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Formation and Characterization of Polyelectrolyte Complexes containing Pectin and Zein

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Abstract

The formation of pectin-zein polyelectrolyte complexes was investigated, with emphasis on the effect of pectin type and zein concentration. The pectin-zein complexes were formed by mixing pectin solution with zein solution at pH 4, where zein and pectin had opposite charges. The formation of the complexes was evidenced by an increased turbidity, a slight decrease of negative charges, and an aggregation of polymers, as observed by digital images, microscopic images, and scanning electron micrographs. Fourier transform infrared spectra suggested the possibility of weak physical interaction between pectin and zein. The pectin-zein complexes demonstrated better oil/water interfacial tension lowering properties than pectin alone. The results suggest that these pectin-zein complexes could be used to improve the physical properties and stability of emulsions. Also, pectin and zein showed to be promising materials, thus extending the range of applications of these natural polymers.

Keywords: Pectin, zein, polyelectrolyte complex, characterization

Introduction

Mixing opposite charged polyelectrolytes, e.g., protein and polysaccharide, in solution will result in spontaneous association, due to formation of reversible electrostatic bonds. These interactions lead to the formation of polyelectrolyte complex networks with non-permanent structures. These polymeric networks, or hydrogels, are generally well tolerated and biocompatible [1,2]. The complexes can be formed when protein and polysaccharide are combined, through either covalent bonding or electrostatic interaction [3]. In the case of electrostatic interaction, the efficiency of protein-polysaccharide complexes depends not only on the distribution of ionized groups onto the protein surface and stability of the protein structure, but also on the flexibility, charge distribution, and density of polysaccharide [4]. It is important to choose the type of protein or polysaccharide, and the conditions which allow them to express opposite charges.

Different polyelectrolyte complexes between protein and polysaccharide have been used in a wide range of applications, such as the food, cosmetic, and pharmaceutical industries, due to the advantages of protein (fast adsorption) and polysaccharides (steric repulsion or viscosity enhancement) [2]. Numerous studies have shown improved emulsion stability, attributable to the presence of interactions between the protein and polysaccharide; for example, β-lactoglobulin + carrageenan [5], pectin + gelatin [6], flaxseed gum + whey protein [7], sodium caseinate + chitosan [8], etc. This is mainly driven by attractive electrostatic interactions at the interface, which can be controlled to increase the thickness of the surface layer surrounding the droplets and to create a multilayer surface. Changes in pH conditions can

particularly affect the interactions occurring between polysaccharides and proteins at the interface. At a pH below the isoelectric point of proteins, the adsorbed proteins on the oil droplet are positively charged, and negatively charged polysaccharides will form complexes at the interface. However, limited reports have studied the complex formation between proteins and polysaccharides and characterized the protein-polysaccharide complexes.

Zein is an interesting protein that could be used to form complexes with polysaccharide. It is a prolamine, a major storage protein of corn. Zein contains many hydrophobic amino acid residues, including many sulfur-containing amino acids, but is deficient in ionizable and polar amino acids. The zein proteins are hydrophobic and insoluble in water and, thus, ethanol at high concentrations (60 - 95 %) is required to maintain their molecular conformations [9]. The properties of zein are not only dependent on the amino acid composition, but also their molecular structures on the nanometer scale [10]. Due to its water insolubility, zein has been used in the food and packaging industry to form a moisture barrier. In pharmaceutical application, zein nanoparticles have been studied for sustained drug delivery applications for drug molecules [11].

Pectin, a naturally occurring water-soluble polysaccharide, is a well-known food additive, which is mainly used for its gelling and stabilizing abilities. Due to its biocompatibility, biodegradability, and non-toxicity, pectin represents an attractive biopolymer for a variety of pharmaceutical and biomedical applications [12]. It also has several unique properties that have enabled it to be used potentially as an emulsifier and a carrier for drug delivery to the gastrointestinal tract, such as matrix tablets, gel beads, and film-coated dosage forms [13,14]. Chemically, pectin contains linear chains of (1-4)-linked α-D-galacturonic acid residues. The linear structure of pectin is partly interrupted by (1-2)-linked side chains consisting of L-rhamnose residues and some other neutral sugars [12]. The galacturonic acids have carboxyl groups, some of which are naturally presented as methyl esters and others, which are reacted with ammonia to produce carboxamide groups. Pectin is divided into 2 major groups on the basis of their degree of esterification (DE) of the galacturonic acid residues. Pectin with DE less than 50 % is so-called low methoxy pectin (LMP), while that with DE more than 50 % is so-called high methoxy pectin (HMP).

Recently, a combination of zein and pectin has been used as drug delivery carriers, in the form of complex hydrogel beads [15], microspheres [16], or nanoparticles [17,18]. The pectin-zein polyelectrolyte complexes have also been used to stabilize oil-in-water emulsions [19]. In our previous report, the effect of zein concentration on the formation of pectin-zein complexes was preliminary investigated [20]. However, the formation of complexes and their characteristics have not been investigated in detail. Therefore, in this research, pectin-zein polyelectrolyte complexes were prepared, and the physical properties of pectin-zein complexes were characterized.

Materials and methods

Materials

High methoxy pectin with a molecular weight of 200 kDa and a degree of esterification (DE) of 70 % (lot number 00501087), and low methoxy pectin with a molecular weight of 70 kDa and a DE of 38 % (lot number 00412072), were a gift from Herbstreith & Fox KG (Germany), and are referred to as HMP and LMP, respectively. Commercial zein (Zein F4000, Freeman Industries LLC, USA) has a maximum molecular weight of 35 kDa, and mostly of 19 and 22 kDa, due to the content of the higher molecular weight β-zein fraction, which is assumed to consist of disulfide-linked α-zein. Refined rice bran oil was purchased from Thai Edible Oil Co., Ltd. (Thailand). All other chemicals were of analytical or pharmaceutical grade, and used as supplied without further purification. Deionized water was used throughout the study.

Preparation of pectin solution

Stock solution (4 % w/w LMP or HMP) was prepared by dissolving pectin powder in distilled water and stirring at an ambient temperature (25 °C) for at least 2 h to ensure complete hydration. The pectin solution was centrifuged (model Universal 320R, Hettich, Germany) at 8,500 rpm for 30 min to remove any insoluble particles. Only supernatant was used.

Preparation of zein solution

Stock solution of zein (10 % w/w) was prepared by dispersing zein powder (10 g) in aqueous alcohol solution (consisting of 80 % v/v ethanol), adjusting to 100 g, and stirring at ambient temperature (25 °C) for at least 2 h to ensure complete hydration. The solution was then treated in the same manner as described in the preparation of pectin solution.

Preparation of pectin-zein complexes

Pectin-zein complexes were prepared by mixing 2 % w/w pectin (LMP or HMP) and 0 - 0.5 % w/w zein. The pH of the solutions was adjusted to the desired pH (pH 4) with sodium hydroxide. The resulting solutions were mixed for 1 min using a vortex mixer and then stored at ambient temperature (25 °C) for 24 h prior to analysis.

Zeta potential measurement

The zeta potential of biopolymer solutions (pectin and zein) at various pHs (pH 3 to 7) and mixtures of 2 % w/w pectin (HMP or LMP) and zein at various concentrations was measured with a zeta potential analyzer (model Zetaplus, Brookhaven, USA). The pectin-zein complexes were dispersed in 5 mM acetate buffer (pH 4), with gentle stirring before measurement. The electric field applied was 1 V. The average and standard deviation of the measurement of 3 batches of samples were reported.

Turbidity measurement

The turbidity of pectin, zein and mixtures of pectin and zein at various pHs (pH 3 to 7) was determined using a UV-visible spectrophotometer (model T60, PG Instrument, USA) at 600 nm. Quartz cuvettes, with a cell path length of 1.0 cm, were used. Distilled water was used as a blank reference. The transmission and absorbance data (at 600 nm) were analyzed (n = 3).

Optical and scanning electron microscopy

The microstructure of pectin-zein complexes was observed using a light microscope (model CX41, Olympus, Japan) with low magnification (40 - 100×). The pectin-zein complexes were dropped on a glass slide and covered afterward with a coverslip, after which photos of sample were taken.

The morphology of pectin-zein complexes was also investigated by a scanning electron microscope (SEM) (model Maxim-2000, CamScan Analytical, England), under an accelerating voltage of 15 keV. All samples were dropped on SEM stubs and allowed to dry as polymeric film on the stub. After that, the samples were coated, in a vacuum, with a thin gold layer before investigation.

Viscosity measurement

The viscosity of pectin (HMP and LMP) was measured using a cone and plate viscometer (model DV-III Ultra, Brookfield, USA) with spindle No.51 at a speed of 50 rpm, at ambient temperature (25 °C). Each measurement was performed in triplicate.

Surface and interfacial tension measurement

Interfacial tension is determined by fitting a shape of drop (in a captured video image) to the Young-Laplace equation, which relates interfacial tension to drop shape. Equilibrium surface tension (liquid-air interfacial tension) was determined by static drop shape analysis (model FTA 100, Data Physics Corporation, USA) operated with the FTA32 v2.0 software. All measurements were conducted at ambient temperature (25 °C). Pendant aqueous solution drops were formed in the air using a needle with inner diameter of 0.635 mm. The surface tension of pectin (HMP or LMP) and zein solutions at various concentrations was determined immediately after drop formation. The mean values of 3 measurements were reported.

The oil-water interfacial tension was also determined using the drop shape analysis method. An aqueous droplet containing 0.5 - 2 % w/w pectin (HMP or LMP) was automatically formed at the tip of the needle, which was immersed in a glass cuvette containing the oil phase (rice bran oil). The droplet shape was automatically analyzed to record the changes in the interfacial tension over time. The

interfacial tension of the system containing pectin-zein complexes, prepared at pH 4 or 7, was also determined. All the measurements were carried out in triplicate.

Fourier transform infrared (FTIR) spectroscopic analysis

The FTIR spectra of samples were recorded by FTIR (model 4700, Thermo Nicolet, Japan). All samples (pure pectin (both LMP and HMP), pure zein, physical mixture of zein and pectin (1:4), polyelectrolyte complexes of 2 % w/w pectin and 0.05 % w/w (or 0.25 % w/w) zein) were prepared using the KBr disc method. Each sample was blended with KBr powder and compressed into a disc with a pressure of 5 tons for 60 s before being placed in a sample holder. The spectral value of the samples was obtained by scanning from 4000 to 400 cm^{-1}. FTIR spectra of the samples were obtained using a software package (Omnic FT-IR software, version 7.2a, Thermo Electron Corporation, USA).

Statistical analysis

Data were analyzed using SPSS version 11.5 for Windows (SPSS Inc., USA). The results were represented as mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to determine difference among the groups, and pairs were compared using either the Scheffé or Games-Howell test. The statistical significance was set at $p < 0.05$.

Results and discussion

Characterization of zein and pectin solutions

The zeta potential of zein and pectin solutions at various pHs was determined as shown in **Figure 1a**. The zeta potential of zein was about zero at pH ~ 5.5, indicating the isoelectric point (pI) of zein. At pH below pI value (pH < 5.5), zein was positively charged, while at pH above pI (pH > 5.5) it was negatively charged. This effect is attributed to a change in the ionization of amino and carboxyl groups at low pH ($-NH_3^+$; -COOH) and high pH ($-NH_2$; $-COO^-$). The zeta potential of pectin solutions was negatively charged at all pHs (from pH 2.5 to 7). As shown in **Figure 1a**, the negative charge of pectin increased with an increase of pH, due to dissociation of carboxyl groups to negative charge (e.g. COO^-). HMP showed less negative charge than LMP, as it contained fewer carboxyl groups in its structure. The quantity of carboxyl groups in pectin structure was calculated and found to be different (i.e. 47 % and 20 % for LMP and HMP, respectively) [21].

The particle size of zein solution at various pHs was determined as shown in **Figure 1b**. The results demonstrated that the particle size of zein increased sharply when pH was close to pH ~ 5.5, i.e., the particle size changed from 2 μm at pH 4 to 45 μm at pH 5.5. This indicated that the extensive droplets aggregation may occur at pH close to the pI of zein. This is confirmed by the photograph of zein solution at various pHs (**Figure 2a**). The zein solution was turbid, with some precipitates at pH ~ 5.5. A clear solution of zein was observed at pH below 4.5 and pH above 7. Therefore, it is confirmed that the pI value of zein was at pH 5.5.

Figure 2b shows the turbidity of zein and pectin solutions at various pHs. The results showed that zein solutions were more turbid than pectin solutions. High absorbance at pH ranging from 4.5 to 6.5 was observed. Moreover, the absorbance of zein solution at pH 4.5 - 6.5 tended to increase with an increased concentration of zein. This may result from the increased amount of the insoluble form of zein [22]. The turbidity of pectin solution at various pHs was also investigated. The results showed that the turbidity of HMP was not significantly different at the investigated pHs (from 2.5 to 7.5). However, the turbidity of LMP, at low pH (2.5 - 3.5), was more than that of HMP, and gave a clear solution at pH 4 and above. It is possible that the ionization of carboxylic acid is suppressed at low pH, resulting in a reduction in hydration of the carboxylic acid group.

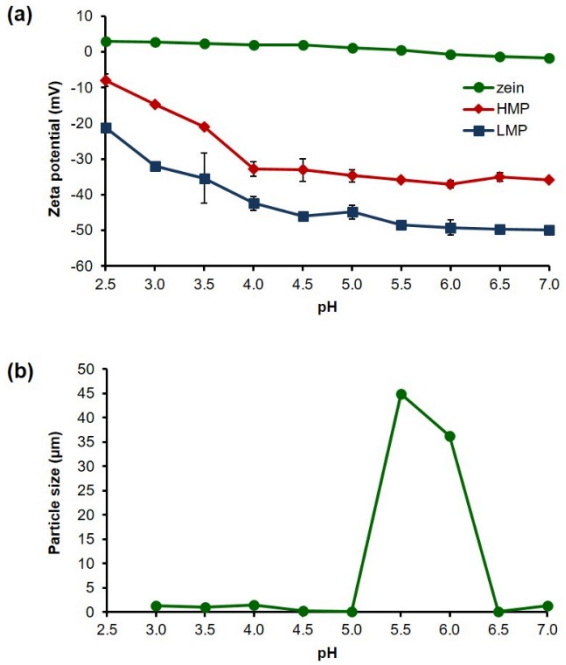

Figure 1 (a) Zeta potential of 1 % w/w LMP, 1 % w/w HMP and 0.125 % w/w zein and (b) particle size of zein solution (0.125 % w/w), at various pHs.

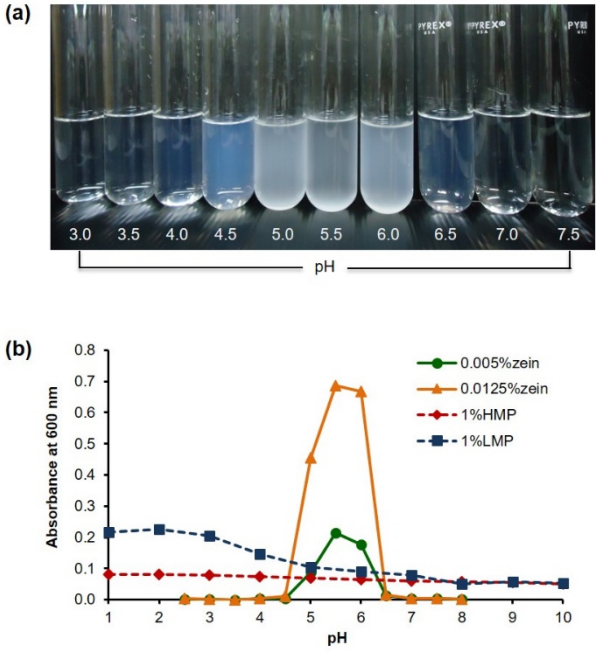

Figure 2 (a) Photograph of 0.125 % w/w zein solution and (b) turbidity of zein and pectin solutions at various pHs.

Figure 3a shows the viscosity of pectin (LMP and HMP) solution at various concentrations. The viscosity of pectin solution increased when the concentration of pectin was increased. This may result from the increase in hydrogen bonding of water with hydroxyl groups of pectin structure and the distortion in the velocity pattern of the liquid by hydrated molecules of the solute [23]. The surface tension of pectin (LMP or HMP) at various concentrations was investigated, as shown in **Figure 3b**. The results demonstrated that, at low concentration, the surface tension of LMP and HMP solutions was not significantly different. However, the surface tension of LMP solution tended to decrease at higher concentrations (1 % w/w or more). This may be due to the fact that concentration of LMP is already in excess, above or close to the interfacial saturation [4]. However, the expansion of the air-water interface is not practical in the preparation of o/w emulsion, so the interfacial tension between oil and water was also investigated.

Interfacial tension measurements using rice bran oil were then performed on pectin. These measurements were done to compare them against surface tension data (**Figure 3b**) and to gain information about the behavior of pectin at the oil-water interface. **Figures 3c** and **3d** show the time evolution of the interfacial tension for rice bran oil and 0.5 - 2 % w/w LMP or HMP. The interfacial tension slightly decreased with an increase of time, and a constant value was observed at more than 600 s. This may be due to the time consumed for adsorption of macromolecules on the interface followed by rearrangement in the surface film [24]. The oil/water interfacial tension readings at 1800 s (near equilibrium) of HMP were 17.2, 16.5, and 15.4 mN/m for 0.5, 1, and 2 % w/w HMP, respectively, while those of LMP were 14.0, 14.7, and 13.4 mN/m for 0.5, 1, and 2 % w/w LMP, respectively. It could be seen that the interfacial tension of pectin decreased with the increased pectin concentration, resulting from the more surface active character of pectin molecules. An exception is for 1 % w/w LMP, which is slightly higher than that of 0.5 % w/w LMP.

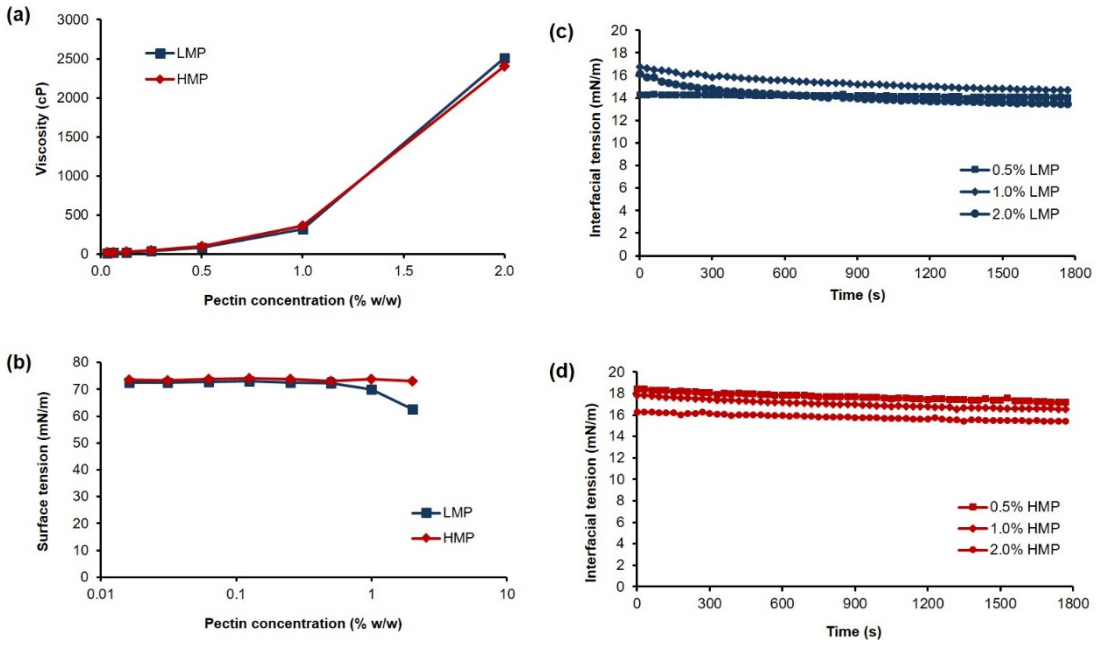

Figure 3 (a) Viscosity and (b) surface tension of pectin (LMP or HMP) at various concentrations; and time evolution of the interfacial tension for rice bran oil and 0.5 - 2 % w/w (c) LMP or (d) HMP, at 25 °C.

Characterization of pectin-zein polyelectrolyte complexes

1. Zeta potential

Zeta potential is an analytical tool that could point to the existence of strong charge-charge interactions between 2 biopolymers. Therefore, experiments were carried out under conditions where proteins and polysaccharides had opposite charges [25], i.e., at pH 4. **Figure 4a** shows the zeta potential of mixtures prepared from various concentrations of zein (0 - 0.01 % w/w) and 2 % w/w pectin (LMP or HMP), at pH 4. The results showed that, for HMP, the zeta potential increased from –17 mV to –13mV in the presence of zein concentration in the range of 0.001 - 0.004 % w/w, resulting from neutralization of cationic zein molecules to pectin surface [25]. The zeta potential of the polyelectrolyte complexes in the presence of 0.006 - 0.010 % w/w zein was slightly more negative than in the absence of zein. It is possible that the low surface charge of zein molecules could form bridges between pectin molecules and show the negative charge [3]. Furthermore, the zeta potential of LMP in the presence of zein was highly negative and close to that of pure LMP solution (–45 mV). This may be because the concentration of zein is not enough for adsorption to the surface of LMP molecules. Therefore, it is suggested that the concentration of zein primarily influenced the formation of zein-pectin complexes, especially those using HMP.

2. Turbidity

The solutions containing 2 % w/w pectin and different concentrations of zein were left to settle overnight, and formation of complexes was assessed by turbidity measurement at pH 4. The pure zein solution was clear, with about 100 % transmittance. The turbidity of pectin-zein solutions increased with the increased concentration of zein (**Figure 4b**). This may be due to the increased amount of insoluble pectin-zein polyelectrolyte complexes in the system, resulting from the strong interaction between the 2 oppositely charged biopolymers [21].

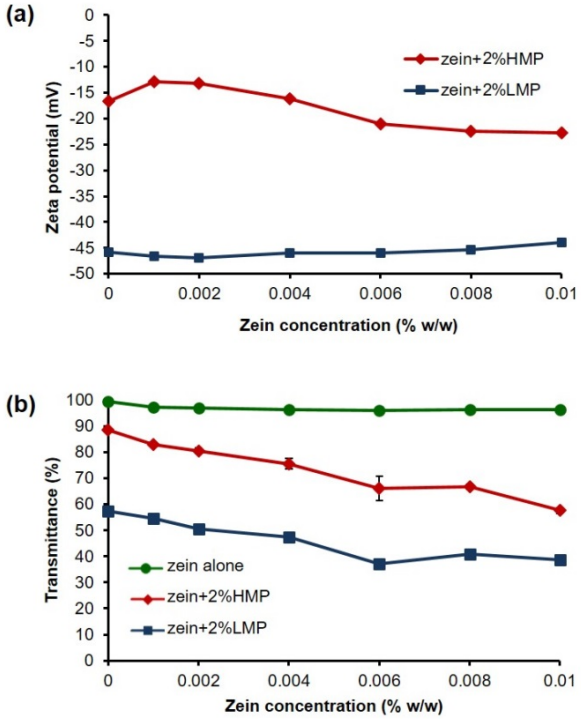

Figure 4 (a) Zeta potential and (b) turbidity of mixtures prepared from various concentrations of zein (0 - 0.01 % w/w) and 2 % w/w pectin (LMP or HMP), at pH 4.

3. Morphology

To confirm the complex formation between pectin and zein at pH 4, zein with various concentrations was mixed with pectin solution and then investigated visually, as shown in photographs of the pectin-zein polyelectrolyte complexes (**Figure 5a**). It is clearly observed that the solutions were turbid. It is possible that the bridging flocculation occurred in the mixed solutions. Moreover, the turbidity of pectin-zein polyelectrolyte complexes increased when zein concentration was increased, resulting from the increased amount of pectin-zein complexes, as discussed above. It is apparent that a mixture of LMP and zein was extensively aggregated, whereas a mixture of HMP and zein was fairly homogeneous. This is probably due to the electrostatic bridging phenomena of LMP, leading to the formation of large particle size. **Figure 5b** shows optical microscopic images of pectin-zein polyelectrolyte complexes containing 2 %w/w pectin (LMP or HMP) and zein (0.05 or 0.25 % w/w). The particulates and fibrous materials were observed in the mixtures. It is likely that the formation of insoluble complexes between protein (i.e., zein) and polysaccharide (i.e., pectin) occurred via electrostatic interaction [4].

The surface morphology of the pectin-zein polyelectrolyte complexes was also investigated by SEM, as shown in **Figure 6**. The SEM micrographs demonstrated the smooth surface of pectin film, both LMP and HMP. In the presence of zein, the round-shaped, submicron-sized globules spreading over surface film were observed. Moreover, the surface of pectin-zein complexes was rougher when the concentration of zein was increased. It is suggested that the formation of pectin-zein complexes resulted from electrostatic interaction between pectin and zein [4].

4. FTIR spectroscopy

FTIR spectra of pectin-zein complexes are shown in **Figure 7**. The obtained spectra were analyzed by comparing the following characteristic regions; O-H stretching (3,100 - 3,600 cm^{-1}), C-H stretching (2,800 - 3,000 cm^{-1}), carboxylic group stretching (1,200 - 1,800 cm^{-1}), and the fingerprint region (under 2,000 cm^{-1}), which reflects the monosaccharide composition of the analyzed pectin [26]. FTIR spectrum of zein showed O-H stretching from H-bond between 3,200 and 3,400 cm^{-1}, and the peaks at 1,550 - 1,500 cm^{-1} and 1,700 - 1,600 cm^{-1} corresponded to the characteristic transmission of primary amide and secondary amide, respectively, which are typical protein transmission bands [27]. **Figure 7a** showed that no new transmission peak or shifting was found from the FTIR analysis. The fingerprint region of the pectin-zein complexes was also similar to that of pectin. However, there were some differences in the FTIR spectra between the physical mixture and the pectin-zein complexes. After pectin-zein complex formation, the peak intensity at 1,735 - 1,750 cm^{-1}, corresponding to ester carbonyl stretching (C=O) of pectin, increased, while the peak intensity between 1,615 and 1,630 cm^{-1}, corresponding to carboxylate ion stretching (COO^{-}) band, decreased, compared to the physical mixture of pectin and zein at the same ratio. These observations suggest the possibility of weak physical interaction between pectin and zein. Moreover, the increase in zein concentration led to the change in peak intensity at 1,735 - 1,750 cm^{-1}, indicating attraction between pectin and zein resulting from the hydrogen bonding. In addition, the broad band spectrum of OH stretching increased with an increase in gelatin concentration. Similar results were observed in case of HMP (**Figure 7b**).

5. Surface and interfacial tension

Figure 8a shows the surface tension of zein and HMP-zein polyelectrolyte complexes prepared from 2 % w/w HMP and various concentrations of zein (0.0001 - 0.1 % w/w), at pH 4. The results demonstrated that the surface tension of zein solution rapidly decreased from 75 to 55 mN/m with an increased concentration of zein from 0.01 to 0.05 % w/w. Similarly, in the case of pectin-zein complexes, surface tension tended to decrease when zein concentration was increased. It is likely that zein, which has an amphiphilic character, can adsorb at the air-water interface and thus decrease the surface tension [28]. However, the decrease in surface tension of pectin-zein complexes was less extent than that of zein solution alone. This may be due to the fact that pectin molecules with low surface activity were located at the air-water interface, replacing some molecules of the zein [29]. This finding indicated that the

concentration of pectin-zein complexes could influence the adsorption properties at the interface between the oil and water phases.

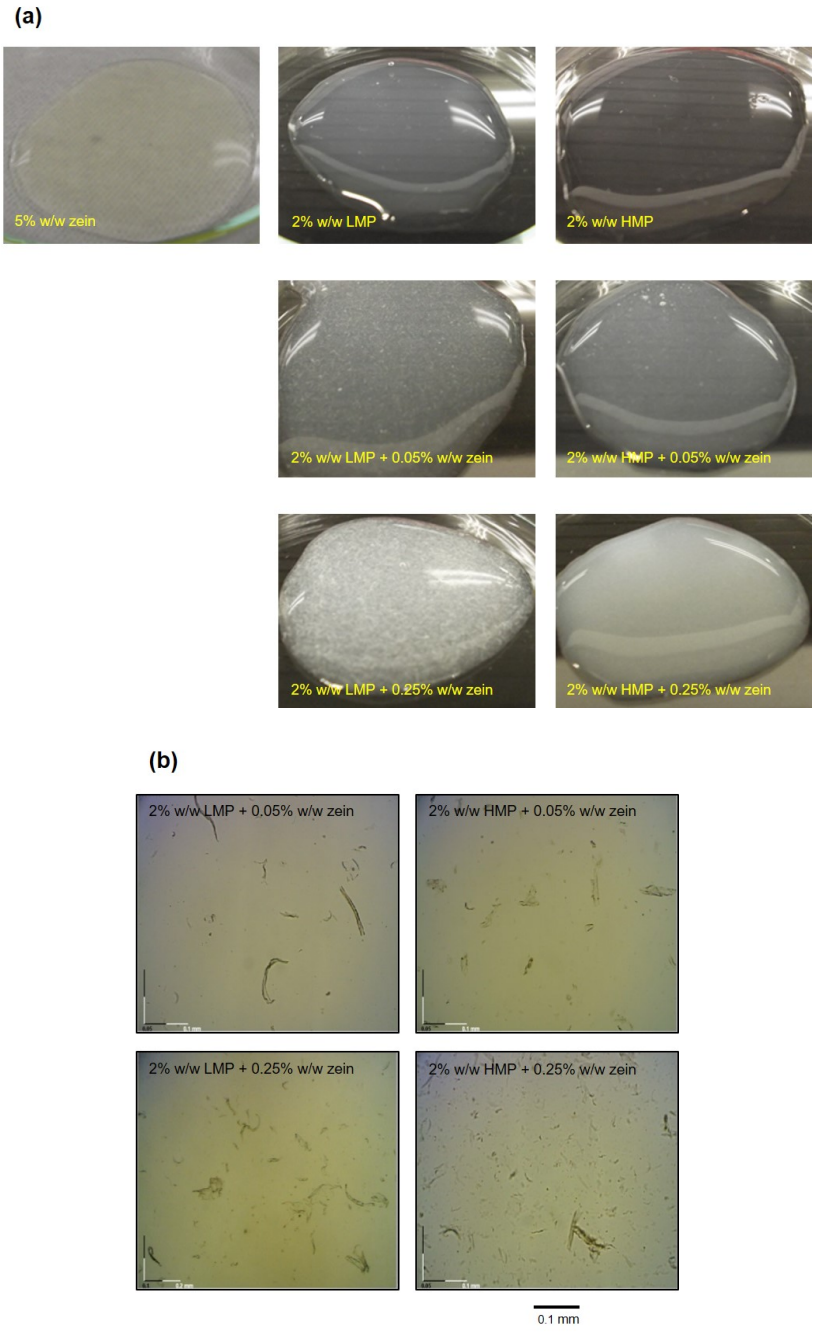

Figure 5 (a) Photographs of zein, pectin and pectin-zein polyelectrolyte complexes containing 2 % w/w pectin (LMP or HMP) and zein (0.05 or 0.25 % w/w), at pH 4; and (b) optical microscopic images of pectin-zein polyelectrolyte complexes containing 2 % w/w pectin (LMP or HMP) and zein (0.05 or 0.25 % w/w), at pH 4.

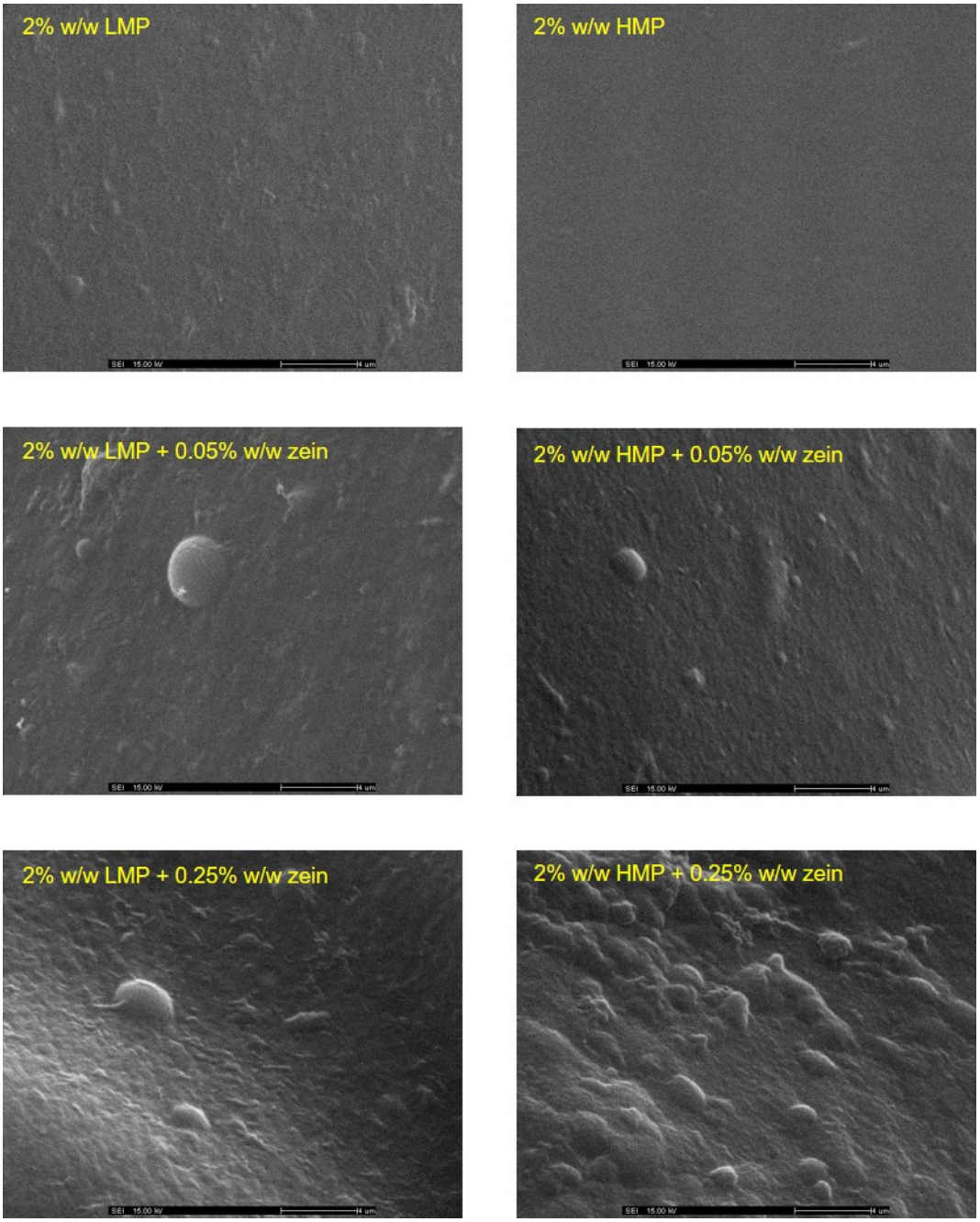

Figure 6 SEM micrographs (5000×) of pectin-zein polyelectrolyte complexes containing 2 % w/w pectin (LMP or HMP) and zein (0.05 or 0.25 % w/w), at pH 4.

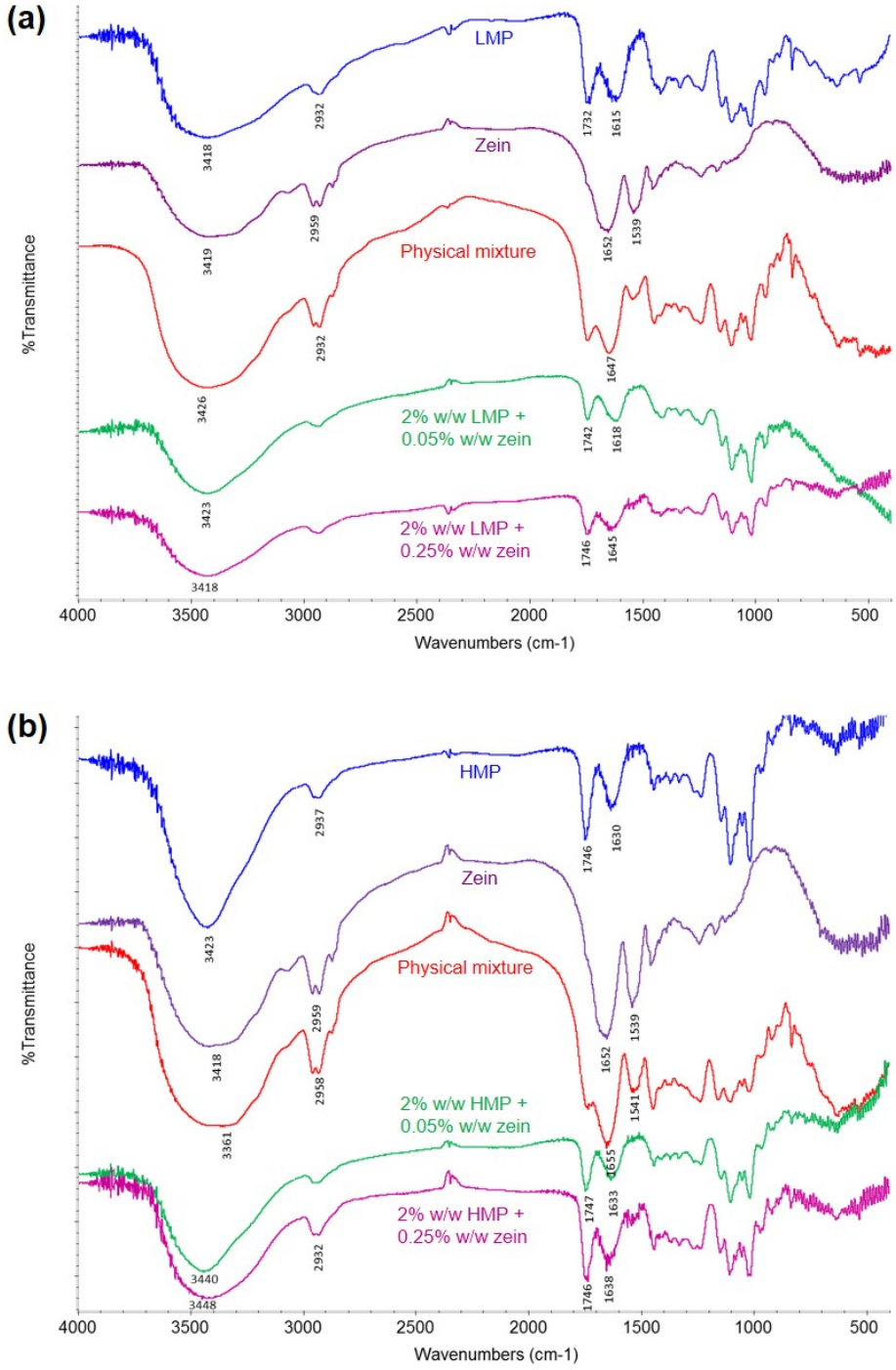

Figure 7 FTIR spectra of pectin, zein, physical mixture of zein and pectin (1:4), polyelectrolyte complexes of 2 % w/w pectin, and 0.05 % w/w (or 0.25 % w/w) zein; (a) LMP and (b) HMP.

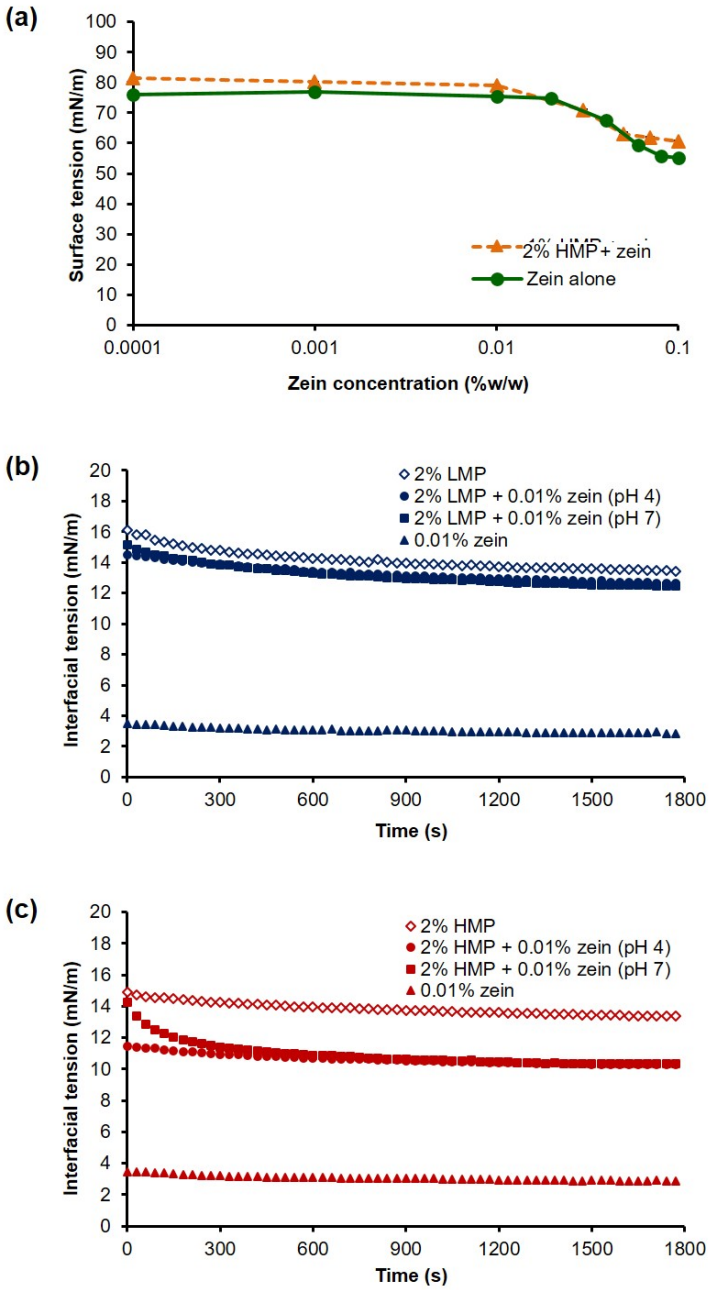

Figure 8 (a) Surface tension of zein alone and HMP-zein polyelectrolyte complexes prepared from 2 % w/w HMP and various concentrations of zein (0.000 - 0.1 %w/w), at pH 4; and time evolution changes of the interfacial tension for rice bran oil containing zein, pectin, or a mixture of zein and (b) LMP or (c) HMP, at 25 °C.

Figures 8b and **8c** demonstrate the time evolution of the interfacial tension for rice bran oil containing pectin, zein, and a mixture of pectin and zein. It could be seen that zein needed less time to achieve equilibrium, while pectin and pectin-zein mixtures needed time for adsorption of macromolecules

on the interface followed by rearrangement at the interface [4]. The interfacial tension readings measured at 1800 s (near equilibrium) for different systems were significantly different; this contrasted with the surface tension data. The lowest interfacial tension of zein, nearly 2.9 mN/m, was due to the effect of solvent (alcohol) used for zein preparation. Pectin, LMP, and HMP showed interfacial tensions of about 13.4 mN/m. The interfacial tension of pectin-zein polyelectrolyte complexes was better than that of pectin alone when using the same pectin concentration. It is also apparent when comparing the polyelectrolyte complexes using LMP and HMP; the interfacial tension at 1800 s of HMP (about 10.3 mN/m) was lower than that of LMP (about 12.4 mN/m). This is because HMP had more hydrophobic groups than LMP, and thus exhibited a good emulsifying property [3]. In order to compare the pH conditions between pectin-zein complexes, interfacial tensions of rice bran oil and zein mixture at pH 4 and 7 were investigated. The results, however, showed that the interfacial tensions at pH 4 and 7 were not significantly different.

Conclusions

In this study, the formation of pectin-zein polyelectrolyte complexes under appropriate conditions was proposed. To gain more understanding about the pectin-zein polyelectrolyte complexes, the properties of the complexes were studied. Various concentrations of zein were mixed with pectin solution at pH 4, and the morphology of pectin-zein polyelectrolyte complexes was observed from digital images, optical microscopic images, and SEM micrographs. When the concentration of zein was increased, the turbidity of pectin-zein mixture increased, but the surface tension of pectin-zein mixture decreased. The interfacial tension of pectin-zein complexes was higher than that of zein alone, and slightly less than that of pectin alone. FTIR results confirmed the possibility of weak interaction between pectin and zein. The complexes obtained could be used to improve the physical properties and stability of emulsions. In future studies, it would be useful to investigate the possibility of using pectin-zein complexes to stabilize oil-in-water emulsions.

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