Study on Pickering Emulsions Stabilized by Tea Polyphenols/Gelatin/Chitosan Nanoparticles

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Abstract. The tea polyphenols/gelatin/chitosan (TGC) nanoparticle was prepared and applied to stabilize Pickering emulsions. The particle size of TGC nanoparticle was about 880 nm with the mass ratio of tea polyphenols to gelatin 1:2. The apparent viscosity of TGC nanoparticle showed typical non Newtonian pseudoplastic behavior, and increased with the increase of tea polyphenols content at the same shear rate. The size distribution of Pickering emulsions stabilized by TGC nanoparticles was highly uniform. The average droplet size of emulsions increased from 46 µm to 80 µm when the mass fraction of oil phase increased from 30% to 60%. The emulsified volume fraction increased with the increase of oil fraction. Nanoparticles provide a strong force on the oil-water interface and effectively inhibit the aggregation and coalescence of droplets. The Pickering emulsion exhibited good storage stability and good stability under high temperature heating.

1. Introduction
Emulsion is a system of unstable thermodynamics and needs to use emulsifier to stabilize the system. The traditional emulsion uses small molecule surfactant. As small molecule surfactant has not only environmental risks, but also medical safety problems such as irritation or hemolysis, some scholars have studied the use of natural or synthetic solid particles as stabilizers. The new emulsion, which is stabilized by solid particles at the water / oil interface, is called Pickering emulsion [1,2]. Pickering emulsion requires less stabilizer with less environmental impact, improves emulsion resistance to coalescence and Ostwald ripening, and has good encapsulation stability, compared with traditional emulsion, therefore, it has great potential in food, cosmetics, medicine and catalysis [3,4]. Proteins are amphiphilic macromolecules with polar and nonpolar groups on their surfaces, which can produce good water solubility and surface activity, and can be used as Pickering stabilizer when the proportion of polar and nonpolar groups on protein surface is appropriate. The stable emulsion [5,6] can be obtained from a single protein particle, however, the stable emulsion of single protein is easy to flocculate, coalescence because the protein is highly sensitive to pH, ionic strength and temperature changes. Protein composite particles can improve the stability of protein in the environment, and their preparation methods mostly use the combination of protein and polysaccharide [7,8].

At present, the study of protein / polyphenol composite particle stabilized emulsion is still lacking. The combination of polyphenols and proteins forms protein aggregates with controllable particle size, and improves the functional characteristics of protein with the physiological activity of polyphenols. Jin et al. [9] studied the self-assembly of protein gelatin and tannic acid to form nano gold complexes as chelating metal particles to stabilize Pickering emulsion. However, the size distribution of the nanoparticles directly formed by protein and polyphenol is wide, and the flocculation precipitation is easy to occur. It was found that the interaction between protein and tea polyphenols was weakened...
after the combination of protein and polysaccharide, which improved the stability and uniformity of nanoparticles. The combination of chitosan and gelatin can weaken the strong interaction between gelatin and tea polyphenols, and improve the swelling and mechanical properties of gelatin [10], so that the formed tea polyphenols/gelatin/chitosan (TGC) nanoparticles have the function of long-term sustained-release.

In this study, TGC nanocomposites were used as stabilizers to prepare Pickering emulsions. By observing the stability mechanism and microstructure of emulsion, the storage stability and thermal stability of emulsion are explored, which will provide a theoretical basis for the design of healthy and high nutrition Pickering emulsion.

2. Materials and Methods

2.1. Materials
Tea polyphenol was obtained from Nanjing Zelang Pharmaceutical Technology Co., Ltd (Jiangsu, China); Chitosan (deacetylation degree ≥ 90%) and gelatin were from Sinopharm Group Chemical Reagent Co., Ltd. (Beijing, China); other reagents are all analytically pure. All the reagents and materials were used as received without any further purification.

2.2. Experiments

2.2.1. Preparation of Tea polyphenols/Gelatin/Gehitosan (TGC) Nanoparticles. A certain amount of tea polyphenols (TP) was dissolved in 5 mg/ml chitosan solution (in 0.2 mol/L sodium acetate buffer solution of pH 5.4), and slowly dropped into 5 mg/ml gelatin (Gel) solution (pH5.4) at 50 °C (the mass ratio of tea polyphenols to gelatin in the system was 1:8, 1:6, 1:4, 1:2, 1:1), and the mixture was continued to stir for 30 min with rotational speed of 300 r/min and the suspension of TGC nanoparticles was obtained. The effects of the mass ratio of tea polyphenols and gelatin on the formation of TGC nanoparticles were investigated by particle size, polydispersity index (PDI).

2.2.2. Determination of Rheological Properties of TGC Nanoparticle Solution. The relationship between the apparent viscosity of TGC nanoparticle solution and the temperature was measured. The diameter of the cone plate clamp with a diameter of 50 mm was used. The temperature of the rheometer was set at 25~80°C, increased linearly and the shear rate was fixed to 100 s⁻¹.

The relationship between the apparent viscosity of TGC nanoparticle solution and the shear rate was measured. The temperature of the rheometer was fixed at 80°C, and the shear rate was 0~1000 s⁻¹, increasing linearly.

2.2.3. Preparation of Pickering Emulsion. Peanut oil was added into the TGC nanoparticle solution (with oil mass fraction of 30%, 40%, 50%, 60% in the system), and the mixture was stirred for 2 min with rotational speed of 10000 r/min and the Pickering emulsion was obtained. Immediately, 10 mL emulsion was placed in capped glass bottle and stored at room temperature.

2.2.4. Storage Stability of Pickering Emulsion. Freshly prepared Pickering emulsions were placed at room temperature. The particle size distribution of the emulsion and the emulsified volume fraction of the emulsion were measured after 30 days storage.

2.2.5. Thermal Stability of Pickering Emulsion. Freshly prepared Pickering emulsions were heated 10 min at 30°C, 50°C and 80°C, separately, then cooled to room temperature, and the particle size distribution and the emulsified volume fraction of the emulsion were determined.

2.2.6. Particle Size Analysis. The sample was analyzed by Mastersizer 3000 particle size distribution analyzer. The parameters are as follows: particle absorption index 0.001, particle refractive index 1.450, dispersant refractive index 1.330, density 0.945, spherical droplet.
2.2.7. Determination of Emulsified Volume Fraction (\(F_{ev}\)). The height of the emulsion phase (\(H_e\)) and the total height of the preparation (\(H_t\)) were recorded. The emulsified volume fraction was reported as: \(F_{ev} \% = \left(\frac{H_e}{H_t}\right) \times 100\).

3. Results and Discussion

3.1. Particle Size Analysis of TGC Nanoparticles

The particle size and PDI of TGC nanoparticles are shown in Table 1. The particle size increased and PDI tended to decrease at first and then increased slightly with the increase of tea polyphenols, however, the particle size exceeded the required size of nanoparticles when Gel:TP mass ratio reached 1:1. The best mass ratio of tea polyphenols to gelatin was 1:2.

The polyphenol hydroxyl structure of tea polyphenols can produce strong hydrogen bond with ketimino group of gelatin, and benzene ring can produce hydrophobic force with proline residue of gelatin [11]. These non covalent bond forces promote the self-assembly of gelatin and tea polyphenols into particles of certain size and shape. However, the interaction between them is too strong, when the concentration of gelatin and the mass ratio of tea polyphenols to gelatin exceed the threshold value, flocculation will occur. Glycosylation of gelatin can increase the flocculation threshold of gelatin with tea polyphenols. Tea polyphenols and gelatin can combine at many points, and the reaction process can be divided into three stages: when the proportion of tea polyphenols is low, it acts as a cross-linking agent, which can not only shrink the extended long chain of gelatin, but also form oligomer between gelatin molecules, so the particles formed are small and the homogeneity is poor; When the proportion of tea polyphenols increased, gelatin had enough binding sites to load more tea polyphenols. Although the size of the particles increased, the tea polyphenols were encapsulated in the particles, and the PDI of the particles decreased. When the proportion of tea polyphenols continued to increase, tea polyphenols could bridge between particles, and the particles gathered and finally precipitated.

| Gel:TP mass ratio | Particle size (nm) | PDI  |
|------------------|--------------------|------|
| 8:1              | 380                | 0.43 |
| 6:1              | 570                | 0.41 |
| 4:1              | 750                | 0.34 |
| 2:1              | 880                | 0.20 |
| 1:1              | 980                | 0.29 |

3.2. Rheological Analysis of TGC Nanoparticles

Figure 1 is the relationship between the apparent viscosity of TPC nanoparticles and the shear rate. The viscosity gradually decreased with the increase of shear rate, showing a typical non Newtonian pseudoplastic behavior (shear thinning). The remarkable shear thinning behavior of TPC nanoparticles was due to typical weak association interaction, which can be explained by the destruction of the entangled polymer network in the shear process, where the broken intermolecular entanglement rate is higher than that of the reformed intermolecular entanglement rate, resulting in lower flow resistance and lower apparent viscosity. Tzoumaki et al [12] have reported similar conclusions that the hydrophobic network structure of the plant glycogen nanoparticles form a weak droplet network. In addition, the apparent viscosity of TPC nanoparticles increased with the increase of TP content at the same shear rate. These results are consistent with the relationship between the apparent viscosity of TPC nanoparticles and temperature (Figure 2). The apparent viscosity of TPC nanoparticles decreased with the increase of temperature.
3.3. Particle Size Analysis and Emulsified Volume Fraction of Pickering Emulsion

Table 2 clearly reflects the effect of oil fraction on the particle size distribution of the droplets and emulsified volume fraction of Pickering emulsions stabilized by TGC nanoparticles. When the mass fraction of oil phase increased from 30% to 60%, the average droplet size increased from 46 µm to 80 µm. Nanoparticles provide a stronger force on the oil-water interface and effectively inhibit the aggregation and coalescence of droplets.

After emulsion preparation, the volume fraction of emulsion phase decreased sharply and remained stable until 1 hours later. The results showed that the emulsified volume fraction increased significantly with oil increase. The emulsified volume fraction increased by 54% with oil fraction from 30% to 50%, and was 88% when the oil fraction increased to 60%. This indicates that gelatin binding to TP forms insoluble complex through hydrophobic interaction, which increases the effective adsorption of particles on the droplet surface and reduces the density difference between droplet and continuous phase, thus increasing the volume fraction of emulsion phase.

| Oil fraction(%) | Particle size(µm) | Emulsified volume fraction (%) |
|----------------|-------------------|-------------------------------|
| 30             | 46                | 48                            |
| 40             | 58                | 65                            |
| 50             | 71                | 74                            |
| 60             | 80                | 88                            |

3.4. Light Microscope Observation of Pickering Emulsion

The microstructure of emulsion droplets was observed by optical microscope, and the results are shown in Figure 3. The droplets were spherical. The droplet size of emulsion increased slightly with the increase of oil mass fraction, and the size distribution was uniform. The size of protein aggregates is suitable for forming Pickering emulsion with homogeneous droplets.
3.5. Storage Stability of Pickering Emulsion

The storage stability of Pickering emulsions stabilized by TGC nanoparticles was characterized by particle size analysis and the emulsified volume fraction, shown in Figure 4. The average particle size of Pickering emulsion increased slightly from 58 µm at first day to 80 µm after 30 d storage. The emulsified volume fraction decreased slightly with storage period. The results showed that the nanoparticles stabilized emulsion had good storage stability.

3.6. Thermal Stability of Pickering Emulsion

Food must be heat treated during processing or consumption. As a protein emulsion system, pasteurization is commonly used for sterilization. Therefore, the effect of temperature on emulsion system stabilized by TPC nanoparticles was explored, shown in Figure 5. The emulsion showed no significant difference at 30°C, 50°C and 80°C, and the structure of the emulsion was still maintained. The average particle size of Pickering emulsion increased slightly from 58 µm at 30°C to 65 µm at 80°C. The emulsified volume fraction decreased slightly with temperature increase. The emulsion has good stability under high temperature heating.

4. Conclusions

In this paper, a tea polyphenols/gelatin/chitosan (TGC) nanoparticle was prepared and the effect of tea polyphenol content on the formed particles was explored. The particle size increased and PDI tended to decrease at first and then increased slightly with the increase of tea polyphenols. However, the particle size exceeded the required size of nanoparticles when Gel:TP mass ratio reached 1:1. The best mass ratio of tea polyphenols to gelatin was 1:2. The apparent viscosity decreased with the increase of shear rate and temperature, increased with the increase of TP content at the same shear rate, which can be explained by the destruction of the entangled polymer network in the shear process. The size distribution of Pickering emulsions stabilized by TGC nanoparticles was uniform. When the mass
fraction of oil phase increased from 30% to 60%, the average droplet size increased from 46 µm to 80 µm. The emulsified volume fraction increased significantly with oil fraction increase, and increased by 54% with oil fraction from 30% to 50%, and was 88% when the oil fraction increased to 60%. Nanoparticles provide a stronger force on the oil-water interface and effectively inhibit the aggregation and coalescence of droplets. The Pickering emulsion had good storage stability and good stability under high temperature heating.

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6. References
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