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Improved viscoelastic zein–starch doughs for leavened gluten-free breads: Their rheology and microstructure

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ABSTRACT

Gluten-free bread was prepared from commercial zein (20 g), maize starch (80 g), water (75 g), saccharose, NaCl and dry yeast by mixing above zein's glass transition temperature (T_g) at 40°C. Addition of hydroxypropyl methylcellulose (HPMC, 2 g) significantly improved quality, and the resulting bread resembled wheat bread having a regular, fine crumb grain, a round top and good aeration (specific volume 3.2 ml/g). In model studies, HPMC stabilized gas bubbles well. Additionally, laser scanning confocal microscopy (LSCM) revealed finer zein strands in the dough when HPMC was present, while dynamic oscillatory tests showed that HPMC rendered gluten-like hydrated zein above its T_g softer (i.e. $|G^*|$ was significantly lower). LSCM revealed that cooling below T_g alone did not destroy the zein strands; however, upon mechanical impact below T_g, they shattered into small pieces. When such dough was heated above T_g and then remixed, zein strands did not reform, and this dough lacked resistance in uniaxial extension tests. When within the breadmaking process, dough was cooled below T_g and subsequently reheated, breads had large void spaces under the crust. Likely, expanding gas bubbles broke zein strands below T_g resulting in structural weakness.

1. Introduction

Gluten-free bread was once regarded as a niche product for people with a rare disorder, celiac disease. However, it has meanwhile been recognized that celiac disease is much more widespread than previously thought, and an average worldwide prevalence of 1:266 has been estimated based on serologic screening studies (Fasano and Catassi, 2001). Once a person is diagnosed with this disease, the total lifelong avoidance of wheat, rye and barley is required (Fasano and Catassi, 2001). Consequently, a growing market has to be supplied with gluten-free bread of acceptable quality. Forbidden ingredients include not only flours from normal bread wheat, rye and barley, but also, for example, from durum wheat (*Triticum turgidum* ssp. *durum* (Desf.) Husnot), ancient wheats like spelt wheat (*Triticum aestivum* ssp. *spelta* (L.) Thell.), or the wheat–rye hybrid triticale (Kasarda, 2001; Kasarda and D'Ovidio, 1999).

Gluten-free breads based on isolated starches, or on flours from gluten-free cereals like rice, sorghum or maize have been described in the literature for many decades (Hart et al., 1970; Jongh, 1961; Nishita et al., 1976; Olatunji et al., 1992; Ranhotra et al., 1975). Numerous studies published more recently have aimed at improving the original formulations and procedures (e.g. Kadan et al., 2001; McCarthy et al., 2005; Schober et al., 2007). However, the basic flaw remains that the gluten-free doughs are soft and batter-like, which typically requires baking in pans (Cauvain, 1998). The batter-like consistency makes these systems more sensitive to collapse, resulting in large holes in the center of the bread crumb or dense areas at the bottom of the crumb (Schober et al., 2005, 2007). Also, shaping such batter-like dough, for example, for the production of soft pretzels, baguettes or braided breads, remains virtually impossible.

It has long been known that mixtures of maize prolamin (zein), starch and water can form a dough with properties similar to wheat dough, provided that they are mixed above room temperature (Lawton, 1992; MacRitchie, 1980). While MacRitchie (1980) mentioned 60 °C as mixing temperature, Lawton (1992) reported that 35 °C was sufficient. When dibutyl tartrate as a second plasticizer besides water was added, Lawton (1992) found that 28 °C was the minimum temperature for the development of viscoelastic zein–starch doughs in a farinograph, and this temperature was close to the glass transition temperature (T_g) of hydrated zein. However, within Lawton's study, it was also found that dibutyl

Abbreviations: db, dry basis; FITC, fluorescein 5(6)-isothiocyanate; $|G^*|$, complex shear modulus (absolute value); HPMC, hydroxypropyl methylcellulose; LSCM, laser scanning confocal microscopy; SDS, sodium dodecyl sulfate; SE-HPLC, size-exclusion high-performance liquid chromatography; T_g, glass transition temperature.

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tartrate had only a limited effect, and thus the minimum temperature for zein dough development without added dibutyl tartrate might be similar to 28 °C, but this was not systematically studied.

An area that, up to now, has not been successfully addressed is the practical use of such zein dough in gluten-free breadmaking. In this situation, it is highly questionable whether dibutyl tartrate should be added. Its use has been suggested as a plasticizer in capsules used for pharmaceutical applications (Bykov et al., 2000), but in staple food like bread, it is necessary to use additives that are more commonly used in food applications, and thus are permitted additives in various countries.

Hydroxypropyl methylcellulose (HPMC) is a widely used food ingredient. It is a hydrocolloid that has at the same time surface-active (emulsifying) properties (Dickinson, 2003). Thus it might influence the properties of zein dough and bread by its water-binding and thickening abilities, but also by its amphiphilic nature. HPMC has been successfully used in batter-based gluten-free breads (Hart et al., 1970; Nishita et al., 1976), its thermorheological behavior has been characterized (Hussain et al., 2002; Schober et al., 2007), and its interaction with regular wheat gluten has been studied (Rosell and Foegeding, 2007). Nevertheless, it appears that the various mechanisms, which might contribute to the positive effects of HPMC on regular and gluten-free breads, are not fully understood.

The objectives of the present study are to develop a formulation and procedure for zein-based gluten-free bread, making use of the improving effects of HPMC. Emphasis is put on studying the practical feasibility of such bread in a regular bakery environment, and on understanding the underlying physicochemical principles. For this purpose, a wide range of techniques is used, including rheological tests at small and large deformation (dynamic oscillatory tests and microextension tests) for the mechanical properties; laser scanning confocal microscopy (LSCM) for the microscopic changes in the three-dimensional protein structure; size-exclusion high-performance liquid chromatography (SE-HPLC) for the molecular basis, and all these methods aim at explaining the results of breadmaking experiments.

In wheat research, it is common practice to study not only dough, but also isolated gluten. Fundamental rheology has been applied to gluten by various researchers including Janssen et al (1996a,b); Pedersen and Jørgensen (2007) and Schober et al. (2002, 2006). Studying gluten rather than dough is a simplification, as starch–starch and starch–protein interactions do not affect the rheological characteristics in the case of gluten (Amemiya and Menjivar, 1992; Pedersen and Jørgensen, 2007; Schober et al., 2002). In an analogous way, in the present study for fundamental rheological tests hydrated, aggregated, viscoelastic zein (prepared above its T_g) is included. This is the analogous substance to wheat gluten, and we therefore call it 'zein gluten' in this study. It should be emphasized, though, that maize is safe for celiac patients (Kasarda, 2001) and that the term 'zein gluten' is only used because of the functional analogy to wheat gluten.

2. Experimental

2.1. Materials

Ingredients for doughs and breads included zein (maize prolamine) and maize starch (unmodified regular corn starch) from Sigma (St. Louis, MO). Moisture contents (AACC Method 44-15A, AACC, 2000) were 5% and 11% for zein and maize starch, respectively, crude protein content (N×6.25, AACC Method 46-30) of the zein was 89% db. HPMC (Methocel K4M, food grade) was from Dow Chemical Co. (Midland, MI), xanthan gum (TICAXAN xanthan 200 powder) was from TIC gums (Belcamp, MD). Table salt (NaCl), granulated sugar (saccharose), and active dry yeast were purchased locally. Distilled water was used for all experiments.

Fluorescein 5(6)-isothiocyanate (FITC, mixed isomers) for microscopy was from Sigma, as were all chemicals used for SE-HPLC. The latter were of the highest available purity (SigmaUltra).

2.2. Methods

2.2.1. General formulation

Preliminary experiments with 10, 15 and 20 g zein plus maize starch to yield a sum of 100 g, showed improved bread quality with increasing zein. The combination 20 g zein (89% db protein, N×6.25 and 5% moisture) and 80 g maize starch results in 16.9% protein (N×6.25) in the zein–starch mixture, comparable to a wheat flour with high protein content.

The HPMC level (2%, zein–starch basis) was the same as in previous studies on gluten-free batter-based breads (Hart et al., 1970; Schober et al., 2007). The water level (75%) was chosen to yield a consistency of zein dough with added HPMC comparable to wheat dough.

2.2.2. Breadmaking

The basic breadmaking procedure involved pre-mixing of the dry ingredients (20 g zein, 80 g maize starch, 2.0 g HPMC, 5.0 g sugar, 2.0 g table salt and 1.0 g active dry yeast). The dry ingredients were then pre-warmed to 40 °C for >1 h. Then, 75 g distilled water (40 °C) was added and the dough mixed manually at 40 °C (in a beaker sitting in a water bath) with the help of a spatula. The covered dough was allowed to rest for 20 min at 40 °C. Then, it was manually re-kneaded until it was visibly homogenous and smooth, rounded and allowed to rest for 5 min at 40 °C. Afterwards, it was sheeted manually, the sheet rolled up and placed seam down in a greased pan (inside diameter top 14 × 8 cm, bottom 13 × 6 cm, depth 5.5 cm). Final proof was 35 min at 40 °C in a proof cabinet at a relative humidity of 94%. Baking was done for 20 min at 225 °C in an electric reel oven (National MFG, Lincoln, NE).

After 1 h of cooling, the breads were weighed and their volume determined by rapeseed displacement. Specific volume was calculated as loaf volume (ml)/loaf weight (g). Breads were then sliced into 12.5 mm slices using an electric bread slicer (Model 711, Oliver, Grand Rapids, MI). Loaf height was measured as the height of the highest (typically center) slice. Afterwards, the crumb grain of both sides of the 3 most central slices was recorded with a C-Cell (Calibre Control International, Warrington, UK).

This basic formulation and procedure was modified to test for the effects of hydrocolloids, water level, temperature changes and mechanical treatment below T_g. Modified treatments were as follows: (1) HPMC was omitted. (2) HPMC was omitted and water reduced to 70 g. (3) HPMC was omitted and instead xanthan gum (0.5, 1.0 and 2.0 g) added. (4) The dough after rounding was not rested for 5 min at 40 °C, but for 10 min at room temperature (≈25 °C), then sheeted as usual. (5) After the initial 20 min rest at 40 °C, re-kneading and rounding, the dough was flattened, sealed in a plastic bag, and cooled for 60 min in a 15 °C environment to a core temperature of 18–19 °C, then re-warmed in its plastic bag for 60 min at 40 °C, re-kneaded until smooth, rounded, and then sheeted as usual; in half of these treatments, the dough was additionally mechanically treated (cut with a spatula, flattened and kneaded) after the 60 min rest at 15 °C, the other half (control) was not mechanically disturbed while below T_g. (6) In preliminary tests for (5), the dough was only cooled for 50 min at 15 °C (core temperature 19 °C), not mechanically treated while below T_g, and then re-warmed at 40 °C for 30 min (core temperature 38 °C).

2.2.3. Foam formation

HPMC or xanthan gum was mixed with water at a concentration of 2% in a high-speed blender (Blender 7012, Waring, New Hartford, CT). Mixing involved pre-mixing (gradual increase to maximum

speed), scraping, and then 5 min rest for swelling, followed by 2 min mixing at maximum speed, scraping and another 2 min mixing at maximum speed. Then, 25 ml of the resulting foam or viscous liquid were weighed for density determination. A small sample of the foam/viscous liquid was compressed between 2 microscope slides and a photograph taken for the qualitative evaluation of the size and number of bubbles. With xanthan gum, other concentrations between 0.1 and 3% were also tested.

2.2.4. Fundamental rheology

Fundamental rheology was conducted with zein dough and zein gluten to study their small deformation behavior. For zein dough, the same formulation was used as for breadmaking (with or without HPMC), except that yeast was omitted and only 1/10 of the above amount was prepared. Directly after mixing at 40 °C, the sample was inserted into the rheometer, the lower plate of which was already pre-warmed to 40 °C to maintain constant sample temperature.

For zein gluten, 2.0 g zein (pre-warmed to 40 °C for >1 h) and 4.0 g water (40 °C) were mixed with a spatula at 40 °C. The aggregated gluten was removed from excess water, kneaded by hand until homogenous, formed into a ball and inserted into the pre-warmed (40 °C) rheometer. The effect of HPMC on zein gluten was tested by pre-mixing 0.13 g HPMC with the zein before water addition. This HPMC amount was calculated from the ratio of the amount of zein plus water in zein gluten versus dough. In the presence of 0.13 g HPMC, 2.0 g zein bound the total 4.0 g of added water. In contrast, in the absence of HPMC, 2.0 g zein bound 2.2 ± 0.1 g water. In order to compare zein gluten with and without HPMC also on identical water levels, a further experiment was added (2.0 g zein, 0.13 g HPMC, 2.2 g water). This latter treatment is abbreviated 'low water, HPMC' in the figure legends.

For rheological testing of the zein doughs and glutens, a ViscoAnalyser 50 (Reologica Instruments, Lund, Sweden) equipped with a serrated plate measuring system (25 mm diameter) was used. For gap control, the autotension function of the instrument was applied with a target force of 0.01 N. This function compensates for expansion or contraction of the sample under the influence of heating or cooling by enlarging or reducing the gap while keeping the normal force constant. The sample was loaded on the temperature controlled (40 °C) bottom plate, and the top plate was lowered to a gap between 3.1 and 3.2 mm, so that all of its area touched the sample. Excessive sample was trimmed off with a plastic knife, and the exposed edges were covered with high vacuum grease dissolved in hexane (about 1:5) with a small quantity of hydrophobic dye (Oil Red O) added as described by Schober et al. (2007). In addition to hydrophobic coating, plate and sample were covered with a plastic lid. The inside of the lid contained moistened cotton wool to increase air humidity around the sample. This was required because the zein samples generally showed a strong tendency for surface drying.

A strong time-dependent rheological behavior of zein dough has been described previously, unless the plasticizer dibutyl tartrate is added (Lawton, 1992) and has also been observed by us in preliminary tests with our own dough system. At the same time, zein dough and gluten showed little elasticity in comparison to wheat dough. Therefore, in contrast to common practice, no relaxation time in the rheometer was allowed. Instead, measurements were started immediately, and the time-dependent changes in the sample were recorded.

Stress sweeps were conducted to establish the linear viscoelastic region of the samples. At 40 °C, sample response was clearly linear up to strains of 2×10^{-4} and 1×10^{-3} for zein dough and gluten, respectively.

Dynamic oscillatory testing at 1 Hz at the above strains was conducted with a temperature profile to study changes in the sample due to glass transition. The temperature profile started with a holding period at 40 °C for 10 min, followed by a linear gradient down from 40 to 15 °C in 25 min. A holding period at 15 °C for 10 min followed, and then a linear gradient up from 15 to 40 °C in 25 min. Finally, the temperature was held constant at 40 °C for 10 min.

2.2.5. Microextension tests

Microextension tests were modifications of the procedure described by Kieffer et al. (1981) and Smewing (1995) for wheat dough. The SMS/Kieffer Rig set and the TA.XT.plus texture analyzer were used (Stable Micro Systems, Godalming, UK). In one set of tests (experiment 1), 4 different treatments were compared to test for the effects of rest time and glass-transition (but no mechanical work input) on large deformation behavior of zein dough with added HPMC: fresh zein dough that was kept constantly at 40 °C for 30 min (Dfresh), dough that had been cooled from 40 °C to about 20 °C in a 15 °C environment (D20), dough that had been cooled from 40 °C to about 20 °C and then re-warmed to 40 °C within a total time of 70 min (D20–40), and, as a control, dough that had been kept at 40 °C for a prolonged time (80 min), comparable to the duration of the cooling and re-warming of the D20–40 treatment (DControl). A second test set (experiment 2) tested for the effects of mechanical work input into the dough at 40 and 20 °C. For this latter purpose, the D20–40 treatment was modified in 2 different ways. In the case of D20–40mech, intense working followed after the dough had re-warmed from ≈20 to 40 °C, while in the case of D20mech–40mech, the dough was intensely worked after it had cooled to ≈20 °C, and after it had re-warmed from ≈20 to 40 °C. A third test set (experiment 3) compared dough prepared from zein with different particle size.

In order to conduct experiment 1, zein dough with added HPMC was prepared as for breadmaking, except that yeast was omitted. After the initial 20 min rest at 40 °C, the dough was divided into 4 pieces, and these were rounded into balls by hand. Two were then flattened for better heat transfer, sealed in plastic bags, and allowed to rest for 20 min at 15 °C (D20 and D20–40), a third was also sealed in a plastic bag, but kept at 40 °C (DControl). The fourth ball (Dfresh) was rolled into a longish strand (elongated). This was inserted into the Teflon dough form that had been greased with paraffin oil, equipped with plastic strips for easier dough removal and pre-warmed to 40 °C before, and then the dough form was compressed with the clamp following the details described by Smewing (1995). The dough in the form was allowed 10 min of rest at 40 °C, and then 4 strands from the center were extended at 3.3 mm/s and the force over distance recorded, using the measurement parameters listed by Smewing (1995), except that trigger force was reduced to 0.5 g due to the softness of the strands. The peak force (N), extensibility until rupture (cm) and area under the curve ($N \times$ cm) were determined.

After the 20 min rest at 15 °C, core temperature of D20 and D20–40 was 19 ± 2 °C. Sample D20 was elongated, pressed in a Teflon form, rested for 10 min and measured as described for Dfresh, except that for D20, another Teflon form was used that had been previously cooled to 15 °C, and the 10 min rest was also at 15 °C. Sample D20–40 was first re-warmed (20 min at 40 °C) in its sealed plastic bag, and then elongated, pressed in a Teflon form, rested for 10 min and measured as described for Dfresh. Finally, DControl was also elongated, pressed, rested for 10 min and measured as described for Dfresh. Its total rest time was slightly (10 min) longer than that of D20–40, because it was required to wait for the availability of the 40 °C dough form after its use for D20–40, and totaled 80 min (20 min initial dough rest, 40 min rest while D20–40 was cooled and re-warmed, 10 min wait time, and 10 min rest in dough form). In all cases, the data for the 4 strands were averaged, and the whole experiment repeated 4 times with 4 individual dough batches.

For experiment 2, zein dough was prepared as for experiment 1; however, after the initial 20 min rest at 40 °C, half the dough was discarded and the remainder divided into 2 pieces. These were treated as D20–40, however, in case of D20–40mech, the dough was intensely worked after the 20 min re-warming period from ≈20 to 40 °C before elongation (30 times flattening and folding of the dough piece). In the case of D20mech–40mech, the dough was worked after the 20 min rest period at 15 °C, i.e. when it had reached ≈20 °C (cutting and compressing with a spatula, then manual kneading). After re-warming from ≈20 to 40 °C, it was worked as described for D20–40mech.

For experiment 3, the commercial zein was ground in a coffee grinder (Krups 203, Offenbach, Germany) into fine powder (temperature directly after grinding ≤36 °C). Particle size distribution of this re-milled zein and of the original commercial zein was compared using a laser diffraction particle size analyzer (Beckman Coulter LS 13 320, Fullerton, CA, USA). Extension tests were conducted with zein dough prepared with the original commercial zein and with the re-milled zein as described for Dfresh.

2.2.6. LSCM

LSCM was used to study the microstructure of zein dough and bread crumb. Emphasis was put on visualizing protein structures in zein dough with HPMC undergoing a combination of temperature changes and mechanical treatments similar to those in the microextension tests. Fluorescein 5(6)-isothiocyanate (FITC) was used in all experiments as fluorescence dye binding preferably to proteins.

The crumb from breads with HPMC was studied as described previously (Schober et al., 2007), except that the vertical (z) slice interval was 4 μm and 1.7 μm for the 20× and 40× objective, respectively, and that 5–7 vertical layers were projected into one image (images abbreviated BrHPMC). For microscopy of zein dough, FITC was added directly to the water used for dough preparation (1 mg FITC/100 ml water). This dough was prepared without yeast as described for fundamental rheology. Zein dough containing HPMC was first examined fresh (DHPMCfresh), while kept on a heated (40 °C) clear culture dish system (Delta T3, Bioptechs, Butler, PA). Then the culture dish with the dough sample was cooled on ice to <20 °C without mechanically disturbing the sample, and the structure observed (DHPMC20). In parallel, the remaining zein dough containing HPMC was cooled to <20 °C in a covered beaker in an ice bath, mixed with a spatula to simulate mechanical impact on dough below zein's T_g, and a sample pressed on a cold culture dish (DHPMC20mech). After observing the structure of this sample, the culture dish with the sample was heated to 40 °C and held at this temperature for 5 min to test for the effect of heating above the glass transition temperature (DHPMC20mech–40). Finally, the beaker with the dough sample that had been cooled in the ice bath and re-mixed while <20 °C was transferred into a water bath at 40 °C. After reaching this temperature, the dough was mixed with a spatula and kneaded by hand, placed on a heated (40 °C) culture dish and observed (DHPMC20mech–40mech). This latter treatment thus differed from the previous one in that it was mechanically treated after being re-warmed above T_g.

Additionally, fresh dough without HPMC with normal (7.5 ml) and reduced (7.0 ml) water content was studied (basis 10 g zein plus starch) keeping it at 40 °C on a heated culture dish (DnoHPMCfresh and DnoHPMCredW, respectively). All doughs were examined with the microscope and settings described previously (Schober et al., 2007). The 10× and 20× objectives were used, and 2–7 vertical layers projected into 1 image, corresponding to thicknesses (sums of z-intervals) between 11 and 44 μm.

2.2.7. SE-HPLC

SE-HPLC was used to compare extractability and size distribution of proteins from zein powder and zein glutens with and without added HPMC. In parallel, the liquid phase of these zein glutens (gluten liquid) was examined for the presence of proteins.

Zein gluten was prepared as described for fundamental rheology (2 g zein, 4 g water; 2 g zein, 0.13 g HPMC, 4 g water; 2 g zein, 0.13 g HPMC, 2.2 g water). Zein gluten (2.0 g) was centrifuged at 15,700 × g for 10 min. The liquid supernatant was collected (no supernatant could be gained from the HPMC containing gluten with only 2.2 g water), 30 mg supernatant weighed into a new vial and diluted with 180 μl mobile phase (50 mM Na-phosphate buffer, pH 7.0, with 1% sodium dodecyl sulfate (SDS)). The diluted supernatant was then centrifuged again (15,700 × g, 10 min) and examined by SE-HPLC as detailed below ('Gluten liquid').

A sub-sample of 10.0 mg was taken of the pellet from the first centrifugation step (centrifuged zein gluten), and extracted, with occasional vortexing, for 90 min at 40 °C (to keep it above its T_g) with 1 ml 12.5 mM Na-borate buffer, pH 10.0, containing 2% SDS. All types of zein gluten dissolved completely within about 1 h, therefore no further extraction steps (e.g. with applying sonication or with added reducing agents) were required. This solution was diluted 1 to 3 with mobile phase (200 μl solution + 600 μl mobile phase), centrifuged (15,700 × g, 10 min) and examined by SE-HPLC ('Zein gluten').

Zein (powder, 7.5 mg) was extracted at room temperature (i.e. below T_g) with 1 ml 12.5 mM Na-borate buffer, pH 10.0, containing 2% SDS. It dissolved quickly in less than 30 min. The solution was diluted 1 to 3 with mobile phase, centrifuged (15700 × g, 10 min) and examined by SE-HPLC ('Zein powder').

SE-HPLC was conducted with the above-mentioned mobile phase as described previously (Schober et al., 2007), except that the injection volume was 40 μl.

2.2.8. Experimental layout, data evaluation and statistics

Microextension tests followed randomized block designs with 4 blocks, and experiment 1, 2 and 3 were conducted independently. Most breadmaking experiments also followed randomized block designs with 3–4 blocks, except for the experiments with xanthan gum and the preliminary experiment in which dough was cooled below T_g and then re-warmed (modified treatments 3 and 6), which were done individually. For statistical analysis of randomized block designs, the GLM procedure of SAS (SAS Institute, Cary, NC) was used, and random block effects incorporated in the model. If the F-test indicated significant differences between treatments, all individual treatments were compared applying a comparison-wise error rate of $P < 0.05$.

Qualitative results reported for fundamental rheology were confirmed in duplicate experiments. Fundamental rheological experiments followed a completely randomized design with 2 replicates, and in the case of a significant F-test, the individual treatments were compared (comparison-wise error rate of $P < 0.05$).

Observations in LSCM were verified by looking at various parts of the sample, and only repeatedly confirmed trends are reported and representative images shown.

3. Results and discussion

3.1. Development of leavened zein bread

3.1.1. Breadmaking

Bread produced from zein dough without HPMC addition was only slightly aerated and had a flat top (Fig. 1). Its crumb was dense, hard and relatively brittle already 1 h after baking. The specific volume was 2.72 ± 0.10 ml/g (average ± standard deviation, $n = 3$). Zein dough without HPMC was thus able to hold some gas, but the amount was insufficient for acceptable bread. The addition of

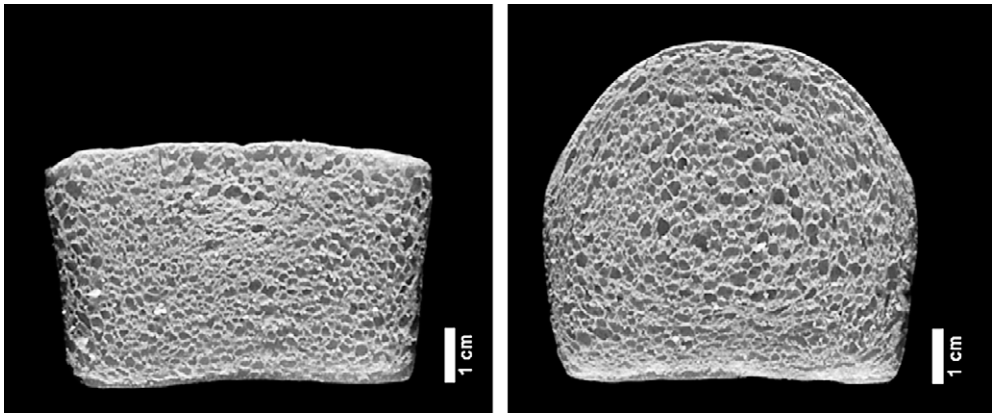

Fig. 1. Crumb images of breads made from zein dough without HPMC (left) and with addition of HPMC (right). The formulation comprised 20 g zein, 80 g maize starch, 75 g water, 5 g sugar, 2 g salt and 1 g dry yeast; 2 g HPMC were added. Mixing and resting was done above zein's T_g at 40 °C.

HPMC clearly improved quality; the bread rose better, resulting in a soft, elastic, well-aerated crumb, a regular, fine crumb grain, and the top was round (Fig. 1). The specific volume was 3.16 ± 0.08 ml/g, which was significantly ($P < 0.05$) higher relative to bread without HPMC. HPMC addition may have several effects (see Section 3.1.3), but the most obvious was the increase in viscosity. Zein dough with HPMC had a consistency similar to wheat dough, while dough without HPMC was very soft, its surface felt wet, and overall it resembled pancake dough rather than bread dough. In order to test if the improving effect of HPMC might have been simply a consistency effect, bread was produced from zein dough without HPMC with a reduced water level (70% water on a zein–starch basis relative to 75% in the regular formulation). This reduction in water made dough without HPMC firmer and closer to wheat bread dough in appearance. However, the specific volume (2.54 ± 0.23 ml/g, $n = 3$) of the resulting bread was even lower than that of bread without HPMC and the regular 75% water. Viscosity increase can therefore be ruled out as a possible cause for the volume increase upon HPMC addition. In order to better understand the structural background of the breads described so far, LSCM was used.

3.1.2. LSCM

Fig. 2 shows fresh zein dough with added HPMC at different magnifications (DHPMCfresh, 10× and 20×), fresh zein dough without HPMC and regular (75%) water content (DnoHPMCfresh), fresh zein dough without HPMC and reduced (70%) water content (DnoHPMCredW), and bread crumb from dough with HPMC at different magnifications (BrHPMC, 20× and BrHPMC, 40×). In the micrographs, zein was brightly stained; in micrographs showing dough, the ungelatinized maize starch granules appeared less intensely stained and could be identified by their round shape and size around 15 μm.

Dough with added HPMC showed zein strands with diameters similar to starch granules, and at larger magnification also two-dimensionally extended zein films were visible (arrow). Similar structure elements, formed by gluten, have been observed in wheat dough by using scanning electron microscopy (Amend and Belitz, 1991). Zein dough without HPMC at the regular water level showed only zein patches, but no strands. Reducing the water level to 70% allowed for the formation of some coarse strands. It is therefore possible that a sufficiently high viscosity, as resulting from HPMC addition or reducing the water level, is required for the formation of zein strands. The results also suggest that the presence of strands alone does not guarantee satisfactory gas holding, as in the case of the treatment without HPMC addition and reduced water content (DnoHPMCredW). From the present results it also appears possible that HPMC facilitates the formation of finer strands. This hypothesis is in line with observations on a macroscopic level. Zein gluten, prepared as described for fundamental rheology, could be more easily extended when HPMC was present, and tended to form more strand-like structures (Fig. 3). The effects of HPMC on zein could possibly be attributed to a lubricating effect, as HPMC forms viscous, lubricant-like solutions. Higher water levels in the zein gluten due to the water-binding of HPMC has also to be considered, and possibly also molecular interactions between HPMC and zein. These aspects will be taken up when discussing fundamental rheology and SE-HPLC.

After baking, the zein strands were no longer visible in the crumb structure (Fig. 2, BrHPMC, 20× and 40×). It appears that instead, zein seems to surround voids which might have been previously occupied by starch granules. Temperature increase upon baking would be expected to initially soften the zein, before at higher temperature crosslinking occurs. Measurements by Madeka and Kokini (1996) suggested that, in sufficiently hydrated zein (≥25% moisture), the crosslinking reaction starts around 65 °C. Below this temperature, entangled polymer flow occurs, and temperature increase reduces the magnitude of storage and loss modulus (Madeka and Kokini, 1996). Softer zein may more easily be extended by expanding gas bubbles and gelatinizing starch, before it gradually sets above 65 °C. Overextension and rupture of the strands and some flow might result in the observed loss of the zein strands and a more irregular arrangement of the zein in the dough.

3.1.3. Effects of HPMC

In order to better understand the effects of HPMC on zein dough and bread, it was omitted and instead a different hydrocolloid, xanthan gum, added. Several levels of xanthan gum were tested to cover a wider range of viscosities. The specific volumes of the resulting breads were low (2.8, 2.2 and 2.1 ml/g for 0.5, 1, and 2% xanthan gum on a zein–starch basis, respectively), confirming the hypothesis that a specific property of HPMC, not mere viscosity increase, was responsible for its improving effects. HPMC differs from xanthan gum and many other hydrocolloids in that it is surface-active (Dickinson, 2003). We could illustrate this difference by mixing 2% HPMC or xanthan gum, respectively, with water in a high-speed blender. HPMC formed a foam (density 0.62 g/cm^3) composed of small individual bubbles, while xanthan gum formed only a viscous solution (density 0.83 g/cm^3) in which few bubbles were trapped (Fig. 4). Varying the xanthan gum concentration between 0.1 and 3% to cover a wider range of viscosities did not principally change this result.

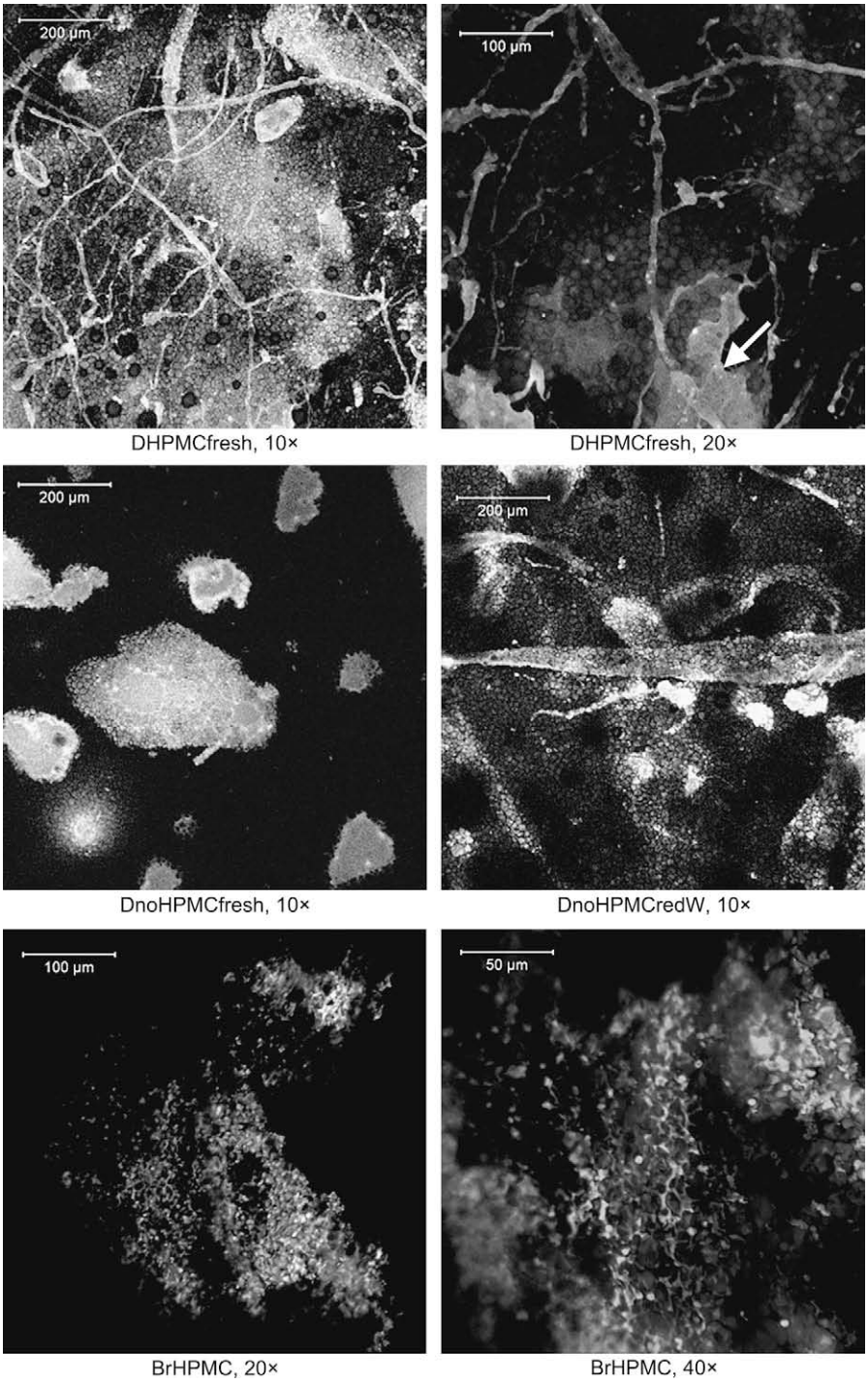

Fig. 2. Micrographs obtained by laser scanning confocal microscopy (LSCM) from samples stained with fluorescein isothiocyanate (FITC) at different magnifications. In the case of the 10×, 20× and 40× objectives, scale bars represent 200, 100 and 50 µm, respectively. Samples were fresh zein dough with HPMC (DHPMCfresh); fresh zein dough without HPMC with normal (75%) and reduced (70%) water content (DnoHPMCfresh and DnoHPMCredW, respectively); and bread crumb of breads from zein dough with HPMC (BrHPMC). The arrow in DHPMCfresh, 20×, indicates extended zein films.

The good gas cell stabilization by HPMC might explain its superior performance relative to other gums in batter-based gluten-free breads. In zein breads, however, additionally zein–HPMC interactions have to be considered. The situation would be expected to be analogous to interactions between HPMC and wheat gluten. Rosell and Foegeding (2007) found that HPMC addition caused increased protein extractability from wheat gluten, and assumed that HPMC might interfere with the interactions of protein chains, hindering their association. If comparable effects occur upon HPMC addition to zein dough, technological consequences could be expected. Solubilized proteins might contribute to gas cell stabilization, as they might be surface-active by themselves (Dickinson, 2003; Gan et al., 1995). Weaker interactions of protein chains might contribute to the above mentioned tendency of zein to form extensible strands upon addition of HPMC. Extraction experiments and SE-HPLC with zein powder, zein gluten with HPMC at 2 water levels, and zein gluten without HPMC were conducted in order to study the

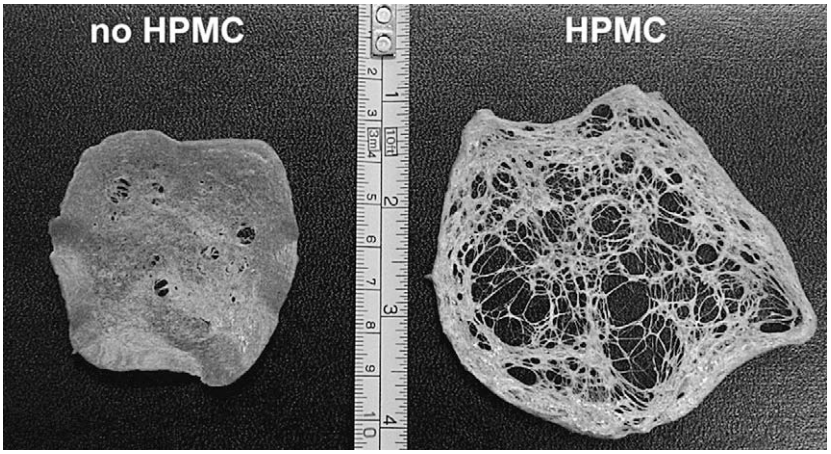

Fig. 3. Hydrated, aggregated, viscoelastic zein above T_g (zein gluten), extended to sheets at 40 °C. (Left: no HPMC, 2 g zein, 2.2 g water; right: 2 g zein, 0.13 g HPMC, 4 g water).

protein phase on a molecular level. The liquid phases of the zein glutens were also examined. Zein powder and all types of zein gluten dissolved completely in the buffer (Na-borate buffer, pH 10, containing SDS) and had qualitatively identical SE-HPLC patterns dominated by monomeric zeins (data not shown). Commercial zein is known to contain mainly α-zeins (21–25 kDa); additionally the presence of small amounts of other monomeric zeins, and dimers has been concluded from SDS-PAGE results (Zhu et al., 2007). No qualitative differences between zein powder and zein gluten suggests that aggregation of zein powder to zein gluten involves no disulfide linkages, but only weaker interactions like hydrogen bonds and hydrophobic interactions, which can be broken under non-reducing conditions by the alkaline SDS buffer. Under the given conditions, it was not possible to detect any effect of HPMC on the properties of zein gluten. This may be because the drastic extraction conditions (i.e. alkaline buffer containing the detergent SDS) allowed complete solubilization, which would however mask possible weaker effects of HPMC on zein gluten. A softening effect of HPMC on zein's rheological properties could however be measured by fundamental rheology (see Section 3.2.4).

The liquid phase of the gluten contained mainly small fractions, eluting later than zeins (data not shown), and little qualitative differences were visible between the liquid phases of zein gluten with and without HPMC addition. There is therefore no evidence that HPMC solubilizes proteins. In the zein bread, stabilization of the gas–liquid interface of gas cells must therefore be an effect of HPMC alone, without contribution of soluble proteins.

3.1.4. A model for zein dough

The simplest model that could be derived from all data measured so far would be a zein dough which contains a network of zein strands in the μm to mm range. This network by itself holds only little gas. However, it traps existing gas bubbles, which are in turn stabilized by HPMC at their interface, similar to the model proposed by Gan et al. (1995) for fermenting wheat dough (the 'liquid lamellae' suggested by Gan et al. would be gas bubble walls stabilized by HPMC in the zein dough). The zein network contributes physical stability on a macroscopic level, so that an extensible, cohesive, viscoelastic dough results rather than simply an HPMC foam as in batter breads. This latter point is most important when it comes to the production of gluten-free products resembling wheat products other than pan breads. Examples would be hearth-type breads, braided breads, various types of rolls, and soft pretzels. It was possible to make a pretzel from the zein dough containing HPMC, applying the same technology as for wheat pretzels (rolling of spindle-shaped strands and slinging; see pictures in the supplementary material online, Suppl_1). Pretzel-making is a most challenging process and illustrates a cohesiveness and extensibility of the zein dough comparable to wheat dough.

3.2. Effects of temperature changes, mechanical impact and rest time

3.2.1. Extension tests

Extension tests (Table 1) were conducted to study the large scale rheological behavior of zein dough with HPMC addition. Experiment 1 showed a clear effect of temperature. Cooling the dough to

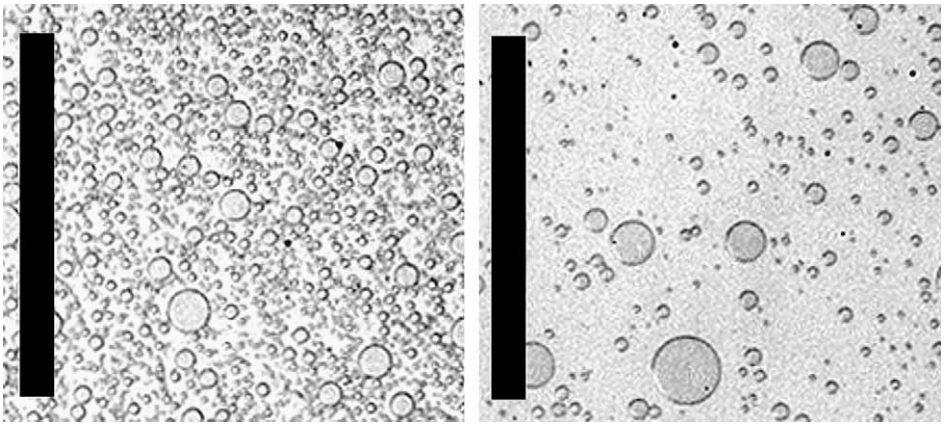

Fig. 4. Photographs of HPMC foam (left) and xanthan gum solution (right); 2% in water each, after high-speed mixing; scale bars represent 5 mm.

Table 1
Microextension tests with zein dough containing HPMC[a,b]

	Experiment 1					Experiment 2		
	Dfresh	D20	D20–40	DControl	LSD[c]	D20–40mech	D20mech–40mech	LSD
Peak force (N)	0.29a	0.18c	0.23b	0.28a	0.04	1.02A	0.11B	0.24
Extensibility[d] (cm)	3.7a	1.9b	3.6a	3.5a	1.1	2.8A	2.6A	0.3
Area ($N \times$ cm)	0.72a	0.17b	0.54a	0.66a	0.26	1.98A	0.15B	0.39

[a] Dfresh = fresh zein dough kept constantly at 40 °C for 30 min; D20 = zein dough cooled from 40 to ≈20 °C; D20–40 = zein dough cooled from 40 to ≈20 °C and then re-warmed to 40 °C (total time 70 min); DControl = dough kept for a prolonged time (80 min) at 40 °C; D20–40mech = like D20–40, but intensely worked after re-warming from ≈20 to 40 °C; D20mech–40mech = like D20–40, but intensely worked after cooling to ≈20 °C and after re-warming from ≈20 to 40 °C.
[b] Within each row and experiment, numbers not sharing a common lower case letter (experiment 1) or upper case letter (experiment 2) are significantly different ($P < 0.05$).
[c] Least significant difference.
[d] Until rupture.

≈20 °C (D20) significantly reduced peak force, extensibility and area under the extension curve in comparison to dough kept at 40 °C (Dfresh, DControl). This result was in agreement with Lawton (1992) who found that zein doughs lost their extensibility when rested at room temperature. The results are also in line with the theoretical expectations, as zein is supposed to be glassy-brittle below its T_g (Madeka and Kokini, 1996).

The 2 treatments Dfresh and DControl involved rest times of 30 min and 80 min at 40 °C, respectively, thus enclosing the duration of the present breadmaking test (60 min, excluding the bake time). No significant differences between these 2 treatments indicated that zein dough with HPMC did not change when stored above its T_g for an extended time period beyond 30 min. This result is not directly comparable to the findings of Lawton (1992) who reported that zein dough, without the additional plasticizer dibutyl tartrate, loses most of its extensibility after resting for 15 min, even if kept warm. The present study excluded measurements within the first 15 min after mixing. Neither do such short rest times appear to be relevant for practical baking, where a minimum proof time is required for leavening, nor do they allow for relaxation of tensions built up during mixing and forming of the dough. Relaxation is important for reproducibility of extension tests and an essential part of extension standard procedures for wheat dough (AACC Method 54-10, AACC, 2000; Kieffer et al., 1981; Smewing, 1995).

With regard to practical baking, it is furthermore important to consider that dough may be mixed and fermented at controlled temperature (e.g. by using an extruder-type temperature controlled continuous mixer and fermentation cabinet at 40 °C). However, short intervals at ambient temperature are very likely to occur, e.g. during rounding and shaping of the dough. The D20–40 treatment would thus reflect dough that had intermediately been cooled below its T_g but later on again heated above T_g. The results for this treatment indicate an intermediate position between dough that was just cooled below T_g (D20) and dough that was kept at 40 °C for short or extended time (Dfresh and DControl). Most characteristic was its peak force that was significantly different from D20 as well as Dfresh and DControl. In order to get further clarification on factors affecting dough that was cooled below T_g and then reheated, additional tests were conducted (experiment 2). Dough that was cooled below T_g, reheated and subjected to intense mechanical treatment after it was above T_g (D20–40mech) showed a very high resistance to extension (peak force), higher than any value from experiment 1. In contrast, its extensibility was very short, while the area under the curve was large. For wheat dough, it has long been known that it can be 'excited' by molding and that upon subsequent rest, resistance to extension decreases while extensibility increases (Munz and Brabender, 1940). In an analogous way, the results for zein dough suggest that elastic structure elements were present that could store mechanical energy. However, due to the very intense mechanical treatment ('exciting'), the stored mechanical energy would not dissipate in the relatively short rest time, i.e. the dough in the case of D20–40mech was not sufficiently relaxed and therefore its resistance to extension was still high and its extensibility short. (It is noteworthy that only in the case of experiment 2 the mechanical energy input after re-warming to 40 °C was severely forced by 30 times flattening and folding the dough piece, while the rest time in the Teflon dough form was optimized for regular conditions as occurring during experiment 1.) The protein networks visualized in Fig. 2 were likely the structure elements in which the mechanical energy was stored, when these networks were oriented and extended during flattening and folding of the dough.

Dough that received the same, intense mechanical treatment after being re-warmed to 40 °C, but that had been additionally mechanically treated while below its T_g (D20mech–40mech) was characterized by opposite extension properties than D20–40mech. It had a very low peak force and area under the curve; only the extensibility was similar to D20–40mech, but lower than all extensibilities measured in experiment 1 except that of D20. These results suggest that this treatment lacks elastic structure elements, so that the mechanical energy incorporated at 40 °C cannot be stored. Unlike the case of D20–40mech, the low extensibility would likely not be explained by tensions in the structure, but by a lack of cohesiveness due to a structural weakness. A more detailed analysis of the underlying structural principles can be provided with the results obtained by LSCM.

3.2.2. LSCM

Dough containing HPMC simply cooled from 40 to <20 °C was dominated by the same protein networks as fresh dough (DHPMC20, Fig. 5). Cooling below T_g alone thus does not notably change the microstructure. However, if the dough was mechanically treated while below T_g, the then glassy-brittle protein network shattered into small pieces, often with a chiseled appearance (DHPMC20mech, 10× and 20×). Reheating this sample above zeins's T_g could obviously not recreate the network (DHPMC20mech–40, 10×), although the image at larger magnification (DHPMC20mech–40, 20×) showed that the individual particles had more roundish edges. This observation would be the expected consequence of zein being above its T_g and thus less rigid and no longer glassy-brittle, but viscoelastic. It would thus flow into these more roundish shapes. It might be expected that the zein pieces would re-aggregate into a network if the dough was kneaded above its T_g. The DHPMC20mech–40mech treatment however showed that this was not the case. It might be argued that the zein pieces were too scattered within the dough, so that the excessive starch and water would keep them separate. Indeed zein seemed to be smeared out amongst starch granules in the DHPMC20mech–40mech treatment (arrow). However, zein in the same ratio to starch and water was able to form a network upon

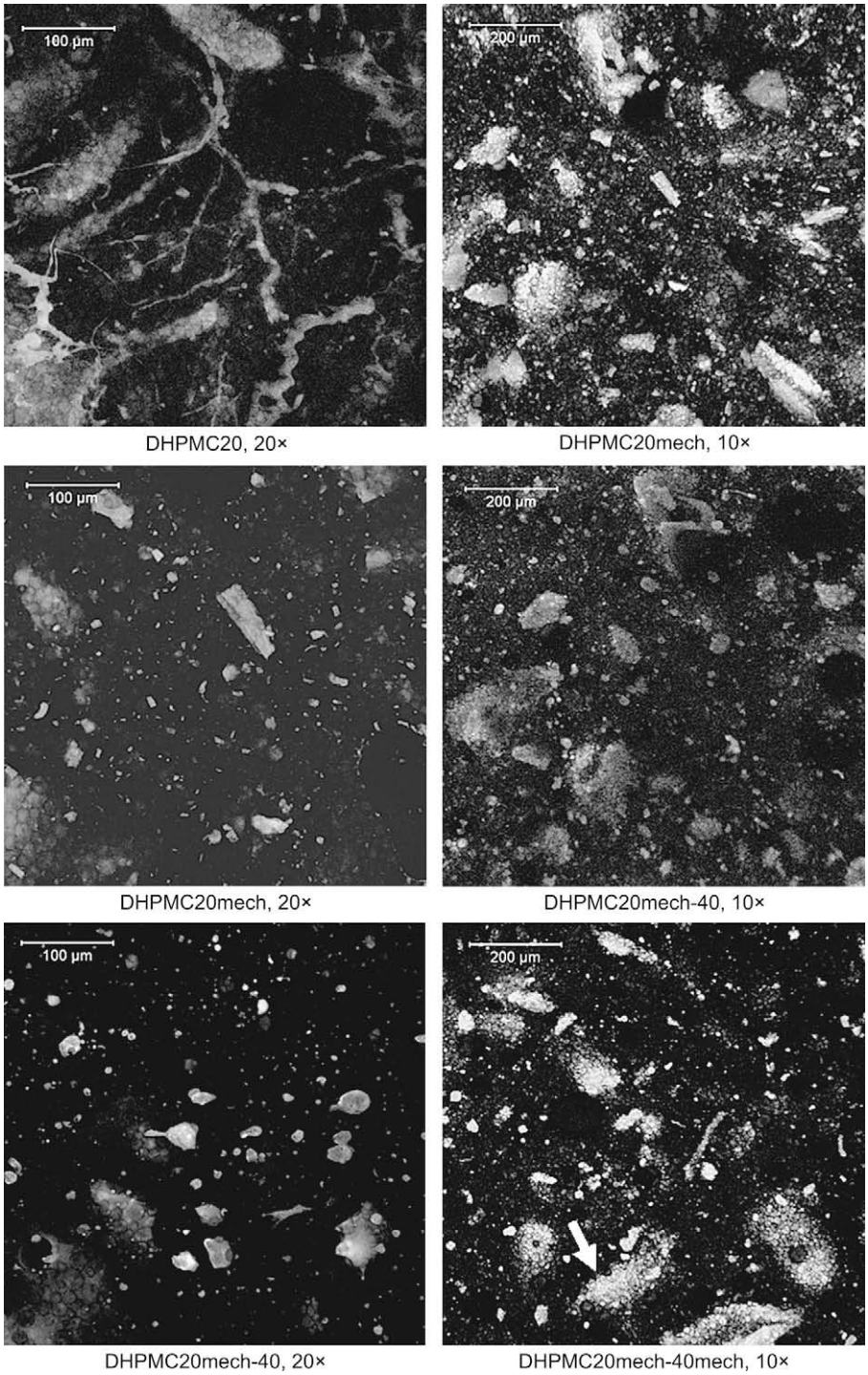

Fig. 5. Micrographs obtained by laser scanning confocal microscopy (LSCM) from samples stained with fluorescein isothiocyanate (FITC) at different magnifications. In the case of the 10× and 20× objectives, scale bars represent 200 and 100 µm, respectively. Samples were differently treated zein doughs with HPMC: zein dough prepared at 40 °C and then cooled to <20 °C without mechanically disturbing it (DHPMC20); zein dough cooled from 40 °C to <20 °C and mixed while cold (DHPMC20mech); zein dough treated as DHPMC20mech and then re-heated to 40 °C without mechanical impact (DHPMC20mech–40); and zein dough treated as DHPMC20mech–40, but remixed and kneaded after being re-heated to 40 °C (DHPMC20mech–40mech). In the latter micrograph, the arrow indicates zein smeared out among starch granules.

mixing when preparing the fresh dough. The only clear difference appears to be that the shattered zein pieces in DHPMC20mech and DHPMC20mech–40 were very small (a considerable amount ranged 10 µm or smaller, see DHPMC20mech, 20× and DHPMC20mech–40, 20×). In contrast, the commercial zein had a particle size distribution that reached into the mm range (50% by volume $<0.49 \pm 0.04$ mm, 90% $<1.28 \pm 0.06$ mm).

3.2.3. Requirement for coarse zein particles

Based on the previous section, the hypothesis was put forward that coarse zein particles are required for the formation of zein strands and consequently strong zein dough. In order to verify this hypothesis, dry zein was re-milled into fine powder (50% $<0.13 \pm 0.00$ mm, 90% $<0.35 \pm 0.00$ mm). Extension tests were conducted with dough prepared from the re-milled zein and

control dough from the normal zein powder. Both doughs were otherwise treated as Dfresh. The values for peak force, extensibility, and area under the curve were 0.12 N, 4.0 cm and 0.31 N × cm for dough from re-milled zein versus 0.33 N, 3.4 cm and 0.80 N × cm for dough from the normal zein ($n = 4$), and all three differences were significant ($P < 0.05$). Re-milling of dry zein therefore caused a similar change to mechanical treatment below T_g in the case of D20mech–40mech, i.e. peak force and area under the curve became significantly smaller. (Extensibility was inconsistent, but the differences in extensibility were relatively small anyhow.) Small particle size of the zein was therefore the likely cause of weak dough in all cases, irrespective of whether these small particles originated from shattering of the glassy zein network in the dough below T_g, or from re-milling of the zein.

The beneficial effect of larger zein particles on dough strength can be interpreted when taking into account that there is an excess of starch granules present in the zein dough. Before mixing, most zein particles, whether smaller or larger, would likely be surrounded by starch. Small zein particles have a large ratio of surface to volume. In case of such small zein particles, the zein portion close to the surface would interact with starch and therefore not be available for interaction with other zein particles. This would leave only a negligibly small portion in the core free for potential interaction with other zein. Upon mixing above zein's T_g, this small portion would likely not be squeezed through the zein–starch layer at the surface of the particle. In contrast, in the case of larger zein particles, the portion of free zein in the core would be larger. If sufficiently large, upon mixing, it could penetrate the zein–starch layer surrounding the particle, get into contact with free zein from other large zein particles, and ultimately form a zein network.

3.2.4. Fundamental rheology

Fundamental rheology was done with zein gluten and zein dough, applying the same temperature profile. This temperature profile was symmetrical and included a holding period above T_g at 40 °C, a downward linear gradient, holding below T_g, an upward linear gradient and holding at 40 °C (Figs. 6 and 7). The data measured with zein gluten and dough were characteristically different from each other. Complex modulus (absolute value, $|G^*|$) and phase angle were largely symmetrical in the case of zein gluten, reflecting the symmetry of the temperature profile (Fig. 6). Small deviations (right shift of the response curves, no plateau of the phase angle upon holding below T_g) can be explained by delayed heat diffusion due to the relatively large sample size. Delayed heat diffusion has also been described by Di Gioia et al. (1999) for dynamic mechanical thermal analysis with corn gluten meal. These authors additionally reported that the glass transition of corn gluten meal was 'almost reversible', but that small changes were detected upon a second heating after intermediate cooling relative to first heating. The largely symmetrical shapes of the $|G^*|$ and phase angle curves in Fig. 6 suggest that the glass transition of zein gluten was largely reversible. In contrast, the curves measured with zein dough were not symmetrical at all, and appeared also noisy (Fig. 7). The noise was likely caused by the small deformations close to the resolution limit of the rheometer, required in order to remain in the linear viscoelastic region of the zein dough at least while above T_g. The lack of symmetry, however, suggests that irreversible changes occur in the zein dough when undergoing the temperature profile and oscillatory deformation. Due to the reversible behavior of zein gluten, it is unlikely that the zein strands within the zein dough by themselves would show a strongly non-reversible behavior. Therefore, in the dough, it must be other interactions than those within the zein phase that determine its rheological behavior. The only major ingredient of the doughs not present in gluten is the maize starch. It appears that starch–protein interactions determine the rheological behavior of the doughs and account for the non-symmetrical shape of the curves. It has been described for wheat doughs that starch–protein and starch–starch interactions contribute to the small deformation dynamic oscillatory flow behavior besides protein–protein interactions (Amemiya and Menjivar, 1992). The present measurements indicate that the same is true for zein doughs. Starch–protein interactions in the broader sense include the arrangement of the zein strands in the starch phase. For example, the zein strands in the zein dough might break apart upon oscillatory deformations below T_g within the temperature gradient (Fig. 7), so that the microstructure would be changed similar to what is shown in Fig. 5 for DHPMC20mech. Above, it was assumed that abundant starch prevents the re-aggregation of small zein

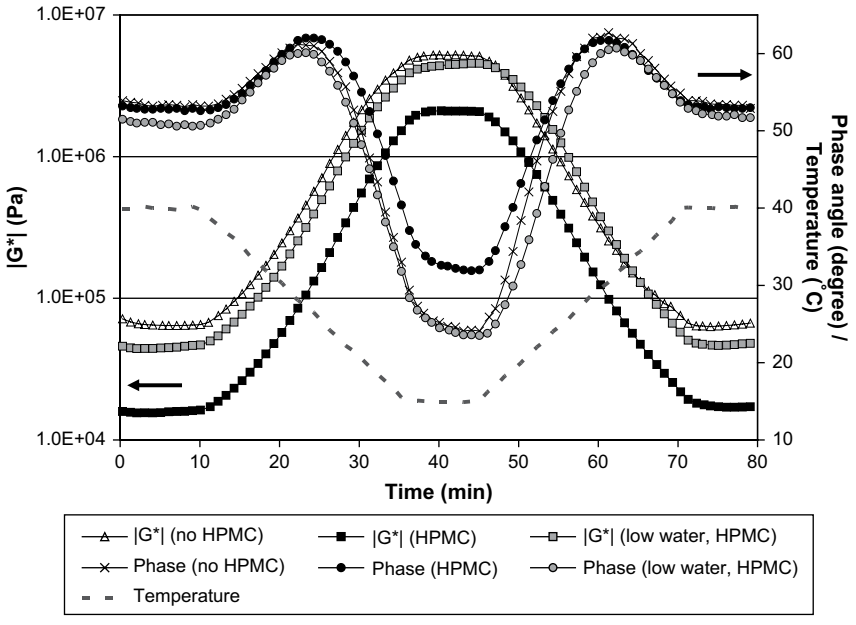

Fig. 6. Dynamic oscillatory tests at 1 Hz and 1×10^{-3} strain with hydrated, aggregated zein prepared above T_g at 40 °C (zein gluten). A temperature profile covering glass transition was applied. Three treatments were compared: no HPMC: 2 g zein, 2.2 g water; HPMC: 2 g zein, 0.13 g HPMC, 4 g water; low water, HPMC: 2 g zein, 0.13 g HPMC, 2.2 g water.

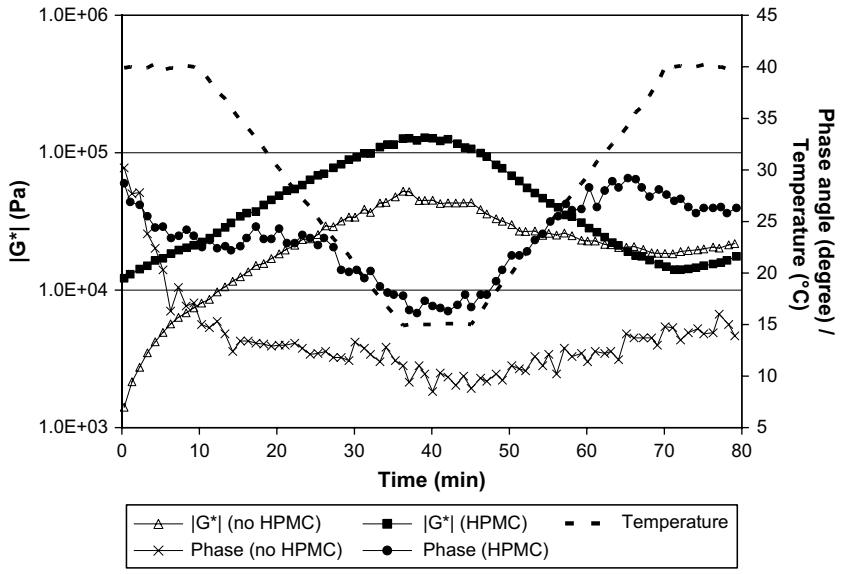

Fig. 7. Dynamic oscillatory tests at 1 Hz and 2×10^{-4} strain with zein dough prepared above T_g at 40 °C without and with added HPMC. A temperature profile covering glass transition was applied. Dough formulation was as for the breads in Fig. 1, but without yeast.

particles above T_g. In a similar way, the arrangement of the zein in the starch could be expected to remain altered even when the zein dough would be reheated above T_g during the subsequent upward temperature gradient. Clearly, this effect would only show in zein dough, not in zein gluten in the absence of starch. However, more research is required to fully understand the structural background of the small scale measurements with zein dough.

The rheological data measured with zein gluten (Fig. 6) allow for some additional conclusions. First, the effect of HPMC addition can be evaluated. Resistance to deformation, as characterized by $|G^*|$, was lower with HPMC addition. Zein gluten with HPMC differed from zein gluten without HPMC in that it did bind more water. In the presence of HPMC, 2 g zein bound the total amount of added water (4 g). However, in the absence of HPMC, 2 g zein could only bind 2.2 g water. The much lower values for $|G^*|$ of the zein gluten with HPMC (Fig. 6, 'HPMC') relative to the zein gluten without HPMC ('no HPMC') may reflect mainly the higher water content of the former. It has been described for wheat gluten, that the dynamic moduli decreased as the water level increased (Janssen et al., 1996a). For an evaluation of the effects of HPMC alone, without the impact of the changed water level, a treatment with HPMC was included where only 2.2 g water per 2 g zein was added ('low water, HPMC'). In the holding periods above T_g, i.e. in the first 10 min and between 70 and 80 min, $|G^*|$ of the 'low water, HPMC' treatment had a lower magnitude than zein gluten without added HPMC. At 9 min, $|G^*|$ was significantly ($P < 0.05$) different for all three treatments. (The time 9 min after the start was picked for statistical comparison, because here, better relaxation and temperature equilibration than directly at the start may be expected). The hypothesis that HPMC softens zein gluten could thus be confirmed. This could possibly be attributed to the above-mentioned effects: lubrication or a possible hindering of the association of protein chains. More research would be required to fully understand all physicochemical mechanisms involved.

Besides studying the effects of HPMC, fundamental rheology with zein gluten also allows for an estimation of T_g. The peak-like maximum of the tangent of the phase angle during temperature gradients has been previously used for this purpose (Di Gioia et al., 1999). Principally, T_g of zein decreases with increasing moisture (Lawton, 1992; Madeka and Kokini, 1996). These studies however also indicate that moisture has the largest effect on T_g at levels below 10–15%, while distinctly above, its effect gets very small. In the present study, zein gluten contained >50% water (2 g zein + 2.2 g water), and zein dough ≈40% water based on the sum of all ingredients. In both, zein dough and gluten, there was thus a high excess of water, and T_g of the zein was therefore assumed to be similar in both systems.

The temperature at the maximum of the tangent of the phase angle differed slightly for the three curves in Fig. 6. The values were 28.2, 26.8, and 27.2 °C during the downward temperature gradient and 30.6, 30.3, and 31.6 °C during the upward gradient for 'no HPMC', 'HPMC' and 'low water, HPMC', respectively. The differences between T_g measured at downward versus upward temperature gradient may in part be attributed to the delayed heat diffusion mentioned above. In the case of both temperature gradients, the temperature in the sample would lag behind the temperature at the lower plate, which is registered by the instrument. The indicated temperature would therefore be too low in case of the downward gradient, but too high in the case of the upward gradient. The best estimation would therefore be obtained by averaging the values measured during the downward and upward temperature gradient. These averaged values are 29.4, 28.6, and 29.4 °C for 'no HPMC', 'HPMC' and 'low water, HPMC', respectively. There is thus no evidence that HPMC affects T_g. The value for T_g of about 29 °C is very similar to the results of Lawton (1992) who estimated T_g of zein dough at around 28 °C based on the temperatures required for development of a viscoelastic dough in the farinograph. An approximate T_g of zein dough around 30 °C has also been supported by observations of Mejia et al. (2007).

It is noteworthy that above T_g, the phase angle of zein gluten (measured at 1 Hz) was above 50° for all three treatments (Fig. 6). This value is much higher than data found for wheat gluten at 1 Hz, in which Dreese et al. (1988) reported a tangent of the phase angle of ≈0.4, corresponding to a phase angle of ≈22° and Pedersen and Jørgensen (2007) phase angles of ≈30°. Janssen et al. (1996b) found that, in reconstituted wheat gluten, the glutenin/gliadin ratio affected the phase angle (higher gliadin contents caused higher phase angles, i.e. lower elasticity). Zein lacks a polymeric fraction comparable to aggregated glutenin or glutenin macropolymer and consists largely of monomeric proteins (see above), which could explain the high phase angle of zein gluten.

3.2.5. Cooling below T_g during baking

Finally, the effect of temperature changes and mechanical impact on zein dough during practical baking was studied. Modified breadmaking experiments were carried out, in which dough was prepared at 40 °C, then cooled to ≈20 °C, and subsequently reheated to 40 °C, before it was re-kneaded and then sheeted, rolled up, and subject to the final proof as usual. Half the doughs were mechanically treated while below their T_g and thus comparable to D20mech–40mech from the extension tests and DHPMC20mech–40mech from LSCM, while the other half was not mechanically treated below T_g (control). In contrast to what might be expected from the results obtained so far, both the mechanically treated doughs and the control doughs appeared subjectively very similar after being re-kneaded before sheeting, rolling and final proof. In line with the subjective feeling the objective results of the baking experiments differed little. Breads with mechanical treatment below T_g reached a specific volume of 3.24 ± 0.05 ml/g, while the control breads averaged 3.25 ± 0.07 ml/g ($n = 4$). Only the bread height was smaller for the mechanically treated breads (5.6 ± 0.1 cm versus 6.1 ± 0.1 cm for the control). This latter difference is of little importance in view of the fact that the breads, whether mechanically treated below T_g or not, tended to have randomly large void spaces under the top crust (Fig. 8 shows an example). These void spaces were also found in preliminary experiments, in which the dough was cooled below T_g and reheated to 40 °C with a slightly different time schedule. No comparable problem was encountered in the regular breadmaking procedure when the dough was kept constantly above T_g. The complete crumb images from the regular breads kept at 40 °C, and the breads cooled below T_g and reheated are shown in the supplementary material online (Suppl_2).

Frequent occurrence of large void spaces suggests that a structural weakness exists in the dough. Upon proofing or baking, the expanding gas would then collect under the crust and force the dough apart, creating the void. This structural weakness can not originate from the mechanical treatment of the doughs below T_g alone, otherwise the voids would not exist in the control treatment that was cooled below T_g and then reheated, but not intentionally mechanically treated below T_g. However, the breadmaking experiments differ from the extension tests and LSCM in that yeast was present. While cooling the dough, zein undergoes glass transition at around 29 °C (see above). At this temperature, however, yeast activity does not stop and carbon dioxide is still produced. As a consequence, the gas bubbles and the zein strands around them are expanded. An increase in volume of the dough during the cooling period from 40 to ≈20 °C was clearly visible, and was thus in the mm range. Therefore, the magnitude of deformation would be sufficient to break the zein strands, whose length and diameter was in the μm range (Fig. 2). As found before, the brittleness below T_g would make zein very sensitive to mechanical impact. In conclusion, the expansion of the zein strands by fermentation gases below T_g appears to be a problem, and could create structural weaknesses. Overall, however, it appears feasible to produce zein bread in a regular bakery environment with an ambient temperature below zein's T_g, as long as the dough is kept most of the time at 40 °C. This comparatively high temperature warrants that the dough does not immediately cool below its T_g when intermediately kept at room temperature. In order to confirm the practicality of the zein bread production, experiments were conducted in which the dough was kept at ambient temperature (≈25 °C) for 10 min before sheeting, and otherwise treated as in the regular breadmaking tests. These experiments resulted in breads with a good crumb structure, no problems with voids and a comparable specific volume to the regular breads with HPMC (3.01 ± 0.11 ml/g, $n = 3$; the crumb images are shown in the supplementary material online Suppl_2). At the end of the 10 min at ambient temperature, the dough surface had cooled to just about T_g (29 °C), while the center was well above T_g at 34 °C.

In conclusion, zein dough can be used for the production of superior gluten-free bread, if HPMC is added. Such dough can be handled similarly to wheat dough and also used for specialties like soft pretzels, not feasible with traditional gluten-free batters. Extension tests, LSCM images and fundamental rheology indicated that the microstructure of the dough is most important, particularly the arrangement of the zein in the dough. Coarse zein particles (in the mm-range) are required for the development of zein strands in the dough upon mixing. Below T_g (29 °C), these strands easily shatter upon mechanical impact. Very likely, structural weaknesses resulting in large holes under the crust are the consequence of such shattered zein strands.

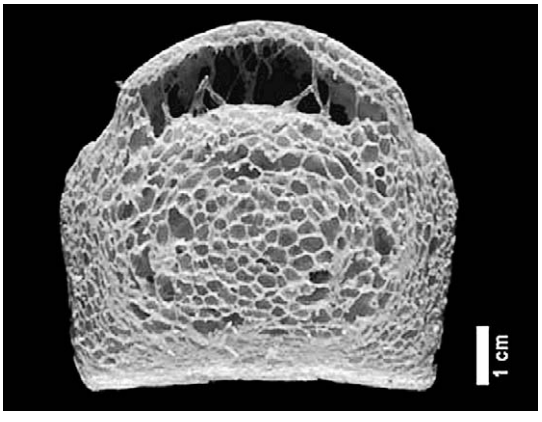

Fig. 8. Crumb image of a bread made from zein dough with HPMC. Formulation and procedure were as in Fig. 1, except that the dough was cooled to ≈20 °C and then reheated to 40 °C during its rest time. (No mechanical treatment below T_g.)

Acknowledgements

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable. We sincerely thank Jhoe de Mesa, Kevin Fay, Brian Ioerger, Rhett Kaufman and Jeff Wilson for their valuable contributions to this paper.

Appendix. Supplementary material

Supplementary material associated with this article can be found at, at doi 10.1016/j.jcs.2008.04.004.

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