Received: 18 January 2023 | Revised: 5 March 2023 | Accepted: 21 March 2023

DOI: 10.1002/cche.10665

RESEARCH ARTICLE

# Bioplastic film making properties of quality protein maize (QPM) zein

**Julia T. Baloyi** | **Janet Taylor** | **John R. N. Taylor**

Department of Consumer and Food Sciences, University of Pretoria, Pretoria, South Africa

**Correspondence**
John R. N. Taylor, Department of Consumer and Food Sciences, University of Pretoria, Private Bag X20, Hatfield 0028, Pretoria, South Africa.
Email: john.taylor@up.ac.za

**Funding information**
National Research Foundation, Grant/Award Number: 119549

## Abstract

**Background and Objective:** Quality protein maize (QPM) has abundant γ-zein, which crosslinks itself and other zeins through disulfide bonding. The work aims to develop zein bioplastics with better functionality by using QPM zein. Physical properties of cast QPM zein films from QPM maize were compared with zein films from regular maize types and commercial zein, all without added plasticizers.

**Findings:** QPM zein contained 3.8% cysteine compared with 1.8%–2.7% in regular maize zeins and 1% in commercial zein and the QPM zein had a much higher proportion of γ-zein. QPM zein films cast from glacial acetic acid (GAA) were opaque but absorbed the least liquid (≈16%) and swelled less (≈11%) after buffer immersion than the other zein films and aqueous ethanol-cast films. Notably, the GAA-cast QPM zein films became highly flexible after ambient storage, whereas the other zein films remained brittle.

**Conclusions:** The low buffer uptake and swelling of QPM zein films is attributed to crosslinking involving the cysteine-rich γ-zein polypeptides. Their flexibility is attributed to better solubilization of the zein in GAA and water molecules bound to the γ-zein polypeptides acting as a plasticizer.

**Significance and Novelty:** QPM zein can enable the formation of flexible zein bioplastics without added plasticizers.

KEYWORDS

bioplastic films, γ-zein, cysteine, quality protein maize (QPM), two-dimensional polyacrylamide gel electrophoresis

## 1 | INTRODUCTION

Quality protein maize (QPM) has better protein nutritional quality than regular maize due to its high lysine and tryptophan contents (Babu et al., 2015). Unlike the original high lysine maize, it has similar endosperm hardness to regular maize due to its high content of cysteine-rich γ-zeins (Wu et al., 2010). Despite these attributes, the utilization of QPM as a food and feed grain is low. Reasons for this include the high price of QPM grain compared with regular maize (Nyakurwa et al., 2017). Therefore, additional applications are required to increase demand for QPM and hence drive greater cultivation by farmers and thereby reduce its cost. A potential application is to use QPM for bioethanol production. The protein-rich distillers

dried grain and solubles coproduct would be more valuable for animal feed because of its better protein quality. Additionally, its zein could have novel applications because of its high content of γ-zein.

Prolamin proteins, in particular zein, are the subject of intensive study as bioplastic materials such as polymeric films for food packaging. This is because of zein's relative hydrophobicity, insolubility, and barrier properties to moisture and solutes (Bayer, 2021; Egea et al., 2021). Unplasticized zein bioplastic films, however, have limited applications in food and nonfood industries, because they are generally brittle and stiff with high water vapor permeability (Dubey & Dubey, 2020; Turasan & Kokini, 2017). Plasticizers are added to induce flexibility in zein films (Huo et al., 2018; Masamba et al., 2016).

Recent research has focussed on crosslinking the protein molecules to improve zein film strength and barrier properties. Using crosslinked plasticized zein films, Turasan et al. (2018) proposed that glutaraldehyde (a crosslinking agent) displaced oleic acid molecules (the plasticizer used) bound at the amine ends of glutamine in zein, inducing cross-linkages between zein polypeptides to form a compact structure that restricts movement of water molecules. Although plasticizers and crosslinking agents improve bioplastic film properties, one drawback is that some agents may be toxic when used in food or biomedical applications (Azeredo & Waldron, 2016; Wang et al., 2021). Moreover, common synthetic plastics such as polyethylene and polypropylene are flexible and do not require the inclusion of plasticizers (Cadogan & Howick, 2012).

The high proportion of the γ-zein class in QPM zein (Holding, 2014) may be beneficial in improving the properties of zein-based bioplastics because of its high content of cysteine residues, which form inter- and intramolecular disulfide crosslinks (Fass & Thorpe, 2018). Kafirin bioplastic films have been shown to have better tensile strength, barrier properties, and film water vapor characteristics than films from commercial zein (Gillgren & Stading, 2008), which is essentially α-zein (Lawton, 2002). These properties of kafirin films have been attributed to disulfide bond cross-linking involving the cysteine-rich β- and γ-kafirins (Anyango et al., 2011; Xiao et al., 2017). With respect to zein, Nonthanum et al. (2012) found that γ-zein pellets formed a continuous film structure after solvent evaporation. This was attributed to cross-linkages between cysteine-rich γ-zein polymers. Therefore, the objective of this work was to determine dry and wet functional properties of unplasticized bioplastic films made from zein isolated from QPM maize.

## 2 | MATERIALS AND METHODS

### 2.1 | Materials

Three maize types were used: (i) whole grain white QPM, Ethiopian variety MHQ 138; (ii) whole grain regular white maize, Ethiopian variety MH 140 (both kindly donated by Dr K. Abegaz, Hawassa University); and (iii) refined white maize meal (80% extraction rate) (Pride Milling) obtained from a local store. They were coded QPM, Eth RM, and SA RM, respectively. Commercial zein was obtained from Sigma-Aldrich (Product code: Z3625).

### 2.2 | Methods

#### 2.2.1 | Zein extraction

The maize grains were milled to a flour of maximum particle size of 0.5 mm using a laboratory hammer mill. Total zein (i.e., comprising α-, β-, γ- and δ-zein) was extracted from the flour (500 g) using 70% (w/w) aqueous ethanol containing 1.0% (w/w) sodium metabisulfite and 0.35% (w/w) acetic acid at a ratio of one part flour to five parts solvent (w/w). The metabisulfite was included to break disulfide bonds to solubilize the cross-linked zein (Anderson & Lamsal, 2011) and the acetic acid was included to enable direct precipitation of the zein without additional pH adjustment (Muhiwa et al., 2017). Extraction was at 70°C for 1 h with continuous stirring. This temperature was used as the efficiency of zein extraction is improved at elevated temperature (Anderson & Lamsal, 2011; Lawton, 2002). The slurry was centrifuged at 1000*g* for 5 min at 25°C. The clear supernatant containing the zein was then decanted into a shallow stainless-steel tray and placed in a fume hood overnight for solvent evaporation at ambient temperature (≈25°C). After which, cold distilled water (8°C) was added to precipitate zein. Then, the wet protein concentrate was filtered under vacuum before air drying in a fume hood overnight at ambient temperature. The zein preparations were then defatted with hexane at 25°C, then air-dried again. After which they were milled into a powder of similar particle size to commercial zein using an air-cooled knife-type laboratory mill and stored in ziplock polyethylene bags at 9–10°C.

#### 2.2.2 | Zein film preparation

Zein films were cast as described by Anyango et al. (2011) but without inclusion of plasticizers. Zein (1.2 g protein equivalent) was dissolved in the casting solvent

(8.8 g) (glacial acetic acid, i.e., pure acetic acid (GAA) at 30°C or 70% (w/w) aqueous ethanol at 70°C) by continuous stirring for 10 min in a covered flask. The zein solution was then cooled to ambient temperature and additional GAA or aqueous ethanol was added to replace the solvent lost during evaporation. Zein solutions (3 g) were weighed into rectangular silicone baking trays (28 mm × 69 mm) then gently swirled for 30 s to distribute the liquid evenly. The trays were placed on a level surface in an oven (not forced draft) and held at 50°C overnight. Zein films were weighed before being photographed by scanning, then stored in ziplock bags at 9–10°C.

## 2.3 | Analyses

### 2.3.1 | Moisture and protein contents

Maize moisture content was determined by AACC Method 44-15A (AACC, 2000). Protein content ($N \times 6.25$) of the maize and zein preparations was determined by a Dumas total combustion method, AACC Method 46-30 (AACC, 2000). Zein preparation yield was calculated as the weight of total protein recovered divided by grain protein content × 100.

### 2.3.2 | Cysteine and methionine contents

The cysteine and methionine contents of the zein preparations were determined using a modified PICO TAG method (Waters Millipore). Zein was first oxidized with performic acid to convert cysteine and methionine quantitatively to cysteic acid and methionine sulfone, respectively. The oxidized zein was hydrolyzed with 6 M HCl for 24 h and then derivatized with phenylisothiocyanate to produce phenyltiocarbamyl amino acids. High-performance liquid chromatography analysis was performed using a Waters Millipore C-18 reversed-phase column (3.9 mm × 150 mm) at a 101.5 ml/min flow rate. Cysteic acid (Sigma-Aldrich, C-7630) and methionine sulfone (Sigma-Aldrich, M-0876) were used as standards.

### 2.3.3 | Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE)

Zein was first solubilized in a buffer solution containing Ampholite and DeStreak Rehydration Solution. Isoelectric focusing (IEF) was performed using a ZOOM® IPG System (Invitrogen) according to the manufacturer's instruction manual. Immobilized pH gradient ZOOM® strips (7 cm; pH 3–10, nonlinear) were hydrated with the protein solution, then placed in a ZOOM® IPG Runner TMMini Chamber and focused on steps at 200 V for 20 min, 450 V for 15 min, 750 V for 15 min, and 2000 V for 45 min. After IEF, alkylation was performed with iodoacetamide. Sodium dodecyl sulfate-PAGE (SDS-PAGE) was carried out in the second dimension at a contact voltage of 200 V, 80 mA, and 10 W for 1 h until bands were closer to the lower level of the gel. Gels were stained with Coomassie Brilliant Blue R-250. After destaining, the gels were photographed using a scanner.

### 2.3.4 | SDS-PAGE

The zeins were analyzed by SDS-PAGE in an unreduced form and after reduction by 2-mercaptoethanol using 4%–12% Novex NuPAGE® polyacrylamide gradient gels at a protein loading of 10 μg, as described by Elhassan et al. (2018).

### 2.3.5 | Film exposure to ambient conditions

Freshly prepared zein films were placed on a polyurethane varnished wooden laboratory benchtop indoors and stored under ambient conditions for 72 h. The mean temperature over the storage period was 26°C and mean relative humidity was 62%. During the storage period, the films were observed visually and at the end of the period their flexibility was assessed by carefully manually manipulating them.

### 2.3.6 | Film immersion in buffer

Buffer uptake by the films was determined as described by Anyango et al. (2011). Weighed films were immersed in 0.2 M sodium phosphate buffer (pH 6.8) at 39°C for 12 h with gentle agitation in a waterbath with a rotating platform. After which, they were removed from the buffer, gently blotted with paper towels to remove buffer on the surface. They were then weighed and photographed using a flatbed scanner. Buffer uptake was calculated as follows:

$$\text{Buffer uptake (\%)} = \frac{\text{Filmweight after immersion (mg)} - \text{Film weight before immersion (mg)}}{\text{Film weight before immersion (mg)}} \times 100.$$

### 2.3.7 | Scanning electron microscopy (SEM)

Zein films (before and after immersion) were dried in a silica gel desiccator for 48 h. Pieces of the dried films (5 mm × 5 mm) were mounted on an aluminum stub with double-sided tape and coated with carbon before viewing using a Zeiss Ultra PLUS Field Emission SEM (Carl Zeiss) at an operating voltage of 3.0 kV.

### 2.3.8 | Statistical analyses

All experiments were performed twice. One-way analysis of variance was performed using STATISTICA 8 software (StatSoft). The means were compared at a 95% level of confidence using Fisher's least significant difference test.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Zein characterization

The amount of zein extracted from QPM was less than from the regular maize types, on both a grain and protein basis (Table 1). The lower zein content of QPM can be attributed to delayed zein synthesis due to the expression of the *opaque-2* genes (Larkins et al., 1984), which results in the proportion of zein in QPM being almost 50% less than that of regular maize types (Holding, 2014).

The cysteine content of the QPM zein was considerably higher (3.8%), compared to the zein preparations from the regular maize types (1.8% and 2.7%) and much higher than that of the commercial zein (1.0%). In contrast, the methionine contents of the regular maize zeins and commercial zein were considerably higher (1.5%–3.1%) than that of the QPM zein, (0.8%). The high cysteine content and low methionine content of QPM zein is as a result of the pleiotropic effect of the *opaque-2* modifier genes that increase the synthesis of cysteine-rich γ-zein (Geetha et al., 1991), while subsequently reducing the expression and accumulation of methionine-rich δ-10 kDa zein (Hunter et al., 2002).

2D-PAGE showed that the QPM zein had less $\alpha_1$-zein (24 kDa), abundant γ-zein (27 kDa), and a fewer higher molecular weight γ-zein spots (~55 kDa), compared with the regular maize zeins (Figure 1). The 27 and 55 kDa γ-zeins are two different forms of γ-zein that are expressed in maize, having similar amino acid composition but differing in their number of amino acid residues, 204 and 278, respectively (Woo et al., 2001). The higher proportion of γ-zein in the QPM zein was expected and is consequence of the *opaque-2* modifier genes that enhance duplication at the 27 kDa locus in chromosome

**TABLE 1** Yield, purity, and cysteine and methionine contents of the zein preparations.

| Zein type | Maize grain Protein content (g/100 g maize) (db) | Zein preparation | | | Amino acid content (g/100 g protein) | |
|---|---|---|---|---|---|---|
| | | Zein extracted (g/100 g maize) (db) | Zein yield (g/100 g protein) | Zein purity (g protein/100 g preparation) (db) | Cysteine | Methionine |
| QPM | $9.72^{b} \pm 0.01$ | 2.90 | 29.8 | $72.9^{a} \pm 1.0$ | $3.80^{d} \pm 0.32$ | $0.84^{a} \pm 0.06$ |
| Eth RM | $10.00^{c} \pm 0.21$ | 5.26 | 52.4 | $81.5^{b} \pm 0.2$ | $1.83^{b} \pm 0.11$ | $1.50^{b} \pm 0.03$ |
| SA RM | $8.35^{a} \pm 0.12$ | 5.70 | 68.3 | $73.0^{a} \pm 0.3$ | $2.69^{c} \pm 0.04$ | $3.09^{d} \pm 0.07$ |
| Commercial zein | NA | NA | NA | $92.4^{c} \pm 0.3$ | $0.98^{a} \pm 0.02$ | $2.02^{c} \pm 0.03$ |

*Note:* Mean ± SD of two replicate determination. Mean values in the same column with a different letter in superscript are significantly different at $p \leq .05$.

Abbreviations: db, dry basis; Eth RM, whole grain regular white maize, Ethiopian variety MH 140; NA, not applicable; QPM, whole grain white QPM, Ethiopian variety MHQ 138; SA RM, refined white maize meal (80% extraction rate).

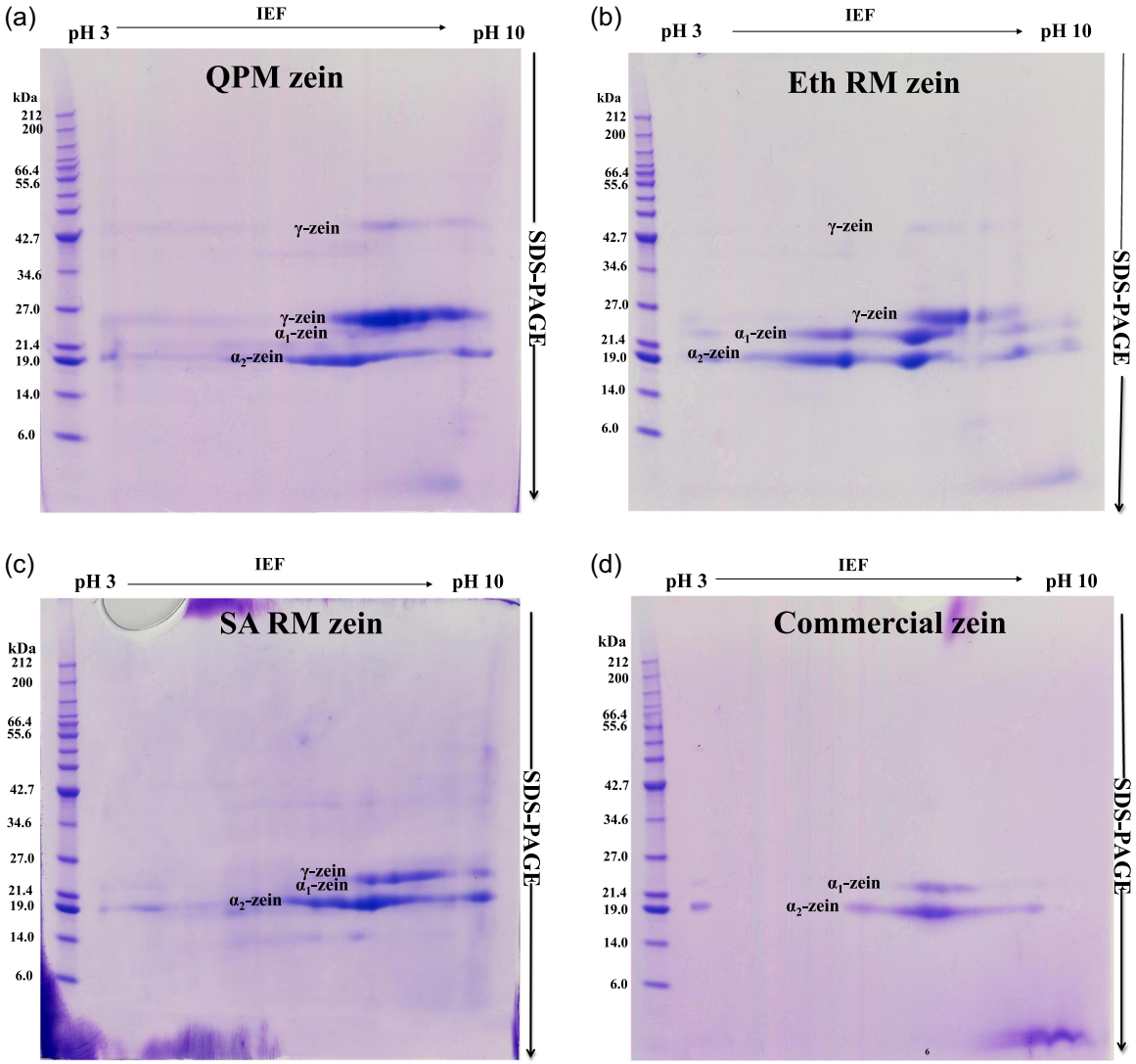


**FIGURE 1** Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) of the different zein preparations. (a) Whole grain white QPM, Ethiopian variety MHQ 138 (QPM zein); (b) whole grain regular white maize, Ethiopian variety MH 140 (Eth RM zein); (c) refined white maize meal (80% extraction rate) (SA RM zein); (d) commercial zein. [Color figure can be viewed at wileyonlinelibrary.com]

7 where the γ-zein locus (QTL) (qγ27) is located (Liu et al., 2016). No γ-zein was evident in the commercial zein. This was due to it being removed during the sulfite steep in the maize wet milling process, where the corn gluten co-product serves as the source of commercial zein (Yang et al., 2005).

## 3.2 | Zein film properties under ambient conditions (before immersion in buffer)

All the zein preparations, including the QPM zein, could form films from both the aqueous ethanol and GAA casting solvents (Figure 2A,B, respectively). However, when first prepared, the films from all the zein types were brittle and fractured very readily (Figure 2Aa–d), as plasticizers were omitted to directly compare the film properties of the different zeins. The fragility of the films precluded the application of testing methods that could quantify their mechanical- and certain other physical properties. The brittleness was due to the zeins being largely in their glassy state, as the glass transition temperature of zein in its dry state is around 160–167°C (Di Gioia et al. (1999). The zein films were ~160 μm thick. However, it was not possible to obtain meaningful comparative thickness data for the different types of films as several contained particles and all were somewhat wrinkled, in addition to being brittle and fragile (Figure 2Aa–d,Ba–d).

The QPM zein films cast from aqueous ethanol were somewhat more yellowish-brown in color and translucent when compared to the Eth RM zein and SA

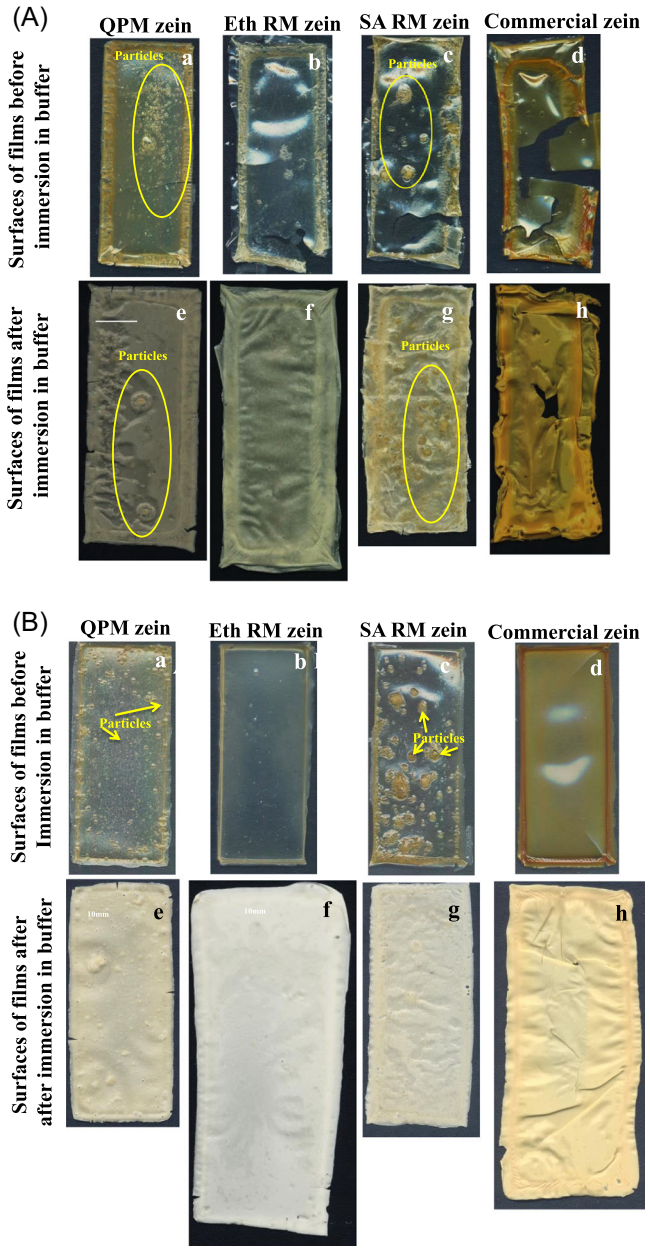


**FIGURE 2** Appearance and relative of size of the cast films from the different zein types before and after immersion in pH 6.8 phosphate buffer for 12 h. (A) Films cast from 70% aqueous ethanol solution. (B) Films cast from glacial acetic acid solution. (a–d) Surfaces of films before immersion in buffer. (e–h) Surfaces of films after immersion in buffer. Eth RM, whole grain regular white maize, Ethiopian variety MH 140; QPM, whole grain white QPM, Ethiopian variety MHQ 138; SA RM, refined white maize meal (80% extraction rate). [Color figure can be viewed at wileyonlinelibrary.com]

RM zein films, with particles within the films (Figure 2A). There were also particles in the SA RM films. The variation in color of the films from the different white maize types was presumably due to them containing differing amounts of carotenoid pigments, which were responsible for the strong yellow color of the commercial zein films. The translucency of the QPM zein films was likely due to poorer solubility of the QPM zein in 70% ethanol due to its high γ-zein content, as γ-zein is more hydrophobic than α- and β-zein (Belton et al., 2006). The particles in the QPM and SA RM films were probably due to nonzein impurities as the protein contents of the preparations of their zein (≈73%) were considerably lower than that of the Eth RM zein (81.5%).

The QPM zein films cast from GAA were more opaque than the Eth RM and SA RM zein films (Figure 2B), and also had particles in them, as with the aqueous ethanol cast films (Figure 2A). The opaqueness of the QPM zein films was likely due to the observed relatively poor solubility of the QPM zein in the GAA casting solvent, as with aqueous ethanol, which was presumably a consequence of its much higher content of γ-zein (Figure 1a).

SEM showed that all zein films had smooth surfaces with particles of differing size on their surfaces (Figure 3a). There was no evident difference between the different zein types. By SEM, all the films cast from GAA also appeared very similar, all having particles on their surface (Figure 3b). As stated, the presence of particles on the surface of the films is likely due to insoluble impurities.

In preliminary work, it was noticed that the QPM zein films became flexible over time. This was studied more systematically. After keeping the films for 72 h at ambient temperature and relative humidity, the QPM zein films cast from GAA had become highly flexible, to the extent that they could be manually bent into an arc and stood on both ends without breaking (Figure 4). However, the regular zein and commercial zein films cast from GAA remained brittle, as did all the zein films cast from aqueous ethanol. The development of a flexible prolamin film without the inclusion of plasticizers is a novel finding, which can be attributed to QPM zein's high γ-zein content in combination with the GAA casting solvent.

γ-Zein, despite its high hydrophobicity (Belton et al., 2006), is soluble in water and salt solutions when reduced into its polypeptide monomers (Wilson et al., 1981), unlike the other zein classes. As explained, the zein was extracted from the three maize types using aqueous ethanol, plus acetic and sodium metabisulfite as a reducing agent to inter- and intermolecular disulfide bonds. When the preparations were subjected to SDS-PAGE in an unreduced form, there was evidence of γ-zein monomers in both the Eth RM zein and QPM zein preparations, the band of $M_r \sim 27$ k (Esen, 1987) (Figure 5a) directly above the strong and very faint 22 k α1-zein monomer bands in the Eth RM zein and QPM zein (Tracks 3 and 4, respectively). These are indicated by dashed arrows. The intensity of the QPM zein γ-zein band was higher than that

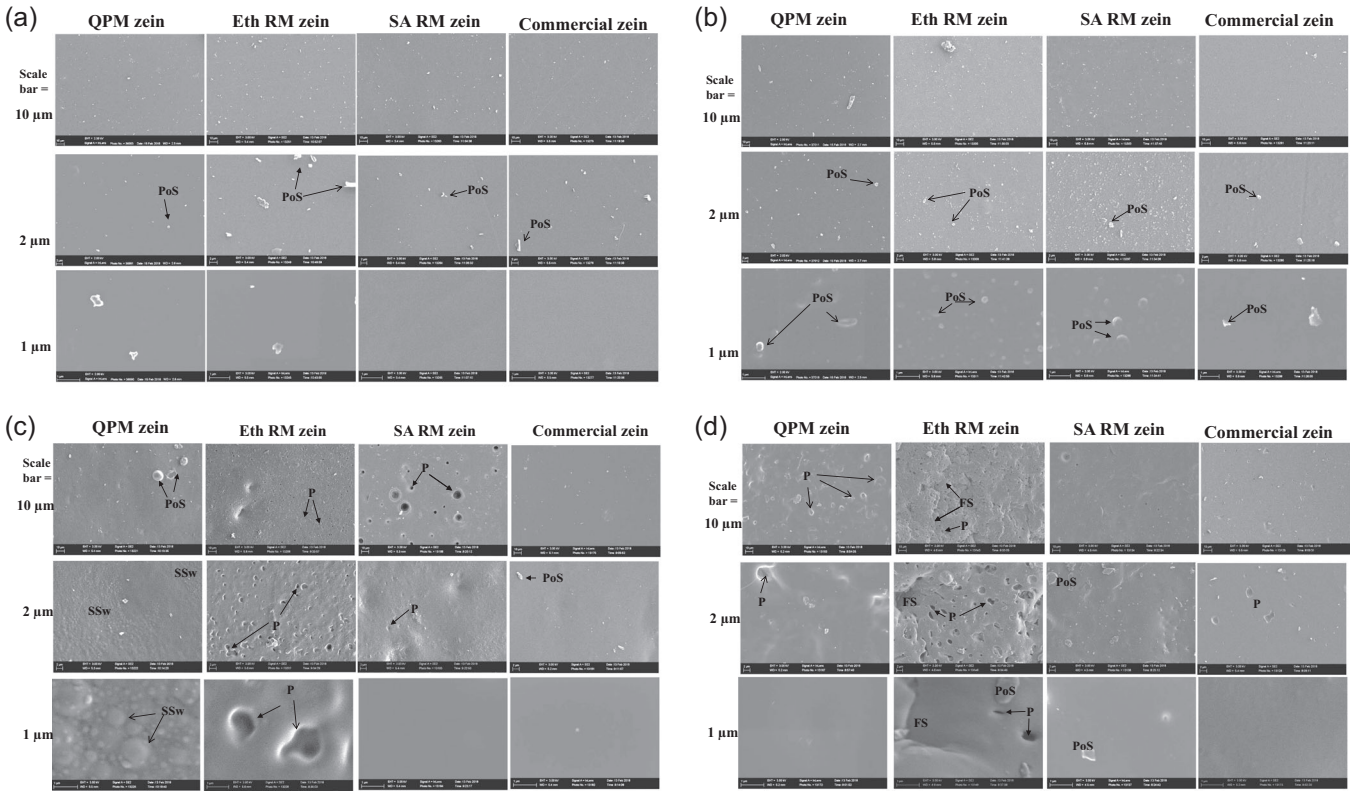


**FIGURE 3** Scanning electron microscopy (SEM) showing the effects of zein type and immersion in buffer on the surface appearance of the cast zein films. FS, folded surface; P, pit(s); PoS, particle(s) on surface; SSw, surface swelling(s). (a) Films cast from 70% aqueous ethanol solution. (b) Films cast from glacial acetic acid (GAA) solution. (c) Films cast from 70% aqueous ethanol solution after immersion in buffer. (d) Films cast from GAA after immersion in buffer.

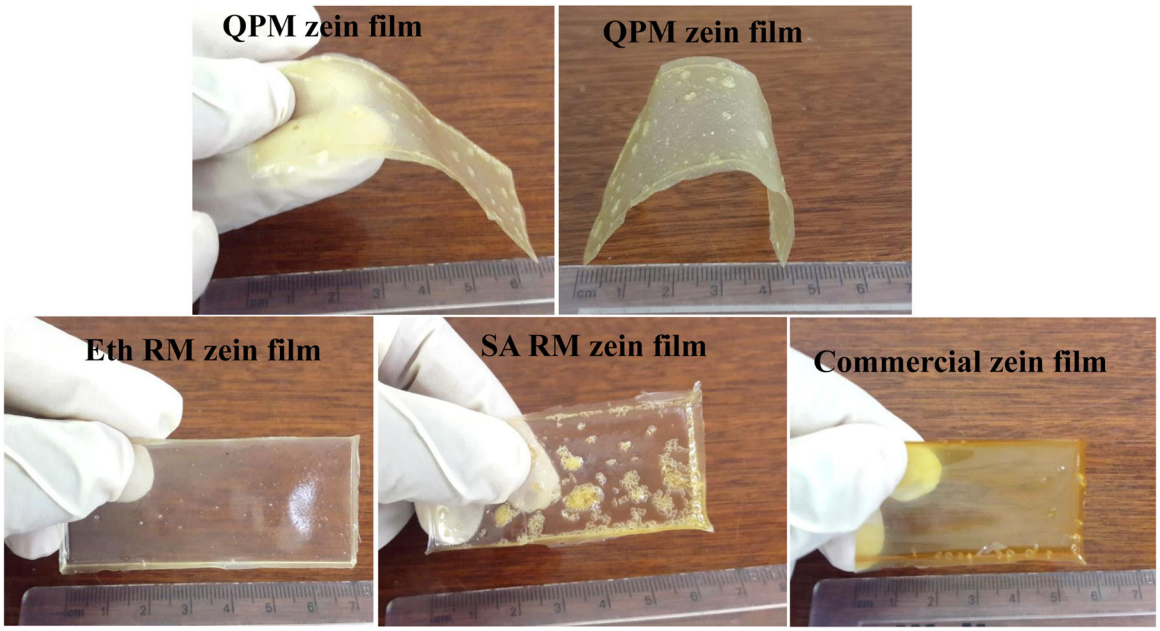


**FIGURE 4** Appearance and flexibility of the zein films cast from glacial acetic acid when kept for 72 h at ambient temperature (average 26°C) and relative humidity (average 62%). Eth RM, whole grain regular white maize, Ethiopian variety MH 140; QPM, whole grain white QPM, Ethiopian variety MHQ 138; SA RM, refined white maize meal (80% extraction rate). [Color figure can be viewed at wileyonlinelibrary.com]

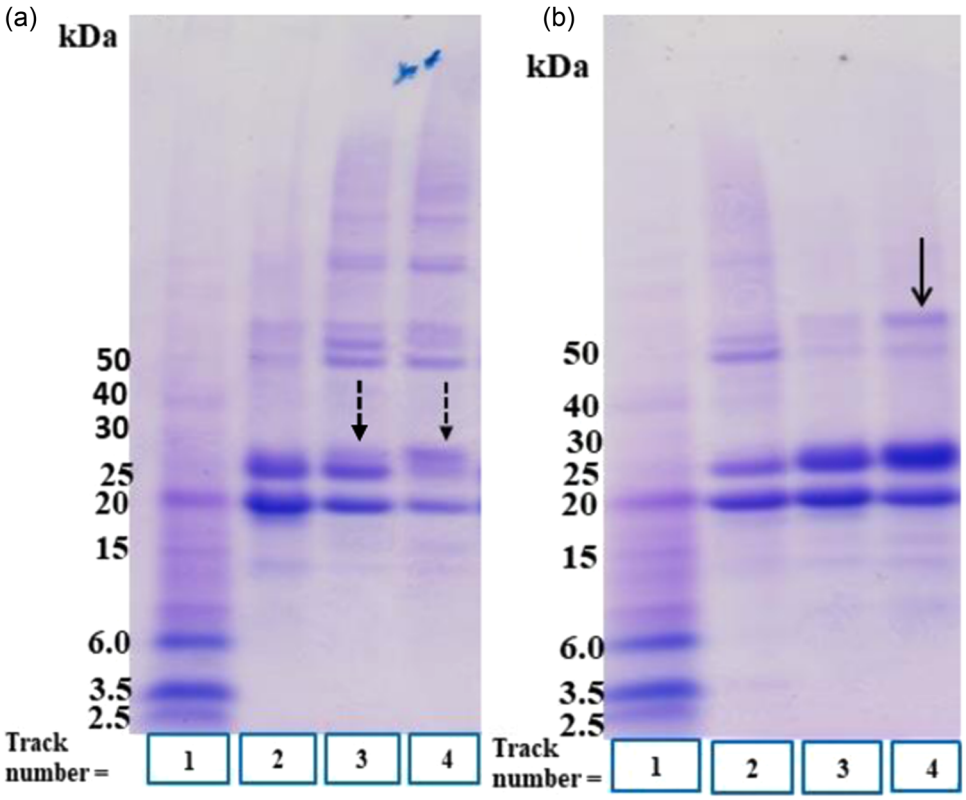


FIGURE 5 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the different zein preparations. (1) Molecular weight standards. (2) Refined white maize meal (80% extraction rate) (SA RM zein) (3) Whole grain regular white maize, Ethiopian variety MH 140 (Eth RM zein). (4) Whole grain white QPM, Ethiopian variety MHQ 138 (QPM zein). (a) Electrophoresis of the zeins in an unreduced form. (b) Electrophoresis of the zeins in a reduced form. Dashed arrows = 27 k γ-zein band (Esen, 1987), Solid arrow = Possibly 50–55 k γ-zein band (Woo et al., 2001). [Color figure can be viewed at wileyonlinelibrary.com]

of the Eth RM zein γ-zein band, which is consistent with the high proportion of γ-zein in QPM zein (Holding, 2014). It is proposed that under the prevailing highish relative humidity (average 62%) conditions, these γ-zein polypeptide monomers bound water molecules from the atmosphere and, as a consequence, the bound water acted as a plasticizer, resulting in the QPM zein films being highly flexible. That this only occurred when the QPM zein films were cast using GAA and not aqueous ethanol can be attributed to the fact that GAA is a better zein solvent than aqueous ethanol due to it causing the molecules to unfold to a greater extent (Li et al., 2012). Consequently, the zein molecules were probably in a more ordered conformation. Shi et al. (2009) using atomic force microscopy observed that the surface morphology of zein films cast from GAA was smoother than that of films cast from aqueous ethanol.

## 3.3 | Zein film properties after immersion in buffer

All the zein films remained whole after immersion in pH 6.8 buffer for 12 h with agitation, that is, they did not disintegrate, and all the films became more opaque (Figure 2A,B). All the zein films cast from 70% aqueous ethanol increased moderately in surface area (Figure 2A and Table 2) and absorbed relatively similar amounts of buffer (Table 2). SEM showed that the Eth RM and SA RM zein films cast from aqueous ethanol exhibited considerable surface pitting after immersion (Figure 3c). This was presumably as a result of zein being leached out by the buffer during immersion. In contrast, the QPM zein films exhibited surface swelling. This may have been a consequence of hydration of individual γ-zein polypeptides, which are water and salt soluble (Wilson et al., 1981)

There were significant differences in the film area and buffer absorbed by the different zein films cast from GAA after immersion (Figure 2B and Table 2). The QPM zein films cast from GAA absorbed the least buffer (~16%) and the film surface area increased the least, ~11%. In contrast, the Eth RM zein and commercial zein films cast from GAA absorbed much higher amounts of buffer (~207% and 123%, respectively, and the surface areas of their films increased very substantially, by nearly 144% and 101%, respectively [Table 2 and Figure 2B]). Notably, these zeins when cast into films using GAA

**TABLE 2** Effects of casting solvent (70% w/w aqueous ethanol or GAA) and zein preparation type (QPM zein, total zein, and commercial zein) on uptake of pH 6.8 phosphate buffer by zein films.

| Solvent used to cast zein films | Zein type | Before immersion in buffer | | | After immersion in buffer | | | Increase in film area (%) | Buffer absorbed by films (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | Film weight (mg) | Film area ($cm^2$) | Film surface density ($mg/cm^2$) | Film weight (mg) | Film area ($cm^2$) | Film surface density ($mg/cm^2$) | | |
| 70% w/w aqueous ethanol | QPM | $517.6^{c} \pm 21.6$ | $21.1^{c} \pm 1.1$ | $26.8^{b} \pm 0.8$ | $732.9^{bc} \pm 19.7$ | $27.3^{c} \pm 0.8$ | $27.6^{a} \pm 1.3$ | $29.8^{bc} \pm 5.9$ | $41.7^{ab} \pm 2.6$ |
| | Eth RM | $402.6^{a} \pm 51.9$ | $17.5^{a} \pm 8.8$ | $22.6^{ab} \pm 1.6$ | $646.9^{ab} \pm 10.6$ | $22.9^{ab} \pm 1.6$ | $28.0^{a} \pm 1.3$ | $36.7^{c} \pm 17.9$ | $62.8^{b} \pm 25.0$ |
| | SA RM | $424.4^{ab} \pm 13.7$ | $18.5^{a} \pm 0.3$ | $23.0^{ab} \pm 1.1$ | $612.3^{a} \pm 36.1$ | $21.1^{a} \pm 0.5$ | $28.7^{a} \pm 1.6$ | $15.6^{ab} \pm 4.0$ | $44.2^{ab} \pm 3.8$ |
| | Commercial | $454.8^{ab} \pm 14.8$ | $17.9^{a} \pm 0.5$ | $25.5^{b} \pm 1.4$ | $781.7^{c} \pm 33.3$ | $21.2^{a} \pm 0.5$ | $36.0^{b} \pm 2.5$ | $17.3^{ab} \pm 5.4$ | $62.9^{b} \pm 13.2$ |
| GAA | QPM | $584.0^{d} \pm 3.0$ | $22.0^{b} \pm 1.1$ | $26.5^{b} \pm 1.4$ | $679.0^{ab} \pm 8.0$ | $24.5^{ab} \pm 0.0$ | $27.6^{a} \pm 0.0$ | $11.2^{a} \pm 5.2$ | $16.2^{a} \pm 1.9$ |
| | Eth RM | $490.0^{b} \pm 5.0$ | $18.0^{a} \pm 0.2$ | $26.6^{b} \pm 0.4$ | $1503.0^{e} \pm 83.0$ | $45.2^{d} \pm 1.0$ | $34.3^{b} \pm 2.0$ | $144.5^{f} \pm 7.5$ | $207.0^{d} \pm 13.8$ |
| | SA RM | $402.0^{a} \pm 25.0$ | $21.0^{b} \pm 1.4$ | $18.8^{a} \pm 0.7$ | $603.8^{a} \pm 23.1$ | $24.6^{ab} \pm 1.3$ | $25.1^{a} \pm 2.0$ | $18.0^{ab} \pm 10.8$ | $50.5^{b} \pm 3.8$ |
| | Commercial | $488.1^{b} \pm 0.6$ | $18.8^{a} \pm 0.2$ | $26.0^{b} \pm 0.0$ | $1089^{d} \pm 12.0$ | $37.7^{c} \pm 3.0$ | $29.0^{a} \pm 3.0$ | $101.6^{e} \pm 17.6$ | $123.0^{c} \pm 2.6$ |

*Note:* Mean ± SD of two independent experiments. Mean values in the same column with different letter in superscript are significantly different at $p \leq .05$.

Abbreviations: Eth RM, whole grain regular white maize, Ethiopian variety MH 140; GAA, glacial acetic acid; QPM, whole grain white QPM, Ethiopian variety MHQ 138; SA RM, refined white maize meal (80% extraction rate).

absorbed far more buffer and swelled to a much greater extent than when cast from aqueous ethanol and the opposite took place with the QPM zein films. SEM showed that the Eth RM zein films had folded surfaces and numerous pits (Figure 3d). The folded surface of the Eth RM zein films was undoubtably a consequence of their very high buffer uptake and swelling.

The notably high expansion and buffer uptake of Eth RM zein and commercial zein films when cast from GAA compared to the QPM zein are likely due to fewer disulfide cross-linkages in the Eth RM and commercial zein and to the greater unfolding of the zeins in GAA (Li et al., 2012). Eth RM and commercial zein had the lowest cysteine contents of the zein, ≤1.83% (Table 1). It has been proposed that disulfide crosslinking brings polypeptides closer together, creating a compact protein network structure that forms a barrier to limit the movement of liquids during immersion (Muhiwa et al., 2017). In contrast, the relative resistance to buffer uptake and expansion of the QPM zein films cast from GAA can be attributed to the QPM zein's high cysteine content, 3.8% (Table 1) due to its high proportion of γ-zein (Figure 1) and consequent more extensive intermolecular disulfide crosslinking in the films. In support of this, Byaruhanga et al. (2006) observed a reduction in free sulfhydryl groups and an increase in disulfide groups when kafirin was heat-treated by microwave energy and a corresponding decrease in the water vapor permeability of the plasticized kafirin films Byaruhanga et al. (2005).

## 4 | CONCLUSIONS

This work shows that the functional properties of bioplastic films made from zein from QPM maize differ from those of commercial zein and total zein from regular maize. QPM zein films take up less liquid and swell less when immersed in aqueous solution. QPM zein films cast from GAA solution without added plasticizer become highly flexible after exposure to highish relative humidity. These properties are attributed to better solubilization of the zein in GAA and the high content of γ-zein in QPM zein, enabling plasticization by water molecules. Because of these flexibility and low swelling properties, QPM zein bioplastics potentially have broader commercial applications than regular maize zein bioplastics.

## ACKNOWLEDGMENTS

The South African National Research Foundation for financial support, Grant number 119549. Dr E. Venter and Ms E. Venter of the University of Pretoria's Laboratory for Microscopy and Microanalysis are also acknowledged for assistance with the microscopy.

## ORCID

*John R. N. Taylor* http://orcid.org/0000-0002-9714-2093

## REFERENCES


AACC. (2000). Method 46-30, Moisture-air oven method and Method 44–15A, Crude protein-combustion, *Approved methods of analysis* (10th Ed.).

Anderson, T. J., & Lamsal, B. P. (2011). REVIEW: Zein extraction from corn, corn products, and coproducts and modifications for various applications: A review. *Cereal Chemistry*, *88*, 159–173. https://doi.org/10.1094/CCHEM-06-10-0091

Anyango, J. O., Taylor, J., & Taylor, J. R. N. (2011). Improvement in water stability and other related functional properties of thin cast kafirin protein films. *Journal of Agricultural and Food Chemistry*, *59*, 12674–12682. https://doi.org/10.1021/jf203273y

Azeredo, H. M. C., & Waldron, K. W. (2016). Crosslinking in polysaccharide and protein films and coatings for food contact–A review. *Trends in Food Science & Technology*, *52*, 109–122. https://doi.org/10.1016/j.tifs.2016.04.008

Babu, B. K., Agrawal, P. K., Saha, S., & Gupta, H. S. (2015). Mapping QTLs for opaque2 modifiers influencing the tryptophan content in quality protein maize using genomic and candidate gene-based SSRs of lysine and tryptophan metabolic pathway. *Plant Cell Reports*, *34*(1), 37–45. https://doi.org/10.1007/s00299-014-1685-5

Bayer, I. S. (2021). Zein in food packaging. In A. Athanassiou (Ed.), *Sustainable food packaging technology* (pp. 199–224). Wiley. ISBN: 978-3-527-34556-4.

Belton, P. S., Delgadillo, I., Halford, N. G., & Shewry, P. R. (2006). Kafirin structure and functionality. *Journal of Cereal Science*, *44*, 272–286. https://doi.org/10.1016/j.jcs.2006.05.004

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. https://doi.org/10.1021/jf052942z

Byaruhanga, Y. B., Erasmus, C., & Taylor, J. R. N. (2005). Effect of microwave heating of kafirin on the functional properties of kafirin films. *Cereal Chemistry*, *82*, 565–573. https://doi.org/10.1094/cc-82-0565

Cadogan, D. F., & Howick, C. J. (2012). Plasticizers, *Ullman's encyclopedia of industrial chemistry* (Vol. 27, 7th ed., pp. 599–618). Wiley-VCH. https://doi.org/10.1002/14356007.a20_439

Di Gioia, L., Cuq, B., & Guilbert, S. (1999). Thermal properties of corn gluten meal and its proteic components. *International Journal of Biological Macromolecules*, *24*, 341–350. https://doi.org/10.1016/s0141-8130(99)00048-3

Dubey, N. K., & Dubey, R. (2020). Edible films and coatings: An update on recent advances. In K. Pal, I. Banerjee, P. Sarkar, D. Kim, W. Deng, & K. Majumder (Eds.), *Biopolymer-based formulations: Biomedical and food applications* (pp. 675–695). Elsevier. ISBN: 9780128168974.

Egea, M. B., de Oliveira Filho, J. G., Braga, A. R. C., Leal, M. C. B. D. M., Celayeta, J. M. F., & Lemes, A. C. (2021). Zein-based blends and composites. In M. R. Samjay, J. Parameswaranpillai, S. Siengchin, & M. Ramesh (Eds.), *Biodegradable polymers, blends and composites* (1st ed., pp. 511–526). Woodhead Publishing. ISBN: 9780128237915.

Elhassan, M. S. M., Oguntoyinbo, S. I., Taylor, J., & Taylor, J. R. N. (2018). Formation and properties of viscoelastic masses made from kafirin by a process of simple coacervation from solution in glacial acetic acid using water. *Food Chemistry*, *239*, 333–342. https://doi.org/10.1016/j.foodchem.2017.06.114

Esen, A. (1987). A proposed nomenclature for the alcohol-soluble proteins (zeins) of maize (*Zea mays* L.). *Journal of Cereal Science*, *5*, 117–128. https://doi.org/10.1016/s0733-5210(87)80015-2

Fass, D., & Thorpe, C. (2018). Chemistry and enzymology of disulfide cross-linking in proteins. *Chemical Reviews*, *118*(118), 1169–1198. https://doi.org/10.1021/acs.chemrev.7b00123

Geetha, K. B., Lending, C. R., Lopes, M. A., Wallace, J. C., & Larkins, B. A. (1991). Opaque-2 modifiers increase gamma-zein synthesis and alter its spatial distribution in maize endosperm. *The Plant Cell*, *3*, 1207–1219. https://doi.org/10.1105/tpc.3.11.1207

Gillgren, T., & Stading, M. (2008). Mechanical and barrier properties of avenin, kafirin, and zein films. *Food Biophysics*, *3*, 287–294. https://doi.org/10.1007/s11483-008-9074-7

Holding, D. R. (2014). Recent advances in the study of prolamin storage protein organization and function. *Frontiers in Plant Science*, *5*, 276. https://doi.org/10.3389/fpls.2014.00276

Hunter, B. G., Beatty, M. K., Singletary, G. W., Hamaker, B. R., Dilkes, B. P., Larkins, B. A., & Jung, R. (2002). Maize opaque endosperm mutations create extensive changes in patterns of gene expression. *The Plant Cell*, *14*, 2591–2612. https://doi.org/10.1105/tpc.003905

Huo, W., Wei, D., Zhu, W., Li, Z., & Jiang, Y. (2018). High-elongation zein films for flexible packaging by synergistic plasticization: Preparation, structure and properties. *Journal of Cereal Science*, *79*, 354–361. https://doi.org/10.1016/j.jcs.2017.11.021

Larkins, B. A., Pedersen, K., Marks, M. D., & Wilson, D. R. (1984). The zein proteins of maize endosperm. *Trends in Biochemical Sciences*, *9*, 306–308. https://doi.org/10.1016/0968-0004(84)90297-4

Lawton, J. W. (2002). Zein: A history of processing and use. *Cereal Chemistry*, *79*, 1–18. https://doi.org/10.1094/cchem.2002.79.1.1

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. https://doi.org/10.1021/jp305709y

Liu, H., Shi, J., Sun, C., Gong, H., Fan, X., Qiu, F., Huang, X., Feng, Q., Zheng, X., Yuan, N., Li, C., Zhang, Z., Deng, Y., Wang, J., Pan, G., Han, B., Lai, J., & Wu, Y. (2016). Gene duplication confers enhanced expression of 27-kDa γ-zein for endosperm modification in quality protein maize. *Proceedings of the National Academy of Sciences*, *113*, 4964–4969. https://doi.org/10.1073/pnas.1601352113

Masamba, K., Li, Y., Hategekimana, J., Liu, F., Ma, J., & Zhong, F. (2016). Effect of type of plasticizers on mechanical and water barrier properties of transglutaminase cross-linked zein–oleic acid composite films. *International Journal of Food Engineering*, *12*, 365–376. https://doi.org/10.1515/ijfe-2015-0289

Muhiwa, P. J., Taylor, J., & Taylor, J. R. N. (2017). Extraction and film properties of kafirin from coarse sorghum and sorghum

DDGS by percolation. *Cereal Chemistry*, *94*, 693–698. https://doi.org/10.1094/cchem-01-17-0003-r

Nonthanum, P., Lee, Y., & Padua, G. W. (2012). Effect of γ-zein on the rheological behavior of concentrated zein solutions. *Journal of Agricultural and Food Chemistry*, *60*, 1742–1747. https://doi.org/10.1021/jf2035302

Nyakurwa, C. S., Gasura, E., & Mabasa, S. (2017). Potential for quality protein maize for reducing protein-energy under nutrition in maize dependent sub-saharan African countries: A review. *African Crop Science Journal*, *25*, 521–537. https://doi.org/10.4314/acsj.v25i4.9

Shi, K., Kokini, J. L., & Huang, Q. (2009). Engineering zein films with controlled surface morphology and hydrophilicity. *Journal of Agricultural and Food Chemistry*, *57*, 2186–2192. https://doi.org/10.1021/jf803559v

Turasan, H., Barber, E. A., Malm, M., & Kokini, J. L. (2018). Mechanical and spectroscopic characterization of crosslinked zein films cast from solutions of acetic acid leading to a new mechanism for the crosslinking of oleic acid plasticized zein films. *Food Research International*, *108*, 357–367. https://doi.org/10.1016/j.foodres.2018.03.063

Turasan, H., & Kokini, J. L. (2017). Advances in understanding the molecular structures and functionalities of biodegradable zein-based materials using spectroscopic techniques: A review. *Biomacromolecules*, *18*, 331–354. https://doi.org/10.1021/acs.biomac.6b01455

Wang, L. P., Wang, H. J., Hou, X., Raza, A., Koyama, Y., Ito, T., & Wang, J. Y. (2021). Preparation of stretchable composite film and its application in skin burn repair. *Journal of the Mechanical Behavior of Biomedical Materials*, *113*, 104114. https://doi.org/10.1016/j.jmbbm.2020.104114

Wilson, C. M., Shewry, P. R., & Miflin, B. J. (1981). Maize endosperm proteins compared by sodium dodecyl sulfate polyacrylamide gel electrophoresis and isoelectric focusing. *Cereal Chemistry*, *58*, 275–281.

Woo, Y. M., Hu, D. W. N., Larkins, B. A., & Jung, R. (2001). Genomics analysis of genes expressed in maize endosperm identifies novel seed proteins and clarifies patterns of zein gene expression. *The Plant Cell*, *13*, 2297–2317.

Wu, Y., Holding, D. R., & Messing, J. (2010). γ-Zeins are essential for endosperm modification in quality protein maize. *Proceedings of the National Academy of Sciences*, *107*, 12810–12815. https://doi.org/10.1073/pnas.1004721107

Xiao, J., Chen, Y., & Huang, Q. (2017). Physicochemical properties of kafirin protein and its applications as building blocks of functional delivery systems. *Food & Function*, *8*, 1402–1413. https://doi.org/10.1039/c6fo01217e

Yang, P., Haken, A. E., Niu, Y., Chaney, S. R., Hicks, K. B., Eckhoff, S. R., Tumbleson, M. E., & Singh, V. (2005). Effect of steeping with sulfite salts and adjunct acids on corn wet-milling yields and starch properties. *Cereal Chemistry*, *82*, 420–424. https://doi.org/10.1094/cc-82-0420

**How to cite this article:** Baloyi, J. T., Taylor, J., & Taylor, J. R. N. (2023). Bioplastic film making properties of quality protein maize (QPM) zein. *Cereal Chemistry*, 100, 805–815. https://doi.org/10.1002/cche.10665