Furthermore, quaternary or tertiary structure of protein was more prone to be affected from heat treatments. Because of that, some proteins might lose their original conformation and had a secondary structure after treatments. Therefore, these peaks might belong to them also.

Peaks between 3000-2840 cm^{-1} region was due to C−H stretching in alkenes (Manoj & Kunjomana, 2012). Broad range peak between 3500–3300 cm^{-1} was attributed to hydroxyl (OH) group (Chuai, Almdal, Poulsen, & Plackett, 2001).

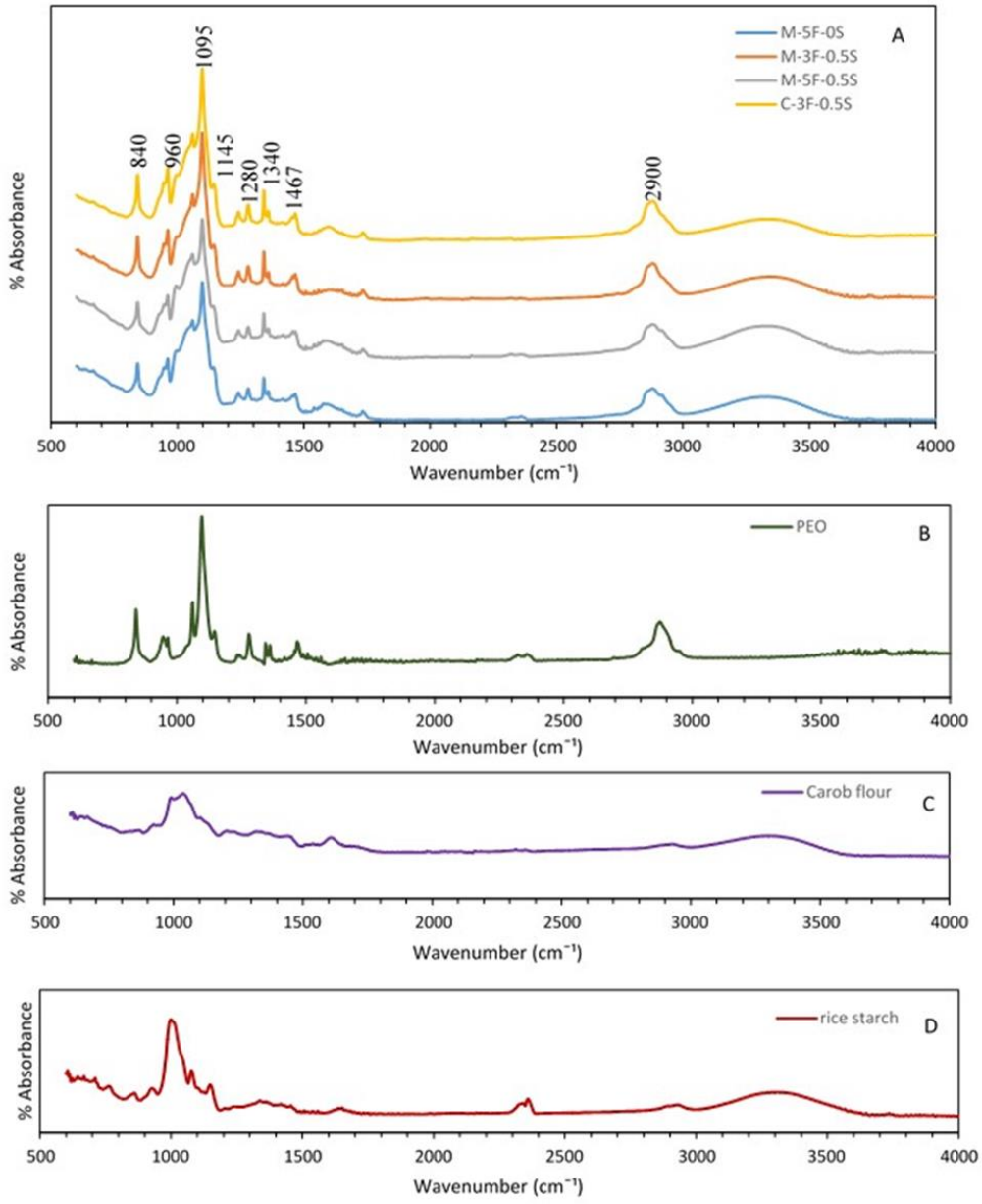

Figure 5. FTIR spectra of nanofibers FTIR spectra of (A) M- 5F-0.5S, M- 5F-0S, M-3F-0.5S and C- 3F-0.5S samples (B) FTIR spectra of PEO (C) FTIR spectra of Carob Flour (D) FTIR spectra of rice starch

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

In this study, it was illustrated that by microwave heating method it was possible to produce bead free and homogeneous electrospun nanofibers with various carob flour concentrations. The usage of microwaves as a pre-treatment of electrospinning increased the free amino groups in the solution which resulted in homogeneous fiber formation and improved film characteristics. Microwave treated samples showed better film characteristics in terms of higher mechanical properties and lower water vapor permeability as compared to conventionally treated sample. M-3F-0.5S sample had higher viscosity and fiber size diameter value than C-3F-0.5S sample. This enlightened that viscosity was a much more important parameter affecting fiber diameter rather than electrical conductivity. It was possible to obtain nanofibers with better mechanical properties by incorporation of rice starch to carob flour. M-3F-0.5S sample can be suggested by its better mechanical properties and higher viscosity. To conclude, it can be recommended that microwave heating method can be an alternative method to be used in preparation of electrospinning solutions.

In future studies, different flour and starch types can be used to prepare different spinning solutions. Microwave pre-treatment of these solutions can result in different interactions and fiber structures. Obtained nanofibers can be used as a layer in multi-layered packages. Natural biopolymer sourced packaging layers can be considered as an important step in preparation of environmentally friend packages. In addition to these, nanofibers can be used in active packaging preparation. By combining natural biopolymer originated nanofibers with active packaging systems, active packages can be obtained with different properties including oxygen scavenging, odor absorber/releaser or freshness and/or ripening indicator etc.

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APPENDICES

A. STATISTICAL ANALYSES

Table A. 1. One-way ANOVA and Tukey's comparison test for available free amino group values of electrospinning solutions (control, microwave treated and conventionally treated) prepared by using 3% PEO and 3% carob flour

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0,05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
sample       3  control; conventionally treated; microwave treated


Analysis of Variance

Source  DF    Adj SS    Adj MS  F-Value  P-Value
sample   2  0,000095  0,000048    30,31    0,010
Error    3  0,000005  0,000002
Total    5  0,000100


Model Summary

        S    R-sq  R-sq(adj)  R-sq(pred)
0,0012538  95,28%     92,14%      81,14%


Means

sample                  N      Mean     StDev         95% CI
control                 2  0,033256  0,000151  (0,030434; 0,036077)
conventionally treated  2   0,03554   0,00203  ( 0,03272;  0,03836)
microwave treated       2  0,042617  0,000752  (0,039796; 0,045438)

Pooled StDev = 0,00125377
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence
```

Table A.1 (Continued)

```
sample                  N      Mean  Grouping
microwave treated       2  0,042617  A
conventionally treated  2   0,03554    B
control                 2  0,033256    B

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

Table A. 2. One-way ANOVA and Tukey's comparison test for viscosity values of electrospinning solutions of M-5F-0.5S, M-5F-0S, M-3F-0.5S and C-3F-0.5S.

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0,05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
sample       4  C-3F-0.5S; M-3F-0.5S; M-5F-0.5S; M-5F-0S


Analysis of Variance

Source  DF    Adj SS    Adj MS  F-Value  P-Value
sample   3  0,011033  0,003678   242,22    0,000
Error    4  0,000061  0,000015
Total    7  0,011093


Model Summary

        S    R-sq  R-sq(adj)  R-sq(pred)
0,0038965  99,45%     99,04%      97,81%


Means

sample     N     Mean    StDev         95% CI
C-3F-0.5S  2  0,14550  0,00226  (0,13785; 0,15315)
M-3F-0.5S  2  0,23885  0,00672  (0,23120; 0,24650)
M-5F-0.5S  2  0,16635  0,00276  (0,15870; 0,17400)
M-5F-0S    2  0,15270  0,00170  (0,14505; 0,16035)

Pooled StDev = 0,00389647
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

sample     N     Mean  Grouping
```

Table A.2 (Continued)

```
M-3F-0.5S  2  0,23885  A
M-5F-0.5S  2  0,16635    B
M-5F-0S    2  0,15270    B C
C-3F-0.5S  2  0,14550      C

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

Table A. 3. One-way ANOVA and Tukey's comparison test for electrical conductivity values of electrospinning solutions of M-5F-0.5S, M-5F-0S, M-3F-0.5S and C-3F-0.5S.

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0,05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
sample       4  C-3F-0.5S; M-3F-0.5S; M-5F-0.5S; M-5F-0S


Analysis of Variance

Source  DF   Adj SS    Adj MS  F-Value  P-Value
sample   3  2,15972  0,719905   387,96    0,000
Error    4  0,00742  0,001856
Total    7  2,16714


Model Summary

        S    R-sq  R-sq(adj)  R-sq(pred)
0,0430770  99,66%     99,40%      98,63%


Means

sample     N     Mean    StDev        95% CI
C-3F-0.5S  2  1,56300  0,00424  (1,47843; 1,64757)
M-3F-0.5S  2  1,50350  0,00212  (1,41893; 1,58807)
M-5F-0.5S  2   2,7100   0,0849  ( 2,6254;  2,7946)
M-5F-0S    2   2,3800   0,0141  ( 2,2954;  2,4646)

Pooled StDev = 0,0430770
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence
```

Table A.3 (Continued)

```
sample     N     Mean  Grouping
M-5F-0.5S  2   2,7100  A
M-5F-0S    2   2,3800    B
C-3F-0.5S  2  1,56300      C
M-3F-0.5S  2  1,50350      C

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

Table A. 4. One-way ANOVA and Tukey's comparison test for *surface tension* values of electrospinning solutions of M-5F-0.5S, M-5F-0S, M-3F-0.5S and C-3F-0.5S.

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0,05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
sample       4  C-3F-0.5S; M-3F-0.5S; M-5F-0.5S; M-5F-0S


Analysis of Variance

Source  DF  Adj SS  Adj MS  F-Value  P-Value
sample   3  0,5772  0,1924     0,87    0,527
Error    4  0,8850  0,2213
Total    7  1,4622


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0,470372  39,47%      0,00%       0,00%


Means

sample     N     Mean   StDev        95% CI
C-3F-0.5S  2  33,4600  0,0566  (32,5365; 34,3835)
M-3F-0.5S  2   33,250   0,311  ( 32,327;  34,173)
M-5F-0.5S  2   33,730   0,778  ( 32,807;  34,653)
M-5F-0S    2   33,960   0,424  ( 33,037;  34,883)

Pooled StDev = 0,470372
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence
```

Table A.4 (Continued)

sample	N	Mean	Grouping
M-5F-0S	2	33,960	A
M-5F-0.5S	2	33,730	A
C-3F-0.5S	2	33,4600	A
M-3F-0.5S	2	33,250	A

Means that do not share a letter are significantly different.

Table A. 5. One-way ANOVA and Tukey's comparison test for average fiber diameter values of nanofibers obtained by electrospinning solutions of M-5F-0.5S, M-5F-0S, M-3F-0.5S and C-3F-0.5S.

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
sample	4	C-3F-0.5S; M-3F-0.5S; M-5F-0.5S; M-5F-0S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
sample	3	6393,78	2131,26	104,92	0,000
Error	4	81,25	20,31		
Total	7	6475,03			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
4,50698	98,75%	97,80%	94,98%

Means

sample	N	Mean	StDev	95% CI
C-3F-0.5S	2	274,87	1,59	(266,02; 283,71)
M-3F-0.5S	2	301,530	0,721	(292,682; 310,378)
M-5F-0.5S	2	243,68	2,34	(234,83; 252,52)
M-5F-0S	2	228,34	8,53	(219,49; 237,19)

Pooled StDev = 4,50698

Tukey Pairwise Comparisons

Table A.5 (Continued)

```
Grouping Information Using the Tukey Method and 95% Confidence

sample     N     Mean  Grouping
M-3F-0.5S  2  301,530  A
C-3F-0.5S  2   274,87    B
M-5F-0.5S  2   243,68      C
M-5F-0S    2   228,34      C

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

Table A. 6. One-way ANOVA and Tukey's comparison test for water vapor permeability (WVP)values of nanofibers obtained by electrospinning solutions of M-5F-0.5S, M-5F-0S, M-3F-0.5S and C-3F-0.5S.

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0,05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
sample       4  C-3F-0.5S; M-3F-0.5S; M-5F-0.5S; M-5F-0S


Analysis of Variance

Source  DF   Adj SS    Adj MS  F-Value  P-Value
sample   3  1,13985  0,379950    83,05    0,000
Error    4  0,01830  0,004575
Total    7  1,15815


Model Summary

        S    R-sq  R-sq(adj)  R-sq(pred)
0,0676387  98,42%     97,23%      93,68%


Means

sample     N    Mean   StDev       95% CI
C-3F-0.5S  2  2,1300  0,0141  (1,9972; 2,2628)
M-3F-0.5S  2  1,6750  0,0212  (1,5422; 1,8078)
M-5F-0.5S  2  2,3200  0,0566  (2,1872; 2,4528)
M-5F-0S    2  2,7250  0,1202  (2,5922; 2,8578)
```

Table A.6 (Continued)

```
Pooled StDev = 0,0676387
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

sample     N    Mean  Grouping
M-5F-0S    2  2,7250  A
M-5F-0.5S  2  2,3200    B
C-3F-0.5S  2  2,1300    B
M-3F-0.5S  2  1,6750      C

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

Table A. 7. One-way ANOVA and Tukey's comparison test for tensile strength values of nanofibers obtained by electrospinning solutions of M-5F-0.5S, M-5F-0S, M-3F-0.5S and C-3F-0.5S.

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0,05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
sample       4  C-3F-0.5S; M-3F-0.5S; M-5F-0.5S; M-5F-0S


Analysis of Variance

Source  DF   Adj SS   Adj MS  F-Value  P-Value
sample   3  13,1538  4,38460   376,36    0,000
Error    4   0,0466  0,01165
Total    7  13,2004


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0,107935  99,65%     99,38%      98,59%


Means

sample     N     Mean    StDev        95% CI
C-3F-0.5S  2  2,30500  0,00707  (2,09310; 2,51690)
M-3F-0.5S  2    3,875    0,177  (  3,663;   4,087)
M-5F-0.5S  2   0,9550   0,0636  ( 0,7431;  1,1669)
M-5F-0S    2   0,6250   0,1061  ( 0,4131;  0,8369)
```

Table A.7 (Continued)

```
Pooled StDev = 0,107935
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

sample     N     Mean  Grouping
M-3F-0.5S  2    3,875  A
C-3F-0.5S  2  2,30500    B
M-5F-0.5S  2   0,9550      C
M-5F-0S    2   0,6250      C

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

Table A. 8. One-way ANOVA and Tukey's comparison test for young modulus values of nanofibers obtained by electrospinning solutions of M-5F-0.5S, M-5F-0S, M-3F-0.5S and C-3F-0.5S.

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0,05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
sample       4  C-3F-0.5S; M-3F-0.5S; M-5F-0.5S; M-5F-0S


Analysis of Variance

Source  DF   Adj SS   Adj MS  F-Value  P-Value
sample   3  4944,10  1648,03   151,09    0,000
Error    4    43,63    10,91
Total    7  4987,73


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
3,30262  99,13%     98,47%      96,50%


Means

sample     N    Mean  StDev        95% CI
C-3F-0.5S  2  69,784  0,841  (63,300; 76,268)
M-3F-0.5S  2   79,91   4,95  ( 73,42;  86,39)
M-5F-0.5S  2   29,56   3,13  ( 23,07;  36,04)
M-5F-0S    2   22,27   2,93  ( 15,79;  28,76)
```

Table A.8 (Continued)

```
Pooled StDev = 3,30262
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

sample     N    Mean  Grouping
M-3F-0.5S  2   79,91  A
C-3F-0.5S  2  69,784  A
M-5F-0.5S  2   29,56    B
M-5F-0S    2   22,27    B

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

Table A. 9. One-way ANOVA and Tukey's comparison test for elongation at break values of nanofibers obtained by electrospinning solutions of M-5F-0.5S, M-5F-0S, M-3F-0.5S and C-3F-0.5S.

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0,05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
sample       4  C-3F-0.5S; M-3F-0.5S; M-5F-0.5S; M-5F-0S


Analysis of Variance

Source  DF  Adj SS  Adj MS  F-Value  P-Value
sample   3  776,42  258,81    22,66    0,006
Error    4   45,68   11,42
Total    7  822,10


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
3,37929  94,44%     90,28%      77,77%


Means

sample     N    Mean  StDev        95% CI
C-3F-0.5S  2  41,685  0,672  (35,051; 48,319)
M-3F-0.5S  2   61,25   6,41  ( 54,61;  67,88)
M-5F-0.5S  2   41,36   1,51  ( 34,73;  47,99)
M-5F-0S    2  34,990  1,344  (28,356; 41,624)

Pooled StDev = 3,37929
```

Table A.9 (Continued)

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

sample     N    Mean  Grouping
M-3F-0.5S  2   61,25  A
C-3F-0.5S  2  41,685    B
M-5F-0.5S  2   41,36    B
M-5F-0S    2  34,990    B

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

Table A. 10. One-way ANOVA and Tukey's comparison test for glass transition temperature values of nanofibers obtained by electrospinning solutions of M-5F-0.5S, M-5F-0S, M-3F-0.5S and C-3F-0.5S.

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0,05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
sample       4  C-3F-0.5S; M-3F-0.5S; M-5F-0.5S; M-5F-0S


Analysis of Variance

Source  DF  Adj SS   Adj MS  F-Value  P-Value
sample   3  4,4663  1,48878    33,79    0,003
Error    4  0,1763  0,04406
Total    7  4,6426


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0,209911  96,20%     93,36%      84,81%


Means

sample     N     Mean   StDev         95% CI
C-3F-0.5S  2   14,730   0,354  ( 14,318;  15,142)
M-3F-0.5S  2  14,4450  0,0354  (14,0329; 14,8571)
M-5F-0.5S  2   13,580   0,212  ( 13,168;  13,992)
M-5F-0S    2  12,8300  0,0707  (12,4179; 13,2421)
```

Table A.10 (Continued)

```
Pooled StDev = 0,209911
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

sample     N     Mean  Grouping
C-3F-0.5S  2   14,730  A
M-3F-0.5S  2  14,4450  A
M-5F-0.5S  2   13,580    B
M-5F-0S    2  12,8300    B

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

Table A. 11. One-way ANOVA and Tukey's comparison test for melting temperature values of nanofibers obtained by electrospinning solutions of M-5F-0.5S, M-5F-0S, M-3F-0.5S and C-3F-0.5S.

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0,05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
sample       4  C-3F-0.5S; M-3F-0.5S; M-5F-0.5S; M-5F-0S


Analysis of Variance

Source  DF   Adj SS   Adj MS  F-Value  P-Value
sample   3  0,33934  0,11311     4,72    0,084
Error    4  0,09595  0,02399
Total    7  0,43529


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0,154879  77,96%     61,42%      11,83%


Means

sample     N     Mean   StDev        95% CI
C-3F-0.5S  2  58,2500  0,0424  (57,9459; 58,5541)
M-3F-0.5S  2   58,565   0,205  ( 58,261;  58,869)
M-5F-0.5S  2   58,655   0,219  ( 58,351;  58,959)
M-5F-0S    2  58,1650  0,0636  (57,8609; 58,4691)
```

Table A.11 (Continued)

```
Pooled StDev = 0,154879
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

sample     N     Mean  Grouping
M-5F-0.5S  2   58,655  A
M-3F-0.5S  2   58,565  A
C-3F-0.5S  2  58,2500  A
M-5F-0S    2  58,1650  A

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

Table A. 12. One-way ANOVA and Tukey's comparison test for melting enthalpy values of nanofibers obtained by electrospinning solutions of M-5F-0.5S, M-5F-0S, M-3F-0.5S and C-3F-0.5S.

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0,05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
sample       4  C-3F-0.5S; M-3F-0.5S; M-5F-0.5S; M-5F-0S


Analysis of Variance

Source  DF  Adj SS  Adj MS  F-Value  P-Value
sample   3  156,57  52,189    20,83    0,007
Error    4   10,02   2,506
Total    7  166,59


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
1,58305  93,98%     89,47%      75,93%


Means

sample     N    Mean  StDev        95% CI
C-3F-0.5S  2   38,36   1,91  ( 35,25;  41,47)
M-3F-0.5S  2  38,801  1,306  (35,693; 41,909)
M-5F-0.5S  2  29,412  0,704  (26,304; 32,520)
M-5F-0S    2   30,09   2,04  ( 26,98;  33,19)

Pooled StDev = 1,58305
```

Table A.12 (Continued)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

sample	N	Mean	Grouping
M-3F-0.5S	2	38,801	A
C-3F-0.5S	2	38,36	A
M-5F-0S	2	30,09	B
M-5F-0.5S	2	29,412	B

Means that do not share a letter are significantly different.