Formation and Properties of Aqueous Compatible Colloidal Blends between Pre-gelatinized Maize Starch and Zein

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This study investigates the formation and properties of compatible binary colloidal blends between pre-gelatinized (uncomplexed and complexed with stearic acid) maize starches (hydrophilic component) and commercial zein (hydrophobic component) with the aim of creating a bipolymeric material with unique properties. The blends were formed under alkaline condition (0.1 M NaOH) with 3:1, 1:1 and 1:3 starch: zein (w/w) ratios. The 3:1 starch-zein blends showed best stability or compatibility. Blends made with starch complexed with stearic acid had better stability than those with uncomplexed starch. Most of the blends were not freeze-thaw stable except for 3:1 starch (complexed with stearic acid)-zein blend. The blends with better stability had higher apparent viscosity, and a microstructure showing uniform mix of starch – zein as observed by confocal laser scanning microscopy. The likely reason for the compatibility of starch and zein is the change in surface activity of zein, possibly due to deamidation, in 0.1 M NaOH as shown by the negative zeta potential values. The pre-gelatinized maize starches also show negative zeta potential values. In conclusion, compatible binary colloidal blends can be produced between pre-gelatinized maize starch and commercial zein using 0.1 M NaOH.

Key Words: colloidal blends; biocompatible; surface activity

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1. INTRODUCTION

The biopolymers starch and zein (maize prolamin protein) have many food and non-food biomaterial applications. Biomaterial application of starch due to its unique physicochemical and functional properties include: carrier for active materials (Kaur et al., 2007 and as biodegradable films (Weber et al., 2002). Zein can also be used for encapsulation to achieve controlled release of drugs (Mathiowitz et al., 1991), as coatings for cosmetic products (Avalle, 1998) and as biodegradable films and plastics (Anderson and Lamsal, 2011). However, materials from individual biopolymers (starch or zein alone) mostly have inferior properties compared to synthetic polymers. For example, starch films are hydrophilic and have poor mechanical properties (Argüello-García et al., 2014). Films produced from 100% zein are also brittle under normal conditions (Soliman et al., 2009). On the other hand, starch and zein have complementary properties, starch having hydrophilic nature and zein is relatively hydrophobic. Also blending starch with zein could reduce the cost compared to zein alone.

Starch-protein blending has shown to improve the material properties of biopolymers. For example, films made from corn starch and casein blend, where both biopolymers are hydrophilic, through intense thermal blending improve the water vapour transmission rate and the tensile strength compared to casein or starch based films alone (Jagannath et al., 2003). However, the blending of starch and zein to produce a stable co-polymer is a challenge due to the relative hydrophobic nature of zein owing to its high contents of non-polar amino acids (Tihminlioglu et al., 2011) and the hydrophilic nature of starch. Corradini et al. (2007) and Habeych et al. (2008) have reported starch-zein incompatibility based on morphological and microscopic evidence, which displayed two separate phases of starch and zein during hot melt blending. To overcome this, Leroy et al. (2012) used an ionic liquid [(1-butyl-3-methylimidazolium chloride

[BMIM](Cl)] to produce a starch-zein thermoplastic blend by melt processing using a twin screw micro-compounder. However, the safety and availability of these ionic liquids is problematic.

The objective of this research was therefore; to investigate the formation of pre-gelatinized maize starch-zein compatible binary colloidal blends in water at alkaline pH (0.1 M NaOH solution). NaOH was used because zein is soluble in water at alkaline pH $\geq$ 11 (Shukla and Cheryan, 2001). Alkaline treatment of starch also favours starch dissolution and expansion of the amylose coil due to charge repulsion (Chen and Jane, 1994).

2. MATERIALS AND METHODS

2.1 Materials

Native starch from white maize was acquired from Tongaat Hulett Starch (Johannesburg, South Africa). The amylose content was about 28.9%, determined according to the method by Imberty et al. (1991). Commercial zein (Z3625) (primarily α-zein) was from Sigma-Aldrich (Johannesburg, South Africa). The zein was defatted with n-hexane (1 part zein: 3 parts n-hexane, w/v) at ambient temperature. Protein content was 90.3% (N $\times$ 6.25) as is basis, determined by a Dumas nitrogen combustion method, AACC International (2000) Method 46-30.

2.2 Methods

2.2.1 Preparation of pre-gelatinized (uncomplexed or complexed with stearic acid) maize starch

Pre-gelatinized maize starch was prepared as described by D'Silva et al. (2011), except that 40 g starch was pasted using a Brabender Viscoamylograph-E (Brabender®OHG, Duisburg,

Germany) instead of Rapid Visco Analyser. Briefly; stearic acid (1.5% w/w in relation to starch) was dissolved in ethanol (3:1 ethanol: starch ratio) and mixed with starch before pasting to prepare complexed starch. The uncomplexed starch was suspended in ethanol. The suspension was thoroughly mixed at 50 ^{o}C in shaking water bath for 30 min. The ethanol was evaporated off. The starch (10% w/v) was then wet heat treated for extended time (120 min) at 90 ^{o}C. The hot paste was frozen in liquid nitrogen, then freeze dried and stored at 10 ^{o}C until further use.

2.2.2 Formation of pre-gelatinized maize starch (uncomplexed or complexed with stearic acid) and zein blends

Pre-gelatinized maize starch (uncomplexed or complexed with stearic acid) and zein were dispersed in water (1:1 starch: zein ratio) and in 0.1 M NaOH, starch: zein ratios of 3:1; 1:1 and 1:3 (w/w), respectively to make a total of 5% (w/v) dispersion. The dispersions were vigorously mixed at 70 ^{o}C for 5 min using an Ultra Turrax T25 (IKA, Staufen, Germany) at 8000 rpm. The samples were cooled to room temperature (22 ^{o}C) for 30 min and immediately analysed as below.

2.3 Analyses

2.3.1 Visual Examination for Blend Stability

Approximately 18 ml of each blend was transferred into a test tube and held at ambient temperature (22 ^{o}C). The blends were visually examined for any separated liquid (blend instability) after 30 min and after 24 h storage and photographs were taken at each observation time.

2.3.2 Ambient temperature (22 °C) storage stability of pre-gelatinized maize starch (uncomplexed or complexed with stearic acid) and zein blends

A sample of each blend (18 g) immediately after preparation was measured into a plastic centrifuge tube and centrifuged at 955 *g* for 10 min at 25 °C. The amount of liquid separated was measured. The remaining part was left at ambient temperature (22 °C) for 24 h and centrifuged at 955 *g* for 10 min at 25 °C. The amount of liquid separated was measured. This procedure was repeated for 5 days and the blend stability was calculated as:

$$\% \text{ liquid separated } = \frac{\text{weight of liquid separated}}{\text{original weight of blend}} \text{ X } 100$$

2.3.3 Freeze-thaw stability of pre-gelatinized maize starch (uncomplexed or complexed with stearic acid) and zein blends

Each blend (18 g) was measured into a plastic centrifuge tube and centrifuged at 955 *g* for 10 min at 25 °C. This was considered as cycle 0. The separated liquid was weighed and the remaining part was frozen at -18 °C for 24 h. It was then thawed out at 30 °C for 1 h, centrifuged at 955 *g*, the separated liquid was weighed and the remaining part frozen at -18 °C. This was repeated for 5 consecutive freeze-thaw cycles and the blend stability was calculated as above.

2.3.4 Degree of Deamidation

Degree of deamidation was determined as described by Cabra et al. (2007). Briefly, zein 0.5% (w/v) was dispersed in water (control), and dissolved in 0.1 M NaOH and 3 M sulphuric acid (standard) by the same process as the blends above. After incubation for 30 min at 22 °C, the

quantity of ammonia released was determined using an Ammonia Enzymatic BioAnalysis Test 106 Kit (Ammonia Assay Kit, Sigma-Aldrich). Degree of deamidation was expressed as the ratio of 107 the amount of released ammonia by deamidation reactions and the total released ammonia when 108 the zein was treated with 3 M sulphuric acid.

2.3.5 Zeta Potential

The 5% dispersions of zein, pre-gelatinized maize starch (uncomplexed or complexed with 111 stearic acid) and starch-zein blends were diluted to 0.5% (w/v). The solvents used were water 112 (control) and 0.1 M NaOH. Zeta potential was measured by laser doppler velocimetry and phase 113 analysis light scattering (M3-PALS) technique using a Malvern Zetasizer Nano ZS Model 114 ZEN3600 (Malvern Instruments, Malvern, U.K.).

2.3.6 Viscometry

Viscometry was conducted using a Physica MCR 101 Rheometer (Anton Paar, Ostfildern, Germany) using a bob and cup. The blends were centrifuged at 166 g to remove the air bubbles and allowed to equilibrate for 10 min at 25°C. Shear rate was increased from 0.1 to 100/s and 119 reduced back from 100 to 0.1/s. The measurements were taken at 25°C. The experimental data 120 were fitted to the Power Law model:

$$\sigma = K (\dot{\gamma})^n$$

Where σ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (1/s), K is the consistency index (Pa.s^n), and n is the flow index where n=1 for Newtonian fluid, n<1 for shear thinning and n>1 for shear thickening materials.

2.3.7 Confocal laser scanning microscopy

Safranin O dye (Sigma-Aldrich) 0.002% of zein content (dry basis) was added to the blends to stain the zein. The dye was solubilised in 0.1 M NaOH before mixing the starch and zein to ensure homogenous distribution. The samples were degassed and a small amount of each blend sample was placed on a concave microscope slide and covered with cover slip. A Zeiss LSM 510 META Confocal Laser Scanning Microscope (Jena, Germany) at 40x magnification was used. Plane neoflar100x and numerical aperture (N.A) 1.4 were used for the blend images. The pixel time for both tracks 1 and 2 was 12.8 µs. Picture size was 512 x 512 pixels. The excitation and emission spectra for the Safranin O dye were 488 nm and 540 nm, respectively.

2.4 Statistical analysis

All data was analyzed by one-way analysis of variance (ANOVA). Significant differences among the means were determined by the Tukey (HSD) test. All experiments were repeated at least two times, unless otherwise stated. The independent variables were type of starch (uncomplexed and complexed with stearic acid), and starch: zein ratio (3:1, 1:1 and 1:3, respectively).

3. RESULTS AND DISCUSSION

Pre-gelatinized maize starch (both uncomplexed and complexed with stearic acid) and zein in 0.1 M NaOH showed a colloidal suspension with no liquid separated after 30 min and after 24 h storage (Figure 1A and F). Pre-gelatinized maize starch (both uncomplexed and complexed with stearic acid)–zein blend (1:1 ratio) in water (control) showed liquid separation immediately after preparation (Figure 1B). This is because of the immiscibility of the pre-gelatinized starch and zein (Habeych et al., 2008) mainly due to the hydrophilic nature of starch and relatively hydrophobic nature of zein. Pre-gelatinized maize starch (both uncomplexed and complexed with stearic acid)–zein blends at all starch: zein ratios in 0.1 M NaOH were found to form a colloidal blend with no liquid separation after 30 min (Figure 1C, D & E – 30 min). However, after 24 h storage at 22 ºC starch (both uncomplexed and complexed with stearic acid)-zein blend at 1:3 ratio in 0.1M NaOH showed liquid separation, while the 3:1 and 1:1 starch: zein ratios blend were stable (Figure 1C, D & E – 24 h). This suggests that compatible pre-gelatinized (uncomplexed or complexed with stearic acid) maize starch-zein binary colloidal blends were formed at starch: zein ratios of 3:1 and 1:1.

During mixing to produce the blends, foam was produced in zein suspension alone and the 1:1 starch (uncomplexed or complexed with stearic acid)-zein blends (Figure 1D & F – 30 min). The foam volume on top of the starch complexed with stearic acid-zein blend (1:1 ratio) was larger than the foam on top of the uncomplexed starch-zein blend (1:1 ratio) (Figure 1D). This suggests that the starch complexed with stearic acid imparted better foaming capacity compared to uncomplexed starch when blended with zein.
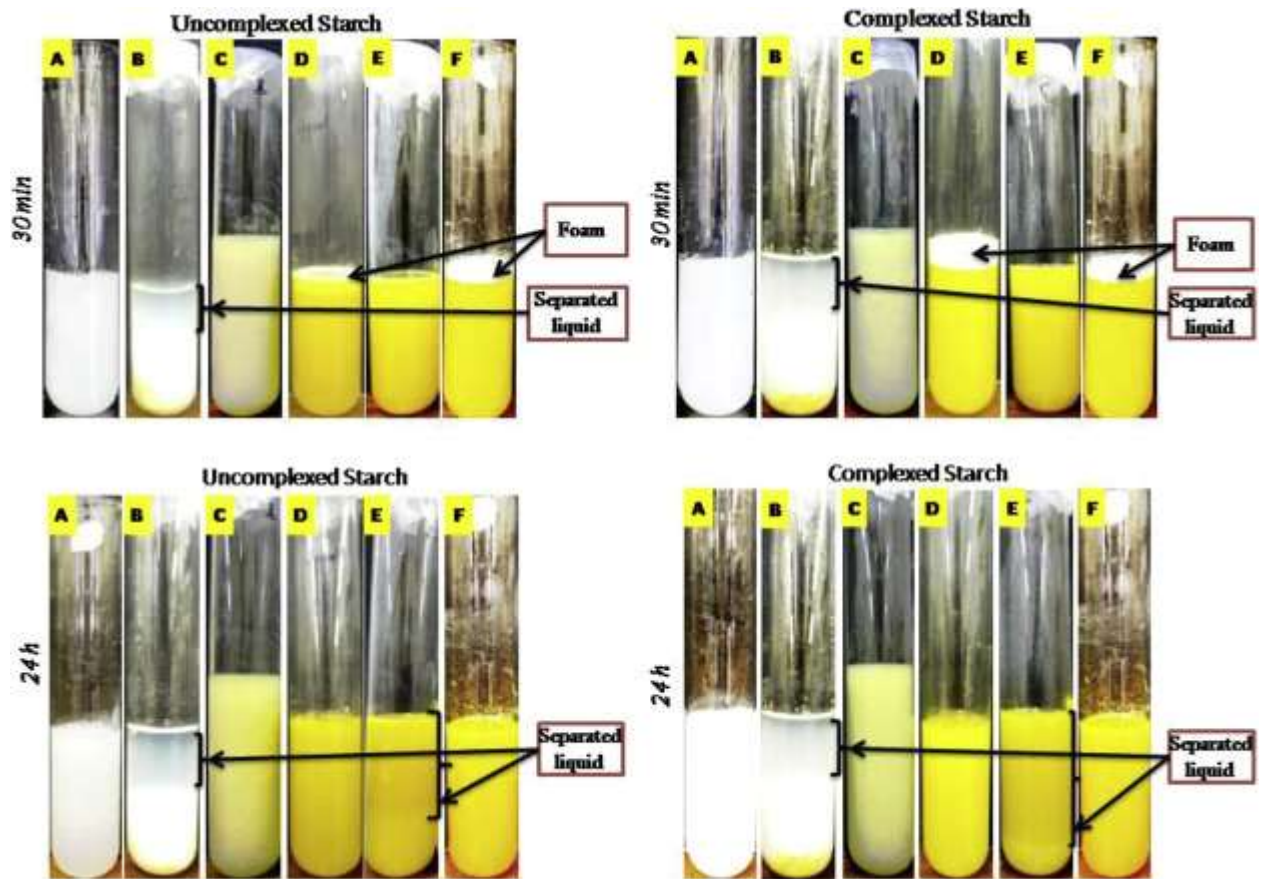
Fig. 1. Images of pre-gelatinized maize starch (uncomplexed and complexed with stearic acid), zein and pre-gelatinized maize starch (uncomplexed and complexed with stearic acid) -zein colloidal blends in 0.1 M NaOH at different starch:zein ratios after 30 min and 24 h storage at ambient temperature (22 °C). A - Pre-gelatinized maize starch (uncomplexed or complexed with stearic acid), B - starch: zein (1:1) in water, C -starch: zein (3:1), D - starch:zein (1:1), E - starch:zein (1:3) and F - zein. * The difference in volume between the samples was due to difference in foaming.

The ambient temperature (22 °C) storage and freeze-thaw stability tests of the colloidal blends are presented in Figure 2a and b. Pre-gelatinized maize starch (uncomplexed and complexed with stearic acid) and pre-gelatinized maize starch (uncomplexed and complexed with stearic acid)-zein blends at 3:1 and 1:1 ratios were stable with no liquid separation during the five days storage (Figure 2a). However, both uncomplexed starch and starch complexed with stearic acid-zein blends at 1:3 ratio and zein alone showed instability with > 70% liquid separated starting from day 1.

Pre-gelatinized maize starch (uncomplexed and complexed with stearic acid) was stable with no liquid separation during the five freeze-thaw cycles (Figure 2b). However, when the starch was blended with zein, only the starch (complexed with stearic acid)-zein blend (3:1 ratio) was relatively stable during the five freeze- thaw cycles with 5% liquid separated at the 5th cycle. Pre-gelatinized uncomplexed maize starch-zein at 3:1, 1:1 and 1:3 ratio blends showed instability starting from the 1st cycle with a separated liquid values of 8.1%, 27.7% and 71.5%, respectively. Pre-gelatinized maize starch complexed with stearic acid-zein blends at 1:1 and 1:3 ratios also showed instability starting from the 1st cycle with 29.5% and 62.7% separated liquid, respectively.

Repeated freeze-thawing cycles are reported to accelerate starch retrogradation in a paste or gel (Jacobson and BeMiller, 1998). The starch retrogradation resulted in syneresis (liquid separation). This liquid separation occurs mainly due to retrogradation of amylose (Morris, 1990). The relative stability of the 3:1 maize starch complexed with stearic acid-zein blend could be related to the non-gelling behaviour of starch complexed with stearic acid (D'Silva et al., 2011).
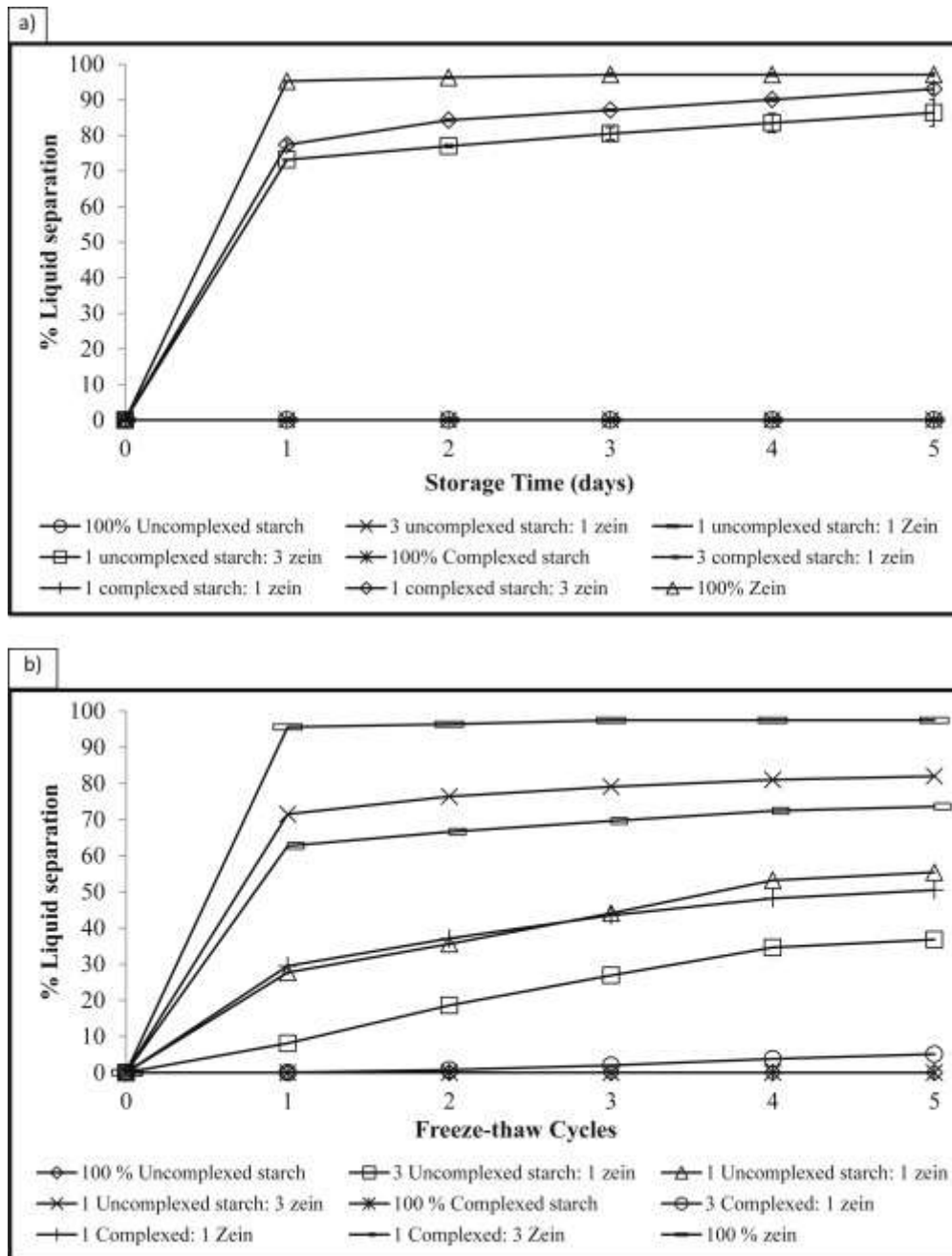
Fig. 2. a) Ambient temperature (22 °C) storage stability and b) freeze-thaw stability of pre-gelatinized maize starch (uncomplexed or complexed with stearic acid), zein and pre-gelatinized maize starch (uncomplexed and complexed with stearic acid) - zein colloidal blends at different starch: zein ratios (3:1, 1:1 and 1:3). Error bars show standard deviations.

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To determine whether the stability of the blends in 0.1 M NaOH was as a result of deamidation of zein, zein amide content and zeta potential of the zein, starch and blends were measured. Zein treated with 0.1 M NaOH had a far higher degree of deamidation (77.3%) compared to zein in water (12.4%). Cabra et al. (2007) had also found more than 60% deamidation of α-zein when treated with 0.5 M, 1.0 M and 1.5 M NaOH in 70% ethanol incubated at 70°C for 30 h. Zhang et al. (2011) also found zein deamidation at alkaline pH (12.5) using NaOH.

The zeta potential values of zein and pre-gelatinized (uncomplexed and complexed with stearic acid) maize starch in 0.1 M NaOH were significantly more negative than the zeta potential of each biopolymer in water (p<0.001) (Table 1). Zein, pre-gelatinized (uncomplexed and complexed with stearic acid) maize starch and blends of pre-gelatinized (uncomplexed and complexed with stearic acid) maize starch and zein had a zeta potential values of ≤ -14.5 mV. Zeta potential is a measure of charges carried by particles suspended in a liquid (mostly water). Zeta potential values greater than -15 mV usually can represent the onset of agglomeration (Riddick, 1968). Almost all the blends and the individual biopolymers in 0.1 M NaOH have a zeta potential value of ≤ -15 mV (more negative) which is below the threshold value of agglomeration. The high degree of deamidation of zein in 0.1 M NaOH (Table 1) could be the cause of the negative zeta potential value of zein in 0.1 M NaOH. During deamidation the amide groups changed to hydroxyl groups and convert glutamine residues (most abundant in cereal proteins) to glutamic acid residues. This increases the number of negatively charged carboxyl groups (COO^-) in the peptide chain (Cabra et al., 2007; Kanerva et al., 2011). Zhang et al. (2011) reported a more negative zeta potential of -21 mV at pH 12.5 compared to -0.94 mV at pH 6.5 for zein. The higher negative zeta potential of pre-gelatinized maize starch (both uncomplexed (- 16.7 mV) and complexed with stearic acid (-18.2 mV)) in 0.1 M NaOH compared to their zeta

Table 1. Zeta potential values of zein, pre-gelatinized maize starch (uncomplexed and complexed with stearic acid) in water and in 0.1 M NaOH, and pre-gelatinized maize starch (uncomplexed or complexed with stearic acid)–zein blends in 0.1 M NaOH.

Treatment	Starch:zein ratio	Solvent	pH	Zeta potential (mV)
Zein	0:1	Water	3.6	43.0**a** (±0.5)
Uncomplexed maize starch	1:0	Water	6.9	−3.6**b** (±0.1)
Complexed maize starch with stearic acid	1:0	Water	7.2	−8.9**c** (±0.3)
Zein	0:1	0.1 M NaOH	12.2	−16.0**de** (±0.1)
Uncomplexed maize starch	1:0	0.1 M NaOH	12.1	−16.7**ef** (±0.2)
Complexed maize starch with stearic acid	1:0	0.1 M NaOH	12.3	−18.2**gh** (±0.1)
Uncomplexed maize starch–zein blend	3:1	0.1 M NaOH	12.1	−19.1**h** (±1.1)
Uncomplexed maize starch-zein blend	1:1	0.1 M NaOH	12.2	−17.7**fgh** (±0.2)
Uncomplexed maize starch–zein blend	1:3	0.1 M NaOH	12.3	−18.6**gh** (±0.9)
Complexed starch with stearic acid–zein blend	3:1	0.1 M NaOH	12.2	−17.5**fg** (±0.8)
Complexed starch with stearic acid–zein blend	1:1	0.1 M NaOH	12.3	−14.5**d** (±0)
Complexed starch with stearic acid–zein blend	1:3	0.1 M NaOH	12.2	−15.9**de** (±0.1)

Means followed by different letters are significantly different ($P < 0.0001$), Tukey (HSD) test. n = 3.

Values in parentheses show standard deviation.

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potential value in water (-3.6 and -8.9 mV) (Table 2) could be due to an alkalization reaction where the hydroxyl groups of the starch molecules are activated and changed into the more reactive and negatively charged alkoxide form (Starch–O⁻) (Lawal et al., 2008).

The visco metry and microstructure of the blends were investigated. Apparent viscosity vs. shear rate graph was plotted (Figure 3) and the viscometry data (shear rate vs. shear stress) was fitted 211 to the Power Law model. Table 2 summarizes the Power Law paramenters of the treatments. The 212 viscometry data showed a good fit to the Power Law model as the coefficient of determination 213 (R^2) for all the treatments were greater than 0.90 (Table 2). All the treatments except zein alone 214 showed shear thinning behaviour with n (flow index value) from the Power Law equation to be < 215 1. This shear thinning behaviour can also be seen from the apparent viscosity vs. shear rate 216 graph, where the apparent viscosity decreased as the shear rate increased. Zein showed a 217 Newtonian behaviour, where the n (flow index value) = 1. The apparent of viscosity vs. shear 218 rate graph of zein was also in agreement to the n-value, where no decrease in apparent viscosity 219 was observed with increase in shear rate. This agrees with research which showed that α-zein 2 - 220 14% (w/w) in aqueous ethanol exhibited Newtonian behaviour (Fu and Weller, 1999).

The blending of zein with pre-gelatinized maize starch reduced the apparent viscosity compared to the pre-gelatinized maize starch (Figure 3). The consistency index (K-value), an indication of shear viscosity (Xie et al., 2009) was also substantially reduced when zein was blended with pre-gelatinized maize starch compared to the pre-gelatinized maize starch (Table 2). Similarly, Corradini et al. (2007) reported a reduction of starch melt viscosity when blended with zein. The apparent viscosity and K-value of the blends decreased with decreasing the proportion of the starch from 3 Starch: 1 zein to 1 starch: 1 zein and 1 starch: 3 zein. However, the n-value

Table 2.

Power law consistency coefficient (K) values (Pa.s^n), flow behaviour index (n) values and R^2 values of 5% (w/v) pre-gelatinized maize starches (uncomplexed or complexed with stearic acid), zein and blends of pre-gelatinized maize starch (uncomplexed or complexed with stearic acid) and zein at different ratios.

Treatment	Starch:Zein ratio	K-value	n-value	R^2-value
Uncomplexed maize starch	1:0	2.2×10^1**b** ($\pm$0.15)	0.42**e** ($\pm$0.00)	0.99
Complexed maize starch with stearic acid	1:0	3.5×10^1**a** ($\pm$2.07)	0.32**ef** ($\pm$0.00)	0.99
Uncomplexed maize starch–Zein blend	3:1	0.6×10^1**d** ($\pm$0.53)	0.40**e** ($\pm$0.01)	0.99
Uncomplexed maize starch–Zein blend	1:1	4.3×10^{-1}**e** ($\pm$0.03)	0.63**c** ($\pm$0.01)	0.99
Uncomplexed maize starch–Zein blend	1:3	2.0×10^{-2}**e** ($\pm$0.00)	0.91**b** ($\pm$0.02)	0.99
Maize starch complexed with stearic acid–Zein blend	3:1	1.1×10^1**c** ($\pm$0.80)	0.30**f** ($\pm$0.01)	0.96
Maize starch complexed with stearic acid - Zein blend	1:1	4.0×10^{-1}**e** ($\pm$0.01)	0.58**d** ($\pm$0.01)	0.99
Maize starch complexed with stearic acid–Zein blend	1:3	1.0×10^{-2}**e** ($\pm$0.00)	0.99**ab** ($\pm$0.01)	0.99
Zein	0:1	1.0×10^{-3}**e** ($\pm$0.00)	1.00**a** ($\pm$0.00)	0.97

Means followed by different letters within a block are significantly different (P < 0.0001), Tukey (HSD) test. n = 3.

Values in parentheses are standard deviations.

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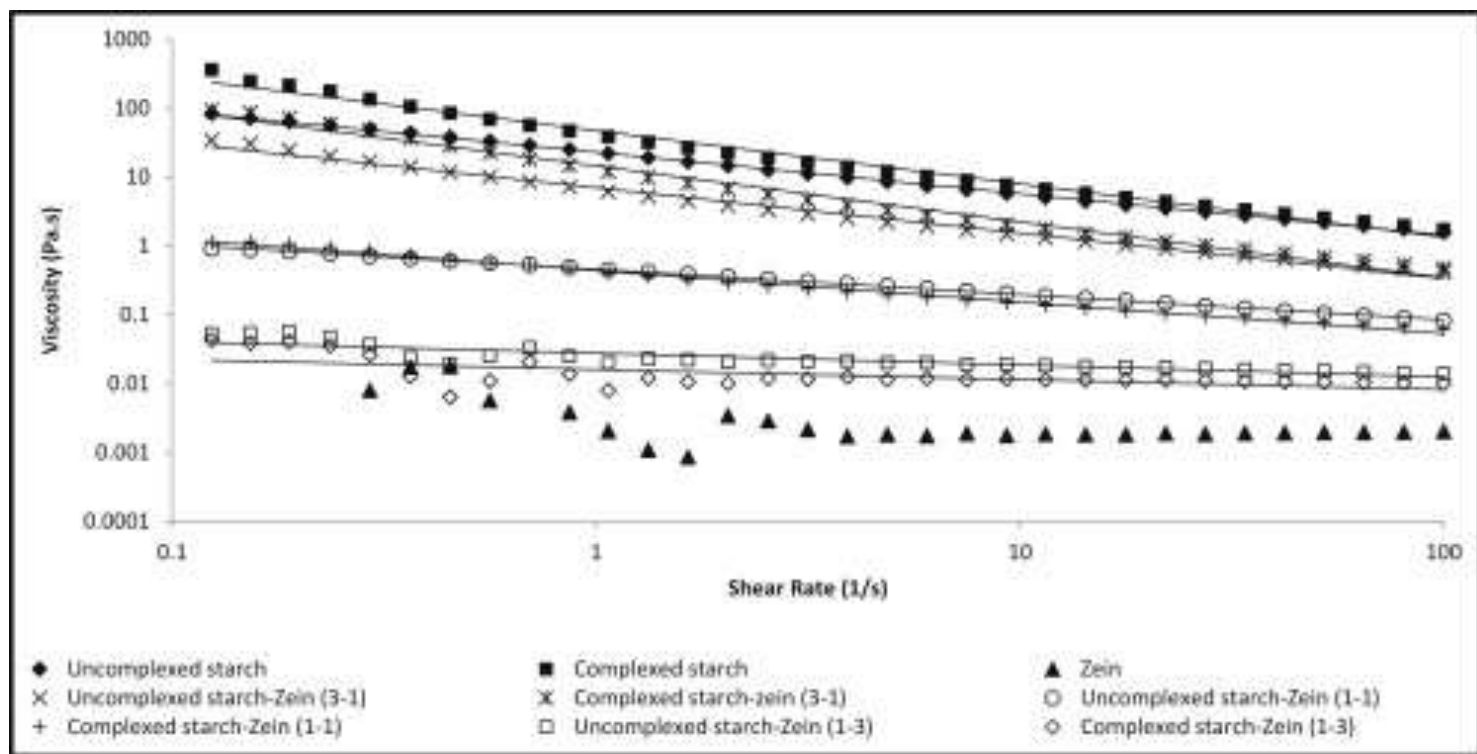
* The viscosity vs. shear rate data of pure zein did not fit to the Power Law trending line.

Fig. 3. Apparent viscosity vs. Shear rate graphs of pre-gelatinized maize starch (uncomplexed and complexed with stearic acid), zein and pre-gelatinized maize starch–zein blends at different starch: zein ratios (3:1, 1:1 and 1:3). *The viscosity vs. shear rate data of pure zein did not fit to the Power Law trending line.

increased with decrease in starch proportion. The K-value of the 3:1 ratio starch complexed with stearic acid-zein blend was significantly higher (P<0.001) than the K-value of 3:1 ratio uncomplexed starch-zein blend. The apparent viscosity and K-value of the blends seemed to be related to the stability of the blends. The 3:1 starch-zein blends were found to be more stable and have higher apparent viscosity and K-values compared to the 1:1 and 1:3 starch-zein blends (Figures 2a and b, Figure 3, Table 2).

Confocal laser scanning microscopy was used to investigate the microstructure of the blends (Figure 4). Three different colour domains were observed: black, light yellow and bright yellow. The starch samples were unstained and showed black colour (Figure 4A, B) and the zein sample showed bright yellow and black colour (Figure 4C). Thus, the black colour can be considered to be starch (unstained) and/or solvent, the bright yellow to be zein (stained with safranin O) and the light yellow is presumed to be starch-zein mixed. The arrows indicate these three different areas, starch/solvent, zein or starch-zein mixture. Starch-zein blends with different starch: zein ratios (3:1, 1:1 and 1:3) showed different microstructures. Type of starch (uncomplexed and complexed with stearic acid) also affected microstructure. The 3:1 ratio uncomplexed starch-zein blend showed some aggregated zein (bright yellow coloured), starch and starch-zein mixed (light yellow coloured) (Figure 4D). However, the 3:1 ratio starch complexed with stearic acid-zein blend showed mostly a mixture of starch and zein as indicated by the light yellow colour (Figure 4G). The uncomplexed starch- zein blend (1:1 ratio) showed some zein aggregation (bright yellow), some starch/solvent (dark) and a mixture of starch and zein (light yellow) (Figure 4E). Less zein aggregation was observed in the complexed starch with stearic acid-zein blend (1:1 ratio) (Figure 4H) compared to the uncomplexed starch-zein blend (1:1 ratio). Both the uncomplexed starch-zein blend (1:3 ratio) and the complexed starch with stearic acid-zein blends (1: 3 ratio) showed zein aggregat ion (bright yellow) where the one with
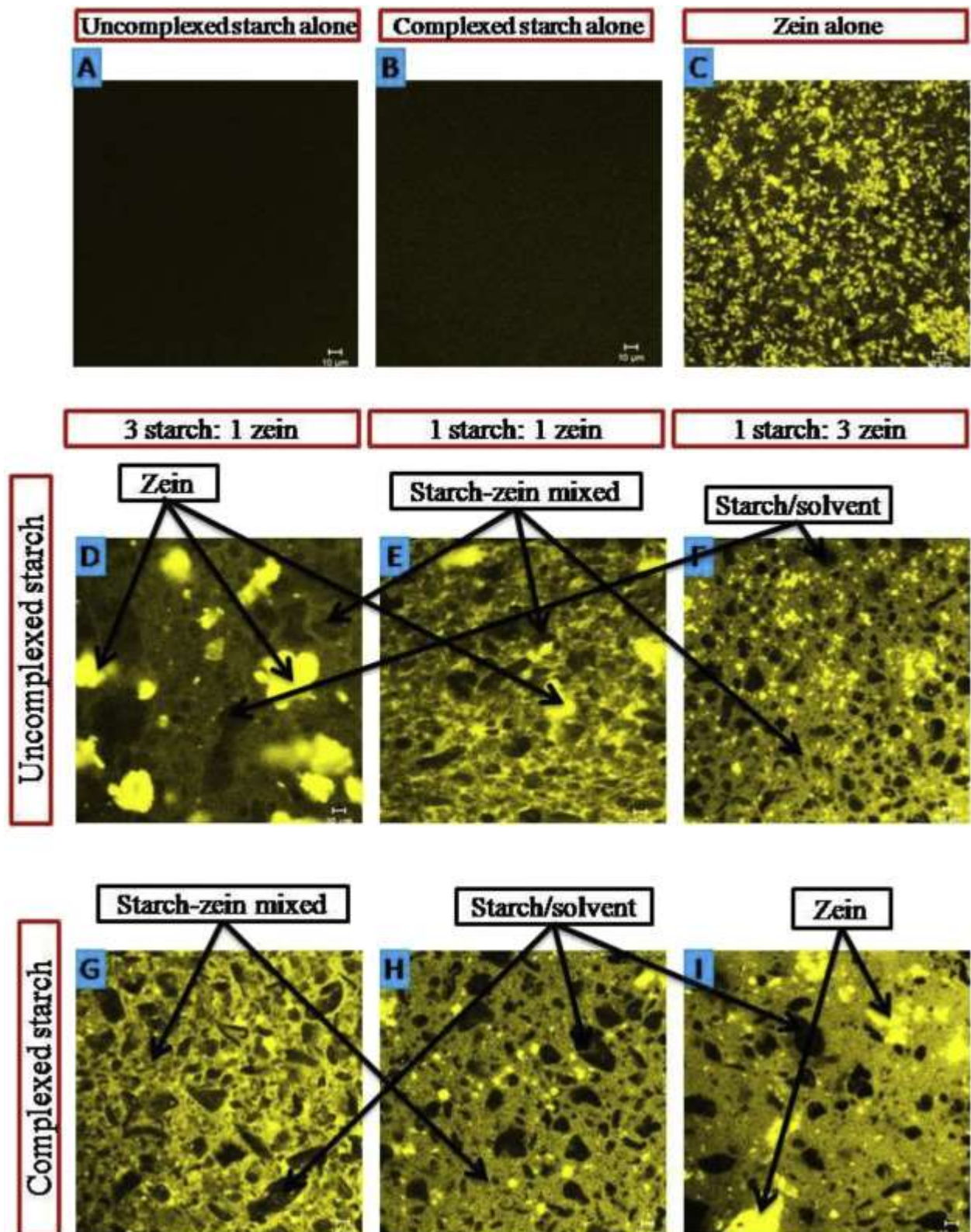
Fig. 4. Confocal laser scanning microscopy of pre-gelatinized maize starch (uncomplexed and complexed with stearic acid), zein and pre-gelatinized maize starch–zein blends. A: uncomplexed maize starch, B: maize starch complexed with stearic acid, C: zein, D: 3:1 uncomplexed starch: zein, E: 1:1 uncomplexed starch: zein, F: 1:3 uncomplexed starch: zein, G: 3: 1 complexed starch with stearic acid: zein, H: 1:1 complexed starch with stearic acid: zein, and I: 1:3 complexed starch with stearic acid: zein.

complexed starch showing more aggregation (Figure 4F, I). Although proportion of starch was lower in the 1: 1 and 1: 3 starch: zein ratios compared to the 3: 1 ratio, there seems to be more and relatively larger black areas (starch/solvent) in the 1: 1 and 1: 3 ratios. This could further indicate liquid separation in the 1: 1 and 1: 3 ratios of starch: zein blends compared to the 3: 1 starch: zein ratio. The black areas in the pure zein could also indicate liquid separation (instability).

The unique microstructures of the blends are related to the flow properties. Blends where the starch and zein just formed a mixture (light yellow), showed higher apparent viscosity and K-value compared to those where the zein aggregated. Colloidal dispersions or emulsions with uniformly distributed particles have higher viscosities than dispersions or emulsions with non-uniform or coarse particle distribution (Pal, 1996). The zein aggregation in the 3:1 ratio uncomplexed maize starch-zein blend could be due to the gelling behaviour of the uncomplexed starch. When the starch gels, the zein molecules may not have been able to disperse uniformly throughout the system. The zein aggregates in the 1: 1 and 1: 3 starch: zein ratios could indicate decrease in stability.

To further discuss the findings; alkaline deamidation of zein increased the total negative net charges of the zein molecules as evidenced by the decrease (more negative) of zeta potential (Table 2). This higher net negative charges increases electrostatic repulsion and decreases hydrogen bonding between the zein molecules (Cabra et al., 2007; Kanerva et al., 2011). It is probable that the repulsion between the negatively charged zein molecules will keep them dispersed in the blend system and prevents aggregation. The starch would increase the viscosity of the aqueous phase because of its hydrophilic nature (Glicksman, 1991) and possible also stabilize the dispersed zein molecules. These two mechanisms, the repulsion between the

negatively charged zein molecules and the stabilizing nature of the starch, could be the main reasons for the formation of compatible blends between pre-gelatinized maize starch and zein under alkaline condition. The sodium ion (Na^+) from NaOH could also interact with the negatively charged glutamic acid residues forming ionic type interactions to further prevent zein aggregation. The alkalization reaction will cause formation of more reactive and negatively charged starch alkoxide (Starch–O^-) (Lawal et al., 2008). This would result in repulsive forces between the starch molecules or would promote ionic interaction with the negatively charged zein molecules, which would further stabilize the system.

The better stability of blends with maize starch complexed with stearic acid compared to blends with uncomplexed maize starch was probably due to the lower retrogradation and syneresis of the starches complexed with stearic acid compared to the uncomplexed starches (D'Silva et al., 2011). Starch complexed with stearic acid is non-gelling as it does not form junction zones (D'Silva et al., 2011). The amylose-lipid complexes are of nanoscale size (Lalush et al., 2005) and could act as nanofillers. Uniform dispersion of nanoparticles leads to a very large matrix/filler interfacial area, and can decrease the molecular mobility and the relaxation behavior, and consequently improves the material properties (Azeredo, 2009).

4. CONCLUSIONS

Compatible binary colloidal blends can be produced between pre-gelatinized (uncomplexed and complexed with stearic acid) maize starches and commercial zein using 0.1 M NaOH. The stability of these bipolymeric blends depend on the starch: zein ratio and type of starch (uncomplexed or complexed with stearic acid). A 3:1 starch: zein ratio produces the most stable binary colloidal blend. Blends with maize starch complexed with stearic acid have better stability

than those using uncomplexed maize starch. The process of producing the compatible pre-gelatinized maize starch-zein colloidal blends is simple and straight forward. The potential of these bipolymeric blends to form biodegradable coatings and films need to be investigated.

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