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

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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 (~28 °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, 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.

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

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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 h. 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 commercial 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 ± 1.0 μm for total zein films and 63.1 ± 1.3 μm 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 μg protein/μl 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 10 × 0.3 numerical aperture objective. The doughs were stretched out as thinly as possible (approx. 15 mm × 5 mm × 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.