UTILIZATION OF PECTIN PRODUCED FROM INFRARED DRIED ORANGE PEEL IN DEVELOPMENT OF ELECTROSPUN NANOFIBERS AND CHARACTERIZATION OF THE NANOFIBERS

KIZILÖTESİ İLE KURUTULMUŞ PORTAKAL KABUĞUNDAN ÜRETİLEN PEKTİNİN ELEKTROEĞRİLMİŞ NANOLİF TASARIMINDA KULLANIMI VE NANOLİFLERİN KARAKTERİZASYONU

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To my little loves Şirin, Eymen and Uras…

ABSTRACT

UTILIZATION OF PECTIN PRODUCED FROM INFRARED DRIED ORANGE PEEL IN DEVELOPMENT OF ELECTROSPUN NANOFIBERS AND CHARACTERIZATION OF THE NANOFIBERS

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In recent years, utilization of infrared (IR; Infrared) drying in food industry is increasing, due to its advantages (efficient, energy saving, low cost, direct heat penetration, fast heating rate, short processing time) over conventional heating. Pectin has been used in many researches in food and pharmaceutical industries due to its biocompatibility, non-toxic structure and biodegradability. Pectin is also used in production of nanofiber by using electrospinning which has advantages (less energy requirement, better control of particle size, cost effectiveness, applicability at room temperature). Wastes of fruit juice industry are important sources of pectin.

In this thesis, pectin was extracted from orange peels infrared dried or oven dried at different conditions. The effects of different drying and extraction conditions on pectin properties were investigated. Nanofibers were produced by using these pectin samples by itself or with PEO or PVA at different electrospinning parameters. The effects of pectin properties and different electrospinning parameters on the characteristics of nanofibers were investigated.

Orange peel samples were infrared dried (600W,700W,800W for 30min) or oven dried (60°C,70°C). Pectin was extracted from dried orange peels at 90°C, by using different extraction conditions (pH1-1.5-2, for 60-90-120min.). Best pectin extraction method was determined by investigating properties of pectin samples and pectin nanofibers. The pectin samples had high purity (galacturonic acid 72.79-98.66%) and high esterification degree (59.22-94.06%). The highest yield of pectin was obtained at pH1-120min for oven dried and pH1.5-120min. for infrared dried samples. Pectin yield values together with the properties of electrospun nanofibers confirmed that pectin extraction at pH1.5-120min. is the key parameter for finest fiber formation with better morphology.

It was not possible to produce nanofibers from pure pectin (extracted from orange peels infrared dried at 800W-30min.) solutions. Nanofibers were produced by using pectin:PEO (3:1, 3:2, 4:1, 4:2, 5:1% (w/w)) or pectin:PVA (2:5, 2:6, 2.5:5, 2.5:6, 3:5, 3:6% (w/w)) solutions in order to determine the co-polymer. Different electrospinning parameters such as flow rate (0.2-0.7ml/h), voltage (15-35kV), distance (8-20cm.) were used. Higher concentrations of pectin was used in pectin+PEO solutions as compared to pectin+PVA solutions. Morphology (SEM), DSC thermogram, water contact angle of the nanofibers were determined. Based on the results, the most appropriate polymer and electrospinning solution concentration was chosen as pectin:PEO 3:1(%w/w,with Triton X-100) and the best electrospinning parameters were chosen as 15cm,0.5ml/h,35kV.

XRD patterns of PEO nanofibers showed that electrospinning process reduced the crystallinity of PEO. However, electrospinning did not cause a change in XRD patterns of pectin. Enthalpy values (89.31-108.95 J/g) of the pectin samples produced in the study were higher as compared to that of commercial pectin (71.07 J/g). Both of the peaks of PEO or pectin were observed in FTIR, DSC thermogram and XRD pattern for pectin+PEO nanofibers, indicating the compatibleness of PEO and pectin in nanofiber production.

Pectin+PEO nanofibers including pectin produced in the study had higher water contact angle value (20.98-50.14°) as compared to that of commercial pectin (15.60°) nanofiber. Pectin extracted from orange peels dried at 700W-30min. showed moderate (50.14°) hydrophilic surface characteristics. Overall results indicated that pectin+PEO nanofibers may have a potential in various applications of food industry.

Keywords: Infrared Drying, Orange Peel, Pectin, Extraction, Yield, Pectin Properties, Electrospinning, Nanofiber, Nanofiber properties

ÖZET

KIZILÖTESİ ILE KURUTULMUŞ PORTAKAL KABUĞUNDAN ÜRETİLEN PEKTİNİN ELEKTROEĞRİLMİŞ NANOLİF TASARIMINDA KULLANIMI VE NANOLİFLERİN KARAKTERİZASYONU

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Son yıllarda, kızılötesi (IR; Infrared) teknolojisinin gıda endüstrisinde kullanımı konvansiyonel ısıtmaya kıyasla avantajlarından (etkin, enerji tasarruflu, düşük maliyetli, doğrudan ürüne penetrasyon, hızlı ısıtma ve kısa işlem süresi) dolayı artmaktadır. Pektin biyouyumluluğu, toksik olmayan yapısı, biyolojik olarak parçalanabilirliği nedeniyle gıda ve ilaç endüstrilerinde birçok araştırmada kullanılmaktadır. Pektin daha az enerji kullanımı, parçacık boyutu dağılımının daha iyi kontrolü, maliyet etkinliği ve oda sıcaklığında uygulanabilirlik gibi çeşitli avantajlara sahip elektroeğirme yöntemi kullanılarak nanolif üretiminde de kullanılmaktadır. Meyve suyu endüstrisi atıkları, pektin için önemli kaynaklardır.

Bu tezde, kızılötesinde veya etüvde farklı koşullarda kurutulmuş portakal kabuklarından pektin ekstrakte edilmiştir. Farklı kurutma ve ekstraksiyon koşullarının pektin özellikleri üzerine etkileri araştırılmıştır. Bu pektin örnekleri, farklı elektroeğirme parametreleri kullanılarak PEO veya PVA ile birlikte nanolif üretmek için kullanılmıştır. Pektin özellikleri ve farklı elektroeğirme parametrelerinin nanoliflerin özellikleri üzerinde etkileri incelenmiştir.

Portakal kabuğu örnekleri, kızılötesinde (600W, 700W ve 800W-30dk) veya etüvde (60 ve 70°C) kurutulmuştur. Pektin, kurutulmuş portakal kabuklarından 90°C'de farklı ekstraksiyon koşulları (pH1-1.5-2, ekstraksiyon süresi 60-90-120dk.) kullanılarak ekstrakte edilmiştir. En iyi pektin ekstraksiyon yöntemi, pektin örneklerinin ve pektin nanoliflerinin özellikleri incelenerek belirlenmiştir. Pektin örnekleri yüksek saflığa (galakturonik asit %72.79-98.66) ve yüksek esterleşme derecesine (59.22-94.06%) sahiptir. En yüksek pektin verimi; etüvde kurutulmuş örnekler için pH1-120dk'da elde edilirken, kızılötesinde kurutulmuş örnekler için pH1.5-120dk'da elde edilmiştir. Pektin verim değerleri ile birlikte elektroeğrilmiş nanoliflerin özellikleri; pH1.5-120dk'da pektin ekstraksiyonun daha iyi morfoloji ile küçük çapta lif oluşumu için anahtar parametre olduğunu doğrulamıştır.

Yalnızca pektin (800W-30dk'da kızılötesinde kurutulmuş portakal kabuklarından ekstrakte edilen) çözeltisinden nanolif üretimi mümkün olmamıştır. Yardımcı polimeri belirlemek için pektin:PEO (% 3:1, 3:2, 4:1, 4:2, 5:1 (w/w)) veya pektin:PVA (% 2:5, 2:6, 2.5:5, 2.5:6, 3:5, 3:6 (w/w)) kullanılarak nanolif üretilmiştir. Akış hızı (0.2-0.7ml/saat), voltaj (15-35kV) ve mesafe (8-20cm) gibi farklı elektroeğirme parametreleri kullanılmıştır. Pektin, pektin+PEO çözeltilerinde pektin+PVA çözeltilerine göre daha yüksek konsantrasyonlarda kullanılmıştır. Nanoliflerin morfolojisi (SEM), DSC termogramı ve su temas açısı belirlenmiştir. Sonuçlar göz önüne alındığında, en uygun yardımcı polimer ve elektroeğirme çözeltisi konsantrasyonu pektin:PEO 3:1 (% w/w, Triton X-100 ile) ve en iyi elektroeğirme parametresi 15cm,0.5ml/sa,35kV olarak seçilmiştir.

PEO nanoliflerinin XRD desenleri, elektroeğirme işleminin PEO'nun kristalliğini azalttığını göstermiştir. Ancak elektroeğirme, pektinin XRD desenlerinde bir değişikliğe neden olmamıştır. Çalışmada üretilen pektin örneklerinin entalpi değerleri (89.31-108.95 J/g), ticari pektininkine (71.07 J/g) kıyasla daha yüksektir. PEO+pektin nanoliflerinin FTIR, DSC termogramı ve XRD desenlerinde PEO veya pektinin her ikisinin piklerinin gözlenmesi, nanolif üretiminde PEO ve pektinin uyumlu olduğunu göstermiştir.

Çalışmada üretilen pektini içeren pektin+PEO nanolifleri (20.98-50.14°), ticari pektin (15.60°) içeren nanolife kıyasla daha yüksek su temas açısı değerine sahiptir. 700W-30dk'da kurutulmuş portakal kabuğundan elde edilen pektin, orta derecede (50.14°) hidrofilik yüzey özellikleri sergilemiştir. Genel olarak elde edilen sonuçlar, pektin+PEO nanoliflerinin gıda endüstrisinde çeşitli uygulamalarda potansiyele sahip olduğunu göstermiştir.

Anahtar kelimeler: Kızılötesi Kurutma, Portakal Kabuğu, Pektin, Ekstraksiyon, Verim, Pektin özellikleri, Elektroeğirme, Nanolif, Nanolif özellikleri

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SYMBOLS AND ABBREVIATIONS

Symbols

w/w	Percent weight of solution
v/v	Percent volume of solution
Ce	Critical polymer concentration

Abbreviations

ADH	Adipic acid dihydrazide
ANOVA	One-way analysis of variance
AOAC	Association of Official Analytical Chemists
ATR-FTIR	Attenuated Total Reflectance-Fourier Transform Infrared
DA	Degree of amidation
DE	Degree of esterification
DM	Degree of methoxylation
DMSO	Dimethyl sulfoxide
DSC	Differential Scanning Calorimetry
GalA	D-galacturonic acid
GLU	Glutaraldehyde
GRAS	Generally Recognized as Safe
FDA	Food and Drug Administration
HGA	Homogalacturonan
IR	Infrared
PEO	Polyethylene oxide
PL	Pectate lyases
PME	Pectin methylesterase

PVA	Polyvinyl alcohol
RG-I	Rhamnogalacturonan-I
RG-II	Rhamnogalacturonan-II
SEM	Scanning Electron Microscopy
XRD	X-ray diffraction
XGA	Xylogalacturonan

1.INTRODUCTION

Infrared (IR) drying has gained a great interest in food industry, due to its advantages over conventional heating. As compared to other conventional heating technologies, IR has many advantages such as higher thermal efficiency, energy saving, fast heating rate and short processing time due to direct heat penetration (Savas and Basman, 2016; Rastogi, 2021).

IR has been used in some food processes such as drying, cooking, baking, thawing, roasting, boiling, pasteurization, sterilization, blanching and inactivation of enzymes. Infrared was used in the production of noodle, bulgur, heat-moisture treated starch, cookies, and bread (Krishnamurthy et al., 2008; Basman and Yalcin, 2011; Ismailoglu and Basman, 2015; Riadh et al., 2015; Ismailoglu and Basman, 2016; Savas and Basman, 2016; Cheaib et al., 2018). Infrared was also used for inactivation of enzymes (lipoxygenase, urease, etc.) (Savas and Basman, 2016). Infrared can be also used for microbial inactivation of liquid and powdered foods (Zeng et al., 2022). Mushrooms, carrots, grapes, peach pulp, pear, jujube, banana and saffron were also dried successfully by using infrared. Both the infrared power and temperature have an effect on the drying kinetics and quality of the food. Several studies in the literature have demonstrated that higher infrared powers can lead to reduced drying times (Huang et al., 2021). IR, as a drying technology, can improve the quality of food products by enhancing nutrient retention (Savas and Basman, 2016; Huang et al., 2021; Zeng et al., 2022). Utilization of infrared facilitates extraction of many food components (polyphenols, flavonoids, lactones, oils, carbohydrates, alkaloids, salidroside, etc.) from the raw materials in larger quantities by disintegrating the structure (Yan et al., 2019; Xiang et al., 2022).

Pectin is one of the most abundant polysaccharides in nature which is found in the primary cell walls and middle lamella of plants. Fruit juice industry waste (apple peel, apple pomace and citrus peel etc.) are important sources of pectin. (Muslu, 2016; Akınalan Balık et al., 2019). In food industry, pectin is generally used in jam and jelly making, due to its gel and thickening property. Pectin has been used in many researches in food and pharmaceutical industries due to its biocompatibility, pH sensitivity, non-toxic structure and biodegradability (Muslu, 2016; Akınalan Balık et al., 2019; Vanitha and Khan, 2019).

Pectin is also used in the production of nanomaterials. Microfiber/nanofibers are produced by using electrospinning. Electrospinning can be used in important applications such as antimicrobial packaging, nutraceutical transport, enzyme immobilization, tissue engineering scaffolds and nanosensors. As compared to the other nanotechnologies, electrospinning has several advantages such as; less energy requirement, cost effectiveness, high surface/volume ratio, large porous structure, better control of particle size, high encapsulation efficiency and applicability at room temperature (Rostamabadi et al., 2020).

Production of electrospun nanofibers from pectin by itself is not possible. Because pectin solutions do not have sufficient viscoelastic structure and have jet stability problems due to its polyelectrolyte structure (Cui et al., 2016; Liu et al., 2016). Therefore, in literature, co-polymers (polyethylene oxide (PEO), polyvinyl alcohol (PVA) and polysaccharides (sodium alginate, pullulan, chitosan, etc.)) are used together with pectin in the production of electrospun pectin nanofibers (Alborzi et al., 2010; Lin et al., 2013; Cui et al., 2017).

To the best of our knowledge, pectin extraction from infrared dried samples (675W, 60-90min.) was used only in a research carried out on peach and pectin properties were reported. Therefore in this study, it was aimed to extract pectin from infrared dried orange peels at different conditions. Pectin samples were used in the development of electrospun nanofibers and nanofibers were investigated in terms of SEM, FTIR, XRD, DSC, water wettability and color.

In the first part of the study, orange peel samples were dried either by using infrared at different powers or oven at different temperatures. Pectin was extracted from dried orange peel samples at different pH and time. Effects of all parameters on pectin yield, galacturonic acid, esterification degree, color and FTIR spectra of pectin were investigated. In the second part of the study, nanofibers where produced from these pectin samples by itself or with PEO and PVA by electrospinning technique. Nanofibers were produced by using pectin:PEO (3:1, 3:2, 4:1, 4:2, 5:1 % (w/w)) or pectin:PVA (2:5, 2:6, 2.5:5, 2.5:6, 3:5, 3:6 % (w/w)) solutions at different electrospinning parameters such as flow rate (0.2-0.7ml/h), voltage (15-35kV), distance (8-20cm). Electrospinning parameters and co-polymer type was determined by considering the nanofiber properties (morphology, DSC thermogram, water contact angle). Based on the results, the most

appropriate polymer and electrospinning solution concentration was chosen as pectin:PEO 3:1(%w/w,with Triton X-100) and the best electrospinning parameters were chosen as 15cm,0.5ml/h,35kV. Characterization of nanofibers was carried out by using SEM, FTIR, XRD, DSC, water wettability and color.

2. GENERAL INFORMATION

2.1. INFRARED

In recent years, application of infrared (IR; Infrared) has become a focus of interest in the food industry because it is a cost-effective process with high heat transfer coefficient and shorter processing time. It is a form of electromagnetic energy transmitted by infrared waves and transferred heat. IR radiation is between the spectrum of visible light (0.38-0.78 µm) and electromagnetic microwaves (1-1000 mm). IR can be divided into three wavelength: near (0.78-1.4 µm), mid (1.4-3.0 µm), and far (3.0-1000 µm) (Fevzioğlu and Başman, 2008; Riadh et al., 2015; Rastogi, 2021).

Infrared radiation is propagated as a wave and when it interacts with a food surface, some of it is absorbed while the rest is reflected. The absorbed part was converted into heat by the vibration of the molecules in the material. The composition and structure of the food and IR wavelength determine the penetration depth of the infrared into the food. Studies have shown that a wavelength of 2.5-3 µm can be effectively absorbed by food (Fevzioğlu and Başman, 2008; Alkaç et al., 2019; Xiang et al., 2022). While the penetration depth is 1.5 mm for carrots and 4.1 mm for raw apples, it is 15-18 mm for potatoes (Krishnamurthy et al., 2008; Yadav et al., 2020).

As compared to conventional heating, infrared heating offers several advantages such as higher heat transfer coefficient and energy efficiency, direct penetration into the product, homogeneous heating in a short time, lower nutrient losses and better control of process parameters (Rastogi, 2021). Because of the advantages, infrared is often preferred in the food industry for many purposes such as; drying of vegetables, fruits and seeds, inactivation of enzymes, frying, cooking, baking, boiling, thawing, pasteurization and sterilization (Savas and Basman, 2016).

Yalcin and Basman (2015) reported that IR treatment caused a decrease in urease activity, trypsin inhibitor activity, and lipoxygenase activity in soybeans.

Infrared drying has been successfully used for the drying of various food products such as mushrooms, carrots, grapes, peach pulp, pear, jujube, banana and saffron. Infrared power and drying temperature affect the drying kinetics and quality of food products.

Studies in the literature have demonstrated that an increase in infrared power can reduce the drying time (Huang et al., 2021). In addition to drying, infrared radiation is also utilized for blanching various food products such as carrots, garlic, bitter gourd, and apple (Zeng et al., 2022).

Infrared is also used in the production of noodle, bulgur, heat-moisture treated starch, cookies, and bread (Krishnamurthy et al., 2008; Basman and Yalcin, 2011; Ismailoglu and Basman, 2015; Riadh et al., 2015; Ismailoglu and Basman, 2016; Savas and Basman, 2016; Cheaib et al., 2018). Utilization of infrared facilitates extraction of many food components from the raw material in larger quantities by disintegrating the structure (Yan et al., 2019; Xiang et al., 2022). Infrared is used for the extraction of polyphenols, flavonoids, lactones, oils, carbohydrates, alkaloids, salidroside from plant materials (Xiang et al., 2022).

Yan et al. (2019) used infrared, hot air, and freeze-drying methods to extract water-soluble polysaccharides from bitter gourd (*Momordica charantia L.*). The polysaccharides obtained by all three methods had similar preliminary structure with different monosaccharide compositions and molecular weights. Infrared dried bitter gourd had higher arabinose and galactose content and lower glucose, xylose, and mannose content as compared to hot-air and freeze dried ones. The polysaccharide obtained from infrared dried bitter gourd did not contain glucuronic acid, whereas no difference was observed in the amount of galacturonic acid content among all samples.

In the study by Nozad et al., (2016), it was found that the essential oil content of infrared dried mint leaves was higher as compared to hot air dried ones.

In a study by Lyu et al. (2019), osmotic dehydration was applied to peach samples by using different sucrose solutions (100, 300, and 500 g/L), at room temperature for 4h. Then the samples were infrared-treated at 675W, 80°C, 60-90min. and DIC (controlled pressure drop) was applied to the samples. The samples were dried under vacuum at 60°C. It was found that the water-soluble pectin content decreased when infrared was applied after osmotic dehydration.

Deng et al. (2018) investigated the effect of short-wave infrared radiation on peanut oil extraction. The extraction yield of oil increased (8.74%) significantly with infrared pretreatment as compared to the control group. This improvement in oil yield can be attributed to the ability of infrared to break down the cell structure and oil body membranes, thereby facilitating the release of oil.

2.2. PECTIN

2.2.1. Properties of Pectin

Pectin is one of the most abundant polysaccharides in nature which is found in the middle layer of the cell wall, primary cell walls and plasma membrane of plants. Industrial wastes of apple pulp, citrus peel and sugar beet pulp are important sources of pectin. Pectin has linear chains of α-1,4-linked D-galacturonic acids and different types of side chains containing rhamnose, xylose, galactose, and arabinose.

As shown in Figure 1, pectin domains are classified as homogalacturonan (HGA), rhamnogalacturonan-I (RG-I), rhamnogalacturonan-II (RG-II), and xylogalacturonan (XGA) depending on the side chains (Li et al., 2021). Homogalacturonan units of pectin are called ‘smooth’ regions whereas other units are called ‘hairy’ regions (Akınalan Balık et al., 2019).

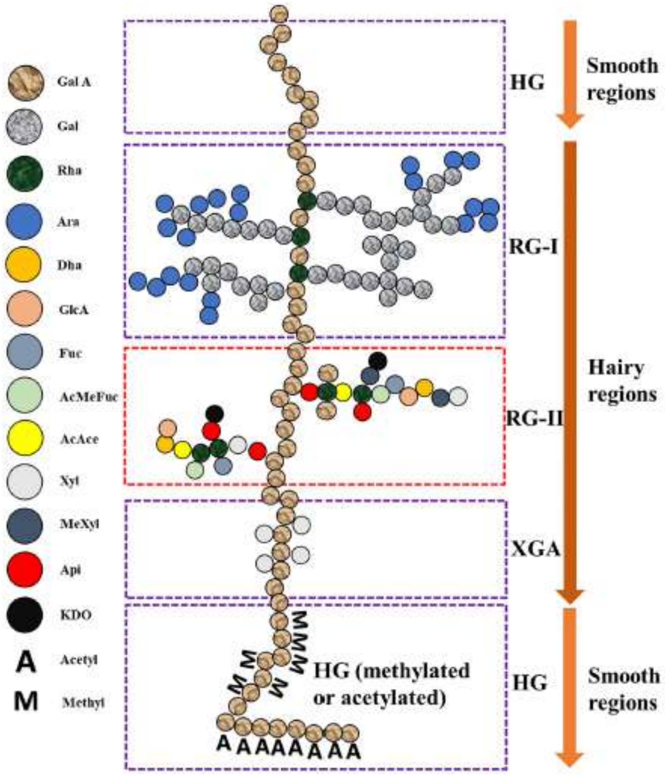


Figure 1. Primary structure of pectin (Li et al., 2021)

HGA domains consist of α-(1 → 4)-D-linked galacturonic acid. Galacturonic acid groups are partially methyl-esterified and sometimes acetyl-esterified/amidated which make pectin cross-linkable by multivalent cations (Cui et al., 2016).

RG domains consist of RG-I and RG-II. RG-I has disaccharide (galacturonosyl and rhamnosyl) and branches of neutral saccharides (araban and galactoarabinan). The branches of RG-II domains exhibit a complex structure, which comprises a minimum of 12 different types of monosaccharides and α-(1 → 4)-D-linked galacturonic acid residues (Cui et al., 2016).

As compared to sodium alginate, chitosan, starch and cellulose, pectin has complex chemical structure and shows adverse effects on chemical reactivity and mechanical strength. Besides this, complex chemical structure of pectin, existing in various saccharide residues, causes positive effects on health. Therefore, pectin can play an important role in biomedicine and drug delivery (Li et al., 2021).

Pectin's chemical structure and functional groups (hydroxyl, carboxyl, methyl, and amide groups) make it suitable for use in biomedicine and drug delivery. Depending on the pH conditions, the carboxyl groups of the pectin molecule can exist in various forms (-COO, -COOH, and $–COOH^{2+}$). Consequently, pectin forms a composite by adjusting pH of the medium with different materials having opposite charges (Li et al., 2021). Pectin's functional groups (-OH and -COOH) have the ability to react with hydroxyl and amino groups, forming a covalent bond. Additionally, its hydroxyl and free carboxyl functional groups can coordinate with polyvalent metal ions (such as Ca^{2+}, Zn^{2+}, and Fe^{3+}). These physical processes and chemical reactions cause in the formation of hydrogels, films, microspheres, and other materials. (Li et al., 2021).

Pectin is mostly soluble in water. In order to precipitate water soluble pectin, ethanol, methanol, propanol or acetone and multivalent cations (Cu^{+2}, Al^{+3}) were added to the solution (Muslu, 2016). The gelation of pectin is attributed to inter- and/or intrachain entanglements between its functional groups through various interactions such as hydrogen bonding, hydrophobic interactions, and ionic bonding (Li et al., 2021).

The degree of esterification (DE) is a measure of the degree of methylation of galacturonic acid residues in pectin, expressed as the molar ratio of the number of methylated galacturonic acid residues to the total number of galacturonic acid residues. This parameter is commonly used to describe the concentration of methoxyl groups in pectin. According to DE value, pectin is classified as lowly esterified pectin (20–40%) and highly esterified pectin (60–75%) (Li et al., 2021). It is the most important parameter affecting surface tension, gelation and emulsion formation (Tiwari et al., 2017).

The solubility and gelation of pectin are affected by its molecular size. Pectin with a higher molecular weight has a longer molecular chain and more reaction sites as compared to low molecular weight pectin. Network structures formed between the pectin molecule and multivalent metal ions can enhance the rheological properties of pectin, such as elastic modulus and viscosity (Li et al., 2021).

The unique properties of pectin, such as its polyelectrolyte structure, biodegradability, biocompatibility, and water solubility, make it suitable for a variety of novel applications, including its use as coatings or edible films for food packaging applications (Akınalan Balık et al., 2019).

2.2.2. Pectinolytic Enzymes

Pectinolytic enzymes known as pectinases affect pectin structure. The most important Pectinolytic enzymes are pectin methylesterase (PME), pectate lyases (PL) and polygalacturonase (PG). These enzymes located in the middle layer of plant cells and in the primary cell wall and cause degradation of pectin structure (Lyu, 2017).

PME catalyzes the removal of methyl esters from the backbone of homogalacturonic acid chains. Generally, PMEs are highly thermolabile, easily inactivated at temperatures below 70°C (Jolie et al., 2010). Jolie et al. (2010) reported that optimum temperature for PME varies between 45°C and 55°C depending on the enzyme source and environmental conditions.

PG converts pectin chains into shorter ones. There are two forms in fruits and vegetables. PG1 is more heat stable and 85°C is required for PG1 inactivation. PG2 is easily inactivated at around 65°C. PL degrades pectin polymers by a ß-elimination mechanism that causes the breaking of homogalacturanan α-(1-4)-D galacturonic acid bonds (Lyu, 2017). The mechanisms of pectinolytic enzymes (pectin methyl esterase, polygalacturonase and pectate lyase) are shown in Figure 2.

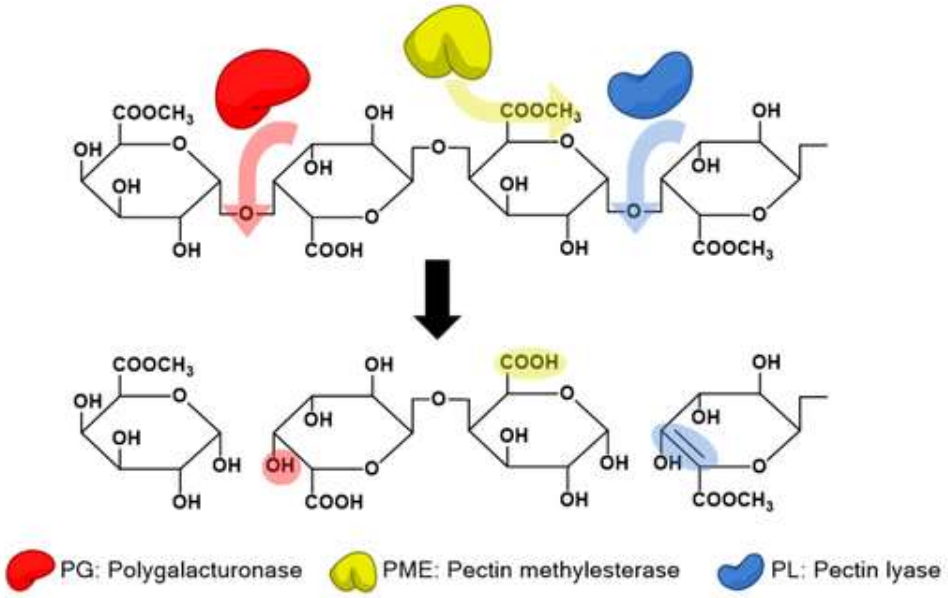


Figure 2. Mechanisms of pectolytic enzymes (Ottone et al., 2020)

2.2.3. Pectin Extraction

Pectin extracted from different sources has different characteristics according to the molecular size, different chemical structure and DE values. Extraction method carried out in acidic conditions at high temperatures also affects the properties of pectin. Conventional extraction procedures de-esterified and depolymerize the pectin and results in break down of the molecular backbone of the pectin. Novel pectin extraction methods minimize the negative effect on pectin chemical structure and composition (Li et al., 2021).

Several factors such as liquid-solid ratio, solvent used for extraction, pH, temperature, time and stirring speed are important in pectin production. In literature, microwave, ultrasound, high pressure, subcritical water, enzyme utilization, electromagnetic induction heating and conventional methods have been used for the extraction of pectin from orange peel (Fakayode and Abobi, 2018). Pectin extraction in hot diluted strong mineral acid solution (sulfuric acid, hydrochloric acid, nitric acid, etc.) is the most commonly used one (Tiwari et al., 2017; Elakkad and Elgamsy, 2019).

Fakayode and Abobi (2018) investigated the effects of extraction temperature (80, 85, 90, 95, 100°C), time (60, 75, 90, 105 and 120 min.) and pH (adjusted with HCl to 1, 1.5, 2, 2.5 and 3) on the yield of pectin extracted from orange peels. The pectin yield was found to be between 12.93-29.05%. Increasing the extraction temperature and time resulted in an increase in pectin yield. Maximum yield (29.05%) was obtained for pH 1.5, 95°C and 105min.

Kamal et al. (2021) extracted pectin from orange peels at different extraction temperatures (80, 90, 100°C), times (90, 105, 120 min) and pH (adjusted with HCl to 1.0, 1.5 and 2). Among all the parameters (extraction temperature, time and pH) tested, the highest yield was obtained as 23.39% for pH 1.5, 90°C and 105min. The highest yield of pectin was obtained for different combinations of extraction temperature, pH, and time. Specifically, the highest yield (21.53%) was obtained at 95°C, pH 1.5, and 60 minutes, while a yield of 21.28% was obtained at pH 1.5, 95°C, and 60 minutes. Higher yield (22.45%) was also obtained at 90 minutes, pH 1.5, and 95°C. Pectin yield increased as extraction time and temperature increased. The highest degree of esterification (77.56%) was observed for pH 2.5, 95°C and 60min. Esterification degree increased when the extraction temperature and time decreased and extraction pH increased.

In a study by Rodsamran and Sothornvit (2019), pectin was extracted from lime peels by using different acids (HCl and citric acid), peel/solvent ratios (1:20 and 1:40), and extraction methods (conventional heating and microwave heating). It was reported that acid type and extraction method were the most important factors on the pectin yield. Pectin yield (23.59%) for conventional heating was higher as compared to the one obtained for microwave heating (14.13%). The highest degree of esterification (91.58%) was obtained when citric acid was used for microwave extraction. In both methods, as compared to extraction with citric acid, lower degree of esterification was obtained for pectin extracted with HCl.

Since hydrochloric acid is a strong acid, it causes the breakdown and demethylation of the polygalacturonic acid chains of pectin. The highest galacturonic acid content (95.93%) was obtained when HCl (peel/solvent ratio 1:20) was used for the extraction of pectin by conventional heating while galacturonic acid content (79.29%) was lowest for extraction with microwave heating.

Kratchanova et al (2004) investigated the effects of different microwave powers (0.45, 0.63, 0.9 kW) and times (5, 10, 15min.) on yield and pectin properties. Orange peels were pretreated by using different powers and time and then dried in an oven at 60°C. While the highest yield was found as 18% (db) at 0.90kW for 10min., the lowest yield was obtained 6% (db) for the control sample (without pretreatment with microwave). It was found that the microwave treated samples had higher galacturonic acid content and esterification degree as compared to control sample.

In a study by Lyu et al. (2019), osmotic dehydration was applied to peach samples by using different sucrose solutions (100, 300, and 500 g/L), at room temperature for 4h. Then the samples were infrared treated at 675W, 80°C, 60-90min. Finally DIC (controlled pressure drop) was applied to these samples and the samples were dried under vacuum at 60°C. Alcohol insoluble residue (AIR) was isolated from peach samples and water soluble pectin (WSP) was extracted from AIR. Water-soluble pectin content of infrared treated samples was found to be 110 mg/g alcohol-insoluble residue (AIR). Water-soluble pectin content was found to decrease when infrared treatment was applied after osmotic dehydration. The infrared treated sample was kept in a solution containing 100g/L sucrose and had a water-soluble pectin content as 100±3 mg/g AIR. water-soluble pectin content was found as 89±3 mg/g AIR and 80±2 mg/g AIR, when the sample was kept in a 300g/L or 500g/L sucrose solution, respectively. The esterification degree of infrared treated pectin sample was lower (57.9%) than that of the control sample (75%).

2.3. NANOENCAPSULATION TECHNIQUES OF PECTIN

Pectin has received considerable attention in food and pharmaceutical researches due to its properties. Pectin is used in nanoparticle production by using various nanotechnological techniques. Various nanoencapsulation techniques used for food bioactive components and nutraceuticals are mentioned below (Jafari, 2017).

Lipid-Based Nanoencapsulation Techniques;

-Nanostructured phospholipid carries (liposomes, phytosomes)
-Nanoemulsions
-Nanolipid Carriers

Biopolymer-Based Nanoencapsulation Techniques;

-Single Biopolymer Nanoparticles

-Complexd Biopolymer Nanocarries (Cosolubility, Complex Coacervation)

-Nanogels

-Nanotubes

Special-Equipment-Based Nanoencapsulation Techniques;

- Electrospinning (fibrous structure)
- Nanospray dryer
- Electrospraying (beaded structure)

Special-equipment-based techniques consume less energy and provide better control over particle size distribution, and structural morphology as compared to other techniques. Electrospinning is one of the techniques based on special-equipment and used in nanofiber production.

Electrospinning technique has high loading capacity, high flexibility, high encapsulation efficiency, ease of application, cost-effectiveness, room temperature applicability (Jafari, 2017; Shishir et al., 2018; Rostamabadi et al., 2020). Nanofibers/microfibers produced by electrospinning can be used for delivery of various bioactive compounds, enzyme immobilization, antimicrobial packaging, tissue engineering and nanosensors due to the unique properties such as high surface-to-volume ratio, large porous structure, better control of mechanical properties, and changeable morphologies (Shishir et al., 2018; Rostamabadi et al., 2020).

2.3.1. Definition and Principles of Electrospinning

Electrospinning process is carried out by using high electrical voltage at a stable flow rate in order to produce charged strands from the polymer solutions and then electrospun nanofibers/microfibers can be collected on a collector with the evaporation of solvent. A standard electrospinning system (either vertically or horizontally) has four main parts as described in Figure 3:

- ✓ A high-voltage power source to form strong electrical field between needle (spinneret) and metallic collector
- ✓ A syringe fitted with a flat-end metal needle (spinneret) containing electrospinning solution
- ✓ A syringe pump to feed and control flow rate of electrospinning solution

- ✓ A metallic collector (called as target) having different geometries

(Haider et al., 2018; Rostamabadi et al., 2020).

Electrospinning process begins with a strong electrical voltage (1-35 kV) applied to the polymer solution. The electrical field, generated by the electrical voltage, causes an induction of charges on the polymer droplet. This causes jet instabilities leading formation of a cone-like shape (Taylor Cone) droplet at the needle tip. Jet formation is available when electrostatic repulsions overcome surface tension of the solution. Solvent is evaporated when the jet elongates towards the metallic collector and the residual solid content are collected on the collector as nanofibers (Ahmed et al., 2015; Quirós et al., 2016; Haider et al., 2018; Rostamabadi et al., 2020).

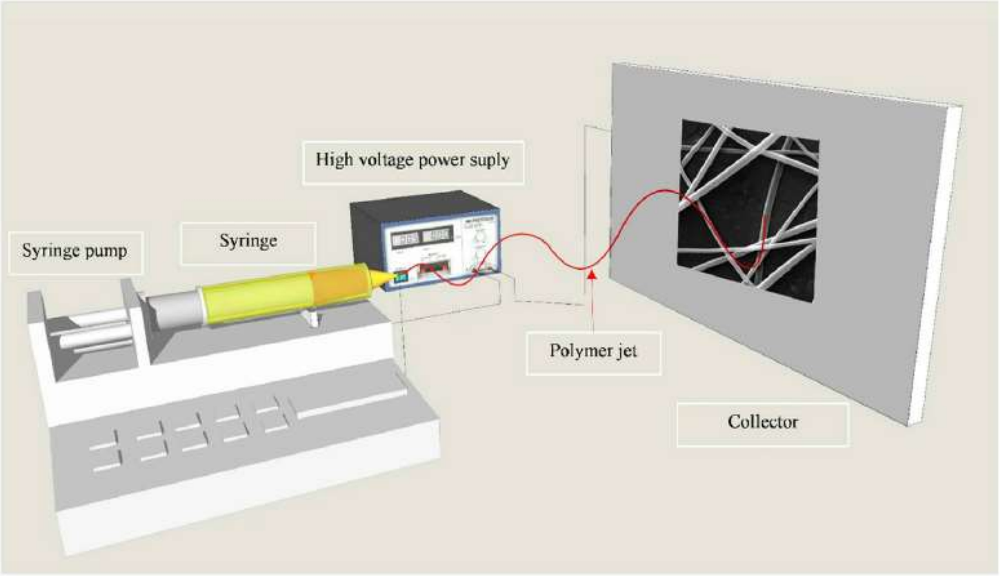


Figure 3. Basic set-up of electrospinning process (Rostamabadi et al., 2020).

2.3.2. Electrospinning Parameters

Electrospun fiber properties are directly affected by three parameters, which are briefly summarized below (Ahmed et al., 2015; Rostamabadi et al., 2020).

- Electrospinning process parameters (electrostatic potential, electrical field intensity, flow rate, distance between syringe and collector)
- Properties of electrospinning solution (viscosity, conductivity, concentration, surface tension, dielectric constant and solvent volatility)

- Environmental conditions such as temperature, humidity, local atmosphere flow, pressure, atmospheric composition.

2.3.2.1. Properties of Electrospinning Solution

Viscosity, which is related to molecular weight of solute and polymer concentration, is the most important parameter that affects product quality and process behavior. High molecular weight (or high viscosity) polymer solutions are necessary in order to obtain suitable polymer chain entanglement (critical polymer concentration, *Ce*) which causes a stable jet (Ghorani and Tucker, 2015; Rostamabadi et al., 2020).

Polymer solutions, having low (<*Ce*, at dilute solutions) and intermediate (up to *Ce*, for semi-dilute solutions) viscosity, generally form nanodroplets or beaded fibers instead of nanofibers, due to insufficient chain entanglements.

Polymer solutions with high viscosity (> *Ce*, entangled solution) give electrospun nanofibers with well-tuned structures due to sufficient chain entanglements (Figure 3.) (Ahmed et al., 2015; Rostamabadi et al., 2020).

Electrical conductivity and surface tension of the polymer solution are the other parameters that affect electrospinning process and electrospinnability of polymer solution. Bead-free fibers can be generally produced from solutions with low surface tension. Increasing the surface tension generally results in bead formation (Ghorani and Tucker, 2015; Rostamabadi et al., 2020). Bead formation is observed when the electrical conductivity of the solution is insufficient.

The electrical conductivity of the solution and the applied voltage are factors that affect the net charge density of the moving jet during electrospinning. As the net charge density increases, the resulting fibers become smaller and more spindle-like. However, conductivity should not be increased above a critical point where there is a balance between the forces by applied electrical field and the forces on surface of droplet (Ghorani and Tucker, 2015; Rostamabadi et al., 2020).

2.3.2.2. Electrospinning Process Parameters

Electrical field intensity can occasionally adjust the fiber diameters by providing surface charge on the electrospinning jet. Application of high voltages beyond the critical value generally results in formation of beaded nanofibers and causes an increase in nanofiber diameter (Haider et al., 2018; Rostamabadi et al., 2020).

Flow rate of the polymer solution determines the morphology of the nanofibers. Flow rate beyond the critical value leads to an increase in nanofiber diameter and bead formation. Minimum flow rate is preferred in order to maintain a balanced flow during jet formation (Haider et al., 2018; Rostamabadi et al., 2020).

Distance between syringe and collector is crucial for nanofiber morphology because it determines the evaporation rate of solvent, jet instabilities and nanofiber collected time. A critical distance between syringe and collector should be provided in order to prepare uniform and smooth electrospun nanofibers. Nanofibers with smaller diameter and less beaded structure can be produced by increasing the distance between the spinneret and the collector. Because of this reason a longer distance allows for a higher stretching and elongation time of the jet before the nanofibers are collected. Shorter distance causes stronger electrostatic field that results in bead formation, due to nanofiber fusion or jet instability (Haider et al., 2018; Rostamabadi et al., 2020).

2.3.2.3. Environmental Conditions

Environmental conditions, expecially temperature and humidity, are important for efficiency of the electrospinning process and fiber morphology. Temperature increases the rate of evaporation of the solvent and decreases the viscosity of the solution, leading a decrease in fiber mean diameter. Electrospinnability of polymers can be affected by the correlation between temperature and conductivity of the solvent and the correlation between temperature and rate of solvent evaporation. Humidity cause changes in diameter of nanofiber by controlling evaporation of the charged jet. Increased ambient humidity results in bead formation and reduced electrospinnability (Ghorani and Tucker, 2015; Haider et al., 2018).

2.3.3. Biopolymers/Polymers Used in Production of Electrospun Nanofibers

Selecting polymers and solvents that are compatible for formation of electrospun nanofibers with enhanced properties (such as higher tensile strength, biocompatibility, degradation behavior) is important for electrospinning process. Synthetic biodegradable/biocompatible polymers such as polyethylene oxide (PEO), polyvinyl alcohol (PVA), poly(-caprolactone) (PCL), poly(lactic acid) (PLA) and polyvinylpyrrolidone (PVP) improve the electrospinnability of the solution. Typically, the use of a single polymer is not sufficient to achieve adequate mechanical properties and degradation processes are necessary for successful electrospinning. Because of this reason, biopolymers can be combined with other polymers to increase the ability of the solution to form fibers (Anu Bhushani and Anandharamakrishnan, 2014; Rostamabadi et al., 2020).

Natural biopolymers (e.g., pectin, cellulose, starch, chitosan, chitin, cyclodextrin, gum, zein, whey protein) can be used in various field especially in delivery of bioactive compounds by using electrospinning technique due to its biocompatibility/ biodegradability, high thermal stability and even anti-bacterial properties. Presence of various functional groups in these biyopolymer offer several opportunities (Rostamabadi et al., 2020).

Because of the polyelectrolyte properties of polysaccharides, nanofibers cannot be produced from polysaccharides such as pectin and sodium alginate without using copolymers. Even if their aqueous solutions can be electrically charged by applying voltage, jet formation cannot be observed because of the chain entanglements necessary to overcome the surface tension.

Pectin solutions do not have sufficient viscoelastic structure and have jet stability problems due to its polyelectrolyte structure and it is not possible to obtain nanofibers from pectin by itself by using electrospinning (Cui et al., 2016; Liu et al., 2016). In literature, PEO (Alborzi et al., 2010; Cui et al., 2017), PVA (Lin et al., 2013), or pullulan (Liu et al., 2016) have been used as co-polymers to prepare electrospun pectin nanofibers. The hydroxyl groups of pectin interact with hydrophilic polymers like PEO and PVA and result in increased chain entanglement between the molecules. As a result, these polymers reduce the electrical repulsive forces and slightly decrease the viscosity of pectin (Alborzi et al., 2010; Cui et al., 2016).

Polyvinyl alcohol (PVA) is considered a non-toxic, biocompatible, and biodegradable polymer that is semi-crystalline and thermoplastic in nature. It has been deemed safe for consumption by the FDA (Food and Drug Administration) and is on the GRAS (Generally Recognized as Safe) list (Williams, 2015; Xu et al., 2022). Polyethylene oxide (PEO) is a semi-crystalline linear polymer that is widely used in various applications due to its biocompatibility and water solubility (Rockwell et al., 2014). PEO is also a FDA-approved polymer (Osorno et al., 2021).

Recently, production of nanofibers using triple blends of alginate-pectin-PEO (Alborzi et al., 2010) or chitosan-pectin-PVA (Lin et al., 2013) has been carried out for biomedical purposes (Cui et al., 2016).

Electrospinning solutions should have a surface tension that enables to form polymer jet. In addition, these solutions should have appropriate electrical conductivity to ensure the formation of fiber jet causing the formation of bead-free nanofibers with fine diameter. It should also have high enough viscosity to prevent the jet from breaking into droplets during the process and to produce continuous smooth fibers, and a suitable polymer concentration to give a bead-free and rod-like morphology (Rostamabadi et al., 2020).

Rockwell et al. (2014) produced nanofibers by electrospinning from citrus pectin (170-230 kDa, 36.4% DE), sugar beet pectin (200 kDa, 55% DE) and apple pectin (30-100 kDa, methoxyl group ≥ 7.1%) by using polyethylene oxide (600 kDa) as a co-polymer. Nanofibers produced from highly esterified citrus and sugar beet pectin had lower crystallinity values as compared to apple pectin. It was found that increased crystallinity of electrospun fibers correlated with smaller fiber diameters and reduced tensile properties.

In a study by Alborzi et al. (2010), apple pomace pectin and sodium alginate (70:30) were mixed in folic acid-NaOH and sodium alginate-pectin solution with low viscosity (250 cPs, LSAP) and medium viscosity (2500 cPs, MSAP) was obtained. Bead-free nanofibers were produced from 4% (w/w) LSAP:PEO (50:50) and 5% (w/w). LSAP:PEO (80:20 to 50:50). Increasing PEO concentration resulted in an increase in fiber diameter, surface tension, and viscosity of solution. However, it causes a decrease in bead formation.

Cui et al. (2017) produced nanofibers from crosslinked pectin+Ca^{+2}, pectin+Ca^{+2}+GLU (glutaraldehyde), pectin+Ca^{+2}+ADH (adipic acid dihydrazide). Three different pectin sources were used: high-methoxylated citrus pectin (856 kDa, 83.6% D-galacturonic acid

(GalA), 70% degree of methoxylation (DM)), low- methoxylated sunflower pectin (208 kDa, 90.4% GalA, 44% DM) and low- methoxylated and amidated apple pomace pectin (355 kDa, 79.4% GalA, 27% DM, 27% DA (degree of amidation)) and also PEO (5000 kDa) was used as a copolymer. Pectin:PEO (95:5) and Triton X-100 and DMSO (dimethyl sulfoxide) were added to the aqueous pectin solution to increase electrospinnability. SEM images showed that crosslinking did not change to the structure of all pectin nanofibers. For all pectin sources, crosslinking with Ca^{+2}+GLU caused a decrease in nanofiber diameter. Crosslinking with Ca^{+2}+ADH caused a decrease in nanofiber diameter for sunflower pectin while an increase was observed for citrus and apple pectin. When X-ray diffraction patterns were examined, it was found that the crosslinking agents slightly affected the crystallinity of the pectin. Crosslinking with Ca^{+2}+ADH improved the mechanical strength of the nanofibers and led to a dramatic increase in Young's modulus of the citrus pectin nanofiber while crosslinking with Ca^{+2} and Ca^{+2}+GLU caused a increase in Young's modulus moderately.

McCune et al. (2018) used lowly esterified pectin, PEO (600 kDa) and Pluronic®F-127 (nonionic surfactant) to produce nanofibers by electrospinning technique. The lowest bead formation was observed when pectin: PEO (60:40 or 65:35) and Pluronic (5%, w/w) was used. Smooth surface and stable nanofiber formation were observed at 0.4 and 0.7 ml/h. Applying voltage in the range of 10-18 kV had no significant effect on the electrospinning process. However, nanofiber formation was not detected above 20 kV. Crosslinking with $CaCl_2$ resulted in macromolecular aggregation of fiber but did not have an effect on nanofiber diameter. Crosslinking with oligochitosan (2-3 kDa) provided a more compact structure and decreased the nanofiber diameter.

In a study by Cui et al. (2016), highly esterified citrus pectin (CP, 66% DE), highly esterified apple pectin (67% DE), lowly esterified amidated apple pectin (39% DE, 20% DA), and lowly esterified sunflower pectin (44% DE) were used. Triton X-100 and DMSO (dimethyl sulfoxide) were added to the aqueous pectin solution to increase electrospinnability. It was aimed to use least PEO in electrospinning solution. Formation of bead-free fibers was achieved at CP:PEO (1000 kDa) ratio of 60:40, 70:30, 80:20 and at CP:PEO (5000 kDa) ratio of 80:20, 90:10, 95:5. The diameter of the fibers decreased as a result of repetitively washing and reducing the PEO concentration to as low as 1.5%.

The removal of PEO by washing was confirmed by disappearance of the peak in DSC thermogram.

Patra et al. (2017) produced nanofibers from citrus pectin and PVA (125 000g mol^{-1}) with homogeneous and bead-free morphology. Smaller nanofiber diameters (400nm) were obtained for pectin:PVA nanofibers as compared to those for PVA nanofibers (600 nm). Higher charge density and crystallinity of pectin led to a decrease in nanofiber diameter and an increase in stretching. It was found that the use of very small amount of pectin (2% w/w) plays an important role in the structural properties of the nanofibers.

Electrospun nanofibers can be used for coating in the food packaging industry. Safari et al. (2020) produced nanofibers from pectin (30-100 kDa, 30%DM), PVA (94-120 kDa) and chitosan (1600 kDa, 86.2% deacetylation). Pectin:PVA (50:50)+chitosan:PVA (50:50) produced more homogeneous nanofiber with fewer beads and smaller diameter as compared to the nanofibers containing chitosan:PVA (75:25)+pectin:PVA (75:25). It was found that the nanofilm (produced from pectin:PVA (50:50)+chitosan:PVA (50:50)) exhibited a significant antibacterial activity against *S. aureus* at 37°C, but had no activity against *E. coli*. Safari et al. (2020) reported that nanofiber film could be used a promising material for active packaging technology in food industry.

Akınalan Balık et al. (2019) produced pectin based electrospun films by using 6.5% (w/w) amidated lowly esterified pectin (27% DE, 20% DA), 3% (w/w) glycerol, and 0.5% (w/w) PEO (2000 kDa). The electrospun pectin-based film was applied as an interlayer between two external layers of electrospun poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) to produce multilayer structures with high barrier properties. The use of electrospun pectin interlayer between two layers of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) was found to be effective in reducing the limonene and water vapour permeability. The barrier performance of PHBV was improved with the incorporation of electrospun pectin interlayer as compared to the multilayer structure based on solution-casted pectin interlayer. These electrospun pectin-based films were utilized in food packaging applications for extending the shelf life of products and as coating materials for aromatic products.

3. MATERIALS AND METHODS

3.1. Materials

Orange samples (*Citrus sinensis*) obtained from a local market in Ankara (Turkey) were used in the study. Pectin from citrus peel (Sigma P9135, Galacturonic acid≥74.0%), PVA (Polyvinyl alcohol, Sigma 363081, 87-89 % hydrolyzed, MW 85,000-146,000), PEO (Polyethylene oxide, Sigma 372803, MW 2,000,000), Triton X-100 (Sigma T8787), D-(+)-galacturonic acid monohydrate (Sigma 92478), crystalline carbazole (Sigma C5132, ≥95%), sulfuric acid (95.0-98.0%), sodium hydroxide, potassium chloride (Sigma P3911, 99.0-100.5%), ethanol (≥ 99.9%) and hydrochloric acid were used in the study.

3.2. Infrared Drying

Orange samples were washed, peeled and cut into 1x1cm pieces. It was observed that cutting into pieces is not the best way for sample preparation since it was not possible to obtain homogeneous thickness for all pieces. Therefore, orange peels were blended by using a mixer (Raks Hanımeli Robot) for 50 sec. The blended orange peels were dried on a tray (26x30cm) in the infrared equipment, as shown in Figure 4.

In preliminary studies, infrared drying at powers of 800W, 900W and 1000W were applied to orange peel samples. When the IR power was higher than 800W, the surface temperature of the samples was 75-85°C. Because of the higher temperature, the upper side of the samples burnt and nonhomogeneous moisture distribution was observed in the inner side. Therefore, in the study, infrared drying was applied at 600W, 700W and 800W.

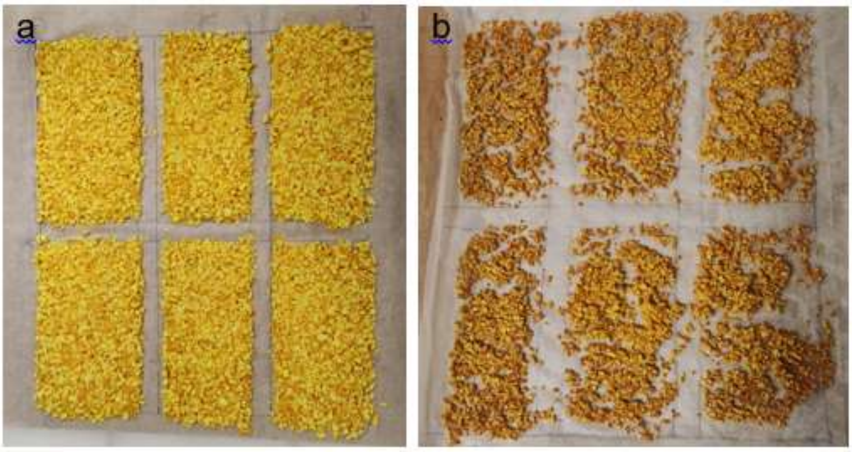


Figure 4. Orange peels before (a) and after (b) infrared or oven drying process

Laboratory scale infrared equipment with a closed drying chamber fitted with twelve 150W halogen lamps (Infrared, BR125 IR; Philips, Eindhoven, The Netherlands) and two aeration channels (12V each) was used in the study. Aluminum reflectors were used on the walls of the equipment to prevent absorption of the light. The lamp system was set to a height of 20 cm from the sample tray. Infrared drying at powers of 600W, 700W and 800W were applied for 30 min. The samples were rotated by 180 degrees at the 15th min. in order to obtain a homogeneous drying. During the infrared application, the surface temperatures of the samples were detected in every 5 min. at six different points by an IR thermometer (Raytek MX6 Infrared Thermometer). IR drying was carried out as eight replicates for each power. In each drying process 24 temperature measurements were taken. The average surface temperatures of the samples dried at 600W, 700W and 800W were 53°C, 57.9°C and 63.1°C, respectively (Table 1). Internal temperature in the infrared cabinet at the beginning and at the end of each infrared treatment are given in Table 1.

Table 1. The surface and internal temperatures during infrared drying

Infrared power-drying time	**Internal Temperature (°C) (beginning-end)**	**Average surface temperature during process (°C)***
600W-30 min.	49-49	53.0
700W-30 min.	58-56	57.9
800W-30 min.	65-64	63.1

*Means are based on eight drying replicates (in each drying process 24 measurements were taken)

Oven drying was carried out for 235 min. and 197 min. until the surface temperature of the blended orange peels was obtained as 60°C and 70°C, respectively. Oven drying was carried out as seven replicates. In each drying process 40 temperature measurements were taken. Average surface temperatures of the samples obtained during oven drying are given in Table 2.

Table 2. The surface temperature and drying time during oven-drying

Oven Temperature (°C) (set)	**Drying Time (min)**	**Average surface temperature during process (°C)***
60	235	57.7
70	197	66.3

*Means are based on seven drying replicates (in each drying process 40 measurements were taken)

After drying, the samples were rested in a fermentation cabinet at 30°C to obtain final moisture contents lower than 12% in all samples.

All replicates for each infrared drying or oven drying were combined in a large batch and ground by using a grinder (Multi Purpose Disintegrator, IC-04A, China). The samples with a particle size larger than 300 μ were also ground by using a blender (Arzum Bebbe, AR 854). The ground samples (<300 μ) were stored at 4°C before analysis.

3.3. Moisture Content

Moisture contents of the dried orange peels and pectin extracted from the dried orange peels were determined at 105°C by using AOAC Method No 20.013 (AOAC, 1990). Analyses were performed in duplicate.

3.4. Pectin Extraction

For extraction of pectin from orange peels, various traditional methods were reported in literature (Kratchanova et al., 2004; Aina et al., 2012; Sayah et al., 2014; Bagde et al., 2017; Fakayode and Abobi, 2018; Tovar et al., 2019; Tsouko et al., 2020; Twinomuhwezi et al., 2020; Kamal et al., 2021). Distilled water (pH 1-3; adjusted by using hydrochloric acid, citric acid, sulfuric acid) was added to dried orange peels and extraction is carried out in a shaking water bath at 70-100°C for 1-3 h. The samples were centrifuged at 7500-10000 rpm for 5-10 min. and filtered by using filter paper. The filtrate was mixed with ethanol at a ratio of 1:1 (v/v) and rested for 1-24 h for pectin precipitation. Pectin was separated by filtration, washed several times with ethanol to remove impurities and then dried in an oven at 35-70°C.

In industry, pectin extraction is usually performed by using strong acids such as hydrochloric, nitric, phosphoric and sulfuric acids. Organic acids such as citric acid cause low hydrolyzing capacity and lower extraction yield, as compared to strong mineral acids such as hydrochloric acid (Rodsamran and Sothornvit, 2019; Belkheiri et al., 2021; Cui et al., 2021). In literature; highest pectin yield (34.81%) was reported for hydrochloric acid as compared to citric acid (Sayah et al., 2014). Rodsamran and Sothornvit, (2019) also reported higher pectin extraction yield for hydrochloric acid (23.59%) as compared to citric acid (19.63%). The highest pectin extraction yield from dried orange peels by using HCl is reported as follows; 27.77% (pH 1, 90°C for 90 min., Fakayode and Abobi, 2018), 23.40% (db, pH 1.6, 95°C for 60 min., Tovar et al., 2019), 15.23% (db, pH 1.54, 100°C for 60 min., Twinomuhwezi et al., 2020) and 21.6% (pH 2, 90°C for 120 min., Kratchanova et al., 2004). Therefore, in the present study hydrochloric acid was used for pectin extraction.

In preliminary studies, various pectin extraction parameters given below were investigated. Pectin yield and color of pectin samples were taken as the main parameters for selection of the extraction method.

- Orange peel / HCl ratio (1:20 and 1:30)
- Extraction in a boiling water bath (90°C for 60, 90 and 120 min.)
- Centrifugation (7,500 rpm for 5 and 10 min.)
- Ethanol precipitation (4°C for 1, 2, 18, 20, 22, 24 h.)
- Washing (1, 3 and 4 times washing with or without magnetic stirring)
- Drying (in an oven (40-50°C), at room temperature)

In the present study, pectin extraction was carried out as follows; distilled water (pH adjusted to 1, 1.5, 2 by using HCl) and dried orange peels (1.5 g, dm) was mixed at a ratio of 1:20 (orange peel / HCl). Pectin was extracted in a shaking water bath at 90°C for 60 min., 90 min., 120 min. The samples were centrifuged at 7500 rpm for 5 min. and filtered by using filter paper. The filtrate was mixed with ethanol at a ratio of 1:1 (v/v) and rested for 20 h for pectin precipitation. Pectin was separated by filtration, washed four times with ethanol to remove impurities and then dried at room temperature overnight. The pectin extraction process was carried out as six replicates. All 6 replicates of each pectin sample were combined in a large batch and ground by using a blender (Arzum Bebbe, AR 854). The samples with a particle size larger than 300 µ were also ground by using a mortar. The ground samples (<300 µ) were stored at 4°C before analysis.

3.4. Yield of Pectin

Yield of pectin extracted under various conditions from dried orange peels was calculated as follows (Kratchanova et al., 2004; Fakayode and Abobi, 2018; Kamal et al., 2021):

$$\text{Yield of pectin (\%)} = \frac{P}{m} \times 100$$

P: the amount of extracted pectin (g, db)
m: amount of dried orange peel (1.5g, db)

Mean values for pectin yield was given as the average of six pectin production replicates.

3.5. Color

Color of the pectin samples extracted from the orange peels dried at different conditions was determined by using Minolta Spectrophotometer (CM-3600d, Japan). The color values were evaluated in terms of CIE L*(lightness/darkness), a*(redness/greenness) and b*(yellowness/blueness). Analyses were performed in duplicate (Dranca et al., 2020).

3.6. Galacturonic Acid Content

Galacturonic acid content was determined by using the method of Ranganna (1979) with minor modifications. Pectin (100 mg) was deesterified with 0.05 N NaOH (100 ml) by mixing for 30 min. 2 ml of the solution was mixed with distiled water in order to make up the total volume to 100 ml. 3 ml of this solution was taken, carbazole (0.1% w/v, 500 µl) was added and white precipitate was observed. Afterwards concentrated sulfuric acid (6 ml) was added to whole of the sample in the tube. After a resting period of 15 min. in a dark place, the absorbance was measured at 525 nm. The calibration curve of D-(+)-Galacturonic acid standard was set in the range of 4-40 mg/L and is given in Figure 5. Analyses were performed in duplicate. In each analysis set commercial pectin sample was also tested in order to test the repeatability.

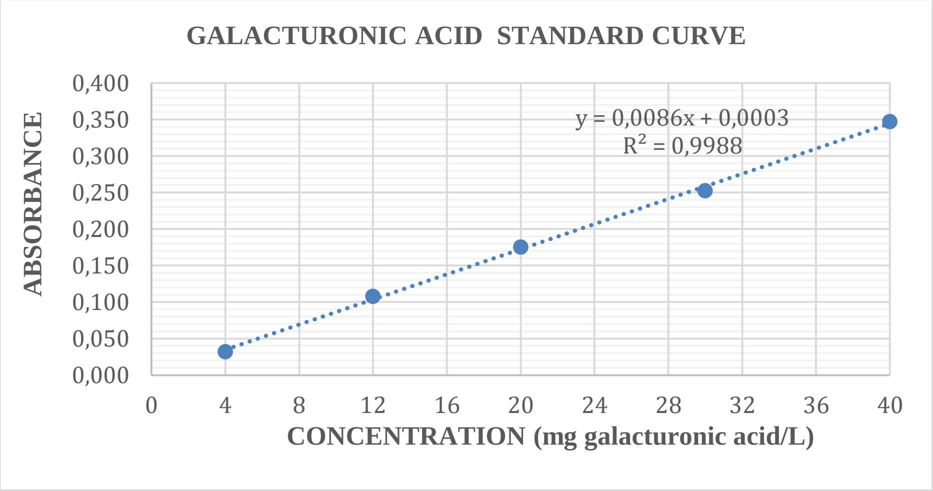


Figure 5. Calibration curve of the galacturonic acid standard

3.7. Degree of Esterification (Titrimetric Method and FTIR)

Degree of esterification (DE) expresses the molar ratio of methyl esters bound to units of galacturonic acid (GalA) (Tiwari et al., 2017).

Degree of esterification by titrimetric method: Method of Tsouko et al. (2020) with some modifications was used for determination of degree of esterification. Pectin (0.1 g) was mixed with ethanol (2 ml) and then dissolved in distilled water (20 ml). The sample was titrated with 0.05 M NaOH by using phenolphthalein as an indicator. The titration volume was recorded as V1 when pink color was observed. Subsequently, for pectin deesterification, excess amount (10 ml) of 0.05 M NaOH was added and the sample was stirred for 20 min. 0.05 M HCl (10 ml) was added for neutralization and stirring was carried out until pink color disappeared. The sample was titrated with 0.05 M NaOH (V2) by using phenolphthalein as indicator. The titration volume was recorded as V2 when pink color was observed. Analyses were performed in duplicate. The degree of esterification was calculated by the formula given below:

$$\mathrm{DE}\ (\%) = \frac{\mathrm{V2}}{\mathrm{V1+V2}} \times 100$$

V1: the number of free carboxyl groups
V2: the number of esterified carboxyl groups

Determination of the degree of esterification by FTIR: Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectra of pectin were recorded by using FTIR spectrophotometer (Nicolet™ iS50, Thermo Scientific™, Waltham, MA) at HUNITEK (Ankara, Turkey, Hacettepe Üniversitesi İleri Teknolojiler Uygulama ve Araştırma Merkezi). Analysis was carried out as 64 scans over a wavenumber range of 4000-500 cm^{-1} with 4 cm^{-1} resolution. FTIR spectra were recorded as duplicated for all samples. Degree of esterification of pectin was calculated using the formula below.

$$R = ((A_{1740}) / (A_{1740} + A_{1630})) \times 100$$

$$\mathrm{DE}\ (\%) = 124.7 \times R + 2.2013$$

A_{1630} and A_{1740} represent the absorption intensities of bands of non-methyl-esterified and methyl-esterified carboxyl groups at 1630 cm^{-1} and 1740 cm^{-1}, respectively (Güzel and Akpınar, 2017; Cui et al., 2020).

3.8. Electrospinning Technique

Pectin solutions were prepared at ratios of 3, 4, 5% (w/w) by using deionized water (60°C) and mixed at room temperature until a clear and homogeneous solution was obtained.

Pectin:PVA solutions were prepared at ratios of 2:5, 2:6, 2.5:5, 2.5:6, 3:5, 3:6 % (w/w) by using deionized water (60°C) and mixed at room temperature until a clear and homogeneous solution was obtained. It was aimed to use pectin with higher concentrations (3, 4, 5% (w/w)) in the pectin: PVA solutions (3:5, 3:6, 4:5, 4:6, 5:5, 5:6% (w/w)). However, no jet formation was observed when the ratio of Pectin:PVA was 3:5 or 3:6 (% w/w), probably due to the higher viscosity and electrical conductivity values (Akınalan Balık et al. , 2019; Safari et al. , 2019). Therefore, it was decided to use lower Pectin:PVA concentrations (2:5, 2:6, 2.5:5, 2.5:6, 3:5, 3:6% (w/w)).

Pectin:PEO solutions were prepared at ratios of 3:1, 3:2, 4:1, 4:2, 5:1 % (w/w) by using deionized water (60°C) and mixed at room temperature until a clear and homogeneous solution was obtained. It was aimed to prepare pectin:PEO at a ratio of 5:2% (w/w), but it was not possible to prepare the solution because of its high viscosity. Firstly, pectin:PEO solutions were prepared without using Triton X-100 but the jet formation was not observed. Therefore, Triton X-100 (1% (w/w)) was added to the mixture as surfactant.

3.8.1. Physicochemical Properties of the Electrospinning Solutions

Viscosity and conductivity are important factors for determination of electrospinning parameters (flow rate, voltage, and the distance between the needle tip and the collector).

Viscosity of the electrospinning solutions (pectin, PEO, pectin:PVA and pectin:PEO) was determined by using Rheometer (Kinexus Pro+, Malvern, UK) at HUNITEK (Ankara, Turkey, Hacettepe Üniversitesi İleri Teknolojiler Uygulama ve Araştırma Merkezi), at 25°C with a shear rate of $50s^{-1}$ (Akınalan Balık et al., 2019; Safari et al., 2020). Analyses were performed in duplicate.

Conductivity of the electrospinning solutions (pectin, PEO, pectin:PVA and pectin:PEO) was measured by a conductivity meter (RS 180-7127, Tayvan) at 25°C. Analyses were performed in duplicate.

3.9. Nanofiber Production by Electrospinning

Electrospinning ESM1100 device (Solvan A.Ş., Ankara, Turkey) was used for production of nanofibers. The system consists of a high voltage power supply (8-35 kV), a rotating cylinder-collector, a syringe pump (NE-300 Syringe Pump, New Era Pump Systems, Inc., New Jersey, USA) with a metal needle syringe.

Electrospun nanofibers were produced at different electrospinning parameters. Electrospinning solutions were placed into a 2.5 ml syringe with 21 gauge stainless steel needle (spinneret). Flow rate of the solution was adjusted between 0.2-0.7ml/h. Various electrical voltages (15-35 kV) were applied between the syringe needle tip and rotating cylinder-collector at a distance of 8-20cm. Based on the preliminary studies, it was decided to use 15cm, 0.5ml/h, 35kV as electrospinning parameters in the nanofibers produced from PEO and various pectin sources.

3.10. Characterization of Pectin Nanofibers

3.10.1. Morphological Analysis (SEM)

Morphologies of the pectin nanofibers were determined by Scanning Electron Microscopy (SEM, Tescan, GAIA3+Oxford XMax 150 EDS, Czech Republic) at HUNITEK (Ankara, Turkey, Hacettepe Üniversitesi İleri Teknolojiler Uygulama ve Araştırma Merkezi). Sample preparation for SEM analysis was carried out as follows; aluminum foil containing nanofibers on its surface was cut randomly and was attached to SEM stub by using carbon tape. The samples were coated with gold-palladium before SEM analysis. Average diameter for 50 bead-free nanofibers of each sample was calculated by using Image J software (Maryland, USA) (Cui et al., 2016; Ye et al., 2016).

3.10.2. Fourier Transform Infrared (FTIR) Analysis

FTIR analysis is used to investigate the chemical structure of the samples. Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectra of powders (pectin or PEO) and nanofibers (pectin+PEO or PEO) were recorded by using FTIR spectrophotometer (Nicolet™ iS50, Thermo Scientific™, Waltham, MA) at HUNITEK (Ankara, Turkey, Hacettepe Üniversitesi İleri Teknolojiler Uygulama ve Araştırma Merkezi). FTIR analysis was carried out only for bead-free nanofibers for which the average diameter distributions were calculated. Analysis was carried out as 64 scans over a wavenumber range of 4000-500 cm-1 with 4 cm-1 resolution (Rockwell et al., 2014; Cui et al., 2016; N. Li et al., 2019).

3.10.3. X-ray diffraction (XRD)

X-ray diffraction (XRD) patterns of powders (pectin or PEO) and nanofibers (pectin+PEO or PEO) were recorded with a XRD diffractometer (Ultima-IV, Rigaku, Japan) at MERLAB (Ankara, Turkey, METU Central Lab). XRD analysis was carried out only for bead-free nanofibers for which the average diameter distributions were calculated. X-ray unit was operated at 40 kV and 30 mA. The radiation used was Cu-K_α Data were collected at 2θ angles of 5–50°. The diffractograms were deconvoluted by using OriginPro7.5 (Rockwell et al., 2014; Cui et al., 2016; Ye et al., 2016).

3.10.4. Differential Scanning Calorimetry (DSC)

Thermal properties of the powders (pectin, PEO or PVA) and nanofibers (pectin+PEO, pectin+PVA or PEO) were determined by using Differential Scanning Calorimetry (DSC) (TA Q20, TA Instruments, USA). 1 mg nanofiber (pectin+PEO, pectin+PVA or PEO) or 5 mg powder (pectin, PEO or PVA) was placed into the aluminum hermetic pan and the pans were hermetically sealed. An empty sample pan was used as reference. Analysis were performed under nitrogen atmosphere by heating the pans from 10 to 300˚C with a heating rate of 10°C/min. DSC was calibrated with indium (Cui et al., 2016; Akınalan Balık et al., 2019). DSC analysis was carried out only for bead-free nanofibers for which the average diameter distributions were calculated.

3.10.5. Water Contact Angle-Wettability

Wettability of pectin nanofibers were measured using a contact angle measurement instrument (Biolin Scientific, Attension Theta, UK) at HUNITEK (Ankara, Turkey, Hacettepe Üniversitesi İleri Teknolojiler Uygulama ve Araştırma Merkezi), operating in static mode at 25°C. Water droplets of 2μL were placed over the samples and the angle was measured immediately (within 10s). The measurements were taken at three different points for each sample and average values are reported (Mouro et al., 2021). Water contact angle of nanofibers was carried out only for bead-free nanofibers for which the average diameter distributions were calculated. Analyses were performed in duplicate.

3.10.6. Color

Color of pectin nanofibers were analyzed using Minolta Spectrophotometer (CM-3600d). The color values were evaluated in terms of CIE L*(lightness/darkness), a*(redness/greenness) and b*(yellowness/blueness). Aluminum foil was used as the target during the calculation of ΔE since the nanofibers were deposited on it. Color values of the aluminum foil were L = 82.55, a*= -0.55, and b* = -1.43

Total color difference (ΔE) was determined by using the following equation:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{0.5}$$

ΔL, Δa, and Δb represent the difference in each color value between the nanofiber and the aluminum foil (Priyadarshi et al., 2021). Color of nanofibers was analyzed only for bead-free nanofibers for which the average diameter distributions were calculated. Analyses were performed in duplicate.

3.11. Statistical Analysis

Duncan’s test was used to determine the differences among main effects (drying method and extraction pH and extraction time) for pectin yield, esterification degree and galacturonic acid content of pectin.

Data related to properties of bulk pectin samples (pectin yield, galacturonic acid, esterification degree), viscosity and electrical conductivity values of electrospinning solutions and water contact angle values of nanofibers were statistically evaluated by one-way analysis of variance (ANOVA). Standard deviations for yield, galacturonic acid, esterification degree, viscosity, electrical conductivity, water contact angle and average diameter distribution were determined by using Microsoft Excel.

4. RESULTS AND DISCUSSION

4.1. Moisture Content of Dried Orange Peels

Orange peel samples were infrared dried at 600W, 700W and 800W for 30 min or oven dried at 60°C and 70°C. Then they were rested in a fermentation cabinet at 30°C until the moisture content is less than 12%. Moisture contents of the dried orange peels are given in Table 3. The moisture contents of the samples were between 11.18-11.88%.

Table 3. Moisture contents of the infrared dried or oven dried orange peels

Drying Method	Moisture Content (%)
IR-600W-30min.	11.38
IR-700W-30min.	11.57
IR-800W-30min.	11.47
Oven-60°C	11.88
Oven-70°C	11.18

4.2. Yield of Pectin Extracted from Dried Orange Peels by Using Different Extraction Conditions

Yield of pectin extracted from infrared or oven dried orange peels by using different extraction conditions were found to be between 1.08-16.99% (Table 4)

Pectin yield increases as the extraction time increases, except for the extraction of infrared dried samples at pH 1. Fakayode and Abobi (2018) and Kamal et al. (2021) also reported an increase in pectin yield by an increase in extraction time.

Among all extraction conditions, extraction at pH 2 resulted in the lowest pectin yield values for all samples (Table 4). Because of the lowest pectin yield values at pH 2, pectin samples extracted at pH 2 were not used in the later stages of the study.

Extraction at pH 1.5 gave lower pectin yield values for oven dried samples as compared to infrared dried samples (Table 4).

Table 4. Yield of pectin extracted from infrared or oven dried orange peels by using different extraction conditions (%, db)*

Extraction Condition		**Pectin Yield (%, db)**				
		Drying Method for Orange Peels				
		IR-600W-30min.	**IR-700W-30min.**	**IR-800W-30min.**	**Oven-60°C**	**Oven-70°C**
pH 1	**60min.**	13.17 ± 0.105	14.85 ± 0.340	12.73 ± 0.327	12.86 ± 0.567	12.75 ± 1.047
	90min.	13.94 ± 0.347	15.15 ± 0.327	12.81 ± 0.144	13.47 ± 0.078	14.10 ± 0.170
	120min.	11.91 ± 0.065	14.35 ± 0.510	12.21 ± 0.216	14.04 ± 0.685	16.99 ± 0.488
pH 1.5	**60min.**	11.80 ± 0.464	7.77 ± 0.000	11.00 ± 0.007	4.15 ± 0.170	4.36 ± 0.148
	90min.	14.10 ± 0.105	10.34 ± 0.353	13.69 ± 0.438	5.36 ± 0.131	5.55 ± 0.214
	120min.	14.84 ± 0.118	11.70 ± 0.634	15.04 ± 0.137	7.42 ± 0.083	8.01 ± 0.179
pH 2	**60min.**	1.48 ± 0.000	1.71 ± 0.065	1.48 ± 0.000	1.40 ± 0.109	1.08 ± 0.087
	90min.	2.85 ± 0.026	2.36 ± 0.065	2.04 ± 0.131	1.79 ± 0.231	1.80 ± 0.083
	120min.	3.52 ± 0.131	3.28 ± 0.065	3.52 ± 0.000	2.45 ± 0.214	2.45 ± 0.061

*Means are based on duplicate analyses

Higher pectin yields were obtained for the samples extracted at pH 1 (90min. for infrared dried, 120 min. for oven dried samples) and pH 1.5-120 min (for infrared dried samples) (Table 4). As pH decreases, demethylation of polygalacturonic acid chains increases (Rodsamran and Sothornvit, 2019). This may result in an increase in pectin solubility leading to an increase in pectin yield.

Among the infrared dried samples extracted at pH 1.5, IR-700W-30min. exhibited the lowest pectin yield. The reason for this can be explained as follows; Pectin methylesterase enzyme (PME) catalyses the removal of methyl esters from the backbone of homogalacturonic acid chains, as mentioned in section 2.2.2. PME is generally quite thermolabile and can be easily inactivated at temperatures <70°C. Optimum temperatures reported for PME (Figure 2) vary between 45 and 55°C, depending on the environmental conditions and enzyme source (Jolie et al., 2010). The average surface temperatures of the samples dried at 600W, 700W and 800W were 53°C, 57.9°C and 63.1°C, respectively. Since the temperature (53°C) at 600W was in the range of optimum temperature for PME, higher pectin yield values were obtained for the samples dried at 600W. Probably, PME was inactivated at 700W and due to the inactivation, lowest pectin yield was obtained for the samples dried at 700W. PME was -also inactive at the surface temperature (63.1°C) obtained for 800W, but higher pectin yield values were obtained for 800W as compared to 700W. The increase in pectin yield for the samples dried at 800W can be explained by the disruption of cellular structures, due to higher infrared power and this resulted in greater pectin extraction efficiency (Lyu et al., 2019; Xiang et al., 2022).

Multiple comparison test results of main factors (drying method, extraction pH and extraction time) for pectin yield are given in Table 5. Multiple comparison test results showed that drying method, extraction pH and extraction time caused significant changes in pectin yield of samples ($p < 0.05$) (Table 5, Appendix 1). It was observed that infrared drying of orange peels significantly increased the pectin yield as compared to oven drying (Table 5). Pectin yield significantly increased, as the extraction pH decreased or the extraction time increased. Significantly highest pectin yield was obtained for the samples extracted at pH 1 or for the samples extracted for 120 min. (Table 5).

Table 5. Multiple comparison test results of main factors (drying method, extraction pH, extraction time) for pectin yield (%, db)*

Main Factors		**Pectin Yield (%, db)**
Drying Method	IR-600W-30min.	9.73 a
	IR-700W-30min.	9.06 c
	IR-800W-30min.	9.39 b
	Oven-60°C	6.99 e
	Oven-70°C	7.45 d
Extraction pH	pH 1	13.69 a
	pH 1.5	9.67 b
	pH 2	2.21 c
Extraction Time	60min	7.51 c
	90min.	8.62 b
	120min.	9.45 a

*For each main factor, values followed by the same letter are not significantly different (α=0.05).

In a study by Rodsamran and Sothornvit (2019), pectin was extracted from lime peels by using acid (HCl, citric acid) and peel/solvent ratio (1:20 and 1:40) and various heating methods (conventional heating at 95°C for 60min. in a water bath) and microwave heating). It was reported that acid type and heating method were the most important factors on the pectin yield. Highest pectin yield was reported as 23.59% by conventional heating. Rodsamran and Sothornvit (2019) reported that microwave heating can be a useful method for extraction of lime peel pectin without any quality loss. Twinomuhwezi et al. (2020) extracted pectin from lemon (*Citrus limon*), tangerine (*Citrus tangerina*) and orange (*Citrus sinensis*) peels. It was reported that the highest pectin yield was obtained at pH 1.27 for all samples. It was reported as 16.61% (db) for lemon, 16.01% (db) for orange and 15.14% (db) for tangerine. Fakayode and Abobi (2018) investigated the effect of extraction temperature (80, 85, 90, 95, 100°C), time (60, 75, 90, 105 and 120 min.) and pH (adjusted with HCl to 1, 1.5, 2, 2.5 and 3) on the yield of pectin extracted from orange peels. It was found that the pectin yield ranged from 12.93-29.05%. Increasing the extraction temperature and time resulted in an increase in pectin yield. Maximum yield (29.05%) was obtained for pH 1.5, 95°C and 105 min. Kamal et al. (2021) also reported that pectin yield increased as extraction temperature and time increased. Among all extraction parameters studied, the highest (23.39%) yield of pectin extracted from orange peels was reported for pH 1.5, 90°C and 105min.

In our study, yield of pectin extracted from dried orange peels by using different extraction conditions were found to be between 1.08-16.99% on dry basis and 1.17-18.37% on wet basis. The highest pectin yield obtained in our study was similar with the pectin yield (16.01%, db) given on dry basis for orange by Twinomuhwezi et al. (2020) for orange. Higher pectin yields were reported by Fakayode and Abobi (2018) (29.05%, pH 1.5 95°C and 105 min.) and Kamal et al. (2021) (23.39%, pH 1.5, 90°C and 105min.). However, it was not indicated whether the results were on a dry basis or not.

4.3. Color Values of the Pectin Samples Extracted from Dried Orange Peels by Using Different Extraction Conditions

Color values of the pectin samples extracted from dried orange peels by using various extraction conditions are given Table 6. L*, a* and b* color values of the commercial pectin sample were 83.09 ± 0.205, 2.41 ± 0.099 and 11.01 ± 0.177, respectively. L* color values of the pectin samples produced in the study were in the range of 49.41-67.16. L* values were found to be lower than that of commercial pectin sample. L* values of some of the pectin samples extracted from infrared dried samples were slightly higher than those of the oven dried samples. Longer extraction time generally resulted in a decrease in the lightness of the pectin samples. This was probably due to increased rate of Maillard reaction and caramelization (Rodsamran and Sothornvit, 2019). a* and b* color values of pectin samples produced in the study were in the range of 1.08-3.89 and 7.11-15.25, respectively (Table 6). a* and b* color values were similar to those of commercial pectin sample.

Table 6. Color values of the pectin samples extracted from dried orange peels by using different extraction conditions*

Extraction Condition			**Color Values of the Pectin Samples**				
			Drying Method for Orange Peels				
			IR-600W-30min.	**IR-700W-30min.**	**IR-800W-30min.**	**Oven-60°C**	**Oven-70°C**
pH 1	**60min.**	L*	62.22 ± 0.735	57.54 ± 0.353	59.75 ± 0.926	58.32 ± 0.191	55.14 ± 0.113
		a*	3.00 ± 0.332	3.12 ± 0.141	2.73 ± 0.021	3.19 ± 0.184	2.97 ± 0.559
		b*	12.74 ± 0.148	12.93 ± 0.969	12.28 ± 0.177	13.50 ± 0.311	10.02 ± 0.445
	90min.	L*	57.05 ± 0.078	55.56 ± 0.651	59.74 ± 0.262	54.20 ± 1.626	52.16 ± 0.050
		a*	3.01 ± 0.028	3.61 ± 1.061	3.16 ± 0.933	3.82 ± 1.025	3.89 ± 0.290
		b*	10.06 ± 0.955	12.24 ± 2.051	11.36 ± 1.570	12.28 ± 1.492	9.60 ± 1.980
	120min.	L*	57.02 ± 0.948	55.51 ± 0.240	53.62 ± 1.301	51.38 ± 0.021	49.41 ± 0.806
		a*	2.60 ± 0.021	2.74 ± 0.962	2.69 ± 0.672	3.42 ± 0.156	3.53 ± 0.184
		b*	9.45 ± 0.311	9.48 ± 0.948	7.11 ± 1.393	9.52 ± 0.127	8.24 ± 0.735
pH 1.5	**60min.**	L*	60.31 ± 0.049	65.20 ± 0.198	67.16 ± 0.226	57.16 ± 1.237	63.74 ± 0.361
		a*	2.20 ± 0.134	1.24 ± 0.134	1.08 ± 0.127	1.67 ± 0.559	1.33 ± 0.212
		b*	13.63 ± 0.438	13.81 ± 0.021	13.78 ± 0.057	10.49 ± 1.549	12.26 ± 0.269
	90min.	L*	56.65 ± 0.764	63.93 ± 1.181	60.90 ± 0.651	56.43 ± 1.506	62.23 ± 0.721
		a*	1.28 ± 0.311	1.39 ± 0.318	2.43 ± 0.042	1.48 ± 0.502	1.81 ± 0.771
		b*	8.88 ± 0.502	13.31 ± 1.004	14.08 ± 0.332	10.07 ± 1.372	12.57 ± 1.301
	120min.	L*	54.93 ± 1.259	62.57 ± 0.099	60.78 ± 0.806	57.00 ± 1.711	60.61 ± 0.304
		a*	2.74 ± 0.339	2.45 ± 0.523	3.26 ± 0.141	2.91 ± 0.255	3.09 ± 0.262
		b*	10.94 ± 0.078	14.55 ± 1.054	15.25 ± 0.693	12.74 ± 0.643	15.21 ± 0.226

Color values of the commercial pectin sample: 83.09 ± 0.205 (L*), 2.41 ± 0.099 (a*), 11.01 ± 0.177(b*)

*Means are based on duplicate analyses

Rodsamran and Sothornvit (2019) extracted pectin from lime peels by using conventional heating (95°C for 60 min.) with different acids (HCl and citric acid), peel/solvent ratios (1:20 and 1:40). L* color values of the pectin samples were in the range of 40.82-49.05. It was reported that pectin extracted by using HCl resulted in higher a* (1.91-2.08) and b* (9.84-10.96) values, as compared to citric acid extraction. As compared to the results reported for lime peel pectin by Rodsamran and Sothornvit (2019), higher L* color values (indicating more lightness) were obtained for the orange peel pectin samples produced in our study. a* and b* color values obtained in the present study were similar to those reported by Rodsamran and Sothornvit (2019). Bagde et al. (2017) reported the colors of pectin extracted from orange peel as pale yellow and the colors of pectin extracted from lemon as white. Twinomuhwezi et al. (2020) also reported the colors of pectin extracted from orange and lemon as pale yellow and from tangerine as brown.

4.4. Galacturonic Acid Contents of the Pectin Samples Extracted from Dried Orange Peels by Using Different Extraction Conditions

Galacturonic acid (GA) contents of the pectin extracted from dried orange peels by using different extraction conditions are given Table 7. Galacturonic acid content is used to indicate the purity of pectin (Rodsamran and Sothornvit, 2019). The galacturonic acid contents of the pectin samples were found to be between 72.79-98.66%. Galacturonic acid content of the commercial pectin was found to be as 87.62%, compatible with the value given on the label (>74%). Most of the pectin samples produced in the present study had galacturonic acid values comparable with that of commercial pectin. For the pectin samples extracted at pH 1, galacturonic acid contents (72.79%-98.66%) were generally higher for the infrared dried orange peels as compared to those for oven dried ones. However, all the pectin samples produced in the study had high purity.

Table 7. Galacturonic acid contents (%) of the pectin samples extracted from dried orange peels by using different extraction conditions*

Extraction Condition		**Galacturonic Acid Content (%)**				
		Drying Method for Orange Peels				
		IR-600W-30min.	**IR-700W-30min.**	**IR-800W-30min.**	**Oven-60^{o}C**	**Oven-70^{o}C**
pH 1	**60min.**	81.80 ± 0.822	72.79 ± 2.878	78.90 ± 0.822	73.66 ± 4.111	76.57 ± 6.578
	90min.	78.31 ± 0.822	74.83 ± 6.578	88.20 ± 1.644	82.67 ± 2.056	75.99 ± 4.933
	120min.	82.38 ± 1.644	83.26 ± 1.233	88.20 ± 0.822	79.48 ± 0.822	78.90 ± 3.289
pH 1.5	**60min.**	87.03 ± 0.822	87.03 ± 4.111	84.42 ± 8.633	82.97 ± 0.822	78.31 ± 1.644
	90min.	88.78 ± 3.289	80.06 ± 7.400	88.49 ± 6.167	94.30 ± 6.167	89.94 ± 2.467
	120min.	75.70 ± 1.233	78.90 ± 1.644	88.49 ± 4.522	98.66 ± 4.111	80.06 ± 2.467

Galacturonic acid content (%) of the commercial pectin: 87.62 ± 3.289.

*Means are based on duplicate analyses.

Multiple comparison test results of main factors (drying method, extraction pH and extraction time) for galacturonic acid content are given in Table 8. Multiple comparison test results showed that drying method and extraction time caused significant changes in galacturonic acid contents of the pectin samples ($p < 0.05$) (Table 8, Appendix 2). Effect of extraction pH on galacturonic acid content of the pectin samples was insignificant.

Table 8. Multiple comparison test results of main factors (drying method, extraction pH, extraction time) for galacturonic acid content (%)*

Main Factors		**Galacturonic Acid Content (%)**
Drying Method	IR-600W-30min.	82.34 bc
	IR-700W-30min.	79.48 c
	IR-800W-30min.	86.12 a
	Oven-60°C	85.29 ab
	Oven-70°C	79.96 c
Extraction pH	pH 1	79.73 a
	pH 1.5	85.54 a
Extraction Time	60min	80.35 b
	90min.	84.16 a
	120min.	83.40 a

*****For each main factor, values followed by the same letter are not significantly different (α=0.05).

The highest galacturonic acid content was obtained for the pectin samples extracted from IR-800W-30min samples (Table 8). The galacturonic acid contents of the pectin samples extracted from Oven-60°C samples were not significantly different than those of IR-800W-30min samples. Higher galacturonic acid values were obtained for the samples extracted for 90 or 120 min., as compared to the ones extracted for 60 min.

Rodsamran and Sothornvit (2019) reported that galacturonic acid contents of pectin samples extracted from lime peel were between 79.29 - 95.93%. Pectin samples were extracted from lime peel by using different extraction methods (conventional heating and microwave heating). Pectin samples extracted by using conventional heating exhibited higher levels of galacturonic acid content (91.00-95.93%) in comparison to samples extracted by using microwave heating (79.29-89.86%). Rodsamran and Sothornvit (2019) reported that microwave heating can be a useful method for extraction of lime peel pectin without any quality loss. It was reported that acid type (HCl and citric acid) and peel/solvent ratio (1:20 and 1:40) did not significantly affect the galacturonic acid content. In a study by Su et al., (2019) galacturonic acid content of pectin extracted from orange peels were reported in the range of 23.4-63.5%. Higher galacturonic acid contents were reported as the pH increased. As compared to the results given by Su et al. (2019) for orange peel pectin, higher galacturonic acid contents (72.79-98.66%) were found in our study.

4.5. FTIR Spectra and Esterification Degree of the Pectin Samples Extracted from Dried Orange Peels by Using Different Extraction Conditions

FTIR spectra of the pectin samples extracted from infrared or oven dried orange peels by using various conditions are shown in Figure 6. FTIR absorption bands of the related peaks given in literature are shown in Table 9.

FTIR spectra of the pectin samples produced in the present study were examined and it was found that all pectin samples had characteristic absorption peaks of pectin similar to the ones given in literature (Table 9). Similar spectra were also obtained for the commercial pectin sample.

Esterification degree was calculated by using the absorption intensities of the bands approximately at 1630 cm-1 and 1740 cm-1 in the FTIR spectra of the pectin samples. Esterification degrees of the pectin samples were given in Table 10.

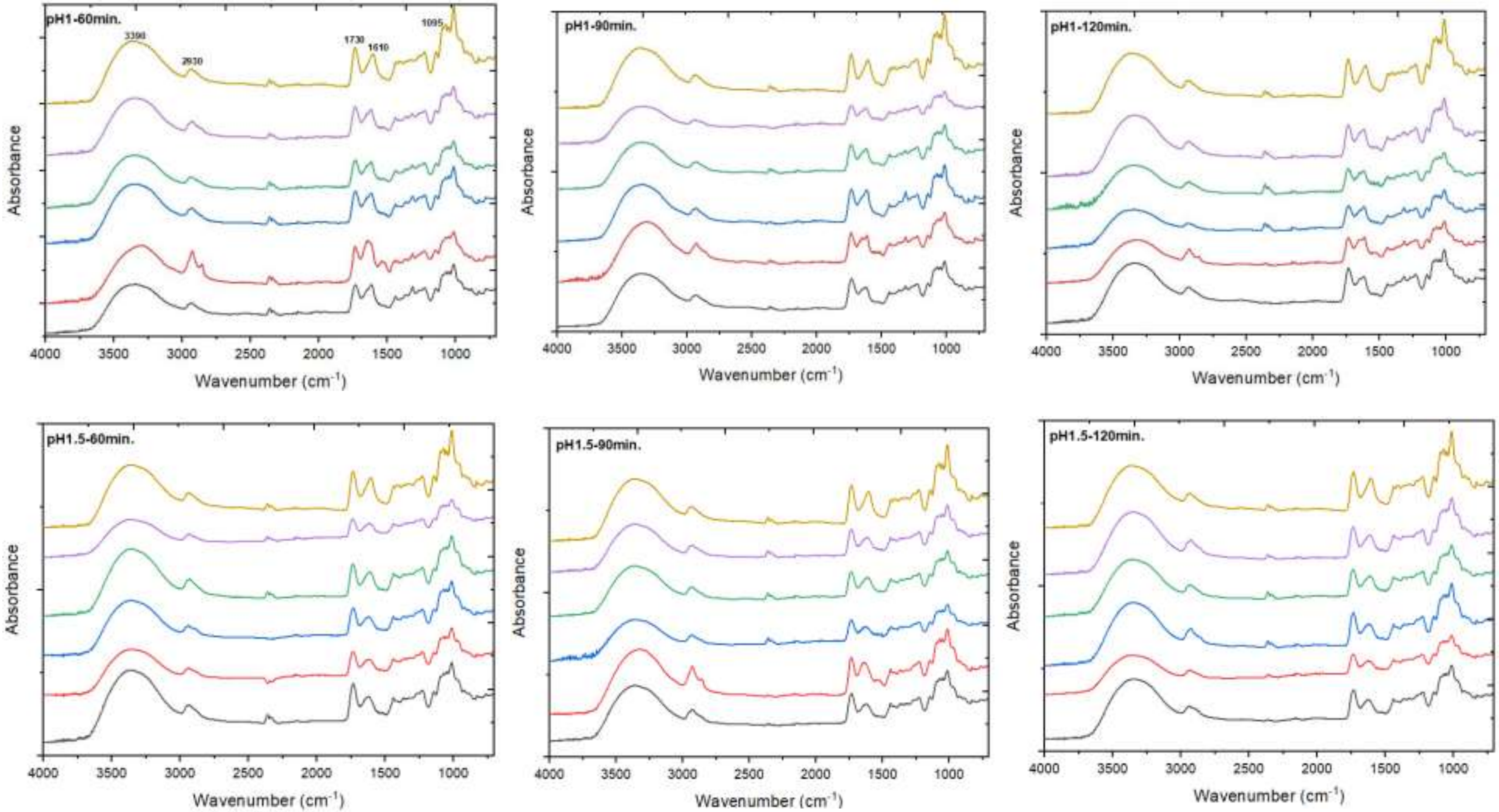


Figure 6. FTIR spectra of pectin samples extracted from infrared or oven dried orange peels at pH1 for 60min, 90min, 120min, at pH1.5 for 60min, 90min, 120min (yellow: commercial pectin sample) (orange peel drying conditions; black:600W-30min, red:700W-30min, blue:800W-30min, green: Oven-60°C, purple:Oven-70°C)

Table 9. FTIR absorption bands of related peaks of pectin given in literature

Type of vibration	**Absorption band location (cm^{-1})**	**Reference**
Inter and intramolecular hydrogen bonding	3600- 3400 cm^{-1}	(Güzel and Akpınar, 2017)
	3600- 3200 cm^{-1}	(Lyu et al., 2019)
	3429 cm^{-1}	(Su et al., 2019)
C-H stretching band	3000–2800 cm^{-1}	(Güzel and Akpınar, 2017)
	2900 cm^{-1}	(Lyu et al., 2019)
	2928 cm^{-1}	(Su et al., 2019)
C=O stretching vibration of esterified carboxyl groups and free carboxyl groups	1740 and 1630 cm^{-1}	(Güzel and Akpınar, 2017)
	1730 and 1654 cm^{-1}	(Rodsamran and Sothornvit, 2019)
Degree of esterification	**1734 and 1616 cm^{-1}**	(Güzel and Akpınar, 2017)
	1800-1500 cm^{-1}	(Lyu et al., 2019)
	1800-1500 cm^{-1}	(Rodsamran and Sothornvit, 2019)
	1745 and 1608 cm^{-1}	(Su et al., 2019)
stretching vibrations of C-OH side groups and the C-O-O glycosidic bond vibrations	1015-1100 cm^{-1}	(Güzel and Akpınar, 2017)
	1100–1000 cm^{-1}	(Lyu et al., 2019)
	1099 cm^{-1}	(Rodsamran and Sothornvit, 2019)

Esterification degree of the pectin samples extracted from dried orange peels by using different extraction conditions were found to be between 59.22-94.06%. Since the degree of esterification values were greater than 50% (reported value for orange pectin in literature, (Rodsamran and Sothornvit, 2019)) pectin samples produced in the present study can be classified as highly esterified pectin.

Table 10. Esterification degree (%, determined by using FTIR Spectra) of the pectin samples extracted from dried orange peels by using different extraction conditions*

Extraction Conditions		**Esterification Degree (%)**				
		Drying Method for Orange Peels				
		IR-600W-30min.	**IR-700W-30min.**	**IR-800W-30min**	**Oven-60°C**	**Oven-70°C**
pH 1	**60min.**	65.31 ± 2.090	59.22 ± 3.531	68.99 ± 0.511	66.51 ± 6.992	70.28 ± 3.179
	90min.	65.46 ± 2.779	66.65 ± 5.438	66.26 ± 1.556	76.81 ± 4.976	63.42 ± 4.090
	120min.	71.73 ± 2.080	63.94 ± 0.059	69.13 ± 0.391	78.60 ± 4.179	70.23 ± 5.244
pH 1.5	**60min.**	94.06 ± 0.125	78.92 ± 9.066	82.41 ± 1.472	73.13 ± 0.286	74.74 ± 0.812
	90min.	77.59 ± 1.659	76.43 ± 4.456	76.32 ± 3.790	68.29 ± 0.872	74.08 ± 0.058
	120min.	79.79 ± 0.593	74.47 ± 4.094	78.01 ± 0.638	78.76 ± 6.642	74.15 ± 0.033

Esterification degree (%) of commercial pectin: 73.86 ± 1.270

*Means are based on duplicate analyses.

Multiple comparison test results of main factors (drying method, extraction pH and extraction time) for esterification degree (determined by using FTIR Spectra) are given in Table 11. Multiple comparison test results showed that drying method and extraction time caused significant changes in esterification degree of pectin samples ($p < 0.05$) (Table 11, Appendix 3).

The highest esterification degree (determined by using FTIR Spectra) was obtained for the pectin samples extracted from IR-600W-30min samples (Table 11). The pectin samples extracted from IR-800W-30min. and IR-600W-30min samples were not significantly different than those of Oven-60°C samples in terms of esterification degree. Although higher esterification degrees were obtained for the samples extracted at pH 1.5, they were not significantly different than those for the samples extracted at pH 1. When extraction time was taken into account, extraction for 120 min gave the highest esterification degree but it was not statistically different than that for 60 min.

Table 11. Multiple comparison test results of main factors (drying method, extraction pH, extraction time) for esterification degree (%, determined by using FTIR Spectra) of the pectin samples*

Main Factors		**Esterification Degree (%)**
Drying Method	IR-600W-30min.	75.65 a
	IR-700W-30min.	69.94 c
	IR-800W-30min.	73.52 ab
	Oven-60°C	73.68 ab
	Oven-70°C	71.15 bc
Extraction pH	pH 1	68.17 a
	pH 1.5	77.41 a
Extraction Time	60min	73.36 ab
	90min.	71.13 b
	120min.	73.88 a

*****For each main factor, values followed by the same letter in the same column are not significantly different (α=0.05).

Su et al. (2019) calculated esterification degree of orange peel pectin by using FTIR spectra. Esterification degree of pectin extracted by using conventional, microwave or surfactant-microwave assisted extraction was found to be 54.8, 64.5 and 73.3%, respectively. Esterification degree (59.22-94.06%, determined by using FTIR spectra) of the orange peel pectin produced in our study was higher as compared to that given by Su et al. (2019).

4.6. Determination of the Best Pectin Extraction Method

Best pectin extraction method was determined by investigating the properties of the pectin samples and pectin nanofibers produced in the preliminary studies.

According to the overall results, all pectin samples extracted by using different conditions had high esterification degree and high purity. As can be seen from Table 8 and Table 11, extraction pH did not have a significant affect on galacturonic acid and esterification degree of the pectin samples. However, extraction pH had a significant effect on pectin yield. The highest pectin yield was obtained for the samples extracted at pH 1 (Table 5). When all extraction conditions were taken into account (Table 4), yield for pectin extracted from oven dried orange peels were higher for pH 1-120 min. and yield for pectin extracted from infrared dried orange peels were generally higher for pH 1.5-120 min. Considering the explanations mentioned above, extractions at pH1-120min. and pH1.5-120min. were selected as the extraction conditions. In the preliminary studies of nanofiber production it was decided to use pectin extracted from orange peels infrared dried at 800W in order to observe the predominant effect of infrared.

Nanofibers were produced from the pectin samples (extracted at pH1-120min. and pH1.5-120min.) by using different electrospinning parameters and the morphologies (SEM images) were investigated.

In preliminary studies, pure pectin solutions (3, 4, 5% (w/w)) extracted from IR-800W-30min dried orange peels were used for nanofiber production. However, it was not possible to obtain fibers from pectin solutions by itself. Instead of continuous jet formation, pure pectin solutions formed droplets during electrospinning, due to its polyelectrolyte structure, insufficient viscoelasticity and chain entanglements (Cui et al., 2016; Liu et al., 2016; Akınalan Balık et al., 2019).

Mixing pectin with PVA or PEO resulted in continuous nanofiber formation by electrospinning due to improved electrospinnability. Pectin extracted from IR-800W-30min dried orange peels at pH1-120min. or at pH1.5-120min. were mixed with PVA at a ratio of 1.2:9.6 (%w/w) and the mixtures were electrospun at different distances (8 and 10 cm), flow rates (0.4 and 0.7 ml/h) and voltages (15 and 30 kV). SEM images, electrospinning parameters and average diameter distributions of the nanofibers are given in Figure 7. The electrospun nanofibers produced from all solutions were continuous, bead-free and had a uniform diameter. As shown in Figure 7, the diameters of pectin+PVA nanofibers produced by using the pectin extracted at pH1-120min. were 184 ± 52nm, 134 ± 21nm and 187 ± 29nm. Smaller diameters (121 ± 22nm, 110 ± 21nm, 107 ± 19nm) were obtained for the pectin extracted at pH1.5-120min.

The preliminary results obtained for electrospun nanofibers confirmed that extraction of pectin at pH1.5 is the key parameter for finest fiber formation with better morphology. Therefore it was decided to use the parameter pH1.5-120min. as extraction condition for bulk pectin production from dried orange peels.

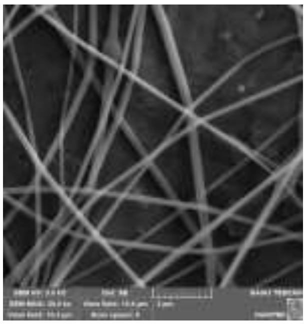
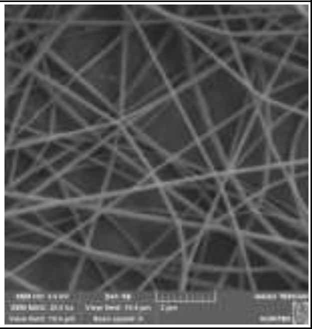
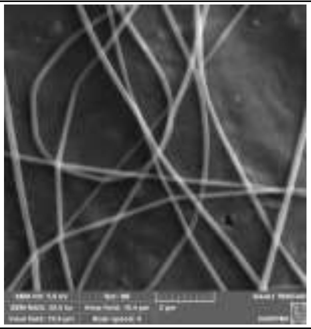
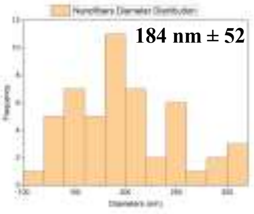
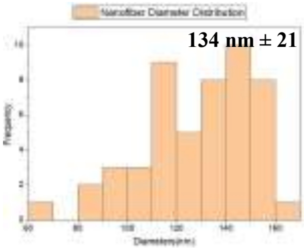
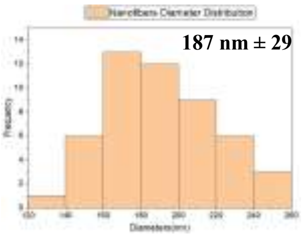
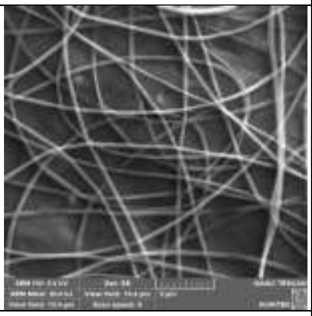
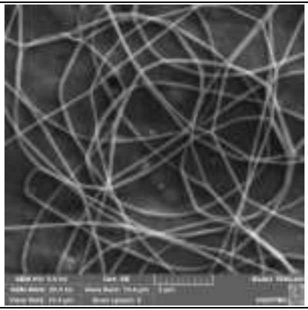
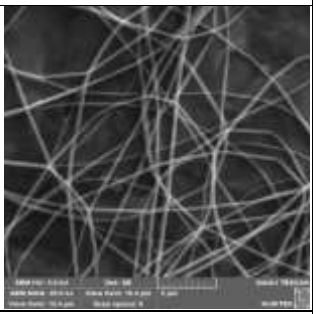
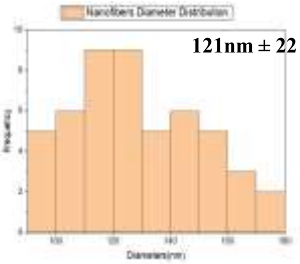
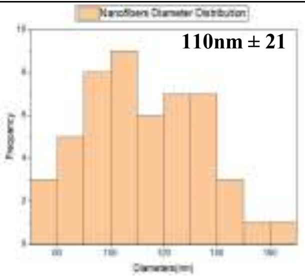
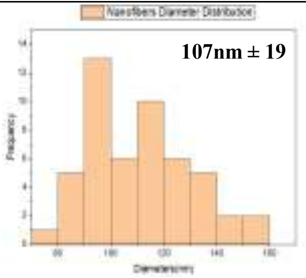
Extraction Conditions	**Electrospinning Parameters**		
	8cm, 15 kV		**10 cm, 30 kV**
	0.4 ml/h	**0.7 ml/h**	**0.7 ml/h**
pH1-120min.			
			
pH1.5-120min.			
			

Figure 7. SEM images of nanofibers produced from pectin:PVA (1.2:9.6 (%w/w)) solutions
(Pectin extracted from IR-800W-30min dried orange peels, at pH1-120min. or at pH1.5-120min.)

4.7. Yield of Bulk Pectin Extracted from Dried Orange Peels, at pH 1.5-120 min

Yield of bulk pectin extracted from infrared or oven dried orange peels at pH 1.5 for 120 min is given Table 12. Pectin yield was found to be between 7.09 – 11.70%. The analysis of variance (ANOVA) results showed that the effect of orange peel drying method on pectin yield was significant ($p < 0.05$).

Table 12. Yield of bulk pectin extracted from infrared or oven dried orange peels at pH 1.5-120 min (%, db)*

Drying method for orange peel	Pectin Yield (%, db)
IR-600W-30 min.**	8.18 ± 0.466^{b}
IR-700W-30 min.	7.81 ± 0.754^{b}
IR-800W-30 min.	11.70 ± 0.147^{a}
Oven-60°C***	8.27 ± 0.263^{b}
Oven-70°C	7.09 ± 0.281^{c}

* Values followed by the same letter are not significantly different (α= 0.05). Means are based on six production replicates

** Pectin was extracted at pH1.5, 120min from orange peels infrared dried at 600W-30min.

***Pectin was extracted at pH1.5,120min from orange peels oven dried at 60°C

Pectin extraction from orange peel infrared dried at 800W resulted in the highest pectin yield (11.70%, db). As mentioned and explained in section 4.2, significantly higher pectin yield values were obtained for the orange peels dried at 800W as compared to those for orange peels dried at 700W and 600W. Pectin methylesterase (PME) is active at the surface temperature (53°C) obtained at 600W but is inactive at the surface temperatures obtained at 700W (57.9°C) and 800W (63.1°C). Since the PME is active during drying at 600W, higher pectin yield values were obtained for the samples dried at 600W as compared to 700W. The increase in pectin yield by infrared drying of orange peels at 800W can be explained by the disruption of cellular structures by infrared, due to higher infrared power, resulting in greater pectin extraction efficiency (Lyu et al., 2019; Xiang et al., 2022).

Infrared drying of orange peels lasted for only 30 min. The oven drying time at 60°C and 70°C was 235 min and 197 min, respectively. It can be concluded that drying of orange peels by using infrared made it possible to produce higher yields of pectin in a shorter time, as compared to oven drying.

Kratchanova et al. (2004) different microwave power (0.45, 0.63, 0.9 kW) and times (5, 10, 15 and 20min) were applied to orange peels and the effects of microwave power and times on yield and properties of pectin extracted at pH 1.5, 80-82°C for 60 min were investigated. While the highest yield was found as 18% at the highest power (0.90kW-10min.), the lowest yield was obtained 6% for the control sample.

In a study by Lyu et al. (2019), it was reported that osmotic dehydration was applied to peach samples by using different sucrose solutions (100, 300, and 500 g/L), at room temperature for 4 h. The samples were then infrared-treated at 675W, 80°C, 60-90min. and controlled pressure drop was applied to the samples. The samples were dried under vacuum at 60°C. Water-soluble pectin content of the infrared-treated samples was found to be 110 mg/g alcohol-insoluble residue. It was found that the water-soluble pectin content decreased when infrared was applied after osmotic dehydration.

In terms of drying method for orange peels, it was more convenient to use the results of Lyu et al. (2019) for comparison of the pectin yield with the results of our study. For infrared treated samples an increase in pectin yield was observed in our study. Lyu et al. (2019) reported a decrease in water-soluble pectin content of infrared treated peach samples.

It can be concluded that pectin extraction efficiency can be increased when novel technologies were used in drying of pectin sources. This is confirmed by the results of the present study for infrared dried orange peels and by the results of Kratchanova et al. (2004) for microwave heated orange peels.

4.8. Color of Bulk Pectin Samples Extracted from Dried Orange Peels at pH 1.5-120 min.

Color values of bulk pectin samples extracted from dried orange peels at pH 1.5-120 min are given Table 13. L*, a* and b* color values of the commercial pectin were 83.09, 2.41 and 11.01, respectively. L* value of the commercial pectin sample was higher than L* values (58.50-63.86) of the pectin samples produced in the study. Infrared drying of orange peels resulted in pectin samples with slightly higher L* values as compared to oven dried samples. a* and b* color values of the pectin samples produced in the study were in the range of 1.91-3.71 and 13.18-16.05, respectively (Table 13). b* color values of the pectin samples produced in the study were found to be slightly higher as compared to that of commercial pectin sample.

As the IR power or oven drying temperature was increased, L* (lightness) value was slightly decreased, a* (redness) and b* (yellowness) values were slightly increased.

Table 13. Color of bulk pectin samples extracted from dried orange peels at pH1.5-120min.*

Pectin samples	**Color Values**		
	L*	**a***	**b***
IR-600W-30min.**	63.86 ± 0.707	1.91 ± 0.248	13.30 ± 0.721
IR-700W-30min.	62.81 ± 0.304	2.94 ± 0.191	15.58 ± 0.502
IR-800W-30min.	60.92 ± 0.883	3.71 ± 0.014	16.05 ± 1.146
Oven-60°C***	59.45 ± 1.775	2.86 ± 0.396	13.18 ± 0.629
Oven- 70°C	58.50 ± 1.054	3.67 ± 0.665	14.83 ± 0.990
Commercial Pectin	83.09 ± 0.205	2.41 ± 0.099	11.01 ± 0.177

***** Means are based on duplicate analyses.
****** Pectin was extracted at pH1.5, 120min from orange peels infrared dried at 600W-30min.
*******Pectin was extracted at pH1.5,120min from orange peels oven dried at 60°C

Wang et al. (2015) reported L*, a* and b* values of the grapefruit peel pectin samples extracted by conventional heating method or ultrasound-assisted heating extraction as 79.98, 0.01, 10.51 or 82.21, -0.73, 16.78, respectively. Rodsamran and Sothornvit (2019) reported L*, a* and b* values of the lime peel pectin samples as 40.82-49.05, 1.91-2.08 and 9.84-10.96, respectively. L* values for the orange peel pectin samples produced in our study were found to be higher as compared to those reported by Rodsamran and Sothornvit (2019) for lime peel pectin and lower as compared to those reported by Wang et al. (2015) for grapefruit peel pectin.

4.9. Esterification Degree (Titrimetric Method) and Galacturonic Acid Content of Bulk Pectin Samples Extracted from Dried Orange Peels at pH 1.5-120 min

Esterification degree (determined by titrimetric method) and galacturonic acid content of bulk pectin extracted from infrared or oven dried orange peels at pH 1.5-120 min. is given Table 14. ANOVA results showed that the effect of drying method on esterification degree and galacturonic acid content of pectin was significant ($p < 0.05$) (Table 14).

Esterification degree of the pectin samples produced in the study was found to be between 53.59-81.25%. Pectin samples extracted from infrared dried orange peels had significantly higher esterification degree as compared to those extracted from oven dried orange peels. Esterification degree of commercial pectin sample was significantly higher than that of Oven-70°C sample. However, it was not significantly different than that of Oven-60°C sample. Pectin samples produced in the present study can be classified as highly esterified pectin since the degree of esterification values were greater than 50% (reported value for orange pectin in literature, Rodsamran and Sothornvit (2019)).

Galacturonic acid contents of the pectin samples produced in the study were found to be between 74.17-80.12% (Table 14). High purity pectin was produced in the present study. Galacturonic acid content of the commercial pectin was found to be as 83.99%, compatible with the value given on the label (>74%). Galacturonic acid values of the most of the pectin samples (except IR-600W-30min., Oven-60°C) produced in the present study were not significantly different than that of the commercial pectin sample. Galacturonic acid content increased as the infrared power increased. However, the increase was not significant.

Table 14. Esterification degree (determined by titrimetric method) and Galacturonic acid content of bulk pectin extracted from dried orange peels at pH1.5-120min (%)*

Pectin Samples	**Esterification Degree (%)**	**Galacturonic Acid Content (%)**
IR-600W-30min.**	80,63 ± 0.884^{a}	74,17 ± 2.946^{b}
IR-700W-30min.	81,25 ± 0.000^{a}	78,33 ± 2.104^{ab}
IR-800W-30min.	76,47 ± 0.000^{b}	80,12 ± 3.788^{ab}
Oven-60°C***	57,89 ± 0.000^{c}	74,46 ± 1.684^{b}
Oven-70°C	53,59 ± 1,353^{d}	76,55 ± 3.788^{ab}
Commercial Pectin	57,43 ± 1,281^{c}	83,99 ± 3.367^{a}

* Values followed by the same letter in the same column are not significantly different (α= 0.05). Means are based on duplicate analyses.
** Pectin was extracted at pH1.5, 120min from orange peels infrared dried at 600W-30min.
***Pectin was extracted at pH1.5,120min from orange peels oven dried at 60°C

The esterification degree results of the present study were compared with the literature in which the esterification degree of the pectin samples was determined by using titrimetric method. In a study by Kamal et al. (2021), the highest esterification degree (77.56%) was obtained for the pectin extracted from orange peel at pH 2.5, 95°C and 60min. The esterification degree for pectin extracted from orange peel at pH 1.5, 95°C and 60min. was reported as 76.02%. Rodsamran and Sothornvit (2019) reported that esterification degree of pectin samples extracted from lime peel was between 70.81-91.58%. It was reported that acid type (HCl and citric acid) and peel/solvent ratio (1:20 and 1:40) did not significantly affect the esterification degree of pectin. Lyu et al. (2019) also reported that esterification degree of pectin extracted from peach samples were lower for infrared applied samples (57.9%), as compared to control sample (75%). The results of the present study were compared with the results of Lyu et al. (2019) since infrared was used in the both of the studies. Higher esterification degree values (53.59-81.25%) were found in our study as compared to those reported by Lyu et al. (2019).

Rodsamran and Sothornvit (2019) reported that galacturonic acid content of lime peel pectin as 79.29-95.93%. Kratchanova et al. (2004) applied microwave power (0.45, 0.63, 0.9 kW) and times (5, 10, 15min) to orange peels and extracted pectin at pH 1.5, 80-82°C for 60 min. Kratchanova et al. (2004) reported that galacturonic acid content of pectin samples (66.5%-70.1%) were higher than control samples (66.4% and 65.7%). Lyu et al. (2019) determined galacturonic acid content by using high-performance anion exchange chromatography. Galacturonic acid content of pectin extracted from peach samples were lower for infrared applied sample (15 mg/g), as compared to control sample (21.8 mg/g). The results of this study were comparable to those of Lyu et al. (2019) as infrared was used in both studies. In contrast to the results of Lyu et al. (2019), infrared resulted in higher galacturonic acid content (74.17-80.12%) in our study.

4.10 Physicochemical Properties of Electrospinning Solutions

In order to observe jet formation in electrospinning, viscosity of the electrospinning solutions should have sufficient chain entanglements and electrical conductivity values should be such that droplet at the needle tip is charged by the applied voltage (Akınalan Balık et al., 2019; Safari et al., 2020). Viscosity and conductivity values can be regulated by changing the polymer concentrations in the electrospinning solutions. When the electrical conductivity and viscosity values are above or below the critical values, jet formation is not seen or nanofibers produced do not have the desired characteristics (such as nanosized morphology, bead-free and homogeneous distribution) (Akınalan Balık et al., 2019; Safari et al., 2020).

4.10.1. Physicochemical Properties of Pure Pectin Solutions

Viscosity and electrical conductivity of the pectin solutions (3, 4, 5% (w/w)) are given in Table 15. The pectin used in the electrospinning solutions was extracted from IR-800W-30min orange peel samples. The viscosity and electrical conductivity values of the pectin solutions were in the range of 0.41-1.98 Pa.s and 1.24-1.66 mS/cm, respectively (Table 15). ANOVA results showed that viscosity and electrical conductivity values of the pectin solutions increased significantly as the amount of pectin in the electrospinning solutions increased ($p < 0.05$).

Table 15. Viscosity and electrical conductivity values of pectin solutions*

Electrospinning Solutions	**Viscosity (Pa.s)**	**Electrical Conductivity (mS/cm)**
Pectin (3% w/w)	0.41 ± 0.049^c	1.24 ± 0.012^c
Pectin (4% w/w)	1.09 ± 0.088^b	1.61 ± 0.009^b
Pectin (5% w/w)	1.98 ± 0.098^a	1.66 ± 0.013^a

* Pectin used in the electrospinning solutions was extracted from IR-800W-30min orange peel samples
Values followed by the same letter in the same column are not significantly different (α= 0.05).
Means are based on duplicate analyses.

Pure pectin solutions (3, 4, 5% (w/w)) were initially studied for nanofiber production but it was not possible to obtain fibers from the pectin solutions by itself. Instead of continuous jet formation, pectin solutions only formed droplets during electrospinning, due to its polyelectrolyte structure, insufficient viscosity and chain entanglements (Cui et al., 2016; Liu et al., 2016; Akınalan Balık et al., 2019).

Akınalan Balık et al. (2019) reported viscosity and electrical conductivity values of the 3, 4, 5% (w/w) amidated lowly esterified pectin (27% DE, 20% DA) electrospinning solutions as 0.09, 0.20, 0.52 Pa.s and 2.68, 3.36, 4.19 mS/cm, respectively. Since the pectin used was amidated and lowly esterified, the results reported by Akınalan Balık et al. (2019) were different than the viscosity and conductivity values of pectin produced in our study. Akınalan Balık et al. (2019) also reported droplet formation during electrospinning instead of continuous jet formation.

When the pectin was blended with PVA or PEO, electrospinnability of pectin was improved and continuous nanofibers were obtained.

4.10.2. Physicochemical Properties of Pectin+PVA Solutions

Viscosity and electrical conductivity of pectin+PVA solutions are given in Table 16. The pectin used in the electrospinning solutions was extracted from IR-800W-30min orange peel samples. At the beginning of the study, it was aimed to use pectin with higher concentrations (3, 4, 5% (w/w)) in the pectin: PVA solutions (3:5, 3:6, 4:5, 4:6, 5:5, 5:6% (w/w)).

However, no jet formation was observed when the ratio of Pectin:PVA was 3:5 or 3:6 (% w/w), probably due to the higher viscosity and electrical conductivity values (Akınalan Balık et al. , 2019; Safari et al. , 2019). Therefore, it was decided to use lower Pectin:PVA concentrations (2:5, 2:6, 2.5:5, 2.5:6, 3:5, 3:6% (w/w)).

The viscosity of the pectin+PVA solutions were in the range of 0.45-1.76 Pa.s and electrical conductivity values were between 0.62-0.82 mS/cm (Table 16). ANOVA results showed that the viscosity and electrical conductivity values of pectin+PVA solutions was significantly different ($p < 0.05$). The viscosity values of the 3:5 and 3:6 (%w/w) pectin:PVA solutions were significantly higher as compared to lower pectin:PVA concentrations. The electrical conductivity values increased as the amount of pectin in the pectin+PVA electrospinning solutions increased.

Table 16. Viscosity and electrical conductivity of Pectin+PVA solutions*

Pectin : PVA	Viscosity (Pa·s)	Electrical Conductivity (mS/cm)
2 : 5	0.57 ± 0.095^{b}	0.62 ± 0.027^{d}
2 : 6	0.51 ± 0.040^{b}	0.65 ± 0.013^{d}
2.5 : 5	0.62 ± 0.036^{b}	0.72 ± 0.007^{c}
2.5 : 6	0.45 ± 0.026^{b}	0.76 ± 0.011^{bc}
3 : 5	1.54 ± 0.213^{a}	0.79 ± 0.005^{ab}
3 : 6	1.76 ± 0.042^{a}	0.82 ± 0.006^{a}

*Pectin used in the electrospinning solutions was extracted from IR-800W-30min orange peel samples
Values followed by the same letter in the same column are not significantly different (α= 0.05).
Means are based on duplicate analyses.

4.10.3. Physicochemical Properties of Pectin+PEO Solutions

Viscosity and electrical conductivity values of the pectin+PEO solutions are given in Table 17. The pectin used in the electrospinning solutions was extracted from IR-800W-30min orange peel samples. The viscosity and electrical conductivity values of the pectin+PEO solutions were in the range of 0.14-0.84 Pa.s and 0.76-1.24 mS/cm, respectively (Table 17). In contrast to pectin+PVA solutions, it was possible to use higher concentrations of pectin (3, 4, 5 % (w/w)) in the pectin+PEO electrospinning solutions for nanofiber production. ANOVA results showed that the viscosity values of pectin+PEO solutions (with or without Triton X-100) was significantly different ($p < 0.05$) (Table 17).

When PEO was used as a co-polymer, it was not possible to produce nanofibers from all pectin+PEO aqueous solutions without Triton X-100 given in Table 17. Therefore, Triton X-100 was added to the solutions in order to reduce the surface tension of the solutions and jet formation was improved. The addition of Triton X-100 caused changes in the viscosity values of the solutions as compared to their counterparts. When the viscosity values of pectin:PEO solutions with Triton X-100 were compared with their counterparts without Triton X-100, significantly higher viscosity values were obtained for pectin:PEO 4:2 solutions and lower viscosity values were obtained for pectin:PEO 5:1 solutions. However, no significant changes were observed in the counterparts of 3:1, 3:2 and 4:1 pectin:PEO solutions.

ANOVA results showed that the electrical conductivity values of pectin+PEO (with or without Triton X-100) solutions was significantly different ($p < 0.05$) (Table 17). The electrical conductivity values significantly increased as the amount of pectin concentration increased in the pectin+PEO solutions (for each PEO concentrations). The electrical conductivity values slightly decreased as the PEO concentration increased in the pectin+PEO solutions with Triton X-100.

Table 17. Viscosity and electrical conductivity of Pectin+PEO solutions (with and without Triton X-100).*

Pectin : PEO	Triton X-100	Viscosity (Pa·s)	Electrical Conductivity (mS/cm)
3 : 1	-	0.14 ±0.011^{d}	0.80 ± 0.003^{g}
3 : 2	-	0.66 ± 0.006^{b}	0.76 ± 0.007^{h}
4 : 1	-	0.23 ± 0.012^{d}	1.07 ± 0.001^{e}
4 : 2	-	0.63 ± 0.070^{b}	1.05 ± 0.001^{e}
5 : 1	-	0.46 ± 0.066^{c}	1.10 ± 0.002^{d}
3 : 1	+	0.17 ± 0.003^{d}	0.89 ± 0.009^{f}
3 : 2	+	0.69 ± 0.089^{b}	0.81 ± 0.006^{g}
4 : 1	+	0.14 ± 0.015^{d}	1.19 ± 0.004^{b}
4 : 2	+	0.84 ±0.053^{a}	1.15 ± 0.005^{c}
5 : 1	+	0.23 ± 0.024^{d}	1.24 ± 0.034^{a}

* Pectin used in the electrospinning solutions was extracted from IR-800W-30min orange peel samples . Values followed by the same letter in the same column are not significantly different (α= 0.05). Means are based on duplicate analyses.

Akınalan Balık et al. (2019) used amidated lowly esterified pectin (27% DE, 20% DA) and PEO (2000kDa) in order to prepare pectin: PEO solutions at a ratio of 3:1, 4:1, 5:1 or 6:1% (w/w). It was reported that viscosity values of the solutions were 2.14, 3.87, 4.98 and 5.41 Pa.s. and electrical conductivity values were 2.63, 3.04, 3.47 and 4.43 mS/cm, respectively. Since the pectin used was amidated and lowly esterified, the results reported for the pectin+PEO were different than those found in our study. Similar to the results obtained in our study for pectin+PEO solutions (with or without Triton X-100), electrical conductivity values generally increased as the concentration of pectin increased in pectin+PEO solutions (for each PEO concentrations).

4.11. Morphology of Nanofibers Produced from Pectin+PVA or Pectin+PEO Solutions

4.11.1. Morphology of Nanofibers Produced from Pectin+PVA Solutions

Pectin extracted from IR-800W-30min. orange peel sample at pH1.5-120min. were mixed with PVA at a ratio of 2:5 or 2:6 (% w/w) and the mixtures were electrospun at different flow rates (0.5ml/h and 0.7ml/h) with a constant distance (10cm) and voltage (30kV).
As mentioned in section 4.10, in preliminary study, it was aimed to use pectin with higher concentrations (3, 4, 5% (w/w)) in the pectin: PVA solutions (3:5, 3:6, 4:5, 4:6, 5:5, 5:6% (w/w)). However, when the ratio of Pectin:PVA was 3:5 or 3:6 (% w/w), no jet formation was observed probably due to the higher viscosity and electrical conductivity (Akınalan Balık et al., 2019; Safari et al. , 2019). Therefore, it was decided to use lower Pectin:PVA concentrations (2:5, 2:6, 2.5:5, 2.5:6, 3:5, 3:6% (w/w)). However, nanofiber formation was only observed for pectin:PVA solutions with a ratio of 2:5 or 2:6 (% w/w). SEM images, electrospinning parameters and average diameter distributions of Pectin+PVA nanofibers are given in Figure 8. The nanofibers produced from pectin:PVA 2:5 (%w/w) solution were continuous and partially bead-free but had nonuniform diameter (108 ± 38nm, 101 ± 34 nm). The nanofibers displayed beaded morphology when pectin:PVA 2:6 (%w/w) solution was used (Figure 8). Because of the beaded morphology, average diameter distributions of nanofibers produced from pectin:PVA (2:6% (w/w)) were not calculated.

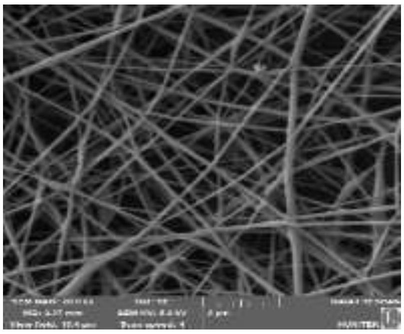
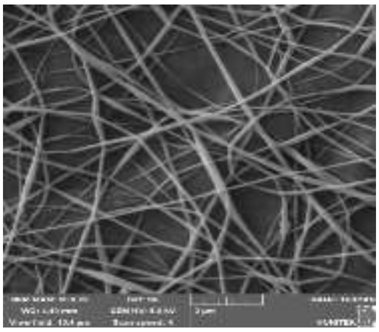
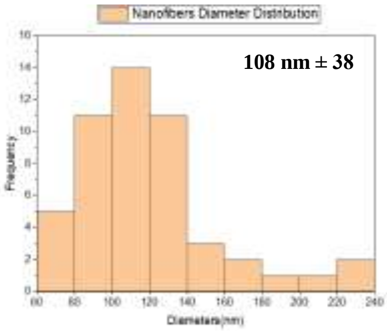
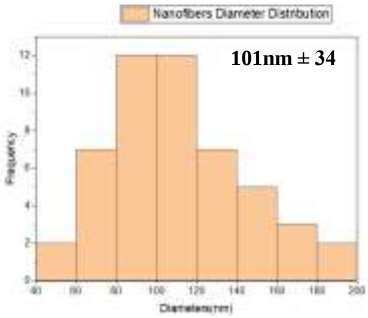
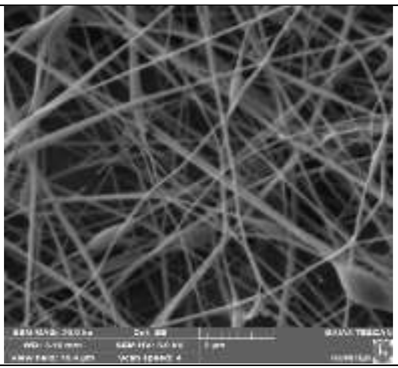
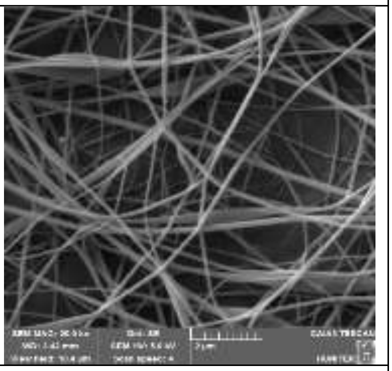
Pectin:PVA	**10cm, 30kV** **0.5ml/h**	**10cm, 30kV** **0.7ml/h**
2 : 5		
		
2 : 6		
2.5 : 5	No fiber formation	No fiber formation
2.5 : 6	No fiber formation	No fiber formation
3 : 5	No fiber formation	No fiber formation
3: 6	No fiber formation	No fiber formation

Figure 8. SEM images of nanofibers produced from pectin:PVA (2:5 or 2:6 (%w/w)) solutions.
(Pectin was extracted from IR-800W-30min dried orange peels at pH1.5- 120min).
(Pectin; Esterification degree 76.47%, Galacturonic acid 80.12%)
(PVA; 87-89% hydrolyzed, MW 85,000-146,000).

Patra et al. (2017) produced nanofibers from citrus pectin:PVA (~125,000g mol^{-1}) solution with a ratio of 2:11 (%w/w). The nanofibers had bead-free morphology and were homogeneous. It was reported that smaller diameters (~400nm) were obtained for pectin+PVA nanofibers as compared to those of pure PVA nanofibers (~600 nm). It was also reported that the use of very small amount of pectin (2% w/w) plays an important role in the morphology of nanofibers.

In a study by Xu et al. (2022), orange peel pectin (galacturonic acid≥74) and PVA (88 % hydrolyzed) was used for electrospun nanofiber production. It was reported that uniform and bead-free nanofibers were produced from pectin:PVA solutions with a ratio between 0:10-1:9. When the pectin:PVA ratio changed from 1:9 to 5:5, diameter of the nanofibers gradually decreased (149 ± 25 nm, 116 ± 26 nm, 113 ± 69 nm, 113 ± 79 nm, and 112 ±78 nm) and nonuniform nanofibers with increased bead formation were observed.

Patra et al. (2017) and Xu et al. (2022) used PVA in combination with pectin for nanofiber production, but the ratio of PVA in pectin+PVA solution were different from the one used in our study. Regarless of the ratio of PVA in pectin+PVA solution, the diameter distributions of nanofibers produced in our study (108 ± 38nm, 101 ± 34 nm.) were similar with the diameter distribution (116 ± 26nm) of pectin:PVA 2:8 (%w/w) reported by Xu et al. (2022) and were smaller than those (~400nm) reported by Patra et al. (2017).

Safari et al. (2020) produced nanofibers from pectin (30-100 kDa, 30%DM), PVA (94-120 kDa) and chitosan (1600 kDa, 86.2% deacetylation). Nanofibers were produced by mixing pectin:PVA (50:50) with chitosan:PVA (50:50) and properties of the nanofibers were compared with the nanofibers produced by mixing pectin:PVA 75:25 with chitosan:PVA 75:25. Nanofibers produced by using mixture of pectin:PVA (50:50) and chitosan:PVA (50:50) showed more homogeneous morphology with fewer beads and the diameters of the nanofibers were smaller.

4.11.2. Morphology of Nanofibers Produced from Pectin+PEO Solutions

Pectin extracted from IR-800W-30min. orange peel sample at pH1.5-120min. were mixed with PEO at a ratio of 3:1, 3:2, 4:1, 4:2 or 5:1% (w/w) and with Triton X-100. Different flow rates (0.2, 0.5 and 0.7ml/h), distance (18cm, 20cm) and constant voltage (35kV) were used as electrospinning parameters.

In preliminary studies, nanofiber formation from all of the pectin+PEO solutions with Triton X-100 was tested at a constant distance of 20 cm (Figure 9). Nanofiber formation was observed only for pectin:PEO solutions with a ratio of 3:2 or 5:1 (% w/w). However, because of the beaded morphology, average diameter distributions of nanofibers were not calculated.

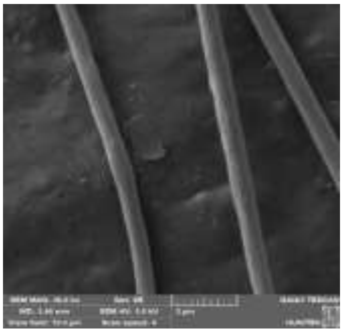
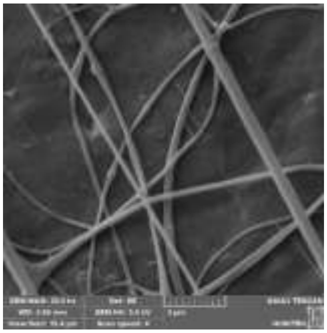
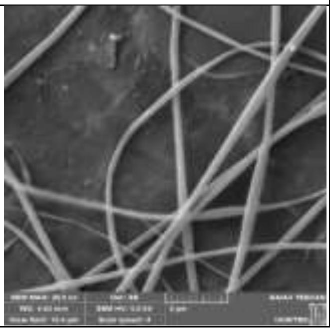
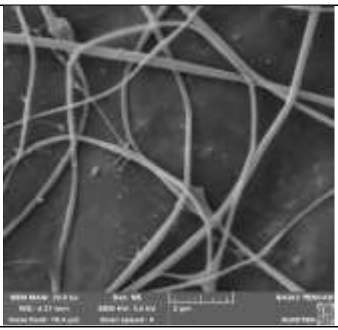
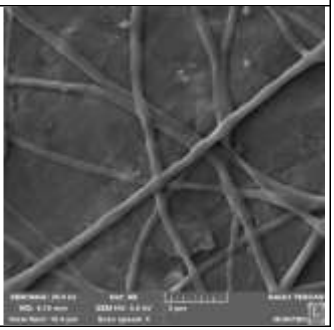
Pectin:PEO	**20cm, 35kV 0.2ml/h**	**20cm, 35kV 0.5ml/h**	**20cm, 35kV 0.7ml/h**
3 : 1	No fiber formation	No fiber formation	No fiber formation
3 : 2	No fiber formation		
4 : 1	No fiber formation	No fiber formation	No fiber formation
4 : 2	No fiber formation	No fiber formation	No fiber formation
5 : 1			

Figure 9. SEM images of nanofibers produced from pectin+PEO solutions (with Triton X-100) at a constant distance of 20 cm.
(Pectin was extracted from IR-800W-30min dried orange peels at pH1.5-120min).
(Pectin; Esterification degree 76.47%, Galacturonic acid 80.12%) (PEO: 2000 kDa).

Nanofiber formation for all of the pectin+PEO solutions with Triton X-100 was tested also at a constant distance of 18 cm (Figure 10). Nanofiber formation was observed at flow rate of 0.5 ml/h., for all pectin+PEO solutions with Triton X-100. When 0.2 and 0.7 ml/h. were used as flow rate, nanofiber formation was observed only for pectin:PEO solution with a ratio of 3:1 or 5:1 (% w/w). Since continuous and bead-free nanofiber formation at all flow rates was obtained only for pectin:PEO solution with a ratio of 3:1 (% w/w), average diameter distributions of nanofibers only for this solution was calculated. As shown in Figure 10, the electrospun nanofibers produced from Pectin:PEO with a ratio of 3:1 (% w/w) had uniform diameters of 240 ± 39 nm, 234 ± 40 nm and 242 ± 49 nm for flow rates of 0.2, 0.5 and 0.7 ml/h, respectively. For all pectin:PEO solutions, except pectin:PEO with a ratio of 3:1, nonuniform nanofiber formation with beaded morphology was observed.

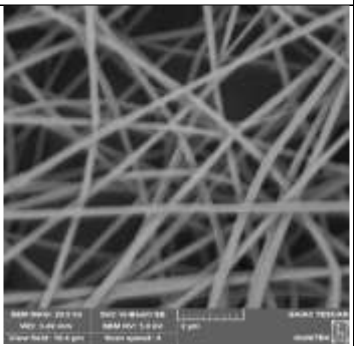
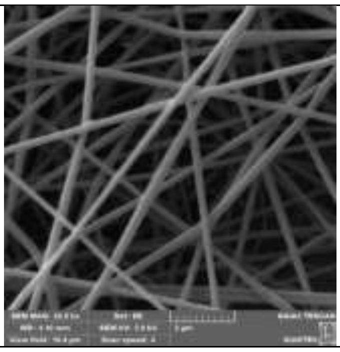
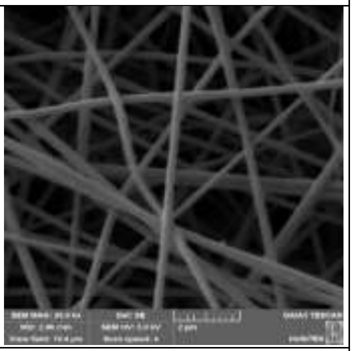
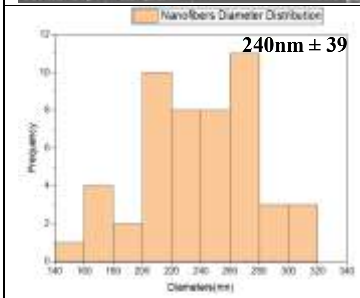
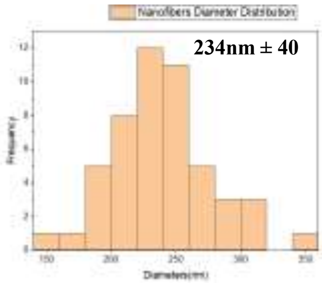
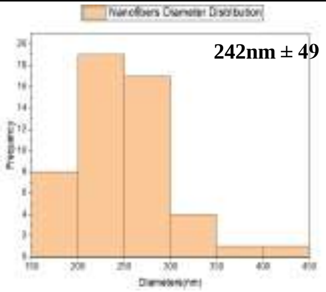
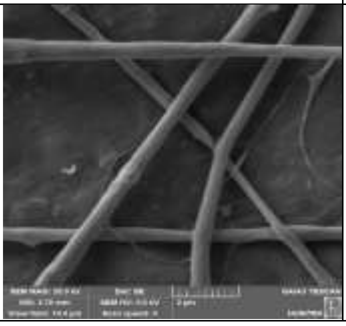
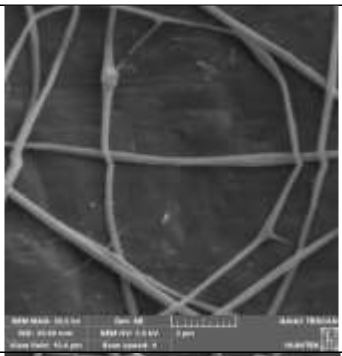
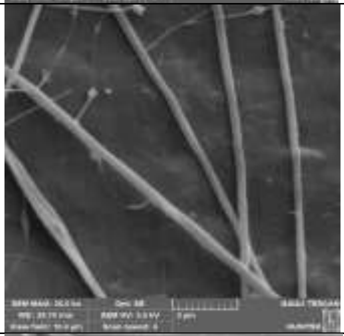
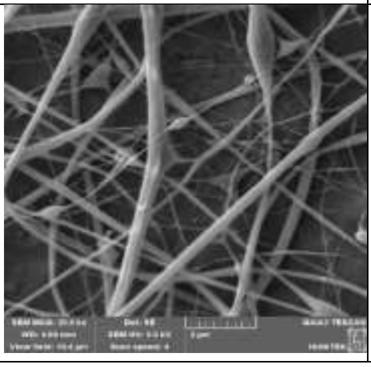
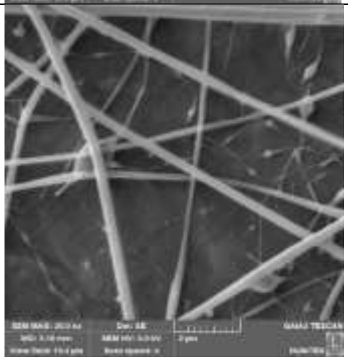
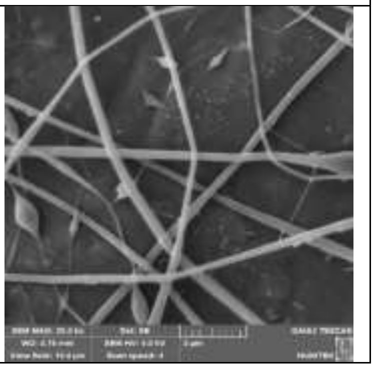
Pectin: PEO	18cm, 35kV 0.2ml/h	18cm, 35kV 0.5ml/h	18cm, 35kV 0.7ml/h
3 : 1			
			
3 : 2	No fiber formation		No fiber formation

Figure 10. SEM images of nanofibers produced from-pectin+PEO solutions (with Triton X-100) at a constant distance of 18cm.
(Pectin was extracted from IR-800W-30min dried orange peels at pH1.5-120min).
(Pectin; Esterification degree 76.47%, Galacturonic acid 80.12%) (PEO: 2000 kDa).
(CONTINUED)

4 : 1	No fiber formation		No fiber formation
4 : 2	No fiber formation		No fiber formation
5 : 1			

Akınalan Balık et al. (2019) produced nanofibers from pectin (%27 DE, %20 DA) and PEO (2000 kDa). Nanofibers were produced by mixing pectin:PEO at a ratio of 3:1, 4:1, 5:1 or 6:1 % (w/w). It was reported that nanofibers were uniform and had bead-free morphology. Diameters of the nanofibers were in the range of 240-265 nm. The result of the study was given in detail in section 4.16. The properties of pectin used in the study of Akınalan Balık et al. (2019) were different than the pectin samples produced in our study. Regardless of the pectin properties, nanofibers were compared in terms of average diameter distribution. Similar diameter distribution (234-242 nm) was found in our study.

4.12. Water Contact Angle of Pectin Nanofibers Produced from Pectin+PVA and Pectin+PEO

Surface hydrophobicity/hydrophilicity of the pectin nanofibers was determined by measurement of water contact angle (Trinca et al., 2017). Water contact angle characterizes the surface wettability of materials. Mouro et al. (2021) stated that water contact angle values between 40°-70°, lower than 20° or higher than 90° are characteristic for moderate hydrophilic surfaces, very hydrophilic surfaces and hydrophobic surfaces respectively.

Water contact angle was measured only for bead-free nanofibers for which the average diameter distributions were calculated. The water contact angle of pectin+PVA and pectin+PEO nanofibers are given Table 18. The water contact angles of nanofibers were in the range of 21.07-35.07°, indicating that the nanofibers produced by using pectin+PVA and pectin+PEO had hydrophilic nature. Among the solutions studied, pectin+PVA resulted in nanofibers with lower water contact angle value (21.07°), indicating more hydrophilic surface. Pectin+PEO nanofibers exhibited water contact angle in the range of 24.67-35.07°. The highest (35.07°) water contact angle was observed for pectin+PEO nanofibers produced at 0.2ml/h.

Table 18. Water contact angle values of Pectin+PVA and Pectin+PEO nanofibers*

Electrospinning Solutions	Electrospinning Parameters	Contact Angle (°)
Pectin**:PVA 2:5 (% w/w)	10cm, 0.5ml/h, 30kV	21.07 ± 0.530
Pectin:PEO 3:1 (% w/w)+Triton X-100	18cm, 0.2ml/h, 35kV	35.07 ± 2.497
Pectin:PEO 3:1 (% w/w)+Triton X-100	18cm, 0.5ml/h, 35kV	24.67 ± 4.739
Pectin:PEO 3:1 (% w/w)+Triton X-100	18cm, 0.7ml/h, 35kV	25.93 ± 4.329

***** Means are based on duplicate analyses.
**Pectin was extracted from IR-800W-30min dried orange peels at pH1.5-120min.

Mouro et al. (2021) used pectin together with PVA (MW 115,000 g/mol) and polycaprolactone in nanofiber production. The water contact angle of nanofibers was reported as 11.21-73.85°. The properties of the polymers used in nanofiber production were not given in detail, but similar hydrophilic character was reported for the nanofibers produced in our study.

4.13. Differential Scanning Calorimetry (DSC) Thermograms of Pectin Nanofibers Produced from Pectin+PVA and Pectin+PEO

Differential Scanning Calorimetry (DSC) is a technique used to analyze thermal changes resulting from interactions between different components (Neufeld and Bianco-Peled, 2017).

DSC thermogram of PVA, PEO and pectin extracted from orange peel samples infrared dried at 800W for 30min. are presented in Figure 11 and thermal properties of the pectin, PVA and PEO are given in Table 19. As shown in Figure 11, peaks observed at 182.10 °C for PVA and 71.60°C for PEO were attributed to the melting of the polymers. In literature, the melting temperature of PEO was reported as 68.4°C by Acosta and Morales (1996). The melting temperature of PVA was reported as 218°C by Kim (2010).
Peak observed at 40°C for PVA was attributed to the glass transition temperature of PVA in our study. Glass transition temperature of PVA was reported as 56°C by Koosha and Mirzadeh (2015). The degradation temperature was observed at 236.19°C for pectin peak. The degradation temperature of pectin was reported as 232°C by Akınalan Balık et al. (2019). A broad peak observed at 178°C for pectin was due to the loss of water associated with hydrophilic groups of pectin (Koosha and Mirzadeh, 2015; Neufeld and Bianco-Peled, 2017; Çay et al., 2014). Neufeld and Bianco-Peled (2017) also reported a broad peak between 130-160°C. The enthalpy values of the peaks were 108.95 J/g (at 236.19°C) for IR-800W-30min pectin sample, 57.70 J/g (at 182.10°C) for PVA, and 169.35 J/g (at 71.60°C) for PEO.

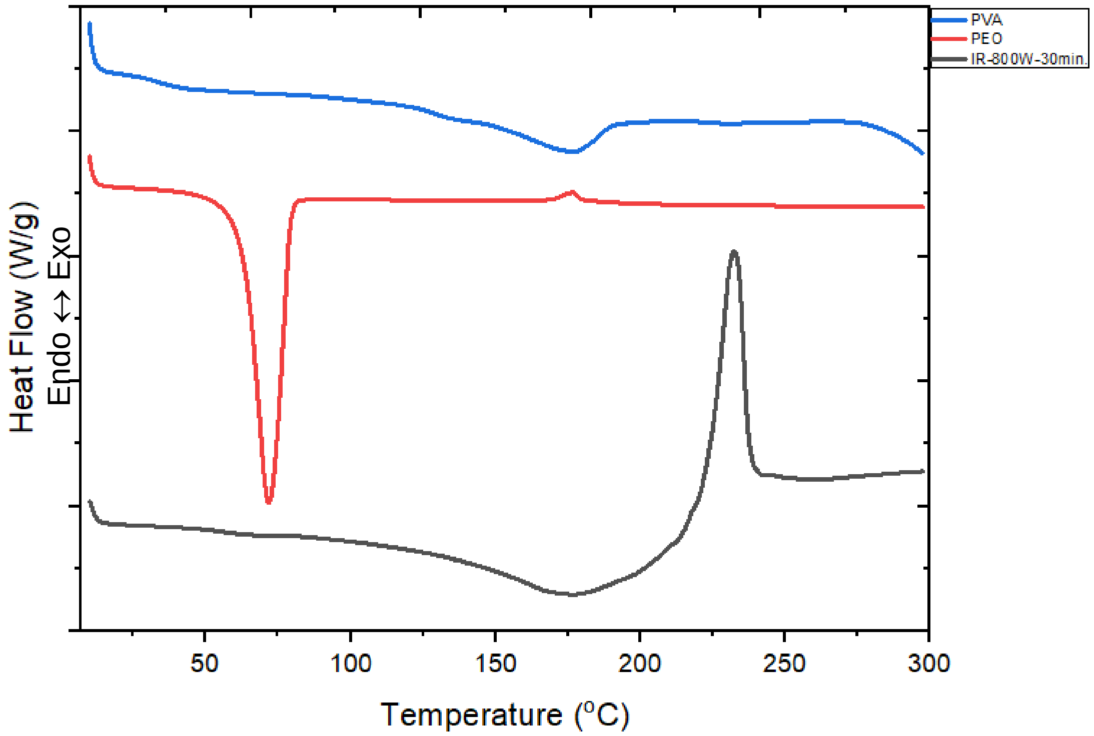


Figure 11. DSC curves of powders; PVA, PEO and pectin
(Pectin was extracted from IR-800W-30min dried orange peels at pH1.5-120min.)

Table 19. Thermal properties of PVA, PEO and pectin powder*

Powder samples	**Tpeak (°C)**	**ΔH (J/g)**
PVA	182.10 ± 8.457	57.70 ± 3.670
PEO	71.60 ± 0.735	169.35 ± 13.930
IR-800W-30min.**	236.19 ± 4.999	108.95 ± 2.051

* Means are based on duplicate analyses.
** Pectin was extracted from IR-800W-30min dried orange peels at pH1.5-120min.

DSC curves of Pectin+PVA and Pectin+PEO nanofibers are presented in Figure 12. DSC analysis was carried out only for bead-free nanofibers for which the average diameter distributions were calculated. Peak observed at 48°C and 191°C (Figure 12) for pectin+PVA nanofiber was attributed to the glass transition temperature and melting temperature of PVA, respectively (Koosha and Mirzadeh, 2015). Peaks observed at 54-56°C for pectin+PEO nanofibers was due to the melting temperature of PEO (Akınalan Balık et al., 2019). As seen in Figure 12, peaks observed at 237°C for pectin+PVA and 247-250°C for pectin+PEO could be attributed to the thermal degradation of pectin (Akınalan Balık et al., 2019). Results showed that degradation temperatures of pectin+PEO nanofibers (247-250°C) were slightly higher than those of pectin+PVA nanofiber (237°C).

Akınalan Balık et al. (2019) produced nanofibers from pectin (%27 DE, %20 DA) and PEO (2000 kDa). It was reported that PEO powder exhibited a peak at 68°C which was attributed to the melting point of the PEO. Peak observed by Akınalan Balık et al. (2019) at 232°C for pectin powder was attributed to the degradation temperature of pectin. It was reported that pectin did not exhibit any crystallization or melting due to the amorphous structure of carbohydrates.

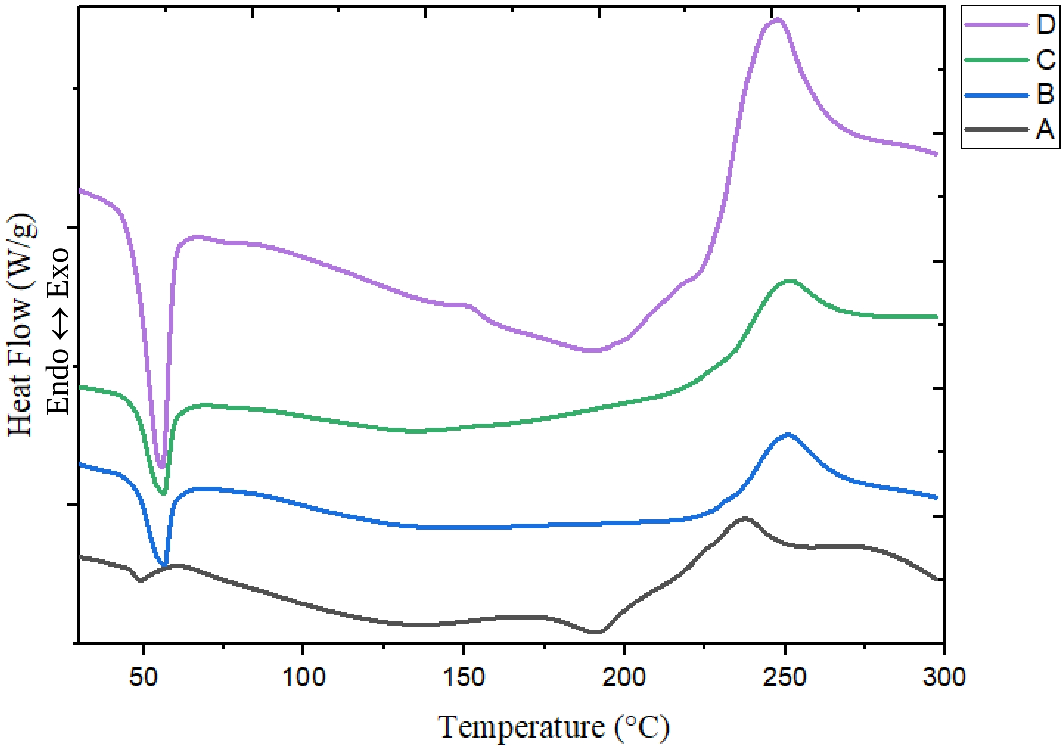


Figure 12. DSC curves of nanofibers (A) Pectin:PVA 2:5 (% w/w) nanofiber. (B-C-D) Pectin:PEO 3:1 (% w/w) nanofibers produced at 18cm and 35kV at a flow rate of (B) 0.2ml/h, (C) 0.5ml/h (D) 0.7ml/h.
(Pectin was extracted from IR-800W-30min dried orange peels at pH1.5-120min.)

In a study by Cui et al. (2016), citrus pectin and PEO (5000 kDa) were used in the production of electrospun nanofibers. Endothermal peak at 69.2 and 78.8°C was observed for PEO and pectin powder, respectively. It was reported that the results showed the semicrystalline nature of pectin and PEO. Pectin+PEO nanofibers only showed a tiny endothermal peak at 49.6°C due to the low amount of PEO in nanofibers.

4.14. Determination of Co-Polymer (PEO or PVA) for Nanofiber Production with Pectin

Co-polymer to be used with pectin for nanofiber production was determined by comparison of the morphology (section 4.11), water contact angle (section 4.12), and DSC (section 4.13) results of the nanofibers produced from pectin+PVA or pectin+PEO solutions prepared with different ratios.

When PEO was used as a co-polymer in electrospun nanofiber production, a higher concentrations (3:1, 3:2, 4:1, 4:2 or 5:1% (w/w, with Triton X-100) of pectin can be used in the solution as compared to utilization of PVA (2:5 or 2:6% (w/w)) as a co-polymer.

When the morphologies (Figure 8) of pectin:PVA (2:5 or 2:6% (w/w)) nanofibers were compared, continuous and partially bead-free nanofiber formation was observed only for pectin:PVA solutions with a ratio of 2:5 (% w/w). But the diameter distribution (108 ± 38nm, 101 ± 34 nm.) was nonuniform.

Among the pectin:PEO (3:1, 3:2, 4:1, 4:2 or 5:1% (w/w, with Triton X-100) nanofibers produced in preliminary studies, continuous, bead-free morphology was only obtained for pectin:PEO solution with a ratio of 3:1 (% w/w). The diameter distribution (240 ± 39 nm, 234 ± 40 nm, 242 ± 49 nm) was uniform. DSC thermograms for pectin+PEO and pectin+PVA nanofibers showed that slightly higher thermal degradation temperatures were observed for pectin+PEO nanofibers (248-251°C) as compared to pectin+PVA nanofiber (236°C). Among the pectin+PVA and pectin+PEO solutions studied, pectin+PEO nanofibers exhibited higher water contact angle values (24.67-35.07°) as compared to pectin+PVA nanofiber (21.07°). This result indicated less hydrophilic character of pectin+PEO nanofibers. Overall results for morphology, DSC thermogram and water contact angle values of the nanofibers indicated that pectin:PEO 3:1 (% w/w, with Triton X-100) was the most appropriate combination among the solutions studied in the present study.

4.15. Physicochemical Properties of Pectin:PEO 3:1 (% w/w) Solutions Prepared by Using Different Pectin Samples

Pectin samples produced by using orange peel samples infrared or oven dried at different conditions were used in order to prepare electrospinning solutions. Commercial pectin sample was used as control. Pectin sample was mixed with PEO at a ratio of 3:1 (% w/w) and Triton X-100 (1% w/w) was added to this solution.

Viscosity and electrical conductivity values of the electrospinning solutions are given in Table 20. The viscosity values of the electrospinning solutions were in the range of 0.05-0.88 Pa.s (Table 20). ANOVA results showed that the viscosity values of electrospinning solutions were significantly different ($p < 0.05$). The viscosity values of the electrospinning solutions prepared by using pectin samples produced from infrared (600W, 700W, 800W) or oven (60°C) dried orange peels were not significantly different. The viscosity values of these solutions were higher than those of other electrospinning solutions.

The electrical conductivity values of the electrospinning solutions were in the range of 0.08-1.30 mS/cm. ANOVA results showed that the electrical conductivity values of the electrospinning solutions were significantly different ($p<0.05$). Pectin addition to the electrospinning solution of PEO resulted in higher electrical conductivity values. The electrical conductivity values of the electrospinning solutions including pectin samples produced by using infrared dried orange peels were significantly lower as compared to those including other pectin sources.

Table 20. Viscosity and electrical conductivity values of electrospinning solutions*

Electrospinning Solutions		**Viscosity (Pa·s)**	**Electrical Conductivity (mS/cm)**
IR-600W-30min.**	**+PEO+Triton X-100**	0.70 ± 0.095^a	0.71 ± 0.001^e
IR-700W-30min.	**+PEO+Triton X-100**	0.79 ± 0.069^a	0.71 ± 0.004^e
IR-800W-30min.	**+PEO+Triton X-100**	0.65 ± 0.015^a	0.74 ± 0.008^d
Oven-60°C***	**+PEO+Triton X-100**	0.88 ± 0.306^a	1.01 ± 0.013^c
Oven-70°C	**+PEO+Triton X-100**	0.18 ± 0.051^b	1.12 ± 0.008^b
Commercial Pectin	**+PEO+Triton X-100**	0.05 ± 0.000^b	1.30 ± 0.006^a
PEO	**+Triton X-100**	0.07 ± 0.007^b	0.08 ± 0.003^f

* Values followed by the same letter in the same column are not significantly different ($\alpha= 0.05$).
Means are based on duplicate analyses.
Pectin:PEO 3:1 (% w/w) + Triton X-100
PEO (1% w/w) + Triton X-100

** Pectin was extracted at pH 1.5, 120 min from orange peel samples infrared dried at 600W for 30 min.

******* Pectin was extracted at pH 1.5, 120 min from orange peel samples oven dried at 60°C

4.16. Morphology of Electrospun Nanofibers Produced by Using PEO and Various Pectin Samples

Various pectin samples were mixed with PEO at a ratio of 3:1(% w/w) and with Triton X-100 (%1 w/w). Electrospinning parameters used were 18cm-0.2 ml/h, 18cm-0.5 ml/h and 15cm-0.5 ml/h at a constant voltage of 35kV. Electrospinning parameters and SEM images of nanofibers are given in Figure 13. When the SEM images of all nanofibers were examined, it was found that nanofibers produced from Oven-70°C or commercial pectin samples at electrospinning parameters of 18 cm-0.2ml/h and 18 cm-0.5ml/h had nonuniform structure surrounded with beads and droplets. Electrospinning parameter of 15cm-0.5ml/h resulted in continuous, bead-free nanofiber formation with uniform diameter for all electrospinning solutions. Therefore, average diameter distribution (Figure 13) was only calculated for the nanofibers produced at 15cm-0.5ml/h. The average diameter distribution (Figure 13) was in the range of 177 ± 28 nm and 288 ± 42 nm. The average diameter distribution of IR-600W-30min., IR-700W-30min. and IR-800W-30min. was 283, 288 and 212 nm, respectively. It is noteworthy that among the IR pectin samples studied, fine nanofibers were obtained for the IR-800W-30min. pectin+PEO sample. The average diameter distribution of this sample was similar to that of pure PEO nanofibers. Finest fibers (177 nm) were obtained for the electrospinning solution prepared by using Oven-60°C pectin sample. It can be concluded that the differences between drying method of orange peel samples resulted in differences in average diameter distribution of nanofibers

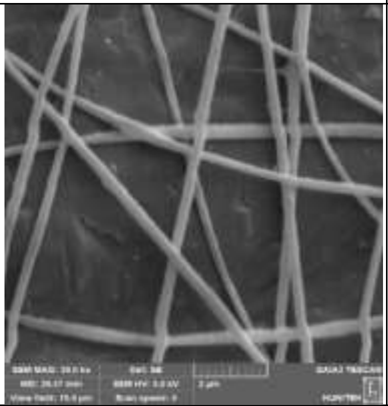
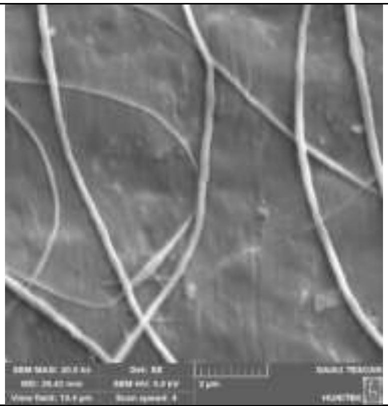
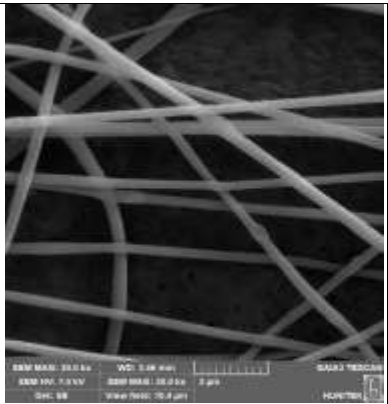
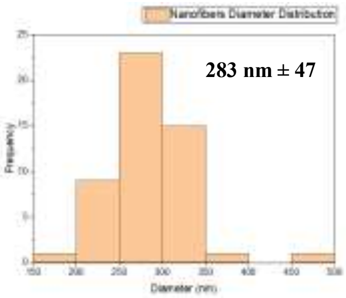
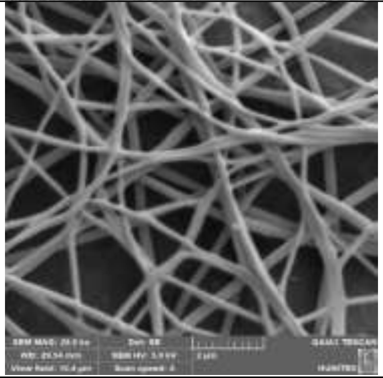
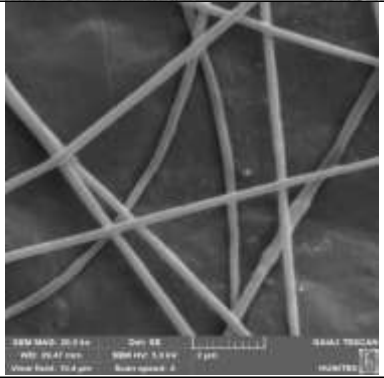
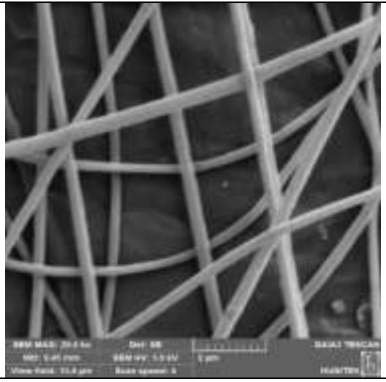
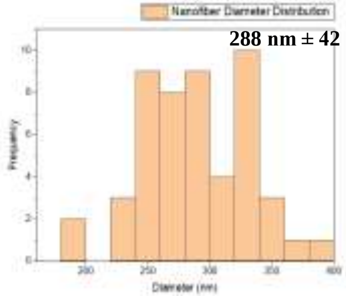
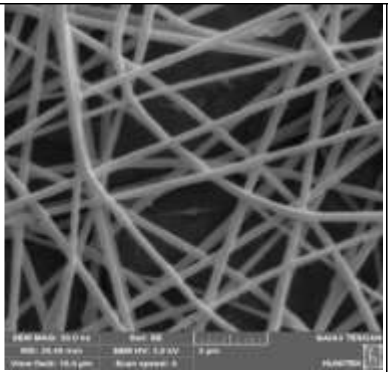
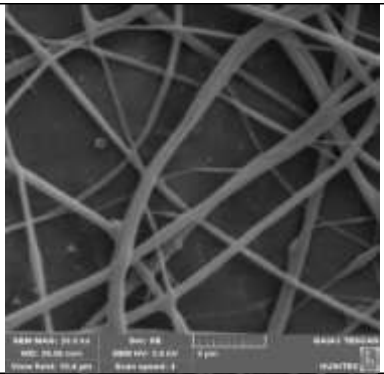
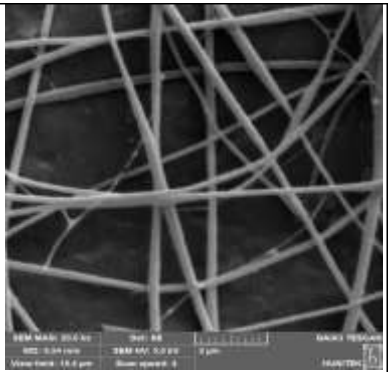
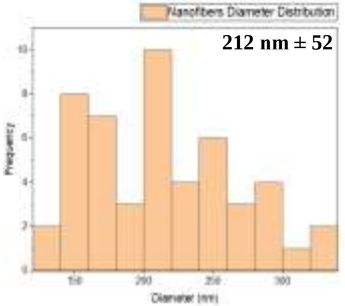
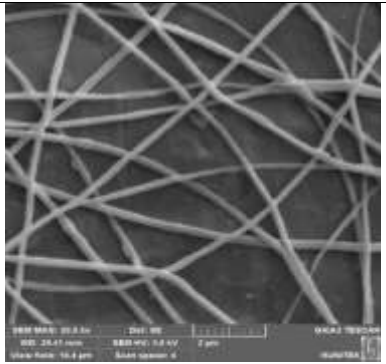
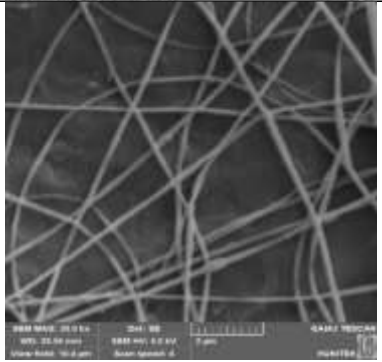
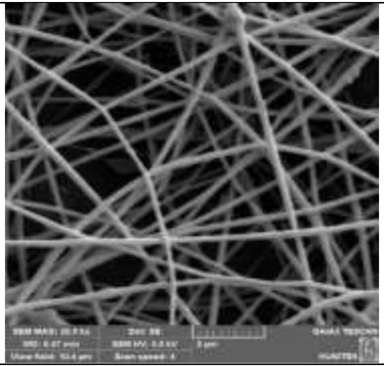
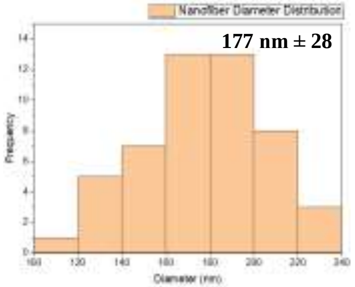
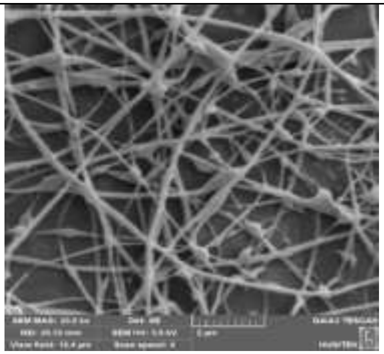
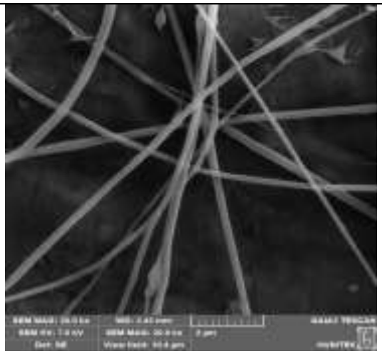
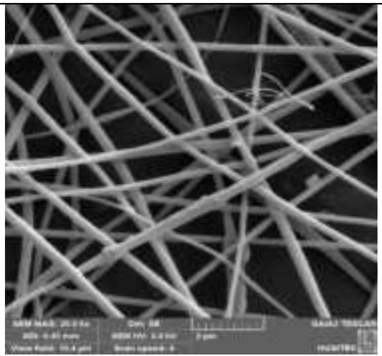
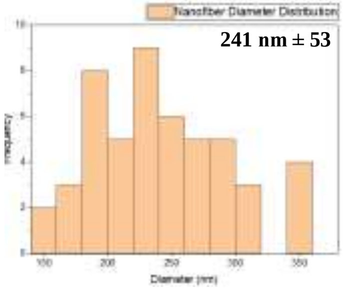
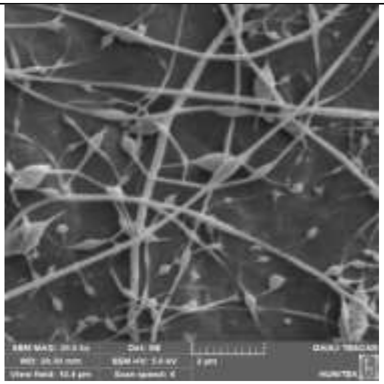
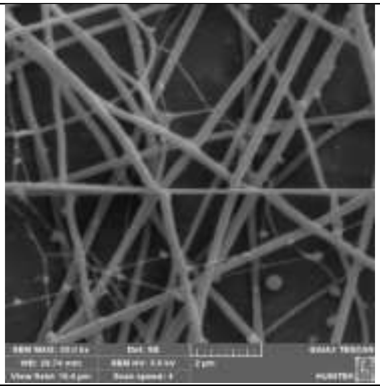
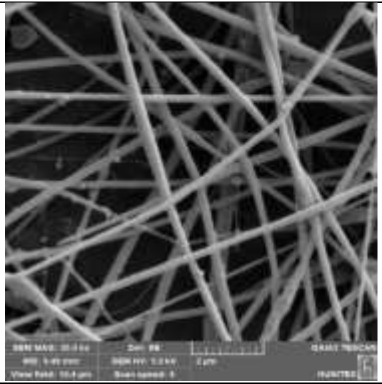
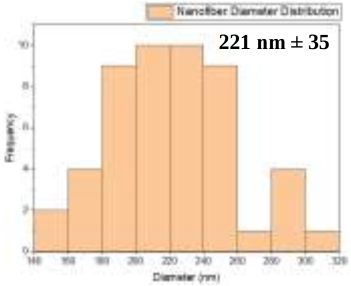
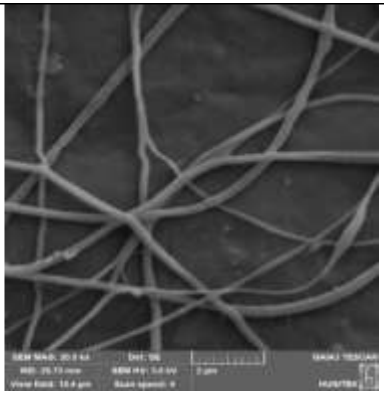
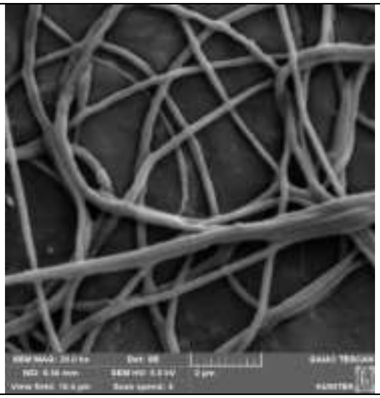
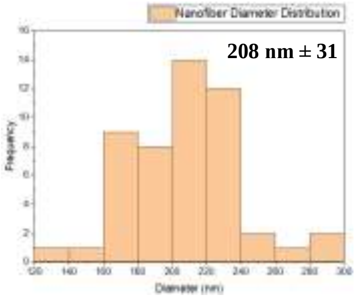
Nanofibers	18cm, 35kV		15cm, 35kV	
	0.2ml/h	0.5ml/h	0.5ml/h	
IR-600W-30min. + PEO +Triton X-100				
IR-700W-30min. + PEO +Triton X-100				

Figure 13. SEM images of nanofibers produced from pectin:PEO (3:1 %w/w, with Triton X-100) solutions or PEO (1% w/w, with Triton X-100) solution. (PEO: 2000 kDa).
(CONTINUED)

IR-800W-30min. **+ PEO** **+Triton X-100**				
Oven-60°C **+ PEO** **+Triton X-100**				

Oven-70°C **+ PEO** **+Triton X-100**				
Commercial **Pectin** **+ PEO** **+Triton X-100**				

PEO **+Triton X-100**		**No fiber formation**		

Akınalan Balık et al. (2019) produced nanofibers from pectin (%27 DE, %20 DA) and PEO (2000 kDa). Nanofibers were produced from pectin:PEO at a ratio of 3:1, 4:1, 5:1 or 6:1 (%, w/w). It was reported that nanofibers were uniform and had bead-free morphology. The electrospinning parameters used in the nanofiber production were 20 cm, 0.2ml/h, 18-22kV. Diameters of the nanofibers were in the range of 240-265 nm. It was reported that increase in pectin concentration did not affect nanofiber diameter.

Rockwell et al. (2014) produced nanofibers from PEO (600 kDa) and various pectin sources (citrus pectin,170-230 kDa, 36.4% DE; sugar beet pectin, 200 kDa, 55% DE; apple pectin, 30-100 kDa, methoxyl group ≥ 7.1%) Nanofibers were produced from pectin: PEO at a ratio of 1:1 (%, w/w). Diameters of bead-free and homogeneous nanofibers were 493, 581 and 121 nm for citrus pectin+PEO, sugar beet pectin+PEO and apple pectin+PEO, respectively.

The results of the present study were compatible with the literature (Rockwell et al., 2014; Akınalan Balık et al., 2019). The properties of the pectin used in our study were different than those of the pectin used by Akınalan Balık et al. (2019). Also, the properties of PEO used in our study were different than those of PEO used by Rockwell et al. (2014). Nanofibers were compared in terms of average diameter distribution without taking pectin and PEO properties into consideration. Average diameter distribution (177-288nm) of the nanofibers produced in our study were similar to those (240-265 nm) reported by Akınalan Balık et al. (2019) and smaller than those (493 nm) reported by Rockwell et al. (2014).

4.17. Fourier Transform Infrared (FTIR) Analysis of Electrospun Nanofibers Produced by Using PEO and Various Pectin Samples

FTIR analysis is used to examine the functional groups of the polymers used in nanofiber production. It helps to confirm the presence of these polymers in the nanofiber and provides insights into any potential chemical interactions that may occur between the polymers (Asghari et al., 2022).

FTIR spectra of the powders and nanofibers are shown in Figure 14 and Figure 15, respectively.FTIR analysis was carried out for the samples (pectin:PEO 3:1(% w/w, Triton X-100)) electrospun at 15cm, 0.5 ml/h and 35kV, due to the better morphology of the nanofibers.

In the present study, characteristic absorption peaks of the pectin observed at 3405 cm^{-1} and 2939 cm^{-1} could be attributed to the inter and intramolecular hydrogen bonding and stretching vibration of C–H, respectively (Table 9) (Güzel and Akpınar, 2017; Su et al., 2019). The peaks observed at 1734 cm^{-1} and 1615 cm^{-1} for pectin could be assigned to the C=O stretching vibration of esterified carboxyl groups and free carboxyl groups, respectively (Table 9). The peaks detected at 1100 cm^{-1} could be attributed to the stretching of C-C bonds (Güzel and Akpınar, 2017; Rodsamran and Sothornvit, 2019; Su et al., 2019; Cui et al., 2016). As shown in Figure 14 and Figure 15, all pectin powders and pectin+PEO nanofibers had characteristic absorption peaks of pectin.

In the present study, the peak observed at 2886 cm^{-1} for PEO powder could be attributed to C-H stretching. This peak of PEO was previously reported at 2871 cm^{-1} by Cui et al. (2016) and at 2891 cm^{-1} by Asghari et al. (2022). In the present study, an enhanced peak at 2870 cm^{-1} was observed in the spectra of pectin+PEO nanofibers, due to the presence of PEO (Cui et al., 2016). PEO showed vibrations of C-O-C stretching at 1147 and 1095 cm^{-1} due to its crystalline phase (Gondaliya et al., 2011). The other characteristics peaks of PEO were observed at 1242, 1357 and 1467 cm^{-1}, indicating CH_2 twisting, CH_2 wagging and CH_2 scissoring, respectively. The other characteristic peaks of PEO was also reported as 1145, 1095, 1279, 1360 and 1466 cm^{-1} by Gondaliya et al. (2011).

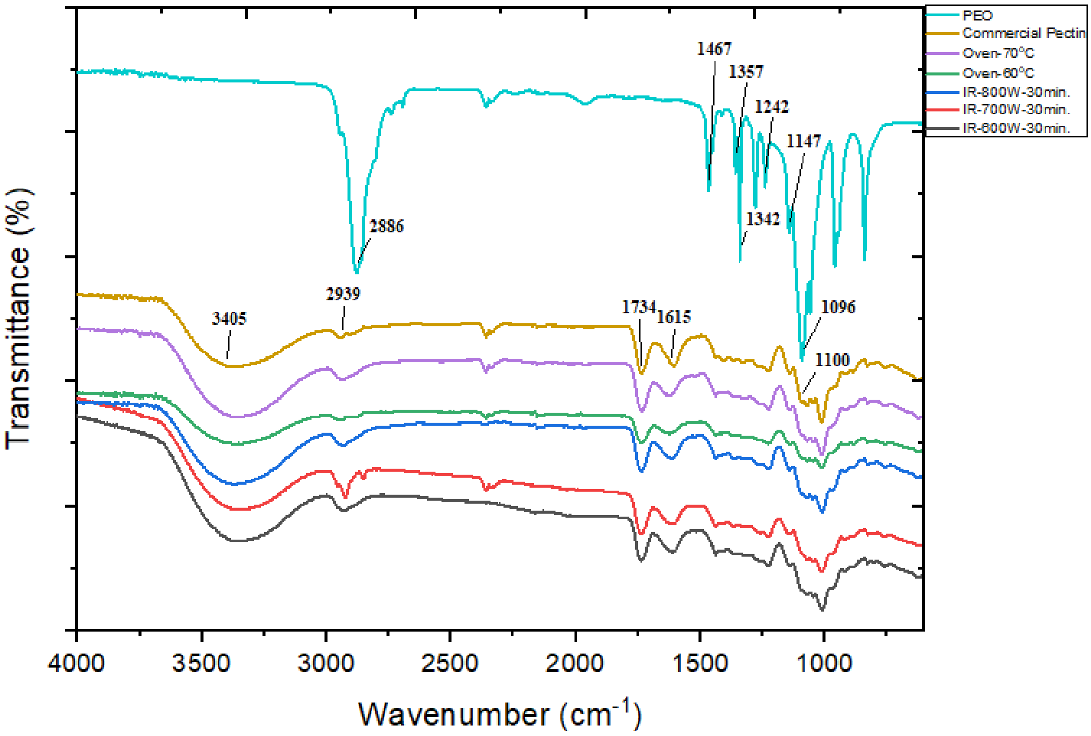


Figure 14. FTIR spectra of PEO and pectin powders

IR-600W-30min: Pectin was extracted at pH1.5, 120min from orange peels infrared dried at 600W-30min.

Oven-60°C: Pectin was extracted at pH1.5,120min from orange peels oven dried at 60°C.

Because of the compatibleness of PEO and pectin, both of the characteristic peaks of PEO or pectin were also observed in pectin+PEO nanofibers. The miscibility of pectin+PEO blends was also reported by Cui et al. (2016) and Akınalan Balık et al. (2019).

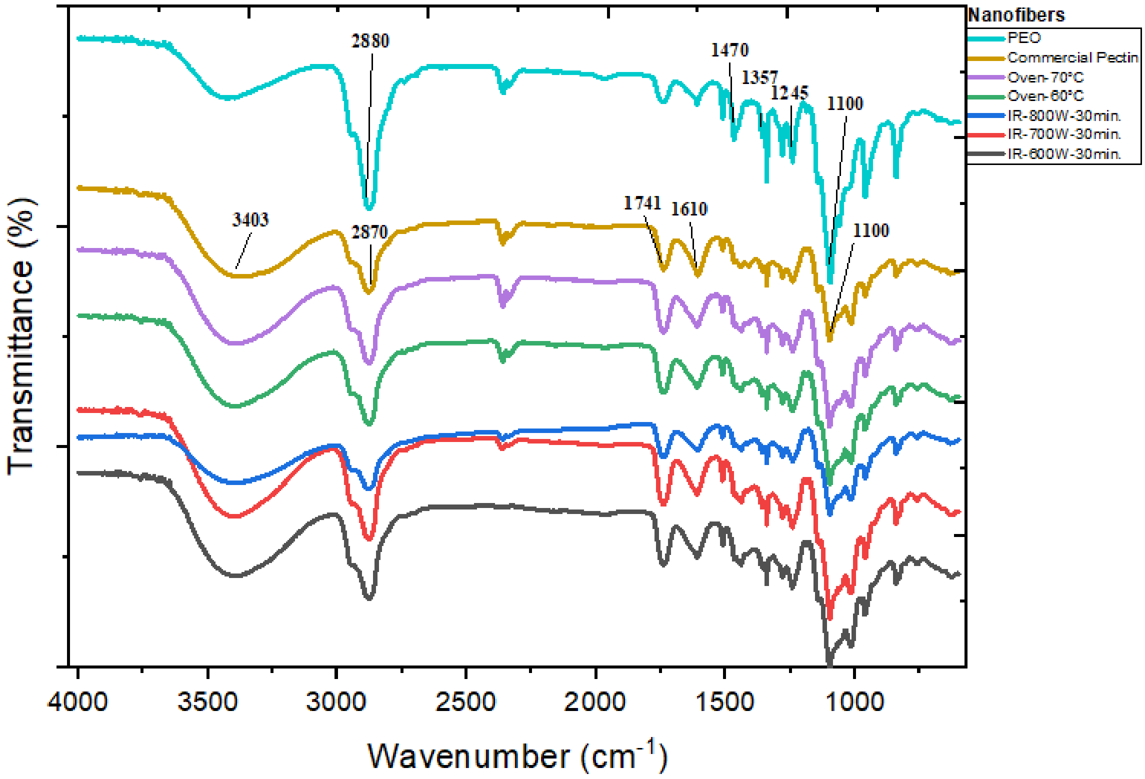


Figure 15. FTIR spectra of PEO nanofiber and pectin+PEO nanofibers

Pectin+PEO nanofibers were produced from pectin:PEO 3:1% (w/w) + Triton X-100 (1% w/w)
PEO nanofibers were produced from PEO (1% w/w) +Triton X-100 (1% w/w)
IR-600W-30min: Pectin was extracted at pH1.5, 120min from orange peels infrared dried at 600W-30min.
Oven-60°C: Pectin was extracted at pH1.5,120min from orange peels oven dried at 60°C.

4.18. X-ray diffraction (XRD) Patterns of Electrospun Nanofibers Produced by Using PEO and Various Pectin Samples

The XRD pattern of the powders and nanofibers are shown in Figure 16 and Figure 17, respectively. XRD was carried out for the samples (pectin:PEO 3:1(% w/w, Triton X-100)) electrospun at 15cm, 0.5 ml/h and 35kV, due to the better morphology of the nanofibers. The potential differences in the crystallinity of each polymer in the nanofiber and the compatibility of the polymers were examined.

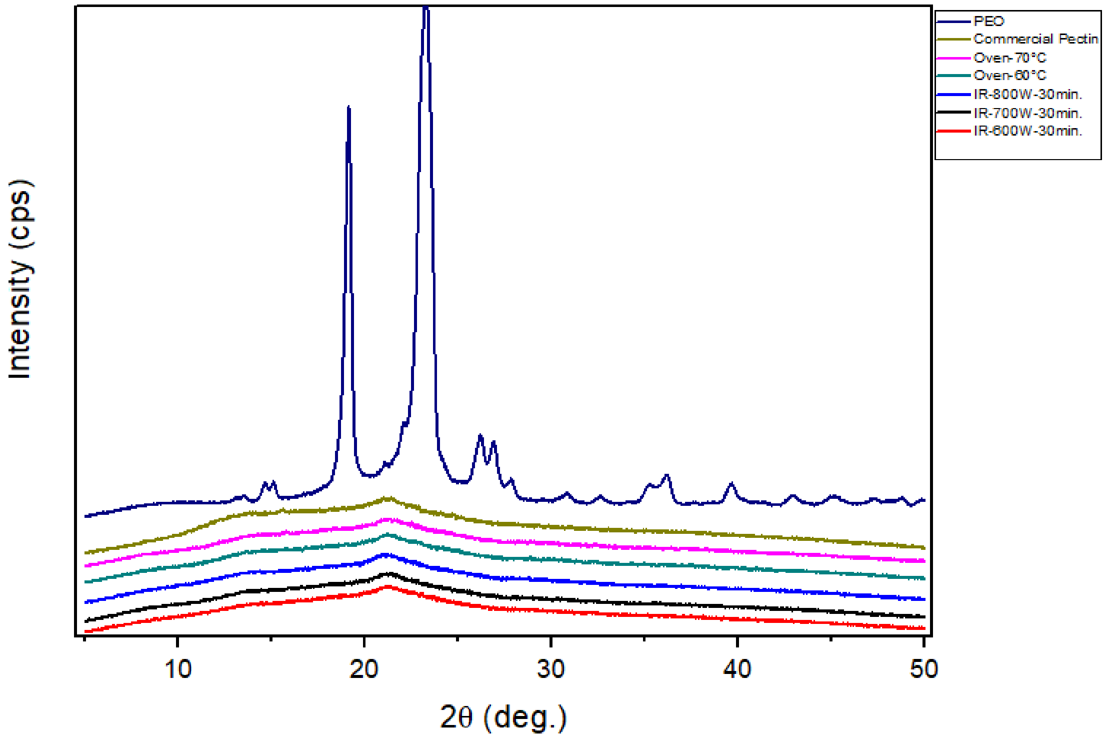


Figure 16. XRD patterns of PEO and pectin powders

IR-600W-30min: Pectin was extracted at pH1.5, 120min from orange peels infrared dried at 600W-30min.
Oven-60°C: Pectin was extracted at pH1.5,120min from orange peels oven dried at 60°C.

As shown in Figure 16, PEO powder showed many sharp peaks between 13.08°-49.79°. However, less number of sharp peaks (6.18°, 7.46°, 20.53°, 41.82°, 44.36°) were observed for PEO nanofibers (Figure 17). The characteristic peaks of PEO were reported as 19.36°, 23.51° by Feng et al. (2019). As compared to PEO powder, the crystallinity decreased in PEO nanofiber. This indicated that the electrospinning process had an effect on crystallinity. This was also reported by Feng et al. (2019).

X-ray diffraction patterns of pectin powder and pectin nanofibers showed characteristic peaks at 8.41°, 13.42°, 14.54°, 20.6°, 20.97°, 21.04°, 24.08° and 27.29°, indicating the crystalline behavior of pectin (Figure 16 and Figure 17) Characteristic peaks of pectin were reported as 12.6°, 14.0°, 20.4°, 21.6° by Cui et al. (2016), and 9.20°, 12.69°, 14.54°, 18.46°, 28.27°, 40.16° by Feng et al. (2019), and 9.7°, 13.9°, 18.9°, 29.4°, 39.4° by Priyadarshi et al. (2021). The peaks observed in our study were similar to the ones reported in literature. The peaks at approximately 12.6° and 20.4° were assigned to pectin's homogalacturonan regions. The angles can shift to higher or lower values depending on the number of pectin domains and bound water in the pectin samples (Cui et al., 2016). In contrast to the results obtained for PEO nanofibers, electrospinning process did not have an effect on XRD patterns of the pectin.

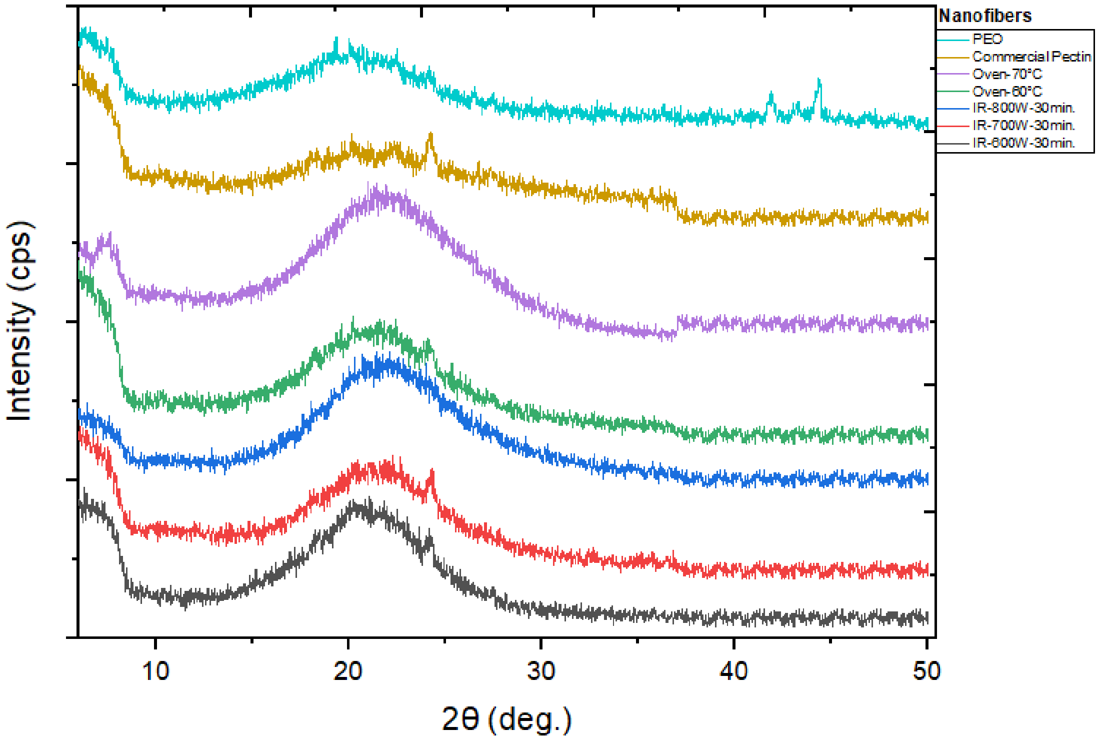


Figure 17. XRD patterns of PEO nanofiber and pectin+ PEO nanofibers

Pectin+PEO nanofibers were produced from pectin:PEO 3:1 (%w/w)+Triton X-100(1% w/w)
PEO nanofibers were produced from PEO (1% w/w) +Triton X-100 (1% w/w)
IR-600W-30min: Pectin was extracted at pH1.5, 120min from orange peels infrared dried at 600W-30min.
Oven-60°C: Pectin was extracted at pH1.5,120min from orange peels oven dried at 60°C.

4.19. Differential Scanning Calorimetry (DSC) of Electrospun Nanofibers Produced by Using PEO and Various Pectin Samples

DSC curves and thermal properties of PEO and pectin powders are presented in Figure 18 and Table 21, respectively. As shown in Figure 18, peak observed at 71.60°C for PEO was attributed to the melting of the sample. The melting temperatures of PEO was reported as 68.4°C by Acosta and Morales (1996). The peak indicating degradation temperature of pectin was observed in the range of 224.79-248.36°C for all pectin samples (Table 21). The degradation temperature of pectin was reported as 232°C by Akınalan Balık et al. (2019) and al 205°C by Neufeld and Bianco-Peled (2017). As shown in Figure 18, a broad peak observed in the range of 160-178°C for pectin was due to the loss of water associated with hydrophilic groups of pectin (Çay et al., 2014; Koosha and

Mirzadeh, 2015; Neufeld and Bianco-Peled, 2017). Neufeld and Bianco-Peled (2017) reported a broad peak between 130-160°C. The enthalpy for the peaks of PEO and pectin powders were in the range of 71.07-169.35 J/g. The enthalpy of the commercial pectin sample was found to be 71.07 J/g. As compared to the commercial pectin sample, enthalpy values were higher for the pectin samples produced in the study. Similar enthalpy values were obtained for Oven-60°C, Oven-70°C, IR-600W-30min. and IR-700W-30min. Among all pectin samples, the enthalpy of IR-800W-30min. sample was the highest. Higher enthalpy values might be attributed to improved thermal stability.

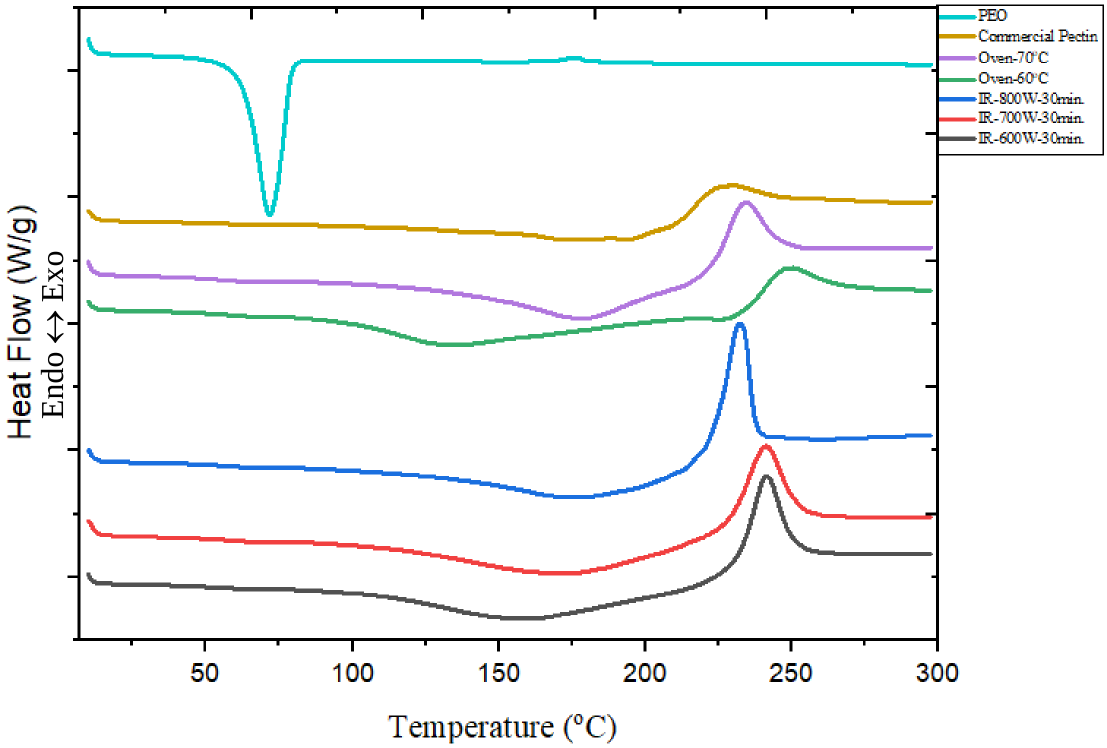


Figure 18. DSC curves of PEO and pectin powders.

IR-600W-30min: Pectin was extracted at pH1.5, 120min from orange peels infrared dried at 600W-30min.

Oven-60°C: Pectin was extracted at pH1.5,120min from orange peels oven dried at 60°C.

Table 21. Thermal properties of PEO and pectin powders *

Powder samples	**Tpeak (°C)**	**ΔH (J/g)**
IR-600W-30min.*	244.04 ± 3.854	89.31 ± 9.440
IR-700W-30min.	243.32 ±3.026	92.65 ± 5.310
IR-800W-30min.	236.19 ± 4.999	108.95 ± 2.051
Oven-60°C***	248.36 ± 0.233	92.93 ± 8.273
Oven-70°C	233.35 ± 0.983	91.49 ± 2.645
Commercial Pectin	224.79 ± 2.369	71.07 ± 4.695
PEO	71.60 ± 0.735	169.35 ± 13.930

* Means are based on duplicate analyses.

****** Pectin was extracted at pH1.5, 120min from orange peel samples infrared dried at 600W for 30min

*******Pectin was extracted at pH1.5, 120min from orange peel samples oven dried at 60°C.

DSC curves of PEO nanofiber and pectin+PEO nanofibers are shown in Figure 19. DSC analysis was carried out for the samples (pectin:PEO 3:1(% w/w, +Triton X-100)) electrospun at 15cm, 0.5 ml/h and 35kV, due to the better morphology of the nanofibers. Peaks were observed between 54-58°C for pectin+PEO nanofibers. This was due to the melting temperature of PEO (Akınalan Balık et al., 2019). As seen in Figure 19, peaks, observed between 229-249°C for pectin+PEO nanofibers, could be attributed to the thermal degradation of pectin.

Because of the compatibleness of PEO and pectin, both of the peaks of PEO or pectin were also observed in pectin+PEO nanofibers. The miscibility of pectin+PEO blends was also reported by Cui et al. (2016) and Akınalan Balık et al. (2019).

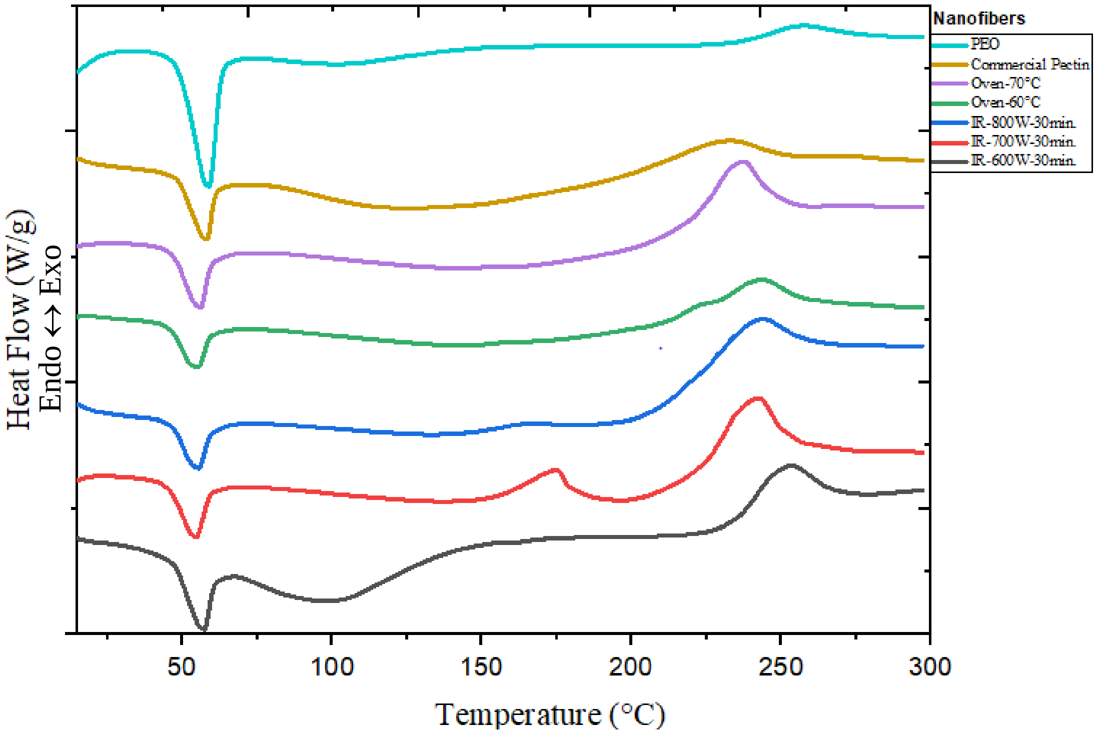


Figure 19. DSC curves of PEO nanofiber and pectin+ PEO nanofibers

(Pectin+PEO nanofibers were produced from pectin:PEO 3:1 (% w/w) + Triton X-10
PEO nanofibers were produced from PEO (1% w/w) +Triton X-100 (1% w/w)
(IR-600W-30min: Pectin was extracted at pH1.5, 120min from orange peels infrared dried at 600W-30min.
Oven-60°C: Pectin was extracted at pH1.5,120min from orange peels oven dried at 60°C.)

4.20. Water Contact Angle of Electrospun Nanofibers Produced by Using PEO and Various Pectin Samples

The surface hydrophobicity/hydrophilicity of the pectin nanofibers was evaluated by measuring the water contact angle, which provides knowledge about materials' surface wettability (Trinca et al., 2017). As stated by Mouro et al. (2021), water contact angle values below 20° or above 90° are characteristic for very hydrophilic surfaces and hydrophobic surfaces, respectively. Whereas water contact angle values between 40°-70° are indicative of surfaces with moderate hydrophilicity.

The water contact angle values of the PEO nanofiber and pectin+PEO nanofibers are given in Table 22. Determination of the water contact angle value was carried out for the samples (pectin:PEO 3:1(% w/w, +Triton X-100)) electrospun at 15cm, 0.5 ml/h and 35kV, due to the better morphology of the nanofibers.

The analysis of variance (ANOVA) results showed that the water contact angle values of pectin+PEO nanofibers were significant ($p < 0.05$) (Table 22). The water contact angle of pectin nanofibers were in the range of 15.60-50.14°. The lowest water contact angle value (15.60°) was obtained for the commercial pectin+PEO nanofibers. However, it was not significantly different than that (20.98°) of IR-800W-30min. IR-800W-30min. (20.98°) and Oven-70°C (24.34°) sample were classified in the same statistical group in terms of water contact angle value. Significantly higher water contact angle values were obtained for IR-600W-30min. (30.76°) and Oven-60°C (31.86°) sample. However, the difference between water contact angle values of IR-600W-30min. and Oven-60°C samples were not significant. All pectin+PEO nanofiber samples, except IR-700W-30min, had hydrophilic surface characteristics. Among all nanofibers studied, significantly highest water contact angle value (50.14°) was obtained for the pectin+PEO nanofibers produced from IR-700W-30min. Since the value was greater than 40°, IR-700W-30min. displayed moderate hydrophilic surface characteristics. This indicated less hydrophilic surface characteristics as compared the other nanofiber samples. Less hydrophilic surface characteristics could be preferred in order to prevent moisture transfer between food and the environment, thereby extending the shelf-life of the food (Rather et al., 2021)

Probably, infrared drying of orange peels at 700W for 30min. created a difference in the properties of the pectin produced. It seems that the utilization of this pectin in nanofiber production together with PEO improved the surface characteristics of the nanofibers, probably due to the differences in the properties of pectin.

Ezati and Rhim (2020) reported the water contact angle of pure citrus pectin (esterification degree>67%) film as 56.2°. The result found for IR-700W-30min. sample (50.14°) in our study was found to be similar to that of the one reported by Ezati and Rhim (2020).

Table 22. Water contact angle of PEO nanofiber and pectin+PEO nanofibers *

Nanofibers		**Contact Angle (°)**
IR-600W-30min. **	+ PEO+Triton X-100	30.76 ± 3.582^{bc}
IR-700W-30min.	+ PEO+Triton X-100	50.14 ± 0.738^{a}
IR-800W-30min.	+ PEO+Triton X-100	20.98 ± 2.850^{de}
Oven-60°C ***	+ PEO+Triton X-100	31.86 ± 3.893^{b}
Oven-70°C	+ PEO+Triton X-100	24.34 ± 1.716^{cd}
Commercial Pectin	+ PEO+Triton X-100	15.60 ± 0.142^{e}
PEO	+ Triton X-100	31.03 ± 4.417^{bc}

* Values followed by the same letter are not significantly different (α= 0.05).
Means are based on duplicate analyses.
****** Pectin extracted at pH1.5-120min. from orange peel samples infrared dried at 600W for 30min
*******Pectin extracted at pH1.5-120min. from orange peel samples oven dried at 60°C.

4.21. Color Values of Electrospun Nanofibers Produced by Using PEO and Various Pectin Samples

Color values and ΔE values of the PEO nanofiber and pectin+PEO nanofibers are given Table 23. Color values were determined for the samples (pectin:PEO 3:1(% w/w, +Triton X-100) or PEO (1% w/w)) electrospun at 15cm, 0.5 ml/h and 35kV, due to the better morphology of the nanofibers. Aluminum foil was utilized as the target during the calculation of ΔE since the nanofibers were deposited on it. Color values of the aluminum foil were L* = 82.55, a*= -0.55, and b* = -1.43. L*, a* and b* and ΔE values of the PEO nanofibers were 89.51, -0,28, -1.18 and 7.07, respectively. IR-800W-30min. and commercial pectin nanofibers had slightly lower L* color values as compared to that of pure PEO nanofiber. Lower L* color values were obtained for IR-600W-30min. (82.28), IR-700W-30min. (81.21), Oven-60°C (81.17) and Oven-70°C (80.69). ΔE values of these samples were also lower as compared to those of commercial pectin, IR-800W-30min. and PEO nanofibers. It was reported by Pishyar et al. (2022) that the lower lightness might be an alternative packaging for different types of foods to protect the foods against the negative effects of light.

a* and b* color values of the nanofibers changed from -0.44 to 0.07 and from -1.51 to 0.09, respectively. It was found that b* (yellowness) color value of commercial pectin nanofiber was slightly higher as compared to that of other nanofibers.

In a study by Priyadarshi et al. (2021), citrus pectin was blended with pullulan in different ratios for fabrication of films by solvent casting method. L*, a*, b* and ΔE values of the pure pectin based film were 90.94, -0.45, 6.38 and 2.11, respectively. In our study, lower L* values for all pectin+PEO nanofibers and lower ΔE values for IR-700W-30min. (1.66) and Oven-60°C (1.56) nanofibers were obtained.

Table 23. Color values of PEO nanofiber and pectin+PEO nanofibers *

Nanofibers		**Color values**			
		L*	**a***	**b***	**ΔE**
IR-600W-30min.**	**+ PEO +Triton X-100**	82.28 ± 3.486	-0.44 ± 0.148	-1.51 ± 0.672	2.52 ± 0.396
IR-700W-30min.	**+ PEO +Triton X-100**	81.21 ± 1.732	-0.35 ± 0.163	-1.13 ± 0.523	1.66 ± 1.287
IR-800W-30min.	**+ PEO +Triton X-100**	87.87 ± 1.916	-0.26 ± 0.141	-0.83 ± 0.686	5.37 ± 1.980
Oven-60°C***	**+ PEO +Triton X-100**	81.17 ± 1.237	-0.27 ± 0.021	-1.06 ± 0.346	1.56 ± 1.018
Oven-70°C	**+ PEO +Triton X-100**	80.69 ± 1.252	0.06 ± 0.071	-0.20 ± 0.247	2.42 ± 0.827
Commercial Pectin	**+ PEO +Triton X-100**	87.66 ± 0.792	0.07 ± 0.035	0.09 ± 0.156	5.37 ± 0.792
PEO	**+ Triton X-100**	89.51 ± 1.527	-0.28 ± 0.346	-1.18 ± 1.322	7.07 ± 1.577

* Means are based on duplicate analyses.

****** Pectin extracted at pH1.5-120min. from orange peel samples infrared dried at 600W for 30min

*******Pectin extracted at pH1.5-120min. from orange peel samples oven dried at 60°C.

5. CONCLUSION

In this thesis, pectin was extracted from orange peels infrared dried or oven dried at different conditions. The effects of different drying and extraction conditions on pectin properties were investigated. Nanofibers were produced by using these pectin samples by itself or with PEO or PVA by using different electrospinning parameters. The effects of pectin properties and different electrospinning parameters on the characteristics of nanofibers were investigated.

Orange peel samples were infrared dried at 600W, 700W and 800W for 30min or oven dried at 60°C and 70°C. Pectin was extracted from dried orange peels at 90°C, by using different extraction conditions (pH 1-1.5-2, extraction time 60-90-120min.).

Effects of different drying methods applied to orange peels and different pectin extraction conditions on pectin yield, galacturonic acid, esterification degree, color and FTIR spectra of pectin samples were investigated. Best pectin extraction method was determined by investigating the properties of the pectin samples and pectin nanofibers. The pectin samples had high purity (galacturonic acid 72.79-98.66%) and high esterification degree (59.22-94.06%). The highest yield of pectin was obtained at pH 1-120min for oven dried orange peels and at pH 1.5-120 min. for infrared dried orange peels. The results obtained for electrospun pectin+PVA nanofibers confirmed that extraction of pectin at pH1.5 is the key parameter for finest fiber (121 ± 22 nm, 110 ± 21 nm, 107 ± 19 nm) formation with better morphology. Considering the overall evaluation of the results, pH 1.5-120 min was chosen as the best extraction condition.

Overall results showed that infrared drying significantly caused an increase in yield (11.70%, db), galacturonic acid content (74.17-80.12%) and esterification degree (76.47-81.25%) of pectin as compared to oven samples. Infrared made it possible to produce higher yields of pectin in a shorter time, as compared to oven drying. The disruption of cellular structures due to higher infrared power resulted in greater pectin extraction efficiency. It can be concluded that pectin extraction efficiency can be increased when novel technologies were used in drying of pectin sources. As the IR power or oven drying temperature was increased, L* (lightness) value was slightly decreased, a* (redness) and b* (yellowness) values were slightly increased.

It was not possible to observe jet formation by electrospinning of pure pectin solutions due to its polyelectrolyte structure, insufficient viscosity, and chain entanglements. However, mixing pectin with PEO or PVA improved electrospinnability and resulted in continuous nanofiber formation.

Nanofibers were produced by using pectin+PEO (3:1, 3:2, 4:1, 4:2, 5:1 (% w/w) + Triton X-100) or pectin+PVA (3:5, 3:6, 2.5:5, 2.5:6, 2:5, 2:6 (% w/w)) in order to determine the co-polymer that will be used in the rest of the study. Different flow rate (0.2-0.7ml/h), voltages (15-35kV) and distances (8-20cm.) were used as electrospinning parameters. It was possible to use higher concentrations of pectin in pectin+PEO solutions as compared to those of pectin+PVA solutions. Higher concentrations of pectin used in pectin+PEO solutions and overall results for morphology (SEM), DSC thermogram, and water contact angle values of the nanofibers showed that the most appropriate copolymer was PEO and the most appropriate electrospinning solution concentration was found to be pectin:PEO 3:1 (% w/w, with Triton X-100).

Based on the preliminary studies, it was decided to use 15cm, 0.5ml/h, 35kV as electrospinning parameters in the production of pectin+PEO nanofibers by using all pectin samples produced in the study.

XRD patterns of PEO nanofibers showed that electrospinning process reduced the crystallinity of PEO. However electrospinning did not cause a change in XRD patterns of pectin. Enthalpy values of the pectin samples produced in the study were higher (89.31-108.95 J/g) as compared to that of (71.07 J/g) commercial pectin. Both of the peaks of PEO or pectin were observed in FTIR, DSC thermogram, and XRD pattern for pectin+PEO nanofibers, indicating the compatibleness of PEO and pectin in nanofiber production.

Pectin+PEO nanofibers including pectin produced in the study had higher water contact angle value (20.98-50.14°) as compared to that of commercial pectin (15.60°) nanofiber. Pectin extracted from orange peels dried at 700W-30min. showed moderate (50.14°) hydrophilic surface characteristics.

Infrared drying of orange peels at 700W for 30min. may have affected the properties of the produced pectin. Using this pectin in combination with PEO for nanofiber production improved the surface characteristics of the nanofibers, possibly due to differences in the properties of pectin. Less hydrophilic surface could be beneficial for extending the shelf-life of food by preventing moisture transfer between the food and the environment.

Lower lightness (L*) color values were observed for IR-600W-30min, IR-700W-30min, Oven-60°C, and Oven-70°C samples, as compared to commercial pectin, IR-800W-30min sample and PEO. Lower L* color values might be an alternative for packaging of different types of foods to protect the foods against the negative effects of light.

Considering the overall results, infrared drying of orange peels was found to have an effect on the morphology, surface properties, and thermal stability of the produced pectin+PEO nanofibers. Further studies in this field may provide additional information on potential use of these nanofibers in various fields such as food industry.

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APPENDIX

Appendix 1. Multiple comparison test results of pectin yield.

Tests of Between-Subjects Effects

Dependent Variable: Pectin Yield (%)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2516,014[a]	44	57,182	603,980	,000
Intercept	6542,000	1	6542,000	69099,144	,000
Drying method	107,803	4	26,951	284,664	,000
Extraction pH	2034,956	2	1017,478	10747,001	,000
Extraction time	56,954	2	28,477	300,785	,000
Drying method * extraction pH	266,098	8	33,262	351,329	,000
Drying method * extraction time	9,488	8	1,186	12,527	,000
Extraction pH * extraction time	23,109	4	5,777	61,022	,000
Drying method * extraction pH * extraction time	17,605	16	1,100	11,622	,000
Error	4,260	45	,095		
Total	9062,274	90			
Corrected Total	2520,274	89			

a. R Squared = 0,998 (Adjusted R Squared = 0,997)

Appendix 2. Multiple comparison test results of galacturonic acid content of pectin.

Tests of Between-Subjects Effects

Dependent Variable: Galacturonic Acid Content of Pectin (%)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2255,205[a]	29	77,766	5,158	,000
Intercept	409722,510	1	409722,510	27174,625	,000
Drying method	436,428	4	109,107	7,236	,000
Extraction ph	506,923	1	506,923	33,621	,000
Extraction time	162,611	2	81,306	5,393	,010
Drying method * extraction ph	239,739	4	59,935	3,975	,011
Drying method * extraction time	405,998	8	50,750	3,366	,007
Extraction ph * extraction time	116,860	2	58,430	3,875	,032
Drying method * extraction ph * extraction time	386,645	8	48,331	3,206	,009
Error	452,322	30	15,077		
Total	412430,036	60			
Corrected Total	2707,527	59			

a. R Squared =0,833 (Adjusted R Squared = 0,671)

Appendix 2. Multiple comparison test results of esterification degree of pectin.

Tests of Between-Subjects Effects

Dependent Variable: Esterification Degree of Pectin (%)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2890,477[a]	29	99,672	7,642	,000
Intercept	317900,134	1	317900,134	24373,536	,000
Drying method	244,264	4	61,066	4,682	,005
Extraction ph	1280,849	1	1280,849	98,203	,000
Extraction time	85,436	2	42,718	3,275	,052
Drying method * extraction ph	522,171	4	130,543	10,009	,000
Drying method * extraction time	301,901	8	37,738	2,893	,016
Extraction ph * extraction time	215,359	2	107,679	8,256	,001
Drying method * extraction ph * extraction time	240,499	8	30,062	2,305	,047
Error	391,285	30	13,043		
Total	321181,897	60			
Corrected Total	3281,762	59			

a. R Squared =0,881 (Adjusted R Squared = 0,766)