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Electrosprayed WPC/PEO Mats Coated to Fresh Figs

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Abstract: Electrohydrodynamic atomization that is also defined as electrospraying is a method of producing mats that are composed of nano- or micro-scaled droplets. Unlike nanofibers that are obtained by electrospinning, the droplets are in spherical forms, and this is the result of the struggle between the applied electrostatic forces and the surface tension of the liquid (meaning polymer solution). This study aims to prepare and characterize electrosprayed biopolymer mats. The 1:2 blend of HWPC (hydrolyzed whey protein concentrate): PEO (poly (ethylene oxide)) is the polymer solution prepared in 2.5% aqueous acetic acid solvent. Physicochemical, morphological and structural analysis are applied to both solution and the electrosprayed mat. Increased viscosity in the protein solution by the addition of PEO enhanced the formation of regular beads observed through SEM images. Image J Visualization and Measurement Software was occupied to determine the diameter distributions of the droplets forming the mat. The mean diameter was found as 1.02±0.55 µm. ATR-FTIR spectroscopy analyses revealed remarkable structural changes in protein and interaction between protein and PEO in the electrosprayed mats. The HWPC/ PEO electrosprayed mat coating revealed one log decrease in the microbial load of fresh figs at the end of fourteen-day storage (4ºC). The findings of the presented research are promising for the application of this electrosprayed biopolymer mat for food coating purposes.

Keywords: electrospraying, nanoparticle, WPC, PEO

1. Introduction

To increase both the stability and the shelf life of foods by nano-scaled materials has been in the focus of researchers from various disciplines for years. In this search, it was revealed that nanomaterials are designed as systems that give good responses to prolong the shelf life of foods. Improvement of surface area, permeability, and some other abilities of food packages are actualized by the usage of nanofibrous or nanodroplet mats. Electrospinning or electrospraying were two similar methods used for this purpose. Electrohydrodynamic atomization or electrospraying is a method of producing ultra-fine droplets from a polymer solution with the help of electrostatic forces (Gómez-Mascaraque and Lopez-Rubio 2019). The continuity of the jet is disturbed in electrospraying to form nano-scaled droplets. The method’s theoretical foundations were laid by Gilbert, and Rayleigh, Zeleny, and Taylor were the ones explaining the method mathematically and experimentally (Jingwei et al. 2015). From the 2000s that experimental studies have been accelerated, spraying has continued to maintain its importance in diversified fields such as textiles (Jadhaw et al. 2011), cosmetics (Bae et al. 2019), biomedical (Wang et al. 2019) and food applications (Lim et al. 2019). Method is based on the principle of formation of droplets by instability of the polymer solution desired to be sprayed in an equipment equipped with a high voltage power supply, syringe pump and collector under the influence of electrostatic field, surface tension, viscoelastic and even negligible air drag and gravity forces. High surface tension and low viscosity are the key factors for the formation of spraying (Lim et al. 2019).

Surfaces with nano-scale unit structure (droplet) have one or more of the very critical features for food packaging such as exhibiting a higher surface area, effectively protecting food from dirt and dust, creating a stable barrier against the passage of oxygen, light, and microorganisms, and maintaining moisture balance (Vasile 2018). In addition, due to the flexibility provided by the electrospraying, multifunctional structures can be obtained by a wide variety of polymers.

Polyethylene oxide (PEO) is a synthetic polymer that is biocompatible, nontoxic, water-soluble, and it tends to form H bonds with the materials used together (Reneker and Chun 2003). Whey protein concentrate (WPC) is a wellrecognized natural biopolymer used for carrying and delivery purposes of bioactives. Whey based proteins are also used to fabricate both electrospun and electrosprayed micro-/ nano-particles especially with the help of carrier polymers (Soleimanifar et al. 2021).

Whey-based films and coatings in different formulations have been reported to be successfully used for coating of fruits and vegetables (Kandasamy et al. 2021). Various bioactives were encapsulated and protected within whey electrosprayed nanospheres as well (Lopez-Rubio and Lagaron 2012) Perishable nature of some fruits, eg. figs requires preservation after harvesting to maintain it in the market. Thus, nanoscale coating matrices can promise for longer post-harvest storage opportunity for this kind of fruits (Moccia et al. 2021).

This study aims to investigate the formation of nano- and micro-scaled droplets from WPC and PEO for food application. Within the scope of the presented study, the morphological and structural features of electrosprayed droplets were analyzed through scanning electron microscopy (SEM) and Fourier transform infrared spectrometer (FT-IR). Some physical properties of spraying solution and sprayed mats such as viscosity, electrical conductivity, thickness, and weight per unit area were also determined. Finally, the effectiveness of the obtained mats coated over fresh figs were evaluated.

2. Materials and Method

2.1. Materials

WPC/PEO electrosprayed mats were prepared using PEO (MW of 100.000, Sigma-Aldrich, USA), and WPC (80 %, Alfasol, Kimbiotek, Turkey). All the reagents were used without further purification. Alcalase enzyme (2.4 AU-A/g) was kindly supplied by Novozymes A/S (Bagsvaerd, Denmark).

2.2. Preparation of Electrospraying Solution

The 20 wt% WPC was dissolved in ultra-pure water by mechanical stirring for 1h at RT and stored at 4 ºC for an overnight to achieve complete dissolution. WPC solution was then subjected to enzymatic hydrolysis by mixing 5 v/w% (enzyme/protein) alcalase at 50 ºC for an overnight. The 10 wt% PEO was dissolved in ultra-pure water via magnetic stirring for an overnight at room temperature (RT). To obtain a homogeneous solution, one-part WPC and two parts PEO solutions (1:2) were stirred for another overnight at 1000 rpm (RT).

2.3. Viscosity and Electrical Conductivity Measurements of Electrospraying Solution

Prior to electrospraying, viscosity and electrical conductivity of the solutions were determined. Viscosity measurements were performed using a rotational viscometer (Brookfield DV2T, MA, USA) with the spindle RV-5 rotating at a rate of 100 and 200 rpm. Each measurement was made in duplicate and average viscosities were calculated. Electrical conductivities of WPC, PEO and WPC/PEO solutions were measured through an electrical conductometer (HACH, HQ440d multi, USA).

2.4. Electrospraying

The WPC/PEO solutions were filled into a 20 ml syringe with a 21 G needle connected to a high voltage power supply for electrospraying. The voltage applied was 8 kV and flow rate of the solution was 0.24 ml/min. The distance between the syringe and collector was kept as 21 cm and the electrosprayed (ES) nanobeads were collected on a grounded plate coated with Al foil.

2.5. SEM Analysis

The morphology of WPC/PEO based mats were characterized by SEM ((FEI QUANTA 250 FEG) with an acceleration voltage of 10 kV and a secondary-electron detector. For this purpose, the samples were coated with gold and fixed onto metallic stubs with double-sided carbon tape.

2.6. Thickness and weight of ES mats

The thickness of ES mats was measured using a digital micrometer (Mitutuoyo, Japan) with the sensitivity of 0.001, at ten different points and the average thickness was recorded. Ten pieces of the 2x2 cm2 cuts of the mats were weighed and the average weight of the ES mats were recorded using an analytical balance (Radwag, Poland) with a sensitivity of 0.1 mg.

2.7. FT-IR Analysis

FTIR characterizations of WPC/PEO samples were carried out between 400 and 4000 cm-1 wavenumbers with Perkin Elmer UATR Spectrum Two FTIR. The resolution was 4 cm^{-1} and the number of scans collected was 32.

2.8. Coating of Fresh Figs by Electrosprayed WPC/PEO Mats

The fresh figs were tied to an apparatus with a strand and hanged up in front of the stable collector. The electrospraying was continued for 4 hours to coat all surfaces with sprayed nano droplets. Then, the coated and uncoated fresh figs were stored at +4ºC for 14 days. Besides visual evaluation, microbial load of the coated and uncoated figs was investigated at the end of this period. Samples prepared in peptone water (0.1%) from both coated and uncoated figs were inoculated on the plate cout agar (PCA) and the colony forming units per ml (CFU/ml) were determined after 48 h incubation at 35 ºC.

3. Results

3.1. Properties of Electrospraying Solutions

Viscosity and electrical conductivity measurements of the HWPC, PEO and HWPC/PEO solutions were presented in Table 1. Except for pure HWPC, viscosity values were recorded at 100 rpm with torque varying between 65% and 95%. Since it was not achieved at 100 rpm, viscosity measurement for HWPC was performed at 200 rpm.

Unprocessed WPC had very low viscosity (data not shown). Viscosity of WPC protein was increased in case of enzymatic hydrolysis. However, it was not enough to prepare electrosprayed mats having uniformly distributed droplets. Although low viscosity is a prerequisite for electrospraying, the ~1500 cP of viscosity seemed still too low to spray droplets. Thus, it was aimed to blend with an auxiliary polymer, PEO to enhance viscosity, facilitating a desirable electrospraying process. The final viscosity of the electrospraying solution was measured as 3701 cP. Although viscosity values in the range of 800-4000 cP are required for the electrospinning process and fiber formation (Chakraborty et al. 2009), lower viscosities are enough to produce droplets by electrohydrodynamic atomization. Since there is a requisite for long polymer chain entanglements that contribute to the viscosity to ensure the continuity of the fiber structure and high aspect ratio, the viscosity limit is a more crucial parameter for the electrospinning of nanofibers than the electrospraying of droplets.

Due to its high mineral content, the conductivity of HWPC (20%) was remarkably high when compared to the PEO solution. Most probably, this prevents its spraying ability alone. Morota et al. (2004) were not able to electrospray the PEO aqueous solutions whose conductivities were changed with $CaCl_2$ at an electrical conductivity value above 5 mS/cm The blended solution exhibited an electrical conductivity of 3.41 mS/cm value which is in the sprayable range.

Table 1 Viscosity and electrical conductivity of biopolymer solutions

Sample solutions	Viscosity (cP)	Electrical conductivity (mS/cm)
HWPC	1491 ± 173	6.520 ± 0.339
PEO	3419 ± 43	0.371 ± 0.010
HWPC/ PEO	3701 ± 101	3.410 ± 0.021

3.2. Thickness, Weight, and Morphology of Electrosprayed Mats

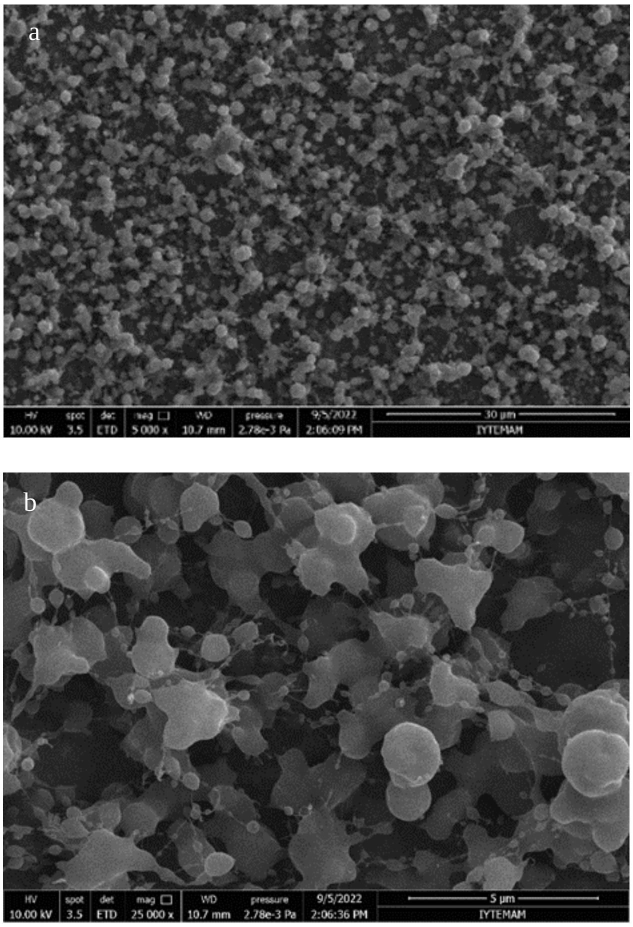
Physical parameters, thickness and weight per unit area of electrosprayed mats produced with an electrospraying of about 4 hours were given in Table 2. As known, the thickness and the weight per unit area of the electrosprayed mats are directly affected by the spraying duration. The results show that a very thin mat structure can be produced in about four hours. Moreover, it can be coated on food without almost any change on the weight of the food which is important in terms of transportation.

Table 2 Thickness and weight per unit area of ES mats

Sample	Thickness (µm)	Weight per unit area (g/m^2)
HWPC/ PEO ES mat	1.8 ± 0.7	0.040 ± 0.009

SEM images of HWPC/PEO mats were given in Figure 1. Increased viscosity in the protein solution by the addition of PEO enhanced the formation of regular beads observed through SEM images. Image J Visualization and Measurement Software was occupied to determine the diameter distributions of the droplets forming the mat. The mean diameter was found as 1.02±0.55 µm. Similar diameters have been obtained with aqueous solutions of different polymers in the literature (Ambrus et al. 2013; Suksamran et al. 2013; Tian et al. 2013).

Fig. 1 SEM images of HWPC/PEO mats (a) X5K (b) X25K



As a result of the interactions between the viscous, surface tension, and electrostatic forces, nanoscaled droplets were obtained as seen in the SEM images. As droplets having diameters of 1 um or less are considered as nanoscaled 0D structures, it can be concluded that droplets having an average diameter of 1 um was achieved in this study.

3.3. Structure of Electrosprayed Mats

FTIR spectra of Al foil and electrosprayed HWPC/PEO mats on Al foil were given in figure 2. ATR-FTIR spectroscopy analyses revealed remarkable structural changes in protein and interaction between protein and PEO in the electrosprayed mats. The peaks at ~ 2882, 1467, 1360 and 699 cm-1 were assigned to PEO. The signals at ~1632 and 1547 cm-1 were attributed to amide I and amide II vibrations.

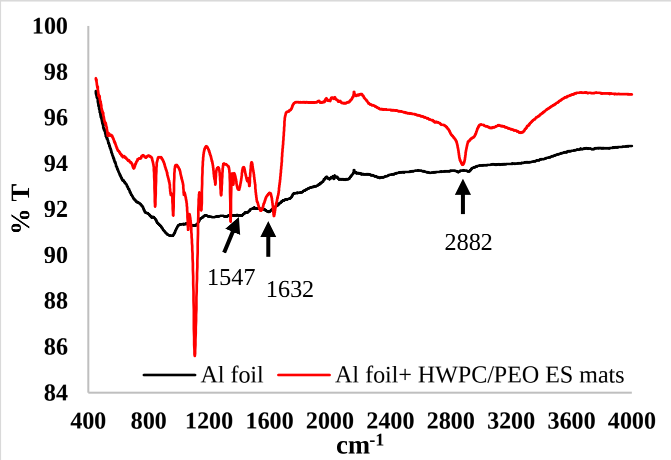


Fig. 2 FT-IR Spectra of naked Al foil and Al foil +HWPC/PEO electrosprayed mats

3.4. Evaluation of Fresh Figs Coated by Electrosprayed WPC/PEO Mats

The four-hour coating provided a thin shield over fresh figs (Figure 3a) partially protecting them from moisture loss (data not shown). Visual evaluation during storage showed that microbial decay in uncoated figs was started earlier than the coated ones. Based on plate counts, electrosprayed HWPC/PEO mat coating alone exhibited almost one log decrease in microbial load (Figure 3b). Besides, it is expected to reach more reduction in case of including antimicrobial agents within mat matrix. Microbial safety of fresh figs was assessed when coated with edible polysaccharide films as well (Paolucci et al. 2020). Although it is not our scope in this study, it was reported that nutraceutical characteristic of fresh figs was preserved while coated with an edible film ((Moccia et al. 2021).

b (uncoated) CFU/g	(coated) CFU/g
$1.056x10^9$	$1.98x10^8$

Fig. 3 Fresh figs a) uncoated (upper) and coated (lower) by HWPC/PEO mats (1st day); b) with CFU/g in uncoated and coated cases.

5. Conclusion

With the given formulation of hydrolyzed WPC and PEO, electrosprayed mat consisting well-distributed nanoparticles were successfully formed. Viscosity and conductivity of the polymer blend solutions critically affect their electrospraying ability. Coating application of electrosprayed HWPC/PEO mats over fresh figs was desirably achieved. Nanodroplets accumulating within the matrix through a certain thickness is promising for making this electrosprayed mat an eligible candidate for food coating purposes. Relevant research work is currently ongoing with the other applications.

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