**Zein and tannic acid hybrid particles improving physical stability, controlled release properties, and antimicrobial activity of cinnamon essential oil loaded Pickering emulsions**

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**Abstract**

Pickering emulsion loading essential oil has demonstrated a promising strategy as delivery system in food preservation, but localization in stability and antimicrobial activity limits application. In this study, Pickering emulsions co-loaded with tannic acid and cinnamon essential oil (ZTC) have been developed based on zein and tannic acid complexes (ZT) mediated interfacial engineering. Fourier transform infrared, fluorescence spectroscopy, and molecular docking results indicated tannic acid altered the structural of zein. Interfacial tension results indicated that tannic acid accelerated the adsorbed speed of zein particles by decreased interfacial tension (11.99-9.96 mN/m). ZT5 formed a viscoelastic and dense layer in oil-water interface than that for other ZTs, which improved stability and control release performance of ZTC. Furthermore, the ZTC showed an effective antimicrobial activity against spoilage organisms *Pseudomonad paralactis* MN10 and *Lactobacillus sakei* VMR17. These findings provide new insight for developing co-loaded multiple antimicrobial agents within Pickering emulsion as a delivery system.

**Keywords**: Function Pickering emulsion; Tannic acid; Zein; Interfacial engineering; Antimicrobial activity

**1. Introduction**

Microbiology in food preservation has always been a concerned topic, emerging tremendous research attention. Natural antimicrobial substances, typically represented by essential oils (EOs), are well-known for their natural source and environmental compatibility (da Silva et al., 2022; Vilela et al., 2018). Many studies found that plant-derived EOs are effective in defending a wide range of bacteria and fungi (da Silva et al., 2022). For example, cinnamon essential oil (CEO), extracted from cinnamon branches, leaves and bark, has been shown to possess strong antimicrobial activity on spoilage organisms (Fan et al., 2023; Shao et al., 2021; Ran et al., 2023), which could be used to prolong the shelf life and maintain quality of fresh agricultural products. However, the volatility, instability, and environmental sensitivity to light, high temperature, and oxygen of EOs lead to decreased antimicrobial function, which limits their application in food preservation. Thus, it is necessary to develop a sustainable technique to overcome the limitations of instabilities and improve the antimicrobial performance of EOs (Ma et al., 2022; Zhang et al.,2019).

Pickering emulsion is one of the most important delivery systems for loading and encapsulating EOs, have been extensively used in food, pharmaceutical, and cosmetic applications (Mwangi et al., 2020). Compared to conventional emulsions, Pickering emulsion is stabilized by solid particles instead of synthetic surfactants, which emphasizes environmental compatibility of the delivery systems. In addition, the colloidal particles anchor at oil-water interface of Pickering emulsions droplet, forming a barrier to block the coalescence of droplets and Ostwald maturation phenomenon. Moreover, the stability and encapsulation effect of Pickering emulsion could be optimized elaborately by designing the behavior of particles at the oil-water interface (Liu et al., 2023).

Recently, interfacial engineering has been developed to enhance the stability of Pickering emulsion systems (Reitzer et al., 2018). Zein, the main storage protein in corn, contains a high percentage of hydrophobic amino acid residues, with a unique self-assembly character. Zein has been studied as a stabilizer for Pickering emulsion systems attributed to its emulsifying properties and ability to self-organize at the oil-water interface (Souza, Ferreira, & Soares, 2022). However, the single zein particles failed to effectively stabilize the dispersed systems due to the adequate interfacial properties. Some modification strategies have achieved in improving interfacial properties and led to an enhancement in emulsion stabilization, as establish molecular interactions between zein and polyphenols for example (Ge et al., 2022; Xu, Wei, & Xue, 2023; Wang et al., 2022a; Zhu et al., 2021). Particularly emphasis be given to the function of polyphenols at oil-water interface. Tannic acid (TA) is a kind of plant-derived polyphenol with multiple hydroxyl groups (around 25 hydroxyl groups) in its structure, providing abundant interaction sites with biomacromolecules (such as protein, polysaccharides and mixtures). TA can form complexes with proteins through its hydroxyl groups and phenolic rings, which improves the interfacial functionalities of proteins. For example, Zhu et al. (2021) found that the addition of TA improved the emulsifying properties of gliadin. TA and soy isolate protein complex as Pickering emulsion stabilizer was able to inhibit the accumulation of oil droplets and improved the physical stability of the emulsions (Li et al., 2023). Both noncovalent and covalent interactions between zein and TA are known to affect the interfacial behavior of complexs. Moreover, TA has been recognized for its antimicrobial and antioxidant bioactivity (Zhang et al., 2023). Therefore, it can be hypothesized above that TA might improve not only the stability property of zein-based Pickering emulsion but also the antimicrobial effects of EOs loaded Pickering emulsions.

In this work, we constructed Pickering emulsions co-loaded with TA and CEO (ZTCs) based on the zein-TA (ZT) complexes mediated interfacial engineering. The impact of structural features and interfacial properties generated by interactions between zein and TA were investigated by Fourier transform infrared, fluorescence spectroscopy, molecular docking analysis, wettability, and interfacial tension. Furthermore, the stability, rheological, control releases properties of prepared emulsions was investigated. Finally, the antimicrobial effect was evaluated against food spoilage organisms. The findings in our work will provide novel insight into developing Pickering emulsions loaded with binary bioactive compounds and for their subsequent application in food preservation.

**2. Materials and methods**

*2.1. Materials and reagents*

Zein (CAS 9010-66-6) was purchased from Sigma-Aldrich (St. Louis, MO, USA). TA (CAS 5424-20-4, purity ≥96%) was obtained from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). CEO was sourced from Jiangxi Taicheng Natural Perfume Co., Ltd. (Ji’an, China). 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and bromophenol blue (BPB) were sourced from Sigma-Aldrich (St. Louis, MO, USA). All reagents and chemicals in this work were analytical grade.The strain *Pseudomonad paralactis* MN10 (*P. paralactis* MN10) and *Lactobacillus sakei* VMR17 (*L. sakei* VMR17) were provided by the Meat Laboratory of the Institute of Food Science and Technology at the Chinese Academy of Agricultural Sciences (Beijing, China).

*2.2. Preparation of the ZT complexes*

ZT complexes were prepared using the procedure of Wang et al. (2022a) with slight modifications. Briefly, 1 g of zein powder and 0.2 g of TA were dissolved in 100 mL of an ethanol water solution (70%, v/v), then the solutions were adjusted to pH 5, pH 7, and pH 9 with 1 M HCl and 1 M NaOH forming ZT complexes dispersions. The dispersions were noted as ZT5, ZT7, and ZT9, respectively. Before further characterization, the dispersions were storage at 4℃ and part of them were freeze-dried to obtain ZT complex powders.

*2.3. Characterization of the ZT complexes*

2.3.1. Average size, zeta-potential and polydispersity index (PDI)

The average size, zeta-potential and PDI of ZT complexes were measured using a Zetasizer Nano ZS (Malvern Instrument Inc., Malvern, UK). Before measurements, each ZT complex dispersion solution was diluted 100-fold with ultrapure water.

2.3.2. Scanning electron microscope (SEM)

The morphology of ZT complex were observed using a SEM (SU 1510, HITACHI, Japan) at 10.0 kV. Briefly, a droplet of the ZT complex dispersions (about 10.0 μL) were directly dropped on a silicon slide (5.0 mm × 5.0 mm), then mounted on a copper tape and coated with gold for microscopic observation at 50,000-magnification.

2.3.3. Fluorescence spectroscopy

The fluorescence spectra of the ZT complexes were performed on a F-2500 spectrofluorometer (Hitachi, Japan). Each ZT complex dispersion was diluted with ultrapure water before measurements. The excitation wavelength, emission wavelength range, and excitation slit widths were 280 nm, 290-400 nm, and 2.5 nm, respectively.

2.3.4. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectroscopy of the ZT complexes were detected using an FTIR spectrometer (Tensor 27, Bruker, Germany). The ZT complex powders were mixed with KBr and pressed steadily, then the FTIR spectra were acquired three times and averaged. Peakfit 4.12 software (SPSS Inc., Chicago, IL, USA) was used to calculate the secondary structure of the samples using the amide I band (1660 - 1700 cm-1).

2.3.5. Surface hydrophobicity and free sulfhydryl content

The surface hydrophobicity was determined using a BPB method according to the procedure of Wang et al., (2022a) with some modifications. To be specific, 10.0 mg of ZT complex powder was dissolved in 1.0 mL of phosphate buffer saline (PBS; 50 mM, pH 7) and added to 200 μL BPB (1 mg/mL), then the mixtures were centrifuged at 4,000 g for 15 min. The absorbance of the supernatant was determined using a microplate reader at 595 nm (Spark, Tecan Ltd, Switzerland).

The free sulfhydryl content was measured using the methods of Cheng et al. (2023) with some adjustments. 0.5 mL of ZT complex dispersion solution, 50 μL of DTNB (4.0 mg/mL), and 5 mL of Tris-glycine buffer solution (0.09 mol/L Tris, 0.09 mol/L glycine, 4 mmol/L ethylene diamine tetraacetic acid, 8 mol/L urea at pH 8.0) were mixed and incubated in the dark of 60 min. Then, the absorbance of the mixture was detected at 412 nm using a microplate reader. The free sulfhydryl content was calculated using Equation (1)

-SH(μmol/g)= (1)

where A is the absorbance at 412 nm, D is the dilution factor, and C is the sample concentration in mg/mL.

2.3.6 Molecular docking

AutoDock 4.0 and AutoDock vina 1.1.2 (The Scripps Research Institute, La Jolla, CA, USA) were used to provide binding information between zein and TA using the procedure described by Wang et al., (2022a). The structure of zein was obtained by homologous modeling using Alphafold 2. The 3D structures of TA were download from Pubchem (<https://pubchem.ncbi.nlm.nih.gov/>). PyMol 2.5 (<http://www.pymol.org/>) was used to analyze the interactions between zein and TA.

*2.4. Evaluation of ZT complexes as potential Pickering emulsion stabilizer*

2.4.1. Wettability

The wettability of ZT complexes was measured using a contact angle meter (OCA40, DataPhysics Instruments Ltd., Germany) according to Liu et al., (2022). The ZT complex powders were compressed into tablets of 1 mm thickness and 10 mm diameter. 5 μL of distilled water was dropped on the pellet by a high precision injector. The profiles of the drop were recorded by a high-speed camera 3 times.

2.4.2. Interfacial tension

The interfacial tension of ZT complexes was determined using a surface tension analyzer (Sigma 701, Biolin Scientific, Sweden) at 25 ℃ (Liu et al., 2023). The calibration factor of corn oil was determined before taking the measurements. Briefly, 30 mL of the ZT complex solutions and 30 mL of corn oil were added into a standard vessel (70 mm), standing for 30 min. The dynamic interfacial tension of the two liquids was record.

2.4.3. Interfacial dilational rheology

The interfacial dilational rheology of ZT complexes were probed at the oil/water interface using a OCA50 optical contact angle meter (Dataphysics, Germany) (Dai et al., 2023). The ZT complex solution was placed in a syringe, then the liquid drop oscillated in corn oil at an 0.1Hz and amplitude of 10%. Three sinusoidal oscillation cycles were preformed by a time corresponding to 30 cycles. The interfacial dilatational modulus (E), dilatational elasticity modulus (Ed), and dilatational viscosity modulus (Ev) were caculated from the oscillation cycle by SCA20 software.

*2.5. Preparation of ZTCs*

The ZTCs were prepared using the method of Cheng, et al., (2020) and Xie, et al., (2022) with some modifications. Briefly, the ZT complex dispersions were mixed with 10% (v/v) CEO and sheared by a high-speed machine (HR-500, Shanghai Huxi Industry Co., Ltd, China) at 8,000 rpm for 2 min at room temperature. Pickering emusions were obtained by adding ultrapure water into oil-dispersion mixture at a ratio of 50:50 (v/v). The final concentrations of zein, TA, and CEO were 0.05% (w/v), 0.01%(w/v), and 5%, respectively. The ZTCs stabilized by ZT5, ZT7, and ZT9 were recorded as ZTC5, ZTC7, and ZTC9, respectively.

*2.6. Characterization of ZTCs*

2.6.1. Droplet size and zeta-potential

The droplet size and zeta-potential of the ZTCs were measured using a Zetasizer Nano ZS (Malvern Instruments Inc., Malven, UK). Before measurements, the samples were diluted 100-fold with ultrapure water.

2.6.2. Transmission electron microscopy (TEM)

The microstructures of ZTCs were observed by TEM (H-7500, Hitachi, Japan). Before observation, 10 μL of emulsion were pipetted onto a carbon-coated copper grid and staind with 15 g/L phosphotungstic acid for 30 s.

2.6.3. Confocal laser scanning microscopy (CLSM)

The microstructures of the ZTCs were observed using CLSM (Leica TCS SP8, Leica microsystems Inc., Wetzlar, Germany) according to our previous method (Fan et al., 2023). Nile red (0.1 wt%) and Nile blue (0.1 wt%) were used in the pre-stained oil phase and aqueous phase in the dark, respectively. The excitations wavelengths of Nile red and Nile blue were 488 nm and 633 nm, respectively, and the images of the emulsion were recorded by CLSM.

2.6.4. Stability evaluation

The stability of the ZTCs was evaluated by Turbiscan TOWER Stability Analyzer (Formulaction Inc., France), and the results were presented as profiles of the Turbiscan Stability Index (TSI). 20 mL of prepared emulsion was placed in a glass test tube and scanned with pulsed near-infrared light (λ = 880 nm) every 1 h, continuously for 24 h.

2.6.5. Rheological properties

The rheological properties of fresh Pickering emulsions were measured by rheometer (MCR502, Anton Paar, Germany) with a 50 mm diameter parallel plate and a gap of 1 mm at 25 °C. Before measurements, the linear viscoelastic region (LVR) of the emulsion was investigated by a strain sweep test ranging from 0.01% to 100% with 10 rad/s of frequency. Then, the viscosity was measured as the shear rate increased from 0.1 to 100 s-1. For the frequency sweep mode, the storage modulus (G′) and loss modulus (G′′) were obtained with the strain of 0.1%.

2.6.6 Encapsulation efficiency (EE)

The EE (%) of the CEO in the Pickeirng emulsion was measured spectrophotometrically and calculated using following equation (2) according to Liu et al. (2024) with a slight modifaction. Briefly, the eumslion (2 mL) was added an equal volume of N-hexane. After centrifugation at 5,000 rpm for 10 min, the concentration of CEO in the supernatant was measured using CEO calibration curve and the EE (%) of CEO in the Pickeirng emulsion was calculated according to the following equation:

EE (%)= (2)

The measurement was repeated three times for each sample group.

2.6.7 Release behavior

The release behavior of CEO from Pickering emulsion was carried out through a dynamic test method. 2 mL eumslion was added an equal volume of N-hexane and set aside at room temperature. At each specific time, 200 uL of the N-hexane phase was removed to measure the absorbance of CEO. The cumulative release rate was calculated as following equation (3)

Cumulative release rate (%)= (3)

*2.7. Antimicrobial assays of* *the ZTCs*

2.7.1. Antimicrobial properties

The plate counting method was used to test the antimicrobial effect of the ZTCs (Meng et al., 2023), and the viability of bacteria was used to evaluate the antimicrobial properties. Briefly, 100 μL of emulsion were added to 10 mL of liquid medium, then 100 μL of tested bacteria (approximately 106 CFU/mL) were added to the liquid medium, and the liquid medium containing the emulsion and bacterial was incubated at 30℃ for 12 h. Thereafter, 100 μL of bacteria suspension was spread on the surface of the agar plate uniformly, and incubation at 30°C for 24 h. A control group consisting of a bacterial suspension with equal volume normal saline solution was also included in the test. The viability of surviving cells was counted.

2.7.2. Minimum inhibitory concentration (MIC)

The MIC of ZTCs were conducted against *P. paralactis* MN10 and *L. sakei* VMR17 using the reported echelon dilution method with some modifications(Zhou, et al., 2018). Briefly, 600 μL of emulsion were added to 6 mL of liquid medium as the first broth tube, then 2-fold dilutions were used to prepared a series of liquid medium tubes; the final concentrations of emulsion of a series of broth tube were 100, 50, 25, 12.5, 6.25, and 3.13 μL/mL, respectively. Then 100 μL of tested bacterial suspension (approximately 106 CFU/mL) were added to each tube. The broth tubes were incubated at 30°C for 24 h. The MIC was defined as the concentration in the lowest serial dilution of emulsions that resulted in the lack of visible microorganism growth in the tubes (Hu, et al., 2023).

2.7.3. Antimicrobial mechanism

The mechanism of ZTCs against *P. paralactis* MN10 and *L. sakei* VMR17 was examined by observing microstructure of cell structure using a SEM (SU 1510, Hitachi, Japan). The logarithmic growth phase cells (approximately 106 CFU/mL) were treated with MIC of ZTCs, and were incubated for 4 h at 30℃. Then, the sediment bacteria was collected by centrifugation at 4 ℃, and fixed with 3.5 wt% glutaraldehyde. Before observation, a gradient dehydration process was applied to the fixed bacteria, using ethanol solutions of 30%, 50%, 70%, 90%, and 100%.

*2.8. Statistical analysis*

Result were expressed as mean ± standard deviation in triplicates for all experiments. Statistical analysis was conducted by SPSS 26.0 software (SPSS Inc., Chicago IL, USA), and Duncan's multiple tests at 95% confidence intervals were used to assess significance of differences.

**3. Results and discussion**

*3.1. TA altered the apparent of zein*

SEM provided information about the morphological feature of ZT complexes. According to Fig. 1A, ZT complexes are presented as spherical particles, which are promoted by the self-assembly process of zein (Wang et al., 2022a). Compared with ZT complex prepared in different pH conditions, the appearance of ZT9 was relatively rough, which could be attributed to different interactions of zein and TA at pH 9. Previous studies also reported that the zein-polyphenol complexes prepared in an alkaline environment appeared to have smooth surface (Liu et al., 2017). The self-assembly process of zein and the interaction force between zein and polyphenol mainly involved hydrogen bonding, hydrophobic and electrostatic forces. However, these interaction forms weakened in alkaline environment, which finally resulted in the obvious change in the ZT complex’s morphology.

The average size, zeta-potential and PDI of different ZT complexes were closely associated with their characteristics. As shown in Table S1, the hydrodynamic average size of ZT5, ZT7 and ZT9 were 293.53 nm, 192.30 nm and 149.77 nm, respectively. The small size of ZT complexes was beneficial to adsorb on the oil-water interface quickly. Notably, the particle size of ZT5 was larger than the others. This phenomenon could be attributed to pH 5 is close to isoelectric point of zein, which more easily to form large particles and decrease of charge repulsion when upon binding with TA. The smaller zeta-potential absolute value (-22.70 mV) provided the evidence. In addition, polyphenols could drive the protein chains folding or unfolding by regulating the interaction force, which could alter the particle size of the complex (Zhang et al., 2023). These observed results indicate that pH conditions modulated the appearance and particle size of ZT complexes.

*3.2. TA altered the conformation of zein*

3.2.1. FTIR spectroscopy

To analyze the interactions between zein and TA, the FTIR spectroscopy of ZT complexes was recorded (Fig. 1B). It was seen that the broad characteristic peak in the wavelength region of 3200-3400 cm-1 was shifted after the formation of ZT complexes compared to the zein, demonstrating that the interaction between zein and TA was highly related to hydrogen bonding coupled with N-H stretching. The characteristic peak at 2980 cm-1 was caused by a C-H stretching vibration. It can be seen a slight shift, indicating that there was hydrophobic interaction between the zein and TA. By contrastive analysis of three ZT complexes, it can be seen that ZT5, which was prepared in acidic pH conditions (pH 5), had strong hydrogen bonding and hydrophobic interactions between the zein and TA while ZT9 (prepared in alkaline pH conditions) was weaker. These differences are mainly attributed to the non-covalent or covalent interaction between zein and polyphenols (Liu et al., 2017). The analysis of FTIR in the amide I band region (1600-1700 cm-1) provided more information about the secondary structure change of zein and their TA complex (Wang et al., 2022a). As shown in Fig. 1C, the major secondary structures of zein were α-helix, which was also observed by Wang, et al. (2022b). Upon binding with TA, a slight increase in α-helix in ZT complexes were recorded. The α-helix was mainly correlated to inter-molecular hydrogen bonds formed between C=O and N-H. There are a large number of phenolic hydroxyl groups in TA, which provide more hydrogen bond site between zein and TA. thus affecting the α-helix content of zein. The similar result was observed by (Wang et al., 2022a) in zein-ferulic acid complexes. Wen et al. (2023) considered that these changes in secondary structure may contribute to enhanced the interfacial performance of proteins.

3.2.2. Fluorescence spectroscopy

To further investigate the interactions between zein and TA, fluorescence spectroscopy was used to probe the tertiary structure of the ZT complexes (Fig. 1D). Since zein lacks tryptophan (Typ), tyrosine (Tyr) and phenylalanine (Phe) residues were the intrinsic fluorophores in zein. The fluorescence intensity of zein (Z5, Z7, and Z9) increased with increasing pH, and the emission peak showed a slight red shift with decreasing pH, indicating the chromophores environmental polarity was changed by pH condition. Zein was dissolved in aqueous ethanol solution (70%, v/v) to form ZT complexes. In this higher ethanol content solution system, the complexes exposed more hydrophobic amino acids to adapt to the non-polar environment (Li et al., 2023). In the case of ZT complexes, the fluorescence intensity increased with the addition of TA. These results suggested that the addition of TA led to the exposure of intrinsic fluorophores, whereas a opposite result was reported by Liu et al. (2017) in zein-chlorogenic acid system, it might be the effect of polyphenol types. In addition, the stronger fluorescence intensity was observed in ZT5, revealing that the interaction between the intrinsic fluorophores of zein and TA was greater in an acid microenvironment.

3.2.3. Surface hydrophobicity and sulfhydryl content

The hydrophobic sites of proteins could bind to the BPB. The increased BPB bounding amount was reflective of the conformational and hydrophobicity changes of proteins. As shown in Fig. 1E, the BPB bound number of ZT5 increased compared to zein, implying that more hydrophobic amino acid residues were exposed in ZT5 structure, the solution polarity altered embedded state of hydrophobic amino acid residues of zein. Another reason for this result was that TA could drive exposed hydrophobic groups of zein aggregation by hydrogen bond and hydrophobic interaction in acid condition. Wang et al., (2023) reported similar results by analysing the interaction between soy isolate protein and TA complexes. In addition, the free sulfhydryl content was also used to reflect the structure change of ZT in our work (Fig. 1F). The significant differences (P < 0.05) of free sulfhydryl content were detected between different ZT complexes, demonstrating the pH conditions changed the conformation of ZT complexes. The increased content of free sulfhydryl was due to rupturation of the S-S bond. The free sulfhydryl content of ZT9 was higher than that of ZT5 and ZT7, which might be associated with the unfolding of the protein in alkaline condition (Pi et al., 2023; Wei et al., 2023). To some extent, the phenomenon in surface hydrophobic and free sulfhydryl content had an influence on the wettability of protein-polyphenol complexes, which finally changed the interfacial properties (Chao et al., 2023).

3.2.4. Molecular docking simulation

Combining experimental data with computational models could provide detailed information on functional groups and molecular forces (Li et al., 2023). A theoretical analysis was carried out by docking the zein with TA and optimized binding sites as illustrated in Fig. 1F. The dominant binding forces between zein and TA were hydrogen bonds and hydrophobic interactions, which were consistent with the FTIR results. TA was located in the hydrophobic pocket of zein, forming strong hydrophobic interactions. The phenolic hydroxyl group of TA interacts with the amino acid residues of zein by hydrogen bonding. Table S2 provided the number of hydrogen bonds and involved amino acid residues. Specifically, TA formed five hydrogen bonds with amino acid residues of zein in pH5 and pH7, involved ARG147, GLN117, SER114, PHE156, and GLN153 of zein in pH5; GLN153, GLN154, ARG147, ALA144 and GLN141 of zein in pH7. In pH9, there were three hydrogen bonds formed between TA and zein involved GLN153, GLN141 and ARG147. The binding affinity of zein and TA was arranged in the order ZT7(-8.3 kcal/mol) > ZT9(-8.5 kcal/mol) > ZT5(-8.6 kcal/mol), implying the favorable affinity of zein and TA in pH5 compare to pH7 and pH9. These results could help us to understand the structural change information of ZT complexes before emulsification and mainly adsorbs at the oil-water interface (Gong, et al., 2022)

*3.3. Potential of ZT complex stabilizing Pickering emulsions*

3.3.1. Wettability

The wettability of ZT complexes was evaluated by determining the contact angle. It was observed in Fig. 2A, that the contact angles of ZT5, ZT7 and ZT9 were 74.38°, 73.15° and 45.63°, respectively. Notably, the contact angles had no significant difference (*P*>0.05) between the three pairings (zein, ZT5, ZT7), implying that zein, ZT5 and ZT7 exhibit similar hydrophobicity. According to the stabilization mechanism of the Pickering emulsion, the emulsion was more stable when the contact angle of stabilizer close to 90°. Previous studies reported that zein nanoparticles had ability to stabilize Pickering emulsion due to their hydrophobic features (Souza et al., 2022). Although most polyphenols are hydrophilic, the introduction of TA under acidic conditions maintained the potential of ZT complexes as Pickering emulsion stabilizers. These results were attributed to the hydrogen bonds between the zein and TA, which reduced the exposure of hydrophilic groups of the zein (Zhu et al., 2021). These observations indicated that the addition of TA contributed to the improved performance of ZT complexes as stabilizer in Pickering emulsions, especially in acidic condition.

3.3.2. Interfacial tension

To further investigate the stabilizing potential of the ZT complexes in loading the CEO to form a Pickering emulsion, the interfacial tension of ZT complexes were determined. Theoretically, there are two stages when colloid emulsifiers stabilized Pickering emulsion. First, the colloid emulsifiers reduce the interfacial tension at the droplet surface rapidly due to the formation of the interfacial layer. Then, the colloid emulsifiers anchored at oil-water interface, with the interfacial tension decreases gradually as a relatively stable value (Liu et al., 2022). As displayed in Fig. 2B, the interfacial tension of ZT complexes decreased more than that of zein, implying that the interfacial activity of complex was improved by the addition of TA, which was consistent with the research of Chen et al. (2020). As reported by Xu et al. (2023), the zein/polyphenol complexes decrease the interfacial tension due to the formation of a homogeneous and cohesive film, which could improve the stability of the emulsion. The initial interfacial tension value in the presence of ZT9 (9.96 mN/m) was significantly lower than that of ZT5 (11.31 mN/m) and ZT7 (10.49 mN/m), indicating that ZT9 had the strongest interfacial adsorption capacity. As the measurement time prolonged, the interfacial tension of ZT complexes tends to remain stable, demonstrating that they anchor at the oil-water interface. These results indicate that ZT complexes have great potential as Pickering emulsion stabilizers.

3.3.3. Interfacial dilational rheology

The interface dilatational viscoelasticity of ZT complexes particles is crucial to examine the formation of Pickering emulsion interfacial layer, including dilatational modulus (E), dilatational elasticity modulus (Ed), and dilatational viscosity modulus (Ev), as this property could reflect the mechanical strength of interfacial layer. The time-dependent dilatational elasticity and viscosity modulus of ZT complexes were shown in Fig.2C. In measurement range, the Ed was dominant compared Ev, and indicating that all ZT complexes could form viscoelastic interfacial layers. To be more specific, the Ed of ZT9 was large than ZT5 and ZT7, implying superior viscoelasticity and stronger mechanical strength of the interfacial layer formed by ZT9 compared to ZT5 and ZT7. The resulting slope of E-π curve further indicated the equilibrium state of complexes at the oil-water interface. In Fig.2D, the slope of all samples is greater than 1, signifying an ideal adsorption state. The slope of E-π curve of ZT complexes were around 1.91, 2.89, and 3.64, respectively, highlighting the intensive adsorption of ZT complexes at interface. Meng et al. (2024) elucidated that this enhanced mechanical strength of the interfacial film could be attributed to the addition of polyphenol that increasing flexibility of protein structure, effectively preventing the coalescence of droplet. The result of interface dilatational viscoelasticity confirms that the mechanical strength of the interfacial layer is enhanced, which can respond to the interfacial tension in section 3.3.2.

*3.4. Characterization of ZTCs*

3.4.1. Morphological observation

After evaluating the potential of ZT complexes as Pickering emulsion stabilizers, the ZTCs were constructed by loading CEO. As displayed in Fig. 3A, the morphology of ZTCs were presented using a TEM. The interfacial layer of emulsions was obviously observable, which was characterized by a slightly dark outer region, implying the formation of a Pickering emulsion. The absorption of ZT complexes onto droplets resulted in a thicker interface of steric hindrance that prevented stabilizer from desorbing (Wei et al., 2019). The droplets stabilized by ZT5 or ZT7 were spherical, while the droplet stabilized by ZT9 did not have a well-defined shape, which could be attributed to the promoted flocculation of small droplets by strong electrostatic attraction. In addition, the CLSM also provided the microstructures of ZTC with three different ZT complex (Fig. 3B). The images of overlay showed the stacked state of CEO and ZTs phase, which were shown in yellow (superimposed color of green and red). The overlapping fluorescence micrograph showed that the CEO was wrapped by dense ZTs complexes. Similar phenomena were also observed in CEO Pickering emulsion stabilized by zein zein/carboxylated cellulose nanocrystals composite nanoparticles (Qin et al., 2024). The CLSM images of ZTCs showed spherical dispersion of emulsion droplets with different sizes. Notably, it was obvious that a clear boundary of the ZTC5 wrapping layer, indicating ZT5 adsorpted onto the oil-water interface stably. However, ZTC9 emulsion system had a lot of dissociative ZT9 particle, suggesting poor stabilization of ZT9. Consistent with the TEM observations, the droplets of ZTC5 displayed dispersive and uniform characteristics, while the droplets of ZTC9 showed a partial aggregation. These results suggest that the structures of ZTCs could be regulated by interfacial compositions.

3.4.2. Droplet size and zeta-potential

The droplet size and zeta potential of ZTCs are listed in Table S1. The average droplet size of ZTCs was between 300 and 400 nm, and also in line with the TEM report. Similar results were observed in an algal oil Pickering emulsion stabilized by zein-gallic acid nanoparticles (Xu et al., 2023). As larger particles adsorb at the oil-water interface for longer periods of time, the diameter of the emulsion droplets increases. The zeta potential defined the net surface charge of oil droplets which represented aggregation possibility. The zeta potentials of ZTC5, ZTC7, and ZTC9 were 24.67 mV, 19.42 mV, and -12.03 mV, respectively. The highter absolute value of zeta potential improved its properties against flocculation because of the electrostatic repulsion among the emulsion droplets (Xu, et al., 2023).

3.4.3 Physical stability

The physical stability of a series of ZTCs was evaluated by TSI measurements. An increase in TSI values expressed a decline in emulsion stability (Fan et al., 2023; Liu et al., 2023). The dynamic curve of TSI values is displayed in Fig. 4A. During the test period, the ZTCs displayed slow-growing TSI values, illustrating the suitability of protein-polyphenol complexes as stabilizers, which was probably related to the cross-linked network of the continuous phase at the oil-water interface. Xu, et al. (2023) observed a similar trend in TSI values of emulsion stabilized by gelatin and catechin complexes. Comparably, the emulsion stabilized by ZT5 showed a lower TSI value in the middle and late measurement, indicating better stability. Based on the stabilization potential of ZT complexes prepared in different pH conditions, ZT5 possessed appropriate wettability and anchored to the oil-water interface to form a homogeneous network film as a barrier, thus reducing the aggregation and coagulation of droplets and showing better stability.

The rheological properties reflected the stability and functionality of the emulsions. Fig. 4B illustrated the viscosity with the shear rate of CEO loaded Pickering emulsions stabilized by ZT5, ZT7, and ZT9. It was obvious that the viscosity of all emulsions decreased gradually with an increase in shear rate from 0.1 to 100 s-1, implying that the prepared emulsions showed shear thinning behavior and were defined as typical non-Newtonian fluid, a well-known rheological property of complex fluids (Chen et al., 2023). Interestingly, ZTC5 exhibited significantly higher viscosity than that of ZT7 and ZT9. The viscosity was related to the tight distributions of particles in continuous phase. The reason for this high viscosity could be case that the strong intermolecular force in ZT5, which made ZT5 anchored at the of oil-water interface easily, lead to more ZT5 particles in the continuous phase. This result was in agreement with the study reported by Xu et al. (2023). It was reported that high viscosity can reduce the collision frequency of droplets in the continuous phase, which was beneficial to improve the stability of emulsions (Liu, et al., 2023). These results about rheological properties of ZTCs provides a supplementary explanation for the stability.

3.4.4 EE and release performance

The EE of emulsion are improtat to efficient dilievery system from the perspective of economically and functional, which could culminate beneficial effects of bioactive agents. Fig.4C showed the EE percentage of emulsion stabilized by ZT complex. The emulsion prepared from ZT5 complex showed a significantly higher (*P* < 0.05) value (86.83%) in comparison with ZTC7 (77.24%) and ZTC9 (76.14%), indicating ZT5 complex confers more protection on the encapsulated by provides a higher viscoelastic oil-water interface. This result was consistent with rheological properties of prepared Pickering emulsion. Moreover, this excellent encapsulation performance of ZTC5 also reflected in release behavior (Fig.4D). All Pickering emulsions (stabilized by single zein particle and by ZT complex) exhibited a control release characteristic, which showed a slowly rising cumulative release rate in initial period. With the extension of time, ZTC5 remained a low cumulative release rate in comparison with ZTC7 and ZTC9, implying that the higher viscoelastic oil-water interface barrier (ZT5) has a delayed effect on the release of active substances in the interior. The oil-water interface is the key to blocking the migration of volatile molecules (Liu et al., 2023). There are abundance hydrogen bond reciprocity between tannic acid and various volatiles molecules at the oil-water of the droplet, reducing the content of free CEO in the aqueous. However, zein and tannic acid bonded covalently under alkaline conditions, with a large number of hydrogen bonding sites were buried, thus ZC and ZTC9 showed a similar release curve.

*3.5. Formation mechanisms of ZTCs*

The possible formation mechanisms of ZTCs are schematically described in Fig. 5. The ZT complexes at the oil-water interface was the dominant factor that determined the formation of the Pickering emulsion in this work. However, the different interactions between zein and TA in different conditions changed the conformation of the ZT complexes. The ZT complexes exhibit adjusted wettability and interfacial tension, which affected the properties of the complex as a Pickering emulsion stabilizer (Zhao et al., 2024). Subsequently, there were two steps of the Pickering emulsion formation: adsorption and stabilization. As evidenced by interfacial tension, the interfacial adsorption capacity of the ZT complexes decreased in the following order: ZT9 > ZT7 > ZT5, which could be attributed to the high charge on ZT9. With the prolonging of time, the adsorption of ZT complexes reached equilibrium and the emulsion reached a stable state. At this moment, the ZT complexes anchors to the oil-water interface to form a film, which against the occur of aggregation and flocculation. Among the three ZT complexes, the contact angle of ZT5 particles was closest to 90° and the particle size was relatively larger, indicating that ZT5 had a strong anchoring effect and the formed interface film was thicker. The microstructure and stability characterization proved this view. Xu et al. (2023) demonstrated that the composite types between zein and polyphenols had a significant impact on the properties of prepared Pickering emulsions. Zhu, et al. (2021) made the same point and suggested that it can be achieved by tuning the molecular interaction between proteins and polyphenols.

*3.6. Antimicrobial activity of ZTCs*

3.6.1 Antimicrobial activity testing

The antimicrobial effect of ZTCs against foodborne spoilage bacteria were confirmed by agar plate tests which are displayed in Fig. 6. ZT complexes showed antimicrobial effects as described digitally by viability in Fig. 6B, which may be attributed to efficient adhesion to bacterial membranes. It is worth noting that ZT complexes prepared in different pH conditions performed different inhibition effects. A number of phenolic hydroxyl groups in TA contribute to the bacteriostatic properties of ZT complexes, however, polyphenols of TA are easily oxidized under alkaline conditions which decreases the bio-abilities (Nassarawa, et al., 2022). The bacteriostasis between emulsions was different due to the encapsulation efficiency of the prepared Pickering emulsion, which will inevitably affect the antibacterial efficiency. The ZTC5 stabilized by ZT5 had a strong anchoring effect and a thicker interface film, which improved the encapsulation efficiency of CEO in the Pickering emulsion. Subsequently, we evaluated the MIC of ZT complexes and ZTCs (Table S3). The MIC value of ZTC5 against *P. paralactis* MN10 (A) and *L. sakei* VMR17 were both 12.5 μL/mL, which were lower than that of ZC (emulsion stabilized by zein). This result indicated that the ZTCs had enhanced antimicrobial activity.

3.7.2. Antimicrobial mechanism

We employed SEM to observed the microstructure of *P. paralactis* MN10 and *L. sakei* VMR17, and ZTC5 was selected as the emulsion model at the previously established MIC values (Fig. 6C). In the control group, the bacterial cell had the typical structure of themselves, however, folds and depressions on the cell surface were observed in the treated group. There are a lot of active compounds in CEO, which could interact with the cell membrane of bacteria, leading to the disruption of the cell structure (Zhou, et al., 2018). Further, EOs' lipophilicity facilitates their targeted diffusion and interaction with the membranes and intracellular components of cells. In addition, the role of TA in the oil-water interface should not be overlooked either. When emulsion droplets adsorbed around the bacteria, the phenol hydroxyl group of TA changed the permeability of the cell, leading to the disruption of the cell structure. These results demonstrated that the ZTCs caused the permeabilization of the cells and disrupted the membrane integrity.

Despite the great promise of zein and TA complexes in functional Pickering emulsion, considering the complexity of food emulsion, the ongoing challenge is to maintain the stability of zein and TA complexes-based Pickering emulsions in multi-component food systems and at various conditions, such as temperatures, ionic strengths, and pH values. Additionally, more studies on applications of these hybrid particle still expected, for example, combining bio-based materials to develop food active packaging.

**4. Conclusions**

In the present work, the Pickering emulsion co-loaded TA and CEO had been successfully prepared based on the ZT complexes mediated interfacial engineering. The addition of TA in zein at different pH conditions was regular the structural properties of zein, and hydrogen bonding and hydrophobic effects were the main driving forces. Introducing TA decreased the interfacial tension of the ZT complexes, implying the dominant role of TA in improving the interfacial stabilization of the ZT complexes. The TEM images confirmed the formation of a stronger interfacial layer film of ZTC5, corresponding to the high physical stability. The ZTC showed an effective antimicrobial property against spoilage organisms *Pseudomonad paralactis* MN10 and *Lactobacillus sakei* VMR17 by destroying cell structure. These findings provide new insight for developing co-loading multiple antimicrobial agents within Pickering emulsion as a delivery system via interfacial engineering.

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**Figure Caption**

**Fig. 1** Characteristics of zein and their complexes with tannic acid (ZT). Microscopic morphology was observed by scanning electron microscope at a magnification of × 20 k (A). FTIR spectroscopy (B), secondary structures contents (C), fluorescence spectroscopy (D), surface hydrophobicity and sulfhydryl content (E). The molecular docking of zein and tannic acid at different pH conditions (F). ZT5, ZT7 and ZT9 represent zein combine with TA at pH 5, pH 7 and pH 9. Different letters above the bars in same measurement indicate significant differences among fractions (*P* < 0.05).

**Fig. 2** Potential of zein and their complexes with tannic acid (ZT) stabilizing Pickering emulsion. Water contact angle (A), interfacial tension (B), time-dependent dilatational elasticity modulus (Ed) and dilatational viscosity modulus (Ev) (C), and dilatational modulus as a function of surface pressure (π) (D) of different ZT as Pickering emulsion stabilizer.

**Fig. 3** Microscopic morphology of Pickering emulsions co-loaded tannic acid and cinnamon essential oil, which observed by transmission electron microscope (A) and confocal laser scanning microscope (B). ZTC5, ZTC7, and ZTC9 represented the CEO loaded Pickering emulsion stabilized by ZT5, ZT7 and ZT9, respectively.

**Fig. 4** Stability evaluation of Pickering emulsion co-loaded tannic acid and cinnamon essential oil by Turbiscan stability index values (C). Rheological properties of Pickering emulsion loaded tannic acid and cinnamon essential oil, viscosity as a function of shear rate (D). Encapsulation efficiency of Pickering emulsion co-loaded tannic acid and cinnamon essential oil (A) and release behavior of CEO from emulsion. Different letters above the bars in same measurement indicate significant differences among fractions (*P* < 0.05).

**Fig. 5** Schematic illustration of two formation steps of Pickering emulsion co-loaded TA and CEO. ZT5, ZT7, and ZT9 were represent zein and tannic acid complexes prepared in pH 5, pH 7 and pH 9; ZTC5, ZTC7, and ZTC9 were represent tannic acid and cinnamon essential oil co-loaded Pickering emulsion stabilized by ZT5, ZT7, and ZT9.

**Fig. 6** Antimicrobial effect (in vitro) of Pickering emulsion co-loaded tannic acid and cinnamon essential oil against *P. paralactis* MN10 and *L. sakei* VMR17. Survival clone on agar culture plates (A), viability of tested bacteria (B), and changes in cell structure of *P. paralactis* MN10 and *L. sakei* VMR17 after treated with minimum inhibitory concentration of ZTC5 by scanning electron microscope with 15000-magnifications (C). Different lowercase letters indicate significant differences (*P* < 0.05).

**Fig. 1**

**Fig. 2**

**Fig. 3**



**Fig. 4**



**Fig. 5**



**Fig. 6**

