Effect of Zein Concentration on the Formation of Pectin-Zein Complexes

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Abstract. Biopolymeric particles can be formed by electrostatic complexes of globular proteins and anionic polysaccharides mixtures at pH values above pK$_a$ of anionic polysaccharide and under the isoelectric point of protein. The purpose of this study was to investigate the effect of protein (i.e., zein) concentration on the formation of complexes between zein and polysaccharide (i.e., amidated low methoxy pectin) in aqueous solution. The zeta potential, turbidity and morphology under different conditions were observed to provide insights into the physicochemical properties of zein-pectin complexes. The biopolymeric particles were obtained at pH 4 and concentration of zein and pectin of 0.001 and 1% w/w, respectively. As the zein concentration increased, the turbidity of zein-pectin solutions increased, resulting from the formation of zein-pectin complexes. The zeta potential of the systems became less negative when the zein concentration increased. The results suggested that concentration of zein primarily influenced the formation of zein-pectin complexes.

Introduction

Recently, biopolymers have been receiving increased interest from researchers because of their non-toxicity, biocompatibility and biodegradability. Many studies have shown that polysaccharides, for example, alginate and pectin could be used as drug carriers (1-3). Pectin, a naturally occurring biopolymer, is a complex mixture of polysaccharides that makes up about one third of the cell wall dry substance of higher plants. Basically, it is a polymer of α-D-galacturonic acid with 1-4 linkages. The galacturonic acid of the backbone is partially methyl-esterified. The number of methyl group is indicating pectin properties (4).

When proteins and polysaccharides are mixed together in an aqueous environment, two different types of interaction can arise: thermodynamic incompatibility or thermodynamic compatibility mainly depending on the electrical charges on both biopolymers. Whether the mixture is under one or the other condition, it will present completely different functional properties. In general, thermodynamic compatibility, also known as associative phase separation or complex coacervation, usually occurs at relatively lower concentrations (< 3 to 4% w/w total solids), and when both molecules carry net opposite electric charges (5). This occurs at a pH between the proteins’ isoelectric point (pI) and the pKa of the polysaccharide. Under such conditions, molecules spontaneously attract each other and separate into two phases, one rich in both protein and polysaccharide and the other one depleted in biopolymers but rich in solvent. The protein and polysaccharide in the biopolymer rich phase are held together by electrostatic forces and can take the form of a coacervate or a precipitate.

In this study, the effect of protein (i.e., zein) concentration on the formation of complexes between zein and polysaccharide (i.e., pectin) in aqueous solution was investigated. The physicochemical properties of biopolymeric complexes, e.g., turbidity, zeta potential and morphology under different conditions were also studied.
Experimentals

Stock solutions of biopolymer were prepared by dissolving 3 grams of amidated low methoxy pectin (pectin cu020, Herbstreith & Fox KG, Germany) in 100-mL distilled water and 1 gram of zein (Freeman Industries, USA) in 100-mL aqueous alcohol (ethanol:water, ratio 3:1) and stirred for at least 6 h to ensure complete hydration. The solutions were first centrifuged (model 6500, Kubota, Japan) at 7,500 rpm for 20 min for zein solutions and 8,500 for 30 min for pectin ones to remove any insoluble particles and then only the supernatants were used.

Biopolymer mixtures containing pectin (1% w/w) and zein (0-0.5% w/w) were prepared by mixing different ratios of the stock solutions, adjusted to pH 4 with sodium hydroxide prior to mixing. The resulting solutions were mixed for 1 min using a vortex mixer. The turbidity of the colloidal system was measured using UV–visible spectrophotometer (model T60, PG instruments, UK) at the wavelength of 600 nm. The zeta potential of biopolymeric particles was measured using zeta potential analyzer (model Zetaplus, Brookhaven, USA). The morphology under different conditions was observed under an optical microscope (model CX41, Olympus, Japan).

Results and Discussion

Aqueous solutions containing 1% w/w of pectin and different concentrations of zein were prepared and assessed for their physicochemical properties. The solutions were left to settle overnight and the formation of the complexes was assessed by turbidity measurement at pH 4. The pure zein solutions obtained were clear solution with 100% transmittance. At the concentration up to 0.01% w/w zein, no change was noted on the turbidity of the zein-pectin solutions. The turbidity of zein-pectin solutions increased with the increase of the concentration of zein (Fig. 1), resulting from the formation of zein-pectin complexes. When the concentration of zein was more than 0.1% w/w, the pectin-zein solutions were very turbid with 0% transmittance, resulting from the formation of insoluble complexes.

![Figure1. Turbidity of the biopolymeric particles prepared from various concentrations of zein and 1% w/w pectin, at pH 4.](image-url)
The overall surface charge of pectin-zein solutions was determined by measuring the zeta potential at pH 4 (Fig. 2). The value of zeta potential of 1% w/w pectin was about 35 mV. The zeta potential value was less negative when the zein was added and seemed to be constant at the zein concentration of 0.000001-0.05% w/w. These results suggested that zein concentration of less than 0.05% w/w, the addition of zein did not cause a decrease in the charge, resulting from too low concentration of zein. The zeta potential value was significantly increased from -32.59 mV to -1.21 mV with the increased concentration of zein from 0.05 to 0.5% w/w. This indicated that zein, which is cationic protein molecule, adsorbed to the surface of pectin (anionic polysaccharide). These results confirmed that electrostatic interactions play a major role in the formation of pectin-zein complexes.

![Graph showing zeta potential vs. zein concentration](image)

Figure 2. Zeta potential of the biopolymeric particles prepared from various concentrations of zein and 1% w/w pectin, at pH 4.

Figure 3. The morphology of the biopolymeric particles prepared from 1% w/w pectin and 0.1% w/w zein, at pH 4.

To confirm that complexes form between the zein and pectin at pH 4, the various zein concentrations were mixed with pectin solution and the surface morphology of the samples was then investigated by optical microscopy. Fig. 3 shows the morphology of the biopolymeric particles.
prepared from 1% w/w pectin and 0.1% w/w zein, at pH 4. It is clearly observed that the solution was turbid and bridging flocculation occurred. The size of the biopolymeric particles was increased as concentration of zein was increased (data not show). This is because of the relatively large electrostatic attraction between the negatively charged pectin molecule and positively charged zein molecules.

Conclusions

The effect of zein concentration on the formation of pectin-zein complexes in aqueous solution was investigated at pH 4. The turbidity, zeta potential and morphology under different conditions were observed and provided insights into the physicochemical properties of pectin-zein complexes. The turbidity of zein-pectin solutions increased with the increase of the concentration of zein, resulting from the formation of zein-pectin complexes. The zeta potential value was significantly increased from -32.59 mV to -1.21 mV with the increased concentration of zein, indicating that zein interacted with pectin via electrostatic interactions. The future work on the biopolymeric particles of pectin-zein may be useful as delivery system or fat replacer in the food, pharmaceutical, cosmetic and other industries.

References