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

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Highlights

• Food compatible kafirin and zein viscoelastic masses can be formed by coacervation.

• The residual acetic acid concentration in the masses can be as low as 0.1%.

• Kafirin and zein viscoelastic masses retain their functionality on storage at 4 °C.

• Kafirin viscoelastic masses have a higher elastic component than those of zein.

• A minimum protein concentration of 5–10% is needed for viscoelastic mass formation.

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%).

Key words : kafirin ; zein ; viscoelastic mass ; rheology

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 crosslinking 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 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 Xio 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, Dronzek and Bushuk, 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 a 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 x 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 seconds) and Stress-recovery (percentage stress-recovery at 11.6 seconds 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/l, 1.7 mM) (Figure 1). This very low acetic acid concentration can be considered as food compatible as it is within the range of acetic acid concentrations found in sourdough breads, 0.7 g/l (1.1 mM) to 7.0 g/l (11.7 mM) (Pontonio et al., 2017).

SEM of kafirin viscoelastic masses formed at acid concentrations decreasing from 20% to 5% showed evidence of extensive intertwined, broad fibrils, indicated by solid arrows (Figure 1). At lower final acid concentrations (1% to 0.1%), intertwined masses of fibrils were present but the individual fibrils appeared somewhat finer and less cohesive, indicated by dotted arrows. However, under the same conditions zein could only form handleable viscoelastic masses at a final acetic acid concentration of 5% (w/w) or less. With zein, progressive further reduction of the final acid concentration from 5% to 0.1%, produced a more dough-like material, which could be stretched. Final acid concentrations above 5% resulted in a sticky, taffy-like mass of zein, to the extent that stress-relaxation values could not be measured. This material did, however, show clear evidence of fibril formation up to a final acetic acid concentration of 10% when examined by stereomicroscopy and SEM (Figure 1). At a final acid concentration of 10%, the zein taffy appeared dough-like by stereomicroscopy but was still too soft to be handled. At 20% final acetic acid concentration, the zein material resembled a shiny, partially solubilised mass when viewed by stereomicroscopy . This same treatment, when viewed by SEM appeared as a soft mass, with no distinct features. In general, when observed by SEM of zein viscoelastic masses were softer and more fluid-like than their equivalent kafirin viscoelastic masses at the same final acetic acid concentrations, which was borne-out by rheological analyses (see below). When made with final acetic acid concentrations of 0.5% and 0.1%, the zein viscoelastic
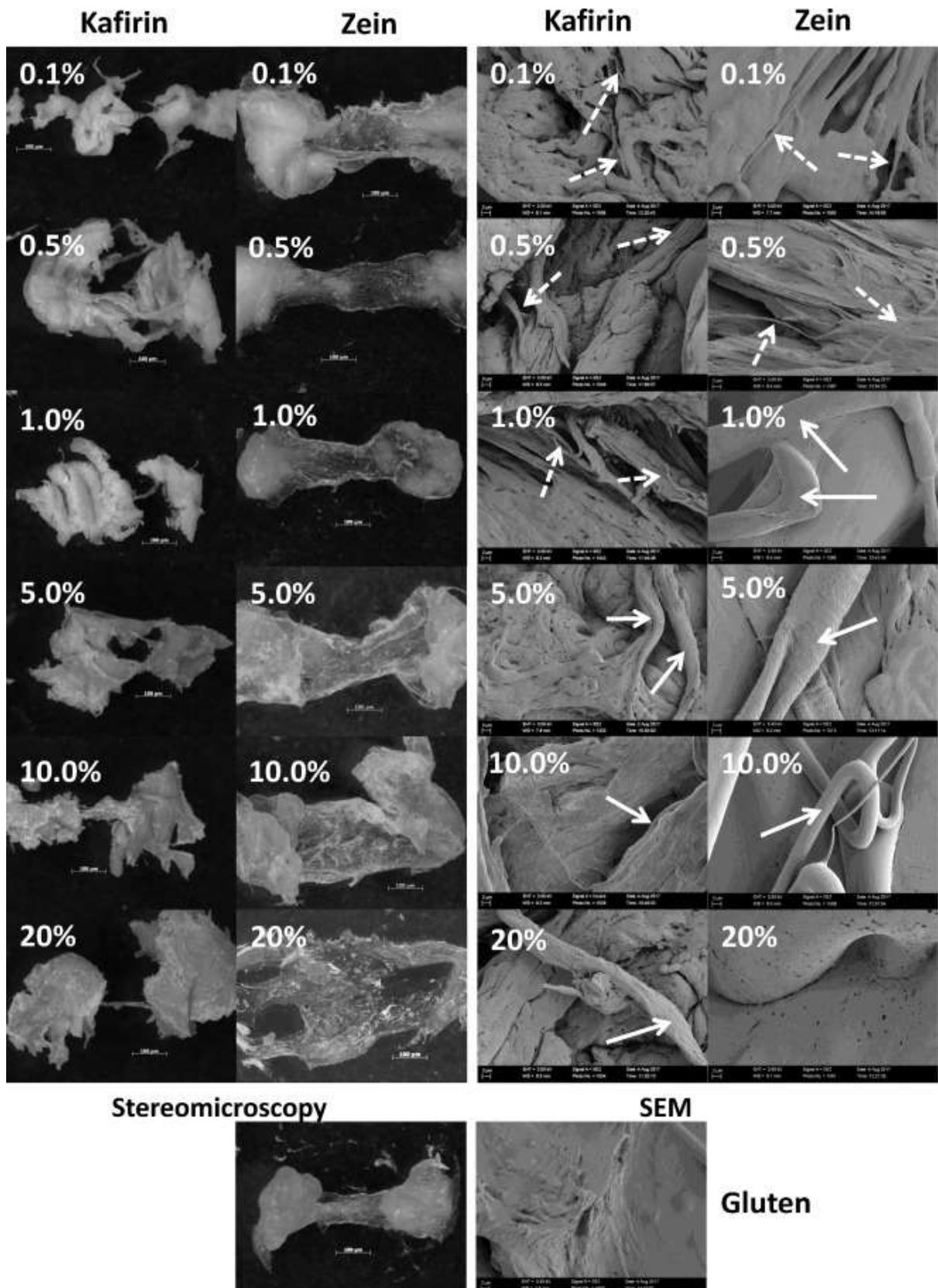
Figure 1: Stereomicroscopy (left, bar=100 μm) and scanning electron microscopy (right, bar = 2 μm) of kafirin and zein viscoelastic masses formed at different final acetic acid concentrations. Solid arrows indicate thick bands of fibrils, dotted arrows indicate finer fibrils

masses resembled the gluten viscoelastic mass, with many interconnected fibrils (Figure 1). In contrast, only at final acetic acid concentrations of 5% and 10% did the kafirin viscoelastic masses somewhat resemble gluten.

3.2. Rheological properties of masses formed at different acetic acid concentrations

Whilst the ability to form fibrils has been identified as a factor critical to dough formation (Lawton, 1992; Schober et al., 2010, 2011), ideally the prolamin fibrils once formed must be able to form a cohesive mass with rheological properties similar to that of gluten in order to be functional in a gluten-free dough system. Our previous work has shown that coacervated zein (total zein) viscoelastic masses demonstrated predominantly viscous flow characteristics (Taylor et al., 2018), whereas coacervated kafirin rheology was more similar to that of gluten, with both viscous flow and elastic recovery properties (Elhassan et al., 2018; Taylor et al., 2018).

All kafirin and zein viscoelastic masses were softer than gluten, even after repeated compression and after 16 days storage at 4°C, regardless of the final acid concentration of the preparation (Table 1). For example, the F Max of kafirin viscoelastic mass (20% final acetic acid concentration) at day 0 was 0.6 N increasing to 4.1 N by day 16 storage, whereas the zein viscoelastic mass was too soft to allow measurement at this final acid concentration. Comparative gluten values were 3.5 N on day 0 and 6.9 N after 16 days storage. On any individual day of testing, as the final acetic acid concentration was reduced, the kafirin viscoelastic masses generally retained the same degree of firmness or became slightly softer but zein viscoelastic masses consistently became firmer with decreasing final acetic acid concentration. Within the same acid concentration, both the kafirin and zein viscoelastic masses became firmer with storage. At the lowest final acetic acid concentration, 0.1% the F Max of kafirin viscoelastic mass was 0.3 N at day 0, increasing to 4.2 N after 16 days storage, whereas for zein the F Max was 0.2 N increasing to 2.4 N after 16 days.

The increase in mass firmness with decreasing acid concentration found in zein is in agreement with previous work. Sly et al. (2014) working with commercial zein found that increasing concentrations of both dilute acetic acid and lactic acid resulted in softer viscoelastic masses. Furthermore, it has been shown that viscoelastic masses made from total zein films cast from glacial acetic acid, that had residual acid washed away, were firmer and less extensible than viscoelastic masses made from similar films where residual acetic acid remained (King et al., 2016). Thus, in this present work it appears that at least for zein, as the final acetic acid concentration decreased its plasticising effect

Table 1: Effect of different final acid acetic concentrations on the stress relaxation of kafirin and zein viscoelastic masses prepared by coacervation at day 0 and after storage at 4°C for 2, 8 and 16 days. Protein concentration during preparation 28.6%

Final acetic acid conc (%)	Final pH	F Max (N)				Ft (N)				% Stress Recovery			
		Day 0	Day 2	Day 8	Day 16	Day 0	Day 2	Day 8	Day 16	Day 0	Day 2	Day 8	Day 16
Gluten													
Not applicable	Not applicable	3.538 ± 0.0373	3.747 ± 0.105	5.510 ± 0.518	6.874 ± 0.108	1.695 ± 0.200	2.110 ± 0.022	1.659 ± 0.040	2.976 ± 0.125	47.9 ± 3.0Db	42.2 ± 4.7Bb	30.2 ± 2.1Ba	43.3 ± 2.5Bb
Kafirin													
20	2.3	0.608 ± 0.081	2.379 ± 0.144	3.378 ± 0.086	4.144 ± 0.134	0.350 ± 0.028	1.701 ± 0.054	2.283 ± 0.054	2.751 ± 0.023	57.8Ea± 3.0	71.6Gc ± 0.1	67.6Hb ± 0.1	66.4Gb ± 1.6
15	2.4	0.656 ± 0.005	2.355 ± 0.076	3.256 ± 0.063	4.177 ± 0.020	0.305 ± 0.015	1.453 ± 0.030	1.938 ± 0.066	2.473 ± 0.140	46.5Da ± 2.6	61.8Fb ± 3.3	59.5FGb ± 3.2	59.2EFb ± 3.6
10	2.5	0.378 ± 0.051	1.640 ± 0.045	2.420 ± 0.004	2.830 ± 0.026	0.241 ± 0.036	1.015 ± 0.030	1.535 ± 0.051	1.810 ± 0.080	63.6Fa ± 1.0	61.9Fa ± 0.1	63.4GHa ± 2.2	64.0FGa ± 2.2
5	2.7	0.412 ± 0.021	2.053 ± 0.021	2.608 ± 0.076	4.896 ± 0.312	0.131 ± 0.009	1.076 ± 0.151	1.405 ± 0.208	2.465 ± 0.153	31.7BCa ± 0.6	52.4DEb ± 6.8	53.8DEFb ± 6.4	50.4CDb ± 0.1
3	2.8	0.231 ± 0.001	1.333 ± 0.049	2.172 ± 0.098	3.702 ± 0.088	0.072 ± 0.004	0.744 ± 0.016	1.298 ± 0.125	1.966 ± 0.321	31.2BCa ± 1.7	55.8EFb ± 0.9	59.9FGb ± 8.5	53.0DEb ± 7.4
2	2.8	0.385 ± 0.043	1.755 ± 0.026	2.481 ± 0.175	3.649 ± 0.113	0.108 ± 0.028	0.798 ± 0.145	1.185 ± 0.098	1.770 ± 0.255	27.7Ba ± 4.1	45.5BCb ± 8.9	48.0CDb ± 7.3	48.5BCDb ± 0.9
1	3.0	0.345 ± 0.047	1.537 ± 0.080	2.207 ± 0.238	3.426 ± 0.035	0.104 ± 0.013	0.753 ± 0.081	1.110 ± 0.127	1.643 ± 0.003	30.2BCa ± 0.5	48.9CDb ± 2.7	50.3CDEb ± 0.3	48.0BCDb ± 0.6
0.5	3.2	0.335 ± 0.007	1.260 ± 0.013	1.815 ± 0.004	3.086 ± 0.628	0.115 ± 0.028	0.658 ± 0.059	1.025 ± 0.140	1.492 ± 0.074	34.4Ca ± 9.2	52.2DEb ± 5.2	56.5EFGb ± 7.6	49.1BCDb ± 7.6
0.2	3.3	0.360 ± 0.069	2.171 ± 0.076	2.364 ± 0.033	3.523 ± 0.028	0.107 ± 0.006	0.954 ± 0.001	1.135 ± 0.045	1.589 ± 0.011	30.0BCa ± 4.0	44.0BCb ±1.6	45.0Cb ± 1.2	45.1BCb ± 0.1
0.1	3.5	0.302 ± 0.025	2.334 ± 0.122	3.233 ± 0.129	4.207 ± 0.190	0.087 ± 0.021	1.026 ± 0.049	1.733 ± 0.035	2.058 ± 0.263	28.5Ba ± 4.4	44.0BCb ± 0.2	53.6DEFc ± 1.1	48.8BCDbc ± 4.1
Zein													
5	2.5	0.088 ± 0.007	0.183 ± 0.035	0.370 ± 0.016	0.687 ± 0.069	0.004 ± 0.001	0.039 ± 0.001	0.090 ± 0.029	0.195 ± 0.046	4.5Aa ± 1.2	20.3Ab ± 8.1	24.3Ab ± 8.9	28.8Ab ± 9.6

3	2.6	0.159 ± 0.043	0.155 ± 0.001	0.432 ± 0.001	0.589 ± 0.043	0.007 ± 0.006	0.025 ± 0.002	0.098 ± 0.020	0.142 ± 0.020	3.7Aa ± 3.0	15.9Ab± 1.4	22.7Abc ± 4.6	24.3Ac ± 5.1
2	2.8	0.131 ± 0.003	0.172 ± 0.020	0.476 ± 0.040	0.724 ± 0.019	0.012 ± 0.001	0.035 ± 0.008	0.125 ± 0.003	0.195 ± 0.016	8.8Aa ± 0.4	20.2Ab ± 2.6	26.4ABc ± 1.6	26.9Ac ± 1.5
1	2.9	0.217 ± 0.087	0.221 ± 0.004	0.546 ± 0.051	1.143 ± 0.178	0.018 ± 0.004	0.048 ± 0.009	0.146 ± 0.023	0.307 ± 0.045	8.4Aa ± 1.8	21.5Ab ± 3.8	26.7ABc ± 1.7	26.9Ac ± 0.2
0.5	3.3	0.154 ± 0.045	0.317± 0.050	0.577 ± 0.024	1.166 ± 0.047	0.011 ± 0.005	0.059 ± 0.012	0.153 ± 0.120	0.317 ± 0.042	6.6Aa ± 1.2	18.4Ab ± 0.9	26.5ABc ± 3.2	27.1Ac ± 2.5
0.2	3.3	0.173 ± 0.039	0.356 ± 0.058	0.830 ± 0.129	1.671 ± 0.193	0.015 ± 0.007	0.071 ± 0.013	0.180 ± 0.009	0.401 ± 0.030	8.6Aa ± 1.5	19.8Ab ± 0.6	22.0Ab ± 4.5	24.1Ab ± 1.0
0.1	3.4	0.171 ± 0.019	0.413 ± 0.072	1.106 ± 0.149	2.449 ± 0.343	0.014 ± 0.003	0.072 ± 0.021	0.286 ± 0.030	0.565 ± 0.063	8.4Aa ± 2.6	17.2Ab ± 2.1	25.9ABc ± 0.8	23.1Ac ± 0.7

F Max. =Maximum force
Ft = Force at which fresh gluten dough had relaxed to 36.8% of its maximum force 11.6 s after F Max
 % Stress Recovery at 11.6 seconds after F Max

Mean values with different upper case letter in a column differ significantly from each other p<0.05
Mean values within a protein type with different lower case letter in a row differ significantly from each other p<0.05

n = 2

was reduced and the viscoelastic masses became firmer. Similar work has not previously been carried out with kafirin.

We have previously shown that percentage stress-recovery of kafirin viscoelastic masses, at a final acid concentration of 33%, exhibited a very high elastic component, which was retained after 16 days storage (Elhassan et al., 2018; Taylor et al., 2018). In this study, as the final acid concentration decreased from 20% to 0.1%, there was a gradual reduction in the percentage stress-recovery for kafirin, from 57.8% (20% final acid concentration) to 28.5% (0.1% acid) on day 0, and from 66.4% (20% acid) to 48.8% (0.1% acid) on day 16 (Table 1, Figure 1S). This may also be related to the reduction in plasticising effect of acetic acid with its corresponding reduction in concentration in the viscoelastic masses. However, the role of the differing pH of the viscoelastic masses, which increased from pH 2.3 at 20% acetic acid to pH 3.5 at 0.1% acetic acid (Table 1) cannot be entirely ignored. Zhang et al. (2011) observed that α-zein became slightly firmer when the pH was increased from pH 2.7 to pH 3.3. The kafirin viscoelastic masses formed at lower final acid concentrations had stress-recovery behaviour that was closer to that of gluten. Potentially, manipulation of final acid concentrations could enable kafirin viscoelastic masses with rheological properties similar to gluten to be formed at a food compatible final acid concentration.

For kafirin viscoelastic masses produced at all final acid concentrations, the elastic component increased on storage. This may have been due to the formation of additional disulphide linkages on storage. It has been suggested that hydrophobic interactions are responsible for kafirin and zein gluten-like functionality and that disulphide bonding is in fact detrimental to this process (Schober et al., 2011; Smith et al., 2014). However, more recent work has shown that the γ-subclass, with its propensity to form disulphide linkages, is necessary for retention of viscoelastic mass softness of kafirin and zein and for elastic recovery of kafirin (Taylor et al., 2018).

 Zein behaved differently to kafirin, even at the very low final acid concentrations (5% to 0.1%), where viscoelastic masses could be formed. Zein viscoelastic masses were even softer than kafirin viscoelastic masses as described, 0.09 N (5% acid) -0.17 N (0.1% acid) on Day 0, and remained soft even after 16 days storage (0.69 N (5% acid) - 2.45 N (0.1% acid) (Table 1). Furthermore, similarly to our previous work (Taylor et al., 2018) these zein viscoelastic masses exhibited much greater viscous flow characteristic than the kafirin viscoelastic masses (Figure 1S). However, in contrast to kafirin, reduction in final acid concentration did not affect stress-recovery of the zein viscoelastic masses on Day 0, as these values were not statistically different, ranging from 4.5-8.8%. On storage, stress-

recovery increased to between 23.1-28.8%, again with no statistically significant effect of final acid concentration.

Thus it can be concluded that when kafirin and zein are dissolved in glacial acetic acid, there is an irreversible change occurring at a molecular level. This change, which affects their functionality to the extent, that apart from water, no additional plasticiser is required in for them to exhibit viscoelasticity is probably due to protonation of the prolamins, causing partial unfolding of the prolamin structure, resulting in more solvent accessible areas and allowing hydration on the molecular surface (Li, Xia, Zhang, Wang, and Huang, 2012).

A model which explains the difference in the stress-recovery behaviour of kafirin and zein viscoelastic masses made by coacervation from acetic acid is proposed (Figure 2). It is known that kafirin contains more cysteine than zein (Belton et al., 2006), which enables kafirin to form more disulphide bonds. This is illustrated in Figure 2A, where the kafirin viscoelastic mass has more disulphide bonds than the zein viscoelastic mass and without the application of force. Figure 2B shows what happens when the same magnitude of force is used to compress both the zein and kafirin viscoelastic masses. It is suggested that the compression force will be sufficient to break the weak hydrogen bonds but the strong covalent disulphide bonds will remain intact. As the zein viscoelastic mass has fewer disulphide bonds than the kafirin, it will deform more than the kafirin viscoelastic mass and much of the energy will be dissipated. With kafirin, the higher number of disulphide bonds will offer more resistance to compression, and more energy will be stored. On removal of the force, (Figure 2C) the energy will be released and the higher number of disulphide bonds within the kafirin viscoelastic mass will contribute towards the viscoelastic mass relaxing almost back to its original shape and size, and the hydrogen bonds will reform. With zein due to its lower number of disulphide bonds, when the force is removed the lower amount of stored energy will also be released but will be insufficient to enable the zein viscoelastic mass to its original size and shape before the hydrogen bonds reform.
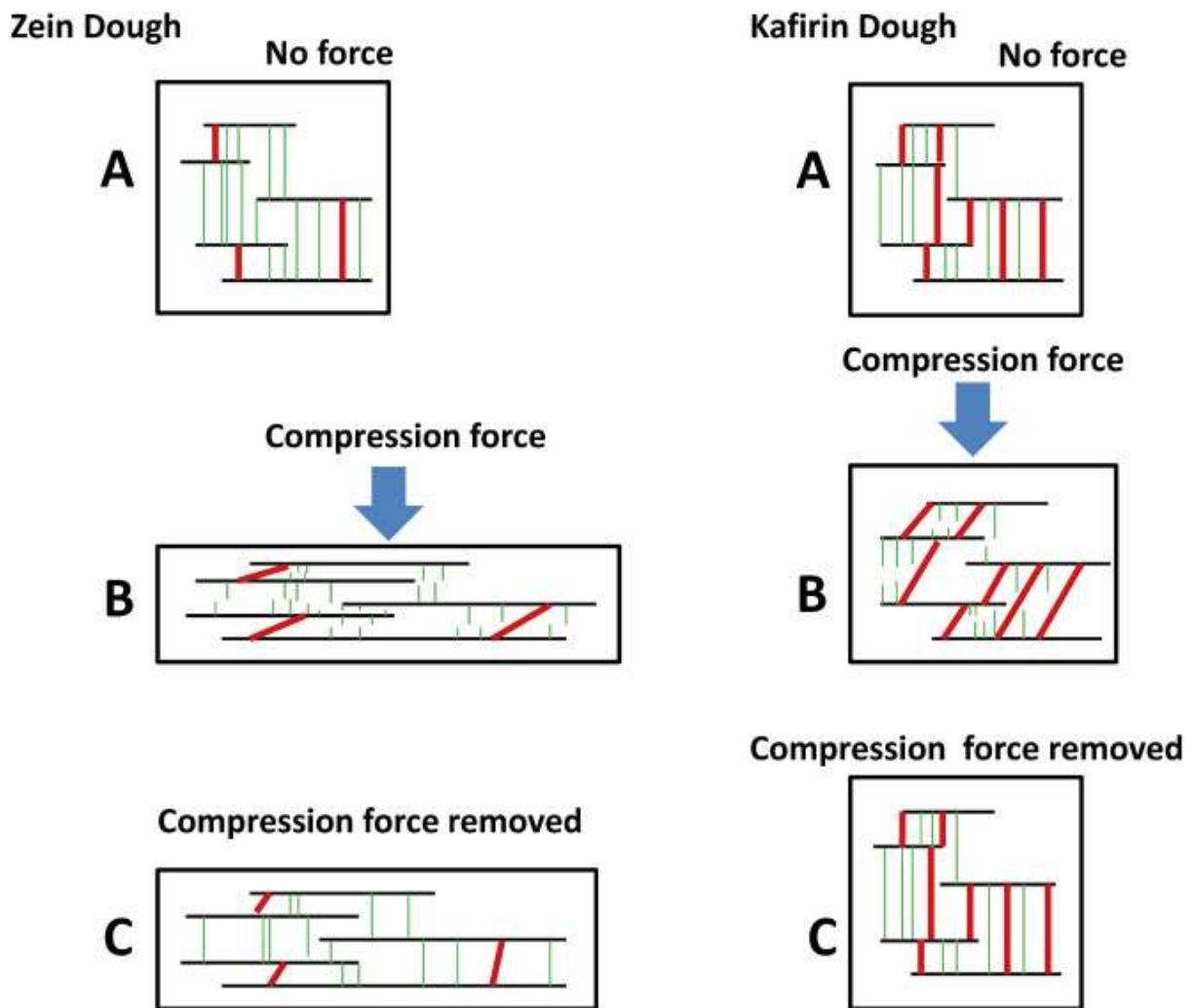
Figure 2: Model to explain the differences in kafirin and zein stress-recovery behaviour when compressed. Disulphide bonds-Thick red lines, Hydrogen bonds-Thin green lines.

A-Viscoelastic masses at rest with no force applied

B-Compression force applied to viscoelastic masses

C-Compression forces removed from viscoelastic masses

3.3. Effect of protein concentration during coacervation

Since viscoelastic masses could be formed at very low final acetic acid concentration from kafirin and zein solutions in glacial acetic acid of high protein concentration (28.6%), the effect of reducing protein concentration on viscoelastic mass formation at low final acid concentration (5%) was investigated. Viscoelastic masses could be formed from both kafirin and zein with decreasing protein concentrations from 28.6% down to 10%. Figure 3 shows CLSM and SEM micrographs of the effects of coacervation of 15, 10 and 5% solutions of kafirin and zein on the characteristics of the materials

Table 2: Effect of protein concentration during preparation of kafirin and zein viscoelastic masses by coacervation on their stress relaxation at day 0 and after storage at 4°C for 2, 8 and 16 days. Coacervation to a final acetic acid concentration of 5%.

Protein Type	F Max (N)				Ft (N)				% Stress Recovery			
	Day 0	Day 2	Day 8	Day 16	Day 0	Day 2	Day 8	Day 16	Day 0	Day 2	Day 8	Day 16
Gluten												
	3.538 ± 0.0373	3.747 ± 0.105	5.510 ± 0.518	6.874 ± 0.108	1.695 ± 0.200	2.110 ± 0.022	1.659 ± 0.040	2.976 ± 0.125	47.9 ± 3.0Eb	42.2 ± 4.7Bb	30.2 ± 2.1Ba	43.3 ± 2.5Bb
Kafirin												
28.6%	0.412 ± 0.021	2.053 ± 0.021	2.608 ± 0.076	4.896 ± 0.312	0.131 ± 0.009	1.076 ± 0.151	1.405 ± 0.208	2.465 ± 0.153	31.7Ca ± 0.6	52.4BCb ± 6.8	53.8Cb ± 6.4	50.4BCb ± 0.1
15% n	0.508 ± 0.127	2.42 ± 0.075	3.810 ± 0.111	5.767 ± 0.093	0.197 ± 0.001	1.317 ± 0.037	1.781 ± 0.058	3.253 ± 0.225	38.9Da ± 0.7	54.4Cc ± 3.2	46.7Cb ± 0.2	56.4Cc ±3.0
10%	0.482 ± 0.071	2.772 ± 0.284	3.079 ± 0.076	5.103 ± 0.427	0.180 ± 0.054	1.178 ± 0.090	1.797 ± 0.081	5.103 ± 0.427	36.8CDa ± 5.9	42.9Ba ± 7.6	46.7Ca ± 0.2	46.8Ba ± 6.5
Zein												
28.6%	0.088 ± 0.007	0.183 ± 0.035	0.370 ± 0.016	0.687 ± 0.069	0.004 ± 0.001	0.039 ± 0.001	0.090 ± 0.029	0.195 ± 0.046	4.5Aa± 1.2	20.3Abb ± 8.1	24.3Ab ± 8.9	28.8Ab ± 9.6
15% n	0.184 ± 0.034	0.547 ± 0.021	1.298 ± 0.037	2.438 ± 0.235	0.030 ± 0.010	0.128 ± 0.018	0.415 ± 0.011	0.723 ± 0.051	16.1Ba ± 2.4	23.5Ab ± 4.2	32.0ABc ± 1.8	29.7Ac ± 0.9
10%	0.235 ± 0.042	0.623 ± 0.045	1.247 ± 0.052	2.865 ± 0.151	0.023 ± 0.016	0.129 ± 0.021	0.377 ± 0.025	0.808 ± 0.221	9.3Aa ± 2.8	20.6Ab ± 4.7	30.2ABc ± 0.7	28.0Abc ± 6.2

F Max. =Maximum force

Ft = Force at which fresh gluten dough had relaxed to 36.8 % of its maximum force 11.6 s after F Max

% Stress Recovery at 11.6 seconds after F Max

[a]Mean values with different upper case letter in a column differ significantly from each other p<0.05

[b]Mean values within a protein type with different lower case letter in a row differ significantly from each other p<0.05

n = 2

produced. Viscoelastic masses were not clearly produced from 5% protein solutions, although with kafirin there was some indication of fibril formation when observed by CSLM (Figure 3A). With coacervation of 10 and 15% protein solutions, the kafirin viscoelastic masses appeared to comprise of more ordered parallel fibrils than the zein masses when viewed by CLSM (Figure 3A). When viewed at much higher magnification using SEM, the kafirin materials comprised a mass of rough surfaced fibrils (Figure 3B). In contrast, the zein viscoelastic masses appeared to comprise a smooth, more uniform "dough-like" material with some broad fibrils, especially when formed from a 15% protein solution. . These differences in appearance were reflected in their stress-relaxation behaviours. Kafirin viscoelastic mass softness on day 0 was similar regardless of the protein concentration used to produce the masses, varying between 0.4 and 0.5 N (Table 2). After 16 days storage, the kafirin viscoelastic masses had become firmer (4.9-5.8 N) and closer in firmness to the gluten standard (6.9 N). In contrast, the zein viscoelastic masses were softer than both gluten and kafirin viscoelastic masses on day 0 (0.09-0.24 N), increasing to 0.69-2.9 N by day 16.
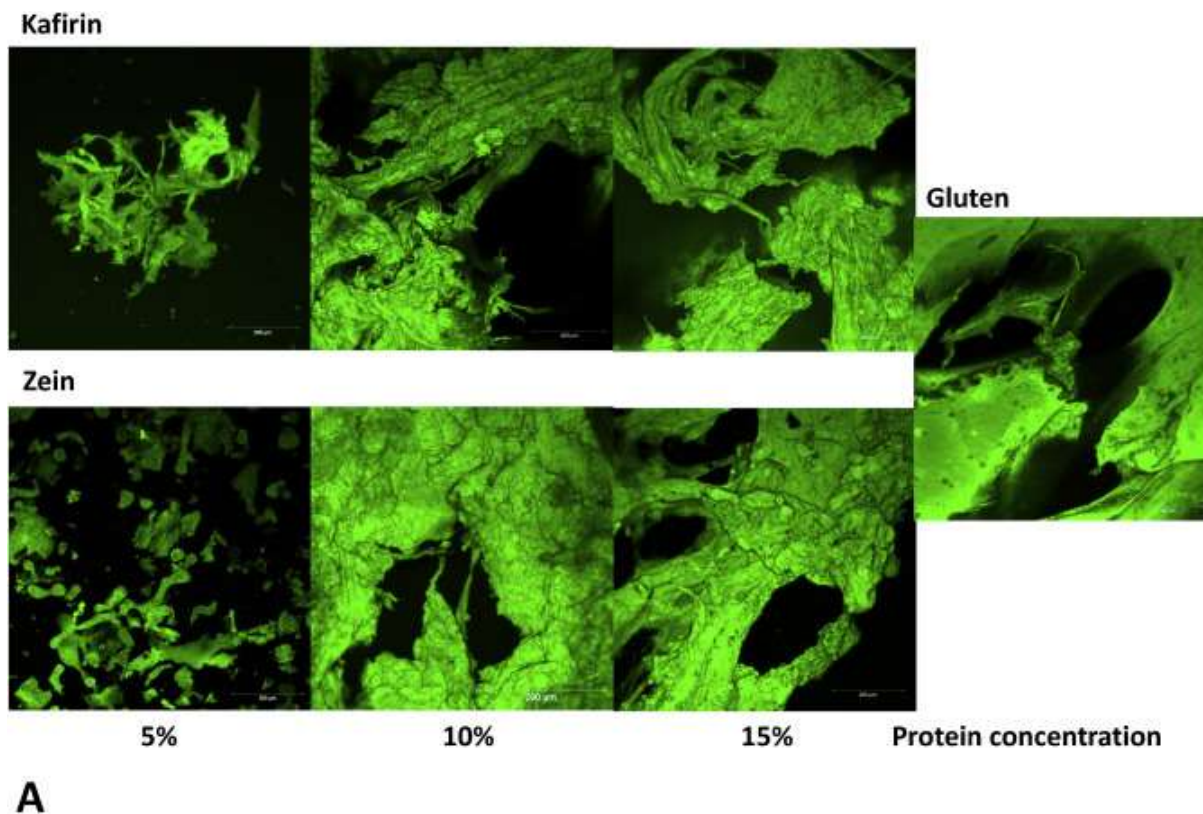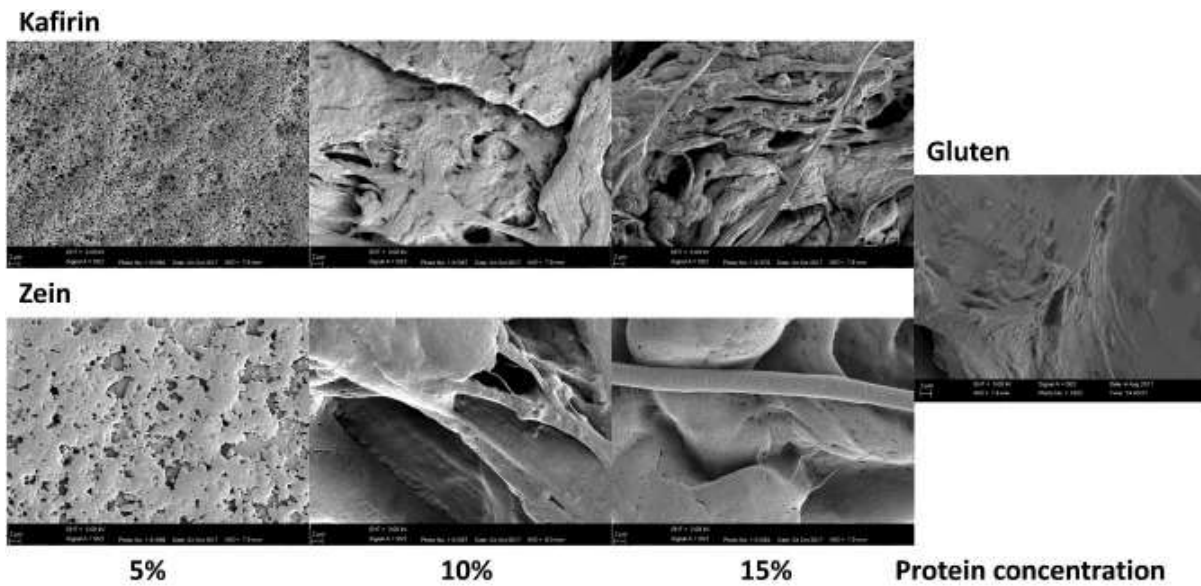

Figure 3A: Confocal laser scanning microscopy of kafirin and zein viscoelastic masses prepared by coacervation at different protein concentrations (bar=200 μm)

Figure 3B: Scanning electron microscopy of kafirin and zein viscoelastic masses prepared by coacervation at different protein concentrations (bar=2 µm)

Protein concentration did not affect the stress-recovery of kafirin viscoelastic masses over the range where viscoelastic masses were formed, 28.6 to 10% protein. The stress-recovery values were lower (31.7-38.9%) than gluten (47.9%) on Day 0 regardless of protein concentration, and becoming slightly higher (46.8-56.4%) than gluten (43.3%) after 16 days storage (Table 2). The stress-recovery of the zein viscoelastic masses was considerably lower (4.5-16.1%) than both kafirin and gluten viscoelastic masses on day 0, increasing to values close to gluten on storage. The higher stress-recovery of kafirin (23.5-34.2%) compared to zein (4.5-13.8%) can be attributed to greater polymerisation of the kafirin protein compared to zein, as described above.

As indicated above, at a 5% protein concentration in glacial acetic acid, the kafirin and zein did not form fibrils that could be kneaded into a viscoelastic mass on coacervation. In fact, a suspension of non-cohesive, aggregated protein was formed with both kafirin and zein. Hence, stress-relaxation analysis could not be performed on these preparations. When these materials were viewed using SEM, a fine sponge-like matrix was observed for kafirin aggregates, whereas zein aggregates looked like a discontinuous mat of a fused matrix (Figure 3B). The kafirin aggregates were similar in appearance to kafirin structures formed by coacervation at relatively high protein content (15.2%) and high final acetic acid content (40%) (Taylor et al., 2009). These kafirin structures were prepared with gentle stirring with a magnetic stirrer and were described as a continuous open matrix

resembling an expanded foam. In contrast, the zein aggregates from this study more closely resembled kafirin aggregates that had been prepared with low protein content (2%) and low acid content (5.4%) but with the application of high shear using an Ultra-Turrax blender (Taylor et al., 2009).

The fact that no fibrils were formed by coacervation with water from a glacial acetic acid solution of low prolamin protein concentration, indicates that there is a minimum concentration of protein that is required to enable intermolecular association (probably by hydrogen bonding) between the molecules to form fibrils. The degree of shear applied during water addition must also be low to allow fibril formation since higher shear appears to result in aggregation of the molecules into particles rather than fibrils.

4. Conclusions

Kafirin and zein viscoelastic masses can be formed by coacervation with water from solutions of the proteins in glacial acetic acid down to very low final acetic acid concentrations, down to 0.1% acetic acid. These low final acetic acid concentrations, since similar to the acid levels of sourdough fermentations would permit yeast and chemical leavening of doughs and it is likely that baked products made from them would be sensorially acceptable. Even at this very low final acetic acid concentration the masses largely retained their functionality when stored at 4 °C for an extended period of time. This implies that when kafirin and zein are dissolved in glacial acetic acid, there is an irreversible change occurring at a molecular level. This change affects their functionality, to the extent that apart from water, no additional plasticiser is required for them to exhibit viscoelasticity. Furthermore, it appears that kafirin and zein are binding water so strongly that they do not become glassy when stored below their glass transition temperatures for several days. Hence, after coacervation from glacial acetic acid kafirin and zein appear to be more hydrophilic in nature and exhibit functionality that is more similar to gluten. However, there are differences in the rheological properties of kafirin and zein viscoelastic masses when formed by this coacervation process. Both kafirin and zein were softer than gluten but kafirin displayed a higher elastic component than zein, which exhibited more viscous flow characteristics. This is probably due to the greater degree of disulphide bonding present in the kafirin viscoelastic masses.

Additionally, this study has shown that there is a minimum concentration of protein, between 5 and 10%, that must be present for fibrils to form from these prolamins, which can be kneaded into viscoelastic masses. This should be taken into consideration when undertaking the next phase of this

work, which is to produce dough systems incorporating these viscoelastic masses and with the inclusion of yeast or a chemical leavening agent could be used to make gluten-free baked products.

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Declaration

The authors declare that they have no conflicts of interest.

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Supplementary material

Figure 1S: Effect of final acetic acid concentration on the stress-recovery behaviour of kafirin and zein viscoelastic mases

Kafirin, Closed square, fine dots-Day 0, Open square, solid line-Day 2, Closed triangle, large dashes-Day 8, Open triangle, small dashes- Day 16.

Zein, Closed circle-Day 0, Open circle- Day 2, Closed diamond- Day 8, Open diamond- Day16

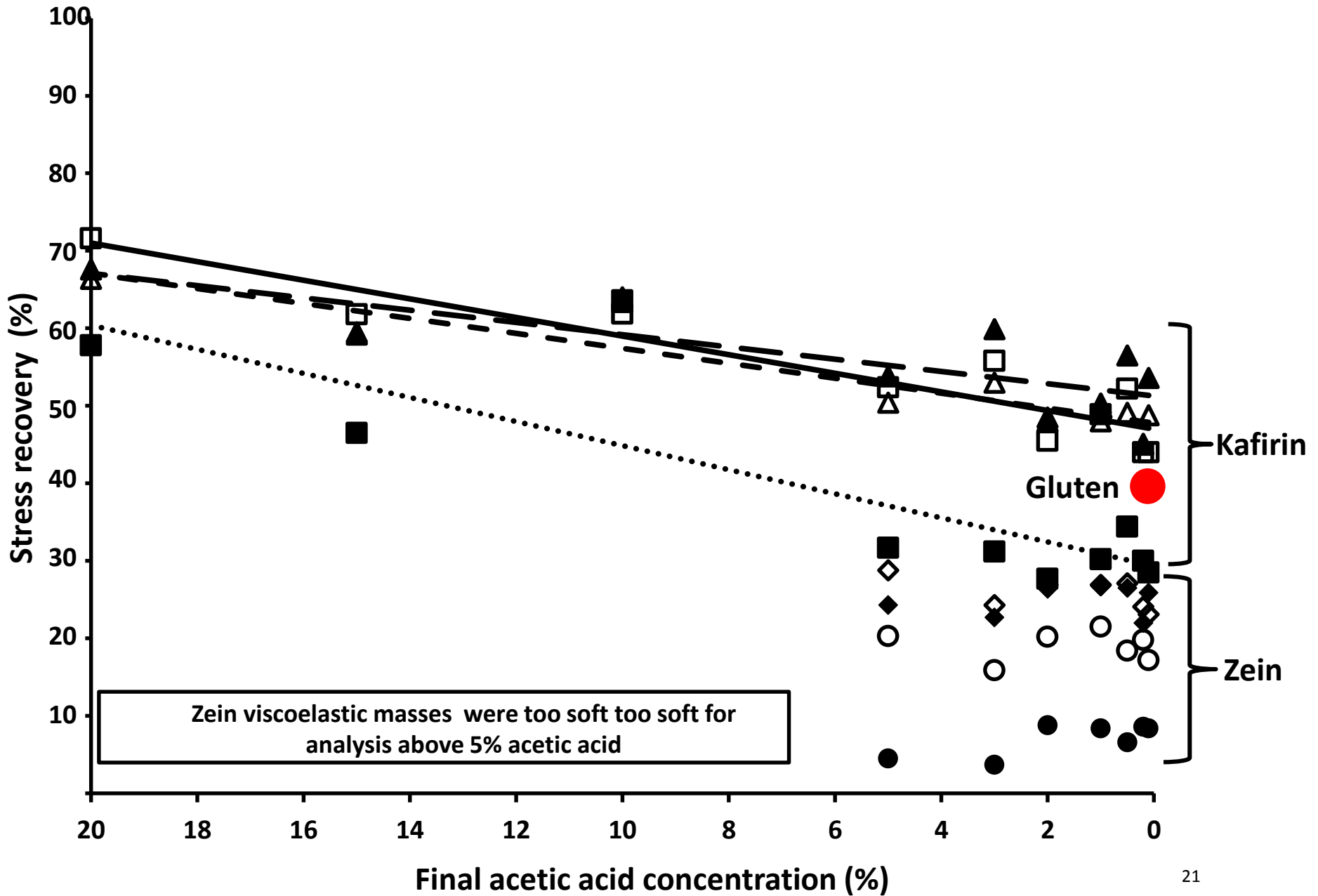**Figure 2S**: Effect of final protein concentration on the stress-recovery behaviour of kafirin and zein viscoelastic masses