ENCAPSULATION OF GRAPE SEED EXTRACT IN RYE FLOUR AND WHEY PROTEIN-BASED ELECTROSPUN NANOFIBERS

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ENCAPSULATION OF GRAPE SEED EXTRACT IN RYE FLOUR AND WHEY PROTEIN-BASED ELECTROSPUN NANOFIBERS

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ABSTRACT

ENCAPSULATION OF GRAPE SEED EXTRACT IN RYE FLOUR AND WHEY PROTEIN-BASED ELECTROSPUN NANOFIBERS

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The main objective of this research was to encapsulate grape seed extract (GSE) into electrospun nanofibers produced from different blends of rye flour, whey protein concentrate (WPC) and polyethylene oxide (PEO). The effects of rye flour concentration (4 and 6% (w/v)) and heating methods (conventional and microwave) on the properties of solutions and nanofibers were studied. Rheology results showed that microwave heated solutions containing 6% (w/v) rye flour had higher viscosity. According to the scanning electron microscope (SEM) images, microwave pretreatment provided beadless and more homogeneous fibers as compared to the ones obtained from conventionally heated solutions. GSE addition had an increasing effect on viscosity and diameter size of microwave heated samples. The physical and thermal properties of GSE encapsulated nanofibers pretreated by microwave heating were determined by X-ray diffraction (XRD), water vapor permeability (WVP), differential scanning calorimeter (DSC), thermogravimetric analyzer (TGA), and Fourier transform infrared (FTIR) analyses. WVP values ranged between 1.09×10^{-10} g m^{-2} s^{-1} and 1.94×10^{-10} g m^{-2} s^{-1} and increased with GSE addition. The GSE addition made strong interactions within polymer matrix which improved thermal

stability of films. Although GSE was not so stable at high pH environment, antioxidant activities of GSE containing fibers with 4% (w/v) and 6% (w/v) rye flour were found to be 41.62 and 42.78%, respectively. GSE loading efficiency of electrospun nanofibers was improved from 54.16 to 61.15% with increasing rye flour concentration. The results showed that rye flour is a good candidate for encapsulation of GSE by electrospinning and obtained fibers could be considered as sustainable active packaging materials.

Keywords: Rye flour, Grape seed extract, Electrospinning, Encapsulation, Microwave

ÖZ

ÜZÜM ÇEKİRDEĞİ EKSTRAKTININ ÇAVDAR UNU VE PEYNİR ALTI SUYU PROTEİNİ BAZLI ELEKTROEĞRİLMİŞ NANOLİFLERE ENKAPSÜLASYONU

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Bu çalışmanın temel amacı, üzüm çekirdeği ekstraktını (GSE) çavdar unu, peynir altı suyu konsantresi (WPC) ve polietilen oksitin (PEO) farklı karışımlarından üretilen elektroeğrilmiş nanoliflere hapsetmektir. Çavdar unu konsantrasyonunun (%4 ve %6) ve farklı ısıtma yöntemlerinin (konvansiyonel ve mikrodalga) çözeltilerin ve nanoliflerin özellikleri üzerindeki etkisi araştırılmıştır. Reoloji sonuçları, mikrodalgayla ısıtılan ve %6 çavdar unu içeren çözeltilerin daha yüksek viskoziteye sahip olduğunu göstermiştir. Taramalı elektron mikroskobu (SEM) ile elde edilen görüntülere göre, mikrodalgayla yapılan ön işlem, konvansiyonel metodla ısıtılan çözeltiden üretilen liflere göre boncuksuz ve daha homojen nanolif oluşumu sağlamıştır. Üzüm çekirdeği ekstraktı ilavesi, mikrodalgayla ısıtılan numunelerin viskozitesini ve çapını artırmıştır. Üzüm çekirdeği ekstraktı kapsüllenmiş ve mikrodalga ön işlemi görmüş nanoliflerin fiziksel ve termal özellikleri X-ışını kırınımı (XRD), su buharı geçirgenliği (WVP), diferansiyel taramalı kalorimetri, termal gravimetrik analiz (TGA), ve Fourier dönüşümü kızılötesi spektroskopisi (FTIR) analizleriyle belirlenmiştir. Su buhari geçirgenliği 1.09×10^{-10} g m^{-2} s^{-1} ile 1.94×10^{-10} g m^{-2} s^{-1} arasında değişen değerler alıp, üzüm

çekirdeği ekstraktı ilavesi olan filmlerde artış göstermiştir. Üzüm çekirdeği ekstraktı ilavesi polimer matriksi içerisinde güçlü etkileşimlere yol açmıştır ve bu şekilde filmlerin termal dayanıklılığı iyileşmiştir. Üzüm çekirdeği ekstraktı yüksek pH değerlerinde çok stabil olmamasına rağmen, üzüm çekirdeği ekstraktı içeren ve %4 ile %6 çavdar unu kullanılarak hazırlanan liflerin antioksidan aktiviteleri sırasıyla %41.62 ve %42.78 olarak bulunmuştur. Elektroeğrilmiş nanoliflerin üzüm çekirdeği ekstraktı yükleme verimliliği, çavdar unu konsantrasyonunun artmasıyla birlikte %54.16'dan %61.15'e çıkmıştır ve böylece yükleme verimliliğinin çavdar unu konsantrasyonuyla beraber arttığı görülmüştür. Sonuçlar, çavdar ununun elektroeğirme uygulaması ile üzüm çekirdeği ekstraktının kapsüllenmesi için iyi bir aday olabileceğini ve ele edilen liflerin sürdürülebilir ambalaj malzemesi olarak düşünülebileceğini göstermiştir.

Anahtar Kelimeler: Çavdar unu, Üzüm çekirdeği ekstraktı, Elektroeğirme, Enkapsülasyon, Mikrodalga

To my beloved family

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ABBREVIATIONS

ΔH_m : Melting enthalpy

AA : Antioxidant activity

DSC : Differential scanning calorimeter

FTIR : Fourier-transform infrared spectroscopy

GSE : Grape seed extract

LE : Loading efficiency

PEO : Polyethylene oxide

SEM : Scanning electron microscopy

T_g : Glass transition temperature

T_m : Melting temperature

TGA : Thermogravimetric analysis

TPC : Total phenolic content

XRD : X-ray diffraction

WVP : Water vapor permeability

CHAPTER 1

INTRODUCTION

1.1 Electrospinning

Electrospinning is an electrohydrodynamical process which produces fibers with diameters in the nanometer scale, smaller than approximately one thousandth the size of a human hair. After many years of the first electrospinning patent in 1931 (Tucker et al., 2012), fabricating nanofibers through a high voltage electric field gained a great importance because electrospinning offers some advantages such as relatively low cost and ambient process conditions, which is a crucial point for heat sensitive materials (Seethu et al., 2020). Since electrospun nanofibers exhibit high porosity, large surface area to volume ratio and homogeneity, this novel and cost-effective technique has applications in many diverse areas, including drug delivery, tissue engineering, biosensors, and packaging (Moomand & Lim, 2015; Shao et al., 2018). Especially the active packaging, which utilizes bioactive substances to enhance the shelf life of the food product by incorporating these compounds into packaging materials, benefits from electrospinning technology (Altan et al., 2018; Cerqueira et al., 2016).

A typical laboratory setup of the electrospinning can be divided into three components which are a high voltage DC power supply, a digitally controlled syringe pump, and a grounded metal collector plate (Kumar & Sinha-Ray, 2018). Figure 1.1 shows a schematic representation of a electrospinning configuration. In this setup, a syringe filled with polymer solution is placed horizontally and controlled with a digital pump to adjust the flow rate of the solution. As the syringe is pumped, the polymer solution goes out through the needle tip of the syringe. Due to the applied

high voltage (5-30 kV), an electrostatic field is generated between the needle connected to positively charged electrode and the collector plate connected to negatively charged electrode. The polymer solution droplet becomes charged and distorted by repulsive forces to a cone shape, which is named as Taylor cone (Britain, 1969). When the critical voltage is surpassed, surface tension is overcome by the repulsive forces and polymer jet is ejected towards the collector plate. As the jet moves to the collector, the solvent in the polymer solution evaporates and solid nanofibers are collected on the plate. Randomly oriented solid nanofibers are assembled as a non-woven mats (Kumar & Sinha-Ray, 2018; Torres-Giner, 2011).

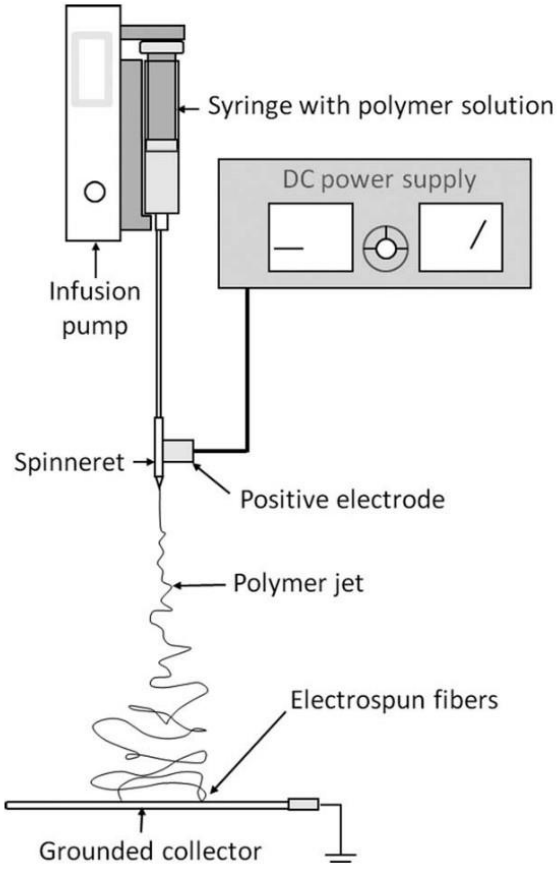

Figure 1.1 Simple representation of electrospinning setup. Reprinted from "Effects of poly(ethylene oxide) and pH on the electrospinning of whey protein isolate," by A. C. Vega-Lugo, 2012, *Journal of Polymer Science, Part B: Polymer Physics, 50(16),* 1189. Copyright [2012] by Wiley Periodicals, Inc.

1.1.1 The Parameters Affecting Electrospinning Process

Although electrospinning has a quite simple setup and straightforward operation, the process is extensively governed by several parameters which determine the overall characteristics of the nanofiber products. These parameters can be studied under three main categories: electrospinning polymer solution properties, processing parameters, and ambient conditions (Angammana & Jayaram, 2011). To produce homogeneous nanofibers with desired characteristics, these parameters should be chosen accordingly. Unstable jet formation might occur if the optimum conditions are not met, which would lead to fabricate nanofibers containing beads and non-uniformity (Drosou et al., 2018; Ghorani & Tucker, 2015).

1.1.1.1 Solution Properties

The properties of the polymer solution have an important effect on the nanofiber morphology obtained by electrospinning. Solution viscosity, polymer concentration and molecular weight, electrical conductivity, and surface tension are the main characteristics that are proven to have a significant influence on the electrospun fibers by several studies (Oguz et al., 2018; Vega-Lugo & Lim, 2012; Xu et al., 2012). Electrospinning process requires elongation of polymer solution and it is not possible with very low viscosity. On the other hand, a thick solution with high viscosity cannot be ejected from the needle tip as desired (Aydogdu, Yildiz, Ayhan, et al., 2019). The optimum viscosity of the solution can be obtained by changing the amount or the type of the polymer used since the viscosity is strongly dependent on those properties. In a study where polyethylene oxide (PEO) and whey protein isolate (WPI) were used for electrospinning, bead formation was observed when lower concentration of PEO was used, which decreased the viscosity of solution (Colín-Orozco et al., 2015). In addition, changing the polymer composition by adding functional materials may affect the process in different ways. When curcumin was added to the amaranth protein isolate and pullulan blend solution, an increase in the

fiber diameter was observed which was explained by enhanced interactions due to hydrogen bond formations (Blanco-Padilla et al., 2015).

The nanofiber formation could be successful if the solution is stretched by the repulsion of the charges at the jet surface which is created by the force between electric field and surface charge. Therefore, solutions with zero conductivity cannot form any fiber by electrospinning. As the solution has more charges, the stretching and elongation of the jet becomes higher (Angammana & Jayaram, 2011). Increasing electrical conductivity by adding salt to the solution is reported to yield in nanofibers with smaller diameters and less bead formation during electrospinning (Tam et al., 2017). However, too high electrical conductivity, which was stated as 1 S/m (Fernández de la Mora, 2007), might create a bending instability which prevents a stable jet formation by breaking solution into droplets (Vega-Lugo & Lim, 2012). Therefore, finding the optimum conductivity of the solution is crucial in terms of electrospinnability and producing homogeneous nanofibers.

When the critical voltage is applied in the electric field, the electrostatic force exceeds the surface tension of the charged polymer droplet and solution jet is ejected from the tip of the Taylor cone (Ghorani & Tucker, 2015). Therefore, electrospinning solution is desired to have lower surface tension in order to prevent bead formation. Surface tension of the solution changes with the type and the concentration of the polymer used. Adding surfactant to the polymer solution is found to be a successful way to reduce surface tension of the solution and produce nanofibers with less or no bead (Aceituno-Medina et al., 2015; Oguz et al., 2018). However, it was shown that surface tension is not the only factor determining the morphology of the fibers. The combined effects of surface tension, conductivity and viscosity should be considered since they all change the molecular entanglement and the results of electrospinning differently (Vega-Lugo & Lim, 2012).

1.1.1.2 Process Parameters

Process parameters are the other important variables that affect the characteristics and morphology of nanofibers. These parameters include applied voltage, flow rate of the solution, and distance between the needle tip and the collector plate (Ramakrishna et al., 2006). As mentioned before, working principle of electrospinning is dependent on the electric field created by applied voltage. In this regard, there must be a sufficient voltage to form an electrostatic force to surpass the surface tension of polymer solution droplet. Since each solution could have different surface tension to be overcome, required applied voltage and electric field may change. In general, voltage of 6 kV and higher would be able to initiate solution droplet distortion to Taylor cone shape (Sir & Taylor, 1964). However, as the applied voltage increases, driving force would increase due to constant surface tension. Higher driving force will cause an accelerated solution drawn from the tip of the needle which would result in unstable Taylor cone and undesired bead formation in nanofibers (Ghorani & Tucker, 2015). It was shown that up to certain point, the increase in applied voltage favors the smaller fiber diameter but after that critical value was passed, high voltage caused bead formation in pullulan-carboxymethylcellulose sodium nanofibers (Shao et al., 2018).

As one of the process parameters, flow rate has an influence on the electrospinning process since there should be continuous solution fed to the electric field. Higher flow rates might result in solution accumulation at the tip of needle which includes larger volume of solvent to be evaporated. When the evaporation is failed due to limited time of electrospinning, sticky fibers and beads would be observed in the final result (Zong et al., 2002). It was reported that although increasing flow rate produced fibers with larger diameter, flow rate was not a significant variable when high voltage was applied (Luo et al., 2012). Therefore, the combined effect of the parameters should be considered for electrospinning process.

During electrospinning, the polymer solution is ejected as jet elongates from needle tip to collector. Therefore, that distance has an important role for the whole process.

Generally, it varies between 10-30 cm (Kumar & Sinha-Ray, 2018). If the tip to collector distance is not long enough, sufficient evaporation of solvent could not be reached. As being in correlation with other process parameters, similar undesired results might be obtained such as non-homogeneous fibers and fused junctions due to short drying time. Also, very long collector distance may reduce the electric field strength which would affect the process and the fiber morphology negatively (Ghorani & Tucker, 2015).

1.1.1.3 Environmental Conditions

Ambient parameters such as temperature and humidity could also have an impact on the electrospinning process due to having strong relationship with solvent evaporation and solution properties (Ghorani & Tucker, 2015). It was argued that solvent evaporation rate is directly affected by electrospinning chamber temperature and it increases as the temperature gets higher. Also, increasing temperature reduces the viscosity of polymer solution. These two changes have different impacts on fiber morphology since they are inversely related to each other (De Vrieze et al., 2009). Even though the influence of humidity on the fiber morphology would change depending on the polymer type and its chemical structure, it can be adjusted to alter fiber diameter size as well as pore formation in the nanofiber matrix. If humidity is high, water condensation could occur between the fibers which would create pores (Torres-Giner, 2011).

1.1.2 Electrospinning Applications

The electrospinning technique and electrospun nanofibers offer various advantages such as low cost, simplicity, large surface area to volume ratio, and high porosity. Therefore, its applications have been used in diverse areas such as energy storage, medical fields, optical sensors, filtration, and textile industry (Ramakrishna et al., 2006). The successful medical applications were studied including wound dressing

(Locilento et al., 2019), and drug delivery (Nazari et al., 2017). Electrospun nanofibers were also used as membranes which showed improved mechanical and surface properties (Bahi et al., 2017). It was shown that using electrospun nanofibers increased the overall efficiency in batteries thanks to high aspect ratio of the fibers (Cheah et al., 2011). In addition to these areas, there is an increasing interest in utilization of electrospinning for encapsulation of bioactive materials and packaging purposes.

1.1.2.1 Food Packaging Materials

Increasing environmental and human health concerns lead scientists to search petroleum-free food packaging materials. To reduce the dependency on fossil-based fuel and establish environmental sustainability, biopolymers and biodegradable polymers came into focus in the packaging material studies. In addition to their renewable nature, biopolymers and biodegradable polymers often exhibit other important properties such as biocompatibility and antibacterial activity (Schiffman & Schauer, 2008). Accordingly, usage of biopolymers such as polysaccharides and proteins became a recent trend to replace synthetic polymers with natural ones (Aman Mohammadi et al., 2018).

Several studies showed that biopolymer-based films could be produced from food-grade polysaccharides and proteins, which are renewable sources. So far, biodegradable polymers, including but not limited to, cellulose, alginate, chitosan, starch, wheat, pullulan, whey, silk and gelatin have resulted in functional electrospun nanofibers (Mendes et al., 2017; Torres-Giner, 2011). However, because of some disadvantages that bio-based and biodegradable polymers have as packaging material like insufficient thermal and mechanic properties and weak barrier properties, their blends with other functional materials or multilayered forms are being developed recently (Cerqueira et al., 2016). For example, starch-based nanofibers often showed brittleness and poor processability (Liu et al., 2017). On the other hand, protein-based films have shown exceptional gas permeability and several

functional properties (Hammann & Schmid, 2014). Besides having outstanding emulsification, gelation, and foaming functionalities, whey proteins are successful at encapsulating the active compounds as electrospinning material (Drosou et al., 2018). However, hydrophilic nature of protein films causes poor water vapor permeability (Hammann & Schmid, 2014). For food packaging purposes, combining polysaccharides and proteins in the solution material might bring out their advantages and discard the drawbacks of the films. For example, it was observed that thermal stability of the whey protein isolate nanofibers were improved when pullulan was added to the solution (Drosou et al., 2018). As a biodegradable and biocompatible material, polyethylene oxide (PEO) could help to form more homogeneous nanofibers by enhancing the electrospinnability of solutions since using PEO along with biopolymers had a decreasing effect on the repulsive forces between molecules which hindered the possibility of sufficient molecular entanglement during electrospinning (Vega-Lugo & Lim, 2012). In another study, the addition of PEO provided homogeneous and beadless nanofibers due to the increased molecular entanglement by PEO and amino acid in lentil flour interaction while pure lentil flour resulted in nanofibers with beads (Aydogdu, Sumnu, et al., 2019).

As an innovative technology, electrospinning is widely being used to produce nanofiber films due to its superior attributes over other biopolymer-based film production techniques. In the study where electrospun nanofibers and films prepared by solvent casting method from polyurethane and clay compared, it was found that nanofibers were smoother and more uniform (Saha et al., 2014). As well as the non-presence of agglomeration, the large surface area to volume ratio of nanofibers provided better drug release than film samples. In traditional solvent casting method, plasticizers are often required since some materials exhibit inflexibility and brittleness. However, addition of plasticizers could result in toxicity and higher cost. In that matter, electrospinning method might be suggested for films with enhanced mechanical characteristics without using plasticizers. For example, when poly(ε-caprolactone) was used as polymeric material, it was found that electrospinning

improved the mechanical properties of films compared to ones produced by solvent casting method by helping plasticization (Ghosal et al., 2018). In addition to mechanical and thermal properties, water and oxygen barrier characteristics play important role in evaluating a material for packaging purpose. Water permeability of a film usually is linked to the hydrophilic or hydrophobic behavior of the materials which is controlled by water contact angle (Wen, Zhu, Feng, et al., 2016). Polylacticacid (PLA) nanofiber films produced by electrospinning were found to have larger water contact angle than casting PLA films, which indicated more hydrophobic behavior of electrospun nanofibers made of the same polymer that would reduce the water vapor permeability (Toncheva et al., 2011). To enhance water vapor and gas permeability of packaging, multilayer systems are being widely used and researched recently. By layering multiple films made from different materials, their individual characteristics are combined. As implementing this method, drawbacks of electrospun nanofiber films would be eliminated by other polymer film layers and advantages that nanofibers offer such as encapsulating active material would be kept. For instance, water vapor permeability of wheat gluten films were enhanced when it was covered with alpha-tocopherol encapsulated electrospun zein nanofiber layer (Fabra et al., 2016). The result of this study showed that active packaging, which provides quality preservation and safety of the product, could be obtained with improved barrier properties by using electrospinning method. In terms of protecting the product, opacity of the packaging is an important to prevent exposure to the light. When hydroxypropyl methylcellulose (HPMC), soy protein and PEO were used as nanofiber material and collected onto PLA films, it was observed that opacity values were increased compared to PLA films (Aydogdu, Yildiz, Ayhan, et al., 2019). Several studies found that electrospinning was a very effective method to produce active packaging by incorporating bioactive materials into polymer nanofiber mats. Antioxidant and antimicrobial compounds could be loaded into packaging films to extend the shelf life of the food products by inhibiting oxidation and microbial growth (Altan et al., 2018). This novel packaging method was applied on the shrimps and it was found that cinnamon nanophytosomes loaded

polyvinyl alcohol based electrospun nanofibers prevented microbial growth and extended the shelf life (M. Nazari et al., 2019). As compared to solvent casting method, electrospinning was more effective as antimicrobial active packaging method for bread preservation when cinnamon essential oil was encapsulated into PVA and β-cyclodextrin polymers (Wen, Zhu, Wu, et al., 2016). By showing controlled release of gallic acid, zein based nanofibers was evaluated as both antioxidant and antimicrobial packaging and it showed a great potential as active packaging with its low water activity, thermal and chemical stability (Neo et al., 2013). Those studies indicate that electrospun nanofiber films are promising candidates for being reinforcement of several characteristics of packaging materials and their utilization should be one of the focus topics of food packaging research.

1.1.2.2 Encapsulation of Bioactive Materials

Natural bioactive materials, especially phenolic compounds, have several beneficial properties such as antioxidant, anti-microbial, and anti-inflammatory activities (Locilento et al., 2019). Despite the broad range of properties, they are likely to be susceptible to environmental factors like light, temperature, or oxygen, which cause degradation and limits bioavailability of the bioactive compounds. For this reason, encapsulation technique has been used to protect and stabilize the bioactive compounds by coating them with another substance as a physical barrier (Nedovic et al., 2011). Since it has many application fields, several encapsulation techniques are being used such as spray drying, coacervation, or melt injection. As one of these encapsulation techniques, electrospinning has been gaining an extensive attraction because of its advantages over other methods like simplicity, cost-effectiveness, high surface over volume ratio (Anu Bhushani & Anandharamakrishnan, 2014). In addition to those, encapsulation by electrospinning is highly preferred because of processing temperature. Heat-sensitive compounds can be incorporated to the matrix at ambient temperature by electrospinning without being exposed to any destroying effect (Seethu et al., 2020). For example, a successful encapsulation of gallic acid,

which is a heat sensitive bioactive material, into lentil based films by electrospinning was carried out and active packaging materials were produced as preserving antioxidant properties of the gallic acid with 74% antioxidant activity and 62% loading efficiency (Aydogdu, Yildiz, Aydogdu, et al., 2019). In another study, flaxseed oil is entrapped in the flaxseed mucilage nanofiber film with 82.7% encapsulation efficiency (Hadad & Goli, 2019). By performing electrospinning at ambient temperature, sensitive flaxseed oil could be protected from undesirable environmental conditions. As well as processing temperature, electrospinning offers controlled release advantage for encapsulation systems because of the high surface over volume ratio of mats composed of ultrathin fibers (Hu et al., 2014). Especially for the bioactive materials with chemical instability or poor solubility, electrospun nanofibers is a great option for incorporation process. It was shown that controlled release of curcumin was successful from the electrospun fibers made of the combination of amaranth protein isolate and pullulan (Blanco-Padilla et al., 2015). The result was promising in terms of offering a biopolymer-based film loaded with curcumin which maintained its antioxidant activity after an in vitro digestion process to be used in the food industry. Additionally, electrospun nanofibrous web structure was found to have a high potential for drug delivery. As compared to solving casted films, drug loaded polyurethane/clay nanofibers showed higher drug release behavior which makes electrospun nanofibers a good candidate for medical applications (Saha et al., 2014). In recent studies, antioxidants and antimicrobials, preferably plant-derived agents, have been encapsulated into polymeric materials for active packaging applications to extend the shelf life of food products. Raw shrimps were preserved longer when they were packed with cinnamon nanophytosomes loaded polyvinyl alcohol electrospun nanofibers (Nazari et al., 2019).

Grape seed extract is a mixture of various polyphenols, including catechin, epicatechin, and gallic acid (Marqués et al., 2013). Being a waste and byproduct of wine and fruit juice industry makes grape seed more preferable among other phenolic and antioxidant sources due to its low cost and sustainability (Faki et al., 2019). It

showed excellent cytocompatibility and antioxidant effect when used in silk fibroin nanofibers and did not change the morphology of the fibers (Lin et al., 2016).

1.1.3 Usage of Flour in Electrospinning

Recent studies have been shown that natural biopolymers, especially carbohydrates and proteins, could result in remarkable products to be used in various fields by electrospinning process. Unlike most of the synthetic polymers, natural biopolymers are renewable and biodegradable, therefore they have been preferred as a nanofiber material for several industries (Schiffman & Schauer, 2008). Utilization of natural polymer sources through electrospinning, which is a low cost and environmentally friendly technology, could help establishing sustainable alternatives for some currently present applications such as wound dressing (Iacob et al., 2020; Locilento et al., 2019), wearable biosensor (Kim & Kim, 2020), active packaging (Aydogdu, Yildiz, Aydogdu, et al., 2019; Sogut & Seydim, 2018), and drug delivery systems (Blanco-Padilla et al., 2015). Besides their ecological benefits, naturally derived biopolymers also have some inherent properties that offer biocompatibility and antimicrobial activity. In literature, different studies on biopolymer based electrospun nanofibers can be found which include usage of chitosan, starch, alginate, cellulose, pullulan as polysaccharide material and gelatin, collagen, zein, whey, silk, soy as protein source for electrospinning applications (Mendes et al., 2017).

Being a low-cost, food-grade polysaccharide and protein source, flours have been considered as a strong option for nanofiber material. Based on the flour type and composition, they could be mixed with other compounds to combine the advantages of different biopolymers. For example, lentil flour, which contains high amount of polysaccharide and protein, was used as a complete biopolymer source as an electrospinning material (Aydogdu, Yildiz, Aydogdu, et al., 2019). In order to improve the electrospinnability by reducing the repulsive forces and enhancing entanglement between molecules, PEO, which is biocompatible and nontoxic

polymer, was added to the lentil flour solutions. As well as lentil flour, pea flour was also used in electrospinning as another legume. High protein (22% (w/w)) and polysaccharide content (55% (w/w)) make pea flour a significant candidate for nanofiber fabrication. Its blend with hydroxypropyl methylcellulose (HPMC) produced successful beadless electrospun nanofibers which were suggested as a promising option for food packaging applications (Oguz et al., 2018). As another protein packed member of the legume family, soybean was also evaluated for electrospinning method. The drawbacks of protein utilization in electrospinning, including brittleness and poor barrier properties, were outcome by the addition of HPMC and poly (lactic acid) (PLA). As a result of mixing of soy protein with biocompatible and biodegradable HPMC in the electrospinning solution and combining the produced films with PLA sheets as a bilayer structure, nanofiber films were obtained with enhanced thermal and physical properties (Aydogdu, Yildiz, Ayhan, et al., 2019). In another study, rice flour-based electrospun nanofibers could be produced by polyvinyl alcohol (PVA) addition since fabrication of nanofibers solely from rice was challenging due to its high starch content resulting in high porosity, swelling and poor processability (Woranuch et al., 2017). PVA and rice flour interaction through hydrogen bonding allowed the formation of homogeneous nanofiber with high thermal stability and good morphology to occur. Some plant flours exhibit other beneficial inherent properties which would make them valuable candidates for different nanofiber application areas. For instance, *Colocasia esculenta* flour (CE) is a good collagen and carbohydrate source with essential amino acid content. Its utilization in a blend with antibacterial and biocompatible chitosan via electrospinning method produced nanofiber structure which could be used with wound dressing or drug delivery purpose due to porous structure of nanofibers having antimicrobial activity (Wardhani et al., 2019). As another plant-based polymer source, carob flour was used to prepare electrospun nanofiber films. High fiber and sugar content of carob flour was supported with rice starch and PEO to fabricate beadless and homogeneous fibers. It was shown that natural biopolymers obtained from carob and rice were beneficial sources for obtaining nanofiber films

with good physicochemical characteristics which can be further investigated as promising packaging material (Uygun et al., 2020). As a biodegradable and low-cost option, cereals, for example, oats, rye, wheat, and rice, are found abundantly due to being staple foods in several countries (Bach Knudsen et al., 2017). Among them, rye has the highest dietary fiber content (Andersson et al., 2009) and contains different types of bioactive compounds (Jonsson et al., 2018). In addition, high starch (66-73%) and pentosans (4-7%) content give rye flour a considerable water-binding capacity (Rosentrater & Evers, 2018). This feature of rye flour might be advantageous for the production of nanofibers since holding more water would change the viscosity of the polymer solution, which is found to be one of the critical parameters for the electrospinning process (Oguz et al., 2018). Also, rye grains contain many important phytochemical compounds such as free phenolic acids, folate, and tocols with high antioxidant activity (Kulichová et al., 2019). To the best of our knowledge, rye flour has never been used as nanofiber material.

1.1.4 Usage of Whey Protein in Electrospinning

Whey proteins are derived from milk and gained attention as a by-product of cheese processing. They are mainly composed of β-lactoglobulin, α-lactalbumin, which is a small globular protein formed of essential amino acids (Chatterton et al., 2006). Their functionalities along with being a valuable biopolymer source make whey proteins a great option for the food industry. These functionalities include solubility over wide pH range, water binding capacity, gelation and emulsification ability, foaming, and absorption (Sullivan et al., 2014). In addition, whey proteins exhibit antimicrobial, anticarcinogenic and antiviral properties (Chatterton et al., 2006). Therefore, they are highly preferred and utilized in several fields such as nutritional applications, food processing, delivery of pharmaceutical materials and bioactive compounds (Zhong et al., 2018). As being supplied in whey protein isolate (WPI) and whey protein concentrate (WPC) form with grades of 90% (w/w) and 80% (w/w), respectively, WPC could be a more cost-effective option with an economic

perspective (Pereira et al., 2017). One of the most recent trends for whey protein utilization is electrospinning technology. This novel method has been used to produce nano scale fibers from whey protein, which could be evaluated as packaging material, food coatings, drug delivery and release vehicle, enzyme immobilization, or synthetic meat (Drosou et al., 2018).

In recent years, environmental and economic concerns have been leading many researchers to study on natural biopolymer based nanofibers to decrease the dependency on non-biodegradable and non-renewable polymer sources (Torres-Giner, 2011). Depending on the processing technique and the types of polymer material used, biopolymer processing could be challenging due to many reasons. For electrospinning, properties of polymer solution and its response to the electric field is crucial to the fabricated nanofiber quality. Electrospinnability of whey protein solution is highly dependent on the solution properties since the state of whey protein could be affected by different factors such as pH, temperature, presence of other materials, or external forces (Zhong et al., 2018). According to the studies, production of nanofibers made only from aqueous solution of whey protein was not successful because of insufficient molecular entanglement or interactions (Sullivan et al., 2014). Even though wide range of pH treatment was used to unfold and denature the globular proteins, whey protein could not form a stable polymer jet (Vega-Lugo & Lim, 2012). In order to provide an enough molecular interaction, soluble and spinnable polymers was added to the whey protein solution in the studies. Polyethylene oxide (PEO) is one of the most preferred polymers to be used with whey protein since it is biocompatible, nontoxic and biodegradable. By acting as a carrying agent or a scaffold, PEO helped whey protein form noncovalent bonds and increase physical chain entanglement which would provide continuous fiber fabrication by electrospinning. Also, PEO addition prevented whey protein agglomeration and provided sufficient viscosity levels to the solutions that played an important role in electrospinnability of the samples (Zhong et al., 2018). In another study, electrospun nanofibers made of whey protein and PEO were found to be thermally stable and able to keep its fiber morphology at temperature levels higher

than melting point of PEO (Sullivan et al., 2014). To benefit from remarkable gas barrier properties of protein films and to overcome their weak water vapor barrier characteristics, pullulan was used as a biocompatible and spinnable polysaccharide source to be blended with whey protein (Drosou et al., 2018). In that study, pullulan and whey protein electrospun nanofibers were successfully obtained and an increase in the intermolecular interactions between them was observed by FTIR spectroscopy. The results of different works in the literature showed that whey protein could be suggested as a promising biopolymer for electrospun nanofibers applications.

1.1.5 Grape Seed Extract

Plant-derived extracts have been used in various fields because of their rich antioxidant and antimicrobial content. Especially phenolic compounds obtained from natural sources have gained attention due to their beneficial features such as antimicrobial, antioxidant, and anti-inflammatory effects (Locilento et al., 2019). Grape seed extract (GSE) is an important example for them since it is a waste byproduct of wine and juice production industry which makes grape seed is highly preferable as a phenolic and antioxidant source with low cost and high sustainability. The composition of GSE includes high amount of catechin, epicatechin, gallocatechin, epigallocatechin, gallic acid, polymeric and oligomeric procyanidins which inhibit oxidation and bacterial growth by their high antioxidant and antimicrobial capacities (Gibis et al., 2012; Mandic et al., 2008). Therefore, GSE has a wide range of application areas studied in the literature. For example, due to its high proanthocyanidins content, which is a natural dentin modifier, grape seed extract was encapsulated into polylactide polymeric microencapsules to be used in dental resin as a restoration material. By using double emulsion and solvent evaporation, microencapsulation of GSE was successfully carried out and preservation of GSE was observed with time dependent release (Bedran-russo & White, 2017). As another encapsulation technique, high pressure homogenization was used to incorporate polyphenolic GSE into liposomes which were found to be

oxidatively stable compared to liposomes without GSE (Gibis et al., 2012). By this way, GSE could be used in the food products to improve nutrient quality without getting into interactions with other components. It was shown that GSE incorporated edible coating produced from chitosan and gelatin was an effective way to preserve fresh pork (Xiong et al., 2020). In that study, GSE was found to extend shelf life of the pork by preventing protein and lipid oxidation as well as inhibiting microbial activity by its antioxidant and antimicrobial properties. In order to eliminate some undesired characteristics of GSE while utilizing it in the food products such as bitterness, astringency or polymerization at high temperature, it was encapsulated into whey protein concentrate/maltodextrin/gum arabic blend by ultrasonification method. Thus, microcapsules with low release rate and high encapsulation efficiency were obtained (Yadav et al., 2020).

As the most recent encapsulation technology, electrospinning has been studied to coat bioactive compounds with nanostructures made from polymer or biopolymer materials. Due to its favorable conditions for highly sensitive bioactive materials, such as ambient processing temperature or high surface to volume ratio which slow down the release rate of the material, electrospinning has been used for encapsulation of several substances into polymer matrix. Recently, grape seed extract was nano-encapsulated into different materials. It was shown that the type of the polymer used in electrospinning had different effects on antioxidant properties of GSE. While GSE embedded gelatin based electrospun nanofibers showed decreased total phenolic content and antioxidant activity, these values were almost unchanged for the GSE added nanofibers obtained from polyvinyl alcohol (Faki et al., 2019). Since GSE is much more effective than vitamin C or E as a free radical scavenging agent, it has been evaluated in the nanofibrous materials used in biomedical fields such as wound dressing or tissue regeneration. For example, nanofibers from silk fibroin and polyethylene oxide was loaded with GSE by electrospinning and it showed remarkable cytocompatibility by inhibiting oxidative stress on skin cells (Lin et al., 2016). Another successful application demonstrated that incorporating GSE into polylactic acid and polyethylene mats resulted in highly biocompatible electrospun

nanofibers with nearly 90% encapsulation efficiency (Locilento et al., 2019). The developed nanofibrous material were found to be very promising for drug delivery and wound dressing applications.

1.2 Microwave Heating

Microwaves are defined as the electromagnetic waves having frequency range of 0.3 and 300 GHz (Gustaw & Mleko, 2007). Other than communication and medical applications, it is used as a heating method for especially food materials since 1940s (Romano & Apicella, 2015). Microwave heating mechanism can be explained as the penetration of energy, which is generated by a magnetron placed in the oven, to the bulk food material and heating it volumetrically. The principle behind this heating mechanism involves the rotation of dipole and the polarization of ionic molecules (Verma et al., 2020). When dielectric materials are exposed to the alternating electromagnetic field, dipole moments are created which make the molecules rotate and generate heat due to molecular friction. In a similar way, ionic polarization results in collisions between ions having accelerating movements that causes heat generation in the food (Gustaw & Mleko, 2007). Although there are several factors affecting the microwave processing such as frequency, thermal properties of food or temperature, the outcome of the microwave heating process is largely dependent on the dielectric properties of materials, which are dielectric constant and dielectric loss factor. These properties are influenced by different characteristics of food like composition, density, or temperature. For example, having water as a major constituent, most food products can be heated efficiently by microwave due to polarity of water molecules since water provides greater dielectric loss (Verma et al., 2020).

Microwave heating has many advantages over conventional heating, especially on time and energy saving, selective heating, and process control (Sumnu, 2001). Since the heating occurs throughout the object, unlike the conventional heating where heat is conducted from surface to interior, much shorter processing times are required by

microwave heating (Verma et al., 2020). Also, it is an advantageous heating method in terms of energy as considering that electromagnetic energy is mostly converted into heat (Oliveira & Franca, 2002).

In addition to its superiority over conventional heating, microwave heating has appreciable effects on the structural and functional characteristics of both carbohydrates and proteins. It was previously studied that microwave treatment increased the denaturation proportion of whey proteins in the milk samples compared to conventionally heated ones (Villamiel et al., 1996). The concentration of heat at the center of the food, consequently the nonuniform distribution of temperature was stated as the reason for the acceleration of this chemical reaction. Additionally, stronger and fine-stranded whey protein isolate gels were formed with microwave application at pH values far from isoelectric point of the whey protein isolate compared to the ones conventionally heated for the same time interval (Gustaw & Mleko, 2007). It was observed that for short processing time, microwave heating was able to result in protein denaturation, unlike the conventional heating. As well as protein denaturation, starch gelatinization is also largely affected by microwave heating mechanism, Since microwave causes internal heating, amylose from lotus seed starch was effectively leached and built complexes with green tea polyphenols, which could not be succeeded by ultrasonic treatment (Zhao et al., 2019). The shape distortion of the starch granules was increased as the microwave power increased due to rapid built-up pressure in the granules. Round shape of starch molecules turned into distorted and irregular forms instead of swelling because of rapid granule expansion during microwave treatment which is followed by leaching out of polymers. Stable hydrogen bonds were formed because of fast migration of amylose and polyphenols and their tendency to interact with each other during microwave processing. By this way, enhanced interaction between different materials, such as starch and polyphenols, could be formed in a short time by microwave treatment. In another study, the improving effects of microwave heating on electrospinning of carob flour and rice starch were shown. It was reported that microwave preheated solutions gave films made of more homogeneous and bead-free nanofibers

comparing to conventionally heated samples. The mechanism behind that was explained by the increased internal pressure during microwave heating which is most likely to have a positive effect on releasing amino groups from the polymer solution. The rise in free amino groups led to more viscous solutions, consequently more electrospinnability and homogeneous nanofibers were obtained (Uygun et al., 2020). As considering the importance of viscosity parameter in electrospinning process, microwave heating could play a significant role as a controllable and fast pretreatment method for electrospinning solutions.

1.3 Objective of the Study

Electrospinning and electrospun nanofibers have been studied and become the topic of many research in recent years due to offering several advantages as a novel technology. Especially in active packaging area, electrospun nanofiber film is considered as a promising candidate for replacement of traditional petroleum-based materials since it is possible to produce environmentally friendly biopolymer-based films with extraordinary physical and thermal properties by electrospinning technique. In addition to its cost-effectiveness and simple operating advantages, electrospun nanofibers can be used for encapsulation of sensitive bioactive compounds because of ambient processing temperature and high surface area to volume ratio.

One of the objectives of this study was to produce nanofiber films from biodegradable materials. Based on a preliminary literature research, it was found that there could be some disadvantages of using solely polysaccharides or proteins on film properties and they could be overcome by combining them in the electrospinning solution. For this purpose, rye flour and whey protein were chosen to be used as biopolymer. To obtain more homogeneous structure and prevent bead formation, PEO was added to the solution mix as a biodegradable and biocompatible source. High starch and pentosan content and low cost make rye flour is a good option for sustainable film material. To the best of our knowledge, rye flour has never

been used as nanofiber material. According to previous studies, whey protein brings physical improvements to the electrospun nanofibers.

Even though encapsulation of bioactive substances into biopolymer matrix by electrospinning has been gaining attention, there is still a literature gap on the usage of natural and sustainable bioactive compounds in the active packaging research. In the literature, there is no study on encapsulation of grape seed extract by electrospinning method which could be used as active packaging material as inhibiting the oxidation of the food product by its antioxidant property.

To enhance the film properties, preheating was applied to the electrospinning solutions. Microwave heating is known with its short processing time, less energy consumption and process controllability.

However, information on microwave pretreatment of flour- and protein-based nanofiber films obtained via electrospinning is very limited. Also, to the best of our knowledge, there is no study on the effects of microwave pre-heating on electrospinning of nanofibers containing phenolic compounds. Thus, the major aim of this study was to fabricate grape seed extract–loaded nanofibers from rye flour–whey protein biopolymers by electrospinning. In addition, the effects of microwave heating on the physical properties of the solutions and nanofibers were also examined.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Rye flour was purchased from Smart Chemical Trading Co. Inc. (Izmir, Turkey) and whey protein concentrate (WPC) (80% protein on a dry weight basis) was supplied from Proteinocean Gıda Co. Inc. (Ankara, Turkey). Polyethylene oxide (PEO) having a molecular weight of 900 kDa was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Polyethylene sorbitan monooleate (Tween 80) (density: 1.064 g/m^3, viscosity: 400-620 cps at 25°C) was provided from Merck (Darmstadt, Germany). Grape seed extract was bought from Arpas Arifoglu Trading Co. Inc. (Istanbul, Turkey).

2.2 Solution Preparation

Solutions of 2% (w/v) were obtained by dissolving PEO in distilled water at room temperature and at 400 rpm for overnight using a magnetic stirrer (Daihan Scientific, Seoul, Korea). Rye flour (4% and 6% (w/v)) and whey protein (4% (w/v)) were added to PEO solutions at different concentrations and mixed with high-speed homogenizer (IKA T25 Digital Ultra Turrax, Staufen, Germany) at 10000 rpm for 3.5 min for complete homogenization. Then, 8M NaOH solution was added to the solutions to adjust pH to 12 by using a pH meter (SG2 SevenGo pH, Mettler Toledo, USA). Conventionally heated solutions were prepared by heating up to 80°C in a water bath (GFL, Type 1086, Germany). Then, heating was continued on magnetically stirred hot plates at 80°C and 750 rpm for 2 h. Others were heated by using a microwave oven (Kenwood MW-767, Hampshire, UK) at 450 W for 2.5 min

to reach 80°C. After heating was complete, solutions were cooled down to room temperature. Tween 80, a non-ionic surfactant, at a concentration of 2% (w/v) was added to the solutions afterwards to decrease the surface tension so that surface tension of the polymer solution could overcome by the electric field to obtain nanofibers (Vega-Lugo & Lim, 2012).

2.3 Solution Properties

2.3.1 Rheological Properties

The rheological properties of the solutions were measured by a controlled strain rheometer (Kinexus, Malvern, UK) equipped with cone and plate geometry (4° cone, 40 mm diameter and 1µm gap). The shear stress data were obtained at a controlled shear rate between 0.1 and 100 s^{-1} at 25°C. Shear stress data were recorded with respect to shear rate and measurements were conducted in duplicates. The collected data was fitted to Power Law model (Eq. (1)).

$$\tau = K\,(\dot{\gamma})^{n} \tag{1}$$

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

2.3.2 Electrical Conductivity

Electrical conductivity of the solutions was measured at 25 ± 1°C using conductivity meter (InoLab®Cond 7110, Wissenschaftlich-Technische Werkstatten GmbH, Wheilheim, Germany) in duplicates.

2.3.3 Total Phenolic Content (TPC) of Solutions

TPC of the electrospinning solutions with and without GSE addition were determined by the modified Folin-Ciocalteau method (Luca et al., 2013). Ethanol solution (70% (v/v)) was used to dilute the sample solutions. Folin-Ciocalteau reagent (0.2 N) of 2.5 mL was added to 0.5 mL of the sample. After storing the vortexed mixture for 5 min in a dark place, 2 mL of 75 g/L sodium carbonate solution was added. The final mixture was kept in the dark for 2 h. By using a spectrophotometer (UV 2450, Columbia, USA), absorbance of the solutions was recorded at 760 nm. Gallic acid was used to create a calibration curve. TPC values were presented as milligrams of gallic acid equivalent (GAE) per gram of dry weight of sample.

2.4 Electrospinning Process

The polymer solutions were electrospun by using Nano-Web 103 (Mersin, Turkey). Each solution was loaded into a 5 mL syringe having 11.53 mm inner diameter and a 21-gauge metallic needle. The needle was connected to the positively charged electrode after the syringe was mounted on the syringe pump horizontally. Fibers were collected onto the aluminum foil covered metal collector, which was connected to the negatively charged electrode of the high voltage power supply. The collector was placed 30 cm away from the needle tip. The flow rate of the solution and the voltage were kept constant at 0.6 mL/h and 12 kV, respectively. Experiments were carried out at 25 ± 1°C and 25–35% relative humidity. Films were symbolized according to heat treatment, rye flour and GSE concentration and the nomenclature is given in Table 2.1. For example, M4R20 denotes film with microwave treatment, 4% (w/v) rye flour and 20% GSE.

Table 2.1 Nomenclature of the nanofiber sample

Nomenclature	Heating method	PEO (w/v %)	Rye flour (w/v %)	WPC (w/v %)	GSE (w/w %)
C4R0	Conventional	2	4	4	0
C6R0	Conventional	2	6	4	0
M4R0	Microwave	2	4	4	0
M6R0	Microwave	2	6	4	0
M4R20	Microwave	2	4	4	20
M6R20	Microwave	2	6	4	20

2.5 Characterization of Films

2.5.1 Morphological Analysis

Fiber morphology of the RF/WPC/PEO films was observed from the images taken by using scanning electron microscope (SEM) (Nova NanoSEM 430, Oregon, USA). Approximately 100 fibers on the SEM images of each sample were selected randomly to determine the average diameter by using Image J analysis software (Maryland, USA).

2.5.2 Water Vapor Permeability

Determination of water vapor permeability (WVP) of nanofiber films was made according to a modified version of ASTM E-96 standard method (Bertuzzi et al., 2007). The measurement cups having diameter of 0.04 m were filled with 30 mL of water. The films were sealed to the cups by using screws and any leakage was prevented by rubber joint. Then, the cups were placed and stored in the desiccator equipped with silica gels. Until steady state was reached, each cup was weighed with 2 h intervals. From the plot of the weight loss versus time, the slope was used to determine the water vapor transmission rate for each sample (WVTR; g m^{-2} s^{-1}. Then, water vapor permeability was calculated by using the equation below;

$$WVP = \frac{(WVTR)\times\Delta x}{(P_1 - P_2)} \quad (2)$$

where, P_1 is the partial pressure of water vapor at the inner surface of the film (Pa) and P_2 is the partial pressure of the water vapor at the outer surface of the film (Pa). Δx is the thickness of the film (m). During the measurement, Relative humidity (RH) and temperature inside the desiccator were recorded using a digital hydrometer (ThermoPro TP50, USA). RH inside the cup was assumed as 100%. Measurements were performed in duplicates.

2.5.3 X-Ray Diffraction

X-ray diffractometry (XRD) for the films was obtained by using Ultima IV X-ray diffractometer (Rikagu, Japan). Operation conditions were determined as voltage of 40 kV and current of 30 mA under Cu source. 2theta range for the measurements was 5-70º for all samples with a scanning rate of 2º/min.

2.5.4 Differential Scanning Calorimetry (DSC)

Differential scanning calorimeter (Pyris 6 DSC, PerkinElmer, USA) was used to determine thermal analysis of films. Approximately 5 mg of sample from each film was placed in an aluminum pan, then sealed. An empty pan was used as reference. Each pan was cooled down to -60°C/min first and then heated up to 100°C with a rate of 10°C/min. The DSC thermograms were used to determine glass transition temperature, melting temperature, and melting enthalpy of each sample. The DSC measurements were conducted in duplicates.

2.5.5 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis of the samples was conducted by using Pyris STA 6000 simultaneous thermal analyzer (PerkinElmer, USA). Approximately 5 mg nanofiber, rye flour, whey protein, and PEO were heated from room temperature to 500°C with a heating rate of 10°C/min with nitrogen. Measurements were performed in duplicates.

2.5.6 Fourier-transform Infrared (FTIR) Analysis

FTIR analyses of the electrospun nanofibers, rye flour, WPC, and PEO were performed by using FTIR spectrophotometer (Pyris STA 6000, PerkinElmer, USA)

which characterizes gas evolved from TGA. Data were recorded in the range of 4000-500 cm^{-1} at 2 cm^{-1} resolution.

2.5.7 Total Phenolic Content (TPC) of Electrospun Fibers

TPC of electrospun nanofibers were found by using the modified Folin-Ciocalteau method (Luca et al., 2013). The same procedure with the TPC determination of electrospinning solution was applied. Instead of sample solutions, 0.1 g nanofiber was dissolved in 70% (v/v) ethanol solution in the first place. Absorption of final solutions were measured by using a spectrophotometer (UV 2450, Shimadzu, USA). The measurements were done in duplicates. The loading efficiency (LE) of GSE into nanofibers was found by using the formula below:

$$LE\ (\%) = \frac{TPC\ of\ GSE\ loaded\ nanofibers}{TPC\ of\ GSE\ loaded\ solutions} \times 100 \quad (3)$$

2.5.8 Antioxidant Activity of Electrospun Nanofibers

The antioxidant activity of GSE-loaded nanofibers was measured with a modified version of the method in which 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was used (Luca et al., 2013). Nanofiber film sample of 0.1 g was mixed with 2 mL of 70% (v/v) ethanol solution and waited for 2 h for complete dissolution. The extract was obtained by filtering through 0.45 µm filter. The filtered sample of 0.1 mL was added to 3.9 mL of 0.6 mM DPPH solution and kept in the dark for 1 h. The absorption (A_{sample}) was recorded at 517 nm by using a spectrophotometer (UV 2450, Shimadzu, USA). Control sample was prepared by mixing 0.1 mL of 70% (v/v) ethanol solution with 3.9 mL of 0.6 mM DPPH solution. The absorbance of the control ($A_{control}$) was measured at 517 nm. Methanol was used as blank. The measurements were carried out in duplicates. The antioxidant activity (%AA) of the fibers was calculated according to the Eq. (4).

$$AA\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (4)$$

2.6 Statistical Analysis

Statistical analysis was conducted by using Minitab software (Minitab Inc., State College, USA). Analysis of variance (ANOVA) was used to observe if there were any significant differences between treatments. Tukey's Multiple Comparison Test was performed for the data with significant differences ($p \leq 0.05$).

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Physical Properties of Solutions

3.1.1 Rheological Properties

Electrospinning process can only be successful in production of homogeneous and beadless nanofibers if elongation of the solution is enough to be extended by the electric field (Stijnman et al., 2011). Since the process and ambient parameters were kept constant during the experiment, rheological properties such as viscosity were expected to have an important effect on bead formation and diameter of the fibers. While too low viscosity restricts the elongation and continuous fiber formation, too high viscosity makes the ejection of the polymer solution difficult. Therefore, the optimum viscosity of the solution is required to be adjusted by changing the polymer and solvent type and concentration (Aydogdu, Sumnu, et al., 2019). Table 3.1 shows the consistency index (k), flow behavior index (n) and apparent viscosity of the solutions. All the electrospinning solutions obeyed the Power Law model and showed high coefficient of determination (r^2 = 0.999) values. Also, they have flow behavior index value (n) ranging between 0.776 and 0.953 which indicates shear-thinning property as being smaller than 1. Conventionally heated solutions, containing 2% (w/v) PEO, 4% (w/v) WPC, and 4% (w/v) and 6% (w/v) rye flour (C4R0 and C6R0), have lower k values and higher n values as compared to the microwave heated solutions with the same composition (M4R0 and M6R0). Moreover, they showed lower apparent viscosity at 60 s^{-1}. While conventionally heated samples resulted in nanofibers with beads, microwave treated ones formed homogeneous nanofiber without beads (Fig. 3.1). Similarly, in a previous studdy it was shown that carob flour-based electrospun nanofibers had higher viscosity and

more homogeneous nanofibers when pretreated by microwave heating as compared to the conventionally heated ones (Uygun et al., 2020). It was explained by the internal pressure formed by microwave heating which accelerated the protein unfolding and amino group releasing, therefore, the solution viscosity was increased. As the composition of the solutions containing whey protein is considered, it is possible to say that microwave heating could yield more impact in terms of viscosity. When the effects of rye flour concentrations were studied, it was seen that the increasing rye flour content resulted in higher viscosity for both conventionally and microwave heated samples. High starch content in rye flour makes the solution more viscous as starch granules are exposed to heat and swell during gelatinization process. Another factor that makes the difference between viscosity values was the addition of grape seed extract into the microwave heated solutions. Microwave heated solutions containing 2% (w/v) PEO, 4% (w/v) WPC, and 4% (w/v) and 6% (w/v) rye flour and 20% (w/w) GSE (M4R20 and M6R20) had higher viscosity than solutions with no GSE (M4R0 and M6R0) (Table 3.1). This result could be related to the polyphenol content of the grape seed extract which could contribute to the crosslinking and entanglement of polymer chains. In another study, it was found that addition of tea polyphenols increased the solution viscosity where the pullulan and carboxymethylcellulose were used as the electrospinning solution materials (Shao et al., 2018).

Table 3.1 Rheological properties, electrical conductivity values, and average diameter of fibers

Sample	k (Pa s^n)	N	Apparent viscosity at 60 s^{-1} (Pa s^n)	Electrical conductivity	Average fiber diameter (nm)
C4R0	0.331 ± 0.002^{d}	0.953 ± 0.005^{a}	0.262 ± 0.002^{c}	3.97 ± 0.01^{a}	295 ± 58^{c}
C6R0	0.592 ± 0.050^{cd}	0.925 ± 0.004^{ab}	0.401 ± 0.029^{b}	3.83 ± 0.06^{ab}	329 ± 74^{ab}
M4R0	0.618 ± 0.052^{c}	0.884 ± 0.035^{ab}	0.379 ± 0.008^{b}	3.74 ± 0.04^{b}	310 ± 52^{bc}
M6R0	1.152 ± 0.121^{b}	0.852 ± 0.011^{bc}	0.577 ± 0.037^{a}	3.65 ± 0.03^{b}	338 ± 62^{a}
M4R20	1.421 ± 0.063^{a}	0.799 ± 0.025^{cd}	0.569 ± 0.022^{a}	3.44 ± 0.03^{c}	302 ± 45^{c}
M6R20	1.601 ± 0.058^{a}	0.776 ± 0.011^{d}	0.617 ± 0.003^{a}	3.18 ± 0.07^{d}	327 ± 60^{ab}

*Columns with different lowercase letters are significantly different ($p \leq 0.05$).

3.1.2 Electrical Conductivity

Electrical conductivity is another important parameter since electrostatic charges are required in the electrospinning solution so that fiber from the syringe would be transferred through the electric field and collected on the plate. Conductivity value of the solution should be high enough to form a sufficient elongation, which helps to fabricate uniform nanofiber. On the other hand, too high conductivity would result in unstable jet formation that cannot reach up to collector plate (Seethu et al., 2020). Electrical conductivity values of solutions were displayed in Table 3.1. It can be observed that conductivity values of solutions containing 6% (w/v) rye flour were lower than those with 4% (w/v). This could be related to increase in polymer interaction in the solutions with more biopolymer content. Similarly, lower conductivity results were obtained when sugar concentration (Luo et al., 2012) and pullulan content (Drosou et al., 2018) increased in electrospinning solutions. The reason for lower electrical conductivity of microwave treated solutions might be linked to intermolecular interactions that increased with more unfolded proteins coming from WPC since microwave heating could promote the release of free amino groups by internal heating principle (Uygun et al., 2020). Moreover, GSE incorporation to the solutions reduced conductivity values. It was previously studied that polyphenol addition could decrease electrical conductivity, which was associated with increase in molecular entanglement in polymer solution (Shao et al., 2018). As being another decisive factor on fiber morphology, electrical conductivity showed a negative correlation with fiber diameter. Larger diameter values were obtained when solution had higher viscosity and lower conductivity combination for electrospinning process (Table 3.1). Also, microwave heating had a contribution to the increase in fiber diameter by resulting in solutions with higher viscosity and decreased conductivity as compared to conventionally heated samples.

3.2 Characterization of Electrospun Nanofibers

3.2.1 Fiber Morphology

The fiber formation and film properties were directly affected by solution properties, ambient conditions and process parameters (Liu et al., 2017). In this study, the impact of solution properties was investigated by keeping the ambient conditions and process parameters constant. The morphology of the electrospun nanofibers was examined to evaluate the effects of heating method, rye flour concentration and antioxidant material addition, which also played a significant role on the rheological properties and the electrical conductivity of solutions. Figure 3.1 display the SEM images and the size distribution of the nanofibers. Conventionally heated solutions yielded a few bead formations on the fibers. As explained previously, microwave heating could promote the unfolding of proteins, which increases the solution viscosity and decreases electrical conductivity. High electrical conductivity of conventionally heated solutions might be exposed to higher attractive forces on the way to the collector, which forms faster travel of polymer jet. Thus, there is not enough time for solvent to evaporate, and beads are formed due to sticky nanofibers (Aydogdu, Yildiz, Aydogdu, et al., 2019). Similarly, electrospun fibers from flaxseed mucilage having larger diameter were obtained when electrical conductivity of solutions were lower (Hadad & Goli, 2019). Table 3.1 shows that average fiber diameter of the nanofibers varied between 295 and 338 nm. Solutions with higher viscosity and lower electrical conductivity yielded fibers with larger diameter. When rye flour concentration and GSE addition were considered, it was shown that both factors had a positive effect on the solution viscosity and their nanofibers had larger diameters. The positive correlation between viscosity and fiber diameter were studied in a different research before. Diameter of electrospun zein fibers were shown to increase as consistency index of solution increased due to enhanced molecular entanglement in the solution (Moomand & Lim, 2015). Likewise, the increasing viscosity of amaranth-pullulan solutions with increasing amaranth content

resulted in electrospun nanofibers with larger diameter (Blanco-Padilla et al., 2015). Another study, in which lentil flour and hydroxypropyl methylcellulose were used for electrospinning, indicated that fiber diameter was higher when viscosity of the solution was increased by higher lentil flour concentration (Tam et al., 2017).

According to the morphology results interpreted from SEM images, it was determined that microwave heated solutions yielded more homogeneous and bead free nanofibers which were decided to be more successful. Therefore, microwave was chosen as the solution heating method rather than conventional method for the following analyses and characterizations of the study including GSE encapsulation.

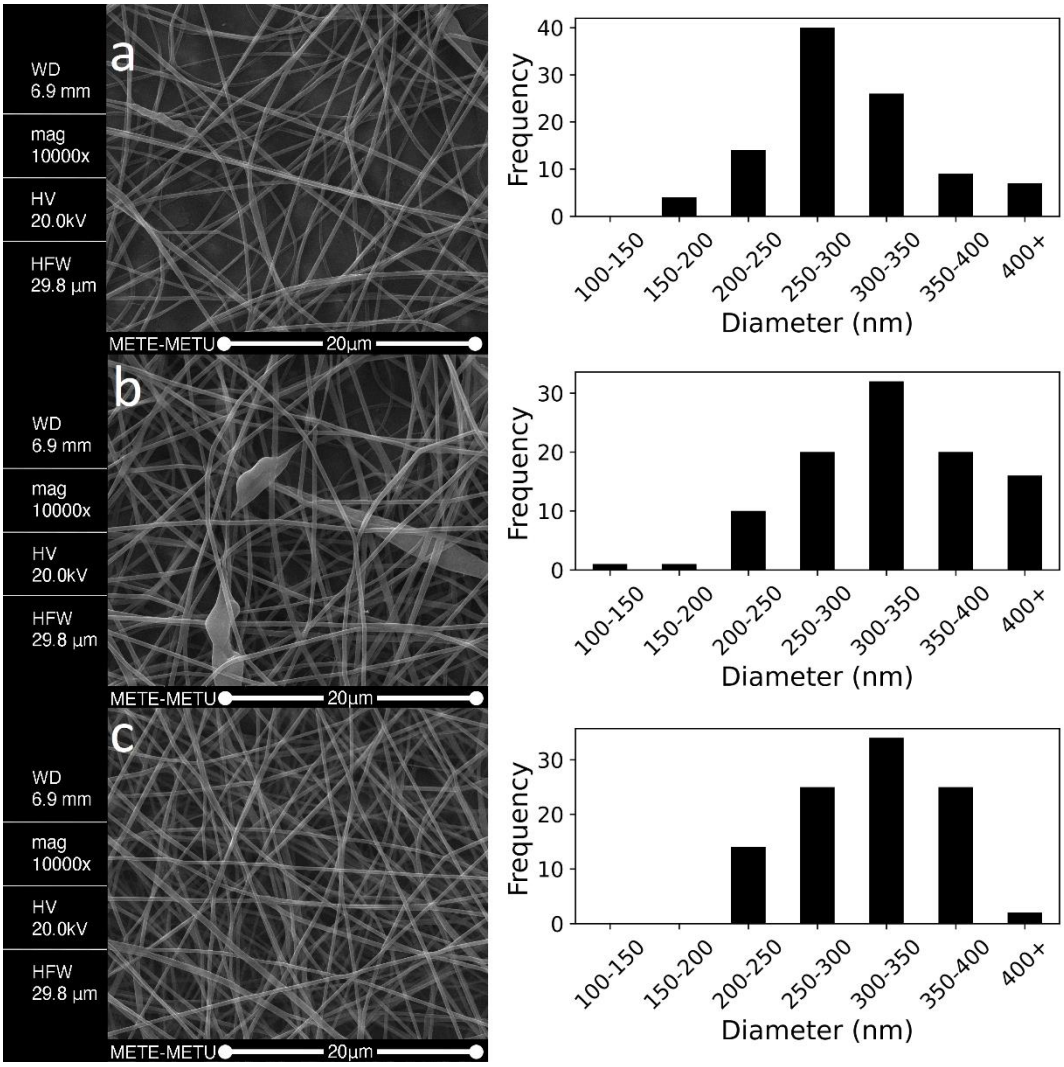

Figure 3.1 SEM images and fiber diameter distributions of the nanofibers: (a) C4R0, (b) C6R0, (c) M4R0, (d) M4R20, (e) M6R0, (f) M6R20

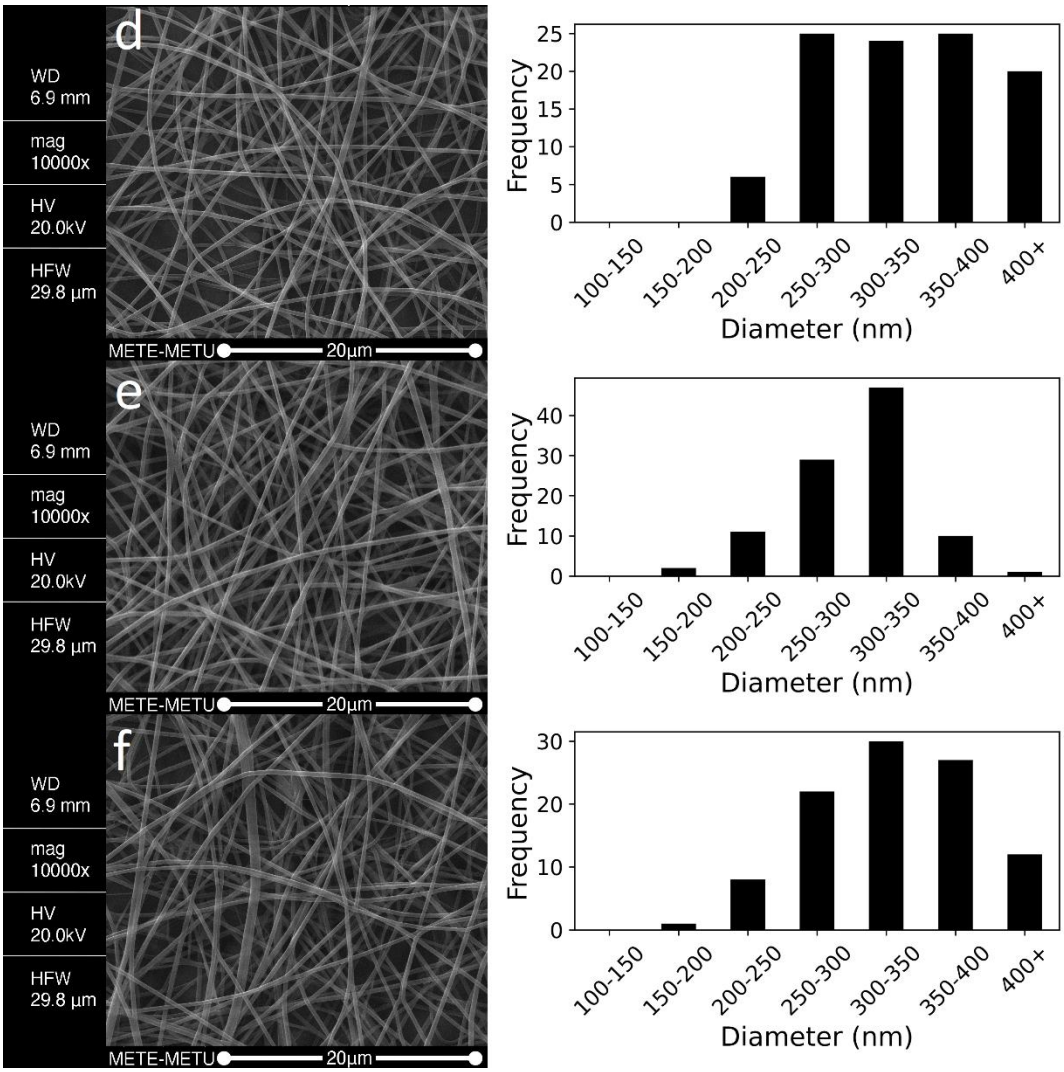

Figure 3.1. (continued)

3.2.2 Water Vapor Permeability (WVP)

Since films separate two environments from each other and control the moisture transfer between them, water vapor permeability is a significant characteristic. Particularly, the film with less WVP is more preferable as a packaging material (Chinma et al., 2012). Table 3.2 shows the WVP values of the electrospun nanofibers which were ranged between 1.09×10^{-10} g m^{-2} s^{-1} and 1.94×10^{-10} g m^{-2} s^{-1}. Increasing rye flour concentration did not have a significant effect on WVP. The effect of antioxidant incorporation on permeability could be observed through the comparison

of WVP values of GSE-loaded and not GSE-loaded samples as keeping the polymer content constant (Table 3.2). The statistical analysis showed that barrier property of the films was significantly different when GSE was added to the samples. WVP of M4R0 film increased from 1. × 10^{-10} g m^{-2} s^{-1} to 1.94 × 10^{-10} g m^{-2} s^{-1} when GSE was incorporated into it. Similarly, WVP of M6R0 film was lower than WVP of M6R20 film. The reason behind that was explained in the study where GSE-loaded chitosan films had higher WVP values than of chitosan films without GSE by hydrophilic nature of GSE (Rubilar et al., 2013). The presence of hydrophilic GSE might let water molecules form hydrogen bonds more which causes a rise in WVP. Moreover, GSE might reduce crystallinity of the films, hence less ordered fibers would probably result in higher permeability. Similarly, it was reported that GSE caused a rise in WVP which could be again related to the hydrophilicity of GSE (Sogut & Seydim, 2018).

Table 3.2 Water vapor permeability and thermal properties of nanofibers

Sample	WVP × 10^{-10} (g s^{-1} m^{-1} Pa^{-1})	T_g (°C)	T_m (°C)	ΔH_m (J g^{-1})
M4R0	1.22 ± 0.06^{b}	-17.76 ± 1.15^{a}	55.40 ± 0.72^{a}	24.95 ± 2.05^{a}
M4R20	1.94 ± 0.07^{a}	-15.63 ± 0.57^{b}	55.87 ± 0.73^{a}	24.17 ± 5.07^{a}
M6R0	1.09 ± 0.09^{b}	-17.66 ± 0.26^{a}	54.17 ± 0.04^{a}	24.11 ± 2.55^{a}
M6R20	1.83 ± 0.03^{a}	-15.27 ± 1.18^{b}	55.58 ± 0.52^{a}	21.35 ± 1.65^{a}

*Columns with different lowercase letters are significantly different ($p \leq 0.05$).

3.2.3 Differential Scanning Calorimetry (DSC) Analysis

The calorimetrically detectable transitions of nanofibers were obtained by DSC. Those including the glass transition temperature (T_g), the melting temperature (T_m), and enthalpy change during melting (ΔH_m) are shown in Table 3.2. The presence of both T_m and T_g for all samples indicated that nanofibers had semi crystalline structure since T_g represented a transition from glassy state to rubbery state. The melting temperature values of nanofibers varying around 55℃ were found to be not significantly different from each other and lower than Tm of PEO which was 71.5℃ according to previous studies (Uygun et al., 2020). Such a decrease have been reported for electrospun nanofibers in which carob flour (Uygun et al., 2020), chitosan (Kuntzler et al., 2018), and soy protein isolate (Xu et al., 2012) were used as polymers along with PEO. The reason behind the T_m depression of PEO might be explained by disruption of crystalline structure of PEO as it was forming strong interactions with the polymers used, which was rye flour and whey protein in this study. The, glass transition temperature of nanofibers increased with the incorporation of GSE since there was a reduction in chain mobility with formation of intermolecular bonds (Wen, Zhu, Feng, et al., 2016). Also, as it was argued previously, hydrophilic nature of GSE might have a decreasing impact on the crystalline structure of the films. Melting enthalpy values were not significantly different from each other.

3.2.4 X-Ray Diffraction (XRD)

According to the XRD result shown in Figure 3.3, all four samples showed similar diffraction pattern with three main peaks. Thus, it can be interpreted that the composite nanofiber samples are semi-crystalline materials both having amorphous and crystalline structures. All samples showed peaks at $2\theta = 19^o$ and $2\theta = 23^o$. Those peaks could be attributed to the crystallinity coming from PEO. In a previous study, pure PEO showed peaks at 19.2º and 23.3º in XRD analysis (Xu et al., 2012). In

between those peaks, another diffraction peak appeared at 2θ = 21.59º, 2θ = 20.8º, 2θ = 22.04º, and 2θ = 21.41º for M6R0, M6R20, M4R0, M4R20, respectively. Therefore, it can be stated that incorporation of GSE reduced the crystallinity of rye flour/WPC/PEO nanofibers since GSE might have an enhancing effect on the interaction of water molecules with polymer chains due to its hydrophilic character (Tavassoli-Kafrani et al., 2018). Similar result was found in the study where silk fibroin and PEO were used as electrospun nanofiber material and the introduction of GSE decreased the crystallinity of the nanofibers (Lin et al., 2016).

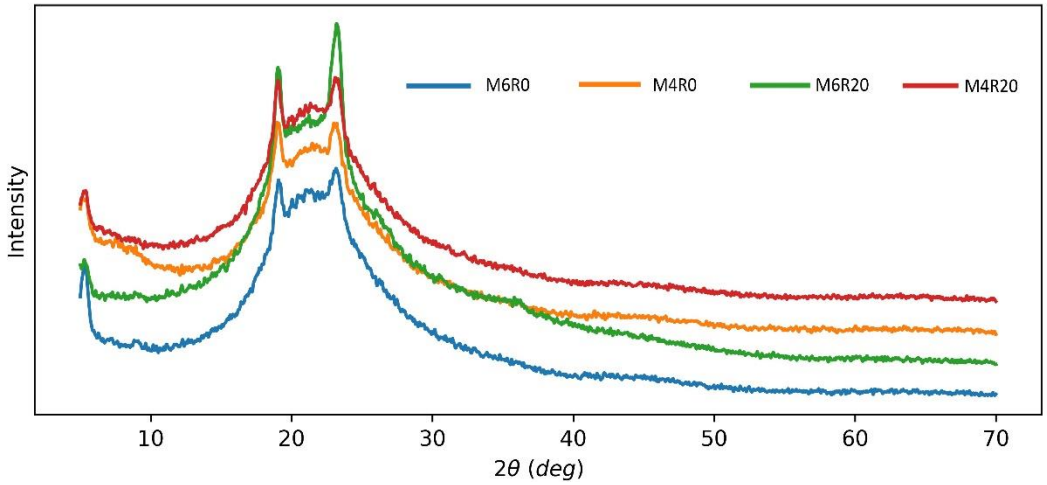

Figure 3.2 X-ray diffractogram of electrospun nanofibers

3.2.5 Thermogravimetric Analysis (TGA)

TGA thermograms in Figure 3.4 shows the weight change profile of the samples as a function of temperature. The TGA of all nanofibers have a minor initial weight loss between 30-100℃ because of vaporization of free water and two stage degradation. The first degradation was occurred between 200℃ and 300℃ which could be associated with polysaccharide degradation coming from rye flour content and whey protein decomposition. While the onset temperature of degradation (T_{onset}) of rye

flour was found near 275-280℃, T_{onset} of WPC was around 270℃. Similarly, in the study of pullulan-whey protein electrospun nanofibers, the first thermal degradation between 250-350℃ was attributed to the polysaccharide degradation (Drosou et al., 2018). The second degradation of nanofibers has T_{onset} around 400℃ which may represent the degradation of PEO since pure PEO showed one single stage degradation near 400℃. Similar result was obtained in the study of PEO-lentil flour electrospun nanofibers, the second T_{onset} was reported as 400℃ (Aydogdu, Yildiz, Aydogdu, et al., 2019). In the first stage degradation, a slight decrease in the weight loss for nanofibers containing GSE could be observed. As explained in the DSC results, it can be argued that GSE had an enhancing effect on intermolecular interactions and thermal stability was improved. As previously reported, TGA curve of GSE displayed two peaks between 450 and 505℃ which were not displayed for that temperature range on the TGA of nanofibers. The absence of thermal degradation peaks after 400℃ could be associated with encapsulation of GSE (Locilento et al., 2019).

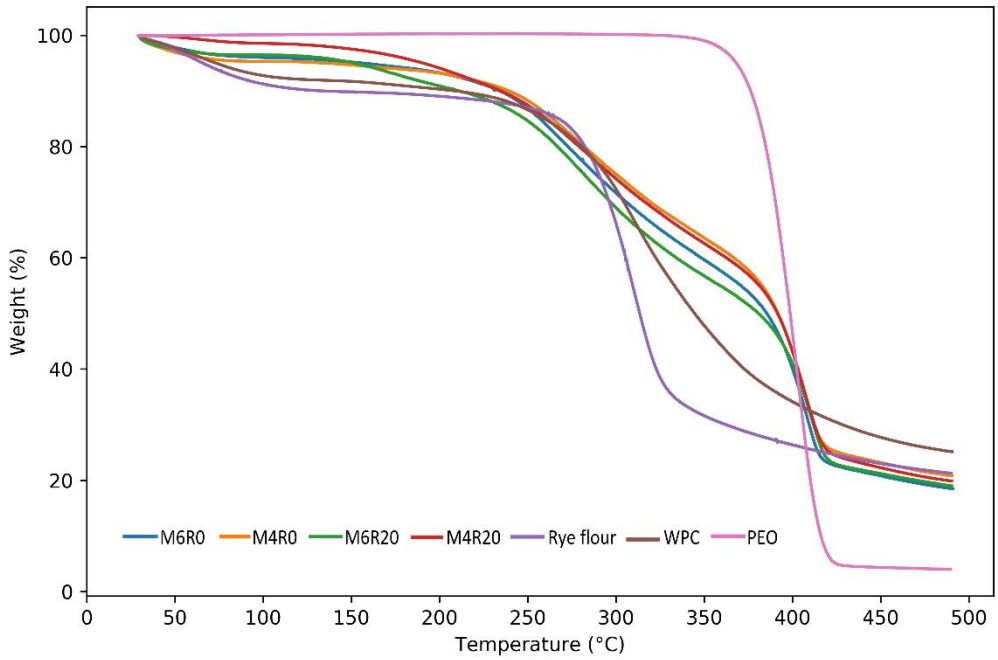

Figure 3.3 Thermogravimetric curves of electrospun nanofibers, rye flour, WPC, and PEO

3.2.6 FTIR Analysis

FTIR measurement is a useful analysis to obtain information on functional groups in the samples and their interaction between the constituents of each nanofiber by examining their characteristic peaks displayed on spectra of each sample. The FTIR spectra of electrospun nanofibers, PEO, rye flour, whey protein concentration was shown in Figure 3.5. Nanofibers had peaks located around 1100 cm^{-1}, which was related to the stretching vibrations occurred at the ether bond found on the backbone of the PEO chain (Vega-Lugo & Lim, 2012). The characteristic peak at near 2800 cm^{-1} originating from stretching of methylene group (CH_2) was also observed at PEO (Sullivan et al., 2014). These are the indications of the presence of PEO in nanofibers after the electrospinning process. The peaks located at 840-960 cm^{-1} band in both

nanofibers and rye flour were attributed to vibrations coming from C−O−C of α-1,4 glycosidic linkages (Kizil et al., 2002). Similar results were observed in which lentil flour (Aydogdu, Yildiz, Aydogdu, et al., 2019) and carob flour (Uygun et al., 2020) were used along with PEO in fabrication of electrospun nanofibers. Spectra for the nanofibers showed characteristic peak around at 1630 cm^{-1} which was associated to the Amide I region and was found in proteins. This peak was also seen at spectrum of WPC due to N−H scissoring. Similarly, in the studies where whey protein isolate and PEO were used for electrospinning process, peak at 1650 cm^{-1} was observed (Colín-Orozco et al., 2015; Sullivan et al., 2014). Differing from nanofibers without GSE addition, GSE-loaded nanofibers had broad new bands around 1350 and 3500 cm^{-1}. These are the characteristic absorption peaks of GSE positioning at 1000-1300 cm^{-1} and 3250-3300 cm^{-1} (Lin et al., 2016). Since GSE has a great amount of phenolic compounds including catechin, epicatechin, and gallic acid, the bands are typically observed at 3300 cm^{-1} and 1283 cm^{-1} due to stretching of different −OH groups and ester C−O stretching, respectively (Locilento et al., 2019). The shifting of the bands could be associated with indication of an interaction between GSE and polymer. These results demonstrate the successful incorporation of GSE into rye flour/WPC/PEO electrospun nanofibers.

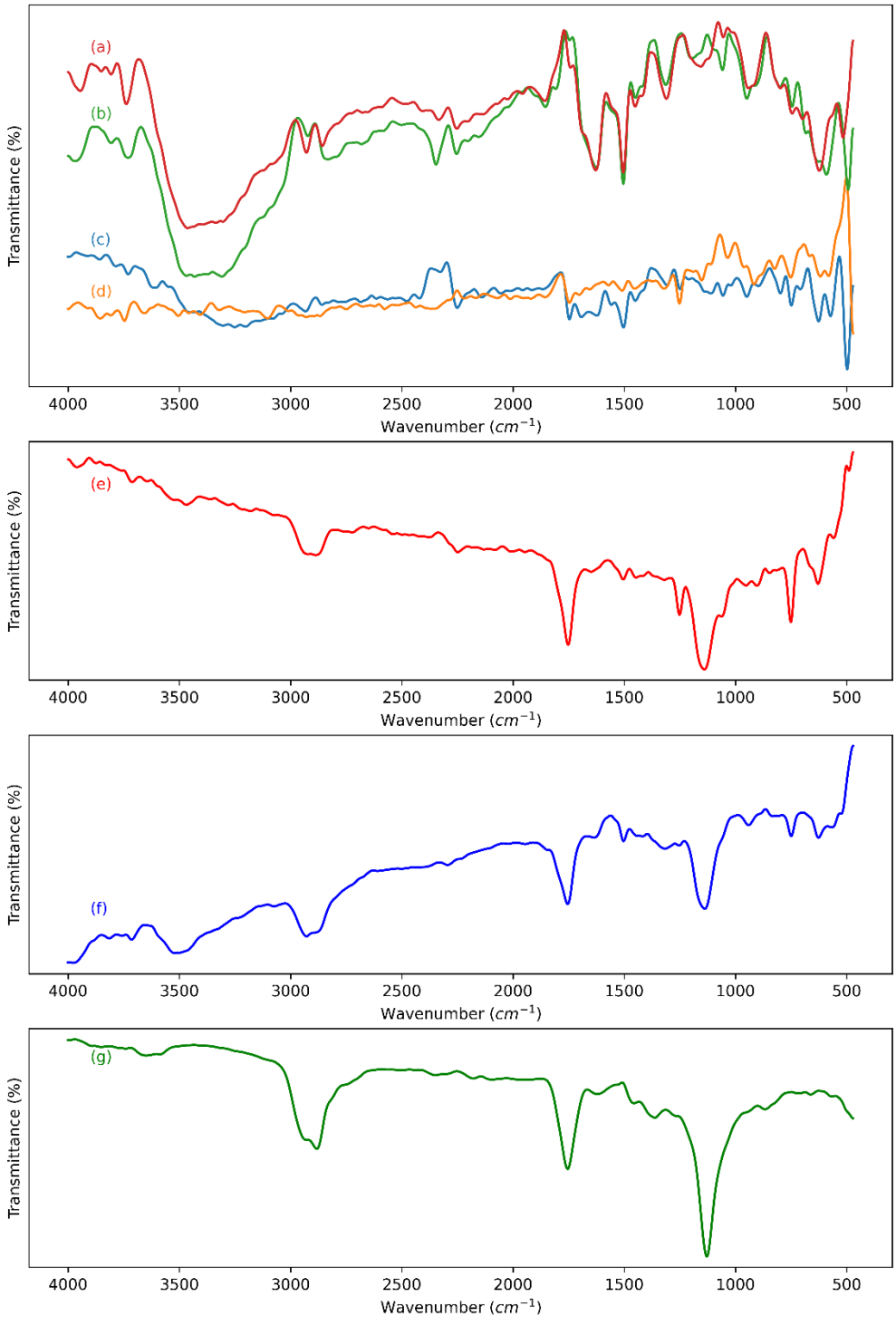

Figure 3.4 FTIR spectra of (a) M4R20, (b) M6R20, (c) M6R0, (d) M4R0, (e) Rye flour, (f) WPC, (g) PEO

3.2.7 Total Phenolic Content (TPC) and Antioxidant Activity of Fibers

Total phenolic content (TPC) of the electrospinning solutions with and without GSE were shown in Table 3.3. Before GSE addition, solutions with 4% (w/v) and 6% (w/v) rye flour had TPC values of 2.98 and 3.19 mg GAE/g dry matter, respectively (Table 3.3). It showed that there were phenolic compounds in the solution materials apart from GSE. Considering that TPC values increased with increasing rye flour content, it could be argued that source of the phenolic compounds was mostly rye flour. Previous studies showed that rye flour grains had TPC content varying between 2.61 mg and 3.37 mg GAE/g dry matter and t-ferulic acid was found to be the most abundant among other phenolic compound in rye grains (Kulichová et al., 2019). Since ferulic acid had thermal stability up to 245℃ (Fiddler et al., 1967) and had a resistance to high pH (Friedman & Jürgens, 2000), heat treatment and alkaline conditions of this study were not destructive for rye flour-sourced TPC in the solutions which did not contain GSE. Also, microwave treatment might have an increasing effect on TPC because such a food process could release phenolics bound in cell walls (Acosta-Estrada et al., 2014). GSE contains high amount of phenolic compounds including mainly catechin, epicatechin and gallic acid (Monagas et al., 2003). TPC of GSE-added solutions increased to 10.61 and 13.98 mg GAE/g dry matter for solutions with 4% (w/v) and 6% (w/v) rye flour, respectively. Despite the high phenolic content of GSE, TPC of the solutions were not elevated considerably. The degradation might be related to high pH of the solutions which was destructive to gallic acid (Aydogdu, Yildiz, Aydogdu, et al., 2019). However, epigallocatechin and epicatechin were found to be relatively stable to alkaline pH, which could be an explanation to the rise in TPC when GSE was added. Table 3.3 shows the loading efficiency and antioxidant activity of electrospun nanofibers. The loading efficiency of the nanofiber containing 6% (w/v) rye flour was higher than of the one containing 4% (w/v) rye flour. Similarly, it was found in the study of carvacrol encapsulation in starch and poly-ε-caprolactone by electrospinning, encapsulation efficiency was increased by increasing polymer concentration (Tampau et al., 2017). GSE has

significant antioxidant capacity due to its oligomeric proanthocyanidin content which prevents oxidization by providing electrons to the free radical (Huh et al., 2004). As displayed in Table 3.3, both of the nanofiber samples showed antioxidant activity around 40%. This showed that antioxidant property of the samples coming mainly from GSE was preserved in electrospun nanofibers. Similarly, in another study, GSE encapsulation to silk fibroin was performed by electrospinning which resulted in films with remarkable antioxidant capacity (Lin et al., 2016).

Table 3.3 Total phenolic content, loading efficiency, and antioxidant activity data for solutions and nanofibers

Sample	TPC of solutions (mg GAE/g dry matter)	TPC of nanofibers (mg GAE/g dry matter)	GSE loading efficiency (%)	AA (%)
M4R0	2.98 ± 0.47^{b}	-	-	-
M6R0	3.19 ± 0.52^{a}	-	-	-
M4R20	10.61 ± 0.73^{c}	5.74 ± 0.11^{b}	54.16 ± 1.14^{b}	41.62 ± 0.58^{a}
M6R20	13.98 ± 0.91^{d}	8.55 ± 0.52^{a}	61.15 ± 0.30^{a}	42.78 ± 0.76^{a}

*Columns with different lowercase letters are significantly different ($p \leq 0.05$).

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In this research, grape seed extract as an antioxidant compound was encapsulated into rye flour and whey protein based electrospun nanofibers successfully. Microwave heating pretreatment to solutions was found to be more effective than conventional heating in terms of obtaining homogeneous and beadless nanofibers with shorter processing time. The effect of GSE incorporation into rye flour and whey protein based electrospun nanofibers material was confirmed by chemical and thermal analyses. The increase in rye flour content resulted in larger fiber diameter. On the other hand, higher rye flour content had a positive impact on loading efficiency of grape seed extract. The addition of GSE provided an increase in fiber diameter due to improved molecular entanglement and intermolecular interactions which yielded in nanofibers with improved thermal stability. Thus, grape seed extract incorporated nanofibers produced in this study from rye flour and whey protein can be suggested as a promising material for biodegradable film with high antioxidant activity and enhanced thermal stability. In particular, using these nanofiber films with antioxidant capacity in combination with another packaging material to form a multilayered packaging can be suggested due to having weak mechanical properties.

Further studies are required to examine the other physical properties of nanofibers, including the release kinetics and stability of antioxidants to evaluate their potential use in active packaging and many other applications.

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APPENDICES

A. Statistical Analyses

Table A. 1 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for consistency index (k) values of solutions containing different amount of rye flour and GSE and heated by different methods

One-way ANOVA: k versus Solution

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Solution	6	C4R0, C6R0, M4R0, M4R20, M6R0, M6R20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Solution	5	2.61477	0.522953	115.56	0.000
Error	6	0.02715	0.004525		
Total	11	2.64192			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0672713	98.97%	98.12%	95.89%

Means

Solution	N	Mean	StDev	95% CI
C4R0	2	0.33120	0.00184	(0.21481, 0.44759)
C6R0	2	0.5921	0.0503	(0.4757, 0.7085)
M4R0	2	0.6180	0.0521	(0.5017, 1.0348)
M4R20	2	1.4209	0.0626	(1.3045, 1.5372)

M6R0	2	1.1518	0.1209	(1.0354, 1.2682)
M6R20	2	1.6010	0.0580	(1.4846, 1.7174)

Pooled StDev = 0.0672713

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Solution	N	Mean	Grouping
M6R20	2	1.6010	A
M4R20	2	1.4209	A
M6R0	2	1.1518	B
M4R0	2	0.6180	C
C6R0	2	0.5921	C D
C4R0	2	0.33120	D

Means that do not share a letter are significantly different.

Table A. 2 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for flow behavior index (n) values of solutions containing different amount of rye flour and GSE and heated by different methods

One-way ANOVA: n versus Solution

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Solution	6	C4R0, C6R0, M4R0, M4R20, M6R0, M6R20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Solution	5	0.047845	0.009569	26.63	0.001
Error	6	0.002156	0.000359		
Total	11	0.050002			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0189575	95.69%	92.09%	82.75%

Means

Solution	N	Mean	StDev	95% CI
C4R0	2	0.95260	0.00552	(0.91980, 0.98540)
C6R0	2	0.92465	0.00445	(0.89185, 0.95745)
M4R0	2	0.8841	0.0354	(0.8512, 0.9169)
M4R20	2	0.7991	0.0249	(0.7663, 0.8319)
M6R0	2	0.85220	0.01089	(0.81940, 0.88500)
M6R20	2	0.77655	0.01068	(0.74375, 0.80935)

Pooled StDev = 0.0189575

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Solution	N	Mean	Grouping
C4R0	2	0.95260	A
C6R0	2	0.92465	A B
M4R0	2	0.8841	A B
M6R0	2	0.85220	B C
M4R20	2	0.7991	C D
M6R20	2	0.77655	D

Means that do not share a letter are significantly different.

Table A. 3 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for apparent viscosity (AV) values of solutions containing different amount of rye flour and GSE and heated by different methods

One-way ANOVA: AV versus Solution

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Solution	6	C4R0, C6R0, M4R0, M4R20, M6R0, M6R20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Solution	5	0.197700	0.039540	84.66	0.000
Error	6	0.002802	0.000467		
Total	11	0.200502			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0216106	98.60%	97.44%	94.41%

Means

Solution	N	Mean	StDev	95% CI
C4R0	2	0.26150	0.00240	(0.22411, 0.29889)
C6R0	2	0.4056	0.0288	(0.3683, 0.4430)
M4R0	2	0.37915	0.00785	(0.34176, 0.41654)
M4R20	2	0.5687	0.0223	(0.5313, 0.6061)
M6R0	2	0.5773	0.0374	(0.5399, 0.6146)
M6R20	2	0.61750	0.00283	(0.58011, 0.65489)

Pooled StDev = 0.0216106

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Solution	N	Mean	Grouping
M6R20	2	0.61750	A
M6R0	2	0.5773	A
M4R20	2	0.5687	A
C6R0	2	0.4056	B

M4R0	2	0.37915	B
C4R0	2	0.26150	C

Means that do not share a letter are significantly different.

Table A. 4 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for electrical conductivity values of solutions containing different amount of rye flour and GSE and heated by different methods

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Solution	6	C4R0, C6R0, M4R0, M4R20, M6R0, M6R20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	5	0.81344	0.162688	79.68	0.000
Error	6	0.01225	0.002042		
Total	11	0.82569			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0451848	98.52%	97.28%	94.07%

Means

Sample	N	Mean	StDev	95% CI
C4R0	2	3.9700	0.0141	(3.8918, 4.0482)
C6R0	2	3.8300	0.0566	(3.7518, 3.9082)
M4R0	2	3.7400	0.0424	(3.6618, 3.8182)
M4R20	2	3.4400	0.0283	(3.3618, 3.5182)
M6R0	2	3.6550	0.0354	(3.5768, 3.7332)
M6R20	2	3.1800	0.0707	(3.1018, 3.2582)

Pooled StDev = 0.0451848

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping			
C4R0	2	3.9700	A			
C6R0	2	3.8300	A	B		
M4R0	2	3.7400		B		
M6R0	2	3.6550		B		
M4R20	2	3.4400			C	
M6R20	2	3.1800				D

Means that do not share a letter are significantly different.

Table A. 5 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for average fiber diameter values of nanofibers obtained from solutions containing different amount of rye flour and GSE and heated by different methods

One-way ANOVA: Diameter versus Sample

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Solution	6	C4R0, C6R0, M4R0, M4R20, M6R0, M6R20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	5	145786	29157	8.29	0.000
Error	594	2089698	3518		
Total	599	2235485			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
59.3128	6.52%	5.73%	4.62%

Means

Sample	N	Mean	StDev	95% CI
C4R0	100	295.14	58.38	(283.49, 306.79)
C6R0	100	329.07	74.37	(317.42, 340.72)
M4R20	100	301.81	45.50	(290.16, 313.46)
M4R0	100	309.94	51.55	(298.29, 321.59)
M6R20	100	327.44	59.97	(315.79, 339.09)
M6R0	100	338.09	62.01	(326.44, 349.74)

Pooled StDev = 59.3128

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
M6R0	100	338.09	A
C6R0	100	329.07	A B
M6R20	100	327.44	A B
M4R0	100	309.94	B C
M4R20	100	301.81	C
C4R0	100	295.14	C

Means that do not share a letter are significantly different.

Table A. 6 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for water vapor permeability values (WVP) of nanofibers obtained from solutions containing different amount of rye flour and GSE

One-way ANOVA: WVP versus Sample

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample	4	M4R0, M4R20, M6R0, M6R20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	3	1.09480	0.364933	77.65	0.001
Error	4	0.01880	0.004700		
Total	7	1.11360			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0685565	98.31%	97.05%	93.25%

Means

Sample	N	Mean	StDev	95% CI
M4R0	2	1.2200	0.0566	(1.0854, 1.3546)
M4R20	2	1.9400	0.0707	(1.8054, 2.0746)
M6R0	2	1.0900	0.0990	(0.9554, 1.2246)
M6R20	2	1.8300	0.0283	(1.6954, 1.9646)

Pooled StDev = 0.0685565

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
M4R20	2	1.9400	A
M6R20	2	1.8300	A
M4R0	2	1.2200	B
M6R0	2	1.0900	B

Means that do not share a letter are significantly different.

Table A. 7 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for glass transition temperature (Tg) of nanofibers obtained from solutions containing different amount of rye flour and GSE

One-way ANOVA: Tg versus Sample

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal

Significance level α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample	4	M4R0, M4R0, M6R0, M6R20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	3	10.355	3.4516	4.47	0.091
Error	4	3.087	0.7718		
Total	7	13.442			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.878493	77.03%	59.81%	8.14%

Means

Sample	N	Mean	StDev	95% CI
M4R0	2	-17.755	-1.318	(-19.480, -16.030)
M4R20	2	-15.625	-0.573	(-17.350, -13.900)
M6R0	2	-17.655	-0.262	(-19.380, -15.930)
M6R20	2	-15.265	-1.181	(-16.990, -13.540)

Pooled StDev = 0.878493

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
M6R0	2	-17.755	A
M4R0	2	-17.655	A
M4R20	2	-15.625	B
M6R20	2	-15.265	B

Means that do not share a letter are significantly different.

Table A. 8 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for melting temperature (Tm) of nanofibers obtained from solutions containing different amount of rye flour and GSE

One-way ANOVA: Tg versus Sample

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample	4	M4R0, M4R20, M6R0, M6R20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	3	10.355	3.4516	4.47	0.091
Error	4	3.087	0.7718		
Total	7	13.442			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.878493	77.03%	59.81%	8.14%

Means

Sample	N	Mean	StDev	95% CI
M4R0	2	-17.755	1.138	(-19.480, -16.030)
M4R20	2	-15.625	0.573	(-17.350, -13.900)
M6R0	2	-17.655	0.262	(-19.380, -15.930)
M6R20	2	-15.265	1.181	(-16.990, -13.540)

Pooled StDev = 0.878493

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
M6R20	2	-15.265	A
M4R20	2	-15.625	A
M6R0	2	-17.655	A
M4R0	2	-17.755	A

Means that do not share a letter are significantly different.

Table A. 9 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for melting enthalpy (ΔH_m) of nanofibers obtained from solutions containing different amount of rye flour and GSE

One-way ANOVA: Enthalpy versus Sample

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample	4	M4R0, M4R20, M6R0, M6R20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	3	14.98	4.992	0.51	0.697
Error	4	39.16	9.791		
Total	7	54.14			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.12904	27.66%	0.00%	0.00%

Means

Sample	N	Mean	StDev	95% CI
M4R0	2	24.95	2.05	(18.81, 31.09)
M4R20	2	24.17	5.07	(18.02, 30.31)
M6R0	2	24.11	2.55	(17.97, 30.26)
M6R20	2	21.35	1.65	(15.20, 27.49)

Pooled StDev = 3.12904

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
M4R0	2	24.95	A
M4R20	2	24.17	A

M6R0	2	24.11	A
M6R20	2	21.35	A

Means that do not share a letter are significantly different.

Table A. 10 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of rye flour and GSE concentration on total phenolic content (TPC) values of solutions

One-way ANOVA: TPC Solution versus Sample

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample	4	M4R0, M4R20, M6R0, M6R20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	3	180.865	60.2883	101.00	0.000
Error	4	2.388	0.5969		
Total	7	183.253			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.772609	98.70%	97.72%	94.79%

Means

Sample	N	Mean	StDev	95% CI
M4R0	2	2.980	0.665	(1.463, 4.497)
M4R20	2	10.605	0.219	(9.088, 12.122)
M6R0	2	3.190	1.032	(1.673, 4.707)
M6R20	2	13.975	0.912	(12.458, 15.492)

Pooled StDev = 0.772609

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
M6R20	2	13.975	A
M4R20	2	10.605	B
M6R0	2	3.190	C
M4R0	2	2.980	D

Means that do not share a letter are significantly different.

Table A. 11 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of rye flour concentration on total phenolic content (TPC) values of nanofiber films

One-way ANOVA: TPC Film versus Sample

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample	2	M4R20, M6R20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	1	7.8680	7.8680	56.35	0.017
Error	2	0.2793	0.1396		
Total	3	8.1473			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.373664	96.57%	94.86%	86.29%

Means

Sample	N	Mean	StDev	95% CI
M4R20	2	5.7400	0.1131	(4.6032, 6.8768)
M6R20	2	8.545	0.516	(7.408, 9.682)

Pooled StDev = 0.373664

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
M6R20	2	8.545	A
M4R20	2	5.7400	B

Means that do not share a letter are significantly different.

Table A. 12 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of rye flour concentration on GSE loading efficiency (LE) values of nanofiber films

One-way ANOVA: LE versus Sample

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample	2	M4R20, M6R20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	1	48.860	48.860	20.87	0.045
Error	2	4.683	2.341		
Total	3	53.543			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.53018	91.25%	86.88%	65.02%

Means

Sample	N	Mean	StDev	95% CI
M4R20	2	54.16	1.14	(49.50, 58.81)
M6R20	2	61.145	0.304	(56.490, 65.800)

Pooled StDev = 1.53018

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
M6R20	2	61.145	A
M4R20	2	54.16	B

Means that do not share a letter are significantly different.

Table A. 13 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for the effect of rye flour concentration on antioxidant activity (AA) values of nanofiber films

One-way ANOVA: AA versus Sample

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample	2	M4R20, M6R20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	1	1.3456	1.3456	2.93	0.229
Error	2	0.9194	0.4597		
Total	3	2.2650			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.678012	59.41%	39.11%	0.00%

Means

Sample	N	Mean	StDev	95% CI
M4R20	2	41.620	0.580	(39.557, 43.683)
M6R20	2	42.780	0.764	(40.717, 44.843)

Pooled StDev = 0.678012

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
M6R20	2	42.780	A
M4R20	2	41.620	A

Means that do not share a letter are significantly different.