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Comparative functional properties of kafirin and zein viscoelastic masses formed by simple coacervation at different acetic acid and protein concentrations

Segun I. Oguntoyinbo[1], John R.N. Taylor, Janet Taylor*

Institute for Food, Nutrition and Well-being and Department of Consumer and Food Sciences, University of Pretoria, Private Bag X 20, Hatfield, 0028, South Africa

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ABSTRACT

Kafirin and zein could be used in making wheat-free leavened dough-based products if their functionality can be modified to more closely resemble gluten. Recently, stable viscoelastic masses were produced from isolated kafirin and total zein by dissolution of the prolamins in glacial acetic acid, followed by simple coacervation with rapid water addition. The methodology was, however, not compatible with food systems, as the final acid concentration was too high (33%). This work revealed that coacervation with reduction in the final acetic acid concentration down to 0.1% still enabled formation of kafirin and zein viscoelastic masses, with functionality retained on storage at 4 °C for an extended period; indicating an irreversible molecular change with dissolution in glacial acetic acid. Kafirin masses were much firmer than zein masses but both were softer than gluten. However, kafirin displayed a similarly elastic high component to gluten, whereas zein exhibited more viscous flow characteristics. This was probably due to the presence of more disulphide bonds in kafirin than zein. A model to explain this behaviour is proposed. Regarding the effect of prolamin concentration in glacial acetic acid, a minimum, between 5 and 10% was necessary for viscoelastic mass formation at low final acetic acid concentration (5%).

1. Introduction

Improvement of the functional properties of non-wheat prolamin proteins such as kafirin and zein has potential to enable them to be used in the making wheat-free leavened dough-based products. Successful application of this approach to bread making would be highly beneficial especially in countries in the semi-arid regions of Africa and Asia where sorghum and maize are widely grown, reducing the need for expensive wheat imports. However, since the first report of the formation of model doughs made from commercial zein and starch, which had wheat flour dough-like viscoelastic behaviour (Lawton, 1992), progress has been slow. Most work has been carried out on commercial zein, which comprises mainly the α-zein subclass, although commercially available it is highly variable between batches (Selling et al., 2005).

Recently, four factors that influence the formation of doughs, (sometimes referred to as viscoelastic masses) from non-wheat prolamin proteins have been identified (Taylor et al., 2018). They are: prolamin composition in terms of prolamin subclasses, secondary structure, glass transition temperature (T_g) and the relative hydrophobicity of the prolamin. With regard to subclass composition, the presence of the γ-subclass and its propensity for disulphide cross-linking is thought to impact negatively on viscoelastic mass formation (Schober et al., 2011; King et al., 2016). However, when kafirin and zein viscoelastic masses were formed by coacervation from glacial acetic acid, it was found that the γ-subclass was necessary to retain softness on storage with kafirin and zein and was important for the retention of elastic recovery of kafirin (Taylor et al., 2018).

Some workers have proposed that the presence of β-sheet formation is necessary for viscoelastic mass formation (Erickson et al., 2012) and that viscoelastic masses can only be formed above the prolamin's hydrated T_g (Lawton, 1992). The inclusion of additional proteins (co-proteins), which can help stabilise a β-sheet formation of zein has been suggested as a way to create a commercial zein dough, which is functionally similar to a wheat dough (Mejia et al., 2007, 2012). In contrast, Taylor et al. (2018) using different acid treatments and a final temperature below the prolamin's hydrated T_g, found that the FTIR spectra of kafirin and zein viscoelastic masses were different from each other and largely independent of subclass composition. All were

* Corresponding author.
E-mail addresses: graceyetty2020@gmail.com (S.I. Oguntoyinbo), john.taylor@up.ac.za (J.R.N. Taylor), janet.taylor@up.ac.za (J. Taylor).
[1] Present address: Moshood Abiola Polytechnic, P. M. B. 2210, Abeokuta, Ogun State, Nigeria.

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predominately α-helical in conformation but the proportion of α-helical to β-sheet varied, dependant on treatment. The amount of α-helical conformation increased with increased acid concentration (Taylor et al., 2018; Elhassan et al., 2018). This was attributed to changes in solvent polarity and was in agreement with the findings of Xiao et al. (2014) working with kafirin. The degree of hydrophobicity of the prolamin affects its ability to hydrate and retain water (Taylor and Belton, 2002). This would affect the prolamin's ability to remain hydrated during viscoelastic mass formation. Furthermore, work by Smith et al. (2014) showed that the addition of salts affected α-zein's surface hydrophobicity, which in turn affected its ability to form viscoelastic masses.

What is also becoming clear is that kafirin and zein, which have been considered to have very similar functional properties (Taylor et al., 2013), are in fact very different in terms of their ability to form viscoelastic masses under certain specific conditions (Taylor et al., 2018). Zein viscoelastic masses demonstrated predominantly viscous flow characteristics, whereas kafirin masses were more elastic.

Up until recently, attempts to form stable viscoelastic masses from kafirin using aqueous systems had not been successful. The masses rapidly lost functionality (Schober et al., 2011). This is attributable to kafirin's higher hydrophobicity and greater propensity to polymerise through disulphide crosslinking (El Nour et al., 1998; Duodu et al., 2003; Belton et al., 2006; Emmambux and Taylor, 2009). Steeping of sorghum flour for an extended period ahead of extracting kafirin enabled the formation of a 'resin', which was extensible (Smith, 2012). This was attributed to partial digestion of kafirins by endogenous sorghum enzymes or by bacterial enzymes produced during steeping. An alternative explanation may be that this was due to the production of lactic acid by action of endogenous, lactic acid bacteria and would be analogous to dilute organic acid treatments (Sly et al., 2014; King et al., 2016). Recently, however, stable viscoelastic masses have been successfully produced from both isolated kafirin and total zein (zein containing the full complement of subclasses present in the maize grain) by dissolution of the prolamins (28.6%) in glacial acetic acid, followed by simple coacervation with rapid water addition to a final acid concentration of 33% (Elhassan et al., 2018; Taylor et al., 2018). The authors attributed the formation of stable viscoelastic masses to the glacial acetic acid enabling complete solvation, protonation, and partial unfolding of the prolamins, which were thought to be present in solution mainly as monomers. These changes were thought to enable fibril and viscoelastic mass formation on water addition.

Whilst interesting, the methodology is far from applicable in a food system due to the high final acetic acid concentration of 33% (w/w). Hence, in order to further develop the coacervation-type process, it was necessary to determine whether kafirin and total zein viscoelastic mass formation and mass functionality was affected by the final concentration of acetic acid, and whether mass formation and functionality was dependent on the concentration of protein present. Additionally, the work resulted in greater understanding of the intrinsic differences in viscoelastic mass formation and functionality between kafirin and zein and deeper insight into how the coacervation process enables kafirin and total zein viscoelastic mass formation.

2. Materials and methods

2.1. Materials

Kafirin and zein (i.e. total zein comprising α-, β-, δ- and γ-zein) (Taylor et al., 2018), were extracted with 70% aqueous ethanol (w/w) containing 0.5% sodium metabisulphite (w/w) and 0.35% acetic acid (w/w) according to the method described by Emmambux and Taylor (2003) using decorticated sorghum from a tan-plant, non-tannin white sorghum cultivar, PANNAR PEX 202/206 and milled whole grain white maize, respectively. The prolamins were air dried at ambient temperature (25° C). Protein content was determined (N x 6.25) by a Dumas combustion method, AACCI standard method 46–30 (American Association of Cereal Chemists, 2000).

2.2. Viscoelastic mass formation

Kafirin and zein viscoelastic masses were prepared by the coacervation method described by Elhassan et al. (2018). In brief, the prolamins were dissolved in glacial acetic acid at 50 °C and then coacervated out of solution in the form of fibrils by rapid addition of cold (15 °C) distilled water. The fibrils formed were then kneaded into a viscoelastic mass using the fingers. The final temperature of the masses was approx. 25 °C, which is below the glass transition temperature (T_g) of hydrated kafirin (approx. 40 °C) (Schober et al., 2008: Schober et al., 2011) and similar to that of hydrated commercial zein (T_g at high water content close to room temperature (25 °C)) (Lawton, 1992). The resulting hydrated solid or viscoelastic masses were stored in ziplock-type bags at 4 °C between testing periods.

To determine the effects of final acetic concentration at a constant protein content of 28.6%, on viscoelastic mass formation and properties, different levels of water addition were investigated to obtain final acetic acid concentrations ranging from 20% to 0.1% (w/w).

To determine the effect of varying the protein concentration on viscoelastic mass formation, kafirin or zein were dissolved in glacial acetic acid at protein concentrations of 28.6%, 15, 10 and 5% (w/w), as described above. Water was then added to a final constant acid concentration of 5% (w/w). The 5% final acetic acid was selected because the stress-recovery of freshly prepared kafirin and zein viscoelastic masses remained constant at acetic acid concentrations below 5% and the masses exhibited formation of uniform, broad fibrils, which has been identified as an important functional characteristic of wheat glutenin (Orth et al., 1973). When fibril aggregates were formed, they were manipulated by hand into a cohesive mass, which was then analysed. Vital wheat gluten was also formed into a viscoelastic mass as described (Elhassan et al., 2018) and used for comparison.

2.3. Analyses

2.3.1. Microscopy

The resulting structures formed were examined by steromicroscopy (Nikon SMZ 800, Tokyo, Japan) and using an Ultra High Resolution Field Emission Scanning Electron Microscope (JEOL 6000F FEGSEM, Tokyo, Japan). Additionally, the structures prepared at the different protein contents were examined using a Zeiss 510 META Confocal Laser Scanning Microscope (CLSM) (Jena, Germany) coupled with a Plan-Neofluar 10 × 0.3 objective, at an excitation wavelength of 488 nm, with natural fluorescence (Taylor et al., 2018). Preparation techniques used were as described.

2.3.2. Rheology of viscoelastic masses

Rheological properties of the viscoelastic masses were determined using the method described by Elhassan et al. (2018) on day 0 and then after storage in polyethylene ziplock type bags at 4 °C on day 2, day 8 and day 16. F Max (the maximum compression force), Ft (the force at the time from F Max at which fresh gluten had relaxed to 36.8% of its maximum force (11.6 s) and Stress-recovery (percentage stress-recovery at 11.6 s from F Max) were measured as described by Singh et al. (2006).

3. Results and discussion

3.1. Microscopy of masses formed at different final acetic concentrations

Kafirin could form cohesive viscoelastic masses by coacervation over the full range of final acetic acid concentrations, decreasing from 20% down to 0.1% (w/w) (1 g/litre, 1.7 mM) (Fig. 1). This very low acetic acid concentration can be considered as food compatible as it is