Utilisation of marama bean [*Tylosema esculentum* (Burchell) A. Schreiber] flour in gluten-free bread making

By

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DECLARATION

I declare that the thesis herewith submitted for the degree PhD Food Science at the University of Pretoria, has not previously been submitted by me for a degree at any other university or institution of higher education

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ABSTRACT

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PhD Food Science

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Interest in gluten-free (GF) bakery products due to wheat allergies and the incidence of coeliac disease, has led to research that continually explores new ingredients and formulations. One aim is to manufacture GF breads as similar as possible to wheat breads. In many African countries the high cost to import wheat particularly into no or low wheat-producing regions, where the climatic conditions do not favour its cultivation, demand that alternative sources of baking flours for bread are required. The cotyledons of marama beans (*Tylosema esculentum*) and starch from cassava roots (*Manihot esculenta* Crantz) contain no gluten. Marama bean has great potential as an oilseed legume. The isolated protein from marama flour has high foaming capacity, and produces a dough with high extensibility and good viscoelasticity, while cassava starch has better performance in gluten-free composite bread formulations than other cereal starches commonly available in Africa. Cassava starch is more similar in functional properties to wheat starch than other tropical cereal starches like maize and sorghum considering its high ratio of amylopectin to amylose which gives it good granule expansion at a low temperature.

The dough properties of composites of defatted marama flour (DMF) and cassava starch (CS) were compared with wheat flour dough with the aim of determining the potential of such DMF-CS composites as a functional nutritious gluten-free ingredient in bread. GF breads from DMF and CS combined in different proportions were studied against wheat breads as standards. Freshly produced breads were assessed for colour, height, specific volume and spread ratio. The crumb texture of breads stored at 5 ° C was measured one day and three days after baking by conducting a texture profile analysis using an EZ-L Shimadzu texture analyser. The measured parameters were expressed as crumb firmness (N) and springiness (%). The crumb structures of GF composite

breads were visualized using stereomicroscopy and a sensory panel described the sensory properties of the breads using the Flash Profiling method.

DMF-CS doughs with similar strength to wheat flour dough were produced. However, the DMF-CS doughs had much shorter Mixolab development times and stability. Alveography revealed that the DMF-CS doughs could inflate a bubble, with the 33:67 DMF-CS ratio having the most similar bubble size, extensibility and deformation energy as wheat flour dough. With the highest proportion of DMF (57:43 DMF-CS), these parameters were lower possibly because of the highly hydrophilic marama protein which tended to form aggregates rather than distribute homogenously throughout the dough as revealed by confocal laser scanning microscopy. Rheofermentometry showed that similar to wheat flour dough, the DMF-CS composite doughs also held gas produced by yeast fermentation. In this regard, DMF appears to have considerable potential as a functional gluten replacement for making protein- and fibre-rich GF bread.

Indeed, DMF-CS breads with brown crusts and a uniform aerated crumb structure were produced. Higher inclusion of DMF in the formulation led to a higher specific bread volume with a lower spread ratio. The DMF-CS bread crumbs were less soft than wheat bread with a more intense fermented, nutty, bean-like flavour as well as chewier texture. Higher inclusion of DMF in the formulation led to more bitter taste because DMF contains bitter compounds such as saponins, gallic and protocatechuic acids. DMF-CS breads presented different, more intense flavour sensory profiles compared to wheat bread which was blander.

This is the first study to clearly demonstrate the utilisation of DFM in gluten-free formulations for bread making. The research covers an interesting and highly relevant topic, which is the overall food supply in Africa through creation of a new ingredient for the bakery industry and manufacturing of a staple food product such as bread. The findings of this work serve an important purpose. Domestication of marama bean plants and successful processing of the seeds to functional flour may revolutionise the bakery industry and could potentially have significant economic impact. More research is needed to optimise the formulations and baking methods along with the use of effective technologies to mask the bitter taste of the DMF-CS bread flavour to enhance consumer acceptance and potential success on the market. Further research possibilities should also include altering recipes to improve the flavour and adopting alternative processing techniques including sourdough fermentation, frozen storage of dough, and partial baking to accomplish longer shelf life. The findings of this work should contribute to stimulating the development of marama bean as a commercial oilseed legume crop. DMF appears to have considerable potential as a highly functional gluten replacement in the production of "additive-free," protein- and dietary fibre-rich gluten-free bread or as a partial wheat flour replacement in composite flour breads.

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DEDICATION

I dedicate this dissertation to my wonderful family. Particularly to my understanding and patient husband, Serge Malandala, and to our precious Malandala children, Emmanuel, Gabriel, Abigael and Dorcas who are the joy of our lives. I must also thank my loving mothers Leontine and Clementine as well as all my family who have helped so much with baby-sitting and have given me their fullest support. Finally, I dedicate this work to my late father, Prof. Patrice Nyembwe, who believed in diligence, science, and the pursuit of academic excellence. And to my sisters: Betty, Sylvie-Rose, Gisele, Doris, Conso, Stella, Laeticia and brothers: Didier, Deo and Joseph.

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1. INTRODUCTION

Before the year 2000, GF products were not commonly sold in South Africa except in specialty health-food stores. While the motivations to consume GF foodstuff are many and not always clear, nowadays an increasing demand for GF products is seen in market trends (Melini, Melini, Luziatelli & Ruzzi, 2017). The numbers of GF products that health food shops and supermarkets display on shelves is increasing all the time. The interest in GF products, particularly GF breads has promoted extensive research to investigate new ingredients. Such ingredients include the utilisation of protein rich legume flours. The formulation of innovative GF bread that is appealing from a technological point of view and that resembles wheat bread is an important goal (Melini et al., 2017).

Marama bean (*Tylosema esculentum* L. Birch) has great potential as an oilseed legume that can be cultivated in hot, arid environments (Gluzar, Taylor & Minnaar, 2017). The marama bean is not grown commercially but collected from the wild in southern Africa where it forms part of the diet of some people (Holse, Husted & Hansen, 2010). Domestication of marama has been initiated through a breeding programme in Namibia (Nepolo, Takundwa, Chimwamurombe, Cullis & Kunert, 2009). Being a good source of protein (29 % - 38 %) and oil (32 % - 42 %) (Holse et al., 2010), the marama seed might be processed into different products including defatted marama flour (DMF). Marama bean flour possesses useful functional properties such as high protein solubility, emulsification, as well as water and oil absorption capacities (Maruatona, Duodu & Minnaar, 2010). In addition, marama protein flour has been identified to have potential for GF applications due to its high foaming capacity and its excellent ability to form an extensible dough (Amonsou, Taylor, Emmambux, Duodu & Minnaar, 2012) with a good balance of viscous flow and elasticity (Gulzar et al., 2017). A high level of extensibility in bread dough prevents sudden breakage in gas cell membranes (Sliwinski, Kolster, Prins & van Vliet, 2004), while viscoelastic behaviour is a critical attribute that allows the capture of carbon dioxide during bread fermentation (Gallagher, Gormley & Arendt, 2004). Legume protein flours are known for their good functional properties that mimic to a limited extent the viscoelastic behaviour of gluten in wheat dough and improve the quality of GF baked goods. For example, Minarro, Albanell, Aguilar, Guamis & Capella (2012) studied the use of soybean flour, chickpea flour and pea protein isolate in GF starch-based formulations. The authors reported that these legume protein flours exhibited some viscoelastic behaviour and provided a fair structure to the dough and texture of the final GF bread. The addition of soy protein isolate to GF dough was reported to increase the elastic balance and reduce the tan δ value (which reflects the balance of viscous flow G" and elastic G' components and represents the overall visco-elastic behaviour of a specific material) (Tunc & Kahyaoglu, 2016). Lower Tanδ values are related to a high degree of crosslinking of protein molecules, leading to a strong protein network and providing structural stability to a dough (Gulzar et al., 2017).

Marama beans are also rich in dietary fibre (19-27 %) (Holse et al., 2010). The carbohydrate fraction of marama beans comprises cell wall polysaccharides, mainly cellulose and pectin (Mosele, 2012). Pectin has been incorporated as a hydrocolloid into GF formulations to increase the dynamic elastic modulus of the batter, improve the quality of bread in terms of volume, and for positive crumb porosity (Lazaridou, Papageorgiou, Belc & Biliaderis, 2007).

Cassava (*Manihot esculenta* Crantz) can tolerate drought conditions and is extensively cultivated as a yearly crop in tropical and subtropical regions for its edible starchy tuberous root, a major source of carbohydrates (Nuwamanya, Baguma, Emmambux, Taylor & Patrick, 2010). Cassava tubers are highly perishable and need to be processed immediately after harvest to various products including cassava flour and starch (Bo &Tunde-Akintunde, 2013). Starch is the main component of cassava tubers with high yield (up to 30 % of the fresh root or 80 % of root dry matter) and purity (Nuwamanya et al., 2010). Cassava starch (CS) has many remarkable characteristics, including high paste viscosity, high paste clarity, and high freeze-thaw stability, as well as a low pasting temperature (average 68 °C) (Nuwamanya et al., 2010). For this reason, countries in tropical Africa are actively promoting the use of cassava as a substitute for wheat flour in bread making and particularly in the form of composites with legume flours (Udofia, Udoudo & Eyen, 2013).

Thus it is hypothesized that a composite made with DMF and CS may have the potential to develop a dough with rheological properties that will result in a GF bread with a crumb structure and textural characteristics comparable to that of wheat bread. This is because defatted marama flour will provide the required viscoelasticity and allow for gas retention due to its rheological behaviour and high functionality. CS has a high ratio of amylopectin to amylose which gives it good granule expansion at a low temperature (Nuwamanya et al., 2010); meaning that cassava starch is more similar in functional properties to wheat starch than other tropical cereal starches like maize and sorghum.

In addition, compositing marama flour with cassava starch to develop GF bread could also address the concern of high cost to import wheat in tropical and subtropical Africa countries where wheat production is limited or not possible due to climatic conditions. For example, in Lesotho wheat has to be imported. As a consequence, the consumption of wheat bread has become expensive and not affordable for poor families (Nkhabutlane, du Rand & de Kock, 2014).

However, no research could be found reporting on the application of marama bean flour or as in this study, DMF, as an ingredient in wheat-free bakery applications. The present research is of particular relevance to persons suffering from gluten intolerance and wheat allergies. To our knowledge, the information on the effect of different levels of inclusion of DMF to CS on dough rheological properties as well as on baking performance of the resulting bread are missing. The insight that is created in parallel, may well open routes into structuring of other types of bakery products as well. For example, this study also addresses the general issue of food supply in Africa communities through creation of a new ingredient which can provide solutions to poverty, malnutrition and hunger. In addition, this study presents insights on the economic potential of a marama bean product that may encourage domestication, cultivation and commercialisation of marama bean. Simultaneously, the work also explores on what current trends can be tapped into, and in what niche markets the potential can be valorised.

The present study investigated the functional properties of doughs consisting of defatted marama flour-cassava starch (DMF-CS) composites for making bread. The interaction between different components e.g. starch, protein and fibre in the bread formulations and their influence on structure, texture, flavour formation, as well as the shelf life of the GF bread were determined and compared to wheat flour bread.

2. LITERATURE REVIEW

The following literature review describes the marama bean and its processing applications and gives an overview of the physicochemical and functional properties of marama flour and cassava starch as potential ingredients in GF bread making. The importance of wheat flour in bakery products is reviewed as well as its impact on those suffering from coeliac disease and wheat-related allergies. The different challenges when considering the absence of gluten in bakery products is covered and the current actions taken to improve the quality of non-wheat as well as wheat baked products through the use of functional ingredients such as legume proteins and other additives as well as enzyme and sourdough technology are reviewed. Then methods applied in scientific literature to determine the quality of bread are also described and critically reviewed.

2.1 Description of marama bean and processing applications

Four species are recognized in the genus *Tylosema*: (i) *Tylosema esculentum* (Burchell) A. Schreiber, (ii) *Tylosema fassoglense* (Kotschy) Torre & Hillc, (iii) *Tylosema argentea* (Chiov) Brenan, and (iv) *Tylosema humifusa* (Pichi-Serm & Roti-Michael) Brena. They are found throughout Africa with the exception of *Tylosema esculentum* (Burchell) A. Schreiber, which is specific only to the semi-arid regions of southern Africa (Figure 1) (Castro, Silveira, Coutinho & Figueiredo, 2005; Jackson, Duodu, Holse, Lima de Faria, Jordaan, Chingwaru, Hansen, Avrelija Cencic, Kandawa-Schultz, Mpotokwane, Chimwamurombe, de Kock & Minnaar, 2010). The genus *Tylosema* belongs to the plant family leguminosae, commonly known as the legume family. Detailed taxonomic classification of various species within the genus Tylosema has been provided by Castro et al. (2005). Here, the discussion is limited to the distribution, habitat of the species, pods and seed morphology from the identified species. *Tylosema fassoglense* occurs mainly in the eastern and central tropical region of Africa. It grows in open woodland on red dolomite soil, in forest and secondary shrub land (Castro et al., 2005). *Tylosema fassoglense* sometimes occurs in cultivated areas, on sandy soil, rocky or clay soil, periodically flooded, at 30-2100 m altitude. The pods are obovate, and oblong to obovate, with length and width varying between 5-10 cm long and 3-5 cm wide. The seeds are suborbicular or ellipsoid, 1.5-2.8 cm long and 1-2 cm wide (Castro et al., 2005). They are chestnut-brown to blackish (Amonsou, 2010). *Tylosema argentea* is distributed in Somalia, Southern Ethiopia and Northern Kenya (Castro et al., 2005). It grows on riversides or rocky soils of old alluvial slopes up to 550 m altitude. The pods are 6 cm long and 4 cm wide. The seeds from this species are ovate-circular with length and width of 13 mm and 10 mm respectively (Castro et al., 2005). *Tylosema humifusa* is limestone soil and plains with open vegetation of grass and low shrubs at 400-1000 mm Somalia and Kenya (Castro et al., 2005). The pods are rhombic, 4.0-4.5 cm long and 2.5-3.5 cm wide. They are brownish with small longitudinal and oriented light-brown grooves. The seeds are subcircular, compressed with length and width of 20 mm and 18 mm respectively (Amonsou, 2010; Castro et al., 2005). Marama bean (*Tylosema esculentum* Burchell A. Schreiber) commonly also called morama bean, tsin bean or gemsbok bean is an important component of the diet in settlements around the Kalahari Desert and the neighbouring sandy regions of Namibia, Botswana, and South Africa (Jackson et al., 2010), mostly in undulating grassveld or savannas (National Research Council, 1979). It is a desiccant-tolerant plant with an ability to grow in high temperatures and dry environments such as the Kalahari area (Holse et al., 2010). The word ''esculentum'' means edible and was given to the plant because not only does it produce seeds and tubers that are eaten but browsing stock and game also consume the tuberous stems (Jackson et al., 2010).

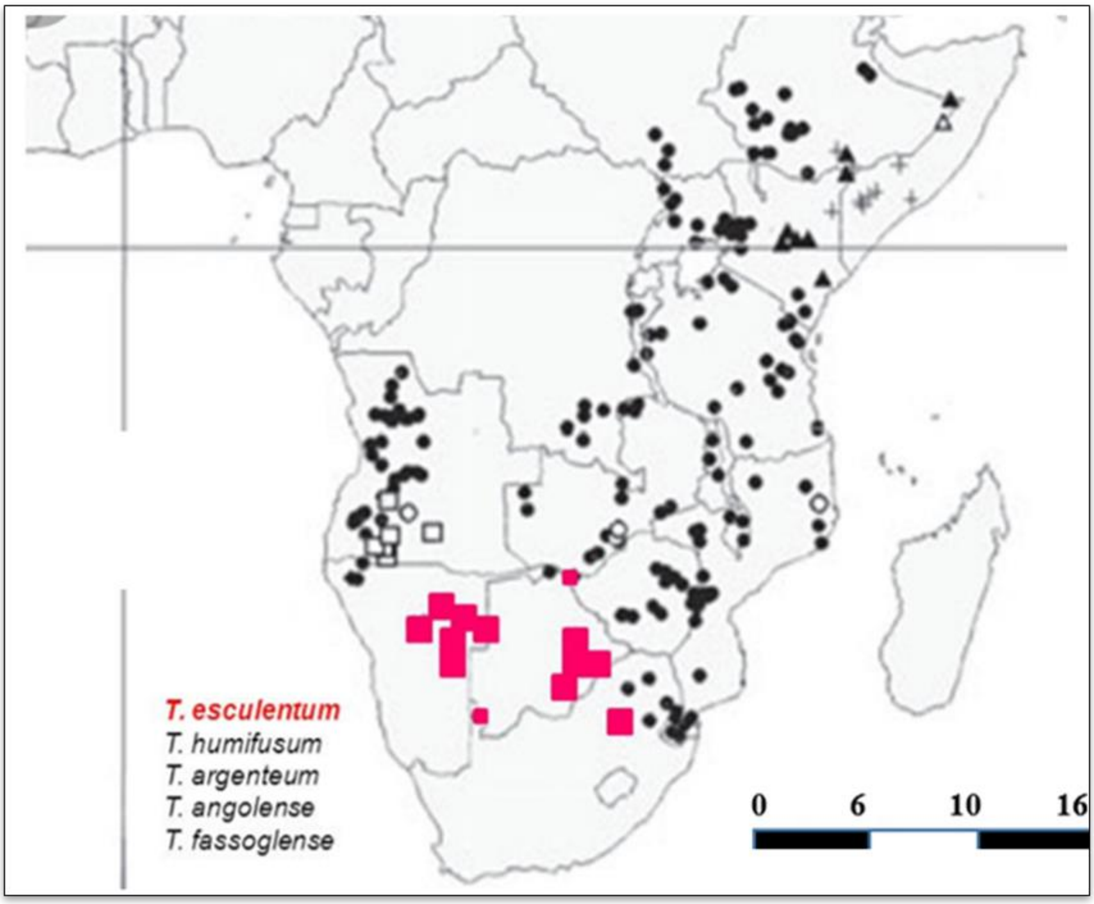


Figure 1: Distribution map of *Tylosema* species in Africa (Castro et al., 2005).

Raw mature seeds of marama beans store well can remain edible for years (National Research Council, 1979). The mature seeds of T. esculentum are encapsulated in woody pods with 1–2 seeds and the pods open at maturity. Their seed coats are reddish to brownish black in colour as shown in Figure 2, and are removed before consumption; while the cotyledons are edible (Figure 2), and eaten boiled or roasted. Traditionally, the roasted beans are cooked with maize meal to prepare porridge or are ground into a powder that is boiled in water to produce a cocoa-like beverage (Nyembwe, Minnaar, Duodu & de Kock, 2015).

Several possible marama bean-processing applications, including protein-rich flours, protein isolate, milk beverage and yoghurt, high-value oil, butter or paste, meat analogue products and snack foods as summarised in Figure 3. The preparation of protein-rich marama flours follows a number of simple unit operations. These include heating, dehulling, defatting (in some cases), and milling (Maruatona, Duodu, and Minnaar, 2010). These operations may impact either positively or negatively on the nutritional, functional, sensory, and phytochemical quality of resultant flours. Defatting is required when protein-rich, stable flours are required. Although full fat flours are deemed to be more energy-dense than fully or partially defatted flours, they have lower protein contents and are prone to hydrolytic and oxidative rancidity. Defatting significantly increases the protein contents of the resulting flours. Dry heating also results in slightly higher protein contents for DMF possibly due to the disruption of lipid bodies of the marama beans upon heating, allowing the oil to be more readily expelled during the defatting step (Maruatona et al. (2010). Dry heating or roasting of marama bean is able to improve protein digestibility and also effectively inactivate heat labile trypsin inhibitors in marama flour. Slight reductions in lysine upon roasting or dry heating of marama beans due to Maillard reaction have also been reported by Mmonatau (2005) and Kayitesi (2009). The use of dry heating processes in the preparation of marama flours can also affect their functional and sensory properties. Heating of marama beans prior to decortication reduced protein solubility and emulsifying capacity of resulting defatted marama bean flours but improved water absorption capacity significantly (Maruatona et al., 2010). Jideani, Van Wyk, & Cruywagen (2009) also found that roasting of marama bean increased the water absorption capacity of full-fat marama flour but reported increased protein solubility and emulsifying activity of the full fat flour upon heating. Differences in results can probably be attributed to the fact that Maruatona et al. (2010) worked with defatted flours, whereas Jideani et al. (2009) worked with full fat flours.

A B

Figure 2: Pictures illustrating marama beans with seed coats (A) and marama cotyledons (B).

Kayitesi (2009) used marama flours to improve the nutritional quality of sorghum porridge, a staple food to millions in Africa. Compositing sorghum porridge with marama bean flours (full fat and defatted) significantly increased protein contents of the porridges. Lysine content of the composite porridges (full fat and defatted) was 3-4 times higher than that of sorghum porridge alone. The total phenolic content and antioxidant activity of composite porridges were also significantly higher than that of sorghum porridge. Porridge composited with full fat marama flour from heated beans was found to be as acceptable as sorghum porridge, but more acceptable than that of composites using defatted flour. A bitter taste and aftertaste were perceived in composite porridges from defatted flours, whereas composite porridges from full fat marama flours were described as buttery/creamy.

Mitei, Ngila, Yeboah, Wessjohann, and Schmidt (2008) developed a high quality marama oil and focused on the characterisation of the fatty acids, phytosterols and vitamin E compounds in the oil. The lipid of marama beans is mainly (75%) unsaturated fatty acids, with the principal fatty acid being oleic acid (43%). The beans furthermore contain linoleic (22%) and palmitic acid (13%) as well as stearic, arachidic, linolenic, arachidonic, erucic, behenic, myristic, palmitoleic, and gadoleic acid in lower concentrations (Mitei et al., 2008). The fatty acid composition resembles that of olive oil (Mitei et al., 2008). Less than 5% of the fatty acids are present as free acids (Jackson et al., 2010), which means that the activity of lipases is negligible in dry marama beans. Marama oil also contains other important compounds. A higher amount of phytosterols have been detected including 4 desmethylsterols (75%) and significant amounts of 4,4-dimethylsterols and 4 monomethylsterols (15.72% total), which may potentially impact its antioxidant potential favourably (Mitei et al., 2009). The oil potentially has both food and nonfood uses, with the latter primarily being for the processing of cosmetics. Extraction can be done using an oil press, or an organic solvent such as hexane as with other oil seeds. The sensory profile of marama oil has been described as resembling almond oil, and being suitable for domestic purposes, having a pleasant nutty flavour with a slightly bitter taste, fresh, grassy and earthy aroma with a thick, creamy and smooth texture. (Tlhong, Sopejame, Mthombeni, Mpotokwane & Jackson, 2009; Mitei et al., 2009). Compared to both sunflower and olive oils, potato chips fried in marama oil were rated as more acceptable by consumers (Tlhong et al., 2009). Therefore, as a cooking oil, marama oil has great potential in terms of consumer acceptability.

The first report of the processing of marama beans into milk has been described by researchers in Botswana (Mpotokwane, Mmonatau, Mthombeni, Mahgoub, Sopejame &Jackson, 2007). Marama milk is a creamy, white water extract of marama beans that closely resembles dairy milk or soymilk in appearance and composition (Jackson et al., 2010). Although not available commercially, marama milk can be consumed as a refreshing and nutritious beverage similar to dairy milk or soymilk. It can be used as an infant supplement providing additional protein, energy, and other nutrients to vulnerable populations where the supply of dairy milk is inadequate. It can also be an intermediate material for other applications such as yoghurt. Marama beans roasted at 150 °C for 20 min, can be eaten as snacks or can be ground into a paste-like butter (Nyembwe, 2014). Marama paste made from roasted cotyledons was described as having an appealing brown colour, nutty aroma and flavour with bitter notes that develop as a function of roasting time. Nyembwe et al. (2015) who focus their research on investigating the bitter elicited compounds of roasted marama beans, found that longer roasting time of beans for 25 min or 30 min at 150° C compared to roasting time of 20 min at 150° C resulted in negative attributes e.g. darker colour, more burnt aroma and more bitter taste. The reasons provided included the potential presence of saponins and an increase in quantities of water-extractable gallic and protocatechuic acids as a result of heating. Compounds resulting from Maillard reactions such as melanoidins, furans and caramel compounds possibly also accounted for more bitterness in the pastes made from beans roasted for longer. This study also indicated the potential of marama bean as a healthy nutritive crop for developing countries, however suggested that the utilisation of marama bean in the food industry will depend on the effectiveness of techniques to mask and effectively control bitterness.

A number of other studies have been initiated to explore the use of marama protein isolate or protein-rich flours in the food system. These included for example production of meat analogues (Jackson et al. 2010) and also the manufacturing of gluten free baked products (Amonsou et al., 2012; Gulzar et al., 2017). The seed coat of marama beans has also been studied (Shelembe, Cromarty, Bester, Minnaar & Duodu, 2012). The author reported that marama seed coat water extract is a valuable source of antioxidants, and can be used in the development of functional foods with potential health benefits

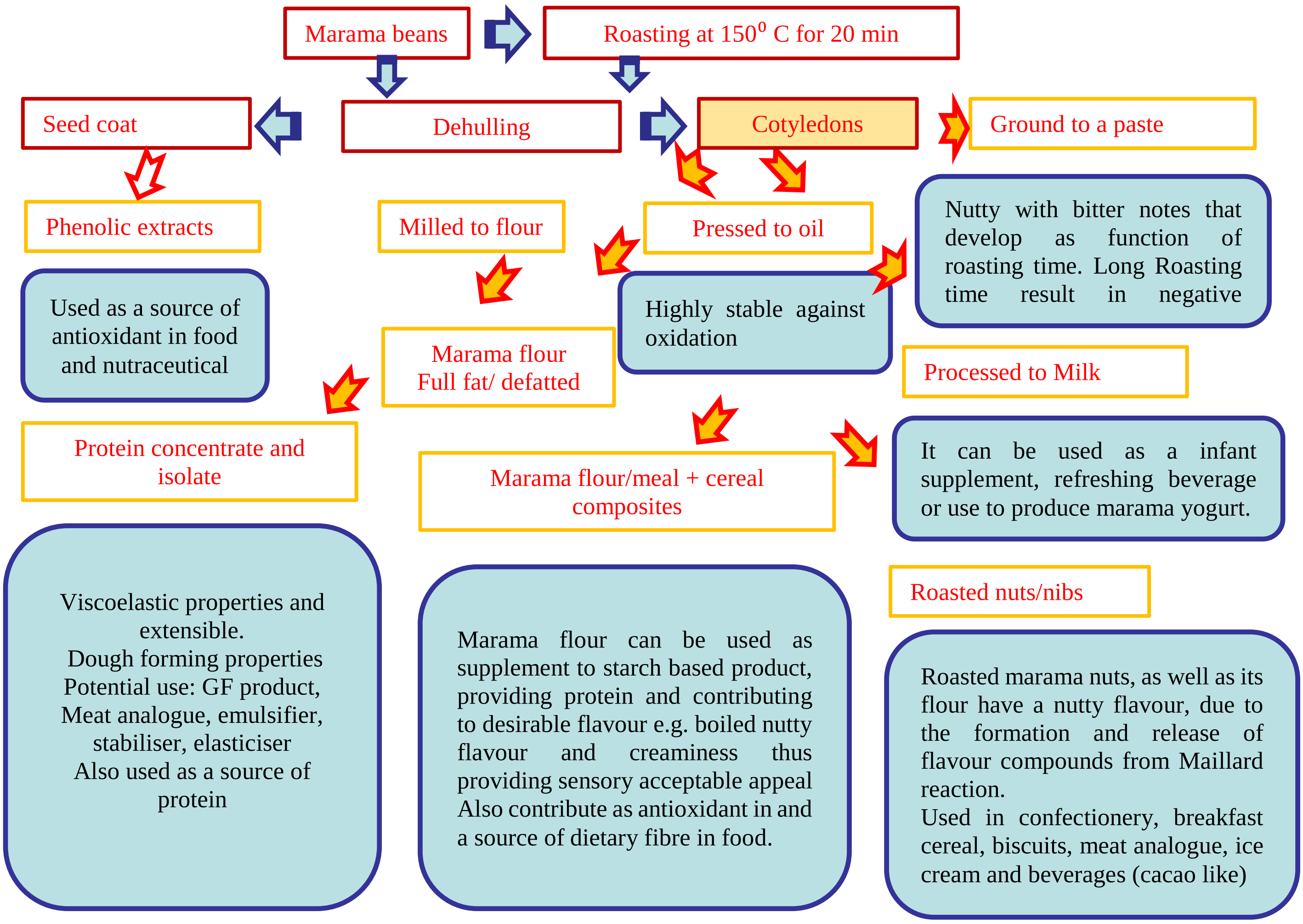


Figure 3: Summary of different processing application of marama bean and potential uses.

2.2. Physicochemical properties of marama flour

Proteins and fat are the major components of marama bean flour (Holse et al., 2010). Marama storage proteins are found in protein bodies. Protein bodies consist of a homogenous proteinaceous matrix material surrounded by a network of lipid bodies, similar to soy beans (Amonsou, 2010). The most abundant protein constituents in marama bean flour are dilute salt soluble globulins (53%), followed by albumins (23%), prolamines (15.5%) and alkali soluble glutelins (0.5 %) (Holse, Larsen, Hansen & Engelsen, 2011).

Marama bean flour is predominantly hydrophilic (Maruatona et al., 2010). Glutamic acid (15.2 g/100 g flour) and aspartic acid (9.4 g/100 g flour) which may include glutamine and asparagine respectively, are the dominating amino acids in the proteins, which are charged polar residues. Marama protein extracts (52.7-56.0 g/100 g flour), was also reported to contain a substantially higher amount of tyrosine (6.2 g/100 g flour) as compared to soybean protein extracts (Amonsou et al., 2012; Maruatona et al., 2010). The authors suggested that the high tyrosine content is possibly due to the presence of the glutelin and prolamin fractions in marama beans. Tyrosine is believed to be involved in polypeptide crosslinking (Amonsou et al., 2012), the high tyrosine content in marama may possibly contribute to the structural stability of its protein (Amonsou et al., 2012). For wheat, Tilley, Benjamin, Bagorogoza, Okot-Kotber, Prakash, & Kwen (2001) suggested that dityrosine cross-links are important in determining glutenin structure and functionality. In addition, the proline content (7.2 g/100 g flour) of marama was also higher compared to soybean flour as well as leucine (7.9 g/100 g protein), arginine (8.0 g/100 g flour) and lysine (5.7 g/100 g flour) (Amonsou et al., 2012). Amonsou et al. (2012) also suggested that the proline content in marama proteins might possibly affect its protein structure, since proline can impart a rigid structure to protein by participating in protein folding and unfolding. Moreover, marama protein contains more aromatic amino acids (15 g/100 g of protein, excluding tryptophan) compared to soybean protein (Amonsou et al., 2012). These, together with the aliphatic amino acids, were suggested to increase the hydrophobicity and stability of marama protein in comparison with soybean protein (Amonsou et al., 2012).

The sulphur-containing amino acids, methionine and cysteine, are present in quite low amounts, as for most legume seeds (Maruatona et al., 2010). Marama contains four times less cysteine (0.4 g/100 g) compared to soy bean (1.7g/100) protein (Amonsou, 2010). Cysteine residues are essential for disulphide bond formation, and increased the intermolecular interaction, while the number and pattern of disulphide cross-links of wheat glutenin polymers could also affect dough strength (Shewry & Halford, 2002). The protein composition of marama bean is also different to other oil seed legumes such as soy bean. As revealed by SDS-PAGE, marama protein contained three major protein bands (Amonsou et al., 2012). The patterns of these bands, under non-reducing and reducing conditions were similar suggesting an absence of disulphide bonds (Amonsou et al., 2012; Maruatona et al., 2010). The vicilin (7S) and acidic 11S subunits were reported to be absent in marama and only a basic (11S) protein and two higher molecular proteins are present in marama (Amonsou et al., 2012). However, the total protein of marama flour and its purified protein extract contained more basic polypeptides compared to soybeans (Amonsou et al., 2012; Maruatona et al., 2010). The protein profile of marama based on the amino acid composition of protein flour and purified protein was reported to contain fewer acidic amino acids than soy and other legumes such as peanuts (Maruatona et al., 2010; Amonsou et al., 2012). From these observations, Amonsou et al. (2012) suggested that the proteome pattern of marama thus seems to be related to its amino composition.

The storage protein composition has been found to significantly influence the functionality of proteins (Amonsou et al., 2012). The absence of vicilin (7S) and the presence of more 11S (legumin) basic proteins in marama may increase the stability of its protein to heat and extreme pH values, as suggested for 11S basic soy glycinin (Amonsou et al. 2012). The author suggested that the stability of the basic 11S soy protein could be associated with its hydrophobicity compared with the acidic subunits and the total glycinin (Amonsou et al., 2012).

A study of seed microstructure showed that marama bean protein bodies were similar to those of soy bean in terms of shape and localisation within the parenchyma cells (Amonsou, Taylor & Minnaar 2011). However, marama protein bodies seemed to contain spherical globoid and druse crystal inclusions, which are absent in soy (Amonsou et al., 2011).

Marama flour is also a rich source of lipids (32.0-41.9 % dry matter) (Holse et al., 2010; Mmonatau, 2005), which is much higher than that of soy beans (21.0 % dry matter) but less than that of peanut (45.0-55.0%) as reported by Salunkhe & Kadam (1989). The cultivation of the bean should therefore be encouraged as it is a potential source of commercial vegetable oil. In terms of the fatty acid composition, the marama bean oil was reported to have palmitic (16:0), stearic (18:0), oleic (18:1n-9) and linoleic (18:2n-6) acids as the principal fatty acids. Oleic acid (47.6%) was the most abundant fatty acid followed by linoleic acid (26.4%) (Holse et al., 2010).

According to a report by Mosele et al., (2011), starch and soluble sugars account for less than 1% of marama bean flour. The major carbohydrates in marama flour have been found to be insoluble polysaccharides mainly cellulose and pectins (Mosele et al., 2011). Holse et al. (2010) reported that the content of total dietary fibre is quite high and varies between 18.7 and 26.8 % dry matter of which the majority is insoluble and only about 4% of the dietary fibers are soluble. Marama cell walls were characterised by a high arabinose content. The arabinose fraction was characterised by arabinan-like linkages and recognised by the arabinan antibody LM6 and LM13 indicating that it is pectic arabinan. Two pools of pectin could be detected; a regular CDTA (1, 2-diaminocyclohexane-N,N,N',N'-tetraacetic acid) or enzymatically extractable pectin fraction and a recalcitrant pectin fraction containing the majority of the arabinans, of which about 40% was unextractable using 6M NaOH. Additionally, a high content of mannose was observed, possibly from mannosylated storage proteins.

Marama bean flour is also a rich source of phytochemicals such as phenolic compounds (van Zyl 2007) and saponins (Nyembwe et al., 2015) known to have potential health benefits. Marama beans contained higher levels of phenolic acids than flavonoids (Van Zyl, 2004). More interestingly is that saponins are also non-volatile, amphiphilic and surface-active compounds with emulsifying and foaming properties (Khokhar & Apenten, 2003; Rochfort & Panozzo, 2007). The amphiphilic behaviour of saponins is a result of opposing lipophilic and lipophobic characteristics of the carbohydrate and aglycone moieties. The amphiphilic character is known to make saponins natural surfactants because the carbohydrate portion of the molecule is water-soluble while the sapogenin portion is fat-soluble (Khokhar & Apenten, 2003). Emulsifying and foaming activities are of great importance in the development of gluten free products as they allow for the interaction between two chemically different phases and are used in baking to increase the stability of thermodynamically unstable systems (Sciarini, Ribotta, Leon & Pérez, 2012a).

2.3 Functional properties of marama protein and defatted marama flour

The term "functionality" as applied to food ingredients, is defined as "any property, aside from nutritional attributes, that influences an ingredient's usefulness in food" (Fennema, 1996). The functional properties of food proteins can be classified into three main groups (Moure, Sineiro, Dominguez & Parajo, 2006): (i) properties related to protein-water interactions (e.g. protein solubility, water hydration, viscosity, gelation, texturisation) (ii) properties related to protein-protein interactions (e.g. gelation and precipitation) and (iii) surface properties (e.g. emulsifying, foaming activities and surface tension). The ultimate success of utilising legume proteins as ingredients depends largely upon the beneficial qualities they impact on foods which depend largely on their functional properties (Maruatona, 2008). Functional properties of proteins, in general, are affected by various intrinsic and extrinsic factors (Moure et al., 2006). Protein molecular structure and size are important intrinsic factors, whereas extrinsic factors include the method of protein extraction, pH, ionic strength, and the components in the food system as well as processing conditions.

Marama protein in water solution formed a highly viscous and extensible dough when compared to soy protein and gluten (Amonsou, 2010). With a dough of 38 % moisture, marama protein extensibility was reported as very high (304 %), twice that of gluten and soy, and this increased considerably (> 3 fold) when the moisture content was increased to 45 %. However, with added peroxidase, the storage modulus (G') of marama protein dough increased with time, suggesting the formation of new and strong protein networks. Amonsou (2010) reported that the highly viscous and extensible rheological behaviour of marama protein is probably related to its high β-sheet conformation, hydrophobic interactions and tyrosine crosslinks. Moreover, with increasing moisture content, to approximately 52 % moisture content, marama protein flowed and it was not possible to measure its extensibility. This was unlike the situation with gluten where it was still possible to form a dough with a well-defined shape at all the moisture contents studied (38, 45 and 52 %). Additionally, in comparison with gluten, marama protein showed less resistance force to extend. Amonsou (2010) reported that the resistance to extension of gluten was related to its high molecular weight glutenin subunits which was stabilized mainly by disulphide bonds. In terms of its viscous flow behaviour and extensibility, marama protein appears similar to wheat gliadin (Amonsou et al., 2012). It is important to note that Amonsou et al (2012) made the marama protein extraction at pH 8. However, to obtain a protein isolate with optimum functional properties, a recent investigation by Gulzar et al. (2017) extracted marama protein under slightly acidic conditions at pH 6. The results revealed that marama protein has excellent food-type functional properties including foaming, emulsifying, oil binding and a good dough visco-elasticity balance, probably because it lacks some 11S polypeptides but has additional high molecular weight proteins. Therefore, marama protein could find wide application as a highly functional protein ingredient especially in GF baked products and dairy-free foam-type food products (Gulzar et al., 2017). It is then fortunate that the findings by Gulzar et al. (2017) were in agreement with a previous study on functional properties of marama flour (Maruatona et al., 2010). Defatted marama bean flour has potential as a functional food ingredient. However, the functional properties of defatted marama flour can be affected adversely by processing such as dry heat roasting of the whole marama bean (Maruatona et al., 2010).

Heat treatment is known to improve the nutritional quality of marama beans by reducing the content of anti-nutritional factors. Maruatona (2008) reported that roasting marama beans at 150 °C for 20 min is required to inactivate the trypsin inhibitor from 251 trypsin units inhibited (TUI/mg flour) to 3 TUI/mg flour. This time/temperature combination was therefore regarded as a minimum requirement for consumption purposes. Dry-heating of whole marama beans reduced the nitrogen solubility index (NSI) by approximately 29 % (Maruatona et al., 2010). NSI is defined as the percentage of water soluble nitrogen in the sample (Soderberg, 2013). The NSI varies widely with moist heat treatment of the meal as it normally indicates the extent of protein denaturation and hence the intensity of heat treatment which has been applied to the starting material (Soderberg, 2013). It is usually the first property measured at each stage of preparation and processing of a protein ingredient due to its significant influence on the other functional properties of proteins (Soderberg, 2013). The decrease in NSI in defatted marama flour prepared from heated marama beans, was suggested to be caused by protein denaturation, followed by a subsequent increase in surface hydrophobicity and aggregation of proteins through hydrophobic, electrostatic and disulphide interactions (Maruatona et al., 2010). Several studies have also reported that heating decreases the protein solubility of legume based ingredients such as low-fat soy flour (Heywood, Myers, Bailey, & Johnson, 2002). Similar results were reported by Yu, Ahmedna, and Goktepe (2007) for peanut flour. The NSI measurement has been found to correlate with protein functionality; a decrease in NSI is generally accompanied by a decrease in functionality (Maruatona, 2008).

In addition, dry-heating of marama beans decreased the oil absorption capacity (OAC) of marama bean flour (Maruatona et al., 2010). The mechanism of oil absorption may be explained as a physical entrapment of oil related to the non-polar side chains of proteins. Both the protein content and the type contribute to the oil-retaining properties of food materials (Ravi & Sushelamma, 2005). The OAC of flours is important for the development of new food products as well as their storage stability particularly for flavour binding and in the development of oxidative rancidity (Siddiq, Ravi, Harte, Dolan & 2010). The greater the amount of heat treatment that is given to a protein, the more hydrophobic the protein becomes, as a result of a greater number of hydrophobic groups being exposed through the unfolding of the protein molecules (Maruatona et al., 2010). Maruatona et al. (2010) suggested that the higher OAC of unheated defatted marama flour may partly be related to the fact that unheated defatted marama flour contained more amino acids with non-polar side chains than had the heated flour, thereby contributing to increased oil absorption.

Emulsifiers belong in the general class of compounds called surface-active agents or surfactants (Stampfli & Nerden, 1995). Emulsifiers are fatty substances possessing both lipophilic and hydrophilic properties. The surface tension between two normally immiscible phases is reduced by emulsifiers; therefore, the two liquids are able to form an emulsion (Stampfli & Nerden, 1995). Dry-heating of marama beans significantly reduced the emulsion capacity (EC) of the defatted marama bean flour. The low EC values observed for the heated defatted marama flour were suggested partly due to the lower nitrogen solubility index of heat treated defatted marama flour (Maruatona et al., 2010). For the baking industry, emulsifiers are believed to improve dough handling including providing greater dough strength; improve the rate of water absorption; provide greater tolerance to resting time, shock and fermentation. Emulsifiers also improve crumb structure: by contributing to finer and closer grain, brighter crumb, increased uniformity in cell size; improved slicing characteristics of bread; increased crust thickness; improved symmetry; improved gas retention resulting in lower yeast requirements, provided better oven spring, faster rate of proof as well as increased loaf volume and longer shelf-life of bread (Stampfli & Nerden, 1995). However, any one emulsifier does not possess all of these characteristics. The efficiencies of the different emulsifiers are closely related to their chemical structure (Stampfli & Nerden, 1995). EC depends directly on protein properties and emulsion formation depends on the rapid adsorption, unfolding as well as reorientation of the proteins at the oil–water interface (Carvalho, Garcia & Amaya-Farfan, 2006). Therefore, proteins with low solubility have a decreased capacity to act as surface-active agents and adsorb at the oil/water interface (Maruatona et al., 2010).

The water absorption capacity of a food component is the ability to retain added water during application of forces such as pressing, centrifugation or heating (Fennema, 1996). Heating increased the water absorption capacity of defatted marama bean flour (Maruatona et al., 2010). The authors explained that this was possibly due to the unfolding of the proteins upon heating, which exposed previously buried hydration sites, thereby making them available to interact with water, resulting in increased water absorption capacity. Water absorption capacity is important for baked cereal product characteristics, such as moistness, limiting starch retrogradation and subsequent product staling (Siddiq, Ravi, Harte, Dolan, 2010).

Moreover, dry-heating of marama beans did not have a significant effect on the foaming capacity of defatted marama bean flour (Maruatona et al., 2010). A protein has to be very soluble to have good foaming properties, because foam capacity requires rapid adsorption of protein at the air–water interface during whipping, penetration into the surface layer and re-organisation at the interface (Were, Hiettiarachchy, & Kalapathy, 1997). Siddiq et al. (2010) reported that selected bean flours such as red kidney, small red kidney, cranberry beans and black beans had relatively higher foaming and emulsion capacities than that of the commercially available all-purpose wheat flour. The foaming and emulsifying characteristics are important parameters when developing formulations for bakery products. Results reported by Siddiq et al. (2010) demonstrated that not only the amount of native protein in the product but probably also the nature of the protein involved influences the foaming stability of the flour.

2.4 Physicochemical and functional properties of cassava starch

Cassava starch is edible and is an important source of calories for over 800 million people in sub-Saharan Africa, Asia, and South America, especially among food-insecure communities (Bechoff, Tomlins, Fliedel, Lopez-lavalle, Westby, Hershey & Dufour, 2018). Being the major component of cassava tubers with high yield (up to 30 % of the fresh root or 80 % of root dry matter) and purity (Nuwamanya et al., 2010). Cassava starch is a neutral (odourless; bland in taste; not sour) product with small particle size and low content of protein, fat and fibre (Bechoff et al, 2018; Nuwamanya et al., 2010). Hence, the composition as well as the physicochemical properties of cassava starch are major determinants of its end uses. The starch in cassava roots accumulates as round truncated with various other shapes and sizes ranging from 2 - 40 µm as seen in Figure 4 (Moorthy, 2002). Dispersibility is a measure of the reconstitutability of starch or starch blends in water (Onitilo, Sanni, Daniel, Maziya-Dixon, Dixon, 2007). The higher the dispersibility, the better the starch reconstitutes in water. Cassava starch has a high percentage of dispersibility ranged between 81.5-89.5 %, thus cassava starch reconstitutes easily to give a fine consistent paste (Onitilo et al., 2007).

Least gelation concentration (LGC) is a measure of the minimum amount of starch or blends of starch that is needed to form a gel. Cassava starch has a value of LGC between 2.0-4.7 %. Like most starches, it is composed of two large molecular weight glucan polymers called amylose and amylopectin which occur in an approximate 20:80 ratio in non-mutant genotypes (Mtunguja et al., 2016). Amylose is a linear polymer of glucose molecules that have few branches, which are separated by large distances and are either very long or very short, allowing the molecule to behave essentially as a linear polymer (BeMiller, 2007). On the other hand, amylopectin is a very large, highly branched molecule that constitutes about three-fourths of most normal starches (BeMiller, 2007). Both glucans are made up of chains of α-1,4 linked glucose units which are intermittently branched by α-1,6 glucosidic linkages. There are branch points placed, on average, every 20 glucose units in amylopectin but only every approximately 4-100 glucose units in amylose. The branching pattern in amylopectin is precise, so that clusters of glucan chains of 3-4 defined lengths are produced in an orderly array which is essential for the formation of the semi-crystalline starch macromolecule (Moorthy, 2002; BeMiller, 2007). The organization of glucose molecules within the starch polymer, the glucan chain length distribution and amylose-to-amylopectin ratio, all influence granule morphology and size (Moorthy, 2002). Cassava starch functionality and hence its end-use is determined by its molecular structure. Gelatinization causes a collapse of starch molecular order and to irreversible changes in water uptake, granule swelling, solubilisation, viscosity, and paste development. These processes and the properties of the resulting gels and pastes formed during subsequent heating, freezing, and thawing, or after enzymatic hydrolysis, can indicate the suitability of starch for various applications.

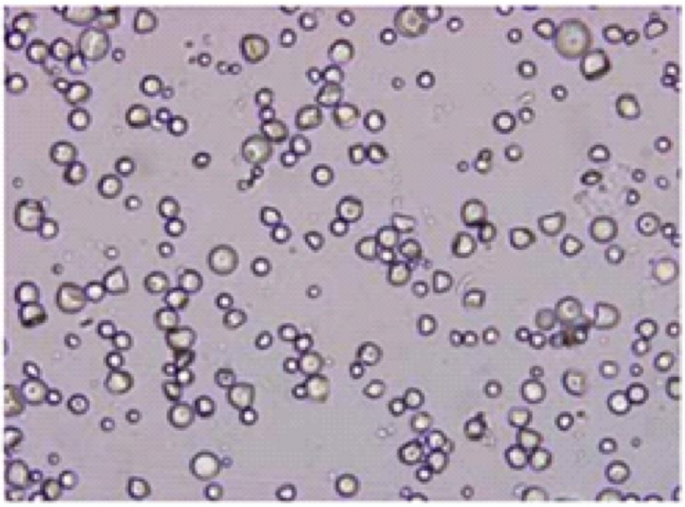


Figure 4: Photomicrograph of cassava starch (Onitilo et al, 2007)

Amylose and amylopectin molecules can swell slightly and return to their original size upon drying. In normal food processing involving heat and moisture, as starch granules are heated in excess water, they can swell as hydrogen bonds in amorphous regions are disrupted, and crystallites are pulled apart while water is absorbed resulting in paste formation (BeMiller, 2007). Gelatinization is the disruption of both the amorphous and crystalline structures that results in the irreversible loss of molecular order of the starch granule, absorption of water and swelling, change of shape and size of starch, leaching of amylose, and loss of birefringence, as well as the formation of a gel or paste (Eliasson & Larsson, 1993). In food processing, it is important to determine the temperature at which gelatinization occurs; however, cassava starch is particularly characterised for its remarkably different properties such as high swelling power which is an indication of the water absorption index of the granules during heating (9.04-16.90%), and good solubility (Zaidul, Norulaini, Omar, Yamauchi & Noda, 2007; Gomes, Mendes da Silva, & Ricardo, 2005; Charles, Chang, Ko, Sriroth, Huang, 2004). Moreover, cassava starch has a high paste viscosity, high paste clarity, and high freeze-thaw stability, as well as a low pasting temperature (average 68°C) (Nuwamanya et al., 2010). Therefore, cassava starch forms a paste much easier compared to starches with high pasting temperatures such as potato (average 72°C) (Moorthy, 2002) and rice (average 69.5°C) (Cameron, Wang & Moldenhauer, 2007). In addition, cassava starch has a high ratio of amylopectin to amylose which gives it good granule expansion at a low temperature, in other words cassava starch is more similar in functional properties to wheat starch than other tropical cereal starches like maize and sorghum (Eduardo, Svanberg, Oliveira & Ahrné, 2013). Thus cassava starch plays an important role in the use of cassava as a food and industrial crop. Physicochemical properties of cassava starch are suitable for supplementation of wheat flour in bread-making without compromising its sensory attributes (Eduardo et al., 2013).

Furthermore, generally, starch pastes after cooling and storage for a certain period of time become less soluble and difficult to dissolve upon heating. The return to an insoluble state with the formation of a precipitate or gel is a phenomenon called retrogradation (BeMiller, 2007). It is the result of crystallization of both amylopectin and amylose, with amylose undergoing retrogradation at a much faster rate. Amylose and amylopectin adjacent linear chains form double helices via hydrogen bonding that result in the formation of crystallites regions in the form of aggregates. The crystallization of partially crystalline polymers such as amylose and amylopectin will occur just above the glass transition temperature, Tg (the temperature at which an amorphous material undergoes a transition from a glassy to a rubbery material) (BeMiller, 2007). The rate and extent of retrogradation depend on several factors including the molecular ratio and structures of amylose and amylopectin, botanical source, temperature, starch concentration, the presence of surfactants and salts (BeMiller, 2007). Cassava starch is reported to have a high rate of retrogradation. Retrogradation is usually associated with many quality defects in food products such as staling of bread and tortillas, syneresis of starch paste after freeze-thaw cycles, loss of viscosity and precipitation in soups and sauces, etc. Nonetheless, intensifying starch retrogradation for value-added products has also been associated with production of resistant starch, preparation of slowly digesting starch, film formation by amylose, etc.

2.5 The role of gluten and its technological importance in bread making

Bread making is a centuries-old traditional craft practised in every country worldwide, and bread in its many forms is traditionally based on flour derived from wheat (Triticum spp.) (Matos & Rosell, 2014). This cereal grain is unique in its ability to form a cohesive and viscoelastic dough, when flour is mixed with water, because of the presence and properties of the gluten complex (Shewry & Halford 2002). Gluten proteins belong to glutelin and prolamin classes and contribute to 80-85% of the total wheat protein content (Minarro, 2013). The gluten protein is composed of two main fractions (according to their solubility in aqueous alcohol): gliadins, the single-chain monomeric proteins that mainly contribute to dough viscosity and extensibility, and glutenins, the latter being multichain polymeric proteins that are responsible for dough strength and elasticity (Wieser, 2007). Gluten is the main structure-forming protein in wheat flour and responsible for the key property of providing flour with a high water absorption capacity and dough with cohesiveness, viscosity, elasticity, and gas-holding ability. The common high volume and porous crumb of bread are thus due to gluten (Minarro, 2013). When mixing flour with water, gluten proteins enable, in fact, the formation of cohesive viscoelastic dough that is capable of forming thin gas-retaining films and, thus, upon application of energy during mixing (kneading), they turn the dough into a stretchable, extensible, coagulable, and protein-starch matrix (Gallagher, Gormley & Arendt, 2003). Glutenins are responsible for holding the gas, produced during fermentation and oven-rise, as well as conveying viscoelastic properties that enable the dough to be elastic and firm. Upon baking, when gluten proteins denature and lose viscoelasticity, the previously formed gluten structure is responsible for maintaining dough structure and the final shape of the baked goods (Arendt, Morrissey, Moore, Dal Bello., 2008). Gluten forms a strong protein network essential for the three-dimensional structure that gives desired viscoelasticity to the dough, and also for the protein-starch interaction that is related to the dough's ability to retain gas during fermentation and partly for the setting of the dough during baking (Hoseney, 1994).

Gluten's major amino acids are glutamine and proline, accounting for more than 50% of the amino acid residues. The high glutamine and hydroxyl amino acids (10%) contents of gluten are specifically responsible for its water-binding properties (Minarro, 2013). Moreover, hydrogen bonding between glutamine and hydroxyl residues of gluten polypeptides contributes to its cohesion–adhesion properties (Arendt et al., 2008). About 30% of gluten's amino acids are hydrophobic, enabling gluten to form protein aggregates by hydrophobic interactions, and to bind lipids and non-polar substances (Damodaran, 2008). Cysteine residues account for 2-3 % of gluten's total amino acids. These residues undergo sulfhydryl-disulphide interchange reactions, resulting in extensive polymerisation of gluten proteins during dough formation (Damodaran., 2008). Though cysteine belongs to the minor amino acids of gluten proteins, it is extremely important for the structure and functionality of gluten (Wieser, 2007). Therefore, gluten protein functionality is thus pivotal to bread quality, and fractionation and reconstitution experiments have clearly shown that any variation in bread making performance is determined by the gluten proteins (Veraverbeke & Delcour 2002).

However, the production of GF bread has, in fact, shown to differ significantly from the production of conventional wheat bread (Figure 7). When raw materials, that is, water, yeast, and GF flours are mixed, the resulting suspension of air retained from the mixing and carbon dioxide obtained from yeast fermentation cannot be entrapped in the gluten network which should allow the dough to expand (Onyango, Mutungi, Unbehend & Lindhauer, 2010). Most gas thus escapes too early, and irregular and unstable cells are formed, contributing to giving bread with reduced volume, lack of cell structure, and a dry, crumbly, and grainy texture. For that reason, the GF dough is much less cohesive and elastic than wheat dough. It has a more fluid-like structure, lacking the protein network that can both hold gas and form the matrix to embed the starch (Melini et al., 2017). It has also a general tendency to contain higher water levels than wheat flour, if an acceptable crumb is to be obtained (Melini et al., 2017). Actually, GF dough is a batter, much less elastic than wheat dough, sticky, and also difficult to be handled, and it can only take the shape of the pan it is baked in, whereas standard wheat dough can be moulded into different shapes of numerous wide-ranging types of products (Figure 7) (Melini et al., 2017). GF batters also require shorter mixing, proofing, and baking times than wheat counterparts (Zannini, Jones, Renzetti & Arendt, 2012). Upon the formation of a complex emulsion and foam system, and, therefore, of a large number of minute air bubbles, it becomes necessary to have the bubbles trapped in the batter by surface-active ingredients, such as emulsifiers, which form a protective film around the gas bubbles and prevent them from coalescing. For that reason, gums, stabilizers, legume protein flour and pregelatinized starch are usually used, so as to provide part of all the gas occlusion and stabilizing mechanisms (Zannini et al., 2012).

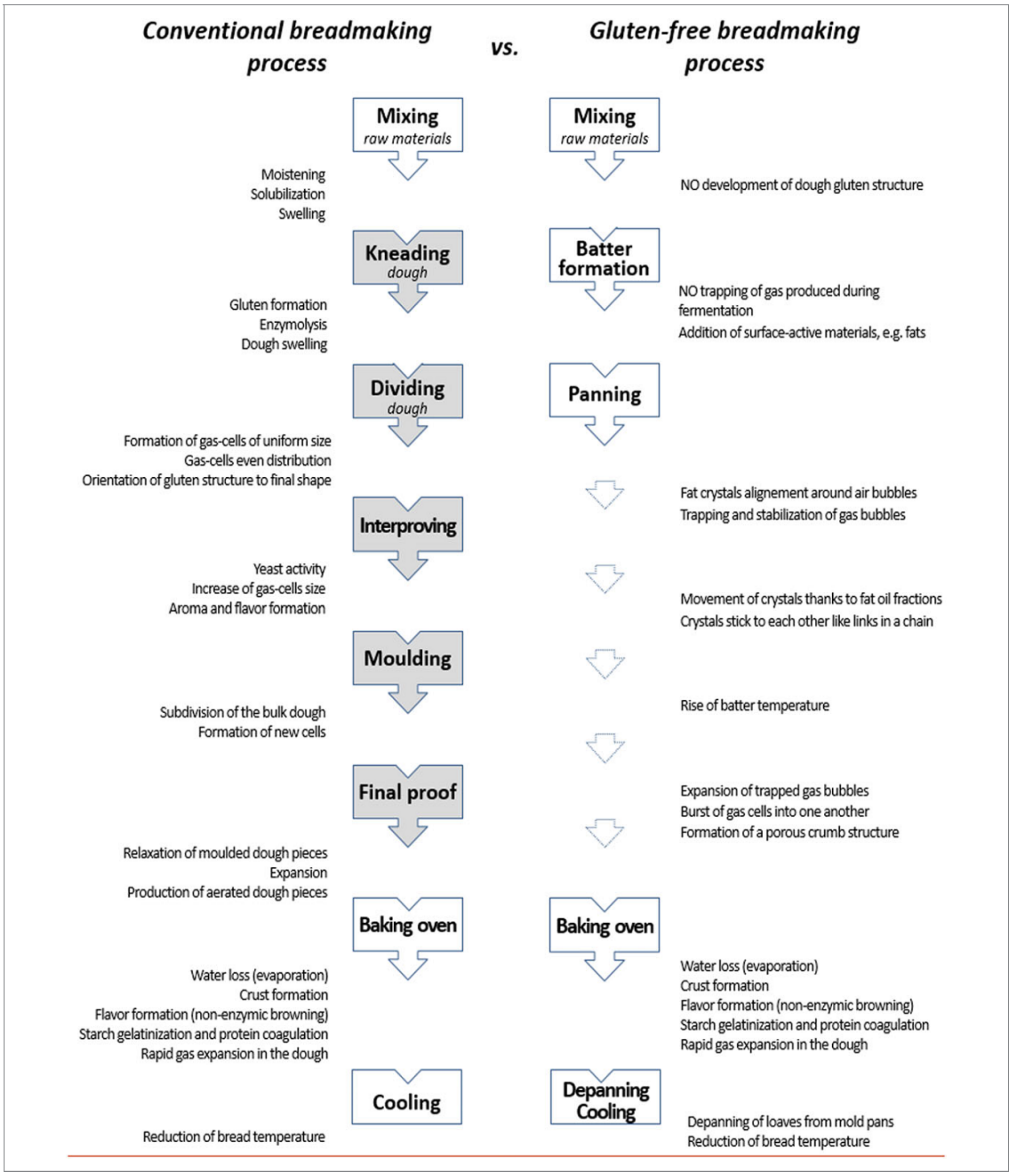


Figure 5: Technology of conventional wheat flour bread compared with GF bread making (Melini et al., 2017).

2.6 Scientific developments in gluten-free bread making

GF breads are made from flours other than wheat, rye, barley or oats (Melini et al., 2017; Matos & Rosell, 2014). The development of GF foodstuffs has attracted great attention as a result of better diagnosis of coeliac disease and a greater knowledge of the relationship between GF products and health (Matos & Rosell, 2014). Although gluten is important for bread quality, this protein can cause health problems in predisposed individuals (Melini et al., 2017; Minarro, 2013, Sciarini, Ribotta, Leo´n & Pe´rez, 2010). Celiac disease is thought to be an autoimmune disorder and may have a familial or genetic component (Melini et al., 2017). In people with celiac disease, inflammation occurs in the small intestinal mucosa when it is exposed to gluten in the diet. The symptoms usually involve the digestive system and cause: abdominal discomfort, bloating, nausea, and loose bowel movements. However, there is a wide spectrum of symptoms that may occur (Melini et al., 2017). Because the intestine becomes inflamed, it may also lose its ability to absorb nutrients from the diet, leading to other associated illnesses (Melini et al., 2017). The principal treatment to gluten intolerance consists of removing gluten from the diet (Ribotta, Ausar, Morcillo, Perez, Beltramo, & Leon, 2004; Sciarini et al., 2010). Moreover, the high demand from non-coeliac patients who perceive that GF diet can help in weight loss or treatment of disorders including chronic fatigue, migraine, fertility problems, has also greatly increased the GF market (Minarro, 2013). Additionally, because of the high cost of wheat importation in tropical and sub-tropical Africa countries where the weather conditions do not favour its cultivation (Nkhabutlane et al., 2014), the consumption of wheat bread has become very expensive. As a result, wheat bread is not affordable by poor families (Nkhabutlane et al., 2014). The increasing interest has prompted extensive research and many studies are still going on into the development of GF foodstuffs that resemble gluten-containing foods.

However, exclusion of gluten from the diet is a challenging task for bakers and cereal researchers, as wheat flour is present in a wide range of products such as bread, biscuits, cakes, pizza and pasta (Matos & Rosell, 2014). The situation is particularly difficult in the case of bread, which constitutes the cornerstone of dietary patterns for many populations (Qarooni, 1996). The absence of gluten in dough production shows great influence on dough rheology, the production process and the quality of the final GF products (Matos & Rosell, 2014). The GF doughs are much less cohesive and elastic than wheat dough. In fact, these GF doughs are often called batters instead of dough (Mariotti, Lucisano, Pagani & Ng, 2009; Matos & Rosell, 2014). Removal of gluten and, consequently the lack of a strong protein matrix able to expand and retain gas, results in weak batters with high permeability to carbon dioxide and big difficulties to maintain the structure, which decrease the volume of baked good (Matos & Rosell, 2014; Minarro, 2013; Gallagher & Gormley, 2002). The absence of gluten also impairs the water holding capacity of breads, which show an early crumbling structure, poor mouthfeel, lack of flavour, quick staling as well as pale colour because of the low protein content in the formulation (Minarro, 2013;). Nkhabutlane et al. (2014) reported that GF breads e.g. sorghum and maize steamed breads prepared according to traditional Basotho procedures were characterized by low loaf volume, denser crumb, heavy and chewy dry texture, more fibrous, more brittle texture and needed a higher compression force to deform. In fact, many GF breads available on the market were often reported as having poor technological quality, and besides great variation in the nutrient composition, with low protein and high fat contents, particularly when compared to their wheat counterparts (Matos & Rosell, 2014).

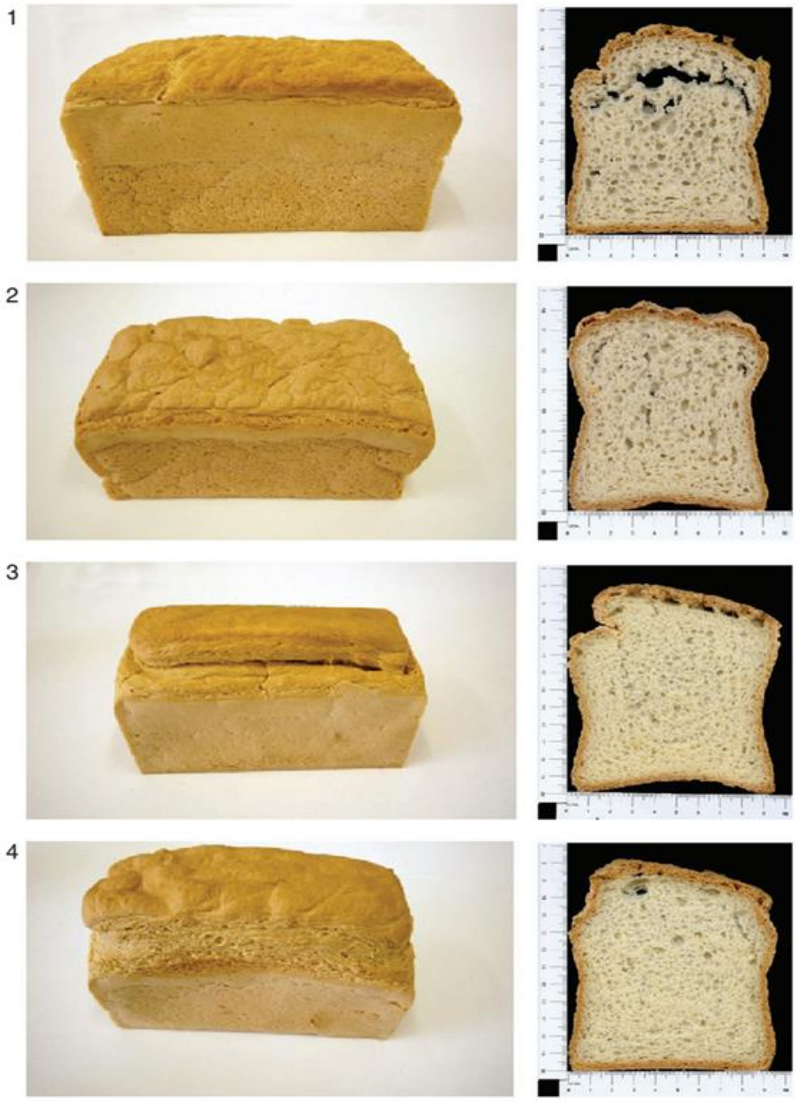


Figure 6: Rice-based GF breads and their crumb cross-sections obtained from different ingredients. Hydroxypropyl methylcellulose (HPMC K4M) (1 and 3); xanthan gum and guar gum blend (2 and 4); pea proteins (1 and 2), albumin (3 and 4) (Matos & Rosell, 2014).

Generally, bread development without gluten has involved the use of diverse functional ingredients and additives (Figure 5) with the purpose of imitating the viscoelastic properties of the gluten, improving the structural acceptability of GF bread products and consequently to obtain wheat bread-like products. Therefore diverse approaches have been developed, such as the use of different naturally GF flours (rice, maize, sorghum, cassava) alone or in combination with starches and other ingredients such as legume protein rich flours (soy isolate or concentrate, pea and chickpea flour) and cross-linking enzymes (glycosyltransferases, transglutaminase, proteases, glucose oxidase, laccase) to help create a protein network; and other ingredients such as hydrocolloids and emulsifiers (Diacetyl Tartaric Ester of Mono and Diglycerides), as well as prebiotics (inulin), which improve the structure, mouthfeel, and shelf-life of GF bakery products.

Hydrocolloids, emulsifiers and proteins isolates act as surface-active ingredients to stabilise the liquid films surrounding gas bubbles in fermenting batters (as reviewed by Taylor, Schober, & Bean, 2006). Starches have a high influence on dough parameters, such as texture, moisture retention, and final quality, as they bind and create a gas-permeable structure (Witczak, Juszczak, Ziobro & Korus, 2012). However, starches also fail to form a continuous phase and thus lack the necessary dough structure for the production of good quality dough (Matosa & Rosell, 2014). Addition of proteins has the supplementary benefit of improving the nutritional pattern of GF products and also to enhance the sensory attributes of the breads through Maillard browning and flavour development. Similarly, to their effect in wheat-based products, emulsifiers have been shown to be good additives for stabilizing gas bubbles during proofing and, consequently, for improving the crumb texture of GF breads. All these ingredients and/or additives modify the GF bread quality.

2.6.1. Legume protein flour

GF loaf breads have been successfully formulated by incorporating protein-rich legume flours such as soy flour, - concentrate or - isolate (Ribotta et al., 2004; Sciarini et al., 2010), chickpea, pea, carob germ and other flours (Minarro, 2013). Legume proteins can improve structural quality by means of gelation, foaming and increasing the elastic modulus of the dough through crosslinking reactions as also observed in table 1 (Matos & Rosell, 2014). Their role in GF formulations is also to provide structural support for starch and hydrocolloids (Mariotti et al., 2009). For example, Matos, Sanz & Rosell (2014) used soy protein isolate and pea protein isolate in rice-based muffins. The incorporation influenced the rheological characteristics as well as mechanical properties by increasing the elastic modulus thereby providing increased elasticity as well as nutritional enhancement. Further, the addition of pea protein in rice flour based muffins caused the lowest hardness and the highest springiness values among samples made from legume proteins (soy protein isolate and pea protein). Again, the pea protein isolate containing muffins were softer compared to muffins with no added protein. These protein isolates led to more structured and solid like batters.

Soy proteins due to their functional properties are beneficial for GF product quality characteristics (Ribotta et al., 2004; Minarro, 2013). Ribotta et al. (2004) reported that the addition of soybean flour improved GF bread quality with regard to good structure and increased loaf volume. This effect seemed to be due to both the structural proteins and the enzymatic activities of the soybean flour (Ribotta et al., 2004). Lipoxygenase and phospholipids contained in soy flour are believed to enhance the mechanical behaviour of the dough (Ribotta et al., 2004). Soy proteins have a high affinity for water, increasing the consistency of the batters and improving its specific volume (Sanchez, Osella, & de la Torre, 2004; Sciarini et al., 2010). The addition of soy protein affects GF bread crumb, resulting in a reduction of firmness and a delay in staling due to the high water absorption capacity of the protein. The high affinity of soy proteins for water results in less available water for starch and therefore a decrease in retrogradation, since starch retrogradation is controlled by its water content (Minarro, 2013). Additionally, Moore, Heinbockel, Dockery, Ulmer, & Arendt (2006) also reported an improvement in GF bread when soy flour was added to a potato starch, corn and white flour based formulation.

Minarro et al. (2012) made different GF corn (“maize”) starch based breads using various legume flours such as chickpea, soy and carob germ flour as well as pea protein isolate. For all formulations, the elastic modulus was greater than the storage modulus and both increased with increasing frequency sweep, suggesting viscoelastic behaviour. The corn starch-chickpea bread exhibited the best physicochemical characteristics with regard to batter quality as well as a good sensory profile (high specific volume, softness, crumb colour etc.). Minarro et al. (2012) reported that due to its specific content of amino acids, chickpea protein presents high foam expansion and stability values compared to other legume flours such as pea and soy protein. The high volume of chickpea bread has been related to the good emulsifying stability index contributed by chickpea flour (Minarro et al., 2012). Foaming capacity and stability are good techno functional properties required in GF bread (Minarro, 2013). Pea protein also possesses functional properties such as gelling, emulsifying and foaming capacity. These functionalities resulted in higher volume of bread (Minarro, 2013). Added legume protein in GF bread acts as emulsifiers by forming a film or skin around oil droplets, preventing structural changes such as coalescence or creaming (Minarro, 2013). Carob germ (*Ceratonia silica*) flour used as a protein supplement in animal feed and for dietetic supplements for humans have been identified as having viscoelasticity similar to wheat gluten and have the potential to be used in GF baked goods to mimic gluten properties (Bengoechea, Romero, Villanueva, Moreno & Alaiz, 2008). Minarro et al. (2012) reported good rheological properties of corn starch batters with added carob germ flour due to remaining gums (galactomannan) from carob germ endosperm, which could contribute to increasing its batter elastic modulus. Nevertheless, GF corn starch bread with added carob germ flour presented poor baking characteristics as compared to GF breads prepared with corn starch and chickpea flour or pea protein isolated (Minarro et al., 2012). In fact, confocal laser scanning microscopy (CLSM) of GF bread crumb made with corn starch with added carob germ flour showed a compact structure without spaces between starch granules (Figure 6 D)., GF breads corn starch with added chickpea flour and pea protein isolate showed a more homogeneous structure (Figure 6 A and B), while corn starch bread with added soybean flour showed a dispersed structure of starch granule (Figure 6 C). These formulations consisting of corn starch with added chickpea, soybean flour or pea protein isolate resulted in an open structure able to incorporate gas which accounted for their volume and textural characteristics (Minarro et al., 2012).

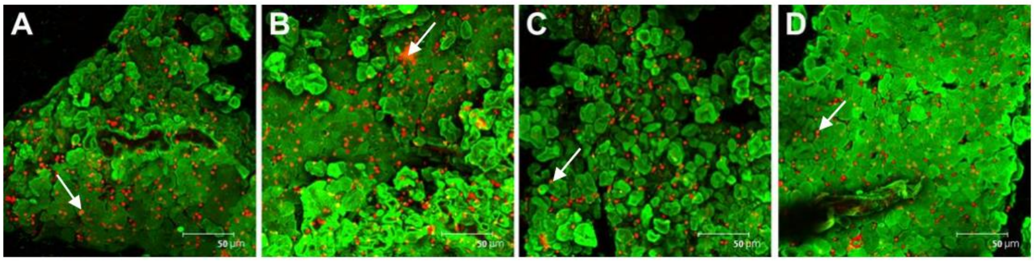


Figure 7: CLSM images of GF bread crumbs prepared with corn starch and different legume flour or protein isolate. A. chickpea flour. B. pea isolate. C. soy flour. D. carob germ flour. The red colour designates yeast cells, orange yellow is the protein (indicated by white arrow) and the bright green is the starch (Miñarro et al., 2012).

2.6.2. Hydrocolloids

Hydrocolloids or gums have colloidal properties and are capable of producing gels in water systems (Zannini, Jones, Renzetti & Arendt, 2012). However, the marama carbohydrate fraction is constituted of cell polysaccharides, mainly cellulose and pectin (Mosele, 2012). Pectin together with hydrocolloids such as carboxymethylcellulose, agarose, agar gum, guar gum, carboxyl-methyl-cellulose, hydroxyl-propyl-methyl-cellulose, xanthan gum and oat beta-glucan have widely been incorporated into GF formulations in place of bread improvers (Lazaridou et al., 2007). Hydrocolloids are employed due to their ability to enhance GF dough characteristic by the formation of three-dimensional polymer networks in aqueous solutions (Arendt, Morrissey, Moore & Dal Bello, 2008). This increases the solution's viscosity, leading to textural improvements and aids in the binding and retention of water (Table 1), (Erickson, Campanella & Hamaker (2012). Schober (2009) reviewed the mechanism by which hydrocolloids improve the texture of doughs and batters. These authors maintain that the increased viscosity of dough or batter following addition, retains gases, which are incorporated during mixing and fermentation, and minimizes the coalescence of gas bubbles. The increased viscosity also prevents separation of starch and other ingredients, thus improving dough homogeneity until gelatinization of the starch during baking. Hydrocolloids are multifunctional ingredients that add flexibility in bread, functioning as a fat replacer, water binder, texturizer and adhesives (Shalini & Laxmi, 2007; Lazaridou et al., 2007). In fact, the study by Lazaridou et al. (2007) on the rheological properties of GF dough supplemented with hydrocolloids as measured by oscillatory and creep measurements showed that the elasticity and resistance to deformation of GF dough formulations followed the order of xanthan gum> carboxymethylcellulose > pectin > agarose > beta-glucan. The type and extent of influence on bread quality produced from these doughs were dependent on the specific hydrocolloid used and its supplementation level. The high rigidity of doughs containing xanthan gum resulted in breads with low loaf volumes and high crumb firmness. Moreover, incorporation of xanthan gum increased the elasticity and the lightness of the crumb. The addition of beta-glucan at a 1% level resulted in increased bread loaf volume, crumb porosity and lightness values of crust, and significantly increased crumb firmness when added at 2% concentration. Agarose when added up to 1% level, showed a favourable effect on loaf volume (Lazaridou et al., 2007). Sodium carboxymethylcellulose and pectin seemed to be the best hydrocolloid improvers of GF breads, since their incorporation into the dough, at 1 % for CMC and 2 % for pectin, resulted in breads with significantly higher volumes and high values of crumb porosity and elasticity (Lazaridou et al., 2007).

Toufeili, Dagher, Shadarevian, Noureddinei, Sarakbi, & Farran (1994) reported that GF pocket-type flat-breads, comparable to regular wheat pocket-type flat bread in term of textural attributes, could be formulated from a bake mix based on pregelatinized rice flour and corn starch that incorporated methylcellulose, gum arabic, and egg albumen as a gluten replacement. Gum arabic had a relatively minor effect on the sensory properties of the final product; its presence in the bake mix imparted "strength" to the dough and improved its tolerance to mechanical handling while methylcellulose and egg albumen were identified as the major determinants of the product's sensory quality. The baked GF breads were compatible with regular wheat bread in the frequency of cracks, separation of layers, rollability, tearing quality, first bite hardness, masticatory hardness, adhesiveness, and cohesiveness. Similar results were also reported by Mahmoud, Yousif, Gadallah & Alawneh (2013) when using gums in the formulation of GF Egyptian *balady* flat-bread. The authors reported that hydrocolloids clearly improved the weight and roundness of the *balady*.

2.6.3. Dairy and eggs proteins

Dairy proteins enhance batter handling properties, as well as bread flavour and crust colour (Table 1), (Zannini et al., 2012). Gallagher et al (2003) investigated the addition of different levels of dairy powders (0, 3, 6 and 9 %) to commercial GF wheat starch. In general, the dairy powders that had high protein contents (skim milk powder, sodium caseinate and milk protein isolates) resulted in breads with low volumes and an increased crumb and crust hardness. However, sensory testing revealed that these breads had an appealing dark crust and a white crumb. Nunes, Moore, Ryan, & Arendt (2009) also investigated the rheological characteristics of GF breads containing low lactose dairy ingredients (whey protein isolate, whey protein concentrate, sodium caseinate, and milk protein isolate). Rheological assessment (frequency sweep and creep recovery) of the batters at 90 % water absorption suggested that addition of whey protein isolates and concentrates significantly decreased the elastic modulus and storage modulus value of the dough, while sodium caseinate and milk protein isolate had the opposite effect with a significant increase in both parameter being measured. However, whey protein demonstrated the ability to increase the specific volume of breads and decrease the hardness over time. Sodium caseinate had a negative impact on the specific volume of the breads which led to an increase in crumb hardness.

Eggs can be added to GF foods to increase nutritional value, improve colour and flavour, and to enhance the product's emulsifying, foaming, coagulation and gelation properties (Table 1), (Arendt et al. 2008). Egg proteins form strong cohesive, viscoelastic films improving gas retention (Zannini et al., 2012). For example, Ziobro, Witczak, Juszczak & Korus (2013) studied the enrichment of GF dough with albumin which leads to a significant increase in the specific volume of loaves; however, the viscoelastic properties of the dough were decreased. The authors also noted that the addition of albumin could adversely affect the acceptability of the smell of resulting breads. However, Moore et al. (2004) found that egg proteins formed viscous solutions in GF bread systems and CLSM of doughs revealed a continuous film-like protein structure, similar to that of wheat gluten. Crockett & Vodovotz (2011) investigated the effect of soy protein isolate and egg white solids on hydroxyl-propyl-methyl-cellulose (HPMC) treated GF dough systems. The purpose of the egg white was to provide additional structure to the dough, whilst soy protein isolate was used to increase disulphide linkages and improve the elasticity of baked goods. These authors found that at concentration of a 5 % soy protein and egg white actually reduced the stability of the dough by decreasing the amount of available water, thus suppressing HPMC functionality by weakening its interactions with the starch matrix. Additionally, other research by Kobylanski, Perez and Pilosof (2004), found that addition of egg whites to maize cassava dough with added HPMC resulted in water binding by egg whites, which in turn reduced the initial gelatinization temperature and improve loaf structure by hindering the gelatinization of starch during baking. However, at a level above 15 %, egg white solids in HPMC-treated cassava dough improve loaf volume and crumb regularity through the formation of an interconnected honeycomb matrix (Crockett & Vodovotz, 2011).

	Gluten-free basis	Gluten-free dough rheology	Gluten free bread quality	
			Volume, Texture and structure	**References**
Soy flour, concentrate or isolate	Corn starch, or rice flour or potato starch	Improve structure , increase the elastic modulus G', provide structural support for starch and hydrocolloids	Good structure and increased loaf volume reduction of firmness and a delay in staling	Ribotta et al., 2004; Sciarini et al., 2010 Moore et al., 2006
Soy flour chickpea isolate, pea and carob germ flour	Corn starch	Enhance the product's emulsifying, foaming, and gelation properties Increasing the elastic modulus	Open and homogenous structure, reduction of firmness, Increase nutritional value, increase volume except for carob germ bread that resulted in poor quality	Minarro, 2013
Soy and Pea protein isolate	Rice flour	Increase the elastic modulus, improve structure and consistency of batters.	reduced hardness and increased springiness	Matos et al., (2014)
Pea protein,	Native maize starch,	Increase the elastic modulus, improve structure and consistency of batters	Soft and aerated crumb	Nunes et al., 2006;
Chestnut flour with a gum blend and DATEM	Rice flour	increase elastic and loss modulus	Soft and aerated crumb	Demirkesen et al., 2010
Pea isolate	corn starch	Increase the elastic modulus, improve structure and consistency of batters	Reduced hardness	Mariotti et al., 2009
xanthan gum, carboxymethylcellulose, pectin, agarose, beta-glucan	Rice flour, or corn starch	Formation of three dimensional polymer networks in aqueous solutions. Increase the solution's viscoelasticity, aids in the binding and retention of water. Increase gas holding properties. Except high rigidity of doughs containing xanthan	Increase flexibility, increase bread loaf volume, crumb porosity and lightness values of crust	Schober (2009); Lazaridou et al. (2007); Erickson, Campanella & Hamaker (2012).
Dairy powders (skim milk powder, sodium caseinate and milk protein isolates)	Wheat starch	Enhance batter handling properties,	Enhance flavour, white crumb and crust colour. Low loaf volume and an increased crumb and crust hardness. Increase nutritional value	Gallagher et al (2003)
Whey protein isolate, whey protein concentrate	White rice flour, or potato starch	decrease the elastic modulus and storage modulus value	Increase the specific volume of breads and decrease the hardness over time. Increase nutritional value	Nunes et al. (2009)
Sodium caseinate and milk protein isolate	White rice flour, or potato starch	increase the elastic modulus and storage modulus value	Sodium caseinate had a negative impact on the specific volume of the breads which led to an increase in crumb hardness.	Nunes et al. (2009)
Eggs and eggs proteins	Corn starch, rice flour	Enhance the product's emulsifying, foaming, coagulation and gelation properties. form strong cohesive, viscoelastic films improving gas retention (Zannini et al., 2012) Albumin decreases the viscoelastic properties of the dough.	Increase nutritional value, improve colour and flavour. Increase in the specific volume and crumb regularity Albumin leads to a significant increase in the specific volume of loaves.and adversely affect the acceptability of the smell of resulting breads	Zannini et al.(2012); Ziobro, Witczak, Juszczak & Korus (2013)
soy protein isolate +egg white solids + HPMC or eggs white + HPMC	Rice flour and cassava starch: Cassava maize flour	Provide additional structure, soy protein isolate increase disulphide linkages and improve the elasticity of baked goods. Depending on concentration, at 5 % soy protein + egg white reduced the stability of the dough by decreasing the amount of available water, thus suppressing HPMC functionality by weakening its interactions with the starch matrix. Or egg whites +HPMC resulted in water binding by egg whites, which in turn reduced the initial gelatinization temperature.	Improve loaf structure by hindering the gelatinization of starch during baking. Improve loaf volume and crumb regularity through the formation of an interconnected honeycomb matrix	Crockett. & Vodovotz (2011)

Table 1: Impact of different protein sources and hydrocolloids on GF dough and bread quality.

2.6.4. Other flours used as functional replacement for gluten

2.6.4.1. Chestnut flour

Chestnut flour contains high‑quality proteins with 4%–7% essential amino acids, 20%–32% sugar, 50%–60% starch, 4%–10% dietary fibre, 2%–4% fat and some vitamins and minerals, such as B‑group vitamins and Vitamin E, phosphorous, magnesium, and potassium. Since the amounts of Vitamin B, iron, folate, and dietary fibre are not sufficient in most GF flour, the use of chestnut flour seems to be advantageous for improving nutritional value (Hosseini, Soltanizadeh, Mirmoghtadaee, Banavand, Mirmoghtadaie, & Shojaee‑Aliabadi. 2018). Unfortunately, the qualities of chestnut bread alone, such as volume and colour, are not suitable because of weak interactions between components of the chestnut dough, inadequate starch gelatinization, and high amounts of sugar and fibre. This flour is more suitable for pastry making (Hosseini et al., 2018). However, blending chestnut flour with other flours such as rice flour and adding some hydrocolloids such as guar gum, xanthan gum, or hydroxypropyl methylcellulose (HPMC) can help to overcome these problems (Hosseini et al., 2018).

Demirkesen, Mert, Sumnu & Sahin (2010) formulated different gluten–free breads with chestnut/rice flour composite with or without a gum blend and the emulsifier Diacetyl Tartaric Ester of Mono and Diglycerides (DATEM). Measurements showed that the formulations with the chestnut/rice flour in ratio of 30/70 had the elastic and loss modulus values increased compared to chestnut/rice flour composite doughs in ratio 10/90, 20/80 or 40/60. Furthermore, gum blend (xanthan–guar) and emulsifier (DATEM) were also added to the chestnut/rice flour composite formulations to obtain the desired physical properties in doughs. Results showed that the breads containing chestnut/rice ratio at 30/70 with the addition of the blends of xanthan–guar and emulsifier had the best quality parameters. Therefore, the formulation consisting of chestnut/rice flour composite in ratio 30/70 can be recommended to be used in GF breads.

2.6.4.2. Pseudocereals

Pseudocereals are dicotyledonae composed mainly of albumins and globulins and contain very little or no storage prolamin proteins; thus, they are good substitutes for cereal in GF foods. The three best-known pseudocereal crops are grain amaranth, quinoa and buckwheat (Zannini, Jones, Renzetti, & Arendt, 2012). These pseudocereals are being sought as alternatives to gluten-containing grains in the GF diet because of their nutritional contribution to the diet of persons with coeliac disease (Hosseini et al., 2018). Amaranth consists of small seeds with a nutritional value better than that of any other vegetable, including cereals, and much higher amounts of fibre and minerals than any other GF grain (Zannini, Jones, Renzetti, & Arendt, 2012). It has a high amount of lysine, arginine, tryptophan, and sulfur‐containing amino acids. Amaranth flour has already been used to enrich cereal‐based foods, including GF pasta. A mixture of popped and raw amaranth flour produces bread loaves with a higher specific volume and more homogeneous crumb than other kinds of GF bread (Hosseini et al., 2018). Quinoa protein is rich in lysine, methionine, and cysteine. Thus, it is a good complement for legumes, which have low methionine and cysteine. In addition, quinoa is a relatively good source of Vitamin E and B‐group vitamins and has high levels of calcium, iron, and phosphorous (Hosseini et al., 2018). Buckwheat seeds contain fagopyritols, a type of soluble carbohydrates. Fagopyritols are a source of D‐chiro‐inositol, a compound that has shown efficiency in patients with noninsulin‐dependent diabetes through improved glycemic control. Buckwheat has a low glycemic index and also shows a beneficial effect on human health, lowering blood pressure and helping cholesterol metabolism. Replacement of corn starch with buckwheat flour in GF bread has been shown to have a positive effect on bread texture and delays staling because of buckwheat flour's lower starch gelatinization enthalpy. Utilization of buckwheat in the production of GF crackers also leads to a product with acceptable sensory qualities. Buckwheat and quinoa breads had a higher volume than other kinds of GF breads (Hosseini et al., 2018). Another study by Turkut, Cakmak, Kumcuoglu & Tavman (2016) developed a GF bread composed of quinoa, buckwheat, rice flour and potato starch. The results showed that all batter formulations independent of the amount of quinoa increased in elastic modulus. However, 25 % quinoa flour bread displayed better results with higher sensory scores and softer texture. The authors also concluded that quinoa and buckwheat flour mixture therefore, were good alternatives for conventional GF bread formulation.

2.6.4.3. Chia flour

The chia (*Salvia hispanica*) seed and flour were one of the main staple foods in Central America. It attracts a great deal of interest due to its nutritional and functional potential in food and pharmaceutical industries (Hosseini et al., 2018). The chia seed is a good source of phenolic compounds, dietary fibre (20%–37%), protein (18%–25%), and oil (21%–33%) with approximately 60%–63% α‐linolenic acid (Hosseini et al., 2018). Sandri, Santos, Fratelli, & Capriles (2017) used chia flour, potato starch, and rice flour in a GF bread formulation by application of mixture design and response surface methodology to achieve the best sensory properties. They found no suitable physical and sensory properties when whole chia flour alone was used. After that, 5%, 10%, and 14% whole chia flour was added to GF bread‐containing rice flour as a main ingredient that led to negligible decrease in crumb moisture, crumb firmness, and loaf volume. Huerta, Alves, Silva & Kubota (2016) observed no significant differences in replacing rice and soy flour with 2.5%, 5.0%, and 7.5% whole chia flour in specific volume, baking loss, and sensory acceptability (scores ranging from 4.5 to 5.5, on a 7‑point hedonic scale) on GF bread in comparison to control. In another study by Moreira, Chenlo & Torres (2013), 2.5%–7.5% whole chia flour was used in chestnut flour‑based GF bread formulation. They found improvements in the dough rheological properties of elasticity, viscosity, and stability up to using 7.5% chia flour. Steffolani, Hera, Pérez & Gómez (2014) found that replacing rice flour with 15% whole chia flour reduced the specific volume, darkened the GF bread colour, and increased the bread hardness but does not have a significant effect on overall acceptability.

2.6.5. Technological approaches in GF bread making

2.6.5.1. Enzyme Technology

The use of enzymes in the baking industry is not a new concept. Enzymes have successfully been applied for many years as natural processing aids and bread improvers in wheat bread manufacturing. However, their use in GF formulations still need to be explored in depth (Hosseini et al., 2018). With the same aim of reinforcing dough structure, enzymes added to GF formulation may promote protein networks to increase dough elasticity and reduce dough deformation (Hosseini et al., 2018; Renzetti & Arendt, 2009). One of the most important mechanisms for engineering food structures with desirable mechanical properties is through the crosslinking and aggregation of the protein molecules. Matosa & Rosell, (2014) reported that enzymes were being added to GF systems as a means of modifying protein functionality through crosslinking, so that a continuous protein network can be created, enhancing the performance of GF flours during bread making. Crosslinking enzymes, particularly transglutaminase (TGase), induces covalent crosslinking between the ɣ-carboxamide group of glutamine residue and the ε-amino group of lysine residues, which is reported to improve firmness, elasticity, water-holding capacity and heat stability in food systems (Hosseini et al., 2018). However, the influence of TGase in GF dough is not always the same. In fact, crosslinking in soy bean enriched rice dough, as a result of TGase has been shown to produce a bread crumb that has an increased hardness but a more continuous structure (Marco & Rossell, 2008b). The improved batter properties do not always translate into increased bread volume (Renzetti et al. 2008, Marco & Rosell, 2008). Beneficial effects of TGase were seen in breads from buckwheat, rice, and corn flour, but not from oat, teff, and sorghum flours (Renzetti et al. 2008). In cases where TGase is ineffective, other enzymes such as glucose oxidase (GO) have been used as a polymerizing agent with varying results, depending on the raw material employed (Sciarini, Ribotta, Leon & Perez, 2012). This enzyme has an oxidizing effect due to the hydrogen peroxide that is released from its catalytic reaction. The GO also promotes protein networks in some GF batters. Zannini, Jones, Renzetti & Arendt (2012) reported the successful used of GO in GF rice breads with increased volume and decreased crumb hardness. GO combined with 2% HPMC improved the bread even more. GO treatment of sorghum and corn also improved the breads, whereas no improvement was observed with buckwheat, teff, and oat breads (Renzetti & Arendt 2009). Enzymes that cause depolymerisation (proteases) have also been used in GF formulations. Protease-treated oat and brown rice batters had lower batter consistency and viscosity, allowing greater expansion and bread volume and reducing crumb hardness and chewiness (Renzetti & Arendt 2009). Both peptidases and glutathione disrupt disulfide linked proteins, present (or formed) in making rice breads. Because highly linked proteins inhibit starch swelling, less agglomerated proteins enhance starch phase continuity and improve bread quality. Another enzyme that affects dough's rheological properties and bread's physical quality is protease. Protease‑treated rice bread had better crumb appearance, high volume, soft texture, and slower staling rate, depending on the amount of enzyme added (Hosseini et al,. 2018). The aggregation of partially degraded storage proteins surrounding the starch granules and protein‑starch interaction may improve gas retention before baking and increase specific loaf volume (Hosseini et al,. 2018). In another study, application of protease of *Aspergillus oryzaeon* the rheological properties of rice dough showed an increase in batter viscosity and a decrease in flour‑settling behaviour because of the aggregation of flour particles after partial cleavage of storage proteins (Hosseini et al,. 2018). The advantages of using enzymes in GF bread formulations is that they are not toxic and require milder processing conditions. However, one of the disadvantages is that they are costly, and many consumers are averse to consuming foods produced with enzymes (Masure, Fierens & Delcour, 2016).

2.6.5.2. Sourdough Technology

The use of sourdough represents an alternative to increasing the quality of both gluten‑containing and GF breads. Sourdough is a mixture of flour and water fermented with LAB and yeasts. Acidification of flour by sourdough fermentation can replace the function of gluten to some extent and enhance the swelling properties of polysaccharides, leading to a better bread structure by enhancing gas retention. It also improves bread volume, crumb structure and texture, flavour and nutritional value in terms of mineral bioavailability, starch digestibility, and concentration of bioactive compounds, and by protecting bread from spoilage and mould (Wang, Lu, Li, Zhao & Han, 2017). Sourdough lactic acid bacteria could break down non-gluten proteins and starch components, thus increasing the dough elasticity and delaying staling. Furthermore, long‑chain sugar polymers called exo‑polysaccharides can be produced by many lactic acid bacteria and act as prebiotics and hydrocolloids to improve the technological as well as nutritional properties of GF breads (Hosseini et al,. 2018). Because, doughs are improved through lactic acid fermentation, proteolysis, exopolysaccharide production, and synthesis of volatile and antimicrobial compounds, it can be said that sourdough technology appears to be a natural and efficient way to improve the quality of GF bread (Wang et al., 2017). Therefore, nowadays researches focused attention on the role of sourdough in GF bread making as an ancient technology to solve a novel issue (Hosseini et al,. 2018). Previously, Moroni, Bello & Arendt (2009), also showed that sourdough fermentation positively influences all aspects of bread quality: texture, aroma, nutritional properties and shelf life. The extent of the effect was really dependent on the microbiota of the sourdough used, and this opens the possibility of obtaining natural products with a reduced use of additives. Indeed, Falade et al. (2014) also used sourdough to prepare GF maize bread. The authors found that the maize sourdough breads had a more open crumb structure with discrete gas and less force was required to compress the maize sourdough breads compared to maize bread without sourdough. Additionally, Edema et al. (2013) found that sourdough fermentation of fonio and sorghum flour improved the dough quality. An increase in viscosity and resistance to the breakdown was observed and that led to a better crumb structure of the GF bread. Sourdough fermentation caused changes in the starch and these changes were apparent as slight granule swelling and probably some leaching of starch molecules. Presumably, the authors suggested that the changes were caused by the action of endogenous amylases from the sourdough microorganisms, bringing about limited starch hydrolysis and probably increasing water absorption.

2.7 Influence of legume flours and the level of inclusion in wheat flour bread

Over the last decades, legumes have come to play a key role in the improvement of techno-functional properties and nutritional enrichment of wheat bread, in terms of increased protein content, protein digestibility, dietary fibre, resistant starch, and a low glycaemic index (Ma, Boye, Simpson, Prasher, Monpetit, & Malcolmson, 2011). Hence, wheat-legume composite flours have been more and more used in bread formulations, despite challenges at the level of dough functional properties and bread sensory characteristics, which sometimes occur when addition exceeds specific amounts (approximately 10% to 30%).

At the technological level, the addition of carob (*Ceratonia siliqua* L.) germ proteins to wheat flour is, for instance, of interest as its caroubin possesses functional properties similar to wheat gluten (Bengoechea et al., 2008). Locust bean gum (LBG) incorporation in wheat bread resulted in a significantly increased elastic character, structure strength, stability during mixing, and decreased starch retrogradation. Lentil (*Lens culinaris* Medik) protein isolates possess good foaming, emulsifying, and fat absorption properties (Minarro, 2013). Wheat bread enriched with lupin proteins (at 5% substitution), especially deriving from full-fat lupin flour or, concentrated lupin flour, and defatted concentrated lupin flour, showed an increase in the stability and the tolerance index of the dough (Paraskevopoulou, Provatidou, Tsotsiou & Kiosseoglou, 2010).

Soybean proteins and soybean flours have also been used to fortify wheat flour blends and to improve the mechanical behaviour of wheat dough by the action of lipoxygenase enzymes and phospholipids; however, this improvement was lost when the soybean flour was heat-denatured (Ribotta et al., 2004). Regarding the inclusion of soy flour in wheat bread, conflicting results have been reported. A 1-3 % addition of soy flour resulted in whiter breads and slowed ageing, while larger amounts (6-8 %) led to breads with less volume and more firmness than those made without soy (Minarro, 2013). A weakening effect of gluten structure was also reported when soy proteins were incorporated in the dough (Ribotta et al., 2004). The lack of interaction between gluten and soy prevents the formation of an elastic matrix, allowing the formation of bread with good qualities (Ribotta et al., 2004; Minarro, 2012). In contrast, Melini et al., (2017) reported that wheat bread formulated with up to 20% full-fat or defatted soybean flour guarantees satisfactory dough performance.

Moreover, the fortification of wheat bread with bean flours (*Phaseolus vulgaris* spp.) also allows an increase in both total protein and fibre contents (Melini et al., 2017). Bean flour blended with cereals produces mixtures with complementary amino acid profiles and improves nutritional quality as the proteins are rich in essential amino acids, isoleucine, lysine, and phenylalanine (Melini et al., 2017).

2.8. Methods to determine the quality of breads

2.8.1. Sensory evaluation of breads

The quality of wheat bread, as well as GF bread, can be assessed on the basis of sensory evaluation by panellists (Correia, Fonseca, Batista, Guiné, 2017). Descriptive sensory analysis can be performed in a laboratory prepared for that purpose. A panel can represent on a scale the intensity of each attribute in the evaluation, where verbal hedonic expressions are translated into numeric values to allow statistical analysis (Correia et al., 2017). The scale of values may vary from 1 point (less intensity) to 10 points (high intensity). The panel evaluates the bread samples according to the following attributes: Appearance: the colour of crumb and crust, roughness, alveolar (uniformity and dimensions), etc. Aroma: fresh bread, fermented, etc. Flavour: bread taste, saltiness, sweetness, fermented, etc. Texture: springiness, firmness, density. All the analysed properties are determined on the same day of evaluation. A quicker sensory evaluation technique is the flash profile method (Lassoued, Delarue, Launay & Michon, 2008). Flash Profile (FP) is a descriptive sensory analytical technique adapted from Free Choice Profiling and Ranking methods for rapid sensory positioning of products (Dairou and Sieffermann, 2002). Trained or semi-trained panels of 6 to 12 assessors can be used for FP (Varela & Ares, 2012). Assessors are allowed to select their own terms to describe and evaluate a set of products simultaneously, which allows better product discrimination (Varela & Ares, 2012). The attributes should be sufficiently discriminant and descriptive to permit ranking the products, yet hedonic terms should be avoided (Valentin, Chollet, Lelievre & Abdi, 2012). Samples are ranked according to each attribute intensity on a scale anchored from low to high, where assessors can apply the same rank value to two or more samples if no difference is perceived (Dehlholm et al., 2012). FP provides a product map in a very short time because the phases of product familiarization, attribute generation and evaluation are integrated into a single step of 2 to 5 hours (Varela & Ares, 2012). It is cost-effective relative to other descriptive analysis methods that require extensive training and a costly set up (Valentin et al., 2012). A repeated blind control can also be included within the sample set to examine individual assessor performance (Ferrage et al., 2010). For example, Muggah, Duizer & Mc Sweeney (2016), used the flash profiling method to provide a description and insight into the sensory characteristics of GF bread types made from tapioca starch, or potato starch composite with rice flour, millet flour and eggs. Attributes that were more frequently used by the assessors to discriminate the bread types included brow crust, light crumb, moist, dense, sweet, bitter, smooth, soft, chewy, dry, grainy and crumbly.

2.8.2. Instrumental assessments

Instrumental measurements are also used to complement the sensory analysis (Correia et al., 2017, Johnson, 2003). The colour of the bread sample can also be evaluated using a colour meter which is a light-sensitive instrument that measures the surface colour of a product. The instrument is standardized against a white tile before each measurement (Johnson, 2003). Colour is expressed in L*, a*, and b*scale parameters where L* is the lightness of the sample, and ranges from 0 (black) to 100 (white), a* ranges from -60 (green) to +60 (red) and b* ranges from -60 (blue) to +60 (yellow) (Johnson, 2003). These colour parameters relate better how the colour is perceived and simplify understanding. Measuring colour using the colour meter gives objective results compared to the visual methods of specifying colour which is subjective (Johnson, 2003). Assessing the bread loaf specific volume textural properties, moisture content as well as crumb microstructure by using image analysis is also important when evaluating the quality of bread as these features affect the consumer acceptability (Correia, Gonzaga, Batista, Beirão-Costa, Guiné, 2015). Bread specific volume is determined by rapeseed displacement method (Campbell, Penfield & Griswold, 1980). Bread specific volume is calculated according to the Approved Methods: 08-01 of the American Association of Cereal Chemists (AACC International, 2000) using the formula;

$$Specific\ volume\ \left(\frac{cm3}{g}\right) = \frac{loaf\ volume}{loaf\ weight}$$

A food texture analyser establishes a standard compression test method for evaluating the textural properties of bread crumb, and can provide a much more accurate measurement, while repeating the same test reliably, time after time (Johnson, 2003). The textural analysis of the bread samples consists of compressing the sample twice to simulate the action of chewing (Johnson, 2003). The test setting parameters are: compression speed, compression distance (corresponding to a deformation of 40% of the height of the sample); recovery time (pause) between the two compressions and acquisition rate (readings are taken per second). The textural properties evaluated on the bread sample included the firmness or hardness (force needed to achieve a given deformation; N) and the springiness (length to which the sample recovers in height during the time that elapses between the end of the first compression cycle and the start of the second compression cycle) (Johnson, 2003). The firming of bread can be significantly influenced by moisture loss and/or redistribution which also cause hardening of the bread (Cauvain & Young, 2008). The moisture content of bread crumb samples can be determined by drying bread samples in an oven at 105 °C for a specific period of time, following the Approved Methods: 44-15 of the American Association of Cereal Chemists (AACC International, 2000) (Demirkesen, Campanella, Sumnu, Sahin & Hamaker, 2010). The moisture content is defined through the following equation: % Moisture = (mw/m sample) X 100, where mw is the mass of the water and m sample is the mass of the sample. There are a number of analytical techniques used to obtain this value. Angioloni & Collar (2009) assessed the quality attributes of commercial wheat pan bread crumb using sensory evaluation, texture profile analysis and colour measurements to better match consumer awareness. Nkhabutlane et al. (2014), assessed the quality attribute of steamed wheat and gluten-free bread made from maize and sorghum flour by determining the bread loaf specific volume, colour parameters of the bread crumb and crust, texture profile of the crumb as well as the bread sensory profile using a panel.

Shelf life can be determined by the change in quality factors of the bread product, whether it is appearance, texture, or odour (Perchonok, 2002). The quality and shelf life of breads are normally limited by moisture loss and staling which is a physico-chemical deterioration that leads to firm and crumbly texture and loss of fresh bake flavour (Dvořáková, Burešová & Kráčmar, 2012). Staling begins immediately as the product is pulled out of the oven and begins to cool. The rate of staling is a function of the product formulation, the baking process, and storage conditions (BeMiller, 2007). Staling of bread is the main cause of the typical short shelf life (3-7 days) of breads (BeMiller, 2007). The major cause of bread staling has been attributed to the gradual transition of amorphous starch (amylopectin) to a partially crystalline, retrograded state (Angioloni & Collar, 2009). Because, firmness is accepted as a measure of freshness and quality of bread, the shelf life of bread can be determined by the measurement of crumb firmness at a specified interval using a food texture analyser (Angioloni & Collar, 2009). Bhattacharya, Erazo-Castrejón, Doehlert, Mc Mullen (2014) reported that reduction in crumb softness over time (firming) is an important manifestation of staling and is often used to test the shelf life of bread, both by consumers and researchers. Dvořáková et al., (2012) used a Texture Analyser TA. XT Plus (Stable Micro Systems, Surrey, UK) to determine the shelf life of breads based on buckwheat and rye mixtures by measuring the textural changes including firmness at interval time 24 and 72 hours after baking. The authors reported that bread crumb of less ratio of rye to buckwheat was dry, crumbly and firmer 72 h after baking due to starch retrogradation and moisture loss. Demirkesen et al. (2010) studied the staling characteristics of GF breads prepared with chestnut and rice flours, and assessed the quality of the GF bread by evaluating the moisture loss and the firmness of the bread crumb.

2.9. Gaps in knowledge

Research has been done on the characterisation of marama bean protein as well as nutritional, physicochemical, functional and sensory properties of the marama bean flour. However, no research has been published on the functional properties of marama bean flour used as an ingredient in GF bread formulations. There is a need to promote the development and the use of marama beans flour, through the production of popular foods such as bread which is an important staple food. The influence of marama flour on the structure, texture, flavour formation as well as shelf life of a GF bread has never been reported. This project will investigate the functional properties of marama flour used in GF dough for making bread. The interaction between components in bread formulations and their influence on the quality of the GF bread will provide more information regarding its potential utilisation in the food industry.

3. HYPOTHESES AND OBJECTIVES

3.1. Hypotheses

1. Defatted marama bean flour composited with cassava starch will produce GF flours that when mixed with water, will develop a dough with sufficient viscoelastic and extensibility properties to retain carbon dioxide during fermentation. These properties might be due to the combined effect of marama protein and soluble fibres in the dough mixture. Amonsou et al. (2012) reported that marama protein can form highly viscous extensible doughs when mixed with water due to its high β sheet content, hydrophobic interactions and tyrosine crosslinks. Proteins from legumes flour e.g. soy and pea are also reported to form a continuous protein network similar to gluten in starch-based GF dough leading to an improvement in dough structure (Moore et al., 2004; Mariotti, Lucisano, Pagani & Perry, 2009). Soluble fibres such as pectins (which are present in marama bean cotyledons) (Mosele et al., 2011) are hydrocolloids that swell in water (Gambus, Sikora & Ziobro, 2007). They can also form structures equivalent to the gluten network in GF dough. Hydrocolloids have been reported to increase the elastic modulus in starch-based GF formulation (Lazaridou et al., 2007). Hydrocolloids also prevent bubble coalescence and keep the starch and yeast from settling (BeMiller, 2007). Mariotti et al. (2009) reported that proteins from legumes (e.g. pea isolate) combined with hydrocolloids in starch-based formulations are able to form a continuous and homogeneous network in the dough, accounting for the increase in their elastic modulus and further improving the ultrastructure of the dough.

2. Compositing defatted marama bean flour with cassava starch will produce GF bread with desirable results in term of crumb structure, reduced firmness, bread volume as well as a delay in staling due to the combined effect of marama flour functional properties such as water absorption and emulsion capacity as well as the presence of soluble dietary fibres. Emulsifiers allow for the interaction between two chemically different phases and are used in baking to increase the stability of thermodynamically unstable systems (Sciarini et al., 2012). Protein from legume flour in GF bread acts as emulsifiers by forming a film or skin around oil droplets, preventing structural changes such as coalescence or creaming (Minarro, 2013; Minarro et al., 2012). This phenomenon is believed to lead to an improvement in bread volume and finer crumb. Additionally, water affinity of protein and soluble dietary fibre (e.g. pectin), in composite DMF-CS composite bread will result in less

available water for cassava starch and therefore, a decrease in retrogradation rate and an improvement in the sensory profile. Gambus et al. (2007) also reported that hydrocolloids improve overall GF bread quality, increased cell average size, lower crumb firmness and delay staling rate over storage. Moreover, Mariotti et al., (2009) stated that the creation of a continuous network by legume protein and a hydrocolloid similar to that of gluten is able to limit starch swelling and gelatinization. Thus, upon baking, this phenomenon could have great importance for shelf-life of the GF bread.

3.2 Objectives

- To determine the effect of adding different proportions of defatted marama flour to cassava starch on dough physicochemical and functional characteristics.
- To determine the effect of adding different proportions of defatted marama flour to cassava starch on the physical and sensory properties of the fresh and stored composite GF bread.

4. RESEARCH

The following research chapters are written in the style of the scientific journal LWT Food Science and Technology. Figures 8 and 9 are flow diagrams of the experimental design.

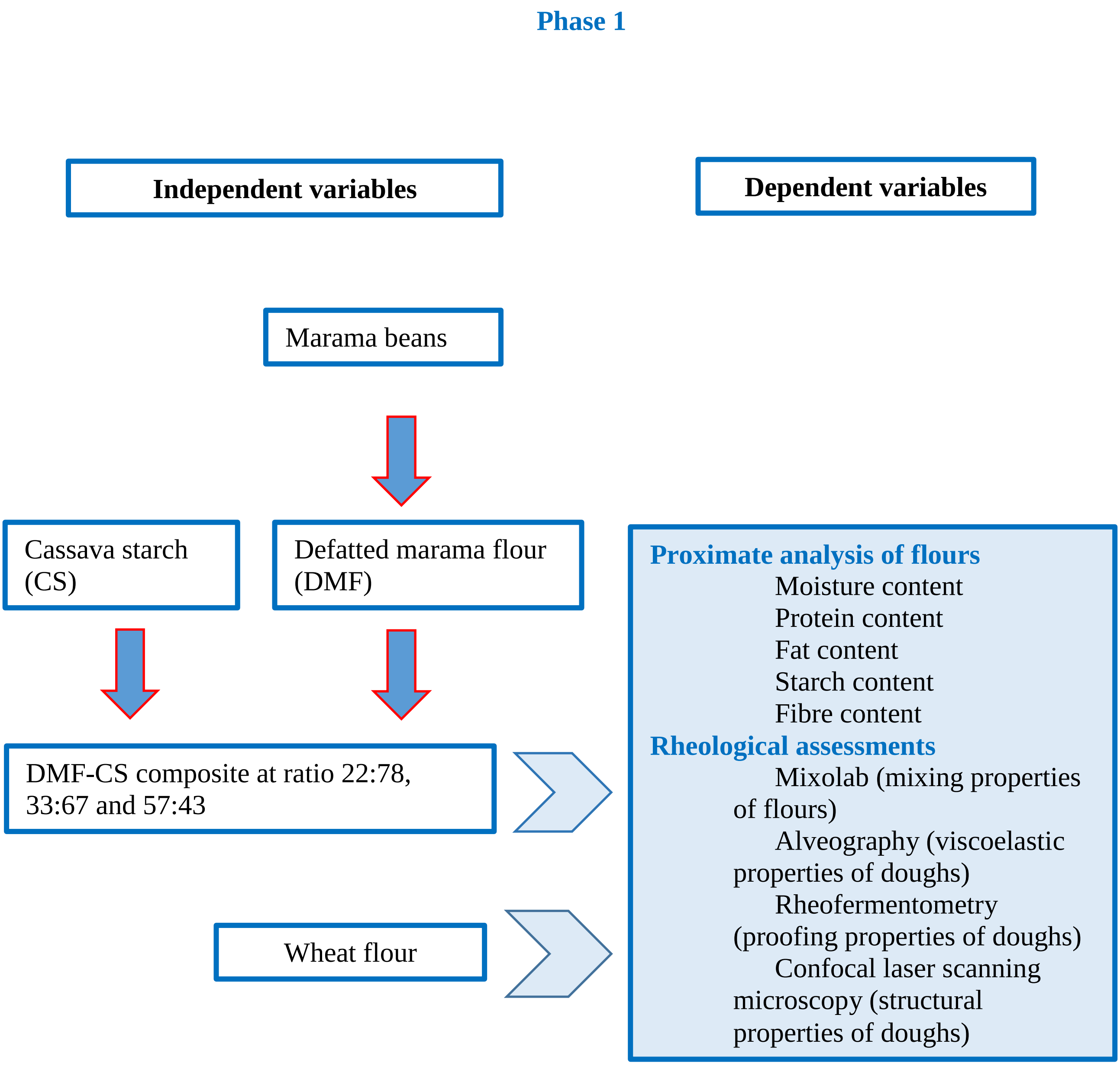


Figure 8: Experimental design to determine the effect of compositing DMF at different ratio to CS on the physicochemical and functional properties of DMF-CS dough.

Phase 2

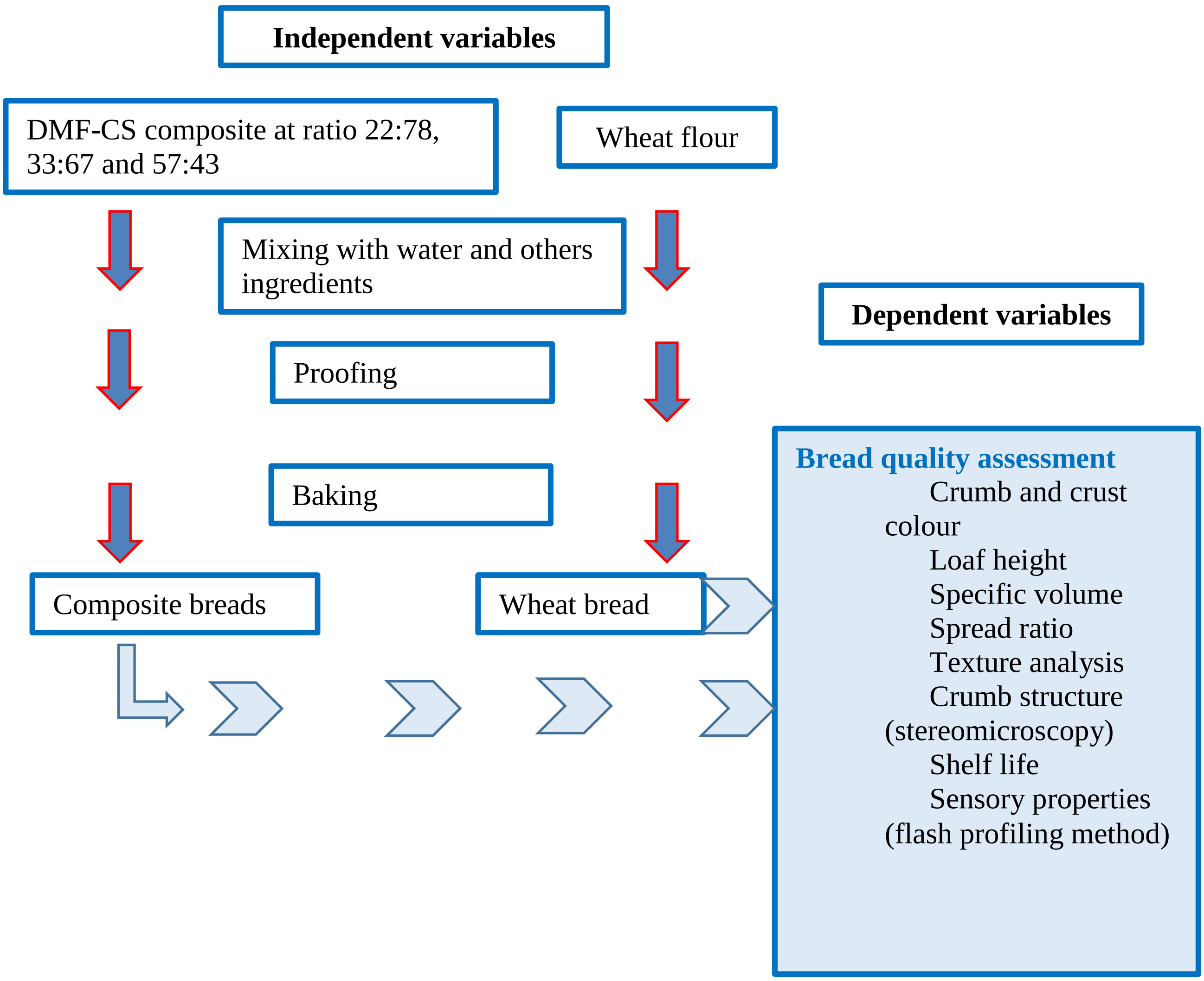


Figure 9: Experimental design to determine the effect of compositing DMF at different ratio to CS on the physical and sensory properties of DMF-CS breads.

4.1 Effect of compositing DMF at different ratio to CS on the physicochemical and functional properties of DMF-CS dough

Abstract

Marama bean is a drought-tolerant oilseed legume. Isolated marama bean protein has high foaming capacity, strong dough extensibility and good elasticity characteristics. The dough properties of composites of defatted marama flour (DMF) and cassava starch (CS) were compared with wheat flour dough with the aim of determining the potential of DMF as a functional nutritious gluten-free ingredient in bread. DMF-CS doughs with similar strength to wheat flour dough were produced. However, the DMF-CS had much shorter Mixolab development time and stability. Alveography revealed that the DMF-CS doughs could inflate into a bubble, with the 33:67 DMF-CS ratio having the most similar bubble size, extensibility and deformation energy to wheat flour dough; with a higher proportion of DMF (57:43) these parameters were lower. Rheofermentometry showed that the DMF-CS composites could also hold gas produced by yeast fermentation. Confocal laser scanning microscopy revealed that as the proportion of DMF to CS was increased, the DMF protein tended to aggregate rather than distribute throughout the dough, probably because the highly hydrophilic marama protein and pectin had a great affinity for each other. Nevertheless, DMF appears to have considerable potential as a functional gluten replacement for making protein- and fibre-rich GF bread.

Keywords: cassava; dough rheology; gluten-free flour; marama bean

4.1.1. Introduction

The highly drought-tolerant leguminous plant marama bean, which is indigenous to south-western Africa, has great potential as a commercial crop (Holse et al., 2010). Marama bean is an excellent source of protein (29-38%) and oil (32-42%), plus dietary fibre (19-27%) but contains little starch; the carbohydrate fraction comprising mainly cellulose and pectin (Mosele, 2012). Also, its defatted flour possesses useful functional properties such as high protein solubility, emulsification, water and oil absorption capacities (Maruatona et al., 2010), like defatted soy flour. Furthermore, isolated marama protein has been found have potential as a functional gluten replacement in gluten-free dough-based products such as bread, as it has high foaming capacity and strong dough extensibility (Amonsou, Taylor, Emmambux, Duodu & Minnaar, 2012b) and good viscoelasticity characteristics (Gulzar et al., 2017). Today, there is increasing interest in gluten-free products which have both similar functional and nutritional attributes as their gluten-containing counterparts (Matos & Rossell, 2015). The defatted marama bean flour (DMF), which is very rich in protein and dietary fibre, would appear to be a useful functional and nutritional ingredient for GF bread making. However, to date the dough properties of DMF, as opposed to isolated marama protein (Amonsou et al., 2012; Gulzar et al., 2017) have not been investigated. Here we describe a study of the bread-type dough properties of composites of DMF and cassava starch with bread wheat flour. Cassava starch was chosen to composite with the DMF because of its better performance in gluten-free composite bread formulations than other starches (Onyango et al., 2011). This is probably related to its relatively high proportion of amylopectin (Defloor, Dehing & Delcour (1998), when compared to starch of other tropical cereals starches.

4.1.2. Materials and methods

4.1.2.1 Materials

Marama beans were collected from Masokaphala area, central district of Botswana (Nyembwe et al., 2015). Wheat flour (Snow Flake, Premier, South Africa, 25.8 % amylose content) and cassava starch (Nature's Choice, Atlantis, South Africa; 18.6 % amylose content), instant yeast and salt were obtained from retail stores.

4.1.2.2 Methods

4.1.2.2.1 Defatted Marama-Cassava Composite Flour preparation

Marama beans were dehulled using a cracker (WMC Metal Sheet Works, Tzaneen, South Africa) and the cotyledons were retained. Defatted marama flour was prepared by coarsely grinding cotyledons (1000 µm mesh) using a Waring blender. Oil was extracted using hexane (flour/hexane 1:5 w/v) for 2 h and the process was repeated three times. The dry residue was then milled again using an ultra-milling centrifugal mill (Retsch ZM 200, Hahn, Germany) to pass through a 500 µm opening mesh. The fat content after defatting was approximately 2g/100g DMF. The DMF was then mixed with cassava starch flour in different proportions to make DMF-CS composite flours in the weight by weight ratios of 0:100, 22:78, 33:67 and 57:43 DMF: CS. These flours and wheat flour as standard were used for subsequent analyses.

4.1.2.2.2 Determination of moisture, protein and fat content.

Moisture and protein content of flours were determined according to: 44-15 A and 46-19 A methods of the American Association of Cereal Chemists (AACC International, 2000) respectively. Crude protein was determined by combustion analysis using a Dumatherm® (Model DT, Gerhardt Analytical Systems, Königswinter, Germany). The conversion factor for DMF-CS composite and wheat flours was (N x 5.71), and for cassava starch (N × 6.25). Crude fat was determined by the Soxhlet extraction method, using petroleum ether (40-60 ^{0}C).

4.1.2.2.3 Determination of starch content

The starch content of the samples was determined using the Megazyme Total Starch assay procedure (Amyloglucosidase/ α-amylase method) (Megazyme Ireland International, Bray, Ireland, 2016). The assay employs thermostable α-amylase to hydrolyze starch into soluble branched and unbranched maltodextrins and amyloglucosidase to quantitatively hydrolyze the maltodextrins to D-glucose. Then oxidised D-Glucose was quantitatively measured colorimetrically.

4.1.2.2.4 Determination of mixing and pasting properties of flours

The Mixolab 2 (Chopin, Tripette et Renaud, Paris, France) was used to measure the rheological behaviour of the doughs. It measures, in real time, the torque (Nm) produced by the dough between the blades. The test is based on preparing a constant hydrated dough mass to obtain a target consistency of 1.1 Nm (+/- 0.05 Nm) during the first test phase.

The conditions used in this work were according to the ICC Standard method 173 (International Association for Cereal Science and Technology, 2011). Two different protocols, namely Mixolab Simulator and Mixolab Standard Chopin+ were used to measure the flour properties in the dough systems. Mixolab Simulator gives the data as a "farinograph-type curve" with parameters such as water absorption, maximum consistency, stability and development time at constant temperature (30 ^{0}C). The conditions used for Mixolab Simulator were 75 g dough weight, constant mixing speed (80 rpm) and a test duration period of 30 min.

The Mixolab Standard Chopin+ protocol is used for more complete characterization of the dough subjected to a dual constraint of kneading and a heating/cooling cycle, while assessing the protein and starch quality in a single test (Koksel, Kahraman, Sanal, Ozay, & Dubat, 2009). The Mixolab

Standard Chopin+ protocol was as follows: A 90 g dough weight was used with initial equilibrium at 30 ^{0}C for 8 min, heating to 90 ^{0}C over 15 min (at a rate of 4 ^{0}C/min), holding at 90 ^{0}C for 7 min, cooling to 50 ^{0}C over 5 min (at a rate of 4 ^{0}C/min) and holding at 50 ^{0}C for 5 min. The mixing speed was kept constant at 80 rpm and the test duration period was 45 min. The results were expressed in a standard curve with fundamental parameters for wheat flour, including: C1, used to calculate water that the flour can absorb to achieve a given consistency during the constant temperature phase; C2 measures protein weakening as a function of mechanical work and temperature; C3 measures starch gelatinization or high paste viscosity; C4 measures the stability of the hot-formed gel and C5 measures starch retrogradation during the cooling period. The quantities of flour (dry basis) and water added were based on the moisture content of the flours. For the Mixolab standard assay, flour (56.7 g and water (38.75 g) were used for the 22:78 and 33:67 DFM-CS composites,53.0 g flour and 40.56 g water for 57:43 DFM-CS composite, 60.6 g flour and 35.47g of water for wheat flour and 61.36 g starch and 36.80 g water for the cassava starch.

4.1.2.2.5 Preparation of doughs

Flour and water (30˚ C) were mixed in quantity as determined by the Mixolab on adapted hydration. A kitchen electric dough mixer, (Kenwood Chef Excel, Maraisburg South Africa) was used to knead the dough for 5 min at speed two (wheat flour and cassava starch). DMF-CS composite flours were also kneaded to a dough using the mixer (speed one) for 1 min and by hand (manually) for 3 min because longer and intense mixing caused the dough to have the consistency of chewed chewing gum. The formed doughs (except for 100 % cassava flour which formed a slurry) were further analysed by alveography, rheofermentometry and confocal laser scanning microscopy.

4.1.2.2.6 Alveography

An Alveograph (Chopin NG Consistograph, Paris, France) was used to analyse the bread making quality of the doughs according to the ICC method 121 (ICC, 1992). First the water absorption values of the flours were determined by the Mixolab Chopin+ protocol as per the ICC Standard method 173, and these absorption values were used in the alveograph test. Dough from wheat flour and composite flours were prepared as described in section 2.2.4. Each dough was sheeted with 12 passes on the Alveograph sheeting plate, then cut into small patties and allowed to rest in the Alveograph chamber (15 min for wheat dough and 3 min for composite doughs). The dough characteristics were automatically recorded by the Alveolink-NG software (Chopin Technologies., Villeneuve La Garenne, France) showing the pressure variation inside the bubbles including the dough resistance to extension (P, mm H_2O) and the dough extensibility (L, mm). The configuration ratio (P/L) and the deformation energy (W, $Jx10^{-4}$), which is the energy required to rupture the test piece, were also recorded.

4.1.2.2.7 Confocal Laser Scanning Microscopy (CLSM)

Confocal laser scanning microscopy (CLSM) was used to analyse the structure of the different doughs. For this purpose, freshly prepared doughs were stretched with five passes using a dough sheeter (Ibili Menaje, Bergara, Spain) to obtain the same thickness of approximately 3 mm. The dough portions (10 mm x 15 mm) were placed on microscope slides and stained using acid fuchsin dye (Falade, Emmambux, Buys, & Taylor, 2014), then dried in an oven at 60 ^{0}C for 1 min. Immediately after drying, the dough pieces were scanned using a Zeiss 510 META system (Jena, Germany) with a Plan-Neofluar 10 × 0.3 objective at an excitation wavelength of 488 nm.

4.1.2.2.8 Dough proofing properties as determined using the Rheofermentometer

The rheological properties of dough during fermentation were determined using a Rheofermentometer F3 (Chopin, Tripette and Renaud, Paris, France) following the Chopin+ method. For this purpose, 250 g flour was mixed with 5 g salt, 3 g yeast and an optimum amount of water based on the Mixolab water absorption data. Dough from wheat flour and composite flours were prepared as described in section 2.2.4. A dough portion (315 g) was placed in the fermentation vessel. The tests were carried out at 28 °C for 3 h, at the same dough consistency and a weight constraint of 2 kg was applied. The rheofermentometer measured and recorded simultaneously as curves the parameters related to dough development [maximum height reached by the dough (Hm), the time at which dough attains the maximum height (T1)] as well as gas production [maximum height of the gas formation (H'm) at time (T'1)], the dough permeability by the time when gas starts to escape from the dough (Tx), and the gas retained (%) in the dough at the end of the assay.

4.1.2.3 Statistical analysis

All experiments were done at least three times. One-way analysis of variance (ANOVA) was performed to determine the effect of flours on moisture, protein and fat content and the effect of dough types on physical and rheological parameters. If the effect was significant ($p < 0.05$) then means were compared using Tukey's Honest Significant Difference test at $p < 0.05$ using IBM SSPS (Version 22.00, New York).

4.1.3. Results and discussion

4.1.3.1 Characteristics and mixing properties of flours analysed using the Mixolab

The protein, fat and starch content of flours are presented in table 2. The protein and fat contents of the flours progressively increased as the proportion of DMF to cassava flour also increased. In terms of starch content, the ratio 22 % of DMF to 77 % CS was the most similar to wheat flour. Since isolated marama bean protein was able to form a viscous extensible dough when mixed with water (Amonsou et al., 2012), an assessment of the mixing and dough properties of DMF-CS composites when compared to wheat flour was assessed using the Mixolab instrument (Figure 1). The rheological behaviour of the doughs was assessed based on Mixolab simulator (Table 2) and Mixolab standard curve data. CS alone did not exhibit any dough-like properties at 30 °C. The mixture resembled a slurry more than a dough. Therefore, for the CS treatment no parameter was recorded in Table 2.

Knowledge of the maximum amount of water absorbed by the flours is crucial for making gluten-free bread, since the water has a plasticizing effect on doughs (Marco & Rosell, 2008a). The Mixolab Simulator water absorption of all three DMF-CS composite flours required to obtain the same dough maximum consistency (C max) as the wheat flour (1.1 ± 0.05 Nm equivalent to 500 Brabender Units) was somewhat higher than the wheat flour (Table 3). The major compounds that enhance water absorption capacity in food systems are proteins and carbohydrates owing to their hydrophilic constituents such as polar or charged side chains (Chinma, Ariahu, & Abu, 2013). CS possesses hydrophilic hydroxyl groups to which water molecules can be bound through hydrogen bonds (Mali, Sakanaka, Yamashita, Grossmann & 2005). Furthermore, CS has been used in gluten-free formulation as a hydrocolloid to increase water absorption due to its hydrophilic nature (Awolu & Oseyemi, 2016). Since this present study used isolated CS, it is likely that the pure starch had more hydroxyl groups available to interact with water than wheat flour, leading to higher water absorption. In addition, DMF has a high water absorption capacity, 1.5 g water/g flour (Maruatona et al., 2010). Marama protein is known to be hydrophilic due to its high content of glutamic acid (8.58 g/100 g DMF) and aspartic acid (5.12 g/100 g DMF) which in proteins constitute charged polar residues (Maruatona et al., 2010). Polar amino acids in proteins have been reported to be primary sites for water interaction (Chinma, Ariahu, & Abu, 2013). Apart from protein, the presence of other components in the DMF including a dietary fibre which comprises cellulose, heteropolysaccharides including arabinoxylans and pectins (Mosele, 2012) also influence water absorption due to the interaction between water and hydroxyl groups of these polysaccharides through hydrogen bonding (Ajila, Leelavathi & Rao, 2008). The dietary fibre content of marama flour has been reported to be 23.6% (Mosele, 2012). Taking the DMF: CS ratios used in the preparation of the composite doughs into consideration, the dietary fibre concentrations in the composite doughs were far higher at the 33:67 and 57:43 ratios than the wheat flour (Table 2). Such high fibre levels should also increase water absorption, due to hydrogen bonding with water by their hydroxyl groups (Ajila, Leelavathi & Rao, 2008).

Table 2: Protein, fat, starch and dietary fibre contents (g/100 g db) of wheat flour, defatted marama (DMF)-cassava starch (CS) composite flours and cassava starch

Flours	Protein	Fat	Starch	Starch amylose content (%)	Dietary fibre
Wheat	12.9^{c}± 0.2[1,2]	2.4^{d}± 0.3	83.5^{d}± 1.1	25.8	3.7[3]
DMF-CS (22: 78)	10.6^{b}± 0.1	0.4^{b}± 0.2	86.1^{d}± 1.2	22.2[5]	2.9[4]
DMF-CS (33: 67)	14.9^{c}± 0.1	0.8^{b}± 0.2	75.3^{c}± 1.3	23.9[5]	9.0[4]
DMF-CS (57: 43)	26.4^{d}± 0.2	1.3^{c}± 0.2	51.9^{b}± 1.4	29.95	27.8[4]
Cassava starch	0.6^{a}± 0.0	0.0^{a} ± 0.0	93.2^{de}± 1.6	18.6	0
DMF	46.3^{e}± 0.9 (50.7)[6]	2.1^{d}± 0.9	9.3^{a}± 1.2	34.8	42.2[4](38.5)[6]

[1]Means of 3 replicates ± standard deviations, [2]Mean values in a column with different superscript letters differ significantly ($p < 0.05$), [3]Product nutritional information data, [4]Calculated by difference from protein, fat and starch contents, [5]Calculated from amylose contents of cassava starch and defatted marama flour starch, [6]Values in parentheses are the means of seven samples of marama bean from Botswana (Holse, Husted & Hansen, 2010)

Furthermore, DMF has a high pectin content, approx. 4.2% (Mosele et al., 2011). Pectin is a strong hydrocolloid (Gambus, Sikora & Ziobro, 2007) due to the many hydroxyl groups in its structure. Lazaridou et al. (2007) found that rice flour with added hydrocolloids (carboxymethylcellulose, pectin and xanthan gum) had elevated water absorption, 63.4-67.0 %. However, as there was no trend in water absorption with relative proportion of DMF and CS in the composite flours despite their great differences in protein, starch and fibre contents, it is probable that as the proportion of DMF increased, the increased water absorption due to the higher protein and dietary fibre contents was cancelled out by the lower starch content.

Table 3: Dough mixing parameters of wheat flour and defatted marama flour (DMF)-cassava starch (CS) composite flours as determined by the Mixolab Simulator test

Dough type	Water absorption (%)	C max. torque (maximum consistency) (Nm)	Development time (min)	Stability (min)
Wheat flour	$57.4^{a} \pm 0.1$	$1.16^{a} \pm 0.02$	$3.30^{b} \pm 0.58$	$7.83^{c} \pm 1.04$
DMF-CS (22:78)	$63.0^{d} \pm 0.1$	$1.15^{a} \pm 0.02$	$1.20^{a} \pm 0.29$	$1.50^{a} \pm 0.00$
DMF-CS (33:67)	$61.7^{bc} \pm 0.1$	$1.14^{a} \pm 0.02$	$1.50^{a} \pm 0.00$	$1.50^{a} \pm 0.00$
DMF-CS (57:43)	$62.6^{cd} \pm 0.2$	$1.15^{a} \pm 0.05$	$3.00^{b} \pm 0.00$	$3.00^{b} \pm 0.50$

Means of 3 replicate experiments ± standard deviations. Mean values in a column with different superscripts letters differ significantly ($p < 0.05$).

Dough development time is known as the time necessary for hydrating all the compounds or the time to reach the maximum level of polymer interaction during the mixing stage (Marco & Rosell, 2008b). The dough development time for composite DMF-CS (57:43) and wheat flour was similar but longer compared to DMF-CS (33:67) and DMF-CS (22:78) (Table 2). A longer dough development time with the highest content of DMF in the composite was due to more protein and possibly more dietary fibre content in the composite flour that required more time for hydration and polymer interaction. In addition, as the dietary fibre in the DMF contained pectin it would hydrate to a greater extent. Pectin would contribute to the consistency of dough by its tendency to gel, therefore resulting in a longer dough developing time (Lazaridou et al., 2007).

Dough stability time is the elapsed time at which the produced torque is kept constant (Marco & Rosell, 2008b). It is also an indication of the flour strength, with longer times indicating stronger doughs (Rosell, Rojas & De Barber, 2001). In this study, the stability time for the DMF-CS composite doughs was significantly shorter compared with the wheat flour dough (Figure 10 and Table 3 and 4). However, the stability time was longer with the 57:43 DMF-CS composite dough than with the composite with lower proportions of DMF (Figures 10, Tables 3 and 4). A likely explanation may be the combined effect of the marama protein and pectin in DMF, with a high proportion of DMF leading to an improvement in dough structure thus increasing the stability. In fact, Mariotti et al. (2009) reported that proteins from legumes such as soybeans combined with hydrocolloids in starch-based formulations form a continuous and homogeneous network in the dough, thus improving the structure of doughs. It is therefore likely that the marama proteins combined with the pectin in the DMF (Mosele et al., 2011) swelled in water and formed a gluten-like dough network. Guarda, Rosell, De Barber & Galotto (2003) showed that hydrocolloids even affected the stability of wheat flour dough. According to the authors, the lowest stability was found in the dough with the lowest hydrocolloid concentration (0.1 %), while dough stability increased when including 0.5 % hydrocolloid (xanthan gum or alginate).

Table 4: Dough mixing parameters of wheat flour and defatted marama flour (DMF)-cassava starch (CS) composite flours as determined by the Mixolab standard test

Dough type	Stability (min)	C1 (Nm)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)
Wheat flour	$9.7^{e} \pm 0.2$	$1.2^{a} \pm 0.0$	$0.5^{b} \pm 0.0$	$2.0^{d} \pm 0.0$	$1.7^{d} \pm 0.0$	$2.4^{d} \pm 0.0$
Cassava starch	$0.8^{a} \pm 0.3$	$1.1^{a} \pm 0.2$	$0.2^{a} \pm 0.1$	$2.4^{e} \pm 0.0$	$1.9^{e} \pm 0.0$	$3.6^{e} \pm 0.0$
DMF-CS (22:78)	$1.5^{b} \pm 0.1$	$1.2^{a} \pm 0.0$	$0.2^{a} \pm 0.4$	$1.3^{c} \pm 0.3$	$0.7^{c} \pm 0.0$	$1.2^{c} \pm 0.0$
DMF-CS (33:67)	$2.2^{c} \pm 0.2$	$1.1^{a} \pm 0.0$	$0.3^{a} \pm 0.0$	$0.9^{b} \pm 0.0$	$0.5^{b} \pm 0.0$	$0.9^{b} \pm 0.1$
DMF-CS (57:43)	$3.9^{d} \pm 0.2$	$1.1^{a} \pm 0.0$	$0.3^{a} \pm 0.1$	$0.8^{a} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.7^{a} \pm 0.0$

Means of 3 replicate experiments ± standard deviations. Mean values in a column with different superscripts letters differ significantly ($p < 0.05$). Meaning of phases for wheat flour: C1 used to calculate flour water absorption to achieve a given consistency during the constant temperature phase; C2 indication of protein weakening as a function of mechanical work and temperature; C3 indication of starch paste viscosity; C4 indication of the stability of the paste; C5 indication of starch retrogradation

The Mixolab allows the mixing and also pasting properties of flours to be determined in a single test. It measures the rheological behaviour of dough during and under different temperature conditions (Koksel, Kahraman, Sanal, Ozay, & Dubat, 2009) to some extent imitating the changes that take place in dough rheological properties during the early stages of bread baking as well as during dough mixing and development. In this study, the initial consistency C1, used to determine the water absorption, was kept constant for all flours, since, as stated, the assessment was done on adapted hydration mode to produce the required torque in bread dough making of approximately 1.10 ± 0.05 Nm (Koksel et al., 2009). Figure 10 showed that CS has negligible resistance to mixing. However, as the proportion of DMF is increased the consistency of the doughs at a progressively higher proportion of DFM becomes more similar to that of the wheat dough, possibly because of the interaction of marama protein and pectin that might have improved the structure of the dough. There was also more reduction in the torque at C2 with all DFM-CS doughs as compared to the wheat dough (Table 4). The reduction in viscosity at this point has been ascribed to protein weakening as a function of mechanical work and temperature (Rosell, Collar, & Haros, et al., 2007). The reduction in torque at C2 values of composites doughs was presumably because of the absence of gluten protein, which is the structure-building component of wheat flour dough (Minarro et al., 2012). In addition, the proteins in DMF flour are mainly globulins and albumins (Amonsou, Taylor, Beukes, & Minnaar, 2012). These might be weaker and less able to resist mechanical deformation and heating as compared to wheat gluten. Edema, Emmambux & Taylor (2013) observed a lower C2 in fonio and sorghum flour doughs presumably because of the absence of gluten as compared to wheat flour. Nevertheless, a slight improvement was noted in the minimum consistency C2 values with higher DMF proportion in the composite dough (Figure 10). Improvement of dough consistency due to the addition of a legume protein source in gluten-free application was already reported. Marco and Rosell (2008) observed an increase in consistency in gluten-free rice flour with the addition of soy protein isolate in the dough. Therefore, it is likely to suggest that increasing DMF flour in the composite dough allowed for more protein and pectin to interact and result in a consistency much closer to the wheat dough as also emphasized earlier.

The second part of the Mixolab curve involves heat treatment and therefore the parameters are related to starch behaviour (Koksel et al., 2009). The values for C3, C4 and C5**,** which relate to paste peak viscosity, stability of hot-formed gel and starch retrogradation respectively decreased with an increase in the proportion of DMF in the DMF-CS composite doughs (Figure 10 and Table 4). Due to its high starch content and hence high swelling at gelatinization, cassava starch had the highest paste viscosity followed by the wheat dough. As the proportion of CS in the DMF-CS composite doughs decreased there was a progressive reduction in paste viscosity and the other post starch gelatinization starch related parameters. In support of this, Xhabiri, Gostivar, Stanojeska & Sinani (2013) reported a decrease in C3, C4 and C5 values being due to less starch in the wheat dough as a result of bran incorporation in. Other research on gluten-free batter made from rice flour or corn starch with soybean flour (Sciarini, Ribotta, Leó, & Pérez, 2010) reported a decrease in pasting property parameters including gelatinization as soybean flour addition increased because of the reduction of starch content in the mixture.

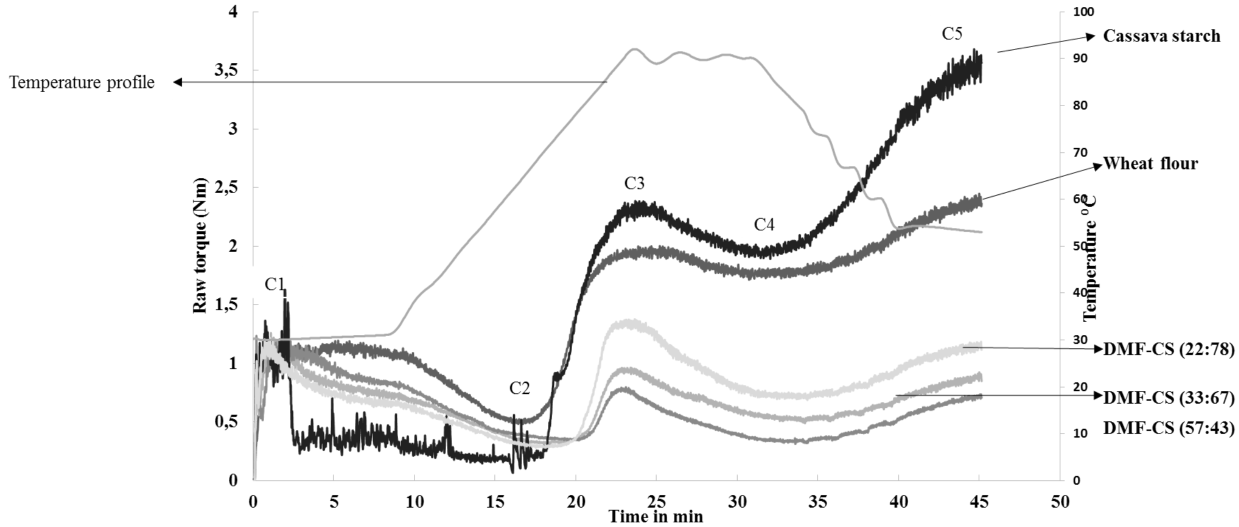


Figure 10: Mixolab profiles of wheat flour and DFM-CS composite flour doughs as determined by the Mixolab standard test.

Meaning of phases for wheat flour: C1 used to calculate flour water absorption a given consistency during the constant temperature phase; C2 indication of protein weakening as a function of mechanical work and temperature; C3 indication of starch paste viscosity; C4 indication of the stability of the paste; C5 indication of starch retrogradation.

4.1.3.2 Alveography of the doughs

The different proportion of DMF to CS had a significant effect on the composite dough alveographic properties as shown in Table 5. As reported earlier, cassava starch alone did not exhibit any dough-like properties. The starch-water mixture formed a slurry that took up water over a period of approximately 15 minutes. For that reason, it was not possible to analyse the cassava starch slurry using the alveograph. On the other hand, the DMF-CF composite doughs exhibited wheat dough-like properties and held gas (Figure 11, Table 5). Dough tenacity (P) or the aptitude to resist deformation is an indication of the ability of the dough to retain gas (Rosell et al., 2001). The P value of the DMF-CS composite doughs was improved as the proportion of DMF increased from 22 % to 33%, making the dough firmer. However, increasing the proportion of DMF to 57%, decreased P significantly ($P < 0.05$), by two and a half times. The dough prepared from DMF-CS (33:67) had the highest tenacity value of all produced doughs. Extensibility (L) represents the potential of the dough to stretch and hold gas and also gives an indication of the handling characteristics of the dough (Rosell et al., 2001). The doughs made from the DMF-CS composite flours were much less extensible compared to the wheat flour dough (Table 4). The 33:67 DMF-CS composite dough had significantly higher extensibility ($p<0.05$) than the 22:78 ratios, but decreased as the ratio increased to 57:43.

Table 5: Alveograph dough rheological properties of the wheat flour and defatted marama flour (DMF)-cassava starch (CS) composite flour doughs

Dough type	Tenacity (P, mm H_2O)	Extensibility (L, mm)	Curve configuration ratio (P/L)	Deformation energy (W, J x 10^{-4})
Wheat flour	$41.7^{a} \pm 2.3$	$133.7^{c} \pm 7.5$	$0.31^{a} \pm 0.10$	$158^{d} \pm 7$
DMF-CS (22:78)	$61.3^{a} \pm 9.5$	$43.0^{a} \pm 9.2$	$1.44^{c} \pm 0.87$	$61^{a} \pm 14$
DMF-CS (33:67)	$103.8^{b} \pm 8.4$	$98.5^{b} \pm 9.4$	$1.05^{bc} \pm 0.56$	$109^{c} \pm 20$
DMF-CS (57:43)	$38.0^{a} \pm 1.0$	$52.7^{a} \pm 5.7$	$0.72^{ab} \pm 0.59$	$90^{b} \pm 15$

Means of 3 replicate experiments ± standard deviations. Mean values in a column with different superscripts letters differ significantly ($p < 0.05$).

As stated, marama protein forms a highly extensible material when hydrated (Amonsou et al., (2012). It was found that the extensibility of marama protein dough increased from two to threefold that of gluten when the moisture content of the dough was increased from 38% to 45%. Of the three DMF levels, the DMF:CS 33:67 composite seemed to absorb the most desirable amount of water for maximum dough extensibility (Table 5, Figure 11). The decreased extensibility at the highest proportion of DMF to CS ratio was probably due to the competition for water between the components such as marama protein, fibre and CS in the mixture. In addition, as the proportion of DFM increased in the composite dough 57:43, it also possible that there was more hydrated marama protein that might have promoted the viscous flow leading to weakening of tensile properties such as extensibility and resistance to extension.

The curve configuration ratio (P/L) provides information on the elastic resistance and extensibility balance of flour (Rosell et al., 2001), and relates to the overall bread-making potential of the flour through a combination of dough strength and extensibility. Composite doughs made from DMF-CS (22:78) and ratio 33:67 had a significantly ($P<0.05$) higher P/L than wheat flour dough. These values (Table 5) were similar to wheat flour dough (P: 1.23) with added xanthan gum (0.1%) (Rosell et al., 2001). The higher P/L can possibly be related to the strong interaction between dietary fibre and marama protein. Guarda, Rosell, Benecticto de Barber & Galotto (2004) reported that hydrocolloids such as xanthan gum increase P/L ratio in wheat doughs due to interaction with the wheat protein. In this study, it may also be that the combined effect of marama protein and pectin that led to improvement in dough structure as explained earlier and resulted in the higher

P/L ratio. Wang, Rosell & Benedicto de Barber (2002) also reported that addition of legume fibre e.g. pea fibre to wheat dough led to an increase of P/L ratio (0.9 vs 0.5). However as DFM proportion rose up to 57:43, the P/L slightly decreased possibly also because of water competition between the flour components in the composite dough.

The deformation energy of DMF-CS 33:67 dough was almost twice that of DMF-CS 22: 78 dough, while further increasing the proportion of DMF to a DMF-CS ratio of 57:43 caused a decrease in the deformation energy (Table 5). Taking into consideration the reduction in deformation energy and tenacity of the DMF-CS 57:43 dough, it is likely that the high level of DMF inclusion might have softened the dough because marama protein and dietary fibre possibly absorbed the much of the water since there was comparatively less cassava starch in the composite. This is probably also the reason why there was lower extensibility in the DMF-CS 57:43 composite dough when blowing bubbles compared to the 33:67 DMF-CS composite dough (Table 5, Figure 11). Holes probably appeared before the softer composite (57:43) dough reached its maximum extensibility.

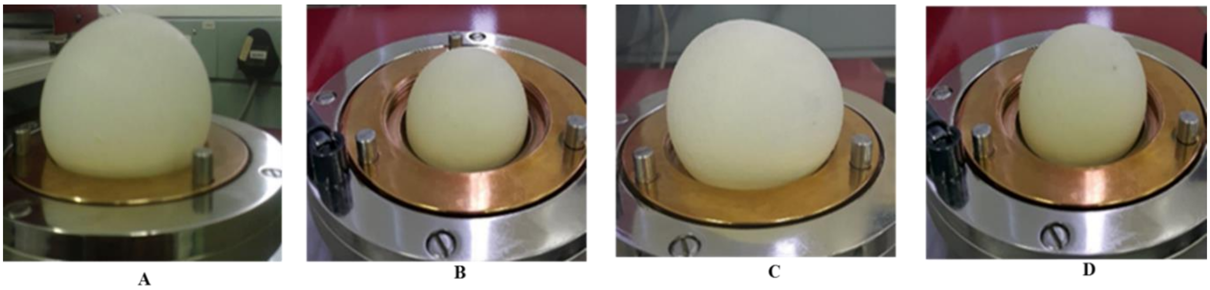
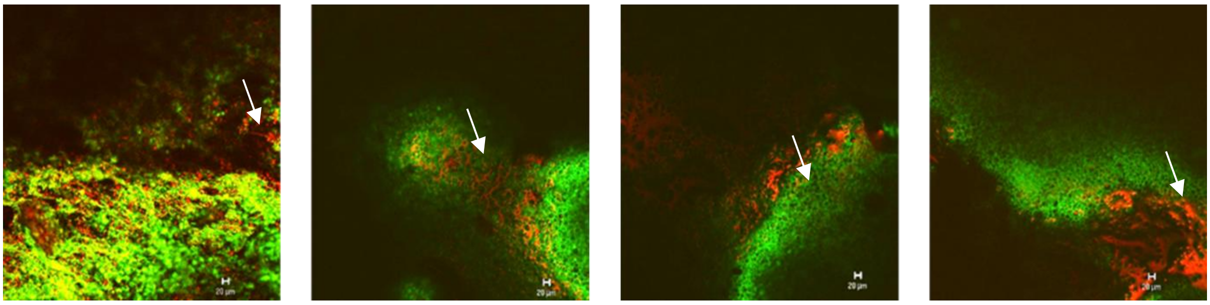


Figure 11: Images of the inflated alveography bubbles of wheat (**A**) and defatted marama-cassava starch (DFM-CS) composite doughs (**B-D**). **B**. DFM-CS (22:78); **C**. DFM-CS (33:77); **D**. DFM-CS (57:43).

4.1.3.3 Confocal Laser Scanning Microscopy (CLSM)

In helping understand the rheological behaviour of the DFM-CS composite doughs these and wheat flour dough were examined using CLSM using acid fuchsin staining to identify the protein (Figure 12). In the wheat flour dough, the protein matrix (red spots) was distributed homogenously throughout the dough. With the DMF-CS composite doughs, as the proportion of DMF increased, the protein matrix in the system became less homogenously distributed and the dough occurred more as aggregates, particularly DMF-CS 57:43 dough as shown in Figure. 12. This non-homogenous distribution of the marama protein can possibly explain the poor extensibility behaviour of the composite dough made with the highest content of DMF. It can be suggested that the reason why the hydrated DMF did not distribute uniformly is possibly because the DM particles had a higher affinity for each other than for the CS, possibly due to the marama protein and fibre's strong affinity for water



A B C D

Figure 12: Confocal laser scanning microscopy (CLSM) images of wheat (**A**) and defatted marama-cassava starch (DFM-CS) doughs (**B-D**) stained with acid fuchsin. **B**. DFM-CS (22:78); **C**. DFM-CS (33:77; **D**. DFM-CS (57:43). Arrows indicate protein matrix.

4.1.3.4 Dough characteristics during proofing

One of the main objectives of wheat dough mixing is to develop a three dimensional viscoelastic structure with sufficient gas-retaining properties to hold the carbon dioxide produced by the yeast during the proofing (fermentation) step of bread making (Gómez, Talegón & De La Hera, 2012). The rheofermentometer instrument provides information regarding the gas production and gas-holding capacity of dough, which is useful for predicting the fermentative properties of dough (Gómez et al., 2012; Wang et al., 2002). Not surprisingly, as shown in table 6, the wheat flour dough had a higher maximum height (Hm) of dough development as compared to the DMF-CS composite doughs, as a result of the distinctive viscoelastic properties of gluten. For DMF-CS composite doughs DMF-CS 22:78 and 33:67 gave similar Hm value, while the DMF-CS 57:43 dough had the lowest maximum height. This agrees with the alveograph assessment (Table 5), where the DMF-CS 57:43 composite dough, i.e. with the highest proportion of DMF showed reduced extensibility, as well as potential to stretch and hold gas. The DFM-CS composite doughs required more time to achieve the maximum height of dough development (Hm) and had significantly ($P<0.05$) lower maximum height of release of carbon dioxide (H'm) compared to the wheat dough. In addition, taking into consideration Tx (the time at which gas starts to escape from the dough) composite DMF-CS doughs took longer for Tx as compared to the wheat dough, which had lower Tx.

Gas production is also affected by the quantity of fermentable sugars present in the dough (Codină et al., 2013). Composite dough should be expected to have lower H'm due to the fact that marama flour contains very tiny quantities of fermentable sugar such as sucrose (123.5 nmol/g) (Mosele et al., 2011) and most of the endogenous amylases would have been probably washed out during the starch isolation process resulting in less fermentable. Flour which shows low amylase activity produces low levels of fermentable sugars and hence yeast fermentation during proofing is limited (Codină et al., 2013). This may also explain the delay in time with regards to Tx and T'1 principally for the DMF:CS 57:43 composite with the highest proportion of DMF.

The gas retention coefficient is related to the ability to stretch the dough in thin membranes, and in turn, it is associated with the quality of the protein network (Wang et al., 2002). The higher gas retention coefficients of the DMF-CS composite doughs compared to the wheat flour doughs shows that DMF-CS doughs can entrap the carbon dioxide produced by yeast during proofing This agrees with alveograph results which revealed that DMF-CS doughs were able to retain gas (Figure 11).

Table 6: Rheofermentometry dough proofing properties of wheat flour and defatted marama flour (DMF) cassava starch (CS) composite flour doughs

Dough type	Hm (mm)	H'm (mm)	T1 (min)	T'1 (min)	Tx (min)	Gas retention coefficient (%)
Wheat flour	$55.9^{c} \pm 3.7$	$61.8^{b} \pm 12.4$	$87.3^{a} \pm 14.2$	$96.3^{a} \pm 18.5$	$55.3^{a} \pm 8.6$	$63.1^{a} \pm 1.2$
DMF-CS (22:78)	$35.6^{b} \pm 3.6$	$33.7^{a} \pm 3.4$	$114.7^{b} \pm 30.9$	$99.3^{a} \pm 33.5$	$85.3^{b} \pm 16.2$	$90.7^{b} \pm 3.1$
DMF-CS (33:67)	$35.1^{b} \pm 5.5$	$40.2^{a} \pm 7.2$	$107.7^{b} \pm 21.8$	$95.3^{a} \pm 27.3$	$78.7^{b} \pm 18.3$	$83.6^{b} \pm 3.5$
DMF-CS (57:43)	$22.6^{a} \pm 5.3$	$32.1^{a} \pm 3.3$	$120.0^{b} \pm 21.6$	$143.7^{b} \pm 32.4$	$87.7^{b} \pm 21.5$	$87.0^{b} \pm 4.6$

Means of 3 replicate experiments ± standard deviations. Mean values in a column with different superscripts letters differ significantly ($p < 0.05$). Hm Maximum dough height; H'm Maximum height of gaseous production; T1 Time at which dough reaches the maximum height; T'1 Time of maximum gas formation; Tx Time at which gas starts to escape from the dough.

4.1.4. Conclusions

Defatted marama-cassava starch composite flour can produce a dough with similar rheological and gas-holding properties to wheat flour dough. This is due to marama protein since marama protein has been found to form a highly extensible dough. However, the inclusion of the marama fibre and cassava starch modify the rheological properties to make them more similar to those of wheat flour dough. The potential of defatted marama flour as either a gluten or wheat flour replacement should be considered in the making of GF bread. More research is needed to optimise requirements for handling such a dough, and to determine the baking performance, sensory quality and shelf life of the composite bread.

4.2. Effect of compositing DMF at different ratio to CS on the physical and sensory characteristics of defatted marama flour-cassava starch composite breads

Abstract

Interest in gluten-free (GF) bakery products due to wheat allergies and the incidence of coeliac disease have led to research that continually explores new ingredients and formulations with the aim to manufacture GF bread as similar as possible to wheat bread. However, many GF breads available on the market lack the sensory properties of their gluten-containing counterparts. GF breads are often inferior in quality with a dense, unleavened appearance, a pale crust colour, dry crumbling crumb and poor mouthfeel as the flours used lack the structure-building characteristics provided by gluten. Marama bean and cassava flours contain no gluten. The investigation aimed to determine if the functional properties of marama flour [particularly defatted marama flour (DMF)], with unique rheological properties of marama bean protein, when composited with cassava starch (CS), have a potential for bread making.

In this study, GF breads from DMF and CS in different proportions were included with wheat breads as standards. Freshly produced breads were stored at 5 °C and the crumb texture was assessed on day number one and three by conducting a texture profile analysis according to the approved method (AACC International, 2009) using the texture analyser EZ – L Shimadzu. The measured parameters were expressed as crumb firmness (N) and springiness (%). The crumb structure of GF composite breads was visualized using stereomicroscopy and a sensory panel evaluated the sensory properties using the Flash Profiling method.

DMF-CS breads with brown crust and a uniform aerated crumb structure were produced. Higher inclusion of DMF in the formulation led to higher specific bread volume. The DMF-CS bread crumbs were less soft than wheat bread with a fermented, nutty, bean-like flavour as well as chewy texture. Higher inclusion of DMF in the formulation led to more bitter taste because DMF contains bitter compounds such as saponins, gallic and protocatechuic acids.

DMF-CS breads presented different sensory profiles compared to wheat bread which was blander. More research is needed to optimise the quality of the DMF-CS breads. The possibilities will include altering recipes and or baking methods along with the use of effective technologies to mask the bitter taste.

Keywords: gluten-free bread, marama bean, cassava, flash profile.

4.2.1. Introduction

New ingredients and formulations for gluten-free (GF) bread are under constant scrutiny with the aim to manufacture GF breads as similar as possible to wheat bread (Melini et al., 2017, Minarro, 2012). Many GF breads available on the market exhibit a dry crumbling crumb, poor mouthfeel, pale crust colour and stale rapidly as the flours used lack the structure-building characteristics provided by gluten (Melini et al., 2017). Researchers have used non-wheat flours or isolated starches composited with polymeric substances such as dairy powders (Gallagher et al 2003; Schober et al 2004), pseudo cereal flours and legume protein flours (Mariotti et al., 2009; Minarro et al., 2012) alone, or in combination with hydrocolloids, to mimic to a limited extent the viscoelastic properties of gluten and to ensure the improvement of quality characteristics of GF bread to be more comparable to those of wheat bread (Gallagher et al., 2003).

Here cassava starch was composited with defatted flour from cotyledons of marama beans (*Tylosema esculentum* L. Birch) in different proportions to prepare GF breads. Marama beans grow wild in desert-like conditions in some parts of southern Africa (Holse et al., 2010) and the cotyledon flour and other constituents may have food application potential (Holse et al., 2010). The rheological behaviour of defatted marama flour (DMF) and cassava starch (CS) composite doughs compared with wheat flour dough was previously reported (Nyembwe, de Kock and Taylor, 2018). A DMF-CS dough with similar strength to wheat flour dough was produced. However, all the DMF-CS doughs had much shorter development time and lower stability. In addition, DMF-CS doughs demonstrated good potential to stretch to a thin membrane and entrap the carbon dioxide produced by yeast during proofing, with the 33 % DMF: 67 % CS dough behaving the most similar to wheat flour dough. Based on those findings it was hypothesised that bread prepared from a DMF-CS composite dough will result in GF bread with a crumb structure and textural characteristics comparable to wheat bread. Wheat bread has a soft, open and sponge-like structure due to gluten which is responsible for the viscoelastic, extensible and gas retaining dough properties (Andersson, Ohgren, Johansson, Kniola & Stading, 2011). Marama protein contributes other beneficial properties such as emulsifying and foaming capacities as well as water and oil absorption abilities (Maruatona, 2010; Gulzar et al., 2017). The functional properties of legume protein flours or protein isolates have been utilised for the development of GF breads that exhibited good baking performance with a soft crumb and high specific volume (Minarro, 2012). The addition of DMF increases the protein and dietary fibre content of the bread and will possibly lead to bread with good baking characteristics. Legume proteins and dietary fibre have been reported to contribute to crumb softness in GF bread due to their hydration properties (Gallagher, Gormley & Arendt, 2003). The use of DMF in bread making is a novel idea. Hence, the objective was to determine the effect of DMF inclusion level (22 %, 33 % or 57 % of flour weight) composited with CS on the baking characteristics and sensory properties of the breads.

4.2.2. Materials and methods

4.2.2.1 Materials

The ingredients used for the bread formulations were: wheat flour (12.9 % protein; Snowflake, Premier, South Africa), cassava starch (0.46 % protein; Nature's Choice, Atlantis, Thailand), instant dry yeast, iodized refined salt, white sugar and margarine (Rama Original, Unilever, South Africa) obtained from retail stores. Marama beans were collected from the central district of Botswana (Nyembwe, Minnaar, Duodu, & de Kock, 2015). The cotyledons were coarsely ground (1000 µm) and defatted with hexane following the method described by Nyembwe et al., (2018) to obtain a fat content of < 2 % in marama flour. After evaporation of hexane, the dry residue was milled into flour using an ultra-milling centrifugal mill (Retsch ZM 200, Hahn, Germany) to pass through a 500 µm mesh. DMF was composited with CS in three different ratios DMF-CS 22:78, 33:67 and 57:43 to achieve flours with a protein content of 10.6 %, 14.9 % and 26.4 % respectively. Diacetyl tartaric acid ester of mono and diglycerides (DATEM) (Ruto Mills, Pretoria, South Africa) was used as bread improver.

4.2.2.2 Methods

4.2.2.2.1 Baking procedure

To bake bread, flour (100 g as is basis) was measured into a mixing bowl with 3 g instant dried yeast, 6 g sugar, 2 g margarine and 0.5 g DATEM. Ingredients were mixed together using a mixer (Chef Excel Mixer-KM 210 model, Kenwood, Johannesburg, South Africa) with dough hook at speed 1 for 30 s. Tap water was then added, 65 g for wheat flour, 76 g for the 22:78 and 33:67 DMF-CS composites and 82 g for 57:43 DMF-CS composite. The quantities of water were previously determined based on the water absorption capacity of the flours by Mixolab standard Chopin+ calculation according to ICC Standard method (International Association for Cereal Science, 2008), in order to achieve a consistency of 1.1 ± 0.05 Nm required for bread making (Nyembwe et al., 2018). The wheat flour dough was mixed for 8 min at speed 2 and shaped manually to a cylindrical dough shape of approximately 130 mm length x 20 mm height x 40 mm width, while composite flour doughs were mixed for 2 min at speed 2, followed by hand mixing for approximately 2 min, until formation of a visible cohesive homogenous dough and then shaped to the same size as the wheat dough. Longer mechanical mixing caused the DMF-CS doughs to have a chewing gum-liked consistency (Nyembwe et al., 2018). The doughs were placed into greased baking tins (190 mm length x 95 mm width x 45 mm depth) and allowed to ferment in a proofing cabinet (Lab Com, Johannesburg, South Africa) for 45 min at 34 °C until the dough doubled in height. The loaves were baked in a convection oven (Unox®, XV303G, Vidodarzere, Padova, Italy) at 150 °C for 40 min until the formation of a brown crust. After baking, the loaves were carefully removed from the baking tins, allowed to cool for two hours and wrapped in plastic film. Each bread type was baked in triplicate.

4.2.2.2.2 Bread physical assessments

Loaf height and width of freshly baked bread was measured using a ruler as seen in Figure 13. A slice (25 mm) of each bread loaf was cut at the centre and the loaf height (H) was measured from the bottom to the highest point of the top crust. Width (W) was taken perpendicularly to the height, by placing the ruler on the two widest points of the bread slice. The ratio (W/H) was calculated as an indicator of bread spreading and the percentage (%) height increase from dough to bread (after baking) was also determined.

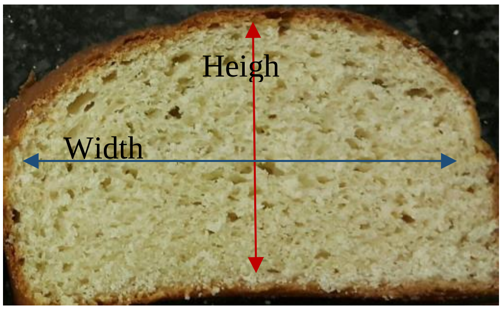


Figure 13: Illustration of the procedure for measurement of bread loaf height and width

Loaves were photographed using a digital camera (Nikon Coolpix L23, Nikon Corp, Kyoto, Japan) and the images of bread slices captured by using a flatbed scanner (HP Scanjet G4010, ComXComputers, Johannesburg, South Africa). Loaf weight was also recorded and the volume (cm^3) was measured using the rapeseed displacement method (Campbell, Penfield & Griswold, 1980). Bread loaves were placed in a graduated glass cylinder (2 L). The rapeseeds were then run into the same container until it was full. The volume of seeds displaced by the loaf was measured and recorded as the loaf volume. The specific volume of bread was then calculated according to the Approved Methods: 08-01 of the American Association of Cereal Chemists (AACC International, 2000) using the formula;

$$Specific\ volume\ \left(\frac{cm^3}{g}\right) = \frac{loaf\ volume}{loaf\ weight}$$

The moisture content of the bread loaf was measured according to Method 44-15A (AACC International, 2000). Moisture was determined as the loss in weight of the bread sample after drying for 3 hours at 103 ^{0}C. The colour of the crumb and crust of fresh bread as well as colour of the respective flours were measured using a Chroma Meter CR-400 (Konica Minolta Sensing, Osaka, Japan). The instrument was calibrated against black and white tiles and the resultant values were scaled on a measure of 0–100, where 100 is the white calibration standard and 0 is the black standard. Colour readings were expressed by Hunter values including light reflectance, expressed in terms of lightness (L*), red/green characteristics (a*) and blue/yellow characteristic (b*).

Bread quality is dependent on the texture characteristics of the bread crumb (Korczyk-Szabo & Lacko-Bartosova, 2013). Texture analysis is primarily concerned with the evaluation of mechanical characteristics where the bread crumb is subjected to a controlled force from which a deformation curve is generated. In this study, the crumb texture of bread samples kept at 5 °C was measured 24 h after baking (day 1) and again 72 h after baking (days 3) by texture analysis using an EZ – L texture analyser (Shimadzu Kyoto, Japan) equipped with a 25 mm diameter Perspex probe. The assessment was done according to method 74-09.01, of the American Association of Cereal Chemists (AACC International, 2009). The measured parameters were expressed as crumb firmness (N) and springiness (%) which is the ratio of the height after compression and recovery, and initial height. A slice (25 mm thick) from the centre of a loaf was positioned between the load cell (50 N) and the plate of the texture analyser. The probe compressed the crumb to a 40 % compression limit (10 mm compression depth) at 10 mm/min speed. The analysis was performed at 3 positions (left, centre and right) of bread slices.

4.2.2.2.3 Stereomicroscopy

Crumb structure of the bread slice was recorded using a stereomicroscope (Zeiss Discovery V20, Jena, Germany) with a field of view of 20.0 mm and 0.6 mm depth of field.

4.2.2.2.4 Sensory evaluation

Descriptive sensory evaluation of the breads was done using the Flash Profiling (FP) method according to Valentin et al. (2012). Flash Profiling is a descriptive sensory analytical technique adapted from Free Choice Profiling and Ranking methods for rapid sensory positioning of products (Valentin et al., 2012). Assessors evaluated the breads in individual standardized booths (ISO 8589:2007) in a sensory laboratory. As a rapid descriptive sensory evaluation method, FP involves ranking products for each attribute on an intensity line scale, with assessors using their own free-choice attributes (Perrin & Pages, 2009; Valentin, et al., 2012). A panel of 10 assessors (3 males and 7 females, aged between 19 and 40 years) with experience of descriptive sensory analysis participated in the evaluation. For comparison, a commercial white wheat bread (Albany, Johannesburg, South Africa) from a retail store with size of approximately 140 mm length x 50 mm height x 55 mm width was also included. The evaluation was done in three sessions. At each session, a set of five different bread slices (15 mm thick) consisting of the wheat bread, the composite breads DMF-CS in ratio 22:77, 33:67, 57:43 and the commercial wheat bread were presented in zip lock plastic bags (100 mm x 110 mm, 40 µm thick). Each bread slice sample was labelled with a randomly selected three-digit code. Bread slices were served simultaneously to assessors. The order of presentation on individual trays followed a Williams' design (5 treatments, Type: Quantitative Descriptive) generated by Compusense® five software (Compusense® Inc, Guelph, Canada). The process of sensory evaluation of breads in this study was done for a period of six hours with two hours per day for three days.

In the first session or the introductory phase, bread samples were presented to each assessor for familiarisation and generation of free-choice terms. Each assessor was asked to smell, observe, feel the texture and taste each slice of bread, then to write down words that describe how the samples differed in terms of aroma, appearance, flavour, texture and the after swallow perceived sensations. During this session, each assessor developed his/her own list of descriptive attribute terms without using hedonic terms. In the second session, each assessor received his/her own attribute list and a global list made from the lists of all other assessors. The goal of the global list was not to obtain consensus, but to allow the assessors to update their own lists if desired, by either adding to their list a few terms thought relevant or by replacing some of their own terms by more adapted terms. In the third session, which was the bread evaluation phase, assessors were asked to evaluate and rank the breads, for each attribute, on an intensity line scale (100 mm) anchored on each side with the words 'low' and 'high', where ties were allowed if no difference was perceived. The actual evaluation phase of all bread types was replicated three times over the two-hour session and assessors were allowed 10 min breaks in between the evaluation of sets of bread samples to avoid sensory fatigue. Filtered tap water was used as a palate cleanser in between samples. The data from the evaluation phase was collected and organised into a suitable matrix for multivariate analysis.

4.2.2.3 Statistical analysis

All experiments were done at least three times. One-way analysis of variance (ANOVA) was performed to determine the effect of bread types on physical quality parameters. Means were compared using Tukey's Honestly Significant Difference test at $p < 0.05$ using IBM SSPS (Version

22.00, New York, USA). Two-way ANOVA with interaction was used to confirm the impact of bread types, and storage time on texture and moisture content parameters. For sensory analysis, collective treatment of the FP data was done using two-way ANOVA with interaction, for the attributes with the frequency of use, $f^* \geq 3$. This analysis included the assessor effect, product effect and the interaction between the two. For each attribute from each assessor one-way analysis of variance (one-way ANOVA) was applied on rank values to determine the significance of attributes. This ANOVA model shows which attributes were important in contributing to sensory differentiation of the bread types under evaluation (de Jesus Ramirez-Rivera et al., 2012). Differences were considered significant at $p < 0.05$. Generalized Procrustes Analysis (GPA) was applied to ten data matrices from ten assessors to obtain consensual configurations among descriptions of bread types by assessors. GPA is a multivariate statistical technique for integrating different groups of variables describing the same observations, and subsequently finding significant variables which explain the greatest variability in the data. GPA carries out a Principal Components Analysis (PCA) for the different matrices and project the results onto a multi-dimensional space to visualize the consensus and also to allow for comparing the proximity between the terms that were used by different assessors to describe products. GPA was performed using XLSTAT® (Addinsoft™, New York, US).

4.2.3. Results

4.2.3.1 Effect of adding different proportions of DMF to CS on the colour of the composite flour, as well as crust and crumb of breads

The colour parameters of the flours as well as the crust and crumb of bread types are summarized in Table 7. Results showed that CS had the highest value for lightness (L^*) and lowest values for redness (a^*) and yellowness (b^*) while DMF was the darkest with lowest L^*, highest a^* and b^* (similar to wheat flour). Also, increasing the proportion of DMF to CS decreased the lightness of the composite DMF-CS flour, while increasing the redness (a^*) and the yellowness (b^*) of the flours. Composite bread DMF-CS (57:43) had a significantly darker crust (Table 7, Figure 14) as indicated by a lower L^* value (Table 7) compared to composite breads DMF-CS in ratio 22:78 and 33:67 which were equivalent to wheat bread. All the bread crusts showed similar redness (a^*). DMF-CS (57:43) bread was the least yellow (b^*). It can also be seen that a higher quantity of DMF in the composite resulted in less cracks on the bread crust (Figure 14). With regards to the crumb colour, composite DMF-CS breads in proportions 33:67, 22:78 were lighter (L^*) compared to wheat bread and composite bread 57:43 DMF-CS (Table 7). The crumb of wheat bread was significantly more red (higher a*) than the composite breads, while the crumb of DMF-CS (57:43) was most yellow (higher b*) (Figure 15).

Table 7: Effect of adding different proportions of defatted marama flour (DMF) and cassava starch (CS) on the colour of flours, bread crumbs and crusts

	L*	**a***	**b***
Flours			
Wheat	$76.70^{b} \pm 0.10$	$0.11^{b} \pm 0.01$	$6.55^{d} \pm 0.01$
CS	$98.51^{e} \pm 0.60$	$0.03^{a} \pm 0.02$	$2.31^{a} \pm 0.01$
DMF-CS (22:78)	$92.53^{d} \pm 0.14$	$0.18^{b} \pm 0.06$	$4.66^{b} \pm 0.03$
DMF-CS (33:67)	$92.20^{d} \pm 0.10$	$0.26^{c} \pm 0.04$	$5.47^{c} \pm 0.01$
DMF-CS (57:43)	$87.40^{c} \pm 1.60$	$0.41^{d} \pm 0.01$	$5.52^{c} \pm 0.02$
DMF	$65.71^{a} \pm 0.42$	$0.58^{e} \pm 0.02$	$6.43^{d} \pm 0.01$
Bread crusts			
Wheat	45.43^{b}(0.56)	12.53^{a} (0.84)	27.64^{b} (0.59)
DMF-CS (22:78)	48.77^{b} (3.02)	14.36^{a} (2.16)	27.02^{b} (2.070)
DMF-CS (33:67)	45.20^{b} (5.37)	10.39^{a} (4.83)	30.66^{b} (19.33)
DMF-CS (57:43)	35.41^{a}(4.63)	13.89^{a} (1.27)	14.54^{a} (2.03)
Bread crumbs			
Wheat	64.55^{a} (0.60)	12.53^{b} (0.84)	27.64^{b} (0.59)
DMF-CS (22: 78)	71.71^{b} (1,15)	2.84^{a} (0.21)	15.09^{a} (0.57)
DMF-CS (33:67)	70.56^{b} (2.50)	2. 45^{a} (0.18)	14.61^{a} (1.05)
DMF-CS (57: 43)	66.84^{a} (1.57)	3.49^{a} (0.02)	38.77^{c} (0.62)

Values represent the means of 3 replicates experiments. ± Standard deviation. ^{abcde}= Mean values with different superscript letters within a column differ significantly ($p < 0.05$). DMF-CS: Defatted marama flour-cassava starch.

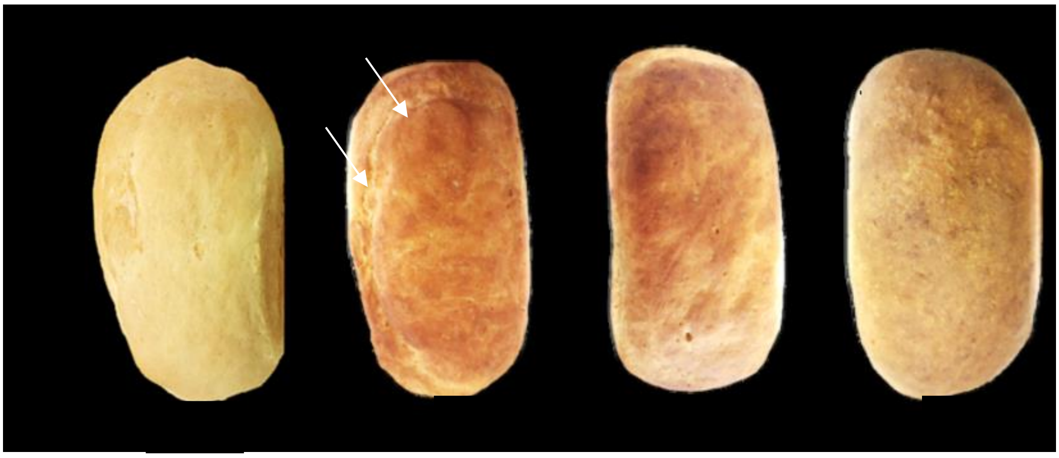
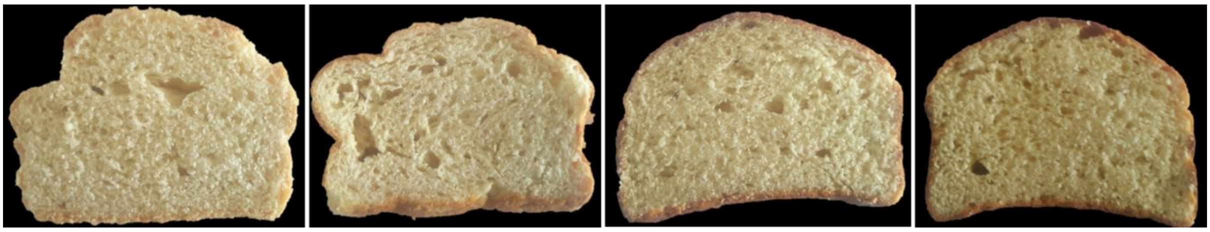


Figure 14: Effect of adding a different proportion of DMF to CS on the colour and appearance of the top crust of bread loaves. Arrows represent cracks on the bread loaf. **A**: wheat bread, **B-D**: DMF-CS composite breads. **B**. DMF-CS (22:78), **C**. DMF-CS (33:77), **D**. DMF-CS (57:43).



A B C D

Figure 15: Effect of adding a different proportion of DMF to CS on the crumb colour of bread loaves. **A**: wheat bread, DMF-CS composite breads. **B**. DMF-CS (22:78), **C**. DMF-CS (33:77), **D**. DMF-CS (57:43).

4.2.3.2 Effect of adding different proportions of DMF to CS on bread height after baking

Table 8 compares the height of doughs before proofing to the height of bread after baking. The results show that all the doughs had similar height before proofing. However, after baking, DMF-CS composite breads in proportions 33:67 and 57:43 were similar but higher than bread 22:78. The former breads rose up to two-fold their initial dough height. The baked wheat bread had the highest height.

Table 8: Effect of adding different proportions of DMF to CS on dough height before proofing and bread loaf height after baking.

Bread Samples	Dough Height (mm) before proofing	Bread Height (mm)	Percentage (%) height increase from dough to bread
DMF-CS (22:78)	$19.5^{a} \pm 0.1$	$35.3^{a} \pm 0.1$	77
DMF-CS (33:67)	$19.8^{a} \pm 0.1$	$44.3^{b} \pm 0.1$	124
DMF-CS (57:43)	$19.7^{a} \pm 0.1$	$44.5^{b} \pm 0.1$	126
Wheat	$20.0^{a} \pm 0.0$	$54.7^{c} \pm 0.2$	174

Value represent the means; n = 3. ^{abc}= Mean values with different superscripts letters within a column differ significantly ($p < 0.05$).
DMF-CS: Defatted marama flour-cassava starch.

4.2.3.3 Effect of adding different proportions of DMF to CS on the Specific volume and width/height ratio of bread loaves

Loaf specific volume is considered as one of the most important criteria in evaluating bread quality since it provides a quantitative measurement of baking performance (Minarro, 2012). In this study, the specific volume of composite breads increased with higher inclusion of DMF in the formulation. As shown in Figure 16, the mean specific volume of composite breads varied from 2.31 cm^3/g for DMF-CS (22:78) to 2.71 cm^3/g for DMF-CS (33:67) and 2.78 cm^3/g for DMF-CS (57:43). Wheat bread had the highest specific volume (2.97 cm^3/g). Width/height (W/H) ratio is an indication of dough spreading during baking. Higher W/H of bread indicates more spreading as a result of the possible viscous flow of the fermenting dough (Dogan, Yildiz & Tasan, 2012). Figure 16 indicates that higher addition of DMF in the composite led to a reduction of dough spreading. Composite breads DMF-CS in ratio 22:78 had a significantly higher dough spread compared to composite bread (57: 43).

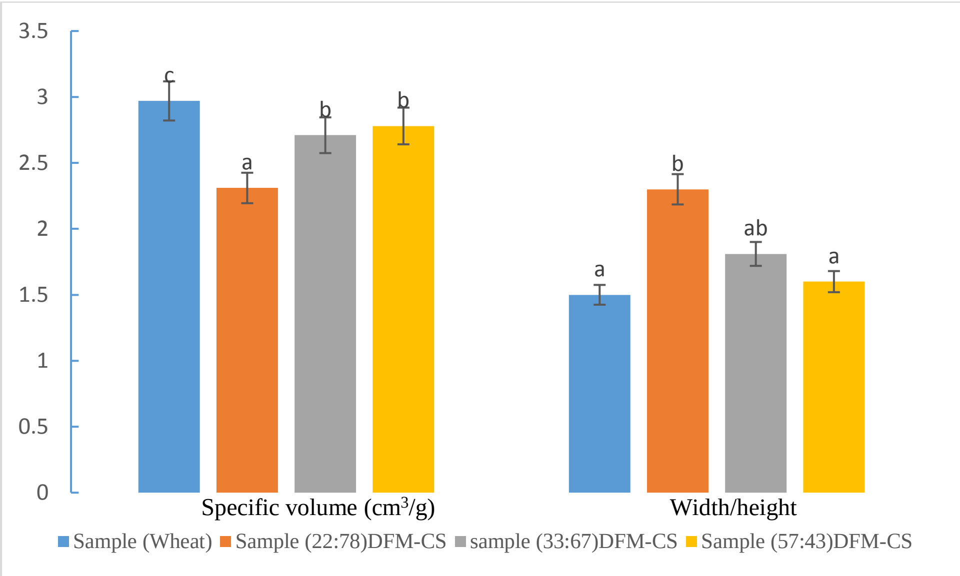
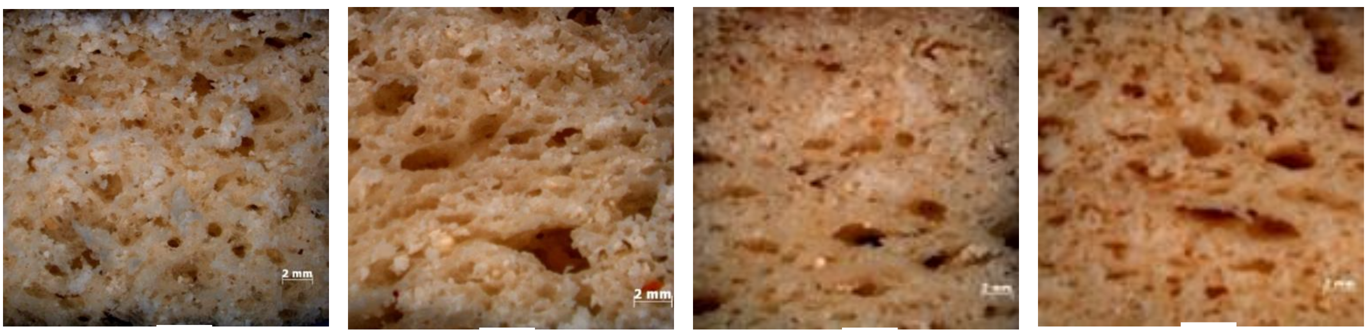


Figure 16: Effect of adding different proportions of DMF to CS on bread loaves specific volume (Cm^3/g) and bread spreading ratio (width/height). Column chart represent the means; n = 3. [ab]= Means value with different letters were significantly different ($p < 0.05$). Error bar represent the standard deviation, DMF-CS: Defatted marama flour-cassava starch.

4.2.3.4 Bread crumb stereomicroscopy

To gain a deeper insight of the functional properties of DMF into a starch-based bread formulation, stereomicroscopy was used to investigate the structure of the bread crumbs. Figure 17 represents the microstructures of the bread crumb of each bread formulation. Wheat bread and the DMF-CS composite breads resulted in aerated crumb structure with a mixture of bigger and smaller gas cells. The micrographs also revealed that the DMF-CS doughs as well as wheat dough were able to incorporate gas and develop a foam structure upon baking.



A B C D

Figure 17: Effect of adding different proportion of DMF to CS on crumb structure of breads. A: wheat bread, B-D: DMF-CS composite breads. B. DMF-CS (22:78), C. DMF-CS (33:77), D. DMF-CS (57:43).

4.2.3.5 Effect of adding different proportions of DMF to CS on the texture and moisture content of bread crumb

Bread crumb is a complex viscoelastic foam material and its texture is an important quality indicator for consumer acceptance (Korczyk–Szabó & Lacko–Bartošová, 2013). Crumb firmness measured in Newton (N) is defined as the maximum force needed to compress the crumb by a pre-set distance. Lower maximum force indicates softer bread crumb texture (Korczyk – Szabó & Lacko – Bartošová, 2013). There was a significant interaction between bread types and days of storage on the crumb firmness ($p = 0.0015$), springiness ($p = 0.0003$) and moisture content ($p < 0.00001$) (Table 9). The crumb firmness, springiness and moisture content of breads on day 1 and 3 of storage are presented in Figure 18, 19 and 20 respectively. Results showed that wheat bread crumb was the softest, but similarly springy as compared to DMF-CS breads over the storage period. Nevertheless, higher inclusion of DMF in the composite formulation also led to a softer bread crumb. Composite breads DMF-CS (22:78) and DMF-CS (33:67) were more firm compared to DMF-CS (57:43) on day 3 of storage. The moisture content in all bread crumbs decreased upon storage. However, the crumbs of composite breads DMF-CS had a significantly higher moisture content as compared to wheat bread. Also, increasing the proportion of DMF in the composite formulation led to bread with higher moisture content.

Table 9: Summary of significance (p-values) for the effects of bread type and storage days on texture parameters and moisture content of breads.

Source of variance	Firmness	Springiness	Moisture content
Bread types	*	ns	*
Days of storage	*	*	*
Bread types x Days of storage	*	*	*

*Significant difference when $p < 0.05$, ns - not significant when $p > 0.05$.

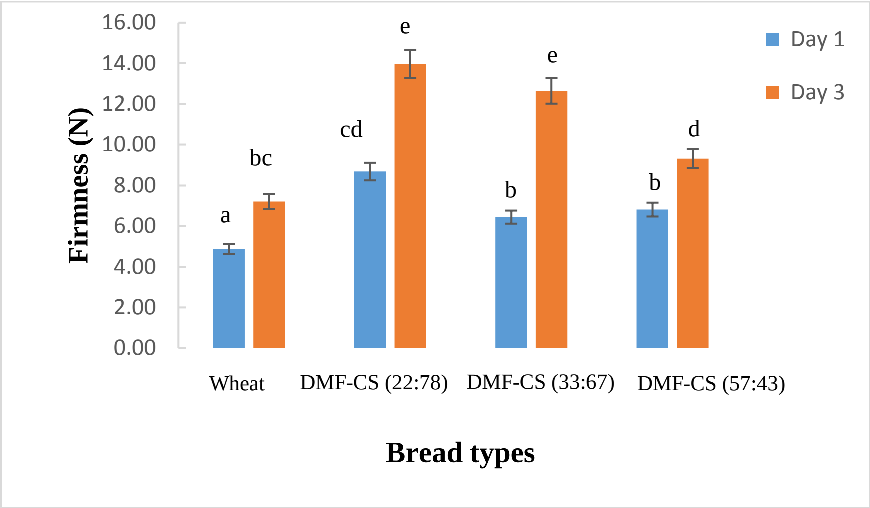


Figure 18: Effect of adding different proportions of DMF to CS on bread crumb firmness on 1 and 3 days of storage. Column chart represent the means; n = 3. abcde= Means value with different letters were significantly different ($p < 0.05$). Error bars represent the standard deviation, DMF-CS: Defatted marama flour-cassava starch.

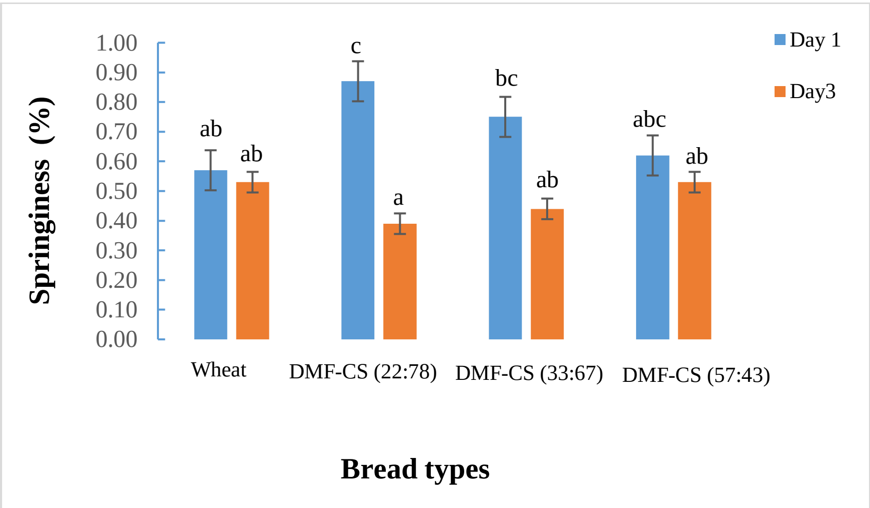


Figure 19: Effect of adding different proportions of DMF to CS on bread crumb springiness on 1 and 3 days of storage. Column chart represent the means; n = 3. abc= Means value with different letters were significantly different ($p < 0.05$). Error bars represent the standard deviation, DMF-CS: Defatted marama flour-cassava starch.

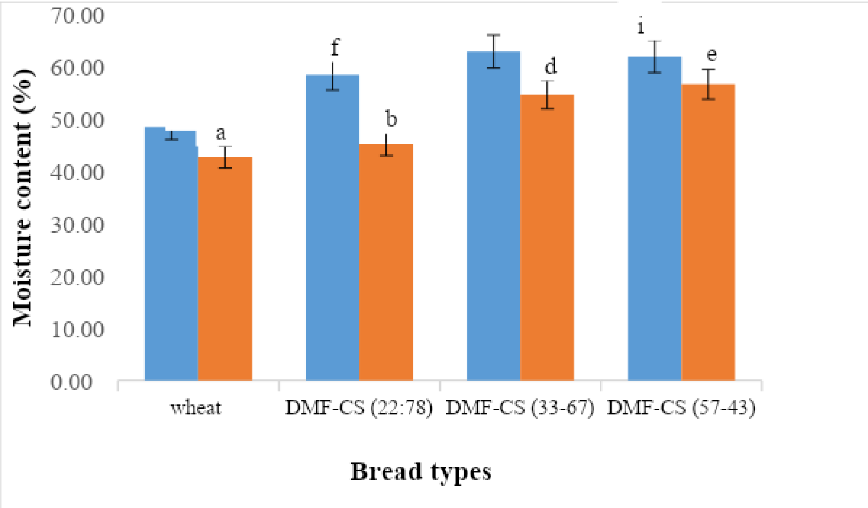


Figure 20: Effect of adding different proportions of DMF to CS on bread crumb moisture content on 1 and 3 days of storage. Column chart represent the means; n = 3. abcdefij= Means value with different letters were significantly different ($p < 0.05$). Error bars represent the standard deviation, DMF-CS: Defatted marama flour-cassava starch.

4.2.3.6 Effect of adding different proportions of DMF to CS on the sensory properties of breads

4.2.3.6.1 Attributes diversity and importance for the sensory discrimination of DMF-CS breads.

The assessors used several terms to characterise the appearance, aroma, flavour and texture of the breads. The number of attributes per assessor varied from 13 to 30 (Table 10). The attributes shown in bold letters are the most important since they contributed to the sensory differentiation ($p < 0.05$) of the breads types. The result also showed that each assessor was able to distinguish the bread types. For each assessor only attributes which significantly discriminated between the bread types (in bold) were used in GPA.

Table 10: Significance levels (p-values) for one-way ANOVA of sensory attributes used by assessors (A) to differentiate wheat and defatted marama flour-cassava DMF-CS bread types using Flash Profiling

Attributes	A 1	A 2	A 3	A 4	A 5	A 6	A 7	A 8	A 9	A 10	Frequency (f*)
Aroma attributes											
Fermented	**0.00**	**0.00**	**0.01**				**0.00**	**0.00**	**0.01**	**0.00**	**7**
Baked	**0.05**	**0.00**				**0.00**	**0.00**	0.94		**0.00**	**6**
Fresh		**0.00**						**0.00**	**0.01**	**0.00**	**4**
Savoury				**0.00**	**0.01**	**0.00**					**3**
Beany	**0.00**				**0.00**					**0.00**	**3**
Wheat bread like	0.25					0.25			**0.00**		**3**
Stale			**0.00**						**0.01**		**2**
Unfamiliar	0.21				**0.00**						**2**
Cake like	0.25	0.08									**2**
Bland					**0.00**						**1**
Raw peanut		**0.00**									**1**
Sweet				**0.00**							**1**
Nutty		**0.00**									**1**
Pungent	**0.00**										**1**
Doughy	**0.03**										**1**
Rich						**0.01**					**1**
Appearance attributes											
Darkness	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**10**
Lightness	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.30	**9**
Porous/airy	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**		**0.00**	**0.00**	**9**
Dene	0.06		**0.00**		0.43	**0.00**		**0.00**	**0.00**		**6**
Moist	0.12										**1**
Fluffy									**0.00**		**1**
Dryness	**0.03**										**1**
Roughness							**0.00**				**1**
Flavour attributes											
Sour	**0.00**	**0.00**	**0.00**		**0.00**	**0.00**		**0.00**	**0.00**	**0.00**	**8**
Sweet		**0.00**	0.25	**0.00**		**0.01**	**0.00**	**0.00**	**0.00**	0.91	**8**
Bitter	**0.00**			**0.00**	**0.00**		**0.00**		**0.00**		**5**
Beany			**0.00**		**0.00**		0.13		**0.00**	**0.00**	**5**
Fermented	**0.00**		**0.00**	**0.00**						**0.01**	**4**
Baked bread like		**0.00**				**0.00**			**0.00**	**0.00**	**4**
Nutty	**0.02**	**0.00**								**0.00**	**3**
Pungent	**0.00**					**0.00**					**2**
Doughy	**0.00**										**1**
Salty				0.28			**0.01**				**2**
Raw peanut		**0.00**									**1**
Bland							**0.04**				**1**

For each assessor, attributes with p-values in bold significantly discriminated between the breads ($P \leq 0.05$). f* is the number of assessors using a particular attribute.

Table 10 (continued): Significance levels (p-values) for attributes used by assessors (A) to differentiate among bread types using Flash Profiling (One-Way ANOVA on each attribute for each assessor)

Attributes	**A 1**	**A 2**	**A 3**	**A 4**	**A 5**	**A 6**	**A 7**	**A 8**	**A 9**	**A 10**	**Frequency (f*)**
Savoury/pleasant				**0.00**	**0.00**						**2**
Doughy	0.15		**0.01**								**2**
Wild leaves		**0.00**									**1**
Texture attributes											
Soft	**0.00**	0.16	**0.00**	**0.01**	**0.00**	0.09		**0.00**	**0.00**	**0.00**	**9**
Firm	0.36	**0.01**	**0.00**	**0.00**	0.82	**0.00**	**0.00**	**0.00**			**8**
Rubbery			**0.00**					**0.00**	**0.03**		**3**
Chewy	**0.01**				0.42					0.08	**3**
Spongy	0.06							**0.00**	**0.00**		**3**
Floury			0.08						**0.00**		**2**
Rough		**0.00**					**0.00**				**2**
Crumbly					**0.01**						**1**
Cake like	**0.01**										**1**
Astringent			**0.01**								**1**
After taste attributes											
Sour	**0.00**	**0.00**	**0.00**					**0.00**		**0.00**	**5**
Bitter	**0.00**		**0.04**	**0.00**	**0.01**	**0.00**	**0.00**		**0.00**		**7**
Fermented	**0.00**		**0.00**	**0.00**							**3**
Buttery	**0.00**						**0.03**		**0.01**		**3**
Salt		**0.00**		0.30							**2**
Off odour/rancid		**0.00**							**0.00**		**2**
Metallic			**0.00**				**0.00**				**2**
Nutty				**0.00**					**0.00**		**2**
Starchy									**0.00**	**0.00**	**2**
Beany					**0.00**					**0.00**	**2**
Sweet		**0.02**					**0.01**				**2**
Baked bread like		**0.00**									**1**
Savoury				**0.00**							**1**
Lingering									**0.00**		**1**
Burnt	**0.00**										**1**
Grainy residue										**0.01**	**1**

For each assessor, attributes with p-values in bold significantly discriminated between the breads ($P \leq 0.05$). f* is the number of Assessors using a particular attribute.

Table 11: shows specific attributes in terms of aroma, appearance, flavour and texture properties that significantly discriminated the bread types ($p \leq 0.05$). Only attributes used by at least three assessors were considered in the collective panel evaluation. The results also show that there was a significant interaction effect between the assessors and the bread types (products) on all sensory bread attributes.

Table 11: Significance values from two-way ANOVA showing assessor effect, product effect and their interaction effect on the sensory attributes of the breads.

Aroma attributes	**F***	**Assessors**	**Products**	**P*Products**
Fermented	7	**0.000**	**0.000**	**0.000**
Fresh	4	**0.000**	**0.000**	**0.000**
Baked bread	4	**0.000**	**0.000**	**0.000**
Savoury	3	**0.000**	**0.001**	**0.000**
Beany	3	**0.000**	**0.000**	**0.000**
Wheat bread like	3	**0.000**	**0.001**	**0.000**
Appearance attributes				
Lightness of crumb	9	1.000	**0.000**	**0.000**
Darkness of crust	10	1.000	**0.000**	**0.000**
Flavour attributes				
Sour	8	**0.000**	**0.000**	**0.000**
Sweet	8	**0.000**	**0.000**	**0.000**
Bitter	5	**0.000**	**0.000**	**0.000**
Wheat bread like	4	**0.000**	**0.000**	**0.000**
Nutty	3	**0.000**	**0.000**	**0.000**
Texture attributes				
Softness	9	**0.000**	**0.000**	**0.000**
Firm	8	**0.000**	**0.000**	**0.000**
Chewy	3	**0.000**	**0.015**	**0.000**
Spongy	3	**0.000**	**0.000**	**0.000**
Rubbery	3	**0.000**	**0.000**	**0.000**
After taste attributes	**F***	**Assessors**	**Products**	**P*Products**
Bitter	7	**0.000**	**0.000**	**0.000**
Sour	5	**0.000**	**0.000**	**0.000**
Fermented	3	**0.000**	**0.000**	**0.000**
Buttery	3	**0.000**	0.060	**0.000**

Attributes with p-values for samples in bold were significant in sensory discrimination of breads at $p \leq 0.05$. Only attributes used by at least 3 Assessors were analysed by two-way ANOVA. f* is the number of assessors using a particular attribute.

GPA reduced the scale effects, allowing to compare the proximity between the terms that were used by different assessors to describe the bread types as well as to obtain a consensus configuration. Figure 21 represents the first and second principal components of the GPA plot showing scores and sensory maps of bread types with loading coordinates for sensory properties. The first two dimensions explained 88% of the total variance of sensory characterisation of breads.

For each assessor, attributes that differentiated the breads types significantly ($p < 0.05$) were used in GPA. F1 clearly separated composite breads from the control wheat and commercial wheat bread. In contrast, F2 separated the wheat control bread from the composites breads and the commercial wheat bread. Composite breads were characterised by dark crusts and porous crumbs and more intense fermented, beany, sour, starchy, nutty and bitter flavour notes, as well as mouthfeel attributes such as rubbery, chewy, firm and grainy. Wheat breads (commercial and control) presented light crusts with soft and spongy crumbs as well as freshly baked wheat bread-like aroma and flavour. It can also be seen (Figure 21) that composites breads formed a consensus group with shared sensory characteristics, while wheat breads (commercial and control) are positioned in an opposite direction with distinctively different sensory attributes. There was fairly good agreement on sensory attribute rankings between individual assessors for the position of DFM-CS composite breads as their judgments are close to the consensus position.

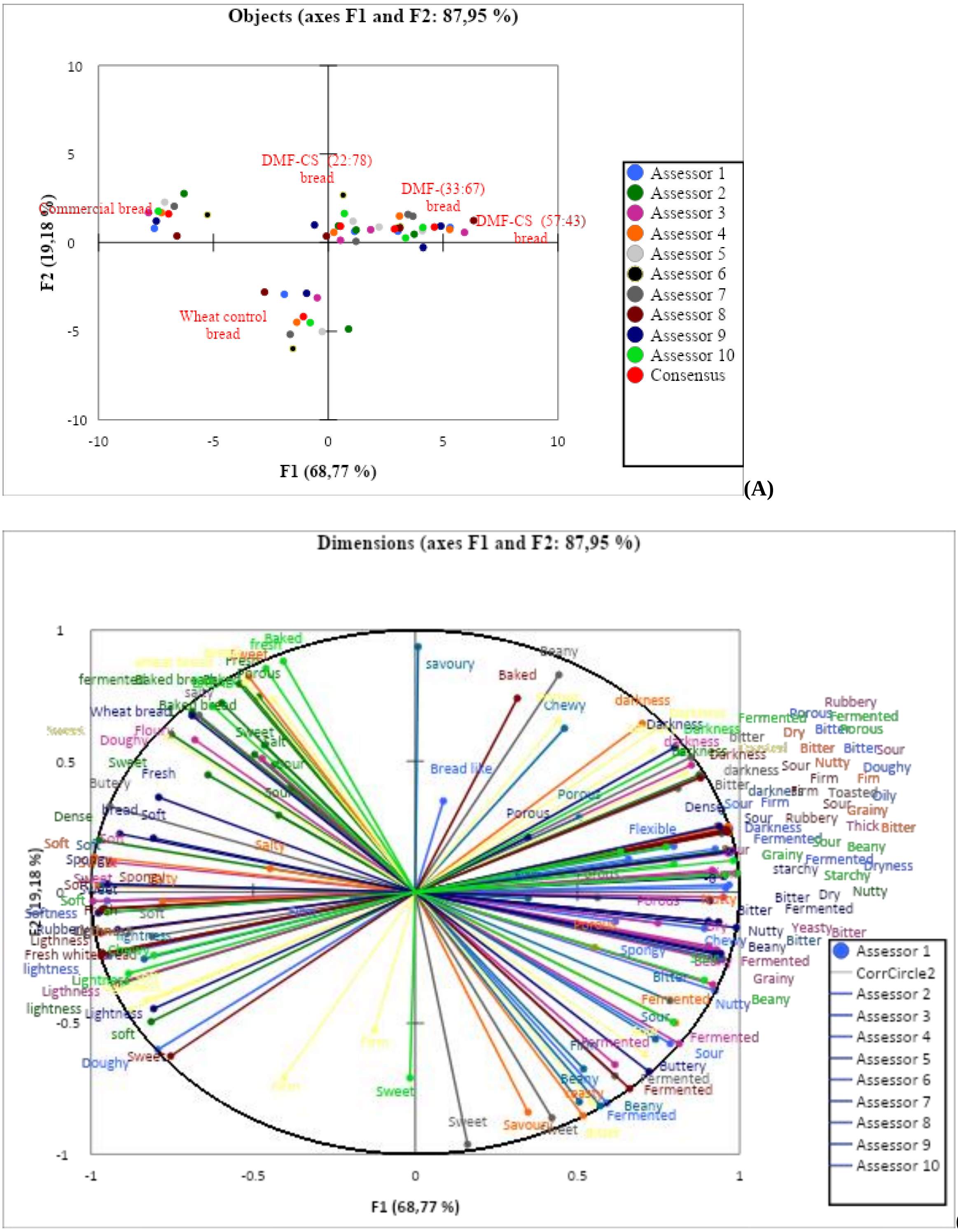


Figure 21: First (F1) and second (F2) principal General Procrustes analysis (GPA) components showing scores and sensory maps of bread types (A) with loading coordinates for sensory properties (B) as evaluated by FP method.

4.2.4. Discussion

The darker crust of DMF-CS 57:43 bread was probably because of the higher content of marama protein in the formulation leading to more Maillard reactions. Gallagher, Gomley & Arendt (2003) reported that bread crust turns browns during baking due to Maillard browning which occurs through the interaction between reducing sugars and amino acids. Colour of bread could come from different sources: intrinsic colour imparted by individual ingredients, or colour development resulting from the interaction of ingredients causing Maillard or caramelisation reactions, besides processing changes associated with chemical or enzymatic reactions (Onyango, Mutungi, Unbehend & Lindhauer, 2010). Colour in baked products is an important attribute and contributes to consumer preferences (Onyango et al. 2010). It is important to note that the darker colour of the DMF-CS bread crust is probably a positive aspect since GF breads tend to have lighter crust colour than wheat bread (Melini et al., 2017).

The crumb colour was probably more affected by the colour of the flour source in composite DMF-CS breads than Maillard reaction. The bread crumb does not reach temperatures as high as the crust, and therefore Maillard reaction and/or caramelisation do not occur to the same extent (Minarro et al. 2012). The lighter crumb of DMF-CS breads was possibly due to the presence of CS in the formulation, which is known to increase lightness in bread. Onyango et al. (2010) reported an increase of bread crumb lightness with increasing CS in sorghum GF bread.

DMF-CS 33:67 and 57:43 composite breads rose up to two-fold their initial height on baking and resulted in less cracks, higher specific volume as well as lower spreading ratio compared to DMF-CS 22:78. Cracks on the bread crust is a reflection of gas escaping from the dough during baking, which causes poor dough expansion (Khuzwayo, 2016). The formulation with higher DFM inclusion would be expected to produce a higher specific volume with fewer cracks on the crust. More DMF in the composite contributed more protein and dietary fibre that increased the strength, extensibility and viscoelasticity of the dough (Nyembwe et al., 2018) and possibly allowed the dough gas cells to sustain more pressure for expansion during baking, thus also reducing the gas cells rupturing that lead to cracks on the bread crust. Nyembwe et al., (2018) predicted that a higher proportion of DFM and consequently higher content of marama protein in the composite would allow for the formation of a more viscoelastic and extensible dough, able to retain more gas during baking thus preventing cracking. The lower bread spreading ratio could also be related to the higher marama protein content in the dough mixture that improved the viscoelastic properties. Dogan, Yildiz & Tasan (2012) reported that in wheat bread, a higher content of gluten protein in the dough mixture increased viscoelastic properties of the dough and reduced the bread spreading ratio upon baking. It is also possible that a higher proportion of marama protein in the composite increased the emulsifying and foaming stability of the dough. These functional properties, together with the high water holding ability of DMF probably favoured the formation of a satisfactory specific volume to provide a soft, springy and suitably aerated bread crumb. The specific volumes of composite breads in this study were similar to GF breads produced with maize starch and pea isolate (2.77 cm^3/g) or maize starch and soy flour (2.76 cm^3/g) (Minarro, 2013). The functionality of legume protein flour in GF bread applications are well known. Marco & Rosell (2008) reported an improvement in rheological behaviour with the addition of soy protein isolate in a GF rice-hydroxypropyl methylcellulose fortified batter formulation. The soy protein isolates increased protein crosslinking, improved the elastic modulus, and led to a GF bread resembling wheat bread in terms of volume, softness and crumb structure. Added legume proteins in GF doughs act as emulsifiers by forming a film or skin around oil droplets, preventing structural changes such as coalescence or creaming. Sciarini et al., (2012) reported that emulsifiers allow for the interaction between two chemically different phases and are used in baking to increase the stability of thermodynamically unstable systems. Boye et al., (2010) reported that the higher volume of GF bread with added chickpea protein isolate, was due to the specific content of amino acids that allowed for a higher foam expansion and stability. In addition, Minarro et al., (2012), also reported the formation of a softer bread crumb in starch based GF bread due to the foaming and emulsifying ability of chickpea flour. Soy flour added to a starch based GF formulation enhanced the crumb softness and bread volume due to higher water absorption properties (Minarro et al., 2012).

Studies also indicated that dietary fibre and/or hydrocolloids improve the volume of GF breads (Tunc & Kahyaoglu, 2016). The presence of fibre and or hydrocolloids as well as emulsifier in a rice GF formulation, was reported to allow the entrapment of air bubbles and holding of water resulting in soft texture and satisfactory specific volume (Marco & Rosell, 2008). The positive effect of dietary fibres and/or hydrocolloids in GF bread could be due to their ability to retain water and also contribute to the formation of a gel network during heating (Tunc & Kahyaoglu, 2016). This network is believed to increase the dough viscosity and also give strength to the expanding cells of the dough; as a result, gas retention during baking improves, and a better bread volume is obtained. Nunes, Moore, Ryan & Arendt (2009) indicated that the strength of the dough and the specific bread volume are related. The dough strength enables the dough to retain gas while elasticity allows for expansion during proofing and baking. Eduardo, Svanberg & Ahrné (2014) found that an increase in the volume of a cassava-maize-wheat composite bread with added hydrocolloids and emulsifier was due to the formation of a gel network during oven heating that strengthened the expanding cells of the dough and resulted in improved gas retention.

All breads types were firmer and less springy on day 3 of storage as a result of moisture loss and starch retrogradation. After three days of storage, wheat bread crumb remained the softest. However, of the three DMF levels, the DMF-CS 57:43 bread had the softest crumb after 3 days of storage. Matos, Sanz & Rosell (2014) reported that crumb firmness can be related to starch retrogradation and bread staling. The moisture content in bread decreased to allow the formation of hydrogen bonds among starch polymers or between starch and proteins leading to hardening. As expected, bread with more DMF had higher moisture content presumably because of the higher water absorption ability of DMF, with its dietary fibres such as pectin known to hold water. Wang, Rosell & Benedito de Barber (2002) reported increased moisture content in wheat bread with the addition of dietary fibres such as carob, inulin and pea fibres.

Greater affinity of proteins and soluble dietary fibres (e.g. pectin) for water in the DMF-CS bread with the highest proportion of DMF possibly resulted in less water available for interaction with CS and therefore contributed to a decrease in retrogradation rate. However, Gambus *et al*. (2007) also reported that pectin is known as a hydrocolloid that improved overall gluten-free bread quality, increased average gas cell size, lowered crumb firmness and delayed staling rate. Moreover, Mariotti et al., (2009) found that legume protein and hydrocolloids formed a network, similar to that of gluten that is able to limit starch swelling and gelatinization in GF formulations. Thus, upon baking, this phenomenon could have great importance for extending shelf-life of the GF bread. Gallagher et al. (2003) reported that crumb firmness in bread may also be due to water migration from crumb to crust. Gluten present in wheat-containing bread slows the movement of water by forming an extensible protein network, thus keeping the wheat crumb structure softer (Gallagher et al., 2004). It is also important to consider the Mixolab findings related to the mixing properties of DMF-CS doughs (Nyembwe et al., 2018). A lower value of C5 already predicted slower starch retrogradation in the composite bread with a higher proportion of DMF to CS because of lower starch content in the formulation.

Besides the physical properties of composite breads such as colour, specific volume and texture which were affected by the proportion of DMF to CS in the formulation as emphasised earlier, sensory assessors also perceived the bread types differently in terms of aroma and flavour. More intense beany, nutty, sour and bitter flavours were noted in DMF-CS 57-43 bread probably due to the higher inclusion of DMF in the formulation. Previously it was reported that DMF was responsible for a strong boiled nut flavour with a slight bitter taste in a composite DMF-sorghum porridge (Kayitesi, 2009). Nyembwe et al. (2015), described a DMF water extract as having a bitter taste due to the presence of bitter compounds such as saponins and phenolic acids (gallic and protocatechuic acids) in the flour. The sour taste can possibly be attributed to phenolic acids such as caffeic acid, ferulic acid and p-coumaric acid in DMF (Nyembwe, 2015). These phenolic acids have been proposed to be responsible for sour taste in protein-rich plant foods (Huang & Zayas, 1991). Assessors perceived the composite breads and the experimental wheat bread as having a more intense fermented aroma as compared to a commercial wheat bread standard possibly due to the different formulations, including yeast types and preparation methods used. Yeasts can liberate aroma precursors such as free amino acids that contribute to the fermented aroma in baked products (Hansen & Schieberle, 2005). In addition, DFM-CS breads had starchier flavour as compared to wheat breads which were blander. The starchy flavour of DMF-CS breads was probably related to the presence of CS in the bread formulation. López, Pereira & Junqueira (2004) reported that CS may influence the flavour of a product with a starchy taste. In addition, the rubberier, chewy mouthfeel of DMF-CS breads was probably also due to the presence of CS in the formulation. López, Pereira & Junqueira (2004) reported that CS bread had a rubbery and gummy like crumb. This is possibly due to the higher amount of amylopectin contained in cassava starch as compared to wheat flour as previously determined by Nyembwe et al. (2018). A higher proportion of amylopectin molecule, in starch, is reported to produce bread with a rubbery and gummy-like bread crumb (Bhattacharya, Erazo-Castrejón, Doehlert, McMullen, 2014).

4.2.5. Conclusions

The effect of DMF inclusion level when composited with CS on physical and sensory quality of breads was studied. A higher inclusion level of DMF in a composite with CS, at a ratio of 33:67 and/or 57:43 seems to have potential to produce GF breads with desirable darker crust, aerated crumb structure, with less soft but springier crumb as compared to wheat crumb over storage. DMF can be considered as a nutritious functional ingredient to address the technological challenges of GF bakery products**.**

5. GENERAL DISCUSSION

The first part of the general discussion is a critical review of the methodology used for this study. It essentially discusses the advantages and disadvantages of the different methods that were applied in this study. Suggestions for applying the methods better in future research are given and different approaches for further improvements of DMF-CS bread including the use of novel alternative techniques for dough preparation, baking, flavour development and shelf life, are proposed. The summary of all the recommendations discussed in the section is also presented in Table 12. The second part critically discusses the findings of the research and also offer an upcoming value chain for marama bean as a source of novel ingredient for gluten-free bread and other bakery products. The proposed value chain can be adopted to assure the availability and sustainability of its flour and the utilisation of other marama based-products.

5.1. A critical review of methodology

5.1.1 Developing the composite gluten-free flour.

Here, marama bean known as *Tylosema esculentum* was the only species used among the four species of Tylosema that are known as food materials in Africa (Jackson et al., 2010). This is because marama bean is an important component of the diet around the Kalahari Desert in Southern Africa where this drought-resistant plant can grow in large populations (Jackson et al., 2010, Mosele et al., 2011) and therefore was available in large enough quantity for this research. The defatted marama bean flour (DMF) is very rich in protein (46 %) and dietary fibre (42 %) and appears to be a useful functional and nutritional ingredient for gluten-free bread making and can have good potential for commercialization (Holse et al., 2010). Interestingly, domestication of marama has been initiated through a breeding programme in Namibia (Nepolo et al., 2009). Consequently, there is a need to encourage the domestication and commercialization to make the DMF more generally available and to create a market.

Cassava (*Manihot esculenta*) is largely cultivated in tropical countries and has the potential to tackle malnutrition due to its starch content that represents an important source of calories for millions of people in sub-Saharan Africa (Bechoff et al., 2018). In fact, cassava has been earmarked as the crop that can spur rural industrial development and raise income for producers, processors, and trade in parts of the world where people are food insecure and have little resources (Bechoff et al., 2018). Consequently, CS was chosen to composite with the DMF because of its better performance in gluten-free composite bread formulations compared to other cereal starches such as maize as reported in section 2.4 (Onyango et al., 2011). Therefore, exploring the potential use of marama bean as a healthy nutritive crop for developing countries in a composite with CS for gluten-free bread making applications could possibly contribute to the development of marama bean as a commercial oilseed legume crop as well as producing valuable income for rural communities gathering marama beans.

In this study, DMF was prepared, and for this purpose, a mechanical cracker was used to dehull marama beans prior to flour manufacturing. The process involved cracking the seed coat first, then manually separating the hulls from the cotyledons. Dehulling marama beans and removing the bean cotyledon pieces in this way was a time consuming and labour intensive process because cracking had to be repeated more than once, particularly for small size marama beans. In addition, it was difficult to remove the cotyledon from the small pieces of cracked marama bean seed coats, thereby resulting in a low yield of marama cotyledons. Sorting the marama beans by size prior to cracking and removing the broken outer shell or hull by blowing a current of an air stream through the bean can effectively simplify the process of dehulling. The technique of blowing air stream to remove hard outer shells is commonly used in cocoa beans (Branch, Paula, Costa, Entzminger, Fredericq, Gilmour, Laird, Matissek, Quintana, Ruiz, & Sigley, 2015).

Defatting is required in the case of leguminous oilseeds such as marama beans when protein-rich, stable flours are required. Full fat marama flour has lower protein content than fully or partially defatted flours, and is prone to hydrolytic and oxidative rancidity (Duodu & Minnaar, 2011). In this study, defatted marama flour was produced following the method as described in the section 4.1.2. The process involved coarsely grinding cotyledons using a Waring blender to produce the full fat flour prior to defatting. The cotyledons were progressively ground and sieved to pass through a 1000 µm mesh. However, the main difficulty encountered was the formation of a paste rather than a flour because marama beans contain a high percentage of fat. This process was also time-consuming, and utilisation of equipment such as a rolling mill that can possibly grind marama bean cotyledons coarsely or roll them into flakes prior to defatting can be considered for further experiments. Furthermore, coarsely grounded marama cotyledons were defatted using hexane to obtain a defatted meal, which was then milled again to pass through a 500 µm mesh to produce DMF. The defatting process was done manually and flour yield losses were the main problem encountered because some flour was decanted with the hexane. Using equipment that can assist with oil extraction such as an oil expeller to prevent flour losses would be strongly recommended for further study. In addition, hexane is a volatile solvent that is used to extract edible oils from seeds such as soybeans as well as nuts and olives. Acute (short-term) inhalation exposure of humans to hexane causes mild central nervous system effects, including dizziness, giddiness, slight nausea, and headache (Vallaeys, Kastel, Fantle, & Buske, 2010). However, it is unclear whether consuming trace residues long term is a health hazard. Therefore, safer and more environmental friendly hexane-free extraction methods such as the use of a cold press to extract oil in soybean, canola seed (Vallaeys et al, 2010) should be considered in the future.

Composite flours are prepared by mixing or blending cereal, root, tuber, or legume flours at a predetermined ratio (Duodu & Minnaar, 2011). These are then used to prepare various food products, including breads and biscuits. An important motivation for the production of composite flour is to improve nutritional quality, because legumes are protein rich relative to starchy food crops and are also generally better sources of essential amino acids. In addition, legumes also offer good functional properties needed in GF bread making (Gulzar et al., 2017).

5.1.2 Dough forming properties of and further improvement of DMF-CS composite dough properties.

Wheat is one of the most common cereals used in the world for bread making because of its viscoelasticity and the quality of the resulting bread (Rathnayake, Navaratne & Navaratne, 2018). However, due to greater public awareness of celiac disease and gluten intolerance as well as consumers demands for healthy food and variety in food products, in many widely consumed staples, such as bread, wheat flour is fully or partially replaced with flour from other cereals, pseudocereals or legumes (Melini et al, 2017). Although wheat bread flour alternatives are readily available in the market, these products are often of inferior quality (Melini et al, 2017). One aim of this project was to predict the suitability of DMF in composite with CS for the production of quality bread. So, wheat flour was chosen as the reference in order to develop a GF dough/bread with similar properties as dough/bread with gluten-containing. Their rheological properties were studied, and compared to the properties of wheat flour which served as a benchmark.

A previous study reported that marama bean protein had visco-elasticity forming properties (Gulzar et al., 2017), the insights from that study were here applied and tested in a GF starch-based food system to develop a new bread. The findings in this thesis support the initial hypothesis of the study, that the DMF composited with CS will produce GF flours that when mixed with water, will develop a dough with sufficient viscoelasticity and extensibility properties to retain carbon dioxide during fermentation. These properties are believed to be caused by the synergetic effect of marama protein and pectin in the DMF with a high proportion of DMF leading to an improvement in dough structure thus increasing the stability as discussed in section 4.1. The potential of marama protein to form viscous and extensible doughs is due to its high β sheet content, hydrophobic interactions and tyrosine crosslinks (Amousou et al., 2012). While, the elasticity of marama protein is due to disulfide bond-linked to high molecular weight proteins. These high molecular weight proteins are also believed to additionally be cross-linked by dityrosine bonds since marama protein is very rich in tyrosine (Gulzar, 2017). Soluble fibres such as pectins swell in water and form structures equivalent to the gluten network, increase the elastic modulus, prevent bubble coalescence, keep the starch and yeast from settling in starch-based GF formulation (Minarro, 2013). However, during the preliminaries works experiences on the dough forming properties of the GF flour, Soybean flour was also included in the study for comparison with marama flour in composite with CS. Only marama flour in composite with CS could produce an additive-free cohesive dough with well-defined shape able to trap enough gas. Soya flour in composite with CS formed a batter instead. In fact, unlike marama protein, soya protein lacks the ability to form a dough in water as reported by Amonsou et al. (2012). So, due to difficulty in forming a dough with soya flour, these experiments were not included in subsequent measurements on equipment such as alveograph and rheofermentometer which analyse dough with viscoelastic properties (Rasper, Pico & Fulcher, 2018). So, the easy ability of marama flour to form a dough in starch-based formulation with no additives (enzyme, hydrocolloid) is an important attribute desirable in the GF bakery industry as most often GF flour formed batter (Melini et al., 2017). More researches are requested to continue investigating the dough forming properties of marama flour in combination with other commercial or locally available GF flours such as sorghum and/or in comparison with other legumes. The choice of the legume flour will depend on whether or not they might show some dough forming properties without any additive. However, the same investigation could also be explored in future on the rheometer which has the advantage of measuring the viscosity and elasticity of batter and dough (Kouassi-Koffi, Muresan, Gnangui, Sturza, Mudura, Kouame, 2015). Therefore, any legume flour of choice such as pea, lupine reported to also increase the elastic modulus by protein crosslink in GF batter (Rathnayake, Navaratne, Navaratne, 2018) may be considered for the assessment. The knowledge acquired from these investigations will contribute to the development and application of marama bean in the food industry.

DMF was composited with CS at different ratios. The choice of these ratios 22 %, 33 % and 57 % of DMF to CS were chosen to produce composite flour with similar or higher protein content as compared to wheat flour. Many trials were done to determine which proportion of DMF to CS should be used. Lower ratio than 22 % of DMF to CS couldn’t show any dough-like properties, while a proportion higher than 57% DMF to CS lead a very sticky dough difficult to analyse using standard rheological measurements.

The knowledge of the maximum amount of water absorbed by the flours is crucial for making gluten-free bread, since the water has a plasticizing effect on doughs (Marco & Rosell, 2008a). The mixolab test was based on preparing a constant hydrated dough mass to obtain the required standard consistency in bread making of 1.1 Nm (± 0.05) during the first test phase. To achieve that, preliminary trials consisting of different combinations in weight (g) of flour and water was done using the mixolab simulator which operates as a farinograph to determine the adequate water absorption. Consistency higher than1.1 Nm (± 0.05) during the first test phase led to a stiff dough which required more water, while lower consistency led to a very soft dough. The advantage of Mixolab 2 (Chopin, Tripette et Renaud, Paris, France) is that it measures, in real-time, the torque (Nm) produced by the dough between the blades. In this study, the test was based on preparing a constant hydrated dough mass to obtain a target consistency of 1.1 Nm (+/- 0.05 Nm) during the first test phase.

An alveograph measures the quality of the flour, by inflating a bubble in a thin sheet of the dough until it burst. The information obtained by such assessment was beneficial in the determination of what the DMF-CS composite flour is useful for as discussed in section 4.1.3. The composite dough made in proportion 33:67 was the closest to wheat in terms of the aptitude to resist deformation, elastic resistance and extensibility balance as well as the deformation energy due to the strong interaction of marama protein and dietary fibre which led to an improvement of structure and stability. However, the, higher proportion of DMF in the DMF-CS composite dough for example 57:43, certainly suggested that there was more hydrated marama protein that might have promoted the viscous flow leading to dough softening, stickiness as well as weakening of tensile properties such as extensibility and resistance to extension. This made the dough with the higher DMF difficult to handle. Thus the 33:67 ratio was chosen as the optimum ratio for further experiments and/or further study because it was able to absorb the most desirable amount of water for formation of an extensible firm dough that was less sticky in the hand.

CLSM was a suitable analytical tool for examining the microstructures of the doughs, as well as studying the possible interactions between marama proteins and CS. The microscopic spatial arrangement of components allowed for more understanding with regard to the links of the physico-chemical interactions such as the less homogenous distribution of the marama protein in the composite dough with the highest DMF to CS ratio (57:43) due to the strong affinity of more hydrated DMF particles for each other than for the cassava starch (Chapter 4.1.3) that lead to a reduction in dough tenacity, extensibility and dough bubble size as measured with alveograph. To allow the visualization of the DMF-CS structure, acid fuchsin staining was used to identify the protein matrix in the dough, and that was found very effective in this research study. However, it would have been more useful to have used a dye to also identify the marama dietary fibre in the dough system. Thus for further studies, marama dietary fibre can be visualized in a different colour than protein to better determine the interaction between the marama protein and dietary fibre.

Rheofermentometer assessment revealed that DMF-CS composite doughs have potential to hold gas and produce a leavened dough. Prior to baking, the effect of proofing time on the composite doughs was also investigated to determine the optimum proofing time at 34 °C for the composite bread with the highest loaf height. When the dough was proofed for 45 min, it doubled in size. However, when it was proofed for more than 45 min, for example, 60 and more minutes, it rose and the crust collapsed, which indicated over-proofing. Proofing of composite bread for 60 min showed undesirable cracks on the crust and also increased the number of visible cracks on the crust. This indicates that a proofing time of 60 min was possibly not ideal for the composite breads.

Another problem encountered was the handling of the DMF-CS composite 57:43 dough which was less firm than DMF-CS composite 33:67 and 22:78 doughs as measured by the alveograph (Chapter 4.1). To resolve the problem, DATEM was added as a dough strengthener. DATEM is a surfactant widely used in commercial wheat bread making for its positive effect including to improve dough tenacity, structure and to increase gluten strength (Gomez et al., 2004). However, DATEM has also been useful in GF bread making. Khuzwayo (2017), reported an improvement in the cohesiveness and strength of maize-zein composite dough. The functionalities of DATEM are attributed to formation of complexes with hydrophobic and hydrophilic interaction with the protein and starch to form more continuous structure during mixing and increase the strength to hold up tightly expanded dough structure (Pan, Luo, Liu, Luo, 2018). For further study, an alternative possible solution to make the handling properties of DMF-CS composite dough 57:43 easier, can be the use of sourdough fermentation. Sourdough fermentation is a process whereby fundamental interactions between lactic acid bacteria (LAB) and yeasts take place. The most frequently used LAB in the sourdough fermentation process are *Lactobacillus sanfranciscensis*, *Lactobacillus plantarum*, *Lactobacillus brevis* and yeast *Saccharomyces cerevisiae* (Wang, Lu, Li, Zhao & Han, 2017). During sourdough fermentation there are biochemical changes occurring in the carbohydrate and protein components of the flour due to the action of microbial and indigenous enzymes (Arendt et al., 2007). Falade et al. (2014) found that maize sourdough exhibited a cohesive dough structure due to endosperm matrix protein degradation. The author also suggested that degradation of the protein possibly enabled the partial starch hydrolysis and also leaching of amylose. The leached amylose would be capable of forming a network which probably resulted in the formation of a cohesive dough structure with sufficient strength to hold gas from fermentation.

5.1.3 Baking performance of the composite DMF-CS gluten-free bread and the way forward.

The current baking experiment was conducted in a convection oven. One of the advantages of baking in a convection oven is that hot air is continually blown through the product during the baking process, creating an evenly baked loaf of bread that is perfectly brown on the outside (Sani, Paip, Kamal & Aziz, 2014). The selected temperature and time combination was 150°C for 40 min because higher baking temperature also proved problematic for DMF-CS composite doughs. As established during trial bakes, elevated temperature caused the bread crust to rapidly develop darker colour while the internal part of the bread was still doughy. The rapid colour development of the bread crust could be possibly related to increasing Maillard reaction as a possible result of the high lysine content of marama protein in the composite doughs. Hallen, Ibanoglu, & Ainsworth (2004) also reported that composite wheat-cowpea bread crust tends to become darker with increased incorporation of cowpea flour. Colour development in a baked product also increases as the intensity of thermal treatment e.g temperature increases through the formation of melanoidins from the Maillard reaction (Borrelli, Visconti, Mennella, Anese & Fogliano, 2002).

In this study, the crumb texture of bread samples that were stored at 5 °C was measured 24 h after baking (day 1) and again 72 h after baking (days 3) by conducting a texture profile analysis. The choice of this temperature of storage was to prevent mould growth as the composite breads had a relatively high moisture content (59-62 g/100g) as compared to wheat flour bread (49g/100g). The high moisture content of DMF-CS bread is due to the fact that DMF-CS composite flour contains more dietary fibre. Dietary fibres are able to absorb more water and swell as explained in section 4.2.3.1. However, it is recommended to store bread at room temperature (approximately 20 °C) to prevent staling as a result of retrogradation (Ronda & Roos, 2011). It is conceivable that the low storage temperature might have promoted staling of the breads as refrigeration temperature normally enhances the retrogradation of starch (Ronda & Roos, 2011). Nevertheless, measurement of crumb firmness over the 3 days of storage indicated that composite DMF-CS breads staled more rapidly as compared to the experimental wheat bread. Therefore, a possible alternative to consider further for extension of DMF-CS composite breads freshness availability may be the application of other technologies as part of the bread-making process such as frozen dough and/or partially or part-baked bread so that consumers can prepare or complete the baking at home when needed. Frozen dough involves inserting a freezing process in the bread-making step prior to baking, while partially baked bread is a semi-finished product with proper crumb texture and minimum crust colouration (Wang et al., 2017). The par-baking process consists of two stages: an initial baking stage until the bread structure is fixed followed by storage, and a second baking stage to create an appropriate flavour and crust colour (Wang et al., 2017). Both technologies, frozen dough and par-baked bread, are suitable for home consumption or for use in food service, since GF bread could be thawed or baked according to the consumer or customer's needs. Moreover, DMF-CS bread could also be stored frozen to extend the shelf life, while frozen temperature (-18°C) slows down the staling process. For example, Ronda and Roos (2011) observed an extended shelf life of GF rice flour-based bread formula containing 2 % hydroxypropymethylcelullose when the GF bread was frozen and stored at -28°C for 7 days, as compared to when the bread was stored at 4 C. After thawing, the GF bread had similar properties including crumb structure and texture to those of GF fresh bread.

Furthermore, active packaging and /or modified atmosphere packaging could also be considered as an effective option for extending the shelf life and preventing any mould development of DMF-CS breads. Capriles & Areas (2015) reported the efficiency of active packaging (the addition of a natural antimicrobial cinnamon essential oil), modified atmosphere packaging (60 % CO_2 and 40 % N_2), and the combination of both techniques on GF bread quality. Results indicated that active packaging increased the shelf life because it inhibited microbial growth while maintaining the GF bread's sensory properties including flavour and texture. Further studies could therefore consider the use of such preservation methods and determine the optimum conditions for extending the shelf life of DMF-CS bread. In addition, another preservation method to consider will be drying of DMF-CS bread in an oven to extract the moisture through evaporation. This specific method will have the benefit of effectively lowering the moisture content which will prevent mould growth and extend the shelf life of DMF-CS breads. However, the dried DMF-CS bread will possibly change in the quality and result in a crispy dry crumb-like rusk for example. Therefore, determining the optimum conditions to apply such a method as well as its effects on the bread quality will also be recommended.

Recently, sourdough fermentation has been applied, either individually or in conjunction with frozen storage, and partial baking, to obtain and maintain better quality characteristics of GF products such as improved flavour, texture and shelf life (Wang et al., 2017). For example, the association of *Lactobacillus plantarum* with *Saccharomyces cerevisiae* caused an increase in carbon dioxide produced and improved the capacity of the sourdough to retain the gas and result in a softer crumb (Gobbetti et al. 2005). Also, the lactic acid produced by *Lactobacillus plantarum* was responsible for a more elastic gluten structure. The application of sourdough may upgrade the characteristics of the composite DMF-CS breads like, higher volume, texture, improved flavour, nutritional value and shelf-life by retarding the staling process and by protecting bread from mould and bacterial spoilage due to the metabolites produced by LAB as suggested by Arendt et al. (2008) and Moroni et al.(2009) for GF breads. Wang, et al. (2017) also reported that sourdough has positive contributions when exploited for the preparation of high quality GF breads. Gobbetti et al. (2005) used sourdough as a way to improve the quality of maize bread because the LAB has been shown to possess both anti-bacterial and anti-fungal properties. Therefore, sourdough addition is an effective procedure to preserve bread from spoilage since it complies with the consumer demands for additive-free products. Falade et al. (2014) also found that the maize sourdough breads had a more open crumb structure with discrete gas bubbles and less force was required to compress the maize sourdough breads compared to maize bread without sourdough.

In this study, the bread quality, as well as the structure of the bread crumb, was also determined by the use of sensory evaluation to obtain information about the sensory properties of bread types. The panel consisted of 10 screened assessors recruited in the Department of Consumer and Food Sciences. To achieve the purpose of bread testing, flash profiling (FP) was chosen because it is a less time-consuming sensory profiling method as compared to quantitative descriptive analysis method due to the absence of a training phase (Valentin et al., 2012). Since FP has no training phase, it is also considered as a more cost-effective method as compared to quantitative descriptive analysis method (Valentin et al., 2012). FP allows diversity of points of view among assessors (Valentin et al., 2012), providing a product map in a very short time space since the steps of product familiarization, attribute generation and ranking are combined (Chollet et al., 2011). Bread types were compared simultaneously to enhance product discrimination as proposed by Delarue & Sieffermann, (2004). This technique was applied in this study as it was able to meet the objectives of the study, including identification of the sensory attributes as perceived by potential consumers while discriminating the GF bread types.

Suitable procedures were also taken into consideration to optimize the sensory evaluation of bread types by FP. The evaluation was conducted with ten panellists using 10-point line scales structured to rank the sensory attributes of DMF-CS composite and wheat breads. To avoid confusion in sensory perception and increase the accuracy of the evaluation, the panellists were selected based on sensory acuity screening results. The screening used the basic tastes identification test and panellists were also told about how to rank and how to use the structured line to be able to identify and quantify correctly the differences between the bread types. For the evaluation, each panellist received a set of samples coded with randomly selected 3-digit numbers and the order of sample presentation was randomized over the panel. Panellists rinsed their mouths with filtered tap water before they began and between each tasting of bread samples to avoid flavour build up in the mouth. The panellists were also asked to take a 1min break after drinking water before tasting the next bread sample. Flash profiling method fitted the objective of the study that was to describe and differentiate the bread types in terms of their sensory properties. Because the all evaluation process is done in a short period of time, there was no need to use a large quantity of samples including the marama bean. The collected data were easy to analyse using Generalized Procrustes Analysis (GPA) which is a multivariate statistical technique for integrating different groups of variables. In this study, GPA explained the greatest variability in the results as presented in section 4.2.3.6. GPA carried out a Principal Components Analysis for the different matrices and projected onto dimensional space that perfectly allowed to visualize the consensus and also compare the proximity between the terms that were used by different assessors to describe products.

A colour meter is a light-sensitive instrument that measures the surface colour of a product. In this study, colour analysis of the bread types was carried out using a colour meter. The instrument was standardized against a white tile before each measurement. Colour expressed in L*, a*, and b*scale parameters relate well to how the colour is perceived and simplify understanding. Measuring the colour of the bread types using the colour meter gave objective results that were in agreement with panellists' results from the sensory evaluation by FP.

The sensory evaluation also revealed that composite bread DMF-CS might look and feel like wheat bread but it smelled and tasted different. However, the fact that DMF-CS breads had attractive brown crusts colour and resulted in similar aerated crumb as wheat bread as discussed in section 4.2.4, represented a positive achievement for the GF bread because many GF breads tend to have a pale crust colour with a compact and crumbly crumb structure (Melini et al., 2017). Besides, the aroma of bread is also considered as one of the main characteristics that influence the consumers' choice (Pico, Martínez, Bernal, Gómez, 2017). There can be no doubt that the ingredients used in the formulation should have affected the final aroma and the taste of DMF-CS bread. The negative sensory attributes such as beany flavour and bitter taste perceived in the DMF-CS breads could represent barriers to consumption to consumers that would expect more sensory properties similar to wheat bread, if DMF would be considered as an alternative of gluten in the baking industry. Thus, understanding the evolution of volatile compounds from dough to crumb may be necessary for further study to develop and/or modified the aroma of bread made from DMF-CS. Since gallic and protocatechuic acids, as well as, saponins are water-extractable components that contribute to bitterness in marama beans (Nyembwe et al., 2015), therefore, attention should also be given to consider the inclusion of processing technology such as soaking of marama bean in water prior to flour making to reduce the content of bitter saponins and phenolic acids in DMF. Drewnowski & Gomez-Carneros (2000) reported that bitterness is a common sensory concern of plant-derived foods due to the presence of different chemical constituents that contribute to bitterness including certain phenolic compounds, peptides, and saponins. Bitter-tasting compounds are therefore often removed from plant foods through debittering processes such as soaking and/or washing in water for a few hours. For example, quinoa seeds (*Chenopodium quinoa* Willd.) are pseudocereals rich in protein, and used as wheat replacer in bread formulations. However, quinoa seed is often washed several times or soaked in water overnight to reduce the saponins content prior to flour making, so that the resulting bread may result in a pleasant taste with slightly bitter aftertaste. Additional research is required to determine how much time and water is needed to reduce the bitter compounds in marama bean. Nevertheless, phenolic acids and saponins are phytochemicals that exert protective or disease-preventing effects. They have been associated with protection from and/or treatment of chronic diseases such as heart disease, cancer, hypertension, diabetes, and other medical conditions (Stantiall & Serventi, 2018). Phenolic compounds, for instance, through their antioxidant activity, are hypothesized to have the ability to reduce the risk of developing certain cancers by potentially protecting body cells against oxidative damage caused by reactive oxygen species (Stantiall & Serventi, 2018). Therefore, to preserve the health benefits that these phytochemicals may possibly offer in their integrity, alternative masking technology such as the use of sweeteners or salt may also be applied. The use of bitter blockers such as sweeteners and salt can also be used to reduce or to mask the bitterness in the DMF-CS composite bread. Beside the role of yeast-food in bread, sugar and honey, for example can be used as flavour enhancers (Heinio, Noort, Katina, Alam, Sozer, de Kock, Herslethe & Poutanen, 2015). Many bakers are turning to honey to build on its ability to mask off-flavours including bitterness and sourness of wholegrain bread such as wheat or rye bread (Heinio et al., 2015). Sweet tasting compounds, such as aspartame and sucralose have been widely used to mask the bitterness of food products such as juice, yoghurt and soy (Szejt1i & Szente, 2005) as well as pharmaceutical products such as quinine (Suzuki, Onishi, Hisamatsu, Masuda, Takashi, Iwata & Machida, 2004). However, more research is still needed to determine how much sugar, sweeteners or honey will be needed to mask the bitterness in DMF-CS breads to levels acceptable by consumers.

In addition to sweeteners, salt also may offer an option in masking the bitter taste as sodium cations have been shown to mask bitter compounds (Heinio et al., 2015). For example, the bitterness and astringency of rice have been shown to be significantly reduced by adding salt to water in cooking (Bett-Garber, Lea, Watson & Champagne, 2013). This is also the case of dark rye bread eaten with salty cream cheese to mask the bitterness and sourness (Song, Perez-Cueto & Bredie, 2018). Thus DMF-CS bread can therefore possibly be eaten with salty relish such as cheese, bacon, salami or salty dishes such as soup or sauce to mask the off flavour including bitterness. Previous research (Nyembwe 2015) on sensory properties of marama bean also suggested using of cyclodextrin to reduce the bitterness of DMF when used as an ingredient, without affecting the phenolic compounds and saponins with their bioactive potential. The same suggestion can also be recommended in this study as a way of reducing the bitterness in the DMF-CS bread which can be a limiting factor for some consumers. Cyclodextrin is a family of cyclic oligosaccharides, are also a common commercial product known for their ability to decrease bitterness due to their hydrophobic cavity and hydrophilic exterior shell (Szente & Szeijtli, 2004). Once bitter eliciting compounds interact with the interior of cyclodextrins, inclusion complexes are formed. As a result, bitterness is decreased because the bitter eliciting compounds are trapped within the cyclodextrin molecule, and therefore are unable to bind to taste receptors cells. For example, cyclodextrins are used to mask the bitterness of citrus juice mainly caused by flavonoids (limonin and naringin) (Drewnowski & Gomez-Carneros, 2000). Because flavonoids are still present in the juice, their bioactive potential is unchanged (Drewnowski & Gomez-Carneros, 2000). In addition to masking the perception of bitterness, it can also be reduced by blocking the human bitter taste receptor hTAS2R39 (Heinio et al., 2015). Three 6-methoxyflavanones known as bitter taste receptor blockers were shown to reduce hTAS2R39 activation by epicatechin gallate which is a bitter flavan-3-ol present in buckwheat. These bitter receptor blockers were characterized as reversible antagonists. Moreover, the complexation of epigallocatechin gallate with food proteins reduced h TAS2R39 activation. Therefore, the next step to take into consideration, is to determine the consumer acceptance of DFM-CS bread with those consumers that seek and are keen to try different sensory properties or seek novel tasting speciality breads to determine appeal as a speciality bread.

Dietary fibre is known as the food fraction that is not enzymatically degraded within the human alimentary digestive tract and its importance is appreciated more and more due to its beneficial effects on the reduction of cholesterol levels and the risk of colon cancer (Wang et al 2017). Melini et al. (2017) reviewed the nutritional quality of GF bread and highlighted some deficiencies in the nutritional profile of GF bread, including low levels of dietary fibre as compared to wheat flour bread. For example, GF bread based on rice flour contained an average dietary fibre of 0.7 versus. 4.3 g/100 g of wheat bread (Stantiall & Serventi, 2018). Here, DMF-CS composites made in proportion 33: 77 and 57: 43 were found as having two to seven times higher content of dietary fibre (9 - 28 g/100g), respectively as compared to wheat flour (chapter 4.1). The dietary fibre content of these DMF-CS composites appeared similar and/or higher than whole grain wheat flour which has a dietary fibre content of approximately 8-11 g/100 g (Melini et al., 2017). DMF seems to be a good source of dietary fibre that can be used in the GF bakery to develop fibre rich bread. However, further research should be conducted to assess the complete nutritional profile of DMF-CS bread. This information will be of value for guidance on the marketing potential of DFM-CS as a nutritionally functional product that can help consumers in the choice of healthy eating. The complete nutritional profile of DMF-CS bread will also help health professionals to give clear and target advice about positive effects of consuming the bread during consultations. Effective information on the expected sensory properties and nutritional benefits will assist in reducing the neophobic tendencies among consumers who are scared to try a new food product. For example, a complete nutritional profile of composite sorghum porridge supplemented with 5 % marama flour was done by Mmonatau (2005). The finding showed that a one-day meal-plan of sorghum porridge with 5% marama flour could contribute significantly to the recommended daily allowance (RDA) in term of protein, dietary fibre, mineral and vitamin content as well as lowering the glycaemic index (GI) of sorghum to an intermediate GI- food. It was therefore recommended that marama bean flour be added to products in the meal plan to complement the general deficiency in Batswana (*people from Botswana)* diets and in school feeding programmes specifically. Another option to consider for further experiments will be the comparison of DMF-CS composite bread with other cereal flour used in bread making including rye or wholegrain wheat to test the effect of dietary fibre and phytochemicals on the functional properties. Such information will be of great importance to categorize the DMF-CS bread in terms of quality among the bread types sold in retail shops.

5.1.4 Porous crumb structure development

The basic ingredients that were used in this study for bread were flour, water, yeast, salt, sugar, and shortening. Normally in bread making, there are a number of processes to convert these ingredients into a well-developed porous structure, where the main processing steps involve kneading, proofing, and baking. Water and flour are the most significant ingredients which may affect considerably the crumb structure properties (Rathnayake, Navaratne & Navaratne, 2018). During mixing and hydration, the proteins combine together and form a viscoelastic network that can retain leavened gas during fermentation and baking (Nwanekezi, 2013). The starch associated with this protein network becomes gelatinized during heating and forms a semi-rigid structure to the product along with the coagulated protein (Rathnayake et al., 2018). In this study, the behaviour during mixing, overmixing, pasting and gelling of DMF-CS composite dough was first determined using the Mixolab® which mimic the thermal conditions that might be expected during the baking process. As reported in section 4.1.2, the Mixolab® allowed the characterization of the physicochemical behaviour of dough submitted to dual mixing and temperature variations. So, when preparing the composite DMF-CS bread, the kneading process had the advantage of getting the ingredients mixed homogeneously, to absorb water by hydrophilic groups of flour protein molecules, for the development of protein network, viscoelastic structure, nuclei for gas entrapment of air into the dough mass. During fermentation, it is reported that the yeast cells utilise the carbohydrates in the absence of oxygen to produce gas such as CO_2 (Rathnayake et al., 2018). The generated CO_2 may partly dissolve within the liquid phase and diffuses to the nuclei generated during mixing stage due to concentration gradient of gas that causes the modifications of the dough structure causing physicochemical changes in protein network by giving the characteristic porosity of the porous crumb which leads to setting the final bread crumb structure within the oven. CO_2 also plays an important role in the expansion of bubbles during baking by releasing from the dough when the bubble walls start to break under pressure, making the porous structure more continuous and open to the outside of the bread. Here, wheat bread and the DMF-CS composite breads resulted in similar open cell crumb structure with a mixture of bigger and smaller gas cells. Based on the finding in this study, it can also be said that the high functionality of the marama protein which relates to its viscoelastic properties, were important for gas retention, and structural stabilization of the bread crumb. In addition, the emulsifying stabilisation and foaming ability of marama flour also contributed in the development of porous crumb. These properties are desirable and needed in the bread-making industry to favour the development of GF bread with improved crumb structure. In fact, other legume proteins such as lupine, and pea protein in GF starch based formulation also caused changes in crumb structure resulting in higher porosity and decrease in cell density due to their high functionality (Ziobro, Juszczak, Witczak, Korus, 2016). Protein from legume flour in GF bread acts as emulsifiers by forming a film or skin around oil droplets, preventing structural changes such as coalescence or creaming (Minarro, 2013; Minarro et al., 2012). The emulsifying and foaming properties decrease the surface tension between two chemically different phases and are used in baking to increase the stability of multiphase systems which increase gas retention and lead to porous and finer crumb structure (Sciarini et al., 2012). An exciting option for future research will also be the comparison of bread crumb structure made from DMF with crumb from flour of pea and lupine in starch based formulation. The effect of mixing and fermentation with variation of parameters such as time and temperature can also be further investigated as they affect the development of crumb structure, texture as well as the loaf volume in bread (Rathnayake et al., 2018).

Table 12: Summary of all proposed recommendations for further study.

1. Commercial cultivation of marama bean is a prerequisite for the application of marama bean flour in food products.
2. Sorting the marama beans by size prior to cracking and removing the broken outer shell or hull by blowing a current of an air stream through the bean can effectively simplify the process of dehulling and save time.
3. The utilisation of equipment such as a rolling mill that can possibly grind marama bean cotyledons coarsely or roll them into flakes prior to defatting.
4. Investigate using equipment that can assist with oil extraction such as an oil expeller to prevent flour losses.
5. The utilisation of safer and more environmental friendly hexane-free extraction methods such as cold pressing to extract oil from marama bean
6. Develop alternative possible solutions such as sourdough fermentation to make the handling properties of DMF-CS composite dough 57:43 easier.
7. Optimise the temperature and time combination for baking the bread e.g. 150°C for 40 min because the higher baking temperature proved problematic for DMF-CS composite doughs.
8. Active packaging and /or modified atmosphere packaging could also be considered as an effective option for extending the shelf life and preventing any mould development of DMF-CS breads.
9. A better understanding is required of the evolution of volatile compounds from dough to crumb to develop and/or modify the aroma of bread made from DMF-CS.
10. Attention should also be given to consider the inclusion of a processing technology such as soaking of marama bean in water prior to flour making to reduce the content of bitter saponins and phenolic acids in DMF.
11. Preservation of the health benefits that phytochemicals may possibly offer in their integrity, by using alternative masking technology such as the use of sweeteners or salt as a way of reducing the bitterness in the DMF-CS bread which can be a limiting factor for some consumers.
12. DMF-CS bread can be eaten with salty relishes such as cheese, bacon, salami or salty dishes including soup or sauce to mask the off flavour.
13. The use of cyclodextrin to reduce the bitterness of DMF when using it as an ingredient, without affecting the phenolic compounds and saponins due to their bioactive potential.
14. Perform a consumer acceptance test on the DFM-CS bread to determine how much the GF bread will be preferred in comparison to bread in retail shop.
15. Continue to investigate the dough forming properties of marama flour in combination with other GF flours and/or in comparison with other legumes. The knowledge acquired from these investigations will contribute to the development and application of marama bean in the food industry.
16. Comparison of the DMF-CS composite bread with bread types other than white wheat bread is needed e.g. rye or wholegrain wheat, pumpernickel and black bread to determine its specialty value. Such information will be of great importance to categorize the DMF-CS bread in terms of quality among the bread types sold in retail shops.
17. Comparison of bread crumb structure made from DMF with crumb from flour of pea and lupine in starch based formulation.
18. Investigate the effect of mixing and fermentation with variation of parameters such as time and temperature as they can affect the development of crumb structure, texture as well as the loaf volume in bread.

5.2 Value of the research on the utilisation of DMF in gluten free formulation for bread making.

Here, the dough properties of composite DMF-CS doughs were compared with wheat flour dough with the aim of determining the potential of DMF combined with CS as a functional nutritious GF ingredient in bread. DMF-CS doughs with similar strength to wheat flour dough were produced although they had shorter Mixolab development time and stability. DMF-CS doughs could successfully inflate into a bubble, with the 33:67 DMF-CS ratio having the most similar bubble size, extensibility and deformation energy as wheat flour dough. DMF-CS composites could also hold gas produced by yeast fermentation. Confocal laser scanning microscopy revealed that as the proportion of DMF to CS was increased, the DMF protein tended to aggregate rather than distributed homogenously throughout the dough, probably because the highly hydrophilic marama protein and pectin had a great affinity for each other as explained earlier. Moreover, DMF-CS breads with desirable brown crust and a uniform aerated crumb structure were also produced. Higher inclusion of DMF in the formulation led to higher specific bread volume with lower spread ratio. The DMF-CS bread crumbs were less soft than wheat bread with more intense sensory flavour profiles compared to wheat bread which was blander. Higher inclusion of DMF in the formulation led to more bitter taste because DMF contains bitter compounds such as saponins, gallic and protocatechuic acids. Hence, unlike previous studies which focused on protein and carbohydrate characterisation of marama bean, as well as nutritional, physicochemical, functional and sensory properties of marama bean, this is the first research investigating the possibility of using DMF in a GF formulation to develop a nutritious composite bread. This research is novel because it described the potential of a new ingredient DMF to the bakery industry. The ingredients could be used for the development of GF bread that have some similar characteristics to wheat bread. Marama bean with its DMF used to make composite flours DMF-CS have the advantage to be available in the southern African countries particularly South Africa, Namibia and Botswana. DMF composite with CS in bread making is a convenient alternative for promoting the use of local crops in sub-Saharan Africa.

The Food and Agriculture Organization (FAO), have been involved in research designed to find ways of partially substituting wheat flour with those from other sources or replacing wheat altogether (Olaoye & Ade-Omowaye, 2011). The technology of composite flours in bread making is useful because it represents an interesting option for lowering the costs associated with the importation of wheat flour in developing countries where wheat is not cultivated for climatic reasons (Olaoye & Ade-Omowaye, 2011). Besides having good nutritional (Holse, 2010) and functional profile to address technological challenges in the bakery industry due to its protein and dietary fibre content, DMF is also a GF ingredient that can potentially address the problem of coeliac disease and wheat allergies. However, another benefit of using DMF in composite with CS as an alternative or partial replacement to wheat flour for bread manufacture is also to minimize the use of imported wheat, because this might enable affordability of the final product by consumers in the long run as marama beans are drought tolerant crops and may require lower input cost (Cullis, Chimwamurombe, Barker, Kunert & Vorster, 2018). Therefore, DMF makes marama bean an important crop for agricultural diversification and that can provide a unique opportunity to combat hunger and nutritional insecurity. Until now, the marama bean has only been found in the wild although a cultivation trial has been initiated in Namibia to carry out selective breeding for marama bean (Cullis et al., 2018). However, it has been reported as one of the world's many neglected plants despite its enormous potential (Cullis et al., 2018). The research in this thesis work was part of an effort to unravel the secrets of the marama bean in an effort of lifting it out of obscurity. The research is also aimed at evaluating the potential of developing the marama bean into new products e.g. DMF-CS bread and other bakery applications, which may contribute to the food supply in some of the world's most challenging agricultural locations to address nutritional necessities. Despite the many attributes of the marama bean, only a few research studies have targeted commercialisation of this unique indigenous natural resource in the countries where it grows naturally (Cullis et al., 2018). However, to have a beneficial effect in reality, as a new ingredient for the bakery industry, marama bean needs to be commercialized to find DMF available on the market like other known protein-rich legume flours made from soybeans, lupin, pea, etc.

Commercial exploitation of the marama bean can also certainly contribute to addressing hunger and malnutrition challenges in Southern Africa, while increasing incomes and improving livelihoods in the areas where the beans grow. The many positive functional and nutritional properties of marama beans through its DMF underline their large potential as a GF ingredient in the food industry. Therefore, this study also used a market-driven approach to develop prototypes of innovative quality marama products such as protein-fibre rich GF doughs as well as healthy DMF-CS breads. Commercialisation of DMF, as well as the DMF-CS composite breads can initially be targeted to niche markets in Southern Africa but may also be sold worldwide in the future. It is then needed to start exploring the commercialisation of marama bean and ensure the sustainability of its flour so that the new develop products e.g. DMF-CS bread can be available on shelves in retail shops and bakeries. To achieve that a value chain should be build up considering many aspects ranging from domestication, cultivation of marama bean and market development as shown in Figure 22. Domestication is the process by which plants are genetically modified over time by humans for traits that are more advantageous or desirable for humans. (Zohary, Hopf, &

Weiss, 2012). Information learned from domestication research aids in the continued improvement of crop species (Sedivy, Wu & Hanzawa, 2016). Normally, domestication tends to decrease genetic diversity, as only certain plants are selected and propagated (Zohary et al., 2012). As an example, almond is considered to be one of the earliest domesticated tree nuts (Delplancke, Alvarez, Espindola, Joly, Benoit, Brouck & Arrigo (2010). Wild almonds are bitter; the kernel produces deadly cyanide upon mechanical handling. Selection of the sweet type, from the many bitter types in the wild, marked the beginning of almond domestication. The wild almond species are bitter and toxic while domesticated almonds are not. Therefore, domesticated marama beans might have improved characteristics or traits such as shorter time to grow to maturity, many seeds per pod, large seed with approximately a similar size and shape, and lower content of bitter and sour substances which are not strongly perceived and thus not objectionable (Cullis et al., 2018).

Marama bean as a wild legume might respond like most plants to stress (Cullis et al., 2018). Wild legumes frequently developed survival mechanisms using a combination of different traits and responses resulting from growth in dry drought-stricken environments. For example, water stress or drought is known to increase the phytochemical concentration in plant tissues as a defence mechanism (Zobayed, Freen, Kozai & 2007). Therefore, it can possibly be expected to develop new marama bean varieties with improved sensory properties e.g. less bitter off flavour, because the domesticated bean will grow in a lower stress environment and probably produce less phytochemicals instead. Breeders can then distribute new varieties of marama bean seeds and promote its cultivation among rural communities and local farmers in sub-Saharan Africa countries. The cultivation of marama beans will have a lot of advantages because this might lead to the production of large quantities of marama beans that can be commercialized and therefore attract the attention of the food industry able to process the bean into different nutritious products ready for use by consumers. For example, marama beans processed in DMF make it an ideal functional ingredient in the food industry for the production of protein-fibre-rich food such as bread, biscuits, pasta, etc. or to fortify staple cereal-based foods including porridges, samp (Holse, 2010). At the same time, DMF is also GF flour, with properties offering many potential applications in the food industry to develop a number of products that could be targeted specifically at vulnerable groups of people with allergies to wheat products and other legumes as well as coeliac patients. Holse (2010), reported that several aspects of the marama bean may be interesting in regards to the market because marama beans seem to be safe for people suffering from lupine or peanut allergy. Hence, these people could consume products containing marama beans of its flours instead. DMF-CS bread is therefore one possibility to produce a ready-to-use marama bean-based products for vulnerable groups of people and for entire communities.

When manufacturing DMF, the high oil content of marama bean can be an obvious source of pressed oil, which may be used for cooking or in salads. In addition, the high lipid and protein content of marama bean make them an ideal food for undernourished people, and at the same time the bean provides many important micronutrients (Holse, 2010). Another potential aspect of the marama bean as reported by Holse (2010) is that, marama bean grows in the wild and hence is organic, and even when domestication is initiated it may be grown by the principles for organic crop production (Holse, 2010). Hence, marama bean products may be marketed as local organic specialties with an exotic story conveyed on the packaging material. In the same perspective, DMF-CS bread could either be sold in specialty stores for example or be used to make snacks like sandwiches in top exotic restaurant, occasional parties and celebrations in southern African countries where people are willing to try new foods, or might be serve at international congresses, weddings, trade fairs and on international flights as an authentic African bread.

The commercialisation of domesticated marama bean could also potentially generate income and contribute to the alleviation malnutrition in parts of the world where people are food insecure and have little resources. These people including small and large scale farmers, independent rural individuals and families growing marama beans, collectors, processors as well as consumers will all be beneficiaries of the commercialisation project. A good example to consider is rooibos tea, an African indigenous plant (*Aspalathus linearis*) that has been turned from a wild harvested crop into a successful commercial product worldwide (Reinten & Coetzee, 2002). This plant was traditionally used as an herbal tea of the Khoi-San people in South Africa. With commercialisation, rooibos tea has been turned into a large scale agriculture crop and today rooibos tea is popular worldwide as a healthy and tasty tea and as an ingredient in cosmetics because of its anti-mutagenic and antioxidant effects. Exports to European countries has greatly improved the financial situation of many poor communities (van Wyk, 2008).

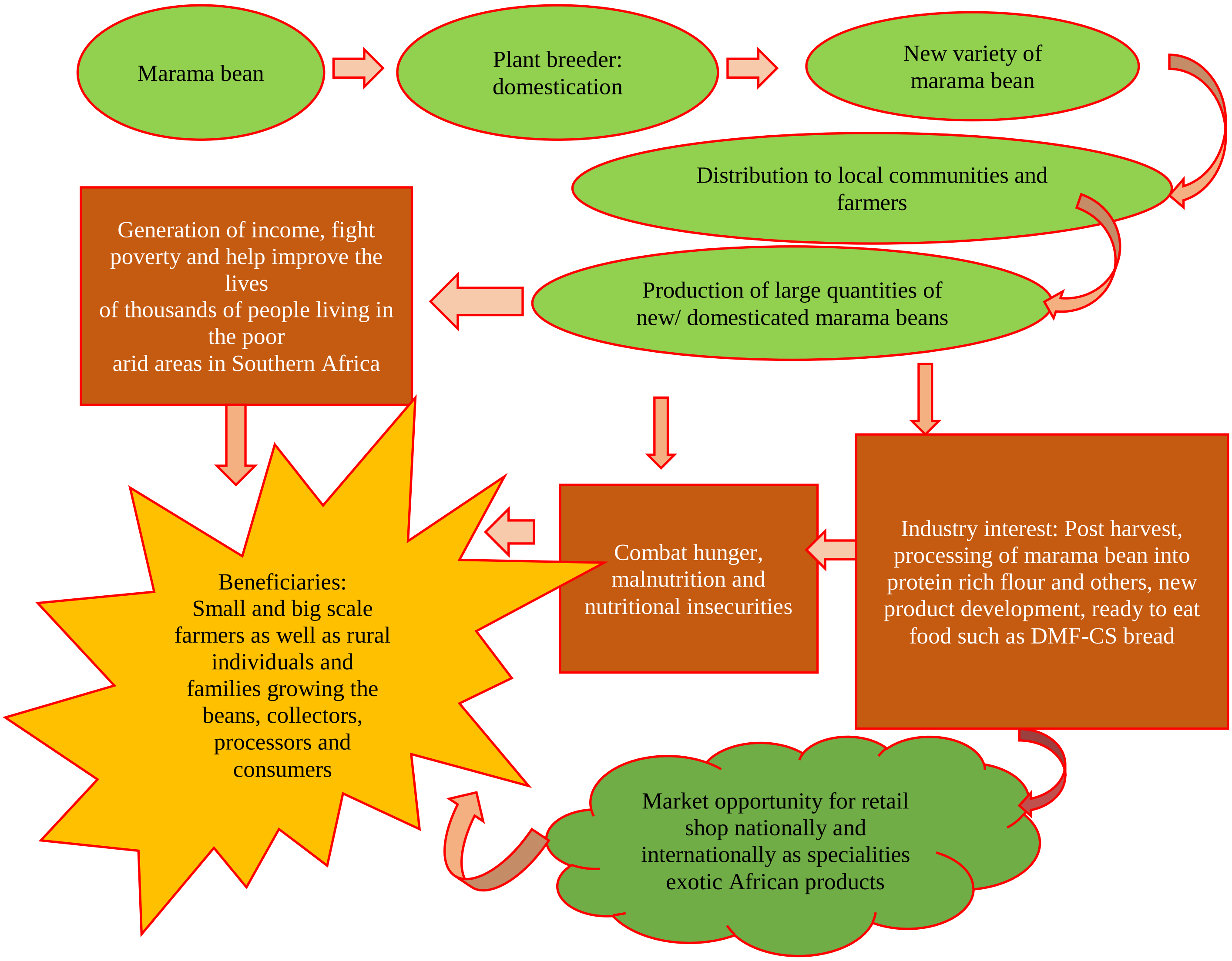


Figure 22: Value chain of the use of marama beans as a functional ingredient for application in gluten-free bread products.

6. CONCLUSIONS

This thesis work contributes new knowledge to the science and technology of gluten-free bread manufacture. To this end a never described before application of defatted flour derived from an indigenous plant in Africa composited with a popular and widely used source of starch namely cassava flour to manufacture bread with textural and appearance characteristics similar to wheat bread was documented. The effect of adding different proportions of DMF to CS on the flour and dough characteristics as well as on the quality of DMF-CS bread was studied. DMF-CS at ratio 33:67 and 57:43 have similar and/or higher protein and dietary fibre content as compared to wheat flour. Composite DMF-CS form doughs with generally similar rheological and gas-holding properties to wheat flour dough. The ratio of 33:67 DMF-CS gives the most desirable results as the composite produced a dough of comparable strength although a less stable dough than wheat flour but with sufficient viscoelasticity and extensibility to retain carbon dioxide gas during fermentation. At ratios of 33:67 and higher 57:43 of DMF-CS, the composite doughs produced breads that presented characteristics desired in the GF bakery industry such as satisfactory specific volume, darker crust, and aerated crumb structure like wheat flour bread. With storage, the crumb texture of DMF-CS breads was springier but less soft as compared to the crumb of wheat bread. The results show that DMF-CS bread looks like wheat bread but with regard to flavour, DMF-CS breads taste and smell differently. The wheat flour bread is blander, while DMF-CS breads have its own authentic sensory profile characterised by starchy, nutty and beany flavour with a sour and bitter taste.

In future studies understanding of the development and evolution of volatile and non-volatile compounds that contribute to the taste and flavour of DMS-CS bread is required. Development and/or modification of the aroma and taste of bread made from DMF-CS to meet the expectations of target consumers can be considered. Attention should also be given to the inclusion of processing technology such as soaking of marama beans in water prior to flour making to reduce the content of saponins and phenolic acids in DMF, which may contribute to the perceived potential undesirable flavour including bitterness and sourness. In addition, the use of alternative masking technology such as sweeteners, salt or pairing the composite bread with a salty relish could be ways of reducing the bitterness in the DMF-CS bread which is a limiting factor for some consumers. Further research could apply technology such as sourdough fermentation, which may have positive effects such as extension of bread shelf life, softer crumb texture, higher specific volume and pleasant flavour. An additional advantage could be improving the handling of DMF-CS composite doughs particularly for the 57:43 ratio by the formation of a more cohesive dough structure.

The findings in the study show that DMF has potential as a new ingredient for the bakery industry and can potentially address the technological challenges in the bakery industry as well as the problems of wheat allergies or intolerance to gluten for coeliacs. DMF can be used for the development of GF bread that has some similar characteristics to wheat bread in terms of nutritional and sensory quality. DMF composite with CS in bread making is a convenient alternative for promoting the use of local crops in sub-Saharan Africa and represents an interesting option for lowering the costs associated with the importation of wheat flour in southern African countries where, for climatic reasons, wheat is not cultivated on a major scale. Thus, DMF makes marama bean an important crop for agricultural diversification and it provides a unique opportunity to combat hunger and nutritional insecurity. More research should target the domestication of suitable varieties of marama bean. The commercialisation of this unique natural resource will assure the availability of DMF on the market and at the same time will also generate incomes and improve the lives of people in the areas where the beans grow. The research in this thesis revealed added more secrets of the potential of the marama bean for novel food product applications. This underutilized wild plant could play a much greater role in future and the research lifts it out of obscurity.

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8. PUPLICATIONS AND CONFERENCE PRESENTATIONS BASED ON THIS WORK

Peer reviewed Journal article

NYEMBWE, P.M., DE KOCK, H.L. and TAYLOR, J.R.N., 2018. Potential of defatted marama flour-cassava starch composites to produce functional gluten-free bread-type dough. *LWT - Food Science and Technology*, 92, pp. 429-434.

Conference Presentations

Patricia Nyembwe & H. L de Kock (2015). Do the sensory properties of marama beans (*Tylosema Esculentum)* hold promise for future utilisation in the food industry? Afrosense Conference, Cape Town, South Africa.

Patricia Nyembwe & H. L de Kock (2017). Crumb texture of defatted marama flour-cassava starch composite breads, 22th SAAFoST Biennial International Congress and Exhibition, Cape Town, South Africa.

Patricia Nyembwe & H. L de Kock (2019). Does bread made from a composite of defatted marama bean flour and cassava starch hold promise for coeliacs? 13th Pangborn Sensory Science Symposium, Edinburg, United Kingdom.

Patricia Nyembwe, J.R.N.Taylor & H. L de Kock (2019). Functional properties of defatted marama flour: a novel ingredient for the bakery industry. 23th SAAFoST Biennial International Congress and Exhibition, Johannesburg, South Africa.