

**Improving the quality of non-wheat bread made from maize
using sourdough fermentation**

By

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DECLARATION

I hereby declare that this thesis submitted at the University of Pretoria for the award of PhD degree is my work and has not been submitted by me for a degree at any other University or Institution of Higher Education.

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Abstract

Improving the quality of non-wheat bread made from maize using sourdough fermentation

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Due to the high cost of wheat importation in countries where the climatic conditions do not favour its cultivation, alternative sources of bread baking flour are required. Maize is a suitable alternative because it is by far the most important crop produced in Africa. However, it lacks gluten, the protein that is formed in wheat dough which is responsible for the desirable quality attributes (high loaf volume, soft and open crumb structure) of wheat bread. Therefore the need arises to improve maize bread quality.

The effects of three types of non-wheat bread methods on the quality of maize bread were investigated. The first was a traditional sourdough method used in Lesotho for making steamed bread. This involved addition of spontaneously fermenting sorghum malt sourdough (equivalent to 15% of the total maize flour) and pre-gelatinization of the starch in the maize flour with boiling water. The second was a Food and Agriculture Organization method which involved pre-gelatinization of the starch in 10% of the maize flour by cooking. The third method was a modern gluten-free sourdough method which involved fermenting 75% of the maize flour with a multiple strains starter culture or *Lactobacillus plantarum* plus the natural flora in the maize. The modern sourdough method produced maize bread with a more open crumb structure and a significant increase in loaf volume compared to the other methods. This was probably related to the high percentage of fermented maize flour in the recipe, which was probably sufficient to modify the dough properties satisfactorily enough to impact positively on the maize bread quality. Based on these findings, the modern sourdough method was investigated further.

Maize sourdoughs were prepared (as described) and compared to chemically acidified maize dough. Sourdough maize bread had an approx. 25-26% increase in loaf volume and a more open crumb structure with large gas cells. This showed that the maize bread quality improvement was not due to low pH. Confocal laser scanning microscopy revealed a cohesive dough structure in the sourdoughs. Larger cells and a more uniform crumb structure were also observed in maize breads with maize sourdough. This indicated an improvement in the maize dough properties with sourdough. Differential scanning calorimetry showed that maize sourdough had a slightly lower peak temperature than straight maize dough, an indication of starch modification. Rheological analysis showed that maize sourdough had a shorter relaxation time, an indication that it was less elastic. Strain sweep analysis revealed that maize sourdoughs had the lowest elastic modulus, also indicating a less elastic dough. Temperature sweep analysis showed an initial less elastic dough and a final high tan delta, suggesting that the maize dough could withstand gas expansion pressure during baking without crumbling.

The dominant lactic acid bacteria in the sourdoughs were identified as *L. plantarum*. In the two sourdoughs, the *L. plantarum* present were gram-positive, catalase negative and exhibited proteolytic activities. However, only the *L. plantarum* in the multiple strains starter culture fermented maize sourdough exhibited amylolytic activities. It is proposed that proteolytic activity of the *L. plantarum* degraded the endosperm protein matrix and hydrolysed the proteins soluble in the dough liquid, thereby allowing increased accessibility of water to the starch granules. It is further proposed that the amylolytic activity of the *L. plantarum* slightly hydrolysed the starch granules, increasing water absorption by the starch granules.

It is proposed that improvement in maize bread quality by sourdough fermentation is due to starch modification (increase water accessibility and water absorption by the starch granules due to the proteolytic and amylolytic activities of the dominant lactic acid bacteria in the sourdoughs) which made the dough less elastic. This in-turn improves the ability of the dough to trap and withstand the pressure of the expanding carbon dioxide in the fermenting dough and bread.

DEDICATION

This thesis is dedicated to my heavenly Father, God Almighty, who has always been the source of my help.

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1. INTRODUCTION

Due to the high cost of wheat importation in countries where the climatic conditions (for example tropical and sub-tropical Africa) do not favour its cultivation (reviewed by Goodall *et al.*, 2012) and also the incidence of gluten intolerance and coeliac disease among consumers (reviewed by Erickson *et al.*, 2012), alternative sources of bread baking flour such as maize flour are required. Maize is a suitable alternative because it is the most important cereal in Africa (FAOSTAT, 2012). Replacing wheat flour with maize flour in bread making will reduce costly wheat imports. However, it is important that the non-wheat bread imitates closely the high loaf volume and open crumb structure of wheat bread which are the qualities that make wheat bread acceptable by consumers. Hence the need arises to improve non-wheat bread quality.

The principal functional protein in wheat flour is gluten (Potter & Hotchkiss, 1996). Gluten possesses unique viscoelastic properties which are crucial for the water holding capacity of the dough and the gas retention during fermentation (reviewed by Arendt *et al.*, 2008). Gas retention properties in turn determine loaf volume and crumb structure of the resulting bread (reviewed by Goesaert *et al.*, 2005). The absence of gluten results in major problems for bakers and currently many gluten-free products available in the market are characterised by low loaf volume and compact crumb structure (reviewed by Arendt *et al.*, 2002). Research is ongoing to improve the quality of gluten-free breads. Research areas include: incorporation of additives such as starches, hydrocolloids and gums (reviewed by Gallagher *et al.*, 2004), protein sources, enzymes, sourdough (reviewed by Moroni *et al.*, 2009), and pre-gelatinized starch (Satin, 1988; Onyango *et al.*, 2011). Of all these additives, the use of sourdough presents a natural and inexpensive alternative (Moroni *et al.*, 2009). In line with this, the need in Africa is for non-wheat breads that are inexpensive, hence, the potential of sourdough fermentation.

Sourdough fermentation involves the mixture of flour and water that is fermented by naturally occurring lactic acid bacteria (LAB) and yeasts (Hammes & Gänzle, 1998). LAB activities in sourdough involve acidification of dough resulting from the organic acids (lactic acid and acetic acid) produced by LAB, which in turn lower the pH of the dough (Corsetti *et al.*, 1998). Acidification of the sourdough and the partial acidification of the bread dough have a direct impact on structure forming components like gluten, starch and arabinoxylans (reviewed by Clarke & Arendt, 2005). Since non-wheat breads do not have

gluten, their structure will probably depend to a large extent on the properties of their starches. According to Hammes & Gänzle (1998), acidification exerts positive effects on the structure of starch granules, leading to increased water-binding capacity. Arendt *et al.* (2008) suggested that acidification of gluten-free flour by sourdough fermentation can replace the function of gluten in a way by enhancing the swelling properties of starch, which may be beneficial to the structure and gas retention ability of gluten-free doughs. This will in turn improve the loaf volume and crumb structure of the gluten-free bread. It is proposed that application of sourdough fermentation in maize bread making will produce desirable results such as higher loaf volume and more open crumb structure.

2. LITERATURE REVIEW

In order to be able to effectively address the problem of improving the quality of gluten-free maize bread, it is necessary to review what the problem really is and also what other researchers have done to try to address this issue. This review will focus on cereals used for bread making, history of bread making, principles of bread making, the prolamin proteins of maize, sorghum and wheat and their importance in dough and bread quality, the application of and research into sourdough fermentation in wheat breads and non-wheat breads, and the adverse effects of gluten in gluten-intolerant and people suffering from coeliac disease.

2.1 Cereals

For thousands of years, cereals have played a vital role in human nutrition. They have been grown by man for thousands of years. Cereals belong to the grass family (Poaceae), which is subdivided into several genera. In temperate zones, cereals such as wheat, barley, rye and oats thrive well (Belderok, 2000). However, in areas where water is limited, for example Africa, maize and sorghum are widely cultivated. Maize is the most important cereal crop in subtropical zones, although it also thrives in temperate zones (FAOSTAT, 2012). Wheat is the most important cereal in breadmaking due to the ability of its gluten protein to form strong viscoelastic dough when hydrated (Goesaert *et al.*, 2005). In many parts of the world, maize, sorghum and pearl millet are used to make dough-type products, such as the *kisra* of Sudan, *injera* of Ethiopia, *tortilla* of Central and South America and the *roti* or *chapatti* of India. However, the dough produced is quite different from wheat flour dough and the major cohesive force appears to be that created by the surface tension of water rather than by the cereal proteins (Hoseney, 1994).

2.2 Bread making

Cooking of cereals preceded their use for bread making. Porridge was probably the first food into which cereals were made into (Belderok, 2000). This technique of preparation is still practiced by many cultural groups, especially in Africa. These practices were later succeeded by flat, unleavened cakes. These primitive forms of bread still persist, and are sometimes linked to ritual significance. People from Sumeria, in the southern part of Mesopotamia, were the first to bake leavened bread. About 6000 years B.C., they started to mix sourdough with unleavened dough. Sourdough is generated during the natural yeasting

process of flour and water, during which carbon dioxide is formed, which in turn causes the dough to rise. The Sumerians transmitted their way of preparing bread to the Egyptians some 3000 years B.C. The Egyptians perfected the system and started to use yeast generated from brewing beer. Moreover, they developed a baking oven which made it possible to bake several loaves at once. The production of wheat loaf bread was achieved successively by the Egyptians, the Greeks and the Romans and was considered by them to be a sign of a high degree of civilization. Bread also represented a substantial part of the daily food in ancient Athens, as well as later in Rome and other Italian towns.

Baked products are one of the most consumed foods in the World (Cho & Peterson, 2010). In fact, about 9 billion kg are being produced annually (Heenan *et al.*, 2008). In wheat breadmaking, the main ingredients include wheat flour, water, salt, yeast with or without fat and sugar. The requirements may differ based on the type of wheat bread. The breadmaking process involves various steps. Cauvain (1998) described these as follows:

- mixing of ingredients together to form a dough
- kneading of the dough to enable the development of the gluten structure and incorporating of air bubbles in the dough
- dividing and shaping the dough
- first proofing to allow modification of the dough
- final shaping to desired shape and size
- final proofing (fermenting and expanding) the dough
- baking

2.2.1 Principles of bread making

Bread making basically involves mixing of the required ingredients (flour, water, yeast and other functional ingredients), expansion of the dough through the production of carbon dioxide from yeast fermentation, and finally baking (Cauvain, 2012). During bread making, complex biochemical and physical transformations occur in the bread dough. These transformations are to a large extent affected by various flour constituents such as the protein and starch components of the flour (Goesaert *et al.*, 2005). The gluten proteins when hydrated are transformed into a continuous cohesive visco-elastic network (Singh & MacRitchie, 2001). This allows the formation of a continuous protein matrix which holds starch and other components (Campos *et al.*, 1997).

According to Goesaert *et al.* (2005), bread making quality of wheat flour largely depends on gluten. Gluten plays a crucial role in determining the gas retention capacity of dough (Gan *et al.*, 1995). Gas retention properties in turn determine loaf volume (Goesaert *et al.*, 2005) and also influences the formation of a crumb structure which after baking confers on the bread the desired qualities quite different from other baked products (Cauvain, 2012). This desirable bread quality depends greatly on the formation of a suitable gluten network. This is essential to trap CO₂ released during yeast fermentation in the bread dough and during the initial stages of baking (Goesaert *et al.*, 2005).

Gluten proteins undergo various changes during the different steps involved in bread making. The nature of these changes, like the native gluten protein structure itself, is still undergoing further research (Goesaert *et al.*, 2005). An important stage in breadmaking is mixing. Mixing is the process that facilitates the formation of a gluten network and air infusion into the dough. The gluten network formed traps and retains the air bubbles for inflation by CO₂ released during yeast fermentation or from lactic acid bacteria during sourdough fermentation (Cauvain, 2012). The gluten network plays a major role in CO₂ retention during fermentation and during the initial stages of baking (Goesaert *et al.*, 2005).

The fermenting dough exists as a dispersion of discrete gas cells in a semi-solid dough phase consisting of starch, gluten and other minor components (Gan *et al.*, 1995). During proofing, a small portion of starch is hydrolyzed into dextrans and sugars by the action of enzymes. Yeast ferments the sugars to produce CO₂ and ethanol (Cauvain, 2012). The carbon dioxide produced goes into solution in the aqueous phase within the dough. It then diffuses through the aqueous phase to the gas bubbles trapped in the gluten network where it evaporates to generate within them an excess pressure that provides the driving force for dough expansion. The CO₂ cannot diffuse out of the gas cells because the aqueous phase surrounding them is saturated. The saturation of the aqueous phase is maintained because the yeast continues to produce more CO₂ as fermentation progresses. The survival of intact CO₂ gas cells and their expansion is of great importance because dough structural stability is maintained by the expansion of the trapped gases. A small proportion of the gas does diffuse to the surface of the dough piece and evaporates into the surrounding atmosphere giving a slow release of gas from the dough (Gan *et al.*, 1995).

In the early stages of baking, yeast activity is at its greatest and large quantities of CO₂ are produced and released from solution in the aqueous phase of the dough. Yeast activity starts to decrease around 43°C and finally ceases at 55°C. The bread dough will only continue to expand if the dough is able to retain a large amount of the total CO₂ gas produced. This is possible only if a suitable dough structure with the appropriate physical properties had been created during dough mixing (Cauvain, 2012). Gluten in wheat flour dough contributes to gas retention by slowing the diffusion of gas through the dough phase (Hoseney & Rogers, 1990). Gelatinization of the starch starts at about 60°C. The starch granules absorb any free water in the dough. Alpha-amylase hydrolyzes a small portion of starch into dextrans and then sugars and attains its maximum activity between 60°C and 70°C (Cauvain, 2012). As a result of these heat-induced changes, the typical sponge structure of baked bread is formed (Goesaert *et al.*, 2005). Under normal baking conditions, the initial loss of CO₂ is slow. Towards the end of oven spring (dough rapid expansion), the rate of CO₂ loss increases. The slow initial loss of CO₂ can be explained by its diffusion to the external surface of the dough followed by evaporation, while the rapid loss have been attributed to the rupture of the starch–protein matrix surrounding the gas cells (Gan *et al.*, 1995). However, Hoseney & Rogers (1990), attributed the rapid loss of CO₂ to an increase in the rate of diffusion through the dough aqueous phase. Rupture of the starch-protein matrix is due to the sharp increase in dough viscosity due to starch gelatinisation (Gan *et al.*, 1995). The rupture of the matrix results in the interconnection of adjacent gas cells. This enables the conversion of the foam structure of dough into an open sponge which enables the direct escape of the CO₂ gas.

The production of wheat-free breads differs significantly to that of standard wheat breads. Most wheat-free doughs tend to contain higher water levels and have a more fluid-like structure (reviewed by Arendt *et al.*, 2008). Also as stated, wheat-free breads do not possess gluten, which plays an important role in gas retention and in turn positively influences dough leavening and the final loaf volume of bread. Therefore, the principles of bread making as discussed cannot be applied to wheat-free breads. In order to understand why gluten is unique in bread making, its structure and functionality will be discussed. Also, the inability of the prolamin storage proteins of maize and sorghum, zein and kafirin respectively, to function like wheat gluten will be discussed with respect to their composition and structure.

2.3 Prolamin storage proteins of wheat and non-wheat cereals

As stated, the distinctive feature that makes wheat unique is the viscoelastic properties of its gluten. The proteins of cereal grains with the exception of wheat do not have dough forming properties to any extent (Hoseney, 1994). Rye and triticale probably come closer than other cereals, but still their doughs are not as strong as that of wheat doughs.

2.3.1 Gluten structure and functionality

Gluten is the rubbery mass that remains when wheat dough is washed to remove starch granules and water soluble constituents. Glutenins and gliadins are the main components of gluten. Goesaert *et al.* (2005) reviewed that glutenins have molecular weights (MW) varying between 80,000 to several millions while MW of gliadin ranges between 30,000 and 80,000. Both gluten fractions play a part in the rheological properties of dough. Gliadins contribute to dough extensibility and viscosity, while glutenins are responsible for the elasticity and strength of the dough (reviewed by Wieser, 2007). The author associated dough strength and elasticity to the development of gluten's polymeric matrix, comprising of high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits linked through intermolecular disulphide bonds. The author further explained that disulphide cross-linkages formed between cysteine residues in the B-domain of γ -type HMW glutenin sub-unit and the C-terminal domain of LMW glutenin subunits have been linked to increased elasticity and are thought to function as 'chain extenders' in the development of glutenin's polymeric network. Shewry & Tatham (1997) explained that non-covalent forces (hydrogen bonding and Van der Waal's forces) are believed to be largely responsible for the viscous nature of gliadin.

According to Weiser (2007), the uniqueness of gluten proteins can be primarily attributed to their amino acid compositions, which are characterized by high contents of glutamine and proline and by low contents of amino acids with charged side groups. Wellner *et al.* (2005) also attributed gluten's unique functionality to its amino acid composition and added that its polymer mobility changes in secondary structure on mixing, and disulphide-sulphydryl interchange occurring between cysteine/cystine groups also play a role. Further, the number and pattern of disulphide cross-links in glutenin polymers has an effect on the strength of the dough (Shewry *et al.*, 2002). Coupling gluten's low charge density with hydrogen bonding and hydrophobic interactions enables close association between gluten polymers and provides a stabilizing force during dough development (Hoseney, 1994).

Belton (2005) further attributed stabilization of wheat dough to β -sheet and disulphide bridge properties of gluten during dough mixing and proofing.

In addition to these properties of gluten, the amount of plasticisers (such as water or oil) and also the temperature at which mixing is done also contributes to the unique quality of the dough. Amorphous proteins such as zein, kafirin, gluten exhibit a change in their physical state from a glassy to a rubbery state when heated above their glass transition temperature (T_g) (Levine & Slade, 1989). Gluten with 16% or higher moisture content has a T_g below room temperature (Hoseney, 1994). Popineau *et al.* (1994) and Belton *et al.* (1995) reported the results of Nuclear Magnetic Resonance (NMR) and Fourier Transform Infrared (FT-IR) spectroscopy analyses as showing that β -sheet structure of HMW glutenin subunits increases when the proteins are in a mixed doughy hydrated state. It is thought that HMW subunits of glutenin initially present in a loop conformation, are extended during gluten fibril formation and form polymeric alignments in which high proportions of β -sheet structures are favoured at the expense of β -turns. For this reason, such polymers are believed to have a high resistance to extension (reviewed by Mejia *et al.*, 2012).

2.3.2 Zein and kafirin composition and structure

The prolamins storage proteins of maize and sorghum, zein and kafirin, respectively, do not exhibit the unique property of wheat gluten. Their functional role in bread making is in part limited by their encapsulation in rigid protein bodies that do not break apart with dough mixing, thus making them inaccessible for any possible functional role (reviewed by Goodall *et al.*, 2012).

Sorghum kafirin resembles maize zein in amino acid composition but they both differ from wheat gluten in amino acid composition (Hoseney, 1994). Both zein and kafirin are encapsulated in protein bodies within the endosperm and have similar chemical composition and properties. Maize zein constituents are grouped under four different subgroups due to their size, amino acid composition and solubility: α - (19 and 22 kDa, 75-85% of total protein), β - (14 and 16 kDa, 10-15%), γ - (28 kDa, 5-10%), and δ - (10 kDa, trace amounts) (Shewry & Tatham, 1990). Alpha-zein is water insoluble. This is due to high proportions of hydrophobic amino acids such as leucine (18-20%), alanine (14%), proline (9-11%), and phenylalanine (4-6%) (Erickson *et al.*, 2012). Also according to these authors, α -zein readily solubilizes in a variety of binary aqueous-alcohol solvents. This has facilitated a great deal of α -zein's structural and behavioural characterization. Internal

structure of the protein body shows the main α -kafirin/zein sub-units at its interior, while β - and γ -kafirins/zein are mainly found at the periphery. Hamaker *et al.* (1987) identified one area in which kafirin and zein differ, their cross-linking behaviour, which specifically impacts on their digestibility. Kafirin is less digestible than zein due to high level of disulphide crosslinking and the arrangement of its subunits in the protein body. A higher proportion of helical conformations and a greater number of hydrophobic residues in kafirin than zein has been reported (reviewed by Belton *et al.*, 2006) as another area in which they differ. Importantly, though kafirin is more hydrophobic than zein, they are both more difficult to hydrate compared to gluten. This was suggested to be related to their composition of mainly α -helical structure, in contrast to gluten which consists of high level of β -sheet and β -turn structure (reviewed by Belton, 1999). Based on the review by Erickson *et al.* (2012), the difference in the structure of gluten and zein/kafirin as stated is believed to be a major reason for the failure of the latter to form an elastic dough under normal condition.

2.3.3 Scientific improvements of non-wheat cereal protein functionality bread making

Various researches have been conducted and many are still going on to devise the most suitable process of modifying or combining non-wheat cereal proteins in a way that will enable them exhibit viscoelastic properties required to produce baked products similar to wheat products.

Schober *et al.* (2008) successfully modified a zein-starch dough for bread making by including the surface-active hydrocolloid hydroxypropyl methylcellulose (HPMC). These authors, while trying to improve the functionality of zein, included 2% of HPMC into the zein-starch dough systems, and obtained improved leavening characteristics due to enhanced gas cell stabilization through the amphiphilic hydrocolloid. In a subsequent study, Schober *et al.* (2010) further improved HPMC containing zein-starch dough by defatting the surface of the zein particles, which facilitated their aggregation and promoted formation of gluten-like strands and formed a stronger dough. Schober *et al.* (2011) studied the impact of different isolation procedures on the functionality of zein and kafirin. Their results suggested that hydrophobic interactions rather than disulphide bonds are the key to gluten-like functionality of zein and kafirin. Bugusu *et al.* (2001) worked on the improvement of sorghum-wheat composite rheological properties and bread making quality through zein addition. They reported that the addition of protein body-free α -zein

(< 25% of total flour weight) at 35°C improved the rheological and leavening characteristics of a wheat-sorghum composite (80:20) flour dough and bread. They suggested that zein, and probably kafirin if freed from the confines of their protein bodies, could improve the poor functionality of wheat-sorghum composite flour dough. Goodall *et al.* (2012) worked on determining whether protein body-free kafirins in high digestibility high lysine (HDHL) sorghum flour can participate as viscoelastic proteins in sorghum-wheat composite dough and bread. Their results showed that kafirin in HDHL sorghum flour can contribute to the formation of an improved protein network with viscoelastic properties that could lead to better quality composite doughs and breads.

MacRitchie (1980) and Bushuk & MacRitchie (1989) first noted zein's ability to form a viscoelastic dough similar to gluten when mixed at temperatures exceeding 60°C. Lawton (1992) showed this functional change as being initiated by mixing temperatures exceeding zein's glass transition temperature (T_g) at sufficient moisture contents similar to the development of viscoelasticity in wheat gluten systems. Below the T_g , amorphous polymers exhibit brittleness and limited mobility (Hoseney, 1994). However, exceeding the T_g through an increase in temperature and/or by plasticizer addition renders the polymer significantly more mobile and consequently more reactive. Madeka & Kokini, (1996) reported that an increase in zein's reactivity above its T_g enhances the proteins propensity for aggregation and cross-linking, thereby allowing for the development of extensive fibrous protein network similar to wheat doughs, as observed by scanning electron microscopy. According to Mejia *et al.* (2007), developing of these aggregate networks conveys viscoelastic functionality to the dough, coinciding with a build-up of β -sheet secondary structures similar to wheat gluten system. Bugusu *et al.* (2001), Lawton (1992), Oom *et al.* (2008) and Schober *et al.* (2008) all found that isolated (protein-body free) zein and kafirin can be mobilized at temperatures above their T_g and have been shown to participate in viscoelastic dough development. Mejia *et al.* (2012) worked on increasing and stabilizing β -sheet structure of zein. Their investigation showed that the addition of a small amount of co-protein to zein produces extended and stable network of β -sheet structures that, at least in the case of the tested high molecular weight glutenin, conferred it similar viscoelastic properties to wheat gluten polymers. These authors stated that this was directly due to conformational change in the structure of zein, because the amount of increase in β -sheet observed is more than that which can be expected from the small amount of co-protein added. Thus, stable β -sheets in the polymer appear directly

related to their viscoelastic properties and relaxation rate. Sly *et al.* (2014) found that chemical acidification of zein at 40°C with lactic acid and acetic acid, as produced during sourdough fermentation, formed dough having viscoelastic property similar to wheat gluten. According to these authors, the acidic condition to an extent reverses the change from mainly α -helical to more β -sheet conformation, which occurs when zein is made into a dough. They also found increase in α -helical conformation. This they attributed to possibly be due to deamination of the zein molecules, which in turn enables the formation of a more uniform dough structure with linear orientation of fibrils. This change in structure immensely improved zein dough properties in terms of extensibility while retaining cohesiveness.

2.4 Sourdough fermentation

As stated, sourdough is a mixture of flour and water that is fermented by naturally occurring lactic acid bacteria and yeasts (Gobbetti *et al.*, 2008). Spontaneous sourdough fermentation is one of the oldest cereal fermentations known to mankind. It is a traditional process for improving bread quality. Its use was to a large extent replaced by yeast fermentation in bread making. However, its use is again becoming popular in bread making (Poutanen *et al.*, 2009). The unique properties of sourdough are due to its microflora which is mainly represented by lactic acid bacteria (LAB) and yeasts (reviewed by Chavan & Chavan, 2011). During the sourdough fermentation, biochemical changes occur in the starch and protein components of the flour due to the action of endogenous microorganisms and enzymes present in the flour (reviewed by Chavan & Chavan, 2011).

2.4.1 Types of sourdough fermentation

Depending on the process applied, sourdoughs have been grouped into 3 types (reviewed by Chavan & Chavan, 2011):

Type I: sourdough that is prepared by using a part of the sourdough from a previous fermentation process (backslopping). This type of sourdough is referred to as traditional sourdough.

Type II: this is an industrial type of sourdough. It involves the use of adapted strains to start fermentation. This sourdough can be liquid, so it is easily pumpable in an industrial bakery.

Type III: this type of sourdough can be dried. It is often used by industrial bakeries since the quality is constant. The use of this type of sourdough prevents variations in the quality of the end product. Variations are usually common when a freshly prepared sourdough is used. This type of sourdough is the most convenient to work with in the present day high-tech bakery industries. Different drying techniques are employed as well as liquid pasteurization, to achieve microbial stability. Spray-drying and drum-drying are the most commonly used drying techniques.

The doughs of Types II and III require the addition of baker's yeast (*Saccharomyces cerevisiae*) as leavening agent, whereas baker's yeast is not required in Type I sourdoughs (reviewed by Chavan & Chavan, 2011).

2.4.2 Sourdough microorganisms

As stated, the technological performance of the bread dough and the nutritional properties, aroma profile, shelf-life and overall quality of the resulting bread are greatly affected by the metabolic activity of the sourdough microorganisms. Also the metabolic activities of these sourdough microorganisms are influenced by flour characteristics and process parameters. Sourdough is characterized by a complex microbial ecosystem, mainly represented by lactic acid bacteria and yeasts (Corsetti & Settanni, 2007). The yeasts are mainly responsible for the production of CO₂. These authors concluded that because the yeast is the major producer of CO₂ in sourdough, it is considered responsible for dough leavening. Lactic acid bacteria are mainly responsible for the production of lactic acid and acetic acid. These authors explained that acetic acid is responsible for a hardening of gluten, whereas lactic acid can gradually account for more elastic gluten. As a consequence, the texture and aroma profile of the bread are also affected. Lactic acid bacteria cause acidification of the dough, proteolysis of protein and moderate hydrolysis of starch (Corsetti *et al.*, 1998). The pH of ripe sourdough varies with the nature of the fermentation process and starter culture used, however, the pH of wheat sourdoughs ranges from 3.5 to 4.3 (reviewed by Chavan & Chavan, 2011). Corsetti *et al.* (1998) explained that wheat sourdough lactic acid bacteria ferment maltose, the most abundant sugar in the flour, and produce lactic acid when expressing homofermentative metabolism. In the case of heterofermentative metabolism, they produce CO₂, acetic acid and ethanol in addition to lactic acid. Gobbetti *et al.* (1994) and Ottogalli *et al.* (1996) stated that the number and quality of sourdough microorganisms, both yeast and lactic acid bacteria depend on several

factors such as the type of raw materials, the amount of water (dough yield), the fermentation temperatures, the environment and the refreshment practices such as backslopping, which involves addition of flour and water to the sourdough so as to keep the lactic acid bacteria and yeast alive. For spontaneous sourdough fermentation, lactic acid bacteria to yeast ratio is approximately 100:1 (Gobbetti *et al.*, 1994). Although a large variety of lactic acid bacteria has been isolated from wheat and rye sourdoughs, only a few *Lactobacilli* species are highly adapted to the sourdough environment and usually dominate industrial and artisan fermentations (reviewed by Gänzle *et al.*, 2008). Obligately homofermentative and facultatively or obligately heterofermentative lactobacilli, are the typical sourdough LAB. *Lactobacillus sanfranciscensis*, *L. plantarum* and *L. brevis* are the most frequently isolated *Lactobacilli*. Several species of yeasts are also found in wheat and rye sourdoughs; *S. cerevisiae* is frequently present or is added. According to Vogel (1997), the quantity of *S. cerevisiae* may be overestimated due to unreliable systems for identifying and classifying yeasts from this habitat. In particular *S. exiguous*, *Candida krusei*, *Pichia norvegensis* and *Hansenula anomala* are yeasts associated with LAB in sourdoughs (reviewed by Gobbetti, 1998). Sanni *et al.* (1997) identified the LAB in maize sourdough to be *L. brevis*, *L. casei*, *L. fermentum*, *Pediococcus acidilacti*, *P. pentosaceus*.

2.4.3 Sourdough fermentation and wheat bread quality

The rate and extent of biochemical changes occurring in the flour greatly influence the properties of the sourdough and ultimately the quality of the final baked product. The flour type, temperature, water content, type and amount of added yeast and bacteria all affect the acidification of the dough and bread quality during sourdough fermentation (Rouzaud & Martinez-Anaya, 1997). Whole meal wheat flour has a high extraction rate therefore nutrients such as B-vitamins and minerals increase as does the buffering capacity of the flour, primarily because of phytic acid from the aleurone layer (reviewed by Hansen & Hansen, 1994). These factors can stimulate the growth and biochemical activity of the microflora in the sourdough followed by a higher production of lactic acids. These authors work confirmed this by showing that sourdoughs made from whole meal wheat flour produced a higher amount of lactic acid than more refined flour (Hansen & Hansen, 1994).

According to Clarke & Arendt (2005), acidification of sourdough and the partial acidification of the final bread dough will have a direct impact on structure forming components like gluten, starch and arabinoxylans. Clarke *et al.* (2004) reported less elastic

and softer wheat dough when sourdough was added to the final bread dough. Acidification due to growth of LAB has an effect on the gluten network of wheat bread dough (Arendt *et al.*, 2007). According to Schober *et al.* (2003), at pH below 4 there is a sizable positive net charge and the increased electrostatic repulsion enhances protein solubility and effectively prevents the formation of new bonds. This results in a weakening of the structure and thus a softening effect. Takeda *et al.* (2001) reported increased solubility of the constituent gluten proteins at acidic pH values. On the contrary, Ryan *et al.* (2006) reported an increase in wheat dough elasticity due to sourdough fermentation of dough. According to Wehrle *et al.* (1997), the addition of acids leads to dough with lower phase angle values and thus more elastic behaviour under optimal mixing conditions. For all viscoelastic materials, the phase angle is between 0 and 90 degrees. The lower the values the more elastic the material (reviewed by Clarke & Arendt, 2005). In addition to the direct impact of decreasing pH values on dough characteristics, secondary effects of acidification and fermentation time may include changes in the activity of wheat cereal or bacterial enzymes associated with changes in the pH of the environment during the fermentation period (Clarke & Arendt, 2005).

Proteolysis during sourdough fermentation is highly dependent on formation of acids. Lactic acid bacteria contribute to overall proteolysis during sourdough fermentation by creating optimum conditions for activity of cereal proteinases (Katina, 2005). Cereal proteinases have been shown to be active at pH 3.7, but show no activity at pH 5.5 (Katina, 2005). Thiele *et al.* (2003) used fluorescence labelling of wheat protein fractions to determine the degree of gluten hydrolysis and depolymerisation during sourdough fermentation. This study showed that microbial fermentation affected the size distribution of the peptides resulting from proteolytic degradation of wheat proteins. The presence of lactobacilli promoted a decrease in the concentration of larger peptides and an increase in that of smaller molecules such as dipeptides and amino acids. It was concluded that dough pH and wheat cereal enzyme activity were mainly responsible for proteolytic degradation of gluten proteins and depolymerisation of the gluten macropolymer observed during sourdough fermentation. Enhanced proteolysis of dough due to proteolytic enzymes in sourdough microorganism or the proteolytic activities of cereal enzymes under the conditions of sourdough fermentation is the major factor leading to the formation of amino acids, the precursors of aromatic substances in sourdough bread (Thiele *et al.*, 2002). In order to generate a sufficient amount of volatile compounds during fermentation, a

multiple-step process of about 12 to 24 hours is required while fermentation with baker's yeast alone is finished within a few hours (reviewed by Chavan & Chavan, 2011). This may result in the weaker flavour of bread fermented with yeast. Therefore, generation of volatiles in sourdoughs is clearly influenced by the activity of the LAB and the sourdough yeast (reviewed by Chavan & Chavan, 2011). The weaker flavour of yeasted bread may also be due to the fact that yeast utilizes a high amount of amino acids for its metabolism. Brummer & Lorenz (1991) reported a richer and more aromatic flavour in sourdough wheat bread compared to wheat bread without sourdough. Thiele *et al.* (2002) stated that bread aroma is significantly enhanced by higher levels of free amino acids in the crumb and especially in the crust. These authors pointed out that wheat sourdough fermentation produces higher levels of amino acids in the dough, while yeast fermentation actually reduces the level of amino acids. Their work determined the amino acid content of dough, and found that ornithine, methionine, phenylalanine, leucine, isoleucine and valine were especially important to wheat bread flavour. Schieberle (1996) identified 14 intense aroma compounds in wheat sourdough bread and attributed the most characteristic aroma of sourdough wheat bread crust to the compound 2-acetylpyrroline. This author observed that a limited extent of proteolysis during sourdough fermentation beneficially improves the bread flavour without adverse effects on texture and volume. It was concluded that the most important factors governing the levels of amino acids in wheat dough are dough pH, fermentation time and the consumption of amino acids by the fermentative microbiota.

Loaf volume is a very important quality parameter of bread. This is dependent on various factors. As stated, the amount of carbon dioxide produced during dough fermentation and the structure of the gluten network is of utmost importance. In the case of sourdough breads, CO₂ is produced by both heterofermentative LAB and yeast and the contribution of each group to the overall gas volume differs with the type of starter culture and the dough technology applied (Hammes & Ganzle, 1998). Clarke *et al.* (2002) suggested that improved volume and shelf-life of sourdough breads is dependent on the nature and intensity of the acidification process. Corsetti *et al.* (2000), Clarke *et al.* (2003) and Gobbetti *et al.* (2005) reported a positive effect of sourdough on bread volume. This was attributed by Gobbetti *et al.* (2005) to a better gas holding capacity of gluten in acidic dough containing sourdough and faster yeast fermentation in the presence of LAB. Corsetti *et al.* (2000) attributed it to solubilisation of pentosans during the sourdough process,

while Clarke *et al.* (2003) attributed it to altered activities of endogenous enzymes due to utilization of sourdough and subsequent low pH.

Improved softness of wheat sourdough breads during storage requires controlled acidity levels. A positive influence is obtained only in moderate acidity (reviewed by Katina *et al.*, 2006). According to Rocken (1996), the use of sourdough produces bread or baked goods indicated to have improved rheology and storage characteristics over products obtained using baker's yeast. Barber *et al.* (1992) using differential scanning calorimetry reported lowest rates of staling during storage for breads produced by spontaneous sourdoughs with low pH and high lactic and acetic acid ratio. Sourdough LAB have also been shown to inhibit or slow down microbial spoilage, therefore improving bread shelf-life (Spicher, 1983). The control and inhibition of spoilage organisms in sourdough during fermentation is primarily due to low pH values (Salovaara, 1998). Positive effects of the use of sourdough on the mould-free shelf-life of wheat bread have also been reported by Lavermicocca *et al.* (2000) through the production of antimicrobial compounds by the sourdough organisms.

Sourdough fermentation has also been reported to have some effect on the nutritional quality of sourdough bread. Lopez *et al.* (2003) found an improvement in the nutritional quality of sourdough baked goods with regard to mineral availability. Phytate is present in all grains and forms insoluble complexes with the minerals in flour, consequently reducing their bioavailability, so excessive amounts of it in the diet can have a negative effect. The low pH values associated with chemically or microbiologically acidified wheat doughs leads to solubilisation of the phytate complex thus increasing mineral bioavailability (reviewed by Clarke & Arendt, 2005). According to Östman *et al.* (2002), the presence of lactic acid during heat treatment promotes interactions between starch and gluten reducing starch bioavailability and consequently the glycaemic index of baked goods. Gobbetti *et al.* (2008) reviewed that organic acids such as those produced during sourdough fermentation have been shown to play a role in the post-prandial glycaemic responses.

2.4.4 Sourdough and non-wheat bread quality

Improving the quality of non-wheat bread is a difficult task due to the absence of gluten. Various types of additives have been included in non-wheat bread recipes to improve its quality (reviewed by Moroni *et al.*, 2009). However, the use of sourdough in non-wheat breadmaking is gradually becoming popular (reviewed by Houben *et al.*, 2010).

Moroni *et al.* (2010) isolated lactic acid bacteria and yeast from teff and buckwheat sourdoughs. Their findings indicated that both flours represent an important reservoir for the isolation of novel and competitive microbial starters for the production of gluten-free sourdough bread. Moroni *et al.* (2011) investigated the impact of sourdough on the biochemical, rheological and textural properties of buckwheat flour, batter and bread. They reported that the addition of sourdough inhibited the production of carbon dioxide by the baker's yeasts during proofing, resulting in lower volume and harder crumb of the sourdough bread. These authors also reported that acidification alone induced hardening of the starch gel, which was responsible for lower volume and irregular crumb grain in buckwheat bread. Coda *et al.* (2011) compared acha (white fonio) and iburu (black fonio) sourdough bread fermented with previously selected autochthonous starters from *Pediococcus pentosaceus* and *L. curvatus*, *P. pentosaceus* and *L. plantarum* previously isolated from acha (*Digitaria exilis*) flour and iburu (*Digitaria iburua*) flour, respectively to wheat sourdough bread started with the same strain and also to breads made with the same formula but using baker's yeast alone. They found that during acha and iburu sourdough fermentation, starter lactic acid bacteria reached almost the same cell density found in wheat sourdoughs. Iburu sourdough bread had the highest total titratable acidity, the lowest pH, and contained the highest levels of free amino acids and phytase activity. Though acha and iburu sourdough breads showed lower specific volume and higher density with respect to wheat sourdough breads, they were preferred for hardness and resilience. Sanni *et al.* (1997) investigated the production of sour maize bread using starter-cultures previously isolated from fermenting maize meal, rye sourdough and fermented maize gruel (ogi). They found that all the bread samples irrespective of the sourdough addition had cracks and were relatively hard. However, an improvement in the shelf-life of the sourdough bread was obtained. Edema (2011) reported that sour maize bread fermented with a mixed culture of *L. plantarum*, *L. brevis*, *Leuconostoc mesenteroides* had the best physical and sensory properties compared to maize bread produced with other test starters. The author recommended the use of these mixed cultures for maize sourdough bread production for good rheological properties, proper acidification and acceptable flavour development. Edema *et al.* (2013) worked on improvement of fonio dough properties through sourdough fermentation. They found that sourdough fermentation substantially improved the dough consistency making it more similar to bread made from wheat flour, as measured by the Mixolab instrument. Sourdough fermentation also increased pasting viscosity, an indication of effects on starch. Scanning electron

microscopy indicated that sourdough fermentation caused some slight swelling and starch leaching from the fonio starch granules. This was confirmed by an increase in damaged starch. It also caused a substantial reduction in starch gel firmness. These authors concluded that sourdough fermentation improves fonio dough and bread quality by bringing about slight changes in the starch granules, which probably increased water absorption and hence improve dough strength and gas holding capacity of the dough. Houben *et al.* (2010) worked on the modification of the rheological behaviour of amaranth and found that acidification of the amaranth dough using lactic acid addition showed a significant positive influence on the rheological parameters of amaranth dough only at the higher stress level. In contrast, sourdough fermentation using *L. plantarum* produced doughs with similar viscoelasticity to that of pure wheat flours. Novotni *et al.* (2012) concluded that the combined application of sourdough and partially baked frozen technology can decrease Glycaemic Index, improve quality and shelf-life of gluten-free bread. Such breads can be recommended as a part of well-balanced gluten-free diet. Galle *et al.* (2012) reported that exopolysaccharides formed *in situ* during sourdough fermentation can be successfully applied in gluten-free sorghum flours to improve their bread making potentials. Schober *et al.* (2007) worked on improving the quality of gluten-free sorghum bread by sourdough fermentation. They found that the addition of 2% HPMC improved a bread based on 105% water, 70% sorghum flour, and 30% potato starch. Nevertheless, a flat top and tendency towards a hole in the crumb remained. These problems were eliminated by sourdough fermentation of the total sorghum flour. Sourdough fermentation caused a stable crumb structure of the breads, in that it prevented the formation of a hole in the crumb and increased loaf height. This effect was explained to be due to the degradation of proteins soluble in the dough liquid during the sourdough fermentation. If these proteins were not degraded, they would aggregate upon baking and interfere with the formation of a starch gel. As a consequence, bread without sourdough fermentation tended to have a large hole in the crumb centre. These authors also reported that size-exclusion HPLC revealed that during sourdough fermentation, proteins from the dough liquid were degraded to peptides smaller than the kafirin monomers (< 19 kDa). Confocal laser scanning microscopy showed aggregated protein and protein bodies in their matrix in bread crumb without sourdough fermentation. In contrast with sourdough fermentation, only small isolated patches of protein bodies embedded in matrix protein remained. They also reported that in oscillatory temperature sweeps, sourdough fermentation caused a significant higher resistance to deformation (G^*) after gelatinization

of the above batter relative to batters without sourdough. Their findings suggested that a strong starch gel, without interference of aggregated protein is desirable for the gluten-free sorghum bread.

2.5 Coeliac Disease (CD)

Production of wheat-free breads with improved quality is also important to gluten-intolerant people and coeliac disease patients (reviewed by Houben *et al.*, 2012). This will enable them to still be able to consume bread, having similar satisfaction as derived from wheat breads, but without its negative effects on their health.

Coeliac disease (CD) is a common heritable chronic autoimmune inflammatory condition of the small intestine caused by permanent intolerance to wheat gluten/gliadin (prolamin) and similar proteins of triticeae cereals, such as wheat, rye, barley and also possibly oats (Van Heel & West, 2006). The wheat gliadins are toxic to persons with CD (Kagnoff *et al.*, 1982). Van de Wal *et al.* (1999) and Dewar *et al.* (2006) have also demonstrated the same for wheat glutenins. During digestion of gluten, prolamin-derived polypeptides rich in proline and glutamine are liberated and elicit T-cell mediated immune responses in coeliac patients (Shan *et al.*, 2002). There is an interplay between innate and adaptive immune responses to ingested gluten in CD (Koning *et al.*, 2005; Jabri *et al.*, 2005). According to Ferretti *et al.* (2012), it has been demonstrated that some gliadin peptides resistant to complete proteolytic digestion may directly affect intestinal cell structure and functions by modulating gene expression and oxidative stress. The gliadin sequence contains regions that play a special role in CD pathogenesis exert a cytotoxic activity or immunomodulatory activity (reviewed by Ciccocioppo *et al.*, 2005). Other regions trigger oxidative stress and induce the release of pro-inflammatory cytokines. Similarly, Cornell & Wills-Johnson (2001) reported that computer modeling studies have shown that two groups of biologically-active peptides derive from α -gliadin, the serine-containing group of peptides appears to be essentially toxic, whilst the tyrosine-containing group has the capacity to trigger immunological reactions in CD patients. Ferretti *et al.* (2012) explained that both these types of activity in CD are possible if there is defective digestion of the active peptides.

CD adversely affects absorption of water and nutrients causing, in some cases, malnutrition (Sollid, 2000). Further, if nutrient deficiency is not treated, CD can result in an increased risk of other autoimmune diseases and malignancy (Lohi *et al.*, 2007). In fact

diarrhoea in CD occurs due to progression of the disease into the distal small bowel (Green and Jabri, 2003). Even in the absence of CD, gastro-intestinal symptoms have been observed with gluten exposure indicating the potential existence of non-coeliac gluten intolerance (Biesiekierski *et al.*, 2011).

Epidemiological data show that CD is a common disease in the world (Table 2.1), affecting not only Europeans and people of European ancestry, but also populations of the developing countries such as the Middle East, South Asia, Africa and South America, where its prevalence is similar to that of Western countries (Cataldo & Montalto, 2007). However, CD shows a variable prevalence in Africa but it has not been ascertained in many African countries. This may be due to the poor living condition and the limited availability of diagnostic facilities resulting in the lack of awareness and low suspicion of the disease (reviewed by Catassi *et al.*, 2012).

Erickson *et al.* (2012) suggested that the quality of life in CD patients could be improved through the development of healthy, palatable gluten-free options with sensory attributes similar to wheat-based products. Although considerable scientific progress has been made in understanding CD and in preventing or curing its manifestations, a strict gluten-free diet is the only treatment for CD to date (Niewinski, 2008).

Table 2.1: Prevalence of CD in some countries

Countries	Prevalence of CD
United Kingdom	1:100
USA	1:133
Australia	1:251
Netherlands	1:198
Switzerland	1:132
Sweden	1:190
Spain	1:118
Italy	1:106
Ireland	1:122
Portugal	1:134
Norway	1:262
Hungary	1:85
Finland	1:99
Estonia	1:88
Czechoslovakia	1:218
Iran	1:164
Israel	1:157
Syria	1:67

Sources: Reviewed by Malekzadeh *et al.* (2005) and Cataldo & Montalto (2007)

2.7 Conclusions

Improving the quality of non-wheat bread is still an ongoing process. This is due to the challenges in attaining the similar desirable qualities, such as high loaf volume and open crumb structure found in wheat bread. Among the various means of improvement, sourdough fermentation alone and in combination with other additives seems to be more promising and practicable. The inclusion of additives to the sourdough process will most likely incur a higher cost on the bread. This will however defeat one of the aims of this present study which is to provide a more affordable alternative source of flour for breadmaking in Africa. Also combining additives with the sourdough fermentation process will probably have an effect on the sourdough, masking its actual influence on the dough properties of the bread, thereby preventing the understanding of the mechanisms involved in sourdough fermentation in improving the quality of the bread. The success of the application of sourdough in non-wheat bread greatly depends on various parameters such as the fermentation time and temperature, the properties of the sourdough microorganisms present and the amount of sourdough used. Therefore, this study will focus on improving the quality of maize bread using an optimized sourdough fermentation method without the aid of additives.

3. HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

1. Application of sourdough fermentation in making maize bread will give desirable results such as improved loaf volume, soft and open crumb structure.

In bread dough, the gas-holding capacity is one of the most important factors affecting the volume of the final product (Wehrle & Arendt, 1998). The gas-holding capacity of the dough depends on the physicochemical structure of the dough (Clarke & Arendt, 2005). Therefore, the effect of sourdough fermentation on the structure-forming component of the dough is very important. Acidification of the bread dough due to the addition of sourdough will most likely have a direct impact on structure-forming components like gluten and starch (Clarke & Arendt, 2005). Since non-wheat breads do not have gluten, their structure will probably depend to a greater extent on the properties of their starches. Arendt *et al.* (2008) suggested that acidification of gluten-free flour by sourdough fermentation can replace the function of gluten in a way by improving the swelling properties of starch, which may be beneficial to the structure and gas retention ability of gluten-free doughs.

Sourdough fermentation may also improve bread quality based on its effects on cereal and bacterial enzymes (Clarke & Arendt, 2005). During fermentation, biochemical changes occur in the carbohydrate and protein components of the flour due to the action of microbial and indigenous enzymes. The rate and extent of these changes greatly influence the properties of the sourdough and ultimately the quality of the final baked product (Clarke & Arendt, 2005). Lactic acid bacteria (LAB) activities in sourdough have been reported by Corsetti *et al.* (1998) to involve acidification of dough resulting from the lactic acids produced by LAB. This lowers the pH, providing a favourable environment for proteolysis of protein by proteolytic enzymes and also moderate hydrolysis of starch by amylase enzymes. Changes in pH level caused by the production of lactic acid (Wehrle *et al.*, 1997), proteolytic (Arendt *et al.*, 2007) and amylolytic (Agati *et al.*, 1998) activities affects the rheological behaviour of dough. These activities affect the technological properties of bread. For wheat breads, bread doughs that are more elastic produce wheat bread with better quality (Tipples *et al.*, 1994).

2. The dominant LAB present in the sourdough will exhibit proteolytic and amylolytic activities.

As stated, proteolysis of protein by proteolytic enzymes and moderate hydrolysis of starch are activities of LAB in sourdough (Corsetti *et al.*, 1998). Sourdough fermentation is said to have an impact on dough rheology. However, sourdough fermentation must have had an effect on the structure-forming component of the dough such as the protein and starch components before it can have an effect on the dough rheology. Enzyme activities of amylases and proteases cause the breakdown of several flour components (Wehrle & Arendt, 1998). Therefore, the presence of proteolytic and amyolytic activities in the LAB, will result in the hydrolysis of the structure forming components of the dough such as the protein and starch. As a result, the dough rheology will be modified.

General research aim

To improve the quality of maize bread with respect to the loaf volume and crumb structure, through the use of a sourdough fermentation in the breadmaking process

3.2 Objectives

1. To determine the effects of various sourdough fermentation processes on the loaf volume and crumb structure of maize bread.
2. To determine how sourdough fermentation brings about improvement in the crumb structure and loaf volume of maize bread
3. To identify and characterize the dominant LAB present in fermented maize sourdoughs

4. RESEARCH CHAPTER

4.1 Research Chapter 1: Effect of different non-wheat bread making methods on the quality of maize bread

4.1.1 Abstract

There is a need in Africa to produce bread from local crops. Maize was used in this work because it is the most important crop produced in Africa. Successes have been reported in the use of sourdough fermentation and pre-gelatinization of gluten-free flour in improving wheat-free breads. Therefore, the effects of three non-wheat bread recipes on the quality of bread made from maize were investigated. The first was a traditional sourdough method used in Lesotho for making steamed bread. This involved addition of spontaneously fermenting sorghum malt sourdough (equivalent to 15% of the total maize flour) and pre-gelatinization of the starch in the maize flour with boiling water. The second was a Food and Agriculture Organization method which involved pre-gelatinization of the starch in 10% of the maize flour by cooking. The third method was a modern sourdough method which involved spontaneously fermenting 75% of the maize flour. The modern sourdough method produced maize bread with a more open crumb structure and a significant increase in loaf volume compared to the other methods. This is probably primarily due to the high percentage of maize flour fermented leading to a more substantial improvement in bread dough properties, which in turn significantly improved maize bread quality.

4.1.2 Introduction

Maize is a potentially suitable alternative to wheat for use in breadmaking in Africa. This is because it is by far the most important crop produced in Africa (about 69.6 million tons) (FAOSTAT, 2012). However, the challenge is to produce bread from maize that will imitate closely the desirable qualities (high loaf volume and open crumb structure) that make wheat bread acceptable by consumers. Wheat gluten is the only protein with the proper functionality to produce high quality breads (reviewed by Mejia *et al.*, 2012). This is attributed to its unique property of forming strong viscoelastic dough when hydrated (reviewed by Goodall *et al.*, 2012).

The use of maize in wheat-free and gluten-free breadmaking is not common. The few investigations have included additives such as egg and maize starch (Sanni *et al.*, 1997), improvers containing enzymes which aid the improvement of the characteristics of baked products such as S500 Acti-plus (Puratos) (Brites *et al.*, 2010), ascorbic acid (Edema, 2011), and hydrocolloids such as hydroxyl propyl methyl cellulose (De la Hera *et al.*, 2013) to aid the final quality of maize bread. The use of additives increases the cost of the final wheat-free bread (reviewed by Moroni *et al.*, 2009), a critical issue where consumers are food insecure. Sourdough fermentation seems to be a promising alternative since it does not involve additional ingredients and is a natural process (reviewed by Moroni *et al.*, 2009). Sourdough is a mixture of flour and water that is fermented by naturally occurring lactic acid bacteria (LAB) and yeasts (Hammes & Gänzle, 1998). Success has been reported in the use of sourdough fermentation on the improvement of the quality of wheat bread and some wheat-free breads (reviewed by Arendt *et al.*, 2007; Edema *et al.*, 2013). Also, pre-gelatinization of starch in wheat-free flour could be a suitable alternative to hydrocolloids to aid carbon dioxide retention in wheat-free bread making. According to Onyango *et al.* (2009), pre-gelatinized starch, aids in the creation of a viscoelastic batter that can trap and retain carbon dioxide produced during proofing of wheat-free bread dough.

This research investigated three different wheat-free sourdough methods that have been used to produce wheat-free breads from other cereals. The work was carried out to determine which of the methods will give maize bread with the most desirable qualities such as high loaf volume and open crumb structure, as a basis for subsequent research.

4.1.3 Experimental

4.1.3.1 Materials

Refined white maize meal (Impala Special Maize Meal, Premier Foods, Isando, South Africa) with a protein content 8.6 g/100 g (db) and a fat content 2.7 g/100 g (db) was milled into a flour using a laboratory hammer grinder (Mikro-Feinmuhle-Culatti MFC grinder, Janke and Kunkel, Staufen, Germany) fitted with a 0.5 mm opening screen. Sorghum grain (cultivar MR Buster) was also milled into flour as described above. Industrial sorghum malt with a diastatic power of 33.4 SDU/g was obtained from United National Breweries, Mandini, South Africa.

4.1.3.2 Methods

Production of sourdoughs for maize bread

Maize and sorghum sourdoughs were produced by mixing a starter [previously fermented maize sourdough (pH: 3.7) or sorghum sourdough (pH: 3.8)] with maize or sorghum flour and water in the ratio of 1:3 (w/v). These sourdoughs were monitored over an incubation period of 5 days at ambient temperature (22°C) and 45°C, after which backslopping (adding fresh maize flour or sorghum flour with water) was done. The backslopped sourdoughs were monitored for a further 5 days at the same temperatures as above. Maize and sorghum sourdoughs were compared in order to determine which of the two will have the best acidification properties over the period of incubation.

The sourdoughs used in the three wheat-free methods were fermented at 22°C until the pH was below 4 (approx. 72 h). Sorghum malt was used to prepare the sourdough used for maize bread produced using a traditional sourdough method practised in Lesotho. Sorghum malt was mixed with water in a ratio of 1:3 (w/v). Final pH was 3.7. Maize sourdough used for maize bread produced according to a Food and Agriculture Organization (FAO) method (Satin, 1988) was prepared as above. Final pH was 3.7. Maize sourdough used for maize bread produced using a modern sourdough method was prepared according to Edema *et al.* (2013). Final pH was 3.8.

Production of maize breads

Maize bread was produced using three different wheat-free methods. Each method involved addition of sourdough and/or pre-gelatinization.

The first wheat-free method was a traditional sourdough method practiced in Lesotho according to Nkhabutlane *et al.* (2013) with some modifications (Fig. 4.1.1). Baking ingredients (salt, sugar, maize flour) were mixed with boiling water and allowed to cool at ambient temperature (22°C). The maize sourdough and/or pre-gelatinized maize flour and yeast were added. All the treatments were incubated at 25°C for 12 h. The doughs were then either baked or steamed. Baking was for 15-20 min at 210°C and steaming was for 2 h over a pan on an electric hotplate.

The second wheat-free method was a FAO method (Satin, 1988) with some modifications (Fig. 4.1.2). This method involved pre-gelatinization of 10% of the maize flour with the total amount of water by cooking for 4 min and then replacing the water that had evaporated. The pre-gelatinized maize flour was allowed to cool before the maize sourdough (equivalent to 10% of total maize flour) and the remaining ingredients (salt, sugar, yeast, and remaining maize flour) were added. Mixing was done manually for 10 min. The dough was scooped into aluminum cans (72 mm diam) to half full (200 g dough). Proofing was for 1 h at 22°C. The breads were baked or steamed as above.

The third wheat-free method was performed according to Edema *et al.* (2013) with some modifications (Fig 4.1.3). Baking ingredients (sugar, salt, soft margarine and instant dried yeast) were added to maize sourdough (equivalent to 75% of total maize flour). First proofing was at 30°C for 20 min. The maize bread dough was remixed and scooped into silicone pans (70 mm top diam and 58 mm bottom diam) to half full (47 g dough). The second proof was at 30°C for 15 min. Baking was at 200°C for 20 min.

Analyses

Bread height was measured using a meter rule. Loaf volume and specific volume were calculated. Crumb structure was photographed with a digital camera.

Statistical analyses

All experiments were performed at least twice. Results were analysed using one-way analysis of variance (ANOVA). Fisher's Least Significant Difference Test (LSD) was used to determine significant differences between the treatments at $p \leq 0.05$.

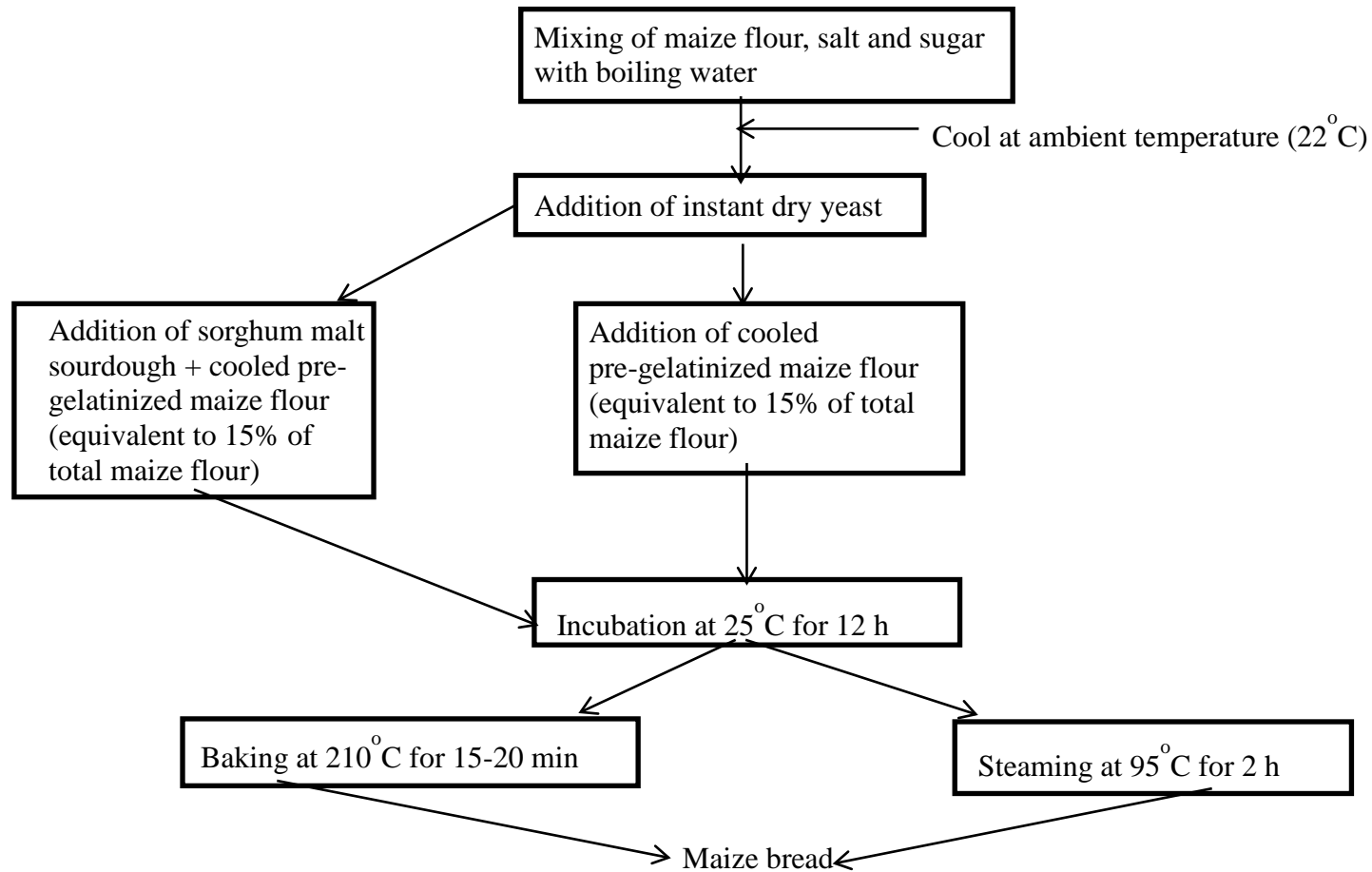


Fig 4.1.1: Procedure for making maize bread using the traditional sourdough method practiced in Lesotho according to Nkhabutlane *et al.* (2013)

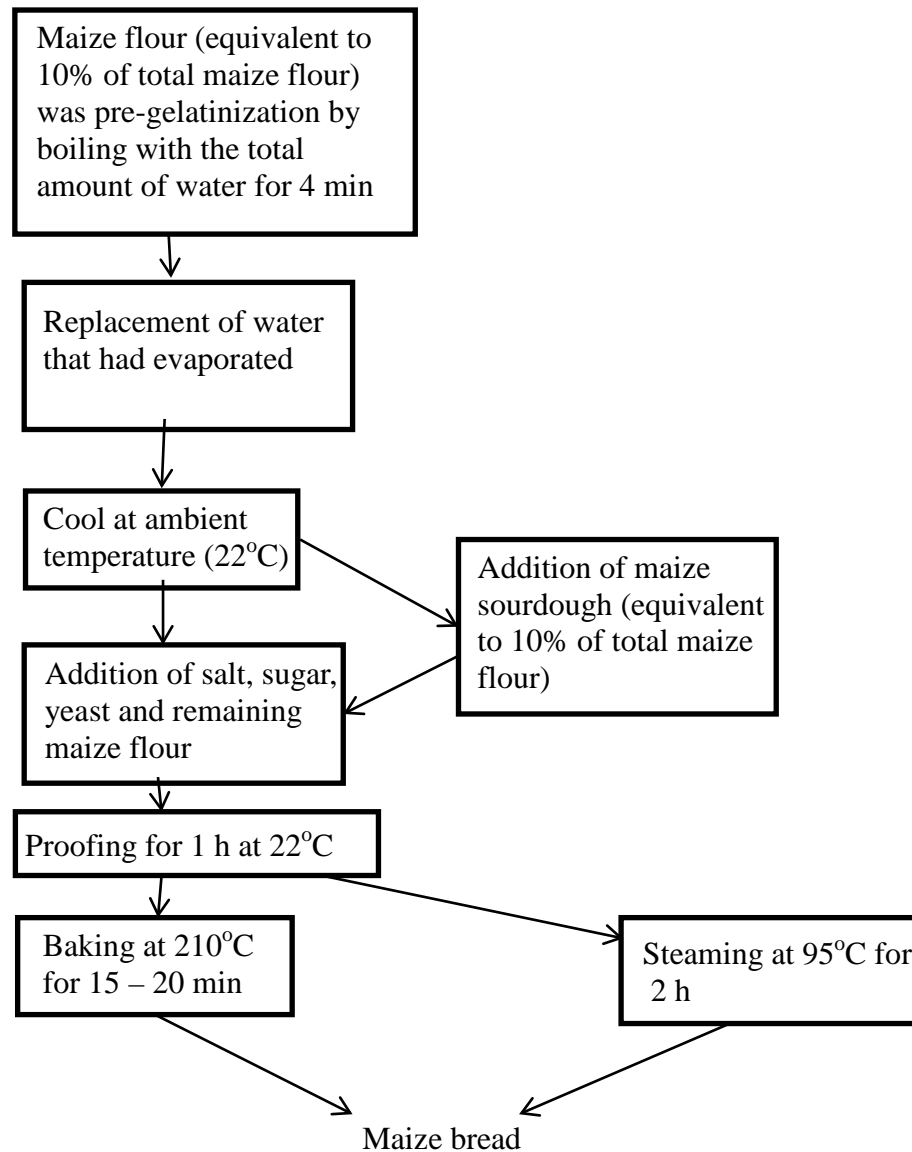


Fig 4.1.2: Procedure for making maize bread using the FAO (Satin, 1988)

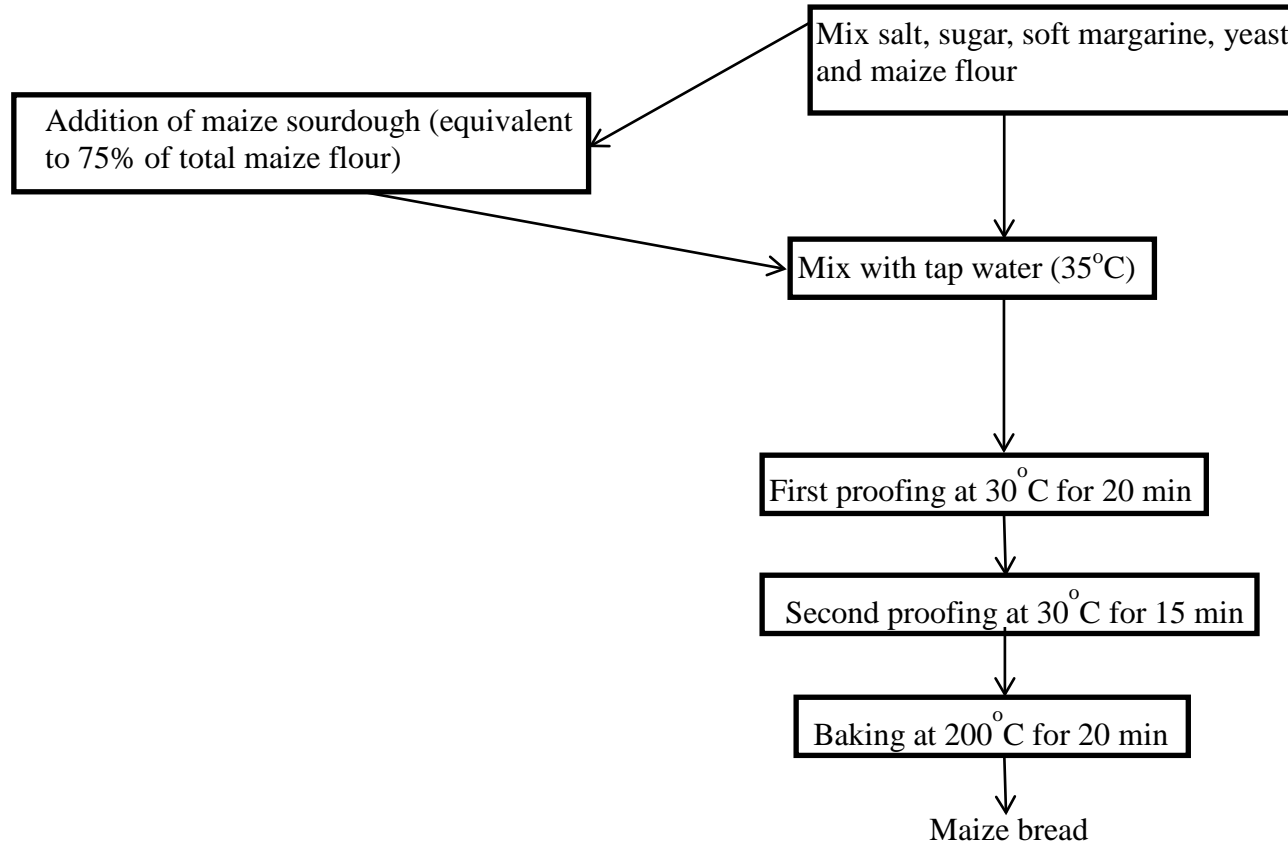


Fig 4.1.3: Procedure for making maize bread using the modern sourdough method according to Edema *et al.* (2013)

4.1.4 Results and discussion

4.1.4.1 Production of maize or sorghum sourdough

At 22°C and 45°C, maize and sorghum sourdough showed similar patterns in pH and titratable acidity throughout the period of incubation (Fig. 4.1.4). At 22°C, there was an initial decrease in the pH and an increase in titratable acidity of both sourdoughs from day 1 to 5, after which an increase occurred in pH and a decrease in titratable acidity on day 6 due to backslopping. There was a slight decrease in pH and increase in titratable acidity on day 7, after which the pH started increasing gradually and titratable acidity started decreasing gradually until day 11. However, maize dough had a slightly lower pH and lower titratable acidity throughout the period of incubation. At 45°C, the pH of both sourdoughs also initially decreased while the titratable acidity initial increased. A gradual increase in pH and decrease in titratable acidity was observed for both sourdoughs from day 2 to 5 after which an increase in pH and a sharp decrease in acidity was observed on day 6 due to backslopping. From day 7 to 11, the pH of sorghum started decreasing slightly with some fluctuations while that of maize was steady. For the titratable acidity, there was a slight increase from day 7 to 8 for both sourdoughs after which a sharp increase was observed for sorghum sourdough followed by a slight decrease on day 11. For maize sourdough, a slight decrease was observed from day 8 to 9 after which the acidity was steady until day 11. Both sourdoughs had higher titratable acidity at 22°C than at 45°C.

Even though both maize and sorghum sourdoughs had similar pH and titratable acidity patterns, maize flour was mainly used in this work to prepare the sourdough because it fermented well and also is the most important cereal in Africa (FAOSTAT, 2012). Incubation temperature of 22°C was chosen because maize sourdough had a higher titratable acidity at that temperature than at 45°C.

4.1.4.2 Maize bread quality

Maize breads produced by the traditional sourdough method, FAO method and modern sourdough method were all compared with their controls (maize bread without sourdough and/or pre-gelatinization). Sorghum malt was used to prepare the sourdough used for maize bread produced using a traditional sourdough method. Malted grain when milled produces flour containing starch that is more susceptible to enzymatic hydrolysis than flours from grains that have not been malted (reviewed by Moroni *et al.*, 2009). This in turn

is believed to improve the acidification ability of the sourdough. Analyses were not done on the breads made from the traditional sourdough method. This was because steamed breads made using this method were all very soft and looked like a lump of gelatinized starch (Fig. 4.1.5). Also, baked breads using this same method had cracks and crumbled when sliced. However, bread made with the combination of sourdough and pre-gelatinization showed very slight expansion sideways. Using the FAO method, steamed maize breads generally had a higher loaf volume compared to the baked maize breads (Table 4.1.1, Fig. 4.1.6). Baked or steamed maize bread made without pre-gelatinization or sourdough addition had higher loaf volume compared to the breads with sourdough or pre-gelatinization. Loaf volume of baked or steamed maize bread made by addition of maize sourdough and pre-gelatinization of part of the maize flour was not significantly different from the loaf volume of baked or steamed maize bread made by pre-gelatinization of part of the maize meal. Concerning the modern sourdough method, bread with sourdough had a significantly ($p \leq 0.05$) higher loaf volume (21% increase) and open crumb structure compared to the bread with no sourdough added (Table 4.1.2, Fig. 4.1.7). Comparing each wheat-free method with its control, it was only the modern sourdough method that produced bread that had a significantly better quality than its control.

The traditional sourdough method and the FAO method did not improve maize bread loaf volume and crumb structure compared to the modern sourdough method, probably due to pre-gelatinization and also the small proportion of sourdough added (10 or 15 % of the total maize flour) compared to the higher proportion of sourdough (75% of the total maize flour) used in the modern sourdough method. Pre-gelatinization probably created an unfavourable environment for gas cell expansion by the yeast provided by the stickiness (high viscosity) of the pre-gelatinized dough. According to Onyango *et al.* (2010), gelatinised starch forms a stiff, inelastic dough that does not favour the expansion of gas cells in the dough. However, in apparent contrast, the same authors, Onyango *et al.* (2009) stated that pre-gelatinized starch, aids in the creation of a viscoelastic batter that can trap and retain carbon dioxide produced during proofing of wheat-free bread dough. In line with this, the pre-gelatinisation process in this work probably made the maize flour more of a stiff dough than a batter, thereby preventing the expansion of gas cells. Moroni *et al.* (2011) who worked on the impact of sourdough on the biochemical, rheological and texture of buckwheat flour, batter and bread suggested that the strengthening of the starch gel observed upon acidification of the batter favoured the rupture of gas cells and impaired

the textural characteristics of buckwheat bread. In line with this, since the purpose of pre-gelatinization was to provide a soft starch gel-like matrix to trap the CO₂ produced during fermentation, probably the dough viscosity achieved was just high enough to do more damage than good by favouring the rupture of the gas cells resulting in a negative effect on loaf volume and crumb structure of the bread. Also, since the pre-gelatinized dough had to be cooled before other ingredients were added, it is possible that starch retrogradation had occurred, defeating the main aim of pre-gelatinization which was to provide a gel matrix to trap the carbon dioxide produced during fermentation. When gelatinized starch cools down, amylose retrogrades, resulting in an increase in viscosity (Zilic *et al.*, 2010). On cooling, retrogradation occurs, the starch granules in the gelatinized paste associate, leading to the formation of a more ordered structure (Hoover, 1995). Due to this change, the starch granules will not be able to effectively absorb water or trap CO₂ produced in the dough. Also, the small proportion of maize sourdough used (10-15% of the total maize flour) was probably not sufficient to modify the dough properties satisfactorily enough to impact positively on the quality. Clarke *et al.* (2002) suggested that improved volume of sourdough breads is dependent on the nature and intensity of the acidification process.

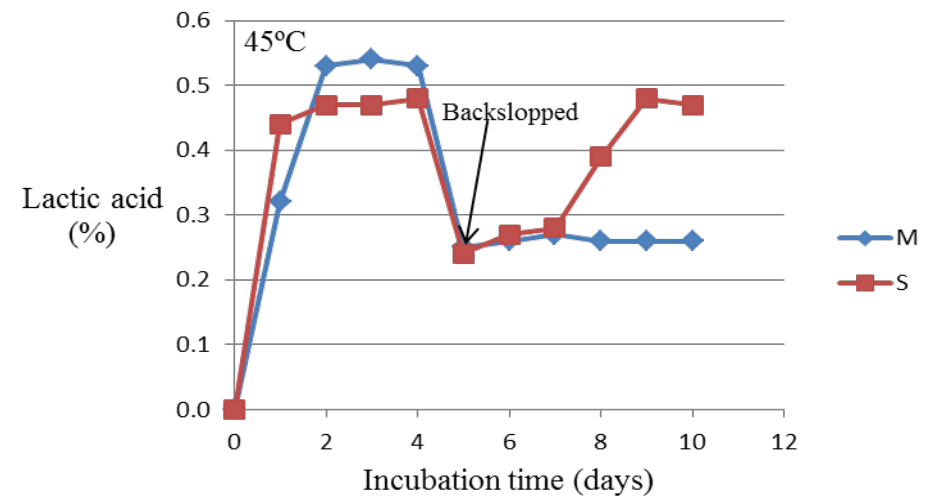
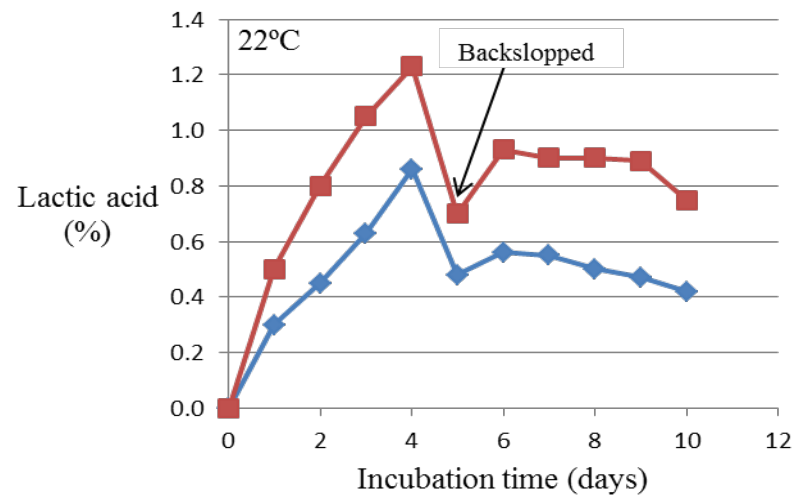
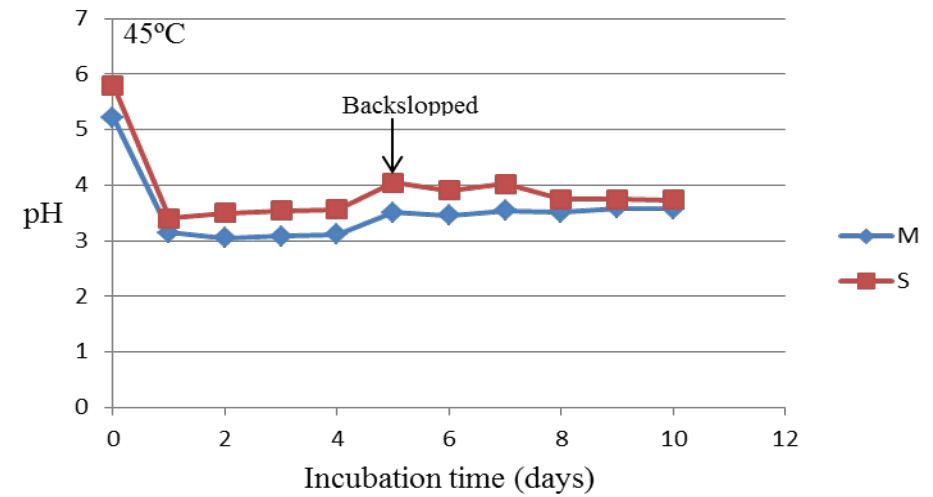
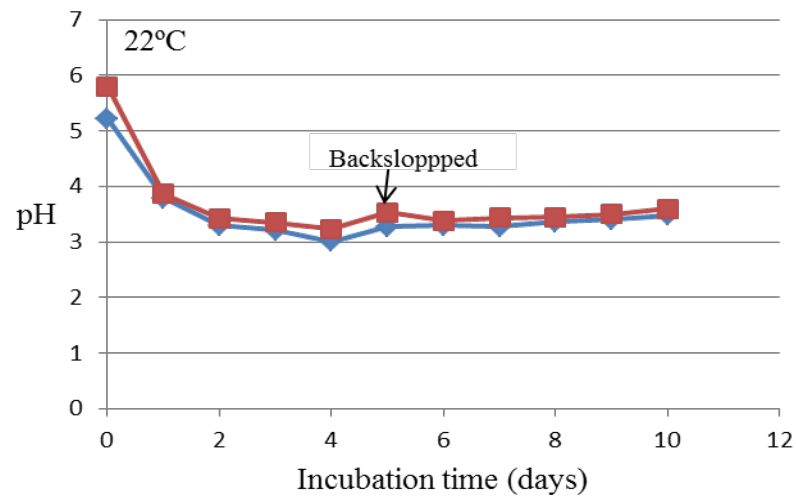


Fig 4.1.4: Effects of incubation time and temperatures (22°C or 45°C) on the pH and titratable acid of fermenting maize flour (M) and sorghum flour (S)

Pre-gelatinization and
addition of sorghum
malt sourdough

Pre-gelatinization
alone

No sourdough or
pre-gelatinized
flour added

A



B

a: steamed, b: baked

Fig 4.1.5: Effects of sourdough fermentation and/or pre-gelatinization of part of the maize flour on the loaf volume of baked or steamed maize bread produced using a traditional sourdough method practiced in Lesotho according to Nkhabutlane *et al.* (2013).

Table 4.1.1: Effects of sourdough fermentation and/or pre-gelatinization, and baking or steaming on the loaf volume and loaf specific volume of maize bread produced according to a FAO method (Satin, 1988)

Treatments	Weight after baking / steaming (g)	Height after baking/steaming (mm)	Loaf volume after baking/steaming (cm ³)	Loaf specific volume after baking/steaming (cm ³ /g)
No sourdough or pre-gelatinization				
Loaves baked	158.4 ^a ± 0.7	59.5 ^c ± 0.1	242.4 ^c ± 2.9	1.53 ^d ± 0.03
Loaves steamed	181.1 ^b ± 2.8	64.5 ^d ± 0.2	262.7 ^d ± 8.6	1.45 ^c ± 0.03
Part of flour pre-gelatinized				
Loaves baked	154.0 ^a ± 1.6	51.5 ^a ± 0.2	209.8 ^a ± 8.6	1.37 ^b ± 0.04
Loaves steamed	181.2 ^b ± 2.7	59.0 ^c ± 0.3	240.4 ^c ± 11.5	1.33 ^{ab} ± 0.04
Part of flour pre-gelatinized + sourdough				
Loaves baked	153.2 ^a ± 0.1	52.5 ^{ab} ± 0.1	213.9 ^{ab} ± 2.9	1.40 ^{bc} ± 0.02
Loaves steamed	178.3 ^b ± 3.6	56.0 ^{bc} ± 0.1	228.1 ^{bc} ± 5.8	1.24 ^a ± 0.01

¹Means and standard deviation n=2. Values followed by different letters are significantly different at p ≤ 0.05

No pre-gelatinization
or addition of maize
sourdough

Pre-gelatinization of part
of the maize flour and
addition of maize sourdough

Pre-gelatinization of part
of the maize flour



A: baked breads, B: steamed breads

Fig 4.1.6: Effects of sourdough fermentation and/or pre-gelatinization of part of the maize flour on the loaf volume and crumb structure of maize bread produced according to a FAO method (Satin, 1988)

Table 4.1.2: Effect of sourdough fermentation on the loaf volume and specific loaf volume of maize bread produced using the modern sourdough method according to Edema *et al.* (2013)

Treatments	Weight after baking (g)	Height after baking (mm)	Loaf volume after baking (cm ³)	Loaf specific volume baking (cm ³ /g)
Maize bread with no sourdough added	46.5 ^a ± 0.5 ¹	24.7 ^a ± 0.2	79.5 ^a ± 6.7	1.7 ^a ± 0.1
Maize bread with sourdough added (75% of total maize flour)	46.0 ^a ± 0.3	30.0 ^b ± 0.1	95.8 ^b ± 2.3	2.1 ^b ± 0.1

¹ Means and standard deviation n=2. Values in the same column followed by different letters are significantly different at $p \leq 0.05$

Maize sourdough added

No sourdough added

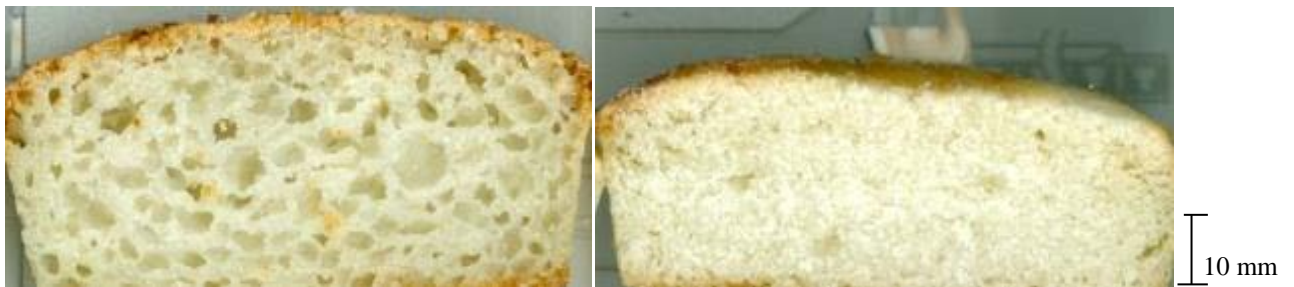


Figure 4.1.7: Effect of sourdough fermentation on the loaf volume and crumb structure of maize bread produced using the modern sourdough method according to Edema *et al.* (2013)

4.1.5 Conclusions

The modern sourdough method produced maize bread with an open crumb structure and a significant increase in loaf volume compared to maize breads produced by the traditional sourdough method practised in Lesotho or the Food and Agriculture Organisation method. This is probably due to the absence of pre-gelatinization and the higher proportion of sourdough used. The high proportion of sourdough probably had a greater impact on bread dough properties which in turn significantly improved maize bread quality.

Based on these findings, the modern sourdough method was used to produce maize bread for subsequent research work.

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4.2 Research Chapter 2: Investigation into how sourdough fermentation improves maize bread quality

4.2.1 Abstract

Following the finding that sourdough fermentation improves maize bread crumb structure and loaf volume, this work investigated the mechanism of improvement. Maize sourdoughs were made by fermenting maize flour with multiple strain starter culture and with *Lactobacillus plantarum*. Sourdough fermentation of maize dough, in combination with the addition of baker's yeast brought about a 25-26% increase in loaf volume of maize bread. Confocal laser scanning microscopy revealed a cohesive dough structure in the sourdoughs. Larger gas cells were also observed in maize breads with maize sourdough. Differential scanning calorimetry showed that maize sourdoughs had a higher endothermic peak enthalpy than chemically acidified or straight maize dough. Rheological analysis showed that maize sourdoughs had a shorter relaxation time. Strain sweep analysis suggested that maize sourdoughs had the lowest elastic modulus, all indicating a softer and less elastic dough. Temperature sweep analysis showed an initial less elastic dough and a final high tan delta, suggesting that the maize sourdough could withstand gas expansion pressure during baking without crumbling. It appears that improvement in maize bread quality by sourdough fermentation is primarily due to starch granule modification which makes the dough more cohesive, soft and less elastic and improves its ability to trap and withstand the pressure of the expanding carbon dioxide during fermentation and baking.

4.2.2 Introduction

The previous chapter showed that a modern-type sourdough process improves maize bread crumb structure and loaf volume. With wheat breads, the positive effects of sourdough on quality has been attributed to the direct influence of low pH on structure forming dough components such as gluten, starch and arabinoxylans (reviewed by Schober *et al.*, 2003). However, wheat-free breads such as maize bread do not contain gluten. Therefore, improvement in the quality of these wheat-free breads may be attributable to the effect of sourdough on the starch component. According to Elkhailifa *et al.* (2005), sourdough fermentation is expected to have an effect on the starch components, due to starch being the primary source of carbon for microbial growth. Arendt *et al.* (2008) suggested that acidification of wheat-free flour by sourdough fermentation can replace the function of gluten somewhat by enhancing the swelling properties of starch. This may be beneficial to the structure and gas retention ability of wheat-free doughs. Although maize does not contain gluten, gluten-like functionality of zein (maize prolamin) dough as a result of acidification with lactic acid and acetic acid has been reported (Sly *et al.*, 2014). Edema *et al.* (2013) attributed the improvement in fonio dough and bread brought about by the use of a sourdough to starch modification (slight granule swelling and probably some leaching of starch molecules) to the activities of endogenous amylases from the sourdough microorganism whose activities were favoured at low pH. However, Schober *et al.* (2007) attributed the improvement in sorghum bread to dough modification. These authors concluded that the major effect of sourdough fermentation was the degradation of proteins soluble in the dough liquid. These authors further proposed that if these proteins were not degraded, they would aggregate upon baking and interfere with the starch gel. As a result, sorghum bread without sourdough fermentation tended to have a large hole in the crumb.

This chapter will investigate the mechanism of sourdough fermentation in improving maize bread quality with particular attention to its effect on the rheological properties of maize dough.

4.2.3 Experimental

4.2.3.1 Materials

Refined maize meal (Impala Special Maize Meal, Premier Foods, Isando, South Africa) with a protein content 8.6 g/100 g (db) and a fat content 2.7 g/100 g (db) was milled into a flour using a laboratory hammer grinder (Mikro-Feinmuhle-Culatti MFC grinder, Janke and Kunkel, Staufen, Germany) fitted with a 0.5 mm opening screen. A *Lactobacillus plantarum* culture (strain B411) was obtained from the Council for Scientific and Industrial Research, Pretoria, South Africa. *L. bulgaricus* and a cocktail of cultures (*Streptococcus thermophiles* + *L. bulgaricus* + *Lactococcus lactis subsp lactis* + *L. lactis subsp cremoris*; and *S. thermophiles* + *L. bulgaricus*) were obtained from Cape Food Ingredients, Noordhoek, South Africa.

4.2.3.2 Methods

Selection of culture for the maize sourdough

Sterilized solutions of MRS broth and skimmed milk were prepared separately. Rejuvenated cells (*S. thermophiles* + *L. bulgaricus* + *L. lactis subsp lactis* + *L. lactis subsp cremoris*, or *S. thermophiles* + *L. bulgaricus* or *L. bulgaricus* or *L. plantarum*) were added to 100 ml MRS broth or skimmed milk. The pH and acidity of the solutions were determined at intervals over a period of 24 h at ambient temperature (22°C).

Preparation of the sourdoughs and chemically acidified dough

L. plantarum fermented maize sourdough was prepared by mixing maize flour (75 g) with sterile distilled water (75 ml) containing *L. plantarum* cells (9.3×10^{10} cfu/ml) in a ratio of 1:1 (w/v). The mixture was fermented at 30°C to a pH range of 3.3-3.6 (approx. 24 h). Multiple strain starter culture fermented maize sourdough was prepared by mixing maize flour (75 g) with sterile distilled water (75 ml). The maize dough was left to ferment for 72-96 h at ambient temperature. A portion of the fermented maize dough was used as a starter (backslopping) for a fresh mixture of maize flour and water. The mixture was fermented at 30°C to a pH of 3.4-3.7 (approx. 48 h). The final cell count of the *L. plantarum* fermented maize sourdough and the multiple strains starter culture fermented maize dough was 6.4×10^{10} cfu/g and 8.6×10^{10} cfu/g respectively. Chemically acidified maize dough was prepared by adding 0.1% lactic acid to the mixture of maize flour and water to pH 3.4. *L. plantarum* sourdough was compared with multiple strain starter culture

fermented maize sourdough to determine if it would produce maize bread with a better quality. Chemically acidified maize dough was compared to these sourdoughs to determine if the improvement brought about by the sourdough was as a result of low pH.

Maize bread making

This was performed as described by Edema *et al.* (2013) with some modifications. The remaining baking ingredients per 100 g of flour (sugar (10 g), salt (1.5 g), soft margarine (5 g) and instant dried yeast (2 g) and water (15 ml) were added to the sourdoughs and the chemically acidified doughs and mixed together. The sourdoughs were also prepared with all the ingredients except yeast. First proofing was at 30°C for 20 min. The maize bread dough was remixed and scooped into circular silicone pans (70 mm top diam and 58 mm bottom diam) to half full (47 g dough). The second proof was at 30°C for 15 min. Baking was at 200°C for 20 min.

Analyses

Maize bread quality

Bread volume was determined. Crumb structure was measured by scanning cut surfaces of the bread using a flatbed scanner. The number and size of cells was determined by using Image J software 1.42 q/ Java 1.6.0_10 (32-bit), Wayne Rasband (National Institutes of Health, Bethesda, ML). Bread firmness was determined by using a TA-XT2 texture analyser (Stable Micro Systems, Godalming, UK) with a 20 mm radius cylinder probe (P/20 L). Pre-Test speed was 1.0 mm/s, test speed 1.7 mm/s to 40% strain.

Stress relaxation of the maize dough treatments

Stress relaxation was measured using a texture analyser (EZ-L, Shimadzu, Kyoto, Japan). A plastic rod (43 mm diam and 10 mm height) was used at a 25% strain to compress the maize dough for 5 sec, after which the dough was allowed to relax over a period of 180 sec. Relaxation time was calculated as the time required for the maximum force of compression to drop to 36.8% of its value, according to Singh *et al.* (2006).

Maize dough rheology under simulated baking conditions

Strain sweep analysis was performed on maize doughs prepared just as it was done for baking. This was done using a Physica MCR 101 Rheometer with Rheoplus software (Anton Paar, Ostfildern, Germany) to determine the linear viscoelastic region of the maize

dough treatments prior to the temperature sweep test. Parallel plate geometry with a 25 mm diam probe and 2 mm gap between the top and bottom plate was used. The strain measured ranged from 0.01 to 100% at constant frequency of 6.3 rad/s (1 Hz) measured at 4°C. Excess dough was removed with a spatula and paraffin oil was put at the edges of the dough to prevent it drying. Temperature sweep analysis was performed to estimate the changes that would occur in dough properties during baking. This analysis was done within the linear viscoelastic range (0.1%) of the maize dough as determined earlier by strain sweep analysis. Frequency was kept constant at 6.3 rad/s (1 Hz) and the temperature range was from 25-150°C for 20 min at a heating rate of 6.25°C/min. Excess dough was scraped off but no paraffin oil was added to the edges because the paraffin oil caused a bubbling effect at higher temperatures.

Damaged starch in the maize dough

Maize doughs and sourdoughs were prepared without yeast and freeze dried. The amount of damaged starch in the treatments was determined using a SDMatic amperometric type instrument (Chopin Technologies, Villeneuve-la-Garenne, France) based on the iodine dye binding principle. The result was expressed in AACC 76-31 method equivalents (AACC, 2000) as calculated by the instrument.

Thermal properties of the maize doughs

These were determined by Differential Scanning Calorimetry (DSC) with STARe software (HPDSC-827, Mettler Toledo, Schwerzenbach, Switzerland). Maize dough treatments were prepared as for baking. Maize dough (45-50 mg) was weighed into a 100 µl aluminium DSC pan. Scanning was from 30 to 120°C at a rate of 10°C/min. Nitrogen, at normal air pressure and 50 ml/min flow rate was used. Onset (T_o), peak (T_p), conclusion gelatinization (T_c) temperatures were measured and enthalpy (ΔH) was calculated.

Structural properties of the maize dough and maize bread

Confocal laser scanning microscopy (CLSM) (Zeiss 510 META system, Jena, Germany) with a Plan-Neofluar 10 × 0.3 objective under natural fluorescence at an excitation wavelength of 405 nm was used. Dough (< 1 g) or maize bread (1mm thick slice) prepared as was for baking, was attached to a slide with double sided tape. Samples were stained with 0.5% acid magenta dye (Maeda *et al.*, 2013). The stained samples were dried in an oven at 60°C for 1 min. Dried samples were mounted on the stage of the CLSM and

viewed. Images were captured using a micro- and macro-photography ultra-high resolution digital camera.

In an attempt to make the gas cells in the bread more distinct when viewed using CLSM, the modern sourdough method was combined with a microwave injera (fermented sorghum flatbread) procedure according to Anyango *et al.* (2011). In this procedure, the chemically acidified treatment was made up of maize flour mixed with water containing lactic acid. The fermented maize batters were made up of maize sourdough (*L. plantarum* or multiple strain starter culture fermented maize sourdough) mixed with maize flour and water. Approximately 1/3 of the fermented batter was mixed with 10 ml of tap water and then added to 40 ml of boiling water. Stirring was done until gelatinization of the starch in the batter was achieved. The gelatinized batter was allowed to cool to 45°C. Commercial active dried baker's yeast (0.5 g) and 1.5 g of sugar was added to the rest of the fermented batter and stirred thoroughly to get a uniform mix. The cooked-cooled (gelatinized) portion was added to the rest of the fermented batter. Water (30 ml) was added and stirred to get a uniform mixture. Incubation for all the treatments was at 40°C for 1 h in a water bath. Steaming was done in a microwave with power output of 800 Watts for 90 seconds.

Statistical analyses

All experiments were performed at least twice. Results were analysed using one-way analysis of variance (ANOVA). Fisher's Least Significant Difference Test (LSD) was used to determine significant differences between the treatments at $p \leq 0.05$.

4.2.4 Results and discussion

4.2.4.1 Selection of starter culture for the sourdough

Of the cultures and media investigated, *L. plantarum* in MRS broth produced the highest titratable acidity (% lactic acid) and the lowest pH throughout the incubation time (Fig. 4.2.1). *L. plantarum* has been shown to be the dominant organism at the end of several natural tropical cereal fermentations. These include maize-derived products like ogi (fermented maize gruel) popularly consumed in West Africa (Steinkraus, 1995). Based on these findings, *L. plantarum* was selected for use as a starter in the preparation of the sourdough referred to as *L. plantarum* fermented maize sourdough in this work.

4.2.4.2 Maize bread quality

Loaf height, loaf volumes and specific volume of maize breads made with sourdoughs: *L. plantarum* fermented maize sourdough or multiple strains starter culture fermented maize sourdough with yeast were significantly ($p \leq 0.05$) higher (by 25-26%) than maize bread made from chemically acidified maize dough with yeast or straight maize dough (maize dough without sourdough or chemical acidification) with yeast or maize sourdoughs without yeast (Table 4.2.1, Fig. 4.2.2). Maize sourdough breads without yeast had the lowest loaf volume, very dense crumb structure and required the highest force to compress.

When viewed CLSM, the maize sourdough breads with yeast also had a more open crumb structure with discrete cells (cells of average diameter 2 mm), whereas a compact crumb structure with ruptured cells (Fig. 4.2.3 A) was observed in the maize breads made by the straight dough process or with chemical acidification, while the maize sourdough breads without yeast had compact crumb structure. Less force was required to compress the maize sourdough breads than required for the maize bread without sourdough (Table 4.2.1). The chemically acidified maize bread required the least amount of force to compress the bread. However, it crumbled more easily than the other maize breads (Fig. 4.2.3A). Similarly, Moore *et al.* (2007) who investigated the effect of lactic acid bacteria on the properties of gluten-free sourdoughs and quality of gluten-free breads, reported that chemically acidified gluten-free bread was more brittle than the biologically acidified one.

As chemical acidification did not improve the quality of maize bread, this suggests that the effect of the sourdough was much more than simple reduction in pH. Open crumb structure

in the sourdough breads (cells of average diameter 2 mm) is a desirable quality attribute in bread. According to Cauvain (1998), cells of relatively small size (approx. 1-2 mm) are desirable in bread. Clarke *et al.* (2002) and Dal Bello *et al.* (2007) both examined the effects of wheat sourdough on wheat bread quality. These authors also reported that biological acidification of wheat dough with sourdough yielded breads with greater specific loaf volumes when compared to either the non-acidified or the chemically acidified treatments. Clarke *et al.* (2002) hypothesized that some physicochemical changes in protein network occurred due to the addition of sourdough. They further explained that these changes may have facilitated a greater expansion of the sourdough bread during proofing due to the softer and more extensible nature of the dough.

Low loaf volume and dense crumb structure of the maize sourdough breads without yeast, chemically acidified maize bread with yeast and straight dough maize bread with yeast suggests that a combination of the addition of yeast and sourdough was responsible for high loaf volume and crumb structure of maize bread. The dense crumb structure observed in the sourdough breads without yeast may probably be due to the loss of the initial little amount of carbon dioxide present in the dough. This CO₂ loss may have led to the collapse of the gas cells. Adding yeast may have probably increased the amount of CO₂ in the dough and complemented for any loss of carbon dioxide during baking. Also, the dense crumb structure may be due to the formation of a very strong starch gel without interference of the gas cells (formed in the dough due to diffusion of CO₂ into the air bubbles). As stated, according to Schober *et al.* (2007), sourdough fermentation facilitated the degradation of proteins soluble in the dough liquid, enabling the formation of a starch gel matrix without interference of the protein.

To determine how sourdough fermentation improves maize bread quality, the rheological, thermal and structural properties of the sourdoughs were examined.

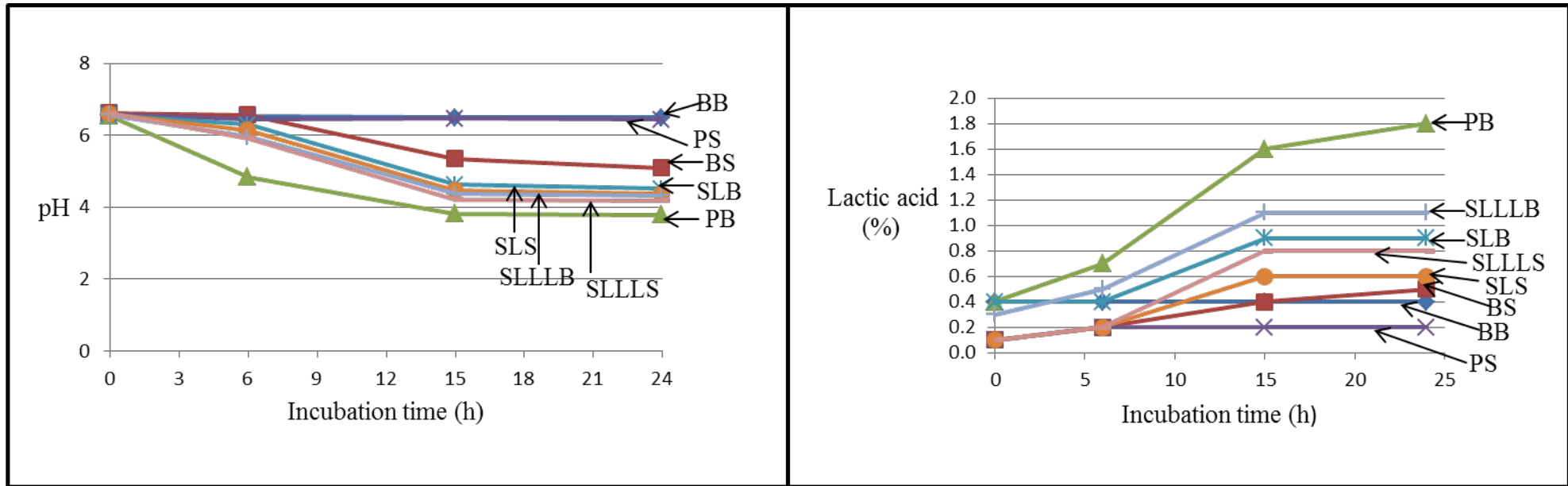


Fig 4.2.1: pH and titratable acidity of *Lactobacillus plantarum* in MRS broth (PB) or in skimmed milk (PS), *L. bulgaricus* in MRS broth (BB) or in skimmed milk (BS), *Streptococcus thermophiles* + *L. bulgaricus* in MRS broth (SLB) or in skimmed milk (SLS), *S. thermophiles* + *L. bulgaricus* + *Lactococcus lactis subsp lactis* + *L. lactis subsp cremoris* in MRS broth (SLLLB) or in skimmed milk (SLLLS), over an incubation period of 24 h at 27°C

Table 4.2.1: Effects of *L. plantarum* or multiple strains starter culture fermentation on the quality of maize bread (loaf volume, force required to compress the maize bread, number and size of the cells)

Treatments	Weight after baking (g)	Height after baking (mm)	Force required to compress maize bread (N)	Loaf volume after baking (cm ³)	Loaf specific volume after baking (cm ³ /g)	Number of cells per mm ²	Average diameter of cells (mm)
Maize bread with yeast	36.3 ^b ± 0.4 ¹	21.0 ^c ± 0.0	38.7 ^c ± 3.5	68.2 ^c ± 0.0	1.9 ^c ± 0.0	ND	ND
Chemically acidified maize bread with yeast	35.2 ^a ± 0.2	20.5 ^c ± 0.6	15.6 ^a ± 3.1	66.6 ^c ± 1.9	1.9 ^c ± 0.1	ND	ND
<i>L. plantarum</i> fermented maize sourdough bread with yeast	36.2 ^b ± 0.2	26.3 ^d ± 1.3	24.9 ^b ± 0.3	85.2 ^d ± 4.1	2.3 ^d ± 0.1	109.0 ^a ± 5.7	1.9 ^a ± 0.2
Multiple strains starter culture fermented maize sourdough bread with yeast	35.8 ^{ab} ± 0.2	26.5 ^d ± 1.0	27.7 ^b ± 2.0	86.0 ^d ± 3.3	2.4 ^d ± 0.1	112.0 ^a ± 14.1	2.0 ^a ± 0.3
<i>L. plantarum</i> fermented maize sourdough bread without yeast	36.2 ^b ± 0.7	13.5 ^a ± 0.6	84.5 ^c ± 2.4	43.9 ^a ± 1.9	1.2 ^a ± 0.1	ND	ND
Multiple strains starter culture fermented maize sourdough bread without yeast	36.1 ^b ± 0.5	17.0 ^b ± 2.3	62.0 ^d ± 1.6	55.2 ^b ± 7.5	1.5 ^b ± 0.2	ND	ND

¹Means and standard deviation n=2. Values followed by different letters in the same column are significantly different at p ≤ 0.05. ND: not detectable

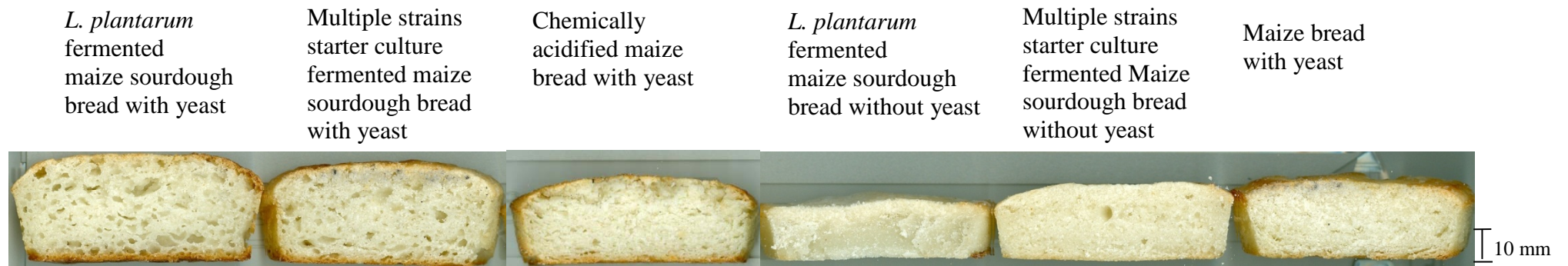


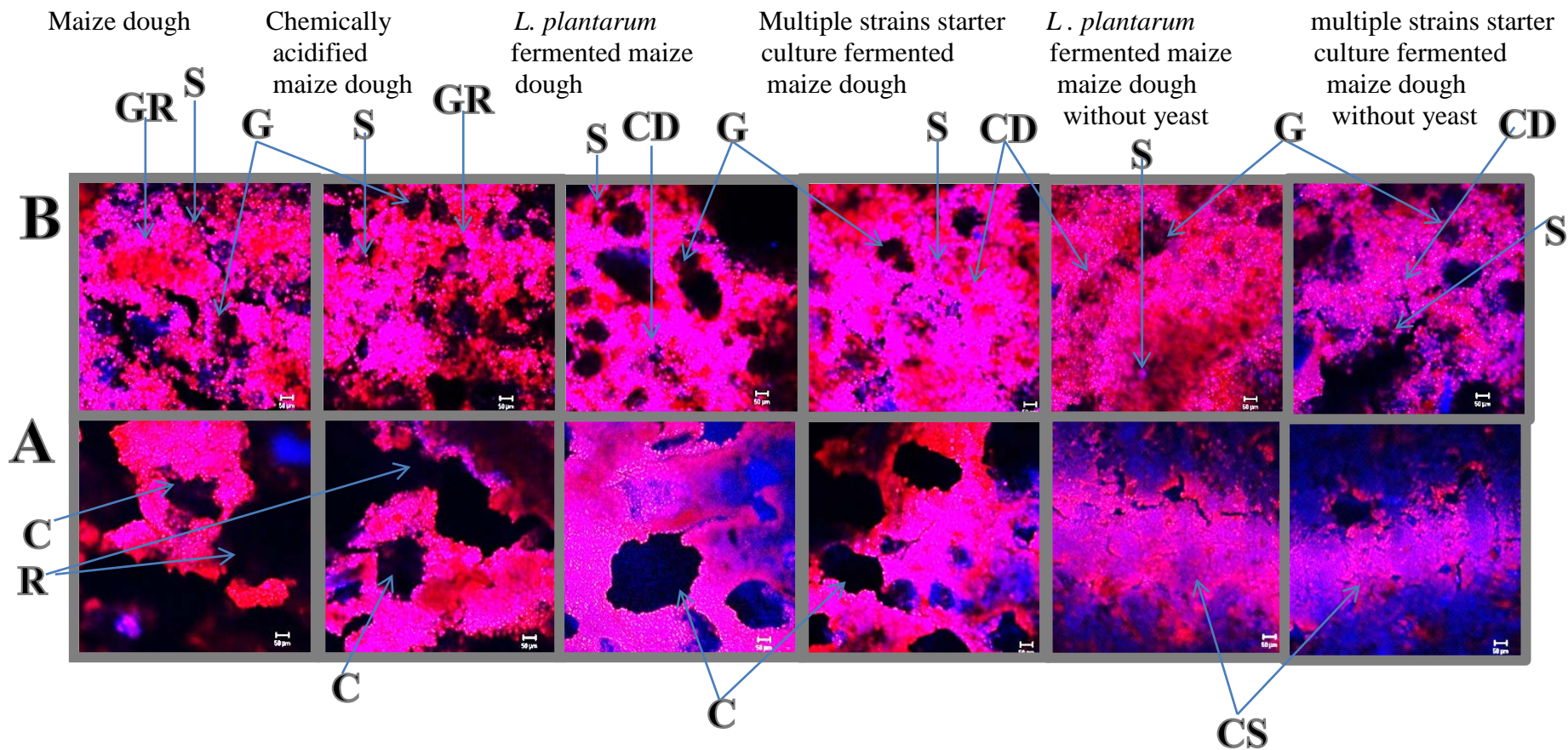
Fig 4.2.2: Effects of *L. plantarum* or multiple strains starter culture fermentation with or without yeast on the crumb structure of maize bread

Dough structural properties

When viewed by CLSM, all the maize dough treatments showed the presence of gas cells (Fig. 4.2.3B). However, the gas cells were more distinct in the maize sourdoughs. The sourdoughs also showed a cohesive (a complete and continuous matrix) dough structure compared to the chemically acidified or straight maize doughs which showed more grainy dough structure. Also, few individual starch granules were visible in the sourdoughs, whereas many discrete starch granules were visible in the chemically acidified and straight doughs.

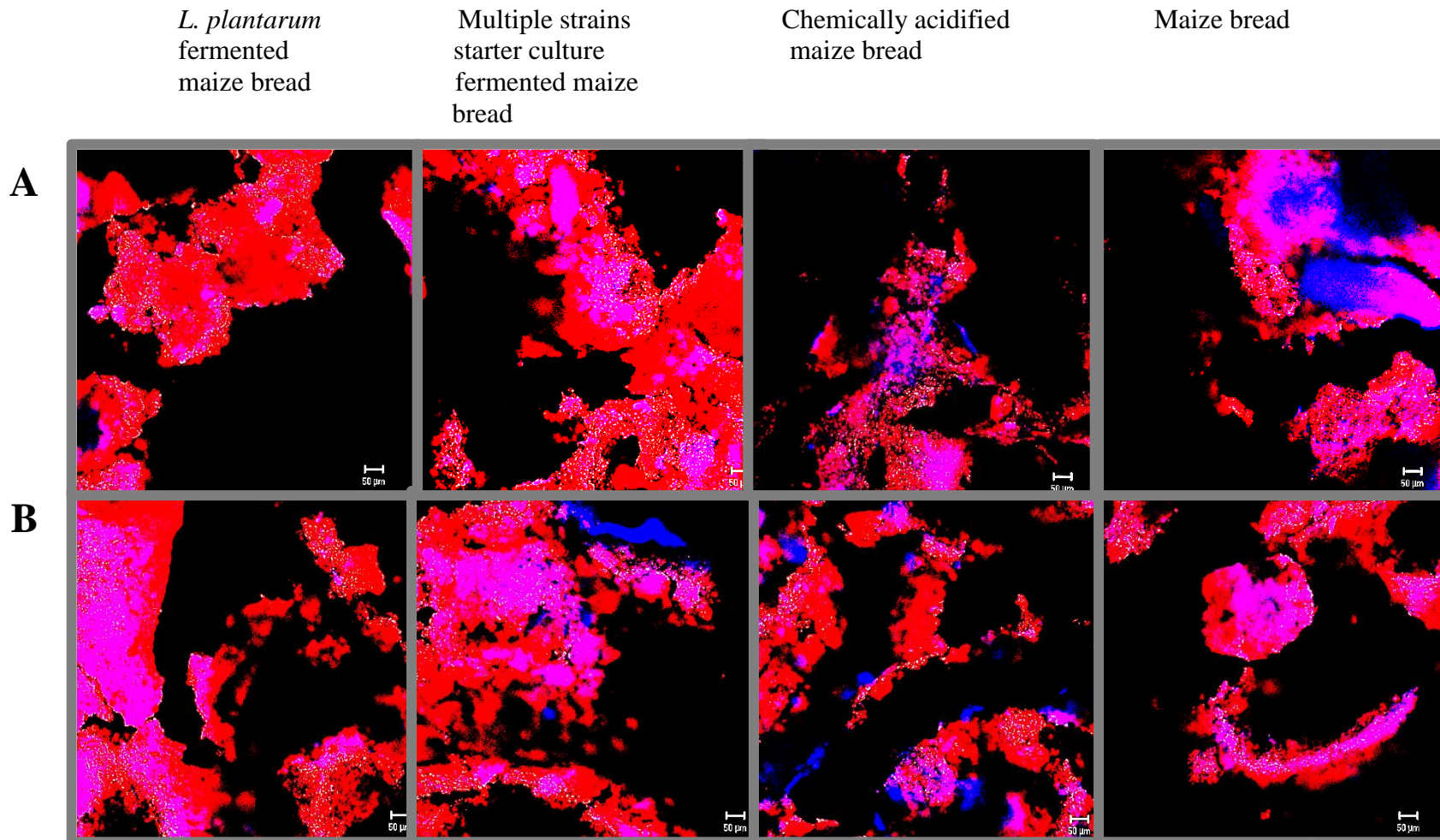
The presence of more distinct gas cells in the sourdoughs is indicative of dough modification improving the dough's ability to withstand the pressure of the expanding CO₂ in the gas cells without collapsing. According to Edema *et al.* (2013), sourdough fermentation improves fonio dough by increasing the water absorption capacity of the dough. This in turn improved dough strength and gas holding capacity of the dough. The cohesive dough structure observed in the sourdough breads may be related to endosperm matrix protein degradation as explained by Schober *et al.* (2007). 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 (Goesaert *et al.*, 2005), which probably resulted in the formation of a cohesive dough structure, as observed. The presence of gas cells in the sourdoughs without yeast was probably due to the carbon dioxide produced by the action of the naturally occurring lactic acid bacteria and yeast in the sourdough as a result of sourdough fermentation.

Combining the modern sourdough method with the microwave injera method did not make the gas cells of the maize breads more distinct when viewed by CLSM. In fact, no gas cells were observed (Fig. 4.2.4). Absence of gas cells when viewed by CLSM was probably due to the bread being more susceptible to crumbling due to its softness. Since, the breads were prepared by steaming in a microwave and not by baking, they probably had a higher moisture content than they would have had if they were baked. The higher moisture content most likely contributed to the softness of the bread and its tendency to crumble. The sample preparation procedure for CLSM involved sticking the maize breads to the slides by pressing them firmly. Pressing was required because the maize bread had to be held inverted in the microscope. Therefore, since the breads were soft, pressing probably altered the microstructure of the bread.



A: maize bread, B: maize dough, G: gas cell, C: cell, R: rupture, CS: compact crumb structure, GR: grainy crumb structure, S: starch granule, CD: cohesive dough structure

Fig 4.2.3: Confocal laser scanning microscopy images showing the effects of *L. plantarum* or multiple strains starter culture fermentation with or without yeast on the microstructure of maize dough and maize bread



A: not pre-gelatinized: part of maize flour not cooked, **B:** pre-gelatinized: part of maize flour cooked

Fig 4.2.4: Confocal laser scanning microscopy images showing the effects of *L. plantarum* or multiple strains starter culture fermentation and pre-gelatinization on the microstructure of microwaved maize bread

Stress relaxation of the maize dough treatments

The sourdoughs with or without yeast required a lower amount of force to compress to 25% strain (Fig. 4.2.5). They also required a shorter relaxation time [the time required for the maximum force of compression to drop to 36.8% of its value (Singh *et al.*, 2006)] than the chemically acidified maize dough or the straight maize dough with or without yeast added.

The lower amount of force required and shorter relaxation time required by maize sourdoughs compared to that required by the chemically acidified and straight dough indicates that the sourdoughs were softer, and less elastic. However, relaxation times of the sourdoughs (irrespective of whether yeast was added or not) being shorter than the chemically acidified and straight dough with or without yeast suggests that the presence or absence of yeast in the sourdoughs is independent of their relaxation times. Keentok *et al.* (2002) associated strong wheat flours with longer relaxation times and weak flours with shorter relaxation times. The terms strong and weak when used to describe wheat flour refers to the quality of the gluten in the flour used for bread production (Tipples *et al.*, 1994). However, this may not be the case for maize gluten-free dough.

Dough rheology under simulated baking conditions

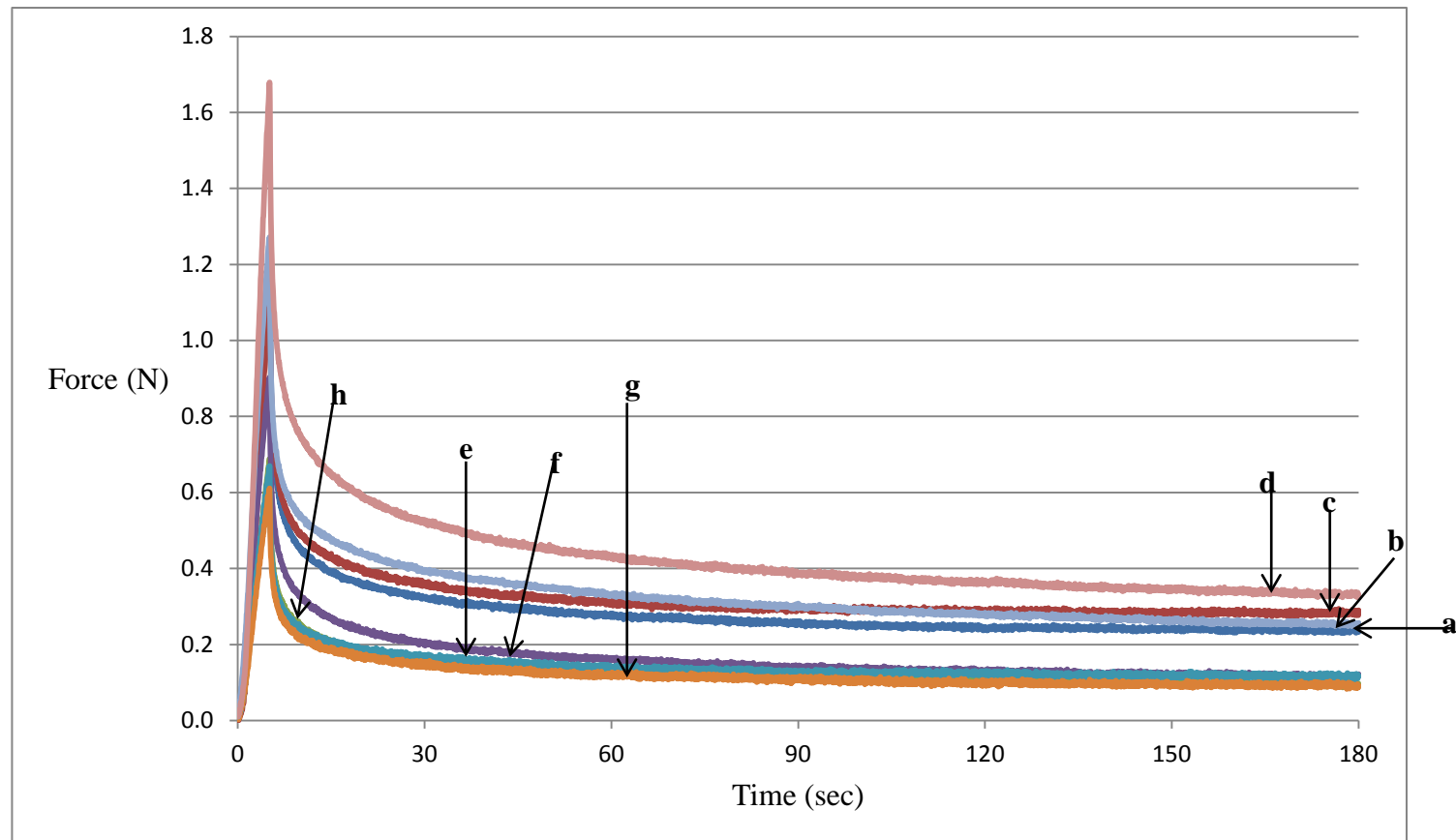
With strain sweep analysis, none of the treatments showed a significant effect on the linear viscoelastic region of the maize doughs (Fig. 4.2.6A I and II, Fig. 4.2.7A I and II). However, all the treatments showed a higher elastic modulus (G') than the loss modulus (G''), with the sourdoughs with yeast having the lowest elastic modulus and loss modulus. Concerning the temperature sweep analysis, though the elastic modulus (G') and complex viscosity (η^*) curves of all the treatments initially diminished in the same pattern, the maize sourdoughs with or without yeast had lower G' and η^* than the chemically acidified maize dough and straight maize dough with or without yeast (Fig. 4.2.6B I and II, Fig. 4.2.7B I and II). As the temperature increased to 85°C through to 95°C, G' and η^* of all the treatments became similar. At around 90°C, G' and η^* of all the treatments started to decrease with *L. plantarum* maize sourdough without yeast having the lowest values at around 130°C. At around 140-150°C, maize sourdoughs without yeast showed lower G' and η^* when compared to chemically acidified maize dough and straight maize dough without yeast. However, there was no clear behaviour pattern of the treatments with yeast added. Maize sourdoughs without yeast when compared to chemically acidified maize

dough and straight dough, had higher $\tan \delta$ at the beginning of the analysis. According to Stathopoulos *et al.* (2008), a higher $\tan \delta$ indicates a greater degree of viscous behaviour (less elastic) while a lower $\tan \delta$ indicates more elastic behaviour. At around 65-75°C, maize sourdoughs showed a considerable increase in $\tan \delta$ compared to that observed in chemically acidified or straight maize dough. However, all the treatments showed similar $\tan \delta$ around 85-90°C. At 149-150°C, $\tan \delta$ of maize sourdoughs started to decrease, while that of the chemically acidified maize dough and straight maize doughs started to increase. $\tan \delta$ for the treatments with yeast showed a lot of fluctuations and no clear pattern was achieved.

Moroni *et al.* (2011) who investigated the impact of sourdough on buckwheat flour, batter and bread, also found higher elastic modulus than viscous modulus. Clarke *et al.* (2002), and Angioloni *et al.* (2006) who worked on the influence of sourdough fermentation on the fundamental rheological properties of wheat dough also found that the addition of sourdough led to a less elastic bread dough.

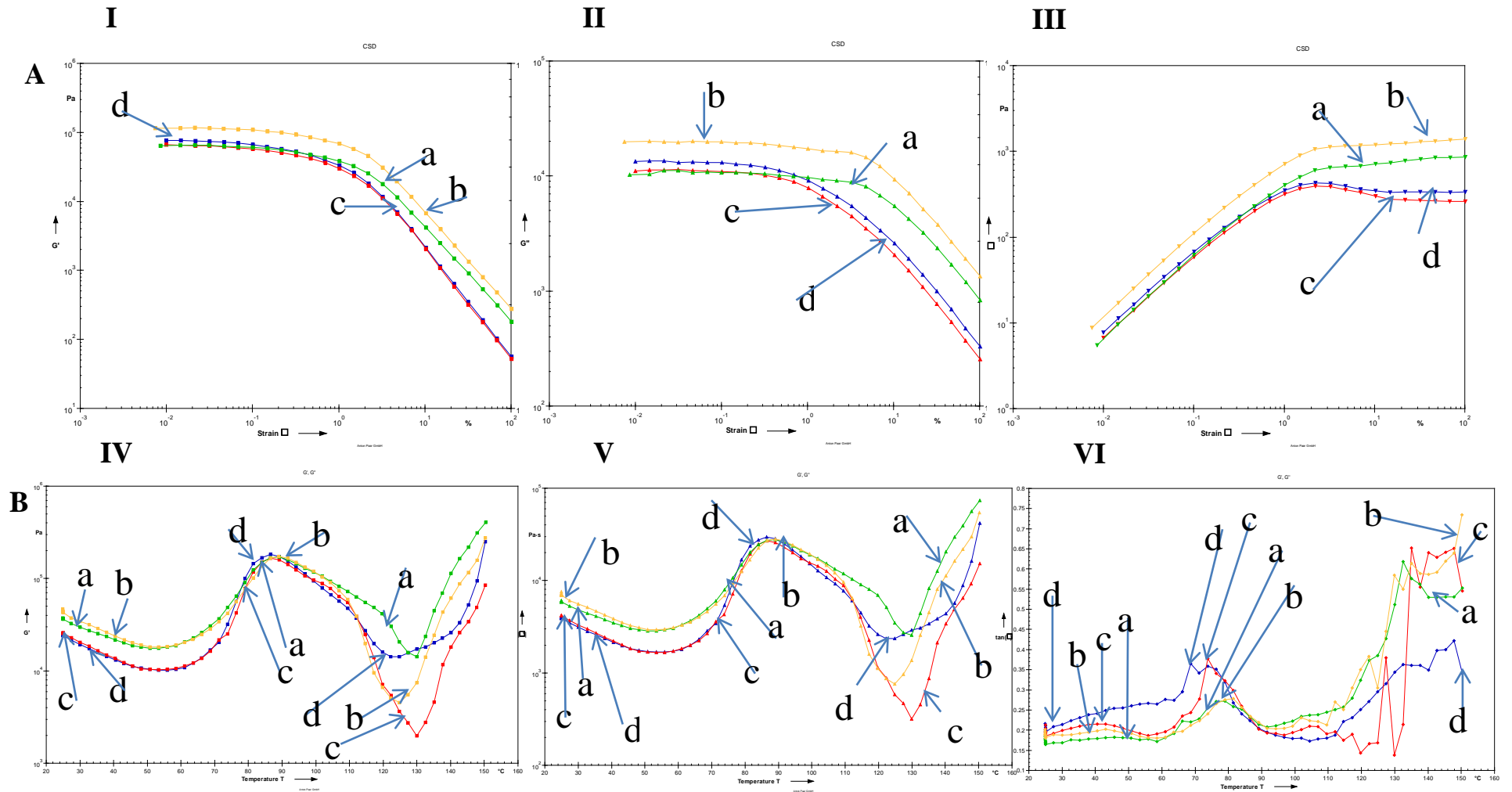
The linear viscoelastic region of the maize dough treatments being similar whether or not sourdough or yeast was added suggests that sourdough fermentation or/and yeast addition had no effect on the linear viscoelastic region of the maize dough. Salvador *et al.* (2006) who investigated the effects of adding NaCl, sucrose and yeast on dynamic rheological characteristics of wheat flour–water doughs, reported that the addition of ingredients such as salt, sugar and yeast at different concentrations did not significantly modify the extension of the linear viscoelastic region. The higher elastic modulus than viscous modulus exhibited by all the maize doughs suggests that the doughs are viscoelastic solids, exhibiting more elastic properties than viscous properties. That maize sourdoughs had lower elastic modulus, loss modulus, and required a lower shear stress than the chemically acidified and straight doughs also indicates that the sourdoughs had softer or less elastic dough. This suggests that sourdough fermentation had an effect on the elasticity of maize dough which was not brought about by chemical acidification. According to Hammes & Gänzle, (1998) and Thiele *et al.* (2002), sourdough fermentation process does much more to influence the properties of the dough than simply produce acid. Clarke *et al.* (2002) assumed that the time frame during which enzyme activity could affect the dough constituents was shorter for chemically acidified dough compared to the biologically acidified doughs. They explained that the changing pH values during sourdough fermentation period may also afford passage through a range of pH values close to the

optimum for various enzymes present in the dough system. Thus, they suggested that the activity of the protease and amylase enzymes present may be influenced to a greater extent by the pH profile of the biological acidification fermentation period in contrast to the almost instant nature of the chemically acidified time frame. Also, the sourdoughs with yeast having the lowest elastic modulus and loss modulus compared to the other treatments shows that yeast also contributed to the softness of the dough. Adding yeast to the already soft sourdough probably further contributed to its softness. Salvador *et al.* (2006) also found that the addition of yeast to wheat dough showed lower storage modulus and loss modulus than samples without yeast. The decrease in $\tan \delta$ of maize sourdoughs without yeast at the end of the temperature sweep analysis probably indicates that the sourdoughs became strong enough to resist rupturing (Fig. 4.2.3A). However, this was not the case for chemically acidified or straight dough maize bread which easily ruptured. Fluctuations were observed in $\tan \delta$ of maize dough treatments with yeast added, making it difficult to achieve a clear behavioural pattern. This may be due to the activities of the yeast present in the dough.



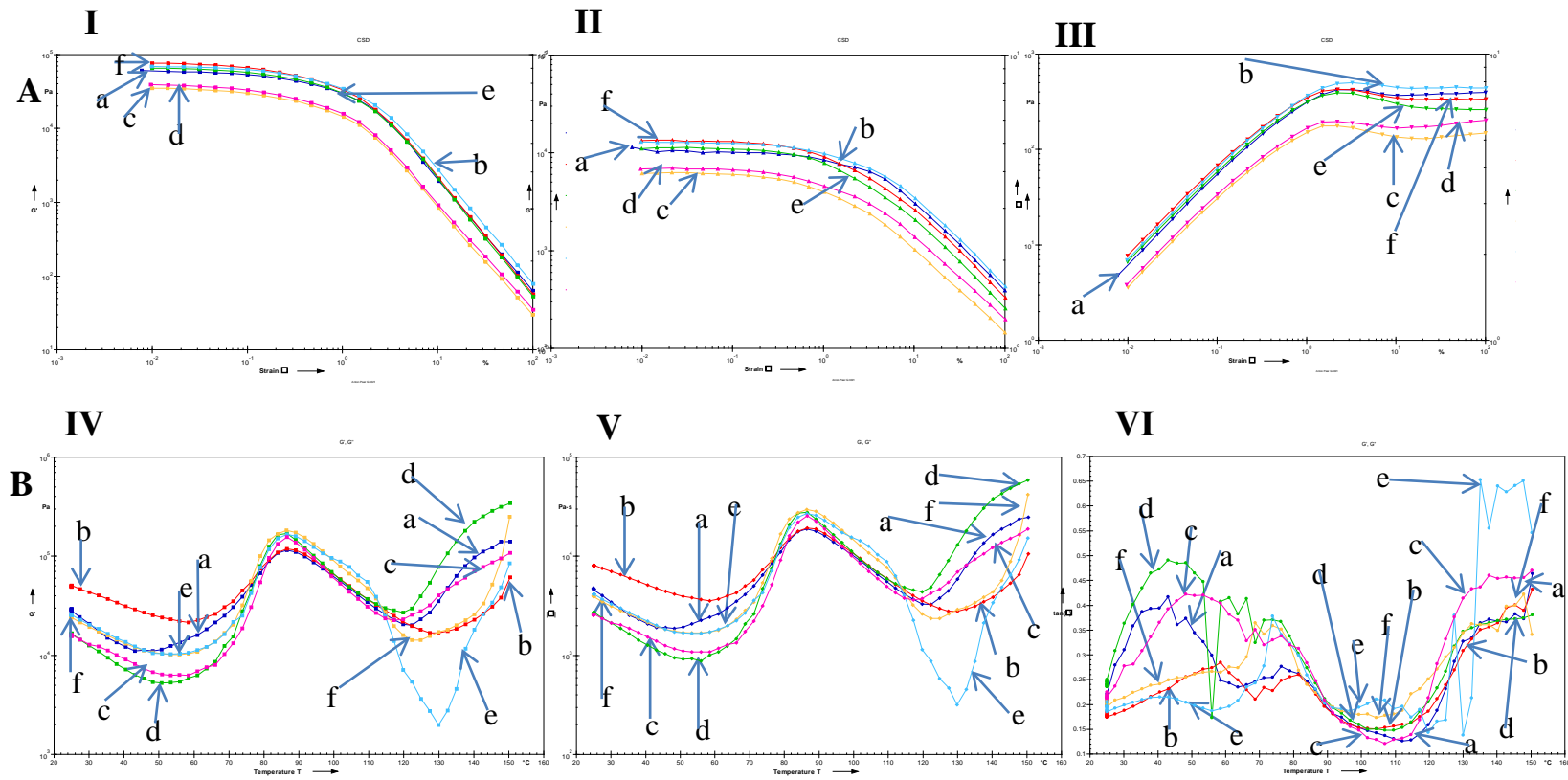
a: maize dough with yeast. **b:** maize dough without yeast. **c:** chemically acidified maize dough with yeast. **d:** chemically acidified maize dough without yeast. **e:** *L. plantarum* fermented maize dough with yeast. **f:** *L. plantarum* fermented maize dough without yeast. **g:** multiple strains starter culture fermented maize dough with yeast. **h:** multiple strains starter culture fermented maize dough without yeast

Fig 4.2.5: Effects of *L. plantarum* or multiple strains starter culture fermentation with or without yeast on stress relaxation of maize dough



a: maize dough. **b:** chemically acidified maize dough. **c:** *L. plantarum* fermented maize dough. **d:** multiple strains starter culture fermented maize dough

Fig 4.2.6: Effects of *L. plantarum* fermentation or multiple strains starter culture fermentation on the rheological properties of maize dough. **A:** strain sweep analysis parameters within a strain of 0.01-100% (I: elastic modulus and linear viscoelastic region, II: loss modulus, III: shear stress), **B:** temperature sweep analysis parameters within a temperature range of 25-150°C (IV: elastic modulus, V: complex viscosity, VI: $\tan \delta$)



a: maize dough with yeast. **b:** chemically acidified maize dough with yeast **c:** *L. plantarum* fermented maize dough with yeast. **d:** multiple strains starter culture fermented maize dough with yeast. **e:** *L. plantarum* fermented maize dough **f:** multiple strains starter culture fermented maize dough

Fig 4.2.7: Effects of *L. plantarum* fermentation or multiple strains starter culture fermentation with or without yeast on the rheological properties of maize dough. **A:** strain sweep analysis parameters within a strain of 0.01-100% (I: elastic modulus and linear viscoelastic region, II: loss modulus, III: shear stress), **B:** temperature sweep analysis parameters within a temperature range of 25-150°C (IV: elastic modulus, V: complex viscosity, VI: tan δ)

Damaged starch in the maize dough

Sourdough fermentation did not have a clear effect on starch damage in the maize dough (Table 4.2.2). Edema *et al.* (2013) found that sourdough fermentation did not have a significant effect on starch damage in sorghum flour. These authors suggested that the increase in broken starch granules was not sufficient to significantly affect iodine absorption, by which starch damage was measured. However, there was a reduction in the amount of starch damage with the multiple strain starter culture fermented maize sourdough. Elkhailifa *et al.* (2005) found that sourdough fermentation decreased the amount of damaged starch in sorghum flour. These authors suggested that this may be due to damaged starch being the preferred substrate for microbial growth during the fermentation process. This probably suggests that reduction in the amount of starch damage with the multiple strain sourdough was due to consumption of its damaged starch by other microorganism present.

Dough thermal properties

The multiple strains sourdough with or without yeast had lower onset endotherm than the other treatments (Table 4.2.3). However, the addition of yeast to this sourdough resulted in an even lower onset and peak endotherm. The chemically acidified maize dough with yeast and the straight maize dough with yeast had lower onset and peak endotherm than the *L. plantarum* fermented sourdough with or without yeast, but they had higher onset and peak endotherms when not combined with yeast. Maize sourdoughs with or without yeast had a higher enthalpy for the endothermic peak, indicating a higher energy requirement to disrupt the starch granules. León *et al.* (1997) working on starch changes occurring in wheat bread baking and storage, and Sanz-Penella *et al.* (2012) working on developing whole wheat bread with improved nutritional quality using sourdough, both reported that the gelatinization enthalpy of fermented doughs were higher than that of the unfermented doughs.

The changes observed in the thermal properties of the doughs brought about by sourdough fermentation also indicate some starch modification. According to Murphy (2000), acid predominantly depolymerises the amorphous regions of the starch granule such that when the starch is heated beyond its gelatinisation temperature, starch granules rupture quickly. If the amorphous region is depolymerised, only the crystalline region is left, therefore, more energy will be required to depolymerise this region. Also, acidification increases the

water binding capacity of starch granules (Hammes & Gänzle, 1998). This suggests that lactic acid produced by lactic acid bacteria during sourdough fermentation of maize dough had an effect on the glycosidic bonds in the starch granule, hydrolysing them and enabling the starch granules to absorb water faster. However, since the chemical acidification did not produce the same effect, part of the effect of the lactic acid fermentation was probably due to the activities of endogenous enzymes in the sourdough microorganisms favoured by the low pH, as proposed by Edema *et al.* (2013). These authors explained starch modification as slight changes in the starch granules, which probably increased water absorption. Similarly, León *et al.* (1997) and Sanz-Penella *et al.* (2012) attributed the higher gelatinization enthalpy required by the sourdoughs to be due to better starch hydration during the period of fermentation.

Table 4.2.2: Effects of *L. plantarum* fermentation or multiple strains starter cultures fermentation on the starch damage of maize dough

Treatments	Starch damage (AACC 76-31)
Maize dough	3.7 ^{ab} ± 0.4 ¹
Chemically acidified maize dough	3.9 ^b ± 0.1
<i>L. plantarum</i> fermented maize sourdough	3.8 ^b ± 0.1
Multiple strains starter culture fermented maize sourdough	3.2 ^a ± 0.1

¹Means and standard deviation n=2. Values in the same column followed by different letters are significantly different at $p \leq 0.05$.

Table 4.2.3: Effects of *L. plantarum* or multiple strains starter culture fermentation with or without yeast on the thermal properties of maize dough

Treatments	Onset temperature (°C)	Peak temperature (°C)	Endset temperature (°C)	Enthalpy (ΔH) (J/g)
Maize dough with yeast	71.5 ^{ab} ± 0.2 ¹	78.1 ^{bc} ± 0.5	84.4 ^a ± 0.1	0.46 ^a ± 0.09
Chemically acidified maize dough with yeast	71.6 ^{ab} ± 0.1	77.8 ^{abc} ± 0.0	84.2 ^a ± 0.1	0.44 ^a ± 0.04
<i>L. plantarum</i> fermented maize dough with yeast	72.7 ^{bc} ± 0.1	78.6 ^c ± 0.4	85.9 ^{ab} ± 0.2	0.89 ^b ± 0.02
Multiple strains starter culture fermented maize dough with yeast	70.6 ^a ± 0.9	77.0 ^a ± 0.4	83.6 ^a ± 1.3	0.56 ^a ± 0.00
Maize dough without yeast	73.4 ^c ± 0.0 ¹	81.1 ^e ± 0.4	88.7 ^{bc} ± 0.0	0.38 ^a ± 0.02
Chemically acidified maize dough without yeast	73.8 ^c ± 0.1	82.1 ^e ± 0.1	89.8 ^c ± 0.1	0.49 ^a ± 0.00
<i>L. plantarum</i> fermented maize dough without yeast	72.9 ^{bc} ± 1.9	79.8 ^d ± 0.4	89.8 ^c ± 0.9	1.06 ^b ± 0.28
Multiple strains starter culture fermented maize dough without yeast	71.4 ^{ab} ± 0.4	77.3 ^{ab} ± 0.8	88.1 ^{bc} ± 3.7	1.13 ^b ± 0.19

¹Means and standard deviation n=2. Values in the same column followed by different letters are significantly different at $p \leq 0.05$

Based on the above findings, it is suggested that sourdough fermentation brought about starch modification which in turn had an effect on the rheological properties of the dough making it cohesive, soft and less elastic. The addition of baker's yeast not only contributed significantly to dough leavening, but also contributes to the softness of the dough. However, sourdough addition did not contribute significantly to dough leavening. Hammes & Ganzle (1997) noted that gas formation by the sourdough microorganism is only of minor importance when baker's yeast is additionally applied in sourdough. Based on the results of Clarke *et al.* (2002) study, they assumed that the amount of gas produced by sourdough organisms does not contribute substantially to the increase in loaf specific volume. It is, however, suggested that, sourdough fermentation in combination with the addition of baker's yeast, improved maize bread quality.

Edema *et al.* (2013) who worked on improvement of fonio dough properties by sourdough fermentation and Schober *et al.* (2007) whose study was conducted to improve the quality and theoretical understanding of gluten-free sorghum bread, found that sourdough fermentation improved the quality of fonio and sorghum breads, respectively. Edema *et al.* (2013) attributed the improvement in fonio bread quality brought about by sourdough fermentation specifically to starch modification (slight granule swelling and probably some leaching of starch molecules) by endogenous amylases from the sourdough microorganism whose activities were favoured at low pH. Schober *et al.* (2007) also attributed the improvement in sorghum bread to dough modification. These authors concluded that the major effect of sourdough fermentation was the degradation of proteins soluble in the dough liquid. They further proposed that if these proteins were not degraded, they would aggregate upon baking and interfere with the starch gel. As a result, sorghum bread without sourdough fermentation tended to have a large hole in the crumb.

4.2.5 Conclusions

Sourdough fermentation of maize dough substantially increases loaf volume and results in a more open crumb structure of the bread when in combination with baker's yeast. Sourdough fermented maize dough is softer and less elastic, but less crumbly than chemically acidified maize dough or straight maize dough. It appears that the improvement in maize bread quality is due to starch granule modification, which although it makes the dough less elastic, improves its ability to trap carbon dioxide produced by the activities of baker's yeast, and withstand the pressure of the expanding gas in the dough. It may be proposed that softer cohesive dough is the key to make good gluten-free maize bread without the use of additives.

The dominant lactic acid bacteria in the maize sourdoughs was then identified and characterised to determine how it contributed to starch modification.

4.2.6 References

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4.3 Research Chapter 3: Identification and characterisation of lactic acid bacteria in maize sourdoughs

4.3.1 Abstract

Lactobacillus plantarum (strain B411) and multiple strains starter culture fermented maize sourdoughs have successfully been used to produce maize bread with improved quality (high loaf volume, open and soft crumb structure). The dominant lactic acid bacteria in these sourdoughs were characterised and identified using MALDI-TOF and found to be *L. plantarum*. These dominant lactic acid bacteria were tested for amylolytic and proteolytic properties. It was only the *L. plantarum* from the multiple strains sourdough that exhibited amylolytic property. However, the dominant lactic acid bacteria in both sourdoughs exhibited proteolytic properties. Greater proteolytic activity was observed in the *L. plantarum* (B411) sourdough. Relating these findings to the improvement in maize bread quality, it is suggested that the amylolytic and proteolytic activities of the lactic acid bacteria brought about starch modification either directly by hydrolysing the starch granules, thereby creating a larger surface area and hence increased water absorption, and/or increasing the accessibility of water to the starch as a result of hydrolysis of the endosperm protein matrix and proteins soluble in the dough liquid, binding to the starch granules. It appears that modification of the starch affected the rheological properties of the maize dough, making it less elastic and improved its ability to trap and withstand the pressure of the expanding carbon dioxide during fermentation and baking.

4.3.2 Introduction

Lactic acid bacteria (LAB) are a group of related bacteria that produce lactic acid from carbohydrates through fermentation as a major metabolic product. LAB have been used to ferment or culture foods for at least 4000 years (reviewed by Reddy *et al.*, 2008). *Lactobacilli* vary in morphology from long, slender rods to short coccobacilli, which frequently form chains. However, under certain conditions some LAB do not display all these characteristics. Thus, the most profound features of LAB are Gram positiveness and inability to synthesize porphyrin groups (Axelsson, 2004). The inability to synthesize porphyrin (for example haem) results in the LAB being devoid of catalase. The taxonomy of LAB has been based on the Gram reaction and the production of lactic acid from various fermentable carbohydrates. Typical LAB are Gram-positive, non-sporing, catalase-negative, devoid of cytochromes, anaerobic but aerotolerant cocci or rods that are acid tolerant and produce lactic acid as the major end product during sugar fermentation (Axelsson, 2004). LAB grow under anaerobic conditions but they can grow in the presence of oxygen. Because of low energy yields, LAB often grow more slowly than microbes capable of aerobic respiration, and produce smaller colonies of 2-3 mm. LAB can grow at a temperature range of 5–45°C (reviewed by Reddy *et al.*, 2008). LAB have complex nutritional requirements for amino acids, peptides, nucleotidebases, vitamins, minerals, fatty acids and carbohydrates (reviewed by Reddy *et al.*, 2008). The LAB are divided into three groups based on fermentation patterns: homofermentative LAB which produce more than 85% lactic acid from glucose and produce lactic acid as the major product of fermentation; heterofermentative LAB which produce only 50% lactic acid together with ethanol and carbon dioxide; and lastly the less well known heterofermentative LAB species which produce DL-lactic acid, acetic acid and carbon dioxide (reviewed by Reddy *et al.*, 2008).

As stated, sourdough is a mixture of flour and water fermented by naturally occurring LAB and yeast. Although these sourdough microorganisms originate mainly from the flours and process equipment, the resulting composition of the sourdough microbiota is determined by endogenous (for example, chemical and enzyme composition of the flour) and exogenous (for example, temperature, redox potential, dough yield and time of the fermentation process) factors (Hammes & Gänzle, 1998). LAB are the dominant microorganisms in sourdoughs, and the rheology, flavour and nutritional properties of sourdough-based baked products greatly rely on the activity of LAB (Gobbetti *et al.*,

2005). LAB in mature sourdoughs occur in high numbers $>10^8$ cfu/g (Ehrmann & Vogel, 2005).

Amylolysis and proteolysis are among the enzymic activities of LAB in sourdough (Corsetti *et al.*, 1998). However, these properties vary among LAB organisms. Amylolytic LAB (ALAB) are a group of LAB that have the ability to partially hydrolyze raw starch through the activities of their α -amylases (Rodriguez-Sanoja *et al.*, 2000). ALAB have been reported in different tropical amylaceous fermented foods, prepared from cassava and cereals such as maize and sorghum (Reddy *et al.*, 2008). ALAB are also involved in cereal-based fermented foods such as European sour rye bread, Asian salt bread, dumplings and non-alcoholic beverages production. ALAB are important because they can metabolise starch into lactic acid in a single step fermentation (Reddy *et al.*, 2008). Lactic acid bacteria also possess a variety of proteolytic enzymes that facilitate their growth (Matar *et al.*, 2001). The proteolytic systems of these LAB hydrolyze proteins to peptides and then to amino acids, which is essential for bacterial growth (Liu *et al.*, 2010).

This chapter will focus on identification and characterisation of the dominant LAB in the *Lactobacillus plantarum* (B411) and multiple strains starter culture fermented maize sourdoughs. In the previous chapter, these sourdoughs were found to produce maize bread with improved quality (high loaf volume, open and soft crumb structure). The role of the dominant LAB in improving the quality of the maize bread will be discussed.

4.3.3 Experimental

4.3.3.1 Materials

As previously, refined maize meal (Impala Special Maize Meal, Premier Foods, Isando, South Africa) with a protein content 8.6 g/100 g (db) and a fat content 2.7 g/100 g (db) was milled into a flour using a laboratory hammer grinder (Mikro-Feinmuhle-Culatti MFC grinder, Janke and Kunkel, Staufen, Germany) fitted with a 0.5 mm opening screen. The *Lactobacillus plantarum* culture (B411) was obtained from the Council for Scientific and Industrial Research, Pretoria, South Africa.

4.3.3.2 Methods

Preparation of the sourdoughs

L. plantarum fermented maize sourdough was prepared by mixing maize flour with sterile distilled water containing *L. plantarum* (B411) cells (9.3×10^{10} cfu/ml) in a ratio of 1:1 (w/v). The slurry was fermented at 30°C to a pH range of 3.3-3.6 (approx. 24 h). Multiple strains starter culture fermented maize sourdough was prepared by mixing maize flour with sterile distilled water. The maize dough was left to ferment for 72-96 h at ambient temperature. A portion of the fermented maize dough was used as a starter (backslopping) for a fresh mixture of maize flour and water. The mixture was fermented at 30°C to a pH of 3.4-3.7 (approx. 48 h).

Selection and purification of isolates in the maize sourdoughs

L. plantarum fermented maize sourdough and multiple strains starter culture fermented maize sourdough were plated on mMRS agar (de Man, Rogosa and Sharpe agar modified with 1% (w/v) maltose and 5% (w/v) yeast extract (Coda *et al.*, 2011), pH adjusted to 5.6 with 0.1M HCl). Six isolates each were randomly selected from the plate with the highest dilution (10^{-9}) for each of the sourdoughs. These isolates were streaked on mMRS agar until a pure culture was obtained.

Morphological properties of the colonies

This was done by physically observing the size, shape and appearance of the colonies with the eyes.

Biochemical analyses

Catalase test

This was performed to determine if the isolates were catalase positive or negative. It involved preparing a smear of the isolate using 3% (v/v) hydrogen peroxide (Olutiola *et al.*, 2000). Oxygen bubble formation is an indication of the presence of catalase.

Gram's test

Eighteen to 24 hour old isolates were heat fixed on a slide. Staining was done with crystal violet solution for 2 min and rinsed off with Gram's iodine solution. The slides were washed with 95% alcohol and rinsed under gentle running water. Counter staining was done with safranin. The slides were then washed, blotted dry and viewed under the microscope (Olutiola *et al.*, 2000). A purplish colour is an indication that the isolate is Gram positive, while a pinkish colour is an indication that the isolate is Gram negative.

Amylolytic activity of isolates

Isolates were tested for their ability to hydrolyze starch. Starch agar was prepared (beef extract (3 g), soluble starch (10 g), agar (12 g) in 1 L of distilled water). The isolates were grown on the starch agar for 48 h at 30°C, after which the plates were flooded with Lugol's iodine solution. Clear zones around the colonies are a indication of starch hydrolysis (Hashim *et al.*, 2004).

Proteolytic activity of isolates

Isolates were tested for proteolytic activity. MRS-caseinate agar (MRS agar (62 g), sodium caseinate (10 g), tri-sodium citrate (3.8 g), calcium chloride (2.2 g) in 1 L of distilled water) was prepared. The isolates were grown on the starch agar for 48 hr at 30°C (Williams & Banks, 1997). White zone formation around the colonies is an indication of proteolytic activity (Vermelho *et al.*, 1996).

Identification of the isolates

The isolates were identified using a MALDI Biotyper 3.0, Bruker Daltonik, Bremen, Germany (Standing *et al.*, 2013). The MALDI Biotyper identifies microorganisms using MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight) mass spectrometry measuring the unique protein fingerprint of an organism. Specifically, the MALDI Biotyper measures highly abundant proteins that are found in all microorganisms

(such as ribosomal or nucleic acid-binding proteins) and uses it as a biomarker (Pineda *et al.*, 2003). The characteristic patterns of these proteins are used to reliably identify a particular microorganism by matching the respective patterns with an extensive open database (MALDI Biotyper Real Time Classification 3.0) to determine the identity of the microorganism down to the species level.

4.3.4 Results and discussion

Morphology of the colonies

All the isolates from the *L. plantarum* or multiple strains starter culture fermented maize sourdoughs were puntiform, dome shaped with entire edge (Tables 4.3.1 and 4.3.2). They also had opaque and smooth surface.

Biochemical tests

Catalase test

All the isolates were catalase negative (Tables 4.3.1 and 4.3.2). This means that they did not produce porphyrin and suggests that they were all LAB (Axelsson, 2004). According to this author, the inability of LAB to synthesize porphyrin groups is one of their profound characteristics. This inability results in the LAB being devoid of catalase.

Gram's test

The isolates from both the *L. plantarum* and multiple strains starter culture fermented maize sourdough were purplish when viewed by light microscopy (Fig. 4.3.1). This indicates that they were Gram positive. Also, isolates from the *L. plantarum* sourdough appeared rod-like in shape. In contrast, the isolates from the multiple strains sourdough appeared more like cocci, joint together like short chains. A key feature of LAB are that they are Gram positive (Axelsson, 2004).

Amylolytic activity of isolates

All the isolates from the multiple strains sourdough showed clear zones when Lugol's iodine was poured over the growth area (Fig. 4.3.2), an indication of amylolytic activity (Hashim *et al.*, 2004). Isolates from the *L. plantarum* sourdough did not show clear zones, indicating the absence of amylolytic activity. According to Wehrle & Arendt (1998), the rapid drop in pH level in sourdough can cause reduced amylolytic activity, whereas the slower drop in the pH level in spontaneously fermented dough permits further starch degradation. Since the *L. plantarum* sourdough was incubated for a shorter time period (24 h) than for the multiple strains sourdough (48 h), it can be speculated that this may be the reason for the absence of amylolytic property in the isolates from the *L. plantarum* sourdough. However, this speculation was disproved when the actual *L. plantarum* culture used as a starter culture for the *L. plantarum* sourdough did not exhibit amylolytic property.

Proteolytic activity of the isolates

All the isolates from the *L. plantarum* sourdough showed white zones around the colony growth, an indication of proteolytic activity (Vermelho *et al.*, 1996) (Fig. 4.3.3). However, only one of the isolates from the multiple strains sourdough showed white zone around the colony. This suggests lower proteolytic activity in the multiple strains sourdough isolates than in the isolates from the *L. plantarum* sourdough.

Identification of isolates from the sourdoughs

All the isolates were identified as *L. plantarum*. As stated, *L. plantarum* has been shown to be the dominant organism at the end of the fermentation of maize-derived products like ogi (fermented maize gruel popularly consumed in West Africa) (Steinkraus, 1995). There are generally four factors that account for the dominance of lactobacilli in a sourdough, namely their highly adapted carbohydrate metabolism, their growth requirements for temperature and pH that match the conditions encountered during sourdough fermentation, their possible stress responses, and their excretion of antimicrobial compounds which may inhibit the growth of other microorganisms (reviewed by De Vuyst & Neysens, 2005). Some LAB occurring in sourdoughs are sensitive to low pH and therefore will not survive for long. More acid-resistant species will be able to survive for longer and eventually, may become dominant (Clarke & Arendt, 2005). Since the pH of the sourdoughs were low (< 4), a more suitable environment was probably created for the *L. plantarum* to thrive better than the other microorganisms in the dough. The dominance of *L. plantarum* at the late stages of cereal fermentation has been attributed to its high acid tolerance (Oyewole & Odunfa, 1990; Hounhouigan *et al.*, 1993), or perhaps better substrate utilization (Oyewole & Odunfa, 1990). Weckx *et al.* (2010) attributed the dominance of *L. plantarum* in sourdoughs not only to its high acid-tolerance but also to its ability to transport and metabolize different plant carbohydrates.

Table 4.3.1: Characterisation and identification of isolates from *L. plantarum* fermented maize sourdough

<i>L. plantarum</i> fermented maize sourdough	Isolate codes	Morphology of the isolates	Gram staining test	Catalase Test	Amylolytic property	Proteolytic property	Organism (best match) as Identified by the MALDI-TOF
	ISMA	Puntiform, dome shaped with entire edge. Opaque and smooth surface	+	-	-	+++	<i>Lactobacillus plantarum</i>
	ISMB	Puntiform, dome shaped with entire edge. Opaque and smooth surface	+	-	-	+++	<i>Lactobacillus plantarum</i>
	ISMC	Puntiform, dome shaped with entire edge. Opaque and smooth surface	+	-	-	++	<i>Lactobacillus plantarum</i>
	ISMD	Puntiform, dome shaped with entire edge. Opaque and smooth surface	+	-	-	++	<i>Lactobacillus plantarum</i>
	ISME	Puntiform, dome shaped with entire edge. Opaque and smooth surface	+	-	-	++	<i>Lactobacillus plantarum</i>
	ISMF	Puntiform, dome shaped with entire edge. Opaque and smooth surface	+	-	-	+	<i>Lactobacillus plantarum</i>

Proteolytic activity: +: zone formed < 5 mm, ++: zone formed > 5 < 10 mm, +++: zone formed >10 mm

Table 4.3.2: Characterisation and identification of isolates from multiple strains starter culture fermented maize sourdough

Multiple strains starter culture fermented maize sourdough	Isolate codes	Morphology of the isolates	Gram staining test	Catalase Test	Amylolytic property	Proteolytic property	Organism (best match) as Identified by the MALDI-TOF
	ISMG	Puntiform, dome shaped with entire edge. Opaque and smooth surface	+	-	+	-	<i>Lactobacillus plantarum</i>
	ISMH	Puntiform, dome shaped with entire edge. Opaque and smooth surface	+	-	+	-	<i>Lactobacillus plantarum</i>
	ISMI	Puntiform, dome shaped with entire edge. Opaque and smooth surface	+	-	+	-	<i>Lactobacillus plantarum</i>
	ISMJ	Puntiform, dome shaped with entire edge. Opaque and smooth surface	+	-	+	+	<i>Lactobacillus plantarum</i>
	ISMK	Puntiform, dome shaped with entire edge. Opaque and smooth surface	+	-	+	-	<i>Lactobacillus plantarum</i>
	ISML	Puntiform, dome shaped with entire edge. Opaque and smooth surface	+	-	+	-	<i>Lactobacillus plantarum</i>

Proteolytic activity: +: zone formed < 5 mm

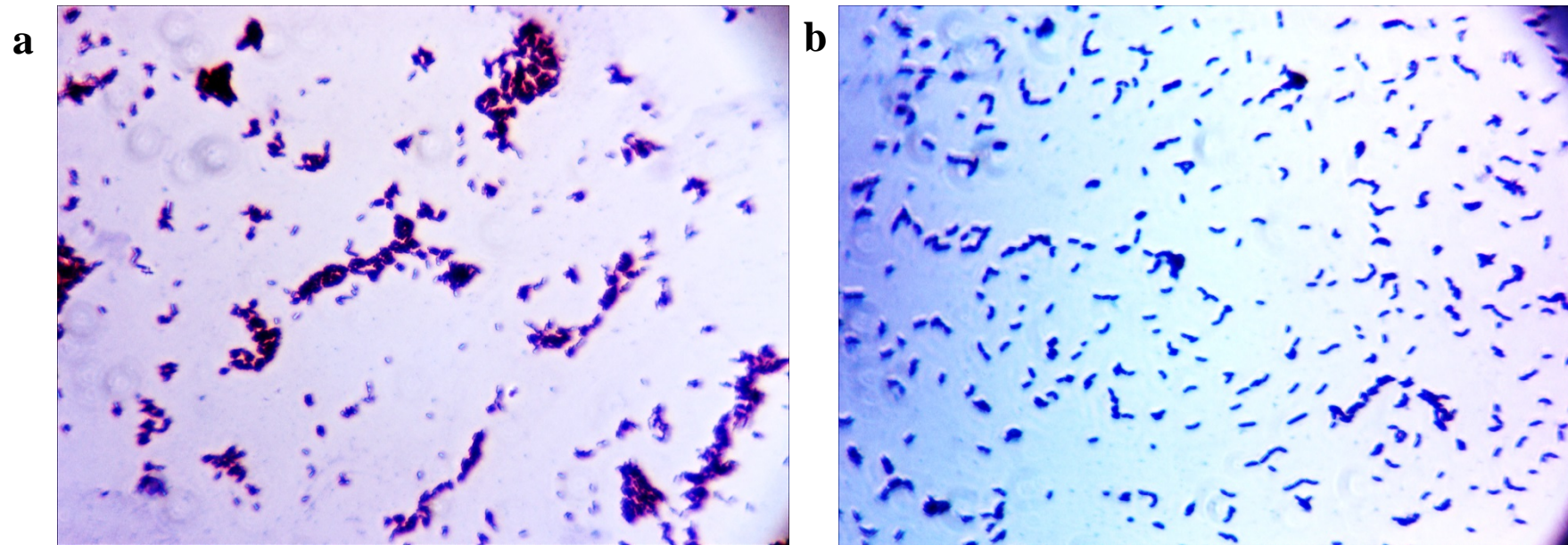


Fig 4.3.1: Light microscopy images showing Gram positiveness of isolates from, a: *L. plantarum* fermented maize sourdough, and b: multiple strains starter culture fermented maize sourdough

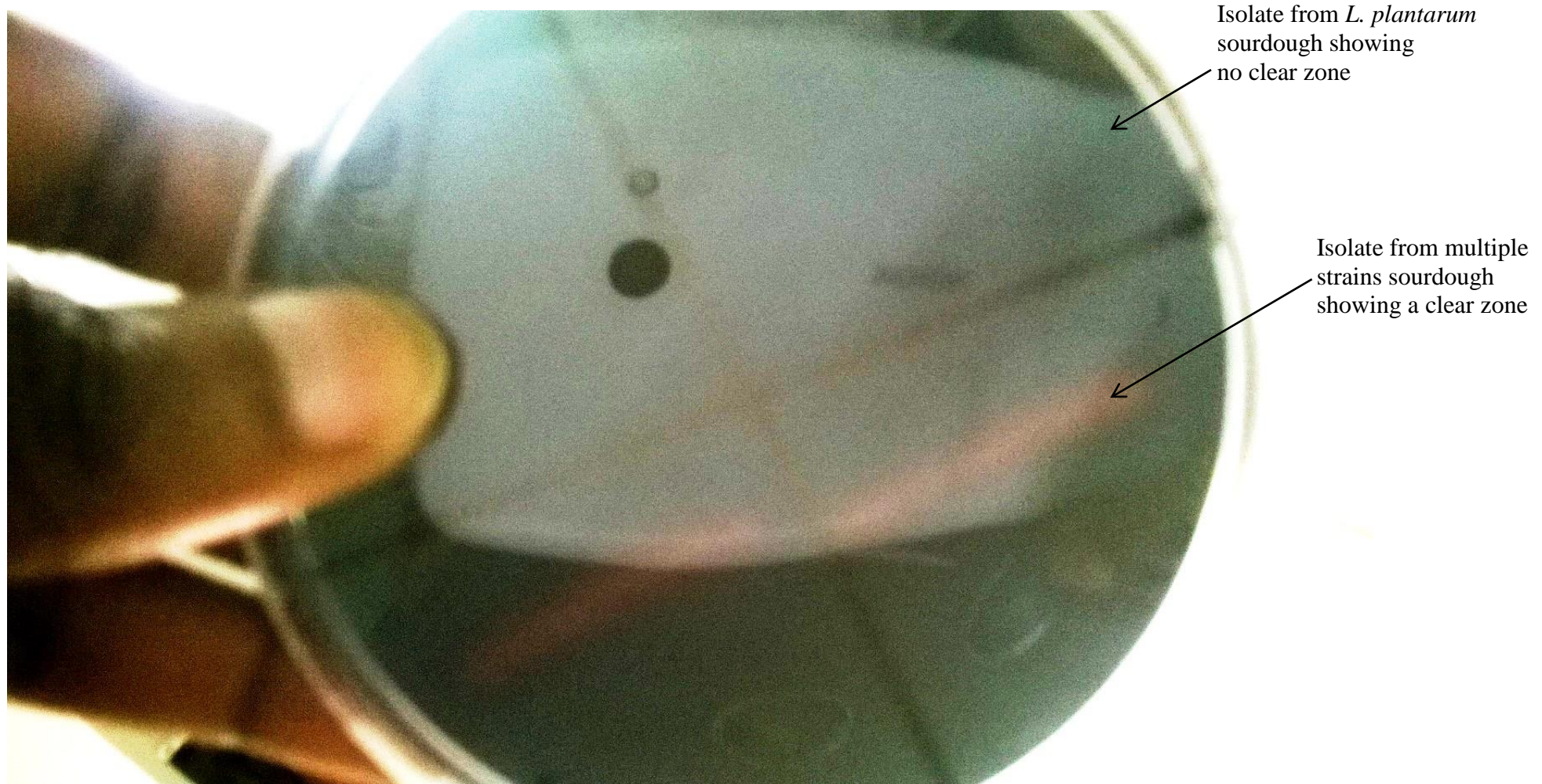


Fig 4.3.2: Detection of amylolytic activity of the isolates from *L. plantarum* and multiple strains starter culture fermented maize sourdoughs

White zones are indications of proteolytic activity

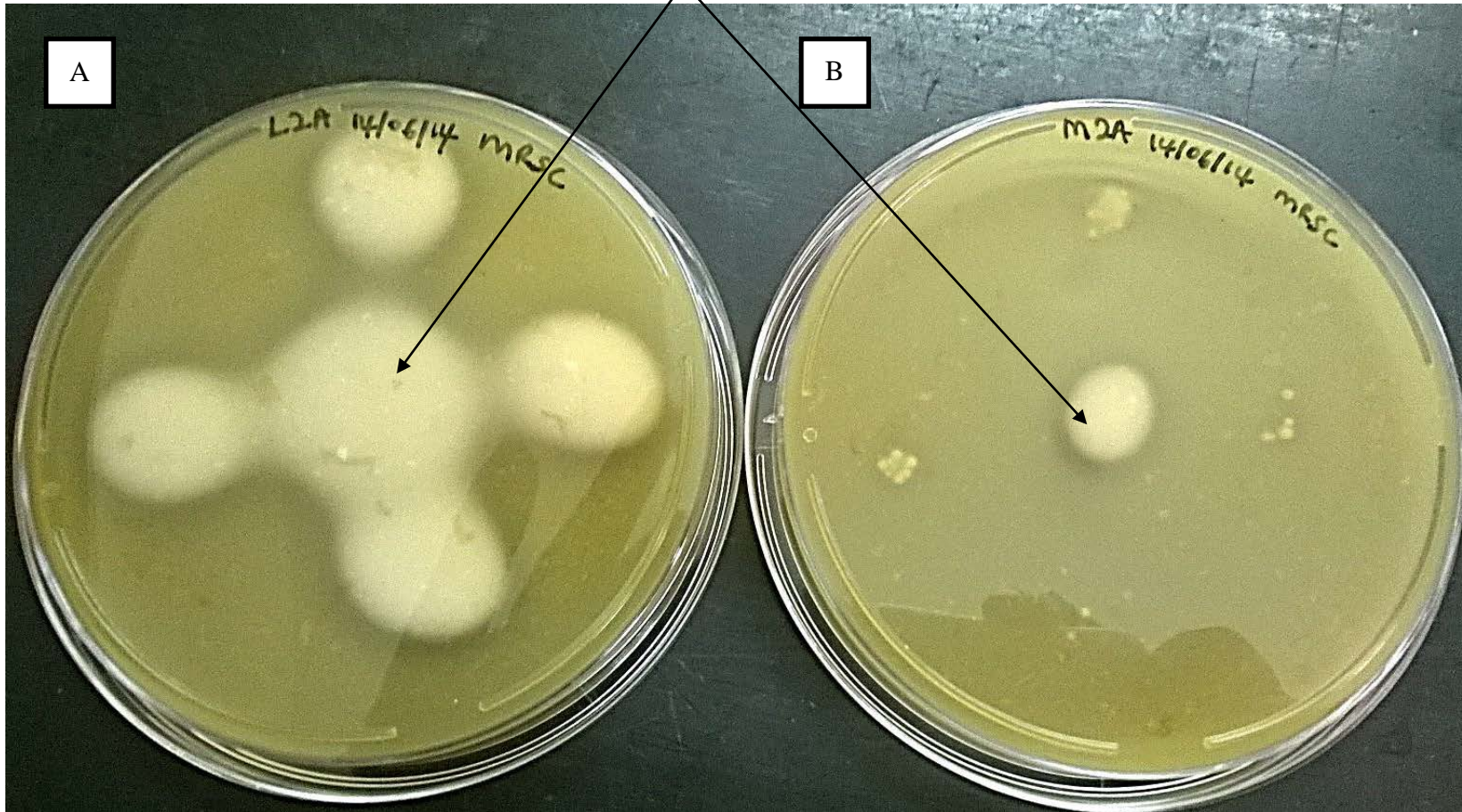


Fig 4.3.3: Proteolytic activity of the isolates from, a: *L. plantarum* fermented maize sourdough, and b: multiple strains starter culture fermented maize sourdough

4.3.5 Probable relationship of the proteolytic and amyolytic activities of LAB to starch modification

The results from Chapter 2 suggest that improvement in maize bread quality by sourdough fermentation is primarily due to starch granule modification. The proteolytic and/or amyolytic activities of the dominant LAB in the sourdough may be related to starch granule modification. However, the way in which each activity brings about this modification differs.

As stated, amyolytic LAB are a group of LAB that have the ability to partially hydrolyze raw starch through the activities of their α -amylases (Rodriguez-Sanoja *et al.*, 2000). It is speculated that hydrolysis of starch probably brought about starch modification by increasing the rate of water absorption by the starch granules. This effect probably influenced the rheological properties of the maize dough and eventually improved maize bread quality. Edema *et al.* (2013) attributed the improvement in fonio bread quality brought about by sourdough fermentation specifically to starch modification (slight granule swelling and probably some leaching of starch molecules) by endogenous amylases from the sourdough microorganism whose activities were favoured at low pH.

As stated, the proteolytic systems of LAB hydrolyze proteins to peptides and then to amino acids, which are required for growth of the LAB (Liu *et al.*, 2010). Though starch cannot be hydrolyzed by the proteolytic activities of the LAB, the proteolytic activities of LAB can be linked to starch modification. It can be proposed that proteolytic activities of LAB hydrolysed the endosperm protein matrix and proteins soluble in the dough liquid, binding to the starch granules allowing increase accessibility of water to the starch granules, hence a form of starch modification. This effect probably influenced the rheological properties of the maize dough and eventually improved maize bread quality. Schober *et al.* (2007) attributed the improvement of sorghum bread to the effect of sourdough fermentation on the endosperm protein matrix and proteins soluble in the dough liquid. Sourdough fermentation brought about degradation of these proteins, preventing them from interfering with the starch gel.

4.3.6 Conclusions

The dominant LAB in the *L. plantarum* and in the multiple strains sourdough fermented maize sourdoughs were identified to be *L. plantarum*. *L. plantarum* in the *L. plantarum* sourdough exhibited strong proteolytic activity but no amylolytic activity. However, that of the multiple strains sourdough exhibited both activities but had a lower proteolytic activity. The role of the dominant LAB in improving the quality of maize bread may be attributed to their amylolytic and proteolytic activities in the maize sourdough. These activities probably brought about starch modification by improving water absorption by the starch granules and/or increasing water accessibility to the starch granules as a result of degradation of proteins binding to the starch granules. Starch modification thereby, positively influenced the rheological properties of the maize dough and bread properties.

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5. GENERAL DISCUSSION

This general discussion will comprise a critical review of some of the methodologies applied in this work, a model to explain the role of sourdough fermentation in improving wheat-free bread loaf volume and crumb structure, and lastly, look at future research including issues such as the acceptability of sourdough maize bread.

5.1 Methodology: critical review

The effects of three different types of wheat-free methods on maize bread quality were investigated (Chapter 1). These were the traditional sourdough method practiced in Lesotho, the FAO method and the modern sourdough method. These methods involved starch pre-gelatinization and/or sourdough fermentation. Pre-gelatinized starch is meant to produce a starch gel matrix to trap the carbon dioxide produced in the dough during fermentation (Onyango *et al.*, 2009), while sourdough fermentation is expected to improve the properties of the dough (Chavan & Chavan, 2011). The two processes involved in the wheat-free methods are natural alternatives to the use of hydrocolloids. Hydrocolloids are currently used to improve the viscoelastic properties of wheat-free batters because of their potential to form three-dimensional polymer network in aqueous solutions (reviewed by Moroni *et al.*, 2009). Some of the hydrocolloids that have been used in gluten-free breads include: hydroxyl propyl-methyl-cellulose, carboxyl- or methyl-cellulose, locust bean and guar gum, xanthan gum and pectins (reviewed by Moroni *et al.*, 2009). Gums and hydrocolloids like hydroxyl propyl methyl cellulose appear to improve gas retention and water absorbing characteristics of bread dough (reviewed by Anton & Artfield, 2008; McCarthy *et al.*, 2005). Based on these functions of hydrocolloids in bread dough, it can be said that the three wheat-free methods investigated in this work made use of starch as a hydrocolloid.

The three wheat-free methods investigated were probably not as well compared with each other as they could have been. Different baking pans were used for each method. Though the investigation was aimed at the principles involved in sourdough fermentation and pre-gelatinization, using the same baking pans with the same diameter may possibly have influenced the breads produced in a different way. Using larger pans may not have produced the same degree of improvement in maize bread quality as found. Therefore the applicability of this method at the industrial level may be an issue. According to Schober *et al.* (2007), the size of the pan has an influence on the final bread quality. They explained

that during proofing and baking, before and during starch gelatinization, the batter is very soft. These authors further explained that mechanical support of the batter comes from the side walls of the pan. The batter closer to the centre of the pan is only supported by surrounding batter. In the case of a larger pan of similar shape than a smaller one, the increase in batter volume and mass is proportional to the third power of the length, while the increase of the area of the supporting side walls is only proportional to the length squared. This means that in larger pans, the batter has to relatively support more weight by itself. This is likely to facilitate collapsing, especially of the crumb centre in larger batter-based breads.

Concerning the sourdough microorganisms, it was only the dominant lactic acid bacteria in the *L. plantarum* and multiple strains starter culture fermented sourdough that were isolated and characterised for proteolytic and amylolytic activities. However, a definition of sourdough fermentation is a mixture of flour and water fermented by lactic acid bacteria and yeast (Hammes & Gänzle, 1998). Some yeasts possess the ability to secrete proteases and amylases (Walker, 1998) which if present in the sourdough could also have acted on the starch or protein component of the dough. For example, Mohamed *et al.* (2007) found amylolytic yeast in traditional Moroccan sourdough. Therefore, ideally, the dominant yeast in the sourdough should also have been isolated and tested for proteolytic and amylolytic activities. If this was done, it would have been verified whether the proteolytic and amylolytic activities of the dominant LAB in the sourdoughs, were actually solely responsible for any modification of the starch, or whether the sourdough yeast also contributed to this starch modification. Another way of verifying if the dominant LAB isolated from the sourdoughs were responsible for starch modification and improvement in maize bread quality would have been to use the isolated dominant LAB as starters in the preparation of another maize bread. If maize breads with improved qualities are produced by this process, then it can be verified that these LAB are actually responsible for the improved maize quality.

Dough development and gas production of the dough were investigated using a Rheofermentometer as also done by Dal Bello *et al.* (2007). The Rheofermentometer simulates what happens in the dough during proofing. This includes the development of the dough and the total amount of carbon dioxide produced, lost and retained. If using the Rheofermentometer to stimulate what happens in the dough during proofing was successful, this would have provided insights into how sourdough fermentation improves

maize dough bread quality. However, this part of the research was not successful. The results could not be verified due to malfunctioning of the Rheofermentometer. Therefore the results had to be discarded. This malfunctioning of the Rheofermentometer was probably due to the nature of the sourdough which was very soft compared to more elastic wheat doughs.

Confocal laser scanning microscopy was used to study the microstructures of the maize doughs and maize breads. The process of sticking the maize breads to the slides involved pressing them firmly on the tape. This was because they had to be held inverted in the microscope. This could have altered the microstructure of the bread. Also, differentiating between the starch granules and protein was difficult, probably due to the stain (0.5% acid magenta dye) used. The colour attributed to protein and starch granule by the staining method according to Maeda *et al.* (2013) seemed to be unclear when applied to this work. Therefore starch and protein could not be adequately distinguished. Using separate stains to identify the starch granule and protein will probably have been a better option.

5.2 The role of sourdough fermentation in improving maize bread loaf volume and crumb structure

In addition to the direct and indirect effects of acidification of the dough system by the lactic acid from the LAB fermentation, the specific roles played by the microorganisms must also be considered. The proteolytic activity of selected lactic acid bacteria on wheat flour proteins has been well documented (Clarke *et al.*, 2004). In Chapter 2, it was established that the low pH resulting from production of lactic acid by the lactic acid bacteria in the sourdough was not the reason for improved quality of maize bread, on the basis of comparison with a chemically acidified dough. Improvement in maize bread quality by sourdough fermentation was suggested to primarily be due to starch granule modification causing the dough to be more cohesive, soft and less elastic, which improved its ability to trap and withstand the pressure of the expanding carbon dioxide during fermentation and baking. In line with this, it may be suggested that the effect of sourdough on the starch component and the resulting effect on the rheological properties of the dough are very important factors. In wheat breads, a more elastic dough is desirable for improved wheat bread quality. Bread dough made from strong flours produced from hard wheat tends to be less viscous, more elastic and produce wheat bread of good quality (Tipples *et al.*, 1994). However, dough from weak flours produced from soft wheat tends to be more viscous, less elastic and therefore produce small loaves of inferior crumb structure. In this

work, it was found that a less elastic (softer) dough is required for improved maize bread quality. Since sourdough is made up of a population of different microorganisms, improvement in the dough properties and quality of the bread should, to an extent, be dependent on, or related to, the properties or activities of the microorganisms present in the sourdough. As explained, it appears that improvement in rheological properties of the dough positively influenced the bread quality in terms of loaf volume and crumb structure. Therefore, the effect of these sourdough microorganisms on dough rheology may be the key to its improvement. The rheology of sourdough-based baked products relies greatly on the activity of LAB, which is the dominant type of microorganism in sourdough (Gobbetti *et al.*, 2005). Proteolysis and amylolysis are among the various enzymatic activities of LAB in sourdough (Corsetti *et al.*, 1998). According to Agati *et al.* (1998), amylolytic LAB may positively modify the rheological properties of the resulting dough. Also, proteolysis has been said to have an impact on dough rheology (reviewed by Arendt *et al.*, 2007). Di Cagno *et al.* (2002), who worked on the effect of proteolysis by LAB on wheat flour protein fractions involved in human cereal intolerance, found that proteolysis by lactobacilli had a positive influence on the softening of the dough during fermentation, as determined by rheological analyses.

As shown in Chapter 3 (Fig. 4.3.2), only the dominant LAB in the multiple strains sourdough exhibited amylolytic activity. As indicated above, it is hypothesized that starch granule modification influenced the rheological properties of the dough by making it less elastic. Reduction in the elasticity of the dough may be due to greater water absorption by the starch granules. Starch granule amylolysis has an important effect on the water-holding ability and porosity of the dough as well as on bread softness (Barrera *et al.*, 2007). Relating the amylolytic property of the LAB to the dough thermal properties results (Chapter 2), it is suggested that the possession of amylolytic activity by the dominant LAB in the multiple strains sourdough had an effect on the thermal properties of the maize dough. This sourdough had the lowest endothermic onset and peak temperatures, an indication of the possibility of an effect of sourdough fermentation on the properties of the starch granule (starch modification). According to Salmenkallio-Marttila *et al.* (2001), starch modification by amylolytic enzymes during fermentation alters the gelatinization properties of the starch granules. Additionally, this sourdough had the lowest level of starch damage. This suggests that the starch was slightly hydrolysed due to the amylolytic activities of the dominant LAB in the multiple strain sourdough. Agati *et al.* (1998)

hypothesised that the presence of amylolytic lactic acid bacteria in maize-based products such as mawe or ogi (fermented maize products) may assist in increasing the rate of acidification, by increasing the availability of energy sources such as glucose or maltose, from starch for the use of other LAB present in the fermenting system. In line with this, the reduced amount of damaged starch in the multiple strains sourdough (Chapter 2, Table 4.2.2) may be due to the suitability of the damaged starch for microbial growth during the fermentation process. This is in agreement with Elkhailifa *et al.* (2005) who suggested that damaged starch may be the preferred substrate for microbial growth during the fermentation process. Damaged starch has a large hydration capacity and its consumption by microorganisms leads to a decrease in dough consistency (Barrera *et al.*, 2007). Therefore, the rheological properties of dough are slightly modified by the microbial consumption of damaged starch. Though the *L. plantarum* in the *L. plantarum* sourdough did not possess amylolytic property (Chapter 3, Fig. 4.3.2), the presence of amylolytic strains of other LAB present in the sourdough cannot be ruled out since the flour was not sterilized before it was inoculated with the *L. plantarum* B411 culture. These amylolytic strains if present may have had an impact on the dough properties before being inactivated at the final stages of fermentation due to biological changes occurring in the fermenting medium. According to Hammes *et al.* (1990), the application of LAB as starter organisms ensures the dominance of the starter during the whole fermentation process. Therefore it is possible that the dominant strain of *L. plantarum* in the *L. plantarum* sourdough was very similar to the *L. plantarum* B411 culture used. This could be the reason why the *L. plantarum* in this sourdough did not exhibit amylolytic activities even though the same unsterilized maize flour was used for the preparation of the sourdough as it was done for the multiple strains sourdough.

The role of the dominant LAB in improving maize bread quality can also be viewed from the perspective of the proteolytic activity of these LAB. The proteolysis that occurs during sourdough fermentation is among the key phenomena that affect the overall quality of sourdough bread (reviewed by Gänzle *et al.*, 2008). As shown in Chapter 3 (Fig. 4.3.3), the dominant LAB which were *L. plantarum* in the maize sourdoughs both exhibited proteolytic activities. However, the proteolytic activity was stronger in the *L. plantarum* fermented sourdough than the slight activity in the multiple strain sourdough. Modification of the proteins in dough can affect the behaviour of the other flour components such as starch (Renzetti & Arendt, 2009). In view of this, protein-starch interaction is an important

issue to consider in terms of having an influence on the altered rheological properties of the dough, which positively influenced maize bread quality. Specifically, Renzetti & Arendt (2009) suggested that changes in protein-protein and protein-starch interactions can influence the rheological properties of dough. The starch granules in maize corneous endosperm are completely enclosed in a very compact protein matrix, which can restrict starch granules from full expansion during cooking (Correia *et al.*, 2010). According to Elkhalfifa *et al.* (2005) who investigated the molecular and structural changes occurring in the protein and starch components of sorghum flour when fermented to prepare typical non-malted Sudanese foods, the proteolytic events occurring during fermentation have a profound impact on the structure of the protein matrix which disappears in the fermented material, leading to the release of the starch granules that were enclosed in the coated structure. In agreement with this, it is suggested that proteolysis by LAB brought about the degradation of the endosperm protein matrix and hydrolysis of proteins soluble in the dough liquid, which probably freed the starch granules allowing them more access to water, in turn bringing about starch modification and positively influencing the rheological properties which ultimately led to improved bread quality. Moroni *et al.* (2011), who worked on the impact of sourdough on buckwheat flour, batter and bread, found that sourdough fermentation induced a drastic decrease in the degree of elasticity of buckwheat batter, similar to the findings of this study. They explained that the presence of organic acids most likely enhanced the solubility of the proteins and induced modifications of the protein-starch interactions, resulting in increased water-holding capacity of the batters. They further suggested that the hydrolysis of the globulin proteins and liberation of smaller polypeptides during fermentation was most likely responsible for weakening the extent of the protein-protein and protein-starch interactions in the dough.

The probability of better water accessibility of the starch in the sourdoughs enhanced by the amylolytic and/or proteolytic activities of the dominant LAB in the sourdoughs can be supported based on the high enthalpy for the endothermic peak required by these sourdoughs (Chapter 2, Table 4.2.3). León *et al.* (1997) and Sanz-Penella *et al.* (2012) attributed the higher enthalpy requirement of starch granules in sourdough to be due to better starch hydration during the period of fermentation. In line with this, the high enthalpy of the *L. plantarum* and multiple strains sourdoughs may also be attributed to better starch hydration. This suggests more water accessibility to the starch granules in the sourdoughs, hence, further supporting the hypothesis as explained.

Sourdough breads baked without baker's yeast required a higher amount of force to compress compared to the sourdoughs with baker's yeast, chemically acidified maize bread with yeast and the straight dough maize bread with yeast (Chapter 2, Table 4.2.1). However, when combined with yeast, the breads were softer than the other treatments. The proteolytic activities of the dominant LAB in these sourdoughs may have contributed to the high force requirement of the doughs when compressed. This was probably due to the degradation of the protein matrix and hydrolysis of protein soluble in the dough liquid, therefore facilitating the formation of a strong starch gel as explained by Schober *et al.* (2007). Presumably, the starch gel formed became very strong after cooling due to starch retrogradation (Zilic *et al.*, 2010). Also, among the sourdoughs, maize bread produced with *L. plantarum* sourdough required a higher force. This can be related to its strong proteolytic activity (Chapter 3, Fig. 4.3.3). Schober *et al.* (2007) emphasised the effect of degradation of proteins, due to sourdough fermentation, had on the batter consistency and final quality of sorghum bread. These authors proposed that if these proteins were not degraded, they would aggregate upon baking and interfere with the starch gel, leading to the formation of an undesirable bread crumb. Also, Renzetti & Arendt (2009) who worked on the effect of protease treatment on the rheological properties and baking quality of brown rice bread reported that proteolysis resulted in improved continuity of the starch phase, which they suggested might be partially responsible for improved brown rice bread quality.

Improved dough properties due to starch modification by amylolytic activities of LAB, increased water accessibility to the starch granule and improved dough strength due to the proteolytic activities of LAB as discussed above, will only positively affect maize bread quality when baker's yeast is added. This is due to the baker's yeast being the main producer of the carbon dioxide required for dough leavening. The dominant LAB in the *L. plantarum* and multiple strains sourdoughs which was identified to be *L. plantarum* probably cannot produce sufficient carbon dioxide required for dough leavening, and instead improves the dough properties. *L. plantarum* is facultative heterofermentative and therefore cannot make the dough rise as much as other lactobacilli such as *L. sanfranciscensis* and *L. brevis* which are obligate heterofermentative (reviewed by De Vuyst & Neysens, 2005).

Researchers have proposed various mechanisms by which sourdough fermentation improves wheat-free breads. Some have attributed it to its influence on the protein

structure (Schober *et al.*, 2007), while the others have attributed it to its effects on the starch (Hüttner *et al.*, 2010; Edema *et al.*, 2013). However, the findings of this research suggest that improved maize bread quality is due to the effect of the proteolytic and amylolytic activities of the dominant sourdough LAB on the protein and starch component of the dough. These enzymatic activities are influenced by the low dough pH due to lactic acid production by LAB in sourdough. According to Clarke *et al.* (2002), the changing pH values during the sourdough fermentation period may afford passage through a range of pH values close to the optimum for various enzymes present in the dough. These authors further explained that the activity of the proteolytic and amylolytic enzymes present may be influenced to a greater degree by the pH profile of the biological acidification fermentation period in contrast to the rather instantaneous nature of the chemically acidification process. These enzymes, which play an important role in terms of their impact on dough constituents, achieve optimum activity at pH 4–5 for the proteolytic activities and pH 3.6–6.2 for the amylolytic activities (Belitz & Grosch, 1992; Clarke *et al.*, 2002). A schematic representation of the probable role of the proteolytic and amylolytic activities of sourdough lactic acid bacteria in improving the gas retention ability of maize dough is shown in Fig. 5.1. As shown, sourdough fermentation brings about a reduction in the pH of the dough. Reduction in pH is due to the production of lactic acid by lactic acid bacteria. The low pH activates the proteolytic and amylolytic activities of the lactic acid bacteria. This leads to the degradation of the endosperm protein matrix and hydrolysis of the soluble proteins in the dough liquid. As a result of the former, the starch granules in the maize endosperm flour are exposed and hence hydrolysed by the amylolytic enzymes of the LAB. Hydrolysis of proteins makes water more assessible to the starch granules, while hydrolysis of the starch granules facilitates increased water absorption. Increase in accessibility of water to the starch granules and increased water absorption by the starch granules would result in starch modification. Starch modification, as explained, becomes a very important process in the contribution to improved bread quality, since the role of starch during baking is to bind the water and create a gas permeable structure (reviewed by Houben *et al.*, 2012). The starch molecule backbone has numerous hydroxyl groups projecting into the surrounding space. These hydroxyl groups have a particular affinity for other hydroxyl groups, in particular water molecules. A strong interaction and affinity is formed through hydrogen bonding between the very large starch molecules and the very small water molecules when the starch granules are hydrated (Murphy, 2000). Relating the process described to the present work, it can be suggested

that as a result of water absorption by starch granules, water binds to the starch molecules via hydrogen bonding and a starch paste is formed. Due to the hydrogen bonding, the ability of the dough to trap and withstand the pressure of the expanding carbon dioxide is improved. Therefore, as tiny gas bubbles are incorporated into the dough due to dough mixing, the improved dough properties enhance the stabilization of these gas bubbles. As carbon dioxide produced during fermentation diffuses into the gas bubbles and starts expanding, the improved dough properties prevents the gas cells from collapsing as pressure builds up in them due to the expansion of carbon dioxide in them. Stabilization of gas cells and expansion of carbon dioxide in the gas cells results in high loaf volume and open crumb structure when the bread dough is baked.

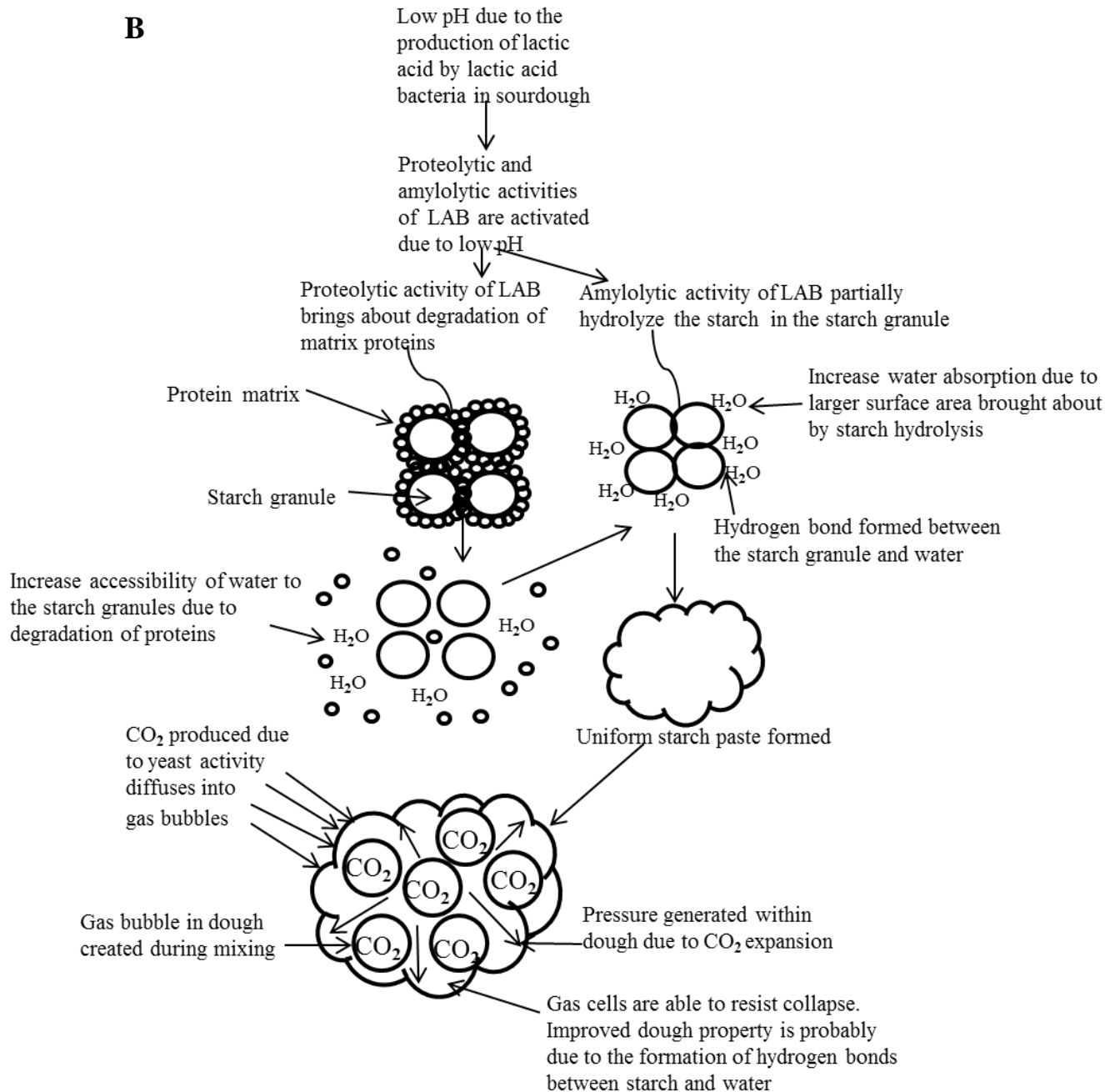


Fig 5.1: Schematic representation of the probable role of the proteolytic and amylolytic activities of sourdough lactic acid bacteria in improving the gas retention ability of maize dough. A: No sourdough fermentation. B: Sourdough fermentation

5.3 Future research and development of maize bread

Maize bread with improved quality developed in this work could be the answer to the problem of wheat importation in countries whose climatic conditions do not favour the cultivation of wheat. The use of maize will also benefit local farmers. Acceptability of the maize sourdough breads is a major issue. Bread consumers in Africa are used to the sensory properties of bread made from wheat. These consumers are familiar with the lightness and fluffiness of wheat breads which is not the case for the maize breads. Therefore, convincing the consumers to try the maize bread may be difficult and require a lot of effort. Also, since the maize breads were produced using sourdough, the acidic taste of the maize bread may not be acceptable to the consumers. An informal sensory evaluation was conducted to determine the acceptability of the maize sourdough bread by wheat bread consumers. Most of the sensory panellist did not like the sour taste of the maize sourdough bread. However, a large proportion of those that liked the maize sourdough bread were from West Africa where *ogi* (fermented maize gruel) is popularly consumed. A formal sensory evaluation should be conducted on the maize sourdough breads to determine the sensory characteristics and acceptability among bread consumers.

Further research may include using the isolated *L. plantarum* from the sourdoughs as a starter for the production of maize sourdough bread. This will help in ensuring that the maize bread quality is maintained. The application of exogenous enzymes in improving maize bread quality may also be a good option. The proteolytic and amylolytic activities of the LAB as discussed are activities of enzymes in the LAB. Therefore, the addition of these enzymes directly may also improve the quality of maize bread produced by a straight dough process. Also, the sourdough yeast should be isolated and characterised as it was done for the LAB. This will give insights into the contribution of the sourdough yeast to the improved maize bread quality. If a positive contribution was found, then the sourdough yeast can also be used as a starter for the production of maize sourdough bread.

6. CONCLUSIONS

Using the modern sourdough method, maize bread with improved quality such as increased loaf volume and open crumb structure was successfully produced. The failure of the traditional sourdough and Food and Agriculture Organization methods to improve maize bread quality was probably primarily due to the low percentage of the total maize flour fermented (10-15%) compared to the high percentage of the total maize flour fermented (75%) in the modern sourdough method. Further investigations into improved maize bread quality produced using the modern sourdough method showed that the improvement was not due to the low pH. Confocal laser scanning microscopy revealed a cohesive dough structure in the sourdoughs and also, larger gas cells in the sourdough breads. The higher endothermic peak enthalpy of the sourdoughs suggests better starch hydration, indicating starch modification. Lower relaxation time required by the sourdoughs suggests that they were softer and less elastic. Strain sweep and temperature sweep analysis also showed that the sourdoughs were less elastic. The sourdoughs being less elastic than the other treatments, is probably a result of starch modification. The dominant lactic acid bacteria in the sourdoughs were *L. plantarum*. The proteolytic and amylolytic activities of the *L. plantarum* is probably responsible for starch modification. It is proposed that the low pH due to production of lactic acid by lactic acid bacteria during sourdough fermentation activated the proteolytic and amylolytic activities of the LAB in the sourdoughs. The proteolytic activity of the LAB degraded the endosperm protein matrix and hydrolysed the proteins soluble in the dough liquid, enabling better accessibility of water to the starch granules, while the amylolytic activity of the LAB slightly hydrolysed the starch granules, increasing water absorption by the starch granules due to larger surface area to volume ratio. As a result, the dough's ability to trap and withstand the pressure of the expanding carbon dioxide in the fermenting dough and bread is enhanced.

Formal sensory evaluation should be conducted on the maize sourdough breads. This should involve a descriptive panel which will determine the sensory characteristics of the bread and the acceptability among bread consumers. Based on the results of the sensory evaluation, the sourdough maize bread procedure applied in this work may be optimized to suit the bread consumers' preference without compromising the original quality of the maize bread. It is further recommended that the application of exogenous protease and amylase enzymes be investigated instead of relying on the bacteria.

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8. PRESENTATIONS AND PUBLICATION MADE BASED ON THIS RESEARCH

Adediwura T. Falade, Elna M. Buys, John R.N. Taylor. The effect of different non-wheat bread recipes on the quality of wheat-free maize bread. Poster presentation at the 20th SAAFoST Biennial International Congress and Exhibition, CSIR International Convention Centre, 7-9 October 2013.

Adediwura T. Falade, Elna M. Buys, John R.N. Taylor. The effect of different non-wheat bread recipes on the quality of wheat-free maize bread. Oral presentation at the New Voices in Cereal Science and Technology Symposium, Cereal Science and Technology Southern Africa, 3 September 2014.

Falade, A.T., Emmambux, M.N., Taylor J.R.N. 2014. Improvement of maize bread quality through modification of dough rheological properties by lactic acid bacteria fermentation. Paper accepted for publication in the Journal of Cereal Science on 29th August, 2014.