AGEING OF KAFIRIN DOUGH RELATED TO SPECIFIC PROTEIN FRACTIONS

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INTRODUCTION

The rapid urbanization in southern Africa has changed the way of eating and living which has lead to a reduced consumption of traditional foods based on sorghum and millets and an increased consumption of wheaten bread. Since wheat cannot be economically cultivated in much of southern Africa due to the dry climate it is imported, an import which can be damaging to the local economies (Taylor 2004). It is therefore of interest to replace the wheat with a cereal crop better adapted to the climate, such as sorghum (Taylor et al. 2006). The problem is the unique properties of wheat gluten which is the main structure forming protein in wheat flour, a property which the proteins in sorghum lack. Gluten gives the dough the viscoelastic properties required to produce porous bread with good volume (Belton 1999). However, Oom et al. (2006) showed that it was possible to produce a viscoelastic dough using the purified sorghum prolamin, kafirin. The dough is similar to dough produced from zein, the prolamin of maize, but ages rapidly becoming stiff and brittle. This may be one reason why kafirin, unlike zein, does not form a dough when mixed with starch. The ageing of the kafirin dough is thought to be the result of the high content of cysteine-rich β- and γ-kafirins which form disulphide bonds giving a highly crosslinked system (Oom et al. 2006).

The aim of this study was to determine how reducing the content of certain kafirin fractions, β- and γ-kafirins, would affect the ageing of the dough.

MATERIALS AND METHODS

Kafirin fractionation by differential solubility

The fractionation was based on the differential solubility of prolamin fractions in aqueous ethanol (Parris et al. 2001).

Kafirin was dissolved in 15 ml 95% (v/v) aqueous ethanol per gram of protein and then stirred for 1 h at 75°C. The solution was centrifuged for 10 min and the supernatant poured into Petri dishes and air dried for two days. The dried protein was collected from the dishes and weighed. This is referred to as the 95% fraction. The pellet was dissolved in 60% ethanol and treated in the same way as the previous fractionation. This fraction is referred to as the pellet fraction.

Preparation of kafirin dough

Kafirin dough was prepared according to the method described Oom et al. (2006). A protein dough is sometimes also referred to as a "resin".

Characterization of kafirin fractions

SDS-PAGE was performed for characterization of the kafirin fractions. The samples were run under non-reducing conditions and a NuPage 4-12% Bis-Tris gel, MES running buffer and a Mark 12 protein standard (NP0335BOX, NP0002 and LC5677, Invitrogen, www.invitrogen.com) was used. The gels were stained with a Colloidal Blue Staining Kit (LC6025, Invitrogen). All steps were performed according to the protocols provided by Invitrogen.

Rheological characterization of dough ageing

The ageing was studied using oscillative measurements with a 30 mm diameter plate-plate geometry and a 3 mm gap. A constant frequency, 1 Hz, and stress, 50 Pa, was used and the test was run for three hours at 20°C. The stress was well within the linear viscoelastic region for the dough. The measurements were conducted on a Stresstech Rheometer (Reologica Instruments, Lund, Sweden). To protect the dough from excessive oxidation and drying a thin layer of paraffin oil was used to cover the edges.

RESULTS AND DISCUSSION

SDS-PAGE

Gel electrophoresis was performed to identify which protein fractions were present when making the doughs and to correlate this to the ageing behavior of the dough. Figure 1 shows the gel from an SDS-PAGE experiment under non-reducing conditions. Lane 1 contains the protein standard, lane 2 the non-fractionated kafirin, lane 3 the pellet fraction and lane 4 contains the 95% fraction.

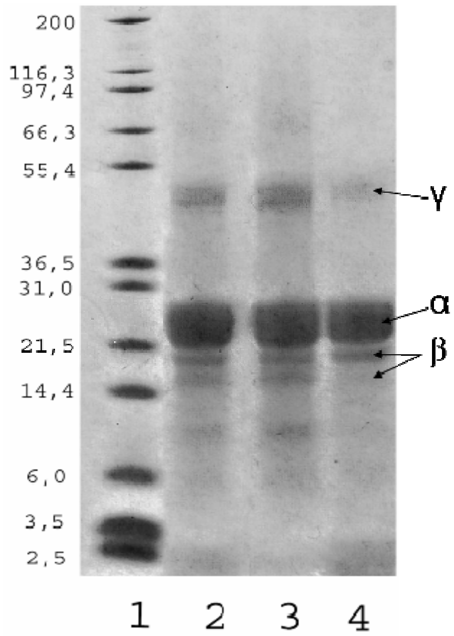


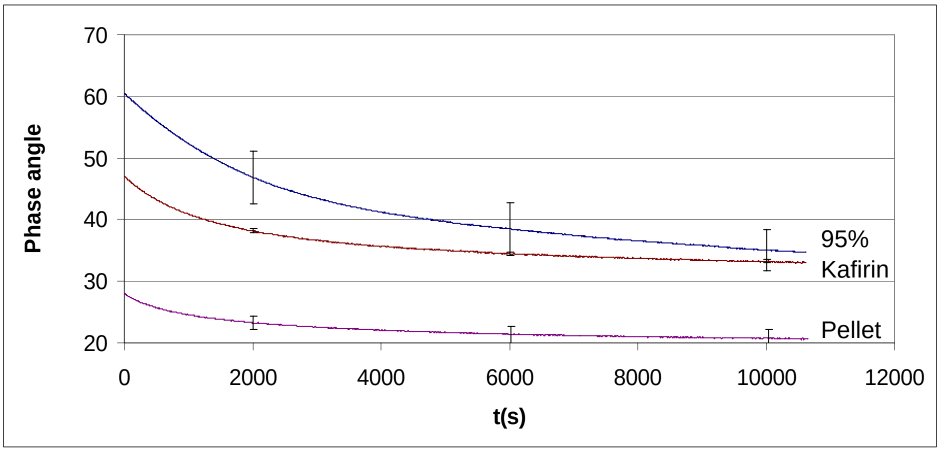
Figure 1. Results from kafirin fractionation analysed with SDS-PAGE

The results show a reduction of the cysteine rich 16-18k β-kafirins as well as the 49k γ-kafirins for the 95% fraction in lane 4 compared to the non-fractioned kafirin in lane 2. However, the relative content of 20k β-kafirin does not seem to have changed. The pellet fraction in lane 3 shows a slight increase in the content of β- and γ-kafirins. This should mean a decreased content of cysteine rich kafirins in the 95% fraction and an increased content in the pellet fraction.

Rheological characterization

The ageing was studied by following the change of the modulus G' (stiffness) and phase angle over time. The phase angle shows the degree of viscous character with 0º being a Hookean elastic solid and 90º being a Newtonian fluid. The ageing can be seen as an increase of the modulus, meaning a stiffer material, and a decrease of the phase angle, meaning a more elastic-like material. Figure 2 show the results from the time sweeps for the non-fractionated kafirin, the 95% fraction and the pellet fraction. The 95% fraction had a higher phase angle and a lower storage modulus over the time measured compared to the non-fractionated kafirin but it still aged fairly rapidly and in the end had a phase angle only slightly higher than for the non-fractioned kafirin dough. However, the storage modulus increased more linearly and was considerably lower, approximately 45 kPa at the end of the test, than all other kafirin doughs. This indicates a less crosslinked system.

The dough also felt softer after 3 hours and seemed to become even softer when kneaded. This is different from the non-fractionated kafirin dough which became crumbly. That the dough still aged was probably due to the high content of 20k β-kafirin.



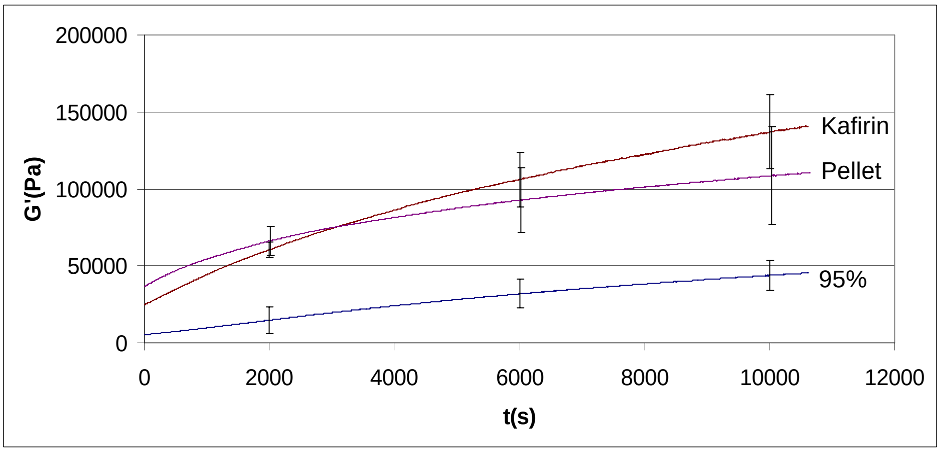


Figure 2. Phase angle and storage modulus over time for the kafirin fractions

The pellet fraction formed a dough which immediately after precipitation by water resembled the dough made with non-fractioned kafirin but then quickly aged and lost its extensibility. This was noticeable before the sample was mounted in the Stresstech rheometer. This can be seen on the low initial phase angle in the time sweep. The final phase angle was also considerably lower then the others, close to 20º. Considering the relatively increased amount of β- and γ-kafirins the formation of disulphide bonds and a highly crosslinked system is likely the reason for the rapid ageing.

CONCLUSIONS

As seen by the results the dough made of the 95%-fraction with reduced amounts of β- and γ-kafirins became softer and aged slightly slower compared to the non-frationated kafirin while the dough made from the pellet fraction with a high β- and γ-kafirin content aged more rapidly. This supports the theory that the ageing is caused by the presence of the cysteine rich β- and γ-kafirins which form disulphide bonds giving a highly crosslinked system.

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