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RESEARCH

Electrosprayed Zein and Quercetin Particles: Formation and Properties

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Abstract
The aim of this study was to physically modify zein (8–20% wt) using electrospraying (ESP) and to evaluate the changes in its morphology, chemical structure, and physical properties. The apparent viscosity and surface tension of zein solution affect the particles produced by ESP. The produced particles were spherical and in the sub-micron size (average size 2000 nm) and exhibited lower contact angle and surface hydrophobicity compared to commercial zein, which is attributed to changes in the secondary structure during processing. The addition of quercetin (0.1–0.4% wt), further improved the microstructure and interfacial properties. Wettability (6.24–17.86%) and interfacial tension of ESP-zein particles can be proportionally altered through the addition of quercetin. The molecular docking results suggest that hydrogen bonding and hydrophobic interactions exist between quercetin and zein, which may be responsible for the regulation of ESP-zein by quercetin. Such particles with tunable physical properties are strong candidates for the development of future food products.

Keywords Zein · Electrospraying · Micro/nanoparticle · Microstructure · Quercetin

Introduction

With enormous environmental pressures, there is a need for a shift from an over-reliance on the consumption of animal-based proteins to the use of plant-based proteins (Silva et al., 2021a, 2021b). Animal-based protein production is inefficient, and it is difficult to meet the needs of a continuously growing global population. Further emphasizing the use and consumption of plant-based proteins will also lead to a wider distribution of plant-based proteins globally, thus ameliorating current environmental press and future food scarcity issues (Sha & Xiong, 2020). Plant-based proteins are highly hydrophobic, and unprocessed plant-based protein particles usually have large size, high polydispersity, and irregular shape (Chi et al., 2003). Whether added as a food ingredient to high-protein beverages or used as emulsion stabilizers, proteins particle are required to have controlled size and morphology as well as suitable hydrophobicity (Sağlam et al., 2014). Therefore, physical or chemical modification of plant proteins using some processing techniques can promote better utilization of plant proteins in the food industry.

Zein is a low-cost, environmentally friendly plant-based protein isolated from corn processing. Zein is classified into four fractions including α, β, γ, and δ based on amino acid composition and molecular weight (*Mw*), of which α-zein has the highest content (70–85%) with *Mw* of 22 and 19 kDa (Gao et al., 2021). The high content of hydrophobic amino acids in zein results in poor water solubility of zein, and often, solvents such as 50–80% aqueous ethanol are used to make them dispersible (Esen, 1986). The low nutritional value of zein and problems with color, odor, and water solubility have led to its being marketed as a low-value protein feed or discharged as waste (Shukla & Cheryan, 2001).

Physical modification applied to plant proteins such as zein includes heat treatment, microwave treatment, ultrasonic treatment, and high-pressure treatment (Akharume et al., 2021). However, these treatments usually require additional energy or temperature and are accompanied by irreversible denaturation and inactivation of the protein (Venkateswara Rao, CK, Rawson, DV, & N, 2023). The high hydrophobicity of zein also limits its widespread use in these treatments. Moreover, classic precipitation treatment methods for zein, including anti-solvent precipitation and solvent

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evaporation, are usually suitable for small amounts of zein at laboratory level (Yang et al., 2022). Therefore, there is a requirement for a novel and promising processing method or technology that can prepare controlled sub-micron structures from plant protein more efficiently and rapidly than conventional processing methods (Silva et al., 2021a).

Electrospraying (ESP) is considered a novel nanofabrication technology in the food field for the development of functional products and packaging materials (Tomadoni et al., 2022). When a high field voltage is applied to a syringe containing a polymer solution, the polymer solution is ejected from the needle, forming a charged droplet, and the solvent evaporates rapidly under the high electric field, leaving dry particles or fibers with micro- and nanostructures on the collector (Marques et al., 2019). In this process the shape and features of the sample obtained can be controlled by adjusting the voltage, the flow rate, and the distance between the needle and the collector (Silva et al., 2021a). Different from electrospinning, the polymer solution used for ESP is usually a low-concentration liquid with low viscosity and surface tension. Under strong electrostatic forces, the continuity of the solution is broken, and individual, finely charged droplets are formed, which are finally deposited on the collector. The advantage of ESP is that the desired micro- and nanostructures can be obtained simply, quickly, and at low cost, which is of great interest in the field of food processing (Paximada et al., 2020). The improvement of the physicochemical properties of plant proteins by electrospraying is limited, and more precise modulation can be achieved by combining two or more treatments. Studies have shown that the interaction between plant proteins and polyphenols, especially non-covalent interactions, can affect the function (e.g., solubility, hydrophobicity, emulsification, microstructure) of proteins, and this can also be modulated by the type and amount of polyphenols added (Paximada et al., 2017, 2021). In addition, the introduction of polyphenols will also enable the plant protein particles to acquire some beneficial biological activities, expanding the scope of the application of plant protein particles. Quercetin is a class of dietary polyphenols that are widely available in nature and have good health benefits such as antioxidant and anti-inflammatory activities, and previous studies have shown that quercetin can bind to plant proteins through non-covalent interaction forces (Yufang Wang & Wang, 2015).

To the best of our knowledge, there is extensive literature on the specific physical properties (viscosity, size, electrical conductivity) before ESP; however, there is no study understanding the effect of ESP processing on protein structure, surface hydrophobicity, and contact angle. In this study, the effect of ESP on the physical properties and functionality of particles before and after ESP treatment was investigated. The materials used were zein or quercetin. In order to evaluate the effect of ESP treatment on the size, morphology, and physicochemical properties of zein, we determined the apparent viscosity and surface tension of the zein solution. We also investigated the effect of microstructural and interfacial properties after polyphenol (quercetin) addition. Finally, possible interactions between zein and quercetin were explored by means of molecular docking. This study will provide a combined ESP treatment and polyphenol compounding approach to further enhance the properties of plant proteins.

Materials and Methods

Materials

Commercial zein powder (from maize) and 8-anilino-1-naphthalenesulfonic acid (ANS, ≥ 97%) were purchased from Sigma-Aldrich (Darmstadt, Germany). Quercetin (Que, ≥ 95%) was purchased from Cayman Chemicals (USA). Other reagents used in this study such as anhydrous ethanol, phosphate-buffered saline buffer (PBS), 0.1 M dilute hydrochloric acid solution, and sodium hydroxide solution were analytically pure.

Zein Solution Preparation

An appropriate amount of zein powder was accurately weighed and dissolved in 80% aqueous ethanol to form 8, 12, 16, and 20 wt% zein stock solutions, which were fully dissolved by continuous magnetic stirring at room temperature for 4 h. The mixture of quercetin and zein was prepared by weighing appropriate amounts of quercetin and zein and dissolving them in 80% ethanol under continued stirring, wherein the concentration of zein was 8 wt% and the concentrations of quercetin were 0.1, 0.2, and 0.4 wt%. The samples were stored at 4 °C until further use.

Determination of Zein Solution Viscosity

The apparent viscosities of zein solutions with different concentrations were determined by a rotational rheometer (Kinexus, Malvern Panalytical, UK) equipped with a plate-plate geometry. The gap value was set to 0.5 mm, and the shear rate was varied from 10–100 s^{-1}, with the number of points to be 10 and at a temperature of 25 °C (Yang et al., 2020).

Determination of Surface Tension

The surface tension of the samples was determined by a surface tensiometer equipped with EZ-Pi Plus high-resolution sensors (Kibron Inc., Helsinki, Finland). After calibration

with ultrapure water (calibration value of 72.80 mN/m), 2 mL of sample was added to the sample cup for analysis.

Electrospraying Processing

The zein solution is being processed by electrospraying setup (E-Fiber EF100, Bollate, Italy). The entire electrospraying process is composed of four parts including the syringe pump, collector, high-voltage power supply, and fume hood. Zein solutions at different concentrations (8–20 wt%) and zein-quercetin mixture (quercetin concentrations were 0.1%, 0.2%, and 0.4 wt%) were pumped at feed rates 20–40 mL/h. High voltages of 20–25 kV were applied on the tip of the needle, and the plate collector was fixed at a distance of 25 cm from the tip of the needle. The plate collector was covered with aluminum foil to collect the deposited particles in the form of a film. The electrospraying process was conducted at a relative humidity of 50% and a temperature of 20 °C. The coding of the samples together with the processing conditions is listed in Table S1.

Scanning *Electron* Microscope Observation

The microstructure of commercial zein, quercetin (Que), ESP-zein, and ESP-zein + que at different concentrations was visualized using a scanning electron microscope (SEM, Carl Zeiss EVO MA15, Germany). The samples were coated with gold under vacuum. The micrographs were observed at an accelerating voltage of 20.00 kV, and pictures were taken at × 1000 and × 10,000; the distribution of particles in the SEM was counted by ImageJ software and then fitted using the GaussAmp formula.

Particle Size and Zeta-Potential Analysis

The commercial zein, ESP-zein, and ESP-zein + que were dissolved in 50% aqueous ethanol to form a 1 mg/mL solution with continuous stirring until complete dissolution. Measurements of particle size and potential were conducted using a Malvern Zetasizer (ZEN3600, Malvern) at room temperature (25 ℃).

Circular Dichroism (CD) Spectrum Analysis

Changes in the secondary structure of commercial zein and ESP-zein (dissolved in 50% ethanol) were determined by circular dichroism (Chirascan, Applied Photophysics, UK). The sample concentration was 0.2 mg/mL, the scanning range was 180–260 nm, the Bandwidth was 2.0 nm, a Cuvette with 1.0 mm path length was used, and the temperature was 20 °C (Whitmore & Wallace, 2008). Quantitative analysis of the secondary structure of proteins is done through an online website (http://dichroweb.cryst.bbk.ac.uk).

Contact Angle Measurement

The contact angle of different concentrations of commercial zein, ESP-zein, and ESP-zein + que was determined using a drop shape tensiometer (OCA25, DataPhysics, Germany), equipped with a high-speed camera, a micro-syringe, and a Peltier cooling 254 system, ensuring that measurements can be obtained at a constant temperature of 25 °C. The particles were pelletized into a tablet using a hydraulic press to create a suitable substrate surface and placed in a rectangular optical glass cell. Using a 0.52-mm syringe filled with water, the change in contact angle was continuously monitored by video before and after the droplets came down.

Surface Hydrophobicity Determination

The surface hydrophobicity of commercial zein and ESP-zein was determined according to a previously reported method with slight modifications (Feng et al., 2019). Briefly, 8-anilino-1-naphthalenesulfonic acid (ANS) was dissolved in 0.01 M PBS buffer (pH 7) to form 0.64 mg/mL of ANSA stock solution and stirred thoroughly until complete dissolution. Twenty microliters of ANS was added to 2 mL of protein solution (0.1 mg/mL), shaken well, and reacted for 15 min. The fluorescence intensity of the mixture was measured by a FluoroMax spectrometer (HORIBA Scientific, France) at an excitation wavelength of 370 and an emission wavelength of 400–600 nm.

Interfacial Tension Measurement

The oil–water interfacial tension of commercial zein, ESP-zein, and ESP-zein + que was determined by a drop shape tensiometer (OCA25, DatePhysics Instruments GmbH, Germany). Briefly, the samples were accurately weighed and dissolved in the aqueous phase to make a 5 mg/mL solution. A 500-μL glass syringe with a 1.65-mm needle was used, and the syringe containing the sample solution was fixed to the instrument. A small dish containing vegetable oil was placed under the syringe, and the needle was immersed below the oil surface. The injection volume was 10 μL at a rate of 2 μL/s, and the change in interfacial tension was continuously recorded after the drop appeared. The Young–Laplace equation was numerically solved and fitted for the droplet profile assessment. All measurements were conducted at 25 °C, achieved by using a Peltier cooling system.

Molecular Docking

The 3D molecular models of quercetin and zein were downloaded in xml format from PubChem (https://pubchem.ncbi.nlm.nih.gov) and UniProt (https://www.uniprot.

org), respectively. After being converted to pdb format by Open Babel, the structure was checked using Polymol. Subsequently, the pdbqt file was exported after hydrogenation and charge calculation in AutoDockTool. A suitable box was constructed using Polymol, and the spatial position and dimensions were determined. The energy range for molecular docking was set to 5, 20 dockings were carried out, and the conformation with the highest affinity was selected for the analysis of interaction forces.

Statistical Analysis

All experiments were repeated at least three times. Results are expressed as mean ± standard deviation (SD) and were statistically compared using one-way analysis of variance (ANOVA) and *t*-test, and $p < 0.05$ was considered significant. The correlation analyses were processed through an online website (https://www.chiplot.online/).

Results and Discussion

Viscosity and Surface Tension

Viscosity and surface tension of the protein solution greatly affect the ESP final product with high-viscosity solutions favoring the production of fibers, while low-viscosity solutions are better suited for the production of micro- and nanoparticles (Silva et al., 2021b). The viscosity of different concentrations of zein solutions is shown in Fig. 1A. The viscosity increases as zein concentration increases, from 0.008 Pa·s (8% zein) to 0.04 Pa·s (20% zein) at a shear rate of 10 s^{-1}. The overall concentration of the solution is still at a relatively low level. At a low shear rate (1–10 1/s), the viscosity of the solution decreases with the increase in shear rate, showing shear thinning behavior. At higher shear rates (10–100 1/s), the viscosity of the zein solution is almost independent of the shear rate and exhibits a behavior similar to that of Newtonian fluids. Previous reports have also shown that aqueous ethanol solutions of commercial zein exhibit Newtonian fluid behavior (Fu & Weller, 1999). During the ESP process, it is necessary to allow the solvent to evaporate sufficiently to obtain a dry sample on the collector. This requires the protein solution to have a low surface tension so that the applied voltage can overcome it to form a Taylor cone (Marques et al., 2019). As shown in Fig. 1B, the zein solution has a low surface tension (26.8–28.5 mN/m), much lower than that of pure water (72.8 mN/m, 20 °C). This is mainly attributed to the fact that the solvent for zein is an aqueous ethanol solution, which itself has a low surface tension (30.7 mN/m), and the addition of zein only slightly increases the surface tension of the solution. These results indicate that 8–20% aqueous ethanol solutions of zein are well suited for ESP processing.

SEM Observation

Different concentrations of zein solutions were subjected to ESP treatment, and the dried samples obtained were observed of their microstructure by SEM. The commercial

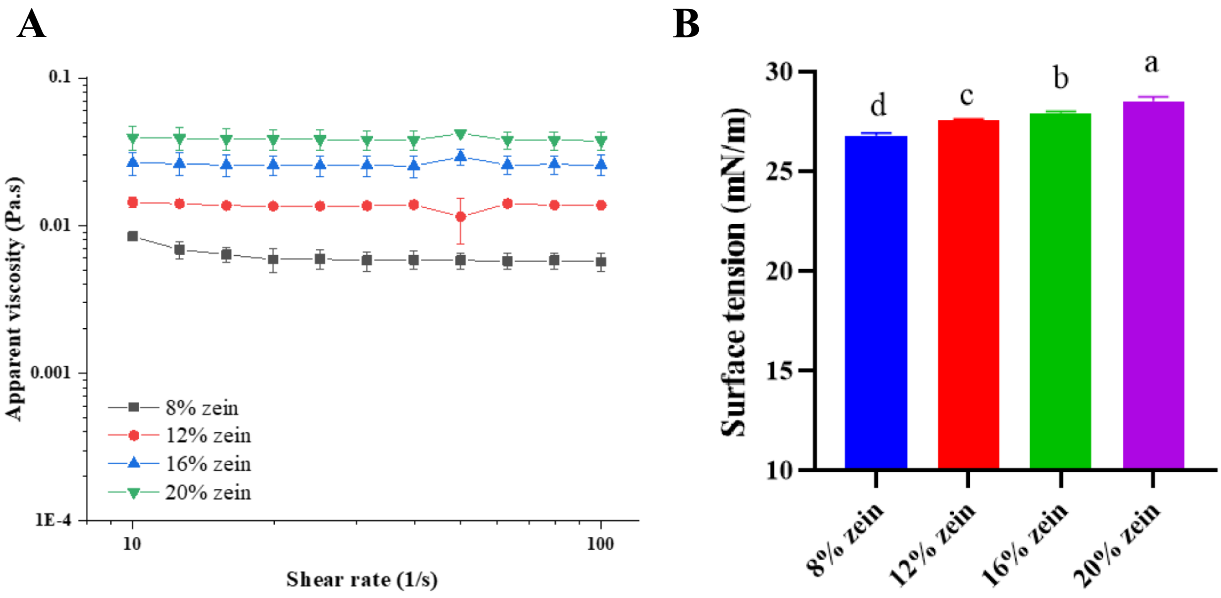

Fig. 1 Apparent viscosity as a function of shear rate 10–100 s^{-1} (**A**) of zein at different concentrations at 25 °C. **B** Surface tension of zein solution with different concentrations at room temperature

zein showed a distinct large granular and irregular structure (Fig. 1C, D), whereas small spherical particles with average diameters below 1 μm can be obtained after the ESP treatment, and 20% ESP-zein has the largest particle diameter (Fig. 2). This is similar to previous reports that electrohydrodynamic processing can alter the microstructure of zein (Liu et al., 2018). This is due to the zein solution being stretched and twisted under the high electric field during the ESP process, forming small charged droplets, and microstructures formed after rapid evaporation of the solution (Wu & Clark, 2008). During the ESP process, some larger particles are produced, but this phenomenon improves as the concentration of zein increases. This may be due to the fact that the lower concentration of zein solution has lower viscosity and surface tension and is more unstable in high-voltage fields, leading to localized aggregation of particles. The particles of 12% ESP-zein are rounder and have a smooth surface with no depressions. Sixteen percent ESP-zein has a small number of large particles, which can be attributed to the high concentration, which produces particles that are stacked on top of each other and aggregated. In addition, many particles with pore-like structures were observed in 16% ESP-zein, this may be due to the rapid evaporation of the solution in a high-voltage field, which may be beneficial for its application in encapsulating functional compositions (Alinaqi et al., 2021). In general, the particle size of ESP-zein particles has a tendency to increase with concentration over a certain concentration range. These results indicate that ESP treatment can be used to obtain dry zein micro- and nanostructures without thermal treatment or energy.

Determination of Physical Properties of ESP-Zein

Dynamic light scattering (DLS) was used to determine the particle size and Polydispersity Index (PDI) of ESP-zein prepared at different concentrations. As shown in Fig. S1A, the commercial zein has a very high particle size (3491 ± 182 nm), and the PDI is close to 1, indicating a very broad particle size distribution. After ESP treatment, all of their particle sizes decreased significantly ($p < 0.05$), and their distributions were more homogeneous. ESP-zein has a much lower Polydispersity Index (PDI) than commercial zein. However, there was no significant difference in the hydrodynamic average particle size of ESP-zein between these different concentrations, which is slightly different from that observed by SEM. This may be related to interactions between the solvent and functional groups on the particle surface, such as hydrogen bonding. These interactions can change the chemical properties of the particle surface, thus affecting the dispersion and particle size. In addition, the solvated layer can also lead to larger particle sizes as determined by DLS than by SEM (de la Calle et al., 2019). The effect of ESP treatment on the ζ-potential of zein was further determined. Commercial zein carries a slight positive charge in aqueous ethanol solution, which may be caused by the ionization of free amino groups on the chains of zein such as proline and glutamine (Silva et al., 2021b). Interestingly, after ESP treatment, the ζ-potential value decreased significantly, suggesting that ESP treatment may alter the structure of zein, leading to a decrease in the amount of ionizable amino in aqueous ethanol solutions. Particles with smaller particle sizes have more obvious advantages in the development of functional products such as encapsulating materials and emulsion stabilizers (Panagiotopoulou et al., 2022).

Secondary Structure Analysis (CD Spectra)

To investigate the effect of ESP processing on the structure of zein, CD spectra were used to analyze the differences in the secondary structure of commercial zein and ESP-zein at different concentrations. The CD spectra of zein before and after ESP treatment are shown in Fig. 3A. Both the commercial zein and ESP-zein have three distinct characteristic peaks at 192 nm, 208 nm, and 222 nm, which are characteristic of proteins with α-helical structures. However, obvious peak intensity differences appeared between commercial zein and ESP-zein, suggesting that ESP treatment did have an alteration on the structure of zein. The secondary structures of both were further quantitatively analyzed, and the results are shown in Fig. 3B. Commercial zein contains 36% α-helix, 17% β-sheet, 19% β-turn, and 28% random coil. The results were similar to previous reports (Selling et al., 2007). While 8% ESP-zein showed a slight decrease in the content of α-helical structure, on the contrary, the content of β-sheet was elevated. Sixteen percent ESP-zein showed a further decrease in the content of α-helix, which was replaced by an elevation in the content of random coil. These results indicate that the effect of ESP treatment on the secondary structure of zein is mainly represented by a decrease in α-helical structure and an increase in β-sheet or random coil structure. The α-helix structure is stabilized by hydrogen bonding, and it is possible that the high-voltage electric field during ESP treatment disrupts the hydrogen bonding in the molecular chain of zein, leading to the reduction of the α-helix structure (Voegler Smith & Hall, 2001). Wang and Padua (2012) also found a transition from α-helical to β-sheet structure in zein during evaporation-induced self-assembly. Similarly, the polarity shift of the zein solution induces a transition from the α-helical structure to the β-sheet structure and the irregularly curled structure (Erickson et al., 2020). All of these structural changes may further affect the hydrophobicity of zein, thus broadening the application of zein.

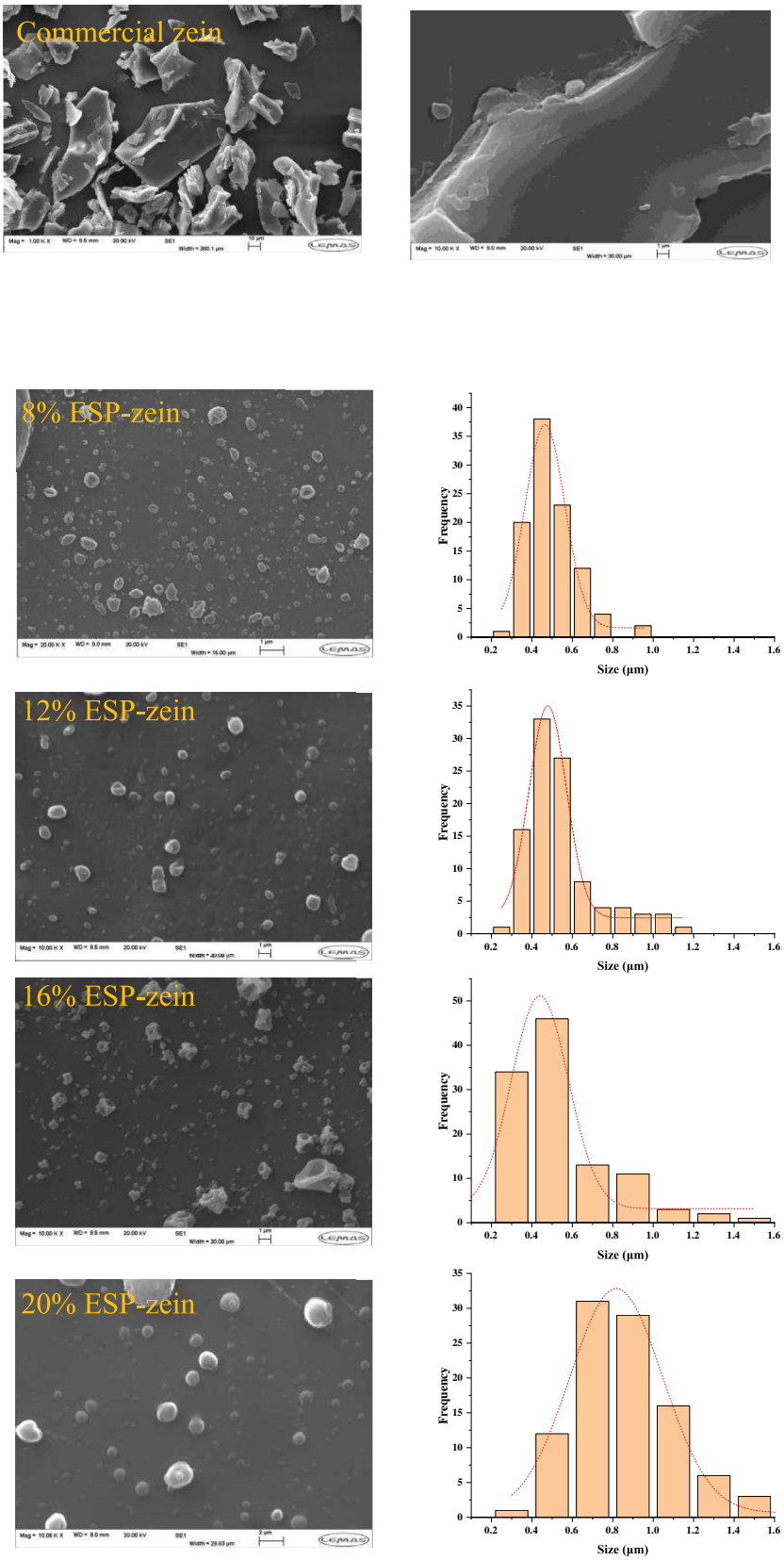

Fig. 2 Representative scanning electron microscopy (SEM) images of commercial zein and electrospraying zein (ESP-zein) at different concentrations

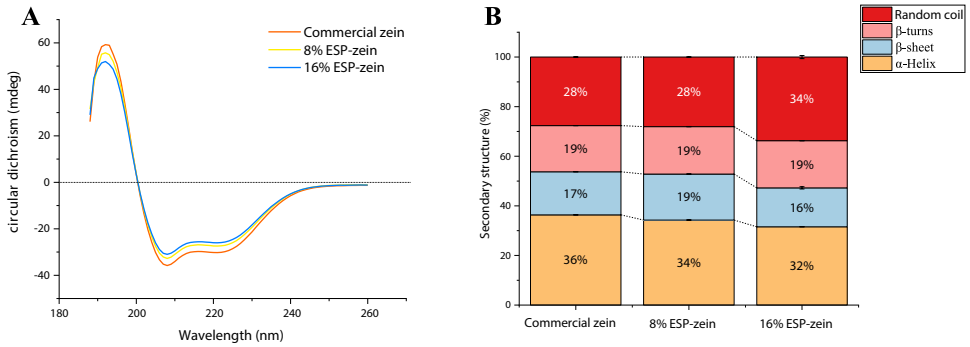

Fig. 3 **A** Circular dichroism (CD) results of zein before and after electrospraying treatment, 190–260 nm, 20 °C. **B** Quantitative analysis of changes in the secondary structure of zein

Measurement of Contact Angle

Zein has poor hydrophilicity due to its high content of hydrophobic amino acids; therefore, improving the hydrophilicity of zein may be beneficial in expanding their applications. As shown in Fig. 4A, B, the water contact angle of 69.8 ± 1.56° for commercial zein was higher than the previously reported results, which may be due to the inconsistent source of zein (Amjadi et al., 2022). Although zein is considered to be a water-insoluble protein, it has a contact angle of less than 90°, which may be caused by the fact that zein still contains a portion of hydrophilic amino acids such as glutamic acid. After ESP treatment, the water contact angle of zein was significantly reduced, especially at low concentrations of ESP-zein. This may be related to the disruption of hydrogen bonding in the molecular chain of zein by ESP treatment, resulting in an increase of free amino or hydroxyl groups in the zein matrix. In addition, the samples formed by ESP at low concentrations of zein were morphologically thinner, which may also account for the lower water contact angle of ESP-zein at low concentrations. In addition, the water contact angle of the samples was continuously examined over time, and it was found that the commercial zein had a high initial water contact angle, but it rapidly collapsed compared to ESP-zein (Fig. 4C). This may be attributed to the reason that zein has better film-forming properties, and the resulting micro- and nanoparticles are uniformly stacked together to form a denser structure during the ESP processing (Singh et al., 2012).

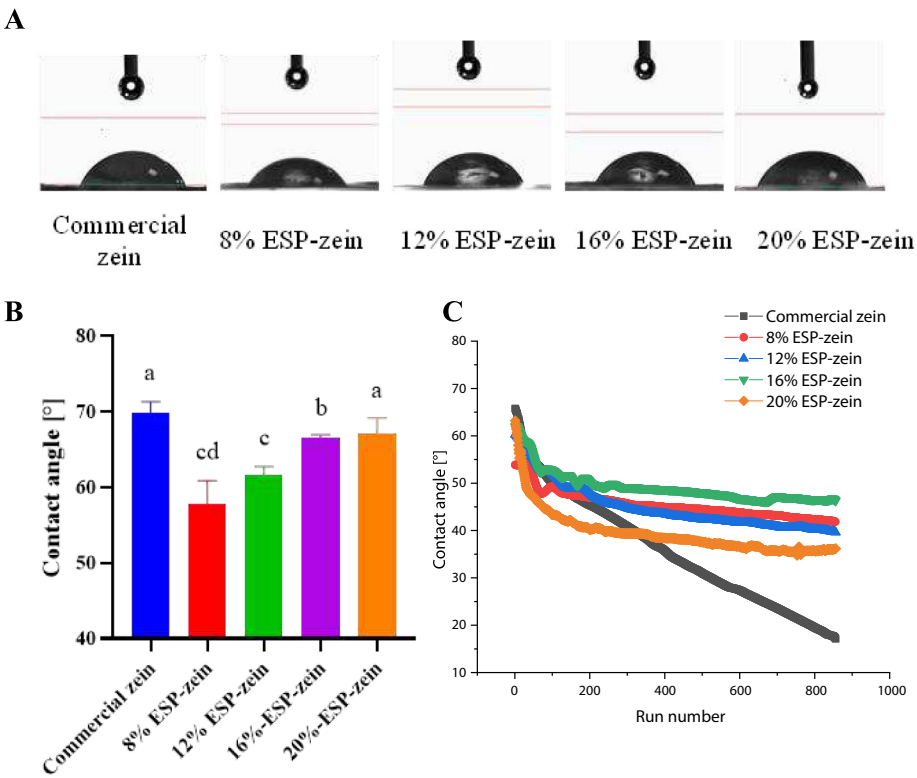

Fig. 4 **A** Representative images of contact angles of zein and ESP-zein (ESP-zein). **B** Contact angle of commercial zein and different concentrations of electrospraying zein. **C** Time-dependent contact angle changes for commercial zein and ESP-zein

Surface Hydrophobicity

ANS, as a sensitive fluorescent probe, was further utilized to determine the surface hydrophobicity of commercial zein and ESP-zein. ANS binds to hydrophobic groups on the surface of the proteins, and the more sites it binds, the stronger the fluorescence intensity (Yang & Zeng, 2018). As shown in Fig. 5, compared to commercial zein, the fluorescence intensity of ESP-zein was decreased upon binding to ANS, which also responds to the fact that ESP treatment inhibits the exposure of hydrophobic regions of the protein and reduces the hydrophobicity of the zein, which is in agreement with the results of the contact angle. Moreover, the shift of the maximum absorption wavelength toward longer wavelengths (from 497 to 500 nm) also verifies this point. As previously mentioned, ESP causes zein to unfold (less α-helix content) and the hydrophobic region to rearrange and contract inward, resulting in fewer sites that can bind to the ANS and reduced fluorescent signal (Cabra et al., 2007). However, the fluorescence of 16% and 20% ESP-zein was found to be lower than that of 8% and 12% ESP-zein. ANS generates fluorescence by binding to hydrophobic regions on the protein surface, which is closely related to the secondary structure of the protein. Previous studies have shown that surface hydrophobicity is significantly and positively correlated with the number of α-helical structures. α-Helical structure usually results in proteins exposing more hydrophobic amino acid side chains, and a decrease in α-helical structure may result in a decrease in the number of hydrophobic amino acids exposed on the surface, which in turn results in a lower ANS fluorescence intensity (Van Oss, 1997; Z. Wang, Li, Jiang, Qi, & Zhou, 2014). The CD results show that 16% ESP-zein has a lower α-helical structure than 8% ESP-zein, which may be the reason for the 16% and 20% ESP-zein having lower fluorescence intensity. The surface hydrophobicity of proteins is closely related to their emulsifying properties, and the exposed hydrophobic regions of proteins can stabilize the oil–water interface, thus contributing to better stabilization of emulsions (Nakai, Li-Chan, & Hayakawa, 1986). However, excessive hydrophobicity can lead to protein aggregation in the aqueous phase, and therefore, appropriate reduction of zein's hydrophobicity may be beneficial for its use in emulsion stabilization.

Interaction Between Zein and Quercetin

Subsequently, in order to further improve the physical properties of ESP-zein particles and to give them some biological activity, quercetin was added to investigate its effect on ESP-zein. It was hypothesized that the microstructure and physical properties of ESP-zein can be affected by the non-covalent interaction between quercetin (Que) and zein. Based on the reasons mentioned below, we conclude that the best concentration was 8% zein. The reasons are as follows: compared to other concentrations, 8% ESP-zein has spherical particles, low particle size, low contact angle, and surface hydrophobicity. Thus, 8% of the zein was used to probe the interaction with quercetin. Molecular docking was used to explore the possible interaction between commercial zein and quercetin. Although some environmental factor (e.g.,

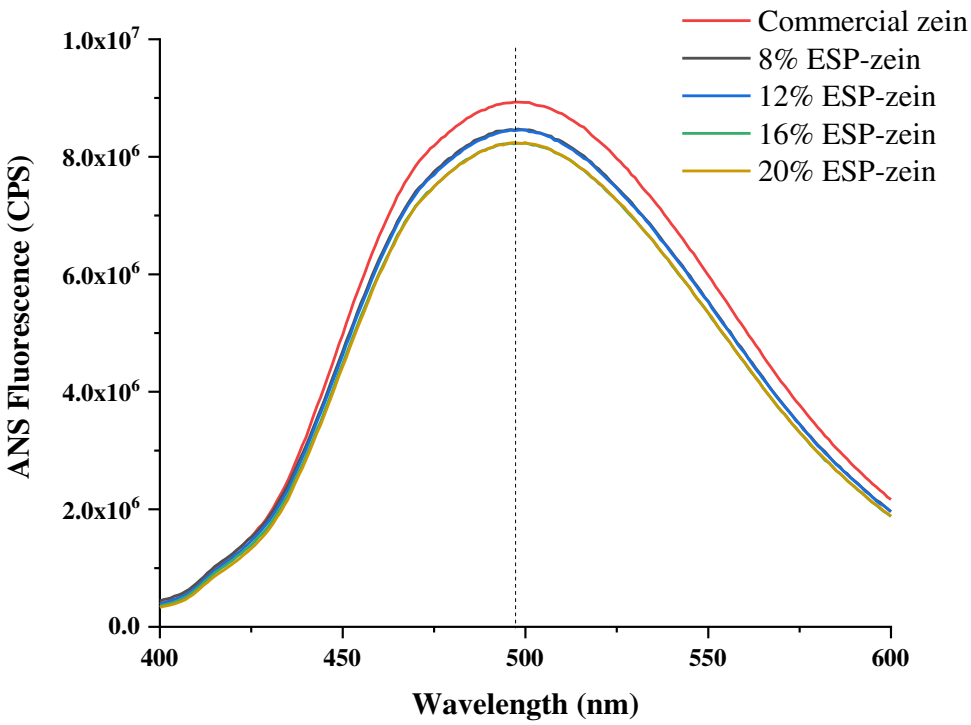

Fig. 5 ANS fluorescence intensity of commercial zein and ESP-zein with different concentrations

solvent, temperature, pH) was not taken into account, it can also clarify the effect mechanisms of quercetin addition on the physicochemical properties of ESP-zein at the molecular level. As shown in Fig. 6, there are several interactions between zein and quercetin, mainly hydrophobic interactions and hydrogen bonds, while electrostatic interactions contribute less. This is mainly because the generation of electrostatic interaction forces requires protonation of the hydroxyl group of quercetin, which usually occurs in alkaline environments (Jaldappagari et al., 2013). Specifically, it involves conventional hydrogen bonds, Van der Waals forces, amide-pi stacked, and pi-alkyl between the phenolic hydroxyl group, benzene ring, ketone group of quercetin, and the GLN-74, LEU-156, SER-160, LEU-78, ALA-127, SER-159, and LEU-81 of zein residue, respectively.

Effect of Quercetin on the Particle Size and Zeta Potential of ESP-Zein

The interaction between plant proteins and polyphenols has received a lot of attention in the food industry because of its ability to form complexes through non-covalent interaction forces such as hydrogen bonding, hydrophobic interaction forces, and electrostatic interaction forces (Ozdal et al., 2013). It provides easy handling, high nutritional value, and low cost and can give new properties to plant proteins and modify their functions (Zhang et al., 2021). After having investigated the effect of electrospraying on the physicochemical properties of zein, we further determined the effect on ESP-zein after the addition of quercetin (a dietary polyphenol). We electrosprayed 8% zein with 0.1–0.4% quercetin. Subsequently, surface tension measurements were carried out on a mixture solution of zein and quercetin to determine its suitability for electrostatic spray treatment. The results showed that mixture solution has low surface tension, and the addition of quercetin slightly increased the surface tension of the solution, but it still applicable to ESP processing (Fig. S2).

After the successful preparation of quercetin-containing ESP-zein particles, the effect of quercetin addition on the particle size and zeta potential of 8% ESP-zein was investigated as shown in Fig. S3. Quercetin alone has a large particle size and a small negative charge. And after mixing quercetin and zein for electrospraying treatment, it

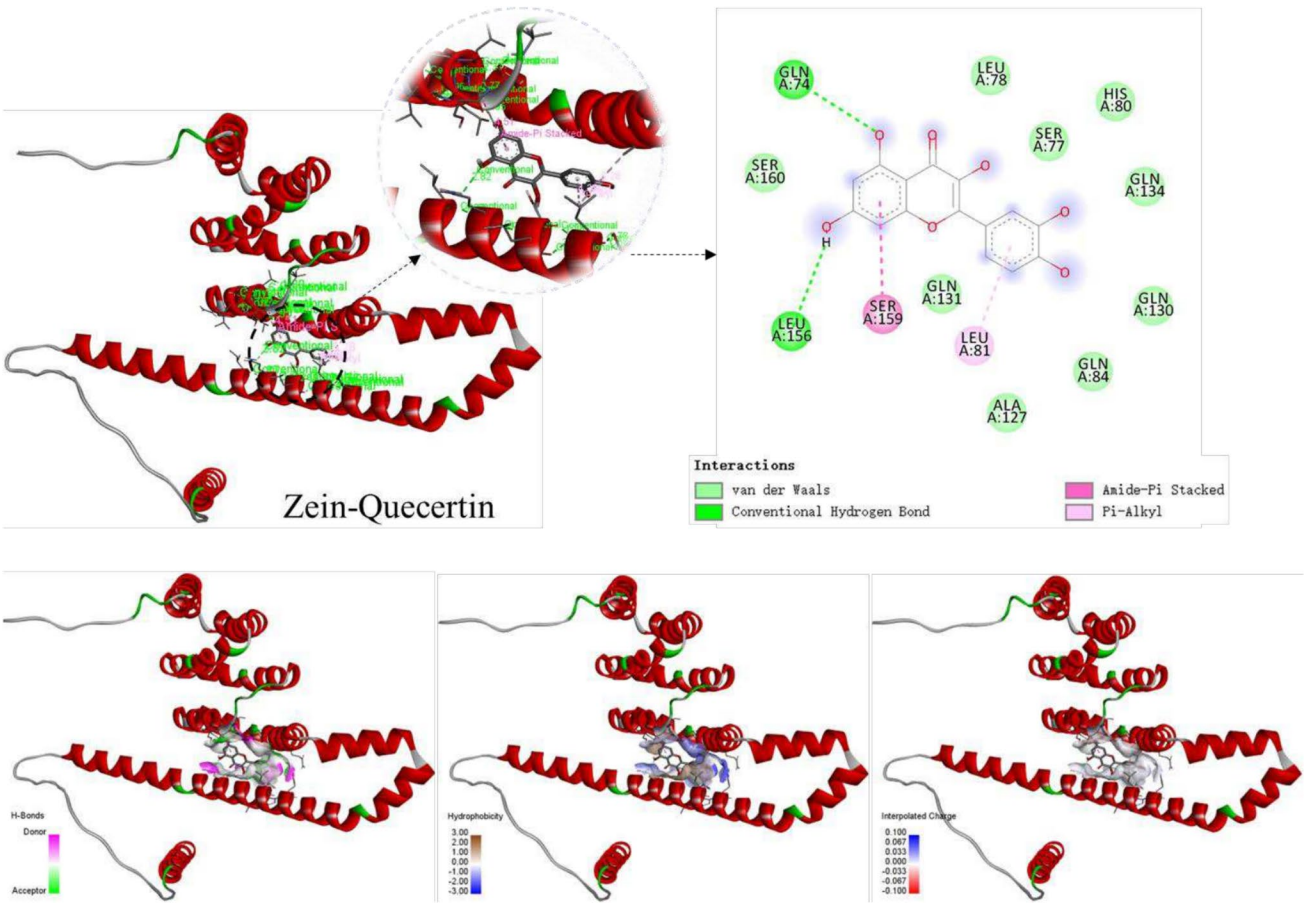

Fig. 6 Interaction analysis between zein and quercetin

can be found that the particle size is significantly reduced. This may be that quercetin is embedded in the interior of the hydrophobic chamber of the zein by hydrophobic interaction during ESP, and actually, ESP is also considered one of the promising methods for the preparation of encapsulated particles (Rodríguez-Félix et al., 2019). However, the addition of quercetin at a low concentration slightly increases the particle size compared to 8% ESP-zein, whereas the addition of quercetin at a high concentration greatly increases the particle size of ESP-zein particles. Moreover, the concentration of quercetin does not have an effect on the zeta potential of ESP-zein.

Effect of Quercetin on the Morphology of ESP-Zein

Scanning electron microscopy (SEM) was used to observe the effect of different concentrations of quercetin on the particle formation of zein during electrospraying. As shown in Fig. 7, quercetin shows a disordered rod-like structure in the SEM image and possesses a large size, whereas, after mixing zein and quercetin for electrospraying, the sample exhibits distinct spherical particles, which is a similar structure to ESP-zein alone, but their size is larger than 8% ESP-zein. Furthermore, after the concentration of quercetin was increased to 0.4%, the ESP-zein obviously displayed larger sizes and even exhibited a partially fibrous morphology. This indicates that quercetin may bind to zein through hydrogen bonding and hydrophobic interaction forces during

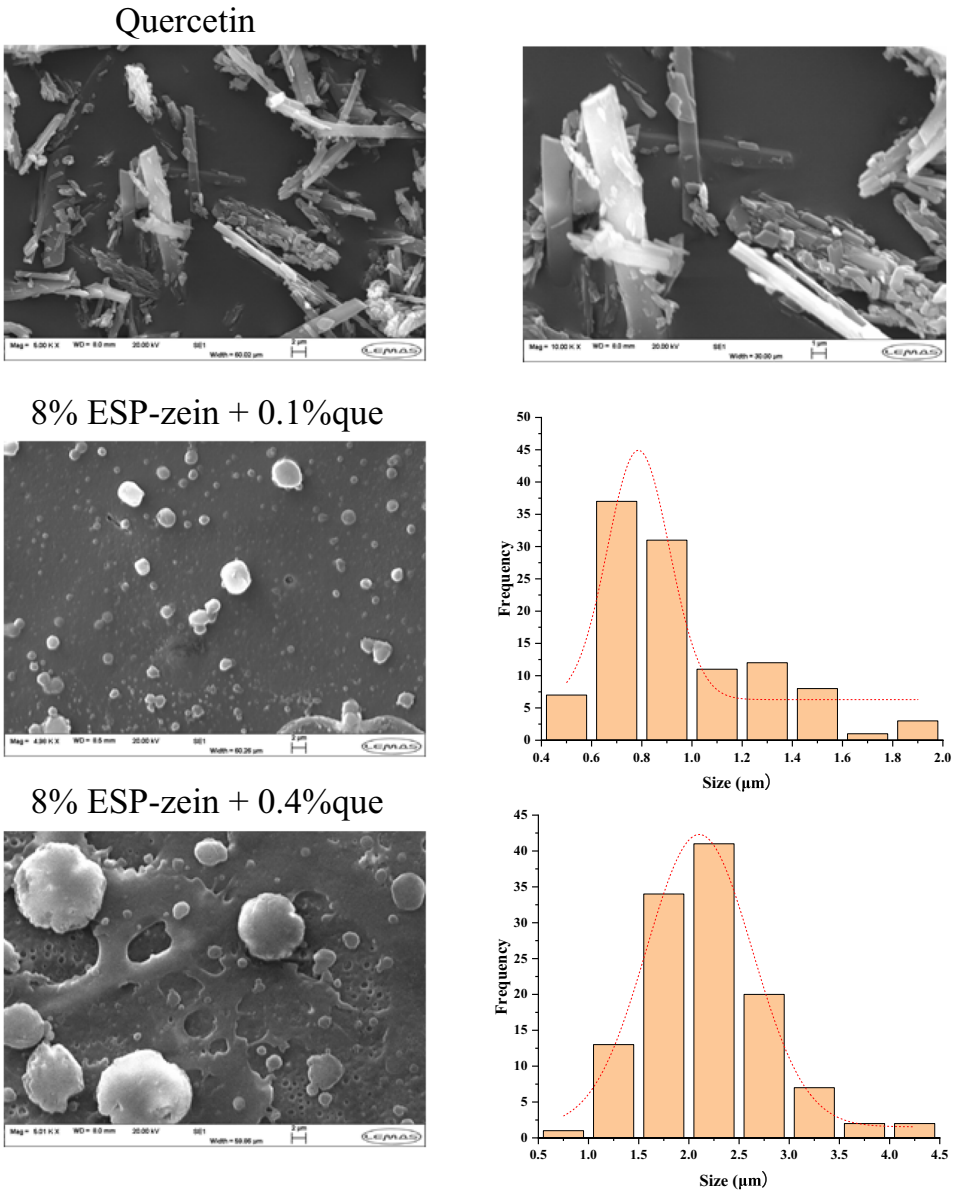

Fig. 7 Scanning electron microscopy (SEM) images and size distributions of ESP-zein with addition of quercetin

the electrospraying process, resulting in the production of larger-sized complexes (Ke et al., 2023). This non-covalent binding may have a certain impact on the interfacial properties of ESP-zein.

Effect of Quercetin on the Interfacial Properties of ESP-Zein

The effect of quercetin addition on the interfacial properties of ESP-zein was investigated by determining the water contact angle and oil–water interfacial tension. As shown in Fig. 8A, after the addition of quercetin, the contact angles of ESP-zein increased in a concentration-dependent manner (6.24–17.86%). This shows that after the addition of quercetin, the non-covalent interaction between the quercetin and zein may increase the exposure of the hydrophobic region of zein. In general, colloidal particles used as stabilizers usually cannot have extremely hydrophilic or hydrophobic properties, which would cause them to aggregate in the water or oil phase rather than stabilize the oil–water interface (Sun et al., 2021). Without considering the excellent antioxidant activity of quercetin, the added amount of quercetin can be used to adjust the wettability of ESP-zein particles, which greatly increases its application prospects in colloids and emulsions.

The changes in oil–water interfacial tension were monitored for commercial zein, ESP-zein, and ESP-zein + que, respectively. As shown in Fig. 8B, these three samples all exhibit a trend in which the interfacial tension gradually decreases with the passage of time and finally levels off. This is because when the colloidal particles come into contact with the oil–water interface, the hydrophobic interaction force will cause the particles to spontaneously rearrange, reduce the interface energy, and finally adhere uniformly to the oil–water interface to form a stable system (Dai et al., 2021). Compared with commercial zein, the interfacial tension of ESP-zein is reduced, indicating that electrospraying technology can reduce the interfacial tension of commercial zein. After the addition of quercetin, the interfacial tension increased. ESP-zein + que shows an interesting phenomenon.

In the initial stage, when the ESP-zein + que particles first come into contact with the oil–water interface, they have an obviously higher interfacial tension than others, but they decrease rapidly with time. It is speculated that quercetin is a hydrophobic molecule that forms a non-covalent bond with the hydrophobic region of zein, which requires more energy to be adsorbed to the oil–water interface during the rearrangement process. Feng et al. (2023) also reported that in the process of preparing nanocellulose-gelatin emulsion, adding nanofibers will increase the interfacial tension of gelatin, but at the same time, the water-holding capacity and stability of the emulsion will be improved.

Correlation Analysis

It was beneficial to link the experimental parameters to the indicators to explore potential correlations and better guide the development of favorable food functional materials. Zein concentration has a positive correlation with both its viscosity and surface tension, which in turn affects the relevant parameters (voltage and flow rate) of the ESP treatment process (Fig. S4A). Higher viscosities require the application of higher voltages to fully evaporate the solvent. The increase in flow rate will greatly improve the efficiency of the ESP process. Variations in the concentration of zein lead to different particle sizes, potentials, microstructure, contact angles, and hydrophobicity of the ESP-zein, which is in agreement with our above results. In addition, there is a correlation between the viscosity and surface tension of the solution and the particle size, PDI, potential, contact angle, and hydrophobicity of the ESP-zein. Apart from the

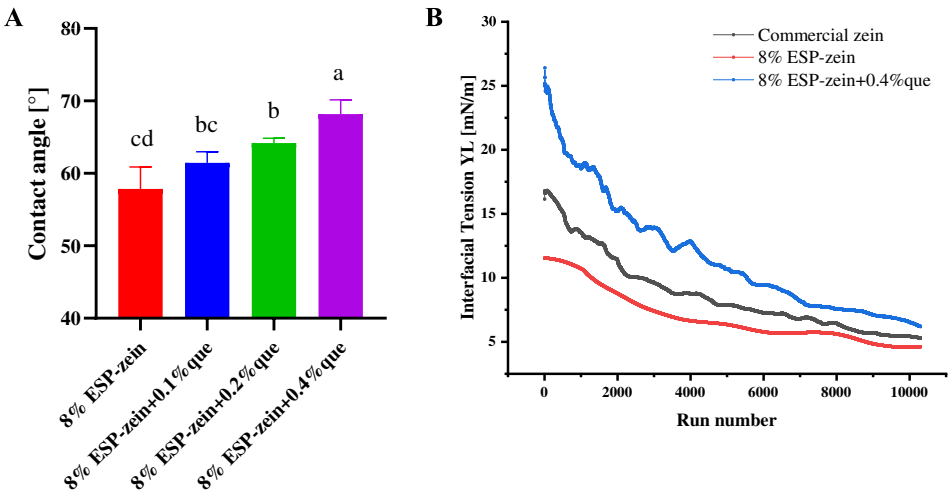

Fig. 8 **A**, **B** Effect of quercetin on contact angle and interfacial tension of ESP-zein

effect of zein concentration, it is still a bit of a consideration whether there is a direct correlation between them. A similar phenomenon was observed after the addition of quercetin at different concentrations (Fig. S4B). The addition of quercetin showed a significant positive correlation with particle size, PDI, contact angle, and oil–water interfacial tension of ESP-zein. Therefore, while improving the wettability and interfacial properties of ESP-zein by the addition of quercetin, the effect of increasing particle size should also be taken into account.

Conclusions

In recent years, plant proteins have attracted widespread interest as they can be used to replace animal proteins and contribute to sustainable development. Zein is a low-cost, environmentally friendly protein by-product isolated from corn processing. Electrospraying is considered to be a promising tool for the development of plant proteins as it can be used to prepare sub-micron particles. We hypothesize that electrospraying has a positive effect on the properties of zein. Herein, the viscosity of different concentrations of zein (8%, 12%, 16%, and 20%) was examined for electrospraying, and zein was found to have an appropriate viscosity and to increase with concentration. Subsequently, zein micro/nano-nanostructures were prepared by electrospraying and characterized. Scanning electron microscopy (SEM) results showed that ESP-zein exhibited granular morphology rather than fiber. The particle size is related to the zein concentration.

Results of dynamic light scattering (DLS) revealed that ESP processing reduced the particle size and Polydispersity Index (PDI) of commercial zein. ESP treatment also causes a decrease in the zeta potential of zein. Further comparison of the secondary structures of ESP-zein and commercial zein revealed that ESP treatment promotes the transformation of the α-helical structure of zein to an irregular curl. Contact angle experiments showed that the water contact angle of ESP-zein was lower than commercial zein. Surface hydrophobicity experiments also found that ESP decreases the hydrophobicity of commercial zein, which is consistent with the results of contact angle experiments. These results suggest that ESP processing has an effect on the physicochemical properties of zein, which may be due to the alteration of the secondary structure of zein as a result of high-voltage treatment. In addition, the addition of quercetin had an effect on the microscopic morphology and interfacial properties of ESP-zein. Quercetin increases the particle size of ESP-zein and improves its wettability and interfacial tension. This may be attributed to the hydrophobic interaction and hydrogen bonding between zein and quercetin. In general, we prepared zein particles with micro- and nanostructures by electrospraying and then regulated their microstructure, wettability, and interfacial tension bidirectionally by the addition of hydrophobic bioactives (quercetin) to meet the application in specific food production. The favorable bioactivities of quercetin, such as antioxidant activity, can also expand the application of ESP-zein particles in the food industry. This study will provide a fundamental understanding of the subsequent application of ESP-zein particles in the food industry such as in Pickering emulsions, intelligent packaging, and food delivery systems.

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Data Availability Raw data available upon request.

Declarations

Conflict of Interest The authors declare no competing interests.

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