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Improvement of zein dough characteristics using dilute organic acids

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ABSTRACT

The replacement of gluten in dough products poses a major challenge. Preparing zein doughs in dilute acetic acid and lactic acid, such as produced during sourdough fermentation, was investigated. Increasing acid concentrations (0.7, 1.3 and 5.4% [v/v]) increased zein extensibility and reduced the stress and related parameters. Preparation of zein-maize starch/-rice doughs in dilute organic acids improved dough properties to the extent that the doughs could hold air and be inflated into a bubble by Alveography. Further, they exhibited similar Stability (P), Distensibility and deformation energy (W) to wheat flour dough. Confocal laser scanning microscopy revealed an ordered linear fibril network in zein and zein-rice flour doughs prepared in the dilute acids, which became uniform with increasing acid concentration. SDS-PAGE showed that the acids did not hydrolyse or polymerise the zein. FTIR indicated that the acidic conditions slightly increased the proportion of α-helical conformation in the zein doughs, possibly as a result of deamination. This conformational change may be responsible for the considerably improved zein dough properties. Zein doughs prepared in dilute organic acids show potential as a gluten replacement in gluten-free formulations.

1. Introduction

Replacement of wheat gluten functionality in gluten-free dough-based formulations such as bread poses a major challenge to food scientists (Gallagher et al., 2004). Viscoelasticity is the critical attribute of gluten that allows the retention of carbon dioxide produced during dough fermentation (Erickson et al., 2011). Zein (maize prolamin) can display viscoelastic functionality similar to gluten in aqueous dough systems, when heated above its glass transition temperature (T_g) (Lawton, 1992; Schober et al., 2008, 2010). These zein doughs exhibit a fibrous network (Lawton, 1992; Schober et al., 2008; Andersson et al., 2011), which also displays similar characteristics to that in gluten dough. However, such zein-based doughs have a limited ability to retain gas (Schober et al., 2008) and are significantly more extensible than gluten-based doughs (Schober et al., 2010). Hydrocolloids such as hydroxypropyl methylcellulose (HPMC) have been found to stabilize the fibrous zein structure in the dough, enabling the bread of fair quality to be produced (Schober et al., 2008; Schober et al., 2010; Andersson et al., 2011).

The incorporation of sourdough (mixed lactic acid bacteria and yeast fermented dough) into a wheat bread system causes a significant increase in loaf volume (Katina et al., 2005). Edema et al. (2013) showed that the inclusion of fonio and sorghum sourdough improved fonio and sorghum dough properties and increased the loaf volume of the resulting breads to a limited extent. Their findings indicated that the sourdough fermentation modified the starch pasting properties. Schober et al. (2007) showed that sourdough fermentation resulted in hydrolysis of proteins in sorghum doughs and caused the endosperm protein matrix in the crumb of sorghum breads to be disaggregated. They proposed that this protein matrix disaggregation enabled the formation of stronger starch gels in such gluten-free breads.

An important related question is whether the dilute organic acids produced in sourdough-type fermentations also affect the functional properties of the non-wheat prolamins. Here, we report on their effects on the functionality of aqueous zein doughs.

2. Experimental

2.1. Materials

Commercial zein (Sigma Z3625) was obtained from Sigma–Aldrich, Johannesburg, South Africa. Maize starch (Maizena, Bokomo Foods, Atlantis, South Africa) and bread wheat flour (SnowFlake, Premier Foods, Isando, South Africa) were obtained

Abbreviations: T_g, glass transition temperature; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; FTIR, Fourier transform infrared spectroscopy; CLSM, confocal laser scanning microscopy.

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from retail stores. Rice flour (Entice 100% Pure Rice Flour) was kindly donated by Daisy Health Foods, Johannesburg.

2.2. Preparation of zein doughs

Zein doughs were prepared essentially according to Schober et al. (2010) except that the zein was used without defatting. Zein (0.5 g as is basis) was pre-warmed to 40 °C. Distilled water, 0.7%, 1.3% and 5.4% (v/v) lactic and acetic acid (1.875 g) were pre-warmed separately. The liquid was added to the zein and vortexed at high speed for 5 s. Excess water or acid was decanted off the dough and the dough was hand-kneaded for 30 s. Zein-maize starch and zein-rice flour doughs (1 g zein: 4 g starch or rice flour, as is basis) were prepared in the same way.

2.3. Zein tensile properties

The tensile properties of the zein doughs were evaluated using a Kieffer rig (Abang Zaidel et al., 2008), mounted on a TA-XT2 texture analyzer (Stable Micro Systems, Godalming, UK). The doughs were pressed into cylindrical, longitudinally split rubber tube moulds (70 mm long and 4 mm diameter) to obtain a uniform size and shape. The moulded samples were placed over the vertical struts (30 mm apart) of the Kieffer rig and clamped in place at both ends. Doughs were extended by means of a hook centred over the sample at a constant rate of 3.3 mm/s over a distance of 150 mm (maximum displacement of the texture analyser). The test was performed at ambient temperature (22 °C) and within 2 min, to prevent the doughs from cooling below their glass transition temperature. The force over distance, peak force (N), extensibility until rupture (mm) and area under the curve ($N \times$ mm) were measured. Rheological parameters were determined using formulae according to Abang Zaidel et al. (2008).

2.4. Alveography

Alveography (Chopin NG Consistograph, Paris) was used to analyse the quality of gluten-free doughs made from zein-maize starch and zein-rice flour mixtures (1:4) prepared with different concentrations of lactic acid and acetic acid, according to ICC Standard 121 (ICC, 1992). Zein (50 g as is basis) was added to 200 g (as is basis) maize starch or rice flour and thoroughly mixed using an electric mixer for 5 min. The "flour" was then pre-warmed to 50 °C in a water bath. The flour was then transferred to the mixing section of the Alveograph. Pre-warmed distilled water (200 g) was slowly added and the dough kneaded for 8 min, formed into round patties and allowed to rest for 20 min before inflation took place. Dough kneading and resting was conducted at a temperature of 35 °C (the highest instrument setting). Alveography was performed and P, L, P/L and W recorded.

2.5. CLSM

Zein mixed in water at 22 °C, zein doughs and zein-starch/rice flour gluten-free doughs prepared at 40 °C were analysed by Confocal Laser Scanning Microscopy (CLSM) using a Zeiss 510 META system (Jena, Germany) with a Plan-Neofluar 10 × 0.3 objective under natural fluorescence at an excitation wavelength of 488 nm. The doughs were prepared as described under 2.2. Thin pieces of freshly prepared dough (approx. 5 × 5 mm diameter and 2 mm thick) as is, and zein dough and zein-rice flour dough stretched in a single direction over a glass slide to approx. 15 × 5 mm × 1 mm, were examined.

2.6. SDS-PAGE

The zein doughs, air-dried at ambient temperature were characterized by SDS-PAGE under reducing and non-reducing conditions using 4–12% polyacrylamide gradient gels (8 × 8 cm × 1.0 mm thick with 15 wells) (NuPAGE® Novex, Invitrogen. Carlsbad, CA). Invitrogen Mark12 Unstained Standard was used. Zein sample loading was 10 μg. Staining was with Coomassie Brilliant Blue R-250. After de-staining, the gels were scanned on a flatbed scanner.

2.7. FTIR

FTIR spectroscopy was performed on zein and freshly prepared zein doughs, as described by Taylor et al. (2009). A Vertex 70v FTIR spectrophotometer (Bruker Optik, Ettlingen, Germany) was used in the attenuated total reflectance (ATR) mode with 64 scans, an 8 cm^{-1} band width, and an interval of 1 cm^{-1} at a wavenumber 400-4000 cm^{-1}. To minimise cooling effects, there was a maximum 30 s delay from the moment the samples were removed following dough formation at 40 °C to the start of the FTIR scan. At least four replicates were performed for each treatment. The FTIR spectra were Fourier deconvoluted with a Lorentzian filter with a band width of 12 and a resolution enhancement factor of 2.

2.8. Statistical analysis

The zein tensile, zein-starch/-rice flour Alveography and FTIR experiments were repeated at least three times. One-way analysis of variance was performed. Means were compared at $p = 0.05$ using Fisher's Least Significant Difference Test (LSD).

Table 1
pH and tensile properties of zein doughs prepared with dilute lactic acid and acetic acid.[a]

Treatment	Acid concentration	Dough pH	Peak force	Extension	Peak stress	Strain at maximum hook displacement (150 mm)		Extensional viscosity	Young's modulus	Area under stress–strain curve
	(% v/v)		(N)	(mm)	(kPa)	(%)	(ε_H)[b]	(η_E, kPa.s)	(E, kPa)	(N, mm)
Distilled water	0	3.90	3.47[a] ± 0.43[c]	270[d]	122.8[a] ± 10.8	1002[a] ± 4	2.31[a] ± 0.00	5625[a] ± 510	29.8[a] ± 0.4	612[a] ± 51
Lactic acid	0.7	3.28	3.25[a] ± 0.67	270	114.9[a] ± 23.5	1000[a] ± 6	2.30[a] ± 0.01	5251[a] ± 756	31.1[a] ± 5.4	612[a] ± 135
	1.3	3.13	2.40[b] ± 0.44	270	84.8[b] ± 15.5	1003[a] ± 2	2.31[a] ± 0.00	3888[b] ± 314	19.8[b] ± 4.0	440[b] ± 69
	5.4	2.47	0.52[c] ± 0.10	270	18.2[c] ± 3.6	1002[a] ± 2	2.31[a] ± 0.00	834[c] ± 165	10.3[c] ± 7.6	118[c] ± 18
Acetic acid	0.7	3.48	3.15[a] ± 0.40	270	111.6[a] ± 14.2	1001[a] ± 4	2.30[a] ± 0.00	5104[a] ± 645	30.7[a] ± 2.6	547[a] ± 91
	1.3	3.40	2.00[b] ± 0.14	270	70.9[b] ± 4.9	996[a] ± 6	2.30[a] ± 0.01	3227[b] ± 204	20.9[b] ± 0.9	316[b] ± 21
	5.4	3.11	0.25[c] ± 0.10	270	9.0[c] ± 3.7	984[b] ± 12	2.29[b] ± 0.01	404[c] ± 169	15.8[c] ± 2.5	48[c] ± 83

[a] Means ± Standard Deviation of three replicates.
[b] Hencky strain/true strain.
[c] Values in columns with different superscript letters differ significantly ($p < 0.05$).
[d] Dough did not break before maximum hook displacement (150 mm).