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Formation and properties of viscoelastic masses made from kafirin by a process of simple coacervation from solution in glacial acetic acid using water

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ABSTRACT

Stable viscoelastic masses have been formed from kafirin in a mainly aqueous system. Kafirin was dissolved in glacial acetic acid (GAA) and simple coacervation was performed by rapid addition of 15 °C water under low shear. Kafirin precipitated out as a network of hydrated fibrils which could be hand-kneaded into a viscoelastic mass. These could be formed from a very wide range of kafirins, including those where β- or γ-subclass expression was suppressed. Kafirin composition influenced the appearance of the masses but did not fundamentally affect stress-relaxation behaviour. Fresh kafirin masses exhibited similar elasticity and viscous flow balance to gluten. They maintained functionality when stored for several days at 10 °C but their elastic component increased. FTIR showed that when kafirin was dissolved in GAA its α-helical conformation increased substantially. Dissociation of the kafirin molecules in GAA, assuming a α-helical conformation may have enhanced water binding, enabling viscoelastic mass formation.

1. Introduction

Climate change is one of the biggest challenges the world is currently encountering with the greatest impacts being seen in sub-Saharan Africa (FAO., 2014) where agriculture is largely directly dependent on rainfall. Sorghum is one of the most drought-tolerant cereal crops and is highly suited for cultivation in the semi-arid regions (Hadebe, Modi, & Mabhaudhi, 2016). It would be advantageous if sorghum flour could be used as the sole functional component to make wheat flour-like bread and other dough-based products, but as yet this has not been possible (Taylor, Taylor, Campanella, & Hamaker, 2016).

The primary reason is that unlike wheat gluten, kafirin, the sorghum prolamin protein, does not by itself form a viscoelastic mass when manipulated in water, a fundamental requirement for making leavened wheat-like bread. Several reasons have been proposed for kafirin's lack of functionality in doughs. Kafirin is more hydrophobic than gluten and kafirins can be cross-linked together by disulphide bonding involving the cysteine-rich β- and γ-kafirin subclasses, resulting in the entrapment of the kafirins in their protein bodies (Taylor et al., 2016). As a consequence they are not functional in bread making. Additionally, research suggests that a β-sheet conformation, as exists in glutenin, is critical for prolamins such as zein (the maize homologue of kafirin) to exhibit viscoelastic functionality (Erickson, Campanella, & Hamaker, 2012). In their native state zein and kafirin are predominantly in the α-helical conformation (Taylor et al., 2016).

Oom, Pettersson, Taylor, and Stading (2008) showed that by plasticizing isolated kafirin (which comprised α- and γ-kafirin subclasses) with oleic acid in a 2:1 proportion a type of viscoelastic mass was formed. However, this viscoelastic mass was more strictly speaking a "resin" as defined by Lai & Padua, 1997 since the plasticizer was a lipid. This kafirin resin exhibited similar extensional viscosity and strain hardening properties as oleic acid plasticized gluten and zein resins but became stiffer much more rapidly. Schober, Bean, Tilley, Smith, and Ioerger (2011) found that isolated kafirin (which was comprised predominantly of α-kafirin) could aggregate into a cohesive mass in water at elevated temperature (55 °C), but it had very poor functional properties and became hard very rapidly, similar to the findings of Oom et al. (2008).

Goodall, Campanella, Ejeta, and Hamaker (2012) investigated the dough making properties conventionally bred high protein digestibility-high lysine (HDHL) sorghum mutants which have modified kafirin expression. They showed that composites of HDHL sorghum flour with wheat flour had improved viscoelastic dough properties compared to normal sorghum-wheat flour composites

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and similar to 100% wheat flour dough. The authors attributed the improved dough functional properties of these HDHL mutants to their kafirin being "freed" from the protein bodies as a result the γ-kafirin being located in the interior of the protein bodies instead of the periphery in normal sorghum (Oria, Hamaker, Axtell, & Huang, 2000), exposing more α-kafirin subclass which is less hydrophobic. Elhassan, Emmambux, Hays, Peterson, and Taylor (2015) furthermore found that sorghum mutants expressing both waxy (high amylopectin) and HD traits had improved flour and dough properties associated with bread making quality, compared to normal and normal starch-HD lines.

Chemical treatment with organic acids has been found to improve the functionality of zein. Sly, Taylor, and Taylor (2014) showed that commercial zein (predominantly α-zein) viscoelastic masses (referred to by the authors as a dough) prepared with dilute acetic acid and lactic acid had greatly improved properties to the extent that zein plus starch or rice flour could form gas-holding doughs as measured by alveography, with properties approaching that of wheat flour dough. Furthermore, King, Taylor, and Taylor (2016) showed that a viscoelastic mass (referred to by the authors as a dough) could also be formed from "total" zein (comprising (α-, β- γ- and δ-zein) by a process of first dissolving it in glacial acetic acid and then casting it into a film.

Notwithstanding these developments, neither process could form a viscoelastic mass with kafirin (Sly, 2014: King, 2016). Earlier, however, Taylor, Taylor, Belton, and Minnaar (2009a) and Taylor and Taylor (2010) showed that by dissolving kafirin in glacial acetic acid and adding water in a controlled manner, kafirin precipitated out of solution as organized structures, a process of simple coacervation. Different types of structures such as microparticles and scaffolds were formed, depending on the exact conditions of the coacervation process.

Based on ongoing research into the kafirin coacervation process, this study applied the process under low shear conditions and as shown here stable, viscoelastic masses can be formed from kafirin. In view of the observations that flours of HD sorghum mutants have improved dough properties compared to normal sorghum, the study investigated the formation of viscoelastic masses and their properties from kafirins isolated from conventionally bred HDHL (normal starch and waxy types) and transgenic HD sorghums with modified kafirin expression.

2. Materials and methods

2.1. Sorghum grain

For kafirin extraction, white tan-plant (non-tannin) whole sorghum grain of two different origins was used:

1) Three conventionally bred HD lines which also expressed the waxy trait (designated WHD1, WHD2 and WHD3), one HD line with normal starch (designated NHD), two waxy-normal protein digestibility lines (designated WN1 and WN2) and two normal starch-normal protein digestibility lines (designated NN1 and NN2). The lines were developed from crosses between parental lines RTx2907 (waxy) and P850029 (HDHL) by Texas A&M University and grown at Halfway, Texas in a controlled field trial. The HDHL line has been identified has having decreased γ-kafirin content, significant down-regulation of 25 kDa α-kafirin, up-regulation of 22 kDa α-kafirin and down-regulation of some β-kafirin subclasses (Benmoussa, Chandrashekar, Ejeta, & Hamaker, 2015).
2) Two transgenic high protein digestibility-high lysine (TG-HD) lines (designated TG-HD1 and TG-HD2) and their normal protein digestibility null controls (designated NC1 and NC2). The lines were produced through the Africa Biofortified Sorghum consortium by DuPont Pioneer in an approved controlled field trial at Johnston, Iowa. The TG-HD sorghum lines have suppressed expression of several kafirin subclasses by means of RNAi (RNA interference) technology. TG-HD1 and TG-HD2 were 75% pure with respect to the ABS032 gene construct which suppresses synthesis of α-kafirin A1 and α-kafirin B1and B2 (the 19 and 22 kDa α-kafirin subclasses, respectively), γ-kafirin 1 and 2, and δ-kafirin 2 (Da Silva, Jung, et al., 2011).

2.2. Kafirin extraction

Total kafirin was extracted from the sorghum grain essentially as described by Taylor, Taylor, Dutton, and De Kock (2005). Clean, whole grain from each sorghum line (220 g) was milled using an air-cooled laboratory hammer mill fitted with a 500 μm opening screen. The milled grain was extracted with 70% (w/w) ethanol plus 0.35% (w/w) glacial acetic acid and 0.5% (w/w) sodium metabisulphite at 70 °C with vigorous stirring for 1 h. The supernatant was collected after centrifugation at 1000g at 25 °C for 5 min. The alcohol was allowed to evaporate from the solute and the precipitated protein washed with cold distilled water (<10 °C). The recovered protein was separated by filtration and air dried at 25 °C.

2.3. Kafirin viscoelastic mass preparation

The method was based on the kafirin microparticle preparation technique of Taylor et al. (2009a) and Taylor and Taylor (2010). Glacial acetic acid (5 ml) was pipetted into a 50 ml beaker containing a magnetic stirrer bar. One gram dry total kafirin preparation was placed in the beaker with constant stirring. The mixture was heated slowly with continuous stirring to 50 °C (within 5 min). The beaker was covered during heating. When the kafirin had dissolved in the glacial acetic acid the stirring was stopped and the beaker was removed from stirrer hotplate. Distilled water (20 ml) at 15 °C was added rapidly (5 ± 1 s) to the kafirin solution without stirring using a 20 ml plastic syringe (without needle). This temperature and volume of cool water was selected as it resulted in a final temperature of the acetic acid in the beaker of 25.0 ± 0.5 °C, very close to the ambient temperature (approx. 24 °C), but substantially below the glass transition temperature (T_g) of water hydrated kafirin of around 40 °C (Schober et al., 2011). The magnetic stirrer bar was then removed. On the rapid addition of the cool water, a soft net of aggregated kafirin formed (Fig. 1S), which was collected with a spatula by gentle stirring. This was kneaded for 20 ± 2 s into a cohesive mass by hand (wearing rubber gloves) with squeezing out of the free liquid in the cohesive mass (approx. 2.5 g), resulting in a final kafirin mass of approx. 2.1 g. The brief hand kneading did not raise the temperature of the kafirin viscoelastic mass.

2.4. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was used to determine which kafirin subclasses were present in the different kafirin preparations. SDS-PAGE was performed under reducing conditions using 4–12% Novex NuPAGE® polyacrylamide gradient gels (8 × 8 cm × 1.0 mm thick with 15 wells) (Invitrogen. Carlsbad, CA). Invitrogen Mark12 Unstained Standard was used. Samples were loaded to 10 μg constant protein. Staining was with Coomassie Brilliant Blue R-250. After