Improvement in maize bread quality through addition of pre-gelatinized starch, sourdough in combination with dough sheeting

by

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DECLARATION

I declare that the dissertation herewith submitted for the degree MSc Food Science at the University of Pretoria has not previously been submitted for a degree at any other university or institution of higher education.

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ABSTRACT

Improvement of maize bread quality through dough sheeting

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Wheat bread is a staple food product that is not easily accessible to many people in sub-Saharan Africa as it is relatively expensive. This is due to the regions climatic conditions which are not generally suitable for wheat cultivation. Hence, most wheat has to be imported. Maize is potentially a suitable alternative for production of bread and other dough-based products since it is widely produced under diverse environments in sub-Saharan Africa. However, maize does not possess unique viscoelastic gas-holding properties like wheat.

The effects of dough sheeting on the quality of maize dough and bread were investigated. Dough sheeting in combination with maize flour starch pre-gelatinization, zein, sourdough fermented maize flour and surfactant (DATEM) addition were investigated. Dough sheeting is a simple technology that has been used for development of dough from wheat flour of low protein quality.

Dough sheeting of maize flour without pre-gelatinization produced a crumbly dough, whereas sheeting in combination with starch pre-gelatinization produced a cohesive dough with dramatically improved dough handling properties. Tensile tests showed development of a smoother texture on maize dough as the number of sheeting passes increased from 5 to 40.

Zein dough addition (mixed above its glass transition temperature in water) in combination with dough sheeting formed a more elastic maize dough. CLSM revealed intermingling of fibrils from the added zein within the maize dough which was presumably responsible for the improvement in viscoelastic properties of the composite. Alveography revealed that maize-zein doughs retained gases well but that increasing sheeting passes reduced stability and extensibility.

Maize bread had undesirable cracks on the crust. With zein addition, there was a reduction in the cracks. DATEM addition improved bread crumb structure, preventing the formation of holes. This was thought to be due to hydrophobic and hydrophilic interactions between starch and protein of maize flour and the DATEM.

Dough sheeting in combination with sourdough addition and pre-gelatinized maize produced maize bread with improved loaf height. Stereomicroscopy of the crumb of maize sourdough bread showed a more continuous crumb structure. It is proposed that the improvement of maize bread by sourdough addition is due to the sourdough inducing softening and modification of starch, making dough less elastic but improved ability of the maize dough to trap carbon dioxide and withstand pressure of expanding gas.

The study shows that dough sheeting together with pre-gelatinization of some of the maize flour improves dough handling and functional properties of maize doughs when applied in combination with other treatments. The best combination for maize bread was found to be dough sheeting at 15 passes, pre-gelatinization together with addition of sourdough and DATEM. Dough sheeting in combination with pre-gelatinized maize flour, addition of maize sourdough and DATEM could form a relatively inexpensive and predominately natural way of producing gluten-free breads.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Maize is a major grain grown worldwide, probably ranking second only to wheat total production (Hager et al., 2012). The production of maize was 71.0 million tons in Africa (FAOSTAT, 2013). Wheat bread is not easily accessible sub-Saharan African because of high import prices due to adverse growing conditions. Wheat gluten which is formed during dough making possesses unique viscoelastic (viscous flow and elastic recovery) (Delcour and Hoseney, 2010) properties that enable wheat flour dough to hold gas produced during the fermentation process, leading to an aerated crumb bread structure (Brites et al., 2010). A challenge is to produce bread from maize that will resemble the desirable qualities of wheat bread such as high loaf volume and open crumb structure (Falade et al., 2014).

The use of cereals such as rice (Hager et al., 2012), sorghum (Bugusu et al., 2001) and maize (Falade et al., 2014) for gluten-free bread making is common. These gluten-free doughs have a batter-like consistency (Schober et al., 2005) since they require more water than wheat flour due to their lower water absorption (Oom et al., 2008). Therefore, innovative ways are required to improve gluten-free batters using maize flour. A few investigations have included additives such as eggs (Houben et al., 2012), milk (Gallagher et al., 2003a; Shin et al., 2010), starch, hydroxypropyl methylcellulose (Schober et al., 2008; Falade et al., 2014) and dilute organic acids (e.g. lactic acid and acetic acid) (Sly et al., 2014). However, the use of these additives affects the sensory properties of the final product (Kenny et al., 2000; Gallagher et al., 2003a).

A method commonly used to improve the functional properties of gluten-free batters is a pre-gelatinization of flour (Brites et al., 2010). It causes an increase in elasticity and viscosity giving body and texture to the product due to gelatinization part of the starch in the flour (Sozer, 2009). This later leads to higher dough viscosity (Brites et al., 2010). Incorporation of maize zein prolamin protein in gluten-free batters has shown some potential in improving the overall functional properties of dough and bread (Schober et al., 2008; Sly et al., 2014). It forms a matrix of protein and starch that is thought to be able to withstand pressure during fermentation thus dough expansion occurs (Bugusu et al., 2002; Sly et al., 2014). Improved dough strength and higher loaf volume was observed when zein was added to a sorghum-wheat composite flour mixed and kept at 35^{o}C (Bugusu et al., 2001).

Dough making by sheeting has been shown to improve dough extensibility and elasticity of wheat dough (Feillet et al., 1977; Kim et al., 2008; Chakrabarti-Bell et al., 2010). In the sheeting process, the dough is kneaded and rolled into a sheet by compression between two rotating cylinders (Petitot et al., 2009). Sheeting can be used to improve the dough quality of weak flours (Patel and Chakrabarti-Bell, 2013). Sourdough fermentation is also a promising alternative to the use of additives in gluten-free systems since it is natural and inexpensive (Falade et al., 2014). Sourdough fermentation is a process where fundamental interactions between the lactic acid bacteria and yeasts occur (Moroni et al., 2009). Sourdough fermented maize dough has been found to be softer and less elastic, but less crumbly than chemically acidified maize dough (Falade et al., 2014). Maize bread made with sourdough had improved quality which was thought to be due to starch granule modification which improved its ability to trap carbon dioxide and withstand the pressure of the expanding gas in the dough. In contrast, in wheat doughs sheeting brings about modifications in the protein network due to the dough being subjected to high mechanical stresses (Feillet et al., 1977). This causes the protein and the starch to become distributed more uniformly throughout the dough (Petitot et al., 2009).

This present work will focus on dough sheeting to improve the rheological properties of maize dough when combined with maize flour pre-gelatinization, sourdough fermentation, incorporation of zein and addition of surfactant dough improvers.

2. LITERATURE REVIEW

The task of producing high-quality leavened cereal products such as breads without gluten has proven to be difficult. When wheat flour is mixed with water, it has the ability to form a strong, cohesive dough that can retain gases and produce light, aerated bread (Delcour and Hoseney, 2010). This property is due to the wheat storage proteins, glutenin and gliadin, which together form gluten. Gliadin is sticky when hydrated and has little or no resistance to extension and appears to be responsible for dough's cohesiveness. In contrast, glutenin is resilient and rubbery but is prone to rupture. Moreover, glutenin plays an integral role in bread structure formation and dough functionality (Erickson et al., 2012). Gluten-free formulations have been investigated with varied results. This literature review will focus on the effects of improvers and gluten replacers on the dough and bread wheat and non-wheat cereals on sourdough fermentation and on the potential of novel dough development methods such as dough sheeting technology in improving dough quality.

2.1. Dough improvers and gluten replacers

2.1.1. Hydrocolloids and Emulsifiers

2.1.1.1. Pre-gelatinized starch and Hydrocolloids

Starch pre-gelatinization is an alternative to using specialized hydrocolloid additives to improve the textural properties of gluten-free batters. It improves dough handling and rheological properties through the partial starch gelatinization of the flour to form a cohesive dough by giving body and texture to the flour (Raina et al., 2005; Sozer, 2009). The gelatinized starch acts as a binder in gluten-free flours since they lack the functionality of wheat gluten in making cohesive dough structure (Sozer, 2009).

Raina et al. (2005) made rice pasta through starch pre-gelatinization. They found that pre-gelatinization of rice flour resulted in a firmer pasta texture. A similar strategy was followed by Brites et al. (2010). They made gluten-free bread from maize by applying flour blanching to increase dough consistency, adhesiveness, springiness and stickiness. The blanching consisted of adding boiling water to maize flour and after mixing it was left to cool, and the rest of the ingredients were mixed, proofed and baked. Blanching of flour gelatinized part of the starch which was responsible for the improved textural properties of dough.

The addition of hydrocolloids has shown to enhance gluten-free dough characteristics thus improving the quality of gluten-free breads (Erickson et al., 2012, Houben et al., 2012). They are used to mimic the viscoelastic (viscous flow) properties of gluten, thereby increasing gas retention during proofing and baking and hence increasing the loaf specific volume (Hager and Arendt, 2013). Hydrocolloids, for example hydroxypropylmethylcellulose (HPMC), stabilize doughs by modifying the texture through improving their water binding capacity due to the hydroxyl groups in the hydrocolloid structure which allows more water interactions through hydrogen bonding (Guarda et al., 2004; Gujral and Rosell, 2004; Houben et al., 2012).

Bread made from maize starch alone was found to have a low loaf volume, bad and pungent smell and hard texture (Acs et al., 1996). It had unfavourable visual and physical traits which made it unpalatable (Figure 2.1A). When binding agents were added (xanthan, locust bean gum and guar gum), a positive effect on loaf volume was observed. Xanthan gum followed by guar gum had the most favourable influence on the volume (Figure 2.1B). Incorporation of 1% of both hydrocolloids resulted in significant increase in bread volume.

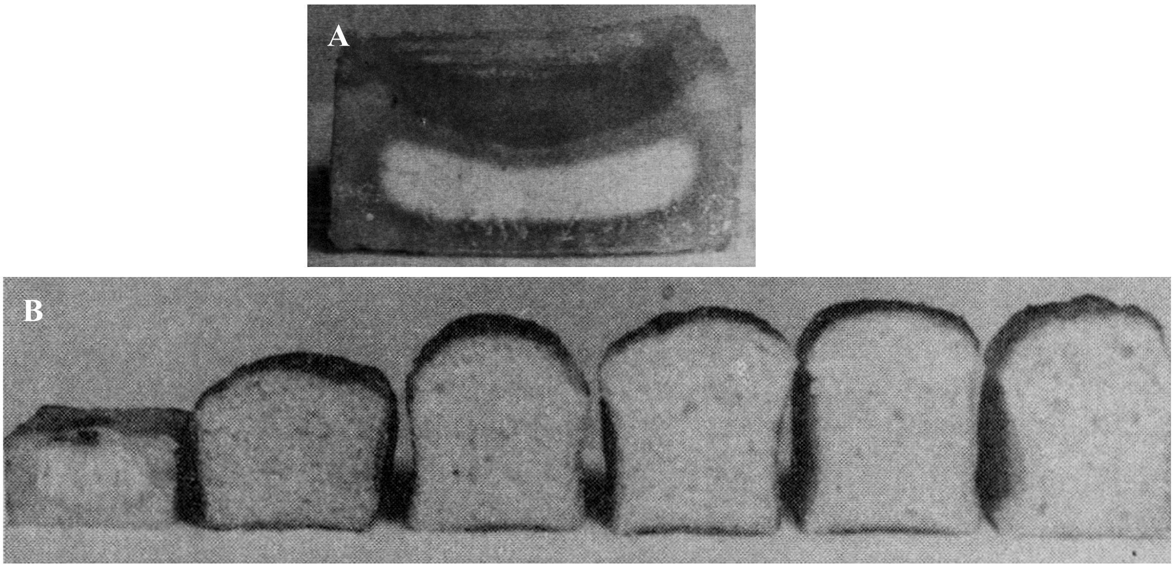


Figure 2.1: Cross section of bread made from maize starch alone with standard additives (salt and yeast) (**A**). Bread images of influence of 0, 1, 2, 3, 4 and 5% xanthan gum on maize starch bread (**B**) (Acs et al., 1996).

The effects of different hydrocolloids on flour are, however very variable (Acs et al., 1996). For instance, xanthan gum had the most favourable influence in maize bread volume compared to guar gum and locust bean gum. Moreover, Hager and Arendt (2013) found that addition of xanthan gum on maize, rice, buckwheat and teff bread increased loaf volume only at low levels (0.30 to 0.52 %). This effect could be due to a thickening effect on the crumb walls surrounding air spaces (Rosell et al., 2001). Dough stability is affected by hydrocolloid addition with a reduction in stability at the lowest hydrocolloid concentration (e.g. HPMC, xanthan gum, carrageenan) (0.1%) and improved dough stability with a 0.5% addition (Guarda et al., 2004).

Schober et al. (2008) found that the addition of HPMC significantly improved the quality of bread made from zein dough and maize starch (unmodified regular maize starch). The resulting bread resembled wheat bread having a regular, fine crumb grain, a round top and good aeration (Figure 2.2). Furthermore, the dough showed zein strands with diameters similar to starch granules. According to Houben et al. (2012), the gel-forming process of hydrocolloids is achieved by connecting to fibril polymer molecules, which are intermolecularly linked to each other through hydrogen bonds or by cross-linking of anionic molecules or multivalent cations (Ca^{2+} or proteins). Zein doughs contain a network of zein strands in the µm to mm range (Schober et al., 2008). This network itself holds only a little gas. However, it traps existing gas bubbles, which are in turn stabilized by HPMC at their interface through hydrogen bonding. Therefore, HPMC has good gas cell stabilization and the positive effects of HPMC on zein could possibly be attributed to a lubricating effect, as HPMC forms viscous, lubricant-like solutions (Schober et al., 2008).

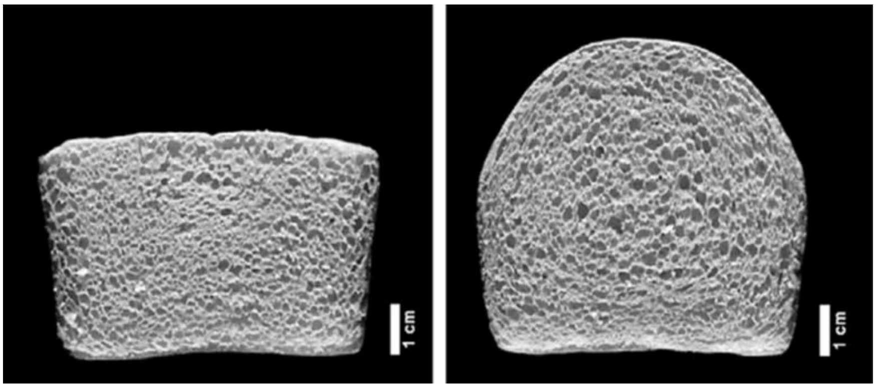


Figure 2.2: Crumb images of gluten-free breads made from zein dough without HPMC (left) and with addition of HPMC (right). The formulation comprised of 20 g zein, 80 g maize starch, 75 g water, 5 g sugar, 2 g salt and 1 g dry yeast; 2 g HPMC were added (Schober et al., 2008).

Similarly, Andersson et al. (2011) found that with the addition of HPMC to a zein-starch system, there was an improvement in the bread volume and height of the gluten-free bread. The HPMC positively affected the rheological properties of zein which yielded similar properties to wheat dough and bread. The bread had open-like crumb structure and increased specific volume.

2.1.1.3. Emulsifiers

Emulsifiers (food surfactants) are food additives that are often used as dough and bread improvers (Nunes et al., 2009a). They are commonly used in bakery products to enhance the structure by increasing the strength of the dough of crumb softness (Stampfli and Nersten, 1995; Sciarini et al., 2012). These emulsifiers are characterized by their amphiphilic nature which allows the molecules to migrate to interfaces between two physical phases lowering surface tension and forming dispersions (Nunes et al., 2009a). Emulsifiers are believed to specifically alter the behaviour of gluten-free batters by acting at the interfaces of the starch, fat or protein (Nunes et al., 2009a). For instance, DATEM (diacetyl tartaric acid ester of mono- and diglycerides) can form a higher number of small cells and stabilises them in bread by forming hydrogen bonds with the protein and starch (Pareyt et al., 2011; Sciarini et al., 2012).

Nunes et al. (2009a) found a significant change in cell size and distribution for white rice flour and potato starch composite breads containing DATEM and lecithin (Figure 2.3). DATEM gave a non-homogenous in crumb at low levels (0.3%). At medium (0.45%) and high levels (0.6%), a decrease in bubble size and a more homogenous crumb structure was observed. Similar results were obtained by Kohler and Grosch (1999) with wheat flour bread. There was an observable increase in loaf volumes of 55 to 60% with 0.1 to 0.5% of DATEM.

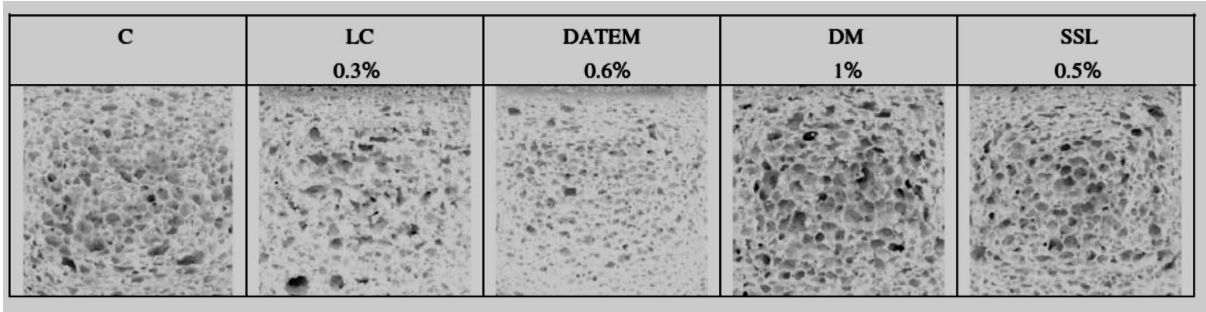


Figure 2.3: Images of gluten-free bread crumb structure at the selected levels of control (C), lecithin (LC), DATEM, distilled monoglycerides (DM) and sodium stearoyl lactylate (SSL) (Nunes et al., 2009a).

In contrast, Sciarini et al. (2012), found that gluten-free breads (rice-cassava starch-soy flour) with DATEM had a lower gas cell number, whereas, breads with SSL presented large cells near the surface. This effect is a characteristic of systems with a rapid water loss and the vapour thus formed exerts certain pressure on the forming of the crumb producing the collapse of the structure (Sciarini et al., 2012).

In wheat dough, Gomez et al. (2004) found that in the presence of emulsifiers (DATEM, SSL, lecithin, polysorbate, distilled monoglyceride, sucrose ester, and enriched lecithin) the wheat flour dough height reached its maximum during proofing. The highest values were obtained with polysorbate, sucrose ester, DATEM and SSL. The dough height increase was related to the strength of the gluten network, since the greatest values were obtained with the emulsifiers that have strengthening action owing to their ability to form complexes with protein and protein-protein aggregation. Also, the emulsifiers slowed down the proofing process, the doughs required a longer time to reach the maximum development. These longer proofing times yielded softer crumbs in the presence of emulsifiers. However, with short proofing times, emulsifiers yielded hard crumbs, significantly harder crumbs than those of the control in the case of lecithin and polysorbate. SSL, sucrose ester, lecithin and enriched lecithin were emulsifiers with greatest crumb softening effects at extended proofing times.

2.1.2. Enzymes

The addition of enzymes to gluten-free doughs can improve dough handling properties and increase the final baking quality (Houben et al., 2012). The water-binding capacity, shelf life, retrogradation and the crumb softness of gluten-free doughs can be influenced positively.

Maize flour treated with transglutaminase (TGase), yielded an increase in specific loaf volume (Renzetti et al., 2008). Moreover, a decrease in crumb hardness was found for lower concentration of TGase (1 TGase unit/g of protein) compared to none at all. TGase activity improved the macroscopic appearance of maize bread (Figure 2.4). It was suggested that a decrease in viscosity brought by TGase addition might facilitate the expansion of batters due to deamidation of glutamine residues.

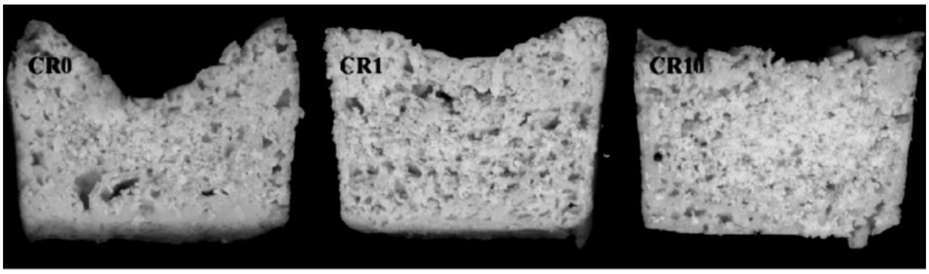


Figure 2.4: Bread slices from yellow maize flour formulations treated with TGase levels (0, 1 and 10 TGase units/g of protein) (Renzetti et al., 2008).

In contrast, addition of glucose oxidase and α-amylase to rice flour-cassava starch-soy flour composite dough resulted in reduced dough viscosity after fermentation (Sciarini et al., 2012). The lower dough viscosity was proposed to be due to the damaged starch fraction being susceptible to α-amylase activity. Moreover, an increase in specific bread volume was observed when α-amylase was added. This effect was thought to be due to the hydrolysis of starch fraction leached as a result of gelatinization during baking. On the other hand, the addition of glucose oxidase produced breads with a specific bread volume similar to the control bread (breads without enzymes) and a high cell number of small size was present in the crumb. A reduction of crumb firmness was observed with enzyme addition.

By comparison, Gujral and Rosell (2004) found that the addition of glucose oxidase in the absence of hydrocolloids (HPMC) increased rice dough consistency with enzyme concentration (0.01 to 0.03%). Furthermore, with the presence of 2% hydrocolloids the dough consistency decreased with increasing level of glucose oxidase. It was thought that the hydrocolloid might be part of the water soluble fraction being affected in some way by the glucose oxidase. The H_2O_2 generated by glucose oxidase in the presence of native peroxidase in the rice flour may have been responsible for the increase in the viscosity.

The proposed mechanism of activity is that glucose oxidase catalyses the oxidation of glucose to glucono-lactone and H_2O_2 (Bonet et al., 2006). The H_2O_2 formed oxidizes sulphydryl groups in proteins, causing protein cross-linking through the formation of disulphide bonds. It also causes the formation of protein/polysaccharide cross-linked part which is responsible for the increased dough consistency in the dough. Alpha-amylase hydrolyses α-(1-4) bonds present in starch, producing low molecular weight dextrins (Goesaert et al., 2005).

2.1.3. Non-gluten proteins

2.1.3.1. Dairy proteins

Dairy proteins are considered as highly functional ingredients that can be readily incorporated into many food products especially gluten-free formulations (Gallagher et al., 2003b). They are used in bread to improve the nutritional and functional properties including flavour and texture enhancement (Gallagher et al., 2003a; Nunes et al., 2009b). They increase water absorption, and therefore, enhance handling properties of batter (Gallagher et al., 2004).

Gallagher et al. (2003a) investigated the effect of addition of rice flour and milk protein to a gluten-free wheat starch isolate. The addition of these ingredients resulted in an increase in loaf volume, more open crumb structure (Figure 2.5) and better external appearance which resembled wheat bread loaves.

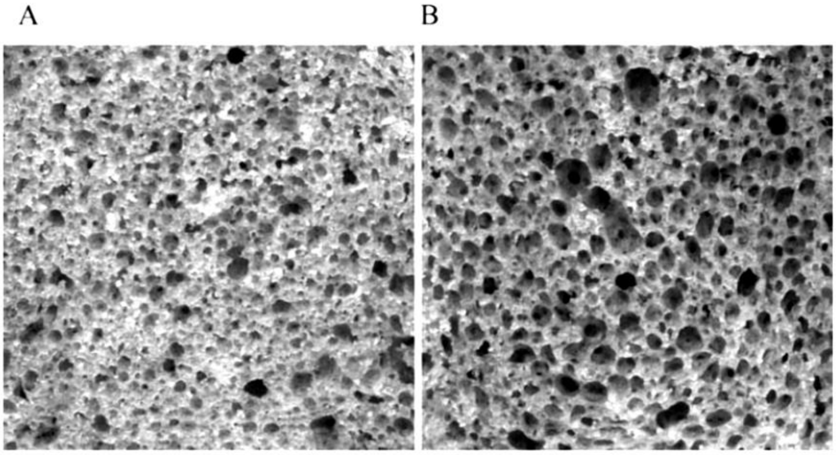


Figure 2.5: Crumb sample images of gluten-free breads. **A**. control (gluten-free wheat starch). **B**. control with rice starch and dairy powder added (Gallagher et al., 2003a).

Further experiments included addition of different levels of dairy powders (e.g. sweet whey, demineralized whey, skim milk replacer, sodium caseinate) and increase moisture content (10 and 20%) to commercial wheat starch gluten free flour (Gallagher et al., 2003b). The resultant batter was more viscous and dough-like. This was presumably due to the dairy powders having a high protein content which contributed to better water absorption and thus minimum migration of moisture to the crust. Moreover, additional moisture in the gluten-free formulation formed bread that had reduced crumb and crust hardness. Generally, breads with the dairy powders were darker when compared to their gluten-free controls and that was attributed to Maillard browning and caramelisation (Gallagher et al., 2003b).

In contrast, Nunes et al. (2009b) investigated the effects of addition of low lactose dairy powders compared to controls without addition of dairy powder and skim milk powder which is high in lactose, on the rheological and baking quality of white rice flour-potato starch breads. They found that whey proteins induced the greatest increase in the specific volume of the breads. They stated that these proteins are characterised by a globular structure, which is susceptible to denaturation at high temperatures (70°C) as well as thermal gelling. The increase in loaf specific volume was attributed to the fact that during baking, these proteins denature and the bonds creating the tertiary structure of the whey globules are destroyed. The proteins unfold and new protein-protein interaction may occur as well as interactions with other components of the batter. This unfolding and interaction with batter components could lead to improved loaf specific volume as observed in the study. Further, it was observed that the highest total cell area and cells/cm^2 for breads was with added sodium caseinate, whey protein isolate (spray dried) and whey protein isolate (membrane technology), indicating a more open crumb structure, whereas the opposite was found for the control with no added dairy powder, skim milk powder and whey protein concentrate (Nunes et al., 2009b).

2.1.3.2. Legume proteins

Legume proteins have been recently incorporated in gluten-free batters to enhance both nutritional and physical characteristics (Marco and Rosell, 2008; Miñarro et al., 2012). Soya proteins have been found to increase crumb texture and bread volume and increase water binding capacity (Shin et al., 2010; Houben et al., 2012).

Miñarro et al. (2012) investigated the characteristics of gluten-free formulations prepared with legume protein sources (chickpea flour, pea protein isolate, soya flour and carob germ flour) as substitutes for soya flour. Breads with legume proteins had a good physicochemical characteristics and adequate sensory profile. The carob germ batters had a thicker structure compared to chickpea, pea and soya batters. The specific volume of chickpea flour bread was the highest, while carob germ bread showed the lowest volume. Microscopy of carob germ breads showed a compact structure without spaces between starch granules (Figure 2.6D). Chickpea and pea breads showed a more homogeneous structure (Figure 2.6 A and B). These formulations resulted in an open structure able to incorporate gas which accounted for their volume and textural characteristics.

In contrast, Matos et al. (2014) used soy protein isolate pea protein isolate in rice-based muffins. The incorporation influenced the rheological characteristics as well as mechanical properties by increasing the elastic modulus thereby providing increased elasticity as well nutritional enhancement. Further, the addition of pea protein in rice flour based muffins caused the lowest hardness and the highest springiness value among samples made from vegetable proteins (soy protein isolate and pea protein). Again, the pea protein isolate containing muffins were softer compared to muffins with no added protein. These protein isolates led to more structured and solid like batters.

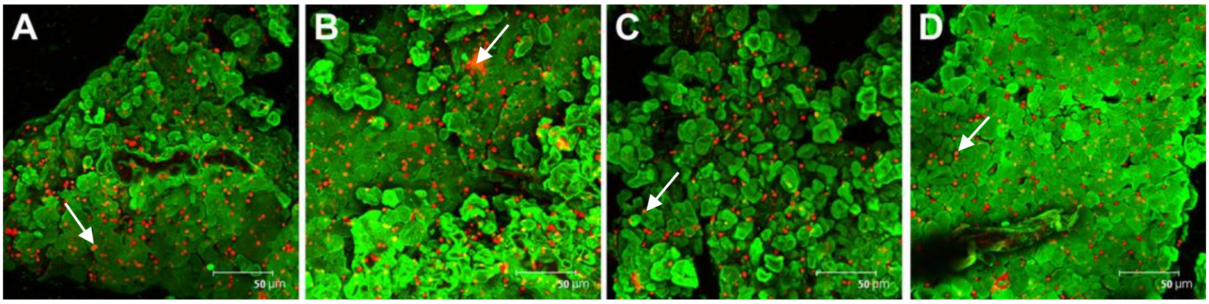


Figure 2.6: CLSM images of legume flour gluten-free breads. **A**. chickpea flour. **B**. pea isolate. **C**. soya flour. **D**. carob germ flour. The red colour indicates yeast cells, orange-yellow is the protein (indicated by white arrow) and the bright green is the starch (Miñarro et al., 2012).

2.1.3.3. Cereal proteins

Cereal proteins such as zein (the maize prolamin protein) can be used to mimic gluten's viscoelastic (viscous flow) nature in gluten-free bakery products (Deora et al., 2014). Zein-starch systems show potential in forming palatable, high-quality gluten-free products. Zein, is able to form a viscoelastic protein network during mixing, if the protein is held and mixed at 35^{o}C (Lawton, 1992). Several studies have been conducted on zein and its ability to form a dough above its glass transition temperature (T_g) for example (Bugusu et al., 2001; Oom et al., 2008; Sly et al., 2014; Andersson et al., 2011). The ability of zein to form a viscoelastic dough is thought to be through non-covalent protein-protein interactions (Smith et al., 2014).

Microscopic observation of maize flour show protein structures and organelles as a continuous phase surrounding the starch granules (Chanvrier et al., 2005). However, this protein phase is not available for dough formation due to the proteins being encapsulated in the protein bodies (the organelles of zein storage) (Oom et al., 2008). Addition of isolated zein protein, which is available for fibril formation, can cause the formation of a matrix between protein and starch of the flour that is thought to be able to withstand pressure during fermentation which contributes to dough strength and dough expansion (Bugusu et al., 2001; Sly et al., 2014).

When zein was mixed above glass transition at 5% and 10% concentration additions, improved rheological and leavening properties of the sorghum-wheat composite flour dough was observed (Bugusu et al., 2001). The improvement of the composite dough properties was attributed to zein being available for participation in fibril formation (Figure 2.7).

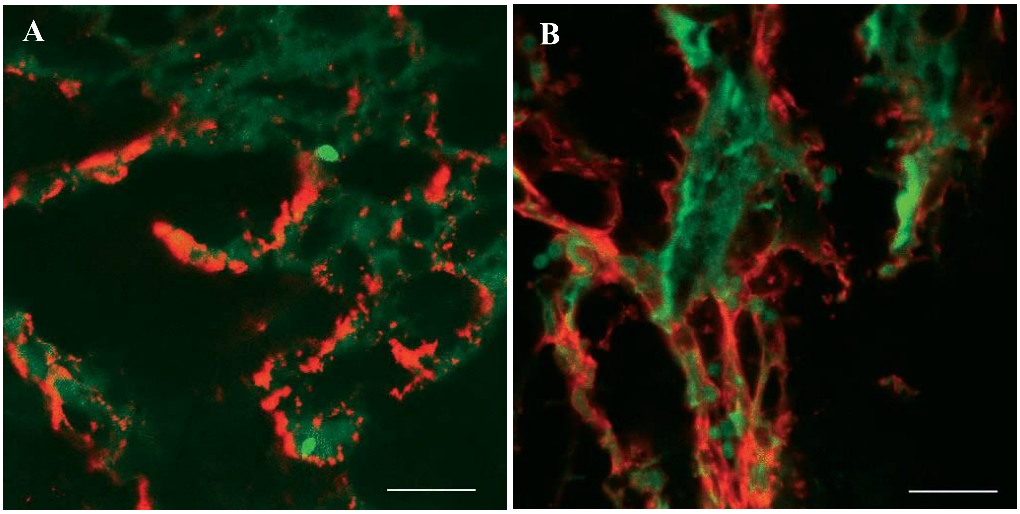


Figure 2.7: CLSM images of sorghum-wheat-zein dough (**A**) and bread (**B**) with added α-zein primary antibody. Green is the gluten network and red is zein fibrils. Size bar= 50 µm (Bugusu et al., 2002).

In the absence of added hydrocolloids (HPMC), Andersson et al. (2011) observed zein-starch dough to have a fibrous surface with low extensibility and brittle when handled at room temperature. This served as confirmation that zein has a T_g above room temperature. Further, there was some phase separation between the zein protein and starch, resulting in a slight amount of starchy liquid surrounding the dough after mixing and these doughs had a sticky and smooth surface. The authors concluded that zein cannot mimic gluten properties on its own, but requires hydrocolloids to positively affect the structural and rheological properties of zein. The microstructure of zein-starch dough prepared above T_g of zein had protein fibres which agrees with the observation of Bugusu et al. (2002).

In comparison, Schober et al. (2008) observed zein patches without addition of hydrocolloids (HPMC) using confocal laser scanning microscopy (CLSM). However, they observed zein strands after addition of hydrocolloids. The results also suggested that the presence of zein strands does not guarantee satisfactory gas holding potential for bread making. After baking, the zein strands were no longer visible in the crumb structure. The loss of zein strands was attributed to overextension and rupture of the zein strands and more irregular arrangement of the zein in the dough.

It is vital for zein to be mixed above T_g in a zein-starch system and this has been shown to lead to an increase in β-sheet fractions (Mejia et al., 2007). This conformal change is similar to gluten in dough extension. In contrast, Sly et al. (2014) found that preparation of zein dough with dilute organic acids (lactic acid and acetic acid) above its T_g improved zein-starch dough functionality. The acidic conditions of the dilute organic acids somewhat reversed the change from the predominantly α-helical to more β-sheet conformation which occurs when zein is made into a dough. Moreover, the authors suggested that the increase in α-helical conformation possibly occurs as a result of deamination of the zein molecules, which in turn enables formation of a more uniform dough structure with linear orientation of fibrils.

2.2. Sourdough fermentation

Sourdough fermentation is one of the oldest technological processes in food technology. It can improve volume, texture, flavour, nutritional value of bread and increase its shelf-life by retarding the staling process and by protecting bread from mould and bacterial spoilage (Arendt et al., 2007, Moroni et al., 2009). Sourdough fermentation is a process whereby fundamental interactions between lactic acid bacteria, (LAB) and yeasts take place. The most frequently isolated lactic acid bacteria are *Lactobacillus sanfranciscensis, Lactobacillus plantarum* and *Lactobacillus brevis*, and *Saccharomyces cerevisiae* is the yeast most frequently present in sourdough with the ratio of LAB: yeast being approximately 100:1 (Gobbetti, 1998).

During sourdough fermentation there are biochemical changes occurring in the carbohydrate and protein components of the flour due to the action of microbial and indigenous enzymes (Arendt et al., 2007). For example, when *Lactobacillus plantarum* was associated with *Saccharomyces cerevisiae*, it caused an increase in carbon dioxide produced and improved the capacity of the sourdough to retain the gas (Gobbetti, 1998). Also, the lactic acid produced by *Lactobacillus plantarum* is responsible for a more elastic gluten structure.

The major problem encountered with gluten-free doughs is that they are much less cohesive and elastic than wheat doughs (Houben et al., 2012). They are smooth and difficult to handle and are more like a cake batter. A possible solution to improve the quality of maize dough is the use of sourdough fermentation. Falade et al. (2014) found that the maize sourdough breads had a more open crumb structure with discrete gas and less force was required to compress the maize sourdough breads compared to maize bread without sourdough. They also showed a cohesive dough structure (Figure 2.8). This was related to endosperm matrix protein degradation as showed by Schober et al. (2007). The degradation of the protein possibly enabled the partial starch hydrolysis and also leaching of amylose. The leached amylose would be capable of forming a network which probably resulted in the formation of a cohesive dough structure.

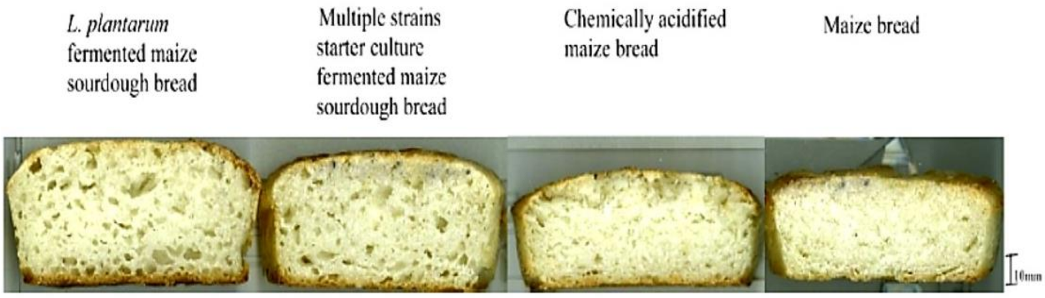


Figure 2.8: Effects of *Lactobacillus plantarum* or multiple strains starter culture fermented maize sourdough on the crumb structure of maize bread (Falade et al., 2014).

Edema et al. (2013) found that sourdough fermentation of fonio and sorghum flour improved the dough quality. An increase in viscosity and resistance to breakdown was observed and that led to a better crumb structure of the bread. Sourdough fermentation caused changes in the starch and these changes were apparent as slight granule swelling and probably some leaching of starch molecules. Presumably the changes were caused by the action of endogenous amylases from the sourdough microorganisms, bringing about limited starch hydrolysis and probably increasing water absorption.

Moore et al. (2007), investigated the effects of addition of lactic acid bacteria on gluten-free (rice-soy-buckwheat-maize) sourdough, batter and bread. They found by CLSM that protein particles were degraded over time within the sourdough, mainly the soy and buckwheat proteins. With the *Lactobacillus plantarum* sourdough (starter) for the gluten-free batters (rice flour, buckwheat, soy flour and maize starch), it was evident from CLSM observation, that there was a contained diffuse mass of large soy globules (white areas) surrounded by starch granules visible as light grey globules. At 0 hours of fermentation, larger particles were dispersed throughout the sourdough structure (Figure 2.9A). After 24 hours, these particles were degraded and the proteins appeared smaller and more scattered with starch granules being more refined particles (Figure 2.9B). It was assumed that the lactic acid bacteria, in conjunction with the naturally present enzymes, may have partially digested the protein-rich particles making the protein more accessible to bind water in the bread batters. The newly accessible protein might have assisted in sticking the remaining proteins together, creating larger aggregates and more stable microstructure.

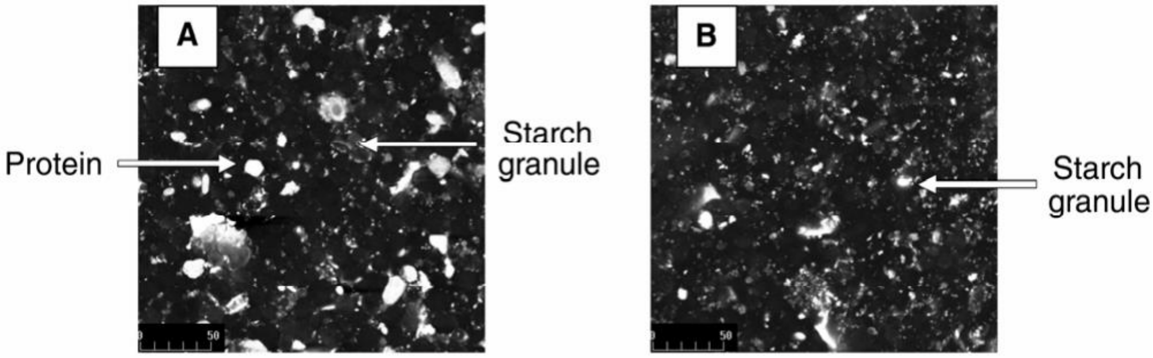


Figure 2.9: CLSM images of *Lactobacillus plantarum* sourdough at 0 hour (**A**) and at 24 hours (**B**). Magnification 40x, bar 100 µm (Moore et al., 2007).

Sly et al. (2014) found a progressive increase in extensibility in zein doughs with both dilute lactic acid and acetic acid concentrations, from 175 mm with dough prepared with water to 341 mm with dough prepared in 5.4% acetic acid. In addition, as stated, the acidic conditions seemed to reverse the predominately α-helical to a more β-sheet conformation which occurs when zein was made into dough. This enabled the formation of a more uniform dough structure with linear orientation of fibrils which improved zein dough properties of extensibility while retaining cohesiveness.

A further advantage of using sourdough as a way to improve the quality of maize bread is that the lactic acid bacteria have been shown to possess both anti-bacterial and anti-fungal properties and sourdough addition is an effective procedure to preserve bread from spoilage since it complies with the consumer demands for additive-free products (Gobbetti et al., 2005).

2.3. Novel physical ways of developing doughs

2.3.1. Dough sheeting

Dough sheeting can be a manual or a mechanized process (Patel and Chakrabarti-Bell, 2013). In both cases, dough is subjected to high mechanical stress which modifies the wheat gluten protein network (Feillet et al., 1977). Sheeting requires much less energy and it imparts lower rate input on the doughs than high speed mechanical dough development mixing (Sutton et al., 2003). In mechanized versions of sheeting, the dough may be carried on a conveyer belt and passed back and forth through one set of rollers, or in large scale operations, doughs may be passed through multiple sets or rollers (Patel and Chakrabarti-Bell, 2013). The roll gap decreases as the dough thins and the roll speed may increase depending on the design of the sheeting process. Generally, the shape of the dough (at a molecular level) changes during sheeting and the rheological properties of the dough determine the stresses and strains during sheeting (Dobraszczyk and Morgenstern, 2003). Furthermore, sheeting can be carried out to redistribute the leavening agents and subdivide the gas cells so as to improve the bread crumb appearance (Scanlon and Zghal, 2001).

During mixing, wheat dough develops a protein network which is associated with changes in the biochemical and rheological properties of gluten. Thereafter, during sheeting the dough becomes more elastic and the solubility of the wheat glutenin proteins increase due to depolymerisation of the glutenin polymer (Kim et al., 2008). Excessive sheeting breaks the protein down and affects the gas cell structure by reducing the bubble size because it causes a gradual decrease in the extensibility and resistance of the wheat dough (Autio and Laurikainen, 1997). Kim et al. (2008) found an increase in elasticity of wheat dough during sheeting (Figure 2.10). These results agree with Sutton et al. (2003), who observed an increase in dough extensional viscosity at the beginning of sheeting of wheat dough and then a decline after 20 to 40 passes.

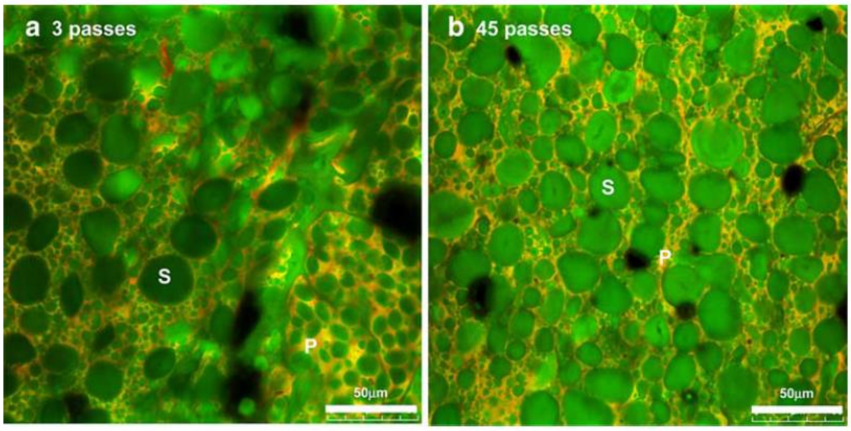


Figure 2.10: CLSM images of microstructure of fresh fettuccini after 3 (**a**) and 45 (**b**) sheeting passes. S-starch granules; P- protein matrix (Kim et al., 2008).

Feillet et al. (1977) investigated the effects of sheeting pasta dough on durum wheat properties. They found that the gluten content was reduced with an increase in a number of sheeting passes but there was a gradual increase in the amount of gliadins. In addition, the solubility of salt-soluble proteins which were not modified during mixing slowly decreased during sheeting from 20.6% (semolina) to 18.4% (30 passes through the rolls). The increase in gliadin content was hypothesized to be due to disulphide (SS) bond breakdown in the glutenin molecules. This breakdown could have arisen either from chemical oxidation or from mechanical stresses developed on the oriented protein chains during sheeting. The highly reactive new polypeptide chains, resulting from the broken glutenin polymers, would be gathered and oriented by sheeting, then cross-linked by disulphide bonds arising from reactions between the new S-H groups. It was also possible for conformational changes in the protein structures to permit hydrophobic groups to interact.

In contrast, Sutton et al. (2003) found that increased sheeting did not increase the rupture of the S-S bonds to form reactive thiol groups. These observations suggested that the overall rupture of disulphide bonds in dough proteins may not be a necessary part of the wheat dough development. It may be that high stresses are not required to develop doughs but instead the nature and direction of the applied stress is important. The application of effective stress is the key to dough development in that in sheeting stress is applied in the most effective direction. This was consistent with the lower energy required to sheet a dough to optimum development.

Petitot et al. (2009) used CLSM to observe freshly sheeted durum wheat pasta and found that the protein matrix was closely associated with starch granules. With increasing sheeting passes (3 to 45 passes), the proteins and starch granules became distributed more uniformly throughout the dough. Hayta and Alpaslan (2001) found that sheeting of durum wheat dough brought about reduction in the gluten content and an increase in gel protein content of dough. This was due to the increase in temperature during sheeting as a result of work input to the dough, resulting in protein denaturation.

2.3.2. High pressure processing

High-pressure (HP) processing is a non-thermal treatment that consists of submitting foods to high pressures, thus creating new structures and textures by inducing starch gelatinization and protein polymerization (Rastogi et al., 2007; Capriles and Arêas, 2014). It is considered as an innovative processing technique for the modification and alteration of cereal ingredients. It has the ability to modify the viscoelastic and structural properties of cereal batters, through gelatinization of starch as well as protein structural changes (Vallons and Arendt, 2009; Vallons et al., 2011; Deora et al., 2014). Further, HP decreases gelatinization temperature and affects the rheological properties of starch-based systems (Rubens and Heremans, 2000). The application of pressure is in the ranges of 1000–100 MPa in gluten-free batters (Deora et al., 2014).

Vallons et al (2011) investigated the effect of HP treatment (300 to 600 MPa) on buckwheat, white rice and teff flours. White rice and buckwheat batters showed an increase in viscosity as the pressure was increased. Teff batter became more elastic, which led to an increased resistance to deformation when it was treated at pressure >200 MPa. The visual appearance of buckwheat revealed changes in the starch granules after HP treatment. The starch granules of rice and teff were small and although the effect of HP on the granules was less obvious, the structure of the batter looked smoother after HP treatment.

HP treatment of sorghum starch was found to cause gelatinization of starch (Vallons and Arendt, 2009). Pressure-induced melting of starch granules started at pressures >300 MPa and complete gelatinization was observed after treatment with 600 MPa. Untreated sorghum starch granules showed characteristic granular shapes and birefringence patterns.

The number of "Maltese crosses" decreased with increasing pressure and temperature above 300 MPa and 60°C, respectively (Figure 2.11). At 400 MPa and 65°C, a significant loss of birefringence was observed. All the sorghum starch granules lost their "Maltese crosses" after treatment with 600 MPa or 75°C. However, the CLSM images suggested preservation of granular structure upon pressure as well as temperature treatment.

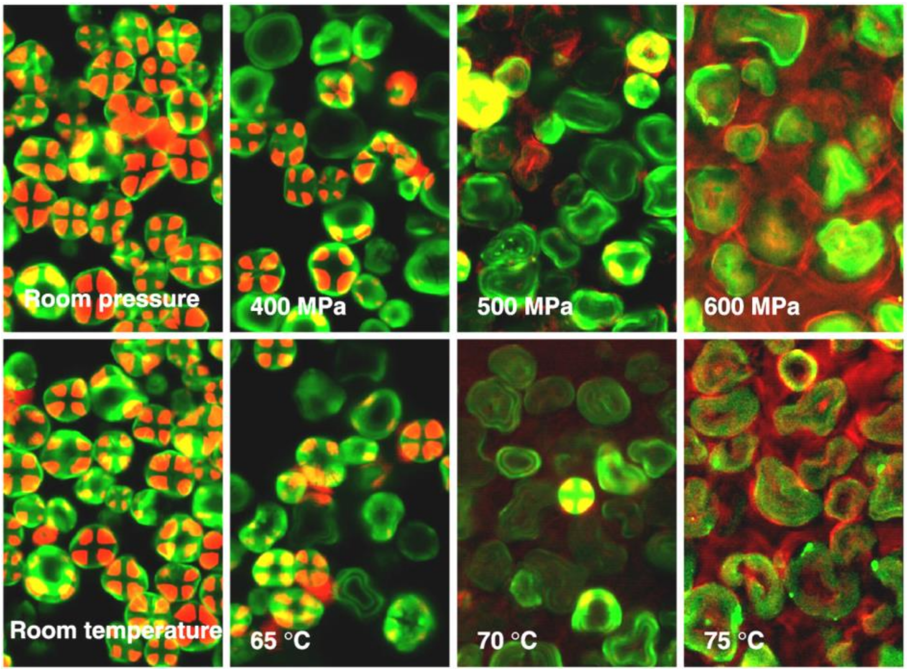


Figure 2.11: CLSM images of sorghum starch at different pressure and temperatures (Vallons and Arendt, 2009).

2.4. Conclusions

Among the various means of improvement of gluten-free batters, starch pre-gelatinization has been utilized to a large extent. It can be induced by addition hot water, extrusion processing or HP processing. Sourdough fermentation has shown success in improving gluten-free batters without use of additional additives. Inclusion of surfactants like DATEM and SSL has shown some success on improving loaf height and crumb texture of gluten-free batters. Furthermore, utilization of cereal proteins such as zein in producing gluten-free bread formulations has been successful through improving structural stability and nutritional quality of breads.

There is limited work on physical ways of improving gluten-free batters. However, sheeting wheat dough has been found to affect the doughs functionality and starch granules. Starch pre-gelatinization in combination with sheeting may improve maize dough handling properties. Furthermore, a combination of sheeting, maize flour pre-gelatinization and sourdough fermentation may improve the dough quality of maize flour. The sheeting and pre-gelatinized starch should improve dough cohesiveness and elasticity of the dough, while sourdough fermentation should improve the dough by softening it for water absorption thus, improving the overall maize dough quality. However, limited data is available on the effects of sheeting on the dough quality of maize flour. Therefore this research should address the possibility of improving the dough quality of maize flour by the sheeting process in combination with starch pre-gelatinization, incorporation of zein, sourdough fermentation and inclusion of surfactants (DATEM).

3. HYPOTHESES AND OBJECTIVES

3.1. Hypotheses

Hypothesis 1:

Application of an increasing number of sheeting passes to maize flour dough will improve maize dough functionality (viscosity and cohesiveness). Wheat flour dough can be developed by sheeting repeatedly between rolls (Moss, 1980). Sheeting has mostly been used for wheat flour of low protein quality for dough development (Bushuk and Hulse, 1974). However, limited research has been done with non-wheat flours. Andersson et al. (2011) stated that zein fibre formation may be induced through an increased input of mechanical energy by mixing systems. Sheeting forms as alternative means of applying work to dough. The adjustment of roll-spacing allows dough to be worked without the tearing action associated with high-speed mixing (Kilborn and Tipples, 1974). In the sheeting process, the dough is kneaded and rolled into a sheet by compression between 2 rotating cylinders. The dough is subjected to high mechanical stresses which produce modifications in the protein network (Feillet et al., 1977). With increasing sheeting passes, there is uniform dispersal of gluten protein and starch granules throughout the wheat dough in the direction of sheeting (Petitot et al., 2009). It has been found that during sheeting, wheat dough became more elastic through the increase in rupture stress and strain. This indicates development of a protein network which may result in increased exposure of the starch granules to digestion in pasta (Kim et al., 2008).

Hypothesis 2:

Combining pre-gelatinized starch with sheeting will improve maize dough bread making quality by forming a cohesive dough. The addition of pre-gelatinized starch forms an alternative to using additives for gluten-free batters. It improves dough development and extensibility through the partial starch gelatinization of the flour to form a cohesive dough. It gives body and texture to the flour (Raina et al., 2005; Sozer, 2009; Brites et al., 2010). The gelatinized starch acts as a binder in gluten free flours since they lack the functionality of wheat gluten in making cohesive dough structure (Sozer, 2009).

Hypothesis 3:

Addition of sourdough fermented maize in combination with sheeting will bring about improvement in dough functionality of the maize flour. Sourdough has a variety of microorganisms that induce softening of dough through proteolysis (Di Cagno et al., 2002). It has been shown that sourdough fermented maize substantially increased the loaf volume and result in a more open crumb structure of the bread (Falade et al., 2014).

Hypothesis 4:

Addition of zein dough to maize flour with sheeting will improve maize dough functionality. Incorporation of zein will cause the formation of a matrix between maize flour protein and starch that is thought to be able to withstand pressure during fermentation, which contributes to dough strength and dough expansion (Bugusu et al., 2002; Sly et al., 2014). When zein was mixed above glass transition temperature at 5% and 10% concentration, the rheological and leaving properties of the sorghum-wheat composite flour dough was improved (Bugusu et al., 2002). The improvement of the composite dough properties was attributed to zein being available for participation in fibril formation.

3.2. Objectives

Objective 1:

To determine the effects of increasing number of sheeting passes of maize flour dough on maize dough functionality.

Objective 2:

To determine the effects of combining starch pre-gelatinization with sheeting on maize dough functionality.

Objective 3:

To determine whether addition of sourdough fermented maize in combination with sheeting brings about an improvement in maize dough functionality.

Objective 4:

To determine the effects of addition of commercial zein (α-zein) in combination with sheeting on maize dough functionality.

4. RESEARCH

Figure 4.1. parts A and B are flow diagrams of the experimental design.

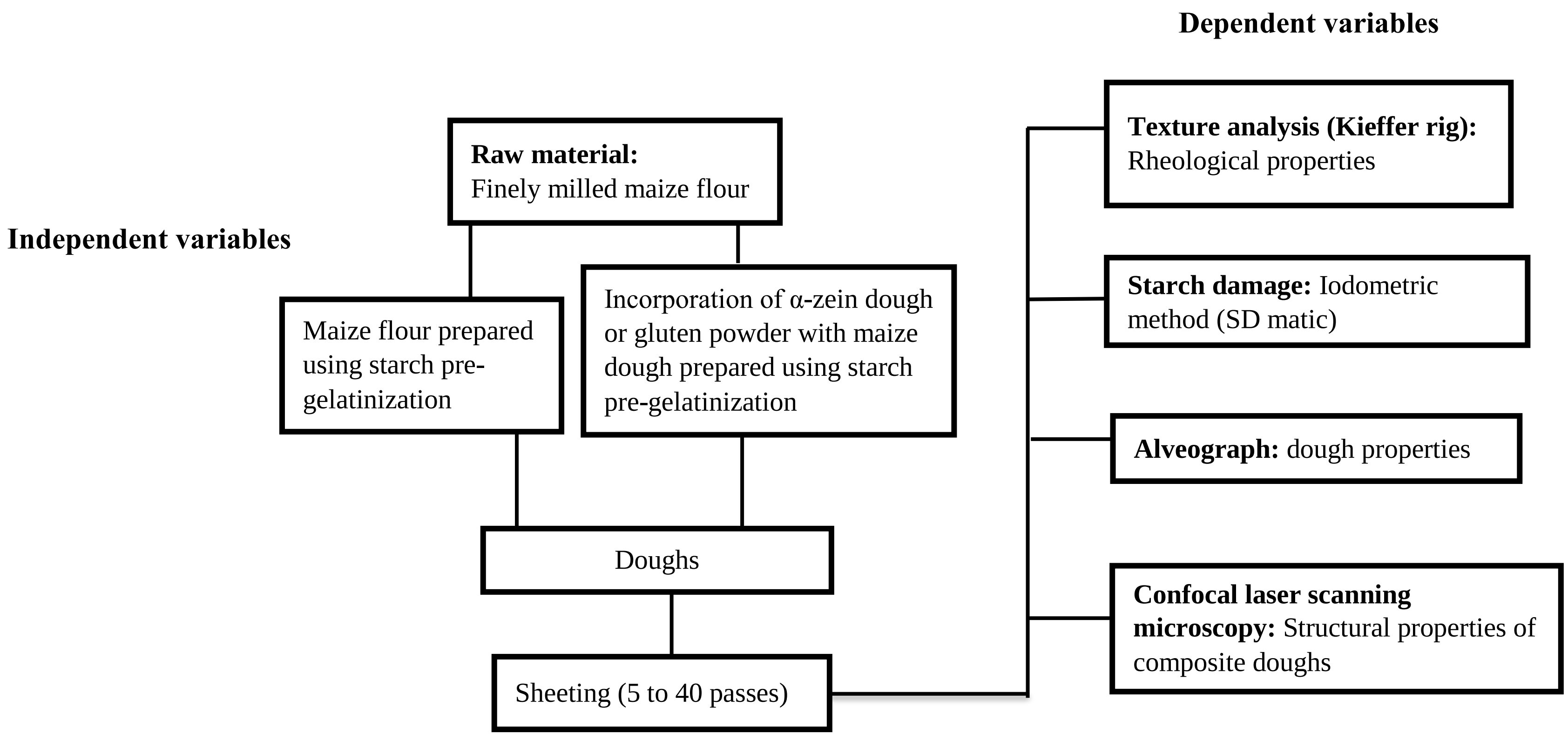


Figure 4.1. A: Experimental design to determine the effects of sheeting of maize dough prepared using starch pre-gelatinization in combination with incorporated α-zein dough or vital gluten powder on maize dough rheological properties

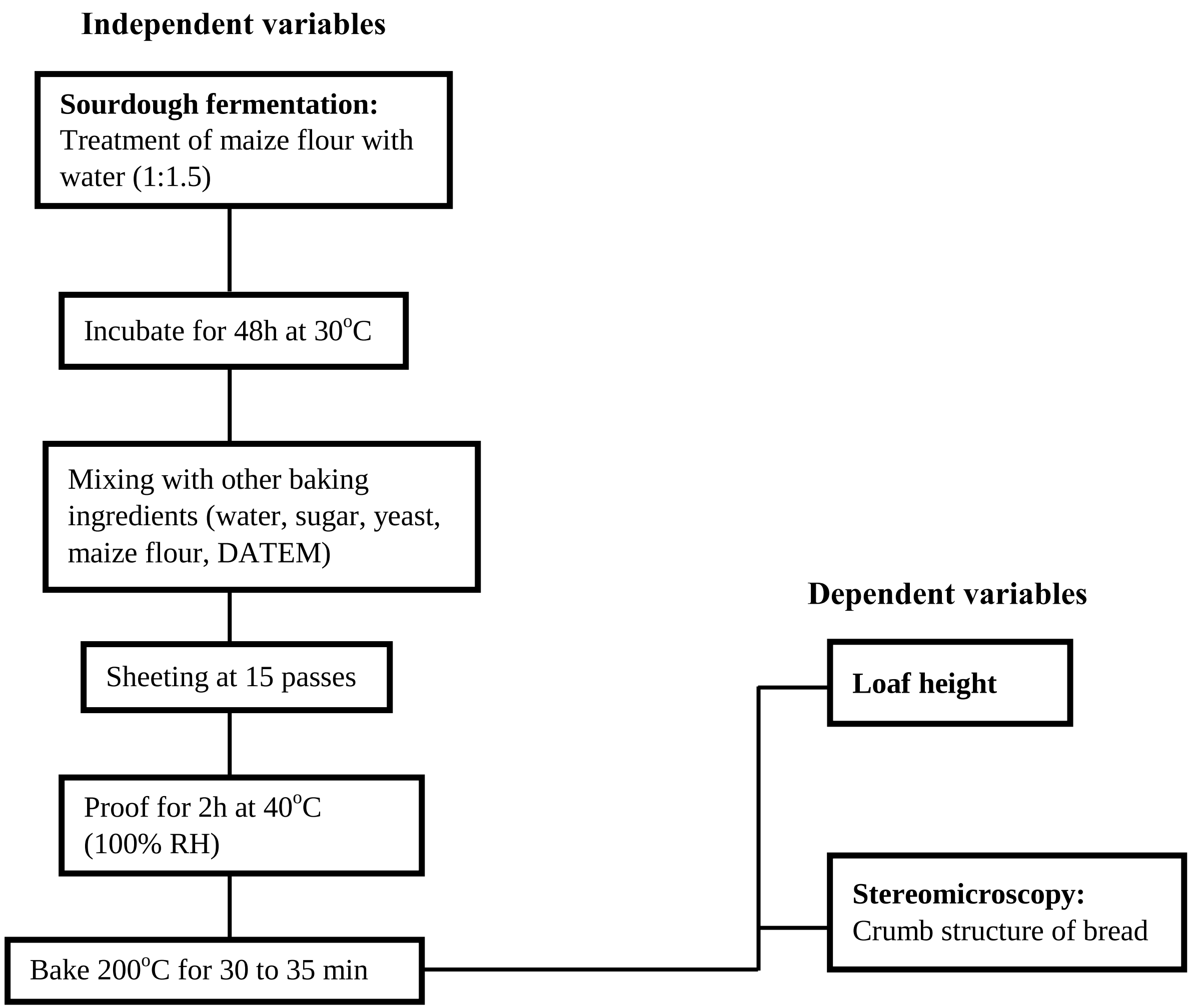


Figure 4.1.B: Experimental design to determine the effects of sheeting of maize dough prepared using starch pre-gelatinization in combination with incorporated α-zein, vital gluten, DATEM (diacetyl tartaric acid ester of mono- and diglycerides) or sourdough on bread quality

IMPROVEMENT IN MAIZE BREAD QUALITY THROUGH DOUGH SHEETING IN COMBINATION WITH VARIOUS DOUGH TREATMENTS

ABSTRACT

Maize is produced under diverse environments in Africa and could be used for bread making to reduce cost of importing wheat. However, it lacks the unique viscoelastic properties of wheat gluten. Dough sheeting has shown to improve wheat flour of low protein quality. This investigation showed that maize bread of improved quality could be made from maize flour with the use of dough sheeting in combination with other treatments. Sheeting and pre-gelatinization of maize flour produced a cohesive dough. Tensile tests showed improved extensibility and strength for maize dough up to 15 sheeting passes. Alveography revealed that increased sheeting passes reduced the strength and extensibility of composite maize-zein dough. CLSM revealed intermingling of zein fibrils with the maize flour which caused presumably improved viscoelastic properties. Furthermore, zein improved gas holding. Addition of sourdough to maize produced bread with improved crumb texture. Stereomicroscopy showed elongated gas cells in the crumb of maize sourdough breads. The addition of DATEM produced a homogenous crumb texture for maize, maize-zein and maize sourdough breads. Dough sheeting in combination with pre-gelatinized maize flour (30 %), addition of sourdough fermented maize flour (80%) and DATEM (5%) has potential as a technology to produce good quality maize bread.

4.1. INTRODUCTION

Maize flour is potentially a suitable alternative to wheat for bread making in Africa because it is very widely cultivated under diverse environments (du Plessis, 2003). However, it does not possess the unique viscoelastic properties and ability to retain gas during proofing like wheat flour (Dobraszczyk et al., 2004). The production of a high quality gluten-free bread product with similar rheological properties as wheat bread poses a challenge to cereal food scientists (Gallagher et al., 2004). Gluten-free breads also have poor nutritional quality (Huttner and Arendt, 2010).

Thus far, gluten-free bread making researchers aimed to improve the dough protein network by the use of enzymes such as transglutaminase (Renzetti et al., 2008) and glucose oxidase (Bonet et al., 2006). Flour water absorption has been improved by use of hydrocolloids such as HPMC (Andersson et al., 2011; Erickson et al., 2012), pre-gelatinization (Raina et al., 2005: Brites et al., 2010), sourdough fermentation (Moroni et al., 2009; Falade et al., 2014) and high pressure processing (Rastigo et al., 2007, Deora et al., 2014).

Dough sheeting has shown potential in improving dough functional properties of weak wheat flours (Patel and Chakrabarti et al., 2013). This is achieved by hydrolyses of starch and protein (Feillet et al., 1977) through compression of the dough between two rotating cylinders (Petitot et al., 2009). Pre-gelatinization of flour improves functional and dough handling properties through increasing elasticity and viscosity (Sozer, 2009; Brites et al., 2010). Incorporation of zein has shown potential in improving gluten-free dough viscoelasticity when produced above zein's hydrated T_g (Schober et al., 2008; Andersson et al., 2011). It forms a matrix that is thought to be able to withstand pressure during fermentation between protein and starch of flour (Bugusu et al., 2002). Sourdough fermentation is a natural alternative to improve volume, texture, flavour and nutritional value of gluten-free bread (Arendt et al., 2007; Edema, 2011). This is achieved by starch granule modification, thus leading to the ability of the dough to trap carbon dioxide and withstand pressure during proofing (Falade et al., 2014).

Studying changes in maize dough rheology and structure through dough sheeting in combination with pre-gelatinization of maize flour, addition of sourdough fermented maize, zein and the surfactant DATEM should provide fundamental knowledge for its use in novel, gluten-free dough systems and bread.

4.2. MATERIALS AND METHODS

4.2.1. Materials

Maize flour (11.7 g/100 g moisture as is basis, particle size <250 µm) was attained by milling white super grade (highly refined) maize meal (Spar Super Maize Meal, Spar South Africa, Pinetown, South Africa) bought from the local store using a CD1 laboratory reduction roller mill (Chopin Technologies, Villeneuve-la-Garenne Cedex, France). Wheat bread flour (14.1 g/100 g moisture as is basis) (Snowflake, Premier Foods, Isando, South Africa) was obtained from the local store. Commercial zein (α-zein) (Sigma Z3625) was obtained from Sigma-Aldrich, Johannesburg, South Africa. Vital gluten was kindly donated to Novozymes SA, Benmore, South Africa. DATEM was kindly donated by Ruto Mills, Pretoria, South Africa.

4.2.2. Methods

4.2.2.1. Proximate analysis

Moisture and protein contents of the maize and wheat flours, doughs and bread samples were determined essentially according to the Approved Methods: 44-15A and 46-19 respectively, of the American Association of Cereal Chemists (AACC International, 2000). Moisture content was determined by loss of weight of the samples after drying at 103^{o}C for 3 h. Crude protein (N × 6.25) was determined by a Dumas Combustion procedure. For wheat flour a conversion factor of N × 5.7 was used. The combustion is at high temperature in the presence of oxygen which leads to the release of carbon dioxide, water and nitrogen (Jung et al., 2003). All forms of nitrogen in the sample are converted to nitrogen oxides through combustion at 800 to 1000°C. These are then reduced to nitrogen gas (N_2) which is quantified by a thermal conductivity detector.

4.2.2.2. Dough preparation

Wheat, maize and maize-zein dough preparations for Texture analyses and Alveography are discussed in Table 4.1.

Table 4.1: Wheat, maize and maize-zein dough preparations for texture analyses and Alveography

Sample	Texture Analyses	Alveography
Wheat	Not applicable	Wheat bread flour (100 g as is basis) was mixed with 60 ml water. Sheeted at 5 passes
Maize	Pre-gelatinized maize flour (3 g in 8 ml distilled water using a microwave oven at 800W for 30 seconds) was mixed with maize flour (7 g). Sheeted from 5 to 40 passes	Pre-gelatinized maize flour (30 g in 80 ml distilled water, microwave oven for 2 mins at 800 W) was mixed with maize flour (70 g), water lost due to evaporation and total water added to composite (i.e. 94 ml). Sheeted at 5 passes
Maize-zein composite	a. Zein (1 g in 3.6 ml distilled water) prepared in a 50^{o}C water bath for 15 mins. Then mixed with pre-gelatinized maize flour (2.7 g in 7.2 ml distilled water) and maize flour (6.3 g) at room temperature (22^{o}C). Sheeted from 5 to 40 passes b. Above method (a.) used with modifications. All the ingredients were pre-warmed for 15 min at 50^{o}C followed by mixing over the water bath. Sheeted from 5 to 40 passes	a. Zein (10 g in 36 ml distilled water) prepared in a 50^{o}C water bath. Then mixed with pre-warmed maize flour (63 g) and pre-gelatinized maize flour (27 g in 72 ml distilled water) over a 50^{o}C water bath. Sheeted from 5 to 40 passes. b. Further treatment: zein dough was prepared with 3% lactic acid with the above described method (a.) and sheeted for 5 and 10 passes. c. Further treatment: DATEM (0.25 g) was incorporated into the maize-zein composite doughs prepared as described above (a.). Sheeted for 15 passes.
Maize-zein (without dough sheeting)	Not applicable	a. Zein (25 g in 90 ml distilled water) was prepared in a 50^{o}C water bath for 20 min. Followed by mixing pre-warmed the maize flour (157.5 g) and pre-gelatinized maize flour (67.5 g in 180 ml

		distilled water). Pre-warmed samples were mixed for 6 min by hand in a mixing bowl and thereafter rolled using the rolling pin of the Alveograph. b. Further treatment: Above method used (a.). After mixing the pre-warmed samples by hand, the composite dough was further kneaded in the kneading section of the Alveograph at 35^{o}C for 10 min. Dough kneading and resting was conducted at 35^{o}C (highest Alveograph temperature).
Maize-gluten	Pre-gelatinized maize flour (2.7 g in 7.2 ml distilled water) was mixed with 6.3 g maize flour and 1 g vital gluten by hand. Sheeted from 5 to 40 passes	Pre-gelatinized maize flour (13.5 g in 36 ml distilled water) was hand mixed with 5 g vital gluten and 63 g maize flour to form a dough. Sheeted for 15 passes.

4.2.2.3. Sheeting of dough using a dough sheeter

A dough sheeter (Ibili Menaje, Bergara, Spain) was set to position 1 by pulling it outwards and turning it so that the two smooth rollers are completely opened (~3 mm gap). A piece of maize, maize-zein or maize-gluten dough was passed through the sheeter while turning the handle, folding it to double the thickness, turning the sheet by 90^{o}.This operation was repeated with the number of sheeting passes 5, 10, 15, 20, 25, 30, 35 or 40. After the required number of passes, for uniform thickness, the dough was passed through the rollers once with the regulators set on number 2 (~2 mm gap). To cut the dough into fettuccine strips using the cutter of the machine, the handle was inserted in the hole for the cutting rollers turning it slowly and passing the dough through so as to obtain fettuccine pasta (width 5 mm). Thereafter, the pasta was then cut into 70 mm long strips for texture analysis. Sheeting was performed at ambient temperature (22^{o}C).

4.2.2.4. Texture analysis

The maize-zein composite doughs were cut into fettuccine strips and placed in zip-lock bags and further incubated in the water bath for 30 min prior to texture analysis The tensile properties of the maize doughs were measured as described by Sly et al. (2014) using a Kieffer rig mounted on a TA-XT2 texture analyzer (Stable Micro Systems, Godalming, UK). Doughs for texture analyses were prepared by sheeting as described in section 4.2.2.2. Maize doughs which had been passed through the dough sheeter were placed over the vertical struts (30 mm apart) of the Kieffer rig and clamped in place at both ends. The doughs were extended by means of a hook centred over the sample at a constant rate of 3.3 mm/s over a distance of 150 mm (maximum displacement of the texture analyzer). The force over distance, peak force (N), extensibility until rupture (mm) and area under the curve (N x mm) were measured. The peak stress (kPa), strain at maximum hook displacement, extensional viscosity (kPa.s), Young's modulus (kPa), area under the curve (N x mm) were calculated. Rheological parameters were determined using formulae according to Abang Zaidel et al. (2008).

4.2.2.5. Alveograph

An Alveograph (Chopin NG Consistograph, Paris, France) was used to evaluate the quality of the various doughs. The analysis was performed according to the ICC standard 121 (ICC, 1992), in combination with the Alveograph NG Consistograph instructional manual (Chopin, 2010). Doughs for Alveography were prepared by sheeting and by hand mixing as described in section 4.2.2.2. The sheeted doughs (3 mm thickness) were folded at 90^{o} to increase the thickness to 7 mm prior to analysis. Alveogram curves showing the following parameters were recorded. The deformation energy (W, J x 10^{-1}), tenacity or resistance to extension (P, mm H_2O), dough extensibility (L, mm) and curve configuration ratio (P/L) of the dough (Wang et al., 2002).

4.2.2.6. Confocal Laser Scanning Microscopy (CLSM)

Dough structure was analysed by CLSM (Zeiss 510 META system, Jena, Germany) with a Plan-Neofluor 10 x 0.3 objective under natural fluorescence at an excitation wavelength of

405 nm and 543 nm with acid fuschin staining. Dough samples were prepared as described in section 4.2.2.2 and sheeted for 15 passes and compared with un-sheeted doughs (doughs prepared by hand). The dough (<1 g) was hand stretched out as thinly as possible and attached to a microscope slide and was either viewed under autofluorescence or stained with 1 drop of acid fuschin (Falade et al., 2014).

4.2.2.7. Starch damage

Damaged starch was measured in wheat and maize flour and dough using a SD Matic instrument (Chopin Technologies, Villeneuve-la-Garenne Cedex, France) using ICC standard 172 (ICC, 2011) in combination with the SD Matic instructional manual (Chopin, 2004). The instrument measures starch damage by an amperometric method. It measures absorption of iodine (AI %) which is proportional to starch damage. The effects of sheeting of maize and wheat doughs on starch damage was determined using doughs which had been dried for 3.5 h at 50^{o}C.

4.2.2.8. Bread making

4.2.2.8.1. Bread preparation from wheat, maize and composites

Wheat bread flour (50 g as is basis) was measured into a mixing bowl with 1 g instant dried yeast, 1.5 g sugar and 20 ml water. For maize bread, pre-gelatinized maize flour (15 g in 40 ml water) with 35 g maize flour, 1.5 g sugar and 1 g instant dried yeast was used. The water lost during pre-gelatinization was added back. Zein (5 g as is basis) was pre-warmed with 18 ml distilled water in a water bath at 50^{o}C for 20 min. Maize flour (13.5 g as is basis) was pre-gelatinized in 36 ml water using a microwave oven at 800 W for 2 min and allowed to cool to 25^{o}C, prior to being pre-warmed at 50^{o}C for 20 min with 31.5 g maize flour. The water lost due to evaporation during pre-gelatinization was replaced. The pre-warmed samples were mixed in a mixing bowl over a 50^{o}C water bath with 1.5 g sugar, 1 g instant dried yeast. Maize-gluten bread was prepared as described with the exception that 5 g vital gluten powder was used without prior pre-warming, unlike the maize-zein composite.

Bread doughs were sheeted for 15 passes, tightly rolled by hand to reduce the air gaps between the sheets, cut into half and placed in bread tins treated with a baking pan release spray (74 mm length, 55 mm width, 66 mm depth). Further treatments for the bread were performed. Addition of DATEM (0.25 g) in maize, maize-zein and maize-zein prepared with lactic acid (3% v/v) doughs was investigated. Additional sugar and yeast were also investigated to obtain the optimum maize bread making recipe. Two g and three g sugar, respectively were found to be optimum.

The doughs were placed in the bread tins and then placed in polyethylene bags and allowed to proof at 40^{o}C in 100% relative humidity for 2 or 4 h over a water bath. Height of risen bread doughs was measured. The doughs were baked at 200^{o}C until they formed a brown crust (approximately 35 min). The loaves were carefully removed from the baking tins and allowed to cool. Bread height was determined using a ruler. Loaves were photographed. Crumb structure was measured after 24 h of storage at 6^{o}C by scanning cut surfaces of the bread using a flatbed scanner.

4.2.2.8.2. Production of sourdough for maize bread

Maize sourdough was produced by mixing maize flour and water in a ratio of 1:1.5 (w/v). The mixing was done by hand using spatula for 5 min to form a thick paste. The sourdough was incubated for 72 h at 30^{o}C. This starter culture was used to enumerate lactic acid bacteria and yeast at 0, 24, 48 and 72 h of sourdough fermentation. Lactic acid bacteria (LAB) colonies were enumerated on MRS agar using the pour plate method to determine the colony forming units per millimetre (cfu/ ml) at 30°C after 48 h of incubation (Collins et al., 2004; Edema and Obimakinde, 2014). Furthermore, yeast colonies were enumerated on PDA agar using pour plate method to determine the cfu/ ml at 30°C after 72 h of incubation (Collins et al., 2004). The colony counts were determined using a colony counter.

After backslopping (adding starter culture to fresh maize flour with water) was done, the backslopped sourdough (20%) was incubated for a further 48 h at the same temperature as above. The final pH was 3.4. Maize bread was prepared by mixing backslopped maize flour (30 g (60%) or 40 g (80%) with maize flour (35 g), pre-gelatinized maize flour (15 g in 40 ml distilled water), yeast (2 g) and sugar (3 g). Maize-zein sourdough dough was prepared by mixing pre-warmed zein (5 g in 18 ml distilled water), pre-gelatinized maize flour (13.5 g in

36 ml distilled water), 31.5 g of maize flour and 40 ml sourdough over a 50°C water bath. Afterwards, yeast (2 g) and sugar (3 g) was added to the dough. In another treatment, DATEM (0.25 g as is basis) was incorporated to the maize sourdough prepared using the above described method.

The doughs were sheeted for 15 passes, cut in 70 mm length and rolled and placed in bread tins. The bread tins were placed in polyethylene bags and proofed at 40°C over a water bath for 2 h. The breads were baked at 200°C until they form a brown crust (approximately 35 min). The loaves were carefully removed from the baking tins and allowed to cool. Bread height was measured using a ruler. Crumb structure was viewed by scanning cut surfaces of the bread using a flatbed scanner after 24 h of storage at 6°C. The crust of the breads was photographed.

4.2.2.8.3. Stereomicroscopy

Bread crumb structure was analyzed using a stereomicroscope (Zeiss Discovery V20, Jena, Germany) with a field of view of 20.0 mm and 0.6 mm depth of field.

4.2.2.9. Statistical Analyses

All experiments were repeated at least three times. One-way analysis of variance (ANOVA) was performed. Means were compared at $p = 0.05$ using Fisher’s Least Significant Difference Test (LSD).

4.3. RESULTS AND DISCUSSION

4.3.1. EFFECTS OF SHEETING ON DOUGH EXTENSIBILITY AND STRENGTH OF WHEAT AND MAIZE FLOURS

4.3.1.1. Moisture and nitrogen contents of flours and prolamin proteins

The nitrogen and moisture contents of wheat and maize flours differed significantly ($p<0.05$) (Table 4.2.1). Wheat flour had the highest moisture and nitrogen content when compared to maize. The low nitrogen (protein) content of the maize flour can be attributed to maize high starch content (Oladunmoye et al., 2010). Vital gluten had a lower nitrogen content when compared to α-zein (Table 4.2.2) because the protein preparation was less pure. However, it had a higher moisture content. Schober et al. (2008) found similar moisture and nitrogen content for zein to this previous work. The reason for zein and vital gluten having far high nitrogen contents when compared to their respective flours is due to maize flour and wheat flour have other constituents such as starch, ash and fat.

Table 4.2.1: Moisture and nitrogen contents of wheat and maize flours[a]

Flour	Moisture (g / 100 g)	Nitrogen content (g / 100 g)
Wheat flour	$14.1^{\mathbf{b}}\pm0.1^{b}$	$1.94^{\mathbf{b}}\pm0.0$ ($2.26^{\mathbf{b}}\pm0.0$)
Maize flour	$11.7^{\mathbf{a}}\pm0.1$	$0.94^{\mathbf{a}}\pm0.0$ ($1.06^{\mathbf{a}}\pm0.0$)

[a] Mean ± Standard Deviation of 2 samples
[b] Numbers in columns with different superscript letters differ significantly ($p<0.05$)
() Values within brackets are on a dry matter basis

Table 4.2.2: Moisture and nitrogen contents of α-zein and vital gluten[a]

Prolamin proteins	Moisture (g / 100 g)	Nitrogen content (g / 100 g)
α-zein	$4.2^{\mathbf{a}}\pm0.1$	$14.7^{\mathbf{b}}\pm0.0$ ($15.4^{\mathbf{b}}\pm0.0$)
Vital gluten	$11.8^{\mathbf{b}}\pm0.1$	$12.6^{\mathbf{a}}\pm0.0$ ($14.3^{\mathbf{a}}\pm0.0$)

[a] Mean ± Standard Deviation of 2 samples
[b] Numbers in columns with different superscript letters differ significantly ($p<0.05$)
() Values within brackets are on a dry matter basis

4.3.1.2. Sheeting of maize doughs

Maize flour dough prepared by hand mixing followed by one sheeting pass formed a crumbly dough (Figure 4.2a). When the maize dough was sheeted for 6 passes with a reduced roll gap size (approximately 2 mm) compared to maize dough with a roll gap size of 3 mm gap, there was no change in the dough appearance (Figure 4.2b). This can be attributed to the lack of water absorption by the maize flour. It has been reported that zein is relatively hydrophobic and unable to interact with water as it is encapsulated in protein bodies (Oom et al., 2008). Dough sheeting of maize flour in combination with pre-gelatinization of maize flour produced a cohesive maize dough with dramatically improved dough handling properties (Figure 4.2c). With continued sheeting (40 passes) the maize dough texture became smoother (Figure 4.2d). Combining the two treatments, pre-gelatinization and dough sheeting, incorporated the pre-gelatinized maize starch and maize flour to form a cohesive dough.

Addition of zein to maize dough together with pre-gelatinized maize flour when prepared at ambient temperature (22^{o}C) formed a dough where the zein was not well incorporated (Figure 4.2e). With additional dough sheeting, there was better distribution of zein dough throughout the maize dough (Figure 4.2f). However, a well incorporated maize-zein composite was not obtained. Preparation of zein and maize dough above zein's hydrated glass transition temperature (T_g) (i.e. at 50^{o}C), formed a composite with zein being well incorporated (Figure 4.2 g and h). A yellow coloured dough was formed and that was due to the colour of the commercial zein. The maize-zein composite dough showed a similar trend as maize dough with regard to the effects of dough sheeting. With increasing dough sheeting passes, a smooth maize-zein dough was formed. The same applied to incorporation of vital gluten powder into maize dough (Figure 4.2 i and j) and wheat dough (Figure 4.2 k and l).

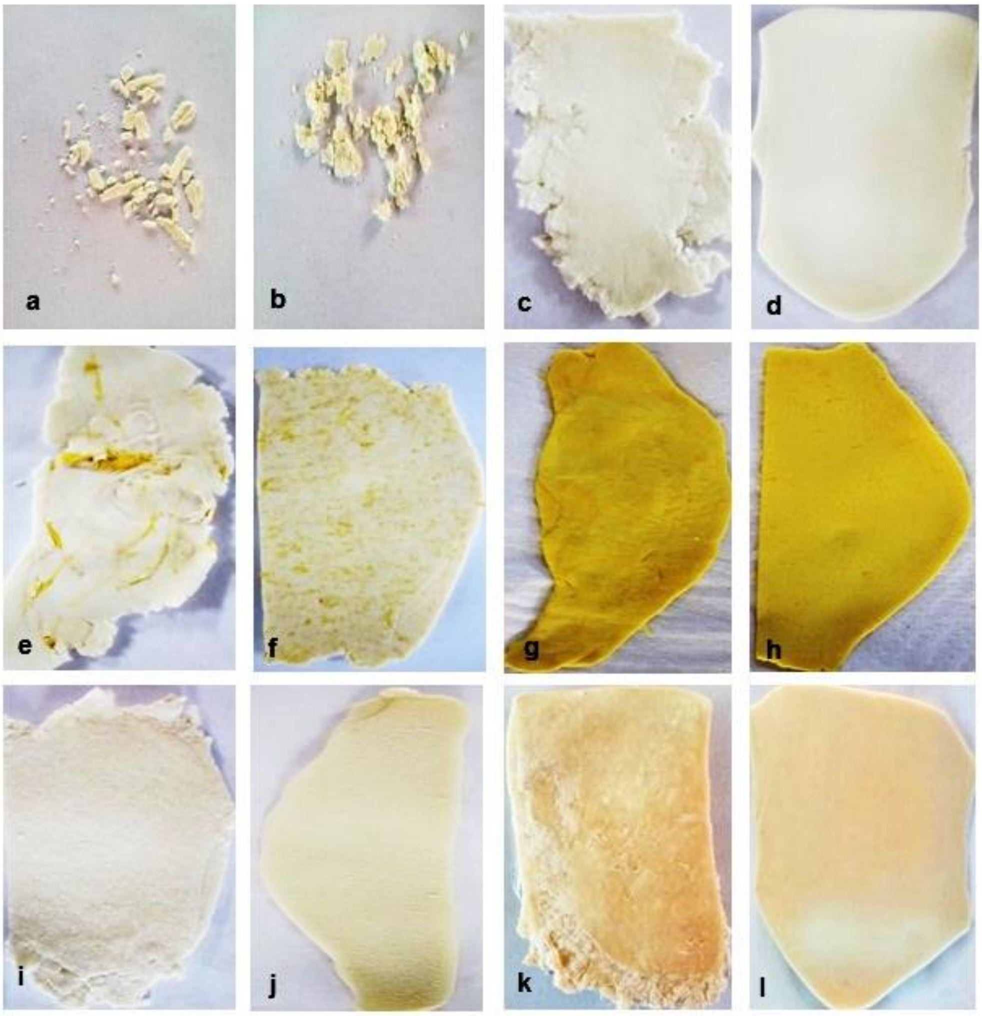


Figure 4.2: Photographs illustrating sheeted maize and wheat flour doughs prepared using pre-gelatinization of maize flour and incorporation with α-zein dough or vital gluten powder. **a**. maize after one sheeting pass. **b**. maize at 6 sheeting passes. **c**. maize prepared using starch pre-gelatinization at 5 sheeting passes. **d**. maize prepared using starch pre-gelatinization at 40 sheeting passes. **e**. maize-zein prepared below T_g at 5 sheeting passes. **f**. maize-zein prepared below T_g at 40 sheeting passes. **g**. maize-zein prepared above T_g at 5 sheeting passes. **h**. maize-zein prepared above T_g at 40 sheeting passes. **i**. maize-gluten at 5 sheeting passes. **j**. maize-gluten at 40 sheeting passes. **k**. wheat at 5 sheeting passes. **l**. wheat at 40 sheeting passes.

Stress is an important factor that contributes to thermal expansion of bread during baking to give good loaf volume (Bugusu et al., 2001). Stress is the force per unit area and is calculated by dividing the force by the average cross-sectional area (Bourne, 2002). According to Bugusu et al. (2001), higher stress indicates a stronger dough that can extend more and has greater potential to hold the expanding gas cells during fermentation. Strain, is a dimensionless measure that refers to the change in size or shape of a material when subjected to a stress (Bourne, 2002).

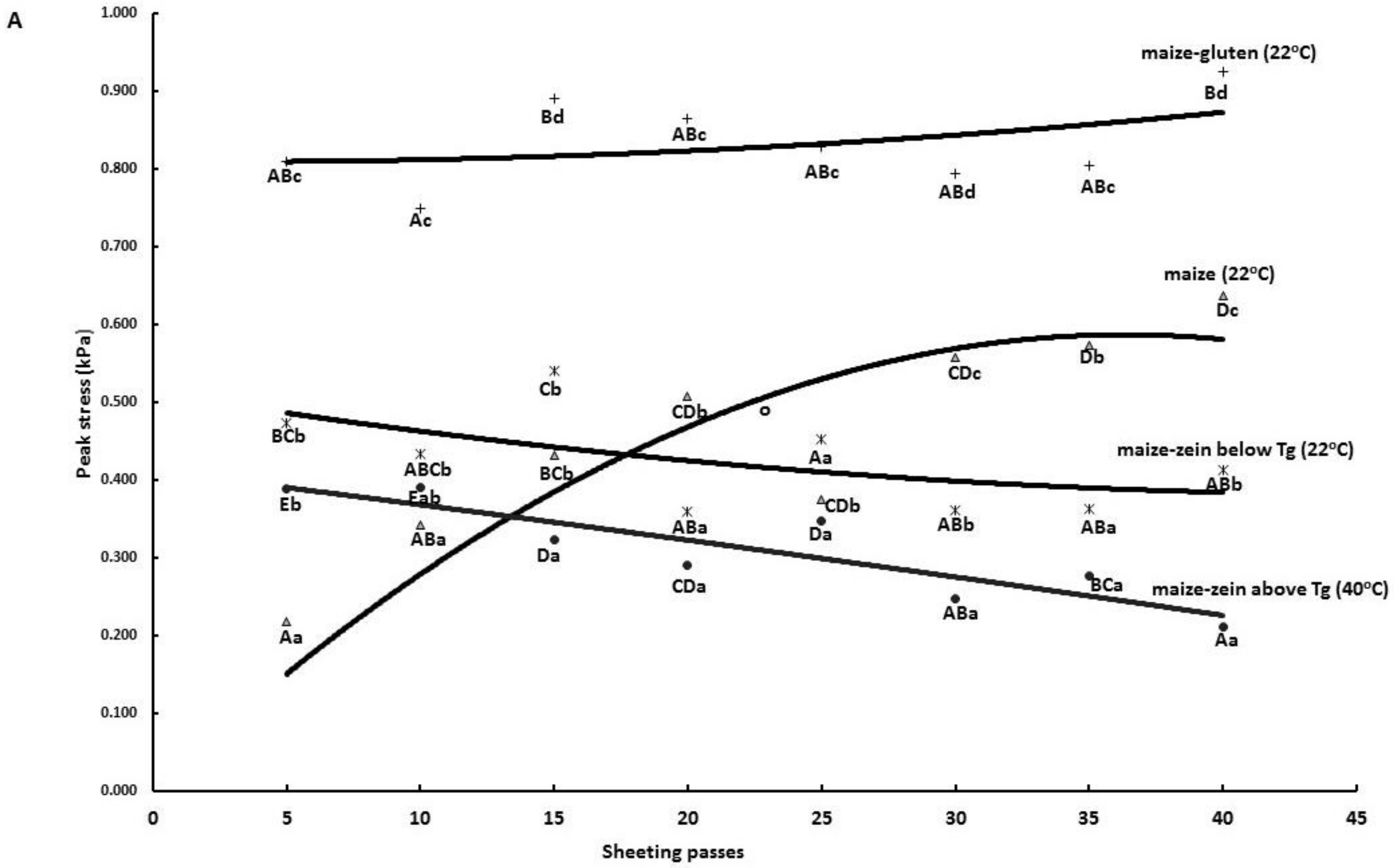
The effects of adding zein dough or gluten powder to maize dough with pre-gelatinized maize flour on peak stress and strain are shown in Figure 4.3A. Pre-gelatinization of the maize flour played an important role in the formation of the maize dough. Pre-gelatinized starch has been found to act as a binder, improving the functional properties of gluten-free doughs (Sozer, 2009). Stress values increased significantly ($p<0.05$) for the maize dough with increasing number of dough sheeting passes. Further, addition of gluten resulted in higher stress than with the other doughs. Also, there was an increase in stress with increasing sheeting passes for the maize-gluten composite. It has been suggested that gluten absorbs added water rapidly (Raina et al., 2005). In this regard, the maize-gluten composite dough formed a strong cohesive dough. On the other hand, addition of zein to maize caused a slight decrease in stress with increasing sheeting passes. Mixing the composite below (22°C) and above (40°C) T_g had no significant difference in stress, it gradually decreased with increasing sheeting passes.

With regard to strain, strain values increased slightly with increasing sheeting passes for the maize-gluten dough (Figure 4.3B). In contrast, addition of zein to maize dough caused a slight decrease in strain with increasing dough sheeting passes, more especially with the maize-zein dough prepared above zein's T_g (40°C). This can be attributed to the zein fibrils losing their elasticity with increasing sheeting passes, causing further change in the maize-zein dough structure.

Thus, incorporation of zein caused an observable improvement in maize flour dough texture. Furthermore, the preparation of the maize-zein composite above T_g improved the mixing and extensibility of the dough. The extensibility of maize-zein composite dough was affected by the increasing sheeting passes. This indicated that the zein had been over-worked and probably lost its elasticity. Autio and Laurikainen (1997) working on wheat dough showed that with repeated sheeting there was a gradual decrease in extensibility and resistance of the dough.

Preparation of the maize-zein dough at 22°C formed a brittle dough with visible zein particles. This was because the zein was not well incorporated with the maize flour dough. Although the zein dough was prepared above T_g, the mixing of other components at ambient temperature greatly affected the incorporation. Since the temperature of other components was low, it caused the zein temperature to decrease and that affected the incorporation of the zein with the maize. Bugusu et al. (2001) found that preparation of wheat-sorghum-zein composite above zein T_g, produced a well incorporated composite. Also, Lawton (1992), observed that the dough formed by zein in a starch-based system was not as strong as wheat dough and attributed this to formation of fewer intermolecular cross-links (covalent bonds). In that case, the increase in stress observed for maize-zein composite dough above T_g suggests that there were more cross-links formed by zein which contribute to the dough strength.

In this present work, zein was mixed above its T_g at 40°C. It had adhesive properties and sticky characteristics. This caused it to incorporate well with the maize dough prepared with pre-gelatinized maize.


A
Peak stress (kPa)
Sheeting passes
maize-gluten (22°C)
maize (22°C)
maize-zein below Tg (22°C)
maize-zein above Tg (40°C)
1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0
5
10
15
20
25
30
35
40
45
ABc
Ac
Bd
ABc
ABc
ABd
ABc
Bd
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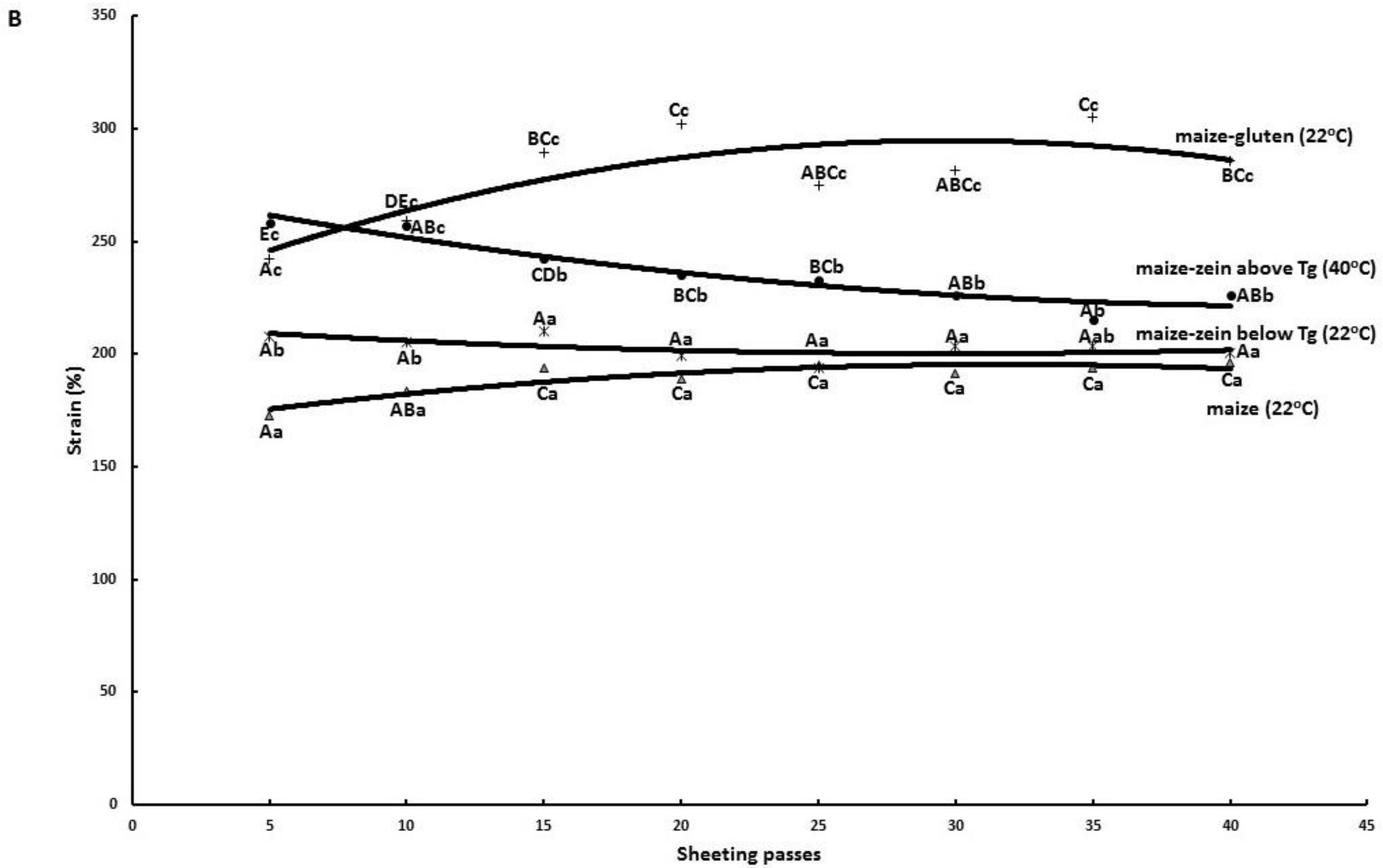


Figure 4.3: Effects of sheeting of maize doughs prepared using starch pre-gelatinization and incorporation with vital gluten powder and α-zein dough prepared above and below zein's T_g on the Peak stress (**A**) and Strain (**B**) of the doughs. Values at sheeting intervals between the treatments (^{abcde}) and different sheeting passes for each individual treatment (^{ABCD}) with different superscript letters differ significantly ($p<0.05$).

4.3.1.3. Alveography

As expected, wheat dough was able to hold air and inflate into a dough bubble when subjected to Alveography (Figure 4.4M). Similar results were observed for the maize-zein dough (Figure 4.4 B to H). However, a major difference was that the maize-zein composite did not prematurely tear nor collapse unlike the maize dough or wheat dough (Figure 4.4 D and M). Maize flour dough, however formed only a small dough bubble (Figure 4.4A). The maize-zein composite formed by hand mixing (Figure 4.4B) was able to inflate a dough bubble which was similar in size to the composite at 5 sheeting passes (Figure 4.4D). However, the dough bubble visually had a coarser and a much thicker texture than when the maize-zein composite was sheeted. When the maize-zein composite was kneaded in the mixing section of the Alveograph, a sticky dough and a small inflated dough bubble (Figure 4.4C) was formed when compared to hand kneaded maize-zein composite dough.

Sheeting of maize-zein composite was able to form a dough bubble larger in size than maize flour dough until 15 passes. With more than 15 dough sheeting passes the dough bubble reduced in size (Figure 4.4 G and H). This indicated that excessive sheeting had an effect on the extensibility of the maize-zein dough as was found by texture analysis (Figure 4.3). With continued dough sheeting, the maize-zein doughs were over-worked and that reduced their extensibility.

The effect of preparation of zein with lactic acid (3%) was investigated. Lactic acid can act as a plasticizer that is meant improve the extensibility of zein (Lai and Padua, 1997; Oom et al., 2008; Sly et al., 2014). The lactic acid treatment produced a cohesive maize-zein dough (Figure 4.4 I and J). It could inflate and maintain a dough bubble without collapsing. Moreover, it had a much thinner texture at both 5 and 10 sheeting passes when compared to sheeting at 5 passes for the maize-zein composite without lactic acid.

The addition of DATEM improved the dough properties of the maize-zein composite. With added DATEM, the inflated dough bubble developed a tear and then collapsed (Figure 4.4L). In this respect it behaved like wheat dough and had a much thinner texture compared to the maize-zein composite without added DATEM. The maize flour-gluten composite was only able to form a dough bubble (Figure 4.4K) that was similar in size to the maize-zein composite at 40 sheeting passes.

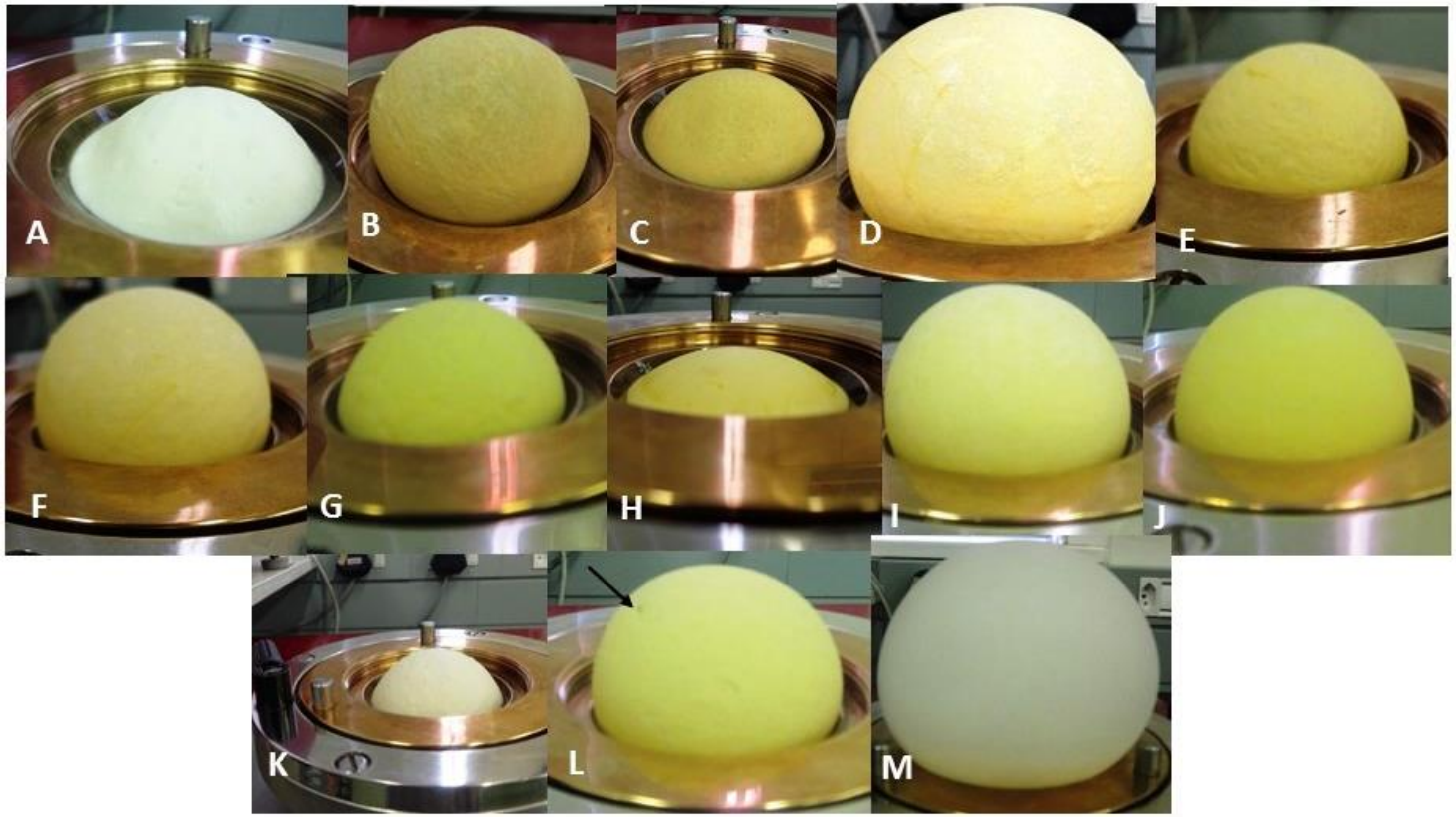


Figure 4.4: Effects of sheeting of maize dough prepared using maize flour pre-gelatinization in combination with incorporated zein dough or vital gluten powder on the extensibility of the doughs. **A**. maize at 5 sheeting passes. **B**. hand kneaded maize-zein composite. **C**. dough bubble of kneaded maize-zein composite in the mixing section of the Alveograph **D**. maize-zein at 5 sheeting passes. **E**. maize-zein at 10 sheeting passes. **F**. maize-zein at 15 sheeting passes. **G**. maize-zein at 30 sheeting passes. **H**. maize-zein at 40 sheeting passes. **I**. maize-zein treated with 3% lactic acid at 5 sheeting passes. **J**. maize-zein treated with 3% lactic acid at 10 sheeting passes. **K**. maize-gluten composite at 15 sheeting passes. **L**. maize-zein with added DATEM at 15 sheeting passes. **M**. wheat at 5 sheeting passes. Arrow indicates development of a tear

The maize-zein composite at 5 dough sheeting passes gave higher Alveograph parameters (P, and W) than the wheat bread dough including the curve configuration ratio (P/L) (Table 4.3). The curve configuration ratio gives information about the elastic resistance and extensibility balance of the dough (Rosell et al., 2001). With increasing dough sheeting passes, the L value of maize-zein dough was reduced. This was probably due to the zein being over-worked with reference to wheat doughs (Delcour and Hoseney, 2010). The maize-zein composite at 5 to 15 dough sheeting passes required more force (P) than wheat to blow a dough bubble. However, the maize-zein dough did not collapse like wheat. This was because zein cooled below its T_g and hardened during Alveography.

The maize-zein composite dough was relatively strong when zein dough was prepared with lactic acid and showed some consistency at 5 to 10 dough sheeting passes. The stability (P) of the maize-zein composite dough without lactic acid was reduced with increased dough sheeting passes, presumably due to zein losing its elasticity. It has been suggested that the acidic conditions caused by lactic acid somewhat reverses the change from predominantly α-helical to more, β-sheet conformation, which occurs when zein is made into a dough (Sly et al., 2014). The increase in α-helical conformation possibly occurs as a result of deamination of the zein molecules. This in turn could enable the formation of a more uniform dough structure with linear orientation of fibrils.

Gujral and Singh (1999) stated that over-mixing leads to formation of a wet sticky wheat dough which poses problems during handling. A wet and sticky dough was obtained after kneading of the maize-zein composite in the Alveograph mixer. The maize-zein dough behaved like a batter which then affected the strength and extensibility of the maize-zein dough. The strength was low and extensibility was high when compared to maize dough (Table 4.3).

DATEM, a surfactant widely used in commercial wheat bread making (Gomez et al., 2004), improved the cohesiveness of the maize-zein composite. As stated, it made it behave like wheat dough since it developed a tear (Figure 4.4L) unlike the other maize-zein composite dough bubbles. Further, behavior of DATEM gave similar Alveography values to the maize-zein composite prepared with lactic acid. Thus, it appears that DATEM acted as a plasticizer which made zein more extensible. This has been reported to be through hydrophobic and hydrophilic interaction of the protein with DATEM to form a more continuous structure during mixing and dough sheeting (Krog et al., 1977).

Table 4.3: Effects of sheeting of maize dough prepared using maize flour pre-gelatinization incorporated with α-zein dough or vital gluten powder prepared above zein's T_g on the extensibility of the doughs with sheeting[a]

Figure 4.3 codes	Type of dough	Sheeting passes	Stability	Extensibility	Curve configuration ratio	Deformation energy
			(P, mmH$_2$O)	**(L, mm)**	**(P/L)**	**(W, J x 10^{-4})**
A	Maize	5	47.3^{a}±3.8	35.7^{ab}±21.7	1.37^{c}±0.27	61.0^{a}±24.9
B	Maize-zein (hand kneaded)	NA	255.7^{e}±2.0^{b}	73.3^{ab}±9.0	3.53^{e}±0.56	641.7^{bc}±87.0
C	Maize-zein (kneaded)	NA	33.3^{a}±6.0	83.0^{ab}±51.1	0.48^{a}±0.21	57.7^{a}±38.1
D	Maize-zein	5	225.0^{e}±17.7	109.3^{bc}±4.2	2.06^{d}±0.22	679.0^{e}±27.8
E	Maize-zein	10	131.0^{bc}±7.9	172.0^{cd}±18.3	0.76^{abc}±0.05	639.0^{de}±124.5
F	Maize-zein	15	152.0^{cd}±3.0	120.3^{bc}±15.3	1.28^{bc}±0.17	476.7^{cd}±38.7
G	Maize-zein	30	60.0^{a}±4.4	95.7^{ab}±16.3	0.64^{ab}±0.11	174.3^{ab}±14.6
H	Maize-zein	40	43.3^{a}±9.3	118.7^{bc}±10.0	0.36^{a}±0.05	176.3^{ab}±65.3
I	Maize-gluten	15	64.0^{a}±1.7	76.0^{ab}±6.0	0.84^{abc}±0.05	98.7^{ab}±7.8
J	Maize-zein (lactic acid)	5	164.3^{d}±7.6	128.3^{bcd}±30.3	1.33^{c}±0.29	506.3^{de}±99.2
K	Maize-zein (lactic acid)	10	150.0^{cd}±16.0	125.7^{bcd}±12.5	1.19^{bc}±0.04	454.7^{cd}±86.9
L	Maize-zein (DATEM)	15	178.7^{d}±10.3	195.7^{d}±56.7	0.97^{abc}±0.28	461.3^{b}±104.0
M	Wheat	5	102.3^{b}±2.1	108.3^{abc}±5.9	0.95^{abc}±0.05	297.7^{bc}±11.7

[a]Means ± Standard deviation (n=3)
[b]Values in columns with different superscript letters differ significantly (p<0.05)
NA = Not Applicable

With gluten powder addition to the maize dough, better water absorption was observed visually. However, the maize-gluten dough was not stable (P) nor extensible (L) based on the Alveograph values. Furthermore, gluten addition resulted in a very poor dough bubble (Figure 4.4K) when compared to zein addition (Figure 4.4F). With wheat flour, upon flour hydration and mixing, a gluten network develops (Codina and Pâslaru, 2008) which reduces dough mobility due to uptake of water. The wheat dough retains large amounts of water due to the high protein content of gluten, meaning a decrease in mobility of the system of the dough. Hence, in this study, a continuous matrix might have developed and entrapped the maize flour particles, which would have resulted in a strong cohesive dough that was not extensible.

4.3.1.4. Starch damage

Maize flour had more damaged starch than wheat flour (Table 4.4). This can be attributed to the maize grain being generally harder to mill than wheat grain due to its vitreous starchy endosperm (Oladunmoye et al., 2010). Furthermore, starch damage may be influenced by method of milling (Evers et al., 1984) and flour particle size. An increase in flour particle size causes a decrease in damaged starch (Wang and Flores, 2000). Flour starch damage optimizes hydration and promotes fermentation activity during bread making (Medcalf and Gilles, 1965). Sheeted maize dough also had more measured damaged starch than wheat dough. The pre-gelatinization of maize flour causes damaged starch because gelatinization disrupts the starch granules. With further dough sheeting (40 passes) of the maize dough, more damaged starch was measured. This observation is in agreement with Petitot et al. (2009) findings in wheat dough that with higher sheeting passes, moderate damaged starch is produced. However, maize flour had more measured damaged starch than maize dough. This could be due to the pre-gelatinization masking some of the damaged starch so that it could not be measured.

Table 4.4: Effects of sheeting of wheat dough and maize dough prepared using pre-gelatinization of maize flour on the level of starch damage[a]

	Starch damage	
	Sheeting passes	**AACC 76-31**
Wheat flour	Not applicable	$5.18^{\mathbf{c}}\pm0.13^{b}$
Wheat dough	5	$0.36^{\mathbf{a}}\pm0.00$
Wheat dough	40	$2.96^{\mathbf{b}}\pm0.03$
Maize flour	Not applicable	$6.02^{\mathbf{c}}\pm0.04$
Maize dough	5	$3.05^{\mathbf{a}}\pm0.03$
Maize dough	40	$5.39^{\mathbf{b}}\pm0.01$

[a] Means ± Standard deviation (n=2)
[b] Values in columns with different superscript letters differ significantly ($p<0.05$)

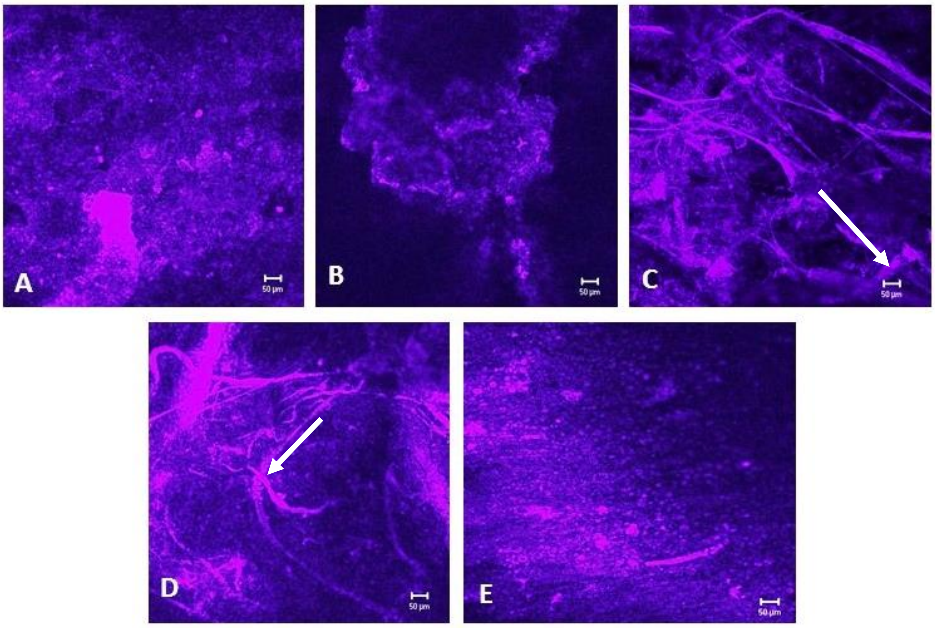
4.3.1.5. Confocal Laser Scanning Microscopy (CLSM)

CLSM clearly showed autoflorescence of zein (blue), as well as changes in appearance in the doughs when subjected to sheeting (Figure 4.5.1a). The acid fuschin stain was used to better identify the protein in the dough because it attaches to protein and fluoresces pink/red in colour (Dürrenberger et al., 2001) (Figure 4.5.2).

With application of dough sheeting, the fibrils in the maize-zein composite dough changed from longer and thick networks to that of a finer fibril network (Figure 4.5.1bC). Moreover, the fibrils were reduced in size (width). In the wheat flour dough fibrils aligned in the direction of sheeting (Figure 4.5.1bE). This phenomenon was also observed Sly et al. (2014) for zein doughs, where stretching in a single direction resulted in alignment of all the fibrils in the direction of stretching. This trend was also observed when the doughs were stained with acid fuschin (Figure 4.5.2bE). Using the stain acid fuschin proved to be challenging to identify starch granules and protein fibrils. Perhaps, it would have been better to use two different stains to identify starch and protein fibrils, as used by Kim et al. (2008). They used two fluorescent dyes, fluorescein which stained starch and Rhodamine B which stained protein fibrils.The zein fibrils were reduced in width after dough sheeting (Figure 4.5.2bC). Sheeted maize dough showed a network of gelatinized starch enveloped maize flour particles (Figure 4.5.1b), whereas the maize-gluten composite had a uniform distribution of maize starch and gluten throughout the dough. This is due to the added gluten (Figure 4.5.2bB).

Addition of sourdough resulted in thin and long fibrils in the maize-zein composite dough (Figure 4.5.1aD). This can be attributed to the acidic nature of the sourdough which affected the zein during incorporation. The observed improvement in rheological properties, for the maize-zein dough by addition of zein can therefore, be attributed to the isolated zein, being available for participation in fibril formation thus coating the maize flour particles in a loose network. As explained, in a study by Bugusu et al. (2002) it was observed that in a wheat-sorghum-zein bread that the gluten network was coated by the zein fibrils. They observed that there appeared to be some intermingling between zein and gluten. They proposed that zein strengthened the bread structure which resulted in improved loaf volume. However, similar to this present work, Sly et al. (2014), CLSM of stretched zein-rice flour composite dough (i.e. in the absence of gluten), observed a matrix of zein protein around the starch granules.

a)



b)

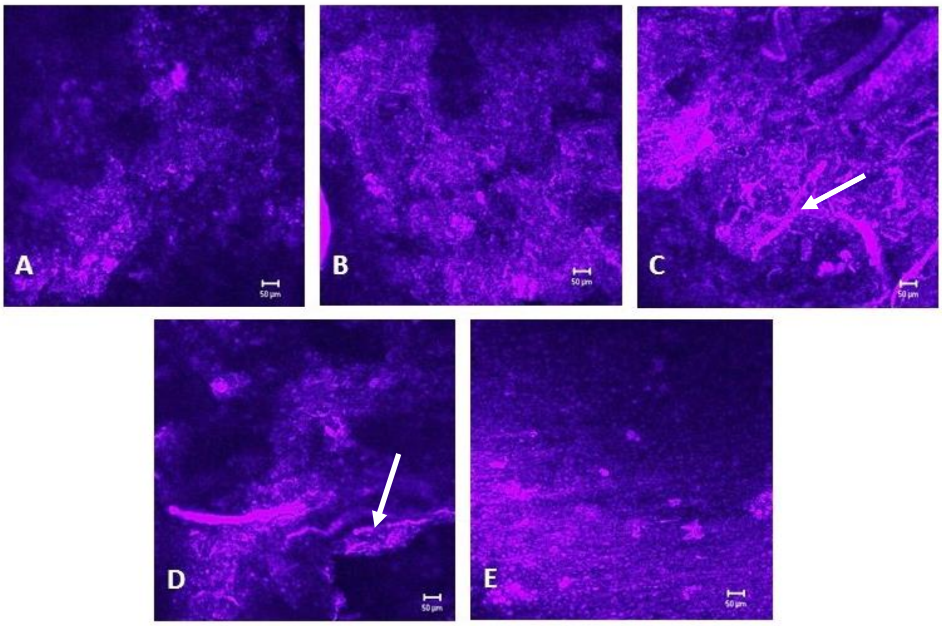
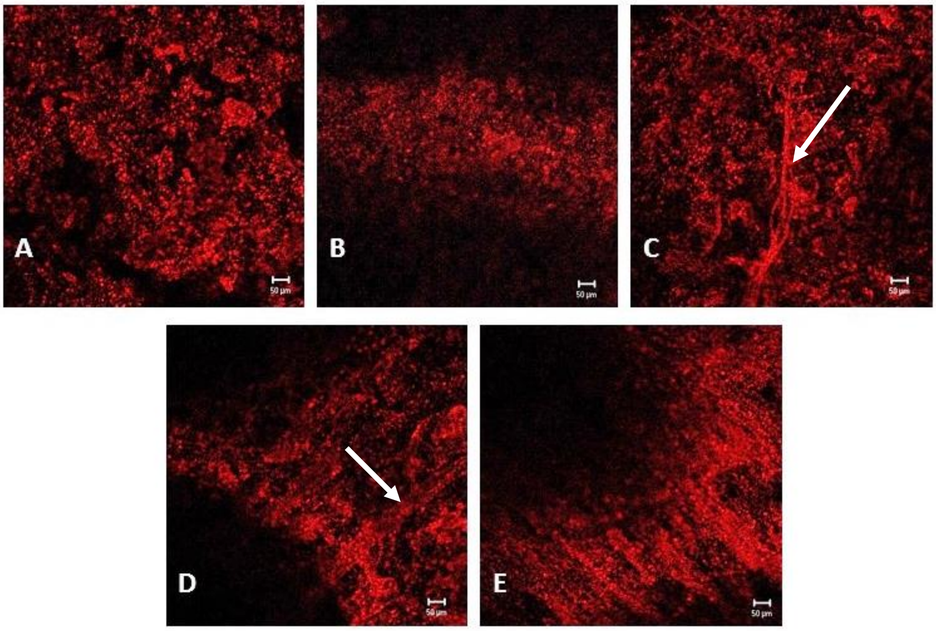


Figure 4.5.1: CLSM of non-stained un-sheeted (**a**) and sheeted (**b**) of maize dough prepared using pre-gelatinization of maize and incorporating α-zein dough or vital gluten powder. **A**. maize. **B**. maize-gluten. **C**. maize-zein. **D**. maize-zein sourdough. **E**. wheat. Arrows indicate zein fibrils.

a)



b)

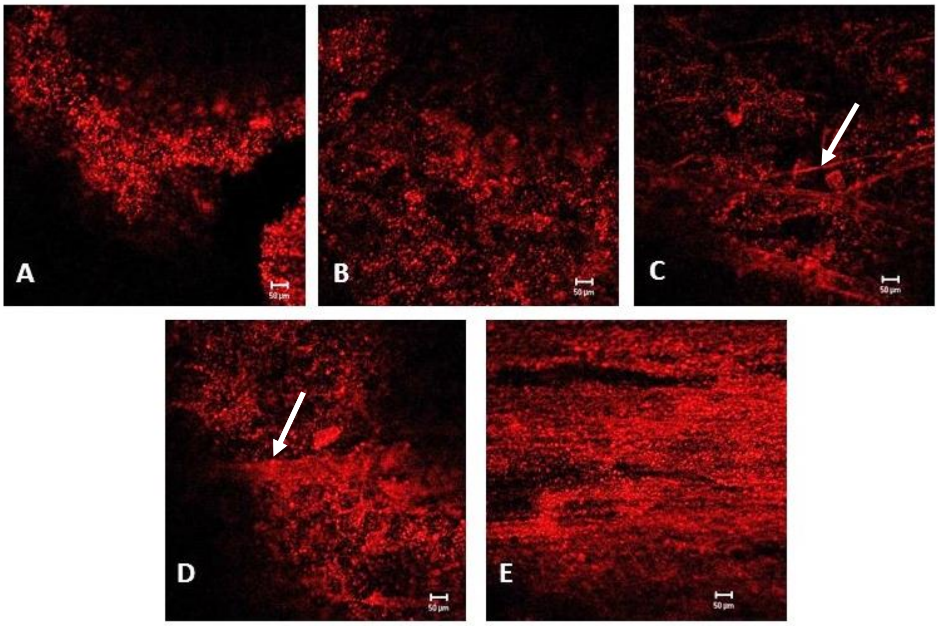


Figure 4.5.2: CLSM of acid fuschin stained un-sheeted (**a**) and sheeted (**b**) of maize dough prepared using pre-gelatinization of maize and incorporating α-zein dough or vital gluten powder. **A**. maize. **B**. maize-gluten. **C**. maize-zein. **D**. maize-zein sourdough. **E**. wheat. Arrows indicate zein fibrils.

4.3.2. EFFECTS OF SHEETING ON BREAD MAKING QUALITY OF MAIZE DOUGHS

4.3.2.1. Moisture and nitrogen content of wheat and maize breads

Wheat and maize breads with various improvement treatments differed significantly ($p<0.05$) in moisture and nitrogen contents (Table 4.5). Maize sourdough breads had a higher moisture content compared to wheat bread. This is probably because the sourdough process softened the starch (Edema et al., 2013) increasing its moisture holding. The maize-gluten composite bread had the lowest moisture content. With inclusion of either zein or gluten in the maize bread, there was an increase in nitrogen content. This can be attributed to zein or gluten being relatively pure protein preparations. Addition of sourdough to the maize-zein bread resulted in a reduction in the nitrogen content. This can be attributed primarily to the higher moisture content of these breads.

Table 4.5: Moisture and nitrogen contents of wheat and maize breads with different treatments[a]

Type of bread	Moisture (g / 100 g)	Nitrogen (g / 100 g)
Wheat	27.7$^{\mathbf{b}}$±0.1^{b}	1.69$^{\mathbf{b}}$±0.1 (2.34$^{\mathbf{b}}$±0.1)
Maize	33.2$^{\mathbf{cd}}$±1.3	0.88$^{\mathbf{a}}$±0.0 (1.32$^{\mathbf{a}}$±0.0)
Maize-zein	33.0$^{\mathbf{c}}$±1.9	2.21$^{\mathbf{c}}$±0.0 (3.30$^{\mathbf{c}}$±0.0)
Maize-gluten	20.8$^{\mathbf{a}}$±0.1	2.26$^{\mathbf{c}}$±0.0 (2.85$^{\mathbf{c}}$±0.0)
Maize sourdough (40%)	35.5$^{\mathbf{cde}}$±0.8	0.99$^{\mathbf{a}}$±0.0 (1.53$^{\mathbf{a}}$±0.0)
Maize sourdough (60%)	37.9$^{\mathbf{e}}$±0.2	1.11$^{\mathbf{a}}$±0.0 (1.79$^{\mathbf{a}}$±0.0)
Maize sourdough (80%)	37.3$^{\mathbf{de}}$±0.2	1.06$^{\mathbf{a}}$±0.0 (1.69$^{\mathbf{a}}$±0.0)
Maize-zein sourdough (80%)	36.5$^{\mathbf{cde}}$±1.7	1.65$^{\mathbf{b}}$±0.3 (2.60$^{\mathbf{b}}$±0.0)

[a] Means ± Standard deviation (n=2)
[b] Values in columns with different superscript letters differ significantly ($p<0.05$)
() Values within brackets are dry matter basis

4.3.2.2. Maize sourdough production

4.3.2.2.1. Sourdough fermentation and enumeration of LAB and yeast in maize sourdough

With fermentation at 30°C, there was an initial reduction in the pH and an increase in titratable acidity for the maize sourdough from 0 to 24 h (Figure 4.6.1). This fermented maize flour was used as a starter to backslop maize flour. The backslopped maize sourdough followed the same trend as the starter culture. The pH decreased while the titratable acidity increased. The final pH of the backslopped maize sourdough was pH 3.4 and it was used to prepare maize sourdough bread.

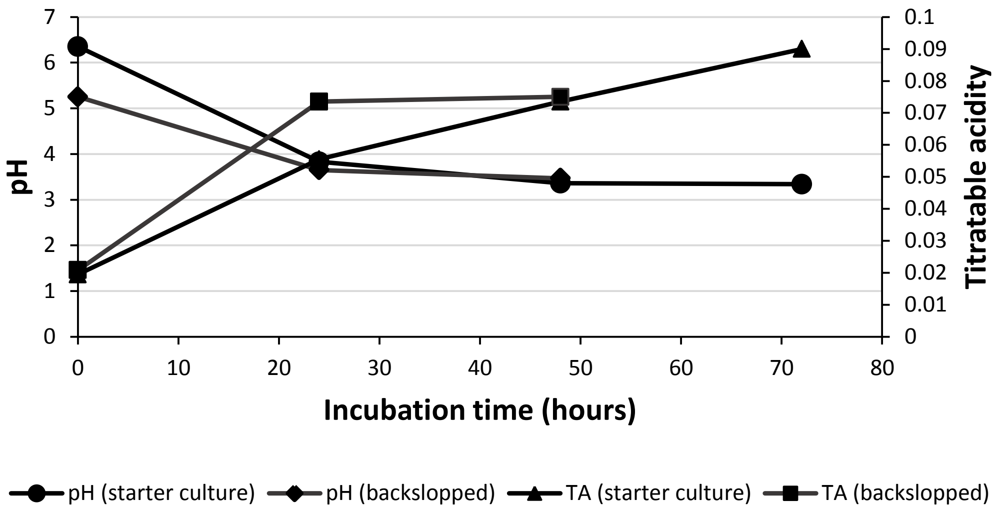


Figure 4.6.1: pH and titratable acidity of spontaneously fermented maize flour of the starter culture sourdough over a 72 h incubation and backslopped sourdough over 48 h incubation at 30°C.

During sourdough fermentation, biochemical changes occur in the starch and protein components of the flour due to the action of microbial and indigenous enzymes (Arendt et al., 2007). The reduction of pH and increase in titratable acidity during sourdough fermentation gives an indication of growth of lactic acid bacteria (LAB) and yeast. The low pH found in this study falls within the range of 3.05 to 3.65 which was observed by Edema and Sanni (2008) on maize sourdough.

The synergistic interaction between LAB and yeast metabolise starch, amino acids and produce carbon dioxide (Gobbetti and Corsetti, 1997). The LAB hydrolyse starch and the sugars produced are utilized by the yeast (Gobbetti et al., 1998). The hydrolysis of starch has been found to induce softening of maize dough (Falade et al., 2014). This was found to produce maize sourdough bread with improved loaf volume.

The LAB colony count increased with increasing fermentation time at 30°C and resulted in 2.01×10^9 cfu/ml after 72 h (Figure 4.6.2). Vogelmann et al. (2009) studied the adaptability of lactic acid bacteria and yeasts in sourdoughs prepared from different cereals. They found the LAB cell colony count for maize increased from day 2 to day 4 of fermentation from 8.7×10^8 to 2.2×10^9 cfu/g. The LAB count in this study fell within this range.

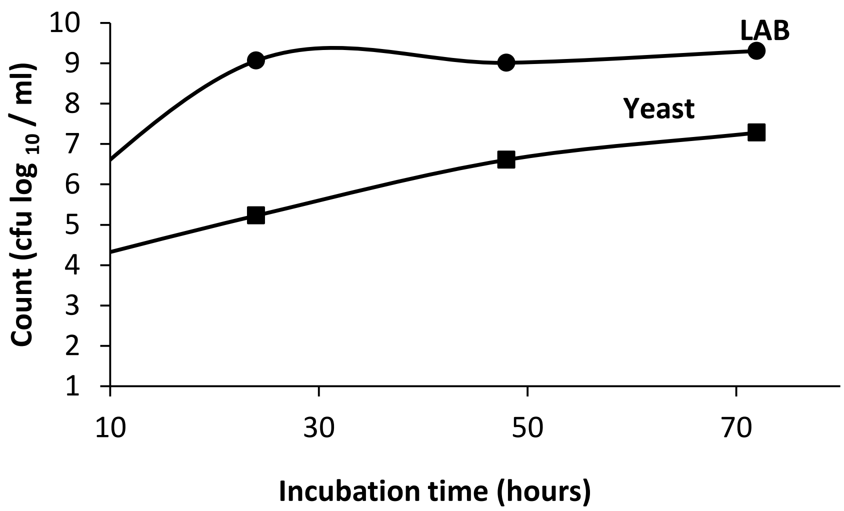


Figure 4.6.2: Plate counts of lactic acid bacteria and yeasts during spontaneous fermentation of maize flour sourdough (starter culture) over a period of 72 h at 30^{o}C.

In this study, the yeast colony count resulted in 4.1×10^6 cfu/ml after 72 h of fermentation at 30°C (Figure 4.6.2). In comparison, Vogelmann et al. (2009) found a yeast colony count from day 2 to day 4 of fermentation ranging from 1.9×10^5 to 9.1×10^6 cfu/g.

Representative samples of lactic acid bacteria were obtained for each colony type at each incubation time. At 0 h of fermentation (H01), fibrous to creamy white colonies were obtained (Table 4.6). These colonies were consistent with Collins et al. (2004) and Breed et al. (1957) findings of *Lactobacillus spp*. and *Micrococcus spp*. Presumptively, a *Pediococcus* *spp*. was obtained at 24 h of fermentation. This mucoid colony disappeared after 48 h of fermentation. This may be due to the *Lactobacillus spp*. and *Micrococcus spp*. being dominant.

In recent studies, *Lactobacillus fermentum*, *L. brevis* and *L. plantarum* were identified in spontaneously fermented maize flour (Edema and Obimakinde, 2015). Furthermore, Falade et al. (2014) used *L. plantarum* as a starter culture for maize sourdough fermentation. These are common lactic acid bacteria in maize sourdough starter cultures.

The morphology of yeast colonies at 24 h of fermentation (H2405) was smooth, round, creamy, white and convex (Table 4.7). These yeast colony morphologies were consistent with the findings of Collins et al. (2004) and Campbell et al. (2013). Furthermore, another mucoid yeast colony was observed and was presumed to be either *Aureobasidium spp* or *Cryptococcus spp*. This mucoid yeast colony disappeared at 48 h. It can be assumed that the *Candida spp* and *Saccharomyces cerevisiae* were dominate after 48 h. In spontaneously fermented maize flour, Edema and Sanni (2008) identified *Saccharomyces cerevisiae*. Whereas, Obiri-Danso (1994) identified *Candida tropicalis*, *C. kefyr* and *C. krusei* in fermented maize dough.

The high levels of LAB and yeasts at the end of 3 days and the low pH give an indication of the biochemical changes that took place during sourdough fermentation. The starter was backslopped, therefore, it can be assumed that the dominate microorganisms in the starter were present in the sourdough. Therefore, biochemical modifications such as acidification and amylolytic activity would take place in the backslopped sourdough, thus leading to improved loaf volume for the maize bread.

Table 4.6: Identification of presumptive lactic acid bacteria using colony morphological description

Isolate code	Morphological description	Presumptive bacteria	Reference
H01	Filamentous/fibrous, white, circular	*Lactobacillus spp.*	Collins et al. (2004)
H03	Circular, clear colour	*Micrococcus spp.*	Breed et al. (1957)
H05	Creamy white, circular, flat-shaped, small	*Lactobacillus spp.*; *Micrococcus spp.*	Collins et al. (2004); Breed et al. (1957)
H2401	Creamy white, circular, flat-shaped	*Lactobacillus spp.*	Collins et al. (2004)
H2403	Clear white, circular, flat-shaped	*Lactobacillus spp.*	Collins et al. (2004)
H2405	Mucoid, clear white/ grey	*Pediococcus spp.*	Collins et al. (2004); Breed et al. (1948)
H4801	Creamy white, circular, flat-shaped	*Lactobacillus spp.*	Collins et al. (2004)
H4803	Creamy white centre with clear surrounding, circular	*Micrococcus spp.*	Breed et al. (1957)
H7201	Creamy white, circular, flat-shaped	*Lactobacillus spp.*	Collins et al. (2004)
H7203	Creamy white centre with clear surrounding, circular	*Micrococcus spp.*; *Lactobacillus spp.*	Breed et al. (1957)

Table 4.7: Identification of presumptive yeast using colony morphological description

Isolate code	**Morphological description**	**Presumptive bacteria**	**Reference**
H09	Creamy, white, smooth, round, convex	*Candida spp*.	Campbell et al. (2013)
H2405	Smooth, round, creamy white, convex	*Candida spp.*	Collins et al. (2004)
H2407	Mucoid, white and flat-shaped	*Aureobasidium spp.*; *Cryptococcus spp.*	Collins et al. (2004); Campbell et al. (2013)
H4801	Smooth, round, white, creamy, convex, glossy	*Saccharomyces cerevisiae*	Campbell et al. (2013)
H4807	Smooth, creamy, matte-like, white	*Candida spp.*	Collins et al. (2004)
H7205	Smooth, round, white, convex	*Candida spp.*	Campbell et al. (2013)

4.3.2.3. Bread making

The effect of proofing time on maize doughs was investigated to determine the optimum proofing time for the highest maize bread loaf height. When wheat dough was proofed for 2 hours, it doubled in size (Table 4.8). However, when it was proofed for 4 hours it rose and the crust collapsed, which indicated over-proofing. Proofing of maize bread for 2 hours showed undesirable cracks on the crust (Figure 4.7B). Proofing for 4 hours increased the number of visible cracks on the crust (Figure 4.7E). This indicates that a proofing time of 4 hours was not ideal for the maize bread. A similar trend was observed for maize-zein composite bread. When maize-zein dough was proofed for 4 hours cracks on the bread crust developed (Figure 4.7F). The cracks on the crust are a reflection of high gas escape from the dough during baking, which caused poor dough expansion. The reduction in cracks are a reflection of the use of zein improving dough gas retention within 2 hours but not with dough which had been over-proofed for 4 hours. Therefore, the optimum proofing time was determined to be 2 hours.

Bread crust turns brown during baking due to Maillard browning, which occurs through the interaction between the sugars and protein (Ames, 1990). Maize bread crust took a longer time to brown than wheat bread crust. This may be due to its lower protein content compared to wheat bread (Table 4.5). The yellow colour of zein in the maize-zein composite posed a challenge in determining time to brown. However, it somewhat took a similar time to brown as maize bread crust.

A non-homogeneous crumb was observed for the maize (Figure 4.7 B and E) and maize-zein composite breads (Figure 4.7 C and F). This was possibly due to dough sheeting. However, wheat bread crumb was homogeneous despite dough sheeting (Figure 4.7 A and D).

Table 4.8: Effects of different treatments on sheeted wheat, maize, maize-zein on dough height before proofing and bread loaf height after baking[a]

Bread type	Treatment	Dough height before proofing (mm)	Loaf height after baking (mm)
Wheat	Proofed for 2h at 40°C	$13.0^{Xa}\pm2.3^{b}$	$48.0^{XYbc}\pm1.2$
Wheat	Proofed for 4h at 40°C	$17.3^{XYab}\pm2.6$	$54.0^{Zc}\pm7.1$
Wheat	Additional yeast & sugar	$18.5^{Yabc}\pm1.9$	$41.8^{Xb}\pm6.2$
Maize	Proofed for 2h at 40°C	$20.5^{BCbc}\pm0.6$	$26.5^{ABa}\pm0.6$
Maize	Proofed for 4h at 40°C	$21.8^{BCbc}\pm1.3$	$25.3^{ABa}\pm2.1$
Maize	Additional sugar	$17.8^{ABCabc}\pm4.1$	$22.3^{Aa}\pm2.1$
Maize	Additional yeast	$17.5^{ABabc}\pm2.4$	$22.8^{Aa}\pm1.9$
Maize	Additional sugar & yeast	$23.5^{Cc}\pm3.1$	$29.8^{Ca}\pm3.2$
Maize	Addition of gluten (10%)	$13.5^{Aa}\pm2.4$	$27.5^{ABCa}\pm3.1$
Maize	Addition of DATEM	$22.8^{BCbc}\pm2.2$	$26.5^{ABa}\pm2.6$
Maize-zein	Proofed for 2h at 40°C	$20.0^{KLbc}\pm2.3$	$31.5^{Ma}\pm1.7$
Maize-zein	Proofed for 4h at 40°C	$18.3^{Kabc}\pm2.6$	$24.0^{KLa}\pm2.4$
Maize-zein	Additional sugar	$20.8^{KLbc}\pm1.0$	$23.3^{Ka}\pm3.3$
Maize-zein	Additional yeast	$23.5^{Lc}\pm1.0$	$23.3^{Kaa}\pm3.3$
Maize-zein	Additional yeast & sugar	$19.0^{Kabc}\pm0.8$	$25.3^{KLMa}\pm1.5$
Maize-zein	Lactic acid	$20.5^{KLbc}\pm1.7$	$29.0^{KLMa}\pm3.6$
Maize-zein	Addition of DATEM	$20.5^{KLbc}\pm0.6$	$29.5^{KLMa}\pm2.5$
Maize-zein (lactic acid)	Addition of DATEM	$22.0^{KLbc}\pm2.3$	$30.3^{KLa}\pm2.2$

[a] Means ± Standard deviation (n=2)
[b] Values in columns for all bread samples (^{abc}), wheat (^{XYZ}), maize (^{ABC}) and maize-zein (^{KLM}) with different superscript letters differ significantly ($p<0.05$)

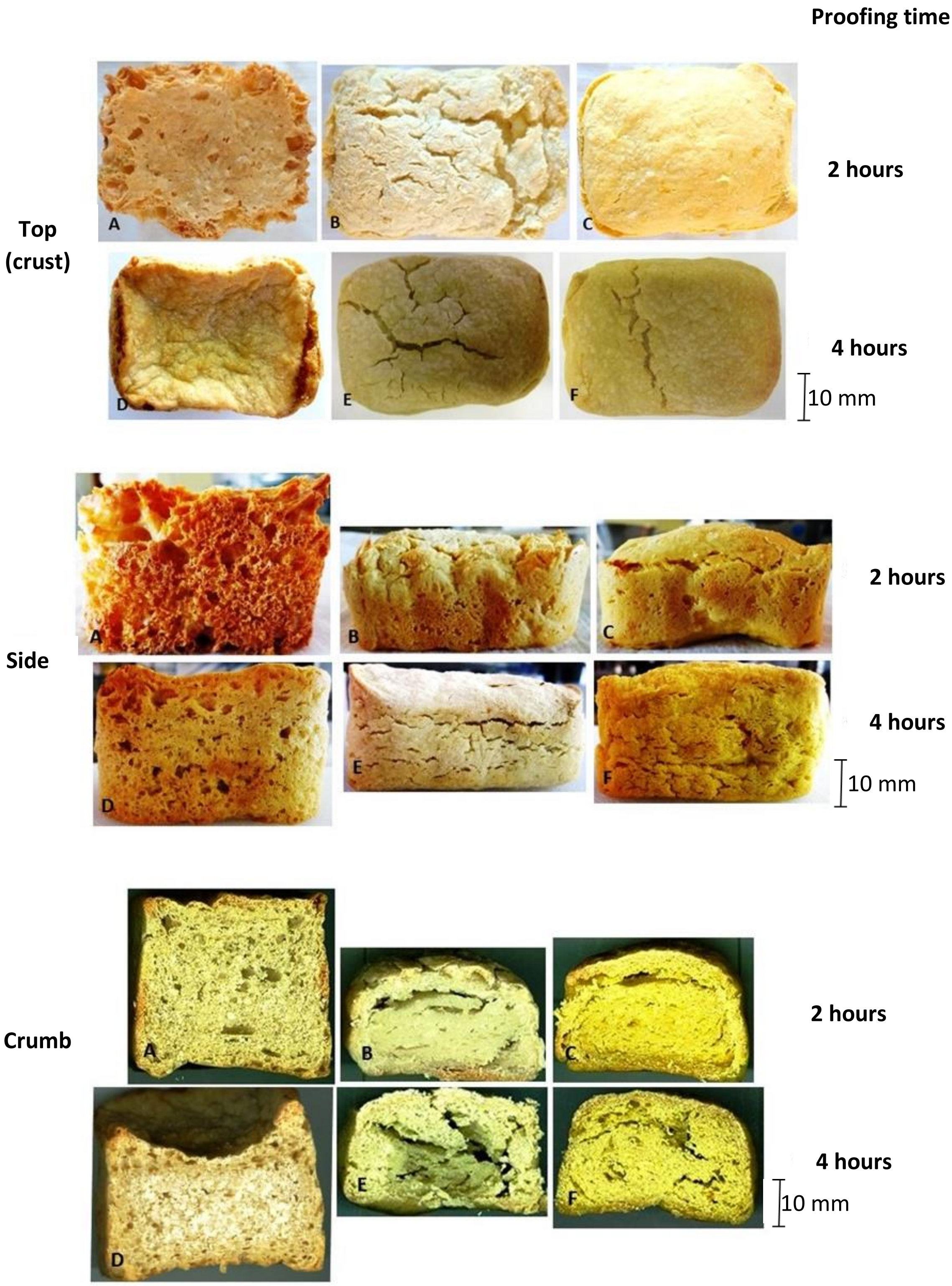


Figure 4.7: Effects of proofing time on loaf appearance, side and crumb texture of wheat, maize and maize-zein breads prepared by dough sheeting with maize flour pre-gelatinization. **A**. wheat proofed for 2 hours. **B**. maize proofed for 2 hours. **C**. maize-zein proofed for 2 hours. **D**. wheat proofed for 4 hours. **E**. maize proofed for 4 hours. **F**. maize-zein proofed for 4 hours.

The effects of additional sugar or yeast on loaf appearance, side and crumb texture was investigated to find the optimum sugar and yeast level. This is because yeast metabolizes sugars into carbon dioxide gas, which diffuses into bubbles incorporated during mixing and causes the bubbles to inflate and the dough to rise (Chiotellis and Campbell, 2003). No significant increase in loaf height for the maize and maize-zein bread was found when additional sugar was added (Table 4.8). Instead, there were undesirable cracks on the crust of the maize bread, which was assumed to be caused by loss of gas, thus affecting the rising of bread during proofing or baking (Figure 4.8.1B). The crust of the maize-zein composite bread was not greatly affected (Figure 4.8.1D) unlike the maize bread. That may be due to the zein coating the maize flour particles to form a maize-zein network. However, the crumb texture of both the maize and maize-zein breads were non-homogenous with tendency to form a hole in the crumb.

With additional yeast, there was no significant improvement in the loaf height with either the maize or the maize-zein composite (Table 4.8). However, the loaf height was much higher when compared to additional sugar alone. Again, there were cracks on the crust of maize bread (Figure 4.8.2B) which was similar to the bread with additional sugar alone. However, the cracks were less pronounced. The crumb texture of maize bread did not, however, have a more continuous crumb. Again, this may be due to the dough sheeting causing a discontinuous structure. With dough sheeting, there is a subdivision of gas cells in the dough (Scanlon and Zghal, 2001) and that may have affected rising of the dough.

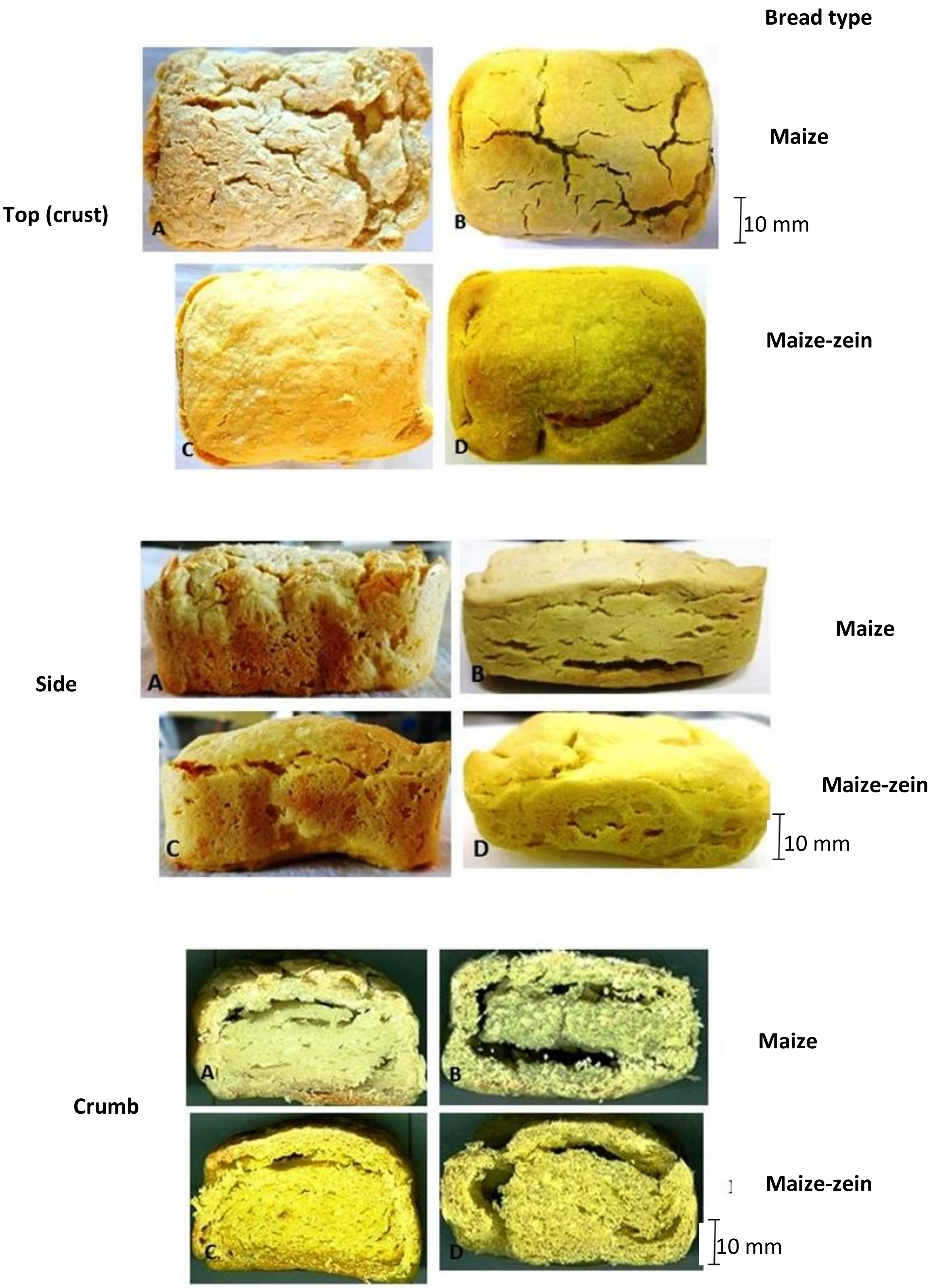


Figure 4.8.1: Effects of additional sugar on the loaf appearance, side and crumb texture of maize and maize-zein breads prepared using dough sheeting and maize flour pre-gelatinization. **A**. maize. **B**. maize with additional sugar. **C**. maize-zein. **D**. maize-zein with additional sugar.

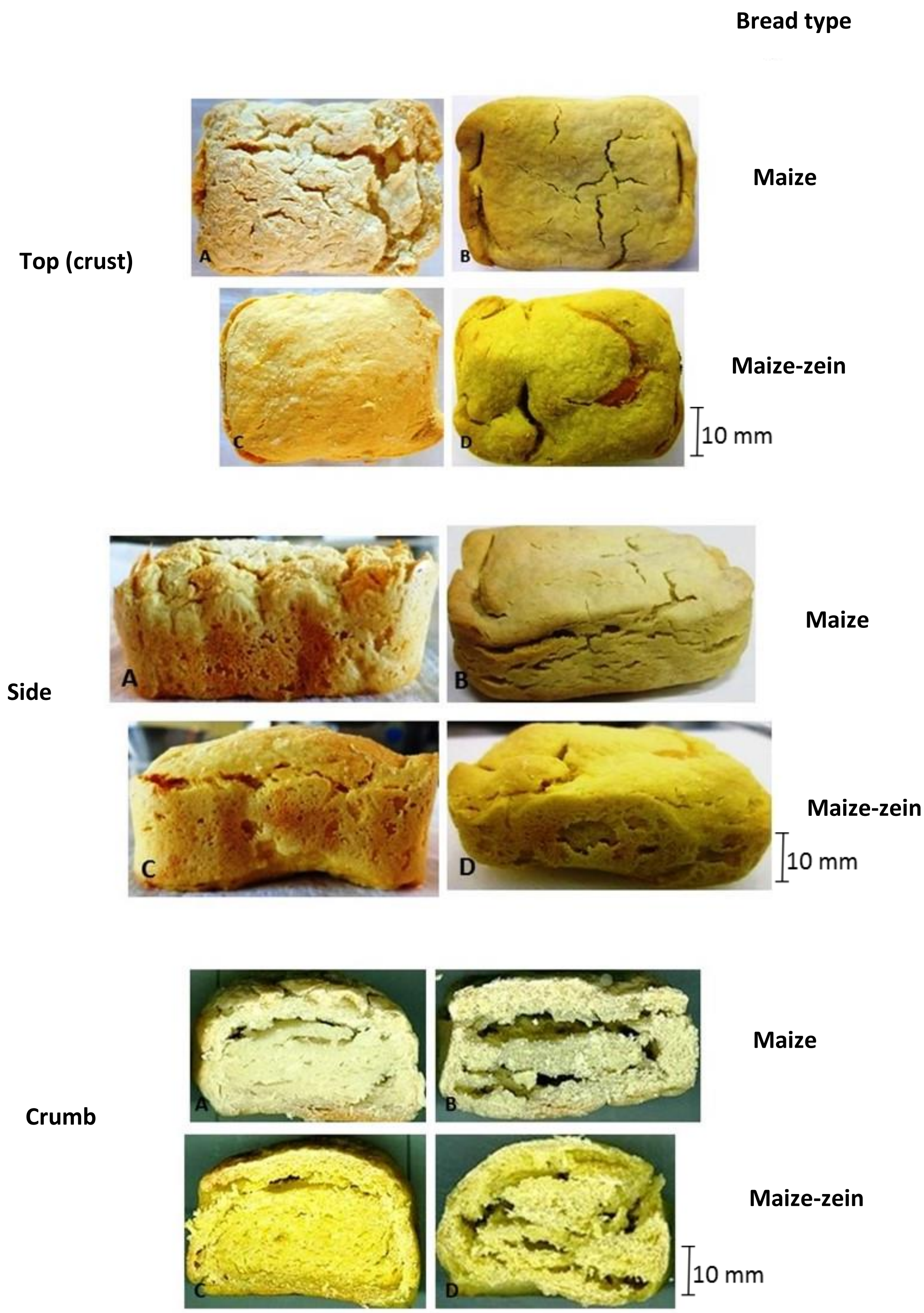


Figure 4.8.2: Effects of additional yeast on loaf appearance, side and crumb texture of maize and maize-zein breads prepared by dough sheeting and maize flour pre-gelatinization. **A**. maize. **B**. maize with additional yeast. **C**. maize-zein. **D**. maize-zein with additional yeast.

With additional sugar and yeast in wheat, maize and maize-zein bread significantly improved the loaf heights (Table 4.8). Wheat bread had a more pronounced loaf height increase than the other breads. However, during proofing, the wheat dough rose and later collapsed because of additional yeast and sugar. Maize bread also had a significant increase in loaf height with additional sugar and yeast.

With additional sugar and yeast alone, there were cracks on the crust of the maize bread (Figure 4.9 B and E). However, there were no undesirable cracks on the crust of the maize-zein composite bread (Figure 4.9C). Nevertheless, the crumb structure of the wheat bread showed a more continuous matrix than the maize and maize-zein breads (Figure 4.9D). The maize and maize-zein breads had holes in the crumb. This may have caused loss of gas during proofing, thus affecting the rising of the breads.

With addition of gluten to maize dough the bread resembled wheat bread in terms of colour and dome-like shape (Figure 4.10B). However, the bread had a lower height than the wheat bread (Table 4.8). The maize-gluten composite had a flying top which gives an indication of loss of gas during baking. Using zein prepared in lactic acid solution (pH 4.8) gave a bread with a lower loaf height than maize-zein bread alone (Table 4.8). Maize-zein bread made at a lower pH through preparing zein in lactic acid, caused the crumb texture of the maize-zein composite bread to have a semi-continuous matrix unlike at normal pH (Figure 4.11D).

The inclusion of DATEM in the maize-zein composite dough resulted in a bread with a rather a more homogeneous crumb texture (Figure 4.11C). Further, there was a reduction in visible cracks on the crust of the maize bread and a homogeneous crumb was observed. This was also observed for the maize-zein prepared with lactic acid (Figure 4.11E). Moreover, there were no cracks on the crust for the maize, maize-zein and maize-zein prepared with lactic acid breads. The loaf height of the maize-zein composite bread slightly increased with addition of DATEM (Table 4.8). In maize bread with added DATEM, there was no significant increase in loaf height. It is proposed that DATEM gave improved crumb structure by reduction of gas cell size in the bread as suggested by Nunes et al. (2009a).

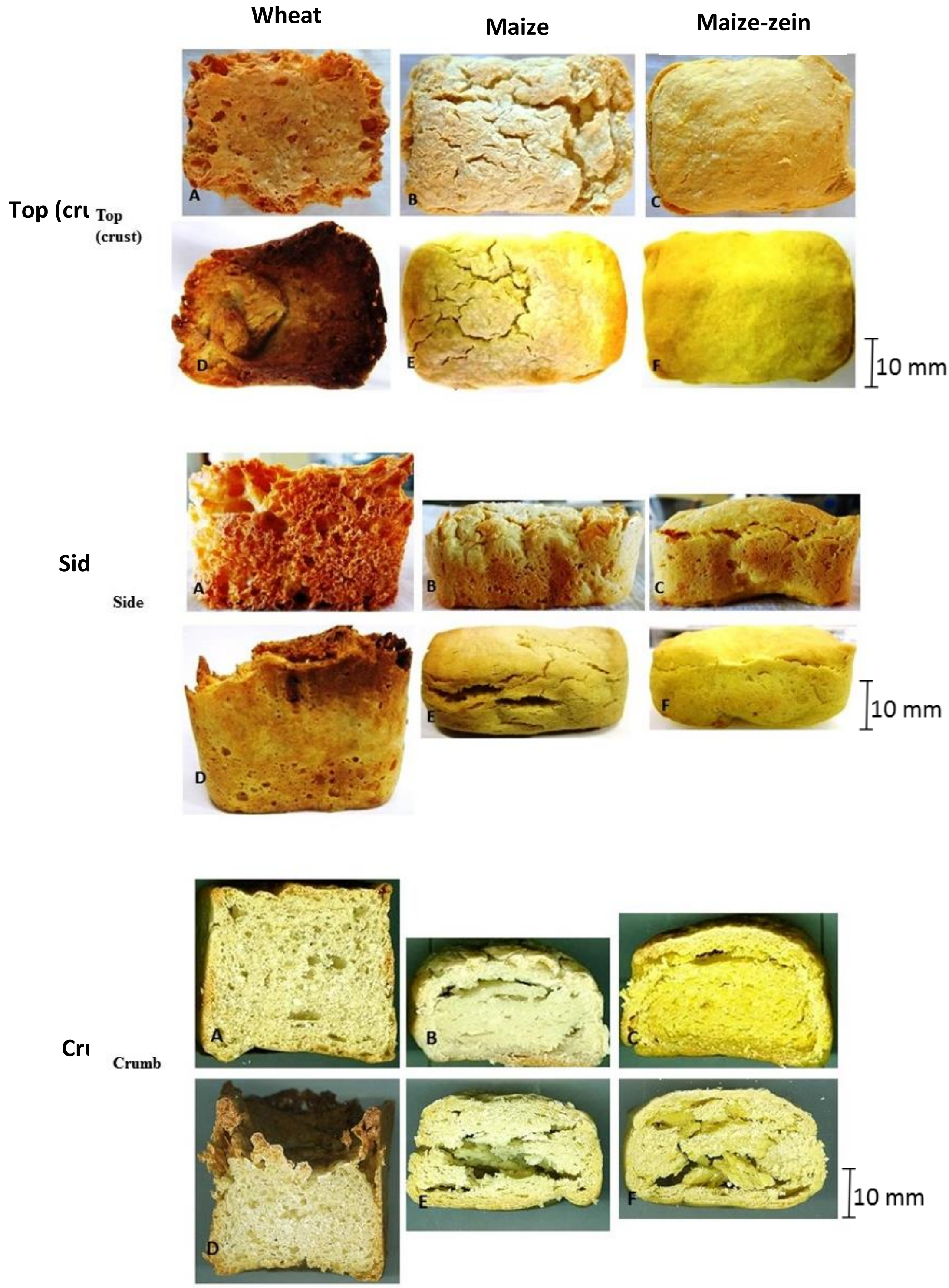


Figure 4.9: Effects of additional yeast and sugar on loaf appearance, side and crumb texture of wheat, maize and maize-zein breads prepared using dough sheeting with maize flour pre-gelatinization **A**. wheat. **B**. maize. **C**. maize-zein. **D**. wheat with additional yeast and sugar. **E**. maize additional yeast and sugar. **F**. maize-zein additional yeast and sugar.

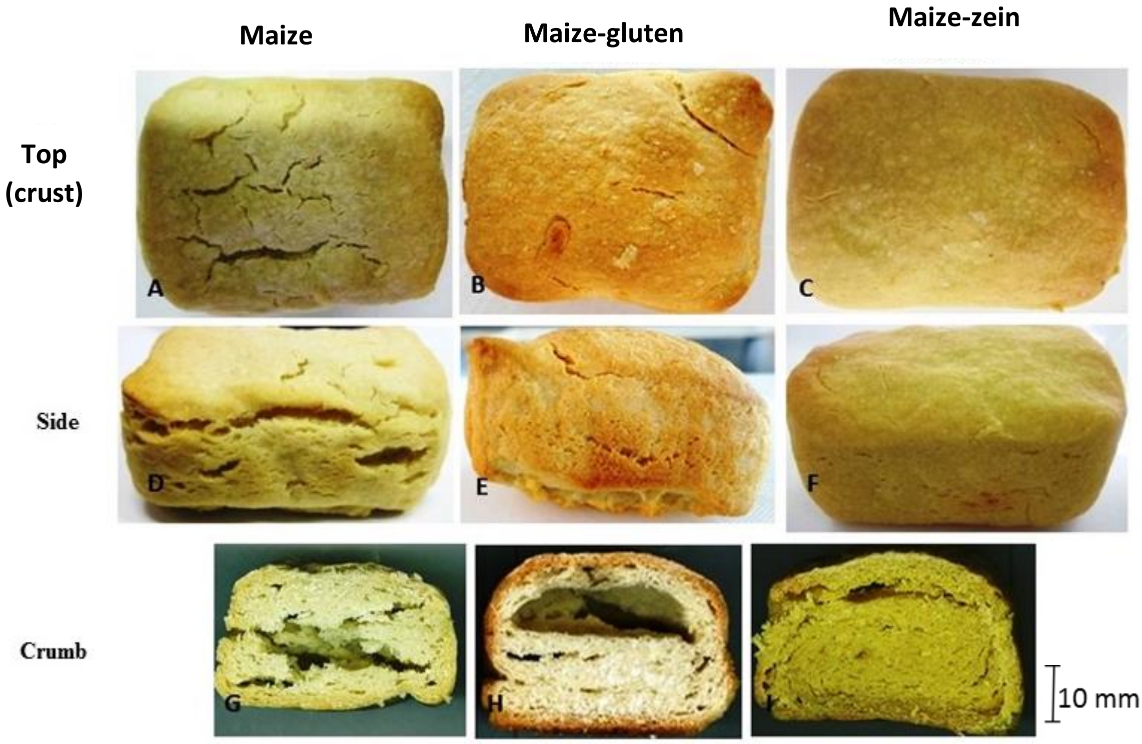


Figure 4.10: Effects of addition of gluten powder or zein dough on loaf appearance, side and crumb texture of maize breads prepared using dough sheeting and maize flour pre-gelatinization. **A**. maize crust. **B**. maize-gluten crust. **C**. maize-zein crust. **D**. maize side. **E**. maize-gluten side. **F**. maize-zein side. **G**. maize crumb. **H**. maize-gluten crumb. **I**. maize-zein crumb.

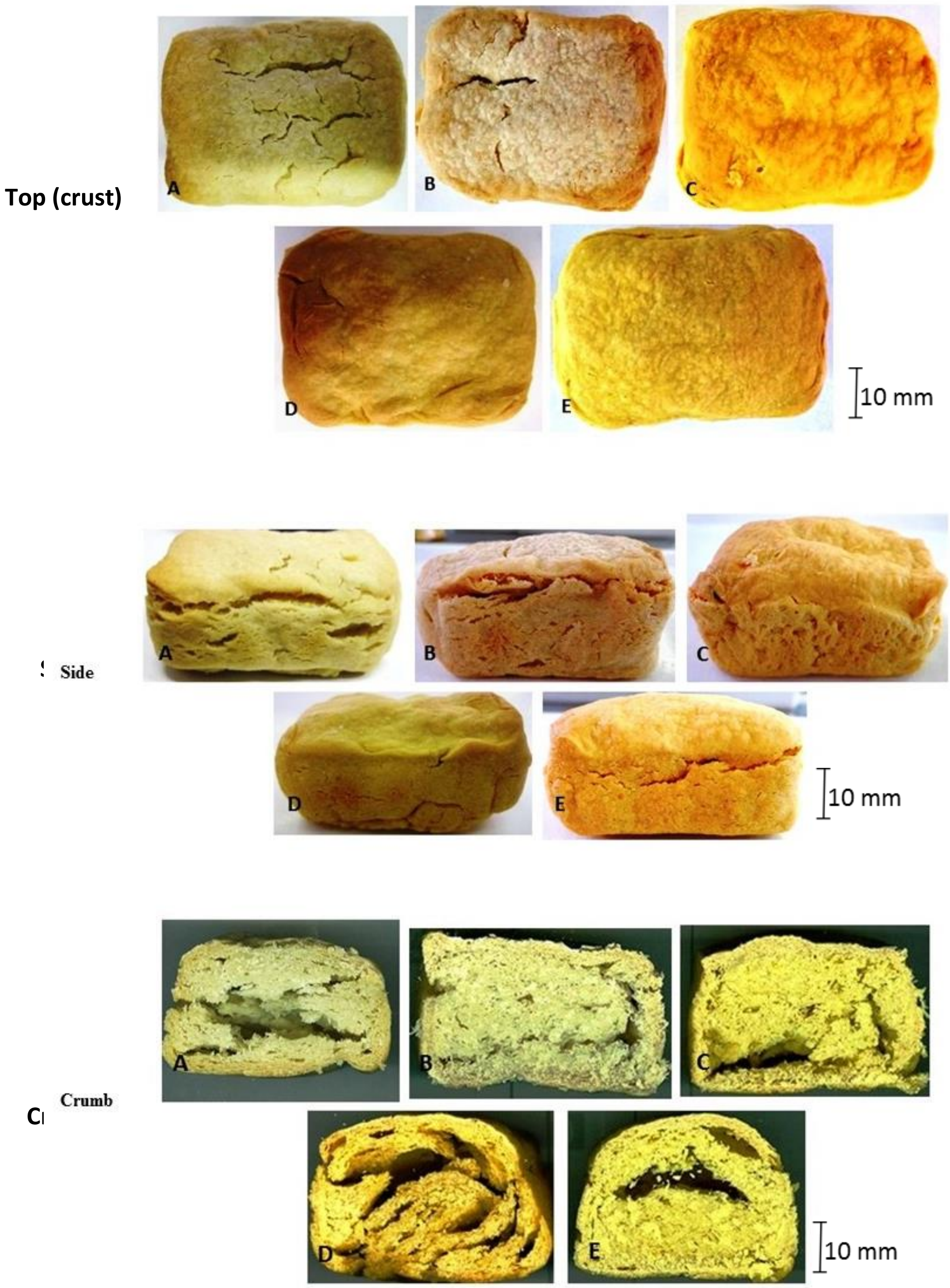


Figure 4.11: Effects of addition DATEM and zein prepared with lactic acid (3%) on the loaf appearance, side and crumb of maize and maize-zein breads prepared with dough sheeting and maize flour pre-gelatinization. **A**. maize. **B**. maize with added DATEM. **C**. maize-zein with added DATEM. **D**. maize-zein treated with lactic acid. **E**. maize-zein treated with lactic acid and with added DATEM.

The maize breads with added sourdough were not directly comparable because the amount of flour in the doughs was not the same. The inclusion of sourdough in maize and maize-zein doughs affected the appearance of the crust and crumb of the breads. However, addition of 60% or 80% sourdough and DATEM did not significantly ($p \geq 0.05$) increase the loaf height of maize bread (Tables 4.9 and 4.10).

The addition of sourdough formed cracks on the crust of maize sourdough breads (Figure 4.12). This may have been due to the sourdough modifying the starch (Falade et al., 2014) and causing the bread to hold more water (Table 4.5). The maize 60% sourdough bread had a discontinuous crumb structure (Figure 4.12B). However, with addition of DATEM there was a much more homogenous crumb (Figure 4.12C). Similarly, inclusion of DATEM formed far more continuous crumb texture in maize 80% sourdough bread (Figure 4.12E). Smaller and elongated gas cells were observed in maize-zein sourdough bread (Figure 4.12F). With maize-zein 80% sourdough bread, there was evidence of unincorporated zein in the bread crumb.

The pH of the maize breads with added sourdough was 3.8 for both 60 and 80 % sourdough. As the amount of sourdough was increased, the pH decreased somewhat. The low pH as a result of addition of sourdough induced softening of the maize dough and worked together with the yeast to increase the loaf height. There have been reports on an increase in resistance to extension and stiffness of wheat dough at low pH (Gujral and Singh, 1999) and also that the reduction in pH results in the reduction in –SH groups in proteins (Tsen, 1966). Gujral and Singh (1999) observed a decreased in wheat bread volume with an increase in lactic acid fermentation. The reduction in extensibility was thought to be due to lactic acid causing less dough expansion during fermentation, and rupture of gas cells, rather than expansion, during baking. This would result in loss of aeration (Bennett and Ewart, 1962).

Table 4.9: Effects of addition of 60% sourdough and DATEM on maize dough height before proofing and bread loaf height after baking[a]

Bread type	Treatment	Dough height before proofing	Loaf height after baking
Maize	Addition of 60% sourdough	$25.3^{\mathbf{a}}\pm2.2^{b}$	$32.8^{\mathbf{a}}\pm2.2$
Maize	Addition of 60% sourdough & DATEM	$27.3^{\mathbf{a}}\pm1.3$	$35.0^{\mathbf{a}}\pm0.8$

[a] Means ± Standard deviation (n=2)
[b] Values in columns ($^{\mathbf{a}}$) with different superscript letters differ significantly ($p<0.05$)

Table 4.10: Effects of addition of 80% sourdough and DATEM on maize and maize-zein dough height before proofing and bread loaf height after baking[a]

Bread type	Treatment	Dough height before proofing	Loaf height after baking
Maize	Addition of 80% sourdough	$18.5^{\mathbf{a}}\pm1.7^{b}$	$39.3^{\mathbf{a}}\pm1.5$
Maize	Addition of 80% sourdough & DATEM	$21.5^{\mathbf{b}}\pm1.7$	$40.0^{\mathbf{a}}\pm1.4$
Maize-zein	Addition of 80% sourdough	$25.5^{\mathbf{c}}\pm0.6$	$42.5^{\mathbf{a}}\pm2.9$

[a] Means ± Standard deviation (n=2)
[b] Values in columns ($^{\mathbf{ab}}$)with different superscript letters differ significantly ($p<0.05$)

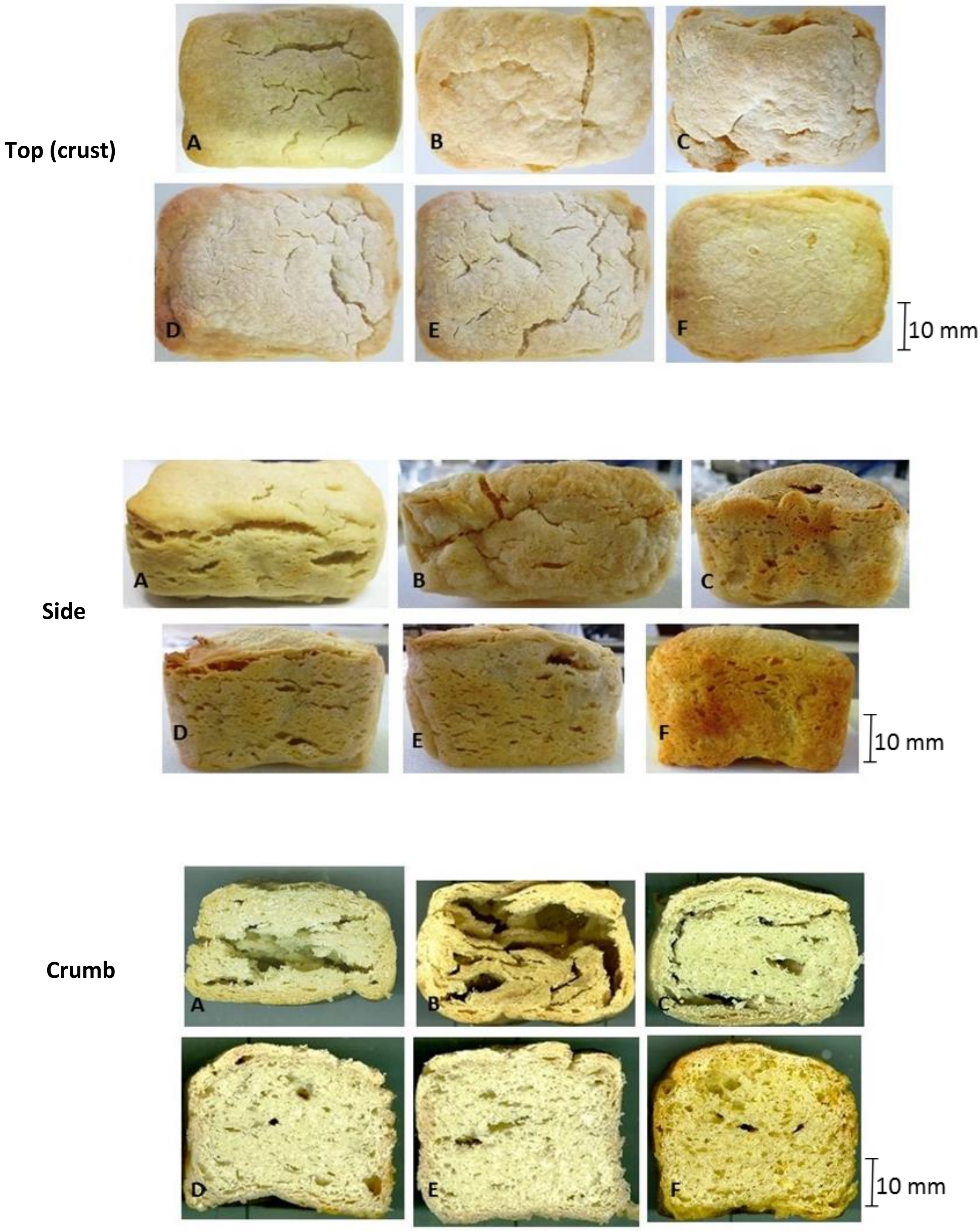


Figure 4.12: Effects of sourdough addition on loaf appearance, side and crumb texture of maize and maize-zein breads prepared using dough sheeting and maize flour pre-gelatinization. **A**. maize. **B**. maize with 60% sourdough. **C**. maize with 60% sourdough and DATEM. **D**. maize with 80% sourdough. **E.** maize with 80% sourdough and added DATEM. **F**. maize-zein with 80% sourdough.

4.3.2.4. Stereomicroscopy of the crumb structure of maize breads

Bread prepared from sheeted maize dough using maize flour pre-gelatinization had more open and defined gas cells in the crumb than maize flour bread prepared by hand dough mixing (Figure 4.13B). The white particles were assumed to be clumps of starch. They were well defined in the maize bread with pre-gelatinized maize. This was perhaps due to the pre-gelatinization step prior to bread making. According to Scanlon and Zghal (2001), during baking there is partial melting of hydrated starch granules but not their complete homogenization.

Incorporation of vital gluten powder or zein dough improved the gas holding capacity of the maize dough bread (Figure 4.14 B and C). The incorporation of zein resulted in bigger gas cells than gluten. Further, they were more defined than with gluten inclusion. There was also evidence of unincorporated zein in the maize-zein bread.

Additional yeast and sugar improved the crumb texture, especially for the wheat bread (Figure 4.15D). There were well defined large gas cells in the wheat bread. Moreover, melted starch was observed. There was no evidence of sheeting in the wheat bread crumb unlike in maize-zein bread where the gas cells were elongated and assumed to be in the direction of dough sheeting.

The inclusion of DATEM in maize doughs resulted in improved bread crumb texture. It formed elongated and smaller gas cells in combination with dough sheeting (Figure 4.16C) than maize bread alone (Figure 4.16A). With DATEM inclusion there were small pre-gelatinized starch clumps present that were uniform or homogenous, unlike when DATEM was not added. Further, the maize-zein composite bread had better incorporation of zein with inclusion of DATEM. A homogenous crumb was formed with smaller more spherical gas cells.

With wheat bread, Zghal et al. (2001) observed an increase in crumb uniformity and density with increasing sheeting passes (up to 5) and alteration in the distribution of cell sizes. As stated, the addition of DATEM resulted in maize bread with a more uniform crumb and gas cells in the direction of dough sheeting. This is similar to what Nunes et al. (2009a) observed where there was decreased gas cell size and a more homogeneous crumb with addition of medium and high levels (i.e. 0.45 to 0.6%) of DATEM in white rice flour and potato starch bread. They considered that this was due to the DATEM lowering the surface tension, leading to the incorporation of air and production of smaller bubbles during mixing.

Addition of sourdough (60%) resulted in a crumb structure that had larger gas cells (Figure 4.17B) compared to maize sourdough with added DATEM (Figure 4.17C). As previously mentioned, dough sheeting redistributed gas cells. There was no evidence of melted starch, perhaps due to the sourdough modifying the starch (Edema et al., 2013; Falade et al., 2014). With an increasing concentration of sourdough of up to 40%, Crowley et al. (2002) observed a negative effect on wheat sourdough bread characteristics such as texture, crumb shrinkage and chewiness during storage. As were stated, in this study, addition of 60 and 80% sourdough formed a close crumb texture with small gas cells and a homogenous crumb. Also, in contrast, Gocmen et al. (2007), observed an open and uniform crumb structure in wheat flour bread as the percentage of sourdough (20 to 40%) was increased. Maize flour dough was used this study which does not possess gluten, thus the difference in observations between this study and Gocmen et al. (2007) work.

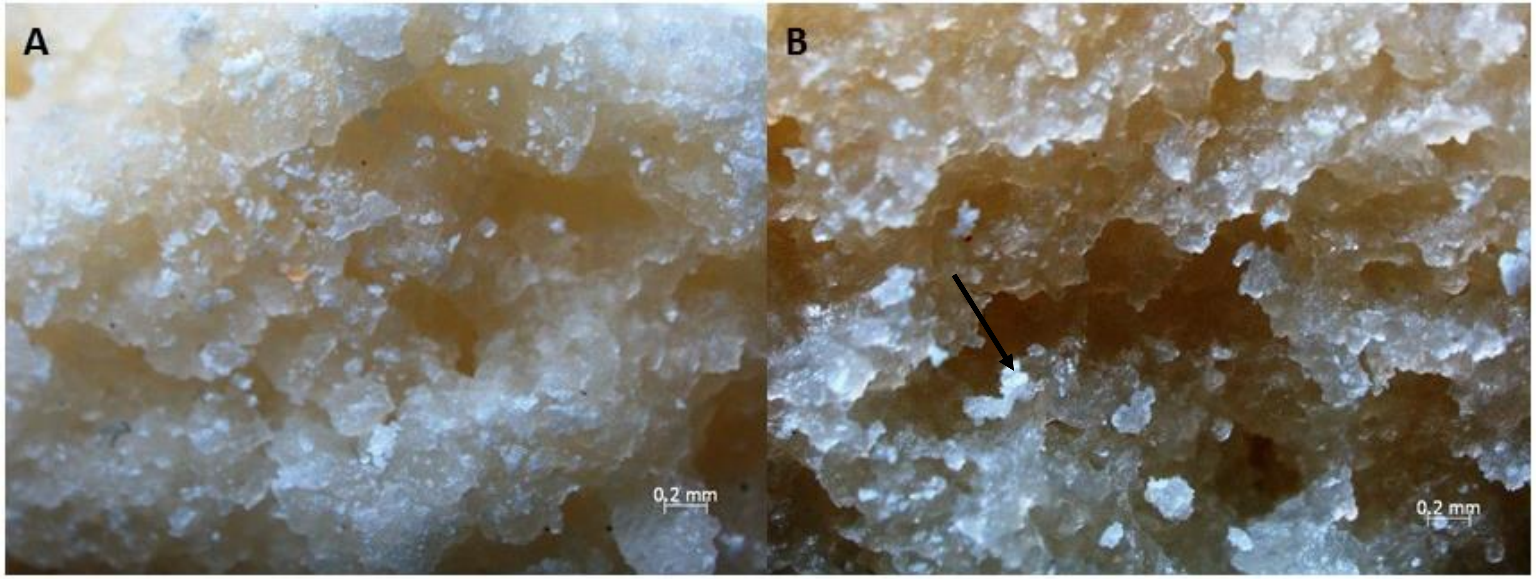


Figure 4.13: Effect of dough sheeting of maize dough prepared using maize flour pre-gelatinization on the bread crumb structure. **A**. hand mixed maize flour dough. **B**. sheeted maize flour dough bread. Arrow indicates starch clumps.

Figure 4.14: Effects of incorporation of vital gluten powder or zein dough in maize dough in combination with pre-gelatinization and sheeting on bread crumb structure. **A**. maize. **B**. maize-gluten. **C**. maize-zein. Arrow indicates unincorporated zein.

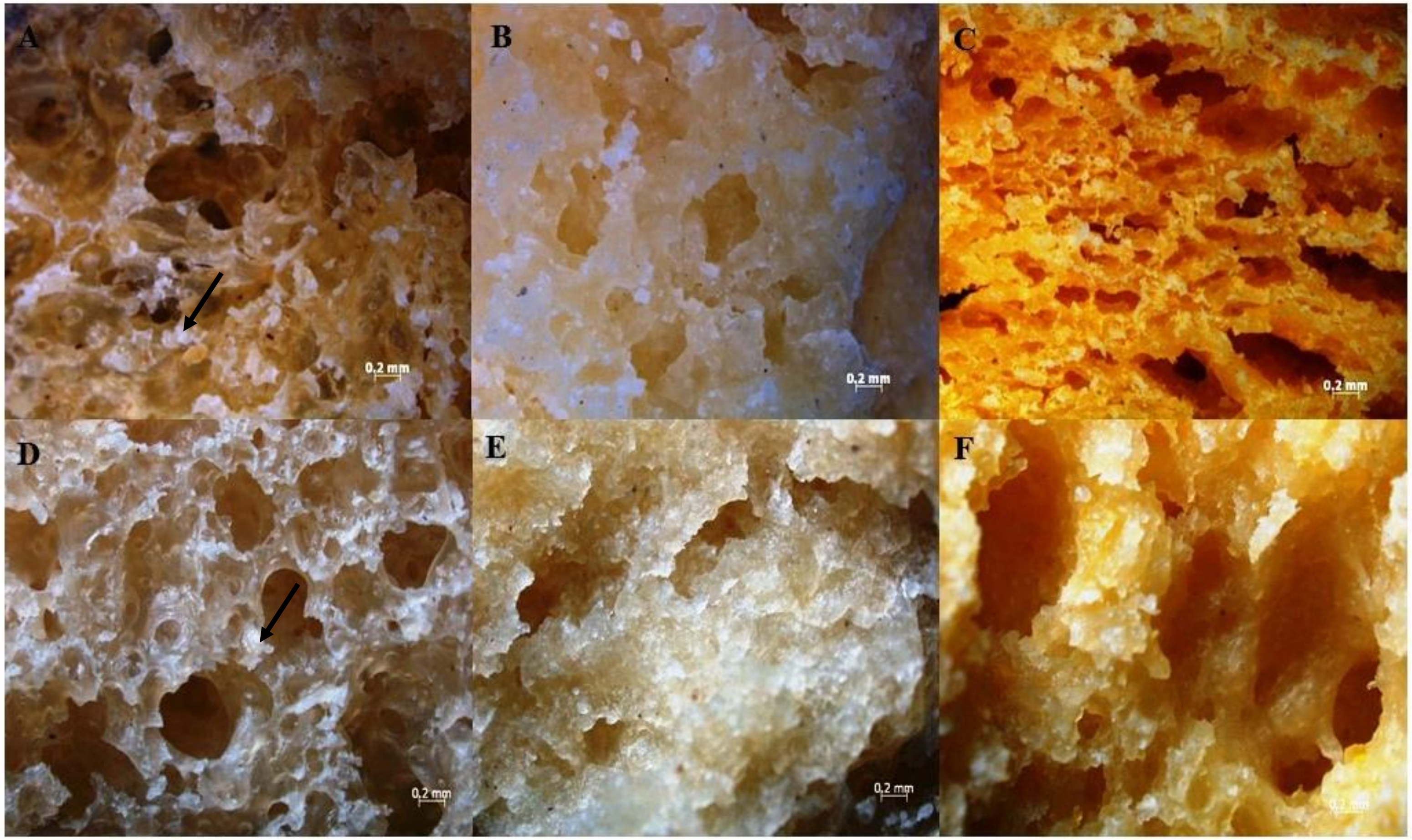


Figure 4.15: Effects of additional yeast and sugar in wheat, maize and maize-zein doughs proofed for 2 h at 40^{o}C in combination with sheeting on bread crumb structure. **A**. wheat. **B**. maize. **C**. maize-zein. **D**. wheat with additional yeast and sugar. **E**. maize with additional yeast and sugar. **F**. maize-zein with additional yeast and sugar. Arrows indicate melted starch.

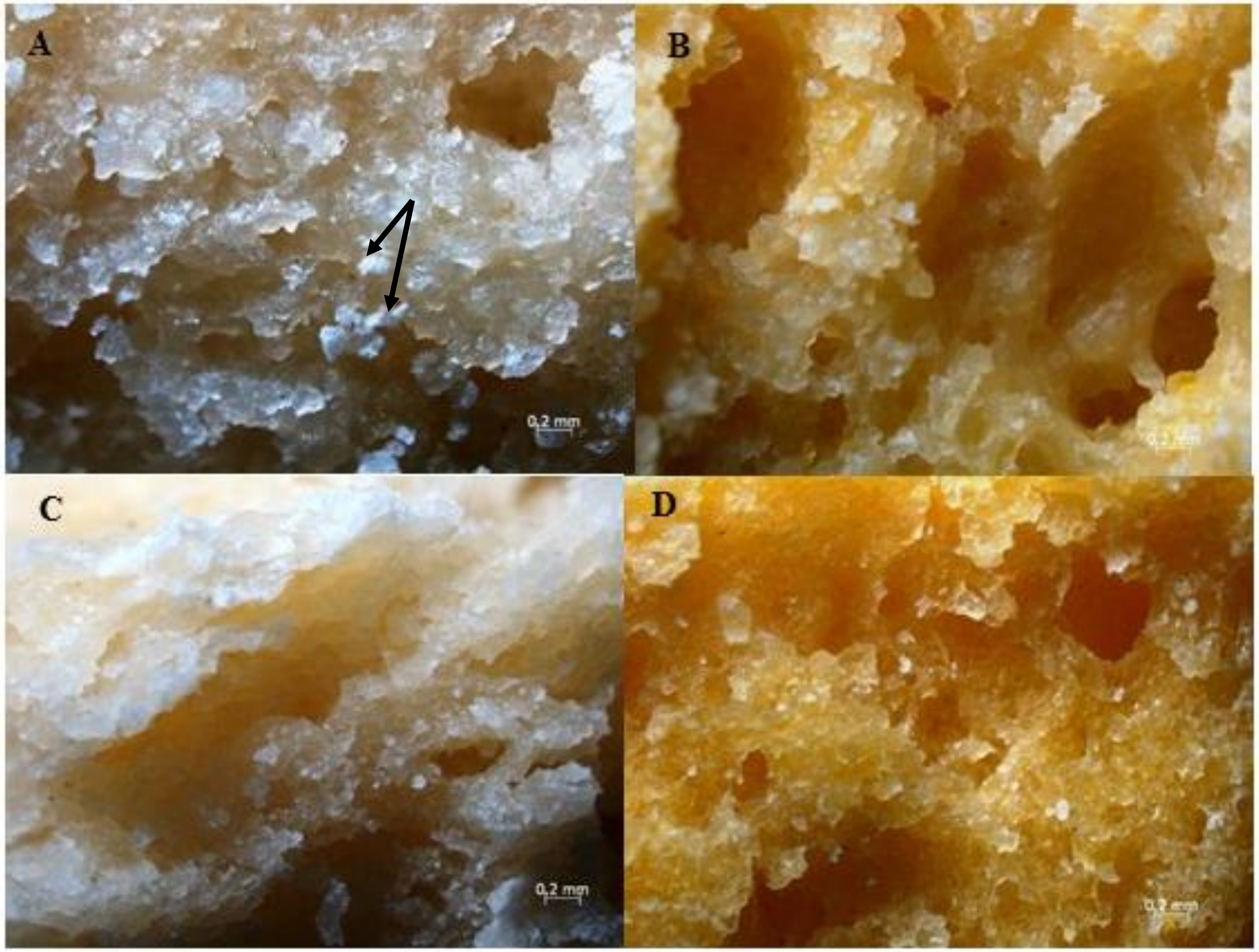


Figure 4.16: Effects of addition of DATEM in maize and maize-zein doughs in combination with sheeting on the bread crumb structure. **A**. maize. **B**. maize-zein. **C**. maize with added DATEM. **D**. maize-zein with added DATEM. Arrow indicates pre-gelatinized starch clumps.

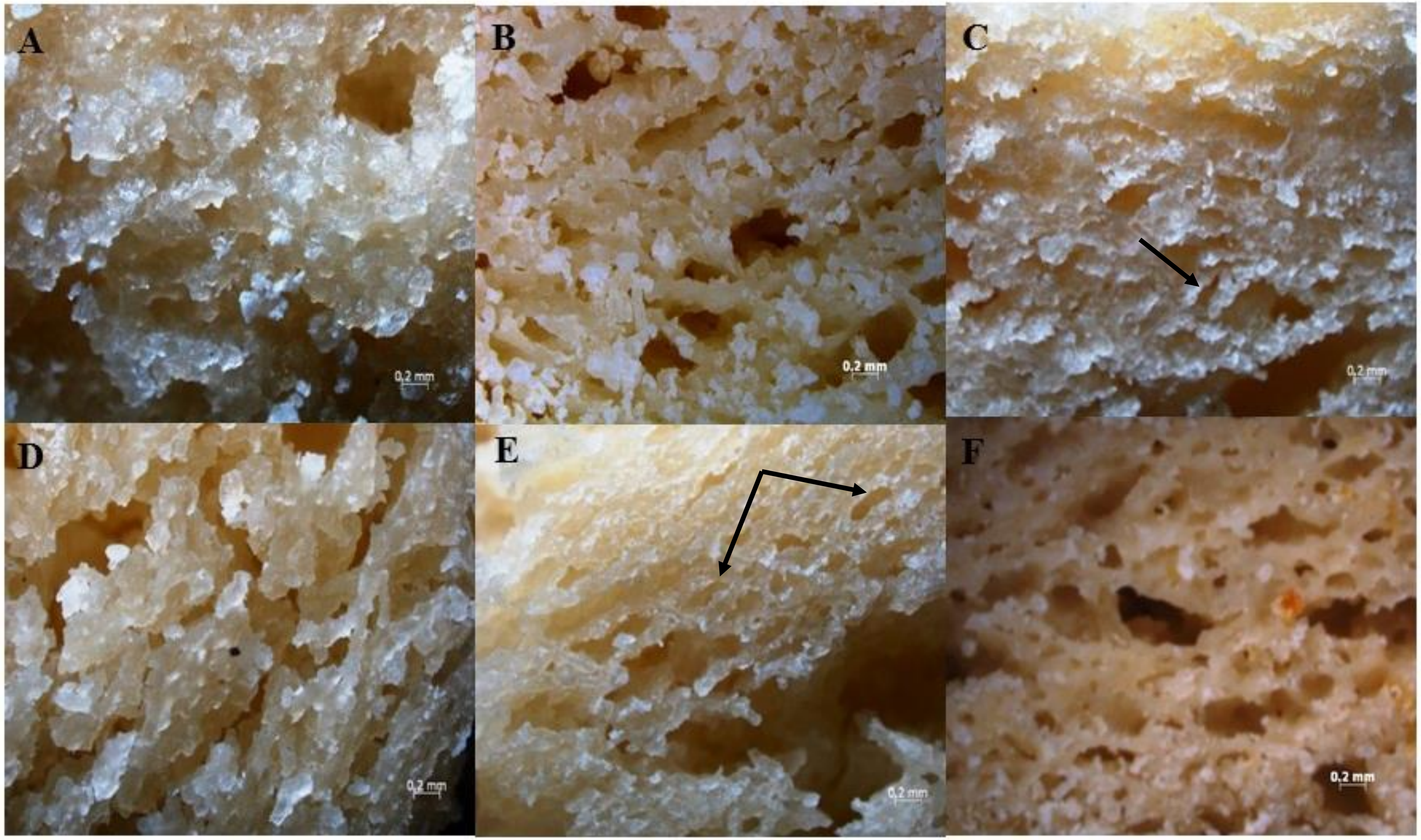


Figure 4.17: Effects of sourdough and DATEM addition in combination with sheeting on maize bread crumb structure. **A**. maize. **B**. maize sourdough (60%). **C**. maize sourdough (60%) with added DATEM. **D**. maize sourdough (80%). **E**. maize sourdough (80%) with added DATEM. **F**. maize-zein sourdough (80%). Arrows indicate elongated gas cells.

4.4. Conclusions

Dough sheeting in combination with pre-gelatinization of maize flour produces a cohesive maize dough with improved dough handling and functional properties. A smooth textured maize dough is produced with increasing number of sheeting passes. The inclusion of commercial zein further improves dough handling, strength and extensibility of maize dough through intermingling of fibrils of the added zein with maize flour particles in the dough. With inclusion of sourdough fermented maize flour there is a significantly improved loaf height and crumb texture of maize bread and zein inclusion improves gas holding potential. Furthermore, DATEM addition produces maize-zein and maize sourdough bread with improved loaf height and a homogeneous crumb texture.

Dough sheeting in combination with pre-gelatinized maize flour shows potential to improve dough handling properties of maize flour. To obtain maize bread with improved crumb texture and loaf height the inclusion of sourdough fermented maize flour and DATEM is advantageous.

5. GENERAL DISCUSSION

This general discussion will firstly comprise of a critical review of some of the methodologies as applied in this work. Secondly, the findings on the effects of dough sheeting in combination with maize flour pre-gelatinization, addition sourdough fermentation, zein and DATEM with respect to improving maize dough and the bread loaf height and crumb structure will be explained. Lastly, based on these findings, future research directions into improving maize and other gluten-free breads will be examined.

5.1. Methodology applied: critical review

The effects of dough sheeting in combination with maize flour pre-gelatinization, the addition of zein, sourdough fermentation and DATEM were investigated. Dough sheeting is a highly efficient form of mechanical dough development (Bushuk and Hulse, 1974) which involves passing dough between two rotating cylinders (Petitot et al., 2009). Also importantly, an advantage of dough sheeting over conventional dough mixing is that dough could be developed with sheeting rolls using only 10 to 15% of the net energy required with a dough mixer (Kilborn and Tipples, 1974).

In this work, it was found that dough sheeting better incorporated the maize flour and pre-gelatinized maize together to form a cohesive dough. However, without pre-gelatinization of maize flour, the resultant dough was crumbly. Dough sheeting in combination with maize flour pre-gelatinization is an inexpensive way of improving maize dough handling properties without addition of hydrocolloids. Pre-gelatinization of maize only requires heating of maize flour in water at high temperature in order to form a gel. These should form affordable maize bread.

In this study, dough sheeting was a manual process. Therefore, the amount of force applied to the dough and roll speed could not be measured. Instead, the roll gap of the dough sheeter was kept constant. In a mechanised process investigated by Patel and Chakrabarti-Bell (2013), the dough sheeter they used was an ABBM model (Veryst Engineering, USA) which had ultrasonic sensors that measured the roll force and dough sheet thickness. The quality of this research could have been improved if the force of the sheeter was measured. With the use of the ABBM model, more information would have been gathered about the rheology, elasticity and sheeting behaviour of maize dough.

Sourdough fermentation is an inexpensive and natural alternative to the use of hydrocolloids. In this study, the maize sourdough breads could not be compared because they had different amounts of flour. To avoid this problem the dough weight before proofing should be kept constant in order to produce comparable breads. Furthermore, to improve the quality of this research, MALDI-TOF and biochemical tests need to be performed to identify specific LAB and yeasts in spontaneously fermented sourdough.

Hydrocolloids such as HPMC are used in gluten-free batters to modify the dough texture. This is through improving their water binding capacity, due to the hydroxyl groups in the hydrocolloids which allow more interactions by hydrogen bonding (Guarda et al., 2004; Houben et al., 2012). It can therefore be said that the pre-gelatinized maize flour was used as a naturally occurring hydrocolloid in this study.

A major methodological challenge experienced was the maintenance of zein at 40^{o}C, i.e. above its T_g (Schober et al., 2008). This required an organised and rapid processing technique as the ambient temperature was 22°C, below zein's T_g (Lawton, 1992). The use of a water bath as well as pre-warmed equipment such as mixing bowls and spatulas assisted in reducing the heat loss. However, the dough sheeting for the maize-zein composite dough could not be performed above zein's T_g. Instead, it had to be performed at ambient temperature. To further reduce heat loss prior to texture analyses the dough samples were placed back in a 50^{o}C water bath for a period of time and then placed in an insulated (polystyrene) container. In an ideal situation, a large scale incubator would have been used.

Confocal laser scanning microscopy was used to study the microstructures of the maize doughs with added zein. These doughs were also stained with acid fuschin (Dürrenberger et al., 2001) to differentiate protein fibrils as a pink/red colour. The process of sticking the dough onto the microscope slide involved firmly pressing the doughs without the use of tape since the dough was originally sticky. Unfortunately, the maize-zein doughs tended to dry out rapidly and fall from the inverted slide during the CLSM. This was as a result of the CLSM being performed at ambient temperature and the zein falling below its T_g. Also, differentiating starch granules and protein fibrils was a challenge with the acid fuschin stain. Perhaps, the use of two different stains to identify starch granules and protein would have been better, as used by Kim et al. (2008). They used two fluorescent dyes, fluorescein which stained starch components and Rhodamine B which stained protein structures.

Stereomicroscopy was a particularly useful technique as it showed the effects of dough sheeting in combination with other treatments on the bread crumb textures. It was simple to perform with little sample preparation. It revealed that there was a tendency for a hole to form in the maize bread with dough sheeting. With the addition of zein or sourdough and DATEM, stereomicroscopy revealed a homogeneous crumb texture. Furthermore, it showed that zein had gas-holding properties with the formation of larger gas cells in the bread crumb than in maize bread alone.

With regard to the measurement of the rheological properties of the sheeted doughs, according to Dobraszczyk and Morgenstern (2003), there is no standardized test for measuring the rheological properties of a dough sheet. Further, the instrumentation used in this work, the Alveograph, was originally designed for wheat flours. The Alveograph was not well suited for maize-zein doughs because zein would fall below its $T_{g.}$ As a consequence, the maize-zein dough bubble would harden and not collapse unlike with wheat dough. Notwithstanding this, Alveography revealed a higher curve configuration ratio (P/L) for maize doughs than wheat dough. The curve configuration ratio gives information about the balance between the elastic resistance and extensibility of the dough (Rosell et al., 2001). This means the maize doughs were strong but not as extensible as wheat dough.

5.2. The role of dough sheeting in combination with other treatments for improving maize dough handling and functionality

Generally with wheat doughs, dough sheeting subjects the dough to high mechanical stress which modifies the protein network (Feillet et al., 1977). It further aligns the starch granules and gluten protein fibrils in the direction of sheeting (Kim et al., 2008; Petitot et al., 2009). In the present study, dough sheeting was performed at ambient temperature with a constant roll gap and varying number of sheeting passes to find the optimum dough sheeting passes for maize dough.

Table 4.11 and 4.12 summarise the effects of dough sheeting in combination with pre-gelatinized maize flour and other treatments on maize dough and bread quality. The sheeting of maize flour without pre-gelatinized maize formed a crumbly dough even when the applied force was increased through reducing the roll gap. Pre-gelatinization of part of the maize followed by sheeting produced a cohesive maize dough that became smoother in texture with a high number (40) of sheeting passes. The pre-gelatinization of starch improved dough viscosity and water absorption without the addition of hydrocolloids. It can be said that pre-gelatinization facilitates the development of a cohesive crumb network that can trap gas bubbles and prevent loss of gas and crust collapse during baking (Onyango et al., 2011). The dough sheeting evenly distributed the pre-gelatinized maize starch throughout the maize dough. It can be assumed that a network of gelatinized starch enveloped maize flour particles during the sheeting process. During sheeting, the dough is rotated after each fold. With wheat flours this folding forms layers which tend to become cross-linked and form a network of interconnected protein and starch (Kilborn and Tipples, 1974).

In addition, increasing number of sheeting passes causes an increase in damaged starch (Petitot et al., 2009). In the present study, damaged starch increased with dough sheeting passes for maize dough as well as wheat dough. As damaged starch plays a role in water absorption (Medcalf and Gilles, 1965) it would further improve maize dough functionality.

Zein dough addition to maize dough above zein's T_g produced a viscoelastic maize dough. It appeared that the application of dough sheeting caused an even distribution of zein dough throughout maize dough. It followed the same trend to maize dough, whereby a smoother texture was obtained at 40 sheeting passes. By Alveography, the maize-zein dough composite had improved strength and extensibility when compared to maize dough. CLSM revealed a network of maize flour particles, pre-gelatinized maize flour and zein fibrils for the composite maize-zein dough. With increasing sheeting passes, the zein fibrils were reduced in size (width) which indicated a reduction in elasticity of zein. Similarly, Schober et al. (2008) observed that overextension of the zein dough revealed loss of zein fibrils or strands and a more irregular arrangement of the zein in the dough.

Table 4.11: Summary of the effects of maize dough sheeting prepared with pre-gelatinized maize flour in combination with other treatments on maize dough quality

	Dough sheeting alone	**Dough sheeting plus pre-gelatinized maize flour**	**Dough sheeting plus addition of zein dough at ambient temperatures**	**Dough sheeting plus addition of zein dough above its T_g**	**Dough sheeting plus addition of vital gluten powder at ambient temperature**	**Dough sheeting plus addition of DATEM in maize-zein**
Dough handling properties	Crumbly	Cohesive	Cohesive, undissolved zein	Cohesive, viscoelastic	Cohesive	Cohesive, viscoelastic
Dough extensibility (Alveograph)	Not applicable	Not extensible	Not applicable	Increased with sheeting and decreased	Not extensible	Extensible with development of a tear
Dough strength (Alveograph)	Not applicable	Decreased	Not applicable	Increased with sheeting and decreased	Decreased	Relatively strong
Level of starch damage	Not applicable	Increased with dough sheeting	Not applicable	Not applicable	Not applicable	Not applicable
Microstructure	Not applicable	Network of gelatinized starch enveloped maize flour particles	Not applicable	Zein fibrils around maize flour particles and gelatinized maize aligned in the direction of sheeting	Uniform distribution of maize flour particles and gluten	Not applicable

Table 4.12: Summary of the effects of maize dough sheeting prepared with pre-gelatinized maize flour in combination with the other treatments on maize bread quality

	Proofing time (2 or 4 hours)	**Addition of zein**	**Additional yeast and sugar**	**Addition of vital gluten**	**Addition of sourdough fermented maize flour**	**Addition of DATEM to maize sourdough**	**Combination of addition of zein and sourdough**
Loaf height	Increased/decreased	Slight increase	Slight increase	No significant increase	Increase	Increase	Increase
Crumb texture	Non-uniform	Semi-uniform	Non-uniform	Non-uniform with flying top	Uniform with high addition	Uniform	Uniform
Crumb structural properties	No data	Gas holding potential with larger gas cells than maize flour alone	Open and defined gas cells with starch clumps	Defined gas cells with clumps of starch	Elongated small gas cells	Elongated smaller gas cells than sourdough alone	Elongated gas cells with unincorporated zein

The number of dough sheeting passes influences dough resistance and extensibility (Moss, 1980). In this present study, the optimum number of dough sheeting passes was found to be 15 for maize and maize-zein dough. The sheeting passes formed maize or maize-zein doughs that were strong and extensible. Dough sheeting above 15 sheeting passes, as seen by Alveography and tensile tests, caused a reduction in strength and extensibility of the maize and maize-zein doughs. In contrast with wheat dough, Sutton et al. (2003) observed the peaking of dough rupture stress at 10 sheeting passes before a decline in dough strength occurred. In contrast, Kilborn and Tipples (1974) found optimum dough sheeting to be between 15 and 20 folds.

In bread making, addition of zein dough seemed to reduce the undesirable cracks in the maize bread crust. However, the loaf height was not significantly improved. Possibly, the commercial zein was not sufficiently cohesive for the gas cells to be adequately expanded by the yeast. Similarly, Schober et al. (2008) found that the addition of zein to maize starch was able to hold some gas, but the amount was insufficient to form an acceptable zein-starch bread.

The inclusion of maize sourdough in combination with dough sheeting in maize dough prepared using pre-gelatinization of maize flour produced bread with a much more homogeneous crumb and also improved loaf height. The resultant maize sourdough bread had an appealing appearance and odour compared to maize-zein composite bread. The finding that maize sourdough improves maize bread quality is in agreement with the work of Falade et al. (2014) who worked on maize bread produced using conventional dough mixing. The addition of sourdough is a natural and inexpensive way of improving maize bread with no extra ingredient cost, unlike with addition of zein.

The effects of sourdough derive from the complex metabolic activities of yeast and lactic acid bacteria, such as acidification, production of exopolysaccharides, proteolytic-amylolytic and phytase activity, and production of antimicrobial substances (Moroni et al., 2009). The lactic acid bacteria in sourdough play a vital role in maize dough improvement. Predominantly, the genus *Lactobacillus* has been frequently identified in sourdough (Sterr et al., 2009). In maize sourdough, *Lactobacillus plantarum* was shown to be the dominant organism during fermentation and was chosen as a starter culture by Falade et al. (2014). In this study, *Lactobacillus spp*., *Micrococcus spp*. and *Pediococcus spp*. were presumptively the lactic acid bacteria present (Breed et al., 1948; Breed et al., 1957; Collins et al., 2004) in the spontaneously fermented maize flour based on colony morphology. Furthermore, *Candida spp*. and *Saccharomyces cerevisiae* were presumptively the yeasts present (Collins et al., 2004; Campbell et al., 2013) in the spontaneously fermented maize flour based on colony morphology. Maize sourdough fermentation was found by Falade et al. (2014) to induce softening of maize dough and worked together with yeast to increase the loaf height. It caused modification in starch, which made the dough less elastic but improved the ability of maize to trap carbon dioxide and withstand the pressure of the expanding gas.

Addition of DATEM to maize, maize-zein and maize sourdough doughs resulted in breads with a continuous crumb texture and reduced the tendency of formation of a hole in the crumb of bread. This is in agreement with the findings of Nunes et al. (2009a) who observed a more homogeneous crumb structure with addition of DATEM to rice flour-potato starch composite bread. However, Sciarini et al. (2012) found a lower gas cell number in the crumb of rice flour-cassava starch-soy flour composite bread with DATEM addition. The improvement in wheat bread crumb brought about by DATEM addition has been reported to be through the interaction of DATEM with protein to form a more continuous structure during mixing through hydrophobic and hydrophilic interactions (Krog, 1977).

The best maize bread making combination was maize flour pre-gelatinization, proofing for 2 h at 40^{o}C with dough sheeting at 15 passes, additional sugar (6 %) and yeast (4 %) with 80% of the maize flour in the form of sourdough and DATEM addition. It formed a bread approaching the quality of wheat bread. Although, the maize-zein dough had improved extensibility it incurred an extra cost because zein is an expensive commercial product. However, the research clearly shows that zein can be incorporated with maize flour by sheeting dough mixing to provide a functional viscoelastic protein which improves dough and bread quality.

5.3. Way forward

This study has demonstrated that dough sheeting has potential to improve maize dough bread making properties when applied in combination with the other treatments. Future work should aim to find ways to further improve the texture of the maize bread. This could be achieved by addition of shortening (baking fat) which has shown an increase in machinability or specifically slicability of wheat bread (Mondal and Datta, 2008). Shortening was not added in this present study because it could have masked the effects of DATEM.

The use of other surfactants such as sodium stearoyl lactylate (SSL) could be an alternative. SSL and DATEM are common anionic surfactants used to improve bread texture that act as dough strengtheners (Gomez et al., 2004) and have shown to improve bread crumb and crust texture (Eduardo et al., 2014), softness and increase loaf volume (Nunes et al., 2009a). SSL has shown to have the greatest crumb softening effects at extended proofing times (Gomez et al., 2004). The softening of dough imparted by SSL has been explained by the volume effect and inclusion of more air during mixing so that in consequence smaller gas cells are formed and in turn, a finer crumb is obtained (Crowley et al., 2000).

If zein is to be used, then the use of plasticizers to make zein more extensible may be required. Plasticizers such oleic acid are able to form a more viscoelastic zein at lower T_g (Oom et al., 2008). This may increase zein viscoelasticity during proofing by gas production by yeast. The sensory properties of maize dough with added zein were poor. The bread had a pungent, rancid and unappealing smell with a bright yellow colour caused by the commercial zein. Ultimately, this will be deemed unacceptable to consumers. Focus should be directed towards improving the appearance, smell and taste of the maize-zein bread perhaps by using zein isolated from white maize.

Starches from different botanical origins could be used to improve the quality of maize bread. Cassava starch has shown some potential to improve sorghum bread quality (Onyango et al., 2011). It has been reported that cassava starch produces a stiff elastic mass during gelatinization, which yields a greater cohesion than starches of other cereals and tubers (Onyango et al., 2009). Further, cassava starch can trap gas bubbles in dough due to its gummy and sticky properties (Taylor et al., 2006). The later could help with the formation of flexible gas cells that hold carbon dioxide during proofing and baking thus enhancing bread volume and texture.

An interesting study would be to apply dough sheeting and the related technologies investigated here to other gluten-free cereals such as sorghum and the millets. Then a comparison can be made with maize in order to find the best non-wheat flour to be used in sub-Saharan Africa for bread making in situations of, for example, drought.

6. CONCLUSIONS AND RECOMMENDATIONS

Dough sheeting of maize flour without pre-gelatinization produces a crumbly maize dough. However, when pre-gelatinization of maize flour is applied in combination with dough sheeting, a cohesive dough is produced. This means that pre-gelatinization of flour is a pre-requisite into producing a cohesive gluten-free dough in the absence of added hydrocolloids. Further, continued dough sheeting reduces the strength and extensibility of the maize dough. With the sheeting equipment used, the optimal dough sheeting passes for maize flour dough is 15 with a roll gap of 3 mm. This number of sheeting passes produces a cohesive dough that is extensible and strong. During dough sheeting, the dough is folded after rotating. This folding probably forms an alignment of polymers at right angles to each other that are bonded together through non-covalent bonds to the extent that there is a uniform distribution of flour particles and gelatinized starch in the maize dough.

The addition of zein dough and vital gluten powder in maize dough prepared with maize flour pre-gelatinization improves maize dough functionality and dough handling properties. The preparation of zein above its T_g in water and incorporating it with the maize dough improves it even further. It produces a viscoelastic maize-zein composite dough. With dough sheeting there is excellent incorporation of the zein into the maize dough. However, repeated sheeting causes a decrease in maize-zein dough extensibility and strength based on the Alveograph findings. The preparation and mixing above zein's T_g perhaps increases the molecular mobility of the zein causing it to incorporate well with the maize dough in order to form a cohesive viscoelastic dough. The viscoelastic functionality of zein is highly temperature dependent and is lost upon cooling below its T_g as observed with Alveography where the maize-zein dough bubble does not collapse. In bread making, zein seems to reduce the level of undesirable cracks on the crust of the maize bread and improves gas holding capacity of maize-zein bread. It is recommended that focus should be in improving maize-zein bread quality and sensory properties.

The addition of sourdough to maize dough prepared with pre-gelatinized maize flour improves the loaf bread height and texture. However, with increasing amount of sourdough added, undesirable cracks on the crust of the maize sourdough breads appear. This is thought to be due to the sourdough being mostly water based, thus more moisture is lost. DATEM addition produces maize, maize-zein and maize sourdough breads with a far more homogenous crumb. This particularly results in interaction of DATEM with the protein and starch through hydrophobic and hydrophilic interactions to form a homogenous crumb. Further, an improvement in loaf bread height is obtained in the maize, maize-zein and maize sourdough breads. DATEM also reduces the undesirable cracks on the crust of the maize and maize sourdough breads.

Dough sheeting in combination with pre-gelatinized maize flour, addition of sourdough fermented maize flour and DATEM could form a relatively inexpensive and predominately natural way of producing gluten-free breads.

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