Loading of cinnamon essential oil into electrospun octenylsuccinylated starch-pullulan nanofiber mats: Electrospinnability evaluation, structural characterization, and antibacterial potential

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Abstract

The application of cinnamaldehyde essential oil (CEO), which is naturally antibacterial, has been limited due to its high volatility and insolubility in water. This issue may be addressed by nanoencapsulation through electrospinning. In this study, octenylsuccinylated starch (OSS) with different molecular structures blended with pullulan was developed to load CEO for enhanced antibacterial potential. OSS with a smaller molecular size and a higher degree of substitution (DS) is beneficial for the electrospinning of OSS/PUL into high-quality nanofiber mats. The electrospinning process of OSS/PUL aqueous dopes is mainly influenced by moderate apparent viscosity, increased conductivity, and reduced surface tension. CEO loading through physical adsorption significantly changed the fiber microstructure and displayed antibacterial effects against *Staphylococcus aureus* (*S. aureus)*, *Escherichia coli* (*E. coli)*, and *Aspergillus flavus* (*A. flavus)* for electrospun OSS/PUL nanofiber mats. This study demonstrates that electrospinning starch-based nanofiber mats with loading of essential oils could hold a potential application in active food packaging and wound dressing.

Keywords: Octenylsuccinylated starch; Electrospinning; Cinnamon essential oil; Nanofiber mat; Electrospinnability; Antimicrobial.

1. Introduction

Many pathogenic microorganisms have developed antibiotic resistance, mainly due to the overuse of antibiotics. Therefore, it is very important to find antibacterial active substances that can replace those antibiotics (Maliszewska & Czapka, 2022). Given the concerns over food safety and environmental impact, natural, highly effective and non-toxic antibacterial substances are preferred to use (Sung et al., 2013). Essential oils from plants are a type of natural active substance possessing non-toxic, biocompatible, antioxidant and antibacterial properties (Dai et al., 2023). These oils are generally recognized as Generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration (FDA), indicating that they can be directly used in the food industry (Wen et al., 2016).

Cinnamon essential oil (CEO) is a frequently used natural essential oil that is obtained by distilling cinnamon leaves to produce a clear oil, and its main ingredient is cinnamon aldehyde (Barbosa, Yudice, Mitra, & Rosa, 2021; D'agostino, Tesse, Frippiat, Machouart, & Debourgogne, 2019). The bacteriostatic mechanisms of cinnamon essential oil has been extensively studied (Vasconcelos, Croda, & Simionatto, 2018), including perturbing the cell membrane and altering the lipid profile of the cell membrane (Burt & Reinders, 2003; Wendakoon & Sakaguchi, 1995), the inhibition of motility and biofilm formation (Venkitanarayanan, 2011), the inhibition of quorum sensing (a bacteria intercellular communication system) (Brackman et al., 2011), and others. However, due to its high volatility, insolubility in water, and peculiar odor, it is often necessary to encapsulate CEO using nanotechnology to improve its water-dispersibility and storage stability, mask the peculiar odor, and achieved controlled release (Lin, Dai, & Cui, 2017). Compared with poly (butylene adipate-*co*-terephthalate) capsules (da Silva Barbosa et al., 2021) and cellulose nanocrystals/chitosan composite films (Liu et al., 2022), electrospinning represents one of the most promising encapsulation methods due to its convenience and inexpensiveness.

Electrospinning is an emerging nanotechnology that utilize electrostatic force to stretch polymer fluid, thereby reducing the diameter of polymer fibers (Reneker &

Chun, 1996). Compared with other nanotechnologies, electrospinning allows for easy control in the preparation of continuous and uniform nanofibers, and the resulting nanofibers are characterized by the large specific surface area and extreme porosity, making them particularly suitable for the encapsulation and controlled release of bioactive substances (Lim, Mendes, & Chronakis, 2019). Numerous studies have shown that electrospinning is an effective platform for improving the storage stability and biological activity of essential oils (Ansarifar & Moradinezhad, 2022; Cui, Yuan, Li, & Lin, 2017; Ghasemi, Miri, Najafi, Tavakoli, & Hadadi, 2022; Ribeiro-Santos et al., 2017; Tampau, González-Martínez, & Chiralt, 2018). Therefore, the use of electrospinning for preparing nanofibers shows great potential for applications in food antimicrobial or active packaging (Wang et al., 2023).

Biopolymers such as alginate, cellulose, chitosan and starch can serve as raw materials for electrospinning (Angel, Li, Yan, & Kong, 2022). Among them, starch can act as a wall material to encapsulate bioactive molecules with high loading capacity and efficiency using electrospinning, producing nanocapsules or nanofibers with controlled diameters and lengths (Dierings de Souza, Kringel, Guerra Dias, & da Rosa Zavareze, 2021). Starch, as one of the most abundant, renewable polysaccharide resources on Earth, is composed of α-1,4-linked and α-1,6-linked glucose units and its macromolecules can be divided into amylose and amylopectin, wherein amylose or amylopectin content in starch plays a vital role in determining its distinct physical and chemical properties (Wei et al., 2022; Zhong et al., 2020; Zhong, Qu, et al., 2022). Previous research showed that a high amylose content promotes the formation of nanofibers through electrospinning, since amylose is more likely to form molecular entanglement required by fibers (Cao et al., 2022; Fonseca et al., 2019; Zhong, Tai, et al., 2022). In contrast, amylopectin, due to its branches and bulkiness, is counterproductive to electrospinning (Kong & Ziegler, 2012). Therefore, normal native starch, which contains 70%-80% amylopectin, is insoluble in cold water and requires the use of organic solvents, such as formic acid or dimethyl sulfoxide, for complete dissolution, which limited the application of electrospinning fibers in the food industry (Kong & Ziegler, 2014; Lancuški, Vasilyev, Putaux, & Zussman, 2015).

Chemically modified starches such as octenyl succinic anhydride (OSA) modified starch, also known as octenylsuccinylated starch (OSS), originates from OSA modification combined with the degradation of waxy starch using acid or enzymes methods (Dierings de Souza et al, 2021). Compared with native starch, OSS can easily form molecular entanglements that promote fiber formation in aqueous solutions (Liu et al., 2021; Sweedman, Schäfer, & Gilbert, 2014; Tizzotti, Sweedman, Schäfer, & Gilbert, 2013). Furthermore, blending OSS with linear polymers such as pullulan (PUL, a type of linear polysaccharide consisting of maltotriose units) (Viswanathan et al, 2006) in an aqueous solution could lead to the formation of nanofibers using electrospinning, which are safe, "green", and non-toxic (Li, Kong, & Ziegler, 2021; Liang & Gao, 2023; Liang, Pan, & Gao, 2021). According to a previous report, OSS/PUL blended aqueous solutions represent a feasible option for "green" electrospinning, which could be used to adsorb the odors of oyster peptides (Li et al., 2022).

Although an OSS/PUL system used for "green" electrospinning has been explored in our previous study (Li et al., 2021), there is still uncertainty regarding the electrospinnability of OSS and its relationship with molecular structure. Additionally, and the loading evaluation of CEO into the above-mentioned electrospun starch-based nanofibers remains uncharted territory. We hypothesize that the ability to blend electrospinnable OSS with PUL is linked to their molecular structure (molecular size and linear structure), which could further affect their CEO loading capacity and antibacterial potential. Therefore, this study selected six OSS varieties with different molecular structures to use as raw materials for electrospinning with PUL. The study investigated their blending electrospinnability map and performed structural characterizations using scanning electron microscopy (SEM), ^{1}H nuclear magnetic resonance (NMR) spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and texture profile analysis to evaluate their antibacterial potential against *Staphylococcus aureus (S.aureus)*, *Escherichia coli (E.coli)* and *Aspergillus flavus (A.flavus)* with CEO loading, which could provide new insights into electrospun starch-based nanofiber mats.

2. Materials and methods

2.1. Materials

OSS products originated from potato starches, namely CF03 (OSS1) and CM1120 (OSS2), were kindly supplied by Kartoffelmelcentralen (KMC, Denmark). OSS3, OSS4, OSS5, and OSS6 originated from waxy maize starches were prepared in Fuyang Biotechnology Co., Ltd (Shandong, China) according to our previous method (Zhao et al., 2018). PUL, with a molecular weight of 1.35×10^5 g/mol was purchased from Kangnaxin Biotechnology (Shandong, China). CEO (≥75% cinnamaldehyde) was purchased from Xin Essence Flavor Co., Ltd. The other reagents are of analytical grade.

2.2. Molecular characterization of OSS

Molecular structures of OSS samples were characterized using high-performance size-exclusion chromatography (Liu et al., 2021) and ^{1}H nuclear magnetic resonance (NMR) spectra according to our previous study (Liu et al., 2021). This process enables the acquisition of characteristic parameters, including the molecular size, molecular weight (M_w), and degree of substitution (DS) of OSS, could be obtained. The details can be seen in the supplementary materials.

2.3 Dope preparation and characterization

Different proportions of OSS (0-120%, w/v) and PUL (0-20%, w/v) were dispersed in ultrapure water, and the mixture was then heated in a boiling water bath to prepare a spinning solution according to a previous report (Li et al., 2021). Before electrospinning, their apparent viscosity, surface tension, and conductivity were measured. To test their apparent viscosity, a stress-controlled rheometer (MCR302, Anton Paar GmbH, Graz, Austria) with a parallel steel plate geometry with a diameter of 50 mm and a gap of 1.0 mm was used. Flow curves of apparent viscosity versus shear rate were obtained at 25 °C and shear rates of 0.1-100 s^{-1} (Cao et al., 2022). Surface tension was measured by the interfacial tensiometer (New Boundary Scientific Instrument Co., LTD., Ningbo, China) using the suspended drop method (Berry, Neeson, Dagastine, Chan, & Tabor, 2015). Conductivity was measured by a

conductivity meter (Mettler Toledo Instrument Co., Ltd., Shanghai, China). More details are shown in the supplementary materials.

2.4 Electrospinning

The above spinning solution was loaded into a 5 mL syringe (Yuegang Medical Equipment Co., Ltd., Changzhou, China), and 23-gauge blunt needles (Nano Apparatus Technology Co., Ltd., Changsha, China) were used as the spinneret. The electrospinning parameters were based on a previous study with some modifications (Li et al., 2022). The parameters were set at 18 kV voltage, 15 cm collecting distance, and 0.4 mL/h feed speed. Nanofibers were generated by electrospinning equipment (JDF05, Nano Apparatus Technology Co., Ltd., Changsha, China) at a temperature of 28 °C and 25% relative humidity, with the assistance of air conditioning and a dehumidifier. Nanofiber mats are collected in an electrically grounded drum rotating at 50 rpm for 12 h and subsequently stored in a dryer at 25 °C for further analysis.

2.5 Structural characterization of nanofiber mats

2.5.1 Microstructure

Scanning electron microscopy (SEM, TM-3000, Japan) was used to investigate the morphology of nanofibers, which were fixed and gold-sputtered (Tian et al., 2022). The nanofiber mats were sectioned into small pieces and mounted on a conductive adhesive. They were then sputter-coated with gold using an ion-sputtering device. The morphology of the nanofibers was imaged using a scanning electron microscope at a magnification of 10,000×. Their fiber diameter distribution and porosity were analyzed by Image J software (National Institutes of Health, Bethesda, MD, USA) according to a previous study (Li et al., 2022).

2.5.2 FT-IR analysis

The samples were analyzed using an ATR-FTIR facility (PE SP2, Perkin-Elmer, America) with a wavenumber range of 4000–800 cm^{-1} and a resolution of 4 cm^{-1} (Li et al., 2022).

2.5.3 Mechanical strength

The mechanical properties of the samples were tested using a texture analyzer (TA.XT plus, Stable Micro Systems, UK), with a testing velocity of 0.5 mm/s. The nanofiber mats were cut into strips (5 × 2 cm, thickness of 0.2 mm) using a paper cutter. The stress-strain curves obtained were used to analyze their elastic modulus, tensile strength, and elongation at the break (Zhang, Wang, Li, Zhang, & Weiss, 2021).

2.6 Loading of CEO into nanofiber mats

The nanofiber mats were cut into round sheets with a diameter of 20 mm, using a hole punch, weighed, and then soaked in CEO for 3 min. Then, the excess CEO was filtered out with a vacuum pump (Haoqing Instrument Co., Ltd., Shanghai, China), and re-weighed until reaching a constant value.

2.7 Characterization of CEO-loaded nanofiber mats

2.7.1 Microstructure

The methodology outlined in section 2.5.1 was used with SEM images captured at a magnification of 10,000 ×.

2.7.2 CEO loading rate

The mass of the nanofiber mats loaded with CEO was measured, and the loading rate of CEO was calculated (Li et al., 2018) using the following formula:

$$\text{CEO loading rate (g/g)} = (m_t - m_0)/m_0 \quad (1)$$

wherein m_0 (g) is the original mass of nanofiber mats, and m_t (g) is the mass of the nanofiber mat after CEO loading for 3 min.

2.7.3 Antimicrobial evaluation

The antibacterial effectiveness of the nanofiber mats with CEO loading was evaluated using the inhibition zone method (Zhou, Abdel-Samie, Li, Cui, & Lin, 2020). The microorganisms used in the test were *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive), and *Aspergillus flavus* (fungus), which were

activated in Trypticase Soy Broth at 37 °C for 12 h prior to testing. The circular nanofiber mat, with a diameter of 20 mm, was sterilized under an ultraviolet lamp for 1 h before conducting the inhibition zone method. A bacterial suspension of 100 μL (1×10^8 CFU/mL) was then coated on the corresponding agar plate. Next, the nanofiber mat was placed on the surface of the plate, and the dish was cultured upright at 37 °C for 24 h. Finally, the diameter of the antibacterial zone was measured to evaluate the mat's antibacterial activity.

2.8 Statistical analysis

Data were expressed as mean ± standard deviation. SPSS 16.0 (IBM, Inc., Armonk, NY) was used for significance analysis, and one-way analysis of variance (ANOVA) with Duncan's multiple range test (P <0.05) was used for data analysis.

3. Results and discussion

3.1 Molecular structure of OSS

The SEC weight distributions w(log R_h) of OSS as a function of hydrodynamic radius are presented in Figure 1A. In this study, OSS1 and OSS2 were OSA-modified potato starch, while OSS3, OSS4, OSS5, and OSS6 were OSA-modified waxy maize starch with different molecular sizes (Chen, Xie, Zhao, Qiao, & Liu, 2017; Liu et al., 2021; Zhao et al., 2018). There are two main peaks in OSS1 and OSS2, with R_h values of 11.5 nm and 10 nm observed for the first peak and 47.09 nm and 38.81 nm for the second peak, respectively, highlighting the differences in molecular structure between amylose and amylopectin (Tian et al., 2022). Notably, OSS2 exhibited a shoulder peak at R_h=22.82 nm, possibly due to limited alkali degradation of potato starch (Tian et al., 2022). OSS3, OSS4, OSS5 and OSS6 displayed a predominant peak in SEC, likely stemming from waxy starch; however, OSS from waxy starch was subjected to acid or enzyme degradation to different extents to obtain suitable sizes of OSS3-OSS6 (Han, Zhang, Li, Li, & Wu, 2019). The average R_h values of these starches, namely OSS3, OSS4, OSS5, and OSS6, were 49.71 nm, 3.72 nm, 3.74 nm, and 11.94 nm, respectively. The average molecular weights of the OSS samples were ranked in the following order:

OSS3 > OSS1 > OSS2 > OSS6 > OSS4 > OSS5. The detailed molecular size and molecular weight results of OSS can be seen in Table S1.

^{1}H-NMR was used to determine the degree of substitution (DS) of modified starch. In this work, OSS showed the signal of the CH_3 group at around 0.85 ppm in the ^{1}H NMR spectra, representing the successful grafting of hydrophobic OS groups into the starch skeleton (Wang, Fu, Tang, Huang, & Zhang, 2017). As shown in Table S1, the DS of OSS increased in the order of OSS1 (0.48%) < OSS3 (1.47%) < OSS2 (2.64%) < OSS5 (2.96%) <OSS4 (3.19%) < OSS6 (4.29%). The DS values were influenced by the average number of octenyl succinate (OS) derivatives per glucose unit along the starch backbone (Sweedman, Tizzotti, Schäfer, & Gilbert, 2013). The different DS values of OSS1 and OSS2 could result from the different surface holes formed by the different varieties of potato starch, which, in turn, influence the surface area for chemical reactions (Wang et al., 2013). Other factors that could affect the DS include the chemical synthesis conditions, such as reaction time (2-4 h) and temperature (30-40°C) (Sweedman et al., 2013).

3.2 Electrospinnability of OSS with PUL

The linear structure and good water solubility of PUL provides significant utility for electrospinning, and its use in combination with other polymers can improve electrospinnability by changing their surface tension, apparent viscosity, and conductivity (Li et al., 2021; Li et al., 2022; Liang & Gao, 2023). For determining the appropriate processing parameters for electrospinning, the electrospinnability map of OSS/PUL aqueous dopes was plotted in Figure 2. Generally, OSS is hard to form fiber in an aqueous solution using common electrospinning parameters, but its electrospinnability could be improved by the addition of PUL, which has a unique linear molecular structure that aids in forming fibers without the use of toxic or corrosive organic solvents (Li et al., 2021; Li et al., 2022). A narrow concentration range of 0-14% (w/v) of some commercial OSS products from Ingredion and PUL with molecular weights of 1-2×10^5 g/mol was used before (Li et al., 2021); however, the limited OSS concentrations and unclear OSS molecular structures are not helpful for obtaining a

complete electrospinnability map to understand their electrospinning behavior. With the permission from solubility, a wider range of OSS concertations was used in this work for obtaining the whole electrospinnability map. The concentration of PUL used in this work stayed between 0 and 20% (w/v), with 20% being the minimum concentration required for successful electrospinning, as determined via a pilot study, to produce bead-free nanofibers. From Figure 2, “unable to form fiber mat” (no fibers), “fiber mat with droplets” (usually with beaded fibers), and “good fiber mat without droplets” (usually with bead-free fibers) were expressed using the colors of red, blue and green, respectively. The typical SEM images of these different fiber mats electrospun from the OSS4/PUL aqueous dope with varying polymer concentrations were further exhibited in Figure 3 as a model.

The electrospinnability maps of OSS1/PUL, OSS2/PUL, and OSS3/PUL were shown in Figure 2A, 2B and 2C, respectively. These OSS/PUL aqueous dopes were unable to form good nanofiber mats with 2-20% PUL addition and 4-24% OSS concentrations due to the occurrence of a large number of droplets during the electrospinning process, which may be attributed to the relatively high surface tension and high apparent viscosity (Kong & Ziegler, 2012; Poudel et al., 2020) (see Table S2).

From the electrospinnability map of OSS4/PUL, OSS5/PUL, and OSS6/PUL (see Figure 2D, 2E and 2F), good fiber mats could be formed when the concentrations of OSS and/or PUL were increased to certain values. When the concentration range of OSS4, OSS5, and OSS6 was set to 0-120% (w/v), and the PUL concentration was set to be at least 6% (w/v), a continuous and stable jet could be formed leading to good fiber mats. Fiber mats with droplets could be produced with a lower concentration of OSS (4%) and PUL (4%), consistent with those reported by Li et al. (2021). The electrospinnable areas of OSS/PUL aqueous dopes follows the order of OSS4/PUL > OSS5/PUL > OSS6/PUL. Among these OSS samples, OSS4 presented a wider electrospinnability area, which should result from combined effects of e.g. molecular size, average molecular weight, and DS, making it more suitable for industrial production.

In order to further understand the fiber formation mechanism of OSS/PUL

aqueous dopes by electrospinning, Figure 3 shows the SEM images of electrospun OSS4/PUL nanofibers across varying polymer concentrations. As the concentrations of OSS4 (20-100%, w/v) and PUL (2-20%, w/v) increased, the status of fiber was changed from no fibers (Figure 3-D1) to beaded fibers (Figure 3-D2), then to uniform and continuous bead-free fibers (Figure 3-D3). This result also indicate that a relatively small addition of PUL played a crucial role in establishing sufficient molecular entanglement for electrospinning (Li et al., 2021). The presence of PUL significantly decreased the number of beads, with the minimum required concentration of PUL in the aqueous dopes decreasing as the concentration of OSS increased (Li et al., 2021; Wang & Ziegler, 2019). The electrospinnability map reveals that achieving successful electrospinning for 16%, 20% and 40% (w/v) aqueous OSS dopes requires a minimum addition of 10%, 8%, and 6% (w/v) PUL, respectively. However, excessive OSS concentration, even with PUL added, can result in the ejection of beaded fibers from the nozzle, leading to the formation of poor fibers.

3.3 Electrospinning dope properties

The electrospinning process of polymer solutions relied on a combination of factors, including molecular structure and entanglement, and the properties of the dopes, such as apparent viscosity, surface tension, and conductivity (Duan et al., 2006; Yu, Fridrikh, & Rutledge, 2006). In order to explain the effect of different OSS components on the electrospinnability of OSS/PUL aqueous dopes, a fixed OSS concentration of 20% (w/v) and a fixed PUL concentration of 10% (w/v) were selected, allowing the characterization of their dope properties.

Among the crucial parameters for electrospinning, rheological properties of polymer dopes hold significant importance (Kong & Ziegler, 2012). The apparent viscosity of OSS/PUL aqueous dopes was measured at different shear rates ranging from 0.1 to 100 s^{-1}, as depicted in Figure 4A and presented in Table S2. In Figure 4A, the OSS1/PUL aqueous dope exhibited notably higher apparent viscosity, with the flow curve indicating non-Newtonian behavior (apparent viscosity deceased with the increasing shear rate) at low shear rates (<100 s^{-1}). Similarly, the OSS2/PUL and

OSS3/PUL aqueous dopes showed this shear-thinning behavior, albeit less pronounced than OSS1/PUL. On the contrary, the OSS4/PUL, OSS5/PUL, and OSS6/PUL aqueous dopes all showed Newtonian behavior. OSS/PUL aqueous dopes, which are suitable for fiber formation by electrospinning, were found to meet the following conditions: being not or weakly shear thinning at shear rates below 100 s^{-1}, which is similar to the conclusions reported in a previous study (Stijnman, Bodnar, & Hans Tromp, 2011).

Another critical factor for successful electrospinning is the conductivity of electrospinning dopes, affected by the conductivity of solvents, polymers, and additives (e.g. salts) (Bhardwaj & Kundu, 2010). The conductivity of OSS/PUL aqueous dopes is shown in Figure 4B and Table S2. Increased conductivity has been shown to effectively suppress bead formation during the electrospinning of fibers (Fong, Chun, & Reneker, 1999). This principle holds true in our study, where the conductivity of the OSS4/PUL, OSS5/PUL and OSS6/PUL aqueous dopes surpasses that of the other three formulations, which may be due to the higher moles of OSA group with a negative charge, contributing to the enhanced electrospinnability of the OSS4/PUL, OSS5/PUL and OSS6/PUL aqueous dopes.

The surface tension of polymer dopes acts as a counterforce to the stretching effect induced by applied voltage (Kutzli, Gibis, Baier, & Weiss, 2019; Mitchell, 2015). Overcoming surface tension is crucial for the successful formation of fibers through electrospinning. Figure 4C shows the time-dependence of surface tension for OSS/PUL aqueous dopes. The curves illustrate that the surface tension of these aqueous dopes decreased with increasing time. At the 3000 s mark, the surface tension followed the order of OSS2/PUL, OSS1/PUL, OSS4/PUL, OSS5/PUL, OSS3/PUL, and OSS6/PUL, mainly influenced by the types of polymers and solvents used (Angel, Li, Yan, & Kong, 2022). Higher surface tension in polymer dopes often results in bead formation during electrospinning (Cao et al., 2022). Conversely, reducing surface tension can enhance polymer jet stability through chain entanglement, effectively preventing bead formation and leading to an increased diameter of the fibers (Lim et al., 2019).

3.4 Characterization of OSS/PUL nanofiber mats

3.4.1 Morphology

The morphology (beads and fiber diameter) of nanofiber mats (with droplets having a diameter ≥ 1 mm) can be observed using SEM and a digital camera (Cao et al., 2022), respectively. The appearance of electrospun nanofiber mats and the SEM images of OSS/PUL nanofiber mats are shown in Figure 5. No fiber and nanofiber mats were observed for the OSS1/PUL aqueous dope. The OSS2/PUL and OSS3/PUL aqueous dopes could be electrospun into short and discontinuous nanofibers, wherein their higher viscosity produced massive droplets on the surface of the nanofiber mat (see Figure 5A). Additionally, some beads also appeared among the fibers (see Figure 5B). The average fiber diameter values, without beads, for the electrospun OSS3/PUL and OSS2/PUL fiber mats were 242 nm and 205 nm, respectively. In contrast, the OSS4/PUL, OSS5/PUL and OSS6/PUL aqueous dopes were conducive to the formation of nanofiber mats with continuous, uniform, and bead-free morphologies, as evident in the SEM images (see Figure 5B). Their average fiber diameters measured 211 nm, 244 nm, and 230 nm (see table S3), similar to the results reported in a previous study (Li et al., 2021). Therefore, the electrospun OSS4/PUL, OSS5/PUL, and OSS6/PUL nanofiber mats were selected for further characterization.

3.4.2 FTIR analysis

ATR-FTIR analysis serve as a valuable tool to discern and examine the chemical characteristics of the surface structure in polymer materials (Liu et al., 2013; Liu, Gao, Sangwan, Yu, & Tong, 2014). The FTIR spectra for OSS (OSS4, OSS5, and OSS6), PUL, OSS/PUL nanofiber mats, and CEO-loaded nanofiber mats are displayed in Figure 6. The spectrum for the pure PUL exhibited the stretching vibration of O-H bonds at ca. 3345 cm^{-1}, accompanied by the stretching vibrations of –CH2–, O–C–O, α-(1, 6)-glycosidic, and α-(1, 4)-glycosidic bonds, at ca. 2926 cm^{-1}, ca. 1642 cm^{-1}, ca. 931 cm^{-1}, and ca. 755 cm^{-1}, respectively. For OSS, the bands at 3436-3428 cm^{-1} can be assigned to O-H bending, while those at 2931–2927 cm^{-1} represents C–H stretching vibrations. The wavenumber range between 1200 cm^{-1} and 1000 cm^{-1} can be associated with the stretching of C–O, C–C, and C–O–H and the bending of C–O–H (Zhang et al.,

2013). Notably, peaks at ca.1733–1727 cm^{-1} and 1569 cm^{-1} indicate the existence of OS groups (Li et al., 2022).

OSS/PUL nanofiber mats exhibited a broad band at 3359-3352 cm^{-1}, with the peak at ca. 3346 cm^{-1} shifting to a lower wavenumber, indicating that intermolecular hydrogen bonds were formed between OSS and PUL (Li et al., 2022). The characteristic bands of CEO were observed at ca.1670 cm^{-1} and ca.1625 cm^{-1}, signifying the presence of carbonyl group (C=O) and benzene ring, respectively. Additionally, the band at 1120 cm^{-1} corresponds to the C–O–H stretching of other trace phenolic compounds in CEO (Correa-Pacheco et al., 2020). From Figure 6, the characteristic absorption peaks of CEO were also observed in the three OSS/PUL-CEO nanofiber mats, confirming the successful incorporation of CEO into the electrospun nanofiber mats.

3.4.2 Mechanical properties

Evaluating the mechanical properties of electrospun nanofiber mats is crucial for assessing their suitability for potential industrial applications, considering factors such as durability and integrity (Yildirim-Yalcin, Tornuk, & Toker, 2022). Their tensile strength, elastic modulus, and elongation at break could be obtained from the strain-stress curve of OSS/PUL aqueous mixtures (see Figure 7). The tensile strength is the maximum stress that a fiber mat can withstand while being stretched before breaking, a factor reliant on interactions between the fiber mat constituents (Ghiasi, Golmakani, Eskandari, & Hosseini, 2020).

The nanofiber mat electrospun from the OSS4/PUL aqueous dope showed the higher tensile strength of 1.71 MPa, surpassing OSS5/PUL and OSS6/PUL (1.13 MPa and 1.42 MPa, respectively) (see Figure 7B). Elastic modulus is a measure of the material stiffness (Yao, Bastiaansen, & Peijs, 2014). The OSS4/PUL nanofiber mat had the maximum elastic modulus (38.06 MPa), indicating that this fiber mat was more resistant to deformation, while the OSS5/PUL nanofiber mat had the minimum elastic modulus, meaning that it was more flexible and ductile.

Elongation at break, which reflects the ability to withstand applied stress breakage, depends on interactions within nanofiber mats (Choi et al., 2022). The

elongation at break of the electrospun OSS6/PUL nanofiber mat was 5.33%, slightly higher than that of OSS4/PUL and OSS5/PUL. This phenomenon may be related to the R_h of OSS, wherein OSS6 possessed the highest R_h resulting in the greatest elongation at break among the above three OSS. Similar to starch films, the mechanical properties of electrospun starch-based nanofiber mats were significantly influenced by the inherent structure of starch itself.

3.5 Formation mechanism of electrospun OSS/PUL nanofiber mats

In this study, OSS with different molecular structures were chosen as model materials to explore the structure-driven formation mechanism of electrospun OSS/PUL nanofiber mats (Figure 8). OSS exhibited greater water solubility than native starch, making the production of electrospun starch-based nanofibers more environmentally friendly and efficient. The aqueous dope of pure OSS could not generate well-formed nanofibers through electrospinning. However, the addition of even a small amount of PUL could significantly enhance nanofiber formation by facilitating molecular entanglement with OSS in an aqueous medium (Li et al., 2021). In addition, the generation of starch-based nanofibers is closely related to the molecular weight distribution of amylose and amylopectin of OSS.

For OSS1, OSS2, and OSS3 with high average molecular weight and larger molecular size, when the OSS concentration is high enough, only a small amount of OSS can interact with PUL to achieve the sufficient molecular entanglement, while the remaining OSS molecules tend to self-assemble, forming a dense gel network. This process increases the apparent viscosity, negatively impacting the electrospinning process (Härdelin, Perzon, Hagström, Walkenström, & Gatenholm, 2013; Kutzli, Gibis, Baier, & Weiss, 2018; Vasilyev, Vilensky, & Zussman, 2019).

Surface tension and conductivity of polymer dopes play equal vital roles in electrospinning. A reduction in surface tension is advantageous for successful electrospinning, and the corresponding polymer dopes must also exhibit sufficient conductivity. The inability of OSS1/PUL to form fibers may, in part, be attributed to

insufficient conductivity, preventing the stable formation of a Taylor cone even under a high-voltage electric field, thus hindering fiber production. Therefore, not all OSS variants are suitable for electrospinning, highlighting the importance of selecting OSS with an appropriate molecular structure for successful electrospinning in conjunction with PUL in an aqueous medium.

In additional, electrospun fiber mats possess a three-dimensional network structure resulting from the intertwining of nanofibers, showing an excellent adsorption potential of CEO as a novel porous material. On a macroscopic scale, the physical adsorption process of electrospun fiber mats is affected by factors such as specific surface area, pore structure, surface properties, and adsorbate properties (de Souza et al., 2021). On a microscopic level, it is mainly governed by van der Waals forces, microporous filling, and capillary condensation (Ghasemi et al., 2022).

3.6 Characterization of CEO-loaded nanofiber mats

3.6.1 Microstructure

Figure 9 shows the SEM images of the electrospun OSS4/PUL, OSS5/PUL, and OSS6/PUL nanofiber mats after CEO loading. The coalescence of overlapping or intersecting fibers led to a web-like 3D structure in all the above three electrospun starch-based fiber mats. Additionally, their average fiber diameter increased from 199 nm to 299 nm, 190 nm to 378 nm, and 209 nm to 496 nm for the electrospun OSS4/PUL, OSS5/PUL and OSS6/PUL nanofiber mats, respectively, upon CEO loading. These results are similar to a previous study on the electrospinning of OSS/PUL with limited polymer concentrations (Li et al., 2021), indicating that CEO can be effectively entrapped within the nanofibers, filling their pores.

3.6.2 CEO loading rate

Table 1 shows the CEO loading rates of the electrospun OSS4/PUL, OSS5/PUL, and OSS6/PUL nanofiber mats were 2.64 g/g, 2.73 g/g, and 3.02 g/g, respectively. The effective CEO loading can be attributed to the hydrophobicity of OSS and the high porosity of electrospun nanofiber mats (El-Samak et al., 2020; Sweedman et al., 2013).

Specifically, the hydrophobicity of OSS is positively correlated with its DS. OSS6, with the highest DS and greatest hydrophobicity, demonstrated the highest CEO loading rate. Furthermore, after CEO loading, the OSS6 nanofiber mat showed the most significant change in porosity from 53.69% to 6.15%, and their average fiber diameter increased from 209 nm to 496 nm, further confirming that the DS of OSS influenced the CEO loading rate of the resulting electrospun OSS/PUL nanofiber mats.

3.6.3 Antibacterial activity

The antimicrobial activity of the electrospun OSS4/PUL, OSS5/PUL, and OSS6/PUL nanofiber mats after CEO loading was investigated using *S. aureus*, *E. coil*, and *A. Flavus* as test microorganisms, as shown in Figure 10. All the CEO-loaded fiber mats effectively inhibited the growth of these three microorganisms. Notably, in this study, the diameter of inhibition zones for electrospun OSS/PUL nanofiber mats loaded with CEO against *S. aureus* was higher than those against *E. coil*, implying that these electrospun OSS/PUL nanofiber mats loaded with CEO had better antimicrobial activity against Gram-positive bacteria than Gram-negative bacteria. This difference may be attributed to variances in cell wall structure. Gram-positive bacteria typically have cell walls that allow hydrophobic molecules to easily penetrate, enabling them to exert their effects both on the cell wall and within the cytoplasm (Nazzaro, Fratianni, De Martino, Coppola, & De Feo, 2013; Nikaido, 1994; Trombetta et al., 2005).

Although OSS6/PUL exhibited the highest CEO loading capacity, there was no significant difference in antimicrobial activity among these three OSS/PUL-CEO fiber mats (see Table 1). This result may be related to the saturation point of CEO released from OSS/PUL-CEO fiber mats, resulting in no significant difference in antimicrobial activity (Van de Vel, Sampers, & Raes, 2017). However, OSS6/PUL demonstrated the most potent antifungal activity, followed by OSS5/PUL, suggesting that the antifungal ability of the OSS/PUL-CEO fiber mats increased with the CEO loading. This finding is consistent with a previous study, which showed that the fungal inhibition effect of essential oils is directly proportional to their concentration (Niu et al., 2022). The differential inhibition of fungi and bacteria by the OSS/PUL-CEO fiber mats may be

attributed to the superficial growth of *A. flavus*, making it more susceptible to the volatile compounds released by CEO (Reyes-Jurado et al., 2020).

4. Conclusion

In this study, electrospun OSS/PUL nanofiber mats were successfully produced using six different OSS variants with different molecular structures, alongside PUL, as the raw materials, and water as the only solvent. Among these, OSS1, OSS2, and OSS3, characterized by larger average molecular weights and lower DS values, exhibited suboptimal electrospinning outcomes. OSS4, OSS5, and OSS6 possessing smaller average molecular weights and higher DS values, yielded well-formed nanofiber mats via electrospinning.

For OSS/PUL aqueous dopes, several factors were found to be crucial: 1) The apparent viscosity at a shear rate of 100 s^{-1} should not be excessively high; 2) High conductivity is conducive to electrospinning; and 3) Surface tension, while influential, is not the sole determining factor. In addition, these dope properties further affected the appearance, SEM micro-morphology, fiber diameter distribution, and mechanical properties of electrospun OSS/PUL nanofiber mats.

Furthermore, the CEO-loaded nanofiber mats showed different degrees of inhibition against *S.aureus*, *E.coli*, and *A.flavus,* with notable efficacy against *Staphylococcus aureus*. These findings highlights the essential role of PUL as an electrospinning aid and its concentration in facilitating molecular entanglement of OSS in the dope. This work is beneficial for the development of starch electrospinning and its application in active food packaging.

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References

References

Angel, N., Li, S., Yan, F., & Kong, L. (2022). Recent advances in electrospinning of nanofibers from bio-based carbohydrate polymers and their applications. *Trends in Food Science & Technology, 120*, 308-324. doi:https://doi.org/10.1016/j.tifs.2022.01.003

Ansarifar, E., & Moradinezhad, F. (2022). Encapsulation of thyme essential oil using electrospun zein fiber for strawberry preservation. *Chemical and Biological Technologies in Agriculture, 9*(1), 2. doi:10.1186/s40538-021-00267-y

Barbosa, R. F. d. S., Yudice, E. D. C., Mitra, S. K., & Rosa, D. d. S. (2021). Characterization of Rosewood and Cinnamon Cassia essential oil polymeric capsules: Stability, loading efficiency, release rate and antimicrobial properties. *Food Control, 121*. doi:10.1016/j.foodcont.2020.107605

Berry, J. D., Neeson, M. J., Dagastine, R. R., Chan, D. Y. C., & Tabor, R. F. (2015). Measurement of surface and interfacial tension using pendant drop tensiometry. *Journal of Colloid and Interface Science, 454*, 226-237. doi:https://doi.org/10.1016/j.jcis.2015.05.012

Bhardwaj, N., & Kundu, S. C. (2010). Electrospinning: A fascinating fiber fabrication technique. *Biotechnology Advances, 28*(3), 325-347. doi:https://doi.org/10.1016/j.biotechadv.2010.01.004

Brackman, G., Celen, S., Hillaert, U., Van Calenbergh, S., Cos, P., Maes, L., . . . Coenye, T. (2011). Structure-Activity Relationship of Cinnamaldehyde Analogs as Inhibitors of AI-2 Based Quorum Sensing and Their Effect on Virulence of Vibrio spp. *PLOS ONE, 6*(1), e16084. doi:10.1371/journal.pone.0016084

576 Burt, S. A., & Reinders, R. D. (2003). Antibacterial activity of selected plant essential
577 oils against Escherichia coli O157:H7. *Letters in Applied Microbiology, 36*(3),
578 162-167. doi:10.1046/j.1472-765X.2003.01285.x %J Letters in Applied
579 Microbiology
580 Cao, P., Wu, G., Yao, Z., Wang, Z., Li, E., Yu, S., . . . Li, S. (2022). Effects of amylose
581 and amylopectin molecular structures on starch electrospinning. *Carbohydrate*
582 *Polymers, 296*, 119959. doi:https://doi.org/10.1016/j.carbpol.2022.119959
583 Chen, P., Xie, F., Zhao, L., Qiao, Q., & Liu, X. (2017). Effect of acid hydrolysis on the
584 multi-scale structure change of starch with different amylose content. *Food*
585 *Hydrocolloids, 69*, 359-368. doi:https://doi.org/10.1016/j.foodhyd.2017.03.003
586 Choi, I., Shin, D., Lyu, J. S., Lee, J.-S., Song, H.-g., Chung, M.-N., & Han, J. (2022).
587 Physicochemical properties and solubility of sweet potato starch-based edible
588 films. *Food Packaging and Shelf Life, 33*, 100867.
589 doi:https://doi.org/10.1016/j.fpsl.2022.100867
590 Correa-Pacheco, Z. N., Black-Solís, J. D., Ortega-Gudiño, P., Sabino-Gutiérrez, M. A.,
591 Benítez-Jiménez, J. J., Barajas-Cervantes, A., . . . Hurtado-Colmenares, L. B.
592 (2020). Preparation and Characterization of Bio-Based PLA/PBAT and
593 Cinnamon Essential Oil Polymer Fibers and Life-Cycle Assessment from
594 Hydrolytic Degradation. *12*(1), 38.
595 Cui, H., Yuan, L., Li, C., & Lin, L. (2017). Control of Staphylococcus aureus on soya
596 bean products by D-amino acids/nutmeg essential oil-co-loaded nanofilms.
597 *52*(11), 2393-2403. doi:https://doi.org/10.1111/ijfs.13523
598 D'agostino, M., Tesse, N., Frippiat, J. P., Machouart, M., & Debourgogne, A. J. M.
599 (2019). Essential oils and their natural active compounds presenting antifungal
600 properties. *24*(20), 3713.
601 da Silva Barbosa, R. F., Yudice, E. D. C., Mitra, S. K., & dos Santos Rosa, D. (2021).
602 Characterization of Rosewood and Cinnamon Cassia essential oil polymeric
603 capsules: Stability, loading efficiency, release rate and antimicrobial properties.
604 Food Control, 121, 107605.
605 Dai, H., Chen, Y., Chen, H., Fu, Y., Ma, L., Wang, H., . . . Zhang, Y. (2023). Gelatin

606 films functionalized by lignocellulose nanocrystals-tannic acid stabilized
607 Pickering emulsions: Influence of cinnamon essential oil. *Food Chemistry, 401*,
608 134154. doi:https://doi.org/10.1016/j.foodchem.2022.134154
609 Dierings de Souza, E. J., Kringel, D. H., Guerra Dias, A. R., & da Rosa Zavareze, E.
610 (2021). Polysaccharides as wall material for the encapsulation of essential oils
611 by electrospun technique. *Carbohydrate Polymers, 265*, 118068.
612 doi:https://doi.org/10.1016/j.carbpol.2021.118068
613 Duan, B., Yuan, X., Zhu, Y., Zhang, Y., Li, X., Zhang, Y., & Yao, K. (2006). A
614 nanofibrous composite membrane of PLGA–chitosan/PVA prepared by
615 electrospinning. *European Polymer Journal, 42*(9), 2013-2022.
616 doi:https://doi.org/10.1016/j.eurpolymj.2006.04.021
617 El-Samak, A. A., Ponnamma, D., Hassan, M. K., Ammar, A., Adham, S., Al-Maadeed,
618 M. A. A., & Karim, A. (2020). Designing Flexible and Porous Fibrous
619 Membranes for Oil Water Separation—A Review of Recent Developments.
620 *Polymer Reviews, 60*(4), 671-716. doi:10.1080/15583724.2020.1714651
621 Fong, H., Chun, I., & Reneker, D. H. (1999). Beaded nanofibers formed during
622 electrospinning. *Polymer, 40*(16), 4585-4592.
623 doi:https://doi.org/10.1016/S0032-3861(99)00068-3
624 Ghasemi, M., Miri, M. A., Najafi, M. A., Tavakoli, M., & Hadadi, T. (2022).
625 Encapsulation of Cumin essential oil in zein electrospun fibers:
626 Characterization and antibacterial effect. *Journal of Food Measurement and*
627 *Characterization, 16*(2), 1613-1624. doi:10.1007/s11694-021-01268-z
628 Ghiasi, F., Golmakani, M.-T., Eskandari, M. H., & Hosseini, S. M. H. (2020). A new
629 approach in the hydrophobic modification of polysaccharide-based edible films
630 using structured oil nanoparticles. *Industrial Crops and Products, 154*, 112679.
631 doi:https://doi.org/10.1016/j.indcrop.2020.112679
632 Han, H., Zhang, H., Li, E., Li, C., & Wu, P. (2019). Structural and functional properties
633 of OSA-starches made with wide-ranging hydrolysis approaches. *Food*
634 *Hydrocolloids, 90*, 132-145. doi:https://doi.org/10.1016/j.foodhyd.2018.12.011
635 Härdelin, L., Perzon, E., Hagström, B., Walkenström, P., & Gatenholm, P. (2013).

636 Influence of molecular weight and rheological behavior on electrospinning
637 cellulose nanofibers from ionic liquids. *Journal of Applied Polymer Science,*
638 *130*(4), 2303-2310. doi:https://doi.org/10.1002/app.39449
639 Jha, P. (2021). Functional properties of starch-chitosan blend bionanocomposite films
640 for food packaging: the influence of amylose-amylopectin ratios. *Journal of*
641 *Food Science and Technology, 58*(9), 3368-3378. doi:10.1007/s13197-020-
642 04908-2
643 Kong, L., & Ziegler, G. R. (2012). Role of Molecular Entanglements in Starch Fiber
644 Formation by Electrospinning. *Biomacromolecules, 13*(8), 2247-2253.
645 doi:10.1021/bm300396j
646 Kong, L., & Ziegler, G. R. (2014). Fabrication of pure starch fibers by electrospinning.
647 *Food Hydrocolloids, 36*, 20-25.
648 doi:https://doi.org/10.1016/j.foodhyd.2013.08.021
649 Kutzli, I., Gibis, M., Baier, S. K., & Weiss, J. (2018). Fabrication and characterization
650 of food-grade fibers from mixtures of maltodextrin and whey protein isolate
651 using needleless electrospinning. *Journal of Applied Polymer Science, 135*(22),
652 46328. doi:https://doi.org/10.1002/app.46328
653 Kutzli, I., Gibis, M., Baier, S. K., & Weiss, J. (2019). Electrospinning of whey and soy
654 protein mixed with maltodextrin – Influence of protein type and ratio on the
655 production and morphology of fibers. *Food Hydrocolloids, 93*, 206-214.
656 doi:https://doi.org/10.1016/j.foodhyd.2019.02.028
657 Lancuški, A., Vasilyev, G., Putaux, J.-L., & Zussman, E. (2015). Rheological Properties
658 and Electrospinnability of High-Amylose Starch in Formic Acid.
659 *Biomacromolecules, 16*(8), 2529-2536. doi:10.1021/acs.biomac.5b00817
660 Li, S., Kong, L., & Ziegler, G. R. (2021). Electrospinning of Octenylsuccinylated
661 Starch-Pullulan Nanofibers from Aqueous Dispersions. *Carbohydrate Polymers,*
662 *258*, 116933. doi:https://doi.org/10.1016/j.carbpol.2020.116933
663 Li, S., Wang, C., Fu, X., Li, C., He, X., Zhang, B., & Huang, Q. (2018). Encapsulation
664 of lutein into swelled cornstarch granules: Structure, stability and in vitro
665 digestion. *Food Chemistry, 268*, 362-368.

doi:https://doi.org/10.1016/j.foodchem.2018.06.078

Li, Z., Weng, W., Ren, Z., Zhang, Y., Li, S., & Shi, L. (2022). Electrospun octenylsuccinylated starch-pullulan nanofiber mats: Adsorption for the odor of oyster peptides and structural characterization. *Food Hydrocolloids, 133*, 107992. doi:https://doi.org/10.1016/j.foodhyd.2022.107992

Liang, Q., & Gao, Q. (2023). Effect of amylose content on the preparation for carboxymethyl starch/pullulan electrospun nanofibers and their properties as encapsulants of thymol. *Food Hydrocolloids, 136*, 108250. doi:https://doi.org/10.1016/j.foodhyd.2022.108250

Liang, Q., Pan, W., & Gao, Q. (2021). Preparation of carboxymethyl starch/polyvinyl-alcohol electrospun composite nanofibers from a green approach. *International Journal of Biological Macromolecules, 190*, 601-606. doi:https://doi.org/10.1016/j.ijbiomac.2021.09.015

Lim, L.-T., Mendes, A. C., & Chronakis, I. S. (2019). Chapter Five - Electrospinning and electrospraying technologies for food applications. In L.-T. Lim & M. Rogers (Eds.), *Advances in Food and Nutrition Research* (Vol. 88, pp. 167-234): Academic Press.

Lin, L., Dai, Y., & Cui, H. (2017). Antibacterial poly(ethylene oxide) electrospun nanofibers containing cinnamon essential oil/beta-cyclodextrin proteoliposomes. *Carbohydrate Polymers, 178*, 131-140. doi:https://doi.org/10.1016/j.carbpol.2017.09.043

Liu, J., Song, F., Chen, R., Deng, G., Chao, Y., Yang, Z., . . . Hu, Y. (2022). Effect of cellulose nanocrystal-stabilized cinnamon essential oil Pickering emulsions on structure and properties of chitosan composite films. *Carbohydrate Polymers, 275*. doi:10.1016/j.carbpol.2021.118704

Liu, X.-x., Wang, Y.-f., Zhang, N.-z., Shanks, R. A., Liu, H.-s., Tong, Z., . . . Yu, L. (2013). Morphology and phase composition of gelatin-starch blends. *Chinese Journal of Polymer Science, 32*(1), 108-114. doi:10.1007/s10118-014-1377-1

Liu, X., Ding, S., Wu, J., Liu, G., Wei, J., Yang, F., & Liu, X. (2021). Molecular structures of octenyl succinic anhydride modified starches in relation to their

ability to stabilize high internal phase emulsions and oleogels. *Food Hydrocolloids, 120*, 106953. doi:https://doi.org/10.1016/j.foodhyd.2021.106953

Liu, X., Gao, C., Sangwan, P., Yu, L., & Tong, Z. (2014). Accelerating the degradation of polyolefins through additives and blending. *Journal of Applied Polymer Science, 131*(18). doi:10.1002/app.40750

Maliszewska, I., & Czapka, T. J. P. (2022). Electrospun polymer nanofibers with antimicrobial activity. *14*(9), 1661.

Mitchell, G. R. (2015). *Electrospinning: principles, practice and possibilities*: Royal Society of Chemistry.

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals (Basel), 6*(12), 1451-1474. doi:10.3390/ph6121451

Nikaido, H. (1994). Prevention of Drug Access to Bacterial Targets: Permeability Barriers and Active Efflux. *264*(5157), 382-388. doi:doi:10.1126/science.8153625

Niu, A., Wu, H., Ma, F., Tan, S., Wang, G., & Qiu, W. (2022). The antifungal activity of cinnamaldehyde in vapor phase against Aspergillus niger isolated from spoiled paddy. Lwt, 159, 113181.

Poudel, D., Swilley-Sanchez, S., O'keefe, S., Matson, J., Long, T., & Fernández-Fraguas, C. (2020). Novel Electrospun Pullulan Fibers Incorporating Hydroxypropyl-β-Cyclodextrin: Morphology and Relation with Rheological Properties. *12*(11), 2558.

Reneker, D. H., & Chun, I. J. N. (1996). Nanometre diameter fibres of polymer, produced by electrospinning. *7*(3), 216.

Reyes-Jurado, F., Navarro-Cruz, A. R., Ochoa-Velasco, C. E., Palou, E., López-Malo, A., & Ávila-Sosa, R. (2020). Essential oils in vapor phase as alternative antimicrobials: A review. Critical reviews in food science and nutrition, 60(10), 1641-1650.

Ribeiro-Santos, R., Andrade, M., de Melo, N. R., dos Santos, F. R., Neves, I. d. A., de

Carvalho, M. G., & Sanches-Silva, A. (2017). Biological activities and major components determination in essential oils intended for a biodegradable food packaging. *Industrial Crops and Products, 97*, 201-210. doi:https://doi.org/10.1016/j.indcrop.2016.12.006

Stijnman, A. C., Bodnar, I., & Hans Tromp, R. (2011). Electrospinning of food-grade polysaccharides. *Food Hydrocolloids, 25*(5), 1393-1398. doi:https://doi.org/10.1016/j.foodhyd.2011.01.005

Sung, S.-Y., Sin, L. T., Tee, T.-T., Bee, S.-T., Rahmat, A. R., Rahman, W. A. W. A., . . . Vikhraman, M. (2013). Antimicrobial agents for food packaging applications. *Trends in Food Science & Technology, 33*(2), 110-123. doi:https://doi.org/10.1016/j.tifs.2013.08.001

Sweedman, M. C., Schäfer, C., & Gilbert, R. G. (2014). Aggregate and emulsion properties of enzymatically-modified octenylsuccinylated waxy starches. *Carbohydrate Polymers, 111*, 918-927. doi:https://doi.org/10.1016/j.carbpol.2014.04.088

Sweedman, M. C., Tizzotti, M. J., Schäfer, C., & Gilbert, R. G. (2013). Structure and physicochemical properties of octenyl succinic anhydride modified starches: A review. *Carbohydrate Polymers, 92*(1), 905-920. doi:https://doi.org/10.1016/j.carbpol.2012.09.040

Tampau, A., González-Martínez, C., & Chiralt, A. (2018). Release kinetics and antimicrobial properties of carvacrol encapsulated in electrospun poly-(ε-caprolactone) nanofibres. Application in starch multilayer films. *Food Hydrocolloids, 79*, 158-169. doi:https://doi.org/10.1016/j.foodhyd.2017.12.021

Tian, Y., Qu, J., Zhou, Q., Ding, L., Cui, Y., Blennow, A., . . . Liu, X. (2022). High pressure/temperature pasting and gelling of starch related to multilevel structure-analyzed with RVA 4800. *Carbohydrate Polymers, 295*, 119858. doi:https://doi.org/10.1016/j.carbpol.2022.119858

Tizzotti, M. J., Sweedman, M. C., Schäfer, C., & Gilbert, R. G. (2013). The influence of macromolecular architecture on the critical aggregation concentration of large amphiphilic starch derivatives. *Food Hydrocolloids, 31*(2), 365-374.

doi:https://doi.org/10.1016/j.foodhyd.2012.11.023

Trombetta, D., Castelli, F., Sarpietro, M. G., Venuti, V., Cristani, M., Daniele, C., . . . Bisignano, G. (2005). Mechanisms of antibacterial action of three monoterpenes. *Antimicrob Agents Chemother, 49*(6), 2474-2478. doi:10.1128/AAC.49.6.2474-2478.2005

Van de Vel, E., Sampers, I., & Raes, K. (2017). A review on influencing factors on the minimum inhibitory concentration of essential oils. *Critical Reviews in Food Science and Nutrition, 59*(3), 357-378. doi:10.1080/10408398.2017.1371112

Vasconcelos, N. G., Croda, J., & Simionatto, S. (2018). Antibacterial mechanisms of cinnamon and its constituents: A review. *Microbial Pathogenesis, 120*, 198-203. doi:https://doi.org/10.1016/j.micpath.2018.04.036

Vasilyev, G., Vilensky, R., & Zussman, E. (2019). The ternary system amylose-amylopectin-formic acid as precursor for electrospun fibers with tunable mechanical properties. *Carbohydrate Polymers, 214*, 186-194. doi:https://doi.org/10.1016/j.carbpol.2019.03.047

Venkitanarayanan, M. A. R. A. K. (2011). Effect of trans-Cinnamaldehyde on Inhibition and Inactivation of Cronobacter Sakazakii Biofilm on Abiotic Surfaces. *Journal of Food Protection, 74*(2), 200-208. doi:https://doi.org/10.4315/0362-028X.JFP-10-296

Viswanathan, G., Murugesan, S., Pushparaj, V., Nalamasu, O., Ajayan, P. M., & Linhardt, R. J. (2006). Preparation of Biopolymer Fibers by Electrospinning from Room Temperature Ionic Liquids. *Biomacromolecules, 7*(2), 415-418. doi:10.1021/bm050837s

Wang, C., Fu, X., Tang, C.-H., Huang, Q., & Zhang, B. (2017). Octenylsuccinate starch spherulites as a stabilizer for Pickering emulsions. *Food Chemistry, 227*, 298-304. doi:https://doi.org/10.1016/j.foodchem.2017.01.092

Wang, C., He, X., Huang, Q., Fu, X., Luo, F., & Li, L. (2013). Distribution of Octenylsuccinic Substituents in Modified A and B Polymorph Starch Granules. *Journal of Agricultural and Food Chemistry, 61*(51), 12492-12498. doi:10.1021/jf404162c

786 Wang, H., & Ziegler, G. R. (2019). Electrospun nanofiber mats from aqueous starch-
787 pullulan dispersions: Optimizing dispersion properties for electrospinning.
788 *International Journal of Biological Macromolecules, 133*, 1168-1174.
789 doi:https://doi.org/10.1016/j.ijbiomac.2019.04.199
790 Wang, J., Ren, F., Yu, J., Copeland, L., & Wang, S. (2021). Octenyl Succinate
791 Modification of Starch Enhances the Formation of Starch–Lipid Complexes.
792 *Journal of Agricultural and Food Chemistry, 69*(49), 14938-14950.
793 doi:10.1021/acs.jafc.1c05816
794 Wang, J., Zhao, F., Huang, J., Li, Q., Yang, Q., & Ju, J. (2023). Application of essential
795 oils as slow-release antimicrobial agents in food preservation: Preparation
796 strategies, release mechanisms and application cases. *Critical Reviews in Food*
797 *Science and Nutrition*, 1-26. doi:10.1080/10408398.2023.2167066
798 Wei, F., Ma, N., Haseeb, H. A., Gao, M., Liu, X., & Guo, W. (2022). Insights into
799 structural and physicochemical properties of maize starch after Fusarium
800 verticillioides infection. *Journal of Food Composition and Analysis, 114*,
801 104819. doi:https://doi.org/10.1016/j.jfca.2022.104819
802 Wen, P., Zhu, D.-H., Wu, H., Zong, M.-H., Jing, Y.-R., & Han, S.-Y. (2016).
803 Encapsulation of cinnamon essential oil in electrospun nanofibrous film for
804 active food packaging. *Food Control, 59*, 366-376.
805 doi:https://doi.org/10.1016/j.foodcont.2015.06.005
806 Wendakoon, C. N., & Sakaguchi, M. J. J. o. f. p. (1995). Inhibition of amino acid
807 decarboxylase activity of Enterobacter aerogenes by active components in
808 spices. *58*(3), 280-283.
809 Yao, J., Bastiaansen, C. W. M., & Peijs, T. (2014). High Strength and High Modulus
810 Electrospun Nanofibers. *2*(2), 158-186.
811 Yildirim-Yalcin, M., Tornuk, F., & Toker, O. S. (2022). Recent advances in the
812 improvement of carboxymethyl cellulose-based edible films. *Trends in Food*
813 *Science & Technology, 129*, 179-193.
814 doi:https://doi.org/10.1016/j.tifs.2022.09.022
815 Yu, J. H., Fridrikh, S. V., & Rutledge, G. C. (2006). The role of elasticity in the

formation of electrospun fibers. *Polymer, 47*(13), 4789-4797. doi:https://doi.org/10.1016/j.polymer.2006.04.050

Zeng, K., Zhou, J., Cui, Z., Zhou, Y., Shi, C., Wang, X., . . . Drioli, E. (2018). Insight into fouling behavior of poly(vinylidene fluoride) (PVDF) hollow fiber membranes caused by dextran with different pore size distributions. *Chinese Journal of Chemical Engineering, 26*(2), 268-277. doi:https://doi.org/10.1016/j.cjche.2017.04.008

Zhang, C., Wang, P., Li, J., Zhang, H., & Weiss, J. (2021). Characterization of core-shell nanofibers electrospun from bilayer gelatin/gum Arabic O/W emulsions crosslinked by genipin. *Food Hydrocolloids, 119*. doi:10.1016/j.foodhyd.2021.106854

Zhang, N., Liu, X., Yu, L., Shanks, R., Petinaks, E., & Liu, H. (2013). Phase composition and interface of starch-gelatin blends studied by synchrotron FTIR micro-spectroscopy. *Carbohydr Polym, 95*(2), 649-653. doi:10.1016/j.carbpol.2013.03.045

Zhao, S., Tian, G., Zhao, C., Lu, C., Bao, Y., Liu, X., & Zheng, J. (2018). Emulsifying stability properties of octenyl succinic anhydride (OSA) modified waxy starches with different molecular structures. *Food Hydrocolloids, 85*, 248-256. doi:https://doi.org/10.1016/j.foodhyd.2018.07.029

Zhong, Y., Liu, L., Qu, J., Blennow, A., Hansen, A. R., Wu, Y., . . . Liu, X. (2020). Amylose content and specific fine structures affect lamellar structure and digestibility of maize starches. *Food Hydrocolloids, 108*, 105994. doi:https://doi.org/10.1016/j.foodhyd.2020.105994

Zhong, Y., Qu, J., Li, Z., Tian, Y., Zhu, F., Blennow, A., & Liu, X. (2022). Rice starch multi-level structure and functional relationships. *Carbohydrate Polymers, 275*, 118777. doi:https://doi.org/10.1016/j.carbpol.2021.118777

Zhong, Y., Tai, L., Blennow, A., Ding, L., Herburger, K., Qu, J., . . . Liu, X. (2022). High-amylose starch: Structure, functionality and applications. *Critical Reviews in Food Science and Nutrition*, 1-23.

Zhou, C., Abdel-Samie, M. A., Li, C., Cui, H., & Lin, L. (2020). Active packaging based

on swim bladder gelatin/galangal root oil nanofibers: Preparation, properties and antibacterial application. *Food Packaging and Shelf Life, 26*, 100586. doi:https://doi.org/10.1016/j.fpsl.2020.100586

Table and Figures

1. Materials and Methods

2.2 Molecular characterization of OSS

2.2.1 SEC

SEM was employed to determine the hydrodynamic radius of OSS molecules using an Agilent 1260 series instrument (Agilent Technologies) equipped with a refractive index detector (Optilab T-rEX, WYATT Corp., USA). Size separation was achieved using GRAM PreColumn, GRAM 30, and GRAM 3000 analytical columns (Polymer Standard Services, Mainz, Germany) with a flow rate of 0.3 mL/min. All samples were fully dissolved in DMSO with 0.5% LiBr (w/w), thus providing the optimal conditions for separation. A series of pullulan standards (Polymer Standard Services, Mainz, Germany) with varying molecular sizes were used for the calibration to obtain the relation between the SEC elution volume and the hydrodynamic volume V_h (the separation parameter for SEC). The SEC results are presented as $w(\log V_h)$ plotted against the corresponding hydrodynamic radius R_h, following the equation $V_h = (4/3)\mathrm{Pi}R_h^3$.

2.2.2 NMR

^{1}H NMR spectra were obtained using a Bruker 600 MHz (Bruker, Fallanden, Switzerland). All spectra were manually phased and baseline-corrected. The degree of substitution (DS) for OSS was determined by quantifying the fraction of OS groups through analysis of the proton signal of methyl protons within the OS group (0.85 ppm).

$$DS = \frac{I_{0.85}}{3\left(I_{\alpha-1,6} + I_{\alpha-1,4} + I_{r-e}\right)}$$

Here $I_{r\text{-}e}$ corresponds to the ^{1}H NMR integral of the reducing chain ends (α and β reducing-end signals at 4.91 and 4.28 ppm, respectively), $I_{\alpha\text{-}1,4}$ and $I_{\alpha\text{-}1,6}$ are the ^{1}H NMR integrals of internal (1→4)-α peaks at approximately 5.11 ppm and (1→6)-α linkages around 4.75 ppm, respectively.

Table S1 Molecular size of OSS and PUL.

Samples	**OSS1**		**OSS2**		**OSS3**	**OSS4**	**OSS5**	**OSS6**	**PUL**
R_h (×nm)	11.50 [a]	47.09 [b]	10.00 [a]	38.81 [b]	49.71	3.72	3.74	11.94	n.a.
M_w (×g/mol)	1.17×10^6		8.03×10^5		1.40×10^6	5.09×10^4	4.48×10^4	2.00×10^5	1.35×10^5
DS ($\times10^{-2}$)	0.48		2.64		1.47	3.19	2.96	4.29	n.a.

[a] AM, amylose content; [b] AP, amylopectin content; OSS: OSA starch; PUL: pullulan; n.a.: not available.

Table S2 Apparent viscosity, conductivity and surface tension of OSS and PUL aqueous dopes.

Samples	Apparent Viscosity at 100 s^{-1} (Pa·s)	Conductivity (uS/cm)	Surface tension (mN/m)
20%OSS1/10%PUL	3.00±0.29 [a]	660.60±1.84 [f]	33.99±0.71 [e]
20%OSS2/10%PUL	1.12±0.05 [b]	761.57±1.76 [e]	30.82±0.59 [f]
20%OSS3/10%PUL	1.30±0.07 [b]	824.83±4.70 [d]	45.36±0.14 [b]
20%OSS4/10%PUL	0.10±0.00 [c]	1077.33±1.53 [c]	38.32±0.36 [c]
20%OSS5/10%PUL	0.14±0.00 [c]	884.20±1.08 [b]	37.05±0.78 [d]
20%OSS6/10%PUL	0.29±0.00 [c]	1168.00±2.00 [a]	50.94±0.66 [a]

Note: Values with a different superscript letter in the same column indicate significantly different ($P < 0.05$).

Table S3 Average fiber diameter and average porosity of OSS/PUL nanofibers

Samples	**OSS4/PUL**	**OSS5/PUL**	**OSS6/PUL**
Average fiber diameter (nm)	211±23 [a]	244±32 [a]	230±26 [a]
Average porosity (%)	55.17±1.84 [a]	54.00±0.91 [a]	53.69±1.65 [a]

Note: Values with a different superscript letter in the same row indicate significantly different ($P < 0.05$).