

Formation of a viscoelastic dough from isolated total zein (α -, β - and γ -zein) using a glacial acetic acid treatment

Bianca L. King¹, Janet Taylor¹ and John R.N. Taylor¹

¹Institute for Food, Nutrition and Well-being and Department of Food Science, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa

Corresponding author: Institute for Food, Nutrition and Well-being and Department of Food Science, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa. E-mail: John.taylor@up.ac.za (John R.N. Taylor). Tel: +27 124204296. Fax: +27 124202839.

Keywords: Cast film; Glacial acetic acid; Total zein; Viscoelastic dough

Abbreviations: 2D-PAGE - Two dimensional polyacrylamide gel electrophoresis, CLSM – Confocal laser scanning microscopy, FTIR - Fourier transform infrared spectroscopy, T_g – glass transition temperature

Abstract

Only predominantly α -type zein (commercial zein) has been shown to form a viscoelastic dough. In maize zein comprises α -, β -, γ - and δ -zein (total zein). Because glacial acetic acid can fully solubilise zein, its effect was investigated. Dissolving total zein (comprising α -, β -, γ - and δ -zein) in glacial acetic acid and casting a film, enabled a viscoelastic dough to be formed with water above zein's T_g . The dough was stronger and less extensible than commercial zein dough made without film formation. When residual acetic acid was removed from the total zein film, a dough still formed.. CLSM showed that the total zein dough fibrils were shorter and less well-aligned than those of commercial zein dough and appeared as particles. Disulphide bond cross-linking was probably responsible, for total zein dough stiffness. FTIR showed that total zein wet doughs and total zein slurry with water were predominantly β -sheet, indicating that β -sheet conformation was not directly responsible for dough formation. It is suggested that acetic acid brings about chemical changes in zein, enabling it to better interact with water molecules, counteracting disulphide bonding effects, allowing total zein to form a dough. This is the first report of viscoelastic dough formation from total zein.

1. Introduction

Maize is an alternative cereal to wheat for bread making (Renzetti and Arendt, 2009). It is a C4 cereal and is cultivated in warmer climatic regions where wheat cannot be economically cultivated, and is quantitatively the world's most important cereal crop (FAOSTAT3, 2014; Mason et al., 2015). However, producing maize-based bread is a challenge because maize flour dough does not exhibit viscoelastic and gas-holding properties like wheat dough (Oom et al., 2008).

Nevertheless, the dough making properties of zein, the maize prolamin, can be considered because commercial zein (predominantly α -zein) forms a viscoelastic dough when hydrated above the glass transition temperature (T_g) of zein in water ($\sim 28^\circ\text{C}$) (Lawton, 1992) and when mixed with starch or gluten-free flour can expand a bubble by Alveography (Sly et al., 2014). Furthermore, recent work concerning the reasons for zein's viscoelasticity with respect to formation of a β -sheet conformation (Erickson et al., 2012) and improvement of zein dough properties using dilute acetic acid and lactic acids (Sly et al., 2014) or the oxidising agent hydrogen peroxide (Taylor et al., 2016a) have shown promise.

However, to date virtually all research on zein dough functionality has involved using preparations comprising essentially only the α -zein class (Taylor et al., 2016b). In fact, zein as present in the maize kernel (referred to in this work as total zein) comprises α -, β - and γ -zein (Shewry and Tatham, 1990; Shukla and Cheryan, 2001) and probably δ -zein (Lawton and Wilson, 2003). One exception was the study by Schober et al. (2011). These authors showed that isolation procedures that produced zein comprising predominantly α -zein, with less than 10% β - and γ -zeins, could produce viscoelastic doughs, whereas zeins with high proportions of β - and γ -zeins could produce films when cast from a solution of aqueous

ethanol. Building on the work of Schober et al. (2011) and our work concerning prolamin film formation using glacial acetic acid (Taylor et al., 2005; Taylor et al., 2009) and on improvement in zein dough functionality with acetic acid (Sly et al., 2014), this study was undertaken to determine whether total zein can also form a viscoelastic dough.

2. Experimental

2.1 Materials

Vital wheat gluten was kindly donated by Novozymes, SA (Benmore, South Africa). Commercial zein from yellow maize (code Z3625) was obtained from Sigma-Aldrich, Johannesburg, South Africa. Total zein was prepared from super grade (highly refined) white maize meal (essentially endosperm) (Super Sun, Pretoria, South Africa) that had been milled finer to a maximum of 0.5 mm particle size using a Falling Number Laboratory hammer mill 3100 (Perten, Hägersten, Sweden). Total zein was extracted from the maize meal with 70% (w/w) absolute ethanol containing 0.5% (w/w) sodium metabisulphite and 0.35% (w/w) sodium hydroxide at 70°C for 1 hr. After centrifugation at 1400 g for 5 min and solvent evaporation of the supernatant in a fume hood at ambient temperature (24°C) overnight, the wet protein concentrate was filtered under vacuum, before air drying overnight at ambient temperature in the fume hood.

2.2 Methods

2.2.1 Casting α -zein and total zein into films

Films were cast essentially as described by Taylor et al. (2005). For the majority of the work, films were cast from commercial zein and total zein dissolved in glacial acetic acid. No plasticiser was used in the preparation of the films. Glacial acetic acid (9.6 g) was added to commercial zein or total zein, 1.44 g (protein equivalent) in an Erlenmeyer flask. The flasks were closed with aluminium foil before being heated to 35°C and 65°C for commercial zein

and total zein, respectively. Total zein was heated at 65°C to dissolve it fully. The protein solutions were held at their respective temperatures for 10 min with continuous, rapid stirring to fully solubilise the protein. After which, further glacial acetic acid was added to make up for any lost by evaporation. For the sonication work total zein films were cast from 70% (w/w) aqueous ethanol at 65°C using the same weight of protein and solvent.

The protein solutions (2 ml) were poured into circular silicone dishes (55 mm in diam.) and dried in a fume hood overnight and thereafter in an oven (not force draft) at 50°C overnight. Average film thickness was $77.1 \pm 1.0 \mu\text{m}$ for total zein films and $63.1 \pm 1.3 \mu\text{m}$ for commercial zein films. To remove residual glacial acetic acid, some films were repeatedly rinsed with cold distilled water (8°C), and centrifuged to remove the water until the rinse water reached pH 7-8. On washing, the films became opaque probably due to zein precipitation at the film surface. Rinsed films were dried overnight in a fume hood.

2.3 Analyses

2.3.1 Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE)

Commercial zein and total zein were solubilised at a concentration of 5 $\mu\text{g protein}/\mu\text{l}$ in a solution containing 7 M urea, 2 M thiourea, 4% w/v 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulphonate, 2% v/v immobilized pH gradient (IPG) buffer pH 3-10 and 40 mM dithiothreitol. 2-D PAGE was carried out according to Adebowale et al. (2011). Gels were stained with Coomassie Brilliant Blue R-250 and photographed using a flatbed scanner.

2.3.2 Zein aggregation, cohesiveness and dough extensibility

Aggregation, cohesiveness and dough extensibility behaviour of the commercial zein and total zein samples were determined using a method based on those of Schober et al. (2010) and Sly et al. (2014). Zein powder or zein film (crushed) (0.5 g protein equivalent), and distilled water or 1.3% (w/w) acetic acid (0.8 ml) were weighed into separate centrifuge tubes

and pre-warmed to 50°C in a water bath. On reaching temperature, the liquid was added to the zein. The protein suspension was then vortexed at high speed for 30 s and then manipulated with a spatula to ensure that all the material was completely incorporated into the dough or slurry. After 15 min, doughs were removed and were manually stretched for 5 s and then photographed. Where doughs did not form, the slurries were decanted into glass beakers and photographed.

Additionally, total zein and total zein that had been cast into a film with 70% (w/w) aqueous ethanol was prepared with water at 50°C and then sonicated at 16 Watts (root mean square) using an ultrasonic cell disruptor (Misonix, New York) for 10 min and 5 min, respectively in an attempt to form a dough.

2.3.3 Confocal Laser Scanning Microscopy (CLSM)

The microstructures of commercial zein and total zein doughs, prepared as described (2.3.2), were studied using a CLSM (Zeiss 510 META, Jena, Germany), at an excitation wavelength of 488 nm, and fitted with a Plan-Neofluar 10x 0.3 numerical aperture objective. The doughs were stretched out as thinly as possible (approx. 15 mm x 5 mm x 1 mm) over a glass slide and then viewed using the autofluorescence of total zein and commercial zein to produce scanned images.

2.3.4 Dough Tensile properties

Doughs were prepared from zein or zein films as described (2.3.2). The doughs were then quickly pressed into a longitudinal split, cylindrical rubber tube mould (60 mm long x 3 mm internal diam.) to obtain a dough piece of uniform shape and size. After which the moulded doughs were placed over the vertical struts (30 mm apart) of a Kieffer rig mounted on a TA-XT2 texture analyser (Stable Micro Systems, Godalming, UK) and firmly held at both ends

with the operator's thumb and index finger. The hook extended the doughs at a constant rate of 3.3 mm/s over a distance of 150 mm (maximum displacement of the texture analyser). To prevent the doughs from cooling below the T_g of zein in water, the test was performed within 3 min at ambient temperature. The tensile properties of gluten dough made from 0.5 g gluten (protein equivalent) hydrated with 0.34 g distilled water were also determined.

2.3.5 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was performed essentially as described by Taylor et al. (2009). Samples were scanned in a Vertex 70/70v FTIR spectrophotometer (Bruker Optik, Ettlingen, Germany), using 64 scans, and 4 cm^{-1} bandwidth, and an interval of 1 cm^{-1} at a wavelength of 400-4500 cm^{-1} . To prevent the zein doughs cooling below the T_g of zein in water, the time interval between removing the dough from the centrifuge tube and commencing the FTIR scan was not more than 30 s. At least two independent experiments were performed for each sample. The FTIR absorbance spectra were baseline corrected using a Rubberband correction of 64 baseline points, normalised and Fourier self-deconvoluted using a Lorentzian filter with a resolution enhancement factor of 2 and a bandwidth of 8 cm^{-1} .

2.3.6 Statistical analysis

All experiments were carried out at least two times. One-way analysis of variance (ANOVA) was performed and the means were compared at a 95% confidence level using Fisher's Least Significant Difference Test.

3. Results and Discussion

3.1 2-D PAGE

2-D PAGE was used to analyse the commercial and total zein as it has a better resolving power than 1-D PAGE and enabled the clear separation of γ -zein from α -zein as distinct bands. 2-D PAGE revealed that the commercial zein comprised two rows of polypeptide spots M_r approx. 22 and 27 kDa of quite widely differing pI, plus some spots of much lower

M_r , approx. 10 kDa (Figure 1A). Although somewhat higher in apparent M_r , the two rows the former are consistent with α -zein 19 and α -zein 22, respectively (Lawton and Wilson, 2003). The low M_r protein was probably δ -zein. Thus, as expected the commercial zein was essentially α -zein. In contrast, in addition to the polypeptide spots in the commercial zein, the total zein contained several higher molecular weight spots of apparent M_r approx. 29 kDa of basic pI and lower molecular weight spots of apparent M_r approx. 18 and 15 kD (Figure 1B). The former are consistent with γ -zein and the latter with β -zein (Lawton and Wilson, 2003) and possibly also the 16 kDa γ -zein (Shewry, 2002). Thus, the 2-D PAGE showed that the total zein comprised the all the major zein sub-classes, α -, β -, γ - and δ -zein which are present in maize kernels, and that the commercial zein was predominantly α -zein.

3.2 Total zein dough formation

Unlike the commercial zein (Figure 2a), total zein did not form a viscoelastic dough when prepared in distilled water above the T_g of zein (Figure 2b). Various other treatments were then investigated to make total zein functional in terms of viscoelastic dough formation. It was found that total zein also did not form a viscoelastic dough when treated with 1.3% (w/w) acetic acid above the T_g of zein (Figure 2f). Dilute acetic acid was used as a treatment because Sly et al. (2014) demonstrated that commercial zein dough prepared with dilute acetic or lactic acids caused an increase in the extensibility of the dough, as was found in this present study (Figure 2e).

Next, the effect of first dissolving the total zein in 70% (w/w) aqueous ethanol and then casting it into a film and drying the film to remove the solvent before attempting to make a dough was investigated. This was done because casting a film from commercial zein in aqueous ethanol has been found to result in an increase in β -sheet structure (Chen et al., 2014) and β -sheet secondary structure is indicative of aggregation (Mizutani et al., 2003; Byaruhanga et al., 2006). When the total zein that had been cast into a film using aqueous ethanol was treated with warm water above the T_g of zein, a dough did not form. However,

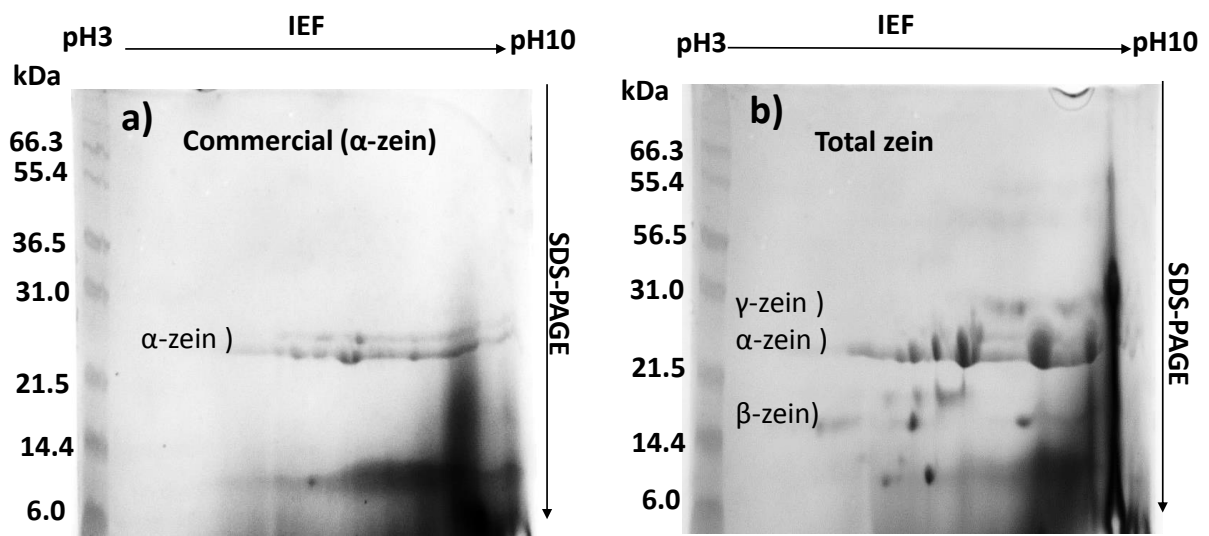


Figure 1: 2-D PAGE of
a) commercial (α -type) zein and
b) total zein

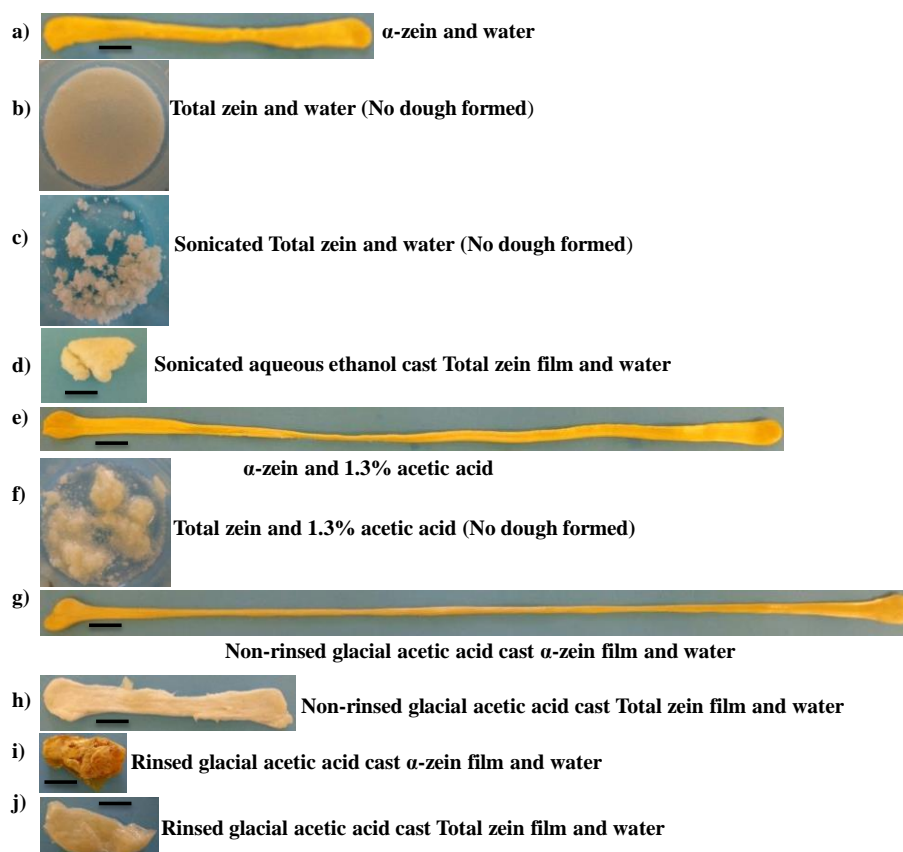


Figure 2: Effects of hydration solvent, film formation, sonication and rinsing out residual glacial acetic acid on α -zein and total dough formation and extension.

- a) α -zein and distilled water,
 - b) total zein and distilled water,
 - c) Sonicated total zein and distilled water,
 - d) Sonicated aqueous ethanol cast total zein film and distilled water,
 - e) α -zein and 1.3 % (w/w) acetic acid,
 - f) total zein and 1.3 % (w/w) acetic acid,
 - g) non-rinsed glacial acetic acid cast α -zein film and distilled water,
 - h) non-rinsed glacial acetic cast total zein film and distilled water,
 - i) rinsed glacial acetic cast α -zein film and distilled water,
 - j) rinsed glacial acetic acid cast total zein film and distilled water.
- Black scale bar represents 10 mm

when this total zein that had been prepared into a film was sonicated with water, a very stiff cohesive and elastic mass was formed (Figure 2d). Notably, however, the total zein preparation did not form a dough when simply sonicated with prior casting into a film. Hence, it appeared that the combination of dissolving total zein in aqueous ethanol, film formation and very high energy input through sonication was required to form a dough from total zein.

Li et al. (2012) found that commercial zein dissolved fully in glacial acetic acid due majorly to protonation and resulted in the formation of protein monomers, whereas in aqueous ethanol there was swelling of the protein, partial unfolding and it remained as multimers. Therefore the effect of dissolving total zein in glacial acetic acid followed by casting it into film was investigated. When this total zein film was treated with warm water above the T_g of zein, a true viscoelastic dough formed (Figure 2h). The same procedure with commercial zein resulted in a highly extensible dough (Figure 2g). The residual acetic acid in the total zein film may have acted as a plasticiser and effectively reduced the zein T_g , facilitating dough formation.

However, it was unclear whether the process of casting a film from total zein that had been dissolved in glacial acetic acid or any residual glacial acetic acid present after film drying was responsible for the total zein forming a viscoelastic dough. Of importance was the fact that the films still smelled of acetic acid after the overnight drying at 50°C. In the case of these unwashed films the residual acid concentration may have been higher than that of 1.3% acetic acid and so the observed effect of dough formation may have been due to a higher acid concentration. However, since the total zein containing all the zein sub-classes did not form a dough with 1.3% acetic acid without film formation this seems unlikely. To determine whether a dough would still form after removal of residual glacial acetic acid, it was completely rinsed out of the total zein film using distilled water. After the washing process, the pH of the wash water was between 7- 8. This compares with the measured pH of 1.3% acetic acid of pH 2.57, showing that the level of residual acid in the washed films very low. After this washing treatment and air-drying of the total zein film material, a viscoelastic dough still formed (Figure 2j). However, this dough was less extensible than the dough made from the total zein film that had not been rinsed with distilled water (Figure 2h). This lack of

extensibility after removal of residual glacial acetic acid was even more evident with commercial zein (Figure 2j). Nevertheless, it appears that it was the action of dissolving the total zein in glacial acetic acid that was the crucial step which enabled the formation of viscoelastic dough from total zein.

3.3 CLSM of α -zein and total zein doughs

As has been found with doughs made from commercial zein (Sly et al., 2014), the total zein doughs also autofluoresced, enabling the dough structure to be studied by CLSM without staining (Figure 3). All the commercial zein and total zein doughs, which had been manually stretched, had a fibrous structure comprising linear fibrils (white arrows). Lawton (1992) and Schober et al. (2010) suggested that the aggregation of zein into strands/fibrils is a key step in dough formation. There were, however, substantial differences in appearance between the treatments. Dough made from non-rinsed (containing residual glacial acetic acid) total zein film had a clearly ordered structure comprising very fine fibrils (Figure 3d). These were, however, much less continuous than the fibrils in the non-rinsed commercial zein film dough (Figure 3b). In turn, the latter were much more uniform with far less discontinuities than commercial zein doughs prepared with water (Figure 3a). Thus, it can be assumed that the presence of residual glacial acetic acid enabled the formation of a uniform ordered fibril structure, as was found by Sly et al. (2014) when commercial-zein was prepared into dough using dilute organic acids. The zein fibrils in commercial and total zein film doughs where residual glacial acetic acid had been rinsed out were much shorter and less well-aligned (Figure 3c,e) compared to their non-rinsed film doughs (Figure 3b,d). This is consistent with their lack of extensibility (Figure 2).

Interestingly, in both the rinsed and non-rinsed total zein film doughs the fibrils were composed of small bead-like structures (Figure 3d,e, white dotted arrows). The beaded fibrils were much coarser in the total zein film dough. Taylor et al. (2009) observed similar

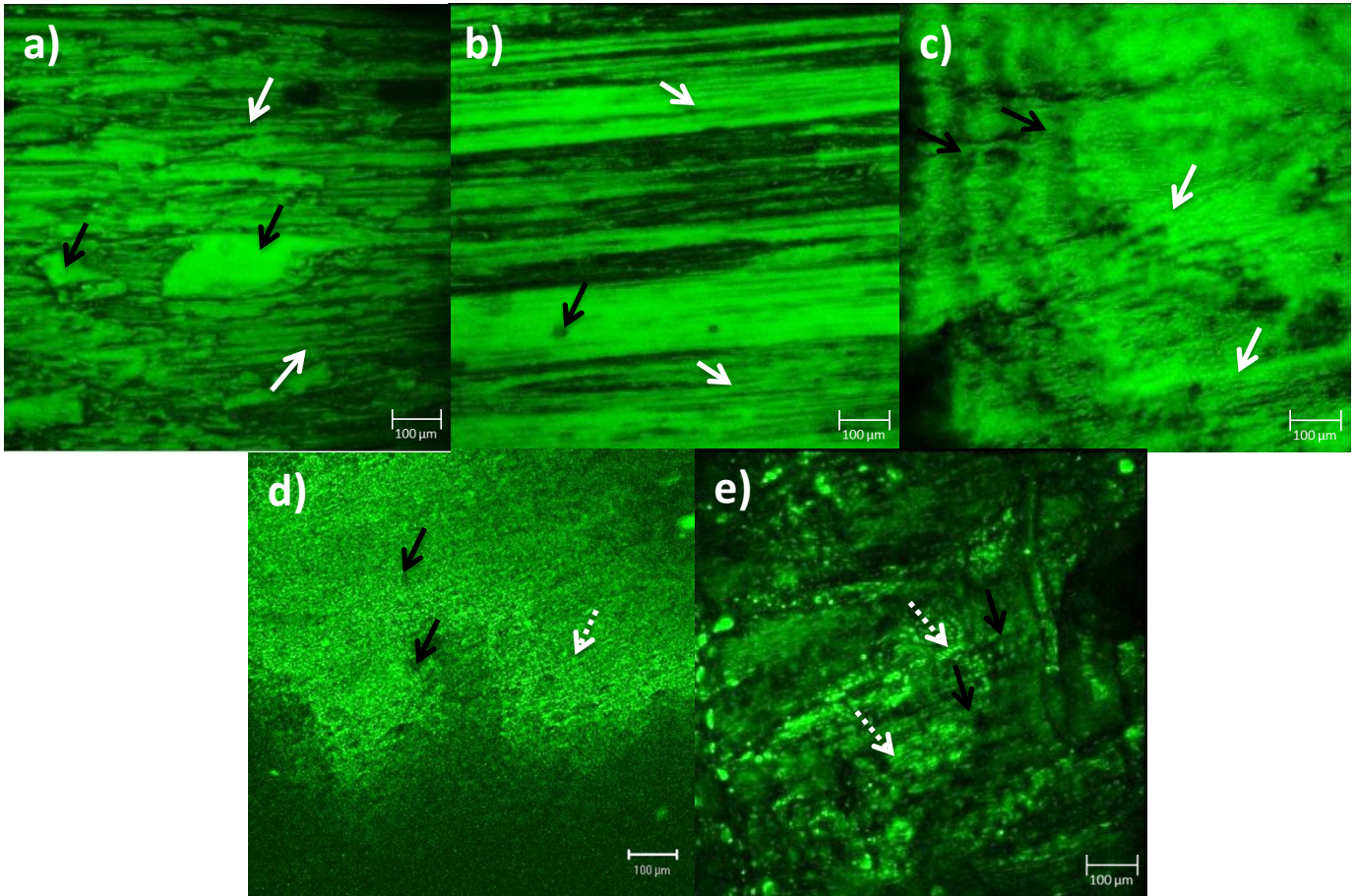


Figure 3: CLSM illustrating the effects of zein type, film formation and rinsing residual glacial acetic acid out with distilled water on the microstructure of manually stretched zein doughs made with distilled water

a) α -zein dough made with distilled water,

b) Non-rinsed α -zein film dough,

c) Rinsed α -zein film dough,

d) Non-rinsed total zein film dough,

e) Rinsed total zein film dough

White arrows: Fibrils are indicated by white arrows, White dotted arrows: Fibrils comprising beads, Black arrows: Discontinuities in fibrils

spherical structures during the formation of cast films from kafirin microparticles using acetic acid that formed short interlinked chains. Moreover, Don et al. (2003) attributed the rheological properties and aggregative behaviour of wheat gluten to spherical glutenin macropolymer, which form particle networks. Such prolamin particles are presumably aggregated prolamin protein nanostructures (Wang and Padua, 2012). That total zein formed fibrils comprising particles and not continuous fibrils may be due to disulphide cross-linking involving the β - and γ -zein.

3.4 Tensile properties of α -zein and total zein doughs

The various zein doughs exhibited a very large spread of tensile properties (Table 1). Doughs made from rinsed total zein (residual glacial acetic acid removed) had the highest peak stress (1,600 kPa) and non-rinsed commercial zein film dough (residual glacial acetic present), commercial zein dough made with 1.3% acetic acid and wheat gluten dough had the lowest peak stress (29-285 kPa). Notably, the rinsed total and commercial zein film doughs had substantially ($p < 0.05$) higher peak stress than their non-rinsed counterparts. Furthermore, the non-rinsed total zein film dough and the rinsed commercial zein film dough had a higher elastic-type component compared to wheat gluten dough but a similar viscous flow component (Figure 4). In turn, commercial zein dough prepared with water had a similar elastic-type component to wheat gluten but was far more extensible.

The tensile data show that when made into films first, total zein dough has much greater strength but statistically similar extensibility to commercial zein dough. Doughs could not be formed from total zein without film formation. However, the data shows that the presence of acetic acid in commercial zein dough made it far more extensible than when water alone was used. This is similar to the effect of commercial zein dough preparation with hydrogen

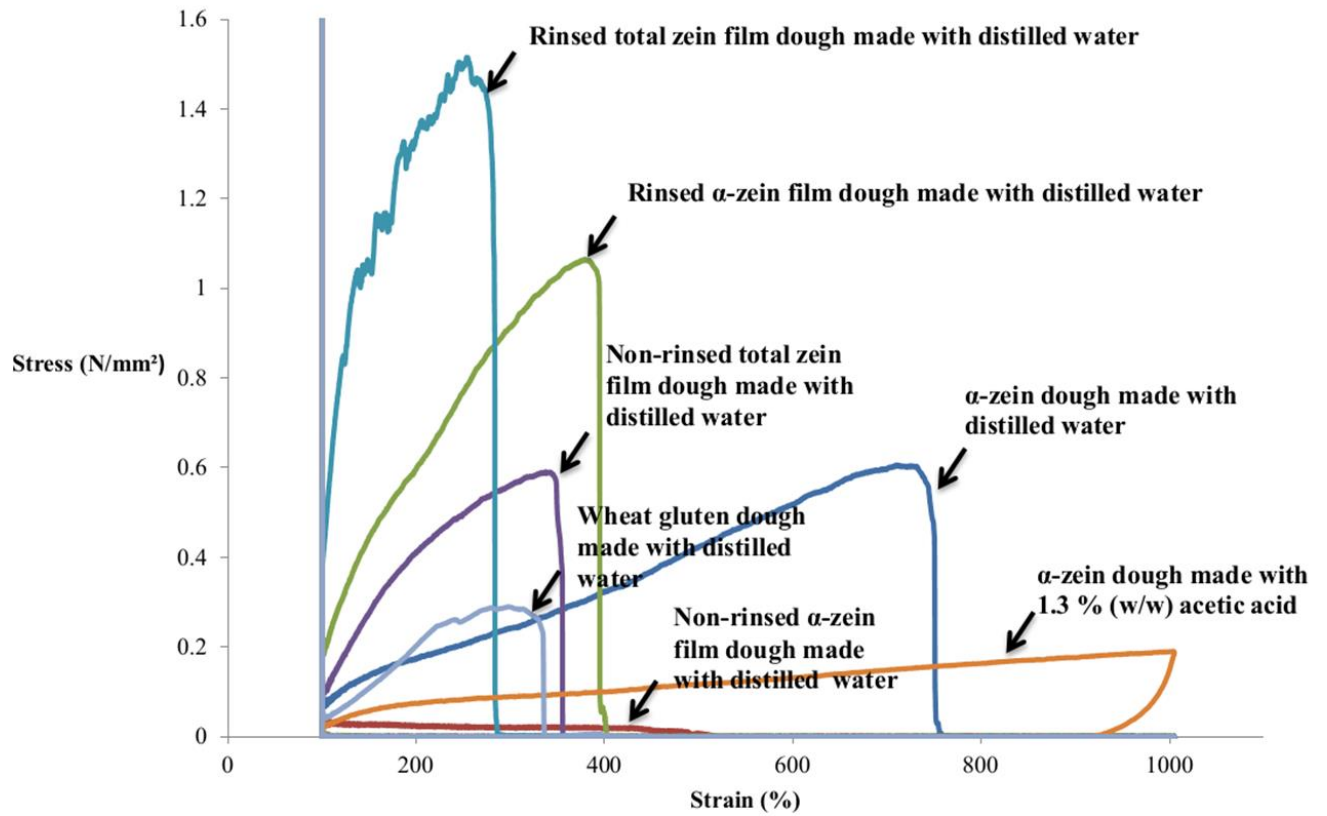


Figure 4: Representative stress-strain curves of the effects of zein type, film formation and rinsing residual glacial acetic acid out with distilled water on zein doughs made with distilled water or 1.3% (w/w) acetic acid

Table 1: Effects of zein type, casting zein into a film and dough preparation solvent on the tensile properties of zein doughs

Protein type	Cast into film	Dough preparation solvent	Peak force (N)	Extensibility (mm)	Peak stress (kPa)	Strain at maximum displacement (%)	Strain at maximum displacement (ϵ_H)	Extensional viscosity from peak stress (η_E , kPa.s)
Gluten	No	Water	2.0 ^{ab} ± 0.1	49 ^a ± 11	285 ^{ab} ± 19	265 ^a ± 35	0.97 ^a ± 0.14	3723 ^a ± 618
α-zein	No	Water	5.1 ^{cd} ± 0.9 ¹	191 ^b ± 29	724 ^{cd} ± 122	738 ^b ± 97	1.99 ^b ± 0.14	24516 ^d ± 5468
Total zein	No	Water	No dough formed (slurry)					
α-zein	No	1.3% acetic acid	1.5 ^a ± 0.3	270 ^c ± 0	213 ^a ± 37	1003 ^c ± 1	2.31 ^b ± 0.00	9780 ^b ± 1690
Total zein	No	1.3% acetic acid	No dough formed (slurry)					
α-zein	Yes	Water	0.2 ^a ± 0.0	50 ^a ± 42	29 ^a ± 5	265 ^a ± 140	0.86 ^a ± 0.56	395 ^a ± 175
Total zein	Yes	Water	4.1 ^{bc} ± 0.4	69 ^a ± 15	579 ^{bc} ± 59	332 ^a ± 50	1.19 ^a ± 0.15	9088 ^b ± 807
α-zein	Yes Residual glacial acetic acid rinsed out	Water	6.7 ^d ± 1.3	66 ^a ± 26	948 ^d ± 181	321 ^a ± 85	1.14 ^a ± 0.28	14979 ^c ± 5870
Total zein	Yes Residual glacial acetic acid rinsed out	Water	11.3 ^e ± 3.6	47 ^a ± 18	1600 ^e ± 513	255 ^a ± 60	0.92 ^a ± 0.23	19484 ^{cd} ± 4240

¹ Means ± Standard Deviation of four independent experiments, ² Calculated from the best fitting graph from four independent experiments, ϵ_H = Hencky strain/true strain, ^{abc} = Values in columns with different superscript letters differ significantly (p < 0.05)

peroxide (Taylor et al., 2016a). Like zein dough preparation with hydrogen peroxide, this is as result of formation of continuous fibrils (Figure 3) as was previously found by Sly et al. (2014) with commercial zein dough prepared with dilute organic acids. Removal of acetic acid prior to dough formation resulted in the formation of stiffer and less extensible zein doughs. This is a result of the fibrils being discontinuous and poorly aligned when the dough was formed and stretched (Figure 3). As the commercial and total zein were not defatted, the presence of lipids in the materials may have somewhat negatively affected the degree of cohesiveness and extensibility of the doughs. Schober et al. (2010) showed that when lipids were removed from the surface of commercial zein particles, doughs were more cohesive, smoother and more extensible.

3.5 FTIR spectroscopy of zein powders, zein films, wet zein doughs and slurries

Considering the Amide I band of the FTIR spectra of the commercial zein and total zein samples, two main peaks were observed at wavenumbers 1650 and 1620 cm^{-1} (Figure 5), the proportions of the peaks differing with the different treatments. The peak around 1650 cm^{-1} can be assigned to α -helical conformations or random coils and 1620 cm^{-1} to antiparallel β -sheet conformations (Duodu et al., 2001). Both the commercial zein and total zein preparations as well as the films formed from them had predominantly α -helical conformation, as indicated by FTIR in the Amide I region (Figure 5a). The commercial zein data are in agreement with the general finding that it is α -helical in conformation (Shewry and Tatham, 1990) and with Hsu et al. (2005) who found that the secondary structure of commercial zein film was predominately α -helical. Table 1 Supplementary shows that when formed into a film there was a slight but not statistically significant decrease in α -helical conformation of the commercial zein and a further slight statistically significant decrease ($p < 0.05$) when residual glacial acetic acid was rinsed out. Nevertheless, α -helical conformation

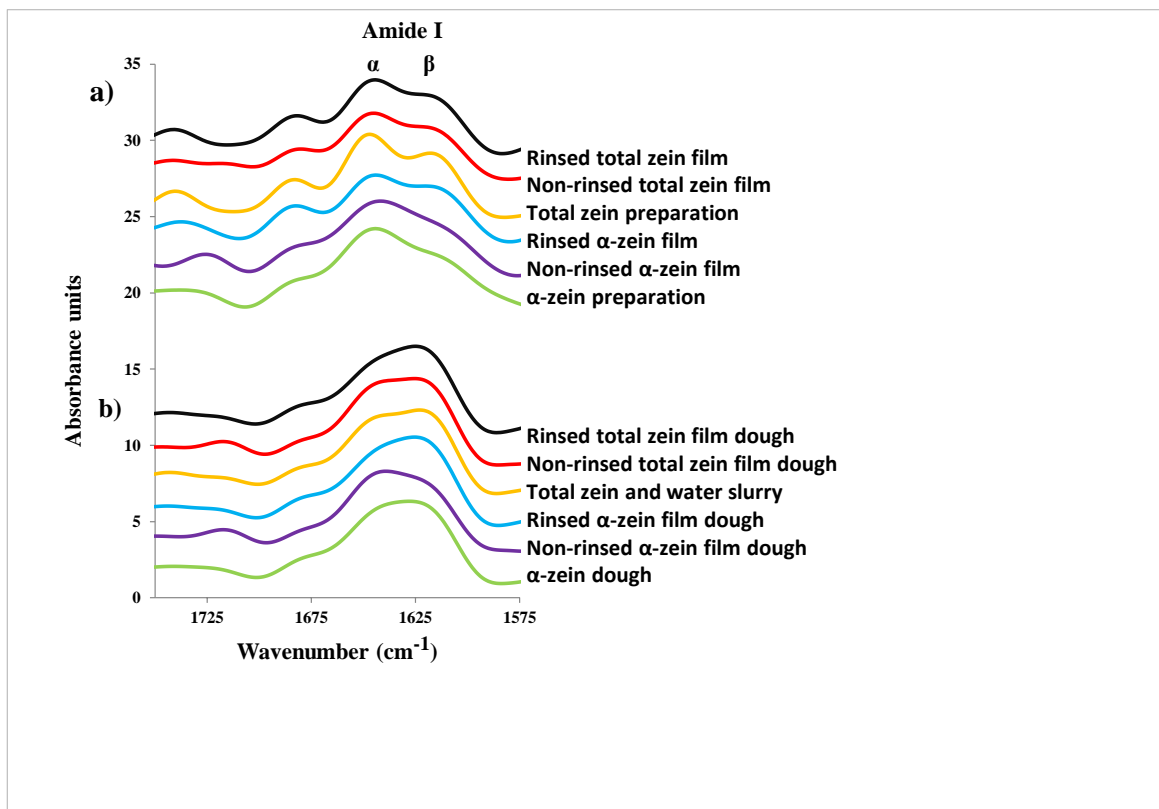


Figure 5: Effects of zein type, film formation and rinsing residual glacial acetic acid out with distilled water on the FTIR absorbance spectra of
a) dry zein powders and zein films and
b) wet zein doughs and slurries

still predominated, with an α : β ratio of 1.19:1. As stated, this increase in β -sheet formation when commercial zein is cast into a film has been described previously (Chen et al., 2014).

The total zein preparation had a higher proportion of β -sheet secondary structure than commercial zein with α : β ratios of 1.29:1 and 1.44:1, respectively (Table 1 Supplementary). However, the β -sheet proportion was slightly but not significantly reduced when the protein was dissolved in glacial acetic acid and cast into a film and slightly but not significantly increased again when the residual glacial acetic acid was removed. Gamma-zein in its physiological state has similar proportions of α -helical and β -sheet structure, 33 and 31%, respectively (Bicudo et al., 2005) and β -zein is predominantly β -sheet in structure (Forato et al., 2003). Hence, the presence of γ - and β -zein in total zein would account for its slightly higher proportion of β -sheet structure.

When both the commercial zein and total preparations and their films were hydrated with distilled water to form doughs or a slurry (when a dough did not form) the relative proportion of α -helical conformation was generally considerably reduced and β -sheet structure predominated, with α : β ratios in the range 0.90-0.97:1 (Table 1 Supplementary). The one exception to this was the non-rinsed (residual glacial acetic acid still present) commercial zein film dough where α -helical structure predominated (α : β ratio 1.12:1), as was previously observed with doughs made from commercial zein in the presence of organic acids (Sly et al., 2014). The increase in β -sheet conformation with dough formation is in agreement with Mejia et al. (2007) who found that when commercial zein was incorporated into a starch-based dough it caused β -sheet conformation to increase to approximately 48% with a concomitant decrease in the relative α -helical conformation. In fact, these workers have

attributed the viscoelastic properties of zein polymers to the presence of a high proportion of β -sheet conformation (Mejia et al., 2012; Erickson et al., 2012).

The observation that commercial zein film dough with residual glacial acetic acid was still predominantly α -helical in conformation and that total zein with distilled water changed to a predominantly β -sheet conformation but did not form a dough suggests that β -sheet conformation is not a prerequisite nor a guarantee that zein will form a dough. Clearly, there is a complex interplay of factors involved in whether zein will form a dough with water, including the type of zein, total zein (β - and γ -zein in addition to α -zein) or commercial zein (predominantly α -zein) and critically also the effect of acetic acid on the zein properties.

We have previously suggested that the increase in commercial zein α -helical conformation in the presence of organic acids (Sly et al., 2014), as observed in the present work with the commercial zein film dough containing residual glacial acetic acid, was possibly a result of deamination of the zein molecules under acidic conditions (Zhang et al., 2011). It appears that with total zein such a change counteracts the effects of the β - and γ -zein in total zein, which otherwise prevents it from forming a dough through disulphide bonded cross-linking.

4. Conclusions

The major finding of this study is that a viscoelastic dough can also be formed from total zein (comprising α -, β -, γ - and δ -zein) as well as from commercial zein (predominantly α -zein).

This was achieved by dissolving total zein in glacial acetic acid and casting a film.

Importantly, even when the residual glacial acetic acid was rinsed out of the film a viscoelastic dough still formed with simple water preparation. This is contrast to the situation with films cast from zein dissolved in aqueous ethanol where it was necessary to impart

considerable energy by sonicating the total zein film material in order to form a dough. The fact that zein is completely solubilised by glacial acetic acid, unlike with aqueous ethanol, and that all the zein doughs were more extensible in the presence of acetic acid implies that acetic acid brings about chemical changes in the zein molecules so that they can better interact with water molecules and hence enable total zein to form a dough.

From a practical standpoint, total zein dough probably would probably be advantageous over α -zein dough in bread making because it is less extensible. This is presumably a consequence of cross-linking through disulphide bonding. Further, as total zein and not solely α -zein is the form of zein present in maize flour, this work suggests that it may be possible to form a dough directly from maize flour with viscoelastic, gas-holding functionality due to the zein present.

Acknowledgements

B.L King is grateful for the provision of South African National Research Foundation and University of Pretoria bursaries. Dr J.O. Anyango for performing the 2-D PAGE. Alan Hall of the Laboratory for Microscopy and Microanalysis is acknowledged for assistance with the CLSM.

References

- Adebowale, A.A., Emmambux, M.N., Beukes, M., Taylor, J.R.N., 2011. Fractionation and characterization of teff proteins. *Journal of Cereal Science* 54, 360-366.
- Bicudo, T.C., Forato, L.A., Batista, L.A.R., Colnago, L.A., 2005. Study of the conformation of γ -zeins in purified maize protein bodies by FTIR and NMR spectroscopy. *Analytical and Bioanalytical Chemistry* 383, 291-296.

- Byaruhanga, Y.B., Emmambux, M.N., Belton, P.S., Wellner, N., Ng, K.G., Taylor, J.R.N., 2006. Alteration of kafirin and kafirin film structure by heating with microwave energy and tannin complexation. *Journal of Agricultural and Food Chemistry* 54, 4198-4207.
- Chen, Y., Ye, R., Liu, J., 2014. Effects of different concentrations of ethanol and isopropanol on physicochemical properties of zein-based films. *Industrial Crops and Products* 53, 140-147.
- Don, C., Lichtendonk, W., Plijter, J.J., Hamer, R.J., 2003. Glutenin macropolymer: A gel formed by glutenin particles. *Journal of Cereal Science* 37, 1-7.
- Duodu, K. G.; Tang, H.; Wellner, N.; Belton, P. S.; Taylor, J. R. N. FTIR and solid state ¹³C NMR spectroscopy of proteins of wet cooked and popped sorghum and maize. *Journal of Cereal Science* 2001, 33, 261–269.
- Erickson, D.P., Campanella, O.H., Hamaker, B.R., 2012. Functionalizing maize zein in viscoelastic dough systems through fibrous, β -sheet-rich protein networks: An alternative, physicochemical approach to gluten-free breadmaking. *Trends in Food Science and Technology* 24, 74-81.
- FAOSTAT3, 2014. Production, Crops. <http://faostat3.fao.org> (accessed June, 2016).
- Forato, L.A., Bicudo, T.C., Colnago, L.A., 2003. Conformation of α -zeins in solid state by Fourier Transform IR. *Biopolymers* 6, 421-426.
- Hsu, B.-L., Weng, Y.-M., Liao, Y.-H., Chen, W., 2005. Structural investigation of edible zein films/coatings and directly determining their thickness by FT-Raman spectroscopy. *Journal of Agricultural and Food Chemistry* 53, 5089-5095.
- Lawton, J.W., 1992. Viscoelasticity of zein-starch doughs. *Cereal Chemistry* 69, 351-355.
- Lawton, J.W., Wilson, C.M., 2003. Proteins of the kernel. In: White, P.J., Johnson, L.A. (Eds.), *Corn Chemistry and Technology*, 2nd ed. AACCI, St. Paul, MN, pp. 313-354.

Li, Y., Li, J., Xia, Q., Zhang, B., Wang, Q., Huang, Q., 2012. Understanding the dissolution of α -zein in aqueous ethanol and acetic acid solutions. *The Journal of Physical Chemistry B* 116, 12057-12064.

Mason, N.M., Jayne, T.S., Shiferaw, B., 2015. Africa's rising demand for wheat: Trends, drivers, and policy implications. *Development Policy Review* 33, 581-613.

Mejia, C.D., Mauer, L.J., Hamaker, B.R., 2007. Similarities and differences in secondary structure of viscoelastic polymers of maize α -zein and wheat gluten proteins. *Journal of Cereal Science* 45, 353-359.

Mejia, C.D., Gonzalez, D.C., Mauer, L.J., Campanella, O.H., Hamaker, B.R., 2012. Increasing and stabilizing β -sheet structure of maize zein causes improvement in its rheological properties. *Journal of Agricultural and Food Chemistry* 60, 2316-2321.

Mizutani, Y., Matsumura, Y., Imamura, K., Nakanishi, K., Mori, T., 2003. Effects of water activity and lipid addition on secondary structure of zein in powder systems. *Journal of Agricultural and Food Chemistry* 51, 229-235.

Oom, A., Pettersson, A., Taylor, J.R.N., Stading, M., 2008. Rheological properties of kafirin and zein prolamins. *Journal of Cereal Science* 47, 109-116.

Renzetti, S., Arendt, E.K., 2009. Effects of oxidase and protease treatments on the breadmaking functionality of a range of gluten-free flours. *European Food Research and Technology* 229, 307-317.

Schober, T.J., Moreau, R.A., Bean, S.R., Boyle, D.L., 2010. Removal of surface lipids improves the functionality of commercial zein in viscoelastic zein-starch dough for gluten-free breadmaking. *Journal of Cereal Science* 52, 417-425.

Schober, T.J., Bean, S.R., Tilley, M., Smith, B.M., Ioerger, B.P., 2011. Impact of different isolation procedures on the functionality of zein and kafirin. *Journal of Cereal Science* 54, 241-249.

Shewry, P.R., 2002. The major seed storage proteins of spelt wheat, sorghum millets and pseudocereals. In: Belton, P.S., Taylor, J.R.N. (Eds.), *Pseudocereals and Les Common Cereals*. Springer, Berlin, pp. 1-24.

Shewry, P.R., Tatham, A.S., 1990. The prolamin storage proteins of cereal seeds: structure and evolution. *Biochemical Journal* 267, 1-12.

Shukla, R., Cheryan, M., 2001. Zein: the industrial protein from corn. *Industrial Crops and Products* 13, 171-192.

Sly, A.C., Taylor, J., Taylor, J.R.N., 2014. Improvement of zein dough characteristics using dilute organic acids. *Journal of Cereal Science* 60, 157-163.

Taylor, J., Taylor, J.R.N., Belton, P.S., Minnaar, A., 2009. Preparation of free-standing films from kafirin protein microparticles: mechanism of formation and functional properties. *Journal of Agricultural and Food Chemistry* 57, 6729-6735.

Taylor, J., Taylor, J.R.N., Dutton, M.F., de Kock, S., 2005. Identification of kafirin film casting solvents. *Food Chemistry* 90, 401-408.

Taylor, J.R.N., Johnson, S.K., Taylor, J., Njila, S., Jackaman, C., 2016a. Oxidation of commercial (α -type) zein with hydrogen peroxide improves its hydration and dramatically increases dough extensibility even below its glass transition temperature. *Journal of Cereal Science* 70, 108-115.

Taylor, J.R.N., Taylor, J., Campanella, O.H., Hamaker, B.R. 2016b. Functionality of the storage proteins in gluten-free cereals and pseudocereals in dough systems. *Journal of Cereal Science* 67, 22-34.

Wang, Y., Padua, G.W., 2012. Nanoscale characterization of zein selfassembly. *Langmuir* 28, 2429-2435.

Zhang, B., Luo, Y., Wang, Q. 2011. Effect of acid and base treatments on structural, rheological and antioxidant properties of α -zein. *Food Chemistry* 124, 210-220.