Influence of Surface Lipids in Commercial Zein on Microstructure and Rheological Properties of Gluten-Free Dough

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ABSTRACT

The influence of the lipids present in commercial zein on the rheological properties and the microstructure of zein-starch doughs supplemented with HPMC were investigated by dynamic and rotational measurements in shear, confocal laser scanning microscopy (CLSM) and hyperbolic contraction flow (HCF).

INTRODUCTION

Gluten intolerance is a disease that affects approximately one per cent of the population and the only cure available is to refrain completely from eating products containing gluten. Increased awareness of the disease has led to an increase in the demand for gluten-free products[1]. Wheat gluten however, is a major structure forming protein and unique in its ability to allow the formation of viscoelastic doughs. As water is added to the flour during mixing, a gluten network held together by hydrogen bonds, disulphide bonds and physical entanglements is formed[2]. The gluten-starch matrix gives a dough with high extensional viscosity and a strain hardening behavior which improves its ability to retain gas during proving and baking as it will help stabilize the cell walls during bubble growth. This makes wheat doughs especially suitable for breadmaking as it makes it possible to produce breads with good volume and an open foam structure[3,4]. Gluten-free breads, on the other hand, are mainly produced from refined flours such as starches, which are lacking structure forming proteins, and are in comparison of poor structural quality[1].

The prolamin zein, which is the storage protein in maize, lacks the properties of wheat gluten and will not form a viscoelastic dough as the zein will not form a network when bound in maize flour. However, using purified zein mixed with starch above the T_g of zein it is possible to form a viscoelastic dough which has rheological properties suitable for breadmaking[5,6]. The zein-starch dough can be improved further by addition of HPMC[7,8].

The effect of surface lipids present in commercial zein has also been considered. Their influence on the breadmaking properties of zein-starch doughs has been investigated by Schober et al.[9]. They found that the removal of surface lipids promoted aggregation of the zein particles which in turn helped in the formation of a protein network during mixing. The work presented here was performed in order to verify these results.

MATERIALS AND METHODS

The zein used was bought from Sigma Aldrich (Stockholm, Sweden) and had a protein content of 94% db. To examine the influence of lipids present in the protein part of the zein was defatted prior to dough preparation. This was achieved by adding a fivefold volume of n-hexane to the zein and stirring with a magnetic stirrer for >3h at room temperature. The n-hexane was then decanted and the process repeated. This was done in total 3 times. The remaining n-hexane was then allowed to evaporate and the protein dry.

Dough preparation

Zein-starch doughs were prepared by manually mixing 2 g zein, 8 g maize starch, 0.5 g suger, 0.2 g NaCl and 0.2 g HPMC. The dry ingredients were preheated in an oven at 40°C for at least 1 h before being transferred to a ReoMixer (Reomix Instruments, Lund, Sweden). 7.5 g destilled water (40 °C) was then added and the ingredients were mixed until optimum dough development according to AACC mixograph method 54-40.02. During mixing the temperature was kept constant at 40°C to ensure that the mixing would be done above the T_g of the zein.

Dynamic and shear measurements

The measurements were performed using an ARES-G2 rheometer (TA Instruments, New Castle, DE, United States) with a 40 mm parallel plate geometry. Frequency sweeps were performed between 0.01 and 10 Hz using a strain of 0.1 % which was within the linear viscoelastic range as determined by an amplitude sweep. Flow curves were measured between 0.001 and 1 s^{-1}.

All measurements were performed in triplicates at 40 °C.

Hyperbolic Contration Flow

The extensional rheology of zein-starch doughs was measured using hyperbolic contraction flow (HCF) as described by Wikström et al.[4] and Stading and Bohlin[10]. The HCF rig was mounted to an Instron Universal Testing Machine 5542 (Instron Corporation, Canton, USA) and the temperature kept constant at 40°C using a controlled circulator Julabo Model FP40 (Julabo Labortechnik, Seelbach, Germany). A nozzle with an inlet radius of 10 mm and an outlet radius of 2.15 mm was used giving a total Hencky strain of approximately 5.

The influence of shear in the HCF measurements was calculated using n and K values obtained from a power law model fitted to the measured flow curves. The shear contribution could then be subtracted as described by Stading and Bohlin[10].

The extensional viscosity was measured at least three times for each extension rate.

Confocal Laser Scanning Microscopy

A Leica TCS SP2 confocal laser scanning microscope (Leica Microsystems, Heidelberg, Germany) was used to study the micrustructure of the doughs. A mixture of Akriflavin (0.1%) and Texas Red (0.01%) dissolved in water was used to dye the starch and protein, respectively.

RESULTS AND DISCUSSION

Figure 1 shows zein before and after defatting. There is a clear difference in particle size with the ordinary zein being in the form of small flakes and the defatted zein in the form of a fine powder. The defatted zein is also slightly less yellow as a result of the defatting.

Figure 1. The pure zein as powder. A shows the zein before defatting, B shows the defatted zein.

Figure 2 shows the mixograph curve for 10 minutes of mixing of zein starch dough with defatted zein. There was no apparent difference between the ordinary zein and defatted zein and therefore only one curve is shown. The only indication to any difference was a slightly faster buildup for the dough with defatted zein. This is likely a result of the smaller particle size of the defatted zein which allows it to be more finely distributed in the flour before addition of water and mixing. This in turn could allow for a quicker formation of a protein network. The optimum dough development was in both cases determined to have been achieved after 4 minutes of mixing and hence this was the mixing time used when preparing the samples for rheological and microstructure studies.

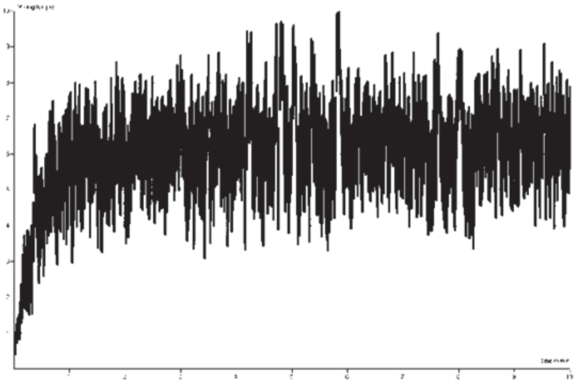


Figure 2. Mixograph curve for zein-starch dough with defatted zein.

In figure 3 the results of the frequency sweeps for the two different doughs are presented. Both doughs are more elastic than viscous in the range of frequencies used but seem to be approaching a crossover point at lower frequencies. There is a small but significant difference between the dough with ordinary zein and the dough with defatted zein with the latter resulting in a slightly higher modulus. The n and K values obtained from the fitting of a power law model to the flow curves did however show a bigger difference. The ordinary zein had n and K values of 1522 and 0.49 respectively while the corresponding values for defatted were 2887 and 0.54 respectively.

Figure 4 shows the extensional viscosity after correction for the shear contribution at three different extension rates for the two doughs. Both show tension thinning behaviour but there is no significant difference between the samples.

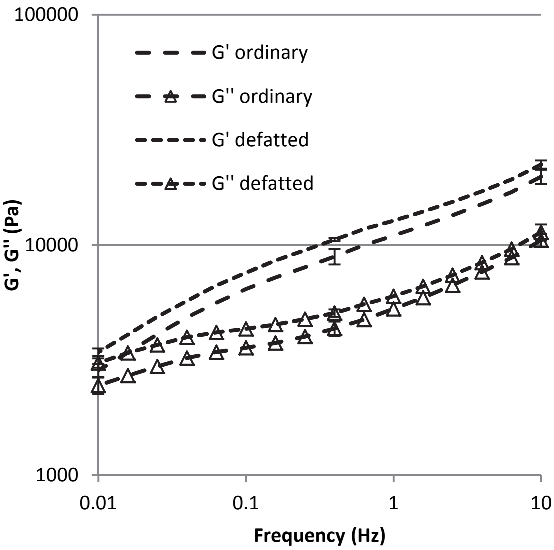
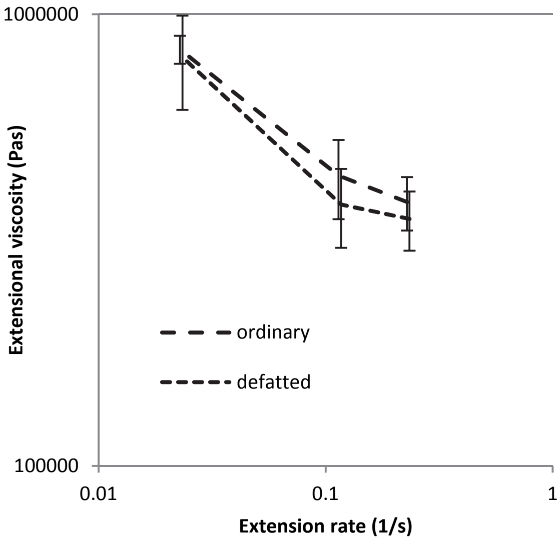


Figure 3. Frequency sweeps for zein-starch doughs with ordinary and defatted zein.



Fiqure 4. Curves showing the extensional viscosity for doughs with ordinary and defatted zein.

Figure 5 shows the microstructure of the two different doughs. Proteins are seen as green (white) and the starch as red (grey). The black areas are water. In both cases the protein forms a well developed network of fine strands. There is however, not any apparent difference between the zein networks in the two samples and it is likely that the differences seen in the rheological properties in this case are not due to differences in the protein network. Instead the slightly lower modulus for the ordinary zein could be a result of a plasticizing effect from the lipids present in the protein.

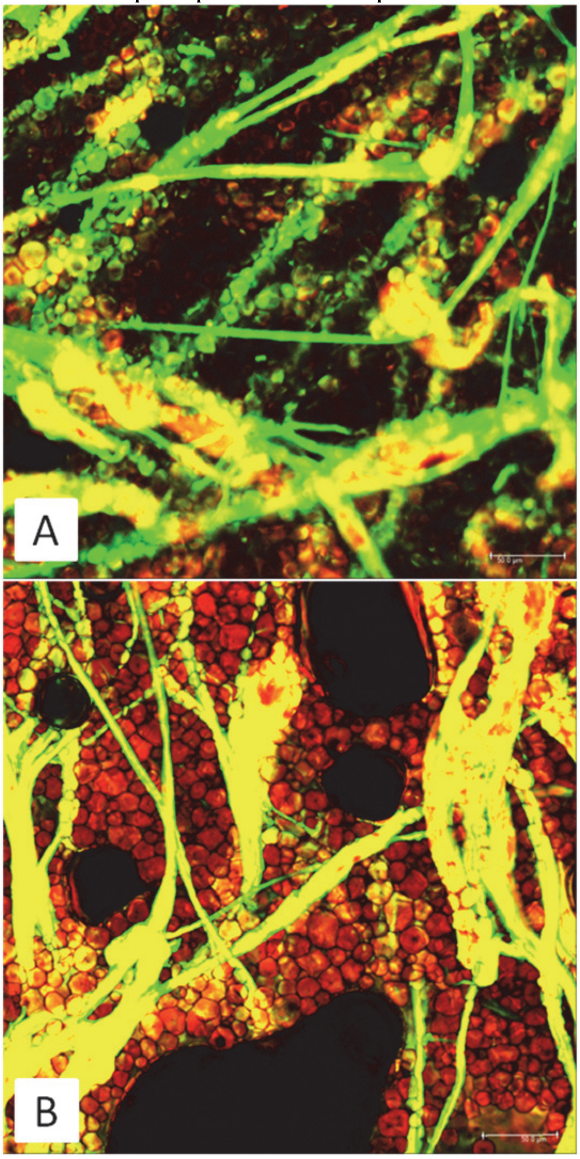


Figure 5. CLSM micrograph of dough with: A, ordinary zein and B, defatted zein. The scale bar is 50 µm.

CONCLUSION

In this study we could see small differences between zein before and after removal of lipids but not the same large influence as was found by Schober et al.[9]. This could be due to the mixing which in the case of Schober et al. was done by hand and it might in that case not have reached optimum dough development. This would indicate that the mixing is a more important factor for the breadmaking performance of zein-starch dough than the presence of lipids in the zein.

REFERENCES

1. Arendt, E.K., Morrissey, A., Moore, M.M. and Dal Bello, F. (2008), “Gluten-free cereal products and beverages”, Elsevier, Oxford, pp. 289-319.

2. Belton, P.S. (1999), “On the elasticity of wheat gluten”, *J. Cereal Sci.*, **29**, 103-107.

3. Dobraszczyk, B.J. and Roberts, C.A. (1994), “Strain Hardening and Dough Gas Cell-wall Failure in Biaxial Extension”, *J. Cereal Sci.*, **20**, 265-274.

4. Wikström, K. and Bohlin, L., (1999), “Extensional Flow Studies of Wheat Flour Dough. I. Experimental Method for Measurements in Contraction Flow Geometry and Application to Flours Varying in Breadmaking Performance”, *J. Cereal Sci.*, **29**, 217-226.

5. Lawton, J.W., (1992), “Viscoelasticity of Zein-Starch Doughs”, *Ceral Chem.*, **69**, 351-355.

6. Oom, A., Pettersson, A., Taylor, J.R.N. and Stading, M., (2008), “Rheological properties of kafirin and zein prolamins”, *J. Cereal Sci.*, **47**, 109-116.

7. Schober, T.J., Bean, S.R., Boyle, D.L. and Park, S. (2008), “Improved viscoelastic zein-starch doughs for leavened gluten-free breads: Their rheology and microstructure”, *J. Cereal Sci.*, **48**, 755-767.

8. Andersson, H., Öhgren, C., Johansson, D., Kniola, M. and Stading, M. (2011), ”Extensional flow, viscoelasticity and

baking performance of gluten-free zein-starch doughs supplemented with hydrocolloids", *Food Hydrocolloids,* **25**, 1587-1595.

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

10. Stading, M. and Bohlin, L., (2001), "Contraction flow measurements of extensional properties", *Transactions of the Nordic Rheology Society,* **8/9,** 147-150.