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Gallic acid encapsulated pea flour-based nanofibers produced by electrospinning as a potential active food packaging material

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Abstract

The main aim of the article was to produce gallic acid-loaded pea flour/polyethylene oxide (PEO)-based nanofibers through the electrospinning method as an alternative active packaging material. Incorporation of gallic acid caused significant changes in both solution properties and fiber morphology. Increasing gallic acid concentration resulted in increasing conductivity and decreasing consistency index, by the way decreasing average fiber size diameter. In thermogravimetric analysis (TGA), gallic acid addition resulted in a decrease in thermal degradation value of nanofibers. In addition to these changes, new chemical bands formed were an indicator of the successful encapsulation of gallic acid in Fourier-transform infrared (FTIR) spectra. Nanofibers also showed promising results in terms of encapsulation of bioactive compounds. Therefore, gallic acid-loaded pea flour/PEO-based nanofibers might be considered as a promising active packaging material.

KEYWORDS
active packaging, electrospinning, encapsulation, gallic acid, pea flour

1 | INTRODUCTION

Grain legumes such as pea, lentil, and chickpea are classified as a primary protein source for almost 30% of the world population. In addition to these benefits, legumes are responsible for the nitrate cycle and green manuring. After bean, the pea is the second most commonly cultivated legume (Coyne et al., 2020). Pea is regarded as a considerable source of some nutrients including protein (20–26% w/w), lipids (1–3% w/w), carbohydrate (46–50% w/w), and fiber (14–18% w/w) (Cipollone & Tironi, 2020). The potential film-forming ability of legume flours obtained from whole-grain materials is derived from high starch and protein content (Galus et al., 2020). To take different advantages of pea flour, it might be utilized as an alternative food packaging material. For example, polysaccharides are used to improve mechanical properties, and proteins are considered as a barrier for gas and aroma at low relative humidity values. Furthermore, films containing both carbohydrate and protein source were shown to have improved mechanical properties (Aydogdu, Yildiz, Ayhan, et al., 2019). Nanoscale fibers were commonly preferred in enzyme immobilization (Porto et al., 2019), textile production (Levitt et al., 2018), biosensors (Mishra et al., 2017), drug delivery (Eslamian et al., 2019), and tissue engineering (Brito-Pereira et al., 2018). Among the methods, electrospinning is the most widely preferred method to obtain nanofiber. It is very advantageous in terms of cost and simplicity. It is possible to obtain well-ordered, homogenous nanofibers with a high surface-to-volume ratio by electrospinning. Furthermore, nanofibers might be produced from many different sources such as natural, synthetic, and hybrid. Whereas soy, casein, zein, whey,

collagen, and gelatin might be listed as the most commonly studied protein source, cellulose, chitosan, starch, alginate, cyclodextrin, and pullulan are the examples of carbohydrate sources.

According to the Plastic Pollution Coalition, in 2019, 40 million tons of plastic waste was produced. It revealed the importance of developing biodegradable and bio-based packaging materials (Sonar et al., 2020). Therefore, using one of these natural sources, mentioned above, is important to produce environmentally friendly films and critical to increase sustainability (Uygun et al., 2020).

One of the naturally occurring polyphenols, 3,4,5-trihydroxybenzoic acid, namely, gallic acid, is commonly presented in different sources such as fruits, vegetables, tea leaves, and longan seeds. It might also be obtained from both solid and liquid by-products of the agri-food industry such as wine. Gallic acid is also referred as a bioactive compound because of its antioxidant, antifungal, anti-inflammatory, and anticarcinogenic characteristics. However, it is very susceptible to high temperature, light, and oxygen, which are common conditions in food processing (Quiles-Carrillo et al., 2019). However, it should also be noted that the polymer matrix is another crucial parameter for stability and encapsulation efficiency. Although the encapsulation of gallic acid with electrospinning technique is a promising method, coating materials used in this technique are so limited. For example, in previous studies, cyclodextrin (Aytac et al., 2016), zein, cellulose acetate (Phiriyawirut & Phaechamud, 2012), and hyroxypropyl methyl cellulose (Aydogdu, Sumnu, & Sahin, 2019) were used as a coating material in electrospinning.

In this study, it was aimed to obtain gallic acid-loaded pea flour-based nanofibers. It was also aimed to analyze morphological, thermal, and chemical characteristics, loading efficiency (LE), and antioxidant capacity of nanofibers to be suggested as active packaging material for food with oxidation stability.

2 | MATERIAL AND METHODS

2.1 | Materials

Pea flour with 55 ± 5% (w/w) carbohydrate, 22 ± 2% (w/w) protein, 12 ± 2% (w/w) dietary fiber 2 ± 2% (w/w) fat, 7–10% (w/w) moisture, and 3 ± 1% (w/w) ash was purchased from Molar Chemical Materials Trading Co. Inc. (Turkey). Polyethylene oxide (PEO) (molecular weight [MW] = 900 kDa) was supplied from Sigma-Aldrich. Polyoxyethylene sorbitan monooleate, Tween 80 (ρ: 1.064 g/cm^3 and μ: 400–620 cps at 25°C), as a surfactant and gallic acid with molar mass of 170.12 g/mol were purchased from Merck (Darmstadt, Germany).

2.2 | Solution preparation

PEO (3.5% w/v) was dissolved in water with a magnetic stirrer (MaxTir 500, Daihan Scientific, Seoul, Korea) until obtaining homogenous solution. Then, pea flour (5.25% w/v) was added in polymer solution, and mixture was homogenized with a high-speed homogenizer (IKA T25 Digital Ultra-Turrax, Staufen, Germany) at 10,000 rpm. To adjust pH of solution to 10.2, 2-M NaOH solution was preferred. After that, solution was heated in water bath until reaching 80°C. To mix and keep solution temperature constant, mixture was stirred at 750 rpm for 1 h.

Then, gallic acid (0.1 g/ml) was dissolved in 80% ethanol aqueous solutions (ethanol:water = 4:1 w/w) and added to pea flour/PEO solution. Gallic acid amount is 5% and 10% (w/w) of solid fibers. Because addition of gallic acid decreased pH of solutions to about 3, the pH of solutions was adjusted again to pH 10.2 with 2-M NaOH solution. The pea flour/PEO solution without gallic acid was used as control.

2.3 | Solution properties

2.3.1 | Rheological properties

To measure rheological behavior of the pea flour/PEO polymer mixture, controlled strain rheometer, the cone with 4° cone angle and plate having 40-mm diameter, was preferred (Kinexus dynamic rheometer, Malvern, UK). Experiment was carried out at room temperature. Change in shear stress values with respect to shear rate varying between 0.1 and 100 s^{-1} was recorded, and collected data were fitted power law equation:

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

where τ, $\dot{\gamma}$, k, and n referred to the shear stress (Pa), shear rate (s^{-1}), consistency index (Pa·s^n), and flow behavior index, respectively.

2.3.2 | Electrical conductivity

The electrical conductivity of solutions was measured by using a conductometer (WTW LF95, Germany) at 25°C in triplicate.

2.3.3 | Total phenolic content of pea flour solutions

Total phenolic content (TPC) of solutions was measured by the Folin–Ciocalteu method (Aydogdu, Yildiz, Aydogdu, et al., 2019). First, pea flour/PEO/gallic acid solutions were diluted using 80% ethanol aqueous solution; 0.5 ml of this diluted sample was taken and mixed with 2.5 ml of 0.2-N Folin–Ciocalteu reagent. The mixture was kept in dark place for 5 min. After addition of 2 ml of 75-g/L sodium carbonate solution into the mixture, solutions were mixed by vortex and stored in dark for 1 h. The absorption of was read at 760 nm by using a spectrophotometer (UV 2450, Shimadzu, Columbia, USA). Calibration curve was prepared with different gallic acid concentrations, and by using calibration curve, TPC of solutions was determined as gallic acid equivalent (GAE) in milligrams per gram dry weight.

2.4 | Electrospinning

The electrospinning process was carried out by using electrospinning device (Nano-Web 103, Mersin, Turkey). The syringe with a metallic 21-gauge steel needle and 11.53-mm inner diameter was placed horizontally into the pump. Positively charged electrode, which was powered by a direct current (DC) high-voltage supplier, was connected with syringe. The flow rate of solutions and the voltage were maintained as 0.6 ml/h and 15 kV, respectively. Nanofibers were collected on to collector covered by aluminum foil. The distance between the collector and needle tip was fixed as 30 cm. Experiments were performed at 25 ± 1°C and 30–40% relative humidity.

2.5 | Characterization of electrospun nanofibers

2.5.1 | Morphological analysis

The morphological characterization of the nanofiber sheets was carried out using field emission scanning electron microscopy (FESEM) (JEOL, Japan) at 10,000× magnification level. Fibers were stuck on metal stubs and then coated with gold palladium (10 nm). Diameter of at least 100 fibers from the scanning electron microscopy (SEM) images of each sample was measured by using ImageJ software (Maryland, USA).

2.5.2 | Total phenol content of electrospun fibers

To determine total phenol content (TPC) of nanofibers containing different amounts of gallic acid, the Folin–Ciocalteu method was used. After dissolving 0.1-g nanofiber in ethanol–water solution (80:20), the same procedure used for the determination of TPC of the solution was followed. The LE of gallic acid was calculated from the Equation (2):

$$LE\ (\%) = \frac{\text{TPC of gallic acid-loaded nanofibers}}{\text{TPC of gallic acid-added solutions}} \times 100. \quad (2)$$

The measurements were carried out in triplicate.

2.5.3 | Antioxidant capacity of electrospun fiber

To measure antioxidant activity (AA) of the nanofibers, 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described by Luca et al. (2013) was followed with some modifications. After dissolving nanofibers in ethanol/water solution (80:20), 0.1 ml of that solution and 3.9 ml of 25-ppm DPPH solution were mixed and stored at dark place for 1 h. The absorbance values (A_2) was measured at 517 nm by using spectrophotometer (UV 2450, Shimadzu, Columbia, USA). For blank, 0.1 ml of methanol (A_1) was mixed with DPPH solution, and the same procedure was followed for the rest. By using calibration curve, concentrations (C_1 and C_2) corresponding A_1 and A_2 were determined, respectively. Then, the AAs were calculated according to Equation (3):

$$AA\left(\frac{\text{mgDPPH}}{\text{g dry weight of sample}}\right) = \frac{C_1 - C_2}{W_{\text{sample}}} \times V, \quad (3)$$

where C_1 is the concentration of DPPH$^{\cdot}$ immediately after the sample (ppm) and DPPH$^{\cdot}$ solution was mixed, C_2 is the concentration of DPPH$^{\cdot}$ 2 h after mixing (ppm), V is the volume of solution (L), and W_{sample} is the amount of nanofiber (g).

2.5.4 | Thermogravimetric analysis

Thermogravimetric analysis (TGA) 2950 (Exstar TG/DTA 6300, RTI Instruments, Inc., Woodland, USA) was used for TGAs. About 5-mg nanofiber and raw materials was heated from room temperature to 500°C at a rate of 10°C/min and with nitrogen at a flow rate of 30 ml/min. Analyses were performed in triplicates.

2.5.5 | Differential scanning calorimetry

The thermal analysis of electrospun nanofibers was conducted by using the differential scanning calorimeter (Pyris 6 DSC, PerkinElmer, Massachusetts, USA), equipped with a cooling system with nitrogen. A piece of sample (4–5 mg) was placed in a hermetically sealed aluminum pan, and sample was heated from room temperature to 250°C at a rate of 5°C/min. As a reference, an empty sample pan was used. The differential scanning calorimetry (DSC) measurements were performed in triplicate.

2.5.6 | Fourier-transform infrared analysis

Fourier-transform infrared (FTIR) analyses of electrospun nanofibers and raw materials (pea flour, gallic acid, and PEO) were carried out by using FTIR spectrophotometer (IR-Affinity1, Shimadzu, Kyoto, Japan) in attenuated total reflectance (ATR) mode using a diamond ATR crystal. The infrared region analysis was recorded with 32 scans over the wavenumber range of 600–4000 cm^{-1}.

2.6 | Statistical analysis

Analysis of variance (ANOVA) was conducted by Minitab (Version 16, State College, PA, USA). If significant differences were found, Tukey's multiple comparison test was used for comparisons ($p \leq 0.05$).

3 | RESULTS AND DISCUSSION

3.1 | Physical properties of solutions

The viscosity of solutions is one of the most important parameters in the success of the electrospinning process because it should be high

enough to show a synergistic effect with electrostatic repulsion to overcome surface tension. Another prerequisite for fiber development is the formation of chain entanglements, which is strongly affected by the concentration of biopolymers and directly associated with the solution's viscosity. Chain entanglements both provide elasticity to polymer melt and help obtain uniform fiber diameter (Pinheiro Bruni et al., 2020).

The rheological properties of solutions are shown in Table 1. All solutions obeyed the power law. The viscosity of solutions depends on the solvent type, the polymer type, and polymer concentration (Kriegel et al., 2009). In this study, because the same amount of PEO and pea flour was used for each sample, the rheological properties of solutions differed with respect to gallic acid concentration.

All sample solutions represented non-Newtonian shear thinning behavior ($n < 1$). A lower flow behavior index indicated possible interaction between protein and polysaccharide molecules via hydrogen bonding (Jia et al., 2020).

The consistency index value (k [Pa·s^n]) was also significantly affected by the presence of gallic acid and its concentration. Increasing gallic acid concentration resulted in a decrease in the consistency index of each sample. During the sample preparation, gallic acid was dissolved in ethanol/water solution and added to the polymer mixture. Therefore, the decreasing trend in consistency index for samples with gallic acid addition might be explained by the dilution effect of gallic acid. However, for samples containing different gallic acid concentrations, a lower k value might be interpreted as looser intermolecular interactions (Deng et al., 2020). Therefore, it might be concluded that the addition of gallic acid decreased the possible hydrogen bonds between hydroxyl groups of starch and etheric oxygen of PEO (Pereira et al., 2011).

In addition to rheological behavior, the polymer mixture's conductivity is another critical factor that affected bead free fiber formation and its size distribution. Conductivity should be high enough to overcome surface tension. By the way, it provides an opportunity for stretching of the polymer solution and starting electrospinning. Up to a certain point, high electrical conductivity promoted thinner fiber formation. However, after that level, it increased electrostatic repulsive forces and bending instability. This made it difficult to obtain homogenous nanofibers (Aydogdu et al., 2018a). The electrical conductivity of all solutions was shown in Table 1. Increasing gallic acid concentration in the solution resulted in an increase in conductivity values drastically. This might be due to the dissociation of gallic acid and the presence of the hydrogen donor (—OH) group (Aytac et al., 2016).

Furthermore, after gallic acid addition, the pH of the solution decreased. In order to set its pH to 10.2 again, 2-M NaOH was added. Therefore, this procedure increased charged molecules in the polymer melt and conductivity. In this study, even the higher electrical conductivity of solutions containing 10% gallic acid did not result in jet instability. Homogenous fibers were obtained from solutions containing 10% gallic acid (Figure 1).

3.2 | Characterization of electrospun nanofibers

3.2.1 | Morphological analysis of nanofibers

One of the essential criteria to obtain homogenous fiber morphology is sufficient interaction between the electrospun polymer mixture. Pea flour is a multicomposite material containing mostly carbohydrates (55% w/w) and protein (22% w/w). Vega-Lugo and Lim (2012) reported that globular protein conformation obstructed physical interaction with carrier polymer during electrospinning. However, the conformation of proteins might alter depending on pH and temperature. Therefore, to increase possible bond formation between materials from different origins, some unique treatments might be required.

The isoelectric point (pI) of pea protein was recorded as 4.5 (Oguz et al., 2017). Protein starts to aggregate, their solubility minimizes, and their total net charge is zero at pI. At alkaline pH, proteins start to unfold, by the way, their polypepetide chain entanglements and chain-chain interaction with PEO increase.

Although the main aim of addition of NaOH to polymer solution was to promote denaturation of proteins, alkali treatments also changed the gelatinization process of starches. At alkaline pH, hydrogen bonding between amylose and amylopectin molecules was disrupted, which decreased gelatinization temperature (Uygun et al., 2020). Therefore, with the help of pH adjustment, heat treatment might accelerate bond formation between PEO and pea flour. By this way, more homogenous solution and possible bond formation were obtained. It can be seen from Figure 1 that all nanofibers prepared with different gallic acid concentrations had a homogenous structure.

As shown in Figure 1b,c, gallic acid addition did not damage the nanofiber structure. This was taken as an indication of successful gallic acid encapsulation by electrospinning.

The average diameters and diameter distribution of the electrospun nanofibers are also shown in Figure 1. In this study, except for the solution characteristics, any parameter that affected

TABLE 1 Rheological properties and electrical conductivities of solutions and average diameter and TPC of solutions

Gallic acid (%)	k (Pa·s^n)	n	Electrical conductivity (μS/cm)	Average diameter (nm)	TPC of solutions (mg gallic acid equivalent [GAE]/g dry matter)
0	2.66 ± 0.01^a	0.85 ± 0.01^b	1419 ± 3^c	297 ± 68^a	9.42 ± 0.49^c
5	2.29 ± 0.06^b	0.88 ± 0.02^a	13,700 ± 126^b	219 ± 48^b	14.48 ± 0.27^b
10	1.52 ± 0.03^c	0.89 ± 0.01^a	38,433 ± 466^a	191 ± 38^c	36.46 ± 0.03^a

Note: Columns having different letters are significantly different ($p \leq 0.05$).
Abbreviations: k, consistency index; n, flow behavior index; TPC, total phenolic content.

fiber morphology was kept constant. Therefore, rheological properties and electrical conductivity were responsible for the average fiber size. As seen from Table 1, there was a parallel trend between increasing consistency index and average fiber size. Higher *k* values were interpreted as more increased molecular entanglements, which resulted in larger fiber diameter (Aydogdu, Sumnu, & Sahin, 2019). Since the solutions with high consistency index elongated less, shortened jet path resulted in high diameters.

Furthermore, a higher consistency index meant a more viscous solution, which decreased mobility of ions and conductivity. As seen in Table 1, there was an inverse relation between conductivity and fiber size. Solutions with higher conductivity formed thinner fibers, which were directly related to experiencing stronger elongation force through the collector plate. The average diameters of nanofibers ranged almost between 190 and 300 nm with a standard deviation of between 38 and 68 nm. The narrow distributions and small standard deviations of nanofibers were the evidence of obtaining uniform nanofibers by electrospinning process.

3.2.2 | TPC and AA of solutions and nanofibers

According to the study conducted by Millar et al. (2019), TPC of pea flour varied from 121.93 ± 1.00 mg to 129.85 ± 2.58 GAE per 100 g.

This phenolic activity comes from the different concentrations of protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, *trans-p*-coumaric acid, *cis-p*-coumaric acid, *cis-p*-coumaric acid derivative, and *trans*-ferulic acid (López-Amorós et al., 2006). Therefore, the reason behind the TPC of pea flour/PEO sample without gallic acid might be the presence of these compounds (Table 1). However, TPC of that sample was lower than the already reported values. During solution preparation, adjusting pH to 10.2 and heating to 80°C for a while might cause degradation of some phenolic naturally present in pea flour. It was also revealed that harsh treatments such as high-pressure steam heating, microwave heating, and roasting decreased the phenolic and AA of flours (Yildiz et al., 2021). On the other hand, as expected, the addition of gallic acid increased TPC of solutions correlated with the concentration.

The TPC of gallic acid-loaded pea flour/PEO nanofibers was shown in Table 2. Increasing gallic acid concentration in formulation resulted in increasing TPC of fibers.

Fibers containing 5% and 10% gallic acid had 92.6 ± 3.1% and 74.4 ± 2.0% loading efficiencies, respectively. Therefore, it might be concluded that gallic acid was successfully encapsulated into pea flour-based nanofibers. It should be noted that one of the main reasons of such high efficiency is the advantageous operation temperature of electrospinning. As a natural phenolic acid, gallic acid is a heat-sensitive compound (Friedman & Jürgens, 2000), so process temperature becomes important during encapsulation. Because it operates at room temperature, electrospinning is the most appropriate technology to handle heat-labile compounds. A similar finding was also recorded in the study of quercetin- and ferulic acid-loaded amaranth isolate/pullulan nanofibers. The LE values were in the range of 84–94% (Aceituno-Medina et al., 2015).

Furthermore, high encapsulation efficiency of nanofibers might also be related to accelerated solidification rate of wall polymers (pea flour/PEO) and high solubility of encapsulated material (gallic acid) within the polymer mixture (Hani et al., 2017). As seen in Table 2,

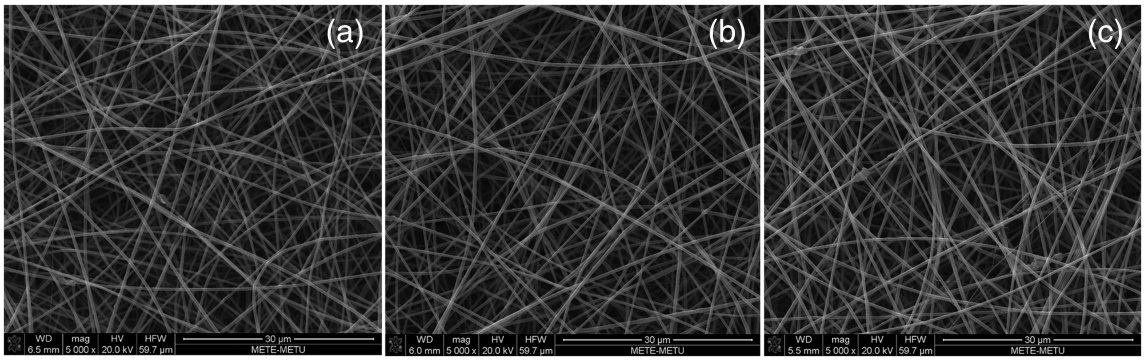

FIGURE 1 Scanning electron microscopy (SEM) images of electrospun nanofibers with different gallic acid concentrations: (a) control, (b) 5%, and (c) 10%

TABLE 2 Loading efficiency and total antioxidant activities of gallic acid-loaded pea flour/PEO electrospun nanofibers

Gallic acid (%)	TPC of nanofibers (mg gallic acid equivalent [GAE]/g dry matter)	Gallic acid loading efficiency (%)	Antioxidant activity (mg DPPH/g dry weight)
5	13.42 ± 0.45[b]	92.6 ± 3.1[a]	5.29 ± 0.24[a]
10	27.15 ± 0.75[a]	74.4 ± 2.0[b]	5.72 ± 0.33[a]

Note: Columns having different letters are significantly different ($p \leq 0.05$).
Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; PEO, polyethylene oxide; TPC, total phenolic content.

fibers with higher gallic acid amounts had lower LE related to average fiber size. Fibers having smaller diameter had higher exposure areas, in other words specific surface areas (Zhang et al., 2018), which caused rapid oxidation of gallic acid. Furthermore, during the electrospinning process, when the polymer is ejected from the needle's tip, water evaporates and solid fibers are collected on the plate. This solidification process is more rapid when fibers' diameters are smaller. Therefore, the time required to form a barrier around the active compound decreases and some of the gallic acid might not be encapsulated efficiently (Hani et al., 2017).

In the structure of gallic acid, there are three —OH groups *ortho*-position bond to the aromatic ring, which are effective for showing AA (Ghitescu et al., 2015). Table 2 shows the AA of gallic acid-loaded pea flour/PEO nanofibers. Similar to the TPC results, it proved that loaded gallic acid preserved its AA after electrospinning. However, as the gallic acid amount increased, AA of nanofibers did not increase significantly ($p \leq 0.05$). This could be related to having lower LE of those nanofibers.

3.2.3 | Thermal analysis of nanofibers

The thermal stability of composite films gave an idea about inheritance character of each component and molecular interaction between them. The thermal behavior of nanofibers, gallic acid, PEO, and pea flour was analyzed by TGA, and weight loss thermograms as a function of the temperature are shown in Figure 2.

Thermal degradation behavior of the films was in between main components, which were PEO and pea flour. The degradation temperature of PEO was around 350°C, and it was found that almost 4% of sample weight remained at the end of the experiment. On the other hand, compared with pea flour and gallic acid, gallic acid-loaded nanofibers achieved relatively higher thermal stability with a degradation temperature as seen in Figure 2. The main reason for that is the strong intermolecular interaction between all components.

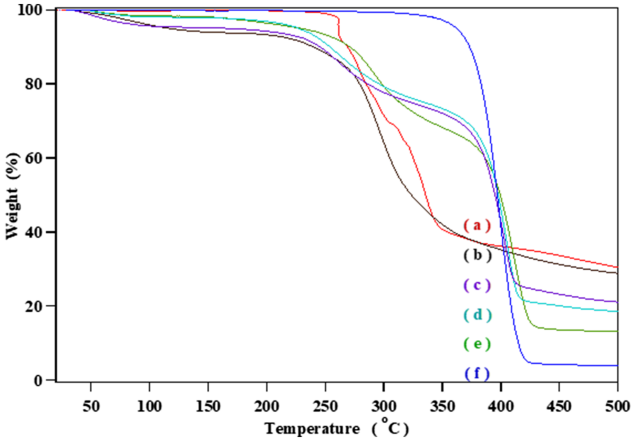

FIGURE 2 Thermogravimetric thermograms of gallic acid and electrospun nanofibers with different gallic acid concentrations: (a) gallic acid, (b) 10%, (c) 5%, (d) control, (e) pea flour, and (f) polyethylene oxide (PEO)

Although the first degradation temperature of control films was 264°C, for the gallic acid-loaded nanofibers, it was recorded as 232°C. Therefore, it might be concluded that the addition of gallic acid into the nanofibers decreased the thermal stability of them. It was interpreted that the addition of gallic acid at different levels in the formulation weakened the internal film structure and lowered the thermal degradation temperature.

Similar behavior was also observed in proso millet starch-based curcumin-incorporated films (Baek & Song, 2019). Furthermore, this decrease was also the evidence of successful encapsulation of gallic acid into nanofibers. The addition of gallic acid to the pea flour/PEO-based nanofibers interfered with interaction between protein–protein bonds and led to a reduction of the thermal stability. After the onset temperature, the first weight losses were associated with decomposition of polysaccharides, which corresponded to the elimination of hydrogen groups, decomposition, and depolymerization of the starch carbon chains (Sanyang et al., 2015). In this study, the first thermal degradation was related to pea flour. In the literature, pea starch degradation onset temperature was recorded as 310°C (Cano et al., 2015). However, the reason behind the decrease in T_{onset} value for pea flour/PEO-based nanofilms could be the significant interaction between components. Because of good compatibility and miscibility between gallic acid and starch molecules, crystallinity levels might suppress, resulting in a reduction in onset temperature.

The second degradation behavior was related to the presence of PEO. As seen in Figure 2, although the onset temperature of the second decomposition was in the range of 384–393°C for nanofilms, pure PEO onset degradation temperature was 400°C (Aydogdu et al., 2018b). PEO had a semicrystalline structure, and blending it with another polymer depressed the crystallinity, by the way decreased its thermal stability. These findings showed some similarities with those reported in the study of Aydogdu et al. (2018b). Nanofibers composed of PEO and hydroxypropyl methylcellulose had lower thermal stability with lower T_{onset} values than pure PEO.

Finally, at the end of the final degradation temperature, residue remained in pea flour/PEO-based films without gallic acid was lower than the nanofibers containing gallic acid. Generally, active components with an aromatic ring might produce more stable end products under nitrogen atmospheres. Similar outcomes were also recorded in curcumin and green tea extract containing starch films (Baek & Song, 2019).

DSC curve of gallic acid, PEO, pea flour, and nanofibers is demonstrated in Figure 3. All nanofibers exhibited an endothermic peak around 62°C, which was related to the melting point of pure PEO. Although the melting point of pure PEO was recorded as 68°C in the study of Aydogdu et al. (2018b), depression of melting point might be associated with disruption of semicrystalline structure. This result was also the evidence of good miscibility and interaction between components as supported by TGA results. Furthermore, similar thermal behavior was also recorded in the research of HPMC/PEO-based nanofilm (Aydogdu et al., 2018b).

Pea flour is mostly composed of starch and protein. As seen, pea flour had wide range endothermic peak, which was the indicator of

higher heterogeneity in the flour. It was mainly composed from protein, starch, and lipid. The wide range peak is the indicator of the melting of starch crystallites, amylose lipid complexes, and melting of amylose and protein denaturation. Although starch gelatinization and protein denaturation temperatures for pea flour were recorded as 62°C (Chung et al., 2008) and 84°C (Shevkani et al., 2015), respectively, there is no endothermic peak representing these thermal behaviors in DSC curve. While preparing solutions, to increase electrospinnability, solutions were heated to 80°C, and their pH values were adjusted to alkaline pH. Therefore, these treatments led to complete denaturation and gelatinization.

As seen in Figure 3, gallic acid exhibited an endothermic melting transition due to its crystal structure at 270°C. However, gallic acid-containing nanofibers had a broader peak with lower intensity compared with gallic acid. This alteration was high probably due to change in the crystal size distribution of gallic acid by dissolution in the electrospinning solution (Musuc et al., 2013).

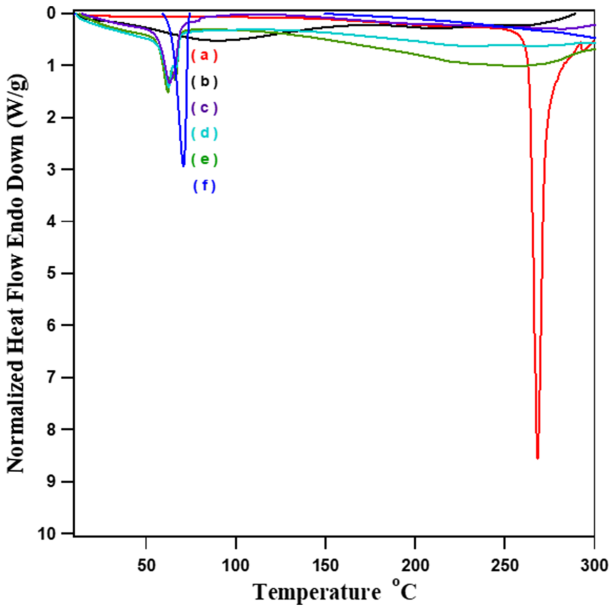

FIGURE 3 Differential scanning calorimetry (DSC) curves of gallic acid and electrospun nanofibers with different gallic acid concentrations: (a) gallic acid, (b) pea flour, (c) control, (d) 10%, (e) 5%, and (f) polyethylene oxide (PEO)

3.2.4 | FTIR analysis

The FTIR spectra of pea flour, gallic acid, PEO, and nanofibers are shown in Figure 4. FTIR spectrum was categorized into three main regions, which are fingerprint (800–1600 cm^{-1}), —CH stretch (2800 and 3000 cm^{-1}), and —OH stretch (3000 and 3600 cm^{-1}) (Uygun et al., 2020). In the spectrum, bands positioned between 600 and 1200 cm^{-1} were because of starch, which is the major constituent of the pea flour. Related to peaks located at these points, between 1100 and 1150 cm^{-1} is attributed to C—O, C—C, and C—O—H stretching and 900–1100 cm^{-1} was attributed to C—O—H bending and glycosidic linkages of starch (Yildiz et al., 2021). The peaks at 1240–1280 cm^{-1} also represent CH_2OH-related mode, which was a typical spectrum for the V form of amylose (Kizil et al., 2002). The band from 1500 to 1700 cm^{-1} was related to amide groups. The band located between 1500 and 1650 cm^{-1} was because of amide I groups (C═O), amide II groups of proteins (C—N stretching), and N—H angular

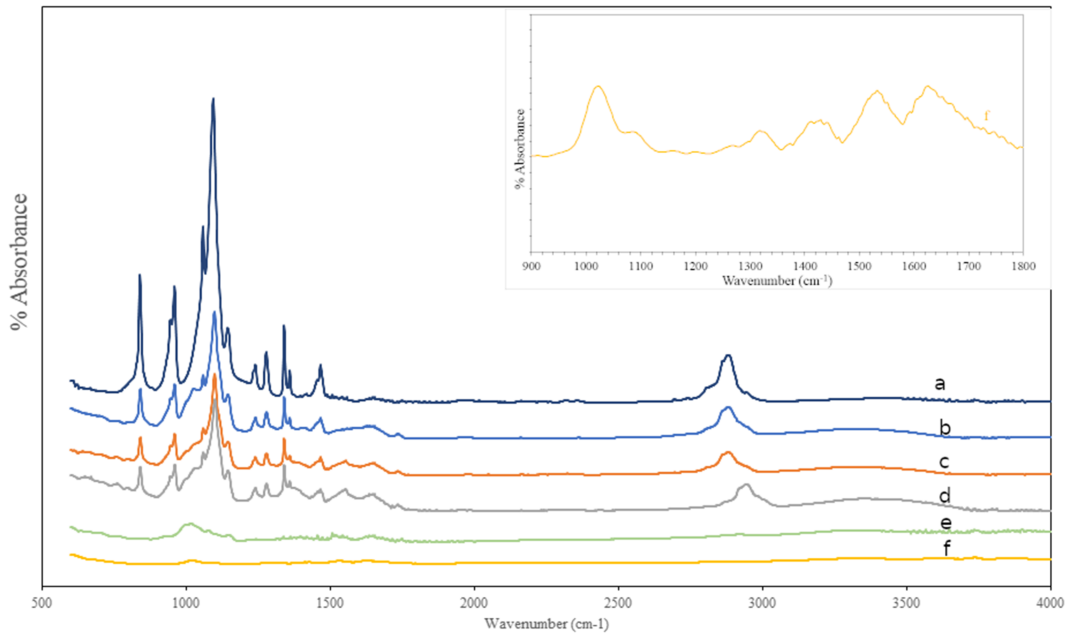

FIGURE 4 Fourier-transform infrared (FTIR) spectra of gallic acid, pea flour, and electrospun nanofibers with different gallic acid concentrations: (a) polyethylene oxide (PEO), (b) control, (c) 5%, (d) 10%, (e) pea flour, and (f) gallic acid

deformation (Yildiz et al., 2021). It should be noted that characteristic peaks, which were corresponding to pea flour, were also observed in nanofiber. Furthermore, at the fingerprint region, nanofibers showed a triplet absorbance (1058, 1110, and 1148 cm^{-1}) with a maximum peak at 1100 cm^{-1}. This peak corresponded crystalline structure of PEO (Aydogdu, Sumnu, & Sahin, 2019). (Yildiz et al., 2021). It should be noted that characteristic peaks, which were corresponding to pea flour and PEO, were also observed in nanofiber. As seen, the changes in the intensity and wavelength in the spectrum were related to destruction of crystalline structure of PEO due to interaction between PEO and pea flour. As the gallic acid amount increased, new peaks were located in spectrum of nanofiber between 1470 and 1759 cm^{-1}. For example, the bands positioned at 1610 and 1703 cm^{-1} correspond to the C═O stretch of conjugated acids that belongs to gallic acid. Finally, gallic acid-containing nanofibers had a peak at 1550 cm^{-1}, and a similar peak at 1540 cm^{-1} was observed in the spectra of pure gallic acid. The other peaks (1024, 1319, and 1423 cm^{-1}) belonging to gallic acid coincided with other bands of PEO and pea flour.

4 | CONCLUSION

This study focused on the gallic acid encapsulation with the electrospinning method to produce a potential active package. It was shown that using a suitable encapsulation method, it was possible to encapsulate heat-sensitive bioactive compounds with a high LE (up to 92.6 ± 3.1%). Furthermore, gallic acid has not only changed solution properties but also altered the morphology of nanofibers. Although increasing gallic acid amount resulted in decreasing both the consistency index and flow behavior index of the solution, the solution's electrical conductivity increased from 1419 ± 3 to 38,433 ± 466 μS/cm. The average diameter of nanofibers decreased from 297 ± 68 to 191 ± 38 nm by 10% gallic acid loading. The fabricated nanofibers were analyzed by TGA, DSC, and FTIR, and the results implied interactions between pea flour, PEO flour, and gallic acid. Nanofibers indicated different thermal properties than pure gallic acid, which could be taken as evidence of gallic acid encapsulation. Therefore, gallic acid-loaded pea flour-based nanofibers with AA can be used as novel active food packaging material to increase the shelf life of food products.

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CONFLICT OF INTERESTS

No conflicts of interest with any one or any other organizations.

AUTHOR CONTRIBUTIONS

Ayca Aydogdu Emir: Conceptualization; methodology; investigation; writing - original draft. **Eda Yildiz:** Methodology; investigation; writing - original draft. **Yildirim Aydogdu:** Investigation. **Gulum Sumnu:** Conceptualization; supervision; writing - review and editing. **Serpil Sahin:** Conceptualization; writing - review and editing.

ETHICAL STATEMENT

Hereby, I, Eda Yildiz, consciously assure that for the manuscript "Gallic acid encapsulated pea flour-based nanofibers produced by electrospinning as a potential active food packaging material," the following is fulfilled:

1. This material is the authors' own original work, which has not been previously published elsewhere.
2. The paper is not currently being considered for publication elsewhere.
3. The paper reflects the authors' own research and analysis in a truthful and complete manner.
4. The paper properly credits the meaningful contributions of co-authors and co-researchers.
5. The results are appropriately placed in the context of prior and existing research.
6. All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
7. All authors have been personally and actively involved in substantial work leading to the paper and will take public responsibility for its content.

The violation of the Ethical Statement rules may result in severe consequences.

I agree with the above statements.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Aceituno-Medina, M., Mendoza, S., Rodríguez, B. A., Lagaron, J. M., & López-Rubio, A. (2015). Improved antioxidant capacity of quercetin and ferulic acid during in-vitro digestion through encapsulation within food-grade electrospun fibers. *Journal of Functional Foods*, *12*, 332–341. https://doi.org/10.1016/j.jff.2014.11.028

Aydogdu, A., Sumnu, G., & Sahin, S. (2018a). A novel electrospun hydroxypropyl methylcellulose/polyethylene oxide blend nanofibers: Morphology and physicochemical properties. *Carbohydrate Polymers*, *181*(June 2017), 234–246. https://doi.org/10.1016/j.carbpol.2017.10.071

Aydogdu, A., Sumnu, G., & Sahin, S. (2018b). A novel electrospun hydroxypropyl methylcellulose/polyethylene oxide blend nanofibers:

Morphology and physicochemical properties. *Carbohydrate Polymers*, *181*(October 2017), 234–246. https://doi.org/10.1016/j.carbpol.2017.10.071

Aydogdu, A., Sumnu, G., & Sahin, S. (2019). Fabrication of gallic acid loaded Hydroxypropyl methylcellulose nanofibers by electrospinning technique as active packaging material. *Carbohydrate Polymers*, *208*-(November 2018), 241–250. https://doi.org/10.1016/j.carbpol.2018.12.065

Aydogdu, A., Yildiz, E., Aydogdu, Y., Sumnu, G., Sahin, S., & Ayhan, Z. (2019). Enhancing oxidative stability of walnuts by using gallic acid loaded lentil flour based electrospun nanofibers as active packaging material. *Food Hydrocolloids*, *95*(April), 245–255. https://doi.org/10.1016/j.foodhyd.2019.04.020

Aydogdu, A., Yildiz, E., Ayhan, Z., Aydogdu, Y., Sumnu, G., & Sahin, S. (2019). Nanostructured poly(lactic acid)/soy protein/HPMC films by electrospinning for potential applications in food industry. *European Polymer Journal*, *112*(December 2018), 477–486. https://doi.org/10.1016/j.eurpolymj.2019.01.006

Aytac, Z., Kusku, S. I., Durgun, E., & Uyar, T. (2016). Encapsulation of gallic acid/cyclodextrin inclusion complex in electrospun polylactic acid nanofibers: Release behavior and antioxidant activity of gallic acid. *Materials Science and Engineering C*, *63*, 231–239. https://doi.org/10.1016/j.msec.2016.02.063

Baek, S. K., & Song, K. B. (2019). Characterization of active biodegradable films based on proso millet starch and curcumin. *Starch/Staerke*, *71*(3–4), 1–8. https://doi.org/10.1002/star.201800174

Brito-Pereira, R., Correia, D. M., Ribeiro, C., Francesko, A., Etxebarria, I., Pérez-Álvarez, L., Vilas, J. L., Martins, P., & Lanceros-Mendez, S. (2018). Silk fibroin-magnetic hybrid composite electrospun fibers for tissue engineering applications. *Composites Part B: Engineering*, *141*-(December 2017), 70–75. https://doi.org/10.1016/j.compositesb.2017.12.046

Cano, A., Fortunati, E., Cháfer, M., Kenny, J. M., Chiralt, A., & González-Martínez, C. (2015). Properties and ageing behaviour of pea starch films as affected by blend with poly(vinyl alcohol). *Food Hydrocolloids*, *48*, 84–93. https://doi.org/10.1016/j.foodhyd.2015.01.008

Chung, H. J., Liu, Q., Hoover, R., Warkentin, T. D., & Vandenberg, B. (2008). In vitro starch digestibility, expected glycemic index, and thermal and pasting properties of flours from pea, lentil and chickpea cultivars. *Food Chemistry*, *111*(2), 316–321. https://doi.org/10.1016/j.foodchem.2008.03.062

Cipollone, M. A., & Tironi, V. A. (2020). Yellow pea flour and protein isolate as sources of antioxidant peptides after simulated gastrointestinal digestion. *Legume Science*, *2*, 1–10. https://doi.org/10.1002/leg3.59

Coyne, C. J., Kumar, S., von Wettberg, E. J. B., Marques, E., Berger, J. D., Redden, R. J., … Smýkal, P. (2020). Potential and limits of exploitation of crop wild relatives for pea, lentil, and chickpea improvement. *Legume Science*, *2*(2), e36. https://doi.org/10.1002/leg3.36

Deng, L., Li, Y., Zhang, A., & Zhang, H. (2020). Characterization and physical properties of electrospun gelatin nanofibrous films by incorporation of nano-hydroxyapatite. *Food Hydrocolloids*, *103*(September 2019), 105640. https://doi.org/10.1016/j.foodhyd.2019.105640

Eslamian, M., Khorrami, M., Yi, N., Majd, S., & Abidian, M. R. (2019). Electrospinning of highly aligned fibers for drug delivery applications. *Journal of Materials Chemistry B*, *7*(2), 224–232. https://doi.org/10.1039/c8tb01258j

Friedman, M., & Jürgens, H. S. (2000). Effect of pH on the stability of plant phenolic compounds. *Journal of Agricultural and Food Chemistry*, *48*(6), 2101–2110. https://doi.org/10.1021/jf990489j

Galus, S., Kibar, E. A. A., Gniewosz, M., & Kraśniewska, K. (2020). Novel materials in the preparation of edible films and coatings—A review. *Coatings*, *10*(7), 1–14. https://doi.org/10.3390/coatings10070674

Ghitescu, R.-E., Popa, A.-M., Popa, V. I., Rossi, R. M., & Fortunato, G. (2015). Encapsulation of polyphenols into pHEMA e-spun fibers and determination of their antioxidant activities. *International Journal of Pharmaceutics*, *494*(1), 278–287. https://doi.org/10.1016/J.IJPHARM.2015.08.020

Hani, N. M., Torkamani, A. E., Azarian, M. H., Mahmood, K. W. A., & Ngalim, S. H. (2017). Characterisation of electrospun gelatine nanofibres encapsulated with *Moringa oleifera* bioactive extract. *Journal of the Science of Food and Agriculture*, *97*(10), 3348–3358. https://doi.org/10.1002/jsfa.8185

Jia, X. w., Qin, Z. y., Xu, J. x., Kong, B. h., Liu, Q., & Wang, H. (2020). Preparation and characterization of pea protein isolate-pullulan blend electrospun nanofiber films. *International Journal of Biological Macromolecules*, *157*, 641–647. https://doi.org/10.1016/j.ijbiomac.2019.11.216

Kizil, R., Irudayaraj, J., & Seetharaman, K. (2002). Characterization of irradiated starches by using FT-Raman and FTIR spectroscopy. *Journal of Agricultural and Food Chemistry*, *50*(14), 3912–3918. https://doi.org/10.1021/jf011652p

Kriegel, C., Kit, K. M., McClements, D. J., & Weiss, J. (2009). Influence of surfactant type and concentration on electrospinning of chitosan–poly (ethylene oxide) blend nanofibers. *Food Biophysics*, *4*(3), 213–228. https://doi.org/10.1007/s11483-009-9119-6

Levitt, A. S., Vallett, R., Dion, G., & Schauer, C. L. (2018). Effect of electrospinning processing variables on polyacrylonitrile nanoyarns. *Journal of Applied Polymer Science*, *135*(25), 1–9. https://doi.org/10.1002/app.46404

López-Amorós, M. L., Hernández, T., & Estrella, I. (2006). Effect of germination on legume phenolic compounds and their antioxidant activity. *Journal of Food Composition and Analysis*, *19*(4), 277–283. https://doi.org/10.1016/j.jfca.2004.06.012

Luca, A., Cilek, B., Hasirci, V., Sahin, S., & Sumnu, G. (2013). Effect of degritting of phenolic extract from sour cherry pomace on encapsulation efficiency—Production of nano-suspension. *Food and Bioprocess Technology*, *6*(9), 2494–2502. https://doi.org/10.1007/s11947-012-0880-z

Millar, K. A., Gallagher, E., Burke, R., McCarthy, S., & Barry-Ryan, C. (2019). Proximate composition and anti-nutritional factors of fava-bean (*Vicia faba*), green-pea and yellow-pea (*Pisum sativum*) flour. *Journal of Food Composition and Analysis*, *82*(July 2018), 103233. https://doi.org/10.1016/j.jfca.2019.103233

Mishra, R. K., Nawaz, M. H., Hayat, A., Nawaz, M. A. H., Sharma, V., & Marty, J. L. (2017). Electrospinning of graphene-oxide onto screen printed electrodes for heavy metal biosensor. *Sensors and Actuators, B: Chemical*, *247*, 366–373. https://doi.org/10.1016/j.snb.2017.03.059

Musuc, A. M., Badea-Doni, M., Jecu, L., Rusu, A., & Popa, V. T. (2013). FTIR, XRD, and DSC analysis of the rosemary extract effect on polyethylene structure and biodegradability. *Journal of Thermal Analysis and Calorimetry*, *114*(1), 169–177. https://doi.org/10.1007/s10973-012-2909-y

Oguz, S., Tam, N., Aydogdu, A., Sumnu, G., & Sahin, S. (2017). Development of novel pea flour-based nanofibres by electrospinning method. *International Journal of Food Science and Technology*, *2013*, 1–9. https://doi.org/10.1111/ijfs.13707

Pereira, A. G. B., Paulino, A. T., Nakamura, C. V., Britta, E. A., Rubira, A. F., & Muniz, E. C. (2011). Effect of starch type on miscibility in poly(ethylene oxide) (PEO)/starch blends and cytotoxicity assays. *Materials Science and Engineering C*, *31*(2), 443–451. https://doi.org/10.1016/j.msec.2010.11.004

Phiriyawirut, M., & Phaechamud, T. (2012). Gallic acid-loaded cellulose acetate electrospun nanofibers: Thermal properties, mechanical properties, and drug release behavior. *Open Journal of Polymer Chemistry*, *2*(1), 21–29. https://doi.org/10.4236/ojpchem.2012.21004

Pinheiro Bruni, G., dos Santos Acunha, T., de Oliveira, J. P., Martins Fonseca, L., Tavares da Silva, F., Martins Guimarães, V., & da Rosa Zavareze, E. (2020). Electrospun protein fibers loaded with yerba mate extract for bioactive release in food packaging. *Journal of the Science of*

Food and Agriculture, *100*(8), 3341–3350. https://doi.org/10.1002/jsfa.10366

Porto, M. D. A., dos Santos, J. P., Hackbart, H., Bruni, G. P., Fonseca, L. M., da Rosa Zavareze, E., & Dias, A. R. G. (2019). Immobilization of α-amylase in ultrafine polyvinyl alcohol (PVA) fibers via electrospinning and their stability on different substrates. *International Journal of Biological Macromolecules*, *126*, 834–841. https://doi.org/10.1016/j.ijbiomac.2018.12.263

Quiles-Carrillo, L., Montanes, N., Lagaron, J., Balart, R., & Torres-Giner, S. (2019). Bioactive multilayer polylactide films with controlled release capacity of gallic acid accomplished by incorporating electrospun nanostructured coatings and interlayers. *Applied Sciences*, *9*(3), 533. https://doi.org/10.3390/app9030533

Sanyang, M. L., Sapuan, S. M., Jawaid, M., Ishak, M. R., & Sahari, J. (2015). Effect of plasticizer type and concentration on tensile, thermal and barrier properties of biodegradable films based on sugar palm (*Arenga pinnata*) starch. *Polymers*, *7*(6), 1106–1124. https://doi.org/10.3390/polym7061106

Shevkani, K., Singh, N., Kaur, A., & Rana, J. C. (2015). Structural and functional characterization of kidney bean and field pea protein isolates: A comparative study. *Food Hydrocolloids*, *43*, 679–689. https://doi.org/10.1016/j.foodhyd.2014.07.024

Sonar, C. R., Al-Ghamdi, S., Marti, F., Tang, J., & Sablani, S. S. (2020). Performance evaluation of biobased/biodegradable films for in-package thermal pasteurization. *Innovative Food Science and Emerging Technologies*, *66*(June), 102485. https://doi.org/10.1016/j.ifset.2020.102485

Uygun, E., Yildiz, E., Sumnu, G., & Sahin, S. (2020). Microwave pretreatment for the improvement of physicochemical properties of carob flour and rice starch-based electrospun nanofilms. *Food and Bioprocess Technology*, *13*(5), 838–850. https://doi.org/10.1007/s11947-020-02440-x

Vega-Lugo, A. C., & Lim, L. T. (2012). Effects of poly(ethylene oxide) and pH on the electrospinning of whey protein isolate. *Journal of Polymer Science, Part B: Polymer Physics*, *50*(16), 1188–1197. https://doi.org/10.1002/polb.23106

Yildiz, E., Bayram, I., Sumnu, G., Sahin, S., & Ibis, O. I. (2021). Development of pea flour based active films produced through different homogenization methods and their effects on lipid oxidation. *Food Hydrocolloids*, *111*(August 2020), 106238. https://doi.org/10.1016/j.foodhyd.2020.106238

Zhang, Q., Wang, X., Fu, J., Liu, R., He, H., Ma, J., Yu, M., Ramakrishna, S., & Long, Y. (2018). Electrospinning of ultrafine conducting polymer composite nanofibers with diameter less than 70 nm as high sensitive gas sensor. *Materials*, *11*(9). https://doi.org/10.3390/ma11091744

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