Properties of thermoplastic maize starch-zein composite films prepared by extrusion process under alkaline conditions

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Highlights

- Large zein aggregates within the starch matrix show incompatibility.
- Sodium hydroxide during extrusion shows small zein aggregates.
- Better plasticization of starch and zein with sodium hydroxide
- Increase in elongation of the starch-zein composite film sodium hydroxide.

Abstract

This work investigates the effect of the addition of NaOH on the compatibility and material properties of thermoplastic starch-zein composite films produced by a twin-screw extruder. Thermoplastic starch-zein composite films were produced by melt extrusion of glycerol-plasticized starch and zein (3:1 ratio) treated with different concentrations of sodium hydroxide (NaOH) (0 M, 0.05 M, 0.1 M, and 0.2 M NaOH). Scanning Electron Microscope and Confocal laser Scanning Microscope revealed that the composite without NaOH formed a phase-separated morphology with large zein aggregates within the starch matrix. However, the increase in NaOH concentration reduced the size of zein aggregates within the starch-zein composite films, with 0.2 M NaOH having the smallest size of zein aggregates. Dynamic mechanical analysis showed a decrease in glass transition temperature (T_g) and storage modulus (E'), suggesting more molecular chain mobility and efficient plasticization of starch and zein. This efficient plasticization was also confirmed by Fourier-Transform Infrared spectroscopy (FTIR). As a result, there was an optimal increase of 28% in elongation at break in the starch-zein composite film with 0.2 M NaOH. In conclusion, compatible thermoplastic starch-zein composite films with improved elongation at break can be produced with a twin-screw extruder by adding 0.2 M NaOH.

Graphical abstract
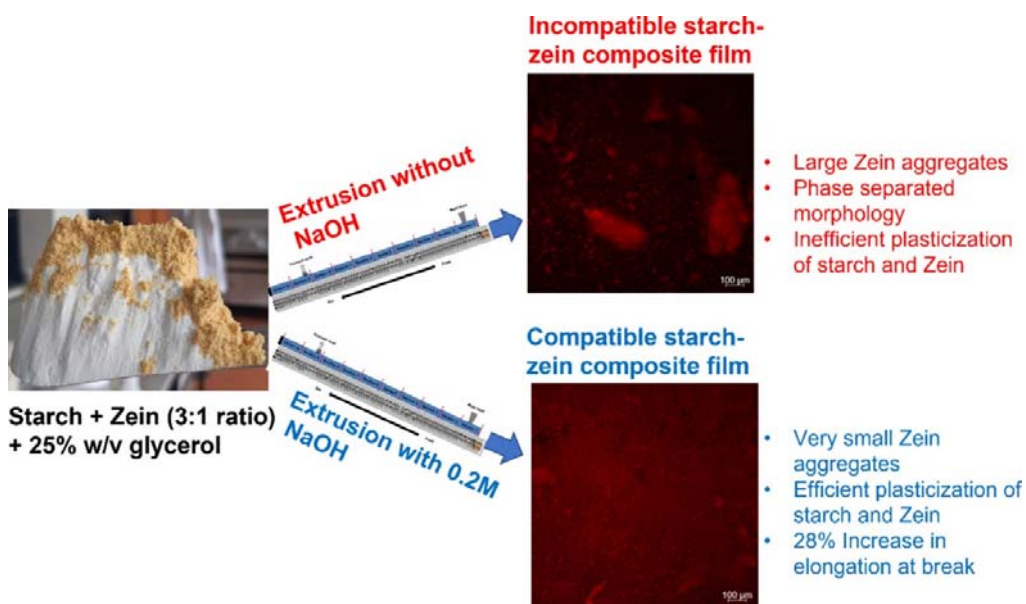
Keywords: Compatibility; Aggregates; Plasticization; Tensile properties

1. Introduction

The non-biodegradable nature of many petroleum-based plastics has motivated research into biodegradable plastics as alternatives. Petroleum-based plastics are resistant to microbial degradation and cause environmental pollution [1], [2]. Like petroleum-based plastic films, biodegradable plastic films must provide good mechanical and barrier properties [3]. Bio-based polymers, such as starch and zein, have gained attention in producing biodegradable plastic films for food applications and in the biomedical field [4]. Unfortunately, starch and zein films have poor mechanical and barrier properties compared to petroleum-based plastic films.

The blending of starch and zein has gained attention because (1) they have complementary properties, starch is hydrophilic, and the zein prolamin protein is hydrophobic, (2) they are both biodegradable, (3) there is a sustainable source of raw material. In addition, the blending of starch and zein will reduce the cost compared to using zein alone. However, starch and zein are immiscible and affect the overall performance of the thermoplastic starch-zein composite films [5], [6]. Chanvrier et al. [5] showed that the mechanical properties of the starch-zein composite films were inferior compared to those of the starch and zein films alone. The composite exhibited a particle-matrix system in which zein appeared as aggregates within the starch matrix, indicating poor adhesion between the two. Leroy et al. [7] reported that the ionic liquid (1-butyl-3methyl imidazolium chloride [BMIM]Cl) resulted in better compatibilization compared to glycerol-plasticized starch-zein composite films.

Teklehaimanot et al. [8] produced solvent-cast compatible starch-zein films using 0.1 M NaOH as a solvent, and these films were reported to have improved tensile strength. In their previous study, Teklehaimanot et al. [9] showed that a compatible colloidal blend of starch and zein could be formed using 0.1 M NaOH as a solvent. This was due to the ionic interaction between

the negatively charged deamidated zein residue, starch alkoxide, and Na$^+$. Therefore, these two studies show that NaOH can induce compatibility between starch and zein. However, solvent casting had some limitations; (1) during film formation, NaOH became concentrated and corrosive, making the film unsuitable for application in food packaging, (2) solvent casting is time-consuming and not scalable for large industrial applications. Therefore, this necessitates the investigation of the production of NaOH-treated compatible starch-zein composite films with typical melt-extrusion methods that can be scaled up to an industrial level. The use of an alkali (NaOH) to produce compatible starch-zein composite films.

Previous studies have demonstrated the processability of starch and zein using melt-processing techniques. For example, single screw extruder [5], Haake Rheomix [6], [10], twin conical screw mini-compounder [7], [11] were previously used to melt-process starch-zein composites. The production of compatible starch-zein composite films treated with NaOH using melt processing techniques has not been previously reported in the literature. The melt-processability of starch-zein composite films treated with NaOH can easily be scalable to the industrial level for application in food packaging and the biomedical field. The use of NaOH offers various advantages to produce compatible starch-zein composite films, it is widely used in the industry for various applications, easy to handle, does not produce toxic fumes during extrusion, and it is relatively inexpensive compared to other chemicals. For example, Leroy et al. [7] used an ionic liquid [BMIM]Cl that is expensive as a plasticizer to produce starch-zein composite films. Therefore, the purpose of this study is to investigate the effect of NaOH concentration (0 M, 0.05 M, 0.1 M, and 0.2 M NaOH) on the compatibility and material properties of thermoplastic starch-zein composite films produced using twin-screw extrusion followed by compression molding. It is hypothesized that the addition of NaOH during extrusion of glycerol-plasticized starch and zein will cause microstructural and molecular changes on zein and starch. Therefore, this will improve the compatibility and the resulting mechanical properties of the thermoplastic starch-zein composite films compared to the composite films without NaOH.

2. Material and methods

2.1. Materials

Commercial maize starch (Amyral©) with 27.23% amylose was obtained from Tongaat Hullet starch (Johannesburg, South Africa). The protein, ash, fat and total starch content were 0.62%, 0.03%, 0,28% and 96.0% respectively per dry basis and 10% moisture. Commercial zein (Z3625) is mainly α-zein and was procured from Sigma Aldrich (Johannesburg, South Africa). Zein was defatted three times at ambient temperature using n-hexane (1 part Zein: 3 parts hexane). The moisture and protein content of zein was 10.21% and 90.1% (N × 6.25), respectively. Glycerol and sodium hydroxide were procured from Merck (Johannesburg, South Africa). All chemicals used were analytical grade.

2.2. Methods

2.2.1. Preparation of thermoplastic starch, zein, and starch-zein composite films

The composition of thermoplastic starch (ST), thermoplastic zein (ZN), and thermoplastic starch-zein composites (ST-ZN) is outlined below. The ST-ZN composite was prepared by mixing starch and zein in a 3:1 ratio. The moisture content of the biopolymers was adjusted to 40% by adding water and different concentrations of NaOH. Thus, the codes are:

- ST H$_2$O is 100% starch with water.
- ST 0.1M NaOH is 100% starch with0.1 M NaOH.
- ZN H$_2$O is 100% zein with water.
- ZN 0.1M NaOH is 100% zein with 0.1 M NaOH
- ST-ZN H$_2$O is 3 starch: 1 zein mixture with water.
- ST-ZN 0.05M NaOH is 3 starch: 1 zein mixture with 0.05 M NaOH.
- ST-ZN 0.1M NaOH is 3 starch: 1 zein mixture with 0.1 M NaOH.
- ST-ZN 0.2M NaOH is 3 starch: 1 zein mixture with 0.2 M NaOH.

About 25% (*w*/w) glycerol was added as a plasticizer in all treatments. The plasticized materials were equilibrated at room temperature for 2 h before extrusion. Thermoplastic materials were produced by extrusion using a twin-screw extruder (Process 11, Thermo Scientific, Waltham, MA, USA) with an L/D of 40. The extruder conditions were optimized: Temperature profile 80|90|110|110|120|120|120|120 °C (feeding to die zone), and the screw speed was 202 rpm. The materials were fed into the extruder assisted using a piston. The extrudates were pulverized in liquid nitrogen using a coffee grinder to produce a powder. The powders were then thermo-compress molded for various characterizations using a Carver compression molder (Carver, USA, mode 973214A) for 6 min at 120 °C at a pressure of 1 MPa.

2.3. Characterization

2.3.1. Tensile properties

The test samples were conditioned at 53% relative humidity (RH) in a desiccator for 48 h before testing. The tensile properties of the type V dog bone test specimen were prepared and tested according to ASTM D638–14 [12] using the instrument (Instron, Norwood, USA) loaded with a tensile grip of a 10 kN load cell at room temperature. The initial distance from the grips was 25 mm. The cross-sectional speed was 5 mm/min. At least 10 samples were tested per treatment, and an average of 5 samples were reported. The ultimate tensile strength and elongation at break were taken from the stress-strain curve. Young's modulus was calculated as the gradient of the linear region of the stress-strain curve.

2.3.2. Scanning electron microscope (SEM)

The compress-molded specimens of thermoplastic starch, zein, and starch-zein composites were freeze fractured in liquid nitrogen to preserve the cross-sectional morphology. The freeze-fractured samples were sputter-coated with carbon. The cross-sectional morphology of the samples was analyzed using the scanning electron microscope SEM (JSM-7500, JOEL, Japan) at an acceleration voltage of 3.0 kV.

2.3.3. Confocal laser scanning microscope (CLSM)

The surface morphology of thermoplastic starch, zein, and starch-zein composite films were analyzed to understand the distribution of zein protein within the starch matrix using CLSM based on a method described by Chanvrier et al. [5] with some modification. Protein staining was performed using 0.01% (*w*/v) fuchsin acid dissolved in water. Staining of the films was performed by a few drops of the dye and left for 5 mins. The stained films were extensively washed with distilled water to remove excess dye. The films were placed on a microscope slide and covered with the glass slides, and the cover slides were sealed with an adhesive tape. The

samples were analyzed using Zeiss LSM 880 confocal laser scanning microscope (Jena, Germany). The 10 × magnification lenses were used. The excitation wavelength of 405 nm was used, and the emitting wavelength was 425–475 nm.

2.3.4. Dynamic mechanical thermal analysis (DMTA)

According to ASTM E 1640–40 [13], the thermomechanical properties were analyzed using PerkinElmer DMA, 800 (USA). The compress molded specimens with dimensions (24.8 mm length × 9.8 mm width × 1.6 mm thickness) were conditioned at 53% RH for 48 h before the analysis. The test was conducted in a dynamic mode using the dual cantilever geometry. The test was conducted in a multi-frequency mode at 1 Hz and 10 Hz in a temperature range −80 °C to 100 °C at a heating rate of 2 °C/min. The storage modulus and glass transition temperature were reported at 1 Hz. The glass transition temperature (T_g) was taken at the peak tan δ.

2.3.5. X-ray diffraction (XRD) analysis

The crystallinity of the films was analyzed using the X-ray diffractometer (XRD, XPERT PRO PANalytical, Netherlands). The instrument was operated at 45 kV and 40 mA in continuous mode using the CuKα with a secondary monochromator ($\lambda = 0.1545$ nm). The diffraction patterns were carried out at room temperature using Bragg-Brenton geometry with a scanning range of $2\Theta = 1$–$40°$ using a scanning rate of $0.01°$/s.

2.3.6. Attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy

The chemical structures of the films were analyzed using attenuated total reflectance Fourier-transform infrared spectroscopy (Perkin Elmer Spectrum 100 spectrometer, USA). FTIR was performed on films with about 0.1–0.2 mm thickness. The films were conditioned at 53% RH for 48 h before analysis. Background canning was performed before the samples. The spectra were collected in the wavelength range of 4000–600 cm^{-1} using 32 scans at a resolution of 4 cm^{-1}. The spectra were collected in duplicates and were baseline-corrected using the spectrum ® software. The secondary structure of zein was analyzed by deconvolution of thermoplastic zein and starch-zein composites in the amide I region using the software fityk 0.9.8.

2.3.7. Thermogravimetric analysis (TGA)

The thermomechanical properties of the samples were analyzed using TGA Q500 (TA Instruments, USA). About 10 mg of the samples were weighed in a platinum pan. The samples were heated from 25 °C to 700 °C at a heating rate of 10 °C/min under nitrogen gas (flow rate = 25 ml/min).

2.4. Statistical analysis

The statistical analysis of the data was conducted using the IBM SPPSS version 20.0 statistical software for Windows (Armonk, NY: IBM Corp.). Multiple analysis of variance was used to analyze the data, and the means were compared at $p \leq 0.05$ using the Turkey test. Experiments were done in duplicates unless stated otherwise. The thermoplastic starch, zein, and starch-zein composites with and without NaOH were independent variables, whereas the data obtained from the analyses were the dependent variables.
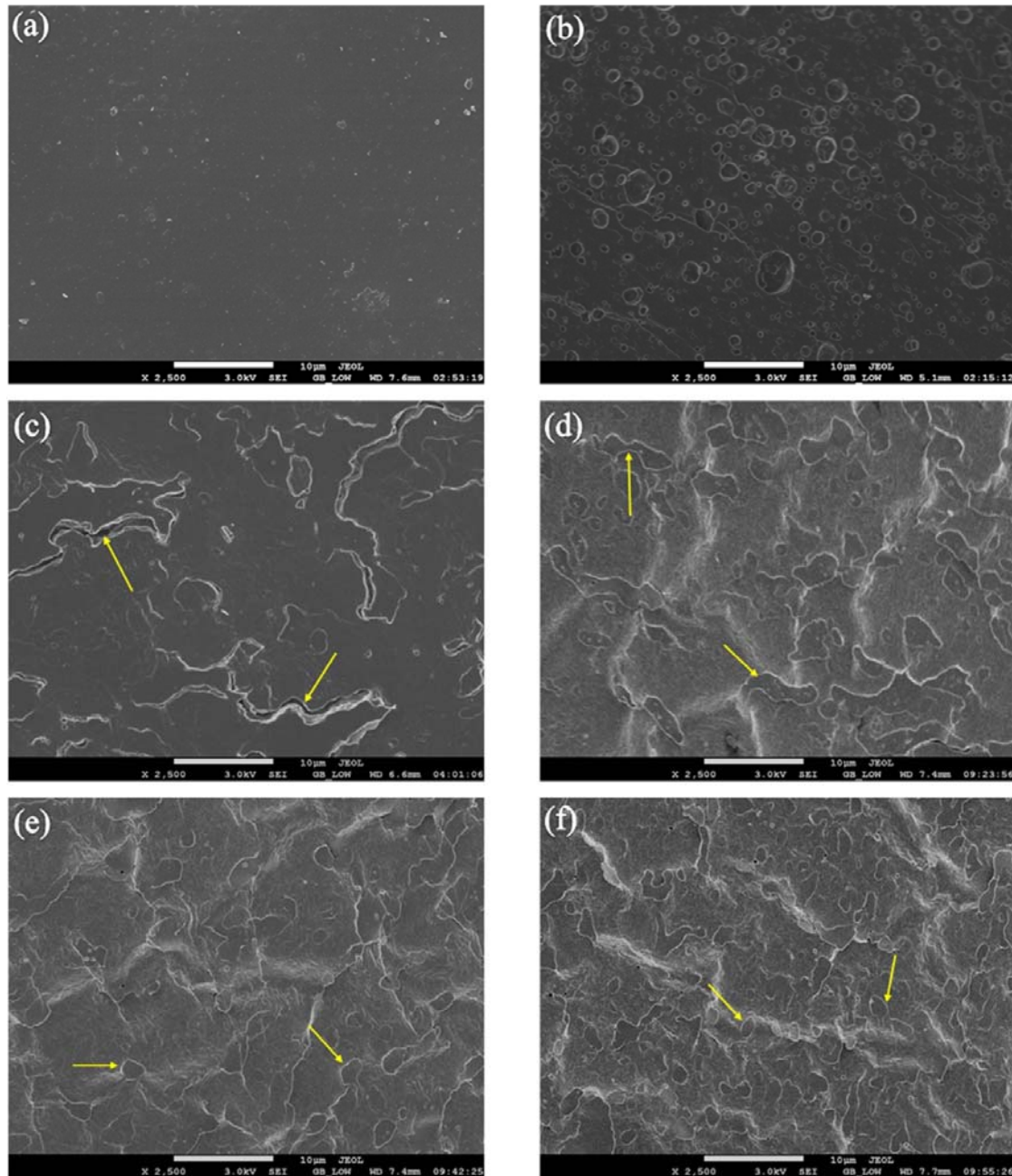
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Fig. 1. Scanning electron microscope of cross-sectional morphology of freeze-fractured (a) thermoplastic starch, (b) thermoplastic zein, (c) ST-ZN H2O, (d) ST-ZN 0.05 M NaOH, (e) ST-ZN 0.1 M NaOH, (f) ST-ZN 0.2 M NaOH. Scale bar = 10 μm.

3. Results and discussion

3.1. Effect of NaOH addition on the microstructure of thermoplastic starch-zein composite films

The cross-sectional microstructure of thermoplastic starch, zein, and starch-zein composite films treated with different concentrations of NaOH are shown in Fig. 1. The cross-sectional microstructure of thermoplastic starch is characterized by a smooth morphology without pores

or cracks but with some particles on the surface (Fig. 1a). Thermoplastic zein was characterized by a surface with many non-homogeneous irregular voids (Fig. 1b) that may be caused by trapped air and water droplets [14]. These voids were absent in thermoplastic starch (Fig. 1a) and suggested that starch had better film formation properties by extrusion compared to zein. The presence of imperfections (voids in this case) may be a source of crack formation, propagation, and failure leading to brittle zein with lower elongation at break [15].

Two phases can be identified in the cross-sectional microstructures of all thermoplastic starch-zein composite films (Fig. 1c-f), suggesting starch as a major matrix and zein as the dispersed phase (irregular aggregates). However, there is a reduction in the size of the zein aggregates within the starch matrix with an increase in the NaOH concentration. The starch-zein composite film with 0.2 M NaOH had the smallest uniform zein aggregate size, suggesting that 0.2 M NaOH induced optimal compatibility between starch and zein. In general, the uniform distribution of reduced aggregate size within a matrix indicates the compatibility between two polymer matrices [16], [17]. This suggests some compatibility between starch and zein with an increase in NaOH concentration. The reasons for this compatibility will be discussed together with the surface morphology as revealed by confocal laser scanning electron microscope (CLSM).

ST-ZN H_2O is starch-zein with water, ST-ZN 0.05 M NaOH is starch-zein composite with 0.05 M NaOH, ST-ZN 0.1 M NaOH is starch-zein composite with 0.1 M NaOH, ST-ZN 0.2 M NaOH is starch-zein composite with 0.2 M NaOH. All films were plasticized with 25% glycerol.

The CLSM images (Fig. 2) showed trends similar to the cross-sectional morphology by SEM. The starch matrix (most likely black), zein (most likely bright red), and starch-zein mixtures (most likely red) can be seen in the images (Fig. 2a-h). The starch-zein composite film in water (without NaOH) showed large zein aggregates (>100 μm) with a clear phase separation between starch and zein (Fig. 1c and Fig. 2e). This is because zein tends to aggregate when mixed with water. Teklehaimanot et al. [9] demonstrated that when zein is mixed with starch in water to form colloidal blends, zein tends to form large aggregates and separates out as determined by CLSM. Batterman-Azcona et al. [18] showed that zein aggregates might form during thermomechanical action due to disulfide bonds formation between zein molecules. The addition of NaOH affected the appearance of zein aggregates in the starch matrix. The increase in NaOH concentration resulted in a reduction in the size of zein aggregates within the starch matrix, with 0.2 M NaOH (Fig. 2h) having the smallest/ no zein aggregates. This suggests compatibility between starch and zein and the formation of a more homogeneous polymer composite system.
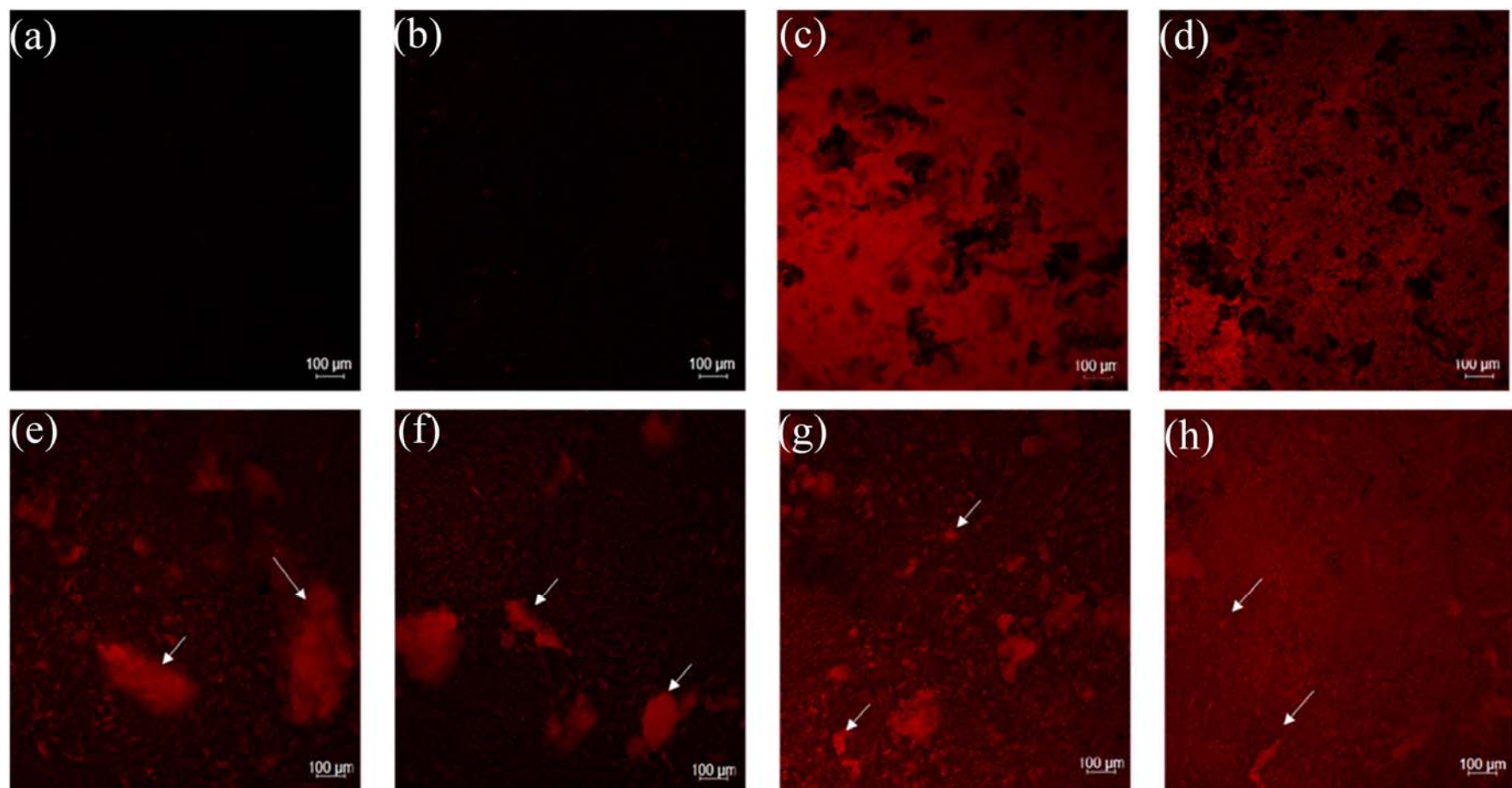
Fig. 2. CLSM images of ST H2O (a), ST 0.1 M NaOH (b), ZN H2O (c), ZN 0.1 M NaOH (d), ST-ZN H2O (e), ST-ZN 0.05 M NaOH (f), ST-ZN 0.1 M NaOH (g), ST-ZN 0.2 M NaOH (h) films. Scale bar = 100 μm. ZN H_2O is zein with water, ZN 0.1 M NaOH is zein with 0.1 M NaOH, ST H_2O is starch with water, ST 0.1 M NaOH is starch with 0.1 M NaOH, ST-ZN H_2O is starch-zein with water, ST-ZN 0.05 M NaOH is starch-zein composite with 0.05 M NaOH, ST-ZN 0.1 M NaOH is starch-zein composite with 0.1 M NaOH, ST-ZN 0.2 M NaOH is starch-zein composite with 0.2 M NaOH. All films were plasticized with 25% glycerol.

The compatibility between starch and zein during NaOH addition during extrusion may be due to the deamidation of the glutamine residue on zein. Deamidation may occur under an alkaline medium on the amide-containing asparagine and glutamine residue [9], [19], [20]. During this process, the amide group on the glutamine residue is cleaved, ammonia is released, and the amide group is converted to a carboxylic group. This carboxylic group is deprotonated to produce a negatively charged glutamate residue on zein [19], [21], [22].

The negatively charged deamidated zein molecules can electrostatically repel each other under alkaline conditions, preventing the formation of zein aggregates and promoting their distribution within the starch matrix as small uniform aggregates. In addition, starch may also undergo alkalization during extrusion under alkaline conditions. The starch hydroxyl groups can become activated and negatively charged to form a more reactive starch alkoxide (Starch O-) [23] that can also contribute to electrostatic repulsion. Therefore, negatively charged zein molecules within the negatively charged starch matrix and sodium ions (Na^+) may promote a more compatible polymer composite system with an even distribution of starch and zein [8]. These results confirm that adding 0.2 M NaOH during extrusion may produce compatible thermoplastic starch-zein composite films. The effect of this compatibility on the mechanical properties will be discussed in the next section.

3.2. Effect of NaOH addition on the tensile properties of thermoplastic starch-zein composite

The stress-strain curves of thermoplastic starch, zein, and starch-zein composite films with and without NaOH are shown in Fig. 3.
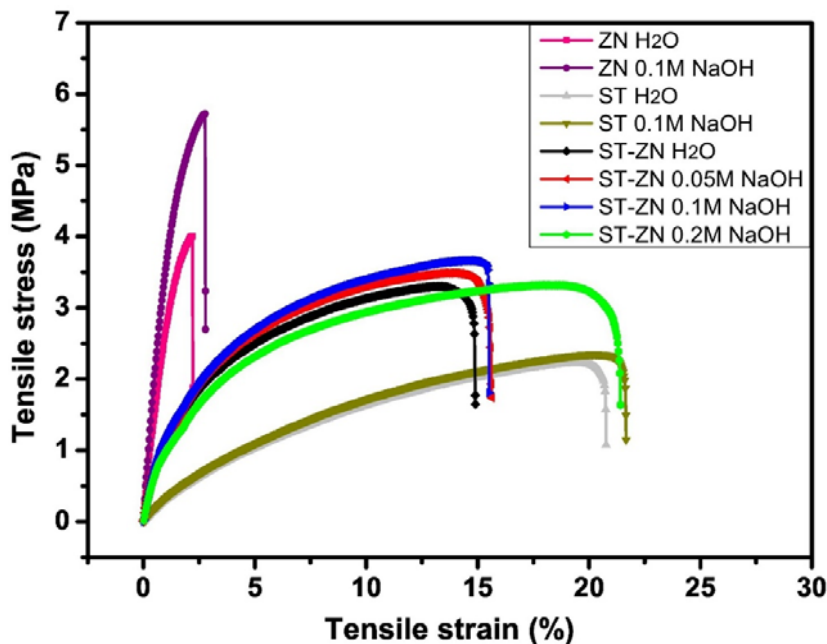

Fig. 3. Effect of NaOH on the tensile properties of thermoplastic starch, zein and starch-zein composite films. ZN H_2O is zein with water, ZN 0.1 M NaOH is zein with 0.1 M NaOH, ST H_2O is starch with water, ST 0.1 M NaOH is starch with 0.1 M NaOH, ST-ZN H_2O is starch-zein with water, ST-ZN 0.05 M NaOH is starch-zein composite with 0.05 M NaOH, ST-ZN 0.1 M NaOH is starch-zein composite with 0.1 M NaOH, ST-ZN 0.2 M NaOH is starch-zein composite with 0.2 M NaOH. All films were plasticized with 25% glycerol.

Thermoplastic zein with and without NaOH showed an elastic region without a yield point, and it did not deform plastically, suggesting that zein is brittle. In contrast, thermoplastic starch with and without NaOH and all thermoplastic starch-zein composite films showed a relatively ductile behavior and deformed plastically before fracture. The addition of zein (25% Wt) to starch increased tensile stress and Young's modulus while decreasing the elongation at break of the starch-zein composite film compared to the starch film alone. This suggests that the plastic deformation of the composite films was reduced by adding zein. This is because zein has a relatively high modulus and rigidity compared to starch. However, the plastic deformation of the compatible starch-zein composite film with 0.2 M NaOH was not significantly affected by the addition of Zein (Fig. 3). This is due to the reduced interfacial tension between the small zein aggregates dispersed within the starch matrix. Generally, the improvement in the compatibility is coupled with a reduction in interfacial tension and improvement in the ductility of the composite films [16], [17].

Starch-zein composite films without NaOH had lower tensile stress and Young's modulus than the composite films with 0.05 M and 0.1 M NaOH. This is most likely due to the poor interfacial adhesion between starch and zein in the composite without NaOH (Fig. 1c). The increase in tensile stress and Young's modulus in starch-zein composite films with 0.05 M and 0.1 M NaOH compared to the composite without NaOH may be related to the morphology (Fig. 1d and Fig. 1e). When a relatively ductile material (starch in this case) is reinforced by a high modulus dispersed phase (zein in this case), starch is highly restrained, and a significant portion of the load is carried by the high modulus dispersed phase leading to an increase in tensile stress and Young's modulus [24].

The elongation at break of the starch-zein composite films with 0.2 M NaOH increased significantly ($p < 0.05$) compared to the composite without NaOH and with 0.05 M and 0.1 M NaOH. As previously shown by morphology (Fig. 1c-f and Fig. 2e-h), the starch-zein composite film with 0.2 M NaOH had the smallest and uniform zein aggregates within the starch matrix. The reduction in the size of zein aggregates within the starch matrix reduced the interfacial tension between starch and zein, leading to significant improvements in elongation at break. Similar observations were made by Cao et al. [16] for compatible polyamide-polyphenylene composites. In addition, there was possibly more efficient plasticization of both starch and zein in the more compatible composite with 0.2 M NaOH leading to an increase in elongation at break. These results further suggest that using a medium in which both starch and zein are dispersible (NaOH) seems to result in more efficient plasticization and an increase in elongation at break. This was suggested by Leroy et al. [7] who demonstrated that the starch-zein composite film plasticized with 1-butyl-3-methyl imidazolium chloride, whereby both starch and zein are dispersible may lead to more efficient plasticization of both starch and zein, resulting in an increase in elongation at break. This will further be explored by the DMA and FTIR results.

3.3. Effect of NaOH on the thermomechanical properties of thermoplastic starch-zein composite film

The effect of NaOH on the damping factor (tan δ) and dynamic storage modulus (E') are shown in Fig. 4a and b, respectively. All the tan δ curves presented at least two tan δ maxima (T_g) (Fig. 4a). Thermoplastic starch films showed the first T_g around −52 °C, and the second T_g occurred over a broad temperature range around 59.4 °C (Fig. 4a). Thermoplastic zein without NaOH showed two T_g's at 65.1 and 89.5 °C that shifted to 58.1 °C and 95 °C in thermoplastic
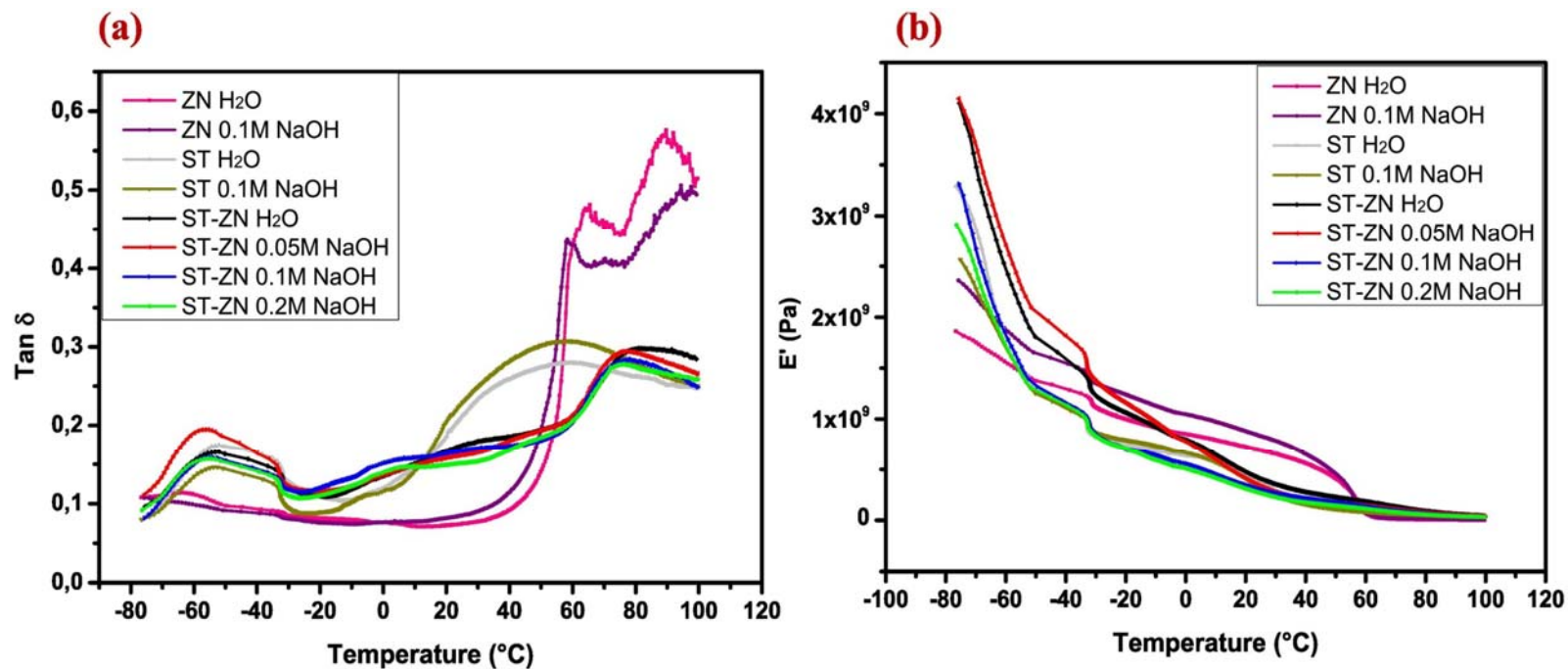
Fig. 4. Effect of NaOH on the thermal transitions (a) and dynamic storage modulus (b) of thermoplastic starch, zein and starch-zein composites. ZN H_2O is zein with water, ZN 0.1 M NaOH is zein with 0.1 M NaOH, ST H_2O is starch with water, ST 0.1 M NaOH is starch with 0.1 M NaOH, ST-ZN H_2O is starch-zein with water, ST-ZN 0.05 M NaOH is starch-zein composite with 0.05 M NaOH, ST-ZN 0.1 M NaOH is starch-zein composite with 0.1 M NaOH, ST-ZN 0.2 M NaOH is starch-zein composite with 0.2 M NaOH. All films were plasticized with 25% glycerol.

zein with 0.1 M NaOH. These two T_g's may correspond to the two zein subunits with different molecular weights [6].

Thermoplastic starch-zein composite films also presented two T_g's. The first T_g was around −52 to −55 °C and was ascribed to the glycerol-rich phase. The second T_g above 70 °C corresponds to starch, zein or starch-zein mixture. Thermoplastic starch-zein composite film without NaOH presented the second T_g at 82.3 °C, and this T_g decreased with an increase in NaOH concentration (Fig. 4a). Starch-zein composite film with 0.2 M NaOH had the lowest second T_g at 76.2 °C compared to all other composite films suggesting more molecular chain mobility. This decrease in T_g with an increase in NaOH concentration can be related to morphology. The reduction in the size of zein aggregates, as shown in Fig. 1, Fig. 2, may allow for better interaction between glycerol, starch, and zein resulting in more molecular chain mobility and a decrease in T_g.

In general, when a polymer composite system is fully compatible or miscible, the composite is characterized by a single T_g, which lies between the T_g of the individual polymers [25]. All the starch-zein composite films showed a single T_g that lies within the temperature range of the starch and zein film alone. This may suggest molecular miscibility between starch and zein in the composites with and without NaOH, or it may be superimposition with the T_g of starch and zein since they occur in the same temperature range. Teklehaimanot et al. [8] reported a single T_g in the starch-zein composite films prepared by solvent casting using 0.1 M NaOH as a solvent that was between the T_g of the starch and zein films alone, this suggested molecular miscibility between starch and zein.

The effect of NaOH on the E' will be discussed in different temperature ranges. At a temperature range, −80 to −60 °C thermoplastic starch films and starch-zein composite films had higher E' compared to zein films (Fig. 4b). At this temperature region, all molecular chains are immobile, therefore the crystalline domains of starch as seen in XRD have a reinforcing effect on the E'. This resulted in higher E' in the starch-zein composite films compared to the amorphous zein films. Crystals in semi-crystalline materials, such as starch and Poly-ε-caprolactone, were previously reported to have a reinforcing effect in composites with an amorphous polymer leading to higher E' [5], [17].

As expected, the decrease in E' with an increase in temperature in all the samples suggests the softening of the materials at higher temperatures. In the temperature range 0 to 50 °C, the starch-zein composite with 0.1 M and 0.2 M NaOH had lower E' compared to the composite without NaOH and with 0.05 M NaOH. This may be related to the surface morphology as shown by CLSM. It is possible that large zein aggregates (>100 μm) in the starch-zein composite films without NaOH and with 0.05 M NaOH resulted in lower chain mobility favoring stiffer behavior and leading to higher E'. The reduction in the size of zein aggregates in the composites with 0.1 M NaOH and 0.2 M NaOH possibly resulted in more molecular chain mobility due to more efficient plasticization. This efficient plasticization of starch and zein could explain the significant increase ($p < 0.05$) in elongation at break in the composite film with 0.2 M NaOH.
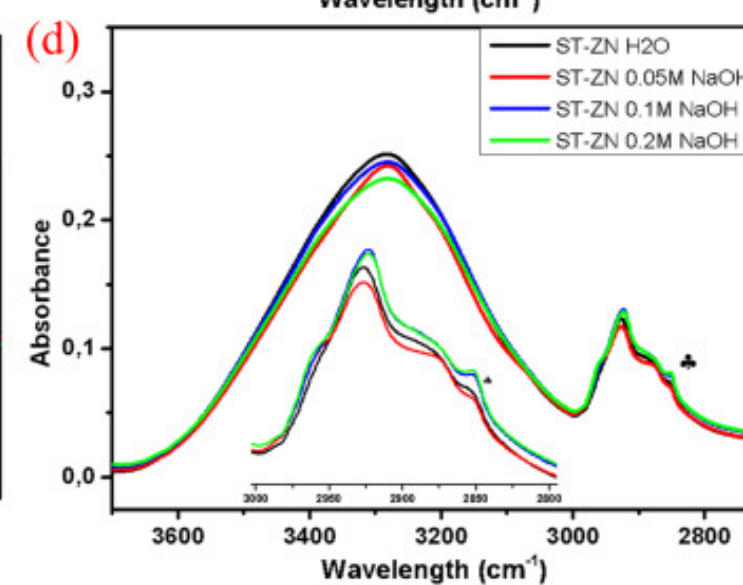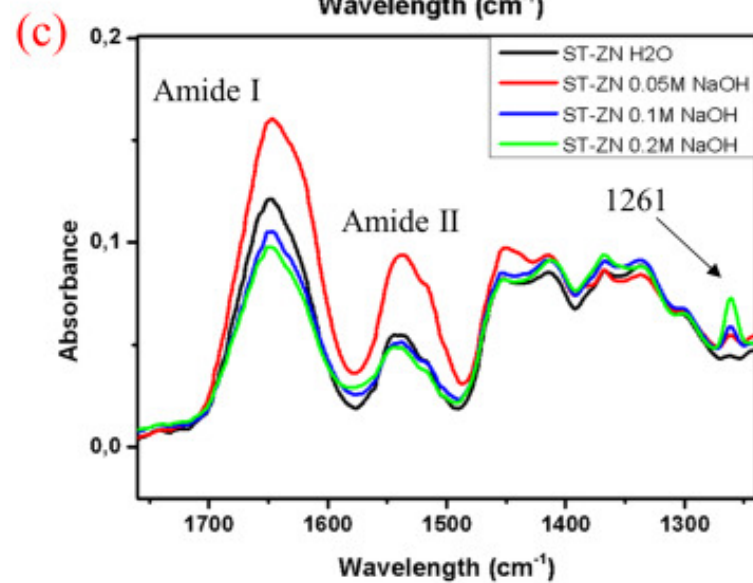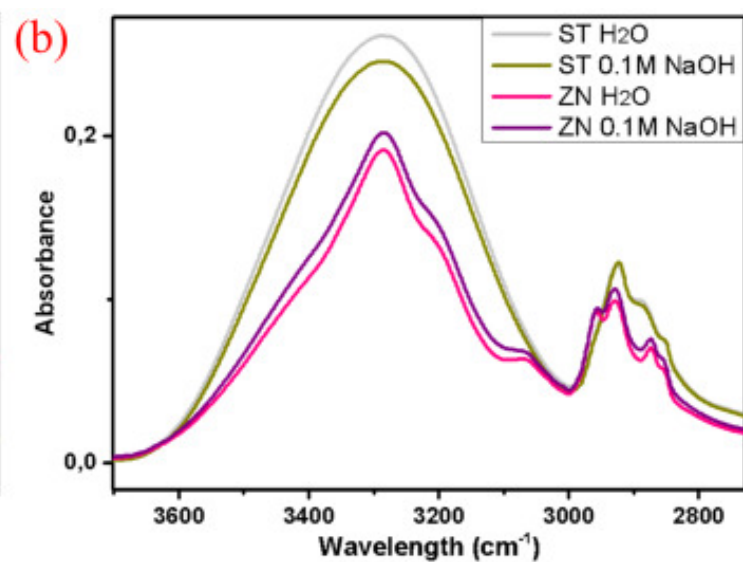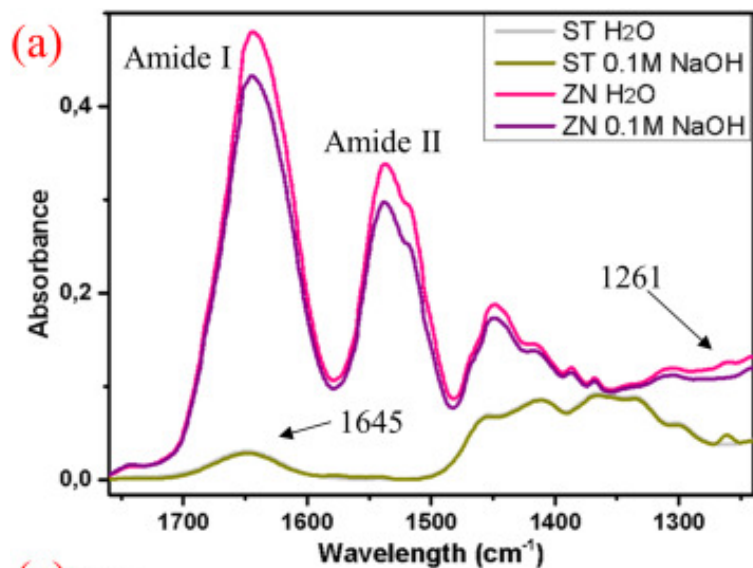
Fig. 5. FTIR spectra for thermoplastic starch, zein, and starch-zein composite films in the wavelength range of (a) and (c) 1760–1240 cm^{-1} and (b) and (d) 3700–2720 cm^{-1}. ZN H_2O is zein with water, ZN 0.1 M NaOH is zein with 0.1 M NaOH, ST H_2O is starch with water, ST 0.1 M NaOH is starch with 0.1 M NaOH, ST-ZN H_2O is starch-zein with water, ST-ZN 0.05 M NaOH is starch-zein composite with 0.05 M NaOH, ST-ZN 0.1 M NaOH is starch-zein composite with 0.1 M NaOH, ST-ZN 0.2 M NaOH is starch-zein composite with 0.2 M NaOH. All films were plasticized with 25% glycerol.

3.4. Effect of NaOH on the chemical structures of thermoplastic starch-zein composite films by attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy

The effect of NaOH on the FTIR spectra of thermoplastic starch, zein, and starch-zein composite films is shown in Fig. 5a-d. The spectra were zoomed in the wavelength range of 1760–1240 cm^{-1} (Fig. 5a and c) and 3690–2730 cm^{-1} (Fig. 5b and d). The bands between 3000

and 2800 cm^{-1} in the starch-zein composites corresponds to the stretching of the C——H bonds, which also constituted the H-C=O bonds appearing as a small peak at 2851 cm^{-1}. These bands suggest a molecular interaction between starch and zein to form carbonyl-amide groups (Fig. 5d) [11]. The increase in NaOH concentration increased the intensity of this band, suggesting more molecular interaction between starch and zein.

The band at 1261 cm^{-1} is related to the C-O-C stretching and C——H deformation and suggests the C——O interaction between the biopolymer and the plasticizer (Fig. 5c) [11]. The intensity of this band increases significantly with an increase in NaOH concentration, with the starch-zein composite film with 0.2 M NaOH having the highest intensity. This suggests optimal plasticization in the starch-zein composite film with 0.2 M NaOH compared to other composite films. As explained earlier, the reduction in the size of zein aggregates favored more efficient plasticization, leading to a decrease in T_g and E' as shown by DMA and an increase in elongation at break, especially in the starch-zein composite film with 0.2 M NaOH. Therefore, we have demonstrated that twin-screw extrusion that is scalable to the industrial level can be used to melt process starch and zein with an addition of NaOH. The resulting film with 0.2 M NaOH had some microstructural and molecular changes that resulted in a more compatible starch-zein film with a 28% improvement in elongation at break compared to composite films without NaOH.

The band between 1600 and 1700 cm^{-1} (peak center 1645 cm^{-1}) corresponds to the stretching of the carbonyl C==O of the amide group and is known as the amide I region (Fig. 5a and c) [26]. The amide I is modulated by the secondary structure of a protein and can be deconvoluted to give the secondary structure of a protein (see Supplementary Table 2). Deconvoluted FTIR spectra revealed that the secondary structure of zein in thermoplastic zein and starch-zein composite films is mainly α-helix, with considerable amounts of β-sheets (Supplementary Table 2). There was a decrease in the α-helices and an increase in the β-sheets of zein in thermoplastic zein and starch-zein composite films with NaOH compared to the composite without NaOH, indicating the deamidation on zein residues. As earlier explained, the glutamine residues on zein (which are essential for forming antiparallel helices in zein) may be deamidated to yield negatively charged zein residues. This weakens the electrostatic balance in the glutamine-rich turns, therefore decreasing the α-helices. This balance is understood to be a major factor that affects the stability of the α-helix [27]. Thus, the decrease in α-helix is an indication of deamidation during which negatively charged zein residues are formed and can electrostatically repel each other to prevent the formation of zein aggregates.

3.5. Effect of NaOH on the crystalline structures of thermoplastic starch-zein composite films

The X-ray diffraction patterns of native starch and zein are shown in Fig. 6a, while the thermoplastic starch, zein, and starch-zein composite films are shown in Fig. 6b. Native zein showed two broad amorphous peaks at 9° and 19° (2θ), and no change was observed in thermoplastic zein films with and without NaOH. Native maize starch was characterized A-

type crystallinity with peaks at 15° and 23° (2θ) and two fused peaks at 17° and 18° (2θ), suggesting that native starch is a semi-crystalline material. [10]. However, thermoplastic starch films and starch-zein composite films had two new prominent peaks at 12.8 and 19.7° (2θ). These were different from native starch (Fig. 6a and Fig. 6b), suggesting that the crystalline structure of native starch was destroyed during extrusion, and a new crystalline structure was formed.
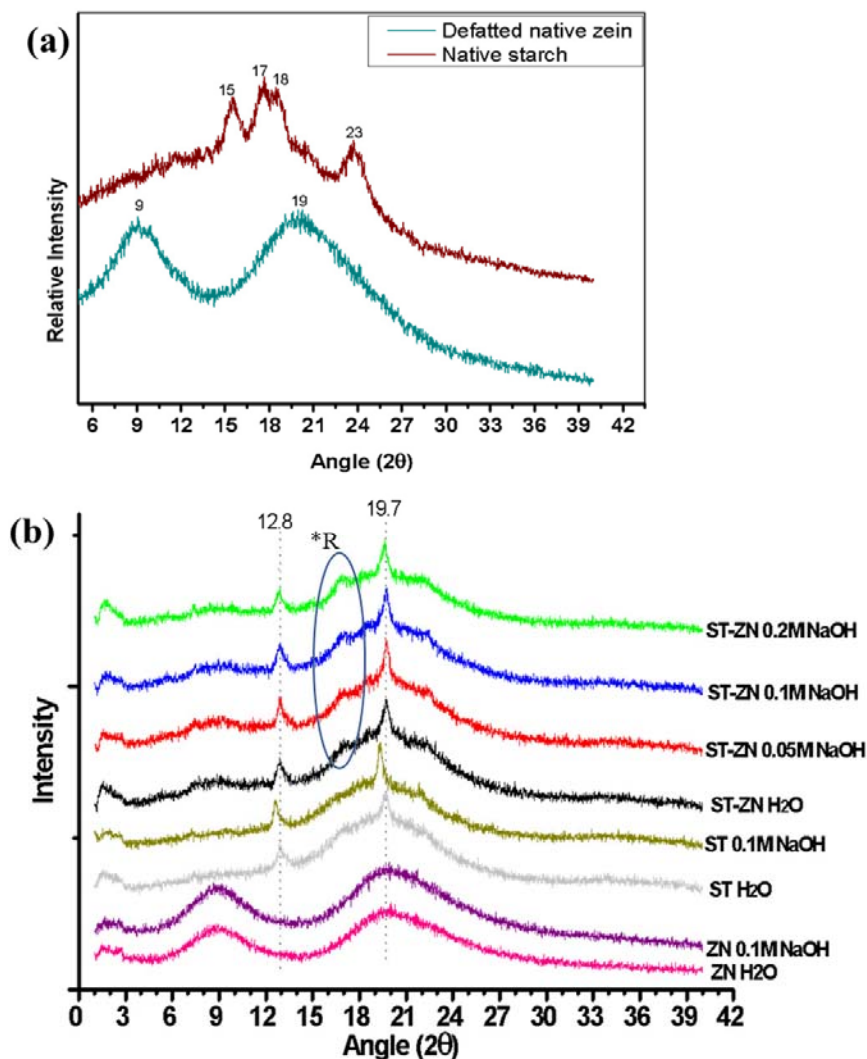

Fig. 6. X-ray diffraction patterns of (a) native starch and native zein powder and (b) thermoplastic starch, zein and starch-zein composites films treated with NaOH. ZN H_2O is zein with water, ZN 0.1 M NaOH is zein with 0.1 M NaOH, ST H_2O is starch with water, ST 0.1 M NaOH is starch with 0.1 M NaOH, ST-ZN H_2O is starch-zein with water, ST-ZN 0.05 M NaOH is starch-zein composite with 0.05 M NaOH, ST-ZN 0.1 M NaOH is starch-zein composite with 0.1 M NaOH, ST-ZN 0.2 M NaOH is starch-zein composite with 0.2 M NaOH. All films were plasticized with 25% glycerol.

These new peaks may be associated with the single helices amylose or V-type complexes. Corradini et al. [10] also reported the formation of V complexes for thermoplastic starch plasticized with glycerol. These authors argued that amylose could associate with glycerol to form V complexes. Vliegenthart et al. [28] also showed that V-type complexes involving amylose and glycerol could develop during the thermomechanical processing of starch during

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extrusion or cooling. However, these peaks shifted to a lower diffraction angle in the thermoplastic starch with 0.1 M NaOH, suggesting a change in the crystalline structure of thermoplastic starch. Qin et al. [29] attributed the shift of these peaks to a lower diffraction angle in the presence of NaOH to the conversion of the V_H type crystalline structures to E_H type crystalline structures. However, NaOH addition did not influence the crystalline structures of thermoplastic starch-zein composite films.

Thermoplastic starch-zein composites films also showed an additional small peak at 16.9° (2θ) (*R) that was not observed in thermoplastic starch. Corradini et al. [10] also reported V-type and B-type crystals for thermoplastic starch-zein composites. These authors argued that when thermoplastic starch is aged above its T_g, it crystallizes into B-type structures with a diffraction peak at about 16.8° (2θ). The short outer chains of amylopectin were responsible for forming these B-type crystals.

3.6. Effect of NaOH on the thermal stability of thermoplastic starch-zein composite films

The TGA and differential TGA (DGTA) curves of thermoplastic zein and starch films with and without NaOH are shown in Fig. 7a and c, while the TGA and DTGA curves of the thermoplastic starch-zein composite films with and without NaOH are shown in Fig. 7b and d, respectively. The TGA curves can be divided into different stages. The mass loss below the To (i.e., 100 to 200 °C) in the thermoplastic starch, zein and starch-zein composites may be attributed to the evaporation and volatilization of low molecular mass compounds, such as glycerol (glycerol has a boiling point of 198 °C at 1 atm) and water (Fig. 7a and c) [30].

Thermoplastic starch showed one major degradation step above 300 °C that corresponds to the degradation of starch. To is the onset degradation temperature, T25% and T50% are the temperatures at 25% and 50% weight loss, respectively. Tmax is the maximum degradation temperature. Thermoplastic starch with 0.1 M NaOH showed a significant decrease ($p < 0.05$) in To, T25%, and T50% and Tmax compared to starch without NaOH (Fig. 7a and Supplementary Table 3). This lower thermal stability may be due to the hydrolysis/depolymerization of starch molecules in the presence of NaOH and extrusion [31], [32].

Above 350 °C, the thermoplastic starch film with 0.1 M NaOH showed a lower degradation rate and higher ash residue at 500 °C (W500) compared to starch film without NaOH. This decrease in the degradation and formation of higher ash residue may be attributed to the carbonization of starch at elevated temperatures (>400 °C) in the presence of NaOH. The decomposition of starch at elevated temperature may occur via glucose ring scissions and the formation of double bonds between the C-atoms [33]. Aromatic rings and/cross-linked structures may form above 300 °C, further heating above 400 °C may lead to carbonization [34].

Thermoplastic zein films showed two degradation steps between 250 and 300 °C (Fig. 7a and b) corresponding to the degradation of two zein subunits with different molecular weights. Thermoplastic zein with and without NaOH had lower thermal stability than starch and the starch-zein composites and had a weight residue at 500 °C above 20%, indicating that zein does not degrade completely. In general, the addition of 25%Wt zein seemed to decrease the thermal stability of the starch-zein composite films compared to starch films. Trujillo-de Santiago et al. [11] also reported an earlier Tmax for starch-zein composite films compared to starch film,
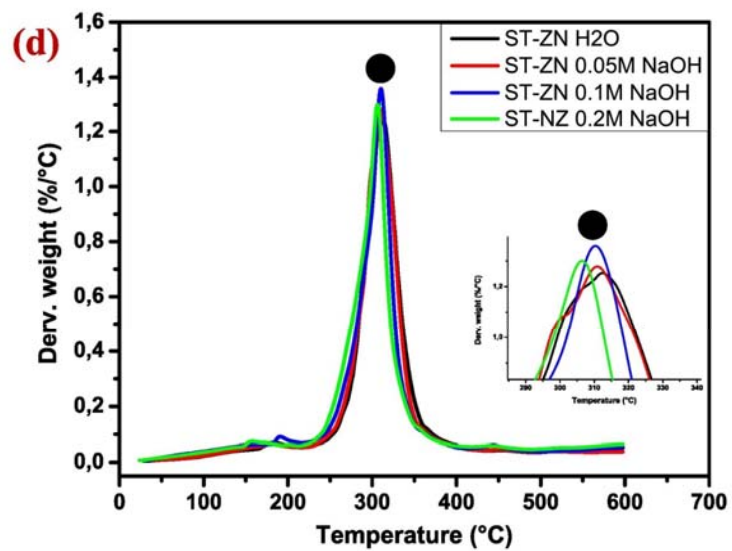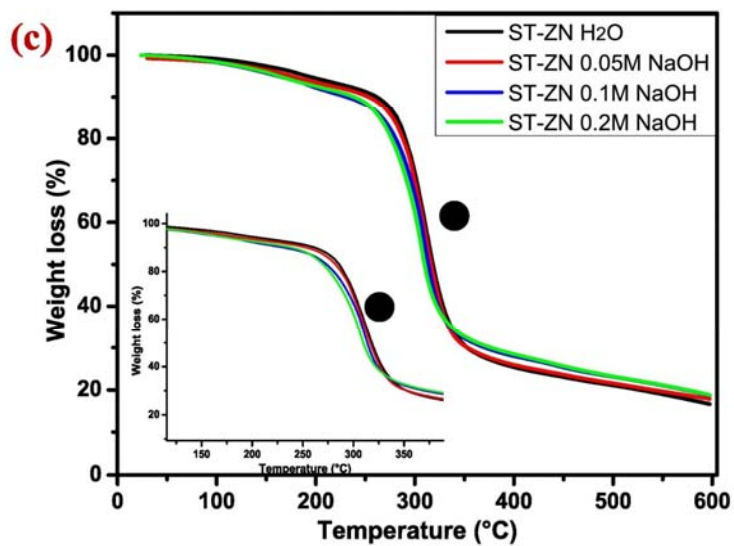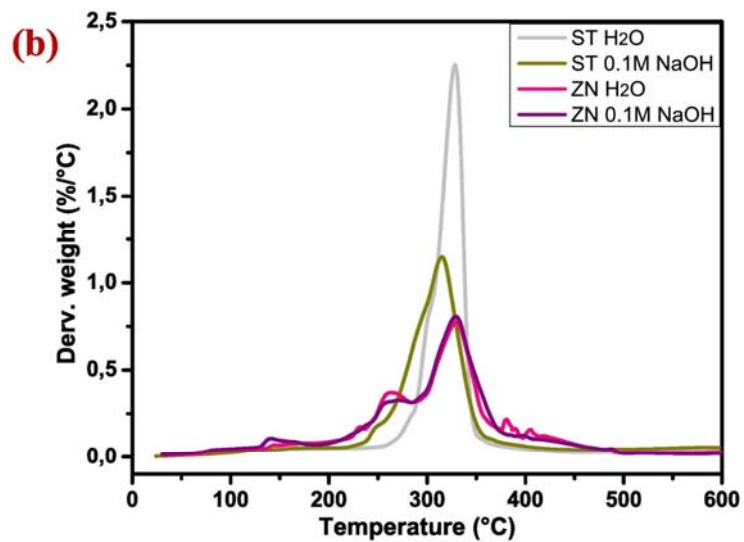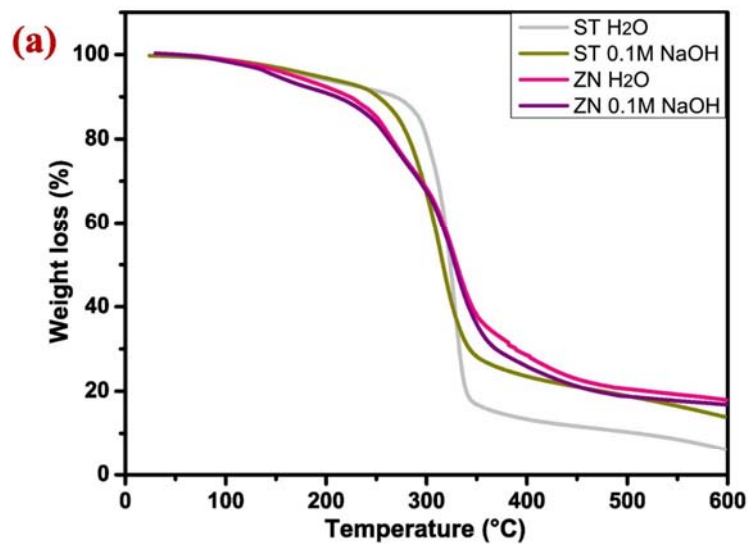
Fig. 7. Effect of NaOH on the TGA curves of (a) thermoplastic starch and zein (c) starch-zein composite. DTGA curves for (b) starch and zein and (d) starch-zein composites. ZN H$_2$O is zein with water, ZN 0.1 M NaOH is zein with 0.1 M NaOH, ST H$_2$O is starch with water, ST 0.1 M NaOH is starch with 0.1 M NaOH, ST-ZN H$_2$O is starch-zein with water, ST-ZN 0.05 M NaOH is starch-zein composite with 0.05 M NaOH, ST-ZN 0.1 M NaOH is starch-zein composite with 0.1 M NaOH, ST-ZN 0.2 M NaOH is starch-zein composite with 0.2 M NaOH. All films were plasticized with 25% glycerol.

suggesting that the addition of zein to starch negatively affected the thermal stability of starch-zein composite films.

The starch-zein composite films showed a major degradation step around 306–311 °C that can be associated with the degradation of starch, zein, or starch-zein mixture (Fig. 7d and Supplementary Table 3). The thermal stability of the starch-zein composite films seemed to decrease with an increase in NaOH concentration, as shown by To, T25%, T50%, and Tmax (supplementary Table 3). This decrease in thermal stability may be related to starch hydrolysis/depolymerization enhanced by higher NaOH concentration. Like starch, above 350 °C, the degradation rate of the thermoplastic starch-zein composite films decreased with an increase in the concentration of NaOH. In addition, the weight residue at 500 °C was above 20%, indicating that the starch-zein composite film does not degrade completely (Supplementary Table 3). This may be due to more carbonization of starch with NaOH or the presence of zein.

4. Conclusion

This study investigated the effect of NaOH concentration on the compatibility and material properties of melt-processed thermoplastic starch-zein composite films. The addition of NaOH reduced the size of zein aggregates within the starch matrix, improving the compatibility between starch and zein. The starch-zein composite film with 0.2 M NaOH had optimal compatibility demonstrated by the smallest size of zein aggregates. This resulted in more efficient plasticization of both starch and zein, as shown by FTIR. In addition, there was a decrease in T_g and E' with an increase in NaOH concentration, suggesting more molecular chain mobility and efficient plasticization compared to the composite film without NaOH. The reduction in the size of zein aggregates and efficient plasticization resulted in an increase in elongation at break, with an optimal increase of about 28% in the starch-zein composite film with 0.2 M NaOH relative to the composite film without NaOH. Therefore, adding 0.2 M NaOH significantly improved the compatibility of starch and zein during extrusion and resulted in thermoplastic starch-zein composite films with improved elongation at break relative to the composite film without NaOH. The film-blowability of thermoplastic starch-zein composites treated with 0.2 M NaOH can be investigated for future work. In addition, the rheological and barrier properties of the melt-processed starch-zein composite films can be explored. These films could possibly find application in food packaging and the biomedical field.

CRediT authorship contribution statement

Mondli Abednicko Masanabo: Conceptualization, experimental design, data analysis, writing of original draft.

M. Naushad Emmambux: Conceptualization, supervision, acquisitions of resources and funding, review of final manuscript.

Suprakas Sinha Ray: Supervision, acquisitions of resources and funding, review of final manuscript.

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Data availability

Data will be made available on request.

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