The effects of laccase and xanthan gum on the quality of gluten-free amadumbe bread

By

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DECLARATION

I hereby declare that the work reported in this thesis and submitted at the Department of Biotechnology and Food Technology at Durban University of Technology for a Master's Degree is my original work. I confirm that it has not been previously submitted for a degree at any Higher Education Learning Institution.

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DEDICATION

This thesis is dedicated to my parents, family and friends for their unrelenting love and support.

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Abstract

Celiac disease (CD) is an auto-immune disorder that is triggered by the consumption of gluten in predisposed individuals. The only remedy that has been proposed thus far is total exclusion of gluten from the diet. This may be the most difficult task to most celiac disease patients for most of the convenient and widely consumed baked products such as bread are prepared using ingredients that contain gluten. The replacement of gluten in the baking industry comes with some implications on the overall quality of the baked products, especially bread. It has been observed that gluten-free baked products currently on the market are of poor texture, less volume, not visually appealing and have a bad taste. Hence, the need for polymeric substances that can mimic gluten properties, yielding baked products with similar characteristics as the gluten-containing counterparts. Various crops such as rice, sorghum, sweet potato and cassava have been used and additives such as hydrocolloids, protein-based ingredients, emulsifiers and enzymes included to improve gluten-free bread quality.

The use of carbohydrate-rich tubers and protein-rich legumes as gluten-free ingredients shows great potential in the food industry. Amadumbe (*Colocasia esculenta*) is a carbohydrate rich tuber which is highly underutilized in South Africa and contains vast amounts of mucilage, a hydrocolloid which can be of great help to improve dough rheology. Hydrocolloids have been reported in literature to have the ability of improving dough water holding capacity and improving dough viscosity hence facilitating gas retention and impacting on the overall quality of the baked product. However, despite the presence of mucilage, amadumbe is very low in protein and it is difficult to produce bread with properties that resemble gluten-containing bread. Hence the need for protein supplementation which may also potentially facilitate protein cross-linking during bread making. Legume proteins from crops such as soy bean and bambara groundnuts contain abundant quantities of lysine, tyrosine and cysteine which could potentially be manipulated through the use of enzymes such as laccase in order to initiate the formation of a network similar to gluten.

The project investigated the effect of laccase and xanthan gum (a hydrocolloid) on the quality of gluten-free bread supplemented with bambara groundnut flour and soy protein isolate as protein sources. Flour blends were prepared using a ratio of 70:30 (amadumbe flour: bambara groundnut flour) and 88:12 (amadumbe flour: soy protein isolate) based on a targeted protein content of 16 g/100 g and the quality properties were determined. Colour analysis showed that amadumbe flour had a higher L^* value compared to the other flours and the blends, showing that amadumbe can be used in applications where food colour contributes to food perceptions. However, when bambara groundnut flour and soy protein isolate were added the L^* value decreased. The nutritional profile of the individual flours and the blends showed that amadumbe flour protein content was improved with the addition of bambara groundnut flour and soy protein isolate in the above-mentioned ratios. The protein content of amadumbe increased from 2.36 g/100 g to 15.87 g when bambara groundnut flour was added and to 16.10 g/100 g when soy protein isolate was added, values that were close to the targeted protein content. Incorporating bambara groundnut flour and soy protein isolate in amadumbe flour resulted in improved water absorption capacity, foam capacity and stability as well as emulsion capacity and stability of the amadumbe flour. However, there was no significant difference in oil absorption capacity between amadumbe flour and the blends. The blends were then used to formulate different bread samples incorporating the enzyme laccase (25 nkat/g flour) and a hydrocolloid, xanthan gum (1%).

Laccase-mediated treatment of gluten-free amadumbe dough resulted in a 30% decrease in the free sulfhydryl groups and a 40% decrease in phenolic content indicating that crosslinking had occurred. Laccase action resulted in a 64% increase in bread specific volume and a 32% decrease in bread crumb hardness. Sensory analysis showed that laccase-treated bread samples were more acceptable compared to the non-treated bread samples in terms of appearance, texture, aroma and taste. The acceptability index varied between 46% and 86.2%. This study showed that there is great potential of laccase in gluten-free bread making.

The addition of 1% xanthan gum to amadumbe dough supplemented with bambara groundnut flour and soy protein isolate resulted in gluten-free amadumbe bread with improved crumb texture and specific volume, and decreased the rate of moisture loss. Sensory analysis also revealed that gluten-free amadumbe bread with added xanthan gum was more acceptable compared to the bread samples without xanthan gum. The acceptability index of the bread samples ranged between 40% and 85%. The resulting bread with xanthan gum showed that hydrocolloids such as xanthan gum can be successfully used in the development of gluten-free baked products.

Overall, this study has shown that the incorporation of laccase and xanthan gum to gluten-free amadumbe bread results in bread with improved and acceptable bread properties.

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ABBREVIATIONS

Anti-tTG: Anti-Tissue Transglutaminase Antibodies

CD: Celiac Disease

EC: Emulsion Capacity

EMA: Anti-Endomysia Antibodies

ES: Emulsion Stability

FAO: Food Agriculture Organization

FC: Foam Capacity

FS: Foam Stability

GF: Gluten-Free

GuHCI: Guanidium Hydrochloride

Ig: Immunoglobulin

OAC: Oil Absorption Capacity

Nm: Nanometre

SH: Sulfhydryl

SPI: Soy Protein Isolate

SS: Disulphide

Tris-Gly: Tris-Glycine

WAC: Water Absorption Capacity

WHO: World Health Organization

PREFACE

This thesis is written using the manuscript format. Chapter one of this thesis is a general introduction. Chapter two provides a literature review of the study Included in the chapter is an overview of celiac disease; its prevalence, diagnosis, treatment, implications of gluten exclusion in the baking industry, what has been done so far, the shortfalls and a proposed solution. Chapter three focuses on the effect of protein supplementation on amadumbe flour functional properties. Chapter four is a study on the effect of laccase on the quality of gluten-free amadumbe bread. The last experimental chapter, chapter five, focuses on the effect of adding xanthan gum on the quality of gluten-free amadumbe bread. Chapter six provides a general discussion, conclusion and recommendations for future work.

Publications and Conferences attended

Publications

Faith Seke, Eric O. Amonsou and Tukayi Kudanga (2017). The effects of laccase on the quality of gluten-free amadumbe bread. LWT Food Science and Technology (Under review).

Conferences attended

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Faith Seke, Tukayi Kudanga and Eric O. Amonsou. The use of hydrocolloids in gluten-free bread making. 9^{th} Annual International South Africa Technology Network (SATN) Conference, Cape Town, South Africa, 12-14 October 2016.

Chapter One

1.0. Introduction

In recent years, there has been an increased interest in gluten-free food products, mainly because of a wider awareness of gluten intolerance and celiac disease (CD). In South Africa, the incidence of celiac disease is yet to be determined, but in Europe and the United States of America, it has been perceived that one in every 133 individuals suffer from celiac disease (Grode *et al.,* 2018) . The only proven remedy for the disease thus far is a strict adherence to a gluten-free diet (Helms, 2005; Rosell *et a.,* 2014; Padalino *et al.,* 2016).

Gluten is a protein fraction found in crops such as wheat and is responsible for the structural quality of most baked products. The replacement of gluten poses a great scientific and industrial challenge for food scientists especially in the baking industry (Gobbetti *et al.,* 2017). It has been observed that gluten-free food products are inferior in terms of physical characteristics and quality. For example, they have a dense appearance and are of poor texture and mouth-feel. These characteristics make it very difficult for consumers to follow a gluten-free diet (Bárcenas and Rosell 2007). The approaches that have been proposed to try and solve this challenge include the use of various additives to a gluten-free starch base in order to improve structure, acceptability and shelf-life (Padalino *et al.,* 2016). These additives include: protein sources such as dairy products and egg-white of which these are amongst the top eight food allergens, hydrocolloids and emulsifiers which are less effective individually unless combined with other additives and crosslinking enzymes for example transglutaminase, glucose oxidase and tyrosinase which are highly substrate specific (Bonet *et al.,* 2006; Gomez *et al.,* 2007; Marco and Rosell 2008b; Rosell *et al.,* 2014; Padalino *et al.,* 2016). Therefore, the need to explore other alternatives that can help solve the problem and at the same time ensure that the baked products are nutritionally sound and safe.

Recently, another crosslinking enzyme, laccase, has gained interest in the food industry. Laccase is a blue multicopper oxidase, which catalyse the monoelectronic oxidation of a broad spectrum of substrates, such as, ortho- and para-diphenols, polyphenols, amino-phenols, and aromatic or aliphatic amines, coupled with a four-electron reduction of oxygen to water

(Kudanga *et al.,* 2011). Its wide substrate range and ability to catalyse coupling reactions makes it a candidate for use in cross-linking gluten-free dough to form networks similar to those found in gluten systems. Renzetti *et al.* (2010) reported an increase in crumb structure and bread volume when laccase was used to make gluten-free oat bread, thus showing the potential of laccase in gluten-free baking. However, the increasing demand for gluten-free bread necessitates the need to investigate laccase-mediated modification of other crops as potential gluten-free ingredients.

Underutilized indigenous crops in South Africa such as amadumbe (*Colocasia esculenta*), together with bambara groundnuts (*Vigna subterranean*)*)* as protein source, can be used as potential gluten-free ingredients. Amadumbe is a carbohydrate-rich tuber and a good source of mucilage (a hydrocolloid) which can help improve the properties of baked products (Nguimbou *et al.,* 2014) as well as offer potential sites for laccase action. Bambara groundnut is a protein-rich legume with a considerable amount of amino acids such as cysteine and lysine which can be oxidised by laccase in oxidative cross-linking reactions. Soy protein isolate which is rich in a potential laccase substrate, tyrosine (Singh *et al.,* 2008), is also a good candidate protein source.

Apart from the use of enzymes, the use of a combination of hydrocolloids such as xanthan gum and mucilage together with a protein base might also have an impact on dough rheology, hence improving the quality of gluten-free bread (Awolu and Oseyemi 2016). The use of xanthan gum in bread dough has been reported to strengthen gluten-free bread dough due to a strong interaction between xanthan gum and proteins (Julianti *et al.,* 2017). Xanthan gum also increases water absorption and enhances the ability of the dough to retain gas hence increasing the specific volume and water activity of the crumb (Padalino *et al.,* 2016) . However, xanthan gum has not been explored with amadumbe as starch base.

The aim of this study was to examine the effect of the enzyme laccase and a hydrocolloid xanthan gum on the properties of gluten-free amadumbe bread supplemented with bambara groundnut and soy protein isolate.

1.1. Contribution to knowledge

This study investigates the effects of laccase and xanthan gum on the quality of gluten-free amadumbe bread supplemented with bambara groundnut flour and soy protein isolate. It has been reported that the functionality of various flours in product development depends on their functional properties, hence it is of paramount importance to study the behaviour of individual flours and their blends before using them (Chapter 3). Previous studies have reported the use of the enzyme laccase mostly in wheat based bread systems; little information has been documented on the effect of laccase on gluten-free bread systems (Chapter 4). The study also seeks to investigate the effect of xanthan gum and laccase enzyme on the properties of gluten-free amadumbe bread including consumer acceptance of the resulting bread. Xanthan gum has been used in the development of gluten-free bread in combination with other hydrocolloids and additives using a variety of crops but no information has been reported on its impact on amadumbe-based bread without other additives (Chapter 5). Overall this study seeks to propose new gluten-free alternatives as well as contribute new knowledge related to enzymatically modified gluten-free systems.

Chapter Two

2.0. Literature Review

The following literature review gives a brief overview of celiac disease, its prevalence, and the biochemistry and physiology of the disease. Also discussed in this chapter are the challenges faced in the food industry in the replacement of gluten in baked products and the ways that have been proposed to improve the quality of gluten-free foods.

2.1. Celiac disease

Celiac disease is an auto-immune disorder that is mainly caused by the assimilation of gluten in inherently predisposed individuals (Green and Lee 2005; Spijkerman *et al.*, 2016). The main causes of celiac disease have been linked to the interface of gluten and the immune factors, environmental and genetic factors. The implication of consuming gluten is that it initiates the modification of protein by the enzyme transglutaminase (Figure 2.1) resulting in an immune system reaction which leads to an inflammatory reaction (Kagnoff 2007; Aronsson *et al.,* 2016). Gluten is broken down into amino acids and peptides by the luminal and brush-border enzymes. Gliadin peptides induce changes in the epithelium and in the lamina propia leading to the damaging of the epithelial cells. This results in an increase in the expression of interleukin-15, which in turn activates intraepithelial lymphocytes (Gianfrani *et al.,* 2005). These lymphocytes lead to the destruction of the enterocytes that express MIC-A (a stress protein) on their surface. This results in the destruction of the villi and the damage of the intestinal mucosal hence interfering with the absorption of nutrients. Villi are the pathway by which gluten is absorbed into the body. When gluten is consumed by a celiac patient it flattens the villi and it is difficult for the individual to process gliadin protein. The condition has several other names, including CD, coeliac sprue, gluten-sensitive enteropathy, gluten enteropathy or, and gluten intolerance (Ciacci *et al.,* 2007; Losowsky, 2008). The indications of celiac disease include chronic diarrhoea, abdominal distension, vomiting, fatigue and weight loss (Fasano and Catassi 2012). However, these may be absent and indications in other organ systems may arise; increased screening is therefore necessary for the asymptomatic (Van Heel and West 2006). The common types of causes, symptoms, and effects are however not necessary for diagnosis given the wide range of possible symptoms (Di Sabatino and Corazza 2009).

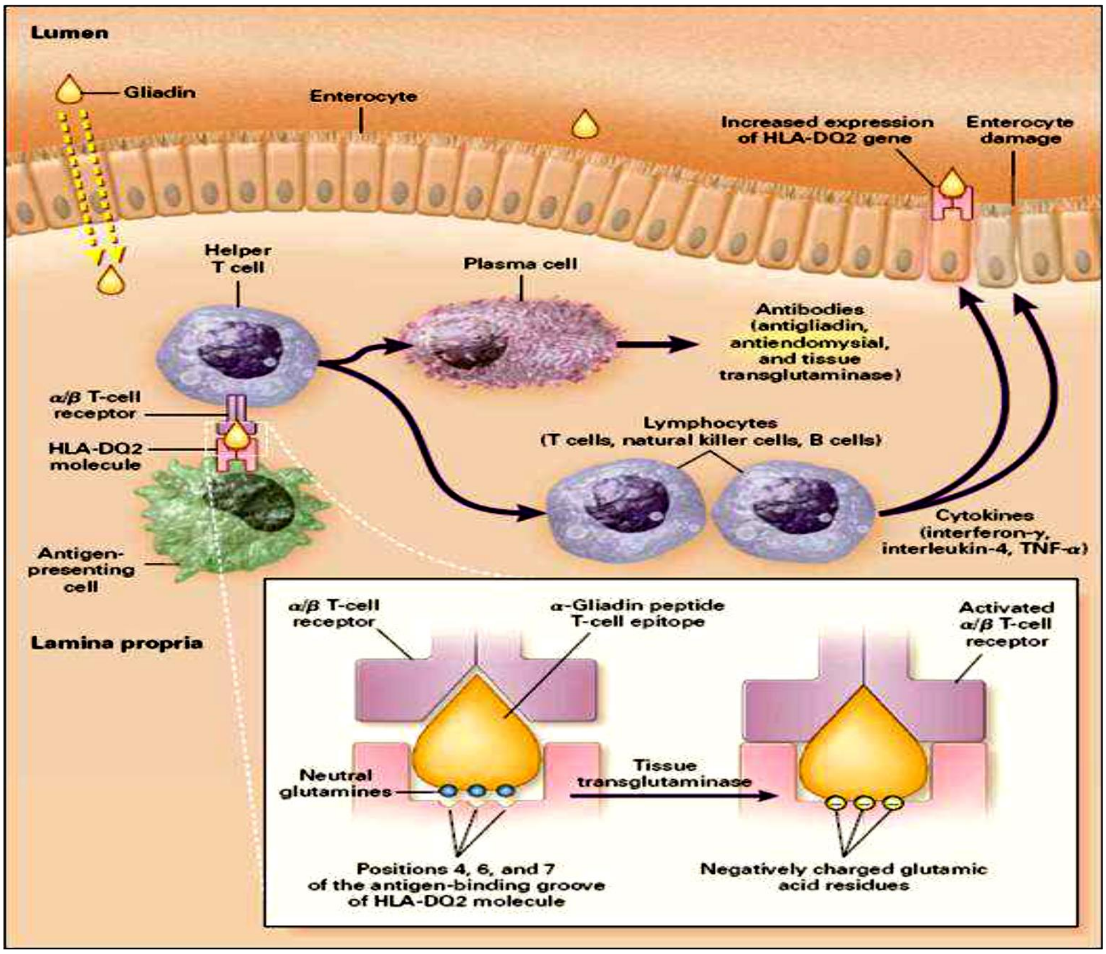

Figure 2.1: Pathogenesis of celiac disease (Farrell and Kelly 2002)

In some individuals, osteoporosis, mouth ulcers, persisting digestive symptoms, fatigue, musculoskeletal pain and fractures may occur due to celiac disease (Faulkner-Hogg *et al.*, 1999). Most celiac disease patients may also suffer from other food allergies, including milk protein, and soy (Selby *et al.*, 1999).

2.2. Prevalence of celiac disease

For decades, celiac disease was regarded as an uncommon disease with a prevalence of 0.03% worldwide (Lohi *et al.,* 2007). The clinical symptoms that were mostly used for the diagnosis of the disease included malnutrition, diarrhoea and weight loss. However, the prevalence has increased and it is estimated that one in 133 individuals have celiac disease (CD) across the world (Grode *et al.,* 2018). Feighery (1999) & Gallagher *et al.* (2004) suggested that the occurrence of celiac disease has been underrated and can be explained through the use of an iceberg model (Figure 2.2). In the model, it shows that a few people are clinically proven to suffer from celiac disease (A), whereas many people are undiagnosed and have no prominent symptoms, hence suffering from the disease in a silent mode (B). Another group of people in the population are also predisposed to develop the disease in the future (C). The rate of detection is increasing due to augmented consciousness of the disorder and improved diagnosis techniques (Gallagher *et al.,* 2004). The diagnosis techniques have evolved from clinical tests to highly sensitive and specific serological tests such as anti-endomysia antibodies (EMA) and anti-tissue transglutaminase antibodies (anti-tTG) (Fasano *et al.,* 2003). These techniques have resulted in a correct diagnosis, hence changing the epidemiology patterns of celiac disease. Celiac disease is mostly associated with a few other autoimmune disorders such as primary biliary, autoimmune thyroid disease, cirrhosis, type 1 diabetes mellitus, Addison disease, and immunoglobulin (Ig) A deficiency. The prevalence of celiac disease is also aggravated in patients with genetic disorders such as Turner syndrome and Down syndrome. Previously CD was recognized as a malabsorption disorder, however, it is now recognized that the presence of obesity does not exclude the possibility of CD.

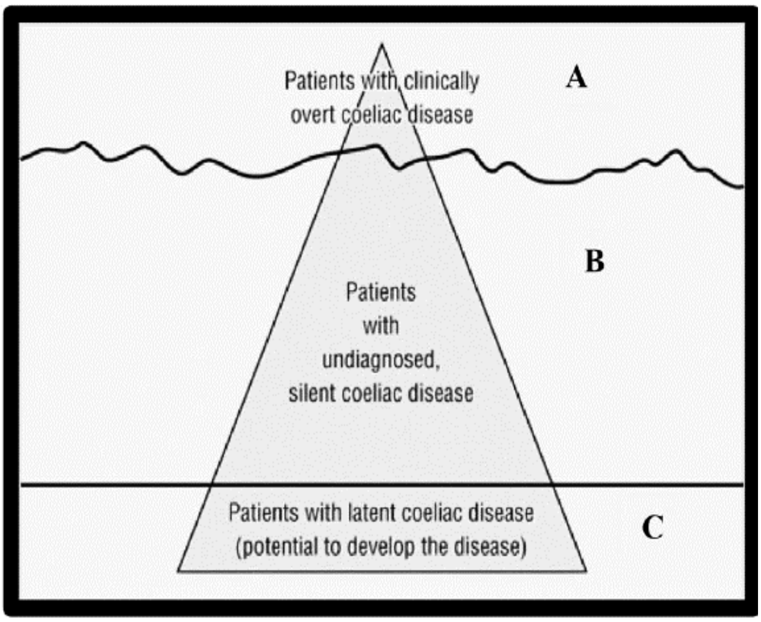

Figure 2.2: The iceberg model, which shows the underestimation of the prevalence of celiac disease (Gallagher *et al.,* 2004)

2.3. Biochemistry and physiology of celiac disease

The Biochemistry and physiology of celiac disease is dominated by prolamins, their chemistry and role in baked products.

2.3.1. Prolamins

Prolamins are the major cereal endosperm storage protein and are responsible for celiac disease, except in oats and rice (D'Amico *et al.*, 2017). Prolamins are hydrophobic proteins containing a large quantity of non-hydrophobic amino acids and are soluble in aqueous alcohol and insoluble in water (Kieffer, 2006; Aluko, 2015). They are good sources of proline and glutamine and contain approximately 30-70% of these amino acids. The prolamins present in cereals such as wheat can be classified into three main groups namely; the high molecular weight prolamins, sulphur-poor, and sulphur-rich prolamins. The prolamin in wheat is called gluten and is divided into two fractions, glutenin and gliadin. Gliadins are monomeric and are classified as α, β, γ and ω-gliadins and the glutenins are polymers, some homologies exist between these fractions (Balakireva and Zamyatnin 2016). It has been reported that these two fractions contain specific peptide sequences that trigger celiac disease. Studies that have been done on the toxicity of specific peptides have shown that in in-vitro tests N-terminal and far C-terminals domains of α-gliadin were observed and peptides found in this region can act as epitopes triggering an immune event resulting in enteropathy (Shewry *et al.*, 2003). In another study, gliadin peptides directly induced early phosphorylation of the protein (Ciacci *et al.*, 2007).

2.3.2. The role of gluten in wheat-based food products

Wheat flour has been identified as the only flour that can positively result in a leavened and open crumb structure bread (Naqash *et al.*, 2017). This is due to the existence of gluten in the flour. Gluten is a protein in control over the structural and functional properties of different baked products such as bread. It has been reported that wheat flour, upon hydration and kneading results in a cohesive and extensible dough as a result of the formation of a viscoelastic network (Gallagher *et al.*, 2004). Both gliadins and glutenins, the two fractions of gluten promote the formation of the viscoelasticity of the network. Gliadins are essentially for dough network's viscosity and glutenins are responsible for the elasticity. It has been proposed that the disulphide bonded glutenin macropolymer leads to the unique physical properties of the dough. The viscoelastic properties result in the increased water holding capacity of the dough as well as gas retaining ability of the dough during dough fermentation and baking (Hager and Arendt 2013). If this network is absent gas retention is reduced hence hindering structure building leading to a dense baked product. This suggests that gluten is the key element to the important rheological properties of bread dough and final baked product.

The viscoelastic network involves a combination of both covalent and non-covalent bonds. Hydrophobic interactions, hydrogen bonds and ionic bonds are responsible for the accretion of glutenins and gliadins, influencing the structure and mechanical properties of the protein network (Wieser, 2007). The 'loop and train' theory has been proposed whereby the linear high molecular weight glutenin subunits are stabilise forming aligned β-sheets as shown in Figure 2.3 (Lindsay and Skerritt 1999). These β-sheets have been linked to the elastic properties of dough, it was proposed that stretching wheat dough results in the destruction of the loops and then the trains as the bonds break. This results in the sliding of the proteins above each other and kneading the dough results in the formation of disulphide bonds (Belton, 1999; Lindsay and Skerritt 1999).

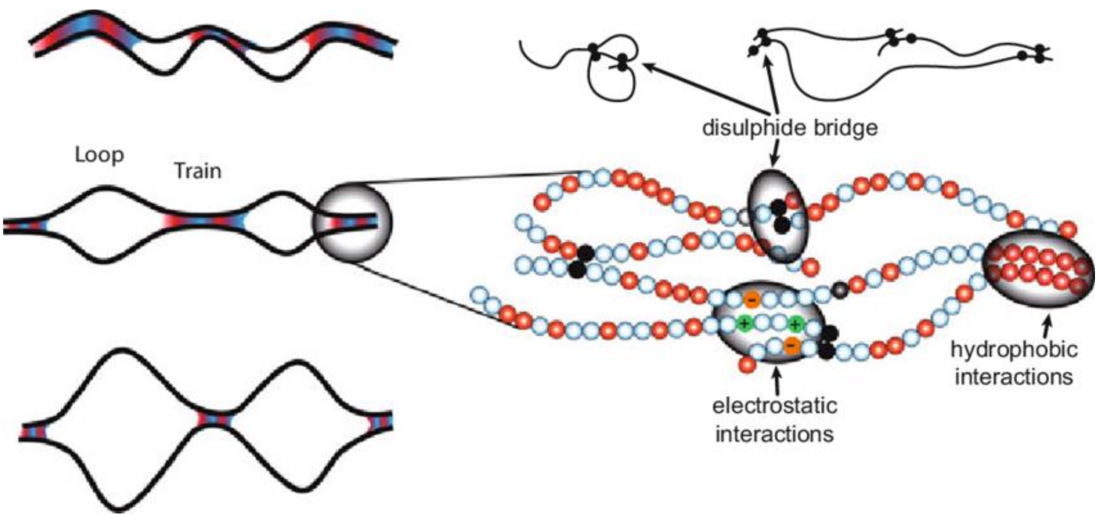

Figure 2.3: The effects of hydration on the loop and train behaviour of high molecular weight subunits (Belton, 1999)

2.4. Importance of thiols and disulphide in dough rheology

The components of a dough system highly affect the system's physical and chemical properties. The presence of proteins containing SS (disulphide) groups and SH (thiol), and lipids containing linoleic and linolenic acids trigger redox reactions (Wieser, 2007; Maforimbo *et al.,* 2008). It has been stated that 30% of the total thiols present in a dough system help in the development of dough and mixing tolerance. In various studies, it was reported that disulphide groups contribute to the stabilisation of the dough at three levels (Edwards *et al.*, 2003; Wieser, 2007). These three levels include within protein molecules, between aggregates of protein and within multi-molecular aggregates of protein. The locality of the thiol groups affects how they work in dough systems. Thiol groups within the interior of protein molecules have been reported to be unavailable in trivial mixing conditions of short time except when exposed to denaturing agents such as physical or chemical elements. The thiol groups that are on the protein surface participate freely in exchange reactions during mixing (Grosch and Wieser 1999). Insolubility, increased viscosity and turbidity are as a result of intermolecular disulphide bonds and studies have reported that the incorporation of reducing agents in dough systems such as sodium sulphite, dithiothreitol and cysteine, reduces disulphide linkages, however, it has been noted that the addition of SH containing amino acids such as cysteine can amplify the extensibility of wheat dough (Dong and Hoseney 1995; Hüttner and Arendt 2010). Oxidation of SH groups to SS bonds can result in dough strengthening, in wheat dough the cleaving of disulphides reduces dough resistance to extension and therefore increases dough extensibility (Gujral and Rosell 2004). When wheat flour is composited with protein rich flours such as, soy it has been reported that the cleaving of protein disulphides (P-S-S-P) results in either complete reduction or single SH/SS interchange, hence the formation of mixed disulphides, P-S-S-R (Chen and Schofield 1996; Wieser, 2012). This may facilitate stress relaxation during dough mixing.

2.4.1. Structural changes during baking

The structural changes that occur during bread making are mainly understood in wheat bread systems. During baking, there is a conversion of a viscous batter to a baked product and this has been explained by the starch gelatinisation theory (Stauffer, 2007). When wheat dough is exposed to heat, it causes gluten to gel releasing bound water to starch hence initiating gelatinisation. The quantity of water, the availability of sugar, salt, emulsifiers and fat determines the extent of gelatinisation. It has been hypothesized that water is released during gelatinisation and results in improved crumb porosity and texture of the resulting baked product (Kumar, 2002). The transformation of dough is affected by the rheological properties of the dough system, with the formation of SS/SH linkages improving the change of the viscous dough to an elastic baked product (Wieser, 2007). The production of gas from yeast or other leavening agents is the main cause of expansion during baking and this can be disturbed by the strength of the material between the gas cells to viscous flow and the extent to which gas cells syndicate is affected by the breaking strength of the dough. Gas production together with the changes in the rheological properties contribute to the overall quality of bread (Cauvain, 2015).

2.5. Baking with Gluten-Free Flours

The use of gluten-free ingredients poses a great scientific challenge to food scientists, especially in the baking industry. It is difficult to get the distinct rheological properties of wheat dough when using gluten-free ingredients whilst ensuring that the final product is nutritionally valuable and exhibits acceptable properties (Torbica *et al*., 2010; Naqash *et al.,* 2017). One of the major problems that have been identified with baking using gluten-free flour is the absence of the matrix formed by the two gluten factors, glutenin and gliadin. Glutenin is responsible for providing higher molecular weight and contributing to elasticity whereas gliadin provides the lower weight component which provides extensibility (Ćurić *et al.,* 2007). Exclusion of gluten during bread making results in a batter rather than a dough and this leads to bread with poor colour and texture as well as quality defects. It has been reported that bread formed from a batter (Figure 2.4) is limited in terms of shape as it takes the shape of the baking pan (Gallagher *et al.,* 2004; Schober *et al.,* 2007). Gluten-free bread has a short shelf life and more prone to stalling because of the absence of a gas cell structure and poor water holding capacity (Schober *et al.,* 2007). Gluten-free baked products are also nutritionally inferior compared to their counterparts. Gluten containing food products are symbolised by a high micro-nutrient content, whilst the gluten-free products are low in vitamin D, magnesium, fibre, calcium and B-vitamins due to their development using refined flours and starches (Moore *et al.,* 2006). Therefore, it is of supreme significance to enrich and supplement gluten-free ingredients. It has been proposed that the use of legumes, tubers and seed flours can be an important move in obtaining acceptable gluten-free baked products. These include millet, amaranth, buckwheat, sweet potatoes, cassava, pea, soy bean, flax, teff and sorghum (Kupper, 2005). Some of these ingredients have been used successfully to recuperate the quality of gluten-free products.

Gluten-free batter Gluten-containing dough

Figure 2.4: The difference between a gluten-free batter and a gluten dough (Schober *et al.,* 2007)

The incorporation of functional ingredients has been reported to enhance volume, mouth-feel, structure, adequacy and durability of gluten-free bread. Some of the functional ingredients include hydrocolloids, emulsifiers and protein isolates and these have been reported to act as surface-active ingredients which stabilise liquid films that will be surrounding gas bubbles in the fermenting batter (Lazaridou *et al.,* 2007). Previous studies have suggested that the addition of protein-rich ingredients in gluten-free systems can help in the formation of a cohesive network which can improve the structural quality of gluten-free bread (Gallagher *et al.,* 2004; Schober *et al.,* 2007). Owing to the functional properties of proteins (gelation, foaming and helping in increasing the elastic modulus), the structure of gluten-free bread can be improved. Proteins have also been used to improve bread sensory properties through Maillard browning and flavour development.

2.5.0 Gluten alternatives in gluten-free systems

2.5.1. Eggs

Eggs are usually added to gluten-free food systems to enhance the nutritional value, colour and flavour of the food product. Eggs also have good emulsifying, foaming, gelation and coagulation properties which can be of great importance in dough systems (Moore *et al.,* 2004).

Egg-white has been used to provide additional structure to gluten-free dough/batter. In one study, egg-white was used together with soy protein isolate and it was hypothesized that soy protein isolates induced the formation of disulphide linkages and facilitated the elasticity of the baked goods. In a study by Crockett *et al*. (2011) the use of eggs in gluten-free bread baking resulted in the formation of a viscous solution where protein scaffolding was observed, resulting in increased loaf volume.

2.5.2. Dairy products

Dairy products have been used to facilitate protein network formation, increase water holding capacity and also to enhance the dough/batter handling properties of gluten-free systems (Nunes *et al.,* 2009). Dairy products also enhance the nutritional properties by increasing the calcium content and protein efficiency ratio. Gallagher *et al.* (2003) reported an appealing bread crust and a whiter crumb when dairy ingredients were used in the production of gluten-free bread. However, it has been hypothesized that since dairy products are abundant in protein, they result in bread with low volume and increased crumb and crust hardness (Moroni *et al.,* 2009). The use of dairy ingredients has also resulted in the formation of a network-like structure, which resembles that of gluten as shown by CLSM analysis (Figure 2.5). However, there is a certain group of people who are allergic to lactose and it has been noted that in people with celiac disease, lactose intolerance is very common. This has been attributed to the damage of the villi hence the inability to produce lactase an enzyme that breaks down lactose. Thus, dairy proteins are not an ideal alternative in gluten-free bread formulations.

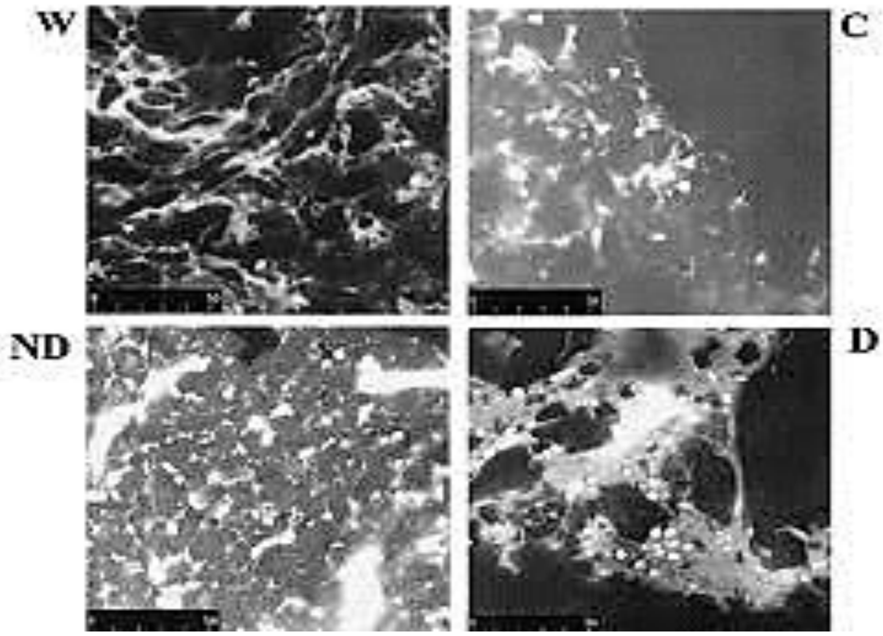

Figure 2.5: Confocal laser-scanning micrographs of wheat bread crumb (W), commercial gluten-free bread crumb (C), non-dairy gluten-free bread crumb (ND), and dairy gluten-free bread crumb (D) stained with Safranin O dye (Moore *et al.*, 2004)

2.5.3. Sourdough process

The sourdough process has been proposed to be another alternative to improving gluten-free bread quality. Acidification of flour through sourdough fermentation has been described as a way of enhancing swelling properties of polysaccharides resulting in the ability to retain gas (Moroni *et al.*, 2009). This process improved crumb structure, increased bread volume, aroma, flavour, nutritional properties and shelf-life. In studies by Schober *et al.* (2007) & Sly *et al.* (2014), sourdough batter was firmer compared to the non-acidified and chemically acidified batter. The authors hypothesized that there was partial digestion of the protein rich particles exposing them, hence binding to water in the bread batter. The exposed protein might also have stuck together forming aggregates and a stable microstructure. In short, the use of acids such as lactic acid resulted in acceptable bread.

2.5.4. Influence of hydrocolloids on dough rheology and gluten-free bread quality

Hydrocolloids are used in bread making because of their ability to improve the rheology and texture of baked goods hence improving the characteristics of the end product. They also play a role in the modification of starch gelatinisation (Gallagher *et al.,* 2003). Hydrocolloids that are often used include hydroxypropylmethylcellulose (HPMC), xanthan gum, guar gum carboxymethylcellulose (CMC) and pectin. It has been reported that there is the formation of a three-dimensional polymer network in aqueous solutions when hydrocolloids are incorporated in dough systems (Hager and Arendt 2013). This increases the batter/dough viscosity, resulting in an improvement in the textural properties and also increasing the water holding capacity of the dough/batter system. Schober (2009) also reported an improved texture of dough/batter following hydrocolloid incorporation. It was reported that due to an increase in viscosity, gas was retained, minimizing coalescence and preventing the separation of starch and other ingredients, thus improving batter/dough homogeneity. Lazaridou *et al.* (2007) piloted a study using a farinograph and rheometer and stated that the addition of xanthan gum in gluten-free dough resulted in a viscoelastic dough with increased strength comparable to wheat flour dough. The type of hydrocolloid used and the supplementation levels were reported to influence bread quality. Collar *et al.* (1999) reported a combined effect of hydrocolloids such as CMC and HPMC with other additives as emulsifiers and enzymes on textural properties of freshly baked bread. Hydrocolloids were noted to increase crumb water activity and this was linked to the increased water holding capacity of hydrocolloids. Hydrocolloids tend to increase volume, producing a softer crumb. Sciarini *et al.* (2010) also reported that guar gum improves bread specific volume and reduces bread crumb hardness. Factors that determine hydrocolloid functionality include source, the method used for extraction, chemical structure and precise relations with ingredients in gluten-free formulations (Guarda, 2004).

2.5.4.1. Xanthan Gum

Xanthan gum is an anion extracellular polysaccharide from the bacterium *Xanthomonas campestris* (Achayuthakan, 2008). In it is soluble in cold water and has an elevated and stable viscosity regardless of pH and a molecular weight (M = 2.5 × 106 g/mol) (Mikuš *et al.*, 2011). Xanthan gum in its solid state has a helical structure, with branches that fold in lying along the backbone. The side chains consist of alpha-D-mannose containing an acetyl group, beta-D-glucuronic acid and beta-D-mannose units which are linked to a pyruvate group as shown in Figure 2.6. When combined with carob gum, guar gum and konjac gum at low concentrations, it possesses some synergic effect (Arocas *et al.,* 2009). As a result, improved viscosity can be observed as well as soft and elastic reversible gels. Xanthan gum distress moisture content, retrogradation enthalpy and texture of bread crust. Xanthan gum is normally combined with emulsifiers in the development of protein-supplemented bread, gluten-free bread, as an emulsion stabiliser and also to extend the durability and lower moisture loss of baked products (Guarda *et al.,* 2004; Gomez *et al.,* 2007; Arocas *et al.,* 2009).

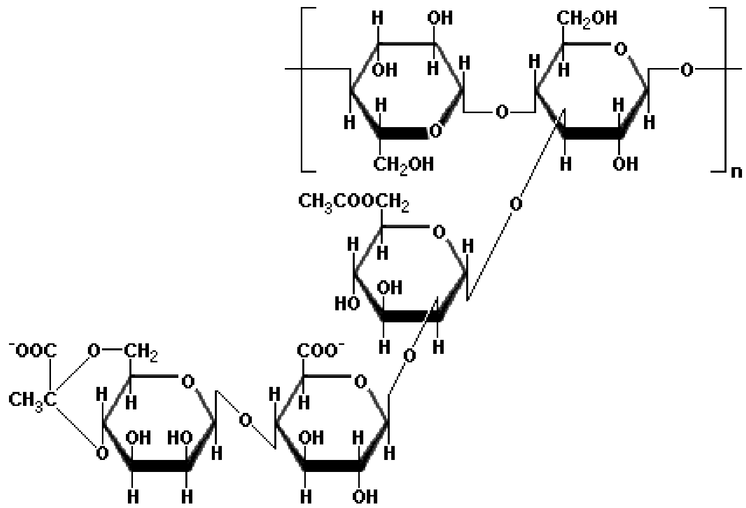

Figure 2.6: The structure of xanthan gum (Sworn, 2009)

However, the incorporation of xanthan gum in dough systems cannot facilitate the formation of a protein network, hence, there is increased moisture from the crumb to the crust, leading to a dry crumb texture, facilitating staling. Hence the need for other alternatives that can result in the formation of a network similar to that formed by gluten-containing crops.

2.5.5. Enzymes in gluten-free products

Another area that has not been fully exploited is the utilization of enzymes as protein network facilitator of covalent cross-links between polypeptide chains. Enzymes generally regarded as safe (GRAS) substitute to chemical complexes (Rosell, 2009). Enzymes are more favourable to chemicals because they function at milder conditions, are highly specific, are less likely to produce any toxins and are completely denatured during bread making (Bonet *et al.*, 2006). The use of enzymes has been linked to improved dough-managing properties, improved fresh product superiority and an extended shelf-life.

2.5.5.0. Crosslinking enzymes

The use of enzymes for protein crosslinking has become very popular and this can be achieved through the formation of crosslinks directly or via enzymatic production of crosslinking agents. The crosslinking agents oxidize reactive structures with subsequent crosslink formation (Buchert *et al.,* 2010). Proteins contain several reactive groups for crosslinking enzymes, such as tyrosine, lysine, glutamine and cysteine residues. The resultant reactions are reliant upon the enzyme type used, accessibility of the target reactive groups and on the process conditions used (Gerrard, 2002). The enzymes used can affect the chemistry of the crosslink that is made and eventually the structure of the biopolymer network. Enzymes that have been used in the development of gluten-free bread include glucose oxidase (Bonet *et al.,* 2006), transglutaminase, α-amylase (Caballero *et al.,* 2007b) and xylanase (Castro *et al.,* 2015). The implication of these enzymes can result in protein-protein, carbohydrate-carbohydrate or protein-carbohydrate adducts. However, these enzymes are highly specific. Elaborated below are some of the commonly used enzymes, glucose oxidase and transglutaminase.

2.5.5.1. Glucose oxidase

Glucose oxidase catalyses the oxidation of β-D-glucose to D-gluconolactone and hydrogen peroxide (Wong *et al.,* 2008). It has been proposed that the formed H_2O_2 oxidises thiol groups of two cysteine to form disulphide bonds (Figure 2.7). The formation of disulphide bonds can help in strengthening dough systems and improving the resultant product. In the baking industry glucose oxidase catalysis has also been linked with the oxidation of the free sulfhydryl units of proteins converting them to disulphide linkages hence having an impact on the rheological properties of dough systems (Bonet *et al.,* 2006) . Glucose oxidase has been used to improve wheat dough properties and reports show that glucose oxidase strengthened wheat dough and improved bread quality. This was attributed to the formation of disulphide and non-disulphide linkages.

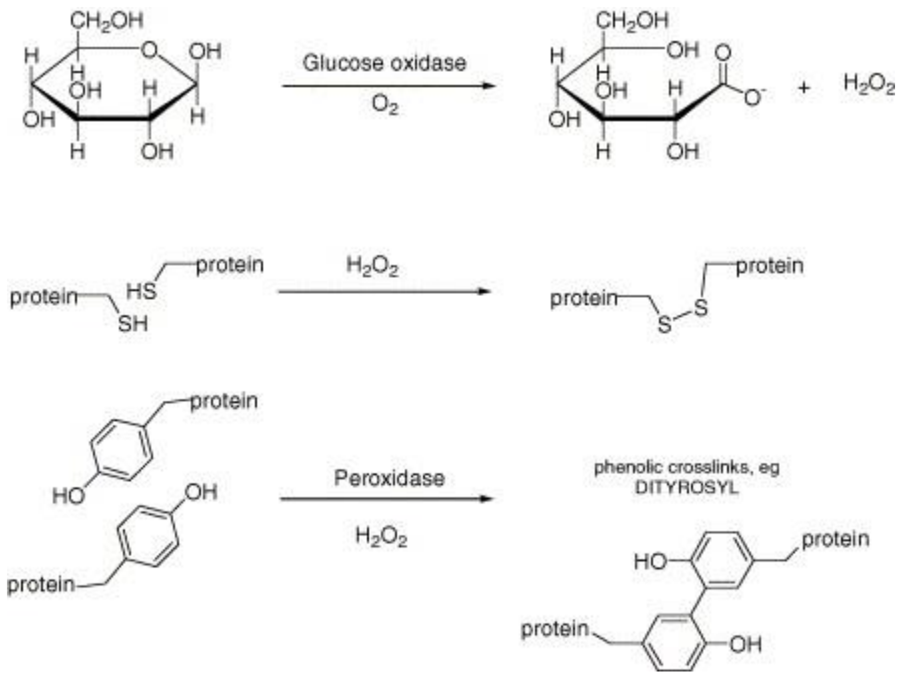

Figure 2.7: The proposed glucose oxidase mechanism (Vemulapalli *et al.*, 1998; Ameille *et al.*, 2000)

2.5.5.2. Transglutaminase

Transglutaminase facilitates the development of covalent linkages between the γ-carboxyamide groups of glutamine residues and the ε-amino group of lysine residues, which improves the water holding capacity, elasticity, firmness and heat stability in food systems (Caballero *et al.*, 2007b). The reaction mechanism is illustrated in Figure 2.8. Water can act as an acyl acceptor in the event that primary amines are absent; this results in the deamination of glutamine residues. Transglutaminases are mostly used in the dairy, pharmaceutical and meat industry and have also been introduced in the baking industry (Moore *et al.*, 2006). Transglutaminase has been used to improve white pan bread properties and it was reported that it reduced the work input required and water absorption resulting in improved bread quality (Gerrard *et al.*, 1998). Transglutaminase has also been used to improve rice flour bread and the results showed an improved hardness and a more continuous structure (Moore *et al.*, 2006; Shin *et al.*, 2010). Renzetti and Arendt (2009) reported an improved overall quality of gluten-free systems and suggested that the addition of transglutaminase promote network formation.

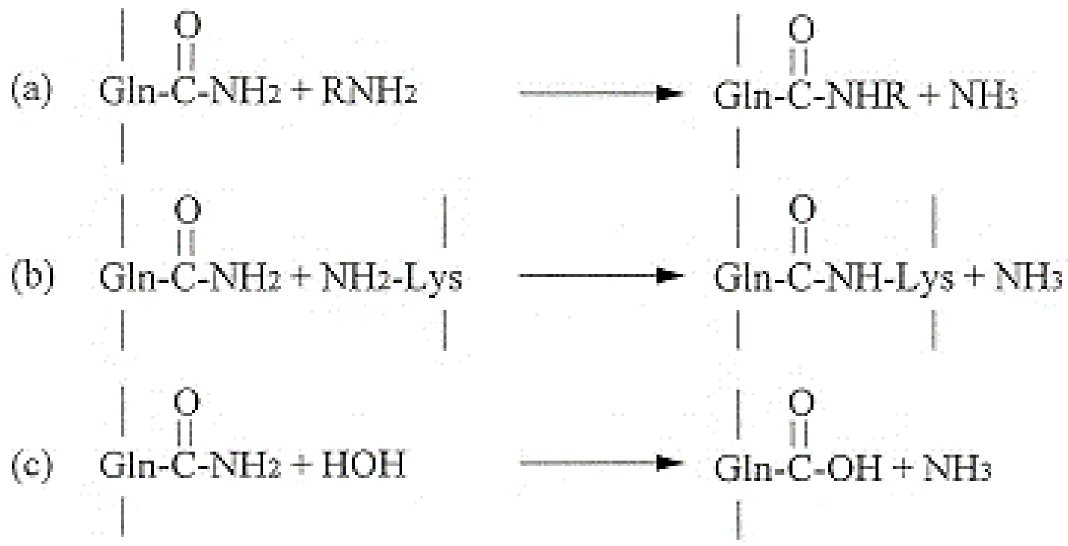

Figure 2.8: Reactions mechanism for transglutaminase: (a), acyl-transfer reaction; (b), crosslinking reaction between Gln and Lys residues of proteins or peptides. The resultant bridge is called ε-(γ-glutamyl) lysine (G–L) bond; (c), deamidation (Motoki and Seguro 1998)

The extent of crosslinking depends mainly on two factors which are the optimum activity and stability conditions and the morphological condition of the substrate molecule at the reaction settings (Buchert *et al.,* 2010). The main factors affecting the formation of inter/intramolecular crosslinks in proteins are the existence and the availability of the amino acid side chains. Studies reported that non-globular proteins are effortlessly reachable to enzyme active sites than globular proteins. However, the above-mentioned crosslinking enzymes are highly specific regarding the substrates they act upon, hence the increasing interest in laccase due to the wide range of substrates it can act on.

2.5.5.3. Laccases

Laccase is an oxidative enzyme that has not been fully exploited especially in the baking industry. Laccase is a multi-copper metalloprotein (Figure 2.9), catalysing the oxidation of phenolic acids, phenolic amines, methoxy substituted phenols and alkylamines (Minussi *et al.,* 2002, Kudanga *et al.,* 2011, Niño-Medina *et al.,* 2017). The oxidation occurs through a single electron removal mechanism with simultaneous reduction of molecular oxygen to water, resulting in the development of free radicals (Pezzella *et al.,* 2015). It is considered to be a dioxygen-binding protein, playing a big role in many biological processes such as activation of the oxygen, binding of molecular oxygen and oxidation of substrate molecules.

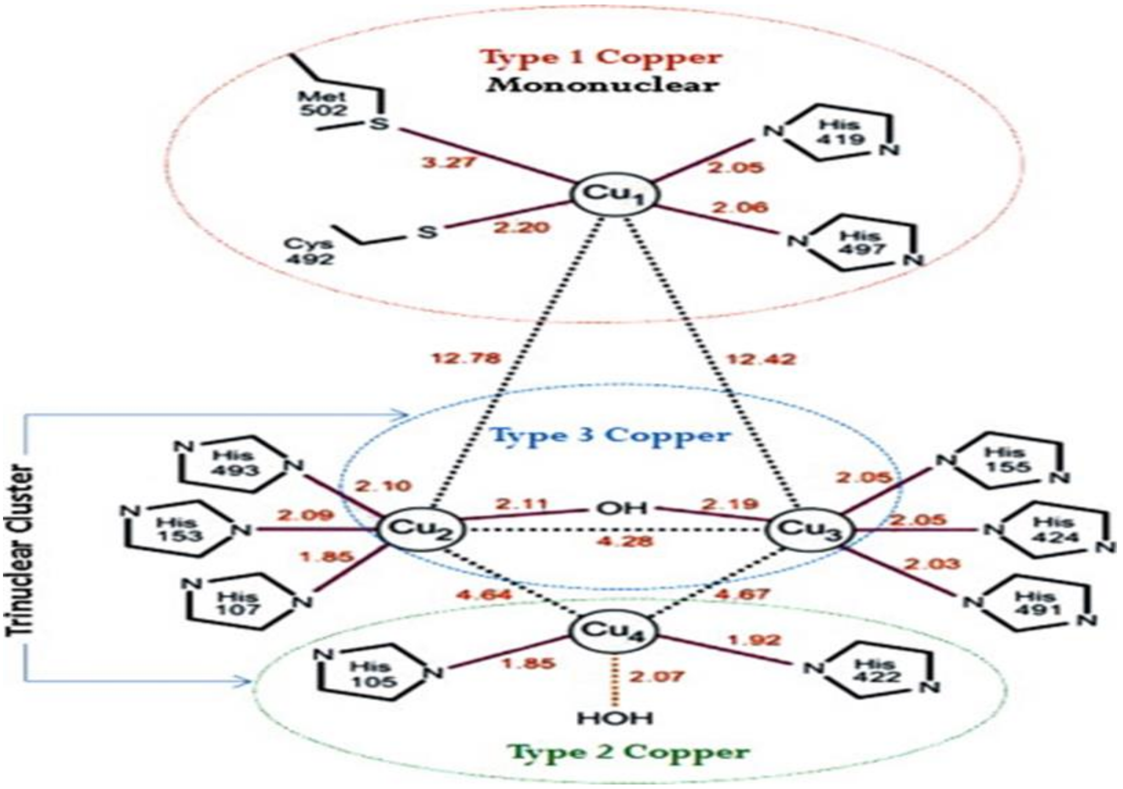

Figure 2.9: The structure of laccase active sites (Zeeb *et al.*, 2014)

Most of the laccases that have been characterized so far are derived from fungi. Laccases are stable in alkaline pH ranges and temperature stability varies with the source; fungal laccases are stable between 30–60ºC and laccase from bacteria the most thermostable (Buchert *et al.*, 2010).

2.5.5.4. Application of laccases

In laccase-catalysed oxidation reactions, the radicals produced can facilitate substrate polymerization due to high reactivity. They have been associated with crosslinking arabinoxylan and pectin through the ferulic acids molecules (Selinheimo *et al.*, 2006; Martínez-López *et al.*, 2013) (Figure 2.10). Laccase can directly oxidize cysteine and tyrosine; however, studies have reported that the linkages mostly formed between tyrosine residues. Due to the ability of laccase to oxidize a variety of phenolic and other non-phenolic compounds by mediator reactions, it has become a desirable enzyme for biotechnological and food production processes.

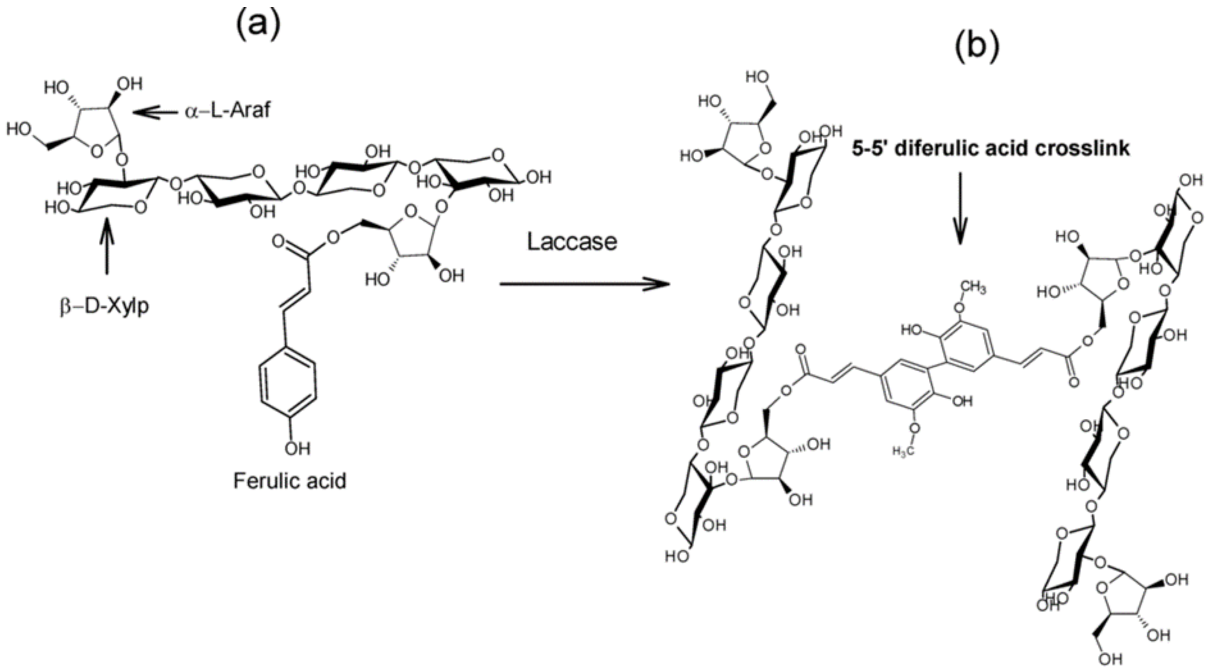

Figure 2.10: Schematic representation of the covalent cross-linking of feruloylated arabinoxylans (Martínez-López *et al.*, 2013)

The main intention in the food industry is to use oxidative cross-linking enzymes in improving the quality of the end products (Osma *et al.*, 2010). An example is in the use in the cereal industry during bread making to improve the quality of weak flours. Studies have shown that the inclusion of laccases in wheat flour processing can result in increased strength as shown in Figure 2.11, reduced stickiness, increased stability of the dough as well as an improvement in the machinability of dough. Also, bread volume tends to increase and crumb structure improves when laccases are used (Labat *et al.,* 2001; Selinheimo *et al.,* 2006; Flander *et al.,* 2008; Castro *et al.,* 2015). In a research by Selinheimo (2006) on enzyme-aided wheat bread making, the influence of laccase on the structure formation of wheat flour dough and bread was compared to that of tyrosinase. Laccase increased the maximum resistance of the dough and decreased the dough extensibility. Laccase effected changes by acting on the protein fraction either directly or by generating ferulic acid radicals (Selinheimo *et al.,* 2006). However, laccase catalysed hardening was predominantly dependent on the arabinoxylan fractions available.

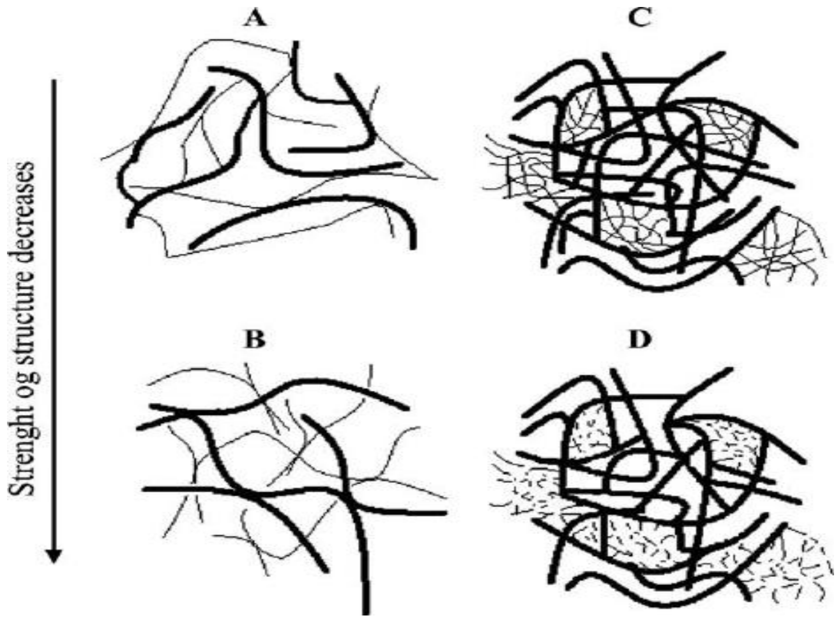

Figure 2.11: Effect of laccase on wheat dough (Buchert *et al.,* 2010)

However, there is little information on the potential of laccase in gluten-free baking. Renzetti *et al.* (2010) reported an improved crumb structure and increased bread volume with laccase addition in gluten-free oat bread recipe, thus showing the potential of laccase in gluten-free baking. However, more research on the effect of laccase on other gluten-free flours could be of paramount importance.

2.6. Amadumbe /Taro (*Colocasia esculenta*)

Tubers are important sources of carbohydrates and are used as a staple food in tropical and subtropical countries (Ugwu, 2009). Different tubers are grown worldwide and these include sweet potato (*Ipomea batatas*), yams (*Dioscorea spp*), potato (*Solanum tuberosum*) and cocoyam (*Colocasia spp and Xanthosoma spp*). *Colocasia esculenta* tubers (Figure 2.12) possess a nutritional composition which is comparable to commonly consumed cereal. They also contain resistant starch and mucilage which have nutritional benefits and can be of great use in the baking industry. The absence of gluten in tubers can make them potential gluten-free ingredients and used in the reduction of incidence of celiac disease (CD) (Rekha and Padmaja 2002).

Figure 2.10: Amadumbe corms collected from Jozini in Kwa-Zulu Natal region in South Africa

2.6.1. Production, utilization, composition, mucilage and current applications

Amadumbe (*Colocasia esculenta*) also known as taro, is one of the root tubers of the tropics and is rather under-utilized in Africa. This has been attributed to the limited information known/ available about its potential hence its production is very low when compared to other tuber crops (Ugwu, 2009; Kaushal *et al.,* 2015). Amadumbe is mainly cultivated because of its corms and is considered the 14th cultivated vegetable/staple around the world (Oscarsson and Savage 2007).

Amadumbe is considered a good source of dietary starch (Mawoyo, 2017). It is easily digestible due to the small size of its starch granules between 0.25 and 0.5 µm hence can be suitable in the preparation of various foods including infant foods (Kaushal *et al.,* 2015). The tuber contains mucilage which is a hydrocolloid and when broken down yields β-galactose and L-arabinose. Mucilage exhibit unique rheological properties and has a potential as food thickeners and stabilisers (Njintang *et al.,* 2008) and could be potentially useful in gluten-free applications.

2.6.2. Nutritional composition of amadumbe

On dry weight basis amadumbe contains about 2.1% protein which is more than that found in yam, cassava or sweet potato; the protein, is rich in essential amino acids threonine, leucine, lysine, valine and phenylalanine (Njintang *et al.,* 2007; Kaushal *et al.,* 2012; Amon *et al.,* 2014). The protein content is highly concentrated towards the periphery of the corm than the centre hence greater care should be employed when peeling the corm. Fat content is very low (0.3-0.6%) in amadumbe and the fat is mainly composed of the lipids of the cell membrane (Bamidele *et al.,* 2014). Both dietary and non-dietary fibre is found in amadumbe and has many desirable functional properties which include helping in glucose metabolism, facilitating alimentary canal functions and slowing down the process of re-absorption of undesirable dietary components (Njintang and Mbofung 2003; Hassan *et al.,* 2015). Ash content of amadumbe ranges from 3.54 - 7.78% and is a good source of minerals such as calcium (31-132mg/100g), iron (8.66-10.8mg/100g), sodium, phosphorus (72.21-340mg/100g) magnesium, zinc, copper and potassium (2271-4276.06mg/100g) (Njoku and Ohia 2007). Amadumbe contains a significant amount of vitamin B complex (thiamine, niacin and riboflavin) and vitamin C. A South African study of traditional foods showed that a boiled extract of amadumbe, had very high antioxidant activity (Lindsey *et al.,* 2002).

However, amadumbe corms have a high moisture content which reduces their shelf-life and one method of preserving the corms is drying them and grinding into flour (Pérez *et al.,* 2007). Different methods can be used to obtain flour from amadumbe corms. Kaur *et al.* (2013) used a method that involved washing, slicing, blanching of the amadumbe corms (at 90°C using distilled water for 2 min), drying at 50°C in a hot cabinet drier for 4 hours and then grinding in a laboratory mixer. The ground materials were passed through a sieve to obtain flour. However, higher temperature is not encouraged due to harmful effects on food components such as proteins, vitamins, colour and other components (Reddy and Love 1999; Dupont *et al.,* 2006). The particle size distribution of the amadumbe flour depends highly on the degree of grinding and the sieve used and it greatly affects the functional properties of the flour and the quality of end products (de la Hera *et al.,* 2013; Protonotariou *et al.,* 2014). Therefore, it is very important to consider the different conditions such as drying temperature, drying time and particle size. Amadumbe contains high amounts of mucilage, making it a possible replacement for corn or wheat flour. Raw and blanched amadumbe flour contain lower protein, fat and starch contents and higher sugar and fibre contents than wheat flour (Godoy *et al.,* 1992). Recent studies have shown that amadumbe flour possesses high water and oil absorption capacities and inferior foam capacity and stability, whip ability and nitrogen solubility comparable to wheat flour making it possible to use amadumbe flour in products that require good viscosity (Kaur *et al.,* 2013). Amadumbe flour has been used to make different snacks and baked products; it has been composited with wheat flour in the production of bread and it was reported that the incorporation of amadumbe flour in wheat flour increased the water holding capacity of wheat dough (Njintang *et al.,* 2008; Sanful, 2011). In another study, amadumbe flour was used to make baked snacks and the results showed that 15% amadumbe flour substitution could result in snacks with improved quality (Njintang *et al.,* 2016).

2.6.3. Mucilage

Mucilages are natural plant products that are sometimes referred to as gums. Though closely related to pectin, they differ in physical properties (Haruna *et al.,* 2016; Jangdey *et al.,* 2016). The differences between mucilages and pectin are that when pectin is exposed to water they gelatinize whereas gums swell and mucilages actually form aqueous dispersions which are slippery. Mucilages are found in different parts of plants, for example, canals, sacs and mucilage secreting hairs (Ameri *et al.,* 2015) and are part of the plant cell walls (Prajapati *et al.,* 2013). Mucilages act as water reservoirs, energy reserves and membrane thickeners in plants.

Mucilages are exopolysaccharides and glycoproteins (Naqvi *et al.,* 2011). The polysaccharides are concentrated with hydroxyl groups which gives mucilages high-water binding capacity. These complex polysaccharides are part of the dietary fibre and absorb large amounts of water and because they store water they assist certain plants by being tolerant to drought conditions (Njintang *et al*., 2008; Nguimbou *et al.,* 2014). Mucilages have been used in the food industry to improve food products. The structure of amadumbe mucilage has not been concluded but it has been hypothesized that it contains a chain of galactose-mannose-arabinose-xylose. Mucilages have a greater potential of being used because they are natural products, they are cheaper and they are freely available (Mishra *et al.,* 2004). They have been used to coat fruits in-order to increase their shelf-life, (Penfield *et al.,* 2001), and also in water purification (Saenz *et al.,* 2004).

2.7.0 Legumes

Legume proteins have been used in the development of baked goods so as to achieve a protein improved product with enhanced amino acid balance (Foschia *et al.,* 2017). One of the most useful properties of legume proteins is the availability of lysine, which is an essential amino acid. However, legume protein is deficient in sulphur-containing amino acids, hence, its combination with cereals such as wheat would be complimentary. The possible use of legumes as protein-enriching agents of baked products, mainly in the form of protein flours, has been reported by several authors (Marco and Rosell 2008a; Sabanis and Tzia 2009; Rizk *et al.,* 2015a; Shevkani *et al.,* 2015; Villarino *et al.,* 2016). Proteins perform a key part in bread making, hence, supplementation of wheat bread with soy bean, cowpea, and proteins affects the rheological properties of the wheat flour bread and other baked products (Aguilar *et al.,* 2015). These outcomes can be quantified by physical dough-testing procedures to assess the bread-making possibility and execution characteristics of the fortified flour. The ability of proteins to build a structure is linked to the swelling ability and emulsifying properties of the protein (Ziobro *et al.,* 2013). Studies have noted that proteins from soy can develop heat resistant gels that have properties resembling gluten properties. Heat application often induces the unfolding of polypeptide chains, hence exposing amino acid residues and sulfhydryl units. These can, however, crosslink through the formation of disulphide linkages or hydrophobic interactions (Miñarro *et al.,* 2012). The resultant dough may possess a three-dimensional network which can help in retaining gas and water, hence impacting on the rheological properties of dough/batter systems. Another legume that has good properties that can be of great importance in bread baking is cowpea, it has good emulsifying properties (Shevkani *et al.,* 2015). It has been stated that emulsifiers facilitate the interaction between two different chemical phases hence stabilising thermodynamically unstable systems. Incorporation of emulsifiers in baking results in strengthened dough systems and softer bread crumb. Another protein source with a great significant functionality is marama bean protein (Amonsou *et al.,* 2012). The dough forming capacity of marama bean dough was investigated and the extensibility of dough made from marama was said to be double that of gluten and soya. With further hydration, it was reported that the extensibility increased threefold (Amonsou *et al.,* 2012; Amonsou *et al.,* 2013). This shows the great potential of legume proteins in gluten-free food products hence a study of other legumes and their potential in gluten-free systems is of great importance.

2.7.1. Functional properties of legume protein

Functional properties are the intrinsic physicochemical properties that influence the behaviour of proteins in food systems (Amonsou, 2010; Mundi, 2012). These properties include water and oil capacity, solubility, foaming and emulsifying properties and rheological properties (Amonsou, 2010). Legumes, including bambara and soy protein, have been exploited as functional ingredients for the preparation of various meals either on their own or as ingredients because of their high nutritional value and availability. Various studies have exploited the protein functionalities of legume crops such as bambara (Adebowale *et al.*, 2011), kidney bean (Wani *et al*., 2013), pea (Barac *et al.,* 2015), soya bean protein (De la Caba *et al.,* 2012; Rebholz *et al.,* 2012) and fenugreek (Feyzi *et al.,* 2015).

2.7.2. Bambara groundnut (*Vigna subterranea)*

Bambara groundnut (*Vigna subterranea)* (Figure 2.11) is a legume which belongs to the family of Fabaceae (Mazahib *et al.,* 2013). Bambara groundnut is regarded as the third most important crop in Africa but due to its low status, it is recognised as a snack and not a lucrative cash crop. Additionally, it has been termed a woman's crop hence it has been given less value and less priority in land allocation. Bambara grows well at an average temperature between 20 and 28 ^{o}C The plant adapts and can thrive in harsh conditions better than most crops, it is a drought resistant crop (Adegbola and Bamishaiye 2011; Hillocks *et al*., 2012). Currently, Africa is faced with food security threats and global warming; bambara might be a crop of hope to help alleviate these challenges because of its drought resistant characteristics (Basu *et al.,* 2007). In South Africa, bambara groundnuts are grown mostly in the Limpopo, Mpumalanga and KwaZulu-Natal provinces. Bambara groundnuts have different seed colours namely white, cream, yellow, brown, purple-red and black as shown in Figure 2.11.

Figure 2.11: Varieties of South African bambara groundnut

2.7.3. Nutritional profile of bambara groundnut

The bambara seed contains sufficient quantities of protein (20.5-26.0%), carbohydrate (50.5-69.3%) and fat (4.3-7.9%) and amino acids are higher than that found in most legumes (Steve *et al.*, 2009; Murevanhema and Jideani 2013, Adegbola and Bamishaiye 2011; Hillocks *et al.*, 2012). Bambara is also a good source of iron, potassium, fibre and calcium. In areas where animal protein is highly expensive bambara can be a great alternative. Bambara remains underutilised despite all these attributes and it is neglected despite the potential it has to play in food security and income generation (Murevanhema and Jideani 2013). Besides the nutritional importance of bambara groundnuts, various problems such as beany flavours, long cooking hours and anti-nutritional factors have been reported and they hinder maximum utilisation of the crop (Honi, 2016). Bambara groundnuts contain low levels of trypsin inhibitor and phenolic compounds. Other anti-nutrients which are contained in bambara groundnut are oxalate, tannic acid, phytic acid, phytin phosphorous, trypsin (Ijarotimi and Esho 2009). It has been stated that the red and brown bambara groundnuts contain the highest levels of tannins with the cream coloured having the lowest (Unigwe *et al.*, 2017).

2.7.4. Uses of bambara groundnut

There are many ways bambara groundnuts are consumed, such as eating it fresh or grilled while immature. The fresh pods can be boiled with salt and eaten as a snack, the nuts can be roasted and crushed to make soup (Murevanhema and Jideani 2013). Recently studies have deduced bambara groundnut potential in the development of various food products such as biscuit and cake production, vegetable milk and yoghurt (Murevanhema and Jideani 2013; Falade and Okafor 2015). In Nigeria bambara groundnut paste is used in the preparation of okpa. Okpa is normally prepared by wrapping bambara paste in banana leaves and boiling. Bambara groundnuts are also famous for their medicinal characteristics, especially in West Africa (Chivenge *et al.,* 2015). In Kenya, boiled bambara seeds are used to treat diarrhoea, the leaves are used to treat wounds and the sap from the leaves is used to treat epilepsy (Adegbola and Bamishaiye 2011). In Senegal bambara roots are taken as an aphrodisiac and the seeds are used to treat cataracts while in Nigeria, the plant is used to treat venereal diseases *(Hillocks et al.,* 2012). In South Africa bambara raw seeds are swallowed to curb nausea during pregnancy (Murevanhema and Jideani 2013).

2.7.5. Bambara in the baking industry

Various studies have reported the use of bambara groundnut flour in the supplementation of other flours such as wheat flour (Abu-Salem and Abou-Arab 2011; Nwosu, 2013). In these studies, the blended flours were used to develop biscuits and the results showed that the biscuits were high in protein. With bambara groundnut incorporation, the diameter of the biscuits was increased and there was an improvement in the flavour and texture of biscuits. These changes were linked to the changes in the functional properties of wheat flour when bambara groundnut flour was added. In another study, bambara groundnut was used to develop bread and the resultant bread exhibited improved protein, ash and crude fibre content (Alozie *et al.,* 2009). In a study by Erukainure *et al.* (2016), it was observed that with bambara groundnut incorporation in wheat flour the development and the constancy time improved. In another study, an increase in bread specific volume was observed with bambara groundnut flour inclusion (Abdualrahman *et al.,* 2012). However, most of the studies that have been done were basically more on wheat flour being supplemented with bambara flour. To our knowledge, not much has been documented on the effect of bambara groundnut supplementation in gluten-free dough systems.

2.7.6. Soy bean (*Glycine max* (L.) Merrill)

Soy bean (*Glycine max* (L.) Merrill) is a legume crop (Figure 2.12) that is rich in proteins (35-45%) and oil (17-25%) and is also a good source of vitamin B1 and vitamin B2 (Abbaspour *et al.,* 2014; Sahin, 2014; Paddon-Jones *et al.,* 2015). It has been reported that soy bean has the highest protein quality for meeting the physiological needs of humans (Wildman, 2016). The high protein content is characterized by a good amino acid balance and has been reported to be the best compared to other plants. According to Food and Agriculture Organization (FAO) and World Health Organization (WHO) protein quality is defined as a protein that is fully digested and has an amino acid composition that is closely related to the amino acid patterns of humans. Soy bean supplies all the nine amino acids that are to be supplied by the diet hence making it a complete protein (Boerma and Specht 2004). However, soy has a minimal amount of methionine hence the combination with cereals or tubers can help in complementing the deficit helping to meet the FAO requirements. Soybean also contains trace amounts of minerals, phytin and phenolics (Vong *et al.,* 2017).

Figure 2.12: Soy bean plant and seeds (Kinsella, 1979)

2.7.7. Health benefits of soy protein

Soy protein has been used in the human diet in different forms that include flours, protein isolates, infant formulas and textured fibres. Various food products have been produced using soy. These include drinks, cheese, vegetarian meat substitutes and tofu and consumption of these food products has been associated with reported health benefits that soy possesses. It has been stated that soy protein can help in lowering plasma cholesterol and preventing cancer (Sun *et al.*, 2004; Wu *et al.*, 2008; Applegate *et al.*, 2017), reducing obesity and diabetes (Ruscica *et al.*, 2016; Panasevich *et al.*, 2017) and also preventing bowel kidney diseases (Kaitha *et al*., 2015; Elamin *et al.*, 2017; Gallant and Spiegel 2017).

2.7.8. Functional properties of soy flour in bread

Soy bean is mostly consumed in Asian diets and is gaining interest in Western diets because of the presumed health benefits linked to lowering risks of heart diseases (Erdman, 2000; Rizza *et al*., 2014; Butteiger *et al.*, 2016). However, in the baking industry soy bean flour is mostly used as a composite with wheat flour and the acceptability index of the resultant baked products is low in terms of the sensory properties and overall quality. This has been attributed to the lipoxygenase activity that might occur during food processing hence causing off flavours in the final product, also the presence of phytochemicals and in most cases a significant decrease of loaf volume when soy flour is added (Shin *et al.*, 2013; Hayward, 2017). It has been hypothesized that there is no interaction whatsoever between soy and gluten proteins hence the resulting adverse effects; the non-gluten networks formed have been reported not to exhibit the elastic properties necessary and hence the inability to retain gas during fermentation (Abass *et al.*, 2018). In a contradictory study, gluten and soy protein interactions were reported to improve dough characteristics and also the addition of emulsifiers improved gas retention and the overall bread quality (Ryan *et al.*, 2002). On the other hand, sulfhydryl and disulphide bonds have been reported to be important in controlling the overall bread quality which includes the loaf volume (Gao *et al.*, 2017). However, the use enzymes have been reported to have the following positive effect during bread making: improvement of the rheological properties through oxidation, increasing dough mixing tolerance and also improving gas retention which resulted in increased bread volume (Liu *et al.*, 2017; Scarnato *et al.*, 2017). Despite the unpleasant organoleptic effects that soy flour imparts on baked products, it has been reported that due to its protein content, it facilitates great water absorption, improves cake/bread tenderness, improves moisture retention, and enhances crumb colour and texture (Veena, 2009). Apart from compositing soy flour with wheat flour, it has been composited with barley and resulted in acceptable organoleptic results (Dhingra and Jood 2002). The functional properties of soy protein flour help in improving dough handling techniques and machinability and (Mert *et al.*, 2016). Nutritionally, soy flour addition in bread making improves the protein content of the baked bread. Furthermore, soy-fortified flour, when used in dough systems, reduces fermentation time required during bread making and in cake making less shortening is required (Vishwakarma, 2016). However, some of the adverse effects of using soy flour highlighted above can be minimised by using soy protein isolate.

2.7.9. Soy protein isolate

Soy protein isolates (SPI) contain approximately 3-5% moisture, 91-95% protein, ~ 0% fat, ~ 0% carbohydrate, and 3-4% ash (Stauffer, 2005). SPI is obtained from defatted soy flakes/flour by extracting the soy flakes/flour using a dilute alkali (pH 8-9) with subsequent centrifugation for the removal of the insoluble materials, oligosaccharides, minerals and producing soy protein. The soy dispersion is then acidified using acids such as hydrochloric acid, sulphuric acid and phosphorus acid to pH 4.5. Acidification results in selective recovery of the proteins as a result of their precipitation and concentration into a curd. The concentrated curd is washed to remove non-soluble protein and then neutralized (pH 7) and dried.

In the baking industry, soy protein isolate has been linked to an improvement of dough rheological properties as well as the quality of the baked product. SPI has been used as an egg substitute during cake making, and SPI alone led to a significant increase in the batter specific gravity and a decrease in specific volume (Lin *et al.,* 2017). Hence, it was proposed that other polymeric substances such as hydrocolloids and emulsifiers could assist in improving the quality of the cake. SPI has also been used in combination with rice flour and a hydrocolloid as a wheat-protein substitute and resulted in crackers with improved puffiness (Morales-Polanco *et al.,* 2017). Due to the substantial protein content and composition of SPI, it has also been used to enrich potato staple foods hence enlightening its potential in food enrichment (Mu *et al.,* 2017). However, despite the use in the baking industry, little research has been done on its potential and functionality in the production of gluten-free baked products when exposed to enzymes such as laccase.

2.8.0. Breadmaking

2.8.1. Basic ingredients of bread

The most significant ingredients in breadmaking are water and flour as they directly affect texture and crumb. The flour is usually 100% and the rest of the ingredients are a percentage of the amount of flour by weight. Usually 50% water results in finely textured and light bread (Cauvain, 2015). In most bread formulas 60%-75% water is used and the higher the water percentage used the more the CO_2 bubbles produced hence a coarser bread crumb. The other ingredients that are used in breadmaking include yeast, sugar, salt and shortening. Bread is a leavened product, a result of the fermentation of flour sugars liberated from starch by the action of natural flour enzymes (Dobraszczyk and Morgenstern 2003; Cauvain and Young 2007). *Saccharomyces cerevisiae* (baker's yeast) is used as a fermentation agent during bread making, as a result sugar is converted to water and CO_2. As water and carbon dioxide produced expand due to exposure to heat, they act as insulating agents hence preventing rapid temperature rise of bread crumb and excessive moisture loss. Sugar is added to dough systems to initiate fermentation, salt is also added to strengthen dough and to assist the action of yeast for controlled expansion of the dough. Shortening is added to increase the machinability or specifically sliceability (Stampfli and Nersten 1995; Brooker 1996; Matuda *et al.,* 2005; Pareyt *et al.,* 2011). Fresh bread is usually brownish and has crunchy crust, fine slicing characteristics, a soft and elastic crumb texture and a moist mouthfeel (Giannou *et al.,* 2003). In order to meet the high levels of demand for high quality, convenience and longer shelf-life products, the use of food additives such as hydrocolloids, enzymes, emulsifiers and anti-stalling agents has been proposed (Pellegrini and Agostoni 2015; Theethira and Dennis 2015; Masure *et al.,* 2016). These have been used to impart greater dough strength, improve rate of hydration, improve crumb structure, improve slicing characteristic, improve gas holding capacity and extend shelf life.

2.9.0. Conclusion

It is a great technological challenge to produce a nutritious, acceptable and good quality gluten-free leavened bread. The use of proteins in gluten-free formulations has proved to be a successful viable alternative, as proteins aid in improving appearance, structural stability and the nutritional value. However, currently proteins need to be used in combination with other polymeric substances such as hydrocolloids and enzymes for them to be fully functional in dough systems.

2.10.0. Aim, hypothesis and objectives

2.10.1. Aim

To investigate the effect of laccase and xanthan gum on the quality of gluten-free amadumbe bread quality.

2.10.2. Hypotheses

1. The incorporation of protein rich flours from legumes such as bambara groundnuts and soy to low protein crops such as amadumbe will improve their functionality.

   Leguminous flours exhibit properties that enhance their wide utilization; these properties include improved emulsion capacity and stability, foam capacity and stability and oil absorption among other properties (Anton *et al.*, 2008). Hence the incorporation of leguminous crops such as bambara groundnuts and soy can improve the functional properties of amadumbe flour. In a study by Ikpeme-Emmanuel *et al.* (2009), soya bean was used to enrich amadumbe flour in the development of infant weaning food resulting in an improvement in the functional properties of amadumbe.

2. Laccase will have an effect on the textural properties, specific volume, moisture content and consumer acceptability of gluten-free amadumbe bread.

   Studies have shown that the inclusion of laccases in cereal processing can result in increased strength, stability and reduced stickiness of the dough. Formation of disulphide bonds by laccase (Flander *et al.*, 2011) and oxidative gelation of ferulic acid esterified to arabinoxylan will improve the dough rheology. This, however, improves the machinability of dough. In addition, bread volume tends to increase and crumb structure improves when laccases are used (Primo-Martin *et al*., 2003; Selinheimo *et al.,* 2006).

3. Xanthan gum will influence the textural properties, specific volume, moisture content and consumer acceptability of gluten-free amadumbe bread.

   Hydrocolloids can be used as a gluten substitute in gluten-free bread; they can be used to improve texture in food, slow down starch degradation, increase moisture retention and extend the overall quality of the product over time (Sidhu and Bawa 2002). An increase in water absorption has been reported when hydrocolloids such as xanthan, alginate, j-carrageenan, and HPMC, were added to rice flour (Rosell *et al.,* 2001). Xanthan gum is normally used together with emulsifiers in the development of gluten-free bread and protein-enriched bread, to stabilise emulsions and to prolong shelf-life of bakery products due to gluten-starch inhibition and lowering of moisture loss of crumb (Guarda *et al.,* 2004; Gomez *et al.,* 2007; Arocas *et al.,* 2009).

2.10.3. Objectives

1. To determine the physical and functional properties of amadumbe flour, bambara groundnut flour, soy protein isolates and their blends regarding their suitability in the production of quality amadumbe gluten-free bread.

2. To determine the effect of xanthan gum on the quality of gluten-free amadumbe bread.

3. To determine the effect of laccase on the quality of gluten-free amadumbe bread.

4. To determine the consumer acceptance of gluten-free amadumbe bread.

Chapter Three

Physicochemical and nutritional properties of amadumbe flour supplemented with bambara groundnut and soy protein isolate flours

Abstract

The physicochemical and nutritional properties of amadumbe flour supplemented with bambara groundnut flour and soy protein isolate, were investigated. Blends were formulated from the flours using a ratio of 70:30 (amadumbe flour: bambara flour) and 88:12 (amadumbe flour: soy protein isolate) based on a target protein concentration of 16 g/100 g flour. Nutritional analysis and amino acid composition were done and the results showed that the protein content of amadumbe flour increased from 2.36 g/100 g to 15.87 g/100 g flour when bambara groundnut flour was added and to 16.10 g/100 g flour when soy protein isolate was added. The amino acid composition of amadumbe flour was also enhanced with the addition of bambara groundnut flour and soy protein isolate. The water absorption of amadumbe flour also improved by 10% and 15%, with the addition of bambara groundnut flour and soy protein isolate, respectively. No significant difference in oil absorption capacity was reported between amadumbe flour and the blends, however, bambara flour exhibited the least oil absorption capacity. The incorporation of bambara groundnut flour and soy protein isolate in amadumbe flour resulted in improved foam and emulsion capacity and also influenced the formation of stable emulsions and foam. These results show the potential of amadumbe blends in the food industry because of good/improved functionality and protein content.

3.1. Introduction

Blends can be defined as a mix of various flours which can include wheat and other flours, or wholly non-wheat flours, for the production of unleavened baked products, leavened bread, porridges, pasta, and snack foods (Chandra *et al.*, 2015). Blending is usually done to cater for economic, nutritional reasons as well as improving flour functional properties. For example, legumes such as cowpea, chickpea and soy bean flour have been exploited to enhance the protein content of the baked products as well as improving functionality of the flours (Singh *et al.,* 2008; Aluko *et al.,* 2009). Some of the blends that have been developed include wheat, cowpea and plantain blends (Akubor, 2003), chickpea-wheat flour blend (Rizk *et al.,* 2015) and small red, black, pinto and navy bean-wheat flour blend (Anton *et al.,* 2008) and in all these studies an improvement in the blend functional properties, status of protein and limiting amino acids was reported. However, there is now a renewed effort to broaden the food base in developing countries by creating new food products without wheat, based on indigenous/traditional raw materials such as sweet potato and cassava (Yadav *et al.,* 2014). However, one of the crops that has recently gained interest due to it nutritional composition is amadumbe.

Amadumbe (*Colocasia esculenta*) is a traditional crop grown in South Africa mainly for subsistence and is highly underutilised. It is a good source of carbohydrates, phosphorous, calcium, Vitamin C, iron, riboflavin, niacin and thiamine which are essential in the human diet. Amadumbe is also a good source of mucilage (75.7 to 137.0 g kg^{-1}, dry weight basis) depending on variety (Nguimbou *et al.*, 2014). Mucilage is a hydrocolloid which can help in improving food texture (Rosell *et al.*, 2001), slow down the starch retrogradation (Arocas *et al.,* 2009), assist in the retention of moisture and prolong the overall characteristics of the product over time (Guarda *et al.*, 2004). However, amadumbe flour is low in protein (2.8%-4.9%, dry weight basis) therefore there is great need to supplement with protein rich crops in order to enhance its dietetic and functional properties (Njintang *et al.,* 2008; Moroni *et al.,* 2009). The incorporation of protein-rich crops such as legumes can help improve the functionality of low protein crops such as amadumbe (Hazen, 2011). Leguminous flours exhibit properties that enhance their wide utilization. These properties include high emulsion capacity and stability, foam capacity and stability and oil absorption among other properties (Anton *et al.,* 2008). In a report by Ikpeme-Emmanuel *et al*. (2009), soya bean was used to enrich amadumbe flour in the development of infant weaning food and reported an improvement in the functional properties of the flour. Hence, the addition of legumes to amadumbe flour could help improve its functionality mainly in the baking industry.
Bambara groundnut (*Vigna subterranea*) is a protein rich-starchy grain also grown in South Africa. Bambara groundnut protein comprises of essential amino acids such as lysine which could complement amadumbe amino acid deficit. Aremu *et al.* (2007) reported that bambara groundnut

protein also possesses functional properties required for an effective utilization in various food products. These functional properties include emulsion capacity and stability and foam capacity and stability. Kiin-Kabari *et al.* (2015) also showed that the addition of bambara groundnut flour to wheat/plantain flour improved foam capacity, emulsion capacity, foam stability and emulsion stability of the wheat/plantain flour. Nnam (2001) also reported an improvement in the nutritional composition of porridge developed from sorghum, bambara groundnut and sweet potato flour. Hence the addition of bambara groundnut flour to amadumbe's flour can help improve amadumbe flour nutritional and functional properties.

Soy bean (*Glycine max* (L.) Merrill) is another legume that is rich in protein, the protein isolate contains approximately 90% of the protein. Soy protein isolate provides a high lysine content (6.83%) and may have a positive impact on the overall product quality (Tömösközi *et al.,* 2001; Singh *et al.,* 2008). Soy protein isolates also provide different physical and functional properties to meet the prerequisites of different food systems (Morales *et al.,* 2015). Crockett *et al.* (2011) noted that the use of the isolate could enhance both the emulsifying capacity and stabilizing properties of food ingredients. Soy protein isolate has also been used in combination with other flours, for example, maize, chickpea and soy protein Rehman *et al.* (2017), soy protein and barley Dhingra and Jood (2004) and cowpea, soy protein and red beans blends (Mbofung *et al.,* 2002). In all the above-mentioned studies, the addition of soy protein enhanced the flour nutritional and functional properties, hence, soy protein isolate can help complement amadumbe flour properties enhancing its functionality.

The aim of the current research was to determine the physicochemical and nutritional properties of amadumbe flour supplemented with bambara groundnut and soy protein isolate. The study is expected to provide information necessary to support for the consideration of amadumbe, bambara groundnuts and soy protein isolate as potential gluten-free ingredients.

3.2. Materials and methods

3.2.1. Materials

Amadumbe corms and bambara groundnuts were procured from Umbumbulu, KwaZulu-Natal province, and the soy protein isolate was commercially sourced from Lionheart Chemical Enterprises (Pty) Ltd, Durban.

3.2.2. Flour preparation

Amadumbe flour was produced as described by Naidoo *et al.* (2015) with some modifications. Briefly, amadumbe corms were cleaned, peeled, sliced (3-4 cm) and dried at 52°C in an air cabinet drier oven for 10 hours and ground into flour using a Warring laboratory mill blender (HGBTWTS3, Torrington, CT, USA).

To produce bambara groundnut flour, the grains were soaked for 12 hours, dehulled and dried at 45°C in an air oven. The dried grains were milled into flour using a Warring laboratory mill blender (HGBTWTS3, Torrington, CT, USA) and the flours were sieved (355 μm) and kept at 4°C for further analysis (Oyeyinka *et al.*, 2015).

3.2.3. Blends preparation

Amadumbe is low in protein hence supplementing with bambara groundnut flour and soy protein isolate could help improve the protein content. The blends were prepared mainly targeting a specific protein content of approximately 16 g/100 g flour, hence the ratios 70/30 (amadumbe flour and bambara groundnut flour) and 88/12 (amadumbe flour and soy protein isolate) were used.

3.3 Analysis

3.3.1. Colour

Colour characteristics colour measurements were determined using a colour flex EZ 0840 hunter lab spectro colorimeter. The sensor recorded the L*, a* and b* values whereby L* (100-white; 0-

black) as a sign of lightness; a* measures chromaticity, with positive values signalling redness and negative values indicating greenness; and b* quantifies chromaticity, with positive values signalling yellowness and negative values signalling blueness. The instrument was calibrated against a standard white-coloured reference tile and the experiment was done in triplicate (AOAC ,2000).

3.3.2. Proximate analysis and amino acid analysis

Moisture, crude fat and total ash contents were determined using AOAC methods (AOAC, 2000). The crude protein content (N × 6.25) was determined using the Kjeldahl method. Total carbohydrate was calculated by difference.

The amino acid content of amadumbe flour, bambara groundnut flour, soy protein isolate and their blends were determined using the Pico-tag method (Bidlingmeyer *et al.*, 1984). The method is grounded on the assumption of reverse phase chromatography through pre-column derivatization following acid digestion. The flours were hydrolysed using 6 M HCl at 116°C for 24 h preceding chromatographic analysis.

3.3.3. Water and oil absorption

Water absorption capacity (WAC) of flours was quantified using the centrifugation method described by Falade and Okafor (2015) with some modifications. Flour samples (3 g) were dispersed in 25 ml of water in pre-weighed centrifuge tubes. The dispersed sample was held for 30 min and then centrifuged for 15 min at 3500 × g. The supernatant was decanted and the sample was reweighed.

The oil absorption capacity (OAC) was also determined using the centrifugation method Falade and Okafor (2015) with some modifications. Flour samples (2 g) was dispersed in 20 ml of sunflower oil in pre-weighed centrifuge tubes. The dispersed sample was vortexed for 1 min and held for 30 min at room temperature (22-25 °C). The tubes were centrifuged for 30 min at 3500 × g. After centrifuging, the separated oil was removed using a pipette and the tubes were upturned to drain excess oil prior to reweighing. The water and oil absorption capacities were expressed as grams per gram of the flour on a dry basis.

3.3.4. Foaming capacity (FC) and foaming stability (FS)

Flour sample (1.5 g) was dissolved in 50 ml distilled water and sonicated for 3 min using an ultrasonicator. The sonicated flour samples were transferred into a graduated cylinder and the volumes of the sonicated samples before and after whipping were recorded. The foaming capacity was expressed as the volume increased due to whipping. For the determination of foam stability, the volume changes in the graduated cylinder were recorded at intervals of 20, 40, 60 and 120 min of storage (Aluko *et al.*, 2009). Triplicate determinations were carried out.

3.3.5. Emulsion capacity (EC) and emulsion stability (ES)

The emulsion capacity (EC) and emulsion stability were determined as described by Adebowale and Lawal (2004) with some modifications. For the determination of emulsion capacity, the flour sample (1.75 g) was homogenised in 25 ml water and 12.5 ml canola oil. The dispersed mix was sonicated and then centrifuged at 1100 g for 5 min. The height of the emulsified layer and total contents in the tube was noted. The emulsion capacity was calculated as follows:

$$EA\% = \frac{\text{height of emulsified layer in the tube}}{\text{height of the total contents in the tube}} \times 100.$$

For the emulsion stability, the emulsion was heated at 80 °C for 30 min then centrifuged at 1100 × g for 5 min. The emulsion stability was calculated as follows:

$$ES\% = \frac{height\ of\ emulsified\ layer\ after\ heating}{\text{height of emulsified layer before heating}} \times 100$$

3.3.6. Statistical analysis

The data obtained was subjected to analysis of variance (ANOVA) to calculate significant differences in the treatment means and LSD ($p < 0.05$) was used for mean separation using Statistical Package for the Social Science software (SPSS) version 21.0 (SPSS, Chicago, IL, USA).

3.4. Results and Discussion

3.4.1. Colour of amadumbe flour, Bambara groundnut flour, soy protein isolate and its blends

Amadumbe flour recorded the highest L^* value and soy protein isolate recording the least (Table 3.1). Amadumbe flour colour properties were consistent with previous findings (Njintang *et al.*, 2008). The addition of bambara flour and soy protein isolate to amadumbe flour resulted in the darkening of amadumbe flour as in its L^* value. The variations of the b^* values in the flours can be ascribed to the carbohydrate and protein content present in the flours. The colour differences amongst the flours could have been highly influenced by the botanic source of the crops and also the components of the flour (Njintang and Mbofung 2003). Usually, the whiter the flour the more appealing the end product, hence, we can conclude that amadumbe flour can be used in the development of appealing food products.

Table 3.1: Colour profile for amadumbe flour, bambara groundnut flour, soy protein isolate and their blends

Sample	L^*	a^*	b^*
Soya protein isolate	$74.23^a \pm 0.02$	$0.43^d \pm 0.15$	$11.53^b \pm 0.23$
Bambara flour	$87.59^b \pm 0.13$	$0.36^c \pm 0.34$	$12.79^c \pm 0.02$
Amadumbe+soy protein isolate flour (88:12)	$89.75^c \pm 0.32$	$0.32^b \pm 0.52$	$12.05^c \pm 0.43$
Amadumbe+bambara flour (70:30)	$90.17^c \pm 0.43$	$0.33^b \pm 0.14$	$13.11^d \pm 0.05$
Amadumbe flour	$90.57^d \pm 0.25$	$-0.30^a \pm 0.21$	$10.01^a \pm 0.04$

Means (n=3) followed by the same letter in the same column are not significantly different at $p < 0.05$. With annotations: (L*) representing lightness, (a*) red-green and (b*) yellow-blue.

3.4.2. Proximate analysis and amino acid composition of amadumbe flour, Bambara groundnut flour, soy protein isolate and its blends

Table 3.2: The ash, carbohydrate, fat, moisture and protein contents of amadumbe flour, bambara groundnut flour, soy protein isolate and its blends

Sample (g/100 g)	Moisture	Ash	Fat	Crude Protein	Carbohydrate*
Amadumbe flour	4.87	2.60	1.03	2.36	89.14
Bambara flour	8.53	4.02	6.01	29.21	52.23
Soya protein isolate	4.93	2.46	0.80	91.14	0
Amadumbe+bambara flour (70:30)	9.38	4.16	3.38	15.87	67.21
Amadumbe+soy protein isolate flour (88:12)	9.57	3.28	0.75	16.10	70.30

*Carbohydrate by difference

Amadumbe flour had the lowest ash content of 2.60% and the flour blends ranged between 3.18% and 4.16% suggesting that the addition of bambara groundnut flour and soy protein isolate enhanced the ash content of amadumbe flour. These values indicate the minerals that are present in the flours and the discrepancy could be due to the various growth locations of the crops as well as varying environmental factors. Many researchers reported different values of 3.10, 3.88, 4.19 and 4.4% as ash content of bambara groundnut nut flour (Abu-Salem and Abou-Arab 2011; Eltayeb *et al.*, 2011; Arise *et al.*, 2015) which is within the same range as the findings of this study. The fat content of the individual flours and the blends varied between 0.75% and 6.01% soy protein isolate having the least fat content. This significant difference between the flours and soy protein isolate might be because of the defatting process carried out during the isolation of soy protein isolate from soy bean. Similarly, Hillocks *et al*. (2012) reported low-fat content. However, soy protein isolate had the greatest protein content compared to the other flours and the addition of soy protein isolate and bambara groundnut flour improved the protein content of amadumbe flour. The amadumbe flour protein content obtained in this study was within the same range as that noted by Naidoo *et al*. (2015). The protein content of bambara groundnut, however, was seemingly higher than that reported in previous studies and for other legumes such as cowpea and chickpea (Adegbola and Bamishaiye 2011; Hillocks *et al.*, 2012; Murevanhema and Jideani 2013; Arise *et* *al.*, 2015). Carbohydrate content varied between 89.14% and 0% with amadumbe flour having the highest and soy protein isolate recording a zero-carbohydrate content. Results obtained in this study agree with previous studies which have reported that amadumbe is a good source of carbohydrates (Naidoo *et al.,* 2015) . Bambara groundnut flour had carbohydrates as the major nutrient of approximately 56% and similar results have also been previously reported (Murevanhema and Jideani 2013; Arise *et al.*, 2015).

The amino acid composition of the individual flours as well as the blends showed some variations. Glutamic acid and aspartic acid were the major amino acids in bambara groundnut flour and soy protein isolate (Table 3.3). These amino acids are also the major amino acids found in other legume seeds such as soybean. This has also been reported in bambara groundnut flour in previous studies (Adebowale and Lawal 2004; Liu, 2012; Arise *et al.,* 2015). The addition of protein-based flours in amadumbe, however, resulted in improved amino acid content hence making it possible for amadumbe flour to be potentially used in applications where a balanced amino acid composition is important. Tyrosine, lysine and cysteine were also observed in the blends in significant amounts, these amino acids are essential in dough strength and can be potential active cites for different enzymes (Figueroa-Espinoza *et al.,* 1998; Bonet *et al.,* 2006; Selinheimo, 2008).

Table 3.3**:** Amino acid composition of amadumbe flour, bambara groundnut flour, soy protein isolates and their blends

Amino acid (g/100 g protein)	Amadumbe flour	Bambara groundnut flour	Soy protein isolate	Calculated Amadumbe+bambara groundnut flour (70:30)	Calculated Amadumbe+soy protein isolate (88:12)
Histidine	0.12	1.68	1.34	0.58	0.27
Serine	0.33	2.17	4.26	0.88	0.80
Arginine	0.34	5.9	1.66	2.01	0.49
Glycine	0.29	2.87	1.48	1.06	0.43
Aspartic acid	0.75	8.49	9.62	3.07	1.81
Glutamic acid	0.55	14.93	14.96	4.86	2.29
Threonine	0.24	2.89	6.27	1.55	0.96
Alanine	0.24	3.23	4.15	1.14	0.71
Proline	0.22	2.42	5.07	0.88	0.80
Lysine	0.24	3.81	7.61	1.31	1.12
Tyrosine	0.20	2.56	2.56	0.91	0.48
Isoleucine	0.18	2.83	5.49	0.98	0.82
Phenylalanine	0.32	4.83	2.48	1.67	0.58
Valine	0.27	3.82	5.17	1.34	0.86
Cysteine	0.32	0.35	3.47	0.33	0.70
Leucine	0.45	6.69	8.87	2.32	1.46

3.4.3. Water absorption capacity of amadumbe flour, Bambara groundnut flour, soy protein isolate and its blends

Water absorption capacity (WAC) is an essential property in bulking and consistency of products as well as in baking applications. The water absorption capacity of amadumbe flour was slightly higher than that of bambara groundnut and significantly higher than soy protein isolate (Figure 3.1). This could be attributed to the presence of high amounts of polar amino acids and hydrophilic carbohydrates in amadumbe flour (Adebowale and Lawal 2004). Nguimbou *et al.* (2014) reported that non-starch components present in the flour such as mucilage could also influence water absorption capacity of amadumbe flour. Mucilage is a colloidal substance with a high affinity for water, forming hydrogen bonds with the hydroxyl groups along the polysaccharide chain and at the anionic groups that are present in the mucilage. Increased association between mucilage and water molecules can lead to an increase in water holding capacity and volume. Previous studies recommended water absorption capacity ranging from 1.49 to 4.72 g/g flour for use in viscous food and results from this study fall within that range (Aluko *et al.*, 2009). Awolu and Oseyemi

(2016) stated that an increase in water absorption capacity is very useful in products which require hydration to enhance handling characteristics such as in baking. Flour with a good water absorption capacity may also prove useful especially in products that require good viscosity for example soups and gravies.

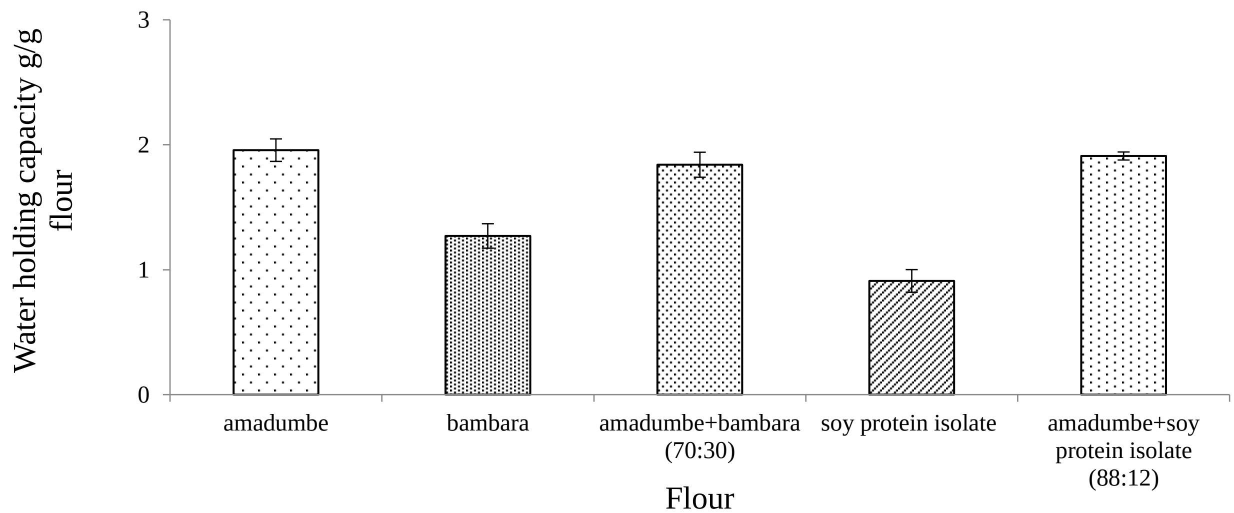

Figure 3.1: Water absorption capacity of amadumbe flour, bambara flour and soy protein isolate and their blends

3.4.4. Oil absorption capacity of amadumbe flour, Bambara groundnut flour, soy protein isolate and its blends

Oil absorption capacity (OAC) is important from an industrial perspective as it can reflect the emulsifying capacity of an ingredient. Oil absorption capacity is important for flavour retention and to enhance the delectableness in bakery products. Soy protein isolate had suggestively higher oil absorption capacity with the least being amadumbe flour (Figure 3.2). Kaur *et al*. (2013) reported an oil absorption capacity of 1 g/g flour which is not significantly different from the findings of this research. The small differences may possibly be attributed to the variations in the presence of nonpolar amino acids which show superior binding of lipids (Adebowale and Lawal 2004). However, in general the incorporation of bambara groundnut flour and soy protein isolate to amadumbe flour resulted in a significant increase in the oil absorption capacity of amadumbe flour.

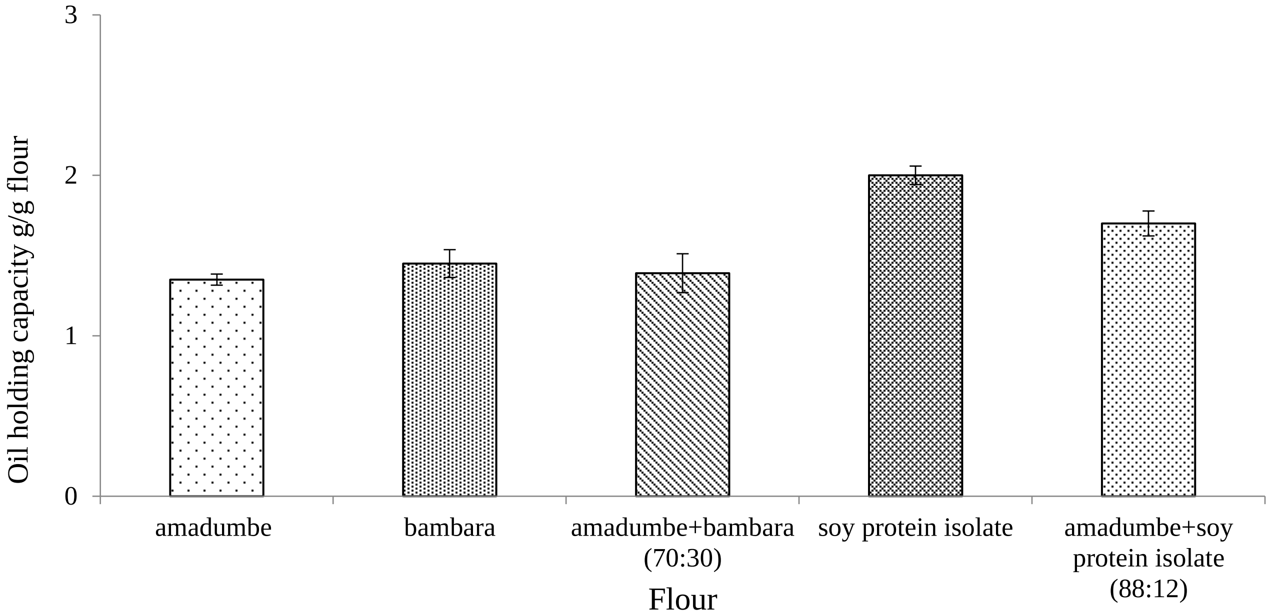

Figure 3.2: Oil absorption capacity of amadumbe flour, bambara groundnut flour, and soy protein isolate and their blends

3.4.5. Foam capacity and stability of amadumbe flour, Bambara groundnut flour, soy protein isolate and its blends

Foam capacity of amadumbe flour, soy protein isolate, bambara groundnut flour and their blends varied significantly with amadumbe flour having the least foam capacity (Table 3.4). This variation could be ascribed to the variances in protein contents of flours. Kumar *et al.* (2015) reported the foaming capacity of amadumbe flour ranging between 9% and 13% for different varieties of amadumbe, which are similar to this study findings. It has been suggested that protein molecules have the tendency to unfold and interact with each other forming multilayer protein films with an improved strength at the air liquid interface. Njintang *et al.* (2007) suggested that the foaming capacity of amadumbe flour can be because of the presence of mucilage (a soluble glycoprotein) content. It was explained that the presence of mucilage in the amadumbe composite formulations could have caused the foam's rheological properties to become complex, resulting in flow to deviate strongly from Newton's law to the nonlinear behaviours. Ptaszek *et al.* (2016) proposed that food products may constitute viscoelastic systems, exhibiting the characteristics of multiphase liquids or solids. The molecular structures of these systems are capable of both storing, as well as dissipating of mechanical energy. However, the addition of soy protein isolates or bambara groundnut flour to amadumbe flour significantly improved the foaming capacity of the flour. Awolu and Oseyemi (2016) also noted an increase in foam capacity of cassava flour with the addition of protein. This may possibly be due to an increase in protein concentration which facilitates protein–protein interaction at the air–water interface and this enhanced formation of a highly viscoelastic multiplayer film that offers resistance to coalescence of bubbles (Adebowale and Lawal 2004). Protein-polysaccharide interactions have been found to affect the foaming ability since nonspecific interactions initiate attractive and repulsive forces that induce the complex development of biopolymers (Tömösközi *et al.,* 2001). This shows that amadumbe composite flours can serve as better whipping agents.

Table 3.4: Emulsion and foaming capacity of amadumbe flour, bambara groundnut flour and soy protein isolate, and their blends

Sample	Emulsion capacity (%)	Foam capacity (%)
Amadumbe	$37.5^{a}\pm0.8$	$12.0^{a}\pm0.6$
Bambara	$38.5^{b}\pm0.7$	$17.0^{c}\pm1.0$
Amadumbe +bambara (70:30)	$44.9^{c}\pm0.6$	$18.7^{d}\pm0.9$
Soy protein isolate	$36.0^{a}\pm1.0$	$14.8^{b}\pm0.8$
Amadumbe + soy protein isolate (88:12)	$44.8^{c}\pm1.0$	$16.5^{c}\pm0.9$

Means (n=3) followed by the same letter in the same column are not significantly different at $p < 0.05$

As a function of time, foam volumes tend to change as observed in Figure 3.3. The foam stability of the flours decreased except for soy protein isolate. Foam stability is essential since the practicality of whipping agents depends on their proficiency to retain the whip over time. Composite flours showed a great ability to maintain the whip over the monitored time. This implies that amadumbe composite flours may be useful as aerating agents in food, which requires the production of stable foam volume when whipping.

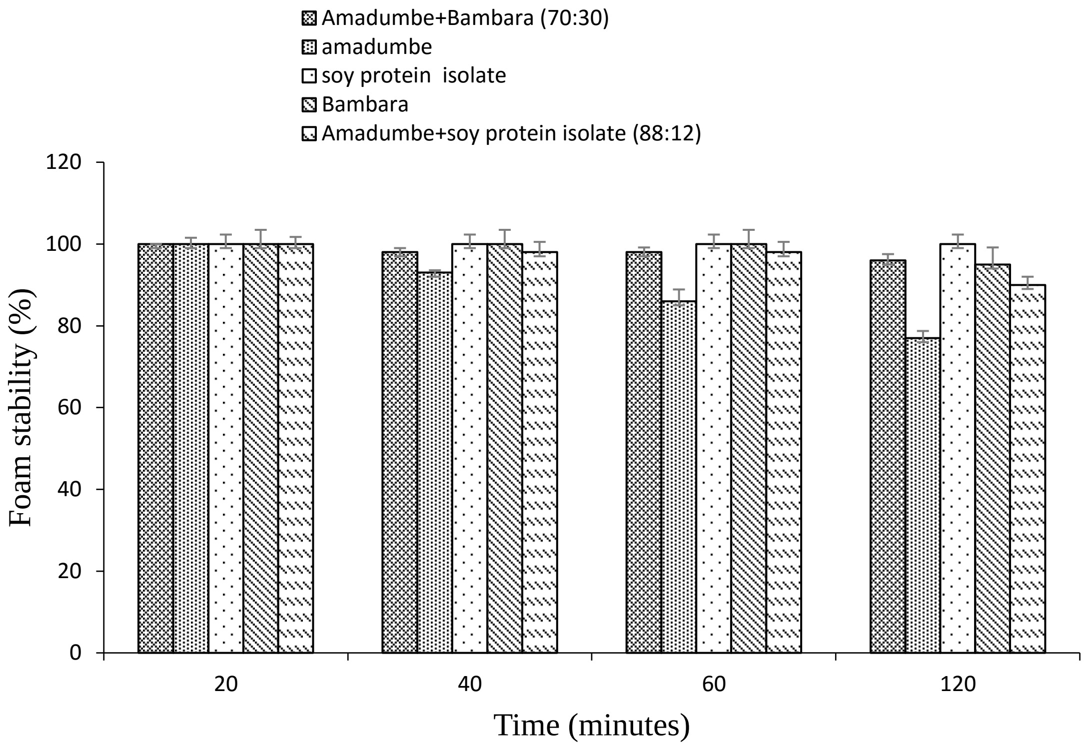

Figure 3.3: Foam stability of amadumbe flour, bambara groundnut flour, soy protein isolate and their blends

3.4.6. Emulsion capacity and stability of amadumbe flour, Bambara groundnut flour, soy protein isolate and its blends

Emulsion capacity (EC) of the flour was recorded, soy protein isolate had the least emulsion capacity whilst there was a significant increase in EC when bambara groundnut flour and soy protein isolate was added to amadumbe flour (Table 3.4). Emulsion capacity is greatly influenced by the emulsification properties of soluble and insoluble protein as well as polysaccharides. The variation between the soy protein isolate and the flour can possibly be due to the difference in protein content. Bambara groundnut flour exhibited the greatest emulsion stability of 100% (Figure 3.4) the least being amadumbe-bambara groundnut flour and soy protein isolate recorded 79%. The increase in protein concentration of a flour has a possible negative influence on the emulsion capacity. Adebowale and Lawal (2004) reported similar findings in which an increase in bambara groundnut flour concentration by 6% resulted in a decrease in emulsion stability. It has been suggested that a decrease in protein concentration can potentially control the rate of adsorption diffusion and high protein concentration acts as an obstruction to adsorption (Zhao *et al.*, 2015). The mechanism behind emulsion capacity and stability is that proteins have the ability to decrease the surface tension of oil droplets while offering electrostatic repulsion on the surface of the oil droplets.

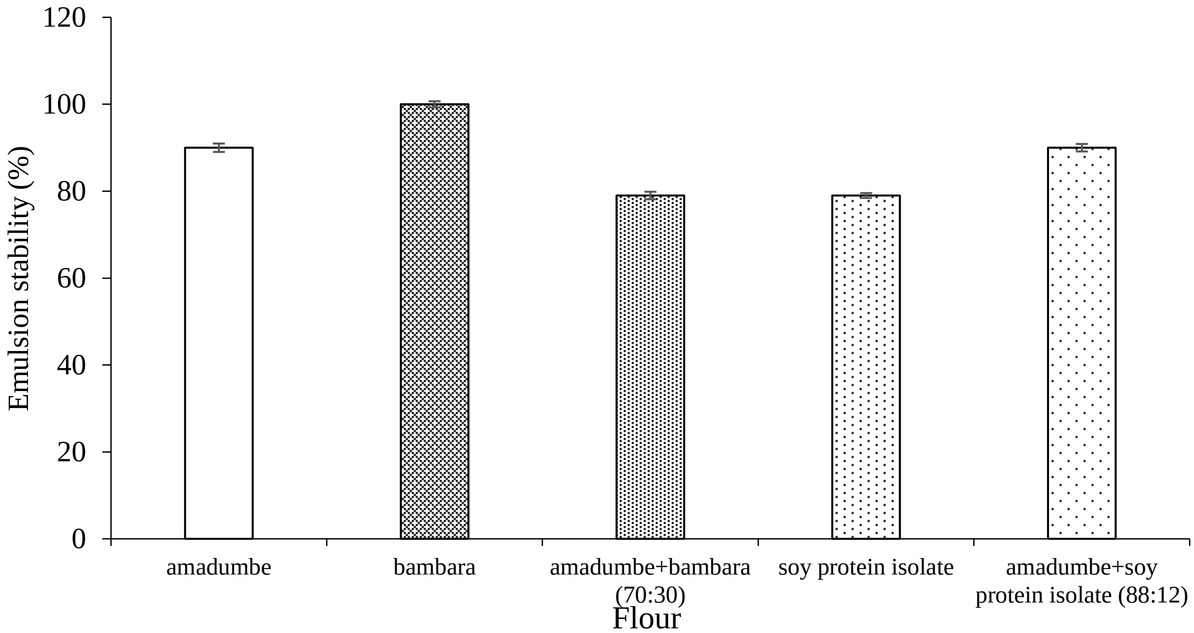

Figure 3.4: Emulsion stability of amadumbe flour, bambara groundnut flour, soy protein isolates and their blends

3.5. Conclusion

The addition of a protein flour base such as bambara groundnut flour and soy protein isolate to amadumbe flour improved the water and oil absorption, foam capacity and stability as well as emulsion capacity and stability properties. The results of this study can help to recommend or suggest the potential food uses of amadumbe, bambara groundnuts and soy protein isolate. The high carbohydrate content of amadumbe flour coupled with the high amounts of some essential amino acids and good functionality exhibited by bambara groundnut flour and soy protein isolate can make the blends potential candidates in the of gluten-free ingredients with characteristics that resemble those of wheat.

Chapter Four

The effect of laccase on the quality of gluten-free amadumbe bread

Abstract

Gluten is an important ingredient required for the overall structure and quality of bread, hence its replacement in the development of gluten-free bread poses a great scientific and industrial challenge in the baking industry. Enzymatic treatments of gluten-free flour have been proposed for improving dough viscosity and creating protein aggregates that could mimic gluten properties. In this study, the effect of laccase on the physical, functional, and sensory properties of gluten-free amadumbe bread was investigated. Bambara or soy protein isolate were added as protein supplements to amadumbe flour and 25 nkat/g flour laccase was added to each sample. Dough treatment with laccase decreased free sulfhydryl groups (-SH) by 30%, while the phenolic content decreased by 40%, which suggest that crosslinking had occurred. Bread prepared from enzymatically modified dough showed 50-64% increase in specific volume and a 32% decrease in crumb hardness and had a high moisture content and reduced moisture loss over time. Sensory analysis showed that amadumbe bread treated with laccase was more acceptable compared to non-treated bread in terms of appearance, texture, aroma and taste with the acceptability index varying between 46 and 86.2%. These results showed the great potential of laccase in gluten-free bread making.

4.1. Introduction

Globally there has been a growing interest in gluten-free food products due to an increased number of celiac patients and the percentage of people who also want to adopt the diet despite not having the disease (Puglise, 2016). Celiac disease (CD) is an auto-immune disorder that is prompted by the consumption of gluten in genetically predisposed individuals (Setty *et al.*, 2008). This results in mutilation of the lining of the small intestine and hence, malabsorption of nutrients (Pietzak, 2012). The only remedy available at present is a strict adherence to a gluten-free (GF) diet ((Padalino *et al.*, 2016). However, this may prove a difficult proposition for celiac patients as most baked products available on the market such as bread are produced with gluten-containing grains, mainly wheat (Alvarez-Jubete *et al.*, 2010).

Gluten is a protein fraction is responsible for the structural quality of most baked products. The replacement of gluten poses a great scientific and industrial challenge for food scientists especially in the baking industry (Gobbetti *et al*., 2017). It has been observed that gluten-free food products are inferior in terms of physical characteristics and quality. For example, they have a dense appearance and are of poor texture and mouth-feel. The approaches that have been proposed to try and solve this challenge include the use of various additives to a gluten-free starch base in order to improve structure, acceptability and shelf-life (Padalino *et al.*, 2016). These additives include: protein sources such as dairy products and egg-white of which these are amongst the top eight food allergens; hydrocolloids and emulsifiers which are less effective individually unless combined with other additives, and crosslinking enzymes for example transglutaminase, glucose oxidase and tyrosinase which are highly substrate specific (Gomez *et al*., 2007, Marco and Rosell, 2008, Bonet *et al*., 2006, Rosell *et al*., 2014). Therefore, there is a need to explore other alternatives.

Recently there has been an increased interest in the crosslinking enzyme laccase, due to its wide substrate range. Laccase is an oxidoreductase enzyme that oxidises a variety of substrates to corresponding radicals by one electron abstraction, and concomitantly reduces molecular oxygen to water. Laccase substrates include phenols, diphenols, phenolic acids, phenolic amines, methoxy substituted phenols, thiols and alkylamines (Kudanga *et al.*, 2011), some of which are present in gluten-free systems. The formed radicals can form C-C, C-N and C-O bonds which can potentially result in the formation of a network similar to that of gluten. In wheat dough systems, laccase have been shown to catalyse the oxidative gelation of feruloylated arabinoxylans by dimerization of their ferulic esters (Buchert *et al.*, 2010, Labat *et al*., 2001). Through the aforementioned mechanism, laccase may produce beneficial effects on the rheological behaviour of dough and the quality of the final product (Rosell, 2009). Therefore, laccase has been used to improve wheat bread properties, and it has been reported that it increased the dough machinability and improved the bread-making quality of wheat flour bread (Selinheimo *et al.*, 2006, Flander *et al*., 2008, Labat *et al*., 2001, Castro *et al*., 2015). The reported increase in crumb structure and bread volume when laccase was used to make gluten-free oat bread by (Renzetti *et al.*, 2010), shows the potential of laccase in gluten-free baking. The increase in demand for gluten-free products necessitates the need to explore other crops as potential gluten-free ingredients.

Of late there has been a shift from using exotic crops to the use of traditional crops such as cassava and sweet potato due to their abundant availability and their nutritional profiles (Yadav *et al.*, 2014). One of the crops that has also gained interest especially in Africa is amadumbe (*Colocasia esculenta*). Amadumbe is a carbohydrate rich crop which contains an abundance of mucilage, a hydrocolloid which can help in improving the functional properties of gluten-free systems (Gomez, 2007). Mucilage can also provide potential active sites for laccase action, for example, phenolics and some amino acids such as tyrosine and cysteine (Andrade *et al.*, 2015). However, amadumbe has a low protein content and therefore requires protein supplementation. It has been proposed that the use of legumes such as bambara groundnuts and soy protein isolate as protein supplements can result in improved gluten-free flour functionality (Anton *et al.*, 2008). Bambara groundnuts and soy protein isolate protein are good sources of amino acids such as tyrosine, cysteine and lysine (Aremu *et al.*, 2007, Tömösközi *et al.*, 2001) which also offer potential sites for laccase action.

The aim of this work was to investigate the effect of laccase on gluten-free amadumbe bread supplemented with bambara groundnut flour and soy protein isolate.

4.2. Materials and method

4.2.1. Materials

Amadumbe corms and bambara groundnuts were procured from Umbumbulu, KwaZulu-Natal province, of South Africa and soy protein isolate was commercially sourced from Lionheart Chemical Enterprises (Pty) Ltd, Durban and Laccase was sourced from Sigma.

4.2.2. Flour preparation

For the production of amadumbe flour, amadumbe corms were cleaned, peeled, sliced (3-4 cm) and dried at 52 °C in an air cabinet drier for 10 h and ground into flour using a laboratory grinder as described by Naidoo *et al.* (2015).

To produce bambara groundnut flour, the seeds were soaked for 12 h, manually dehulled and dried at 45 °C in hot an air oven. The dried seeds were milled into flour using a Warring laboratory mill blender (HGBTWTS3, Torrington, CT, USA) and the flour was sieved (355 µm) and kept at 4 °C (Oyeyinka *et al.,* 2015).

4.2.3. Folin method for total phenolic

The determination of the phenolic content of milled amadumbe dough was done using the Folin-Ciocalteu reagent, according to a method of Nguimbou *et al.* (2014). The reagents were obtained from Sigma (Sigma Chemicals, St. Louis, MO, USA). The phenolic contents were expressed in µg equivalent of ferulic acid per mg flour. Folin-Ciocalteu reagent (75 µl) was added to 1 ml of the sample (1 mg/ml) and mixed thoroughly. The mixture was left to set for 3 min and 750 µl of 20% sodium carbonate was added and allowed to stand for 1 h at room temperature with interval shaking. The absorbance was quantified at 760 nm using the UV-visible spectrophotometer. The total phenolic content was measured as mg of ferulic acid equivalent per gram of dry milled amadumbe dough weight, using an equation obtained from the standard ferulic acid curve. The phenolic content results were presented as mg gallic acid equivalent per g flour.

4.2.4 Quantification of thiols

The modifications in thiol (SH) groups were measured using a modified method of (Gujral and Rosell 2004). The reagents were acquired from Sigma (Sigma Chemicals, St. Louis, MO, USA). Milled amadumbe dough (200 mg) in the presence or absence of laccase (control) was held in 1.0 ml of GuHCI/Tris-Gly buffer and vortexed for 10 min and centrifuged at 16 000 g for 5 min. The supernatant (100 µl) was added to 150 µl of GuHCI/Tris-Gly solution and 50 µl of Elman's reagent. Absorbance was read at 412 nm using the spectrophotometer. Results were calculated against a cysteine standard curve. Values obtained were means of three determinations.

4.2.5. Bread making

The ingredients that were used for each of the gluten-free bread are shown in Table 4.1. The level of laccase incorporation was selected according to optimisation results. Amadumbe flour, bambara flour and soy protein contents were determined using a targeted protein content of 16 g/100 g flour. The dry instant yeast was liquified in a mixture of sugar and water at 20-23 °C and was left to ferment for 30 min before use at 24 °C and 40% relative humidity. The 100 g batter samples of each formulation were put on greased pans (measuring 120 × 50 mm), proofed for 45 min at ± 25 °C and then baked at 175 °C for 40 min (Caballero *et al.*, 2007a). The bread samples were stored at room temperature for further analysis.

Table 4.1: Bread recipe

Ingredients	AB	AS	ABL	ASL
Amadumbe flour (g)	69.3	87.12	69.3	87.12
Bambara groundnut flour (g)	29.7		29.7	
Soy protein isolate (g)		11.88		11.88
Fat (g)	2.5	2.5	2.5	2.5
Yeast (g)	2	2	2	2
Water (g)	200	200	200	200
Salt (g)	1.5	1.5	1.5	1.5
Sugar (g)	3.5	3.5	3.5	3.5
Laccase			25 nkat/g flour	25 nkat/g flour

Relative amounts in percent flour weight basis (% fwb). AS-Amadumbe+soy protein isolate, AB-Amadumbe flour +bambara groundnut flour, ASL-Amadumbe flour+Soy protein isolate+Laccase, ABL- Amadumbe flour +Bambara groundnut flour+ Laccase

4.2.6 Evaluation of bread quality

All loaves were measured 24 h after baking. Crumb and crust colour were determined using a colour flex (A60-1014-593; Hunter Associates Laboratory, Reston, VA, USA) based on lightness (L*), red-green (a*) and yellow-blue (b*) values. To determine crumb moisture content a 10 g portion of the bread was removed from the centre of the bread. The weight of the bread crumb portions was recorded 4 times at 24 h intervals and the differences noted. Crumb hardness was quantified using a Shimadzu EZ Texture Analyzer EZ-LX/E2-SX series (Kyoto, Japan) after 24 h of baking. Bread slices of a thickness of 20 mm was compressed to 50% of its original height at speed of 1 mm/s with a 25 mm cylindrical stainless-steel probe and trigger force of 5 g. The peak force of compression was then reported as hardness (Gujral and Rosell 2004). Loaves were weighed and loaf volume was measured by rapeseed displacement (Gujral and Rosell 2004). Loaf specific volume (cm^3/g) was calculated according to the AACC method 10-05.

4.2.7 Sensory analysis

Sensory analysis was conducted on all the four samples and the level of laccase incorporation was 25 nkat/ g flour. A panel of 60 people was involved in the tasting of the four bread samples. The session was conducted in an environment with less distractions for example less noise, less movements, controlled temperature and intrusive odours. To eliminate bias tasting booths were used. The size of the samples was kept constant across all samples and the panellists were provided with water for pallet cleansing between samples. The 9-point hedonic scale was used to separate the acceptability of the bread. The panellists were asked to rate the samples based on overall acceptability, taste, texture, aroma and appearance.

4.2.8 Statistical analysis

The data reported are averages of triplicate measurements. The data was subjected to analysis of variance (ANOVA) to calculate significant differences in the treatment means and LSD ($p < 0.05$) was used to separate means using the Statistical Package for the Social Science software (SPSS).

4.3 Results and Discussion

4.3.1. Total phenolic content of amadumbe dried dough

The total phenolic content decreased significantly by 40% in both laccase treated dough (ABL and ASL) samples (Figure 4.1). The incorporation of laccase in amadumbe dough induced oxidative mechanisms which resulted in the reduction of phenolic groups of ferulic acid residues (Labat *et* *al.*, 2001; Figueroa-Espinoza *et al.*, 1998). The oxidation mechanism generated feruloyl radicals promoting the reduction of ferulic acid residues. The possibility of the phenoxy radicals to react with other phenoxy radicals or with thiols or tyrosine leading to the formation of conjugates was previously reported (Moore *et al.*, 2004; Selinheimo 2008). Minussi *et al*. (2002) reported that when laccase is used during dough formation, it could result in an increase in dough consistency.

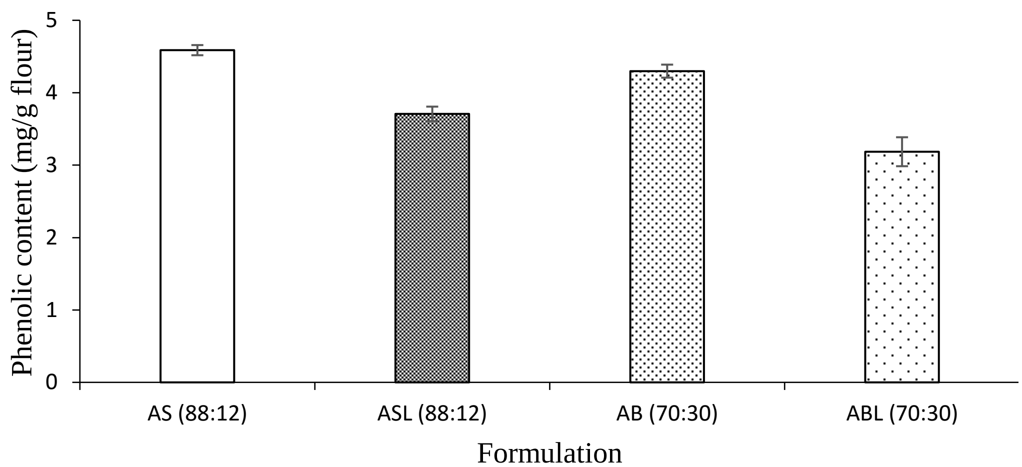

Figure 4.1: Effect of laccase on the phenolic content of gluten-free amadumbe dough
AS-Amadumbe flour+Soy protein isolate (88:12), AB-Amadumbe flour+bambara groundnut flour (70:30), ASL-Amadumbe flour +Soy protein isolate+Laccase (88:12, 25 nkat/g flour), ABL-Amadumbe flour+bambara groundnut flour+Laccase (70:30, 25 nkat/g flour)

4.3.2. Thiol quantification of amadumbe dried dough

The addition of laccase to amadumbe and soy protein isolate composite resulted in a 21.44% decrease in the number of SH groups and a 9.62% decrease was observed when bambara groundnut flour was added (Table 4.1). Laccase has been reported to cause the oxidation of the free sulfhydryl units giving disulphide linkages (SS), consequently improving dough strength, improving oven spring and hence a larger loaf volume (Caballero *et al.*, 2007b). The decrease in thiols can also be attributed to laccase action on the activated double bond of the ferulic acid resulting in the formation of linkages between the adjacent protein molecules and arabinoxylan (Hoseney and Faubion 1981; Gujral and Rosell 2004). In a related study a decrease in the sulfhydryl units was reported in research on the effect of cysteine, tyrosine, lysine and glutathione on the cross-linking of feruloylated arabinoxylans by laccase (Figueroa-Espinoza *et al.*, 1998).

Table 4.1: Quantification of thiol groups of gluten-free amadumbe dough

Sample	Free thiol groups (µg/ml)
ASL	$0.0039^{a}\pm0.01$
ABL	$0.0047^{b}\pm0.10$
AS	$0.0049^{b}\pm0.02$
AB	$0.0052^{c}\pm0.12$

Values are means ± SD (n=3). Means followed by different letters in a column are significantly different at $p < 0.05$. Results were calculated against cysteine standard curve.

AS-Amadumbe flour+Soy protein isolate (88:12), AB-Amadumbe flour+bambara groundnut flour (70:30), ASL-Amadumbe flour+Soy protein isolate+Laccase (88:12, 25 nkat/g flour), ABL-Amadumbe flour+bambara groundnut flour+Laccase (70:30, 25 nkat/g flour).

4.3.3. Crust and crumb colour of amadumbe gluten-free bread

Crust and crumb colour is an important attribute of bread, which contributes to consumer preference. The lightness of the gluten-free bread crust varied widely ranging from 25.30 to 30.67. Sample AB had a lighter crumb and crust colour compared to the other samples (Table 4.2). The lower L^* values may be attributed to the Maillard browning and caramelization which are induced by water distribution and the reaction of amino acids and reducing sugars (Kent and Evers 1994). The L^* value of sample AB decreased with the addition of laccase. This can be attributed to the oxidation reaction that occurs on the phenolic compounds present in the flour, on the contrary, sample AS reacted differently with the addition of laccase, instead of decreasing the L^* value increased. Sosulski (1979); Li *et al.* (2015) suggested that due to the oxidation of polyphenolic compounds solutions are bound to develop darker colours. On the contrary, laccase can also act as a bleach hence we can attribute the colour difference of sample ASL to the bleaching effect of laccase. It has been reported that to a certain extend laccase can act as a bleaching agent through its mediator systems (Almansa *et al.*, 2004). The crumb colour was significantly different across all the bread samples. The L* for the crumb colour ranged between 56.89 and 60.68 and these differences can be attributed to the phenolic and protein content differences in the samples.

Table 4.2: Effect of laccase on crust and crumb colour profile of gluten-free amadumbe bread

Sample	L^*	a*	b*
Crust colour			
AS	25.30^a±0.01	15.68^b±0.02	15.81^b±0.01
AB	30.67^c±0.01	15.74^b±0.01	16.92^c±0.02
ASL	30.27^c±0.01	15.76^b±0.01	16.91^c±0.02
ABL	26.03^b±0.01	12.74^a±0.04	12.38^a±0.01
Crumb colour			
ABL	56.89^a±0.01	5.00^b±0.01	16.13^a±0.03
AS	58.74^b±0.01	4.01^a±0.01	16.40^c±0.01
AB	60.68^c±0.01	4.03^a±0.02	16.32^b±0.1
ASL	60.15^c±0.4	4.09^a±0.11	16.32^b±0.02

Values are means ± SD (n=3). Means followed by different letters in a column are significantly different at $p < 0.05$. With annotations: (L*) representing lightness, (a*) red-green and (b*) yellow-blue.

AS-Amadumbe flour+Soy protein isolate (88:12), AB-Amadumbe flour+bambara groundnut flour (70:30), ASL-Amadumbe flour+Soy protein isolate+Laccase (88:12, 25 nkat/g flour), ABL-Amadumbe flour+bambara groundnut flour+Laccase (70:30, 25 nkat/g flour)

4.3.4. Crumb hardness of amadumbe gluten-free bread

The incorporation of laccase induced a 32% reduction in crumb hardness in both laccase treated samples (ABL and ASL) (Figure 4.2). This result is consistent with the results of a research by, Caballero *et al.* (2007b) and Renzetti *et al.* (2010). There was no significant difference between amadumbe-soy-laccase and amadumbe-bambara-laccase bread samples suggesting that protein type did not significantly affect the functionality of laccase (Figure 4.2). The changes in crumb texture can be attributed to the formation of a gel-like substance due to laccase-mediated cross-linking, hence, increasing the water absorption capacity of the bread. Also, the reduction in bread crumb hardness can be attributed to the expansion of the bread samples. On the contrary, Flander *et al.* (2008) reported an increase in bread firmness when laccase was incorporated in oat bread. These results were attributed to the polymerization of the water extractable arabinoxylan to water-unextractable arabinoxylans and protein cross-linking through disulphide bonds. However, they suggested that the use of low enzyme activity could result in a decrease in crumb hardness.

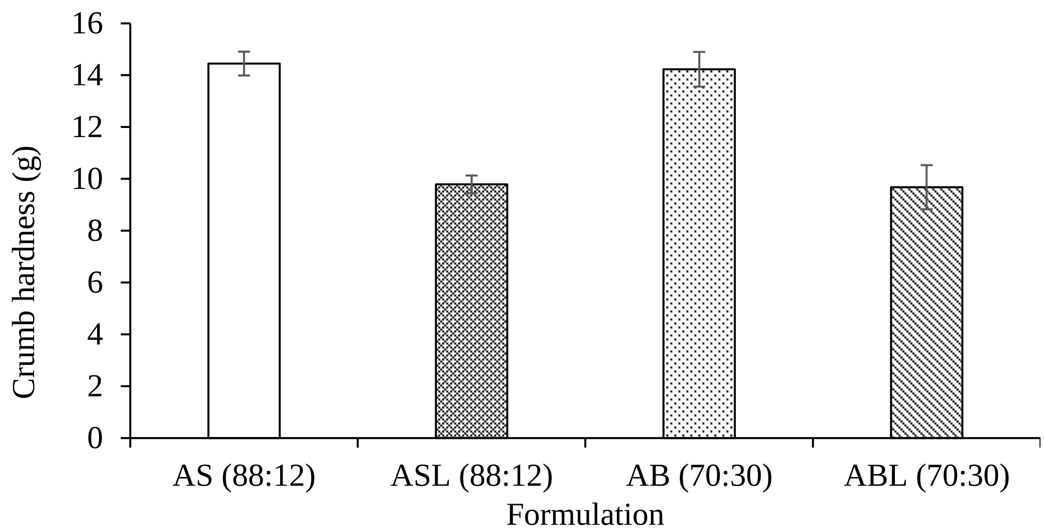

Figure 4.2: Effect of laccase on gluten-free amadumbe bread crumb hardness
AS-Amadumbe flour+Soy protein isolate (88:12), AB-Amadumbe flour+bambara groundnut flour (70:30), ASL-Amadumbe flour +Soy protein isolate+Laccase (88:12, 25 nkat/g flour), ABL-Amadumbe flour+bambara groundnut flour+Laccase (70:30, 25 nkat/g flour)

4.3.5. Bread specific volume of amadumbe gluten-free bread

Bread treated with laccase recorded significantly higher specific volumes compared to non-laccase treated bread and there was 50% and 64% increase in specific volume for AB and AS, respectively when they were treated with laccase (Figure 4.3 and Figure 4.4). This can be attributed to the oxidation process of phenols which led to the formation of a gel-like substance which increased the water holding capacity of the batter (Niño-Medina *et al.*, 2017). This result can also be attributed to the formation of disulphide bonds which reinforce the batter protein network, allowing for gas retention during fermentation and an increase in specific volume. However, there was no significant difference between amadumbe-soy and amadumbe-bambara laccase treated bread (Figure 4.3). This suggests that the addition of different protein sources did not affect the functionality of laccase on the dough system. Studies carried out on oat bread showed an increase in specific volume with the incorporation of laccase (Renzetti *et al.*, 2010). These effects were attributed to the improved batter development and elasticity. On the contrary Flander *et al.* (2008) reported no significant difference between laccase treated and non-laccase treated oat bread.

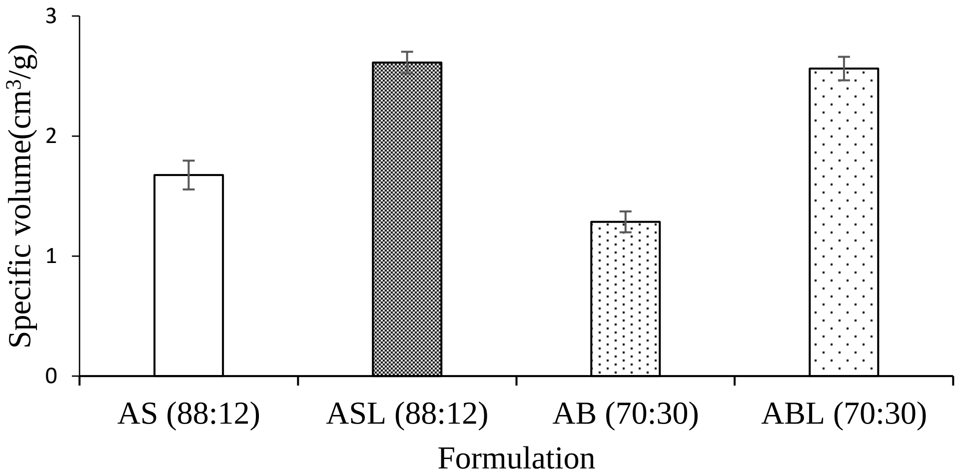

Figure 4.3: The effect of laccase on gluten-free amadumbe bread specific volume**.**

AS-Amadumbe flour+Soy protein isolate (88:12), AB-Amadumbe flour+bambara groundnut flour (70:30), ASL-Amadumbe flour+Soy protein isolate+Laccase (88:12, 25 nkat/g flour), ABL-Amadumbe flour+bambara groundnut flour+Laccase (70:30, 25 nkat/g flour)

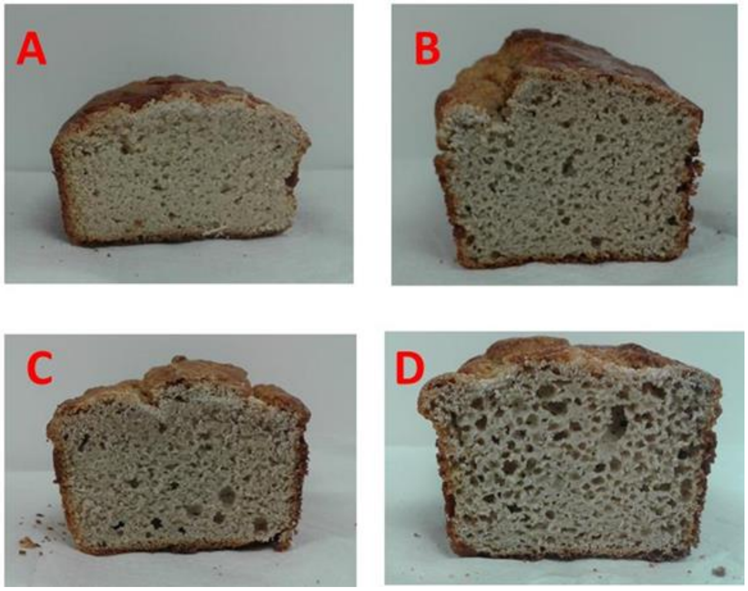

Figure 4.4: Amadumbe-based gluten-free bread; A: Amadumbe flour+Soy isolate protein, B: Amadumbe flour and soy isolate protein+laccase, C: Amadumbe flour+bambara groundnut flour, D: Amadumbe flour and bambara groundnut flour+Laccase

4.3.6. Crumb moisture content of amadumbe gluten-free bread

The addition of laccase to the amadumbe bread resulted in a more humid bread crumb between 24 h through to 96 h of storage compared to the bread samples with no added laccase (Figure 4.5). This can be attributed to the cross-linking of phenols which resulted in the formation of a gel-like substance hence increasing the water holding capacity of the bread (Niño-Medina *et al.,* 2017). This can possibly be due to the phenoxy radicals produced could have gone through a secondary reaction resulting in a loss of water-extractable arabinoxylan through crosslinking and depolymerization reactions. This process can lead to a decrease in bread hardness during storage (Carvajal-Millan *et al.*, 2005).

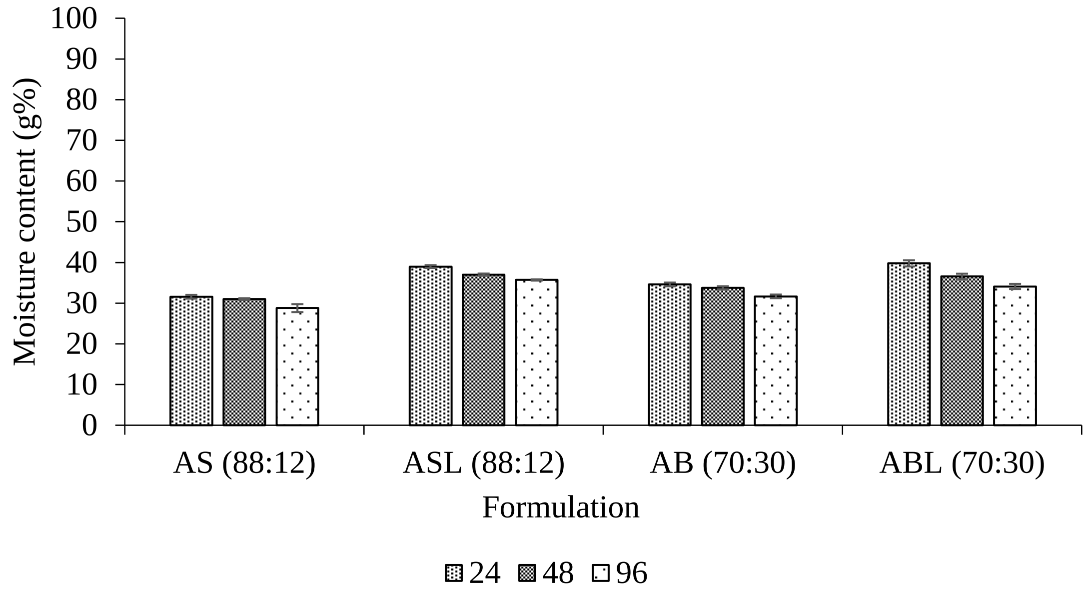

Figure 4.5: Effect of laccase on gluten-free amadumbe bread moisture loss
AS-Amadumbe flour+Soy protein isolate (88:12), AB-Amadumbe flour+bambara groundnut flour (70:30), ASL-Amadumbe flour+Soy protein isolate+Laccase (88:12, 25 nkat/g flour), ABL-Amadumbe flour+bambara groundnut flour+Laccase (70:30, 25 nkat/g flour).

4.3.7. Consumer acceptability of amadumbe gluten-free bread

Sensory evaluation showed that laccase treated amadumbe bread was rated higher than the non-laccase treated bread in terms of texture, appearance, aroma, taste and overall acceptability (Figure 4.6). There was a significant difference in the acceptability of non-laccase treated and laccase treated bread samples; in terms of appearance and texture, laccase treated bread samples were more acceptable. This can be attributed to the effect laccase had on the bread properties such as colour, specific volume and texture. The oxidation of the phenolic compounds (Sosulski, 1979; Li *et al.*, 2015), as well as Maillard reaction and caramelization (Kent and Evers 1994), might have influenced the brown colour. As the specific volume of laccase-treated bread increased, the texture improved. Taste is the most important parameter which affects the acceptability of most food products; the incorporation of laccase in amadumbe bread resulted in a more desirable flavour. Laccase has been reported to reduce bitterness and unpleasant tastes in food (Takemori *et al.*, 1992). Laccase catalyses the oxidation of phenolic compounds in dough systems resulting in a decrease in phenolics which contribute to off flavours, hence resulting in a desirable taste in amadumbe bread (Minussi *et al.*, 2002). Overall, samples having laccase were superior to the samples without laccase with ABL being the most acceptable compared to the other samples (Figure 4.6). However, all the gluten-free amadumbe bread samples were scored by the mean number higher than 6, implying that their sensory properties were acceptable.

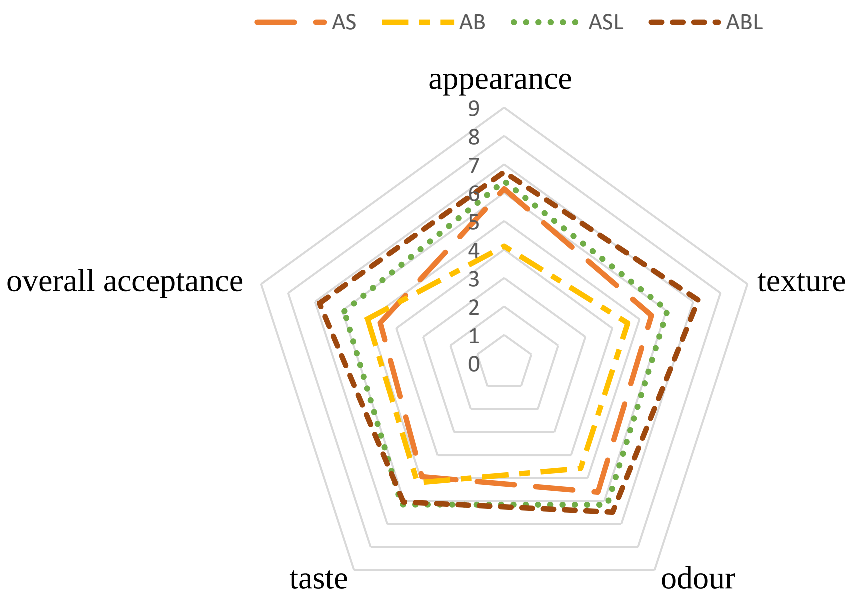

Figure 4.6: Effect of laccase on the sensory characteristics of gluten-free amadumbe bread AS-Amadumbe flour+Soy protein isolate (88:12), AB-Amadumbe flour+bambara groundnut flour (70:30), ASL-Amadumbe flour+Soy protein isolate+Laccase (88:12, 25 nkat/g flour)

4.4. Conclusion

This study showed that laccase treatment improves breadmaking functionality of amadumbe supplemented with bambara groundnut flour or soy protein isolate. The improvements in the amadumbe gluten-free bread are related to laccase-catalysed the formation of disulphide bonds as revealed by a decrease in thiol group. Laccase positively improved the textural, specific volume and moisture activity of gluten-free amadumbe bread. Sensory evaluation showed that the consumers accepted laccase treated amadumbe bread. Therefore, laccase can be incorporated into amadumbe bread formula to improve bread quality and enhance its acceptability.

Chapter Five

Effect of xanthan gum on gluten-free amadumbe bread quality

Abstract

The effect of xanthan gum on gluten-free amadumbe bread supplemented with bambara groundnut flour and soy protein isolate, on fresh bread quality and its influence on bread staling was evaluated. Physical properties such as specific volume, hardness and moisture loss over a period of 96 h storage time. Xanthan gum concentrations of 1% (w/w, flour basis) resulted in a 55% increase in specific bread volume and a 40% decrease in bread crumb hardness. Xanthan gum also reduced the moisture loss during the 96 h storage period by reducing the dehydration rate of the bread crumb. Sensory analysis showed that amadumbe bread with xanthan gum was more acceptable compared to bread with no added xanthan gum in terms of appearance, texture, aroma and taste with the acceptability index varying between 40% and 85%. These results indicate the potential of xanthan gum in gluten-free bread making.

5.1. Introduction

The consumption of bread backdates to the early Egyptian era; it is a staple in most countries worldwide. Amongst the grains used in breadmaking, wheat is the commonest. Wheat contains a protein gluten, which can be divided into two fractions, that is glutenin and gliadin. These two fractions are responsible for the viscoelastic properties of wheat dough which are relevant for the protein-starch interaction linked to the development of gas cells, their stabilisation and retention during the proofing and baking process (Moore *et al.,* 2006; Wieser, 2007). However, besides the importance of gluten for the bread-making process, the presence of gluten in food products may be an issue to some people. A certain percentage of the world's population lacks the ability to digest this protein, they suffer from a disorder known as celiac disease (gluten intolerance). This disorder causes damage to the mucous membrane of the small intestine, resulting in malabsorption of nutrients, hence, weight loss, anaemia and other diseases. The only effective remedy that has been reported is a strict adherence to a gluten-free diet (Padalino *et al.,* 2016). The total elimination of gluten may result in clinical and mucosal recovery (Gallagher *et al.,* 2004). However, it is very difficult to propose a diet for celiac patients, since most baked products such as pizzas, cakes, pasta, bread and biscuits are generally prepared using wheat flour and are consumed on an everyday basis by most people. The exclusion of gluten during baking leads to a technological challenge for bakers; most gluten-free products accessible on the market are inferior, exhibiting poor flavour and mouthfeel (Hüttner and Arendt 2010). For the development of gluten-free breads, different flours, and starches together with other ingredients and additives such as protein-based flours and hydrocolloids have been used to try and mimic the viscoelastic properties of gluten (Rosell *et al.,* 2001; Moore *et al.,* 2006; Moroni *et al.,* 2009; Renzetti and Arendt 2009; Hager and Arendt 2013). Hydrocolloids are water-soluble polysaccharides with different chemical structures providing a range of functional properties that make them suitable for different applications in the food industry (Gomez *et al.,* 2007). Hydrocolloids can be used as a gluten substitute in gluten-free bread. They can also be used to enhance texture in food, slow down starch degradation, improve moisture retention and to extend the overall quality over time (Sidhu and Bawa 2002). Hence, the use of hydrocolloids appears to be a potential alternative in the development of gluten-free bread of high-quality. Some of the hydrocolloids that have been used in the food industry include guar gum, locust bean gum, xanthan gum etc. The hydrocolloid that was used in this study was xanthan gum.

The aim of the current research was to evaluate the effect of xanthan gum on gluten-free amadumbe bread quality.

5.2. Materials and methods

Amadumbe corms and bambara groundnuts were obtained from Umbumbulu, KwaZulu-Natal province, and the soy protein isolate was commercially sourced from Lionheart Chemical Enterprises (Pty) Ltd Durban.

5.2.1. Flour preparation

For the production of amadumbe flour, amadumbe corms were cleaned, peeled, sliced (3-4 cm) and dried at 52 °C in an air cabinet drier for 10 h and ground into flour using a laboratory grinder as described by Naidoo *et al.* (2015).

For the production of bambara groundnut flour, the seeds were soaked for 12 h, manually dehulled and dried at 45 °C in hot an air oven. The dried seeds were milled into flour using a Warring laboratory mill blender (HGBTWTS3, Torrington, CT, USA) and the flour was sieved (355 µm) and kept at 4 °C (Oyeyinka *et al.,* 2015).

5.2.2. Bread making

Four samples of bread were baked, designated AB, AS, ABX and ASX, the variations between the samples being the flour contents as follows: AB (69.3 g amadumbe flour and 29.7 g bambara groundnut flour), AS (87.12 g amadumbe flour and 11.88 g soy protein isolate), ABX (88.12 g amadumbe flour, 11.88 g bambara groundnut flour and 1% xanthan gum), ASX (87.12 g amadumbe flour, 11.88 g soy protein isolate and 1% xanthan gum). The rest of the ingredients were constant in all the bread samples sugar (2.5% flour basis), salt (1.5% flour basis), yeast (2% flour basis) and 200 g water; all blended in a mixing bowl. Oil (2.5% flour basis) was added and mixed with the other ingredients. Then 100 g batter samples of each formulation were put in greased pans (measuring 120 x 50 mm), proofed for 45 min at ± 25 °C and then baked at 175 °C for 40 min. the bread samples were stored at room temperature for further analysis.

5.2.3. Determination of bread quality

Bread quality evaluation was carried out by determining the colour, specific loaf volume, crumb hardness and crumb moisture loss. The specific loaf volume was expressed as the volume/mass ratio (cm^3/g) of bread according to AACC method 10-05. Crumb and crust colour were determined using a colour flex (A60-1014-593; Hunter Associates Laboratory, Reston, VA, USA) based on lightness (L*), red-green (a*) and yellow-blue (b*) values. Crumb hardness was measured in a

Shimadzu EZ Texture Analyzer (EZ-LX/E2-SX series) after 24 h of baking. A bread slice of 20 mm thickness was compressed to 50% of its original height at a crosshead speed of 1 mm/s with a cylindrical stainless-steel probe having a diameter of 25 mm and trigger force of 5 g. The peak force of compression was reported as hardness (Gujral and Rosell 2004). Crumb moisture content was determined by slicing and removing 10 g of the bread crumb from the centre of the bread slice. The weight of the bread crumb portions was recorded for 96 h after removal from the oven and the differences noted.

Sensory analysis was conducted on all the bread samples using 60 untrained panellists. The session was done in an environment with less interruptions for example less noise, less activities, controlled temperature and intrusive odours. To eliminate predisposition tasting booths were used. The size of the samples was kept constant across all samples and the panellists were provided with water for pallet cleansing between samples. The 9-point hedonic scale was used to separate the acceptability of the bread. The panellists were asked to rate the samples based on overall acceptability, taste, texture, aroma and appearance.

5.2.4. Statistical analysis

The data reported in all the tables and figures are an average of triplicate determinations. The data was subjected to analysis of variance (ANOVA) to calculate significant differences in the treatment means and LSD ($p < 0.05$) was used to separate means using Statistical Package for the Social Science software (SPSS).

5.3. Results and discussion

5.3.1. Colour profile of amadumbe gluten-free bread

The acceptability of bread by most consumers is based on how the bread looks like in terms of colour. It has been reported that crust and crumb colour are very important properties that most consumers look at before purchasing bread. The crust lightness of amadumbe bread varied from 25.30 to 30.67 with samples AS having the lightest crust colour and sample AB having the darkest. The addition of xanthan gum to sample AB resulted in a lighter crust colour, however, there was no significant difference between samples AS and ASX (Table 5.1). With regards to the bread crumb colour, sample ABX had a lighter bread crumb compared to the other bread samples. In general, bread with added hydrocolloid shows lighter crusts (Lazaridou *et al.*, 2007). These findings can be attributed to the effect of hydrocolloids on water dispersal, which influences Maillard reaction and caramelization (Mezaize *et al.*, 2009).

Table 5.1: The effect of xanthan gum on gluten-free amadumbe bread crust and crumb colour

SAMPLE	L^*	a^*	b^*
	Crust colour		
AS	$25.80^a \pm 0.01$	$15.68^b \pm 0.02$	$15.81^a \pm 0.01$
ASX	$26.10^a \pm 0.03$	$15.87^b \pm 0.01$	$15.82^a \pm 0.02$
ABX	$26.94^b \pm 0.04$	$14.63^a \pm 0.03$	$15.61^a \pm 0.02$
AB	$30.67^b \pm 0.04$	$15.74^b \pm 0.19$	$16.92^b \pm 0.02$
	Crumb colour		
AS	$58.74^a \pm 0.01$	$4.01^a \pm 0.01$	$16.40^a \pm 0.01$
ASX	$59.71^{ab} \pm 0.02$	$4.14^{ab} \pm 0.01$	$18.32^b \pm 0.02$
ABX	$66.13^c \pm 0.04$	$5.69^c \pm 0.05$	$22.16^c \pm 0.05$
AB	$60.68^b \pm 0.04$	$4.03^a \pm 0.02$	$16.32^a \pm 0.01$

Means (n=3) followed by the same letter in the same column are not significantly different at $p < 0.05$.

With annotations: (L*) representing lightness, (a*) red-green and (b*) yellow-blue.

AS-Amadumbe+soy protein isolate (88:12), AB-Amadumbe+bambara groundnut flour (70:30), ASX-Amadumbe + Soy protein isolate+Xanthan gum (88:12, 1%), ABX-Amadumbe+bambara groundnut flour+Xanthan gum (70:30, 1%).

5.3.2. Bread specific volume of amadumbe gluten-free bread

The addition of 1% xanthan gum to amadumbe bread formulation led to an increase in the specific volume of the bread. The bread volume of samples that had xanthan gum increased by about 55% as shown in Figure 5.1. It has been reported that when xanthan gum is added to dough systems, it can induce an increase in dough viscosity, therefore, conferring greater stability of gluten-free networks during baking. Hydrocolloids have a high-water absorption capacity and during baking, this water is evaporated and results in an increase in specific volume. Xanthan gum might also have provoked a thickening of the crumb walls during baking hence allowing more gas to be retained resulting in an increase in bread specific volume. Shittu *et al.* (2009) reported similar findings when 1% xanthan gum was added to cassava bread. However, contrary to these findings, Lazaridou *et al.* (2007) and Mezaize *et al.* (2009) stated a progressive reduction in bread specific volume when up to 2% xanthan gum was used in rice/corn bread and buckwheat bread respectively. The argument being that an optimum resistance of bread dough to deform or inflate exists.

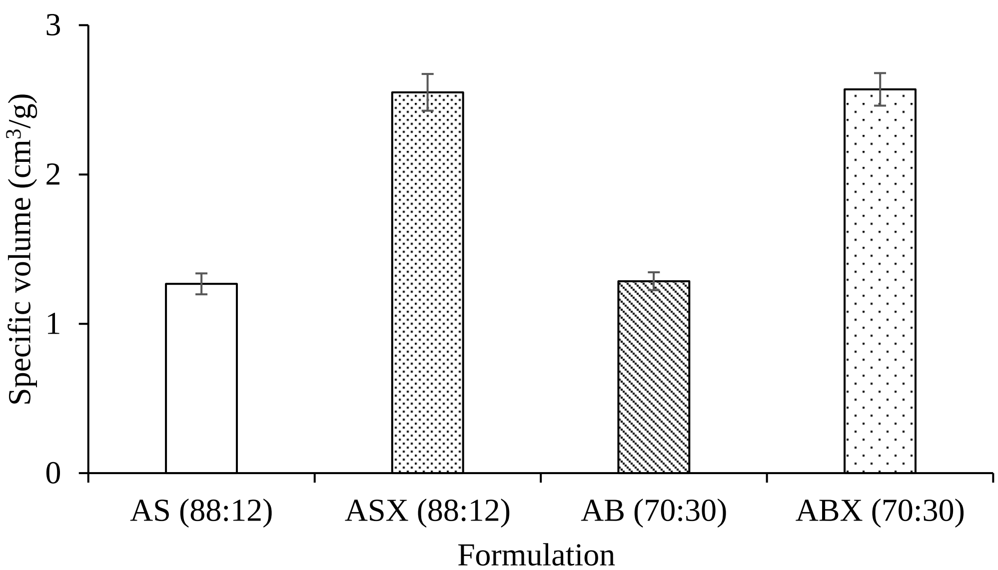

Figure 5.1: The effect of xanthan gum on gluten-free amadumbe bread specific volume
AS-Amadumbe+soy protein isolate (88:12), AB-Amadumbe+bambara groundnut flour (70:30), ASX-Amadumbe + Soy protein isolate+Xanthan gum (88:12, 1%), ABX-Amadumbe+bambara groundnut flour+Xanthan gum (70:30, 1%)

5.3.3. Crumb hardness of amadumbe gluten-free bread

The incorporation of xanthan gum to amadumbe dough resulted in a 24%-32% decrease in amadumbe bread crumb hardness as shown in Figure 5.2. However, there was no significant difference between amadumbe-soy and amadumbe-bambara groundnut with xanthan gum added suggesting that bambara groundnut flour performed similarly with the usually used protein source, soy protein isolate. Gambuś *et al*. (2007) observed that the incorporation of xanthan gum reduced bread specific volume which is contrary to the findings of this study. The argument to their findings was that amylose that leached during baking might have been bound by xanthan gum present in the dough. Therefore, the amount of xanthan gum was too low for efficient self-complexation of this hydrocolloid, thus amylose-xanthan gum cross-links were favoured.

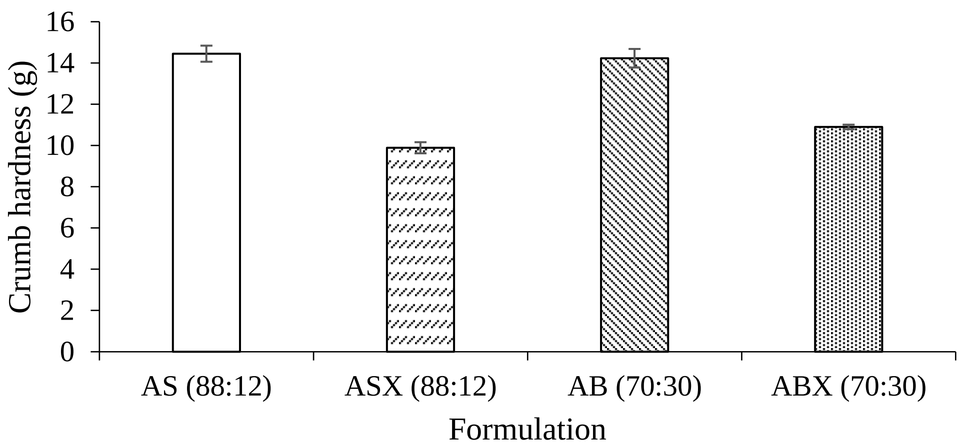

Figure 5.2: The effect of xanthan gum on crumb hardness gluten-free amadumbe bread AS-Amadumbe+soy protein isolate (88:12), AB-Amadumbe+bambara groundnut flour (70:30), ASX-Amadumbe + Soy protein isolate+Xanthan gum (88:12, 1%), ABX-Amadumbe+bambara groundnut flour+Xanthan gum (70:30, 1%)

5.3.4. Crumb moisture content of amadumbe gluten-free bread

Moisture content of the bread samples containing xanthan gum was significantly higher than the bread samples without xanthan gum (Figure 5.3). Similar results have been reported by Guarda *et al.* (2004) in a study which investigated the effect of different hydrocolloids such as HPMC, alginate, xanthan gum and k-carrageenan and reported that these hydrocolloids reduced moisture loss. This may possibly be due to the increased water retention and when hydrocolloids are used. Xanthan gum, at low concentrations, offers storage stability and water binding capacity (Collar *et al.,* 1999). Hydrocolloids are hydrophilic in nature thus increasing the water absorption capacity of dough (Bárcenas and Rosell 2007).

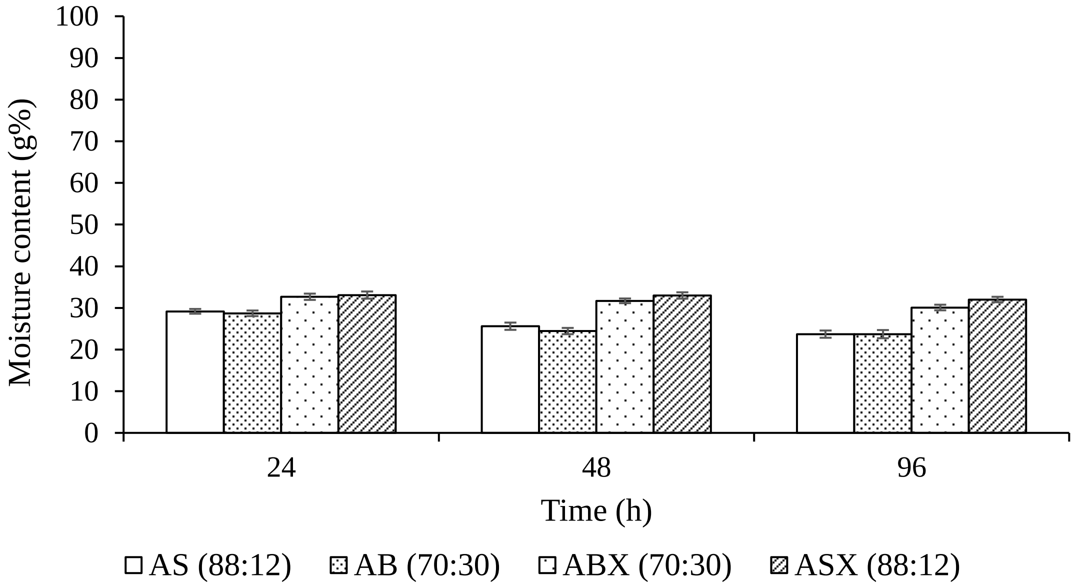

Figure 5.3: The effect of xanthan gum on moisture retention of gluten-free amadumbe bread stored at 24±2^{o}C up to 96 h.

AS-Amadumbe+soy protein isolate (88:12), AB-Amadumbe+bambara groundnut flour (70:30), ASX-Amadumbe + Soy protein isolate+Xanthan gum (88:12, 1%), ABX-Amadumbe+bambara groundnut flour+Xanthan gum (70:30, 1%).

5.3.5. Consumer acceptability of amadumbe gluten-free bread

Amadumbe bread with added xanthan gum rated higher compared to bread samples without xanthan gum, in terms of appearance, texture, taste, aroma as well as the overall acceptability (Figure 5.4). Xanthan gum have been tested for their potential as bread improvers and anti-stalling agents (Guarda *et al.,* 2004). In the study xanthan gum was able to decrease moisture loss and reduced the dehydration rate, hence retarding the crumb hardening. This might also have influenced the texture of bread with added xanthan gum. Owing to the capability of hydrocolloids to increase the water absorption capacity of dough systems, the resultant bread with added xanthan gum had increased volume which also had an impact on the texture. These improvements impacted on the overall quality of the bread hence the improved sensory characteristics. Generally, samples with xanthan gum were scored better than the samples with no xanthan gum added, ASX was more acceptable compared to the other samples (Figure 5.4) with the acceptability index of between 40% and 85%.

Similar results have been reported in previous studies (Collar *et al.* 1999; Moore *et al.* 2004; Bárcenas and Rosell 2007) which confirms the feasibility of using hydrocolloids in bread making.

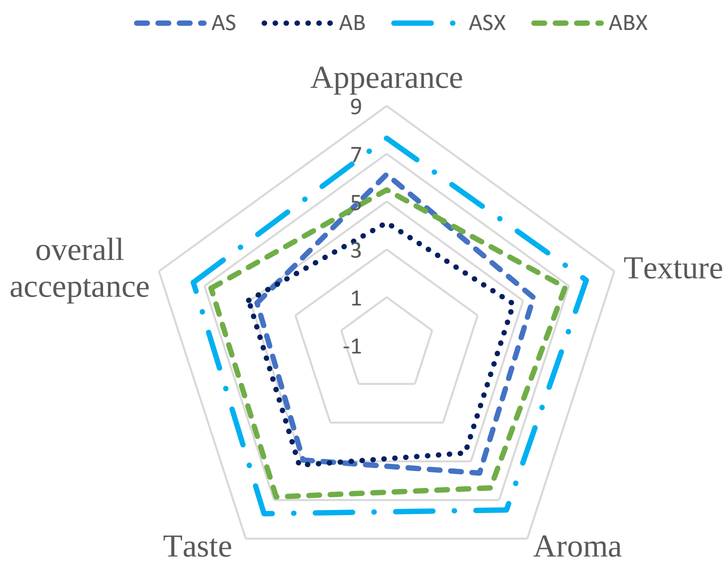

Figure 5.4: Effect of xanthan gum on the sensory characteristics of gluten-free amadumbe bread AS-Amadumbe+soy protein isolate (88:12), AB-Amadumbe+bambara groundnut flour (70:30), ASX-Amadumbe + Soy protein isolate+Xanthan gum (88:12, 1%), ABX-Amadumbe+bambara groundnut flour+Xanthan gum (70:30, 1%).

5.4. Conclusion

This study has shown that xanthan gum helps to improve amadumbe bread properties. The improvements are related to the ability of xanthan gum to increase the water absorption capacity of dough systems, increasing the viscosity and consequently the dough elasticity. The addition of xanthan gum to amadumbe flour supplemented with bambara groundnut flour and soy protein isolate positively improved the specific volume, texture and moisture activity of the resultant bread. The improvements are mainly related to the ability of xanthan gum to increase the water absorption capacity of dough systems. Sensory evaluation showed that consumers accepted bread samples with added xanthan gum. Therefore, xanthan gum can be included in gluten-free amadumbe dough systems, resulting in enhance bread quality and acceptability.

Chapter Six

6.1. General discussion, conclusion and recommendations

In this work, the functionality of amadumbe flour supplemented with bambara groundnut flour and soy protein isolate was studied and the resulting blends were used to produce gluten-free amadumbe bread. The first part of this section discusses the production of the blends and changes in the functional properties of amadumbe flour with protein supplementation. The second section focuses on the development of gluten-free amadumbe bread and the influence of adding the enzyme laccase on bread quality. The third section explains the effect of adding xanthan gum on amadumbe bread quality and the acceptability of the bread.

Blends were produced using ratios of 70:30 (amadumbe flour: bambara groundnut flour) and 88:12 (amadumbe flour: soy protein isolate). Proximate analysis showed that the protein content of amadumbe increased to approximately 16% and ash content to approximately 3.28 and 4.16 % due to the addition of bambara ground flour and soy protein isolate respectively. This trend was also reported in various studies where bambara groundnut and soy where used a protein supplement (Achi, 1999; Ayo *et al.*, 2007; Arise *et al.*, 2015). These findings may possibly be due to the high protein content in bambara groundnut flour and soy protein isolate which is low in tubers hence complementing and improving the amadumbe protein content. The results of this study also indicate an improvement in amadumbe flour functional properties. The changes in the water and oil absorption capacities of amadumbe and the blends could possibly be because of polar amino acids and hydrophilic carbohydrates in the added flours. The incorporation of bambara groundnut flour and soy protein isolate to amadumbe flour also improved the emulsion and foam capacities as well as stabilities. These results showed that there was an improvement in the ability of amadumbe flour to facilitate the formation of stable oil droplets through interfacial membrane development while preventing coalescence of droplets of air bubbles (Mbofung *et al.*, 2002; Adebowale and Lawal 2004; Njintang *et al.* 2007). This may have been as a result of an increased protein composition in the blends. These results showed great potential in the use of amadumbe composite flour in bread making. The amino acid content of the composite flour was useful in providing potential active sites for laccase action for the next objective of the study.

It is a difficult task to obtain a viscoelastic dough when gluten-free ingredients are used, hence, it has been proposed that using a batter instead of a dough can still facilitate bread development. This has been supported by different studies that have been done thus far (Gujral and Rosell 2004; Lee *et al.,* 2007; Renzetti and Rosell 2016). In this study the blends developed in the first section of the study were used to develop gluten-free bread. An enzyme laccase was added to the blends to try and facilitate the development of a protein network similar to that of gluten. Laccase has been used in previous studies and was reported to crosslink various substrates including phenolic compounds, tyrosine and cysteine which were also present in the amadumbe blends. To confirm laccase action, phenolic and thiol content were quantified prior and after laccase addition. The results of these tests showed that there was a decrease in the phenolic content and thiol groups after laccase treatment proving that laccase action had occurred. The decrease in the phenolic content has been reported in previous studies and was attributed to laccase-mediated oxidation of ferulic acid to corresponding radicals (Labat *et al.,* 2001; Moore *et al.,* 2004; Selinheimo 2008). The decrease in the thiols may be attributed to the oxidation of free sulfhydryl units present in our system promoting the formation of disulphide linkages (Caballero *et al.,* 2007b). In a different study, the decrease in sulfhydryl units was attributed to formation of adjacent linkages between proteins molecules and the arabinoxylan matrix due to the activation of the ferulic acid double bond (Hoseney and Faubion 1981; Gujral and Rosell 2004). It is of great interest to note that the choice of protein supplemented had no significant effect on the laccase-assisted changes to bread properties. This indicates that bambara groundnut flour can perform in the same way as the normally used protein source (soy protein isolate). Treating amadumbe-based dough with laccase resulted in bread with a higher specific volume, softer crumb texture and reduced water loss. During bread making with laccase incorporation there is the oxidation of various substances due to wide substrate specificity of the laccase enzyme. This results in the formation of free radicals which can participate in various cross-linking reactions. For example, as mentioned above, the oxidation of free sulfhydryl units results in the formation of disulphide bonds which helps in strengthening the dough system. This reaction promotes the ability of the dough system to retain gas during fermentation and baking resulting in improved bread structure and quality (Caballero *et al.,* 2007b; Renzetti and Rosell 2016; Niño-Medina *et al.,* 2017). These bread properties indicate the acceptability of bread by consumers hence the other part of the objective was to conduct a sensory analysis test to confirm if the amadumbe bread would be acceptable or not. The results of the sensory analysis showed that laccase treated bread was more acceptable compared to the non-laccase treated bread samples.

Hydrocolloids have been traditionally used in the development of gluten-free bread. In this study, xanthan gum was incorporated in the development of gluten-free amadumbe bread. The results showed that xanthan gum incorporation improves loaf volume, bread crumb texture and reduces moisture loss. This was possibly a result of an increase in water absorption capacity that xanthan gum imparts on dough systems (Gambuś *et al.,* 2001; Sidhu and Bawa 2002; Lazaridou *et al.,* 2007; Shittu *et al.,* 2009). It has been reported that the use of hydrocolloids promotes the formation of a stable storage for water and also improves the water binding capacity of food systems (Collar *et al.,* 1999; Bárcenas and Rosell 2007). This might have contributed to the stability in moisture loss in the developed amadumbe bread. To further confirm the acceptability of the amadumbe bread with added xanthan gum, a sensory analysis test was done and the results showed that consumers preferred bread samples with added xanthan gum compared to the bread samples without xanthan gum.

6.2. Conclusion and Recommendations

Generally, the outcomes of this research give an insight into the potential of laccase treatment or xanthan gum supplementation of amadumbe flour enriched with bambara groundnut flour or soy protein isolate, in the baking industry. Due to the low protein content of amadumbe, it is very difficult to use amadumbe in various food development systems hence, it was of great importance to look for a suitable protein supplement so as to improve its functionality. Therefore, the incorporation of bambara groundnut flour or soy protein isolate resulted in an improvement in some of the major functional properties relevant to the baking industry. Protein supplementation resulted in an improved water absorption capacity an important parameter in food industries such as the baking industry where it directly affects dough machinability and the overall quality of bread. The oil absorption capacity, foam capacity and stability and emulsion capacity and stability also improved allowing for the potential use of amadumbe blends in food industries where flavour needs to be enhanced and coalescence is to be avoided. With added laccase to amadumbe flour blends, the resultant bread had properties and a high acceptability index. In general, the inclusion of laccase in amadumbe dough oxidizes the phenols and thiols present in the dough system resulting in dough strengthening and improved machinability resulting in bread with improved bread crumb hardness, increased volume and moisture loss stability. The incorporation of xanthan gum in amadumbe dough resulted in bread with increased specific volume, stable moisture loss and improved bread crumb hardness. The results of this study suggest that xanthan gum can potentially improve the amadumbe dough properties by improving the water absorption capacity of the dough which may improve its viscosity and impact on gas retention and bread quality.

To the best of our knowledge, this study is the first to report the effect of laccase and xanthan gum on the quality of gluten-free amadumbe bread. Despite the positive results obtained, it is necessary to optimise the conditions and parameters so as to recommend the most suitable method for production of the bread. From the findings of this study, more work still needs to be done to further confirm the rheological changes that occur when laccase or xanthan gum are added to amadumbe dough systems. Also, future work needs to include microscopy work to show the differences in microstructure between laccase or xanthan gum-treated amadumbe bread and non-treated samples. It is also recommended to do protein extraction of the laccase treated dough/bread samples and the non-treated and conduct SDS PAGE to confirm disulphide bond formation after laccase action.

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