School of Public Health

Evaluation of Dough Mixing Properties of Sorghum Genotypes using a Micro-Scale Screening Method and Properties of Maize Prolamin Co-Protein Blends

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DECLARATION

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: Koya Ange Pamela Dovi

Date: 31/10/2019

DEDICATION

To God Almighty, to Him be all the glory for making all this possible.

To my loving husband, Aimable and our first son, Joshua, with love. Your love, prayers, patience and tireless support carried me throughout to the end of this research.

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ABSTRACT

Evaluation of dough mixing properties of sorghum genotypes using a micro-scale screening method and properties of maize prolamin co-protein blends

By

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Massive population growth will require increase production of major cereal crops. However, due to increase climate variability the production of the major cereal crops such as wheat and maize is projected to decline. This projection highlights the need for increased production and consumption of cereal crops that are more climate resilient, such as sorghum. Sorghum is rich in nutrients and widely grown in arid regions of the world, including eastern Australia. Bread is one of the most consumed foodstuffs worldwide. However, it is difficult to incorporate sorghum in leavened bread. This is due to the sorghum prolamin, kafirins, which are hydrophobic and encapsulated in rigid protein bodies. The hydrophobic and tightly-packed nature of kafirin bodies prevent both the water absorption and protein inter-chain reactions needed for hydration and development of a viscoelastic gas-holding dough.

There is some but limited evidence that different sorghum varieties have different dough forming properties. However, there have been no broad screening of sorghum genotypes which may have potential use in bread dough. To date, the dough forming ability of Australian sorghum genotypes for bread manufacture appears absent in the scientific literature. Often in breeding programs there is only few grams of sorghum genotypes, therefore a standard micro-scale method is required. However, currently such methods are not available.

In the first phase of this research, a micro-scale screening method suitable for evaluation of mixing properties of 50:50 ratio sorghum-wheat composite flour was developed. This formulation was used in order to identify sorghum genotypes which has less impact on wheat quality dough. Whole grain white sorghum (Liberty) and whole grain wheat flours were mixed using a micro-doughLAB (Perten Instruments of Australia, Sydney, Australia). Initially, total moisture (water absorption) was adjusted to identify the level that gave both a smooth mixing curve and maximum dough resistance to mixing (target peak torque). At this chosen water absorption level (64.0%) and target peak torque (87 mNm), doughs were mixed at each combination of four levels of speed (63, 95, 120 and 150 rpm) and three levels of temperature (30, 35 and 45ºC) during which their mixing quality in terms of peak torque (mNm), dough stability (minutes) and softening (mNm) were recorded. Both mixing speed and temperature significantly affected these quality attributes. From a comparison of individual combinations, 30ºC and 120 rpm was selected as the standard conditions since it resulted in a composite dough developed to maximum resistance close to the target peak torque. The composite dough mixed under these conditions was more stable and softened to a lesser extent than those mixed using the other speed and temperature combinations, suggesting a desired consistency.

The standard micro-doughLAB mixing method was used under the standard method previously established to evaluate the dough forming ability of 25 sorghum genotypes based on their mixing parameters of peak torque, dough development time, dough stability and degree of softening in the whole grain sorghum-whole grain wheat composite system. The evaluation also aimed to identify genotypes, which reach target peak torque, and have long stability and low degree of softening. There was overall effect of genotype on all mixing parameters. In terms of peak torque two genotypes (NGT16N434-2 and NGT17N208-1) had values that were not significantly different from the target peak torque. NGT16N438 had the longest stability ($P<0.05$) whereas NGT16N434-1 had the shortest. Sample NGT17N216 had the lowest degree of softening, however, its peak torque was in the low range. The two samples with peak torque close to the target, however, had intermediate stability and degree of softening. The method was able to identify differences in mixing quality between the sorghum genotypes. However, no individual genotype demonstrated the desirable combination of the target peak torque, long stability and low degree of softening. This research

provides some new information on dough forming ability of sorghum genotypes when mixed under standard conditions, however, analysis of genotypes with great diverse, for example those with modified kafirin subunit is now required to identify those with mixing qualities most suitable for bread making. The standard micro-doughLAB method may help sorghum pre-breeding and commercial breeding programs to screen and select lines for development of new sorghum varieties with more useful functionality for manufacture of leavened bread.

The prolamin proteins have major influence on dough functionality of grains such as sorghum (kafirin) and maize (zein). Therefore studies investigating how to improve dough-like properties of pure prolamin may provide insight of their applications of these grains in leavened bread. However, kafirin and zein do not form a gluten-like viscoelastic gas-holding dough, but there is evidence that the addition of a small protein to commercial zein (essentially α-zein) can enhance its viscoelastic properties. Therefore, zein was chosen as an example of prolamin for which there is a baseline research for it. A limited amount of research has demonstrated that the use of a small amount of the high protein, leguminous seed Australian sweet lupin in wheat-based dough has the potential to improve the dough functional properties.

Therefore, in the second phase, isolated lupin protein was combined with zein viscoelastic mass prepared either in aqueous ethanol by coacervation or dilute acetic acid by hand kneading plus sheeting. The objective of this study was to determine if lupin protein can act as a co-protein to improve the viscoelastic properties of zein.

Zein prepared with aqueous ethanol and combined with lupin protein formed a sediment when coacervated with cold water. This was probably due lupin protein insolubility in aqueous ethanol.

In contrast, combining the zein and lupin viscoelastic masses separately prepared in dilute acetic acid and water, respectably gave very different viscoelastic mass properties. This zein:lupin mass was cohesive with lots of entangled fibres. However, the rheological properties revealed that this mass was far less extensible than zein alone prepared in acetic acid. In addition, when formed into a dough, zein: lupin-starch composite could not hold air nor be inflated into a bubble by Alveography. The absence of observable new molecular weight bands by SDS-PAGE, indicated that zein and lupin protein did not covalently interact to form a copolymer in any of the

formulations. Notwithstanding this, zein-lupin protein dough had some but limited viscous flow and elastic properties. It is therefore proposed to investigate the use of lupin protein as co-protein with total zein (zein comprising all subunits and hence more cysteine residues than commercial zein), which may covalently bond the cysteine in lupin protein to produce a dough with better and more wheat flour-like functional properties.

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CHAPTER 1

General introduction

1.1 Background

The world continues to suffer massive population growth accompanied by climate change. According to the latest revision of the United Nations World Population Prospects (United Nations, Department of Economic and Social Affairs, Population Divison, 2019), the world population is projected to reach 9.7 billion in 2050. Global mean crop yields of maize and wheat is projected to decrease from 3 to 10% per degree of warming (FAO, 2018). These projections highlight the need for increased production and consumption of cereal crops that are more climate resilient, such as sorghum.

Sorghum is a high temperature drought-tolerant grain crop widely grown in arid regions of the world, including eastern Australia (Henzell & Jordan, 2009; Taylor, 2019). Although widely grown, sorghum remains used almost exclusively as feed for livestock in Australia (Taylor, 2019) with a very limited use as the major ingredient in mainstream commercial food products (Sanitarium Health and Wellbeing, Coorabong, NSW, Australia). Despite being readily available, sorghum is rich in nutrients and phytochemicals (Slavin, 2004), however, it lacks functionality in food due the hydrophobicity of its major storage proteins, the prolamins, which called kafirins. For instance, kafirin proteins do not form a viscoelastic, gas-holding dough associated with wheat gluten (as reviewed by Taylor, Taylor, Campanella, & Hamaker, 2016), and hence the incorporation of sorghum into leavened bread presents a major technological challenge.

To evaluate the dough forming potential of sorghum, some previous studies have incorporated it into a wheat dough system and measured the extent of reduction in dough quality. It has been reported that substitution of 30% refined wheat flour with decorticated sorghum flour from a high protein digestibility genotype gives a dough with higher extensibility and bread with higher loaf volume, than when a normal-digestibility sorghum genotype was used (Goodall, Campanella, Ejeta, & Hamaker, 2012). As such, the high-digestibility sorghum genotype may have potential to produce higher quality dough for leavened bread manufacture compare to the normal-digestible genotype (Goodall et al., 2012). In Australia, there is a pre-breeding program based on

primarily agronomic traits (Henzell & Jordan, 2009) and more recently, sorghum genotypes with increased protein content and digestibility have been developed in Australia (Liu et al., 2019). However, research into the end-use functionality of Australian sorghum genotypes for bread manufacture appears absent in the scientific literature.

Empirical mixing tests have been used for more than 80 years to provide basic assessment of wheat dough strength and mixing requirements to assist screening and selection of new wheat varieties for bread making (Haraszi, Gras, Tömösközi, Salgó, & Békés, 2004). These types of tests have potential to evaluate sorghum mixing properties but have rarely been used for this purpose (Schober, Messerschmidt, Bean, Park, & Arendt, 2005). More recently micro-scale methods that mimics the high energy mixing used in modern commercial bakeries have been introduced, for example, the micro-doughLAB (Perten Instruments of Australia, Macquarie Park, Australia). These micro-scale methods appear suitable for evaluation of dough forming properties of very small samples of different sorghum genotypes from breeding programs.

Although, sorghum flour does not form elastic dough under standard bread making conditions, published research demonstrates that kafirins, in presence of organic solvent/water mixtures at elevated temperature, can form elastic material. However, this structure becomes rapidly stiffened on cooling thus losing its elastic functionality (Oom, Pettersson, Taylor, & Stading, 2008). Far more research have studied the potential of the maize prolamin, zein to form a dough (Lawton, 1992; Schober, Bean, Boyle, & Park, 2008; Schober, Moreau, Bean, & Boyle, 2010). Mejia, Gonzalez, Mauer, Campanella, & Hamaker (2012) and Erickson, Renzetti, Jurgens, Campanella, & Hamaker (2014) reported that the addition of a small amount of casein to zein improved and stabilised its dough properties through interaction between the proteins to give a co-protein structure. Therefore, further research using zein can provide valuable informations on potential of other prolamins, such as kafirin. A protein with potential to form a co-protein structure is that from lupin seed. Lupin is a high protein legume adapted to poor and dry soil and is a vital crop for sustainable cereal production in Western Australia due to its N-fixing ability (Johnson, Clements, Villarino, & Coorey, 2017). It has been demonstrated that the addition of lupin protein to gluten improved the dough functional properties (Paraskevopoulou, Provatidou, Tsotsiou, &

Kiosseoglou, 2010); suggesting the formation of a co-protein structure. To date the potential of lupin protein as a co-protein to improve the dough properties of prolamin proteins, such as zein has not been investigated. Commercial zein (essentially α-zein) will be investigate because it is readily available in purified form than α-kafirin, however, it has physical and chemical similarities with it (Belton, Delgadillo, Halford, & Shewry, 2006).

1.2 Aims

1) To understand the variation in dough mixing properties of sorghum genotypes, which may assist in selection of those genotypes most suitable for bread making
2) To understand if the addition of lupin protein to prolamin protein can improve its dough-like functionality.

1.3 Objectives

1) To develop a standard micro-scale method to evaluate the mixing properties of sorghum-wheat composite flour using a micro-doughLAB,
2) To evaluate dough mixing properties of sorghum genotypes using the developed micro-doughLAB standard method,
3) To determine if lupin protein can act as a co-protein to improve the dough-like properties of commercial zein.

CHAPTER 2

Literature review

2.1 Abstract

This review explores the current understanding of the key measures of dough quality and the main stages of dough processing, which affect them. The rheological properties used to assess the dough quality for production of high quality bread are described. The effect of water absorption, mixing and temperature on wheat dough are discussed and contrasted with that containing sorghum or maize flours. Also reviewed is the evidence of lupin protein as a co-protein to improve the viscoelastic properties of maize prolamin, commercial zein as a potential system for improving dough quality.

2.2 Why bread dough?

Wheat is a unique cereal that is suitable for preparation of a wide diversity of leavened products as desired by consumers. Among wheat leavened products, bread is one of the most consumed foodstuffs worldwide (Rosell, 2011). The gluten-forming proteins in wheat give the dough desirable qualities, in terms of elasticity, extensibility and gas retention for making leavened breads. However, there is increased interest in the use of cereals and legumes that do not form gluten for the formulation of bread dough (Cauvain, 2014; Cauvain, 2015). For instance, sorghum is gaining global interest in both developed and developing countries as a suitable flour for partial substitution of wheat in composite doughs for commercial bread making. A main driver for this interest is due to the increased understanding of the health benefits associated with consumption of sorghum grain (Matos & Rosell, 2014; Stefoska-needham et al., 2015; Taylor, 2017). In addition its drought- and high-temperature tolerant nature makes it suitable in arid regions of Africa and India, where it is difficult to grow wheat cost-effectively. Therefore, the use of locally produced sorghum as staple food in bread dough formulation can improve food and financial security (Duodu & Taylor, 2012).

2.3 Dough quality

Dough is a blend of flour and water formed upon mixing in presence of air (Cauvain, 2012; MacRitchie, 2003). In a widely used baking process, mixing transforms the blend of flour and water into a cohesive mass (Eliasson & Larsson, 1993). In this process, the dough is exposed to stress and stretching while mixing, which leads to development of a network structure. The energy generated during the dough making

process is crucial for development of the network structure responsible for the elastic texture. The response of the dough to the physical stresses is called dough rheological properties, which can be measured and used to assess the quality of the dough for production of quality bread (Abdelrahman & Spies, 1986). Key physical attributes used to measure dough quality are given in Table 2.1. High dough quality is essential for its effective handling during processing and for producing the texture and volume of the final bread as desired by consumers (Armero & Collar, 1997; Mironeasa & Codină, 2013).

472 **Table 2.1** Key physical quality attributes of dough

Attributes	Description	Measurement technique	References
Extensibility	Ability of dough to stretch and at the same time resist from breaking during stretching	Expressed in mm and can be measured using a Kieffer extensibilty rig	(Dunnewind, Sliwinski, Grolle & Van Vliet, 2003)
Viscoelasticity	Dough exhibits both viscous and elastic properties.	Small amplitude oscillatory shear test using a rheometer	(Abdelrahman & Spies, 1986; Belton, 2012)
Viscous	Ability of dough to deform upon application of a force	Represented by the loss modulus G″	
Elasticity	Ability of dough to deform and recover its original dimension when force is removed	Represented by the: Storage modulus G′	
	Phase angle (δ) is another function used to describe viscoelasticity of dough. It gives a relative contribution of the viscous and elastic components to the rheological properties	Expressed as: Tangent (δ)= G″/G′ A small phase angle (δ) indicates a dough with higher elasticity	

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Amongst cereals, only wheat has the ability to form high quality dough with the desirable physical attributes described in Table 2.1. High quality dough is able to withstand both the stress during mixing and handling, and also expand by retaining gas bubbles during proofing, resulting in a light leavened bread desired by consumers (Eliasson & Larsson, 1993; Cauvain, 2012). It is the main storage proteins called gliadins and glutenins, which allow the wheat dough to form a high quality dough (Wieser, 2007). Upon hydration of wheat flour and application of mechanical energy through mixing, the gliadins and glutenins form a protein network within the dough called gluten through breaking and forming of chemical bonds (Belton, 2012; Cauvain, 2012).

Doughs produced from non-wheat grains that do not contain gliadins and glutenins are much less cohesive and very crumbly compared to wheat dough. Such doughs are difficult to handle during processing and have poor gas retention properties, and therefore result in bread lacking the desired quality, such as soft and loaf volume of leavened breads (Deora, Deswal, & Mishra, 2014; Matos & Rosell, 2014; O'Shea, Arendt, & Gallagher, 2014).

A number of reviews have described different approaches used to improve the quality of non-wheat doughs (Houben, Höchstötter, & Becker, 2012; Deora et al., 2014; O'Shea et al., 2014). For example, the use of flours and/or starches (for instance: maize, potato, cassava, rice, bean), hydrocolloids (for instance: hydroxypropyl methylcellulose)/ gums (for instance: xantham, guar gum) and proteins from dairy, eggs and soya (Keetels, Visser, Van Vliett, Jurgens & Walstra, 1996; Rosell, Rojas & Benedito de Barber, 2001; Sanchez, Osella & De la Torre, 2002; Moore, Schober, Dockery, & Arendt 2004) However a number of limitations remain for non-wheat breads. Due to inclusion of these purified starches, hydrocolloids and gums, their protein content and nutritional quality can be low, and their micronutrients levels can be reduced (Matos & Rosell, 2014; Taylor et al., 2016).

Alternative processing technologies (sourdough, enzymes and high pressure) to try and mimic the gluten functionality in non-wheat have shown some promise. Non-wheat breads are often expensive due to the use of these complex formulations and non-standard technologies (Matos & Rosell, 2014; Taylor et al., 2016). Therefore, there is

a need to identify low cost materials and design simple formulations and processing methods for manufacture of breads incorporating gluten-free flours.

2.4 Effect of processing on dough quality

The impact of the main stages of dough making of wheat on dough quality will be discussed and contrasted with that using non-wheat flours, such as sorghum.

2.4.1. Effect of water absorption on wheat dough

Water absorption is one of the most important dough quality parameters that affects the dough formation process and is depended on the functional properties of a flour (Haraszi et al., 2004; Fu, Wang & Dupuis, 2017). Water absorption is defined as the amount of water needed by a flour during mixing to give a dough with optimal handling characteristics (Haraszi et al., 2004). The Brabender Farinograph has been the most used instrument to measure water absorption of wheat flour. The two Z-arms of the Farinograph form the dough by squeezing and kneading the dough between the arms (blades) and the mixer body (AACC, 2000). This kind of dough development gives a linear relationship between the dough maximum resistance during mixing and the amount of water added to the flour. Therefore, the resulting Farinograph absorption reflects the amount of water that is required to obtain a standard dough consistency of 500 BU for a constant weight of flour during mixing (Haraszi et al., 2004; Fu, Wang, & Dupuis, 2017). Water absorption is function of flour components, for example the protein content and characteristics, the starch, and non-starch polysaccharides (dietary fibres) among others and varies between wheat varieties (Greer & Stewart, 1959). Thus, the water absorption is one measure of quality of a particular wheat variety for bread making.

2.4.1.1 Effect of water absorption on sorghum doughs

Elkhalifa & El-Tinay (2002), Yousif, Nhepera & Johnson (2012) and Jafari, Koocheki & Milani (2017) used a Farinograph to determine the water absorption required to reach optimum consistency of 500 BU for 5:95, 10:90 and 40:60 sorghum-wheat composite dough, respectively. Elkhalifa & El-Tinay (2002) reported that the 5:95 sorghum-wheat composite dough produced bread with acceptable volume. This was probably due to the high level of wheat flour, suggesting that the gluten was still able to give the dough acceptable gas holding properties. However, Yousif et al. (2012) and

Jafari et al. (2017a) found that substitution of wheat flour with 40% and 10% sorghum flour decreased the composite dough stability and increased its degree of softening during mixing. These findings were attributed to the lower resistance to mixing of the composite dough compared to the wheat-only due to the disruption of the continuous gluten network. Also as the composite dough, in particular 40:60 sorghum-wheat has far less gluten network than that of wheat-only dough, standardizing the water level to achieve the typical wheat consistency of 500 BU probably led to the observed results. Therefore composite wheat flour containing sorghum may require to be mixed to a different consistency than the 500 BU of wheat for dough development to maximum dough quality.

2.4.2 Mixing wheat dough

During the initial mixing of dough, hydration and homogenization of wheat flour components, in particular the proteins and damaged starch occurs. During the following stage of high energy mixing (kneading); stretching, tearing and shearing of the dough results in breaking and making of the chemical bonds needed to develop the elastic gluten network (Kilborn & Tipples, 1974).

The two fraction of gluten have unique properties, gliadins are monomeric proteins linked with one or no intra-chain disulphide bonds, whereas, glutenins are aggregated proteins linked by many of these cross-links (Wieser, 2007). The N and C termini of glutenins contain cysteine residues that are able to form disulphide bonds (Belton, 1999; Belton, 2012). High molecular weight glutenin (HMWG) subunits play a critical role in wheat dough rheology during mixing. Unhydrated HMWG is in a random coil conformation, however, when wheat flour is fully hydrated, molecular realignment of the HMWG allows formation of hydrogen bonds between it and water, resulting in the hydrated extended secondary structure called β-turns (Belton, 1999).

Application of mechanical energy during mixing applies stress to the dough resulting in straightening of the β-turns. This allows closer associations between the protein chains, leading to many inter-chain hydrogen bonds, giving an ordered secondary structure known as β-sheet (Belton, 1999). In addition, the spatial location of cysteine residues on HMWG changes during mixing permitting disulphide cross-links at C and N terminal ends of the HMWG, which strengthens the gluten network. When the mixing ceases or is complete, an elastic force restores the high energy β-sheet to β-

turns conformation of HMWG (Belton, 2012). In comparison to HMWG, hydrated gliadins have little elasticity because they only form very few inter-chain disulphide cross links. However, gliadins contribute more to the viscosity and extensibility to the dough through hydrogen bonds (Wieser, 2007; Belton, 2012).

2.4.2.1 Effect of mixing on sorghum and maize doughs

In sorghum and maize, their proteins, i.e. kafirin and zein, respectively, do not undergo the same transformation as wheat gluten during dough mixing. Kafirin and zein are both hydrophobic proteins that are highly cross-linked by disulphide bonds and concentrated in protein bodies in the starchy endosperm of the grain (Shewry, 2002; Belton et al., 2006). Such protein bodies are not found in wheat grains (Duodu & Taylor, 2012). The secondary structure of kafirin and zein are different from that of wheat glutenins and gliadins. In sorghum and maize flour, the kafirin and zein are found in α-helices that are tightly folded into a rod-like structures, whereas as in wheat the glutenins and gliadins are found more open structures of the β-turns (Belton et al., 2006). The hydrophobic nature, structure differences and tightly-packed nature of zein and kafirin bodies reduces both the water absorption, protein inter-chain reactions and protein-starch needed for the development of a cohesive, extensible and elastic dough.

Conventional dough mixing used in wheat manufacture might not disrupt the protein bodies of kafirin and zein. In contrast, high mechanical extrusion cooking freed zein from the protein bodies (Batterman-Azcona, Lawton, & Hamaker, 1999); the authors suggested that changes in protein folding and secondary structures could have happened along with fibril formation but no evidence was reported. Similarly, Jafari, Koocheki, & Milani (2017b) found that the shearing forces during extrusion cooking freed kafirin from the protein bodies in sorghum flour and could participate in as a viscoelastic protein in sorghum-wheat composite (Jafari et al., 2017a). Therefore, the use of higher energy mixing in dough incorporating gluten-free flours, such as sorghum-wheat composite may assist in releasing proteins from the protein bodies, which may interact to form gluten-like network structure.

2.4.3 Temperature during dough mixing

Temperature during dough mixing plays an important role in the formation of the gluten network. Wheat gliadin and glutenin are in a solid brittle (disordered) state

termed ‘glassy’ when dry and at room temperature. As water is taken up during dough mixing, the glass transition temperature (Tg) occurs at room temperature. As a result, the adsorption of energy makes the gliadin and glutenin mobile and able to interact with water and each other to form the gluten network (reviewed by Hoseney & Rogers, 1990), which gives the wheat dough desirable physical attributes.

2.4.3.1 Effect of temperature on dough formation in sorghum and maize

A number of researchers have studied the effect of temperature on dough forming ability of the maize protein, zein and found that this ability was closely related to the T_g. Lawton (1992) found that commercial zein (essentially α-zein) formed a viscoelastic wheat-like dough when mixed with maize starch and dibutyl tartate (as plasticizer) at 25°C, but at 35°C or above the zein could form such dough without the need of the dibutyl tartate. Observation under scanning electron microscopy showed that the zein had formed an extensive network of fibres. A viscoelastic dough could not be formed below 25°C and viscoelasticity seen at above 25°C was lost when the dough was cooled below this temperature. It was concluded that viscoelasticity of zein was governed by its T_g and the fibre formation was apparently responsible for the viscoelasticity. Sly, Taylor, & Taylor (2014) found that such dough could expand and hold gas. Similarly, Oom et al. (2008) showed that commercial zein plasticized with oleic acid and hydrated at 22°C could be formed into a dough-like structure, called a resin, which was similar to gluten resin. It was also possible to form a zein-starch dough mixed with water at 35°C. These studies demonstrated that the zein could form viscoelastic dough above the T_g or at low temperatures in presence of plasticizers.

This suggests that the use of high temperature dough mixing of prolamin containing flours, including sorghum is worth investigating.

2.5 Use of sorghum in dough

Sorghum is a drought-tolerant gluten-free grain crop widely grown in arid regions of the world, including the drylands of eastern Australia (Henzell & Jordan, 2009; Taylor, 2019). Although widely grown, sorghum remains used almost exclusively as feed for livestock in Australia (Taylor, 2019) with a very limited use as the main ingredient in mainstream commercial food products; one notable exception being the sorghum-based gluten-free breakfast cereal, Weetbix™ (Sanitarium Health and Wellbeing, Coorabong, NSW, Australia). Therefore, there is a need for research into formulation

and processing of sorghum as a major ingredient into a wider range of staples food, including high quality doughs for manufacture of nutritious and consumer acceptable leavened breads.

In arid regions of Africa, however, sorghum is the main ingredient for many traditional staple foods (ICRISAT, 2011). However, due to rapid urbanization, coupled with massive population growth (UN-Habitat, 2014), there have been changes in the way of living and eating habits in Africa. This has led to reduction in production and consumption of sorghum accompanied with a huge demand for convenient wheat-based products; particularly bread (Taylor, Belton, Beta, & Duodu, 2014) (Taylor et al., 2014; Dlamini & Siwela, 2015). There has also been a consumption shift to maize since its introduction to the continent (Dlamini & Siwela, 2015). To meet this demand, Africa is now importing more than half of its domestic wheat utilisation and 20% of its maize (Weigand, 2011), making the continent more food insecure. Therefore, there is a widespread need for formulation and processing of sorghum into high quality doughs for manufacture of nutritious and consumer acceptable leavened breads.

Sorghum grain does not contain gluten-forming proteins, therefore its incorporation of into high quality breads present technological challenges. Some but very little research has investigated the ability of sorghum kafirin to form dough. Oom et al. (2008) showed that kafirin plasticised by oleic acid and hydrated at 22˚C could be formed into a dough-like, resin, which was similar to that of gluten resin. However, the kafirin resin unlike zein and gluten resins became immediately stiff after removal of the stress applied during mixing. This was attributed to the higher levels of disulphide cross-linking in kafirin. It was not possible to make kafirin-starch-water doughs at 35˚C similar to those reported from zein-starch-water, even when mixed at higher temperature of 55˚C with addition of lactic acid as a second plasticizer. The greater hydrophobicity, high levels of disulphide cross-links and secondary structure of kafirin (α-helical) may contribute to the difficulty in forming a viscoelastic dough (Taylor, Anyango, & Taylor, 2013).

2.5.1 Effect of sorghum genotypes on dough properties

Few studies have investigated the potential of sorghum genotypes for instance developed by conventional breeding or by genetic modification for making dough-based products.

Goodall et al. (2012) investigated whether kafirin from a high-digestibility sorghum variety could form viscoelastic sorghum-wheat composite dough. This dough mixed at 35°C had improved rheological properties, in terms of greater maximum resistance to extension than dough containing normal-digestibility sorghum. It was hypothesised that differences in the kafirin protein body structure in the high-digestibility variety (Oria, Hamaker, Axtell & Huang, 2000) allowed release of kafirin that participated in dough formation at temperature above 35°C. As such, high-digestibility sorghum appears to have potential for improved quality dough manufacture.

Elhassan, Emmambux, Hays, Peterson & Taylor (2015) investigated sorghum genotypes with waxy starch (high amylopectin) and high protein digestibility traits on characteristics related to flour functionality. The authors found that genotypes with high protein digestibility had loosely packed starch granules and floury endosperm, regardless of whether they were waxy or non-waxy. In addition, flours from both waxy and high protein digestibility sorghum genotypes had much higher flour solubility than non-waxy-normal protein digestibility genotypes, which was attributed to their less dense endosperm texture. In the next study, Elhassan, Emmambux & Taylor, (2017) investigated sorghum genotypes with increased protein digestibility resulting from supressed synthesis of γ-kafirin. The authors reported that at 30°C the high digestibility flour solubility was higher than that of the normal digestibility flour and their doughs were twice as strong as their null control, which had normal protein digestibility. The improved flour and dough rheological properties were attributed to less compact endosperm due to suppression of synthesis of the hydrophobic γ- kafirin subclass, which modifies protein body and matrix structure and thus increased the hydration of the protein and protein-starch interactions. These studies show that high digestibility sorghum genotypes developed by conventional breeding and by genetic modification may have potential for improved dough manufacture.

Recently, sorghum genotypes with increased protein content and digestibility have been developed in Australia (Liu et al., 2019). The authors designed a synthetic β-kafirin gene with 10 additional proteolytic sites compared to the native β-kafirin gene. When this was transformed into a sorghum breeding line, they found that the synthetic endosperm protein disrupted the protein body and increased the number of sites for proteolytic enzymes during digestion. At the present time, no study has reported the

forming ability of different sorghum genotypes, such as the above mentioned transgenic sorghum lines (Liu et al., 2019).

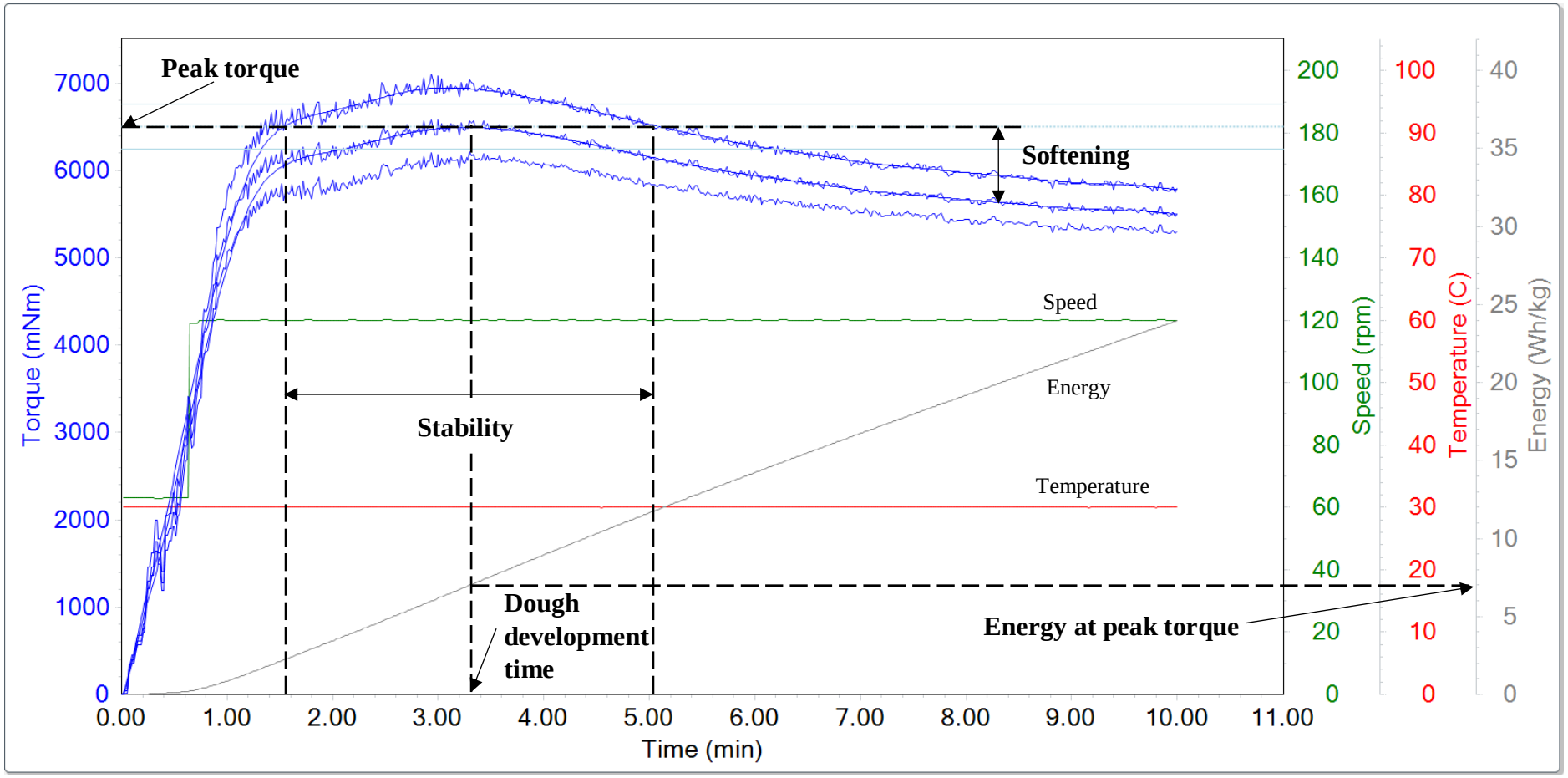
2.6 Methodological approaches to assist dough mixing

The Brabender Farinograph has been used to measure and record the resistance of dough to mixing. The properties of dough are measured by placing a defined weight of flour in a tempered (30°C) mixing bowl equipped with two Z type arms and mixing it with a defined amount of water to the maximum resistance centered on the 500 BU line (AACC, 2000).

There are few studies in the literature that have evaluated the mixing quality of sorghum-wheat flours. Yousif, Nhepera, & Johnson (2012) used a Farinograph equipped with 300 g mixing bowl capacity to determine the water absorption required to reach optimum consistency (500 BU) of sorghum-wheat composite dough. Goodall et al. (2012) in their comparison between high-and normal-digestibility genotypes used a Mixograph equipped with 10, 35 and 100 g mixing bowl capacities to adjust water content of the dough to identify the required water addition for the composite dough reached a maximum resistance. The above mentioned studies did not consider the effect of speed and temperature when mixing the doughs to maximum resistance.

The traditional methods to evaluate the mixing properties of sorghum-wheat composite dough used in the previous studies (e.g. Mixograph and Farinograph) have a key limitation of the need for a large amount of sorghum flour, which is impractical within breeding programs where only few grams of grain may be available for assessment (Tomoskozi & Bekes, 2016). Smaller-scale controlled mixing dough testing equipment such as the 2 g Mixograph (Mixograph, National manufacturing Co., Nebraska, USA) and 4 g micro-doughLAB (Perten Instruments of Australia, Macquarie Park, Australia) are now available. In addition, the Farinograph and Mixograph use gentle mixing actions, which do not mimic high-energy mixers used in modern commercial bakeries. In contrast, the micro-doughLAB does have a mixing action more closely related to high-energy mixing. The micro-doughLAB provides a reliable measure of maximum dough resistance with acceptable precision and better reproducibility than the traditional methods, such as the Farinograph (Dang & Bason, 2013). The micro-doughLAB comprises of a mixing bowl in which z-arm mixer blades

727 rotate to mix flour and water into a dough. An example of a 300 g doughLAB of a
728 wheat flour and measured parameters is shown in Figure 2.1. The summary of the
729 measured parameters and what their relevance in dough manufacture is in Table 2.2.



730

Figure 2.1 Example doughLAB curve of a wheat flour showing commonly measured parameters: peak torque, dough development time (DDT), stability, softening at 5 min. after peak, and accumulated energy at peak torque. Adapted from Dang & Bason (2013).

731 **Table 2.2 Measured parameters from the micro-doughLAB**

Parameters	Units	Definition	Relevance in dough manufacture	References
Optimum water absorption (WA)	%	The volume of water, expressed in ml per 100 g of flour required to achieve target peak torque.	It indicates the amount the water required to add to a flour and develop it to maximum resistance	(AACC, 2000)
Peak torque	mNm	The dough resistance measured and recorded as torque mixed at specific constant speed.	It indicates the maximum dough resistance.	(AACC, 2000)
Dough development time	minutes	The time from zero minute to the point of defined maximum torque.	It indicates the time required to mix the dough up until the point of defined maximum torque.	(AACC, 2000)
Stability	minutes	The length of time the maximum torque remains constant.	Higher stability and low degree of softening indicates the dough's high tolerance to mechanical process	(AACC, 2000)
Softening	mNm	The difference in midline peak torque and at 12 minutes after DDT.	Low degree of softening indicates the dough's tolerance to mixing	(AACC, 2000)

732 **Table 2.1 Continued** Measured parameters from the micro-doughLAB

Parameters	Units	Description	Relevance in dough manufacture	References
Accumulated energy at peak torque	Wh/kg	Accumulated mechanical energy to peak torque	It indicates the amount of energy required to develop a dough to maximum torque (resistance).	(AACC, 2000)

733

2.7 Effect of aqueous ethanol and dilute acetic acid on formation of commercial zein viscoelastic mass

In a study on zein chemical modification, Kim & Xu (2008) reported that when water is added to commercial zein dissolved in aqueous ethanol, phase separation occurs and zein precipitates out as the solvent becomes more hydrophilic. This is a simple coacervation process (Burgess, 1994), which results into a soft fibrous mass of zein that forms a cohesive mass with viscoelastic behaviour when hand kneaded (Oom, Pettersson, Taylor & Stading, 2008; Erickson et al., 2014). Another modification of preparing commercial zein with dilute acetic acid above the T_g of the protein has been reported (Sly et al., 2014; Taylor et al., 2018; Oguntoyinbo, Taylor & Taylor, 2018). Sly et al. (2014) found that commercial zein prepared with dilute acetic acid absorbs just enough of the solvent to hydrate it so that a dough is formed, and the rest of the solvent may be decanted off.

The ability of commercial zein to form viscoelastic mass when prepared in aqueous ethanol or dilute acetic acid above the T_g of the protein is due to its solubility in these solvents (Li et al., 2012). Aqueous ethanol and acetic acid form hydrogen bonds with zein (Li et al., 2012; Smith, Bean, Selling, Sessa & Aramouni, 2014). Nonetheless, the hydrogen of the –COOH group in acetic acid can be released easily unlike that of –OH group in aqueous ethanol, hence protonation of zein in the former solvent is more effective. Li et al. (2012) hypothesised that, zein is more unfolded in acetic acid than in aqueous ethanol due to the protonation of acidic amino acids side chains of the zein, which modified the protein-protein interactions compared to that of ethanol.

2.8 Evidence for lupin as co-protein

To utilize sorghum and maize in dough, it is important to understand how their protein interact to form gluten-like network.

Although commercial zein can readily form viscoelastic mass upon addition of dilute acetic acid, Taylor et al. (2018) found that it was unstable. The authors attributed this instability to the low level of cysteine in α-zein, which prevented formation of large disulphide crosslinks. A study on the addition of a small amount of different protein to commercial zein to stabilize its viscoelastic mass has been reported. Erickson, et al. (2014) found that addition of casein to commercial zein dissolved in aqueous ethanol increased significantly the zein strength and elasticity as compared to zein alone. It

was hypothesised that casein interacted with commercial zein, which contributed to the zein dough strength and elasticity.

A limited amount of research has demonstrated that the use of a small amount of protein from the high protein, leguminous seed Australian sweet lupin in wheat-based dough has the potential to improve its elasticity. For example, Paraskevopoulu et al (2010) found that addition of 5 to 10% lupin protein isolate to wheat dough increased resistance to deformation, stability and extensibility of the dough. The increased dough extensibility and resistance to deformation could have been due to unfolding of the lupin protein during mixing, resulting in intermolecular interactions with the gluten network (Pozani et al., 2002). These qualities suggest good handling properties during processing and good gas retention during proofing and baking.

Researchers have studied and characterised the storage protein of lupin, called globulins. Lupin globulins have four main protein families termed α-, β-, γ- and δ-conglutins, which differ in their molecular features. The α- and β-conglutins are the main proteins present in lupin protein concentrate. The α-conglutins contain disulphide bonds (Blagrove & Gillespie, 1975; Duranti et al., 2008), suggesting that cysteine residues may be available for cross-linking with co-protein.

Lupin as a crop is adapted to poor and dry soil (Johnson, Clements, Villarino & Coorey, 2017). In addition, lupin protein extraction does not involve high temperature and large volume of solvents compared to that of commercial zein (Carter & Reck, 1970; Lawton, 2002; Chew et al., 2003). This means that lupin protein may be cost-effective plant co-proteins than other potential co-proteins.

2.9 Conclusion

The inclusion of sorghum in sorghum-wheat composite flour have potential to form a dough. However, there is need for a micro-scale screening method to evaluate dough forming properties of very small samples of different sorghum genotypes under standard conditions of mixing speed, temperature and water for mixing the sorghum-wheat composite dough to maximum development. The micro-scale screening method may assist sorghum-breeding programs to identify genotypes most suitable for bread making

Research has demonstrated that the use of a small amount of lupin protein in wheat-based dough has the potential to improve its elasticity. However, the potential of lupin protein as a co-protein to improve the viscoelastic properties of prolamin proteins, such as commercial zein has not been investigated. Filling this gap in the research may assist the use of prolamin proteins in leavened products.

PHASE 1

Evaluation of Dough Forming Properties Sorghum Genotypes in a Whole Grain Sorghum-Whole Grain Wheat Composite System

CHAPTER 3

Initial screening of sorghum genotypes by hand mixing for flour and rheological properties related to dough quality

3.1 Abstract

The use of sorghum into leavened dough remains a major technological challenge. The sorghum storage proteins, kafirins are hydrophobic in nature with a tightly-packed three dimensional structure. These properties prevent both the water absorption and protein inter-chain reactions needed for hydration and development of a cohesive, extensible and elastic dough required for leavened bread. A very small number of studies suggest that sorghum varieties may however differ in their dough-forming ability, however these preliminary findings need support from further studies.

Whole grain of four white sorghum genotypes (NGT16N0434, NGT17N208, 134 and Liberty) were supplied by Nuseed (Toowoomba, Queensland, Australia). Doughs were produced by hand from the whole grain sorghum flours composited with a commercial wholemeal wheat flour at a ratio 30:70. It was hypothesised that there will be difference between the quality of the dough made using the different sorghum genotypes, as measured by small deformation rheological properties of G′, G″, phase angle (δ) and |G*| on the composite doughs and compared to those of a wholemeal wheat-only dough.

All sorghum containing doughs had higher G′ and G″ values than wholemeal wheat-only dough, indicating that they were stiffer. However, that containing NGT17N208 had the lowest G′ and G″ values of the sorghum varieties and was thus closer to the value for wholemeal wheat-only. All sorghum containing doughs and wheat-only dough had similar |G*|, possibly due to high content of wheat dominating this parameter. All sorghum composite doughs had a higher phase angle (δ) than the wholemeal wheat-only dough, indicating that they were less elastic. These findings indicate that the composite dough containing the sorghum sample NGT17N208 compared to other sorghums had some rheological properties important in high quality dough, closer to that of wholemeal wheat-only dough.

3.2 Introduction

Sorghum is a drought-tolerant gluten-free grain crop widely grown in arid regions of the world, including the drylands of eastern Australia (Henzell & Jordan, 2009). Although widely grown, sorghum remains used almost exclusively as feed for livestock in Australia (Taylor, 2019) with a very limited use as the main ingredient in mainstream commercial food products; one notable exception being the sorghum-based gluten-free breakfast cereal, Weetbix™ (Sanitarium Health and Wellbeing, Coorabong, NSW, Australia). Therefore, there is a need for research into formulation and processing of sorghum as a major ingredient into a wider range of staples foods, including high quality doughs for manufacture of nutritious and consumer acceptable leavened breads.

However, the incorporation of sorghum into leavened bread remains a major technological challenge. Unlike wheat storage proteins, those of sorghum, called kafirins, are hydrophobic and they are highly cross-linked by disulphide bonds and encapsulated in rigid protein bodies in the starchy endosperm (Shewry, 2002; Duodu et al., 2003; as reviewed by Belton et al., 2006). In addition, the secondary structure of kafirin is predominantly α-helical and tightly folded into a rod-like structures, rather than the more open spirals of the β-turns found in the high molecular weight glutenin subunit in the wheat that is important for gluten development (Belton et al., 2006). In sorghum, the starch granules are surrounded by a hydrophobic protein matrix (Duodu, Taylor, Belton, & Hamaker, 2003), which consist of kafirin protein bodies and glutelins (Taylor, Schüssler, & van der Walt, 1984). The hydrophobic, three dimensional structure and tightly-packed nature of kafirin bodies can reduce the level of water absorption and solubilisation of both the protein itself and the starch it surrounds. As a result, this may prevent both the starch-protein and protein inter-chain interactions needed for hydration and development of a cohesive, extensible and elastic dough required for quality leavened breads (Taylor, Taylor, Campanella, & Hamaker, 2016).

High dough quality is essential for its effective handling during processing and for producing the soft texture and high volume of the final leavened breads as desired by consumers (Armero & Collar, 1997; Mironeasa & Codină, 2013; Deora et al., 2014). The physical attributes of high quality dough can be assessed using rheological properties. Dough rheological properties are expressed in terms of a loss modulus (G″)

and a storage modulus (G′). A G′ value greater than G″ indicates that the storage (equivalent to the elasticity) of the dough dominates the rheological properties. The complex modulus |G*| combines both the G′ and G″ into a single value and indicates the overall strength (stiffness) of the dough. The phase angle (δ) provides a measure of whether the elasticity (G′) or viscous behaviour (G″) dominates the rheological properties. A phase angle (δ) of zero indicates a perfectly elastic material and a phase angle (δ) of ninety degrees suggests a perfectly viscous material (Norton, Spyropoulos & Cox, 2011). These rheological characteristics help us to understand how a dough will respond to mechanical deformation during stretching, mixing and handling (Abdelrahman & Spies, 1986).

There is only a very few studies reported in the scientific literature that have investigated how sorghum varieties may differ in their dough-forming ability. For example, decorticated sorghum flour from a high digestibility variety (Weaver, Hamaker, & Axtell, 1998) with modified kafirin protein body structure (Oria et al., 2000) was compared with normal-digestibility sorghum. It was found that the high digestibility sorghum formed a dough with improved rheological properties, in terms of greater maximum resistance to extension than the normal-digestibility sorghum when evaluated in a sorghum-wheat composite system (Goodall et al., 2012). This effect was attributed to an improved protein network in the high digestibility sorghum-containing dough. Elhassan, Emmambux, Hays, Peterson & Taylor (2015) investigated sorghum genotypes with waxy starch (high amylopectin) and high protein digestibility traits on characteristics related to flour functionality. The authors found that genotypes with high protein digestibility had loosely packed starch granules and floury endosperm, regardless of whether they were waxy or non-waxy. In addition, flours from both waxy and high protein digestibility sorghum genotypes had much higher flour solubility than non-waxy-normal protein digestibility genotypes, which was attributed to their less dense endosperm texture. In the next study, Elhassan, Emmambux & Taylor, (2017) investigated sorghum genotypes with increased protein digestibility resulting from supressed synthesis of γ-kafirin. The authors reported that at 30°C the high digestibility flour solubility was higher than that of the normal digestibility flour and their doughs were twice as strong as their null control, which had normal protein digestibility. The improved flour and dough rheological properties were attributed to less compact endosperm due to suppression of synthesis of the

hydrophobic γ- kafirin subclass, which modifies protein body and matrix structure and thus increased the hydration of the protein and protein-starch interactions. As such, digestibility sorghum genotypes developed by conventional breeding and by genetic modification may have potential for improved dough manufacture. However, there is very little understanding of the variation in dough-forming ability of different sorghum within commercial collections or in the Australian sorghum breeding program mapping population.

In Australia, there is a sorghum development program for identifying new varieties with good agronomic traits and good food grade performance (Australian Government Department of Health, 2017). However, research into their end-use functionality in bread has been very limited. Therefore, in this work we assessed the flour and dough rheological properties of selected white sorghum genotypes in a sorghum-wheat composite system. The aim of this was to identify any variability in the flour properties and dough rheological properties between the sorghum genotypes.

3.3 Materials and Methods

3.3.1 Sorghum samples and flour preparation

Sorghum seed samples comprised of four white tan plant sorghum varieties coded (NGT16N0434, NGT17N208 and 134) and Liberty, a commercial white, non-tannin sorghum. The seeds were produced at Brookstead, West Toowoomba, and supplied by Nuseed, (Toowoomba, Queensland, Australia). They were coarsely milled using a laboratory Cemotec™ 1090 Sample Mill (Foss Analytical, Denmark) to give whole grain coarse flour. The coarse flour was further milled many times in a coffee grinder BCG 200 Breville the Coffee & Spice™ (Breville, New South Wales, Australia) and passed through a 212 µm sieve to give whole grain flour. The extraction rate was 92.9%, 95.6%, 95.7% and 93% for Liberty, NGT17N208, NGT16N0434 and 134, respectively. Other ingredients used were purchased from retail stores in Perth (Western Australia, Australia). These were dry yeast “Tandaco” (Seven Hills, New South Wales, Australia), table salt “Saxa” (Seven Hills, New South Wales, Australia), and a wholemeal wheat plain flour (Anchor Foods, Fremantle, Western Australia, Australia).

3.3.2 Analytical methods

3.3.2.1 Particle size

The particle size distribution of the flours was determined in duplicate by laser light scattering using a Mastersizer 2000 (Malvern Instruments Ltd, Malvern, UK). For each analysis, approximately 2.5 g of sample was dry-dispersed into the apparatus using a Scirocco 2000 dry powder dispersion unit (Malvern Instruments Ltd). Data were calculated by the instrument software as d (0.1), d (0.5) and d (0.9) which represents the maximum diameter of 10%, 50% and 90% of the particles, respectively.

3.3.2.2 Moisture

The moisture content of flours was determined in triplicate by oven drying to a constant weight according to the American Association of Cereal Chemists (AACC, 2000) Method 44-15A (One stage). Data were expressed as g/100 as is basis.

3.3.2.3 Protein content

Protein content (N × 6.25) of the flours was determined by Kjedhal digestion distillation method according to AOAC (2000). Data were expressed as g/100 g on dry basis (db).

3.3.2.4 Starch

Total starch content of the flours was determined according to the Amyloglucosidase/Amylase Method, K-TSTA 50A 02/17 from Megazyme International (Bray, Ireland). In brief, samples were first treated with potassium hydroxide to dissociate any resistant starch, then heated in presence of thermostable α-amylase, which hydrolysed the starch into maltodextrins followed by amyloglucosidase, which quantitatively hydrolysed maltodextrins to D-glucose. The D-glucose produced was measured using glucose oxidase/peroxidase reagent. Data were expressed as g/100 g on dry basis (db).

3.3.2.5 Water absorption index and water solubility index of flour

Water absorption index (WAI) and water solubility index (WSI) of the flours were measured at 30°C as described by Anderson et al (1970). For each sample 2.5 g was suspended in 30 ml water at 30°C stirred intermittently over 30 minutes, and then

centrifuged at 3000 × g for 10 minutes. The supernatant was carefully poured into a tared drying dish and the remaining hydrated flour (gel) weighed. The water absorption index was then expressed as the weight in g of the gel per gram of original dry flour. For the water solubility (%), the amount of dried solids recovered after drying the supernatant was expressed as percentage of the original sample weight.

3.3.3 Composite dough preparation

A ratio of 30:70 whole grain sorghum flour to wholemeal wheat flour was chosen as an adaption from a previous method (Goodall et al. 2012). The micro-scale formulations were used due very small sample size available for the genotypes (Table 3.1). The composite dough formulation comprised 1.2 g sorghum flour, 2.8 g wholemeal wheat flour, 0.08 g yeast, 0.08 g salt and 2.68 g water at 35ºC. For the 100% wholemeal wheat dough control, the micro-scale formulation comprised of 4 g wholemeal wheat flour, 0.08 g yeast, 0.08 g salt and 2.68 g water at 35ºC.

Table 3.1 Formulation of sorghum-wheat composite and wheat-only doughs

Ingredients	Sorghum : Wheat	Wheat
	30:70	0:100
Sorghum (g)	1.20 (17.5)[a]	0 (0.0)
Wheat (g)	2.80 (40.9)	4.0 (58.4)
Yeast (g)	0.08 (1.2)	0.08 (1.2)
Salt (g)	0.08 (1.2)	0.08 (1.2)
Water (g)	2.68 (39.2)	2.68 (39.2)
Total weight (g)	6.84 (100)	6.84 (100)

[a] Figures in bracket are percentage of total dough weight

The dry ingredients were combined in a beaker by stirring with a spatula. Water was then added and mixed manually using one finger until a homogenous mass was formed. This was then kneaded at ambient temperature (25-27ºC) and humidity (37%) with one hand 50 times in attempt to standardise the process. The resulting dough was sheeted by passing 10 times through a Baccarat® pasta machine (Playcorp Pty Ltd, Victoria, Australia) with its aperture set at 5, which is approximately 3 mm. Before each sheeting, the dough was folded once and rotated 90°C to its axis.

3.3.3.1 Determination rheology characteristics related dough quality

Dough rheological properties were measured at 35°C using a TA Instrument AR-G2 controlled stress rheometer (TA Instruments®, Delaware, USA) at Central Chemical Consulting (Malaga, Australia) equipped with a parallel plate of 40 mm diameter. Immediately after sheeting, approximately 2 g of each dough was loaded on the lower plate of the rheometer, and the upper plate was slowly lowered until the gap between the plates was 2 mm, the excess dough was trimmed off with a plastic spatula. The dough was then subjected to varying oscillatory small amplitude deformation tests as described by Fevzioglu et al. (2012). The conditions for the rheological tests were established by identifying the linear viscoelastic region (a strain sweep over a range of 0.1% to 40% at a constant frequency of 1 Hz) and the frequency sweep (0.1 rad/s to 100 rad/s at 0.5% strain amplitude). The dough rheological properties were expressed in terms of the storage modulus (G′), loss modulus (G″), absolute complex modulus |G*| and the phase angle (δ). All doughs were prepared in duplicate and analyses performed once on each sample replicate.

3.4 Statistical analysis

Data is reported as means ± SD. The flours and dough attributes were compared by one-way analysis of variance (ANOVA). Individual means were compared by Tukey post-hoc test. $P < 0.05$ was considered significant. SPSS V24 (SPSS, Chicago, IL, USA) was used for the analyses.

3.5 Results and Discussion

3.5.1 Dough rheological properties

3.5.1.1 Storage and loss modulus

Between the doughs containing the sorghum genotypes, sample NGT17N208 had significantly lower G′ value ($P < 0.05$), however, the wheat-only dough had the lowest value (Figures 3.1). This suggests that the dough containing sorghum NGT17N208 has viscoelastic properties closer to that of the wheat-only dough compared to the other sorghum genotypes. NGT17N208 as well as Liberty also had significantly lower ($P < 0.05$) G″ values than that of the two other genotypes but these values were higher than that of wheat-only dough.

3.5.1.2 Complex modulus

The complex modulus |G*| gives information on how strong a dough is and how its strength changes during mixing (Norton et al, 2001). The |G*| did not differ ($P < 0.05$) between the sorghum containing doughs, nor the wheat-only dough (Figure 3.3). As the frequency increased, the |G*| of all samples increased gradually. This gradual increase of |G*| likely due to domination of the wheat in the composite dough.

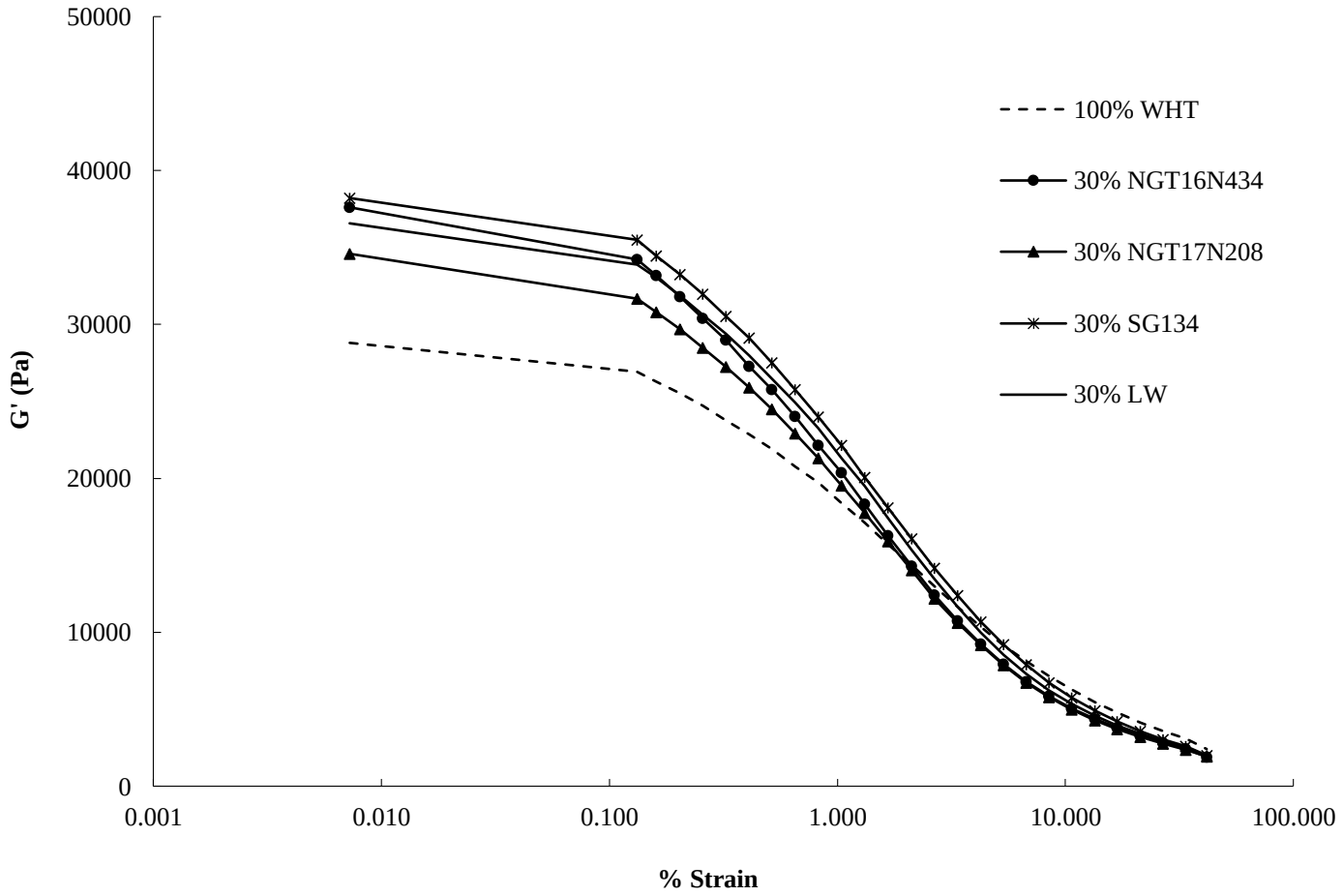
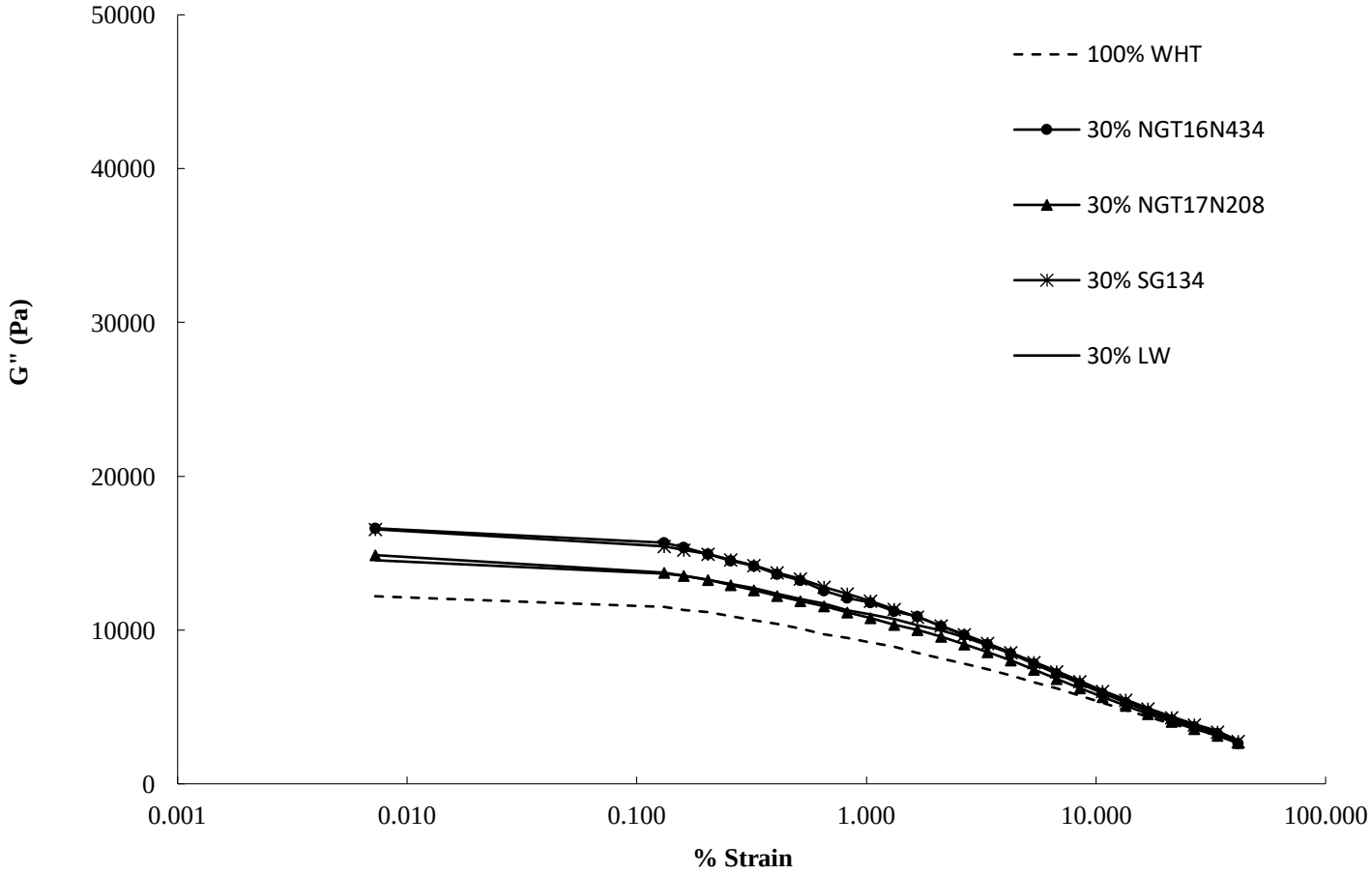


Figure 3.1 Storage modulus (G′) of whole grain sorghum-wholemeal wheat composite doughs compared to wholemeal wheat dough measured at 35°C. WHT: Wholemeal wheat and LW: Liberty white.



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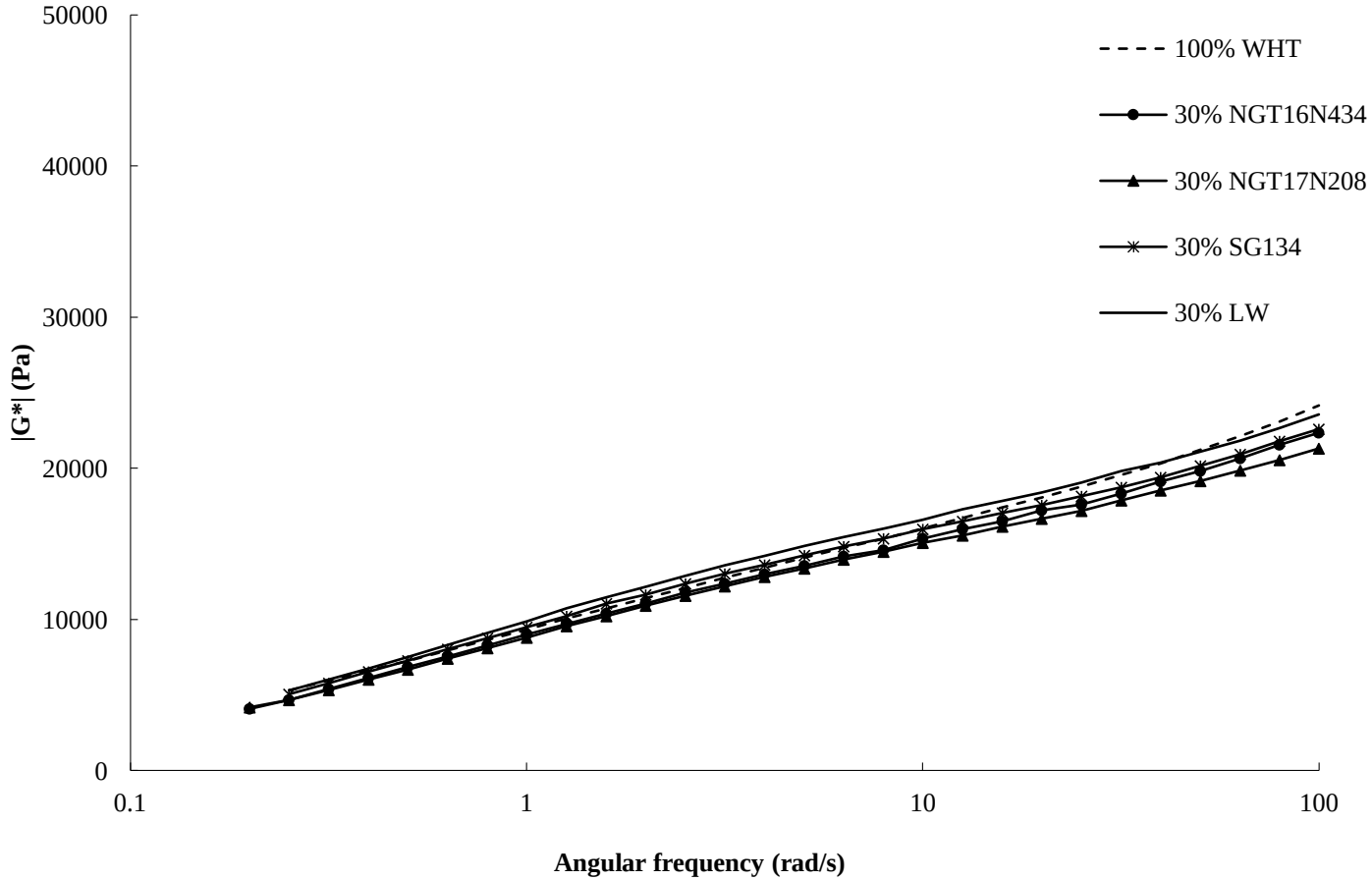
1049 **Figure 3.2** Loss modulus (G″) of whole grain sorghum-wholemeal wheat composite doughs compared to wholemeal wheat dough
1050 measured at 35°C. WHT: Wholemeal wheat and LW: Liberty white.

3.5.1.3 Phase angle

The phase angle (δ) graph gives a relative contribution of the viscous and elastic moduli to viscoelastic behaviour of the dough at different frequencies (Norton et al., 2011). There was no effect of sorghum variety on the phase angle and the wheat-only dough had significantly lower phase angle (δ) than all sorghum composite doughs (Figure 3.4) when subjected to high strain. This indicates that the sorghum composite dough were less elastic in nature than the wheat-only dough. This observation may be due to the hydrophobic and tightly-packed nature of sorghum prolamin proteins, kafirin bodies preventing both the water absorption and protein inter-chain reactions needed for hydration and development of an elastic dough (Taylor, Taylor, Campanella, & Hamaker, 2016).



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1063 **Figure 3.3** Complex modulus IG*I of whole grain sorghum-wholemeal wheat composite doughs compared to wholemeal wheat dough
1064 measured at 35°C. WHT: Wholemeal wheat and LW: Liberty white.

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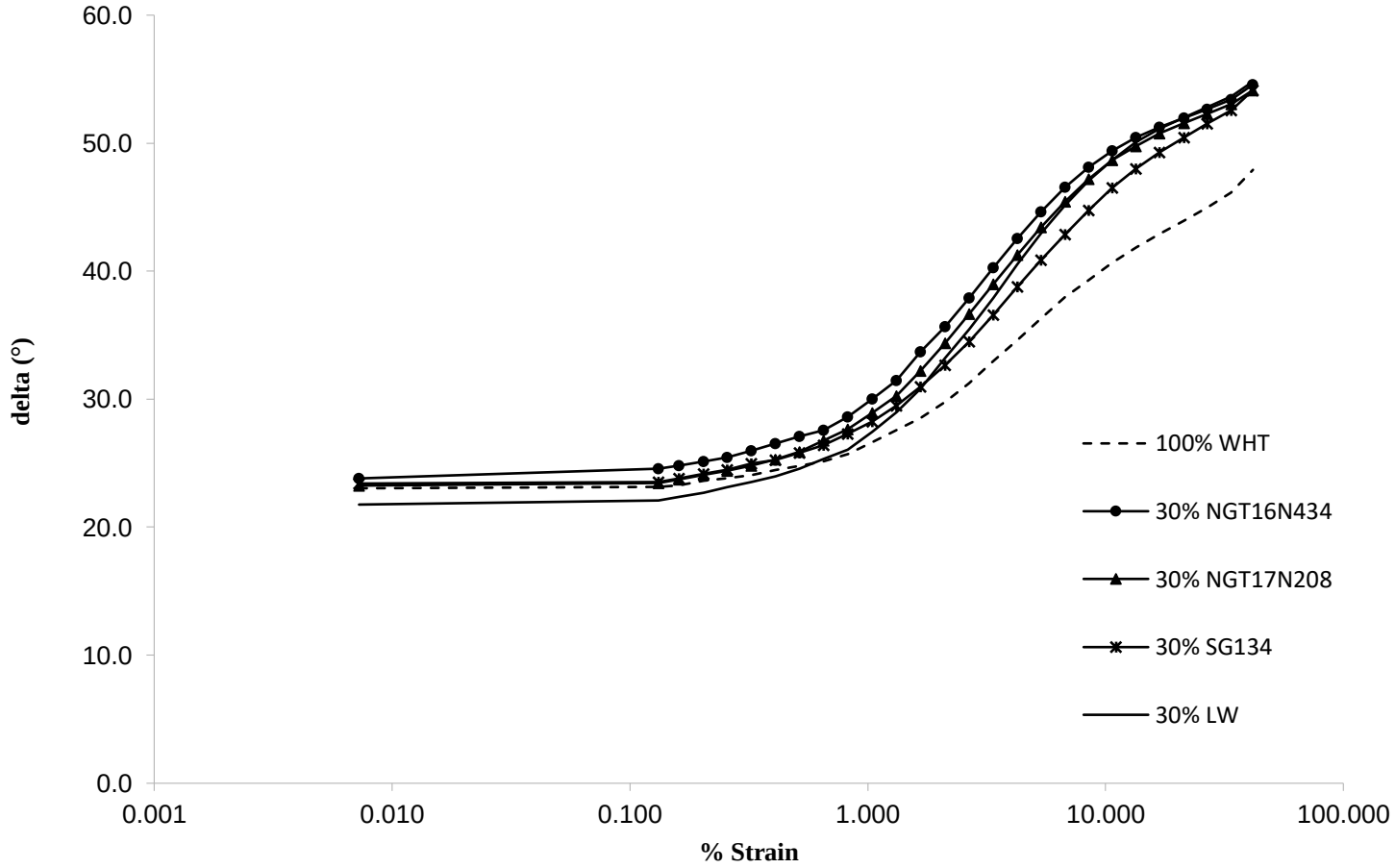

Figure 3.4 Phase angle (δ) of whole grain sorghum-wholemeal wheat composite doughs compared to wholemeal wheat dough measured at 35ºC. WHT: Wholemeal wheat and LW: Liberty white.

3.5.2 Moisture, protein and starch contents of sorghum flours

The moisture content was significantly different ($P < 0.05$) between the sorghum flours (Table 3.2). The same amount of water was added to each flour, meaning that the amount of water content in their doughs differed slightly (data not shown), which may contribute to any difference in their rheological properties.

There was significance difference between the protein content of the sorghum flours ($P < 0.05$) but the range of the values was narrow. Taylor et al. (1984) reported that the protein content of diverse genotypes grown in various locations was 9.0-13.9 g/100 g (dry basis). The narrow range of the protein content of the genotypes in the present study may be due to them being all white varieties grown in the same location.

The starch content of the sorghum flours were not significantly different (Table 3.2). Wang et al. (2008) found that the starch content in seventy sorghum genotypes ranged between 64-74%, the starch content of the sorghum in this study was within this range.

Table 3.2 Moisture, protein and starch of the sorghum and wholemeal wheat flours (g/100g)[1]

Whole grain sorghum flour sample	Moisture	Protein	Starch
Liberty	$8.68^{a} \pm 0.02$	$11.30^{c} \pm 0.06$	$67.35^{a} \pm 2.59$
134	$9.06^{ab} \pm 0.15$	$11.61^{c} \pm 0.02$	$67.03^{a} \pm 1.30$
NGT16N434	$9.14^{b} \pm 0.08$	$10.01^{a} \pm 0.01$	$65.03^{a} \pm 1.20$
NGT17N208	$9.01^{ab} \pm 0.19$	$10.61^{b} \pm 0.04$	$65.10^{a} \pm 0.51$
Wholemeal wheat	$10.22^{c} \pm 0.01$	$13.55^{d} \pm 0.27$	$61.86^{a} \pm 3.11$

[1] Mean ± Standard Deviation, n=3. Mean values in a column with different superscript letters are significantly different ($p < 0.05$). Protein and starch means on dry basis, db.

3.5.3 Particle size

Milling reduces particle size, increasing surface area to volume ratio at the same time damaging the integrity of the starch-protein matrix and thus increases hydration of flour during dough making (Kent & Evers, 1994). Although the particle diameter at each percentage volume differed among samples, the range between the samples was narrow (Table 3.3). This suggests that particle size is not a key factor in this study influencing the variation in dough quality between the sorghum samples.

Table 3.3 Particle size distribution of the sorghum and wholemeal wheat flours[1]

Sample	d (0.1)[2] µm	d (0.5)[2] µm	d (0.9)[2] µm	D[4,3][2] µm
Liberty	$13.41^{b} \pm 0.08$	$92.36^{b} \pm 0.36$	$209.67^{a} \pm 0.28$	$101.68^{a} \pm 0.23$
134	$14.53^{c} \pm 0.06$	$108.41^{d} \pm 0.42$	$239.44^{c} \pm 0.14$	$117.65^{c} \pm 0.25$
NGT16N434	$12.52^{a} \pm 0.01$	$90.71^{a} \pm 0.29$	$214.17^{ab} \pm 0.19$	$101.32^{a} \pm 0.20$
NGT17N208	$14.24^{d} \pm 0.00$	$101.00^{c} \pm 0.03$	$221.63^{b} \pm 0.25$	$109.21^{b} \pm 0.05$
Wholemeal wheat	$24.79^{e} \pm 0.4$	$120.78^{e} \pm 0.07$	$387.79^{d} \pm 4.57$	$174.24^{d} \pm 0.76$

[1] Mean ± Standard Deviation, n=2. Mean values in a column with different superscript letters are significantly different ($p < 0.05$).
[2] d (0.1), d (0.5), d (0.9) are maximum diameters of 10%, 50% and 90% of total volume of particles; D [4,3] is the volume-weighted mean particle size

3.5.4 Water absorption and water solubility of flours

3.5.4.1 Water absorption of flour

There was no effect of sorghum variety on water absorption ($P < 0.05$). This indicates the same flour hydration capacity for all genotypes, meaning that it does contribute to any difference in rheological properties of their doughs. However, the water absorption indexes of all sorghum flours were significantly higher ($P < 0.05$) than that of the wheat (Table 3.4). This means they hold more water in the gel fraction.

3.5.4.2 Water solubility of flour

There was no significant difference ($P < 0.05$) between the water solubility indexes of the sorghum flours. The water solubility of wheat flour was significantly higher than that of all sorghum genotypes, indicating a higher level of soluble solids in the wheat flour (Protonotariou, Drakos, Evageliou, Ritzoulis, & Mandala, 2014).

Table 3.4 Flour water absorption and solubility[1]

Sample	Water absorption index	Water soluble index
Liberty	$1.26^{b} \pm 0.07$	$5.82^{a} \pm 0.02$
134	$1.14^{b} \pm 0.01$	$6.18^{a} \pm 0.03$
NGT16N434	$1.23^{b} \pm 0.01$	$5.84^{a} \pm 0.46$
NGT17N208	$1.29^{b} \pm 0.00$	$5.91^{a} \pm 0.25$
Wholemeal wheat	$0.84^{a} \pm 0.00$	$7.78^{b} \pm 0.37$

[1] Mean ± Standard Deviation, n=2. Mean values in a column with different superscript letters are significantly different ($p<0.05$).

3.6 Conclusion

This study is one of very few studies that have investigated the effect of sorghum genotypes on dough rheological properties using whole grain in a sorghum-wheat composite model. The study reveal some differences between sorghum genotype, in particular the lower G′ value of NGT17N208 containing dough. Therefore this variety is worthy of further investigation for its use in dough manufacture.

In this chapter, dough mixing and kneading were done by hand. And thus, the energy applied during mixing was not necessarily standardised, which may have hidden any small but important differences among sorghum genotypes. Therefore, the next study will use the micro-scale controled mixing equipment the micro-doughLAB (Perten Instruments of Australia, Sydney, Australia) to ensure standardised mixing and kneading. In addition, the next chapter will use this equipment to identify the speed and temperature, which provide dough mixing quality closest to that of the wheat-only dough.

In terms of water addition the same amount of water was added to the wheat-only and the composite flours in this chapter. Therefore in the next study the amount of water to add to the composite flour system will be optimised using the micro-doughLAB to develop it to its full capacity. These optimised conditions may better reveal any difference between the sorghum genotypes.

The level of sorghum in the composite flour used in this study may not be enough to allow differentiation between the genotypes, therefore in the next chapter an increased amount of sorghum will be used.

Sorghum samples were milled using a laboratory Cemotec^{TM} 1090 Sample Mill to give whole grain coarse flour, which was further milled many times using a coffee grinder and passed through a sieve. In the next chapter, to mimic commercial milling more closely all samples (sorghum and wheat grains) will be milled under the same standard conditions using an SR 300 Retsch Mill (Retsch GmbH, Germany).

In this study, salt and yeast were used in the dough formulation. However, because salt and yeast participate in dough formation, they may have hindered any difference in the rheological properties of the different sorghum containing formulations. Therefore, in the next study a simple formulation without salt and yeast will be used to better evaluate the sorghum genotypes on the mixing quality of the doughs.

CHAPTER 4

Development of a micro-scale screening method to evaluate mixing properties of sorghum- wheat composite dough

4.1 Abstract

Research suggests that different sorghum varieties have different dough forming properties but the evidence for this is limited. To evaluate the dough forming potential of very small samples of diverse sorghum genotypes from breeding programs a micro-scale method is required that mimics the high energy mixing used in modern commercial bakeries. However, such a method has not been standardised to screen sorghum genotypes. In this study, whole grain white sorghum (non-tannin) and whole grain wheat were both milled using a standardised procedure. Composite doughs were produced with a 50:50 ratio sorghum to wheat flour using a micro-doughLAB (Perten Instruments of Australia, Sydney, Australia). Initially, total moisture (water absorption) was adjusted to identify the level that gave both a smooth mixing curve and optimum dough resistance to mixing (target peak torque). At this chosen water absorption level (64.0%) and target peak torque (87 mNm), doughs were mixed at each combination of four levels of speed (63, 95, 120 and 150 rpm) and three levels of temperature (30, 35 and 45°C) during which their mixing quality in terms of peak torque (mNm), dough stability (minutes) and softening (mNm) were recorded. Both mixing speed and temperature significantly affected these quality attributes. From a comparison of individual combinations, 30°C and 120 rpm was selected as the standard conditions since it resulted in a composite dough developed to maximum resistance close to the target peak torque 87 mNm. The composite dough mixed under these conditions was more stable and softened to a lesser extent than those mixed using the other speed and temperature combinations, suggesting a desired consistency. This standard method will be used to evaluate the dough forming ability of diverse sorghum genotypes.

4.2 Introduction

Determination of dough quality of cereal flours is of great importance for plant breeders in early stages of breeding programs for the selection of genotypes with good dough forming traits. Dough developed to its maximum resistance during mixing is desirable for good dough-handling properties and bread loaf qualities (Dang & Bason, 2013). This is because correctly developed dough is able to withstand the shear and tensile forces imparted during mixing and handling, and can also expand and retain gas bubbles during proofing, resulting in the light leavened bread desired by consumers (Eliasson & Larsson, 1993; Huang, Yun, Quail, & Moss, 1996; Cauvain, 2012). Therefore, small-scale mixing-quality tests are applied by breeders to new grain genotypes to understand their dough quality.

Sorghum is gaining global interest in both developed and developing countries as a suitable flour for partial substitution of wheat in composite doughs for commercial bread making. This is due to the increased understanding of the health benefits associated with consumption of sorghum grain and the drought- and high-temperature tolerant nature of the crop (Stefoska-needham et al., 2015; Taylor, 2017). There is some but limited evidence that different sorghum varieties have different dough forming properties. It has been reported that substitution of 30% refined wheat flour with decorticated sorghum flour from a high protein digestibility genotype, when mixed above its glass transition temperature (Tg), gives a dough with higher extensibility and bread with higher loaf volume, than when a normal-digestibility sorghum genotype was used (Goodall et al., 2012). As such, the high-digestibility sorghum genotypes appear to have potential to produce higher quality dough for leavened bread manufacture compare to the normal-digestible genotype (Goodall et al., 2012).

Goodall et al. (2012) in their comparison between high-and normal-digestibility genotypes used a Mixograph equipped with 10, 35 and 100 g mixing bowl capacities to adjust water content of the dough so that the composite dough reached a maximum resistance. Similarly, Bugusu, Campanella, & Hamaker (2001) used a 35 g mixing bowl of a Swanson-Working Mixograph to determine the mixing quality of zein in a decorticated sorghum-wheat composite flour. In another study, Yousif, Nhepera, & Johnson (2012) used a Farinograph equipped with 300 g mixing bowl capacity to determine the water absorption required to reach optimum consistency (500 BU) of

sorghum-wheat composite dough. The above mentioned studies did not consider the effect of speed and temperature when mixing the doughs to maximum resistance. The investigation of these parameters will be studied in this chapter to understand the mixing requirements of the sorghum-wheat composite flour.

The traditional methods to evaluate the mixing properties of sorghum-wheat composite dough used in the previous studies (e.g. Mixograph and Farinograph) have a key limitation of the need for a large amount of sorghum flour, which is impractical within breeding programs where only few grams of grain may be available for assessment (Tomoskozi & Bekes, 2016). Smaller-scale controlled mixing dough testing equipment such as the 2 g Mixograph (Mixograph, National manufacturing Co., Nebraska, USA) and 4 g micro-doughLAB (Perten Instruments of Australia, Macquarie Park, Australia) are now available. In addition, the Farinograph and Mixograph use gentle mixing actions, which do not mimic high-energy mixers used in modern commercial bakeries. In contrast, the micro-doughLAB does have a mixing action more closely related to high-energy mixing. In addition, it provides a reliable measure of maximum dough resistance with acceptable precision and better reproducibility than the traditional methods (Dang & Bason, 2013).

The micro-doughLAB comprises of a mixing bowl in which z-arm mixer blades rotate to mix flour and water into a dough. As mixing proceeds, the changing resistance of the dough is measured and recorded as torque (mNm). The time from zero to the point of defined maximum torque is referred to as dough development time (min), and indicates the time required to mix the dough up until the point of defined maximum development. The length of time the maximum torque remains constant is referred to as dough stability (min). The difference (mNm) in midline peak torque and the torque at 12 minutes after DDT is referred to as softening, and indicates the dough's tolerance to mixing. The cumulative energy to reach the maximum torque is defined as "energy at maximum resistance (Wh/kg)" and can be used to scale-up the level of mechanical energy required during commercial mixing (D'Appolonia, 1984; Cauvain, 2009; Elliott, 2010; Dang & Bason, 2013). The micro-doughLAB may be highly suitable to determine WA and dough-mixing parameters required to give a well-developed sorghum-wheat composite dough and then assess diverse sorghum genotypes under these controlled conditions when only a limited amount of each sorghum genotype is available.

Whole grain sorghum is a better source of dietary fibre, health-promoting phytochemicals and essential micronutrients (for example iron) than decorticated sorghum (Dlamini, Taylor, & Rooney, 2007; Hama, Icard-Verniere, Picq, Diawara, & Mouquet-Rivier, 2011). Therefore, in this study whole grain white sorghum flour was evaluated for its water absorption and mixing requirements to form a fully developed sorghum-wheat composite dough. The objective of this study was to develop a micro-scale method for mixing a composite flour of whole grain white sorghum and whole grain wheat to optimum dough development using a micro-doughLAB. The effect of water absorption, mixing temperature and speed on the dough mixing parameters was evaluated and the level of these parameters to give maximum dough development. This standard method will then be used to evaluate the dough forming ability of diverse sorghum genotypes, which may assist sorghum-breeding programs to identify genotypes most suitable for bread making.

4.3 Materials and methods

4.3.1 Preparation of whole grain flours

Clean commercial samples of whole grain non-tannin white sorghum (Liberty) (Truong et al. 2017) kindly supplied by Nuseed (Queensland, Australia) and a hard commercial whole grain wheat (Emu Rock) kindly donated by InterGrain (Perth, Australia) were used in this study (Figure 4.1). The grains were separately milled using an SR 300 Retsch Mill (Retsch GmbH, Germany) fitted with a 250 μm opening screen to give whole grain flour (extraction rate 100%). The falling number, wet gluten, dry gluten, water-binding capacity and gluten index of the wheat was 436 sec, 27.8%, 8.6%, 19.3 and 72.9, respectively. The falling number was determined according to the AACCI Method 56-81.04 and gluten according to AACCI Method 38-12.02. All flours were vacuum packed and stored at 4°C prior to use.

The falling number was determined to measure the degree of alpha-amylase activity in the ground whole grain wheat sample. Alpha-amylase is an endogenous enzyme that increases due to grain germination as result of rain prior to harvest. The enzyme randomly hydrolyses the α-1,4 glycosidic linkages in starch, resulting in short chains (Rosell, Haros, Escrivá & Benedito de Barber, 2001). The effect of excessive amount of alpha-amylase result in flour with less water binding capacity and this decreases the quality of the dough (Dowell et al., 2008). The falling number principle is based on

the ability of the alpha-amylase to liquefy a gelatinized starch. The time required to stir and allow a viscometer stirrer to fall from its top position to a set distance under the influence of gravity through a hot aqueous flour undergoing liquefaction is recorded in seconds, and represents the falling number (AACC, 2000). The falling number of 436 seconds for the wheat sample used in this study indicated no activity of the alpha-amylase, suggesting a very good wheat quality for dough making (Mares & Mrva, 2008).

The wet gluten, dry gluten, water-binding capacity, and gluten index of the ground whole grain wheat were determined to assess the quantity and quality of the gluten in the sample. The results reported suggest that the gluten index of the wheat sample was optimum for dough making (Perten, 1990). The wet gluten, dry gluten, water-binding capacity, and gluten index tests involve separating wet gluten from wheat flour using the glutomatic system. The wet gluten is then centrifuged on a special sieve such that a part of it passes through, with the remainder recovered, weighed and reported as percentage of the total. The wet gluten is then dried under standardized conditions, weighed and expressed as percentages of the sample. The difference between the weights of wet and dry gluten gives the water bound in the wet gluten, which is the water-binding capacity. The gluten index is the ratio of the wet gluten remaining on the sieve (after centrifugation) to the total wet gluten and indicates whether the gluten is weak, normal or strong (AACC, 2000).

Figure 4.1 Whole grain wheat (Emu Rock) and whole grain sorghum (Liberty) used in this study

4.3.2 Composite dough preparation

A ratio of 50:50 of the sorghum flour to the wheat flour was chosen as an adaptation from the previous method of Goodall et al. (2012) to measure the extent to which sorghum varieties disrupted the quality of whole grain wheat dough. This was a higher level of sorghum than used in the previous chapter (Chapter 3) of this thesis in an attempt to increase the disruption of the wheat matrix. Thus, the method should have more power to differentiate between the effects of different sorghum varieties that will be investigated later in this thesis (Chapter 5).

The moisture content of flours was determined as previously described in *section 3.3.2.2* of this thesis.

4.3.3 Determination of water absorption of sorghum-wheat composite dough

The water absorption (WA) is the amount of water required during mixing to reach maximum peak torque (resistance). The WA of the wheat and 50:50 sorghum-wheat flour was assessed in the micro-doughLAB using AACC International (2000) Method 54-70.01 adapted as described by (Gajo & Dang, 2016) (Table 4.1). The amount of water required by the wheat to reach maximum peak torque (130 mNm) was used as a standard to determine that of the sorghum-wheat composite flour.

Table 4.1 Micro-doughLAb method for determining water absorption of sorghum-wheat composite flours

Time (hr:min:sec)	Type	Value
00:00:00	Temperature	30°C
00:00:00	Speed	63 rpm
00:00:30	Speed	120 rpm
00:10:00	End of test	-
Premix time (min:sec)	00:30	
Premix speed (rpm)	63	
Target torque (mNm)	130 ± 5	
Sample weight (g)	4.00 ± 0.01	

The required WA was determined following the standard protocol defined by the micro-doughLAB manufacturer (micro-doughLAB 120 rpm method, unpublished) by weighing equivalent to 4.00 ± 0.01 g (14% moisture basis, corrected for actual sample moisture) of either the wheat or the sorghum-wheat composite flours. The wheat flour had 10.8% moisture content, thus 3.86 g of flour (as is basis) was weighed. The sorghum-wheat composite flour had 10.2% moisture content, therefore 3.83 g of the composite flour (as is basis) was weighed. The micro-scale formulation of the sorghum-wheat composite comprised 1.915 g of the sorghum flour and 1.915 g of the wheat flour. The flour was mixed with a spatulas in a beaker and then transferred to the Micro-doughLAB bowl. They were pre-mixed for 30 seconds at 63 rpm, then water was automatically dispensed from the Micro-doughLAB and mixed for 10 minutes at 120 rpm as described in Table 4.1.

Preliminary mixing trials were performed using a range of WAs between 60% - 65.6% and 60.0% - 65.5% to determine the maximum peak torque (resistance) of the wheat and the sorghum-wheat composite flours, respectively. Tests targeting the peak torque of 130 mNm (Gajo & Dang, 2016) required WA of 65.6% for the wheat and 60.0% for the sorghum-wheat composite flours, respectively. However, by comparing the peak curves, that of sorghum-wheat composite flour was noisier than that of wheat flour, suggesting that the composite flour was probably under hydrated and appeared crumbly (Figure 4.2). Therefore, the WA was increased further until a smooth curve

occurred, which gave a peak torque of 87 mNm at WA of 64.0%. The WA 64.0% was used in all subsequent tests and the target peak torque was set at 87 mNm. Triplicate tests were performed under these conditions to evaluate the precision of the mixing curve data.

4.3.4 Determination of the effect of temperature and mixing speed on mixing quality of sorghum-wheat composite dough

The standardised WA (64.0%) and target peak torque (87 mNm) were used to determine the effect of mixing temperature and speed and their interaction on the mixing properties of the sorghum-wheat composite dough. Mixing properties measured were peak torque (mNm), DDT (min), stability (min), softening (mNm) at 12 min after peak torque and energy (Wh/kg) at peak torque. And the combination of mixing temperature and speed, which gave the target peak torque was identified. Mixing tests using the Micro-doughLAB were performed using AACC International (2000) Method 54-70.01 adapted as described in Table 4.2.

Table 4.2 Micro-doughLAB method adapted from AACC International (2000) Method 54.70.01 for determination of mixing qualities of sorghum-wheat composite doughs

Time (hr:min:sec)	Type	Value
00:00:00	Temperature	30°C
00:00:00	Speed	63 rpm
00:00:30	Speed	120 rpm
00:20:00	End of test	-
Premix time (min:sec)	00:30	
Premix speed (rpm)	63	
Target torque (mNm)	87 ± 3[1]	
Sample weight (g)	4.00 ± 0.01	

[1] For whole grain white sorghum-whole grain wheat composite dough

The flours were mixed at three test temperatures: 30°C according to AACC International (2000) Method 54-70.01, 35°C as used by Goodall et al. (2012), and 45°C as it is above the glass transition temperature of sorghum major proteins 'kafirins' (Schober, Bean, Tilley, Smith, & Ioerger, 2011). Four mixing speeds were used: 63

rpm according to AACC International (2000) Method 54-21.02, 95 rpm was selected as a mid-point between 63 rpm and 120 rpm, 120 rpm according to AACC International (2000) Method 54-70.01 and 150 rpm as a more extreme speed level for this experiment. Tests were repeated three times.

4.4 Statistical analysis

The inter-day precision of the method was assessed by recording the peak torque from triplicates tests each day over three days. Precision data was expressed as relative standard deviation (% RSD).

Mixing results (peak torque, DDT, stability, softening and energy at peak torque) were expressed as means ± standard deviation (SD). The main effects of speed and temperature and their interaction were investigated by two-way analysis of variance (ANOVA). If significant main effect was observed, then one-way analysis of variance was used to separate individual sample means. Individual means were compared by Tukey post-hoc test. $P < 0.05$ was considered significant. The data were statistically analysed using SPSS V24 (SPSS, Chicago, IL, USA).

4.5 Results and discussion

4.5.1 Effect of water absorption on peak torque of whole grain sorghum-whole grain wheat composite flour

The average peak torque of the sorghum-wheat composite dough with 60.0% WA was higher than that with 64.0% WA, however, it resulted in a noisy curve during the initial mixing period compared to that the wheat dough (Figure 4.2). It is likely that the hydration of the composite flour was not sufficient, resulting in high peak torque values and a noisy curve.

The addition of 64.0% water resulted in smooth torque curves, which probably suggest that the composite flour was hydrated and therefore formed a cohesive dough. This water level gave the peak torque at 87 mNm for sorghum-wheat composite flour rather than the target peak torque of the wheat flour (Gajo & Dang, 2016). This means that substitution of wheat with sorghum weakened the wheat dough structure. Similarly, Goodall et al. (2012) found that the addition of sorghum flour to wheat flour negatively affected the dough rheological properties. The reduction in the functionality in the composite dough is related to the hydrophobicity of its major proteins, kafirin and their

tight encapsulation in protein bodies. These prevent both the water absorption and formation of a cohesive, extensible and elastic dough when sorghum flour is mixed with water (Taylor, Taylor, Campanella, & Hamaker, 2016).

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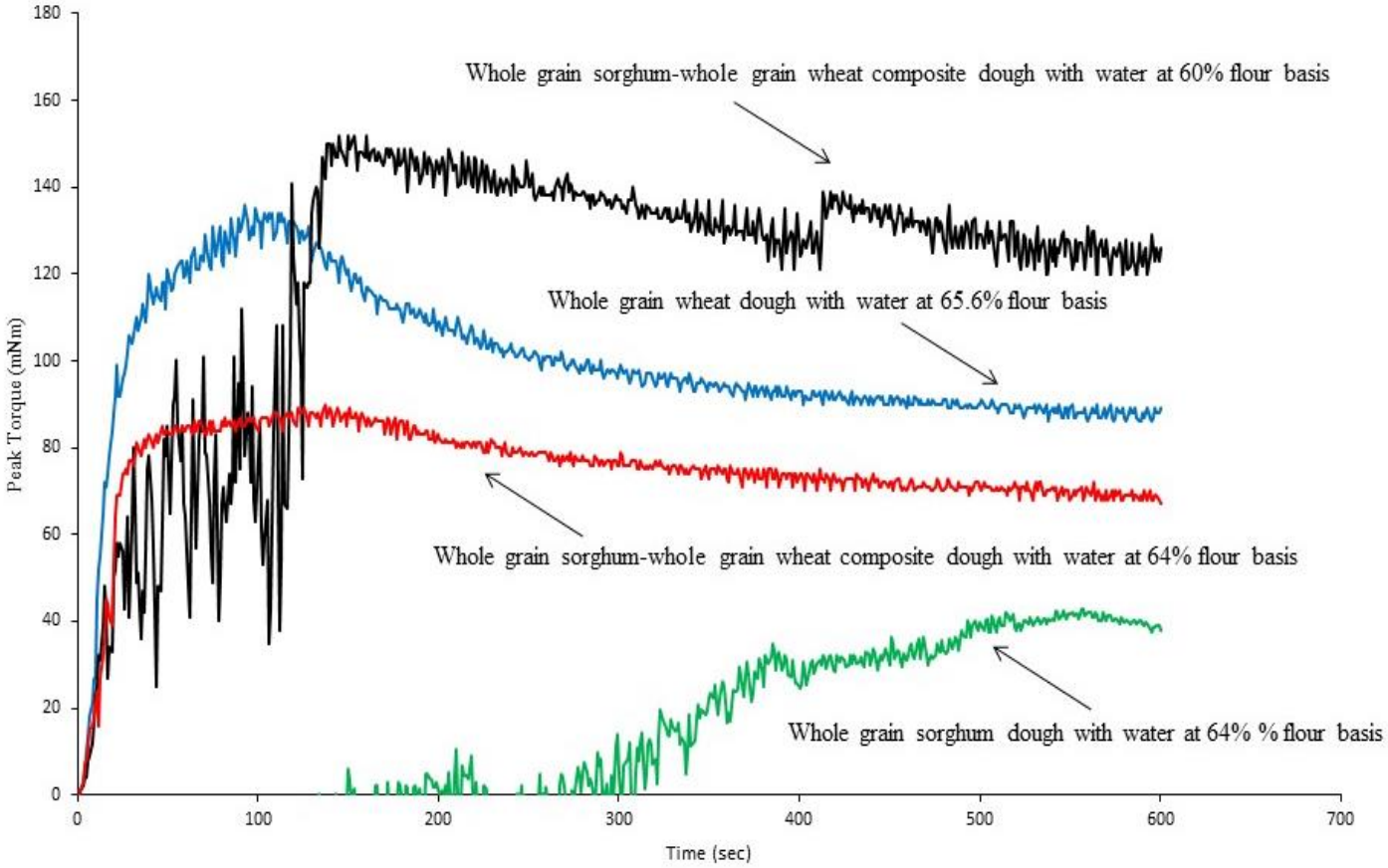

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Figure 4.2 Effect of water absorptions on peak torque of whole grain sorghum dough, whole grain sorghum-whole grain wheat composite dough and whole grain wheat dough mixed at a target torque of 130 mNm using a micro-doughLAB equipped with a 4 g bowl.

4.5.2 Effect of temperature and mixing speed on mixing quality of sorghum-wheat composite doughs

4.5.2.1 Peak torque

The effect of temperature and mixing speed on peak torque of sorghum-wheat composite dough is presented in Figure 4.3. Two-way ANOVA demonstrated that temperature and mixing speed had a significant ($P < 0.05$) main effect but not their interaction ($P = 0.19$).

Mixing at higher temperatures decreased the peak torque significantly ($P < 0.05$) at any given speed (Figure 4.3). Composite dough mixed at 30°C had the highest peak, whereas those mixed at 45°C had the lowest peak at all speeds ($P < 0.05$). It may be suggested that higher temperature possibly induced breakdown in the dough structure. It was hypothesised that high temperature would result in improved peak torque.

Unlike mixing at higher temperatures, higher mixing speeds caused a significant increase ($P < 0.05$) in peak torque at any given temperature. For example, at 30°C, doughs mixed at 63 rpm had the lowest peak torque, whereas those mixed with same temperature at 150 rpm had the highest peak ($P < 0.05$). This is because high speed mixing imparts more energy to the dough, resulting in the breaking and making of more chemical bonds needed for the development of the dough (Kilborn & Tipples, 1974).

The mixing temperature and speed combinations of 30^0C and 120 rpm and 35^0C and 150 rpm gave a range of peak torque values closer to the target peak torque (87 mNm).

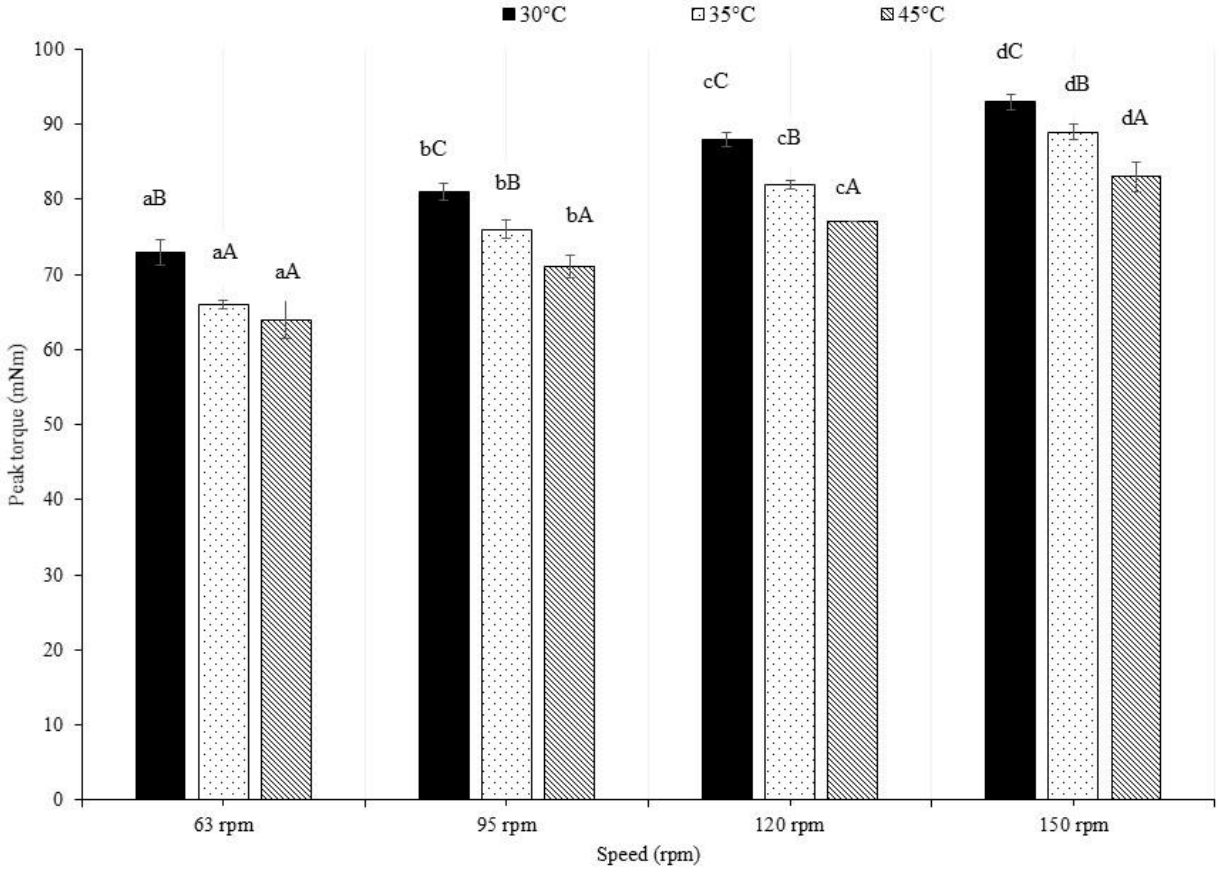

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Figure 4.3 Effect of mixing temperature and speed on peak torque of whole grain sorghum-wholemeal wheat composite doughs mixed using a micro-doughLAB equipped with a 4 g bowl (a- d letters on bar charts with the same mixing temperature at different speeds are significantly different, ($P < 0.05$) and A-C letters on bar charts with same speeds at different temperatures are significantly different, ($P < 0.05$). Vertical bars = ± standard deviation, n=3.

4.5.2.2 Dough development time

Development time (DDT) of sorghum-wheat composite doughs was significantly influenced by mixing temperature, speed and their interaction ($P < 0.05$). Figure 4.4 shows that within any given mixing speed, increasing temperature decreased the DDT, with the lowest value at 45°C ($P < 0.05$). Dough mixed at 30°C and 63 rpm had the longest DDT compared to those mixed at the same temperature using 95 rpm, 120 rpm and 150 rpm (Figure 4.4). This means that dough mixed at 63 rpm required a longer time to achieve target peak torque than when mixed at 95 rpm, 120 rpm, and 150 rpm, respectively. Mixing at 150 rpm significantly shortened the DDT, and using a higher temperature at the same speed shortened it further ($P < 0.05$).

As mentioned, the combinations of temperature and speed that gave peak torque values that were within the target resulted in a wide variation in DDT, ranging from 1.9 to 2.19 min. Mixing at 35°C and 150 rpm resulted in the shortest DDT ($P < 0.05$) due to the breaking and making of more chemical bonds as a result of high energy (Kilborn & Tipples, 1974).

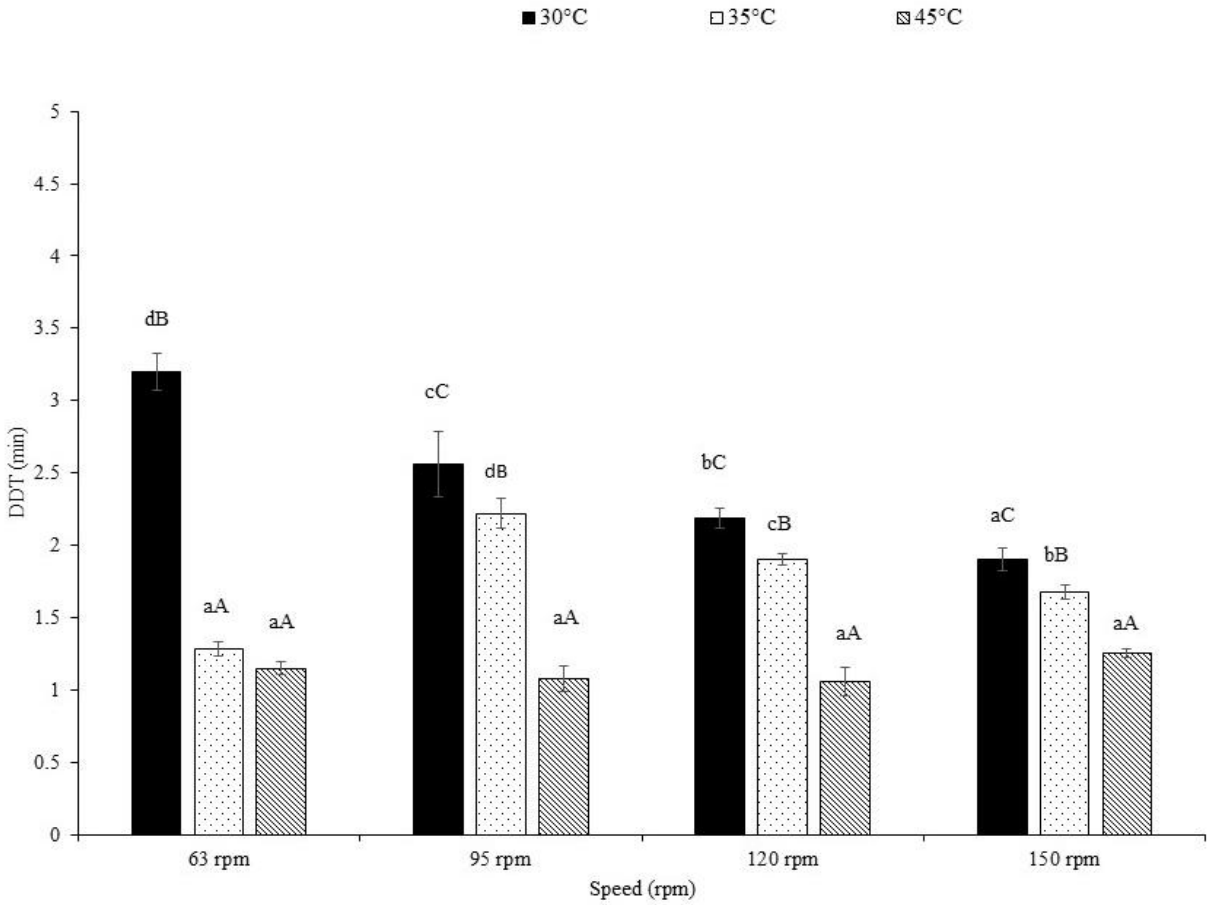

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Figure 4.4 Effect of temperature and speed on dough development time of whole grain sorghum-wholemeal wheat composite doughs mixed using a micro-doughLAB equipped with a 4 g bowl (a- d letters on bar charts with the same mixing temperature at different speeds are significantly different, ($P < 0.05$), and A-C letters on bar charts with same speeds at different temperatures are significantly different, ($P < 0.05$). Vertical bars = ± standard deviation, n=3.

4.5.2.3 Stability

Mixing temperature, speed and their interaction had significant main effects on sorghum-wheat composite dough stability ($P < 0.05$). At all mixing speeds, the stability of the sorghum-wheat composite dough mixed at lower temperatures (30°C and 35°C) was significantly higher ($P < 0.05$) than when mixed at high temperature (45°C) (Figure 4.5). The composite doughs mixed at 30°C and 63 rpm had the highest stability ($P < 0.05$); however, its peak torque (Figure 4.3) was significantly lower than the target peak torque ($P < 0.05$). It was difficult to compare the stability of such dough with a peak torque far less than the target peak torque.

By comparing the stability of whole grain white sorghum-wholemeal wheat composite doughs with peak torque values close or equal to the target peak torque (Figure 4.3), the stability of the composite dough mixed at 30°C and 120 rpm was significantly higher than that mixed at 35°C and 150 rpm ($P < 0.05$) (Figure 4.5). This suggests that dough mixed at 30°C and 120 rpm was more tolerant to mixing than the one mixed at 35°C and 150 rpm (Figure 4.5).

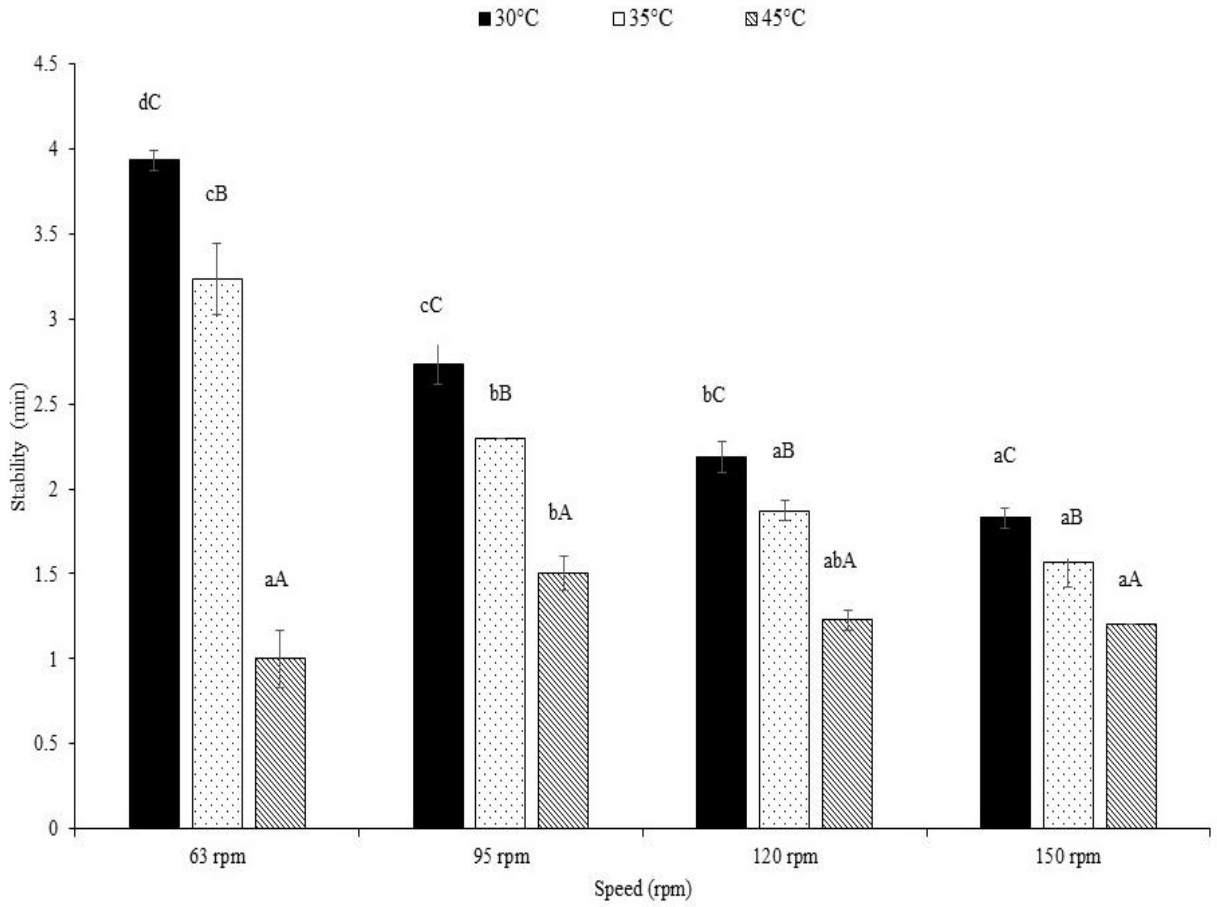

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Figure 4.5 Effect of temperature and speed on stability of whole grain sorghum-wholemeal wheat composite doughs mixed using a micro-doughLAB equipped with a 4 g bowl (a- d letters on bar charts with the same mixing temperature at different speeds are significantly different, ($P < 0.05$), and A-C letters on bar charts with same speeds at different temperature are significantly different, ($P < 0.05$). Vertical bars = ± standard deviation, n=3.

4.5.2.4 Softening

Mixing temperature, speed and their interaction had a significant effect on dough softening of sorghum-wheat composite dough ($P < 0.05$). Softening was significantly greater at higher temperature (45°C) than at lower temperature (30°C) and as the mixing speed increased, softening increased concomitantly and substantially ($P < 0.05$) (Figure 4.6). This means that the higher the mixing temperature and speed, the greater the dough break down.

The sorghum-wheat composite dough mixed at 30°C and 120 rpm broke down to a lesser extent than when mixed at 35°C and 150 rpm. This suggests that the optimum conditions for preventing softening of the sorghum-wheat composite dough are 30°C and 120 rpm.

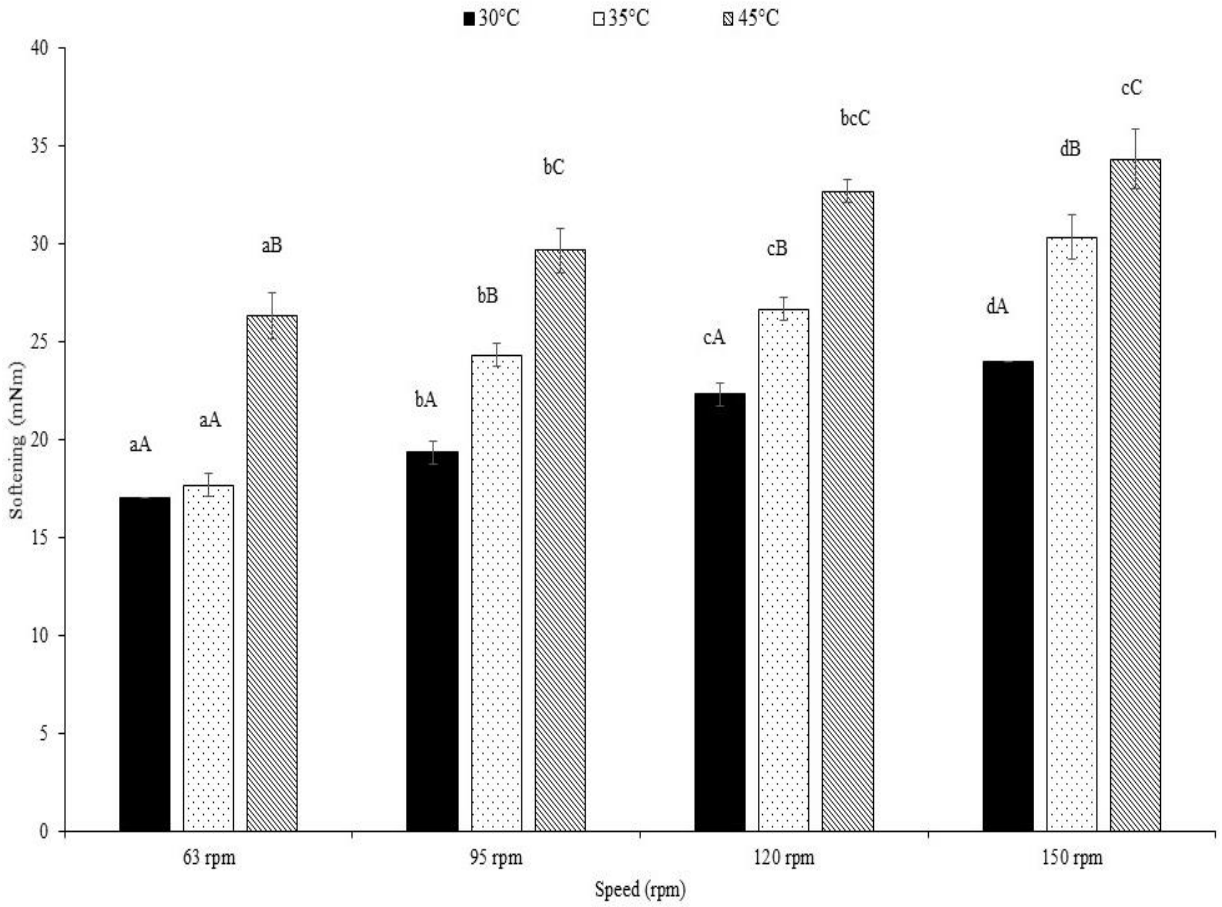

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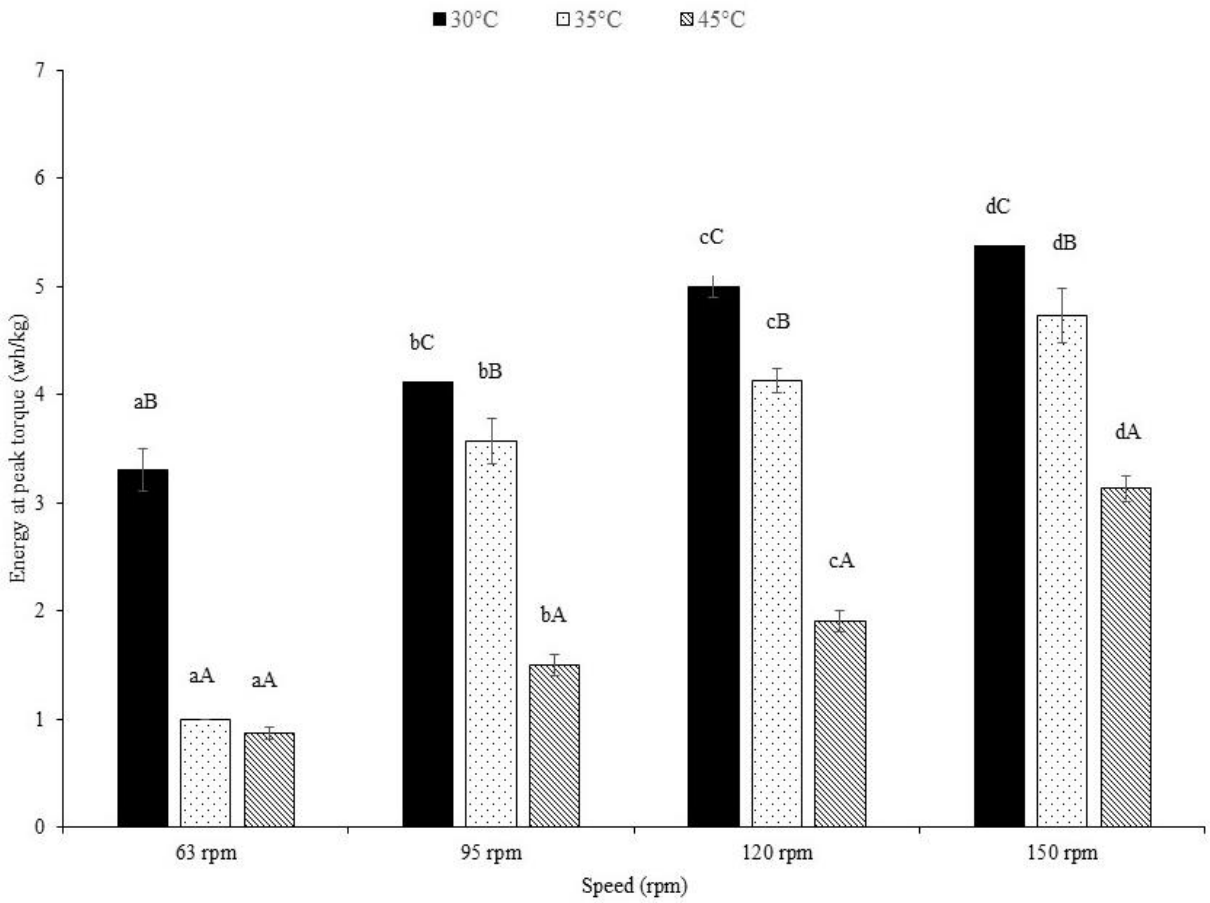
Figure 4.6 Effect of temperature and speed on softening of whole grain sorghum-wholemeal wheat composite doughs mixed using a micro-doughLAB equipped with a 4 g bowl (a- d letters on bar charts with the same mixing temperature at different speeds are significantly different, ($P < 0.05$), and A-C letters on bar charts with same speeds at different temperature are significantly different, ($P < 0.05$). Vertical bars = ± standard deviation, n=3.

4.5.2.5 Energy at peak torque

Mixing temperature, speed and their interaction had a significant effect on energy at peak torque of sorghum-wheat composite dough ($P < 0.05$). Figure 4.7 shows that with any given mixing speed, increasing temperature decreased the energy at peak torque of the composite dough, and the value was lowest at 45°C ($P < 0.05$). In contrast to the effect of temperature, higher mixing speeds significantly increased the energy at peak torque at any given temperature. For example, at 30°C, doughs mixed at 63 rpm had the lowest energy at peak torque, whereas those mixed with the same temperature at 150 rpm had the highest energy at peak torque ($P < 0.05$). As mentioned, this is because high speed mixing imparts more energy to the dough, resulting in the breaking and making of more chemical bonds needed for development of the dough (Kilborn & Tipples, 1974).



1472

Figure 4.7 Effect of temperature and speed on energy at peak torque of whole grain sorghum-wholemeal wheat composite doughs mixed using a micro-doughLAB equipped with a 4 g bowl (a- d letters on bar charts with the same mixing temperature at different speeds are significantly different, ($P < 0.05$), and A-C letters on bar charts with same speeds at different temperature are significantly different, ($P < 0.05$). Vertical bars = ± standard deviation, n=3.

4.5.3 Method precision

The precision of the micro-doughLAB mixing method as described in Table 4.2 was determined by measuring the peak torque in triplicate on each of three days of the sorghum-wheat composite dough over three days. Precision was expressed as relative standard deviation (%). Within day precision was 1.3%, 1.1% and 1.3% for day 1, day 2 and day 3, respectively. The inter-day reproducibility was 1.0%. This demonstrate appropriate precision and reproducible. According to AACC International (2000) Method 54-70.01, precision for peak torque should not exceed 4% among replicates and days. Therefore the standard precision in this study meet the requirements.

Overall, one of the most significant finding to emerge from this study is that mixing whole grain white sorghum-whole grain wheat composite flours to the standardized peak torque of wheat flour resulted in noisy torque curves, probably due to insufficient hydration of the flours. Addition of water to WA 64.0% resulted in smooth torque curves that gave the peak torque at 87 mNm instead of 130 mNm as it is for wheat. A possible explanation to the lower peak torque values may be due to the lack of sorghum functionality (Taylor et al., 2016). The second major finding is that mixing the composite flour at 30°C and 120 rpm using 64.0% water gave the peak torque close to the target peak torque of 87 mNm. Composite dough mixed under these conditions was stable and softened to a lesser extent than other speed and temperature combinations, suggesting that they probably developed to an optimum consistency.

4.6 Conclusion

This study identified the optimum conditions (temperature and speed) for mixing the sorghum-wheat composite dough to maximum development, these conditions were 30°C and 120 rpm using 64.0% water addition. The composite dough mixed under these conditions were stable and softened to a lesser extent, showing a desired consistency. The standard method developed in this chapter has overcame the limitations of the previous chapter (Chapter 4.1). The hand mixing was replaced by the use of the controlled mixing of the micro-doughLAB. Standard conditions of water absorption, time, temperature and speed to give maximum developed sorghum-wheat composite dough has been established.

The standardised method developed in this chapter will next be used to evaluate the dough forming ability of diverse sorghum genotypes in the following chapter.

CHAPTER 5

Evaluation of dough forming ability of different sorghum genotypes

5.1 Abstract

The objective of this study was to evaluate the dough forming ability of 25 sorghum genotypes based on their mixing parameters of peak torque, dough development time, dough stability and degree of softening in the whole grain sorghum-whole grain wheat composite system. The standard micro-doughLAB mixing method developed in the previous study (Chapter 4) was used, that is, a 50:50 ratio of whole grain sorghum to whole grain wheat composite flour was mixed at 64% water (flour basis), 120 rpm speed and 30°C to a target peak torque of 87 mNm. Amongst the genotypes the range of peak torque was 74-100 mNm; DDT was 2.2-3.1 min, stability was 1.6-3.5 min and degree of softening was 13.7-30.1 mNm. There was overall effect of genotype on all mixing parameters. In terms of peak torque two genotypes (NGT16N434-2 and NGT17N208-1) had values that were not significantly different from the target. NGT16N438 had the longest stability ($P < 0.05$) whereas NGT16N434-1 had the shortest. Sample NGT17N216 had the lowest degree of softening, however, its peak torque was in the lowest range. The two samples with peak torque close to the target, however, had intermediate stability and degree of softening. The method was able to identify differences in mixing quality between the sorghum genotypes. However, no individual genotype demonstrated good combination of peak torque, stability and degree of softening. This research provides some new information on dough forming ability of sorghum genotypes when mixed under standard conditions, however, analysis of more diverse genotypes is required to identify those with mixing qualities most suitable for bread making.

5.2 Introduction

The previous chapter showed that the optimum conditions giving peak torque close to target peak (87 mNm) and longest stability to mixing the composite flour of 50:50 whole grain sorghum and whole grain wheat were 30°C and 120 rpm using 64% water addition. In wheat breeding program for instance, phenotyping very large number of samples of limited size is required to identify traits such as good dough mixing properties (Fu, Wang, Dupuis, & Cuthbert, 2017). Empirical mixing tests have been used for more than 80 years to provide basic assessment of wheat dough strength and mixing requirements to assist screening and selection of new wheat varieties for bread making (Haraszi et al., 2004). However, these types of tests have rarely been used to evaluate dough mixing properties of different sorghum genotypes (Goodall et al., 2012).

The flour of white sorghum is considered useful for food products because it has a bland taste and does not impart unusual colour (Taylor, Schober, & Bean, 2006). However, there is lack of research on white sorghum varieties being developed in Australia, in terms of end-use functionality in dough for bread making. Such data will be valuable to select genotypes with good dough forming traits. Therefore, the objective of this study was to evaluate dough mixing properties of 25 sorghum white cultivars using the micro-doughLAB standard method developed in Chapter 4.2. The aim is to identify genotypes with good mixing properties, which might be included in the breeding program.

Figure 5.1 Appearance of Australian white sorghum cultivars used in this research

Figure 5.1 continued

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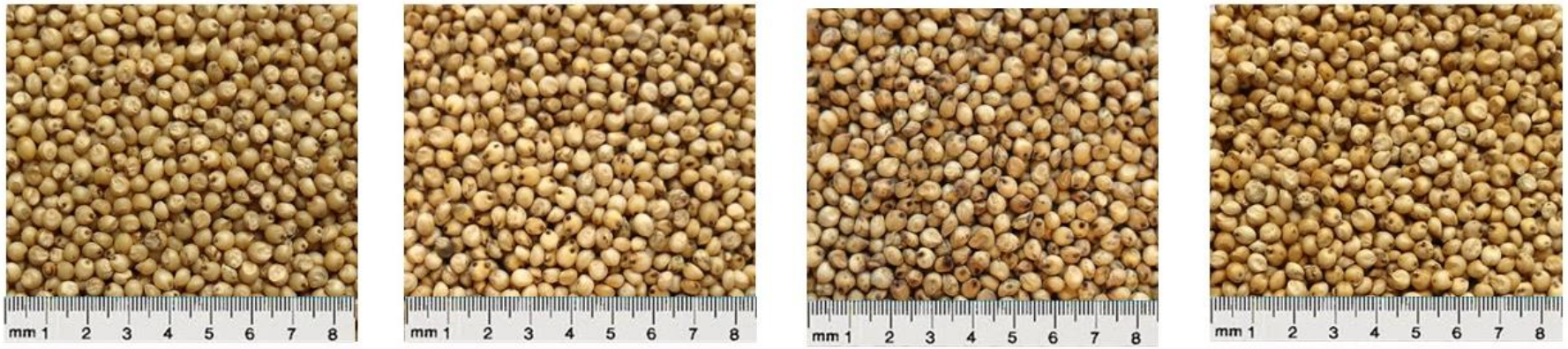

NGT17N184 NGT16N435 NGT16N437 NGT16N434

Figure 5.1 continued

5.3 Materials and Methods

Twenty five white sorghum cultivars (Figure 5.1) grown in Norwin and Clifton and harvested 2017 and 2018 were obtained from Nuseed (Queensland, Australia), and used in this study.

The control genotype Liberty was grown in Norwin and harvested in 2016. One of the genotypes, Liberty was grown in the same location (Clifton) and harvested in two different years (2017 and 2018). NGT16N434 was grown in two different locations (Clifton and Norwin) and harvested two different years in 2017 and 2018. NGT17N208 was grown in two locations (Clifton and Norwin) and harvested in the same year 2018.

The grains were separately milled and their dough mixing properties determined using the standard Micro-doughLAB mixing method as previously in Chapter 4.

5.4 Statistical analysis

Data is reported as means ± SD. The dough mixing parameters were compared by one-way analysis of variance (ANOVA). Individual means were compared by Tukey post-hoc test. $P < 0.05$ was considered significant. SPSS V24 (SPSS, Chicago, IL, USA) was used for the analyses.

5.5 Results and discussion

Amongst the genotypes the range of peak torque was 74-100 mNm; DDT was 2.2-3.1 min; stability was 1.6-3.5 min and degree of softening was 13.7-30.1 mNm (Table 5.1). There was overall effect of genotype on all mixing parameters ($P < 0.05$). The variation in mixing parameters is probably due to differences in composition and structure of the sorghum genotypes.

In terms of peak torque, two genotypes NGT16N434-2 and NGT17N208-1 had values that were similar ($P < 0.05$) from the target (88 mNm). However, they required significantly longer dough development time ($P < 0.05$) compared to the Liberty standard. The stability of these two genotypes was in the high range and their degree of softening was intermediate compared to other genotypes. This, indicates that the dough of these genotypes are likely to withstand mixing and handling processes better other genotypes.

Under these standard conditions of the screening method, sample NGT16N34-1 did not produce good mixing properties. This sample had the highest peak torque (100mNm) but gave a noisy curve (for example Figure 4.2.2) and longest dough development time ($P < 0.05$) compared to other genotypes. In addition, it had the shortest stability and highest degree of softening compared to other genotypes. These mixing properties indicate that NGT16N434-1 was probably not may be completely hydrated under the standard conditions of the method. This genotype grown in Clifton was also significantly different in mixing properties compared to the same genotype (NTG 434-2) grown in Norwin.

The longest stability was demonstrated for NGT16N438 ($P < 0.05$) compared to other genotypes, however, it did not reach the target peak torque.

The lowest degree of softening was found in sample NGT17N216, however, its peak torque was in the low range compared to other genotypes.

1604 **Table 5.1** Mixing quality of 25 whole grain sorghum genotypes mixed with whole grain wheat at 1:1 ratio[1]

Sample	Peak torque (mNm)	DDT (min)	Stability (min)	Softening (mNm)
***Liberty*[2]**	***88.0^{h} ± 1.0***	***2.2^{a} ± 0.1***	***2.2^{ab} ± 0.1***	***22.3^{fg} ± 0.6***
NGT17N216	74.0^{a} ± 1.7	2.8^{fg} ± 0.1	2.5^{abc} ± 0.1	13.7^{a} ± 1.2
18G393	76.3^{ab} ± 0.6	2.7^{def} ± 0.1	2.6^{abc} ± 0.1	17.7^{bcd} ± 0.1
LS	76.3^{ab} ± 1.2	2.5^{bcd} ± 0.1	2.2^{ab} ± 0.1	23.0^{fg} ± 1.0
18G533	77.0^{ab} ± 1.0	2.7^{def} ± 0.1	2.8^{bc} ± 0.1	18.0^{bcde} ± 0.0
NGT17N191	77.7^{abc} ± 0.6	2.7^{def} ± 0.1	2.7^{abc} ± 0.1	18.7^{bcde} ± 0.1
Liberty-1	78.0^{abcd} ± 1.0	2.4^{abc} ± 0.1	2.3^{ab} ± 0.1	20.7^{defg} ± 0.1
18G388	78.3^{bcd} ± 3.1	2.6^{cde} ± 0.1	2.8^{bc} ± 0.1	15.3^{ab} ± 1.2
18G537	78.3^{bcd} ± 1.2	2.4^{abc} ± 0.0	2.3^{ab} ± 0.1	16.0^{ab} ± 1.7
Liberty-2	78.3^{bcd} ± 1.2	2.6^{cde} ± 0.0	2.7^{abc} ± 0.1	18.7^{bcde} ± 2.1
HS	78.3^{bcd} ± 0.6	2.5^{bcd} ± 0.0	2.1^{a} ± 0.1	18.0^{bcde} ± 0.0
NGT16N436	78.7^{bcd} ± 2.5	2.7^{def} ± 0.1	2.8^{bc} ± 0.1	19.7^{cdef} ± 1.5
18G391	79.7^{bcde} ± 1.5	2.4^{abc} ± 0.1	2.0^{ab} ± 0.1	17.0^{abc} ± 1.0
18G390	80.0^{bcde} ± 1.0	2.5^{bcd} ± 0.1	2.4^{abc} ± 0.1	17.3^{bcd} ± 1.2
18G389	80.0^{bcde} ± 1.7	2.6^{cde} ± 0.0	2.5^{abc} ± 0.1	18.3^{bcde} ± 1.5
NGT17N192	80.0^{cde} ± 1.0b	2.7^{def} ± 0.1	3.0^{bc} ± 0.2	18.3^{bcde} ± 1.5
NGT17N208-2	81.3^{cdef} ± 0.6	2.7^{def} ± 0.1	2.7^{abc} ± 0.1	17.3^{bcd} ± 0.1
18G552	81.7^{cdef} ± 0.6	2.5^{bcd} ± 0.1	2.7^{abc} ± 0.1	20.0^{cdefg} ± 0.0
NGT16N438	81.7c^{def} ± 0.6	2.5^{bcd} ± 0.1	3.5^{c} ± 1.7	23.3^{g} ± 0.1
NGT17N217	82.0^{def} ± 1.7	2.3^{ab} ± 0.1	2.2^{ab} ± 0.1	21.3^{defg} ± 1.5
NGT17N184	83.0e^{fg} ± 1.0	2.7^{def} ± 0.1	3.1^{bc} ± 0.1	19.7^{cdef} ± 0.1
NGT16N435	83.3e^{fg} ± 1.5	2.5^{bcd} ± 0.1	2.7^{abc} ± 0.1	20.0^{cdefg} ± 1.0
NGT16N437	84.7^{fg} ± 1.5	2.4^{abc} ± 0.1	2.3^{ab} ± 0.1	22.3^{fg} ± 1.5
NGT16N434-2	86.3^{gh} ± 0.6	2.6^{cde} ± 0.0	3.1^{bc} ± 0.1	19.7^{cdef} ± 1.2
NGT17N208-1	87.0^{gh} ± 0.0	2.6^{cde} ± 0.0	2.9^{bc} ± 0.1	18.7^{bcde} ± 0.1
NGT16N434-1[3]	100.0^{i} ± 1.0	3.1^{h} ± 0.1	1.6^{a} ± 0.4	30.1^{h} ± 1.0

[1] Mean ± Standard Deviation, n=3. Mean values in a column with different superscript letters are significantly different ($P < 0.05$).

[2] Internal standard as used to develop of the standard micro-doughLAB mixing method (Chapter 4).

[3] The mixing curve to peak torque of this sample was noisy, indicating lack of formation of a cohesive dough.

5.6 Conclusion

The mixing attributes of the sorghum genotypes were successfully determined using the Micro-doughLAb mixing method developed in Chapter 4. The new knowledge generated in this chapter was that the mixing attributes of the genotypes differed under the standard conditions. Amongst those genotypes, NGT16N434-2 and NGT17N208-1 had the best combination of mixing attributes, suggesting that they might be more suitable for quality dough manufacture.

One limitation of this study is the lack of highly diverse genotypes, such high protein digestibility lines, genetically modified varieties with modified kafirin expression and protein bodies' structures. I recommend that these genotypes are investigated in the future studies, which is beyond the scope of this study.

The outcome of this study and the proposed further genotype screening may help sorghum pre-breeding and commercial breeding programs to select lines for breeding of new sorghum varieties with more useful functionality for manufacture of quality for leavened bread.

PHASE 2

Effect of lupin protein on commercial zein viscoelastic properties

CHAPTER 6

Characterization of zein-lupin protein copolymer viscoelastic masses made by coacervation from ethanol or dilute acetic acid plus hand kneading and sheeting

6.1 Abstract

Prolamin proteins from sorghum (kafirin) and maize (zein) tend to have limitation in leavened dough for bread products due to their hydrophobic and tightly-packed nature, which prevent both the water absorption and protein inter-chain reactions needed for hydration and development of a viscoelastic gas-holding dough. However, there are evidence that the addition of a small protein to commercial zein (essentially α-zein) can enhance its viscoelastic properties. A limited amount of research has demonstrated that the use of a small amount of the high protein, leguminous seed Australian sweet lupin in wheat-based dough has the potential to improve the dough functional properties. Therefore, isolated lupin protein was combined with zein viscoelastic mass prepared either in aqueous ethanol by coacervation or dilute acetic acid by hand kneading plus sheeting. The objective of this study was to determine if lupin protein can act as a co-protein to improve the viscoelastic properties of zein.

Zein prepared with aqueous ethanol and combined with lupin protein formed a sediment when coacervated with cold water. This was probably due lupin protein insolubility in aqueous ethanol.

In contrast, combining the zein and lupin viscoelastic masses separately prepared in dilute acetic acid and water, respectively gave very different viscoelastic mass properties. This zein:lupin mass was cohesive with lots of entangled fibres. However, the rheological properties revealed that this mass was far less extensible than zein alone prepared in acetic acid. In addition, when formed into a dough, zein: lupin-starch composite could not hold air nor be inflated into a bubble by Alveograph. The absence of observable new molecular weight bands by SDS-PAGE, indicated that zein and lupin protein did not covalently interact to form a copolymer in any of the formulations.

6.2 Introduction

Formation of a viscoelastic mass is a critical attribute that allows a dough to withstand both the stress during mixing and handling, and expand by retaining gas bubbles during proofing, resulting in a light leavened product (Eliasson & Larsson, 1993). Commercial zein (essentially α-zein), the prolamin of maize has potential to form a dough (Lawton, 1992). Above its glass transition temperature (T_g), commercial zein in aqueous systems goes through a glass transition, which enables it to interact with water when mixed thereby forming a viscoelastic mass similar to gluten. An extensible zein fibre network formed during mixing is responsible for the observed viscoelastic behaviour (Lawton, 1992; Schober, Bean, Boyle, & Park, 2008; Schober, Moreau, Bean, & Boyle, 2010).

However, such aqueous zein viscoelastic masses have limited ability to resist extension compared to wheat gluten and lose their extensibility when allowed to rest at room temperature. This is related to the fact that when zein is cooled to 25°C and below, its squeezes out the water and returns from rubbery to its amorphous state, hence the dough becomes hard and loses its viscoelasticity (Lawton, 1992). Also commercial zein polypeptides have only one or two cysteine per subunit (Shewry & Tatham, 1990), suggesting that they cannot form extensive disulphide cross-linking to give extended polymers as in wheat gluten matrix.

Various methods have been studied to improve the functionality of commercial zein for potential use in leavened products. These include addition of hydrocolloids (Schober et al., 2008), chemical modification (Kim & Xu, 2008; Sly, Taylor, & Taylor, 2014; Taylor, Johnson, Taylor, Njila, & Jackaman, 2016; Taylor, Anyango, Muhiwa, Oguntoyinbo, & Taylor, 2018) and addition of co-proteins (Renzetti, Hamaker, Campanella, Jurgens, & Erickson, 2014; Mejia, Gonzalez, Mauer, Campanella, & Hamaker, 2012). These modifications somewhat improve commercial zein functionality, suggesting it could potentially produce high quality products.

The effects of the addition of a small amount of different protein to commercial zein to stabilize its viscoelastic mass have been reported. Erickson, et al. (2014) found that addition of casein to commercial zein dissolved in aqueous ethanol increased significantly the zein strength and elasticity as compared to zein alone. The authors referred to such proteins as co-proteins (Erickson, Campanella, & Hamaker, 2012). It

was hypothesised that casein interacted with commercial zein, which contributed to the zein dough strength and elasticity.

Some studies have demonstrated that the use of a small amount of protein from the protein-rich, legume seed lupin to gluten dough has the potential to improve the dough functional properties (Paraskevopoulou, Provatidou, Tsotsiou, & Kiosseoglou, 2010). This is attributed to unfolding of the lupin protein during mixing, resulting in intermolecular interactions with the gluten network (Pozani, Doxastakis, & Kiosseoglou, 2002). To date this potential has not been investigated with non-wheat prolamin proteins, such as commercial zein.

In this study, the effect of lupin protein on commercial zein viscoelastic mass was investigated. Two different methods of preparation of commercial zein:lupin protein viscoelastic masses were compared. The aim was to determine if lupin protein and commercial zein as co-proteins can interact to form a copolymer, which may have potential use in leavened products.

6.3 Materials and methods

6.3.1 Materials

Commercial zein Z3625 (Sigma-Aldrich, Johannesburg, South Africa), vital wheat gluten (Novozymes, Benmore, South Africa), dehulled Australian sweet lupin grits, variety *Coromup* (Coorow Seeds, Perth, Australia) and maize starch (Premier Foods, Isando, South Africa) were used in this study. *Coromup* was chosen because it has demonstrated good bread making properties and high protein content (Villarino, Jayasena, Coorey, Chakrabarti-bell, Foley, et al., 2015).

Lupin protein was isolated from the grits as described by Chew, Casey, & Johnson (2003). In summary, the grits were soaked in distilled water at ambient temperature for 3 hours and homogenised using a waring laboratory blender (Australian Scientific Pty Ltd, New South Wales, Australia). The pH of the slurry was adjusted to 8 to 9 with 1M $NaOH_{(aq)}$ to solubilise the proteins and centrifuged to remove the dietary fibre. To precipitate the lupin protein, the pH of the supernatant was adjusted to 4.5 using 1M $HCl_{(aq)}$ and centrifuged again. The pH of the protein pellet was then adjusted to 7.0 and freeze dried. Dried lupin protein was ground using a coffee grinder BCG 200 Breville

the Coffee & Spice™ (Breville, New South Wales, Australia), vacuum packed and stored at 10°C until use.

The moisture content of the commercial zein, gluten and lupin protein were determined in duplicate by oven drying to a constant weight according to the American Association of Cereal Chemists (AACC, 2000) Method 44-15A (One stage). Data were expressed as g/100 as is basis. The protein contents of these protein samples were determined in triplicate by the Dumas combustion standard method 46-30 of the American Association of Cereal Chemists (AACC, 2000). The protein contents were calculated using correction factors N ×6.5 for commercial zein, N×5.7 for gluten and N×5.5 for lupin protein (Mossé, 1990). Data were expressed as g/100 on dry basis (db).

6.3.2 Preparation of protein viscoelastic masses

Two different methods of preparations of protein viscoelastic masses and different protein ratios were compared:

Firstly, viscoelastic masses were prepared by the coacervation method of Erickson et al. (2014) with modifications.

- Controls were prepared as follow: viscoelastic mass of zein was made by dissolving 1.45 g of zein (91.8% protein, dry basis) in 8.06 mL 70% aqueous ethanol at 70°C. To prepare lupin control, 1 g (as is basis) of pre-warmed lupin was mixed with water at 40°C. Pre-warmed gluten 1.5 g (as is basis) was mixed with water (40°C) and used as standard.
- Masses made from zein and lupin protein composites were prepared at 4:1, 3:2 and 1:1 zein:lupin protein ratios. For 4:1 zein:lupin, 1.16 g zein was dissolved in 6.44 ml 70% aqueous ethanol at 70°C and 0.07 g lupin protein (71.2% protein on dry basis) dissolved in 2.1 ml pre-warmed distilled water at 70°C. For 3:2 zein:lupin, 0.87 g zein was dissolved in pre-warmed 4.83 ml 70% aqueous ethanol and 0.14 g lupin protein dissolved in pre-warmed 4.2 ml distilled water at 70°C. For 1:1 zein:lupin, 0.72 g zein was dissolved in pre-warmed 4.0 ml 70% aqueous ethanol and 0.35 g lupin protein dissolved in pre-warmed 10.50 ml distilled water at 70°C. The lupin suspensions were poured into the zein solution and immediately mixed with a spatula vigorously.

To coacervate the prepared suspensions, 102.07 g (zein alone), 102.36 g (4:1 zein: lupin protein), 102.65 g (3:2 zein:lupin protein) and 101.91 g (1:1 zein:lupin protein) of cold distilled water (5.8^{o}C) was poured rapidly without stirring to give a total weight of 110 g. Zein alone and 4:1 zein:lupin precipitated into fibres-like structures, which were collected as a soft mass using a spatula, and manipulated by hand until a cohesive mass was formed. However, 3:2 and 1:1 zein:lupin did not formed fibres, instead they both resulted in non-cohesive sediments, which could not be manipulated. Because of this, 3:2 and 1:1 zein:lupin protein sediments were not analysed further.

Secondly, using the method of Taylor et al. (2018) with modifications.

- For 1:1 zein:lupin, 1.09 g zein was dissolved separately in pre-warmed 2.18 g acetic acid 5.4% (w/w) at 40°C and 1.41 g lupin protein in 0.85 g water separately pre-warmed at 40°C. The separately prepared viscoelastic masses of zein and lupin protein were mixed together, hand kneaded and repeatedly passed through a pre-warmed pasta press at 40°C to mix them. Following each pass, the viscoelastic mass was double folded, rotated to 90°C clockwise and passed 5 times into the pasta press (Baccarat®, Melbourne, Australia) to standardize mixing.
- Controls were prepared by dissolving separately pre-warmed commercial zein alone 2.18 g in 4.36 g acetic acid at 40°C, lupin protein alone control, 1 g (as is basis) of pre-warmed lupin protein was mixed with pre-warmed water at 40°C and pre-warmed gluten 1.5 g (as is basis) was mixed with pre-warmed water at 40°C and used as standard. The two controls and gluten standard viscoelastic masses were passed through the pre-warmed pasta press at 40°C.

6.4 Analyses

6.4.1 Confocal Laser Scanning Microscopy (CLSM)

The microstructures of zein, lupin protein, zein:lupin protein and gluten viscoelastic masses were studied using a Zeiss 510 META Confocal Laser Scanning Microscope (Jena, Germany) fitted with a Plan-Neofluar 10×0.3 objective at an excitation wavelength of 405 nm under natural fluorescence (Elhassan et al., 2017). Each piece of approximately (7×3×1 mm) was stretched over a glass slide, then viewed using autofluorescence.

6.4.2 Scanning electron microscopy (SEM)

The surface morphology of zein, lupin protein, zein:lupin protein and gluten viscoelastic masses were studied using a Zeiss Ultra PLUS Field Emission Gun SEM (Oberkochen, Germany). Viscoelastic masses of approximately (6×4×1 mm) were stretched and mounted on an aluminium stab with double-sided tape. They were then air dried in a desiccator for 2 days and half and coated with gold before viewing.

6.4.3 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was used to determine if zein combined with lupin protein formed a true copolymer. Various parts of the zein, lupin protein, zein-lupin protein and gluten viscoelastic masses were characterised by SDS-PAGE under reducing and non-reducing conditions using the method described by (Taylor, Anyango, & Taylor, 2013). A vertical X Cell SureLock^{TM} Mini-Cell electrophoresis unit (Invitrogen Life Technologies, Carlsbad, CA, USA) with a 4-12% Bis-Tris gradient gel of 1 mm thick and 15 wells was used to run the samples. Invitrogen mark12 Unstained Standard was used. Samples were suspended in either reducing or non-reducing buffer then placed in boiling water for at least 15 minutes with vigorous vortexing every 5 minutes to ensure that all proteins were completely dissolved. For every sample, 10 µl protein (1 µg protein/1 µl) and 5 µl molecular weight standard were loaded on the gel. The gels were stained with Coomassie Brilliant Blue R-250 and scanned using a flatbed scanner.

6.4.4 Tensile properties of viscoelastic masses

Tensile properties were analysed using a Kieffer rig-type extensimeter as described by King, Taylor, & Taylor, 2016. Moulded pieces of the viscoelastic masses were placed over a vertical struts (30 mm apart) of the Kieffer rig and firmly held at both ends. The hook extended the viscoelastic masses at a constant speed of 3.3 mm/s over distance of 150 mm (maximum displacement of the texture analyser). The test was performed within 3 minutes at ambient temperature to prevent the masses from cooling below the T_g of zein.

6.4.5 Stress-relaxation of protein viscoelastic masses

The viscoelastic masses were analysed for their stress-relaxation properties compared to that of gluten, as described by Taylor et al. (2018). Approximately 0.5 g (4 mm long

× 5mm thick) of each mass piece was transferred on the base plate of a Shimadzu Scientific Instruments EZ-Test texture analyser (Kyoto, Japan), fitted with a 10 mm cylindrical probe for analysis. A single compression test was carried out with a test speed of 0.5 mm/s, 1 mm distance and a relaxation time of 100s. Tests were repeated at 5, 10 and 15 min on the same viscoelastic mass preparations. F Max (the maximum force at compression), Ft (the force at the time from F Max at which fresh gluten has relaxed to 38.6% of its maximum force (13.4 s) and SR (% stress recovery at 13.4 s from F Max) were measured according to Singh, Rockall, Martin, Chung, & Lookhart (2006).

6.4.6 Alveography

Model doughs made from zein-starch, (zein plus lupin)-starch and lupin-starch mixtures prepared with either acetic acid and/or water were analysed using Alveograph (Chopin NG Constistograph, Paris), as described by Sly et al. (2014):

- For zein-starch dough, 15 g of zein were thoroughly mixed with 15 g of starch using a spatula. The composite was pre-warmed to 50°C. Acetic acid 2.7% (w/w) at 60% flour basis pre-warmed to 50°C was added to the zein-starch composite. The liquid was incorporated and the mixture was stirred with the spatula, and then hand kneaded for approximately 5 minutes, and sheeted as described in *6.3.2*.
- For production of the 1:1 zein plus lupin-starch dough, the zein-starch and lupin-starch doughs were prepared separately. The zein (7.5 g) was mixed with the starch (7.5 g) then 2.7% (w/w) acetic acid at 30% flour basis was added. The mixture was stirred with the spatula and hand kneaded for approximately 5 minutes. The lupin (9.55 g) was mixed with the starch (7.5 g) and pre-warmed to 50°C. Then pre-warmed water (50°C) was added at 30% flour basis and the dough formed as per zein-starch dough excluding sheeting. Both zein-starch and lupin-starch doughs were combined, kneaded and sheeted as described for zein-starch dough. The resulting dough was sticky and broke easily during handling. However, it possible to mould it carefully and analyse it.
- For lupin alone dough, 15 g lupin was mixed with 15 g starch and pre-warmed to 50°C. Water (50°C) was added at 60% flour basis and the dough formed as per zein-starch dough. The lupin-starch dough was very sticky and not cohesive. It was not easy to handle, therefore it could not be analysed.

- Gluten alone dough used as standard was formed by mixing 15 g gluten (as is basis) with 15 g starch and pre-warmed to 50°C. Water (50°C) was then added at 60% flour basis as per zein-starch dough.

It was hypothesised that the stickiness of the zein plus lupin-starch and lupin-starch doughs were possibly due to relatively high amount of protein and low amount of starch. Therefore, the amount of starch was increased to 1:3 protein-starch ratio (zein-starch; zein plus lupin-starch, lupin-starch and gluten-starch) and their dough properties compared.

All doughs were formed into round patties and allowed to rest at 35°C (the highest instrument setting) for 20 minutes before inflation took place. Alveograph was performed and P, L, P/L and W recorded.

6.5 Statistical analysis

Data is reported as means ± SD. The moisture, protein and the protein mass tensile properties were subjected to one-way analysis of variance (ANOVA). For stress relaxation, the main effects of preparation method of protein mass and resting time, and their interaction were analysed by two-way analysis of variance. If significant main effect was observed, then one-way analysis of variance was used to separate individual sample means. Individual means were compared by Tukey post-hoc test. $P < 0.05$ was considered significant. SPSS V24 (SPSS, Chicago, IL, USA) was used for the analyses.

6.6 Results and Discussion

6.6.1 Protein content of commercial zein, gluten and lupin protein

The protein content of commercial zein, gluten and lupin protein are shown in Table 6.1. Among the three samples, lupin protein had the lowest protein content of approximately 71%, which was lower than the 87% reported by Chew et al. (2003) using the same extraction method but different analytical method. The low protein content of the lupin was probably due to the dilution effect of other components from the original kernel present such as fat, fibre and ash (Villarino, Jayasena, Coorey, Chakrabarti-bell, & Johnson, 2015). Unlike the lupin protein, which is a concentrate, the commercial zein is highly purified protein isolate with a protein content above 90%.

Table 6.1 Protein contents of commercial zein, gluten and lupin protein (g/100 g)

Protein	Moisture (g/100 g)	Protein[1] (g/100 g) as is	Protein (g/100 g) on dry basis
Commercial zein	$3.43^{b} \pm 0.20$	$88.65^{b} \pm 0.31$	$91.81^{c1} \pm 0.32$
Gluten	$10.78^{c} \pm 0.18$	$70.63^{a} \pm 0.29$	$79.69^{b} \pm 0.32$
Lupin protein	$2.35^{a} \pm 0.33$	$69.55^{a} \pm 0.40$	$71.21^{a} \pm 0.40$

[1]Calculated using correction factors N ×6.5 for commercial zein, N×5.7 for gluten and N×5.5 for lupin protein

[2] Mean ± Standard Deviation, n=2 for moisture and n=3 for protein. Mean values in a column with different superscript letters are significantly different ($P < 0.05$).

6.6.2 Viscoelastic mass formation

Upon stretching, gluten viscoelastic mass had a visible fibril-like structure (Figure 6.1A) compared to the other protein masses (Figures 6.2 and Figure 6.3). These fibrils were clearly seen by the CLSM (Figure 6.1B, indicated by white solid arrow). On the surface of the gluten mass, two types of spherical shapes were visible using SEM (Figure 6.1C, S: A and B as indicated by black solid arrows). These were presumed to be two types of wheat starch granules. The large disk-like shape is attributed to A and the small polygonal B starch types (Evers, 1971).

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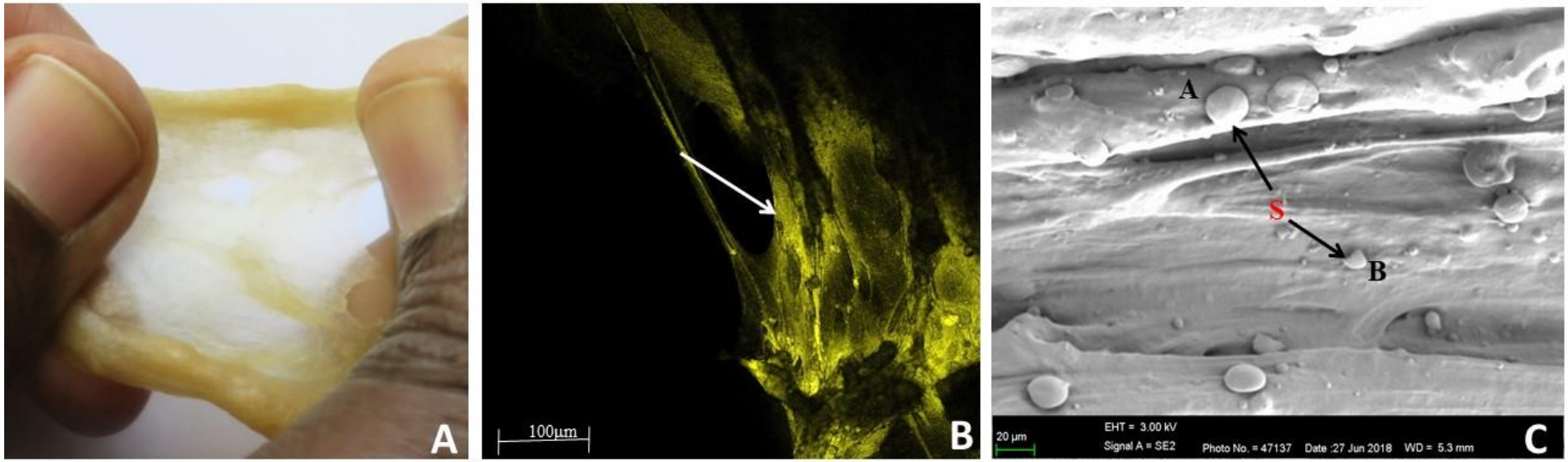

Figure 6.1 Images of gluten viscoelastic masses.

A- Manually stretched,
B-Viewed under confocal laser scanning microscopy-CLSM (white solid arrow indicates fibre formation) and
C-Surface morphology of gluten viscoelastic mass under SEM (black solid arrows indicate two types of starch granules: S = starch granules; both A and B are two types of wheat starch granules).

6.6.2.1 Viscoelastic masses made by coacervation from aqueous ethanol-water

Zein coacervated from aqueous ethanol with cold water gave a soft fibrous mass. After hand kneading, the zein particles merged to form a fibrous structure, possibly as a result of hydrogen bonding (Lai & Padua, 1997; Li et al., 2012). The still warm zein structure displayed visible fibres when stretched (Figure 6.2A). These fibres were clearly seen by CLSM and were approximately 1 to 10 μm in diameter (Figure 6.2D, indicated by a solid white arrow). The zein mass was extensible when stretched and recovered its shape when stretched not past its elastic point. SEM showed that the fibres were present on the surface of the stretched zein viscoelastic mass (marked F, Figure 6.2G, indicated by a solid black arrow). The zein viscoelastic mass became very stiff upon cooling to ambient temperature and broke when stretched.

When making the 4:1 zein:lupin mass, the lupin suspension was poured into the zein solution forming a milky-like suspension. This milkiness was presumed to be a fine lupin protein precipitate due to its insolubility in the ethanol as it is hydrophilic (Sathe, Deshpande, & Salunkhe, 1951). This sample gave a lumpy soft fibrous mass (Figure 6.2B, lumps indicated by black arrows), which broke easily when stretched. CLSM and SEM showed that the fibres in the sample were short with uneven surfaces (Figure 6.2E). The short fibres were probably zein truncated due to disruption by lupin protein particles.

The 1:1 ratio of lupin to zein gave precipitation of both proteins by coacervation with cold water forming a sediment, which was gritty (Figure 6.2C). The high amount of lupin protein probably prevented the formation of the zein fibre completely. As a result the two proteins did not form a copolymer. The microscopy showed that the sediment appeared as scattered particles, which could be an amorphous mixture of both proteins (Figure 6.2F and Figure 6.2H).

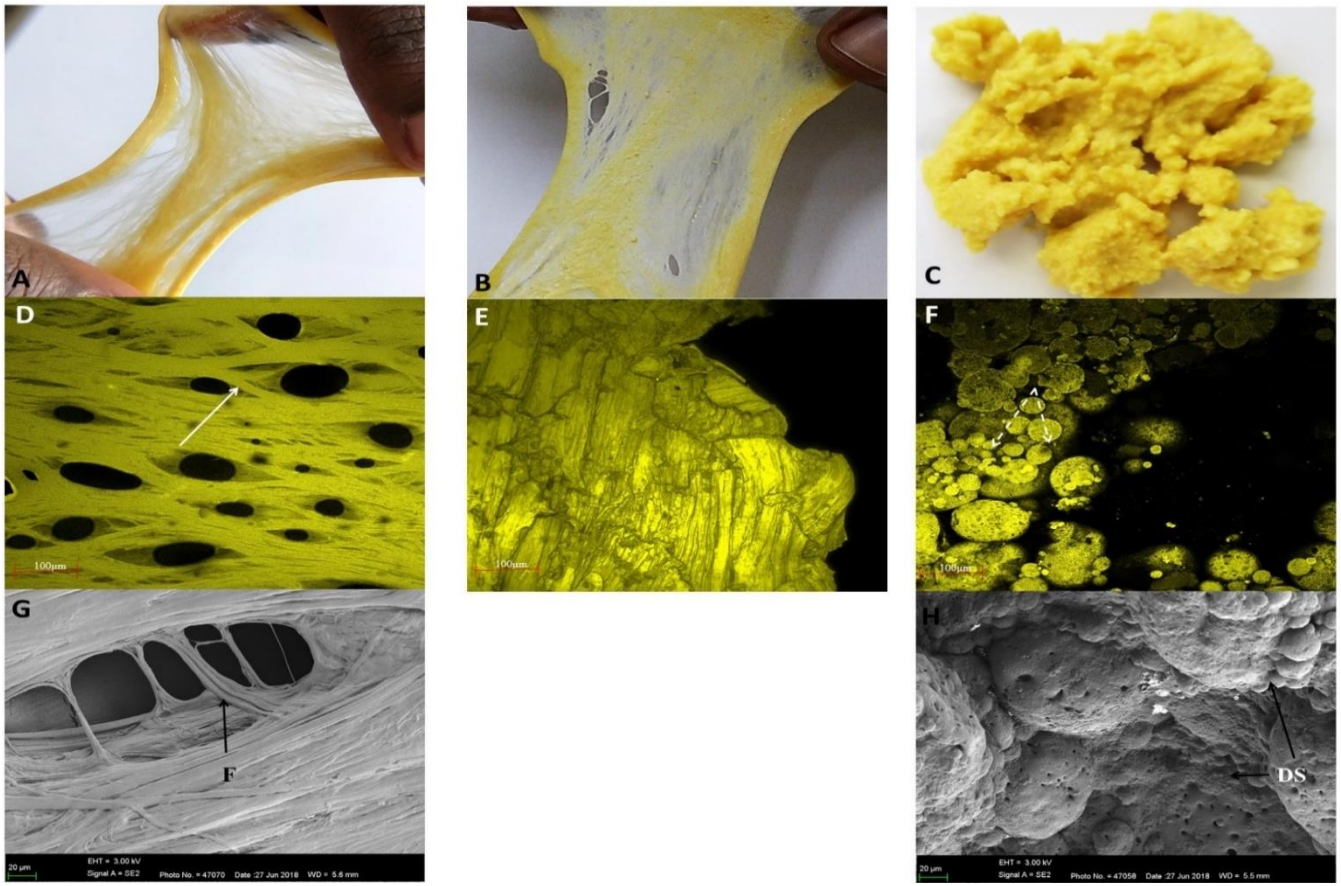

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Figure 6.2 Images of coacervated zein and lupin protein masses from aqueous ethanol-water.
Manually stretched, **A**- zein, **B**- 4:1 zein:lupin and **C**-1: 1 zein: lupin protein masses.
CLSM of coacervated zein and lupin protein masses, **D**- zein, **E**- 4:1 zein:lupin and **F**-1: 1 zein: lupin protein masses. White solid arrow show fibril formation, and dash arrow shows non-cohesive masses of zein and lupin proteins.
SEM of surface morphology of coacervated zein and lupin protein, **G**-, zein and **H**-1: 1 zein: lupin protein masses. Black solid arrow indicated by F show fibre and those indicated by DS show dispersed particles of zein and lupin proteins.
Note that 4:1 zein:lupin protein mass was not viewed under SEM.

6.6.2.2 Viscoelastic masses made with dilute acetic acid plus kneading and sheeting

The zein prepared with dilute acetic acid gave a soft fibrous mass (Figure 6.3A), which in contrast to that coacervated from ethanol was smoother (Figure 6.4A) and did not stiffen immediately upon cooling to ambient temperature. This observation may be due to the protonation of acidic amino acids side chains of the zein, which modified the protein-protein interactions compared to that of ethanol (Li et al., 2012). Similar results showing that zein dissolves in dilute acetic acid were reported by Sly et al. (2014) and Taylor et al. (2018) studying zein viscoelastic mass characteristics using dilute organic acids and factors that influence zein and kafirin viscoelastic mass formation, respectively.

The CLSM of this zein mass indicated fine fibres closely packed together (Figure 6.3D, indicated by white solid arrow) compared to that made of coacervation from ethanol (Figure 6.4E). Numerous and closely associated fibres were previously observed in zein masses prepared using acetic acid (Sly et al., 2014; Taylor et al., 2018). CLSM showed that the stretched zein mass made with acetic acid formed continuous fibrils compared to that coacervated from ethanol, which had holes in the fibre matrix (Figure 6.4E). The formation of the unbroken fibre network may improve extensibility.

Lupin protein mixed with water at 60% weight to volume basis formed an adhesive sticky mass. After rolling the lupin protein mass many times by hand, it became cohesive and recovered its shape when manually compressed (Figure 6.3B), indicating that it was somewhat elastic. The stretched lupin mass did not form fibrils (Figure 6.3B), instead it formed hydrated particles viewed with CLSM (Figure 6.3E). Nonetheless, when observed under the SEM (Figure 6.3H), there was fairly short fibre formed on the surface of the lupin protein mass.

Given that each zein and lupin formed cohesive masses in their respective solvents, these masses were prepared separately and mixed together by kneading and sheeting, which gave a cohesive mixture. As shown in Figure 6.3C, the combined mass was soft and cohesive with lots of entangled fibres. The fibres were more visible than in zein alone using the CLSM and SEM (Figure 6.3F and Figure 6.3I, indicated by solid

arrows). This is in great contrast of the zein:lupin mass made by coacervation from ethanol and water, which formed a non-cohesive mass (Figure 6.4G and Figure 6.4K).

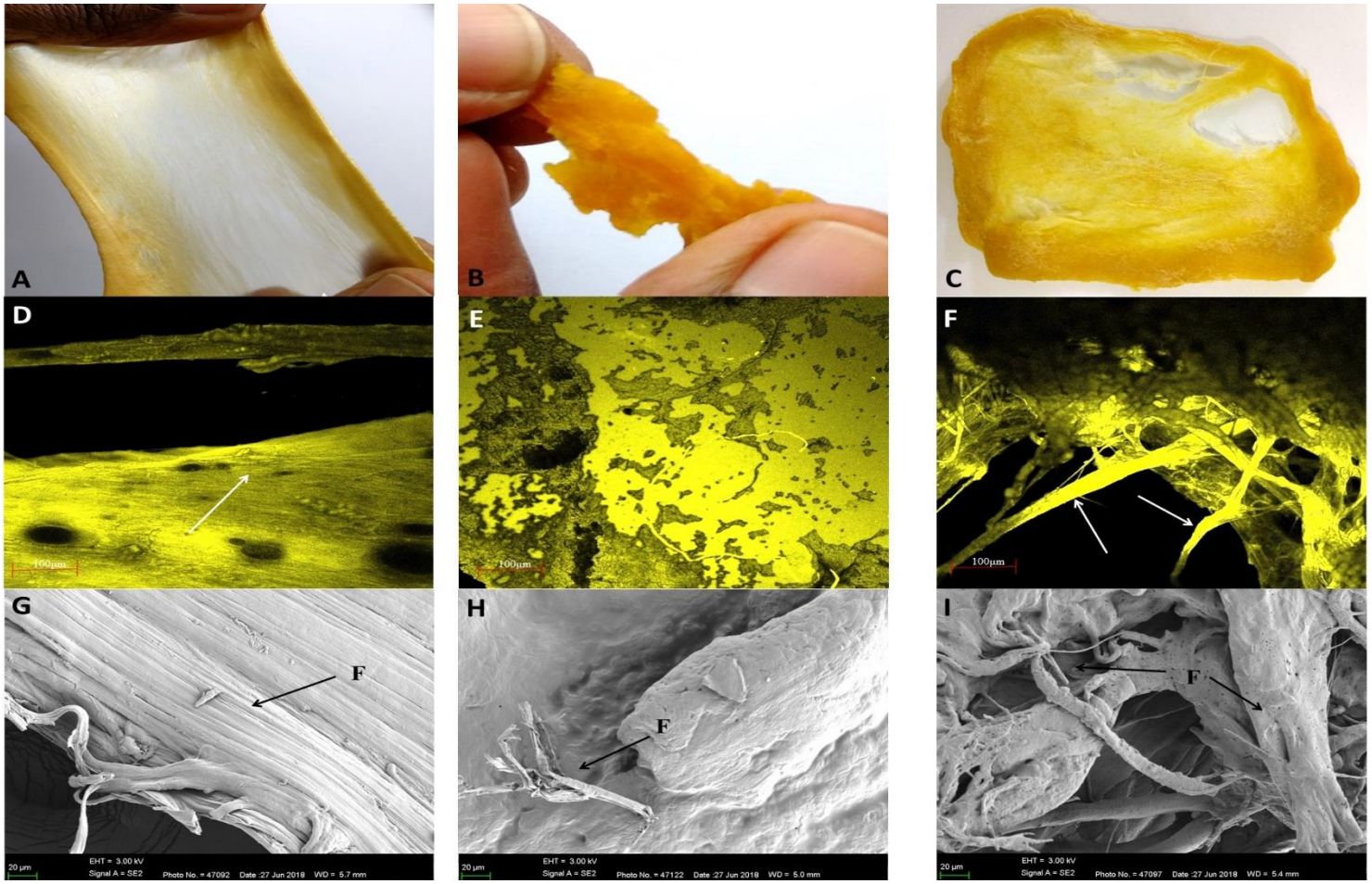

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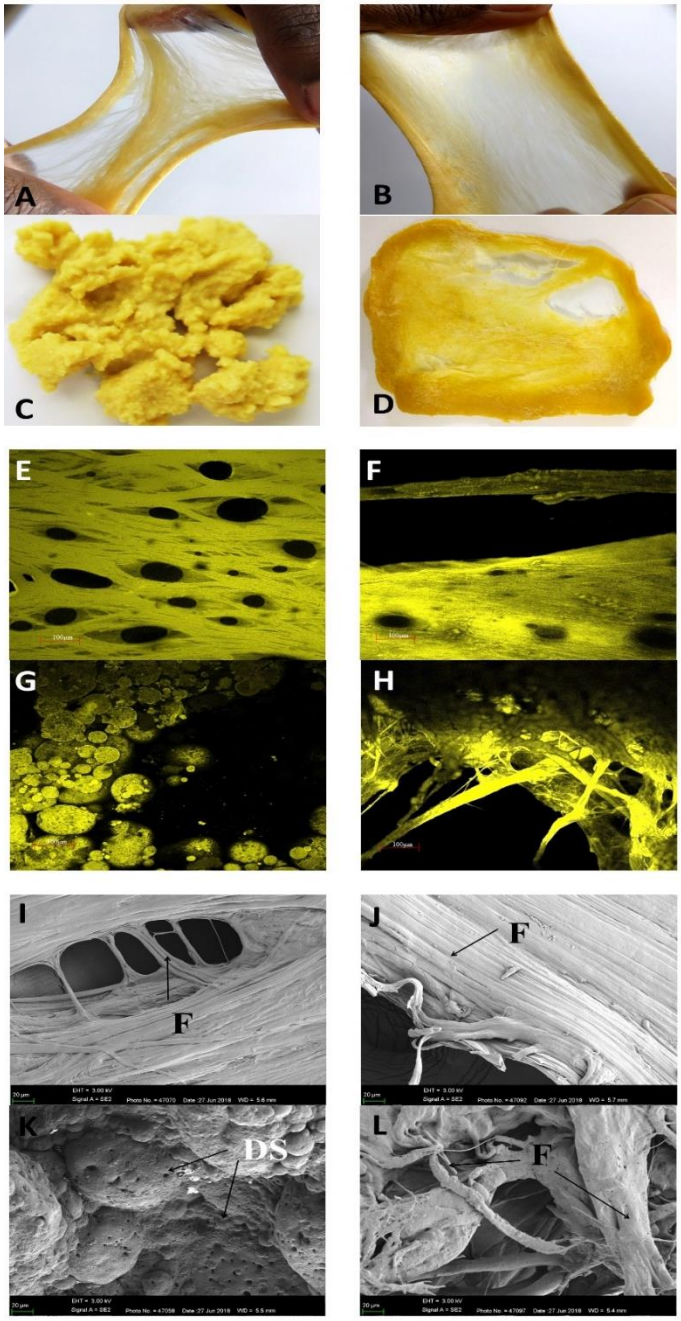
Figure 6.3 Images of zein and lupin protein masses made with dilute acetic acid and water plus kneading and sheeting.
Manually stretched, **A**- zein, **B**-lupin and **C**-1: 1 zein: lupin protein masses
CLSM of zein and lupin protein masses, **D**- zein, **E**- lupin and **F**-1: 1 zein: lupin protein masses. White solid arrows show fibre formation.
SEM of surface morphology of zein and lupin protein, **G**- zein, **H**- lupin and **I**- 1: 1 zein: lupin protein masses. Black solid arrows show fibre formation.



1957 **Figure 6.4** Comparison between zein and lupin protein masses made coacervation from ethanol-water and those made with dilute acetic acid and water plus kneading and sheeting.

-Manually stretched, **A**- zein and **C**-non-cohesive mass of 1: 1 zein: lupin made by coacervation. **B** -zein and **D**- cohesive mass of 1:1 zein:lupin made with dilute acetic acid plus kneading and sheeting.

-CLSM of zein and lupin protein masses, **E**- zein and **G**- non-cohesive mass of 1: 1 zein: lupin made by coacervation. **F**- zein and **H**- cohesive mass of 1:1 zein:lupin made with dilute acetic acid plus kneading and sheeting.

-SEM of surface morphology of zein and lupin protein masses, **I**- zein and **K**- non-cohesive mass of 1: 1 zein: lupin made by coacervation. **J**- zein and **L**- cohesive mass of 1:1 zein:lupin made with dilute acetic acid plus kneading and sheeting.

6.6.3 SDS-PAGE of protein masses

SDS-PAGE was used to determine if zein combined with lupin protein formed a true copolymer. The zein alone (Figure 6.5, lane 2) and 4:1 zein:lupin (results not shown) protein masses coacervated from ethanol had identical band patterns under both non-reducing and reducing conditions. Both the zein and 4:1 zein:lupin protein masses comprised of zein monomers, dimers and trimers, with low levels of the latter (Oom et al., 2008). The identical band patterns of the zein and 4:1 zein:lupin protein masses, means that the latter was essentially zein. This indicates that zein and lupin protein did not interact to any significant extent to form a copolymer.

There was joint presence of zein and lupin protein bands observed in both 1:1 zein:lupin protein sediment coacervated from aqueous ethanol and 1:1 zein:lupin protein mass prepared with dilute acetic acid plus kneading and sheeting, either under non-reducing (compare lanes 3-5 and 9-12, Figure 6.5A) or reducing conditions (compare lanes 3-5 and 9-12, Figure 6.5B). However, the coacervated zein:lupin sediment had fainter bands at approximately 35-75 kDa compared to its counterpart mass prepared with dilute acetic acid (compare lanes 3-5 and 9-12, Figure 6.5A). This indicates less presence of the lupin protein in the former sample, probably due to incomplete coacervation of the lupin protein with the zein, and some left in the solution. On the other hand, the high intensity of lupin protein in the 1:1 zein:lupin protein masses prepared with dilute acetic acid and water was due to direct mixing of both the zein and lupin protein masses.

A copolymer is formed with two or more structural units connected by covalent bonds and its presence is demonstrated by formation of visible higher new molecular weight bands when running an SDS-PAGE under non reducing conditions (Ravve, 1995; Sikkema et al., 2007). The absence of new molecular weight bands in both 1:1 zein:lupin protein sediment coacervated from aqueous ethanol and 1:1 zein:lupin protein mass prepared with dilute acetic acid plus kneading and sheeting, suggests that there was no such interactions to form a copolymer between the zein and lupin proteins. This finding is similar to the absence of disulphide cross-links under-reducing conditions reported by Taylor et al. (2016) in oxidised commercial zein with hydrogen peroxide. The authors attributed this to the lack of cysteine groups in commercial zein for substantial disulphide bonding.

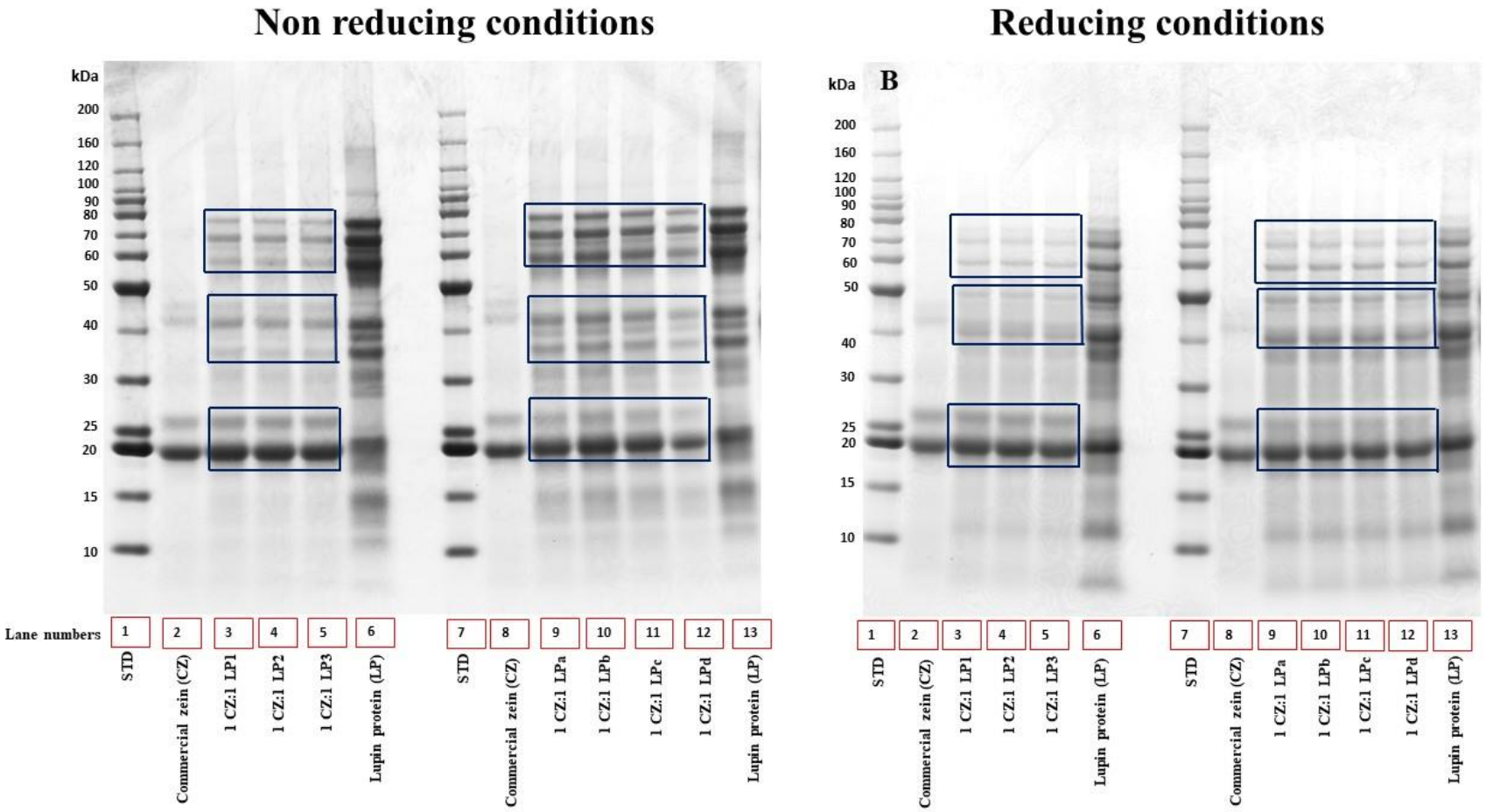


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Figure 6.5 SDS-PAGE of commercial zein (CZ) and lupin protein (LP) masses under (A) non reducing and (B) reducing conditions. Lane 1 and 7 = molecular weight standard; Lane 2= zein coacervated from aqueous ethanol; Lane 8 = zein made with 5.4% acetic acid; Lane 3-5 and 9-12 = various parts of 1: 1 zein: lupin mass, zein prepared with aqueous ethanol, lupin with water, both suspensions mixed and coarcervated with cold water (5.8°C); Lane 6 and 13 = lupin mass; Lane 9-12 = various parts of 1: 1 zein:lupin prepared separately with 5.4% acetic acid and water, respectively, both masses combined by hand kneading plus sheeting. Boxes show bands from either zein or lupin protein in the zein:lupin mass.

6.6.4 Rheological properties of protein masses

Tensile and rheological properties represent mechanical behaviour of materials that is required to give desired properties (Ashter, 2014). Zein mass prepared with aqueous ethanol had higher FMax (firmer) than all the other protein masses even after repeated compression (Table 6.2). This could be due to the 12 fold volume of cold water (5.8°C), which was added to coacervate the zein and cooled it to around 19°C. It has been reported that zein-starch dough returns from rubbery to its amorphous state if rested and cooled below 25°C (Lawton, 1992). Furthermore, drying may have happened during repeated compression measurements contributing to the firm zein mass. On measurement of the tensile properties (Table 6.3), the zein mass broke easily as indicated by its shortest extension (Table 6.3). This breaking of the stretched zein is also probably why it had pinholes viewed by CLSM (Figure 6.2D) and SEM (Figure 6.2G).

On the other hand, the zein mass prepared with dilute acetic acid had lower FMax, indicating that it was very soft (Table 6.2). With time, this zein mass also became firm probably due to water and acetic acid evaporation during measurement but still remained very soft compared to its counterpart prepared with aqueous ethanol. However, on tensile evaluation, the force required to break this zein was not significantly different to that coacervated from aqueous ethanol but it was nearly 3 times more extensible (Table 6.3). The effect of dilute acetic acid on zein extensibility is similar to that reported from zein mass diluted in the same solvent (Sly et al., 2014). These authors found that diluting commercial zein in acetic acid resulted in highly extensible zein mass, which reached the maximum passible extension on the Kieffer rig (270 mm) without breaking. As mentioned, the extensibility of zein made with dilute acetic acid may have been due to the change in protein-protein interaction as result of protonation of acidic amino acids side chains (Li et al., 2012).

Lupin protein mass appeared softer at FMax than both zein masses prepared with either aqueous ethanol or dilute acetic acid (Table 6.2). As stated, this might be due to the presence of more hydrophilic globulins proteins in lupin protein giving high water binding capacity (Sathe et al., 1951). It became firm during repeated compression but remained softer than zein mass prepared with aqueous ethanol and firmer than zein mass prepared with dilute acetic acid.

Data obtained from tensile tests (Table 6.3) showed that both the zein alone and the1:1 zein: lupin made with dilute acetic acid were statistically similar ($P < 0.05$). The extensibility of the 1:1 zein:lupin protein mass made with dilute acetic acid was approximately 3 times less than that of zein alone mass made with dilute acetic acid. This suggest that adding lupin protein to zein did not improve its tensile properties, probably due to lack of covalent molecular interactions between the two proteins. This lack of interaction is also reflected by absence of new molecular weight bands in the SDS-PAGE of the 1:1 zein:lupin protein mass prepared with dilute acetic acid (lanes 9-12, Figure 6.5A).

Stress relaxation is a characteristic behaviour of polymers studied by applying a fixed amount of deformation to a specimen and measuring the force required to maintain it as a function of time (Ashter, 2014). The stress relaxation test is used to provide fundamental comparison of the viscoelastic properties of the materials (Singh et al., 2006). The percentage stress recovery of the zein mass containing lupin protein significantly higher ($P < 0.05$), indicating some elasticity compared the zein alone mass for which the lower value indicated mainly viscous flow properties (Table 6.2). The percentage stress recovery of the mass containing lupin protein was significantly higher (P), indicating some elasticity than that of zein alone mass. This shows that the elasticity displayed by the 1:1 zein:lupin protein mass prepared with dilute acetic acid was contributed from mainly lupin protein without any molecular interactions between the zein and lupin as illustrated with the SDS-PAGE (lanes 9-12, Figure 6.5A).

Although the zein alone mass had higher FMax than the lupin protein mass (Figure 6.2), the percentage stress-recovery of the latter was significantly higher ($P < 0.05$). This indicates that lupin protein mass had some viscous flow and elastic character whereas zein mass had mostly viscous flow character. Similarly, Chakrabarti-Bell et al. (2013) reported that dough made of lupin flour was fragile but remained intact and elastic enough to produce chapatti that puffed (produced one large bubble). The disulphide cross-links present in lupin protein (Blagrove & Gillespie, 1975; Duranti et al., 2008; Wong et al., 2013) could be involved in the observed elasticity.

2052 **Table 6.2** Stress relaxation behaviour of commercial zein, commercial zein: lupin protein, lupin protein and gluten protein masses[1,2]

Mass type	Preparation method	Repeated stress relaxation testing (min)	FMax (N)	Ft (N)	% Stress recovery
Gluten		0	0.19 ± 0.03	0.06 ± 0.01	33.01^{aC} ± 2.39
		5	0.51 ± 0.13	0.18 ± 0.04	36.28^{aD} ± 2.03
		10	1.38 ± 0.46	0.50 ± 0.17	36.31^{aD} ± 1.27
		15	2.12 ± 0.45	0.71 ± 0.16	33.24 ± 1.64^{aD}
Zein	Coacervated with water from 70% (w/w) aqueous ethanol solution	0	2.34 ± 0.06	0.15 ± 0.02	6.34^{abA} ± 0.65
		5	7.34 ± 0.58	0.33 ± 0.17	4.42^{aA} ± 1.94
		10	13.81 ± 0.61	1.35 ± 0.31	9.75^{bA} ± 1.89
		15	31.63 ± 3.85	2.58 ± 0.47	8.14^{bA} ± 0.51
Zein	5.4% acetic acid, hand kneaded and sheeted 5 times	0	0.65 ± 0.11	0.42 ± 0.01	6.36^{aA} ± 0.84
		5	2.02 ± 0.19	0.18 ± 0.02	8.98^{bB} ± 0.77
		10	4.00 ± 0.64	0.38 ± 0.08	9.49^{bA} ± 1.11
		15	6.96 ± 0.28	0.65 ± 0.03	9.28^{bA} ± 0.78
Lupin protein		0	0.32 ± 0.15	0.06 ± 0.03	18.02^{aB} ± 1.24
		5	1.07 ± 0.22	0.22 ± 0.05	21.23^{abC} ± 1.09
		10	2.95 ± 0.94	0.72 ± 0.26	24.34^{bC} ± 1.21
		15	10.38 ± 4.63	2.39 ± 0.77	23.88^{bC} ± 3.24
1:1zein:lupin protein	Coacervated with cold water from 70% (w/w) aqueous ethanol solution		Not determined		
1:1zein:lupin protein	Zein prepared with 5.4% acetic acid and lupin protein with water. Both doughs were combined, hand kneaded and sheeted 5 times	0	0.46 ± 0.17	0.49 ± 0.01	11.35^{aA} ± 4.24
		5	1.12 ± 0.22	0.15 ± 0.03	13.0^{aC} ± 0.72
		10	2.73 ± 1.07	0.44 ± 0.18	16.06^{aB} ± 0.81
		15	5.94 ± 1.94	0.99 ± 0.35	16.63^{aB} ± 0.78

FMax: Maximum force

Ft: Force at which fresh gluten viscoelastic mass had relaxed to 36.8% of its maximum force (13.4 s after F Max)

[1]Mean ± Standard Deviation of 3 preparation replicates, mean values within protein type with different lower case letters in a row are significantly different ($P < 0.05$)

[2]Mean values with different upper case letters in a column are significantly different ($P < 0.05$)

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2054 **Table 6.3** Tensile properties of commercial zein, commercial zein: lupin protein, lupin protein and gluten protein masses[1]

Mass type	Preparation method	Peak Force (N)	Extension (mm)	Peak Stress (kPa)	Percentage strain (%)	Extensional viscosity (η_E, kPa)	Young's Modulus (E, kPa)
Gluten	Water	$10.04^{c} \pm 1.06$	$78.00^{b} \pm 2.47$	$192.20^{c} \pm 19.39$	$263.06^{b} \pm 8.23$	$2489^{c} \pm 311$	$186.23^{d} \pm 24.43$
Zein	Aqueous ethanol	$0.25^{a} \pm 0.07$	$44.59^{a} \pm 3.29$	$6.14^{a} \pm 1.49$	$148.64^{a} \pm 10.98$	$57^{a} \pm 14$	$2.48^{a} \pm 1.05$
Zein	Dilute acetic acid	$1.77^{ab} \pm 0.26$	$154.77^{c} \pm 19.87$	$32.11^{a} \pm 4.85$	$485.90^{c} \pm 66.26$	$721^{a} \pm 119$	$50.37^{c} \pm 7.53$
Lupin protein	Water	$0.93^{a} \pm 0.07$	$52.86^{ab} \pm 9.21$	$20.49^{a} \pm 2.35$	$176.20^{ab} \pm 30.72$	$201^{a} \pm 21$	$11.25^{b} \pm 2.97$
1:1 zein:lupin protein	Aqueous ethanol and water	Did not form a mass, instead formed a sediment, which could not be measured					
1:1 zein:lupin protein	Zein in dilute acetic acid water	$3.39^{b} \pm 0.68$	$56.63^{ab} \pm 9.29$	$71.03^{b} \pm 9.62$	$188.77^{ab} \pm 30.98$	$728^{b} \pm 169$	$45.44^{bc} \pm 17.76$

[1] Mean ± Standard Deviation of 3 preparation replicates, mean values in a column with different superscript letters are significantly different ($P < 0.05$)

6.6.5 Alveography of doughs

Dough tenacity as measured with Alveography is a predictor of the ability of the dough to retain gas (C. M. Rosell, Rojas, et al., 2001). At 1:1 and 1:3 ratios, gluten-starch and zein-starch doughs had the highest tenacity values and were not significantly different ($P < 0.05$) (Table 6.4). This was shown by their large bubble sizes (Figure 6.6A and E for gluten-starch dough, B and F for zein-starch dough).

At 1:1 ratio, lupin-starch dough was very sticky and for this reason it was impossible to test its dough properties. However, 1:3 lupin-starch dough was cohesive but its tenacity value was significantly lower ($P < 0.05$) than those of both gluten- and zein-starch doughs. The 1:3 lupin-starch dough formed a bubble (Figure 6.6G), which broke during inflation. This indicates that this dough had some but limited gas retention ability. Chakrabarti-Bell et al. (2013) found that lupin flour doughs held bubbles the least and were the least elastic compare to 50:50 wheat:lupin flour and wheat flour doughs. However, the authors reported that the lupin flour doughs were nevertheless elastic enough to produce chapattis that puffed.

Adding lupin protein to zein significantly reduced ($P < 0.05$) the tenacity value compare to zein-starch dough alone. The observed tears on the surface of the 1:1 zein:lupin-starch dough may have prevented the growth of the dough bubble (Figure 6.6D), resulting in the low tenacity value. The 1:3 zein:lupin protein-starch dough could not inflate a bubble (Figure 6.6H). These results suggest that the lupin protein may have disrupted the zein network instead of interacting with it.

Extensibility (L), indicates the capacity to extend a dough without breaking it (Rosell et al., 2001). Dough made from 1:1 zein:lupin protein-starch was significantly less extensible ($P < 0.05$) than 1:1 zein-starch dough. As shown in Table 6.4 mixing lupin protein mass with zein mass made with dilute acetic acid decreased its extensibility by 3 times approximately.

A well-balanced ratio between dough tenacity and extensibility (P/L), and a high deformation energy (W) are associated with technological success of leavened products (Cappelli et al., 2018). For refine flours, the optimal P/L reference value is between 0.4 and 0.7, and for unrefined flours the P/L values are often higher (Parenti et al., 2019). In this study, the P/L value of 1:3 lupin protein-starch was within the

range of that of refine flour optimal reference, which may suggest that the use of lupin protein in bread dough has potential to produce leavened products.

The deformation values of the 1:1 and 1:3 zein:lupin protein-starch doughs were inconsistent because as shown in Figure 6.6C and 6.6D the 1:1 zein:lupin protein-starch dough produced small bubble , whereas, the other 1:3 ratio did not produce a bubble. This inconsistency may be due to the imbalance between the amount of proteins and starch or protein, starch and their respective solvents.

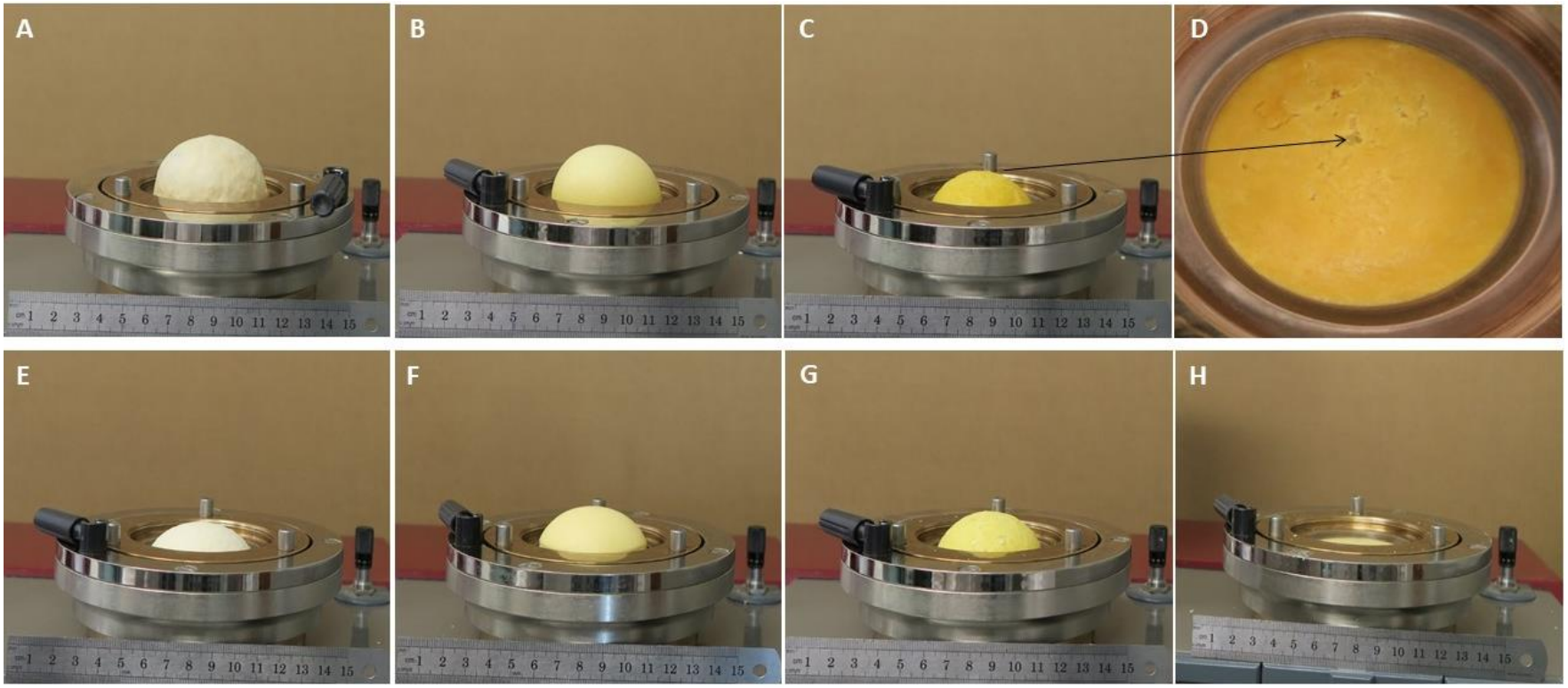

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Figure 6.6 Alveography of commercial zein and lupin protein masses. **A**-1:1 gluten starch, **B**- 1:1 commercial zein starch, **C**- 1:1 commercial zein-lupin protein starch, **D**- Tears on the surface of 1:1 commercial zein-lupin protein starch, **E**- 1:3 gluten starch, **F**- 1:3 commercial zein starch **G**- 1:3 Lupin protein and **H**- 1:3 commercial zein-lupin protein starch composite doughs.
Note 1:1 lupin-starch dough was not cohesive enough for alveography measurement.

2094 **Table 6.4** Alveography dough properties of commercial zein, commercial zein: lupin protein, lupin protein and gluten protein doughs[1]

Protein-starch dough	Protein: Starch ratio	Tenacity	Extensibility	Curve configuration ratio	Deformation energy
		(P, mm H_2O)	(L, mm)	(P/L)	(W, J $\times 10^{-4}$)
Gluten-starch	1:1	$278.0^{b} \pm 26.9$	$28.5^{a} \pm 2.1$	$9.7^{c} \pm 0.2$	$355.0^{b} \pm 67.9$
Zein-starch	1:1	$235.0^{b} \pm 4.2$	$175.0^{b} \pm 8.5$	$1.3^{a} \pm 0.1$	$648.0^{c} \pm 9.2$
Lupin-starch	1:1	Dough was very sticky and not cohesive enough to measure			
Zein:lupin-starch	1:1	$85.0^{a} \pm 2.8$	$13.0^{a} \pm 0.0$	$6.5^{b} \pm 0.2$	$57.5^{a} \pm 0.7$
Gluten-starch	1:3	$129.0^{b} \pm 26.2$	$67.5^{ab} \pm 31.8$	$2.1^{b} \pm 0.6$	$321.5^{a} \pm 153.4$
Zein-starch	1:3	$129.0^{b} \pm 5.7$	$21.5^{a} \pm 0.7$	$6.0^{c} \pm 0.1$	$151.5^{a} \pm 12.0$
Lupin-starch	1:3	$59.0^{a} \pm 14.1$	$110.0^{b} \pm 14.1$	$0.5^{a} \pm 0.0$	$190.5^{a} \pm 157.7$
Zein:lupin-starch	1:3	$24.0^{a} \pm 21.9$	$54.5^{ab} \pm 20.5$	$0.3^{a} \pm 0.1$	$110.0^{a} \pm 87.7$

[1] Mean ± Standard Deviation of 3 replicates measurements, mean values in a column with different superscript letters are significantly different ($P<0.05$).

6.7 Conclusions

This study is the first of its kind that has investigated the effect of lupin protein as a co-protein to prolamin proteins; namely zein.

The study reveals that coacervation with cold water from aqueous ethanol combined with lupin protein completely prevents the formation of a viscoelastic mass. This is probably due lupin protein insolubility in aqueous ethanol.

In contrast, combining the zein and lupin viscoelastic masses separately prepared in dilute acetic acid and water, respectably gave very different viscoelastic mass properties. This zein mass is cohesive with lots of entangled fibres. However, the rheological properties reveal that this mass is far less extensible than zein alone prepared in acetic acid. In addition, when formed into a dough, zein: lupin-starch composite could not hold air nor be inflated into a bubble by Alveography. The absence of observable new molecular weight bands by SDS-PAGE, indicates that zein and lupin protein did not covalently interact to form a copolymer in any of the formulations.

Lupin protein did not appear to function as co-protein with commercial zein (primarily α-zein with very limited amount of cysteine) in this study. Nonetheless, lupin protein formed a sticky yet cohesive mass when mixed with small amount of water. This mass has some but limited viscous and elastic flow properties. The disulphide cross-links present in the lupin protein may have been responsible for the observed viscoelasticity. Therefore, it may be useful to investigate the use of lupin protein as co-protein with total zein (comprising all subunits with more cysteine residues than commercial zein), which might covalently interact with cysteine in lupin protein to form disulphide-crosslinks.

CHAPTER 7

General discussion, overall conclusions, recommendations and future prospects

7.1 General discussion and overall conclusions

7.1.1 Development of a standard micro-scale screening method for evaluation of sorghum-wheat composite flour

The first objective of this study was to develop a standard micro-scale method suitable for evaluation of mixing properties of 50:50 ratio sorghum-wheat composite flour using a micro-doughLAB. The standard micro-scale screening method that mimics the high energy mixing used in modern commercial bakeries is required to evaluate the dough forming potential of only few grams of diverse sorghum genotypes from breeding programs (Dang & Bason, 2013; Tomoskozi & Bekes, 2016). The water absorption level of 64% gave a smooth curve and targeted the peak torque to 87 mNm, indicating a proper hydration of the composite flour. The sorghum-wheat composite flour mixed at 30°C and 120 rpm using the chosen water absorption level of 64.0% and target peak torque of 87 mNm, resulted in a composite dough developed to maximum resistance close to the target peak torque. In this study, the first stage of a potential standard method suitable for mixing the sorghum-wheat composite dough to maximum development was developed. The amount of water, target peak torque, temperature and speed required to mix the sorghum-wheat composite dough to maximum development were identified. This standard method was the used to evaluate the dough forming ability of different sorghum genotypes.

7.1.2 Dough forming ability of different sorghum genotypes

The second objective of this study was to evaluate dough mixing properties of 25 sorghum genotypes using the developed Micro-doughLAB standard method. For more than 80 years empirical mixing tests to evaluate wheat flour have been used. These tests measure the resistance of a dough to mixing and dough quality such as dough development time, stability and degree of softening to assist screening and selection of new wheat varieties for bread making (Haraszi et al., 2004). However, these types of tests have rarely been used to evaluate dough mixing properties of sorghum flour (Goodall et al., 2012). The findings of Chapter 5 demonstrated that all mixing parameters were significantly influenced by sorghum genotypes. Among the 25 sorghum genotypes, samples NGT16N434-2 and NGT17N208-1 had peak torque

values that were not significantly different from the target peak torque. NGT16N438 had the longest stability whereas NGT16N434-1 had the shortest. Sample NGT17N216 had the lowest degree of softening, however, its peak torque was in the lowest range. The two samples with peak torque close to the target, however, had intermediate stability and degree of softening. There was no individual genotype that had good combination of the target peak torque, long stability and low degree of softening. The present study provides new information on dough forming ability of 25 sorghum genotypes when mixed under standard conditions. In addition, the standard Micro-doughLAB method was sensitive enough to identify differences in mixing attributes of the sorghum genotypes.

7.1.3 Zein-lupin protein co-proteins made by coacervation from ethanol or dilute acetic acid plus hand kneading and sheeting

The third objective of this study was to determine if lupin protein can act as a co-protein to improve the dough-like properties of zein. Prolamin proteins from sorghum (kafirin) and maize (zein) tend to have limitation in leavened dough for bread products due to their hydrophobic and tightly-packed nature, which prevent both the water absorption and protein inter-chain reactions needed for hydration and development of a cohesive, extensible and elastic dough (as reviewed by Taylor, Belton, Beta, & Duodu, 2014). However, a limited amount of research has demonstrated that the use of a small amount of the high protein, leguminous seed Australian sweet lupin in wheat-based dough has the potential to improve the gluten dough functional properties (Paraskevopoulou et al., 2010). Therefore in this study two different methods of preparation of commercial zein:lupin protein viscoelastic masses used to determine the effect of lupin protein on commercial zein viscoelastic mass. The results in Chapter 6 reveal that under the two methods used, there was no experimental evidence that a covalently linked copolymer was formed between zein and lupin protein.

7.1.4 Limitations

7.1.4.1 Phase one

Samples and flour preparation

Three genotypes of white sorghum (NGT16N0434, NGT17N208 and 134) were used in Chapter 3 for the preliminary study to assess their flour and rheological properties. In Chapter 5, 25 white sorghum genotypes were used to evaluate their dough mixing properties using the developed Micro-doughLAB standard method. Genotypes NGT16N0434 and NGT17N208 from Chapter 3 were studied again in Chapter 5, whereas, genotype 134 was not included due to shortage of the sample. Despite the difference in the mixing properties of the genotypes studied under standard micro-doughLAB conditions, the genotypes did not demonstrate major diversity in their mixing properties. The genotypes were chosen because of their different agronomic traits (Chris Haire, sorghum breeder, Nuseed, Australia, personal communication, 2016) but have not been previously evaluated for end-use quality. No breeding history was available for the received sorghum grains due to commercial confidentiality. Future work is now required to fully characterise the physical and chemical properties of the grains and their flours to hypothesise the mechanism for the difference in the mixing properties of the genotypes.

The results of the two mixing methods, hand mixing and Micro-doughLAB mixing used for the sorghum-wheat composite flour could not be directly compared due to the following:

- Sorghum genotypes were milled differently for each method. Sorghum samples used in Chapter 3, were milled using a laboratory Cemotec^{TM} 1090 Sample Mill to give whole grain coarse flour, then further milled many times using a coffee grinder and passed through a sieve. The coffee grinder required prolonged milling time to produce fine flour. This milling technique was used due to lack of a standard milling equipment for the preliminary phase of the project. The difference in particle size of the three genotypes (Table 3.3) was probably due to uneven particle shape as a result of the vigorous cutting motions of the coffee grinder blades, which sliced some particle finer than others. In addition to particle size, such milling technique may have affected the amount of damaged starch in the resulting sorghum flours, and thus introduced a

confounding variable. By definition, damaged starch refers to small particles of starch broken from the main starch granules as a result of milling. The amount of damaged starch is important because it influences the flour's ability to absorb water (Kent & Evers, 1994). Therefore, it would have been useful to use the same standard milling equipment for a direct comparison between the results of the hand and micro-doughLAB mixing methods.

- A commercial wholemeal wheat flour was used for the preliminary study (Chapter 4.1). The particle size of the commercial wholemeal wheat flour was significantly different compared to that of the sorghum genotypes (Table 3.3), as the milling conditions were different. In milling industry, wholemeal wheat flour is produced by separating the endosperm from the bran and germ, followed by gradual size reduction and sifting of the endosperm. At the end of milling a certain percentage of the bran and germ is blended back with the endosperm flour (Atwell, 2001). According to Kihlberg, Johansson, Kohler, & Risvik (2004) wholemeal flours are produced by a variety of techniques, which result in flours with widely different bran particle sizes. This suggests that for better comparison, the use of a known variety of wheat milled to the same particle size as sorghum genotype is more desirable. Therefore, to minimize experimental errors due to the use of the commercial wholemeal flour, the hard commercial whole grain wheat (Emu Rock) milled to the same particle size as the sorghum genotypes was used in Chapter 4 and 5 to evaluate dough mixing properties of sorghum genotypes using the developed Micro-doughLAB standard method.
- Two ratios (30:70 and 50:50) of sorghum to wheat were used in the hand and micro-doughLAB mixing methods, respectively. The higher level of sorghum was intentionally chosen to increase the disruption of the wheat matrix and therefore be able to better differentiate between the effects of different sorghum varieties. This approach is advantageous because it may allow to identify any sorghum sample with different mixing properties and thus improve potential use in leavened bread.

Production of sorghum-wheat composite doughs

The mixing properties of the 25 white sorghum genotypes was determined using the developed Micro-doughLAB standard method. The main advantages of the equipment are: (a) a rapid determination of the processing potential of a flour and (b) the

equipment's ability to mimic the high-energy mixers used in modern commercial bakeries, indicating that it is useful for developing practical industrial applications. Furthermore, the Micro-doughLAB is suitable in research for screening and selection of the very limited quantity of grain samples that are only available in the early stage of breeding (Dang & Bason, 2013; Tomoskozi & Bekes, 2016). The equipment measures the resistance of a dough to mixing and gives dough quality measures such as water absorption, dough development time, stability, degree of softening and energy at maximum resistance (Dang, & Bason, 2013). The limitation of the micro-doughLAB is that it is empirical in nature, that is, the data obtained cannot be converted into a well-defined rheological properties, therefore it is extremely difficult to interpret the results fundamentally (Bloksma, 1962; Janssen, Van Vliet, & Vereijken, 1996; Dobraszczyk & Morgenstern, 2003). Therefore, in order to validate the Micro-doughLAB standard method developed in this study, further research to measure tensile properties and/or dough rheology (e.g. using small deformation rheometer) is important to determine if the standard mixing conditions used produced a developed sorghum-wheat composite dough with higher quality compared to those mixed under other conditions.

Another major limitation of using the Micro-doughLAB alone is that, the deformations of the dough during mixing give no information about the slow deformations, such as occur in proofing and baking (Bloksma, 1962). Therefore, since the end use of the sorghum-wheat flour evaluated was for bread making, future research is required to bake sorghum-wheat dough produced to assess bread qualities, in terms of loaf height, loaf volume and specific volume. By doing so, this would have provided a more rigorous method to screen those sorghum genotypes that are more suitable for bread making.

7.1.4.2 Phase two

Protein samples and protein mass preparation

Commercial zein was chosen as a model for kafirin to investigate if it could interact with lupin protein to form a copolymer with improved rheological properties. Different ratios of zein to lupin protein were used for this purpose. Commercial zein (essentially α-zein) was preferred because it is readily available in purified form than α-kafirin,

however, it has physical and chemical similarities with it. Commercial zein polypeptides have only one or two cysteine per subunit (Shewry & Tatham, 1990), suggesting that they did not interact with the cysteine residues in lupin protein to form a copolymer. Total zein and kafirin have substantial amount of cysteine residues (Shewry & Tatham, 1990; Belton, Delgadillo, Halford, & Shewry, 2006), thus for future studies, it would be useful to investigate if they interact with cysteine in lupin protein to form disulphide-crosslinks required to form a copolymer.

The second method of protein mass preparation using dilute acetic acid and water plus kneading and sheeting may be recommended for future studies. This is because the zein mass prepared with dilute acetic acid at 40°C did not stiffen fast during mixing with lupin protein.

7.2 Future Prospects

- To validate the micro-doughLAB standard method, further research is required to measure the rheological properties and bake the dough produced under selected standard mixing conditions. This will confirm if there is any correlation between the mixing attributes and the dough rheological properties as well as the bread quality. This will provide a more rigorous method to screen those sorghum genotypes that are suitable for bread making.
- Investigation of dough forming ability of highly diverse genotypes, such high protein digestibility lines, genetically modified varieties with modified kafirin expression and protein bodies' structures is recommended in the future studies.
- For future research, alternative approaches to investigate the potential of prolamin proteins in leavened dough for bread making using lupin as a co-protein may be to use total zein (comprising all subunits with more cysteine residues than commercial zein) and total kafirin, which might covalently interact with cysteine in lupin protein to form disulphide-crosslinks.

7.3 Recommendations to sorghum researchers and breeders

- The standard micro-doughLAB method may help sorghum pre-breeding and commercial breeding programs to screen and select lines for development of new sorghum varieties with more useful functionality for manufacture of leavened bread.

- The approach used to develop the standard micro-doughLAB method for evaluation of sorghum-wheat composite flour might be applied to get initial information on mixing properties of different composite formulations, including gluten-free which may assist in process development.

REFERENCES

AACC International. (2000). Approved Methods of the American Association of Cereal Chemistry: 44-15A Moisture-air oven method, 46-30 Crude protein-combustion method, 38-12-02 Wet Gluten, Dry Gluten, Water-Binding Capacity, and Gluten Index, 54-21.02 Rheological Behavior of Flour by Farinograph: Constant Weight Procedure, 54-70.01 High-Speed Mixing Rheology of Wheat Flour Using the dough LAB, and 56-81.04 Determination of Falling Number(10th ed.). St. Paul, Minnesota, USA: American Association of Cereal Chemists.

Abdelrahman, A.& Spies, R. (1986). Dynamic rheological studies of dough systems. In H. Faridi & J.M. Faubion (Eds.), *Fundamentals of Dough Rheology* (pp. 87–103). St. Paul, Minnesota, USA: The American Association of Cereal Chemistry.

Armero, E., & Collar, C. (1997). Texture properties of formulated wheat doughs. *Zeitschrift Für Lebensmitteluntersuchung Und -Forschung A*, *204* (2), 136–145. https://doi.org/10.1007/s002170050050.

Ashter, S. A. (Ed). (2014). *Thermoforming of Single and Multilayer Laminates: Plastic Films Technologies, Testing, and Applications* (p. 147-156). Oxford, UK: Elsevier.

Atwell, W., A. (Ed). (2001). *Wheat Flour* (p. 39-40). St. Paul, Minnesota, USA: American Association of Cereal Chemists.

Australian Government Department of Health, Office of the Gene Technology Regulator. (2017). The Biology of *Sorghum Bicolor* (L.) Moench Subsp. *bicolor* (Sorghum). Retrieved from https://www.ogtr.gov.au/Content/Sorghum Biology Version 1.1 July 2017.

Batterman-Azcona, S. J., Lawton, J. W., & Hamaker, B. R. (1999). Effect of specific mechanical energy on protein bodies and α-zeins in corn flour extrudates. *Cereal Chemistry*, *76* (2), 316–320. https://doi.org/10.1094/CCHEM.1999.76.2.316.

Belton, P. S. (1999). Mini Review: On the Elasticity of Wheat Gluten. *Journal of Cereal Science*, *29* (2), 103–107. https://doi.org/10.1006/jcrs.1998.0227.

Belton, P. S. (2012). The molecular basis of dough rheology. In S. P. Cauvain (Ed.), *Bread Making: Improving Quality* (2nd ed., pp. 337–351). Cambridge, UK: Woodhead Publishing Limited.

Belton, P. S., Delgadillo, I., Halford, N. G., & Shewry, P. R. (2006). Kafirin structure and functionality. *Journal of Cereal Science*, *44* (3), 272–286. https://doi.org/10.1016/j.jcs.2006.05.004.

Blagrove, R. J., & Gillespie, J. M. (1975). Isolation, Purification and Characterization of the Seed Globulins of Lupinus angustifolius. *Australian Journal of Plant Physiology*, *2*, 13–27. https://doi.org/10.1071/PP9750013.

Bloksma, A. H. (1962). Slow Creep of Wheat Flour Doughs. *Rheologica Acta*, *47*, 217–230.

Bugusu, B. A., Campanella, O., & Hamaker, B. R. (2001). Improvement of sorghum-wheat composite dough rheological properties and breadmaking quality through zein addition. *Cereal Chemistry*, *78* (1), 31–35. https://doi.org/10.1094/CCHEM.2001.78.1.31.

Cappelli, A., Cini, E., Guerrini, L., Masella, P., Angeloni, G., & Parenti, A. (2018). Predictive models of the rheological properties and optimal water content in doughs: An application to ancient grain flours with different degrees of refining. *Journal of Cereal Science*, *83*, 229–235. https://doi.org/10.1016/j.jcs.2018.09.006.

Cauvain, S. P. (2009). The relevance of testing the manufacture of bread and fermented products. In S.P. Cauvain & L.S. Young (Eds), *The ICC Handbook of Cereals, Flour, Dough and Product Testing Methods and Applications* (p. 131,134). Pennsylvannia, USA: Destech Publications.

Cauvain, S. P. (2014). Getting more from whole grains. *Journal of Cereal Foods World*, *59* (5), 260-263. https://doi.org/10.1094/CFW-59-5-0260.

Cauvain, S. P. (2015). Grains , Pulses , and Innovation, *Journal of Cereal Foods World*, *60*(5), 244–245. https://doi.org/10.1094/CFW-60-5-0244.

Cauvain, S. P. (Ed). (2012). Introduction to bread making. In *Bread Making: Improving Quality* (2nd ed., pp. 1–7). Cambridge, UK: Woodhead Publishing

Limited.

Chakrabarti-Bell, S., Wang, S., Patel, M. J., Weiss, R. M., & Austin, P. J. (2013). Bubbles in chapatti doughs. *Journal of Cereal Science*, *57*(3), 504–513. https://doi.org/10.1016/j.jcs.2013.02.006.

Chew, P. G., Casey, A. J., & Johnson, S. K. (2003). Protein quality and physico-functionality of Australian sweet lupin (Lupinus angustifolius cv. Gungurru) protein concentrates prepared by isoelectric precipitation or ultrafiltration. *Food Chemistry*, *83*(4), 575–583. https://doi.org/10.1016/S0308-8146(03)00156-0.

D'Appolonia, B. L. (1984). Types of farinograph curves and factors affecting them. In B. L. D'Appolonia & W. H. Kuneth (Eds.), *The Farinograph Handbook* (3rd ed., p. 13). St. Paul, Minnesota, USA: American Association of Cereal Chemists.

Dang, J.M.C. & Bason, M. L. (2013). AACCI Approved Methods Technical Committee Report: Collaborative Study on a Method for Determining the Mixing Properties of Dough using High-Energy Mixing. *Journal of Cereal Foods World*, *58*(4), 199–204. https://doi.org/10.1094/cfw-58-4-0199.

Deora, N. S., Deswal, A., & Mishra, H. N. (2014). Alternative Approaches Towards Gluten-Free Dough Development: Recent Trends. *Food Engineering Reviews*, *6*(3), 89–104. https://doi.org/10.1007/s12393-014-9079-6.

Dlamini, N. & Siwela, M. (2015). The future of grain science: The contribution of indigeneous small grains to food security, nutrition and health in South Africa. *Journal of Cereal Foods Wolrd*, (60), 1–4.

Dlamini, N. R., Taylor, J. R. N., & Rooney, L. W. (2007). The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. *Food Chemistry*, *105*(4), 1412–1419. https://doi.org/10.1016/j.foodchem.2007.05.017.

Dobraszczyk, B. J., & Morgenstern, M. P. (2003). Rheology and the breadmaking process. *Journal of Cereal Science*, *38*(3), 229–245. https://doi.org/10.1016/S0733-5210(03)00059-6.

Dunnewind, B., Sliwinski, E. L., Grolle, K., & Van Vliet, T. (2003). The Kieffer

dough and gluten extensibility rig - An experimental evaluation. *Journal of Texture Studies*, *34*(5–6), 537–560. https://doi.org/10.1111/j.1745-4603.2003.tb01080.x.

Duodu, K. G. & Taylor, J. R. N. (2012). The quality of breads made with non-wheat flours. In S. P. Cauvain (Ed.), *Bread Making: Improving Quality* (2nd ed., pp. 337–351). Cambridge:UK: Woodhead Publishing Limited.

Duodu, K. G., Taylor, J. R. N., Belton, P. S., & Hamaker, B. R. (2003). Factors affecting sorghum protein digestibility. *Journal of Cereal Science*, *38*(2), 117–131. https://doi.org/10.1016/S0733-5210(03)00016-x.

Duranti, M., Consonni, A., Magni, C., Sessa, F., & Scarafoni, A. (2008). The major proteins of lupin seed: Characterisation and molecular properties for use as functional and nutraceutical ingredients. *Trends in Food Science and Technology*, *19*(12), 624–633. https://doi.org/10.1016/j.tifs.2008.07.002.

Elhassan, M. S. M., Emmambux, M. N., & Taylor, J. R. N. (2017). Transgenic sorghum with suppressed synthesis of kafirin subclasses: Effects on flour and dough rheological characteristics. *Journal of Cereal Science*, *75*, 69–76. https://doi.org/10.1016/j.jcs.2017.03.020.

Elhassan, M. S. M., Naushad Emmambux, M., Hays, D. B., Peterson, G. C., & Taylor, J. R. N. (2015). Novel biofortified sorghum lines with combined waxy (high amylopectin) starch and high protein digestibility traits: Effects on endosperm and flour properties. *Journal of Cereal Science*, *65*, 132–139. https://doi.org/10.1016/j.jcs.2015.06.017.

Eliasson, A.C. & Larsson, K. (Eds). (1993). Dough. In *Cereals in Bread Making: A Molecular Colloidal Approach* (pp. 261–324). New York, USA: Macel Dekker.

Elkhalifa, A. E. O., & El-Tinay, A. H. (2002). Effect of cysteine on bakery products from wheat-sorghum blends. *Food Chemistry*, *77*(2), 133–137. https://doi.org/10.1016/S0308-8146(00)00325-3.

Elliott, B. (2010). Better modelling of high-speed commercial dough mixing. *Journal of Cereal Foods Wolrd*, *55*, 136–138. https://doi.org/10.1094/CFW-55-3-0136.

Erickson, D. P., Campanella, O. H., & Hamaker, B. R. (2012). Functionalizing maize

zein in viscoelastic dough systems through fibrous, β-sheet-rich protein networks: An alternative, physicochemical approach to gluten-free breadmaking. *Trends in Food Science and Technology*, *24*(2), 74–81. https://doi.org/10.1016/j.tifs.2011.10.008.

Erickson, D. P., Renzetti, S., Jurgens, A., Campanella, O. H., & Hamaker, B. R. (2014). Modulating state transition and mechanical properties of viscoelastic resins from maize zein through interactions with plasticizers and co-proteins. *Journal of Cereal Science*, *60*(3), 576–583. https://doi.org/10.1016/j.jcs.2014.08.001.

Evers, A. D. (1971). Scanning Electron Microscopy of Wheat Starch. III. Granule Development in the Endosperm. *Starch - Stärke*, *23*(5), 157–162. https://doi.org/10.1002/star.19710230502.

Food and Agriculture Organization of the United Nations. (2018). The State of Agricultural Commodity Markets: Agricultural Trade, Climate Change and Food Security. Retrieved from https://www.fao.org/3/I9542EN/i9542en.

Fu, B. X., Wang, K., & Dupuis, B. (2017). Predicting water absorption of wheat flour using high shear-based GlutoPeak test. *Journal of Cereal Science*, *76*, 116–121. https://doi.org/10.1016/j.jcs.2017.05.017.

Fu, B. X., Wang, K., Dupuis, B., & Cuthbert, R. D. (2017). A rapid extensigraph protocol for measuring dough viscoelasticity and mixing requirement. *Journal of Cereal Science*, *76*, 99–107. https://doi.org/10.1016/j.jcs.2017.05.012.

Gajo, K.A.F. & Dang, J. M. C. (2016). Comparison of mixing and pasting behaviours of commercially available wheat and gluten-free flours. Retrieved from https://www.perten.com/Publications/Articles/Comparison-of-mixing-and-pasting-behaviors-of-commercially-available-wheat-and-gluten-free-flours.

Goodall, M. A., Campanella, O. H., Ejeta, G., & Hamaker, B. R. (2012). Grain of high digestible, high lysine (HDHL) sorghum contains kafirins which enhance the protein network of composite dough and bread. *Journal of Cereal Science*, *56*(2), 352–357. https://doi.org/10.1016/j.jcs.2012.04.001.

Greer, E. N., & Stewart, B. A. (1959). The water absorption of wheat flour: Relative

effects of protein and starch. *Journal of the Science of Food and Agriculture*, *10*(4), 248–252. https://doi.org/10.1002/jsfa.2740100409.

Hama, F., Icard-Verniere, C., Picq, G.J.P., Diawara, B. & Mouquet-Rivier, C. (2011). Changes in micro-and macronutrient composition of pearl millet and white sorghum during field versus laboratory decortication. *Journal of Cereal Science*, *54*, 425–433.

Haraszi, R., Gras, P. W., Tömösközi, S., Salgó, A., & Békés, F. (2004). Application of a Micro Z-Arm Mixer to Characterize Mixing Properties and Water Absorption of Wheat Flour. *Cereal Chemistry Journal*, *81*(5), 555–560. https://doi.org/10.1094/cchem.2004.81.5.555.

Henzell, R.G. & Jordan, D. R. (2009). Grain Sorghum Breeding. In M. J. Carena (Ed.), *Cereals: Handbook of Plant Breeding* (3rd ed., pp. 183–197). German: Springer Science Business Media.

Hoseney, R. C., & Rogers, D. E. (1990). The Formation and Properties of Wheat Flour Doughs. *Critical Reviews in Food Science and Nutrition*, *29*(2), 73–93. https://doi.org/10.1080/10408399009527517.

Houben, A., Höchstötter, A., & Becker, T. (2012). Possibilities to increase the quality in gluten-free bread production: An overview. *European Food Research and Technology*, *235*(2), 195–208. https://doi.org/10.1007/s00217-012-1720-0.

Huang, S., Yun, S. H., Quail, K., & Moss, R. (1996). Establishment of flour quality guidelines for northern style Chinese steamed bread. *Journal of Cereal Science*, *24*(2), 179–185. https://doi.org/10.1006/jcrs.1996.0051.

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). (2011). Science-based and sustainable solutions to drought and famine in the Horn of Africa, 2011: Sorghum (*Sorghum bicolor* (L.) Moench). Retrieved from https://www.icrisat.org/what-we-do/SASA/sasa_specialissue-2011.htm.

Jafari, M., Koocheki, A., & Milani, E. (2017a). Effect of extrusion cooking of sorghum flour on rheology, morphology and heating rate of sorghum–wheat composite dough. *Journal of Cereal Science*, *77*, 49–57. https://doi.org/10.1016/j.jcs.2017.07.011.

Jafari, M., Koocheki, A., & Milani, E. (2017b). Effect of extrusion cooking on chemical structure, morphology, crystallinity and thermal properties of sorghum flour extrudates. *Journal of Cereal Science*, *75*, 324–331. https://doi.org/10.1016/j.jcs.2017.05.005.

Janssen, A. M., Van Vliet, T., & Vereijken, J. M. (1996). Fundamental and empirical rheological behaviour of wheat flour doughs and comparison with bread making performance. *Journal of Cereal Science*, *23*(1), 43–54. https://doi.org/10.1006/jcrs.1996.0004.

Johnson, S.K., Clements, J., Villarino, C.B. & Coorey, R. (2017). Lupin:Their Unique Nutritional and Health-Promoting Attributes. In J.R.N. Taylor & J.M. Awika (Eds.), *Gluten-free Ancient Grains, Cereals, Pseudocereals and Legumes: Sustainable, Nutritious and Health Promoting Foods for the 21st Century* (pp. 179–221). Cambridge, UK: Elsevier Science & Technology. https://doi.org/https://doi.org/10.1016/B978-0-08-100866-9.00008-x.

Keetels, C. J. A. M., Visser, K. A., Van Vliett, T., Jurgens, A., & Walstra, P. (1996). Structure and mechanics of starch bread. *Journal of Cereal Science*, *24*(1), 15–26. https://doi.org/10.1006/jcrs.1996.0033.

Kent, N.L. & Evers, A. D. (Eds). (1994). Dry Milling Technology. In *Technology of Cereals: An Introduction for Student of Food Science and Agriculture* (4th ed., p. 149). Oxford, UK: Elsevier Science.

Kihlberg, I., Johansson, L., Kohler, A., & Risvik, E. (2004). Sensory qualities of whole wheat pan bread - Influence of farming system, milling and baking technique. *Journal of Cereal Science*, *39*(1), 67–84. https://doi.org/10.1016/S0733-5210(03)00067-5.

Kilborn, R.H. & Tipples, K. H. (1974). Implication of the mechanical development of bread dough by means of sheeting rolls. *Cereal Chemistry*, *51*, 648–657.

Kim, S., & Xu, J. (2008). Aggregate formation of zein and its structural inversion in aqueous ethanol. *Journal of Cereal Science*, *47*(1), 1–5. https://doi.org/10.1016/j.jcs.2007.08.004.

King, B. L., Taylor, J., & Taylor, J. R. N. (2016). Formation of a viscoelastic dough

from isolated total zein (α-, β- and γ-zein) using a glacial acetic acid treatment. *Journal of Cereal Science*, *71*, 250–257. https://doi.org/10.1016/j.jcs.2016.09.005.

Lai, H. M., & Padua, G. W. (1997). Properties and microstructure of plasticized zein films. *Cereal Chemistry*, *74*(6), 771–775. https://doi.org/10.1094/CCHEM.1997.74.6.771.

Lawton, J. W. (1992). Viscoelasticity of Zein-Starch Doughs. *Cereal Chemistry*, *69* (4) 351-355.

Li, Y., Li, J., Xia, Q., Zhang, B., Wang, Q., & Huang, Q. (2012). Understanding the dissolution of α-zein in aqueous ethanol and acetic acid solutions. *Journal of Physical Chemistry B*, *116*(39), 12057–12064. https://doi.org/10.1021/jp305709y.

Liu, G., Gilding, E. K., Kerr, E. D., Schulz, B. L., Tabet, B., Hamaker, B. R., & Godwin, I. D. (2019). Increasing protein content and digestibility in sorghum grain with a synthetic biology approach. *Journal of Cereal Science*, *85*(August 2018), 27–34. https://doi.org/10.1016/j.jcs.2018.11.001.

MacRitchie, F. (2003). Fundamentals of dough formation. *Cereal Foods World*, *48*(4), 173–176.

Matos, M. E., & Rosell, C. M. (2014). Understanding gluten-free dough for reaching breads with physical quality and nutritional balance. *Journal of the Science of Food and Agriculture*, *95*(4), 653–661. https://doi.org/10.1002/jsfa.6732.

Megazyme International. (2001). Total starch assay procedure (Myloglucosidase/α-Amylose Method), K-TSTA-50A/K-TSTA-100A 08/19. Retrieved from https://www.megazyme.com/documents/Booklet/K-TSTA-100A_DATA.

Mejia, C. D., Gonzalez, D. C., Mauer, L. J., Campanella, O. H., & Hamaker, B. R. (2012). Increasing and stabilizing β-sheet structure of maize zein causes improvement in its rheological properties. *Journal of Agricultural and Food Chemistry*, *60*(9), 2316–2321. https://doi.org/10.1021/jf203073a.

Mironeasa, S., & Codină, G. (2013). Influence of Mixing Speed on Dough Microstructure and Rheology. *Food Technology and Biotechnology*, *51*(4), 509–

519.

Moore, M. M., Schober, T. J., Dockery, P., & Arendt, E. K. (2004). Textural comparisons of gluten-free and wheat-based doughs, batters, and breads. *Cereal Chemistry*, *81*(5), 567–575. https://doi.org/10.1094/CCHEM.2004.81.5.567.

Mossé, J. (1990). Nitrogen to Protein Conversion Factor for Ten Cereals and Six Legumes or Oilseeds. A Reappraisal of Its Definition and Determination. Variation According to Species and to Seed Protein Content. *Journal of Agricultural and Food Chemistry*, *38*(1), 18–24. https://doi.org/10.1021/jf00091a004.

Norton, I.T., Spyropoulos, F. & Cox, P. (Eds). (2011). *Practical Food Rheology: An Interpretive Approach*. Oxford, UK: Wiley-Blacwell.

O'Shea, N., Arendt, E., & Gallagher, E. (2014). State of the Art in Gluten-Free Research. *Journal of Food Science*, *79*(6), 1067–1076. https://doi.org/10.1111/1750-3841.12479.

Official Methods of Analysis of Association of Official Analytical Chemists (AOAC). (1995). 955.04 Kjeldahl Method (16th ed.). Washington DC, USA: Association of Official Analytical Chemist.

Oom, A., Pettersson, A., Taylor, J. R. N., & Stading, M. (2008). Rheological properties of kafirin and zein prolamins. *Journal of Cereal Science*, *47*, 109–116. https://doi.org/10.1016/j.jcs.2007.02.005.

Oria, M. P., Hamaker, B. R., Axtell, J. D., & Huang, C. P. (2000). A highly digestible sorghum mutant cultivar exhibits a unique folded structure of endosperm protein bodies. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(10), 5065–5070. https://doi.org/10.1073/pnas.080076297.

Paraskevopoulou, A., Provatidou, E., Tsotsiou, D., & Kiosseoglou, V. (2010). Dough rheology and baking performance of wheat flour-lupin protein isolate blends. *Food Research International*, *43*(4), 1009–1016. https://doi.org/10.1016/j.foodres.2010.01.010.

Parenti, O., Guerrini, L., Canuti, V., Angeloni, G., Masella, P., & Zanoni, B. (2019).

The effect of the addition of gelatinized flour on dough rheology and quality of bread made from brown wheat flour. *LWT-Food Science and Technology*, *106*(October 2018), 240–246. https://doi.org/10.1016/j.lwt.2019.02.066.

Pozani, S., Doxastakis, G., & Kiosseoglou, V. (2002). Functionality of lupin seed protein isolate in relation to its interfacial behaviour. *Food Hydrocolloids*, *16*(3), 241–247. https://doi.org/10.1016/S0268-005X(01)00094-7.

Protonotariou, S., Drakos, A., Evageliou, V., Ritzoulis, C., & Mandala, I. (2014). Sieving fractionation and jet mill micronization affect the functional properties of wheat flour. *Journal of Food Engineering*, *134*, 24–29. https://doi.org/10.1016/j.jfoodeng.2014.02.008.

Ravve, A. (Ed). (1995). *Principles of Polymer Chemistry*. (p 1-4). New York, USA: Springer Science. https://doi.org/10.1007/978-1-4899-1283-1.

Renzetti, S., Hamaker, B. R., Campanella, O. H., Jurgens, A., & Erickson, D. P. (2014). Modulating state transition and mechanical properties of viscoelastic resins from maize zein through interactions with plasticizers and co-proteins. *Journal of Cereal Science*, *60*(3), 576–583. https://doi.org/10.1016/j.jcs.2014.08.001.

Rosell, C. M. (2011). *The Science of Doughs and Bread Quality*. In V.R. Preedy, R.R. Watson & V.B. Patel (Eds), *Flour and Breads and their Fortification in Health and Disease Prevention* (p. 3-14). London, UK: Elsevier Sience & Technology. https://doi.org/10.1016/B978-0-12-380886-8.10001-7.

Rosell, C. M., Rojas, J. A., & Benedito de Barber, C. (2001). Influence of hydrocolloids on dough rheology and bread quality. *Food Hydrocolloids*, *15*(1), 75–81. https://doi.org/10.1016/S0268-005X(00)00054-0.

Salgó, A., Haraszi, R., Gras, P. W., Tömösközi, S., & Békés, F. (2007). Application of a Micro Z-Arm Mixer to Characterize Mixing Properties and Water Absorption of Wheat Flour. *Cereal Chemistry Journal*, *81*(5), 555–560. https://doi.org/10.1094/cchem.2004.81.5.555.

Sanchez, H. D., Osella, C. A., & De la Torre, M. A. (2002). Optimization of gluten-free bread prepared from cornstarch, rice flour, and cassava starch. *Journal of*

Food Science, *67*(1), 416–419. https://doi.org/10.1111/j.1365-2621.2002.tb11420.x.

Sanitarium Health & Wellbeing Coorabong, NSW, Australia . (2014). Gluten Free WeetbixT. Retrieved from https//www. sanitarium.com.au/products/breakfast/weet-bix-gluten/gluten-free. Accessed 09/09/2015.

Sathe, S. K., Deshpande, S., & Salunkhe, D. K. (1951). Functional Properties of Lupin Seed (Lupinus mutabilis) Proteins and Protein Concentrates. *Journal of Food Science*, *47*, 491–497.

Schober, T. J., Bean, S. R., Boyle, D. L., & Park, S. H. (2008). Improved viscoelastic zein-starch doughs for leavened gluten-free breads: Their rheology and microstructure. *Journal of Cereal Science*, *48*(3), 755–767. https://doi.org/10.1016/j.jcs.2008.04.004.

Schober, T. J., Bean, S. R., Tilley, M., Smith, B. M., & Ioerger, B. P. (2011). Impact of different isolation procedures on the functionality of zein and kafirin. *Journal of Cereal Science*, *54*(2), 241–249. https://doi.org/10.1016/j.jcs.2011.06.007.

Schober, T. J., Messerschmidt, M., Bean, S. R., Park, S. H., & Arendt, E. K. (2005). Gluten-free bread from sorghum: Quality differences among hybrids. *Cereal Chemistry*, *82*(4), 394–404. https://doi.org/10.1094/CC-82-0394.

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. *Journal of Cereal Science*, *52*(3), 417–425. https://doi.org/10.1016/j.jcs.2010.07.004.

Shewry, P. R. (2002). The major seed storage proteins of spelt wheat, sorghum, millets and pseudocereals. In P.S. Belton & J.R.N. Taylor (Eds.), *Pseudocereals and Less Common Cereals: Grain Properties and Utilization Potential* (pp. 1–24). Berlin, Germany: Springer, Heidelberg.

Shewry, P. R., & Tatham, A. S. (1990). The prolamin storage proteins of cereal seeds: structure and evolution. *Biochemical Journal*, *267*(1), 1–12. https://doi.org/10.1042/bj2670001.

Sikkema, F. D., Comellas-Aragonès, M., Fokkink, R. G., Verduin, B. J. M., Cornelissen, J. J. L. M., & Nolte, R. J. M. (2007). Monodisperse polymer-virus hybrid nanoparticles. *Organic and Biomolecular Chemistry*, *5*(1), 54–57. https://doi.org/10.1039/b613890j.

Singh, H., Rockall, A., Martin, C. R., Chung, O. K., & Lookhart, G. L. (2006). The analysis of stress relaxation data of some viscoelastic foods using a texture analyzer. *Journal of Texture Studies*, *37*(4), 383–392. https://doi.org/10.1111/j.1745-4603.2006.00058.x.

Slavin, J. (2004). Whole grains and human health. *Nutrition Research Reviews*, *17*(1), 99-110. https://doi.org/10.1079/NRR200374.

Sly, A. C., Taylor, J., & Taylor, J. R. N. (2014). Improvement of zein dough characteristics using dilute organic acids. *Journal of Cereal Science*, *60*(1), 157–163. https://doi.org/10.1016/j.jcs.2014.02.006.

Stefoska-needham, A., Beck, E. J., Johnson, S. K., Linda, C., Beck, E. J., & Johnson, S. K. (2015). Sorghum : An Underutilized Cereal Whole Grain with the Potential to Assist in the Prevention of Chronic Disease Sorghum : An Underutilized Cereal Whole Grain. *Food Reviews International*, *31*(4), 401–437. https://doi.org/10.1080/87559129.2015.1022832.

Taylor, J. R. N. (2017). Environmental, nutritional and social imperatives for ancient grains. In J.R.N. Taylor & J.M. Awika (Eds.), *Gluten-free Ancient Grains, Cereals, Pseudocereals and Legumes: Sustainable, Nutritious and Health Promoting Foods for the 21st Century* (pp. 6–9). Cambridge, UK: Elsevier Science & Technology. https://doi.org/https://doi.org/10.1016/B978-0-08-100866-9.00008-x.

Taylor, J. R. N. (2019). Sorghum and Millets : Taxonomy, History, Distribution, and Production. In J.R.N. Taylor & K.G. Duodu (Eds.), *Sorghum and Millets: Chemistry, Technology, and Nutritional Atrributes* (2nd ed.,p 1–21). Duxford, UK: Elsevier Science & Technology. https://doi.org/10.1016/b978-0-12-811527-5.00014-9

Taylor, J. R. N., Belton, P. S., Beta, T., & Duodu, K. G. (2014). Increasing the utilisation of sorghum, millets and pseudocereals: Developments in the science

of their phenolic phytochemicals, biofortification and protein functionality. *Journal of Cereal Science*, *59*(3), 257–275. https://doi.org/10.1016/j.jcs.2013.10.009.

Taylor, J. R. N., Johnson, S. K., Taylor, J., Njila, S., & Jackaman, C. (2016). Oxidation of commercial (α-type) zein with hydrogen peroxide improves its hydration and dramatically increases dough extensibility even below its glass transition temperature. *Journal of Cereal Science*, *70*, 108–115. https://doi.org/10.1016/j.jcs.2016.05.025.

Taylor, J. R. N., Schober, T. J., & Bean, S. R. (2006). Novel food and non-food uses for sorghum and millets. *Journal of Cereal Science*, *44*(3), 252–271. https://doi.org/10.1016/j.jcs.2006.06.009.

Taylor, J. R. N., Taylor, J., Campanella, O. H., & Hamaker, B. R. (2016). Functionality of the storage proteins in gluten-free cereals and pseudocereals in dough systems. *Journal of Cereal Science*, *67*, 22–34. https://doi.org/10.1016/j.jcs.2015.09.003.

Taylor, J. R. N., Taylor, J., Campanella, O. H., & Hamaker, B. R. (2016). Functionality of the storage proteins in gluten-free cereals and pseudocereals in dough systems. *Journal of Cereal Science*, *67*, 22–34. https://doi.org/10.1016/j.jcs.2015.09.003.

Taylor, J. R., Schüssler, L., & van der Walt, W. H. (1984). Fractionation of proteins from low-tannin sorghum grain. *Journal of Agricultural and Food Chemistry*, *32*(1), 149–154. https://doi.org/10.1021/jf00121a036.

Taylor, J., Anyango, J. O., & Taylor, J. R. N. (2013). Developments in the science of zein, kafirin, and gluten protein bioplastic materials. *Cereal Chemistry*, *90*(4), 344–357. https://doi.org/10.1094/CCHEM-12-12-0165-IA.

Taylor, J., Anyango, J. O., Muhiwa, P. J., Oguntoyinbo, S. I., & Taylor, J. R. N. (2018). Comparison of formation of visco-elastic masses and their properties between zeins and kafirins. *Food Chemistry*, *245*, 178–188. https://doi.org/10.1016/j.foodchem.2017.10.082.

Tömösközi, S. & Békés, F. (2016). Bread; dough mixing and testing operations.

Encyclopedia of Food and Health, 490-499.

Truong, H. H., Neilson, K. A., McInerney, B. V., Khoddami, A., Roberts, T. H., Cadogan, D. J., … Selle, P. H. (2017). Comparative performance of broiler chickens offered nutritionally equivalent diets based on six diverse, "tannin-free" sorghum varieties with quantified concentrations of phenolic compounds, kafirin, and phytate. *Animal Production Science*, *57*(5), 828–838. https://doi.org/10.1071/AN16073.

UN-Habitat. (2014). The State of African Cities: Re-Imagining Sustainable Urban Transitions. United Nations Settlements Programme, Nairobi, Kenya. Retrieved from https://www.gwp.org/globalassets/global/toolbox/references/the-state-of-african-cities-2014_re-imagining-sustainable-urban-transitions-un-habitat-2014.

United Nations, Department of Economic and Social Affairs, Population Division (2019). World Population Prospects 2019: Highlights (ST/ESA/SER.A/423. Retrieved from https://www.population.un.org/wpp/Publications/Files/WPP2019_Highlights.

Villarino, C. B. J., Jayasena, V., Coorey, R., Chakrabarti-bell, S., Foley, R., Fanning, K., & Johnson, S. K. (2015). The effects of lupin (Lupinus angustifolius) addition to wheat bread on its nutritional , phytochemical and bioactive composition and protein quality. *Food Research International*, *76*, 58–65. https://doi.org/10.1016/j.foodres.2014.11.046.

Villarino, C. B. J., Jayasena, V., Coorey, R., Chakrabarti-bell, S., & Johnson, S. K. (2015). LWT - Food Science and Technology The effects of Australian sweet lupin (ASL) variety on physical properties of fl ours and breads. *LWT - Food Science and Technology*, *60*(1), 435–443. https://doi.org/10.1016/j.lwt.2014.08.028.

Wang, D., Bean, S., McLaren, J., Seib, P., Madl, R., Tuinstra, M., Shi, Y., Lenz, M. Wu, X. & Zhao, R. (2008). Grain sorghum is a viable feedstock for ethanol production. *Journal of Industrial Microbiology and Biotechnology*, *35*(5), 313–320. https://doi.org/10.1007/s10295-008-0313-1.

Weaver, C. A., Hamaker, B. R., & Axtell, J. D. (1998). Discovery of grain sorghum germ plasm with high uncooked and cooked in vitro protein digestibilities. *Cereal Chemistry*, *75*(5), 665–670. https://doi.org/10.1094/CCHEM.1998.75.5.665.

Weigand, C. (2011). Wheat import projections towards 2050: market analysis. U.S. Associates. Retrieved from https://www.wheat/import/projections/towards2050/Weigand/20Jan2011(1).pdf.

Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology*, *24*(2), 115–119. https://doi.org/10.1016/j.fm.2006.07.004.

Wong, A., Pitts, K., Jayasena, V., & Johnson, S. (2013). Isolation and foaming functionality of acid-soluble protein from lupin (Lupinus angustifolius) kernels. *Journal of the Science of Food and Agriculture*, *93*(15), 3755–3762. https://doi.org/10.1002/jsfa.6249.

Yousif, A., Nhepera, D., & Johnson, S. (2012). Influence of sorghum flour addition on flat bread in vitro starch digestibility , antioxidant capacity and consumer acceptability. *Food Chemistry*, *134*(2), 880–887. https://doi.org/10.1016/j.foodchem.2012.02.199.

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APPENDIX

Table 7.1 Summary of the effect of adding lupin protein to commercial zein on the properties of the resulting masses

Protein	Preparation method	Effect on mass formation	Effect on mass properties	Explanation
Zein alone	Dissolved in 70% (w/w) aqueous ethanol solution at 70°C and coacervated with water (5.8ºC)	Formation of a fibrous mass	Extensible when stretched. Became very stiff and broke upon cooling to ambient temperature	Zein returns from rubbery to its amorphous state due to cooling below its T_g temperature
Zein:lupin protein(4:1)	Zein dissolved separately with 70% (w/w) aqueous ethanol solution at 70°C and lupin protein in water. Both protein suspensions combined and coacervatedwith water (5.8ºC)	Formation of a lumpy fibrous mass with short fibres		Lupin protein is insoluble in ethanol, hence disrupt the zein, which result in truncated fibres
Zein:lupin protein (3:2 and 1:1)	Zein dissolved separately with 70% (w/w) aqueous ethanol solution at 70°C and lupin protein in water. Both proteins mixtures were combined and coacervated with distilled water (5.8ºC)	No mass formation, instead gave a gritty sediment	Absence of new molecular weight bands	The high amount of lupin protein prevent the formation of zein fibre completely Zein and lupin protein did form a copolymer

2763 **Table 7.1 Continued** Summary of the effect of adding lupin protein to commercial zein on the properties of the resulting masses

Protein	Preparation method	Effect on mass formation	Effect on mass properties	Explanation
Zein alone	Prepared with 5.4% (w/w) acetic acid at 40°C hand kneaded + sheeted	Formation of a soft fibrous mass with numerous fibres	Very extensible Does not stiffen quickly at ambient temperature	Change in zein protein-protein interactions as a result of protonation of acidic amino acids side chains
Zein:lupin protein (1:1)	Zein prepared with 5.4% (w/w) acetic acid at 40°C. Lupin protein prepared with distilled water. Both protein masses combined, hand kneaded + sheeted	Formation of a fibrous mass	Approximately 3 times less extensible than zein alone prepared with dilute acetic acid Absence of new molecular weight bands	Zein and lupin did not interact a copolymer
Lupin protein alone	Prepared with distilled water at 40°C	Formation of a cohesive mass	With some but limited elasticity	Due to disulphides cross-links present in lupin protein

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National meeting oral presentation from this research:

Dovi, K. A. P., Solah, V., Taylor, J. R. N., Dang, J. M. C. Dang & Johnson, S. K. Development of a micro-scale screening method to understand variation in dough forming ability of sorghum genotypes. *Australian Sorghum Research Group Meeting (AusSoRGM)* organised by DAF/QAAFI, Queensland, Australia, 27-28 July 2017.

International conference poster presentation from this research:

Dovi, K. A. P., Solah, V., Taylor, J. R. N., Dang, J. M. C. Dang & Johnson, S. K. Development of a micro-scale screening method to evaluate mixing properties of whole grain sorghum and whole grain wheat composite flour. *Sorghum in the 21st Century Conference: Food, Feed and Fuel in a Rapidly Changing World*, Cape Town, South Africa, 9-12 April 2018.