Effect of different banana cultivars on the physico-chemical properties of the flour and the quality characteristics of gluten-free bread.

EFFECT OF DIFFERENT BANANA CULTIVARS ON THE PHYSICO-CHEMICAL PROPERTIES OF THE FLOUR AND THE QUALITY CHARACTERISTICS OF GLUTEN-FREE BREAD

by

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DECLARATION BY CANDIDATE

“I hereby declare that the dissertation submitted for the degree Magister Technologiae: Food Technology, at the Tshwane University of Technology, is my own original work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references.”

IJ KONGOLO

Signature: …….

May 2019

DEDICATION

I dedicate this dissertation to my father Prof Pierre Kongolo Kitala and my mother Stephanie Mbayo Kalenga who have been my source of inspiration and planted the seed of learning in me. They gave me unconditional moral, spiritual, emotional and financial support to achieve my goals.

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ABSTRACT

Dessert bananas are the most important subtropical fruit grown in South Africa. However, approximately 20-50% of all bananas harvested are wasted due to post-harvest losses. The production of unripe banana flour (UBF) is one of the ways to valorise the banana crop and reduce waste. The clear benefits of UBF include a high total starch, high resistant starch and high dietary fibre content. In addition, UBF has been identified as a potential commercial ingredient in various food products with increased resistant starch and gluten-free products. Yet, the proportion of fruits processed into flour and the suitability of cultivars used for processing is still relatively unknown. The board aim of the study was to gain a better understanding of the differences between cultivars in terms of their physico-chemical properties. This was achieved by determining the general characteristics, the functional and thermal properties of UBF from 10 different cultivars grown at the Agricultural Research Council Tropical and Subtropical Crops (ARC-TSC). In addition, the potential use of UBF in gluten-free bread production was investigated using sourdough and hydrocolloids.

In the first part of the study, the general, functional and thermal properties of UBFs produced from dessert banana cultivars (n = 10) cultivated in South Africa were analysed and juxtaposed to wheat and maize flour. The functional properties varied significantly ($p \leq 0.05$) between banana cultivars, and from wheat and maize flours, to various extents. Selected properties of UBFs, wheat and maize flours, ranged respectively for: flour colour index (63.16–76.42, 77.34 and 80.96), paste viscosity (405.5–556.6, 124.7 and 115.6 RVU), gelatinization temperature (64.67–71.21, 71.11 and 69.95 °C), gel firmness (7.24–11.44 × 10− 2 N, 3.49 × 10− 2N and 6.56 × 10− 2 N) and resistant starch content (19.9– 47.4, 2.8 and 2.2% w/w). Multivariate analysis (principle component analysis) showed that the UBFs from different varieties were distinguished from each other based on the pasting temperature. The UBFs were distinguished from both wheat and maize flour based on breakdown and peak paste viscosities. The breakdown viscosity was the most positively related measure to the resistant starch content with a linear regression (R^2= 0.898) indicating a significant role played by granule structure in resistance to enzymatic hydrolysis. The present research demonstrates that the selection of appropriate

dessert banana variety is important when replacing staple flours (wheat and maize) with UBF as a functional ingredient.

In the second part of the study, the development of gluten-free bread containing UBF was investigated. The basic formulation containing 60% UBF (Gros Michel cultivar) and 40% white rice flour. Hydrocolloids (guar gum and xanthan gum) and sourdough (fermentation using *Lactobacillus Plantarum* strain R and A) were added to the gluten-free bread formulation, individually or in combination. The rheological behaviour (Mixolab analysis) of the formulations, the baking characteristics (Volume, height, mass after baking, specific volume, moisture, colour and texture) and sensory evaluation (using a 9-point hedonic scale) of baked bread was assessed. During the development of gluten-free bread containing UBF, the formulation required the addition of more water compared to the wheat formulation. The addition of hydrocolloids (individually or in combination) resulted in the improvement of rheological properties of the formulations. This in turn, after baking, resulted in bread with improved volume (ranging from 18% - 49% increase) and better crumb with more uniform gas distribution. The addition of sourdough improved the quality characteristics of gluten-free bread. The baked bread resulted in improved properties mainly due to the effect of acidification of dough rheological properties (Moroni et al., 2009, Naqash et al., 2017). However, it was noticed that a high amount of sourdough addition resulted in less acceptable bread, particularly in terms of aroma and taste. The sourdough used in combination with hydrocolloids resulted in the best baking performance of the bread, compared to the standard wheat formulation (3.07g/cm^3 and 3.59g/cm^3 for the gluten-free bread and standard wheat bread respectively). However, when subjected to consumer acceptability test, a clear preference for gluten free-bread containing sourdough only (without hydrocolloids) was observed.

This is the first study that investigates the characteristics of UBF from cultivars grown on the same orchard plot in South Africa. These cultivars were subjected to identical environmental conditions, thus reducing variabilities based on environmental factors. This study provides new knowledge that characteristics of flour from banana cultivars from the same genomic group are different

independently of the growing conditions the plant was subjected to. Thus it would be important to select the banana cultivar for a specific food application based on the required properties to obtain the best performance. In addition, it was shown that UBF (Gros Michel) can be used successfully as the main ingredient for the development of gluten-free bread, with or without the use of hydrocolloids and sourdough. However, the best baking performance is obtained when sourdough is used in combination with hydrocolloids. Thus further research into the synergistic effect of the addition of sourdough and hydrocolloids on gluten-free bread containing UBF is being investigated to determine whether or not the improvement observed is dose-dependent.

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LIST OF ABBREVIATIONS

ANOVA	Analysis Of Variance
ARC-TSC	Agricultural Research Council-Tropical and Subtropical Crops
AS	Available Starch
CD	Celiac Disease
DP	Degree Of Polymerisation
DSC	Differential Scanning Calorimetry
FAO	Food and Agriculture Organization
GOPOD	Glucose Oxidase/Peroxidase
LAB	Lactic Acid Bacteria
MEA	Malt Extract Agar
MRS	De Man Ragosa And Sharpe
OHC	Oil Holding Capacity
PC1	First Principle Component
PC2	Second Principle Component
PCA	Plate Count Agar
PPO	Polyphenol Oxidase
RBCA	Rose Bengal Chloramphenicol Agar
RDS	Rapidly Digestible Starch
RS	Resistant Starch
RVA	Rapid Visco-Analyser
RVU	Rapid-Visco Unit
SDS	Slowly Digestible Starch
TS	Total Starch
UBF	Unripe Banana Flour
WHC	Water Holding Capacity

CHAPTER 1 : GENERAL INTRODUCTION

1.1 BACKGROUND AND MOTIVATION

Bananas are the second largest fruit crop produced in the world after citrus, contributing ±16% of the total fruit production (FAO, 2013) and are considered a staple food in many countries as they are an important source of energy (Ovando-Martinez et al., 2009). There are over 1200 cultivars of bananas, each having unique genetic and agronomic characteristics (Pillay & Tenkouano, 2011). Edible banana fruit cultivars are a genetic complex based on two wild diploid species originating from South-East Asia: *Musa balbisiana* (BB), a homogeneous hardy plant with a massive pseudo-trunk and *Musa acuminata* (AA), which is highly polymorphous, with spindly plants that grow in clumps (Simmonds & Shepherd, 1955). Banana cultivars are utilized and consumed in different ways. Traditionally cooking bananas (plantains) are consumed cooked (fried, steamed, roasted or baked) while dessert bananas are consumed raw after ripening (Pillay & Tenkouano, 2011). However, worldwide post-harvest losses of bananas are high (approximately 20-50%), due to the fact that all banana cultivars are highly perishable (Maqbool et al., 2010). Appropriate affordable technologies to extend the shelf life of harvested mature (but green) and ripe bananas is limited, especially for rural and small to medium size farmers, where infrastructure is inadequate. Thus, further processing of the harvested bananas is required to extend the shelf life and decrease post-harvest losses.

The production of banana flour, using mature-green (unripe) bananas, is the major method used traditionally in Africa to further process bananas into a shelf-stable product (Aurore et al., 2009). Mature-green bananas have been identified as ideal raw material for the production of banana flour, as the flour is composed of high total starch (±70.0% w/w), resistant starch (±17.5% w/w) and dietary fibre (±14.5% w/w) (Juarez-Garcia et al., 2006). Because of the composition of mature-green banana flour, it has great commercial potential as an ingredient in various food products for increasing the content of resistant starch and dietary fibre in foods. Unripe banana flour has been used for the development of innovative products including gluten-free products such as slowly digestible biscuits, low glycaemic

index pasta (Aparicio-Saguilán et al., 2007) and high-fibre bread (Juarez-Garcia et al., 2006). These are just a few examples of the economic potential of banana flour in new food product development formulations. Compared to commonly used cereals such as wheat and maize for food production, little knowledge is available on the effect of different banana cultivars on the physico-chemical properties of the flours and further processing into foodstuffs (including cooking/baking performance, sensory quality, and shelf life).

Research on the commercial potential and further processing of a wide variety of different banana cultivars is limited. To date, the majority of published research is mainly on the use of banana cultivars for banana flour production, and its use in food applications has been done on a small selection of cultivars namely: *Musa cavendishii* (Wang et al., 2012, Bezerra et al., 2013), *Musa acuminata balbisiana Colla* cv. *awak* (Choo & Aziz, 2010), *Luvhele mabonde* (Anyasi et al., 2015), and *Musa balbisiana* cv *muomvared* (Anyasi et al., 2015). The cultivars studied were selected purely on their availability at the time of the research conducted (i.e. locally available). The Agricultural Research Council-Tropical and Sub-tropical Crops (ARC-TSC) is one of the 56 institutions worldwide involved in the conservation of banana perennial herb. The ARC-TSC hosts a number of experimental farms in South Africa, where more than 60 cultivars of bananas both dessert and plantains are grown on small plots. The trees have been adapted for different growing conditions in South Africa and are used for the conservation of genetic material, for breeding programs, to conduct research on plant productivity characteristics, as well as the identification of various industrial applications.

1.2 PROBLEM STATEMENT

Although processing fresh bananas into shelf-stable flour is recognized as a way of preserving the fruit, the suitability of various banana cultivars for further processing (into flour and foods) is still relatively unknown (Aurore, Parfait & Fahrasmane, 2009). Some studies on banana flour, using a limited number of cultivars, have demonstrated that the cultivar used for developing banana flour can have an effect on the final composition (namely total sugar, dietary fibre and resistant starch) and functional properties of the flour, as well as sensory quality and shelf life of the

resultant processed food (Da Mota et al., 2000, Anyasi et al., 2015). Hence, research on the factors stated above could be valuable to improve further processing methods in order to prepare flour of acceptable quality and guide new food product development using banana flour as a functional ingredient.

In this study, the composition and functional properties of flour prepared from 10 different banana cultivars were evaluated, and the potential of using unripe banana flour in bread was determined.

1.3 OBJECTIVES

- To analyse the moisture, colour, bulk density, ash and total starch (resistant starch and available starch) of different green banana flours (10 cultivars), compared to wheat and maize flour.
- To determine the functional properties: water and oil holding capacity, foaming and emulsifying capacity and stability of different green banana flours (10 cultivars), compared to wheat and maize flour.
- To determine the thermal and rheological (pasting) properties, and set gel texture of the gels for the different banana flours (10 cultivars), compared to wheat and maize flour.
- To determine the effect of the addition of selected hydrocolloids on dough rheological properties of gluten-free bread dough prepared from green banana flour (1 cultivar), compared to wheat flour.
- To develop and assess the baking performance and quality characteristics of gluten-free (sourdough) baked bread prepared from green banana flour, compared to wheat based products.

1.4 HYPOTHESIS

It is expected that the composition and functional properties of flour prepared from 10 different cultivars will vary. A limited number of studies conducted on banana flour have shown that banana cultivars can have an effect on the composition and functional properties of the flour (Da Mota et al., 2000, Anyasi et al., 2015). However, there might be considerable differences between the rheological

properties of the banana flours compared to wheat and maize flour due to variations in the major components (such as starch content and type, proteins and types of dietary fibre) which contribute to the rheological properties of these flours.

It is expected that UBF can be successfully used in bread due to similarities in properties with wheat flour. However, due to the lack of gluten, it will be necessary to use additives such as hydrocolloids as they influence the rheological properties of the dough by increasing the water absorption and elasticity (Torbica et al., 2010). Furthermore, the addition of sourdough to the bread formulation will improve the baking properties of the baked bread as sourdough has been used to improve gluten-free flours (Moroni et al., 2009).

1.5 FORMAT OF THE STUDY

This section presents an overview of the study. The study was divided into three different stages that are represented in Figure 1.1. The first stage focused on sample collection and flour preparation, the second phase details the physico-chemical characteristics of the flours compared to wheat and maize. And the last phase focus on the development of gluten-free bread from one banana flour cultivar.

The results are presented into two research working chapters for better understanding of the findings and a final chapter that summarize the findings and give recommendations. The first research working chapter gives results of all flour related analysis and the second chapter focuses on the application in the development of gluten-free bread.

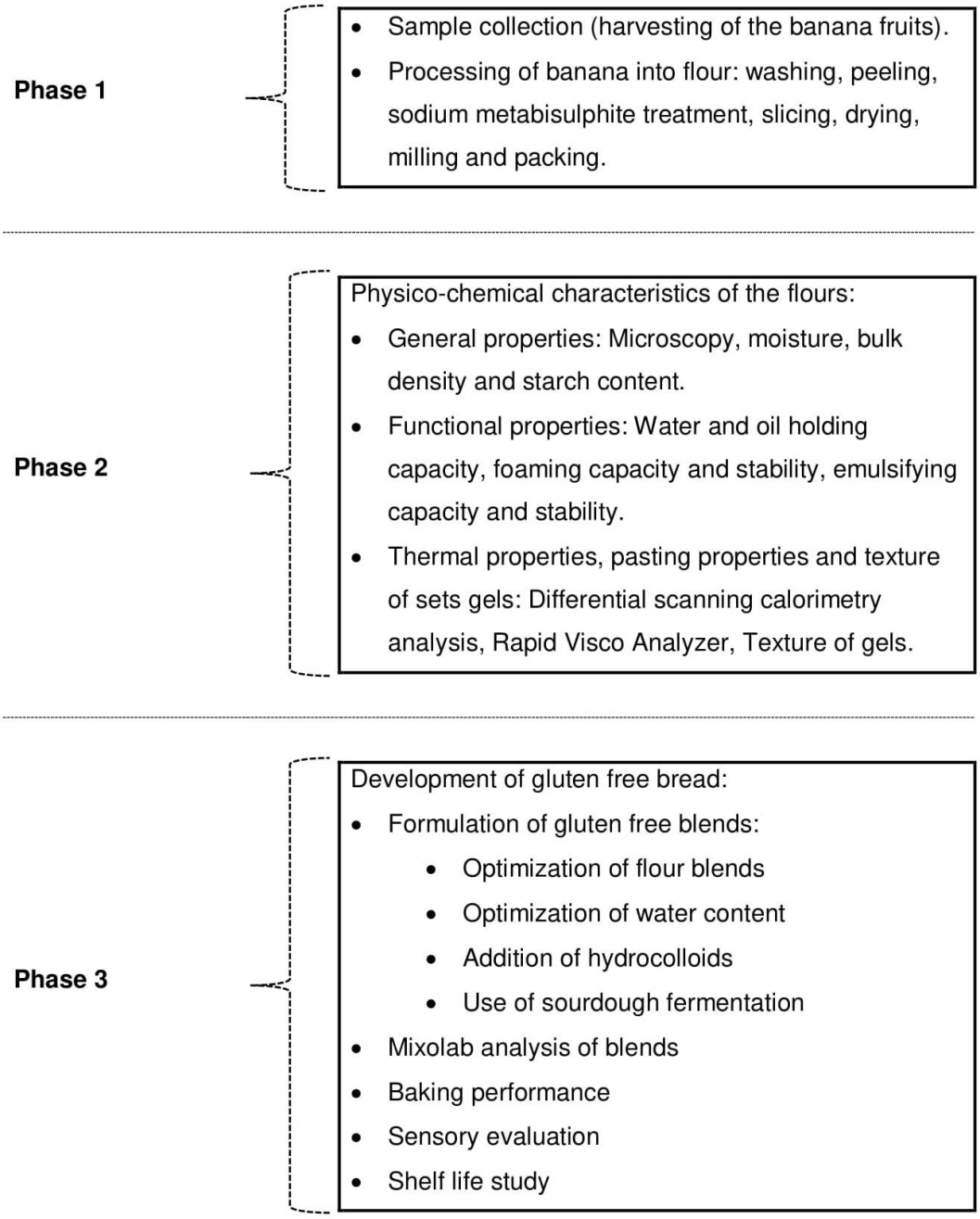

Figure 1.1 Experimental design of the different phases of the study

CHAPTER 2 : LITERATURE REVIEW

This chapter presents a brief overview of relevant literature across the scope. This review starts by section 2.1 giving an overview of banana fruit, its ripening process, consumption, and processing. Section 2.2 emphasizes on starch, functional properties of flours, thermal and pasting properties of flours. Section 2.3 details gluten-free products, gluten intolerance, the ingredients used, as well as technological challenges in their manufacturing. Lastly, section 2.4 summarizes gap in some of the work that has been published to date.

2.1 BANANA FRUIT AND UTILIZATION

2.1.1 Origin and crop cycle

The banana plant constitutes one of the largest fruit-bearing perennial herbaceous plants (ranging from 2-9 m in height), which is typically grown in the equatorial and tropical regions of the world (Morton, 2013). Bananas belong to the genus *Musa* which is part of the family Musaceae. The genus *Musa* is further divided into three sub-genus based on chromosome number (diploid (AA, BB, AB), triploid (AAA, AAB, ABB) or tetraploid (AAAA, AAAB) genomic groups), pseudo stem stature, inflorescence characteristics and seed morphology (Cheesman, 1947, Pillay & Tenkouano, 2011). The morphology of a typical banana plant, bearing fruit, a wild banana fruit, as well as a domesticated or cultivated banana, is illustrated in Figure 2.1.

Worldwide, there are approximately 1200 banana plant varieties that produce seedless, fleshy fruit (bananas) which have been domesticated by subsistence farmers and are used for food across Asia and Africa (Aurore et al., 2009). These domestic plants have evolved from the wild type, seedy, non-edible plant varieties. All banana varieties today originated in South-East Asia, from two wild diploid species ($2n = 22$), namely *Musa acuminata* (AA) and *Musa balbisiana* (BB). Natural occurring mutations and human selection of edible diploids species have also resulted in a vast diversity of triploids ($3n = 33$) and tetraploids ($4n = 44$) edible banana varieties. Of the different varieties, the triploids have more vigorous growth,

larger fruit, and have generally replaced the original diploid varieties within their indigenous environments (Simmonds & Shepherd, 1955, Simmonds, 1962).

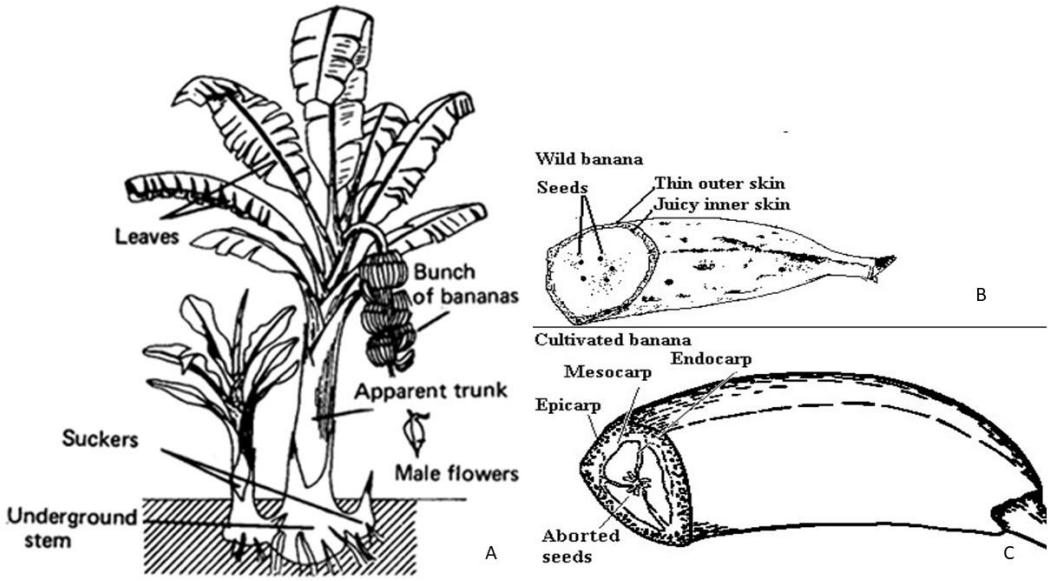

Figure 2.1 The morphology (A)[1] of a typical banana plant, (B)[2] wild banana fruit and (C)[2] cultivated banana fruit

In addition, fruit harvested from the various plant varieties, are classified into dessert banana (eaten after ripening, without cooking), and cooking bananas (eaten after ripening, but need to be cooked), regardless of the genomic group (Aurore et al., 2009). Figure 2.2 shows a large variety of edible banana fruit used for food being sold on a market.

[1] http://www.fao.org/docrep/006/t0308e/T0308E03.htm . [Accessed 20/06/2018]
[2] http://www.uq.edu.au/_School_Science_Lessons/BaProj.html . [Accessed 12/12/2018]

Figure 2.2 Different bananas varieties sold on a local market[3]

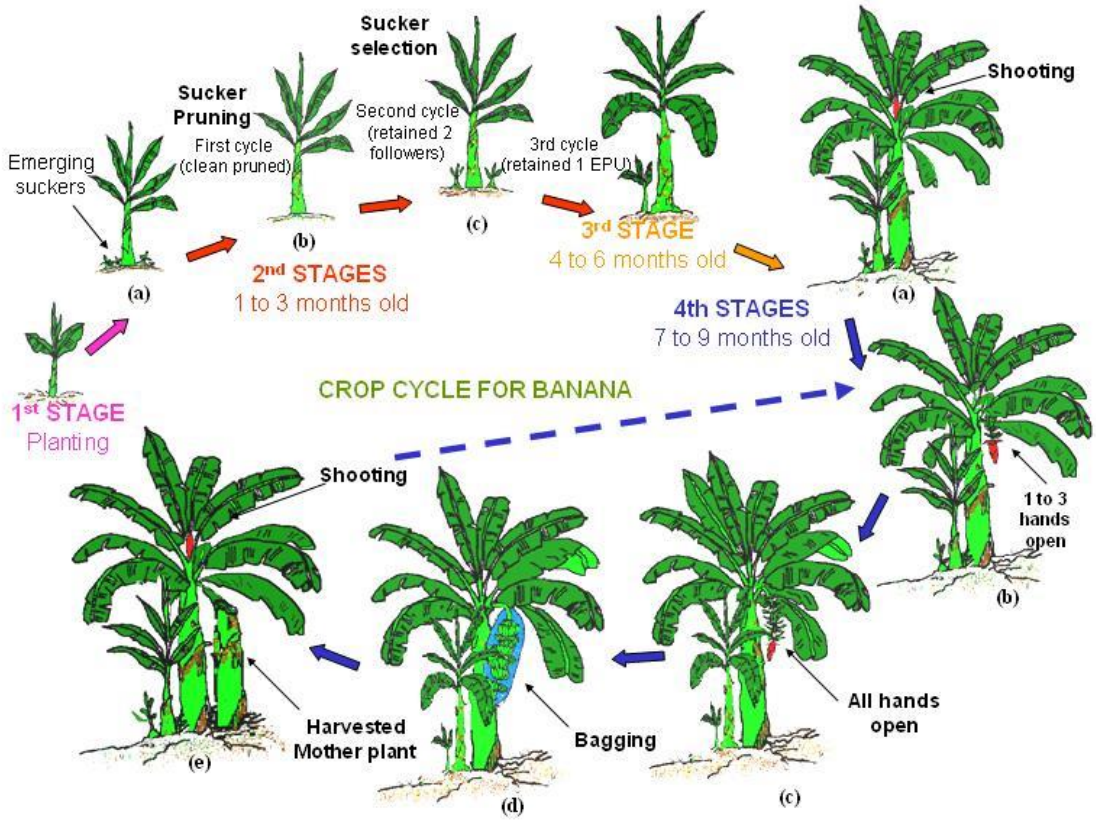

Figure 2.3 Banana crop life cycle from planting to harvesting of the fruits[4]

As illustrated in Figure 2.3, the life cycle of banana crop takes 9-14 months after planting to harvest one fruit bunch of 20-40 kg from each plant. The first stage is the planting of the banana tree which produces suckers next to the mother pseudo

[3] http://www.crop-mapper.org/banana_test/images/banana%20at%20market.jpg. Accessed [25/05/2016]
[4] http://4.bp.blogspot.com/_RrKF5Hd0xis/RuisMPnZl8I/AAAAAAAAAD8/VaIQzkhKvkI/s1600-h/BananaG2.JPG . Accessed [12/12/2018].

stem at the base of the plant, with strong vascular connection to the mother. These can be removed from the parent and planted separately in a process called pruning, where they rapidly develop new leaves and root systems, allowing rapid vegetative propagation and multiplication (2nd stage Figure 2.3). In cultivation, unwanted suckers are removed to avoid weakening the parent plant. As seen in Figure 2.3 (4th stage), the plant produces a flower stalk at the top which is actually a cluster of multiple flowers protected by the large leaf (Heslop-Harrison & Schwarzacher, 2007). The flower stalk blooms, it opens up, revealing first white flowers and then small bananas which grow over time. The fruits are harvested green but mature. Maturity of the fruit is assessed by measures such as change of peel colour from dark green to pale green, the disappearance of angularity, finger length, and diameter (Surya Prabha & Satheesh Kumar, 2015). The plant is then cut to ground level, the leaves removed and destroyed to control disease, and a side sucker allowed to grow up to produce the next crop (Heslop-Harrison & Schwarzacher, 2007).

2.1.2 Ripening of bananas

Banana is a climacteric fruit that can ripen naturally at ambient temperature but which is usually ripened under a controlled atmosphere in ripening houses. Different biochemical and physiological changes take place during the ripening process, therefore it is necessary to understand the ripening process of banana in order to develop the best processing method (Pillay & Tenkouano, 2011).

2.1.2.1 Climacteric nature of respiration

Respiration is the oxidative breakdown of complex substrates like carbohydrates to simpler molecules like carbon dioxide and water (Hubbard et al., 1990). The glycolytic pathway which occurs in the cytoplasm of the cell is the first step of respiration followed by tricarboxylic acid cycle, pentose phosphate pathway and electron transport system. Fruits can be classified into two broad categories: climacteric and non-climacteric according to their respiration pattern (Figure 2.4). In climacteric fruit, respiration rate shows a decreasing trend to the lowest value termed as pre-climacteric minimum followed by a sharp rise in respiration rate to

the climacteric peak. This sudden rise is called respiratory climacteric followed by a decrease in respiration rate in the senescence period. Because banana is a climacteric fruit, it shows a sudden increase of respiration rate and consequently a burst of ethylene production at the onset of the climacteric peak. Enzymes play a key role in the climacteric rise in respiration rate during glycolytic pathway (Hubbard et al., 1990, Lohani et al., 2004).

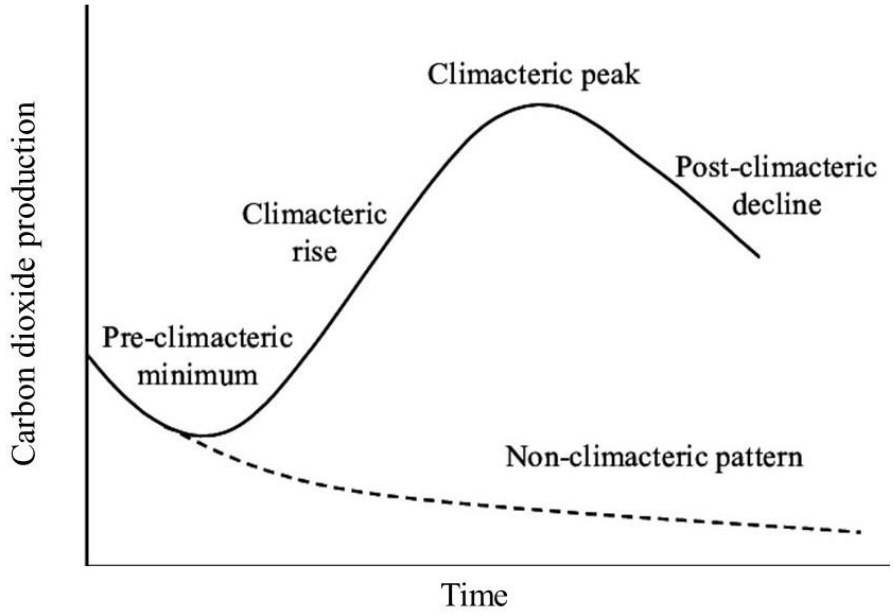

Figure 2.4 Climacteric and non-climacteric ripening process of fruits (Saltveit, 2004)[5]

2.1.2.2 Effect of ethylene

Climacteric fruits ripen in the presence of ethylene which is a colourless, odourless, tasteless gas that has many effects in plant physiology and is active in such a small amount (part per million) that it is considered as a plant hormone. Ethylene is transported by diffusion from its production site to the active site. Ethylene diffusion gradients are determined by the surface to volume ratio, diffusion resistance, and ethylene production rates (Abeles et al., 1992). A thorough understanding of the biochemical basis of ripening is essential to ensure predictable ripening and good quality ripe fruit (Yang et al., 2008). Different biochemical changes that take place during ripening are described in Table 2.1.

[5]https://www.researchgate.net/figure/The-climacteric-pattern-of-respiration-in-ripening-fruit_fig1_267417141.[Accessed 13/05/2019].

Table 2.1 Biochemical changes during banana ripening, adapted from Sen et al., (2012)

Composition	Summary of biochemical changes during ripening
Moisture	Moisture content in pulp increases because of the respiratory breakdown of starch to sugar and migration of water from peel to pulp.
Carbohydrate	Starch decreases (20-30 to 1-2%). Sugar increases (1-2 to 15-20%). The main starch degrading enzymes are phosphorylases, α-amylase, β-amylase, and α-1, 6-glucosidase
Organic acids	Organic acid content increases. Oxalic acid which is responsible for the astringent taste of unripe banana undergoes significant decarboxylation by the action of oxalate oxidase. During the climacteric, malic acid becomes the major acid (65% of total acidity). pH decreases from 5.4 ±0.4 to 4.3±0.3.
Proteins	Increases from 1-2.5% to 3.8-4. 2%. Glutamate oxaloacetate transaminase and glutamate pyruvate decarboxylase, involved in aspartate and alanine synthesis respectively are found to be maximum at the initiation of climacteric.
Lipids	No substantial changes. In the phospholipid fraction, the proportion of linolenic acid increases and the proportion of linoleic acid decreases. Increases in the unsaturation of the phospholipid fraction result in increased fluidity in cellular membranes during ripening.
Pectin	Insoluble protopectin is converted into soluble pectin. Main pectin degrading enzymes are pectin methyl esterase, polygalacturonase, cellulase, and hemicellulase.
Phenolic compounds	Main phenolic compounds are 3, 4-dihydroxyphenylethylamine and 3, 4-dihydroxyphenylalanine. Main browning causing enzymes are polyphenol oxidase and phenylalanine ammonia lyase. Astringency is caused by tannins of the banana peel which gradually disappears with fruit ripening due to tannin depolymerisation.

2.1.3 Consumption and banana based products

Bananas and plantains are considered a staple food in many countries in the world and one of the most important sources of energy (Ovando-Martinez et al., 2009). Bananas and plantains are consumed in different ways in different countries. Traditionally plantains are consumed cooked (fried, steamed, roasted, or baked) while desert bananas are raw in the last stage of ripening process (Pillay & Tenkouano, 2011). The need for processing banana has been slow compared to other crops and has been stimulated by the high amount of postharvest losses (Muyonga et al., 2001, Aurore et al., 2009). The climatic conditions where bananas are grown also account for the losses with optimum storage conditions between 13-14 °C. The shelf life of green, as well as ripe bananas, is very limited due to the lack of appropriate technology.

However, banana is now processed into a few products that are consumed in the world. Dehydration has been used traditionally in Africa to process bananas into flour. In addition to flour preparation, several new products have been developed such as banana chips (either dried to produce flour or fried into oil) (Muyonga, 2000, Molla et al., 2009), banana puree (sterilized ripe banana puree) (Barrett et al., 2004), banana powder (from ripe banana) (Barthakur & Arnold, 1990), banana figs (sun drying of bananas) (Cano-Chauca et al., 2004, Leite et al., 2007), banana flakes, banana jam, banana juice, banana alcohol (beer or wine), banana vinegar, banana sauce, banana fibre, and banana starch. One of the limitations of the different studies is that varieties differences (characteristics) and origin of the fruit used (influence of environmental factors) were not taken into consideration to determine the suitability for specific applications. Banana flour, which can be produced either from ripe or unripe bananas is one of the strategies to increase the utilization of banana, will be dealt with in more details in the following section.

2.1.4 Unripe banana fruit processing into flour

Green banana flour is made from fully mature fruits in the first stage of the ripening chart. The fruit at this stage is still fully green (Figure 2.5). The fruit with the correct amounts of starch and sugars should be used to obtain a good quality flour. If

immature fruits are used, they produce flour which is bitter and astringent due to high tannin content (Pacheco-Delahaye et al., 2008). One strategy to increase the utilization of banana and plantain includes the production of flour from ripe and unripe fruit and to incorporate the flour into various innovative products such as slowly digestible cookies (Aparicio-Saguilán et al., 2007), low glycaemic index pasta (Wang et al., 2012), high-fibre bread (Juarez-Garcia et al., 2006), edible films (Sothornvit & Pitak, 2007), thickeners in soup (Muyonga et al., 2001) and fat replacers in sausages (Alves et al., 2016). Many different methods to prepare flour from unripe bananas have been reported (Ovando-Martinez et al., 2009, Rodríguez-Marín et al., 2013).

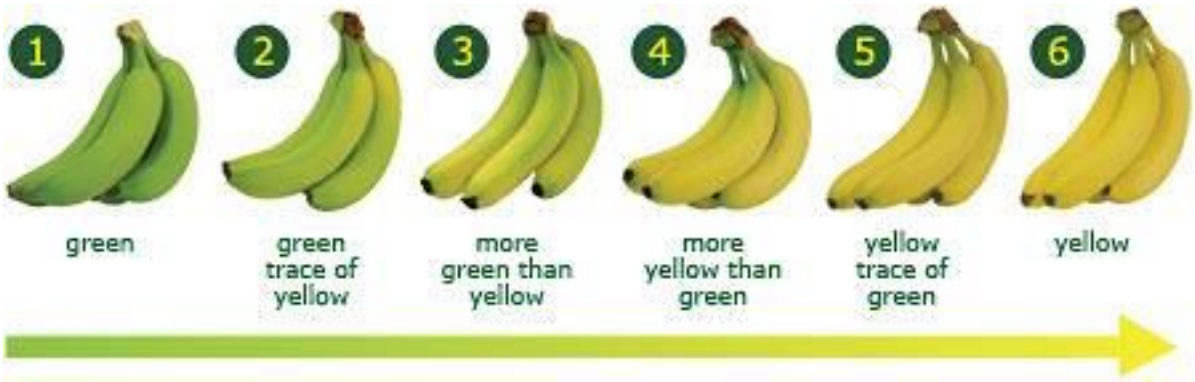

Figure 2.5 Ripening stage of bananas[6]

As a method to preserve the fruit, the production of banana flour is widespread in Africa where traditionally, the banana is peeled, cut into small pieces and sun-dried for a few days (Aurore et al., 2009). The dried chip is then ground into flour in a mortar and pestle. Miniature solar dryers have been developed and are used by small scale farmers and producers. The use of a solar dryer ensures better quality products due to uniform drying, and improved microbiological quality of the chips, compared to open sun drying methods. However, unless the banana is treated for enzymatic browning, the flour obtained is usually brown in appearance, which is undesirable to the consumer (Cano et al., 1997). Enzymatic browning is caused by the action of endogenous enzymes, such as polyphenol oxidase and peroxidase, which is released from the cells during peeling and slicing, and exposure to oxygen (Cano et al., 1997). Polyphenol oxidases (PPOs) are a group of copper proteins,

[6] http://www.jnfoxandsonsltd.co.uk/.[Accessed 20/06/2016]

that catalyse the oxidation of phenolic compounds to quinones, which produce brown pigments in wounded tissues (Queiroz et al., 2008).

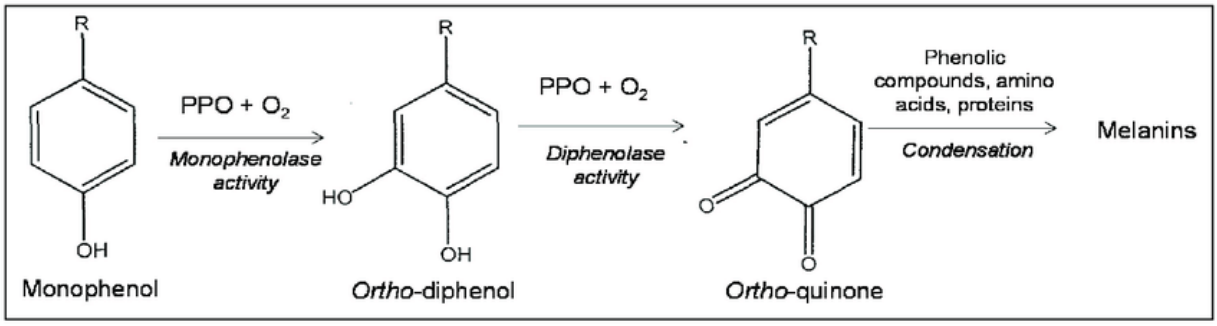

Figure 2.6 Simplified process of enzymatic browning catalysed by PPO (Taranto et al., 2017)

The simplest method of controlling enzymatic browning is by immersing the peeled fruit in ambient water before processing (Talburt & Smith, 1967). However, this method is limited as the fruit will brown on re-exposure to oxygen, once the banana is sliced into chips for drying.

Table 2.2 Various methods, dips and heat treatment used to reduce enzymatic browning of bananas during flour production.

Methods	Description	Reference
Ascorbic acid (dip)	1.0-2.0% v/v solution for 10 min	(Anyasi et al., 2017)
Citric acid (dip)	0.1-0.5% v/v solution for 5 min	(Tribess et al., 2009, Alkarkhi et al., 2011, Rayo et al., 2015)
Lactic acid (dip)	1.0-2.0% v/v solution for 10 min	(Anyasi et al., 2017)
Potassium metabisulphite (dip)	1% w/v Solution for 15 min	(Pelissari et al., 2012)
Sodium metabisulphite (dip)	0.02% w/v solution for 30 min	(Ayo-Omogie et al., 2010)
Blanching (heat treatment)	Soaked in boiling water for 5 min, Steaming for 7 min.	(Muyonga et al., 2001, Campuzano et al., 2018)

Various methods have since been developed to reduce enzymatic browning of bananas before processing, these methods are summarized in Table 2.2. These mechanisms act on one or more of the essential components necessary for the reaction to occur: oxygen, enzyme, copper or phenolic compound substrate (Queiroz et al., 2008). Typically methods can be classified based on the inhibition

mechanism involved, such as reducing agents (sulphur-containing agents), acidulants (ascorbic acid, citric acid, and lactic acid), and blanching (heat treatments) (Queiroz et al., 2008). The principles of these mechanisms are summarized below:

- **Sulphur-containing agents:** Sulphur containing agents (including sodium metabisulphite dip and sulphur dioxide), act on the unstable quinones as a reducing agent, regenerating the polyphenols, which are stable and colourless, preventing the formation of brown pigments (Queiroz et al., 2011). However, studies have shown that sulphur-containing agents are potentially allergenic and cause adverse health effects in sensitive subjects (Freedman, 1977, Tarlo & Broder, 1989, Simon, 1992). Hence alternative methods are used.
- **Acidulants:** Acidulants (including ascorbic, citric and lactic acid) are used for changing the pH of the medium (lower than pH 4.5). The reduced pH, decreases the polyphenol oxidase enzymatic activity (optimum pH ranges from 5 to 7.5), preventing the formation of quinones, and hence the brown pigments (Yoruk & Marshall, 2003). Ascorbic acid (AA), also known as vitamin C, is frequently used to prevent enzymatic browning in fruits and vegetables (Jang & Moon, 2011, Anyasi et al., 2017). This is due to the fact, AA is a powerful antioxidant and organic acid, which results in two inhibition methods being employed during the prevention of enzymatic browning (Ünal, 2007).
- **Blanching**: Blanching (submersion in hot water, or steaming), is a mild heat treatment applied to the peeled (or unpeeled) fruit. The heat denatures the polyphenol oxidase enzyme, preventing the formation of quinones and resulting brown pigment (Cano et al., 1997, Wang et al., 2017). However, blanching has a negative effect on the proximate composition of the unripe banana flour, as nutrients (starch, proteins, and minerals) can be leached from the fruit (Muyonga et al., 2001). In addition, the functional properties (including pasting and gelling properties) can be affected due to partial gelatinization of the starch in the final flour (Muyonga et al., 2001, Ünal, 2007, Campuzano et al., 2018). However, in some circumstances the altered functional properties are desirable. According to Muyonga *et al* (2001), the use of flour obtained by steaming of the banana

fruit before dehydration is beneficial in the production of food products for infants and supplements where lower viscosities are desirable.

2.1.5 Unripe banana flour composition

The nutritional composition of unripe banana flour includes total carbohydrates (ranging from 86.92%), moisture (ranging from 4.17-7.75%), protein (2.6-4.43%), minerals (1.804%) and trace amounts of fat (0.34 -2.7%) (Table 2.3).

Total carbohydrates consist of multiple nutrients, including total fibre (8.490%), starch (78.43%) and trace amounts of simple sugars. The composition of unripe banana flours is dependent on the ripening stage at which the fruits are harvested for processing, as previously discussed (Section 2.1.3)

Of great interest, unripe banana flour is reported to contain significant levels of resistant starch content (ranging from 17.50 to 48.99%) (Juarez-Garcia et al., 2006, Menezes et al., 2011). According to Lockyer and Nugent, (2017), unripe banana fruit, contains the highest level of resistant starch, compared to other fruits. Resistant starch (RS) is defined as that fraction of starch which is not digested in the small intestine, acting similarly to that of dietary fibre (Sajilata et al., 2006). It is the difference between total starch (TS) obtained from homogenized and chemically treated sample and available starch (Nissar et al., 2017).

Table 2.3 Proximate composition of unripe banana flour from different cultivars

Cultivar	***Musa acuminata var Nanicao*** (Menezes et al., 2011)	***Musa acuminata var Nanicao*** (Da Mota et al., 2000)	***Musa paradisiaca L.*** (Ovando-Martinez et al., 2009)	***Musa paradisiaca L.*** (Juarez-Garcia et al., 2006)
Components(g/100g)				
Totals starch	76.77 ± 0.54	76.10 ± 3.70	73.70 ± 1.70	73.4 ± 0.92
Resistant starch	48.99 ± 0.44	ND	42.54 ± 0.43	17.5 ± 0.15
Protein	3.60 ± 0.19	2.60 ± 0.20	4.43 ± 0.90	3.30 ± 0.40
Fat	0.89 ± 0.04	0.82 ± 0.47	0.34 ± 0.02	2.70 ± 0.38
Ash	3.14 ± 0.02	3.00 ± 0.06	2.67 ± 0.01	4.70 ± 0.13
Moisture	6.90 ± 0.09	4.17 ± 0.42	7.75 ± 0.12	7.10 ± 0.05

ND = not determined

2.2 PHYSICO-CHEMICAL PROPERTIES OF FLOUR

The physico-chemical properties of flour are dependent on the starch composition and content of the flour due to the fact that starch is the component found in flour in the greatest amount. In this section, an overview of starch structure and classification is provided to ease the understanding of the functional properties.

2.2.1 Starch

2.2.1.1 Structural units and granules

Native starch consists of two types of glucose polymers, namely: amylose (normally 20-30% of the total starch) and amylopectin (normally 70-80% of the total starch) (Copeland et al., 2009). The starch is stored in plants tissues, in the form of starch granules (Coultate, 2016). Tissues containing starch granules include roots, tubers, bulbs, fruits, and endosperm of seeds (BeMiller & Whistler, 2009).

Amylose is essentially a linear polymer of glucose molecules (Figure 2.7). The amylose polymer chain consists mainly of glucopyranose units linked through α (1→4) glycosidic linkages. On average, amylose has a molecular weight range of approximately 10^5 - 10^6 g/mol which corresponds to the degree of polymerization (DP) of 1 000 to 10 000 glucose units. Less than 0.5% of the glucose units in amylose have α(1→6) linkages, resulting in a low degree of branching (Copeland et al., 2009).

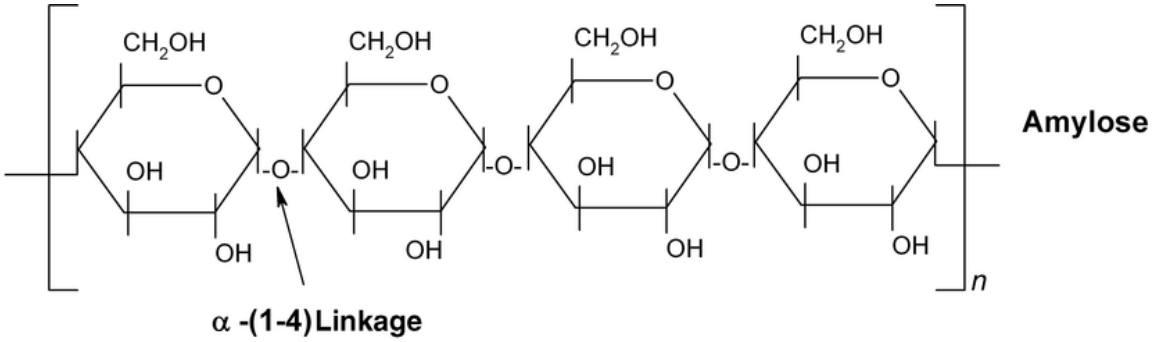

Figure 2.7 Chemical structure of amylose (Visakh et al., 2012)

Amylopectin is a highly branched polymer of glucose molecules (Figure 2.8), containing both linear chains (linked through α 1-4 glycosidic linkages) and α(1-6) glycosidic linkages, which results in branching points, giving the molecule a tree-like structure (Copeland et al., 2009) (Figure 2.9 b). The α (1-6) glycosidic linkages constitute 5% of the amylopectin molecule. Due to the linkages, amylopectin is a much larger polymer, compared to amylose, with a molecular weight of 10^8 g/mol, and a degree of polymerization that may exceed one million glucose units.

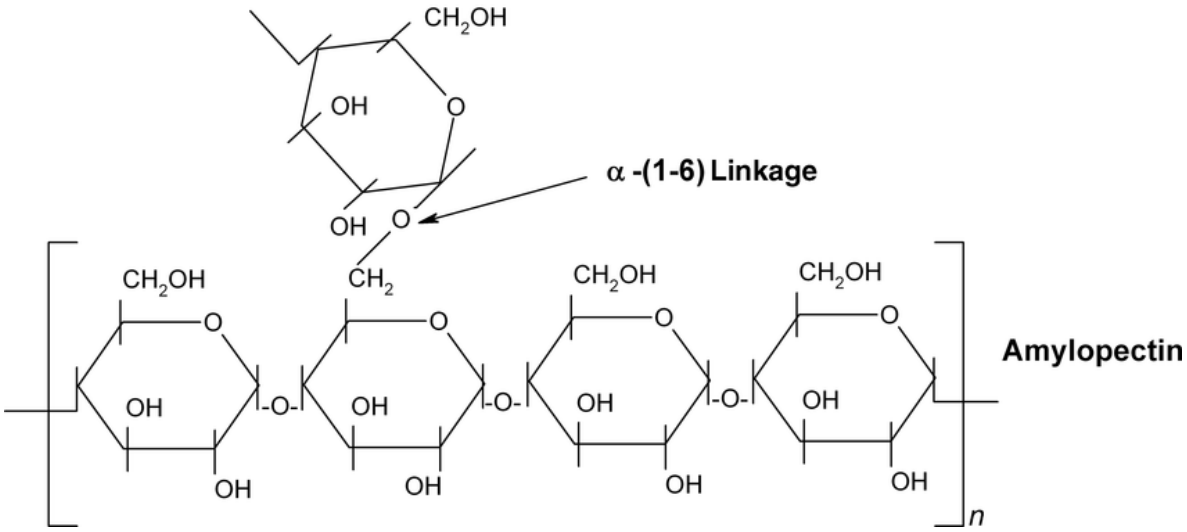

Figure 2.8 Chemical structure of amylopectin (Visakh et al., 2012)

A single amylopectin molecule consists of a branched polymer with different types of polymer chains, which are classified according to their pattern of substitution, namely: A-chains which are defined as unsubstituted (no additional chains linked to it); B-chains which are substituted by other chains (these are major chains, onto which A-chains are linked); and a single C-chain, which carries the reducing glucose unit, and is situated on the centre of the starch granule (Figure 2.9 B).

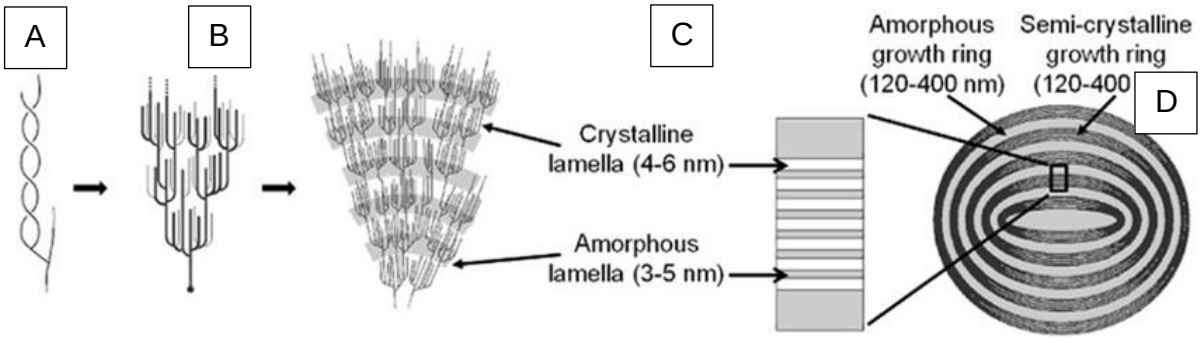

Figure 2.9 The structure of amylopectin and the starch granule (Coultate, 2016)

Starch granules have a hierarchical structure that can be observed readily by light and electron microscopy. Multiple concentric layers of so-called growth rings of increasing diameter extend from the hilum (the centre of growth) towards the surface of granules (Figure 2.9 d). The concentric growth rings, in turn, contain alternating crystalline and amorphous regions of higher and lower density, respectively (Buléon et al., 1998). The crystalline layers are considered to be formed mainly by amylopectin chains packed into a crystalline lattice, whereas the amorphous layers contain the amylopectin branching points and single amylose molecules (BeMiller & Whistler, 2009).

Table 2.4 Starches from different botanical origins

Source	Amylose content (% total starch)	Diameter range(μm)	Shape	References
Maize native	25 – 28	2 – 30	Polyhedral and rounded	(Buléon et al., 1998, Singh et al., 2003, Waliszewski et al., 2003, Zhang et al., 2005, BeMiller & Whistler, 2009)
Maize waxy	0.5	2 – 30	Round polygonal	
Wheat native	25 – 29	16 – 25 0.5 – 16	Lenticular Spherical	
Potato native	18 – 21	5 – 100	Large oval or cuboidal	
Banana native	16 – 40	6 – 80	Irregular in shape, elongated ovals or sphere	

Starch granules range in size from 1 to 100 μm diameter, and the shape can vary from polygonal, spherical, and lenticular, depending on the plant source, the composition of the starch (ratio of amylose to amylopectin molecules) and the micro-organization of the molecules (Table 2.4) (Copeland et al., 2009).

The amylose and amylopectin polymers are packaged in the starch granule in a specific order (discussed above). The ratio of amylose to amylopectin, within a starch granule, will depend on the plant source (for example maize, wheat, banana, cassava, and potato), yet, the majority of starch sources, contain 60 to 90% amylopectin, with 10 to 40% amylose. However, there are plant varieties which contain high amylose starches (70% amylose, with 30% amylopectin), and some

plant varieties contain high amylopectin starches, such as waxy starches with essentially 100% amylopectin (BeMiller and Whistler, 2009). Compared to common starches, banana starch appears microscopically spherical or elongated ovals while wheat starch appears both lenticular and spherical. The granule size of banana starch is bigger than common cereals such as maize (2 – 30 µm) and wheat (0.5 – 25 µm) but smaller than potato starch which is characterized by large granules (Izidoro et al., 2007, Sikora et al., 2010, Soares et al., 2011, Cai et al., 2014).

2.2.1.2 Classification of starch based on digestibility

Starch can be classified into three different types based on their behaviour when incubated with enzymes (BeMiller & Whistler, 2009):

- Rapidly digestible starch (RDS) which consists mainly of amorphous and dispersed starch and is found in high amounts in cooked starchy foods. It is measured chemically as the starch, which is converted to glucose in 20 min of enzyme digestion.
- Slowly digestible starch (SDS) which is digested more slowly in the small intestine. This category consists of physically inaccessible amorphous starch and raw starch in granule form or retrograded form in cooked foods. It is measured chemically as starch converted to glucose after >100 min of enzyme digestion.
- Resistant starch is fermented by the gut microflora. Five different types of resistant starch have been identified based on the structure.

Resistant starch has been classified into 5 different categories that are summarized in Table 2.5.

Table 2.5 Resistant starch types, description and sources.

Type of resistant starch (RS)	Description	Sources
RS 1	Inaccessible to digestive enzymes due to the physical barriers.	Coarsely ground or whole kernel grain products (e.g. bread, seeds, and legumes).
RS 2	Starches protected from digestion due to their crystalline structure.	Raw potatoes, green bananas, high-amylose maize, ginkgo starch.
RS 3	Retrograded starch formed when starchy foods are cooked then cooled	Potatoes, bread (especially stale bread), pasta, rice, cornflakes.
RS 4	Chemically modified starch formed by crosslinking, etherisation or esterification.	Foods containing chemically modified starches, such as cross-linked starch (e.g. some commercially produced bread and cakes).
RS 5	Two different components: amylose-lipid complexes, which either form during processing and reform after cooking or can be created artificially, resistant maltodextrin, which is processed to purposefully rearrange the starch molecules.	Foods containing naturally occurring amylose-lipid complexes, such as bread containing fat as an ingredient, or foods containing artificially made amylose-lipid complexes, such as stearic acid-complexed high-amylose starch.

Adapted from Lockyer & Nugent, (2017)

2.2.1.3 Physiological effects and health benefits of resistant starch

Due to the structure of resistant starches, a large number of physiological effects have been associated with resistant starch which is summarized in Table 2.6 below. Resistant starch, by escaping digestion in the small intestine, is fermented by intestinal flora in the large intestine resulting in the production of by-products like carbon dioxide, methane and hydrogen gases, organic acids and short chain fatty acids; including butyrate, acetate and propionate.

Table 2.6 Physiological effects and benefits of resistant starch in the diet.

Health benefits	Mechanism of action	References
Prevention of colonic cancer	Production of Butyrate in the bowel, through fermentation by probiotic bacteria of the lumen.	(Nugent, 2005, Sajilata et al., 2006, Ashwar et al., 2016, Lockyer & Nugent, 2017)
Hypoglycaemic effect	Lack of available digestible starch and slow digestion rate.	
Prebiotic	Stimulate the growth and activity of probiotic microorganisms in the gastrointestinal tract.	
Hypocholesterolemic effect	Reduce secretion of insulin which stimulates the synthesis of cholesterol.	
Laxative effect	Increase the daily faecal matter and reduces gastrointestinal discomfort.	

2.2.1.4 Determination of resistant starch in foods

Different methods have been developed to measure resistant starch in food (Perera et al., 2010). The main step of any method to measure the content of RS in foods must first remove all of the digestible starch from the product using thermostable a-amylases. The method of (McCleary & Monaghan, 2002) is considered the most reliable at present. It is based on the principle of enzymatic digestion and measures the portions of starch resistant to digestion at 37 °C followed by digestion at 60 °C. The Megazyme® kit for RS determination based on the basis of both AOAC method 2002.02 and AACC Method 32-40.01 is widely used in analytical laboratories. The principle is summarized in Figure 2.10.

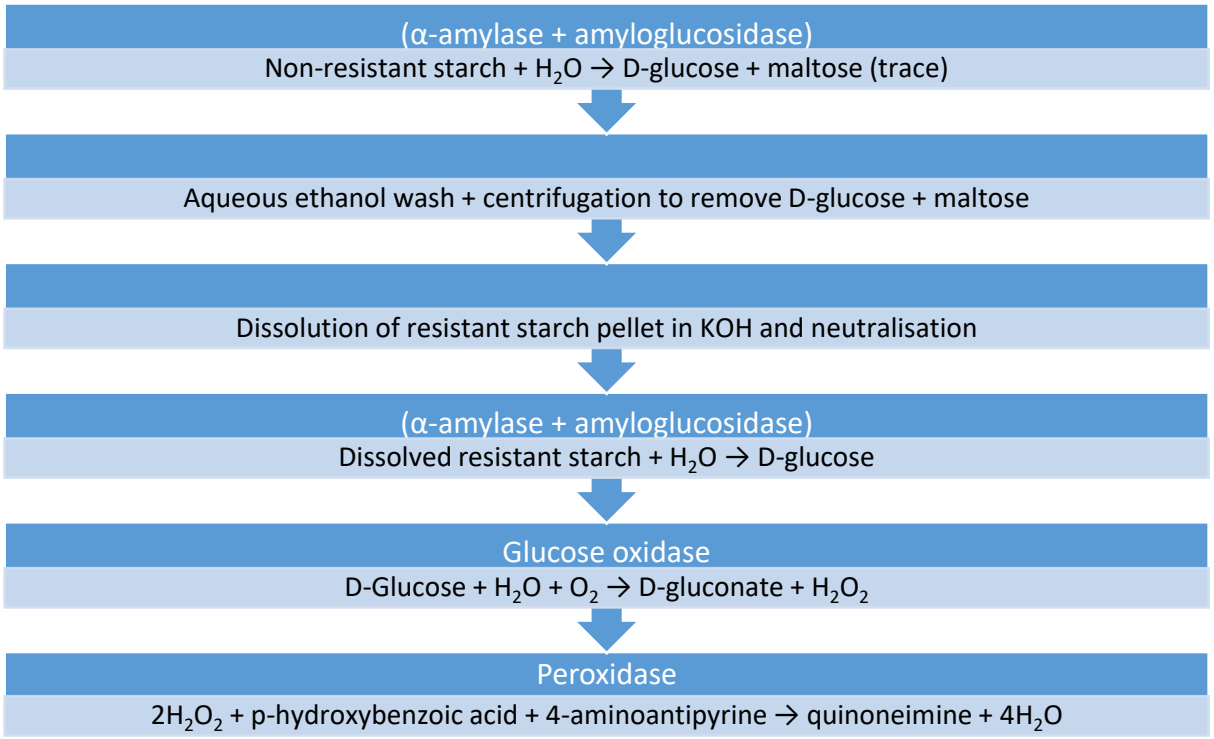

Figure 2.10 Process flow of determination of resistant starch content in food products

2.2.2 Functional properties of green banana flour

In food applications, many different properties are critical in order to predict the behaviour of the flour in terms: of packaging requirements, colour which has an influence on consumer acceptability and behaviour of the flour in presence of water or oil. The following section will discuss bulk density, colour, and the absorption capacity of the flour, emulsifying capacity, stability of emulsion formed, foaming capacity and stability of the foam.

2.2.2.1 Bulk density

Bulk density is a measurement of the weight of a sample per unit volume, expressed as g/mL or kg/L (Mohamed *et al., 2009)*. It is an important property that determines the packaging requirements of food products, especially flours. This assists with appropriate packing, loading and transporting of the packaged food materials (Oladele *et al*., 2007). Studies show that the bulk density of unripe banana flour ranges from 0.66 to 0.86 g/mL (Anyasi et al., 2017), which is comparable to maize

(ranging from 0.69 to 0.83 g/mL) (Samir et al., 1998), and wheat (0.68 to 0.71 g/mL) (Bian et al., 2015b).

2.2.2.2 Colour

Colour is an important property for food materials and systems. Many factors influence the colour of the food such as yellow maize, white maize, sorghum, and rice flours. These factors include inherent pigments in the flour, such as chlorophyll and carotenoids (Coultate, 2016).

The use of unripe banana flour in food products is growing (Aurore et al., 2009). However, the literature indicates that the colour of foods prepared with unripe banana flour can vary considerably depending on the method used to prepare the flour and are generally darker in colour compared to wheat based products (Mohamed et al., 2010). This can have a negative effect on the acceptability of products made from banana flour due to the fact that consumers have a preference for lighter products. The colour of the flour varies depending on the stage at which the fruit was processed into flour as well as the processing method used (Falade & Oyeyinka, 2015). This is due to biochemical (the ripening process in a banana), microbial and physical changes that can occur during growth, maturation, postharvest handling and processing of the raw materials (Pathare et al., 2013). In addition, as previously discussed (section 2.1.3) bananas are highly susceptibility to enzymatic browning.

In the case of physical indicators, the L^*, a^*, b^* has been one of the most used with L^* being the luminance or lightness component, it ranges from 0 to 100, while a^* (green to red, or redness) and b^* (blue to yellow, or yellowness) are two chromatic components, with values varying from -120 to +120 (Quevedo et al., 2009, Falade & Oyeyinka, 2015).

Table 2.7 Functional properties of banana flour, wheat flour, maize flour, soy protein, and egg

	Water holding capacity (g/g)	Oil holding capacity (g/g)	Foaming capacity (%)	Foaming stability (%)	Emulsifying capacity (%)	Emulsifying stability (%)	Reference
Banana flour	2.922 ± 0.004 - 4.604 ± 0.418	1.804 ± 0.002 - 3.115 ± 0.003	7.45 ± 1.54	6.46 ± 1.19	62.97 ± 6.41	ND	(Ayo-Omogie et al., 2010, Alkarkhi et al., 2011, Savlak et al., 2016),
Wheat flour	1.05 ± 0.12 - 1.28 ± 0.13	1.30 ± 0.12 - 1.58 ± 0.10	8.2 ± 0.13 - 20.2 ± 0.19	58.4 ± 0.18 - 79.8 ± 0.09	14.7 ± 0.12 - 28.6 ± 0.23	65.8 ± 0.12 - 82.6 ± 0.09	(Mepba et al., 2007, Bashir et al., 2017, Punia et al., 2017)
Maize flour	1.9 ± 0.1 - 2.19 ± 0.07	1.7 ± 0.1 - 2.1 ± 0.1	ND	ND	ND	ND	(Bolade et al., 2009, Shi et al., 2016)
Soy protein	3.246 ± 0.175 - 3.97 ± 0.23	2.06 ± 0.02 - 7.656 ± 0.349	131.65 ± 1.46 - 293.3 ± 6.1	50.2 ± 0.8 - 84.33 ± 1.05	81.71 ± 0.05 - 128.5 ± 7.6	51.22 ± 1.4 - 82.26 ± 5.64	(Zhao et al., 2018, Ziegler et al., 2018)
Egg	0.96±0.07 - 1.68±0.06	1.35±0.07 - 1.97±0.06	85.6 ± 2.2	80.6 ± 0.6	ND	91 - 96	(Le Denmat et al., 2000, Wong & Kitts, 2003, Duan et al., 2018)

2.2.2.3 Water and oil holding capacity

Water and oil holding capacity can be defined as the maximum amount of water or oil that can be absorbed by a food sample, respectively. According to Kisambira et al. (2015), water holding and oil holding capacity is an indication of the ability of the proteins present in the material or system to prevent fluid loss, especially during processing and storage. Studies conducted on green banana flour show that the water and oil holding capacities range from 1.45 to 2.84 g water/g flour and 1.60 to 1.93 g oil/g flour, respectively (Alkarkhi et al., 2011, Anyasi et al., 2015, Falade & Oyeyinka, 2015). Compared to wheat and maize, banana flour has higher water and oil holding capacity (see Table 2.7). This means that when used in a specific application, banana flour will require a larger amount of water in order to achieve the same consistency as compared to wheat or maize flour.

2.2.2.4 Foaming capacity and stability

The foaming capacity of flour gives an indication as to the increase in volume upon introduction of air or gas into a slurry of a given food (Wong & Kitts, 2003, Foegeding & Davis, 2011). While foaming stability refers to the ability of the foam formed to retain its maximum volume over time (Foegeding & Davis, 2011). These two properties are an indication of the ability of the proteins and non-starch polysaccharides present in the food to reduce the surface tension, the molecular flexibility and physico-chemical properties in a system (Adebowale et al., 2005, Moure et al., 2006, Foegeding & Davis, 2011). Foods with good foaming properties are useful as aerating agents in whipped toppings, creams and sponge cake (Adebowale et al., 2005). Banana flour shows a very low foaming capacity and stability compared to other ingredients in Table 2.7 and which may be a challenge when used in applications where these properties are required.

2.2.2.5 Emulsifying capacity and stability

Emulsification is an important process in the manufacturing of many formulated foods. An emulsion represents a heterogeneous mixture of fat globules in an aqueous phase (Foegeding & Davis, 2011). Emulsions and foams are two-phase systems commonly found in food systems and their formation is significantly

affected by protein and non-starch polysaccharides surface activity. Banana flour has shown higher emulsifying capacity and stability compared to wheat flour (see Table 2.7), but when compared to commonly used good emulsifiers such as soy proteins and egg, banana flour shows lower emulsifying capacity and stability. This means that when replaced in an application where wheat flour is used, banana flour will perform better compared to wheat.

2.2.3 Thermal and pasting properties of heat treated green banana flour

The crystalline order in starch granules is often the basic underlying factor influencing the functional properties of starch (Singh et al., 2003). Most edible starches are subjected to some form of processing, which usually involves heating in the presence of moisture under shear, and then cooling. During heat treatment, the starch granules are gelatinized, losing their crystallinity and structural organization. On cooling, the disaggregated starch molecules first form a gel and then retrograde gradually into semi-crystalline aggregates that differ in form from the native granules (Coultate, 2016). Understanding the steps that occur during gelatinization and retrogradation are critical steps to better predict the functional properties of processed starch. Different methods of analysis have been used to study the gelatinization behaviour of various flours, amongst which the use of the Rapid Visco-Analyzer (RVA) for pasting properties and the Differential Scanning Calorimetry (DSC) for thermal properties. The two methods will be discussed below, as well as textural analysis of set gels that can give an indication of the properties of retrograded starch.

2.2.3.1 Thermal properties (DSC) of green banana flour

Differential Scanning Calorimetry is a thermo-analytical technique which measures heat capacity and monitors physical and chemical changes of a sample as a function of temperature (Gabbott, 2008). During the scanning, changes in the sample that are associated with absorption (endothermic) or release (exothermic) of heat are recorded as a peak or trough. The area under the peak is directly proportional to the enthalpy change and the direction of the peak depending on the nature of the transition (endothermic or exothermic) (Kaletunç, 2009). Differential Scanning Calorimetry is suitable for analysis of food systems because they are often

subject to heating or cooling during processing. In food analysis, melting of solids and denaturation of proteins are endothermic reactions while crystallization of carbohydrates and aggregation of proteins are exothermic reactions (Kaletunç, 2009).

The thermal properties of flour are closely linked to their pasting properties (Dufour et al., 2009a), and gives an indication of the amount of energy needed to cook the flour beyond its gelatinization point. In addition, the behaviour of the food system, when subjected to heating and cooling cycles, can be estimated. When using a DSC, the values obtained from the graph can represent the gelatinization temperature and enthalpies.

The gelatinization temperature and associated enthalpies for unripe banana flour have been reported with ranges of 67.9-76.8 °C and 9.04-13.3 J/g, respectively (Da Mota et al., 2000, Tribess et al., 2009, Pelissari et al., 2013). The gelatinization temperature of the banana flours is higher than that of wheat and maize flour (Singh et al., 2003, Anyasi et al., 2017). These observations probably result from differences in the crystalline amylopectin structure and the ratio of amylose to amylopectin in the samples. The more crystalline the structure, the higher the gelatinization temperature and enthalpies, and vice versa. Figure 2.11 shows that the gelatinization temperature and enthalpy of green banana flour are higher than those for wheat flour. The peak shape of banana flour was sharper than wheat flour. It was suggested that the higher gelatinization temperatures and enthalpies of banana flour result from a longer amylopectin chain length of banana C-type crystallinity flour compared to shorter A-type crystallinity amylopectin chains (Tribess et al., 2009).

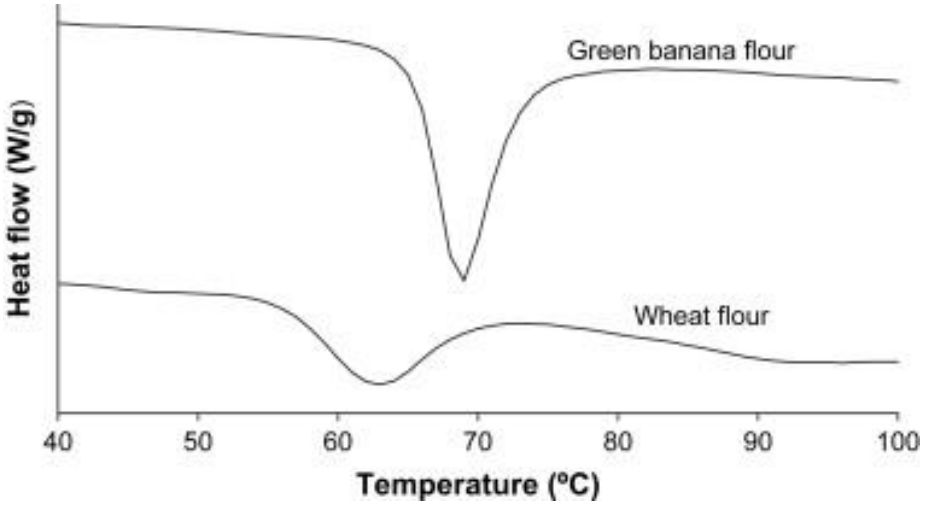

Figure 2.11 Endothermic transitions of green banana flour in comparison with wheat flour, obtained by differential scanning calorimetry (Tribess et al., 2009)

2.2.3.2 Viscosity and pasting properties green banana flour

Gelatinization behaviour and the pasting properties of flour-water and starch-water mixtures are commonly monitored using the RVA, which is a heating and cooling viscometer that measures the resistance of a sample to controlled shear. Gelatinization occurs when native starch is heated in the presence of sufficient moisture (Coultate, 2016). The granules absorb water and swell, and the crystalline organization is irreversibly disrupted. Gelatinization starts at the hilum of the granule and swells rapidly to the periphery (BeMiller & Whistler, 2009). It occurs initially in the amorphous regions of the granule, as opposed to the crystalline regions, of the granule. This is due to weak hydrogen bonding occurring between the loosely available starch molecules in the amorphous region (Copeland et al., 2009). Once the granules start to swell, amylose molecules begin to leach from the granules as they are disrupted under shear and the viscosity of the resulting paste increases to a maximum. This corresponds to the point when the number of swollen (but still intact) starch granules is at a maximum in the paste. The maximum is followed by a decrease in paste viscosity, as the granules rupture and starch molecules are dispersed in the aqueous phase. The rate and extent of swelling and breakdown are dependent on the type and amount of starch, the temperature gradient, shear force and the composition of the mixture, for example, the presence of lipids and proteins (Debet & Gidley, 2007). The gelatinization temperature of most starches is between 60 °C and 80 °C. As the starch paste cools, the viscosity

increases due to the formation of a gel held together by intermolecular and intramolecular interactions involving amylose and amylopectin molecules.

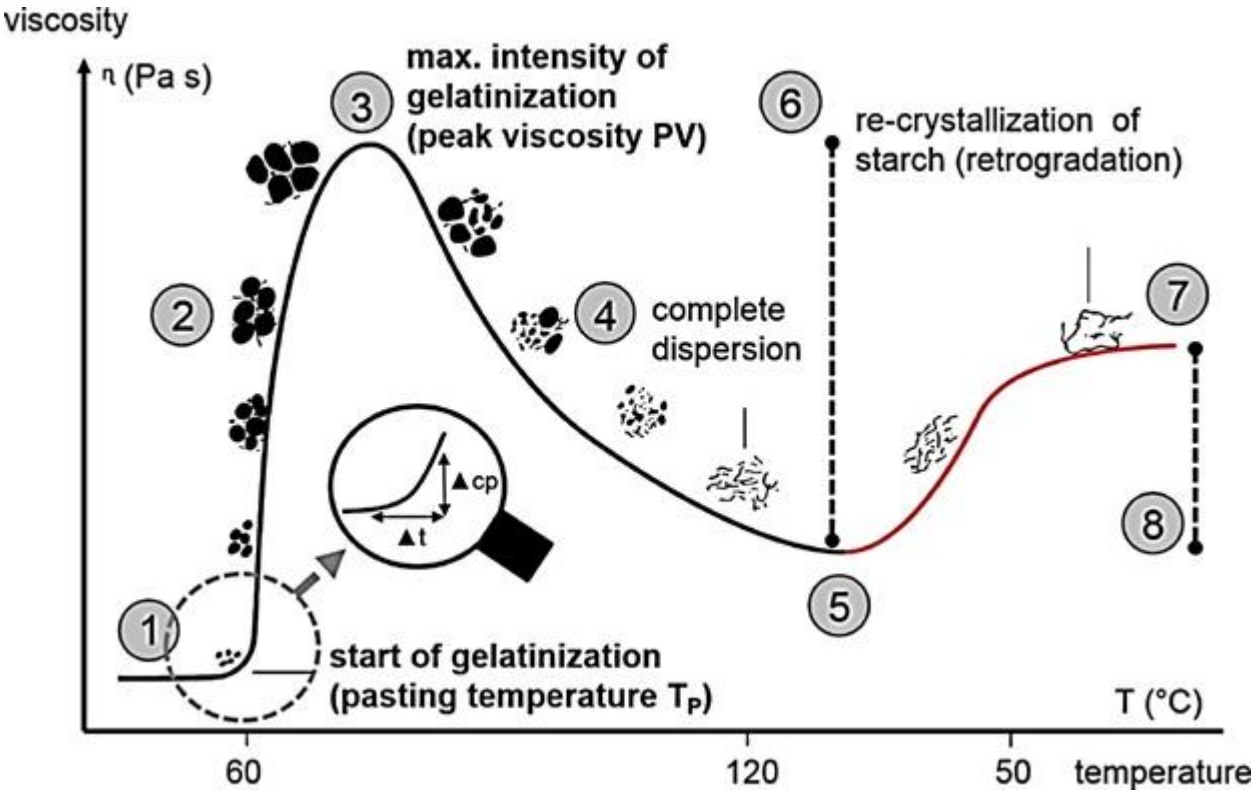

Figure 2.12 Typical RVA graph (1) pasting temperature, (2) hydration of starch granules, (3) peak viscosity, (4) enzymatic and sheer destruction of starch granules, (5) through paste viscosity, (6) breakdown viscosity, (7) final viscosity, and (8) set back viscosity (Schirmer et al., 2015)

The RVA has been used intensively to assess the viscosity, overheating, cooling cycles of various flours. A typical graph is illustrated in Figure 2.12 and the data obtained from the graph are explained below:

- Pasting temperature (1): which gives an indication of the temperature at which a flour enter into the paste phase (BeMiller, 2011),
- Peak viscosity (3): which is the maximum viscosity developed during the heating phase is an indication of the water binding capacity of the starch. It depends on the water holding capacity and well as the presence of other components in the flour (Kiin-Kabari et al., 2015),

- Through viscosity (5): Holding strength indicated the ability of the starch granules to maintain their gelatinized structure when under mechanical shearing stress,
- Breakdown (6): Breakdown viscosity is a measure of the degree of starch disintegration. It is an indication of hot paste stability of the starch. Breakdown viscosity value is a measure of the ease with which the swollen granules can be disintegrated and hence an indicator of the stability of the flour product (Kaur and Singh 2005),
- Final viscosity (7): is the section of the paste gel curve where the gelatinized dispersion of starch viscosity increase during cooling, resulting in the formation of a loose paste or gel (Bakare et al., 2014). The final viscosity of the starch paste is related to the amylose content. This implies that the flour with a higher amylose content gives a higher viscosity and flour with a lower amylose content gives a lower viscosity,
- Setback viscosity (8): is the phase of the pasting curve after cooling the starches to 50 °C. This stage involved reassociation, retrogradation, or reordering of starch molecules. Higher the setback viscosity, greater is the tendency toward retrogradation.

From various research, it has been showed that the pasting properties of banana flours are significantly different from those of wheat and maize flour (Dufour et al., 2009a, Haslinda et al., 2009, Chen et al., 2010, Alkarkhi et al., 2011, Pelissari et al., 2012, Yadav et al., 2016, Campuzano et al., 2018), as shown in Table 2.8.

Table 2.8 Pasting properties (gelatinisation) of banana flour, wheat flour, and maize flour

	Peak viscosity	Through viscosity	Breakdown viscosity	Final viscosity	Setback viscosity	Peak time	Peak temperature	Reference
Banana flour	367.4 - 461.0	263.8 - 308.1	104.7 - 152.9	348.5 - 432.3	40.38 - 169.6	5.13 - 5.14	83.25 - 84.70	(Bakare et al., 2016, Badejo et al., 2017)
Wheat flour	186.2 - 216.6	98.42 - 128.25	67.92 - 118.25	210.91 - 217.08	88.83 - 112.58	6.24 - 9.10	87.20 - 95.0	(Ragaee & Abdel-Aal, 2006, Chen et al., 2010, Bakare et al., 2016, Wang et al., 2018)
Maize flour	97.7 - 143.6	62.9 - 78.5	33.8 - 65.1	147.3 - 167.3	76.0 - 88.8	6.4 - 9.0	71.1 - 74.6	(Sandhu & Singh, 2007, Bolade et al., 2009, Paraginski et al., 2014)

The variations observed in the pasting properties can be explained by the difference in granular size, the amylose to amylopectin ratio, starch molecular characteristics, the volume fraction of suspended solids, and the conditions of the thermal process used to induce gelatinization (Zhou et al., 1998).

2.2.3.3 The texture of set gels

Texture plays an essential role in determining the final acceptance by the consumer and it is one of the predominant criteria for assessing the final product (Antognelli, 1980). The texture of the set gel is usually analysed using a texture analyser. The texture analyser moves in either an up or down direction to compress or stretch a sample. The travelling arm is fitted with a load cell and records the force response of the sample to the deformation that is imposed on it. Force, Distance, and Time data are collected and usually presented as a curve on a graph which, when analysed, indicates the texture of the sample (Bourne, 2002). The data that can be obtained from the graph includes:

- The maximum positive force obtained from the gel,
- The maximum negative force, which is an indication of how the gel will stick to the probe,
- Penetration Energy, the energy needed for the probe to penetrate the gel, it is represented by the positive area.

The properties of the set gel give an indication of the behaviour of the gel upon cooling and over time. Retrogradation is an ongoing process occurring over an extended period of time, depending on the composition of the starch in the flour (Coultate, 2016). This is due to the fact that amylose molecules retrogrades within a few minutes to a few hours, while amylopectin molecules retrograde over a few hours to days (BeMiller, James & Whistler, 2009). Understanding retrogradation of the different starch molecules is important for processed foods, as it is related to the change in the properties of the starch (such as stickiness, ability to absorb water and digestibility) during storage. Retrogradation of amylopectin is probably a more important determinant in the staling of bread and cakes (Coultate, 2016).

2.3 GLUTEN-FREE PRODUCTS

2.3.1 Gluten intolerance and celiac disease

The demand for gluten-free products is growing considerably due to the fact more consumers are becoming aware that they are gluten intolerant, or have been diagnosed with celiac disease (CD).

Celiac disease is an immune-mediated enteropathy caused by the ingestion of grains containing gluten proteins including wheat, barley, rye and possibly oats (Catassi & Fasano, 2008). The disease can be presented at any age and with multiple clinical symptoms, which can be categorized into three types: the typical malabsorption syndrome (chronic diarrhoea, weight loss, abdominal distension), the atypical presentation with major extra intestinal symptoms, and the silent form characterized by the apparent absence of symptoms (Catassi & Fasano, 2008). The consumption of gluten food products in subjects suffering from celiac disease results in the malabsorption of important nutrients including iron, folic acid, calcium and fat-soluble vitamins (Gallagher, 2009). This is due to the inflammatory reaction in the mucosa of the small intestine that leads to the damage of intestinal villi (Figure 2.13). When the villi are damaged or inflamed, they become flattened and are unable to absorb nutrients, leading to malabsorption.

The only treatment for celiac disease is the strict lifelong adherence to a gluten-free diet. Even after many years avoiding gluten, celiac disease patients are still at risk of reactivation of the disease when exposed to a gluten diet (Koehler et al., 2014b). The necessary dietary changes by celiac patients are to maintain a strict gluten-free diet are considerable and may have a significant effect on daily life (Gallagher, 2009, Alvarez-Jubete et al., 2010).

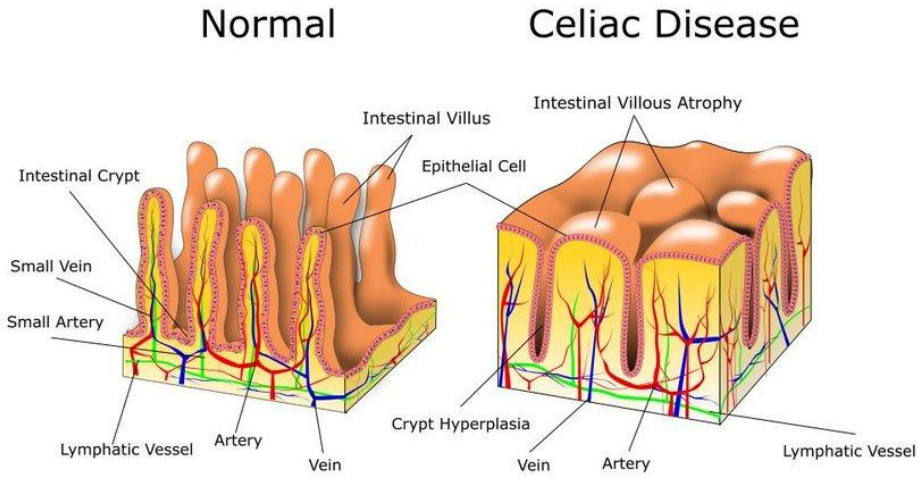

Figure 2.13 Healthy and damaged villi in the intestinal tract (Koh et al., 2019)

Many staple foods in the world that contain gluten should be avoided, this includes but is not limited to:

- Any bread, pasta, breakfast cereal or other food products made with wheat, rye, barley flours and by-products made from these grains,
- Processed foods that contain wheat and gluten-derivatives such as thickeners, stabilizers, and fillers. For example processed sausages, salad dressings, canned soups/dried soup mixes, processed cheese, and cream sauces,
- Medications that use gluten as pills or tablet binders.

The main proteins in cereals include the storage proteins, found in the starchy endosperm of wheat, rye, and barley (Koehler et al., 2014a). These storage proteins contribute about 70–80% of total protein content in the grain and have been classified as albumins, globulins, prolamins, and glutenins, according to the solubility properties, as described by Osborne, 1916 (Osborn fractionation). Of these storage proteins, it is the glutenins which have been reported to be responsible for gluten intolerance and celiac disease.

Wheat gluten proteins play a critical role in baking by giving unique water absorption capacity, cohesivity, viscosity, and elasticity on dough made from wheat flour due to the formation of a hydrated gluten network with water (Wieser, 2007). Despite its insolubility in water, gluten has the ability to absorb twice its dry weight in water.

Thus, the dough retains gas produced during fermentation, and this results in a leavened loaf of bread with an evenly pored, elastic crumb after baking. (Koehler et al., 2014c).

2.3.2 Gluten-free ingredients

Many ingredients have been used as replacers of wheat flour in the manufacturing of gluten-free products. Typically these ingredients include maize flour and maize starch, rice flour, sorghum flour, and millet flour, as well as flours prepared from pseudo-cereals such as amaranth, buckwheat, and quinoa (Koehler et al., 2014c).

Cereals flours such as maize and rice, used in gluten-free products, are generally refined as they are decorticated (removal of the germ and bran). This results in flours with lower content of essential nutrients such as B vitamins, iron, calcium, and fibre, compared to the whole grain flours (Thompson, 1999, 2000). Thus gluten-free products prepared from these flours require enrichment and fortification (Catassi & Fasano, 2008).

On the other hand, pseudo-cereals (amaranth, quinoa, and buckwheat), are not decorticated (due to their tiny grains), and the whole grain flour is higher in protein content, fibre, and minerals, making them suitable flours for improving the nutritional content of gluten-free foods (Alvarez-Jubete et al., 2010). Several studies have reported the successful use of pseudo-cereals in gluten-free bread formulations (Alvarez-Jubete et al., 2010, Demirkesen et al., 2010b, Miñarro et al., 2012, Mariotti et al., 2013, Aguilar et al., 2015, Machado Alencar et al., 2015, Campo et al., 2016, Paciulli et al., 2016, Rinaldi et al., 2017). However, commercialization of these gluten-free products is still limited.

Table 2.9 gives a summary of studies in which unripe banana flour has been used as an ingredient.

Table 2.9 Studies on the use of unripe banana flour as an ingredient in food products

Types of products	Ingredients	Reference
Steamed bread	Soft wheat flour, unripe banana flour, baking powder, yeast, shortening, and castor sugar	(Loong & Wong, 2018)
Bread	Hard wheat flour, unripe banana flour, yeast, granulated sugar, table, salt, baking fat, and vegetable fat	(Adebayo-Oyetoro et al., 2016)
Pasta	Wheat semolina, unripe banana flour	(Ovando-Martinez et al., 2009)
Bread	Banana flour, gluten, onion powder, garlic powder, salt, sugar, baking powder, concentrated milk, butter	(Juarez-Garcia et al., 2006)
Bologna type sausages	Pork meat, banana flour, pork skin, salt, sodium nitrite, sodium tripolyphosphate, sodium erythorbate, monosodium glutamate, garlic, coriander, black pepper, and crushed ice	(Alves et al., 2016)
Bread	Wheat flour, banana flour, sugar, salt, yeast, fat, and ascorbic acid	(Bakare et al., 2016)
Layer and sponge cake	Wheat flour, banana flour, Sugar, sunflower, oil, whole liquid milk, powdered milk, liquid pasteurized egg, baking powder, and emulsifier SuperMixo T500	(Segundo et al., 2017)

For instance, unripe banana flour has been used to increase of the crude fibre content of wheat-based baked bread, as it is believed the high content of resistant starch in the flour is beneficial to intestinal health. Interestingly, research on the use of banana flour in gluten-free formulations is limited.

In the study by Juarez-Garcia et al., (2006) in which the unripe banana flour was used as the main ingredient in the manufacturing of bread. It was found that the unripe banana flour bread presented significantly higher resistant starch and dietary fibre than the control bread (wheat based).

2.3.3 Challenges of gluten-free formulations for bread making

The replacement of wheat gluten in baked foods, presents a major technological challenge, as the gluten is an essential structure-building protein, which is responsible for the elastic characteristics of dough and final baking performance (Wieser, 2007). In addition, gluten contributes to the appearance and crumb structure of many baked products.

All other cereal flours, when used as alternative ingredients for baked products such as bread, yield products of with low volume and small-pored, inelastic crumb after baking (under standardized conditions) (Gallagher et al., 2003, Demirkesen et al., 2010a, Koehler et al., 2014c). Such products are considered inferior to those prepared from wheat flour, containing gluten. The inferior quality of the gluten-free products is due to the inability of the proteins endogenous to the cereal flour used, to form a dough with cohesiveness, viscosity, elasticity, and gas-holding ability (Gallagher et al., 2004). Hence, replacing gluten in baking formulations requires employing a mix of flours, proteins, hydrocolloids, and special technologies (such as sourdough) in an attempt to mimic the functional properties of wheat gluten (Gallagher, 2009).

According to Naqash et al., (2017), gluten-free batters and dough, are more sticky and difficult to handle. These batters require a higher percentage of water in the formulation compared to wheat-based formulations, resulting in a more runny texture, which is comparable to the cake batter. The lack of gluten in gluten-free

formulations requires shorter mixing, proofing, and baking times, compared to wheat doughs. The resulting baked gluten-free bread has poor crumb and crust characteristics (smaller volume, firmer crumb, and softer/leathery crust) as well as poor mouthfeel and flavour (Gallagher et al., 2003), compared to wheat based bread. According to Naqash et al., (2017), gluten-free bread also has a short shelf life and rapid staling rate compared to wheat bread.

To compensate for the lack of wheat gluten in gluten-free breads, additives such as hydrocolloids (xanthan gum and guar gum) have been studied to improve the baking performance and texture of gluten-free breads (Lazaridou et al., 2007, Demirkesen et al., 2010a, Torbica et al., 2010, Hager & Arendt, 2013, Mir et al., 2016, Encina-Zelada et al., 2018, Liu et al., 2018, Morreale et al., 2018). Furthermore, additional fermentation steps, using sourdough technology has also been researched to improve the overall quality of gluten-free bread (Moroni et al., 2009, Coda et al., 2011b, Edema, 2011, Edema et al., 2013, Ktenioudaki et al., 2015, Campo et al., 2016, Rizzello et al., 2016b, Rinaldi et al., 2017).

2.3.4 Rheological behaviour of formulations using Mixolab

According to the manufacturer, the Mixolab is an apparatus used to measure the rheological properties of dough subjected both to mixing and temperature changes. It can allow the prediction of the mixing and baking properties of a specific flour. A typical Mixolab graph is illustrated in Figure 2.14.

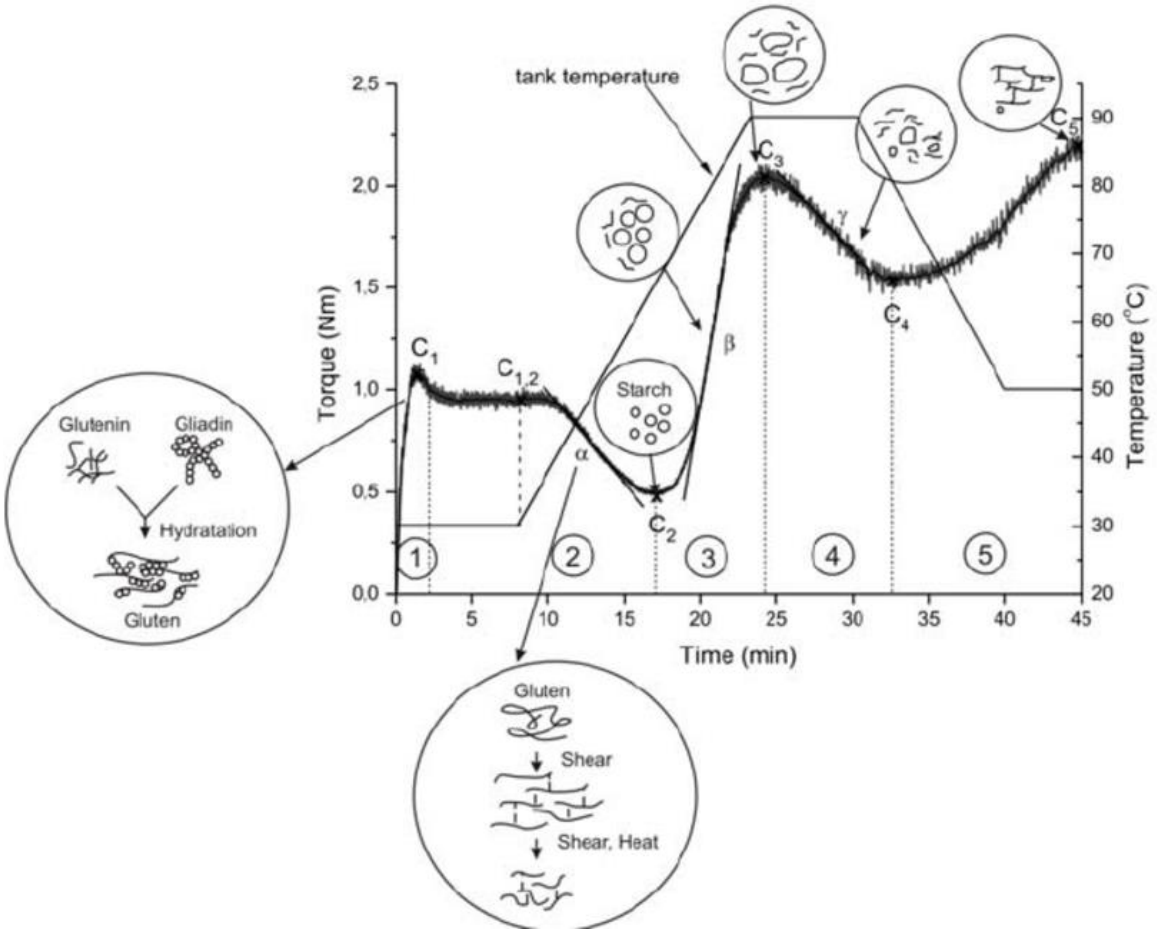

Figure 2.14 Typical Mixolab profile recorded using Chopin protocol (Hadnađev et al., 2011)

1, 2, 3, 4 and 5 are different stages during the mixing process. C1 and C2 give an indication of protein behaviour in the dough while C3, C4 and C5 focus on the behaviour of starch. α, β and γ are slopes and gives an indication of enzymes activities.

The early stages (C1 and C2) mainly represent the behaviour of proteins in the dough while the latter stages (C3, C4, and C5) focus more on the behaviour of starch. The initial mixing (stage 1) during which the hydration of the compounds occurred in combination with the stretching and alignment of the proteins to form a viscoelastic structure. In the 2nd stage, the combined effect of the mechanical stress and the temperature change causes a decrease in the torque due to the beginning of protein denaturation. With the increase in temperature, the behaviour of the proteins is masked by the gelatinization of the starch (3rd stage). During this stage, the swelling and gelatinization of the starch granules occur until the physical breakdown of the granules is accompanied by a reduction in the torque (4th stage).

A further increase in the torque is observed when the temperature decreased (5th stage), and is related to the retrogradation of the starch molecules (Huang et al, 2010).

2.3.5 Hydrocolloids for gluten-free bread

Hydrocolloids, such as guar gum and xanthan gum, are hydrophilic carbohydrate polymers that act as water binders, improving the rheological properties of gluten-free dough and bread texture (Rosell et al., 2001, Lazaridou et al., 2007). They have also been reported to slow down the retrogradation of starch in the bread (Guarda et al., 2004). A variety of hydrocolloids are used in the bakery industry for both gluten and gluten-free products, to improve the texture and appearance properties of the baked foods (Gallagher, 2009). Considerable research has been conducted on the use of hydrocolloids, such as hydroxypropylmethylcellulose (HPMC), carboxymethylcellulose (CMC), locust bean gum, guar gum, carrageenan, xanthan gum and agar (Gallagher et al., 2003, 2004, Lazaridou et al., 2007, Gallagher, 2009, Houben et al., 2012, Hager & Arendt, 2013) for improving the overall quality of gluten-free baked products. Two hydrocolloids of particular interest include xanthan gum and guar gum, due to their availability and relatively affordable prices, compared to the others mentioned above.

Xanthans, known commercially as xanthan gums, have been widely researched for use in gluten-free bread. Xanthan gum is a high molecular weight polysaccharide produced by a pure-culture fermentation of a carbohydrate with strains of *Xanthomonas campestris* (Garcıa-Ochoa et al., 2000). The primary structure of the molecule is composed of a backbone of 1,4-linked β-D-glucose with side chains containing two mannose and one glucuronic acid. When in water, they form solutions with high viscosity that are stable to changes in temperature, pH, or salt concentration (Arendt & Dal Bello, 2011). Xanthan is a non-gelling hydrocolloid but can form gels when combined with other hydrocolloids (such as agarose, locust bean gum or carrageenan). At least ten different categories of xanthans may be available, differing in particle sizes, different viscosity grades, easily dispersible types, rapidly hydrating types, and delayed hydrating types (Arendt & Dal Bello, 2011). When used in gluten-free bread formulations, xanthan gum (level of up to

3%) has been found to significantly increase the loaf volume and improve crumb texture, by loosening of the crumb structure (Guarda et al., 2004, Lazaridou et al., 2007, Demirkesen et al., 2010a).

Guar gum is the ground endosperm of the seeds of the guar bean *(Cyamopsis tetragonoloba)*. The gum powder primarily contains 75-85% polysaccharide (the actual hydrocolloid), 5-6% protein and 8-14% moisture (Mudgil et al., 2014). Guar gum mainly consists of the high molecular weight polysaccharides of galactomannans which are linear chains of (1 → 4)-linked β-D-mannopyranosyl units with 1,6-linked α-D-galactopyranosyl residues (Mudgil et al., 2014). When dissolved in water, the solutions are stable, unaffected by change in pH (Mudgil et al., 2014), and produces the highest viscous solutions, compared to other hydrocolloids at the same concentration. It also exhibits synergism when combined with agar, kappa-type carrageenan and xanthan gum (BeMiller, 2008). When investigated as a water-binding additive in gluten-free formulations, guar gum resulted in crumb structure with an evener cell size distribution, at a level of up to 2% (Gallagher et al., 2004, Lazaridou et al., 2007). However, it was reported that the volume of the gluten-free bread containing guar gum was lower when compared to other gums such as xanthan gum (Gambuś et al., 2001, Rosell et al., 2001, Guarda et al., 2004, Demirkesen et al., 2010a, Torbica et al., 2010).

To date, there is limited studies are available on the use of xanthan gum in combination with guar gum in gluten-free and gluten-containing bread (Mandala & Sotirakoglou, 2005, Matuda et al., 2008, Hejrani et al., 2017, Ahmed & Thomas, 2018). In a study by Hejrani et al., (2017), when guar/xanthan gum was used in combination at a dosage of 0.4% w/w for each in a formulation containing wheat flour, the crumb texture of the bread obtained was improved and the volume increased compared to the control sample. However, a further increase in the concentration of the gums resulted in a decrease in specific volume of the bread.

2.3.6 Sourdough technology

2.3.6.1 Introduction

Sourdough is one of the oldest biotechnological processes used in food production (Hammes & Gänzle, 1998). It is used to improve the texture and aroma of gluten-free baked products. Sourdough is a mixture of flour, water, and other ingredients (sugar, salt) that is fermented by naturally occurring lactic acid bacteria and yeasts. Although these microorganisms originate mainly from the flours and process equipment, the resulting composition of the sourdough microbiota is determined by endogenous (e.g. chemical and enzyme composition of the flour) and exogenous factors (e.g. temperature, redox potential, dough yield and time of the fermentation process) (Hammes & Gänzle, 1998). The dominating microorganisms in mature sourdough are lactic acid bacteria, which are found in numbers greater than 10^8 CFU/g (Ehrmann & Vogel, 2005). Commonly found lactic acid bacteria found in sourdough are obligate and facultative hetero-fermentative lactic acid bacteria including *Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus paralimentarius, Lactobacillus plantarum, Lactobacillus pontis, Lactobacillus sanfranciscensis, Lactobacillus frumenti, Lactobacillus panis,* and *Lactobacillus reuteri* (Gobbetti & Corsetti, 1997, De Vuyst & Vancanneyt, 2007).

2.3.6.2 Types of sourdough

Three different types of sourdough can be distinguished:

- Type I sourdough, carried out at room temperature with a final pH of about 4.0, is manufactured using a traditional technique (without the addition of starter culture), and is characterized by continuous, daily refreshments (with fresh, unfermented dough) to maintain the microorganisms in an active state. This is indicated by their high metabolic activity (Chavan & Chavan, 2011),

- Type II sourdough mainly uses a small portion of fermented dough to acidify unfermented dough, before baking. It is mainly carried out on an industrial scale with the addition of specific lactic acid bacteria strain to start fermentation (Chavan & Chavan, 2011). The sourdough is fermented for 2-5 days at >30°C. To speed up the process, higher fermentation temperatures

(37 °C) are used, until the dough pH is <3.5 (usually after 24 hours of fermentation) (Hammes & Gänzle, 1998). The fermenting organisms are in the late stationary phase of growth and exhibit restricted metabolic activity,

- Type III is a dried sourdough in powder form. It is obtained by drying Type II sourdough that is fermented by defined starter cultures. Different drying techniques can be used such as spray-drying, drum-drying and freeze drying. Sourdough type III is used as an acidifier supplement and aroma carrier during bread making (Chavan & Chavan, 2011). The process also requires the use of baker's yeast *(Saccharomyces cerevisiae)* for additional fermentation and dough development (De Vuyst & Neysens, 2005).

2.3.6.3 Effect of sourdough on bread quality

Overall, the gluten-free bread products available on the market are of low quality, exhibiting poor mouthfeel and flavour (Gallagher *et al.,* 2004). Nevertheless, the current literature is limited with regards to the use of sourdough in gluten-free goods (Coda et al., 2011b, Edema, 2011, Galle et al., 2012, Edema et al., 2013, Rizzello et al., 2016b, Naqash et al., 2017, Rinaldi et al., 2017). The few available results indicate that sourdough has a positive effect on baking quality, particularly regarding volume, texture, and flavour. In the study by Edema et al., (2013), fonio and sorghum were fermented using type I sourdough development, leading to the improvement of the viscosity of the dough, and resulting in bread with better crumb structure.

The influence of sourdough fermented by different lactic acid bacteria strains on the textural quality of gluten-free bread was evaluated during storage and compared to that of chemically acidified or non-acidified doughs (Clarke *et al.,* 2002; Crowley *et al.,* 2002). Sourdough fermentation caused an increase in the dough elasticity and staling was delayed (Ryan *et al.,* 2006). These effects were mainly attributed to the breakdown of non-gluten proteins and starch components by the lactic acid bacteria in the sourdough. Although there are no available studies on the use of sourdough in bread made from unripe banana flour, studies using other flours (sorghum, rice, buckwheat and teff) suggest that sourdough can possibly improve the rheological

and baking properties of gluten-free bread formulations (Coda et al., 2011b, Edema et al., 2013, Campo et al., 2016, Rinaldi et al., 2017). Table 2.10 gives an overview of the effect of sourdough on the sensory attributes, nutritional content and shelf life of baked bread.

Table 2.10 Effect of sourdough on the sensory attributes, nutritional content and shelf life of baked bread (with or without gluten).

Properties	Effect	Mechanism of action	Reference
Texture	Increase of bread volume	Lactic acidification	(Clarke et al., 2002, Crowley et al., 2002) (Katina *et al,* 2005)
	Increase dough extensibility and softening	Lactic acidification	
	Improvement of the texture of whole grain bread	Lactic acidification	
	Improved gas retention	Lactic acidification	
Flavour	Increase the synthesis of acetic acid	Use of various energy sources, interactions with enzymes	(Gobbetti & Corsetti, 1997, Di Cagno et al., 2002)
	Liberation of free amino acids and their derivatives	Secondary proteolysis General catabolism of free amino acids	
Nutrition	Increase levels of various compounds (volatiles and bioactive)	Lactic acidification, degradation of phytate	(Liukkonen *et al,* 2003). (Lopez *et al.,* 2001; De Angelis *et al.,* 2003).
	Retard of the starch bioavailability and decrease glycemic index	Lactic acidification	
Shelf life	To inhibit the endospore germination and growth	Lactic acidification	Corsetti *et al,* 2000; Crowley *et al.,* 2002 (Schntirer and Magnusson, 2005; Dal Bello *et al.,* 2007).
	antifungal metabolites	Lactic acididfication	
	Decrease in the rate of bread staling	Lactic acidification	

2.3.7 Sensory evaluation

The most important factor in the acceptance of a food product is its sensory character, which is the integrated response to the chemical and physical stimuli imparted by the food through its texture, taste, colour, aroma, and other components (Forde and Delahunty, 2004).

Generally, gluten-containing products have better sensory attributes (such as better flavour profile, texture, and taste) compared to gluten-free products (which are typically poor flavour, texture, appearance, and taste) (Gallagher et al., 2004, Gallagher, 2009). As previously discussed, the only treatment for patients with celiac disease is a lifelong diet in which food products containing gluten are avoided (Arendt & Dal Bello, 2011). The technological approach to the production of gluten-free foods that meet the unique nutritional and sensory requirements for patients with celiac disease, includes the use of starches, dairy products, gums and hydrocolloids, including other non-gluten proteins (milk, egg, pea protein, soy protein, proteins from legumes) as alternatives to wheat gluten. These additives are primarily used to improve the structure, mouth-feel, acceptability, and shelf-life of gluten-free bakery products (Arendt & Dal Bello, 2011, Koehler et al., 2014c). Regardless of numerous efforts to improve the sensory attributes and nutritional composition of gluten-free bread, ongoing studies on improved formulations are still required (Gallagher, 2009). One of the routes that should be further investigated, is the use of sourdough as it has been shown to improve the sensory attributes of baked products (Table 2.10).

Sensory evaluation of food products is a subjective test as they rely on panellists' opinion. They provide valuable data on the acceptability of the product, they also allow the producer to evaluate the preference of the consumer to a specific product (Kemp et al., 2011). One of the most common sensory evaluation tests used is the acceptance test using a 9-point hedonic scale (Garruti *et al*., 2012). It is a 9-point scale defining the most suitable acceptability where 1 = Dislike extremely; 2 = Dislike very much; 3 = Dislike moderately; 4 = Dislike slightly; 5 = Neither like nor dislike; 6 = Like slightly; 7 = Like moderately; 8 = Like very much; 9 = Like extremely (Kemp et al., 2011)

2.4 GAPS IN THE LITERATURE

Limited studies are available on the physico-chemical properties of unripe banana flour from different varieties (Da Mota et al., 2000, Dufour et al., 2009a, Anyasi et al., 2015, Anyasi et al., 2017). The selection of banana varieties used in these studies is based on local availability of varieties, even when underutilized varieties were considered. One should consider the use of an extended number of varieties sourced from the same growing site, to reduce the effect of environmental differences in the properties of the processed flours.

Different gluten-free flours (rice, teff, buckwheat, quinoa, fonio) have been used in the development of gluten-free bread suitable for celiac disease patients (Ribotta et al., 2004, Demirkesen et al., 2010b,a, Miñarro et al., 2012, Mariotti et al., 2013, Tsatsaragkou et al., 2014, Aguilar et al., 2015, Machado Alencar et al., 2015, Campo et al., 2016, Marston et al., 2016, Paciulli et al., 2016, Korus et al., 2017, Encina-Zelada et al., 2018). To our knowledge, there is no available study on the use of unripe banana flour in the development of gluten-free bread. In addition, there is no available information on the improvement of unripe banana flour through the utilization of sourdough technology in gluten-free bread formulations. Furthermore, there is no study on the use of xanthan and guar gum in combination for a gluten-free formulation containing unripe banana flour.

CHAPTER 3 : PHYSICO-CHEMICAL PROPERTIES OF UNRIPE BANANA FLOUR

3.1 INTRODUCTION

The production of green banana flour is one of the main ways that have been identified to preserve bananas and minimize post-harvest loses (Aurore et al., 2009). To valorise the crop, Unripe banana flour (UBF) has been added as a functional ingredient in different products that replaces staple flours such as wheat (Aparicio-Saguilán et al., 2007, Agama-Acevedo et al., 2009, Ovando-Martinez et al., 2009, Agama-Acevedo et al., 2012) and maize flour (Utrilla-Coello et al., 2011, Bello-Perez et al., 2015) in order to increase their resistant starch content. However, in order to use unripe banana flour in a particular product, it is important to have a deep understanding and knowledge about its physico-chemical and thermal properties as in most food products, these will affect the functionality greatly.

In addition, most of the studies previously reported, varieties were supplied from the local market where guarantee cannot be given on quality particularly on maturityof the fruit. In this study, 10 cultivars were selected based on yield and availability to be compared to commercial maize and wheat flours. The banana cultivars were provided by The Agricultural Research Council Tropical and Sub-tropical Crops (ARC-TSC) from its experimental farm in Burgershall (South Africa)

This chapter of the study focused on the analysis of the physico-chemical and thermal properties of the unripe banana flours compared to maize and wheat flour. Part of the work reported has been published in the Journal of Food Measurement and Characterization (Appendix 6 and available online at https://link.springer.com/article/10.1007/s11694-017-9481-x).

3.2 MATERIALS AND METHODS

3.2.1 Banana flour and other ingredients

Green banana flours, from ten different varieties (Table 3.1), were harvested from the ARC-TSC farm in Burgershall, Mpumalanga from September 2015 to January 2018 depending on the availability of the cultivars.

Table 3.1 Banana varieties used and their genomic group

Variety	Genome group
FHIA 18	AAAB
Gold Finger	AAAB
Green Red	AAA
Gros Michel	AAA
IBP. 5. 61	AAA
Khai Thong	AAA
Lady Finger	AA
Pisang Lemark	AA
PK 6	AAAB
Pome	AAB

Figure 3.1 gives an overview of the processing of bananas into flour. Briefly, the bananas were harvested from the banana trees (Figure 3.1a) at full maturity (unripe, mature green bananas), and bought to the ARC processing facility. Typically, the bananas were stored in crates (Figure 3.1b) in a cool area, and processed within 24 hours. Processing of the bananas involved, washing (Figure 3.1c) using tap water and peeled (Figure 3.1d) with a knife. To prevent enzymatic browning, the peeled banana were treated immediately with sodium metabisulphite solution (4 ppm, 30 min at ambient temperature) (Figure 3.1e). The treated fruits were sliced into a piece of ± 1 cm thick using an HLC-300 vegetable cutter (Newin Machinery, Zhengzhou, Henan, China) (Figure 3.1f).

Figure 3.1 Flow chart of processing of banana fruit into flour: (a) harvesting, (b) packing into crates, (c) washing, (d) peeling, (e) sodium metabisulphite treatment, (f) slicing, (g) drying, (h) dried banana chips , (i) milling and (j) milled flour

Then packed in a thin layer onto drying trays, and dried at 55°C, the relative humidity of 25% for 15 hours, using a cabinet dryer (AD3000 Agri-dryer, Dryer for Africa, Limestone Hill, South Africa) (Figure 3.1g). The dried banana slices (Figure 3.1h) were then milled using a rotor mill (Retsch RS200, Retsch, Haan, Germany) fitted with a screen (aperture of ± 500 µm) (Figure 3.1i). The flour obtained (Figure 3.1j) was then packed in airtight containers and stored at ambient temperature.

In addition, two commercial cereal flours (wheat flour and maize samp) were purchased from a local supermarket and used for comparison in the study. To ensure particle size uniformity, the wheat and maize samples were milled using the same rotor mill (±500 µm), then packed in airtight containers and stored at ambient temperature.

3.2.2 Chemicals

Table 3.2 summarize the chemicals and enzymes that were used in the microscopy of the starch as well as the determination of starch content. The methods used to prepare each solution are summarized in Appendix 1. The water used for all the experiments was deionized water (dH_2O) (Barnstead Genpure Pro, Thermo Scientific, USA).

Table 3.2 Chemical reagents and enzyme used in microscopy and starch determination analysis

Chemicals/Reagents/enzymes	Supplier
Acetic acid glacial	Rochelle chemicals & lab equipment, Johannesburg, South Africa
Calcium chloride dihydrate	Merck (Pty) Ltd, Johannesburg, South Africa
Ethanol 99.9 %	Illovo Sugar Ltd, Durban, South Africa
Iodine solution 0.05M	Merck (Pty) Ltd, Johannesburg, South Africa
Maleic acid	Merck (Pty) Ltd, Johannesburg, South Africa
Potassium hydroxide pellets	Merck (Pty) Ltd, Johannesburg, South Africa
Sodium azide	Merck (Pty) Ltd, Johannesburg, South Africa
Sodium hydroxide pellets	Merck (Pty) Ltd, Johannesburg, South Africa
Megazyme resistant starch kit (Amyloglucosidase (AMG), Pancreatic α-amylase, GOPOD reagent buffer, GOPOD reagent enzymes, D-glucose standard solution, Resistant starch control)	Megazyme international, Bray, Ireland

3.2.3 General properties of the flours

3.2.3.1 Microscopy of flours

The morphological features and size of the particles were analyzed using an Olympus SC30 light microscope (Olympus, Shinjuku, Tokyo, Japan). A dispersion of flour (0.5±0.1 g) in 5 mL iodine solution (0.05 M) was prepared. A drop was transferred onto a glass slide and covered with a slide cover to expel any excess solution. The prepared slide was then viewed at a magnification of 400 times. The image obtained was then analyzed using Olympus Stream Image Analysis software.

3.2.3.2 Moisture content

The moisture content of the different flour samples was determined using the Kern Moisture Balance Analyzer (Kern MLB_N, Kern, Germany). Each sample (4 ± 0.1 g) was dried at 120 °C until constant weight. The moisture content of the flour was used in other analyses.

3.2.3.3 Ash content

The ash content of the different samples was determined using the AACC method 08 – 01 - 01. Briefly, the flour samples were weighed into crucibles and place into the ashing furnace (Carbolite AAF 1100, Carbolite Gero Ltd, United Kingdom) which was preheated at 550 °C. The ashing was carried out until the residue was white in colour. The ash content was expressed as a percentage of the residue weight over the sample weight.

3.2.3.4 Colour

The colour attributes of each sample were determined using a chroma meter (CR 400, Konica Minolta, Tokyo, Japan). The instrument was calibrated using the white calibration plate. A black plastic cell was filled and levelled with the sample, and the cell was placed under the light source. The L^* (Lightness), a^* (red-green) and b^* (yellow-blue) values were recorded. The values obtained were used for the determination of chroma, yellowness and flour colour index (FCI), according to the following equations:

$$Chroma = \sqrt{a^{*2} + b^{*2}} \quad \text{Equation 1}$$

$$Yellowness = 142.86b*/L* \quad \text{Equation 2}$$

$$FCI = L* - b* \quad \text{Equation 3}$$

3.2.3.5 Resistant, available and total starch content

The determination of starch content was conducted based on the AOAC method 2002.02 (McCleary & Monaghan, 2002). Samples (100 ±5 mg) were weight directly into 15 mL falcon centrifuge tubes (Eppendorf, Johannesburg, South Africa), 4 mL of pancreatic α-amylase and amyloglucosidase (Solution 2, Appendix 1) was added to each tube, tightly capped and mixed using a vortex (Heidolph HEAX Top, Germany). The tubes were then attached horizontally in the direction of the oscillation motion and incubated in a shaking water bath (GLF 1083, Germany) for 16 h at 37°C with continuous shaking (200 strokes/min). During the incubation period, non-resistant starch is solubilized and hydrolyzed to D-glucose by the combined action of the two enzymes. The reaction was terminated by the addition of an equal volume of ethanol (99 % v/v, 4 mL) in the uncapped tubes with stirring on the vortex. The uncapped tubes were then centrifuged at 4°C, 1878 rcf for 10 min (centrifuge 5430R, Eppendorf, Johannesburg, South Africa). The supernatants were carefully decanted into 100 mL volumetric flasks (flask 1) and the pellet was re-suspended with aqueous ethanol (50% v/v, 8 mL), followed by centrifugation as above. The suspension-centrifugation-decantation step was twice. The supernatants collected was added to the 100mL volumetric flask (flask 1).

3.2.3.5.1 Measurement of resistant starch

The resistant starch present in the pellet was dissolved in 2 mL of KOH (2 M). The tube was placed in an ice/water bath and stirred vigorously with a magnetic stirrer for 30 min. The resulting solution was neutralized with sodium acetate buffer (pH 3.8, 8 mL, 1.2 M) and the starch was hydrolyzed to glucose with the enzyme amyloglucosidase. The resulting hydrolyzed solution (0.1 mL) was transferred into a glass test tube, and glucose oxidase/peroxidase (GOPOD) reagent (3 mL) was added and incubated at 50 °C for 20 min. The absorbance was measured at 510 nm using a spectrophotometer (Helios γ, Thermo Fisher Scientific, Massachusetts, USA) against a blank reagent (0.1 mL of 100 mM sodium acetate buffer (pH 4.5) and 3.0 mL of GOPOD reagent). A standard D-glucose was prepared (in quadruplicate) by mixing 0.1 mL of D-glucose (1 mg/mL) (bottle 5, Megazyme

resistant starch kit) and 3.0 mL of GOPOD reagent. The absorbance obtained was used to calculate the amount of resistant starch per 100 g sample.

3.2.3.5.2 Measurement of Non-resistant starch

The volume of flask 1 containing all combined supernatants was adjusted to 100 mL using sodium acetate buffer pH 4.5 (Appendix 1). Aliquots of this solution (0.1 mL) were transferred in duplicate into glass test tubes containing 10 µL of dilute AMG (solution 1) and incubated for 20 min at 50°C. Then GOPOD reagent (Solution 4, 3 mL) was added to the tube and incubated for a further 20 min at 50°C. The absorbance was measured at 510 nm against a reagent blank (0.1 mL of 100 mM sodium acetate buffer pH 4.5 and 3.0 mL of GOPOD reagent). The absorbance obtained was used to calculate the amount of non-resistant starch per 100 g sample.

The amount of resistant and non-resistant starch in the samples were calculated using the following equations:

$$\text{Non-Resistant (Solubilized) Starch (g/100 g sample)} = \Delta E \text{ x } F/W \text{ x } 90 \quad \text{Equation 4}$$

$$\text{Resistant Starch (g/100 g sample)} = \Delta E \text{ x } F/W \text{ x } 90 \quad \text{Equation 5}$$

$$\text{Total Starch} = \text{Resistant Starch} + \text{Non-Resistant Starch} \quad \text{Equation 6}$$

Where:

ΔE = absorbance (reaction) read against the reagent blank.

F = conversion from absorbance to micrograms (the absorbance obtained for 100 µg of D-glucose in the GOPOD reaction is determined and F = 100 (µg of D-glucose) divided by the GOPOD absorbance for this 100 µg of D-glucose.

W = dry weight of sample analysed

= “as is” weight x [(100-moisture content)/100].

3.2.4 Functional properties of green banana flour

3.2.4.1 Bulk density

Bulk density was determined according to the method of (Wani et al., 2015). A measuring cylinder, previously tarred, was filled with each sample up to the 10 mL mark. The weight of the sample was recorded. The filled cylinder was tapped on top of the bench until no diminution of the sample level and the volume was noted. The bulk density was calculated as a ratio of mass per unit volume (g/mL).

3.2.4.2 Water holding capacity

The water holding capacity (WHC) was determined as described by Anyasi *et al.* (2015) with slight adaptations. Approximatively 1 g of sample (W_0) was mixed with 10 mL of deionized water, the slurry was transferred into a pre-weighed (W_1) centrifuge tube (50 mL) and thoroughly mixed for 30 sec using a vortex mixer (Reax top, Heidolph, Schwabach, Germany). The sample was allowed to stand for 30 min and then centrifuged for 20 min at 1057 rcf (5430R centrifuge, Eppendorf, Hamburg, Germany). The supernatant was decanted, and the tube containing the pellet was weighed (W_2). The water holding capacity was calculated as a percentage of water bound per gram of sample, using the following equation:

$$WHC = \frac{W_2 - W_1}{W_0} \qquad \text{Equation 7}$$

3.2.4.3 Oil holding capacity

Oil holding capacity was determined according to the method of Anyasi *et al.* (2015). Approximatively 1 g of flour (W_0) was placed into a pre-weighed centrifuge tube and 10 mL of sunflower oil (V_0) was added. The samples were vortexed for 30 sec and allowed to stand for 30 min. The resulting suspension was centrifuged at 1057 rcf for 20 min (5430R centrifuge, Eppendorf, Hamburg, Germany). The supernatant was decanted and the volume measured (V_1). The oil holding capacity was calculated as a percentage of the difference between the initial volume of oil and the final volume of the supernatant per gram of sample, using the following equation:

$$OHC = \frac{V_0 - V_1}{W_0}$$ Equation 8

3.2.4.4 Foaming capacity and stability

Foaming capacity and stability were determined according to the method described by Wani *et al.* (2013), with the following modifications. An aqueous slurry (2% w/v) was prepared by weighing 0.8 g flour in 40 mL of deionized water and mixed in a glass measuring cylinder (100 mL). The mixture was homogenized with the Ultra-Turrax (T18/10, IKA works GmbH & Co. KG, Staufen, Germany) at 20 000 rpm for 1 min. The volume of the liquid (V_1) and the volume of the foam (V_2) were determined. The foaming capacity was expressed as the percentage volume increase (%, v/v) using the following equation:

$$\text{Foaming capacity } (\%) = \frac{Volume\ after - volume\ before}{volume\ before}\ x\ 100$$ Equation 9

For foaming stability the measuring cylinder holding the homogenized sample was allowed to stand for 60 min, undisturbed, and the volume of the foam (V_3) was determined. Foaming stability was expressed as the percentage of foam remaining after 60 min (%, v/v) using the following equation:

$$\text{Foaming stability } (\%) = \frac{Foam\ volume\ after\ particular\ time}{initial\ foam\ volume}\ x\ 100$$ Equation 10

3.2.4.5 Emulsifying capacity and stability

The emulsifying capacity and stability were determined according to an adapted method of Chavan *et al.* (2001). Emulsions (0.5 % m/v) were prepared by dispersing 0.3 g flour into 60 mL water- oil mixture (45 mL of water and 15 mL of vegetable sunflower oil). The flour-water-oil mixture was placed in a glass measuring cylinder (100 mL) and homogenized with the Ultra-Turrax (T18/10, IKA works GmbH & Co. KG, Staufen, Germany) at 20 000 rpm for 1 min. The total volume (V_1) was observed after 5 sec, and the volume of the emulsified layer (V_2) was recorded. The emulsion capacity was expressed as a percentage using the following equation:

Emulsifying capacity (%) = $\frac{Height\ of\ the\ emulsified\ layer}{Total\ height\ of\ tube\ contents}\ x\ 100$ Equation 11

The emulsion stability was determined by heating the glass measuring cylinder containing the homogenized slurry (emulsion), in a water bath (at 80 °C for 30 min). The total volume (V_3) and the volume of the emulsion (V_4) were recorded after 5 s. The emulsion stability was expressed as a percentage, using the following equation:

$$\text{Emulsifying stability (\%)} = \frac{Height\ of\ the\ emulsified\ layer\ after\ heating}{Total\ height\ of\ tube\ contents}\ x\ 100$$

Equation 12

3.2.5 Thermal and rheological properties of unripe banana flour

3.2.5.1 Thermal properties (DSC)

The thermal properties were determined using a high-pressure Differential Scanning Calorimeter (DSC) (HPDSC-827 equipped with STARe® 127 software, Mettler Toledo, Switzerland). The flours (10 mg) were wetted with distilled water (30 mg) and equilibrated at room temperature (6 h), and measurements were done at a scanning rate of 10 °C/min (30 to 95 °C) with a nitrogen flow rate of 40 mL/min (atmospheric pressure). The DSC was calibrated using indium (peak melting temperature = 156.61 °C, ΔH-enthalpy 28.45 J/g), an empty pan was used as a reference.

3.2.5.2 Pasting properties

The pasting properties of the different flour samples were analyzed using the Rapid Visco Analyser (RVA 4500, Perten Instruments, Stockholm, Sweden). The standard protocol was modified and adapted for use with unripe banana flour. Due to the high viscosity of the unripe banana flour, only 80% of the user manual recommended sample weight was used in the experiment. The amount of flour used for each cultivar varied due to differences in the moisture content of the flour (Table 3.3). Briefly, flour was suspended in water (Table 3.3). The suspension was dispersed at 960 rpm for 30 s then the speed reduced to 160 rpm for the rest of the test. The sample was equilibrated at 50 °C for 2 min, heated to 91 °C in 3 min 30 s

at a rate of 11.08°C/min, held at 91°C for 2 min 30 s, then cooled to 50°C in 3 min 30 s and finally kept at 50 °C for 1 min. The pasting temperature, peak viscosity, peak time, final viscosity, breakdown viscosity and set back viscosity was obtained from the resulting graph. In addition, the resulting gels from the analysis were collected and the gels transferred into plastic sample holders (diameter: 3.5 cm; height: 1.1 cm) and used to determine the texture of the set gels.

Table 3.3 Mass (g) of water and flour used for determining the pasting properties of the different flours.

Sample	Mass of flour(g)	Water(g)
FHIA 18	2.712	25.11
Gold Finger	2.808	24.99
Green Red	2.712	25.11
Gros Michel	2.824	24.97
IBP. 5. 61	2.768	25.04
Khai Thong	2.768	25.04
Lady Finger	2.808	24.99
Pinseng Lemark	2.712	25.11
PK 6	2.800	25.00
Pome	2.712	25.11
Maize	2.912	24.86
Wheat	2.912	24.86

3.2.5.3 Texture of set gels

The texture of the set gels (obtained from the RVA, section 3.2.5.2) were analyzed according to a modified method of Edema et al., (2013). The gels collected and packed into plastic containers were allowed to stand for at least 2 h, at ambient temperature. The properties of the set gels were measured using a TA.XT Plus Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) fitted with a P/2 cylinder probe (2mm Ø Stainless Steel). The firmness, penetration energy, adhesive force, and adhesiveness were obtained from the resulting graph. The test parameters were set to measure force in compression, at a speed of 1.0 mm/s, to a distance of 5 mm into the sample and at a data acquisition rate of 50 pps. The

results were obtained as a graph using Exponent software (Stable Micro Systems, Godalming, Surrey, UK).

3.2.6 Statistical analysis

All measurements were done in triplicate. Statistical analysis was done using SAS Software V8 (SAS Institute, Inc., Cary, NC, USA). One-way ANOVA with Duncan's Multiple Range correlation test for separation of means between samples was used (confidence interval of 95 %). Principal component analysis and linear regression were done using Statistica© Software, version 8 (StatSoft, Inc., Tulsa, Oklahoma, USA).

3.3 RESULTS AND DISCUSSION

3.3.1 General properties of the flours

3.3.1.1 Microscopy of the flours

Microscopic studies of flour suspensions stained with iodine solution are shown in Figure 3.2 and the size of granules is found in Table 3.4.

In banana flour, while being irregular in shape, the starch granules appeared both ellipsoid and spheroids. The granules varied in length in average from 29.9 to 43.7 µm for Lady Finger and Pome respectively. These results are in accordance with previous studies on banana flour and starch in which it was found that banana starch granules varied in length from 21 to 108 µm (Waliszewski et al., 2003, Zhang et al., 2005). Maize and wheat flour, on the other hand, appeared rounded and spherical with an average diameter of 25.0 µm and 29.9 µm respectively. These results fall in the lower range of those of banana flour and were significantly different from the bigger banana starch granules (Green Red and Pome). The size and shape of granules are a useful tool in the identification of plant source (Coultate, 2016).

Table 3.4 Granular size of different flour samples

Sample	Granular size (μm)
FHIA 18	32.6 ± 6.0ab
Gold Finger	37.4 ± 5.7ab
Green Red	43.3 ± 13.3a
Gros Michel	35.9 ± 10.4ab
IBP. 5. 61	32.6 ± 12.1ab
Khai Thong	30.1 ± 4.6b
Lady Finger	29.9 ± 9.6b
Pinseng Lemark	36.1 ± 9.4ab
PK 6	34.4 ± 6.3ab
Pome	43.7 ± 9.4a
Maize	25.0 ± 1.9b
Wheat	29.9 ± 6.6b

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of at least three independent measurements

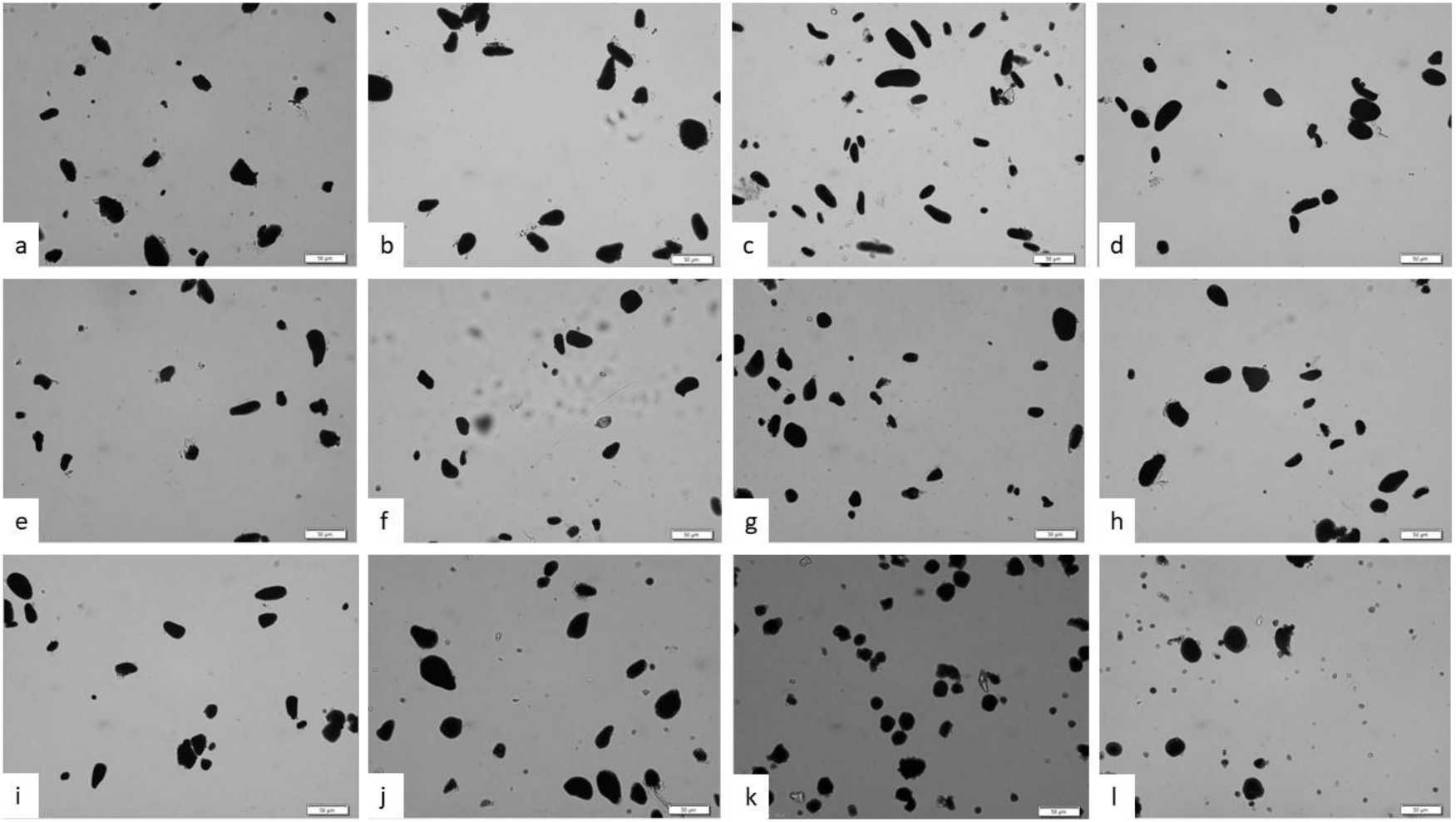

Figure 3.2 Microscopy of flour samples (FHIA 18 (a) , Gold Finger (b), Green Red (c), Gros Michel (d), IBP 5.61 (e), Khai Thong (f), Lady Finger (g), Pisang Lemark (h), PK 6 (i), Pome (j), Maize (k), and wheat (l)) after iodine staining
Olympus light microscope (magnification 400x, scale 50µ)

3.3.1.2 Moisture content

The mean moisture content of the different samples is reported in Table 3.5. The moisture content of banana flours ranged from 3.10 to 6.84%, for FHIA 18 and Gros Michel, respectively. These values were slightly lower than that of wheat (9.66%) and maize (7.88%).

Table 3.5 Moisture content and ash content of banana flour samples compared to wheat and maize flour

Sample	Moisture content (%)	Ash content (%)
FHIA 18	3.01 ± 0.92g	3.05 ± 0.03g
Gold Finger	6.33 ± 0.20d	3.44 ± 0.03e
Green Red	3.07 ± 0.05g	3.38 ± 0.03f
Gros Michel	6.84 ± 0.08c	3.07 ± 0.03g
IBP. 5. 61	4.90 ± 0.012f	3.55 ± 0.02d
Khai Thong	5.07 ± 0.08f	3.97 ± 0.06b
Lady Finger	6.47 ± 0.08cd	2.53 ± 0.01i
Pinseng Lemark	3.12 ± 0.34g	3.90 ± 0.04c
PK 6	6.14 ± 0.09d	4.30 ± 0.03a
Pome	3.05 ± 0.12g	2.83 ± 0.02h
Maize	7.88 ± 0.10b	0.25 ± 0.01k
Wheat	9.66 ± 0.06a	0.73 ± 0.01j

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of three independent measurements.

The low moisture content of the UBF's indicate that the method used to dry the banana chips was effective, and the flour will have an acceptable shelf life as microorganisms will not be able to proliferate during storage. However, it is important to ensure the flours are stored correctly, as dry flours have the potential to pick up moisture, reducing the shelf life, and thus the final product quality. The flours should always be stored in airtight packaging to prevent the increase of moisture content. In addition, the knowledge of the moisture content of a particular sample is required for other analysis such as starch content and pasting properties.

3.3.1.3 Ash content

The ash content of the samples is reported in Table 3.5. The ash content of the banana samples ranged from 2.53 to 4.30% for Lady Finger and PK 6, respectively. These values were significantly higher than those of Maize (0.25%) and Wheat (0.73%). The values reported in this study are comparable to those previously reported by Gibert et al., (2009) for dessert banana grown in Colombia. In their study, the ash content ranged from 2.9 % to 4.3% for bocadillo and Fhia 17, respectively (Gibert et al., 2009). The ash content gives an indication of total mineral content present in the sample which is useful nutritional criteria (Leterme et al., 2006). In addition, minerals assist in body catalytic, structural and regulatory activities as they function as electrolytes where they bring about fluid and acid-base balance (Anyasi et al., 2018). Hence, the high ash content of banana flour will result in higher ash content in the final found product after processing compared to maize and wheat.

3.3.1.4 Flour colour characteristics

The colour characteristics of the UBF from 10 banana varieties compared to maize and wheat flour are shown in Table 3.6. The L* character represents the whiteness (100) or blackness (0); a* represents redness (+a*) or greenness (-a*); while b* represents yellowness (+b*) or blueness (-b*). All the UBFs colour characteristics (L*, a*, b, Chroma, and FCI) significantly ($p< 0.05$) varied with the banana cultivars.

Table 3.6 Colour characteristics of the different banana flours compared to maize and wheat

Flour Type	Colour Values					
	L*	a*	b*	Chroma	Yellowness	FCI
FHIA 18	86.82 ± 5.39ba	0.34 ± 0.02f	10.41 ± 0.51f	10.41 ± 0.51f	17.14 ± 0.47f	76.42 ± 4.93ba
Gold Finger	84.47 ± 0.71b	0.74 ± 0.07e	15.60 ± 2.69b	15.72 ± 0.77b	26.36 ± 4.50cb	66.30 ± 2.8dc
Green Red	76.30 ± 4.58d	3.72 ± 0.38a	13.14 ± 0.59d	13.66 ± 0.66d	24.72 ± 2.45cd	63.16 ± 4.99e
Gros Michel	83.03 ± 1.84bc	1.60 ± 0.10d	11.28 ± 0.15ef	11.39 ± 0.16ef	19.41 ± 0.64fe	71.75 ± 1.96bc
IBP. 5. 61	78.94 ± 2.51dc	1.43 ± 0.15d	12.45 ± 0.27ed	13.66 ± 0.66ed	22.56 ± 1.07d	65.50 ± 3.2de
Khai Thong	78.72 ± 4.79dc	2.37 ± 0.18c	15.54 ± 0.77b	15.72 ± 0.77b	28.25 ± 1.36b	63.18 ± 4.36e
Lady Finger	78.49 ± 3.09dc	2.47 ± 0.37c	13.65 ± 0.50cd	13.87 ± 0.55cd	24.90 ± 1.88cd	64.84 ± 3.57de
P.Lemark	78.34 ± 4.16dc	2.94 ± 0.20b	12.77 ± 0.23d	13.11 ± 0.22d	23.34 ± 1.09d	65.56 ± 4.05de
PK 6	86.78 ± 0.38ba	-1.09 ± 0.04i	20.07 ± 0.10a	20.10 ± 0.10a	33.04 ± 0.02a	65.20 ± 2.7dc
Pome	78.54 ± 2.50dc	2.89 ± 0.17b	14.69 ± 0.06cb	14.97 ± 0.08cb	26.74 ± 0.92cb	63.85 ± 2.54de
Maize	89.86 ± 1.81a	-0.57± 0.06h	8.92 ± 0.40g	8.94 ± 0.40g	14.19 ± 0.68g	80.96 ± 2.41a
Wheat	89.88 ± 2.47a	-0.33± 0.01g	12.51 ± 0.40ed	12.52 ± 0.40ed	19.91 ± 1.01e	77.34 ± 2.18a

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of three independent measurements.

Typically the UBF's were significantly ($p<0.05$) darker (ranging from 76.30 to 86.82 for Green Red and FHIA 18, respectively), more red (ranging from 0.34 to 3.72 for Fhia 18 and Green Red, respectively), and more yellow (ranging from 10.41 to 20.07 for Fhia 18 and PK 6, respectively), compared to wheat and maize flour. A similar trend was observed for the perceived quantitative colourfulness (Chroma) (Table 3.6) (ranging from 10.41 to 20.10 for FHIA 18 and PK 6, respectively), compared the colourfulness of wheat and maize.

The flour colour index of wheat and maize flour was significantly ($p < 0.05$) higher than that of most of the UBFs apart from the one from FHIA 18 cultivar. The FCI correlates with the amount of yellow pigments in the different flours. The differences in the colour characteristics of UBFs compared to maize and wheat could also be explained by the fact that polyphenol oxidase was only deactivated on exposed surfaces during treatment of the fruits with sodium metabisulfite. However cutting of the fruit results in new surfaces, which when exposed to air cause browning. The UBF colour characteristics shown, fall within the range of UBF reported by Falade et al., (2015) (L* (60.96–84.86), a* (0.39–9.01) and b* (11.08–26.88)) and Alkarkhi et al., (2011)(L* (64.37-79.25), a* (1.57-3.67) and b* (14.69-21.69)).

Thus, the UBF used in this study should be carefully considered when used as a flour replacement in a formulation where the final product colour is important. Addition of UBF (Cavendish variety) has been shown to negatively affect the colour characteristics of cassava and fish crackers (Wang et al., 2012), while that from the cooking banana (*Musa paradisiaca*) has been shown to negatively affect pasta colour (Agama-Acevedo et al., 2009).

3.3.1.5 Resistant, available and total starch content of the flours

The resistant, available and total starch content of banana, maize, and wheat flours are reported in Table 3.7. The resistant starch content of the UBFs significantly ($p<0.05$) varied with banana variety from 19.9% dwb for FHIA 18 to 47.4% for PK6 variety (Table 3.7).

Table 3.7 Resistant starch, available starch and total starch content of banana flours compared to maize and wheat flours

Flour Type	Resistant Starch (RS) ($g.100g^{-1}$ dwb)	Available Starch (AS) ($g.100g^{-1}$dwb)	Total Starch (TS) ($g.100g^{-1}$dwb)
FHIA 18	19.9 ± 0.8e	45.7 ± 2.5c	65.6 ± 2.5de
Gold Finger	46.1 ± 1.7a	17.9 ± 2.3hg	64.0 ± 1.1fe
Green Red	34.4 ± 2.6d	30.0 ± 0.7ed	64.4 ± 2.3fe
Gros Michel	36.8 ± 0.5cd	33.4 ± 1.5d	70.2 ± 1.6c
IBP. 5. 61	45.6 ± 1.0a	13.5 ± 0.7i	59.1 ± 1.1g
Khai Thong	42.4 ± 2.4b	19.8 ± 0.2g	61.5 ± 2.3feg
Lady Finger	39.5 ± 2.6cb	29.4 ± 3.3ed	69.0 ± 1.0dc
Pisang Lemark	46.7 ± 2.7a	14.5 ± 1.3hi	61.1 ± 2.5fg
PK 6	47.4 ±1.6a	23.8 ± 2.1f	71.2 ± 3.0c
Pome	39.3 ± 1.7cb	26.4 ± 2.5ef	65.6 ± 1.4de
Maize	2.20 ± 0.6f	88.3 ± 0.4a	90.4 ± 0.6a
Wheat	2.80 ± 0.3f	73.1 ± 1.2b	75.8 ± 1.3b
Resistant Starch*	42.1 ± 1.6b	45.6 ± 4.4c	89.0 ± 3.6a

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of three independent measurements.
*Standard Resistant starch flour from (Sigma Aldridge, Poole, UK) with a resistant starch content of 44 ± 5% w/w on a dry weight basis (dwb).

The RS contents of the banana flours except for FHIA18 and Green Red were comparable to those reported by Bezera et al., (2013) for Cavendish (40.14%), Sarawong et al., (2014b) for Cavendish (47.25%), and Tribess et al., (2009) for Terra (40.9-58.29%). The RS content in the present study was however lower than those reported by Juarez-Garcia et al., (2006) for non-descript *M. paradisiaca* (56.29%). Differences in the results may be due to different processing parameters as well as differences in varieties. As expected, the RS content of the UBFs was significantly ($p< 0.05$) higher than that of the maize and wheat flours with respective value of 2.2% and 2.8%. Varieties from the same genomic groups varied significantly between each other: FHIA 18 (19.9%), PK 6 (47.7%) and Gold Finger (46.1%) are from the same genomic group AAAB, have different resistant starch content, showing that when looking at the variety with highest starch content, PK 6

stands out. The banana flours when compared to maize and wheat flour, have significantly higher RS content up to 16 times more. This shows that banana flours can be used to increase the resistant starch content of food products and consequently the total dietary fibre. Resistant starch which can be defined as the portion of starch and starch degradation products that resist digestion as they pass through the gastrointestinal tract (Fuentes-Zaragoza et al., 2010), seems to reduce the accessibility of the substrate to amylases due to the double-helical, process stability, and rapid stable formation (Fuentes-Zaragoza et al., 2010). Several studies have suggested that consumption of unripe bananas have beneficial effects for human health, a fact often associated with its high resistant starch (RS) content. Although banana represents an alternative source of indigestible carbohydrates, mainly RS and dietary fibre, it is important to keep in mind that, when the unripe banana flour is cooked, its native starch (RS 2) is rendered digestible (Rodríguez-Ambriz et al., 2008). Upon cooling on the cooked gels, the starch granules reorganize and resistant starch is found mainly as RS type 3 which is of particular interest due to its thermal stability (Wepner et al., 1999).

While the Available Starch (AS) and Total Starch (TS) values were relatively higher in maize and wheat flours (Table 3.7), the UBF total and available starch contents significantly ($p<0.05$) varied with banana variety, ranging from 59.1-75.8% and 13.3-45.7% respectively. The TS values were comparable to those presented in studies on other 10 banana varieties from Brazil by Soares et al (2011) (44.4-76.4%) and Da Mota et al., (2000) (61.3-76.5%). Due to the high total starch content, UBF can be investigated as a potential alternative in products where traditionally wheat and maize are used. Overall there is an inverse correlation between the RS and AS content of the different varieties. Considerable variations were noticeable even in varieties part of the same genomic group (FHIA 18, Gold finger and PK 6 are in the same group (AAAB)) showing bananas varieties influence the starch content of flours produced. Variations in the present study with previously reported studies may be due to the difference in the maturation stage of the bananas, variety selection and processing method used to produce the flour.

3.3.2 Functional properties of the flours

The results of functional properties analysis of banana flour compared to maize and wheat are presented in Table 3.8 (Bulk density, water holding capacity, and oil holding capacity) and Table 3.9 (Foaming capacity, foam stability, Emulsifying capacity, and emulsion stability).

Table 3.8 Bulk density, water holding capacity (WOC) and oil holding capacity (OHC) of the banana flours compared to maize and wheat flours

Flour Type	Bulk density (g/mL)	WHC (g/g)	OHC (g/g)
FHIA 18	0.701 ± 0.012fegd	3.59 ± 0.19c	1.95 ± 0.19bac
Gold Finger	0.721 ± 0.021bcd	2.52 ± 0.06f	1.70 ± 0.14edc
Green Red	0.685 ± 0.005g	3.52 ± 0.10c	1.50 ± 0.11e
IBP. 5. 61	0.751 ± 0.006a	2.56 ± 0.09f	1.80 ± 0.28ebdac
Gross Michel	0.720 ± 0.006becd	2.18 ± 0.05h	2.05 ± 0.19a
Lady Finger	0.724 ± 0.010bc	2.11 ± 0.05h	1.65 ± 0.11edc
Khai Thong	0.687 ± 0.006g	4.45 ± 0.18a	1.75 ± 0.10ebdc
Pisang Lemark	0.700 ± 0.014feg	3.96 ± 0.05b	2.00 ± 0.16ba
PK 6	0.711 ± 0.019fecd	2.34 ± 0.04g	1.85 ± 0.19bdac
Pome	0.738 ± 0.004ba	2.76 ± 0.10e	1.65 ± 0.10edc
Maize	0.697 ± 0.026fg	3.10 ± 0.07d	1.65 ± 0.19edc
Wheat	0.690 ± 0.006g	1.12 ± 0.051	1.55 ± 0.10ed

Means with any same letter in a given column are not significantly ($p>0.05$) different
Values reported are means of three independent measurements

3.3.2.1 Bulk density of the flours

Bulk density is an important factor during packaging of flours and affects the functionality of flours especially during mixing with other ingredients. The banana variety used to prepare UBF significantly ($p<0.05$) affect the bulk density (Table 3.8). The bulk density values ranged from 0.685 to 0.751 g/mL. The UBFs were at least equal to or denser ($p<0.05$) than both wheat and maize flour, depending on the banana variety used. The tapped bulk density values reported in the present research were generally higher than reported in previous single or duo variety

studies (0.39 to 0.7 g/mL) (Fagbemi, 1999, Anyasi et al., 2015, Falade & Oyeyinka, 2015, Anyasi et al., 2017). This may stress the importance of variety in affecting UBF bulk density. During handling or transport, the structure of bulk solids collapses significantly because of vibration, when friction between the particles is reduced, the particles rearrange, so bulk density determined by the tapping method, results in improved packing conditions (Bian et al., 2015a).

3.3.2.2 Water holding capacity of the flours

The water holding capacity (WHC) of the UBFs are reported in Table 3.8. They varied significantly ($p<0.05$) within banana variety ranging from 2.11 to 4.45 g/g, for Lady Finger and Khai Thong, respectively. The WHC values observed were higher than those reported previously for single varieties by Falade et al., (2015) (0.98 to 1.69g/g). The WHC of the UBF's was significantly ($p<0.05$) higher than that of wheat flour (1.12 g/g), but fell within the range for maize flour (3.10 g/g). WHC is related to the physical state of starch, dietary fibre and protein in the flour (Waliszewski et al., 2003). In particular, amylose has the capacity to effectively bind water molecules, yielding a higher WHC (Rodríguez-Ambriz et al., 2008). With regards to the wheat, it is possible that the presence of more hydrophobic proteins (gluten) in the wheat flour led to lower WHC compared to the banana flours and the maize flour. In practice, banana flour will require a higher amount of water to reach the same consistency as wheat flour. The water holding capacity of banana flours could be explained by the presence of non-starch polysaccharides which have been reported to increase the water absorption (Serena & Knudsen, 2007). Of particular interest is the presence of soluble non-starch polysaccharides which have been identified in banana flour (Cordenunsi et al., 2008).

3.3.2.3 Oil holding capacity of the flours

There was significant ($p<0.05$) variation in the oil holding capacity (OHC) between the different UBF flours (Table 4.3), ranging from 1.5 to 2.05 g/g. These values relate to the hydrophilic nature of the starch present in the flour and are primarily due to the physical trapping of oil within the starch structure through non-covalent bonds (Zhang et al., 2005, Rodríguez-Ambriz et al., 2008). The OHC of the wheat

(1.55g/g) and maize (1.65 g/g) flours lay within the range of the banana flour values, indicating that the UBF's will function similarly to wheat and maize in food systems such as batters containing high levels of oil. The values observed, were similar to those reported in the literature by Fagbemi (1999) (2.25 g/g) and by Falade et al., (2015).

3.3.2.4 Foaming capacity and stability of the flours

Foaming plays an important role in air containing food systems such as batters, cakes, ice cream, and bread among others. The foaming capacity and stability of the UBFs significantly ($p<0.05$) varied between the different banana varieties (Table 3.9), ranging from 18.1 to 27.5%, while the stability ranged from 8.7 to 58.1%.

Although the forming capacity of the UBF from some varieties was higher than that of wheat flour (24.4%), only the UBF of Gross Michel, Pome and Green Red had similar ($p<0.05$) foam stability to wheat flour. Maize was found to have the lowest foaming capacity (7.5%). These results illustrate that for partial or complete replacement of wheat flour, in foam-based foods, the variety of banana used will play a significant role. In addition, the UBF, irrespective of the cultivar, will probably offer better foam characteristics than maize flour. The foaming capacity values reported here were higher than those previously reported by Fagbemi (1999), probably due to differences in the varieties used in that study.

Table 3.9 Foaming capacity, foam stability, emulsifying capacity and emulsion stability of the banana flours compared to maize and wheat flours

Flour Type	Foaming capacity %	Foam stability %	Emulsifying capacity %	Emulsion stability %
FHIA 18	27.5 ± 2.9a	28.3 ± 2.9fg	55.2 ± 1.2e	35.6 ± 2.0dce
Gold Finger	23.3 ± 1.4bc	8.7 ± 1.5h	75.3 ± 3.9c	40.1 ± 0.4b
Green Red	18.3 ± 2.9d	39.2 ± 1.4cde	89.4 ± 3.4a	45.2 ± 2.6a
IBP. 5. 61	25.0 ± 2.5ba	30.1 ± 2.2fge	63.4 ± 2.4d	37.7 ± 1.4c
Gross Michel	18.1 ± 1.3d	58.1 ± 1.6b	57.6 ± 3.9ed	34.7 ± 0.6de
Lady Finger	20.6 ± 1.3dc	36.1 ± 2.4fde	58.7 ± 2.8ed	33.9 ± 0.5fe
Khai Thong	18.1 ± 1.3d	30.2 ± 2.7fge	93.7 ± 2.9a	46.5 ± 1.0a
Pisang Lemark	22.5 ± 2.5bc	22.4 ± 2.5g	80.7 ± 4.9cb	41.9 ± 0.6b
PK 6	18.3 ± 1.4d	10.8 ± 1.4h	83.1 ± 2.2cb	36.4 ± 0.8dc
Pome	20.6 ± 1.3dc	39.9 ± 3.3cd	81.5 ± 1.3b	37.6 ± 0.8c
Maize	7.5 ± 0.1e	61.1 ± 9.6a	32.0 ± 2.1f	6.0 ± 0.5g
Wheat	24.4 ± 1.3b	46.6 ± 3.0cb	53.2 ± 7.4e	32.5 ± 0.6f

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of at least three independent measurements.

3.3.2.5 Emulsifying capacity and stability of the flours

The emulsifying capacity and stability values of the UBFs are reported in Table 3.9 and varied significantly ($p<0.05$) within the different banana varieties, ranging from 55.2 to 93.7% and 33.9 to 46.5%, respectively. The UBF from all varieties were similar ($p<0.05$) or had greater emulsify capacity than both wheat and maize flour, while the stability was clearly greater ($p<0.05$) than both wheat and maize flour. The values of emulsifying capacity obtained can be explained by the differences observed in the starch composition (Table 3.7), as well as the low protein content of banana flour (Rodríguez-Ambriz et al., 2008, Bolade et al., 2009). When compared to soy protein (Table 2.7, page 26), banana flour has lower emulsifying capacity and stability showing that in an application where these properties are required, the banana flour will not perform as good as soy protein. However, it can be predicted that UBF would probably be a better emulsifying ingredient in a formulation compared to both wheat and maize flour.

3.3.3 Thermal, pasting properties of the flours and texture of the set gels

3.3.3.1 Pasting properties of the flours

The pasting properties of the different flours are reported in Table 3.10. A typical graph of banana flours compared to wheat are presented in Appendix 2. There were significant variations in the pasting parameters observed for the different UBF cultivars.

The UBF pasting temperature values ranged from 63.8 to 71.1°C and were in accordance with those reported for 23 Colombian banana varieties (Dufour et al., 2009a), but higher than that reported for Cardaba variety (Ayo-Omogie et al., 2010). The pasting temperature is an indication of the heat required to cook the banana flour beyond its gelatinization point (BeMiller, 2011, Bakare et al., 2016). The pasting temperature for the maize (69.3 °C) fell within the range of the UBF, but wheat was significantly higher (83.8 °C), which is comparable to values reported by Chen *et al*. (2010). This is probably due to the hindrance of starch granule swelling by the gluten matrix (Chen et al., 2010). The difference between pasting properties is influenced by the starch granular size, the amylose to amylopectin ratio, starch molecular characteristics, the volume fraction of suspended solids, and the conditions of the thermal process used to induce gelatinization (Zhou et al., 1998).

The UBF peak viscosities ranged from 405.5 to 556.6 RVU and were comparable to those reported by Ayo-omogie et al., (2010) (449 RVU) for Cardaba variety, and Da Mota et al., (2000). All the UBF peak viscosities observed were significantly ($p<0.05$) higher than those of the wheat (124.7 RVU) and the maize (115.6 RVU) flours.

Table 3.10 Pasting properties of banana flours compared to maize and wheat flour

Flour Type	Pasting Temperature (℃)	Peak Viscosity (RVU)	Trough Viscosity (RVU)	Breakdown Viscosity (RVU)	Final Viscosity (RVU)	Setback Viscosity (RVU)
FHIA 18	69.2 ± 0.1dc	418.8 ± 3.3g	229.1 ± 4.0b	189.7 ± 1.9h	292.6 ± 2.8c	63.5 ± 1.8d
Gold Finger	68.7 ± 0.3d	503.6 ± 7.7dc	176.2 ± 1.7e	327.4 ± 6.2b	248.7 ± 2.5e	72.4 ± 2.4c
Green Red	63.8 ± 0.1f	405.5 ± 2.5h	137.3 ± 4.7g	268.3 ± 7.7e	213.0 ± 2.4g	75.7 ± 4.1c
Gros Michel	69.1 ± 0.1dc	514.0 ± 7.6c	259.4 ± 2.7a	254.6 ± 4.9f	305.3 ± 0.3b	46.0 ± 2.7f
IBP. 5. 61	67.6 ± 0.7e	556.6 ± 3.2a	205.7 ± 2.5c	350.9 ± 5.3a	263.9 ± 2.0d	58.3 ± 4.4ed
Khai Thong	69.1 ± 0.1dc	476.8 ± 0.9e	181.0 ± 6.0ed	295.8 ± 5.4d	252.8 ± 1.7e	71.8 ± 5.7c
Lady Finger	71.1 ± 0.4c	492.7 ± 6.3d	261.2 ± 3.8a	231.5 ± 9.3g	333.4 ± 5.6a	72.3 ± 1.8c
P. Lemark	68.9 ± 0.4dc	490.9 ± 4.7d	165.1 ± 9.6f	325.9 ± 5.0b	253.2 ± 9.8e	88.2 ± 1.6b
PK 6	69.3 ± 0.4c	450.0 ± 12.8f	185.1 ± 5.7d	264.9 ± 7.6e	241.8 ± 3.3f	56.6 ± 2.4e
Pome	68.6 ± 0.4d	537.4 ± 9.9b	229.7± 7.3 b	307.7 ± 3.4c	300.5 ± 1.7b	70.8 ± 5.7c
Maize	69.3 ± 0.5c	115.6 ± 1.4i	88.60 ± 2.4i	27.00 ± 2.0j	116.5 ± 2.0i	27.9 ± 0.8g
Wheat	83.8 ± 0.1a	124.7 ± 1.7i	36.20 ± 1.1h	88.50 ± 0.7i	133.3 ± 2.5h	97.1 ± 1.4a

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of at least three independent measurements.
1 RVU = 12 centipoise.

Peak viscosity occurs once most of the granule swelling has ceased. Since not all granules are swollen, the starch paste is a mixture of fragments and swollen starch granules, at this stage in the analysis. This causes an increase in the viscosity and further disintegration of the starch granules. The peak viscosity also measures the alpha-amylase activity and other contributory factors such as the inherent susceptibility of the starch to amylase and the starch gel strength (Bakare et al., 2014). Higher peak viscosities indicate the greater ease of swelling of starch granules during heating, which is due to weaker cohesive forces within the granules.

The UBF trough and breakdown viscosities 137.3 to 261.2 RVU and 189.7 to 350.9 RVU, respectively. The trough/minimum and breakdown viscosities are a measure of the stability of a given flour during cooking (resistance to breakdown due to shear). The trough viscosity indicates the ability of starch granules to resist mechanical shear. The smaller the breakdown viscosity, the higher the paste stability (Bakare et al., 2012). The breakdown viscosity values reported in the present work were comparable to those reported by da Mota et al., (2000) (68 to 272 RVU), but higher than those reported by Pelissari et al., (2010) (154 RVU) and Sarawong et al., (2014) (45.3 RVU). The variation in the observed data, as well as that reported in the literature, shows different banana varieties could behave differently during high shear type cooking processes such as extrusion and steam jet cooking. According to Da Mota et al., (2000) banana starch granules can be categorized as those with granules sensitive to mechanical damage and those that are more shear resistant. The UBFs had much ($p< 0.05$) higher trough and breakdown values compared to wheat and maize flours (Table 3.10). This indicates that the UBFs flours may tend to thin relatively rapidly during continuous cooking processes, compared to both wheat and maize flour.

The UBF final viscosity and the setback viscosity values ranged from 213.0 to 333.4 RVU and 46.0 to 88.2 RVU, respectively (Table 3.10). Setback viscosity is the phase of the pasting curve after cooling the starches to 50°C. This stage involved reassociation, retrogradation, or reordering of starch molecules. Also, the water previously bounded in the viscoelastic gel are released at this stage in a process referred to as syneresis. The higher the setback viscosity, the greater is the tendency toward retrogradation. The UBF values were comparable to the final and

setback viscosity values previously reported for other UBF varieties, such as Terra (Pelisa et al., 307 RVU and 83 RVU), Cardaba (Ayo-Omogie et al., 2010) (410 RVU and 131 RVU), for non-descript banana flours (88 RVU and 26 RVU) (Sarawong et al., 2014b) and Cavendish (Bezerra et al., 2013) (418 RVU) for final viscosity. Banana flour have a greater tendency to retrograde compared to maize but less than wheat meaning a product containing banana flour will stale less than a similar product with wheat flour. The wide variation in values observed indicates banana variety differences is probably based on variations of the starch content of different banana flours. The UBF's had significantly ($p<0.05$) higher final viscosities compared to both wheat (133.3 RVU) and maize (116.5 RVU) flour (Table 3.10), and much ($p<0.05$) lower setback viscosity than wheat (97.1 RVU), but much higher than the maize (27.9 RVU). Final viscosity is the section where the gelatinized dispersion of starch becomes viscoelastic on cooling, resulting in the formation of a loose paste or gel. Therefore, banana flour may have a stronger gel upon cooling due to its high viscosities. The pasting properties can be used to determine the application of flours in a specific product. Banana varieties with higher viscosities will be suitable for products where high viscosity is required and can be used in small quantities compared to wheat and maize.

3.3.3.2 Texture of set gels

The gel texture of the UBFs from the different varieties compared to the wheat and maize flours are shown in Table 3.11. A typical graph of UBFs compared to wheat flour is found in Appendix 3.

There were significant ($p<0.05$) variations in the firmness, penetration energy, adhesive force and adhesiveness of the UBF gels from the banana varieties. The UBFs generally had significantly ($p<0.05$) higher penetration related parameters (gel firmness and penetration energy) than both the wheat and maize flour. The higher UBF penetration parameters for most varieties were probably due to a greater ability of the UBFs gels to re-crystallize compared to the wheat flour gels. The amylopectin in the UBF starch of most of the varieties probably had longer polymer chains (due to C-type crystallinity compared to A-type crystallinity (Yuan et al., 1993), which probably enable more crystallization junction zones, hence a stronger three-dimensional recrystallized-amylopectin network compared to both

the wheat and the maize flour starch. Texture plays an essential role in determining the final acceptance by the consumer and it is one of the predominant criteria for assessing the final product (Antognelli, 1980). The adhesive force of the wheat and maize flour gels were within the range observed for the UBFs, while the adhesiveness was much ($p<0.05$) less than that of the UBFs.

Table 3.11 Textural properties of banana set gels compared to maize and wheat

Flour Type	Gel Firmness (g)	Penetration Energy (g.sec)	Adhesive Force (g)	Adhesiveness (g.sec)
FHIA 18	8.47 ± 0.89c	30.98 ± 2.97ba	-3.63 ± 0.29i	-1.47 ± 0.14gf
IBP. 5. 61	11.44 ± 0.83a	33.32 ± 2.41a	-1.63 ± 0.16b	-1.38 ± 0.12ef
Khai Thong	9.04 ± 0.83c	31.65 ± 1.95ba	-1.97 ± 0.18ced	-1.27 ± 0.11ed
Lady Finger	7.24 ± 0.76d	29.51 ± 2.99b	-2.61 ± 0.20g	-1.58 ± 0.16g
Gold Finger	9.85 ± 0.86b	30.25 ± 1.63ba	-2.09 ± 0.21fed	-1.59 ± 0.18g
Green Red	8.79 ± 0.69c	32.21 ± 3.41ba	-2.22 ± 0.22fe	-1.31 ± 0.11e
Gross Michel	9.03 ± 0.72c	26.18 ± 2.31b	-1.17 ± 0.10a	-1.23 ± 0.21a
P. Lemark	10.04 ± 0.82b	32.16 ± 1.41ba	-1.90 ± 0.24cbd	-1.34 ± 0.12ef
PK 6	8.97 ± 0.62c	31.20 ± 1.65ba	-1.71 ± 0.19cb	-1.15 ± 0.13cd
Pome	8.54 ± 0.71c	31.50 ± 2.04ba	-2.89 ± 0.31h	-1.39 ± 0.14ef
Maize	6.56 ± 0.49d	21.11 ± 1.26c	-2.27 ± 0.18f	-0.97 ± 0.09b
Wheat	3.49 ± 0.27e	13.07 ± 1.59d	-1.84 ± 0.20cb	-1.00 ± 0.07cb

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of at least 5 measurements.
Firmness/Hardness = Maximum/Peak force.
Adhesiveness force = Maximum negative force.
Penetration Energy = Positive Area.
Adhesiveness = Negative Area (Work in the negative area).

These results imply that, depending on the banana variety, pre-gelatinized UBF could give greater thickening action than both pre-gelatinized wheat and maize flours, when applied as a thickening agent.

3.3.3.3 Thermal properties

The results of thermal analysis of different banana flours compared to maize and wheat are reported in Table 3.12.

Table 3.12 Thermal properties of banana flours compared to maize and wheat

Flour Type	Gelatinization Temperature (°C)	Gelatinization Enthalpy (Jg^{-1})
FHIA 18	69.44 ± 2.96a	-7.81± 0.89fe
Gold Finger	71.18 ± 0.66a	-6.09 ± 0.25c
Green Red	64.67± 0.04b	-5.42 ± 0.27c
Gros Michel	68.32 ± 0.16a	-8.30 ± 0.50f
IBP. 5. 61	69.17 ± 0.36a	-8.66 ± 0.30f
Khai Thong	69.51± 0.24a	-6.28 ± 0.21dc
Lady Finger	70.74 ± 1.12a	-8.30 ± 0.20f
Pisang Lemark	71.21 ± 1.79a	-5.43 ± 0.56c
PK 6	68.77 ± 0.11a	-10.12 ± 0.01g
Pome	69.81 ± 1.89a	-7.06 ± 0.06de
Maize	69.95 ± 1.41a	-0.68 ± 0.03b
Wheat	71.11 ± 0.74a	-1.60 ± 0.06a

Means with any same letter in a given column are not significantly (p>0.05) different.
Values reported are means of at least three independent measurements.

Thermal properties of bananas flours are closely linked to their pasting properties (Dufour et al., 2009a). The gelatinization temperature of the banana flours was moderately correlated with the pasting temperature (R^2 = 0.62), probably due to the differences in the principles of measurement by RVA and DSC techniques. The gelatinization temperature and associated enthalpies were in accordance with those reported in previous studies on varieties not assessed in the present work with ranges of 67.95-76.8 C and 9.04-13.3 J/g respectively (Da Mota et al., 2000, Tribess

et al., 2009, Pelissari et al., 2012). The gelatinization enthalpies in the present study generally lower probably due to differences in the varieties. The gelatinization temperature of the UBFs had no significant ($p<0.05$) differences with that of wheat and maize flour (see Table 3.12), apart from Green Red UBF, while the enthalpy significantly ($p < 0.05$) varied with banana variety and with both wheat and maize flours. These observations probably result from differences in crystalline amylopectin structure and quantities in the samples. More crystalline quantities would lead to higher gelatinization enthalpies and vice versa. The UBFs from all the banana varieties had higher gelatinization enthalpies peaks compared to both maize and wheat flour. Similar observations were recently made by Tribess et al., (2000) for comparison with wheat flour. It was suggested the higher gelatinization enthalpies of banana flours result from a longer amylopectin chain length of banana C-type crystallinity flour compared to shorter A-type crystallinity amylopectin chains (Yuan et al., 1993, Tribess et al., 2009).

3.3.4 Multivariate exploratory analysis: Principle Component Analysis (PCA)

Principle Components Analysis showed that first and second principle components (PC1 and PC2 respectively) could account for 56.59% and 14.48% of the variation in the results, giving 71.04% (Figure 3.3). Two major clusters, X and Y with a low and high loading on PC1 respectively could be observed. Based on the variables principle component analysis plot and with flour type as a grouping variable, it was clear that cluster X was associated with banana flour (negative factor loadings on PC1) while cluster Y was associated with wheat and maize flours (positive factor loadings on PC1).

There was a close relationship between the breakdown viscosity and resistant starch content. This indicated that it could be possible to predict the resistant starch content in banana flours based mostly, on the breakdown viscosity. Granule structural arrangements are associated with greater resistance to enzymatic hydrolysis and smaller round granules are more susceptible to hydrolysis than larger granules (Soares et al., 2011).

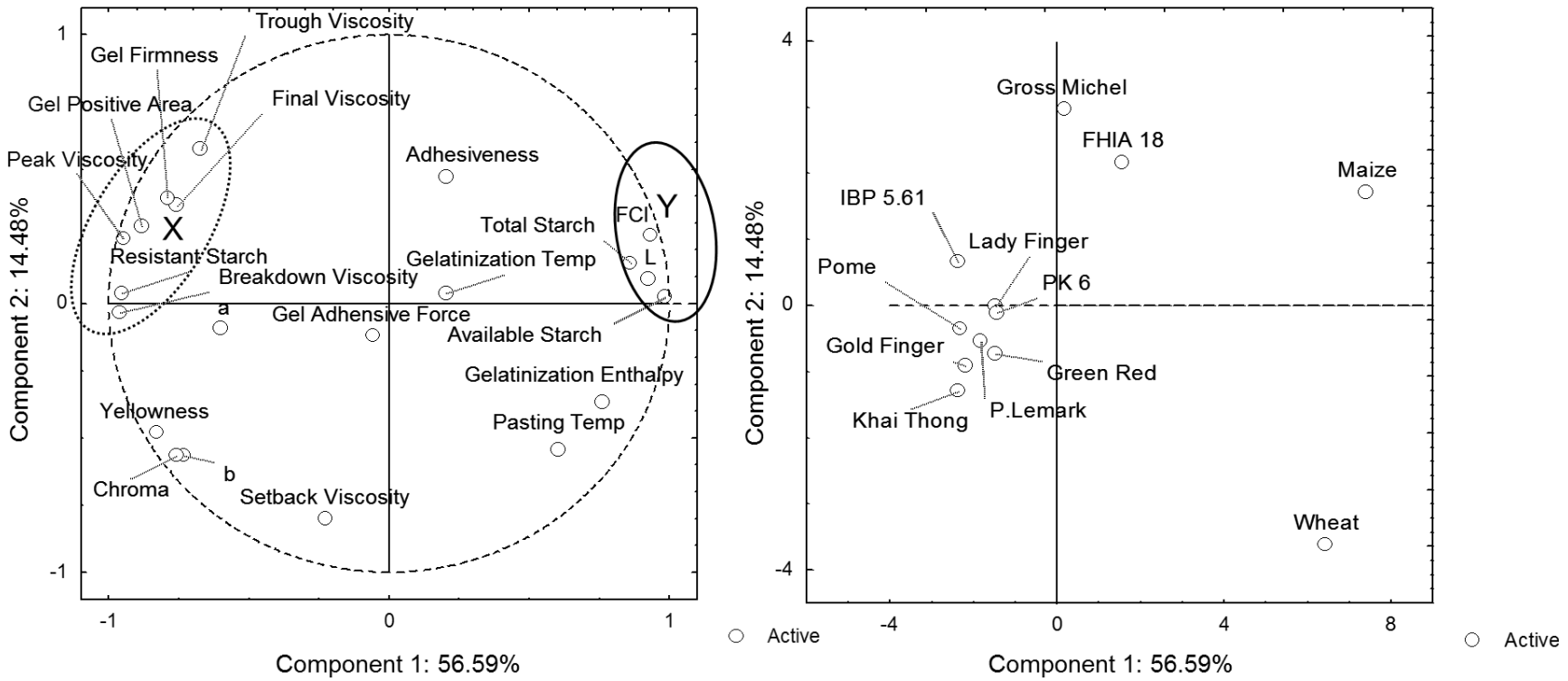

Figure 3.3 Principal component analysis of physico-chemical properties UBFs compared to maize and wheat flour

In addition, the outer structures of banana starch granules are more responsible for resistance to digestion, rather than the internal crystalline structures (Jiang et al., 2015). A linear regression assessment between breakdown viscosity values and resistant starch content of the samples gave the equation:

$$\text{Resistant starch} = 0.157 \times \text{Breakdown viscosity} + 4.730 \qquad \text{Equation 13}$$

With an R^2 value of 0.898

The variables that were spatially separated from the banana types (on both PC1 and PC2), were gel adhesiveness and pasting temperature (in different quadrants, and furthest from axes). These could serve as reliable measures for differentiating between UBFs from different desert banana flours. The use of pasting temperature for differentiation of UBFs from dessert bananas from FHIA hybrids, non-cooking plantains, and cooking plantains was recently recommended by Dufour et al. (2009b).

3.4 CONCLUSION

The aim of this chapter was to determine the physico-chemical characteristics of banana flour in comparison to maize and wheat flour. It was found that there were considerable differences between banana varieties, maize, and wheat in terms of functional properties. Overall banana flour had higher ash content, water holding capacity and oil holding capacity compared to wheat and maize flour. Taking into account the fact that starch granules size, composition, and shape affect the thermal properties of flours, the differences noted between banana flour, maize, and wheat flour can be explained by the properties of the starch granule. Of particular interest, FHIA 18 and Gross Michel showed properties which were different from other cultivars used. Banana flour presented similarities with maize and wheat flour making it a possible replacement in food products where an increase in resistant starch content is required.

CHAPTER 4 : DEVELOPMENT OF GLUTEN-FREE BREAD USING UNRIPE BANANA FLOUR

4.1 INTRODUCTION

Celiac disease is an autoimmune disease that is triggered by the ingestion of gluten-containing cereals (wheat, barley, rye, and possibly oats) by genetically predisposed subjects (Gallagher, 2009). The consumption of gluten leads to the damage of the lining in the small intestine and consequently the malabsorption of nutrients (Arendt & Dal Bello, 2011). Advancements in diagnostics methods for this disease has led to an increase in the number of diagnosed patients. For these patients, a strict gluten-free diet is considered to be the only treatment for this disease (Gallagher, 2009).

Since wheat-based flours are typically used in most dough-based products (bread, pasta, pastries), researchers are continuously searching for alternative gluten-free flours. The most commonly studied gluten-free flours are maize flour and rice flour. Other alternative flour studied include cereals such as sorghum, pseudocereals such as quinoa, buckwheat, amaranth, and nut-based flours such as chestnut.

Of great interest, unripe banana flour is gaining popularity in the research community as this flour has been used in the development of good quality gluten-free pasta (Agama-Acevedo et al., 2009, Zandonadi et al., 2012), slow digestible cookies (Aparicio-Saguilán et al., 2007) as well as gluten-free bread (Sarawong et al., 2014a).

Owing to the fact that gluten plays a critical role in food products such as baked products and pasta, its removal in gluten-free products requires additional ingredients and/or technologies to mimic the visco-elastic properties of gluten proteins (Gallagher et al., 2004). In this study, the use of hydrocolloids and sourdough fermentation was investigated to improve the quality characteristics of gluten-free bread made from unripe banana flour.

4.2 MATERIALS AND METHODS

4.2.1 Ingredients

For the preparation of the gluten-free bread, a single banana variety (Gros Michel) was selected, and used in combination with rice flour in the formulations of gluten-free blends. Other additional ingredients are summarized in Table 4.1 as well as the source from where they were obtained.

Table 4.1 Ingredients used in the development of gluten-free bread and their source

Ingredients	Sources
Rice flour	Lifestyle Food, Dischem, South Africa
Wheat flour	Eureka, Dischem, South Africa
Anchor instant yeast	Checkers, South Africa
Sugar	Pick n Pay, South Africa
Salt	Pick n Pay, South Africa
Alpha-amylase	Anchor yeast, Johannesburg, South Africa
Guar gum	Tate & Lyle, Durban, South Africa
Xanthan gum	Tate & Lyle, Durban, South Africa

4.2.2 Gluten-free bread formulations

The development of gluten-free formulations from unripe banana flour has been subdivided into four different phases namely: optimization of flour blend and water content, the addition of hydrocolloids, sourdough fermentation, and hydrocolloids plus sourdough fermentation. The formulation of the different phases is summarised in Table 4.2. The formulations include flour (gluten-free blend or wheat), water, sourdough (banana), salt, sugar, instant yeast, and hydrocolloids. The hydrocolloids used for the different formulations included guar gum and xanthan gum individually or as combinations.

Table 4.2 Formulations used for the different gluten-free flour blends

Phase 1	**Flour blends**	**FB100**	**FB80**	**FB60**	**FB40**	**FB20**
	Banana flour (g)	100	80.0	60.0	40.0	20.0
	Rice flour (g)	0.00	20.0	40.0	60.0	80.0
	Water (g)	80.0	80.0	80.0	80.0	80.0
	Water optimisation	**FB60W1**	**FB60W2**	**FB60W3**	**FB60W4**	
	Banana flour (g)	60.0	60.0	60.0	60.0	
	Rice four (g)	40.0	40.0	40.0	40.0	
	Water (g)	80.0	100	120	140	
Phase 2	**Hydrocolloids**	**FB60H1**	**FB60H2**	**FB60H3**	**FB60H4**	**FB60H5**
	Dough (FB60W4) (g)	100	100	100	100	100
	Guar gum (g)	2.00	1.50	1.00	0.50	0.00
	Xanthan gum (g)	0.00	0.50	1.00	1.50	2.00
Phase 3	**Sourdough treatments**	**SD1**	**SD2**	**SD3**		
	Dough (FB60W4) (g)	100	100	100		
	Sourdough (g)	12.5	25.0	50.0		
Phase 4	**Sourdough with hydrocolloids**	**SD1**	**SD1H2**	**SD1H4**		
	Dough (FB60W4) (g)	100	100	100		
	Sourdough (g)	12.5	12.5	12.5		
	Guar gum (g)	0.00	1.50	0.50		
	Xanthan gum (g)	0.00	0.50	1.50		

Salt (1.4%), sugar (5%), yeast (2%) and alpha amylase (0.002%) were held constant in all treatments

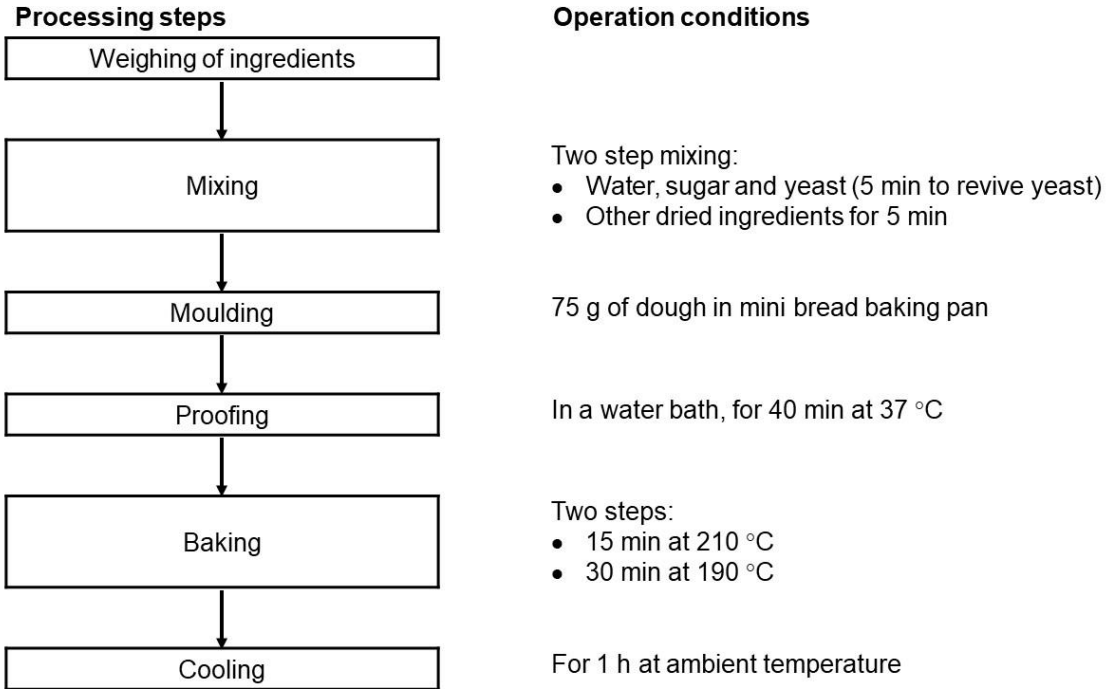

Figure 4.1 Flow chart of the gluten-free bread baking process

The baking process is illustrated in Figure 4.1. In brief, yeast was pre-dispersed with sugar and water for 5 min. Then, flour, salt, hydrocolloids and/or sourdough was manually mixed. After that, the pre-dispersed yeast and the rest of the water were added and mixed for 5 min. The resultant batter (75 g) was poured into mini bread baking pans and fermented for 40 min in a water bath (Scientific, Johannesburg, South Africa) held at 37 °C. Baking was carried out in a multifunction double oven (Gemini Gourmet, Defy appliances, Durban, South Africa) in two steps, namely: at 210 °C for 15 min and then at 190 °C for 30 min. The final baked products were cooled at ambient temperature for 1 h, then packed into Ziploc bags overnight and analysed as described below (Demirkesen et al., 2010; Mohamed et al., 2010; Coda et al., 2011).

4.2.3 Sourdough preparation

The sourdough destined for bread-making was prepared in a three-step procedure:

Step 1: *Lactobacillus Plantarum* strains R and A (Department of Biotechnology and Food Technology, Tshwane University of Technology, South Africa) were incubated in de Man Rogosa Sharpe (MRS) (Merck, Johannesburg, South Africa) broth at 37 °C for 48 h to revive the cultures.

Step 2: Sourdough starter was prepared using banana flour (80 g), glucose (10 g), salt (0.5 g), and water (1 L), and was partially gelatinized by heating for 10 min on a heating plate held at 100 °C.

Step 3: The prepared dough (100 mL) was inoculated with the revived culture (1 mL), and the dough was allowed to ferment (at 37 °C) until pH 3.9 or less, was obtained. The sourdough was refreshed by back-slopping with freshly prepared dough, at a ratio of 1:9 sourdough to the fresh dough, and further incubated (37 °C until pH 3.9 or less).

To ensure a continuous supply of sourdough, the above procedure was done daily, for 5 days and the resulting sourdough refrigerated (for a maximum of 5 days) until use. The fermented dough was then used according to the formulations given in Table 3.4 for baking. A portion of the sourdough was kept to inoculate the next batch of sourdough (Galle et al., 2012, Edema et al., 2013).

4.2.4 Dough rheological properties

All formulations in Table 4.2 were analysed for rheological properties. Rheological behaviour and functional properties of doughs were determined using a Mixolab (Chopin, Tripette et Renaud, Paris, France) and analysed using the standard Chopin+ protocol. The protocol consisted of a heating/cooling cycle, while the sample is being mixed (speed of 80 rpm) at a constant rate. The required amount of flour for each analysis was calculated according to the Mixolab® software (ratio of moisture content and hydration rate of each sample). The effect of variable amounts of water, xanthan gum, and guar gum concentrations was studied using the Mixolab (Marco & Rosell, 2008). The following parameters were obtained from the curve: initial consistency (C1), stability time (min) at which the torque produced was kept constant, minimum torque (Nm) produced by dough passage subjected to mechanical and thermal constraints (C2), peak torque (Nm) produced during the heating stage (C3), the minimum torque during the heating period (Nm) (C4) and the torque obtained after cooling at 50°C (C5) (Bonet et al., 2006, Rosell et al., 2010).

4.2.5 Baking performance and bread quality

4.2.5.1 Specific volume

The specific volume of the baked products was determined according to the AACC Approved Method 10-05.01 (AACC, 2000) and expressed as the weight/volume ratio of the bread (g/mL).

4.2.5.2 Crumb and crust colour

The crumb and crust colour attributes of the baked products were determined using a chroma meter (CR 400, Konica Minolta, Tokyo, Japan). The instrument was standardized using the white calibration plate. A black plastic cell containing the sample was placed against the light source and the L^* (Lightness), a^* (red-green) and b^* (yellow-blue) values were recorded. The values obtained were used for the determination of chroma, yellowness and colour index of the baked bread.

4.2.5.3 Crumb structure

The crumb structure of the baked products was evaluated using image analysis. Images of the sliced baked products (25 mm) were scanned using an Image Scanner, the distribution of gas bubble, and crumb density were determined using a density scale.

4.2.5.4 Texture analysis (baked product firmness)

The bread firmness was determined according to the AACC method 74-09 using the compression test, with the texture analyzer (TA.XT Plus Texture Analyzer, Stable Micro Systems, Godalming, Surrey, UK). The analyzer was fitted with a 36 mm diameter cylindrical probe. The maximum peak force (N) was measured from the penetration curve and expressed as product firmness (N). To determine the staling quality characteristics of the baked products, samples were packaged (polyethylene bags), sealed and stored at ambient temperature for five days, and the firmness and crust hardness analyzed daily.

4.2.6 Sensory evaluation and consumer acceptability

The sensory profile of the baked products was subjected to a bench top sensory evaluation and a consumer acceptance test.

4.2.6.1 Benchtop sensory evaluation

The sensory evaluation was performed using a panel composed of 6 postgraduates' students from the Department of Biotechnology and Food Technology. The panel was instructed to assess the sensory attributes (appearance, texture, taste and overall acceptability), using a 9-point hedonic scale, of all the formulations developed, and based on the results, the most promising formulations were selected for consumer acceptability testing.

4.2.6.2 Consumer acceptability test

The sensory attribute of gluten-free bread was evaluated by 25 untrained panellists using a 9 point hedonic scale (Appendix 5) at the Tshwane University of Technology. The attributes considered were aroma, taste, appearance, texture and overall. The procedure was as follows:

4.2.6.2.1 The panel

The sensory panel was composed of male and female who were at least in second-year national diploma food technology, they have been exposed to evaluating products as part of their course. A consent (Appendix 4) form was given to the panel to sign as an indication of acceptance to partake in the present study, as well as a list of samples and evaluation instructions.

4.2.6.2.2 Sample preparation

The samples to be evaluated were baked at least 2 h before the sensory evaluation and were allowed to cool at room temperature before slicing (1 cm thick) immediately before the test.

4.2.6.2.3 Sensory evaluation

Each sample was attributed with a three digit code. Three samples were testing each time. The samples were placed following three different order on the tray and a cup of water as placed as well. Panellists were instructed to test the sample in no particular order but to rinse their mouth with water between each sample. The 9-point hedonic scale for each parameter, where 1 = dislike extremely and 9 = like extremely was used. The following sensory attributes were evaluated: taste, appearance, texture, flavour and overall (Appendix 5). Products were found acceptable if their mean scores for the acceptability are above 5.

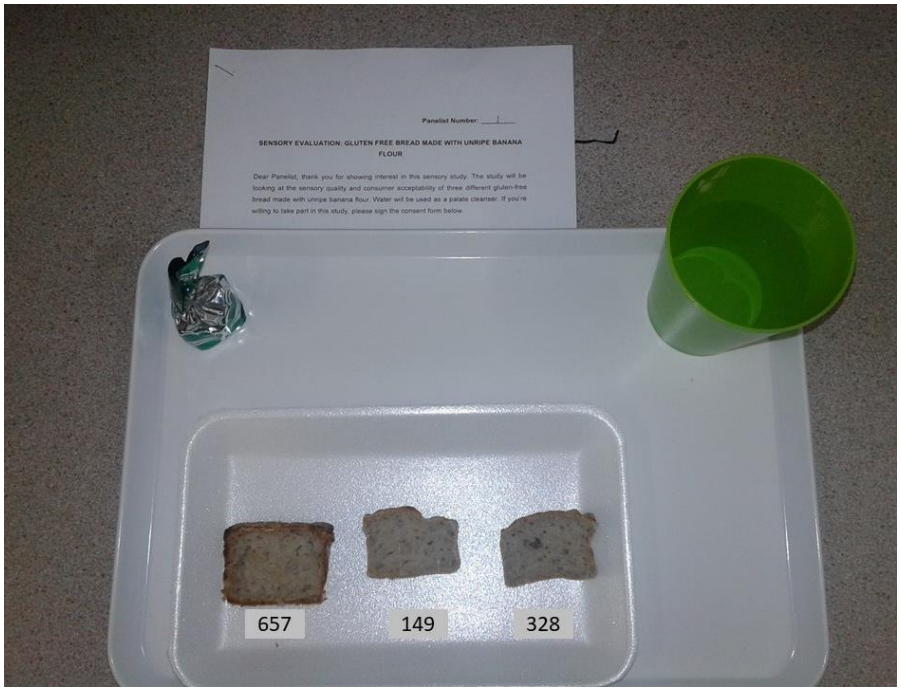

Figure 4.2 Sensory evaluation set up

4.2.7 Shelf life testing

The shelf life of the baked products was done using texture and moisture analysis, as well as microbiological quality. The baked bread, packed in Ziploc polyethylene bags were stored at room temperature (20 °C) for a maximum of five days. Each day sample was analyzed for texture according to the method in section 4.2.5.4 and moisture (section 3.2.3.2).

The microbial composition of the baked bread was analysed for total plate count using Total Plate Count Agar (PCA) (incubate at 28 °C for 48 h), yeast and mould

counts using Rose Bengal Chloramphenicol Agar (RBCA) and Malt extract agar (MEA) both of which were incubated at 25 °C for 7 days. The procedure was as follows:

- For MEA, a sample from the bread was directly transferred in the middle of the plate using a sterile needle. After incubation (25 °C for 7 days), the presence or absence of growth was determined visually.
- For PCA and RBCA, a 1:10 dilution was prepared using 10g of bread sample 100 mL sterile Ringer solution (Merck, Johannesburg, South Africa). The mixture placed in a sterile stomacher bag and macerated with a stomacher machine (Seward, England) for 3 min at 250 rpm to homogenize the sample. A 10-fold dilution series (up to 10^{-6} dilution) was prepared, by aseptically transferring 1 mL of the solution aliquot into 9 mL of sterile Ringer solution in sterile test tubes and vortexed (Heidolph HEAX Top, Germany). One hundred microliters (100 µL) of each dilution was then transferred in triplicate to Petri plates (90 mm, Merk, South Africa). The plate count was visually performed and colonies in the range of 30-300 were recorded and result expressed in Log CFU/mL.

4.2.8 Statistical analysis

All measurements were done in triplicate. Statistical analysis was done using SAS Software V8 (SAS Institute, Inc., Cary, NC, USA). One-way ANOVA with Duncan's Multiple Range correlation test for separation of means between samples was used (confidence interval of 95%). Principal component analysis and linear regression were done using Statistica© Software, version 8 (StatSoft, Inc., Tulsa, Oklahoma, USA).

4.3 RESULTS AND DISCUSSION

4.3.1 Optimization of banana flour blend for bread formulation

In the first 5 formulations (FB100, FB80, FB60, FB40 and FB20), banana flour and rice flour were used in different proportions to create a flour blend, and the water content was held constant at 80% (Table 4.2). Images of the dough and baked bread are given in Figure 4.3.

Compared to the wheat-based dough (standard), all doughs made from banana flour (FB100, FB80, FB60, FB40 and FB20) in Figure 4.3, appeared less elastic, cracking and dry. This can be solved by increasing the amount of water used as gluten-free doughs generally require more water than gluten dough (De La Hera et al., 2014).

After baking, the standard dough resulted in a bread with a smooth crust, a uniform golden brown colour which raised well. Wheat flour is known to produce bread of best quality as the presence of gluten proteins allow to have a dough with good viscoelastic properties. Gluten-free blend on the other side failed to rise and resulted in bread with cracking of the crust, non-uniform colour that failed to rise during proofing. This was mainly associated with the amount of water used which was too low and the lack of gluten protein as gluten plays a role in the structure of the dough by holding carbon dioxide gas.

In addition, the sensory attributes of the gluten-free bread, made using 100% banana flour, particularly the taste was not acceptable with a bitter/astringent after taste. This could be explained by the fact that bananas when still in their green stage contain a high amount of tannins which contribute to the astringency/bitterness of the flour (Barnell & Barnell, 1945).

When used in bread formulation, masking agents are needed due to the green banana flour which has a particular taste that is not common and desirable to the consumer. I

n a study by Loong et al. (2018) in which banana flour was blended with wheat flour to make bread, the panellists preferred the sample which was supplemented with coffee as the coffee masked the banana flour taste. Although the four other

formulations (FB80, FB60, FB40 and FB20) did not result in a better quality bread in terms of baking performance, their sensory attributes were improved. FB60, which contain 60% banana flour and 40% rice flour did not present any bitter/astringent after taste and was thus selected as the basic flour blend.

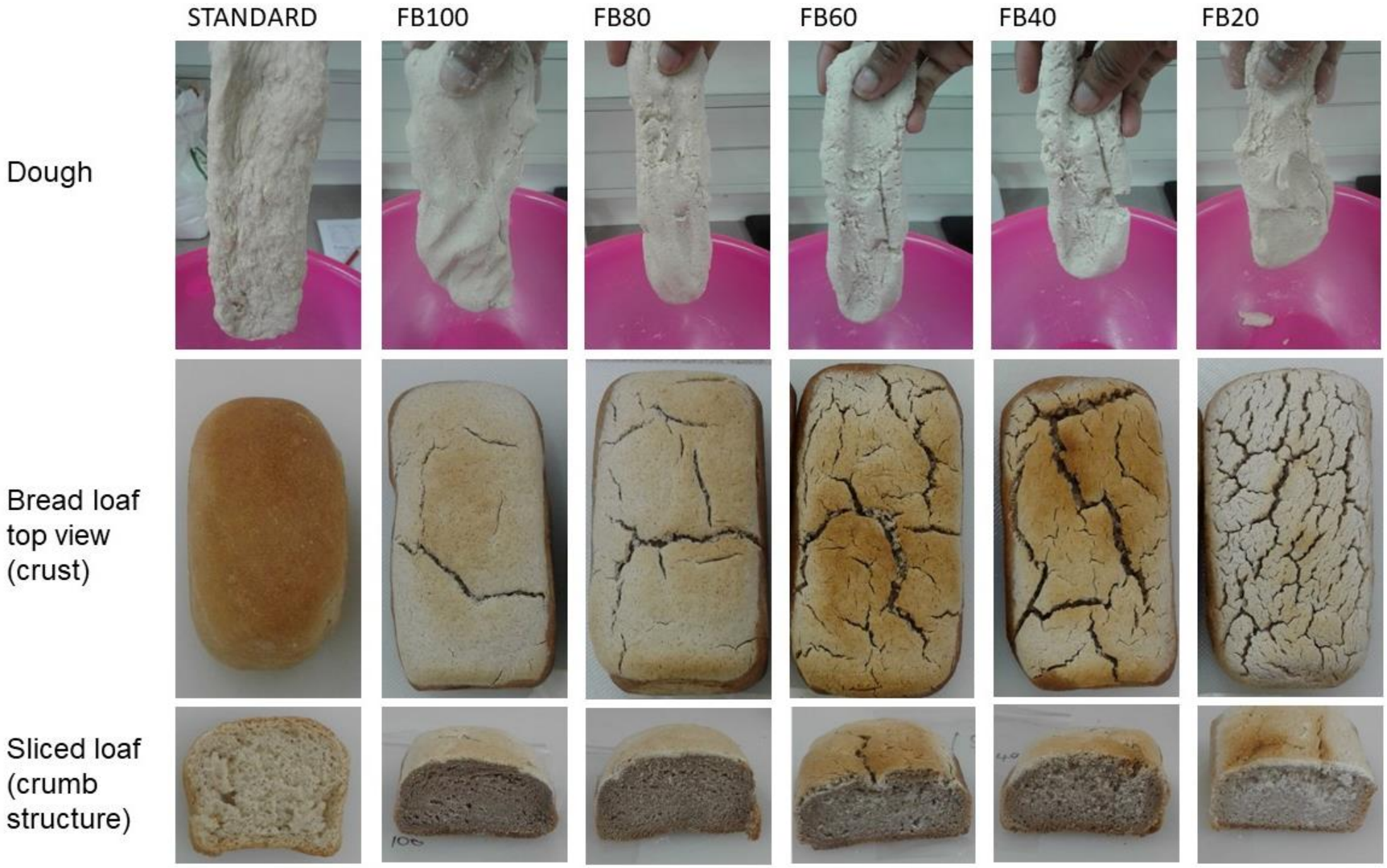

Figure 4.3 Dough and baked bread of the different gluten-free flour blends compared to standard wheat bread

4.3.2 Optimization of water content for bread formulations

Analysis of the results showed that the overall appearance (Figure 4.4) of the gluten-free dough and bread was significantly dependent on the amount of water added in the formulation.

Figure 4.4 Optimization of water content: dough, proofed bread, baked bread and crumb structure

Water plays a critical role in the textural and structural properties of the dough and consequently of the bread (reference). As seen in Figure 4.4, the increase in water content resulted in a batter in term of consistency that is thin and pourable (FB60W2, FB60W3, and FB60W4) as opposed to the dough obtained when from FB60W1. It was also observed that the volume of the bread after proofing and baking increased as well with water content. The need for use of higher water

amount may be due to the fact that water plays a critical in gluten-free bread because it contributes to the extensional properties of the dough during mixing and consequently the ability of the dough to entrap carbon dioxide (De La Hera et al., 2014). The results obtained are comparable to other studies on gluten-free bread where it was found that water significantly improved the quality of baked bread (Schober et al., 2005, Lazaridou et al., 2007, Schoenlechner et al., 2010, Hager & Arendt, 2013, De La Hera et al., 2014).

These results correlated to the results from the rheological behaviour of the formulations that are in Figure 4.5. It can be seen that the increase in hydration ratio considerably improves the rheological behaviour of the formulations.

Although the addition of water improved the quality of the gluten-free dough, it resulted in a bread which collapsed during baking when water content above 100% was used. This can be explained by the fact that banana flour lacks the structural proteins comparable to wheat that can help in maintaining the structure. The addition of hydrocolloids is used to improve the rheological properties of gluten-free dough, as they can mimic the visco-elastic properties of gluten, and are essential to increase gas retention during proofing and baking (Hager & Arendt, 2013). Formulations containing hydrocolloids generally require more water. Because of that FB60W4 was selected to be used as the basis to study the effect of the addition of guar and xanthan gums which is investigated in the following section.

The formulation with the highest water content resulted in bread with the highest volume compared to other formulations (Figure 4.4). This can be explained by the fact that the addition of a large amount of water in gluten-free formulations leads to an improvement of the proofing of the dough and better entrapment of carbon dioxide (Torbica et al., 2010).

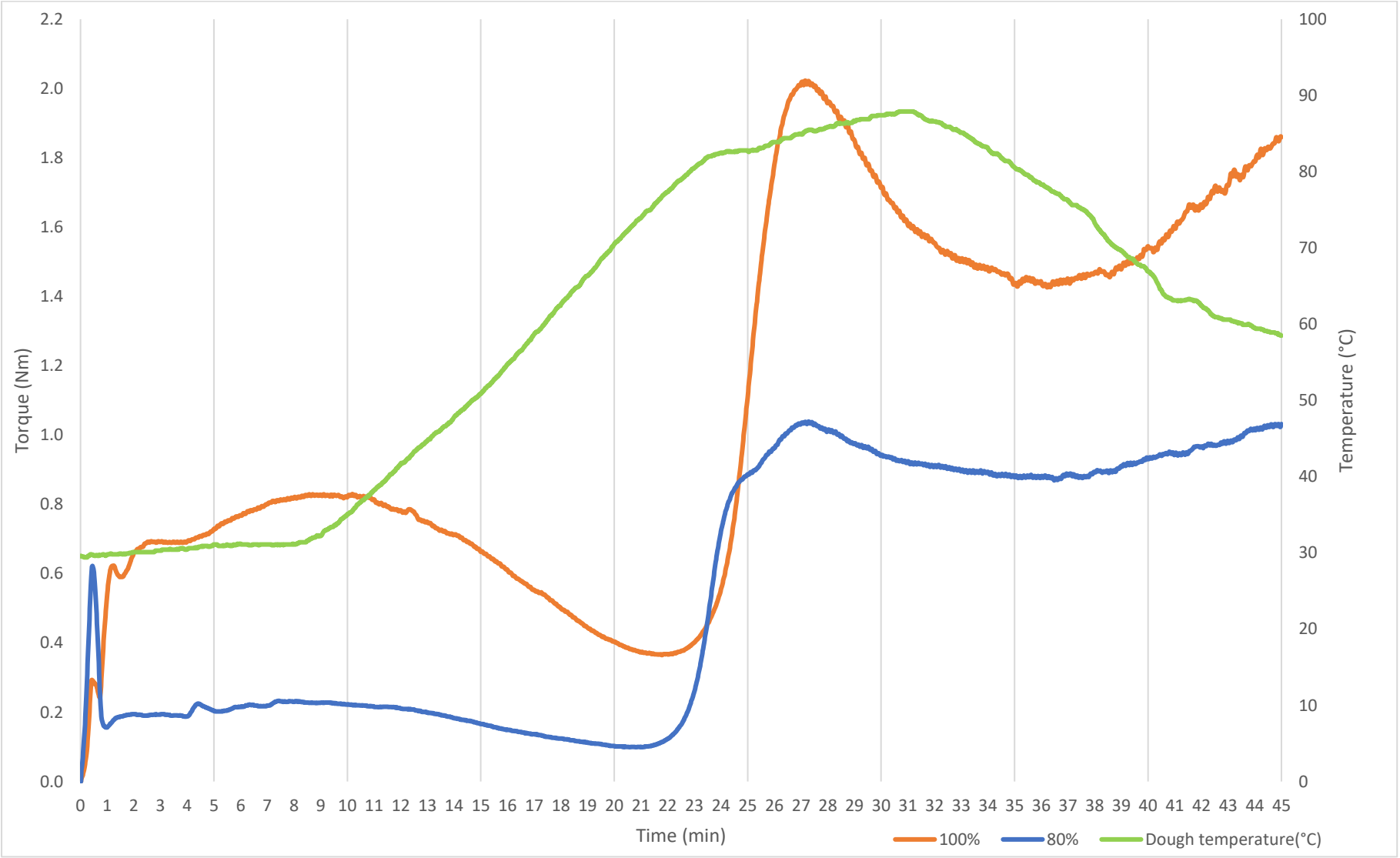

Figure 4.5 Rheological behaviour of formulations made with 80% and 100% hydration ratio

The increase in water content needed for the preparation on the dough might be related to the water absorption of unripe banana flour is higher compared to that of wheat flour (see section 3.3.2.2). Although the increase in content resulted in a proofed batter with a higher apparent volume, the bread obtained collapse during baking resulting in a lower volume of the final product. This is because the absence of gluten proteins prevents the structure from holding carbon dioxide during baking. One of the ways to overcome this challenge is to look into the use of hydrocolloids which have a hydrophilic nature and can form gels when mixed with water (Demirkesen et al., 2010a, Torbica et al., 2010).

4.3.3 Effect of addition of hydrocolloids

The formulations for the dough containing hydrocolloids are presented in Table 4.2 (FB60H1, FB60H2, FB60H3, FB60H4 and FB60H5). The results of the dough consistency and baked bread structure are illustrated in Figure 4.6.

Overall, the batter obtained was thicker for all formulations compared to the gluten-free control (FB60W4), due to the fact that hydrocolloids have a hydrophilic nature allowing them to bind to the available water in the dough. This results in the hydrocolloid forming a gel network, which increases the viscosity of the dough (Lazaridou et al., 2007).

In the case of guar gum, the increased viscosity of the dough was proportional to the increase in guar gum content (ranging from 0.5 to 2.0%, for the different formulations (FB60H4 < FB60H3 < FB60H2 < FB60H1). With FB60H1 having the most viscous with 2.0% guar gum, compared to the control (FB60W4). However, in the case of xanthan gum, the change in viscosity of FB60H5 (2.0% xanthan gum) as less apparent when compared to the control (FB60W4). According to Demirkesen (2010a) studied on gluten-free bread formulations showed that guar gum resulted in a more viscous dough when compared to dough containing xanthan, as well as, a large variety of other hydrocolloids. These results indicate that guar gum forms stronger gels in solution, compared to xanthan gum mainly due to the difference in their chemicals compositions which affect their gelling properties (Martín-Alfonso et al., 2018).

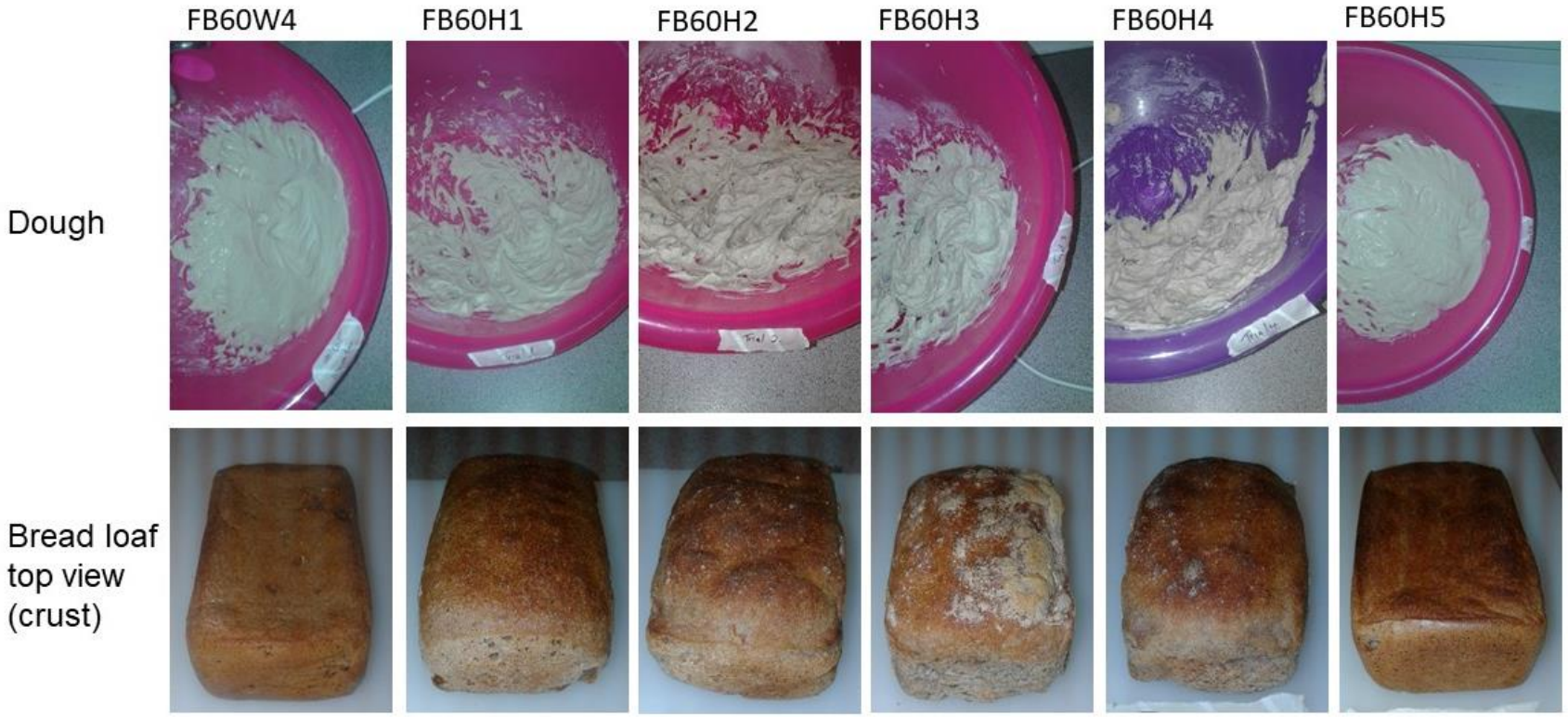

Figure 4.6 Dough consistency and bread prepared from the different formulations containing hydrocolloids

4.3.3.1 Mixolab measurements of flour blends containing hydrocolloids

The rheological properties of the different blends containing hydrocolloids are reported in Table 4.3. Each result will be discussed for each stage in the mixing/heating process.

For stage 1 (C1), there was no significant difference ($p>0.05$) in the torque (ranging from 0.53 to 0.67 Nm, for FB60H5 and FB60H1, respectively) of the different samples containing the different hydrocolloids. In contrast, the wheat flour had a value of 1.03 Nm. Stage 1, gives an indication of the amount of water.

The amplitude (amp) of the gluten-free samples containing hydrocolloids ranged from 0.18 to 0.56 for FH60H4 and FH60H3, respectively. The amplitude gives an indication of the dough elasticity: the higher the value, the more elastic the dough is. All gluten-free formulations resulted in higher torque compared to standard wheat. This could be explained by the fact that banana flour contained various compounds that give elasticity to the dough in addition to the use of hydrocolloids particularly guar gum (Martín-Alfonso et al., 2018). There was no significant difference in the stability of the dough (min) of the different gluten-free formulations. This can be explained by the fact that the protein content of the gluten-free flours used in the mix (banana flour and wheat flour) is lower than that of wheat flour. The stability time refers to the mixing resistance of the dough with longer time associated with “strong” flours. A flour is considered strong when it contains a high amount of protein. In the case of wheat flour, the protein content of 13% is considered a strong flour that is favourable to use in an application such as bread making (Kuktaite et al., 2004).

At the point C2, wheat flour presented higher torque value compared to the gluten-free trials mainly due to the difference in protein content as well as the composition (the type of protein) of wheat flour compared to gluten-free blend. When mixed with water and subjected to mechanical stress, individual cells of gluten protein from wheat flour form a three-dimensional matrix, as a result of the formation of disulphide, ionic and hydrogen bonds between molecules (Wieser, 2007).

Table 4.3 Rheological properties of the blends containing hydrocolloids

Sample	C1			C2	C3	C4	C5
	Torque (Nm)	Amp (Nm)	Stability (min)	Torque (Nm)	Torque (Nm)	Torque (Nm)	Torque (Nm)
Wheat	1.09 ±0.13a	0.11 ±0.02b	10.38 ±0.39a	0.35 ± 0.08a	0.51 ± 0.08c	1.65 ±0.09a	2.33 ±0.17a
FB60W4	0.79 ±0.20b	0.47 ±0.038ab	0.39 ±0.01b	0.10 ± 0.08b	0.74±0.36ab	0.58 ± 0.39c	1.01 ±0.16c
FB60H1	0.64 ±0.10bc	0.34 ±0.28ab	0.36 ± 0.05b	0.10 ± 0.02b	1.13 ± 0.10a	1.00 ± 0.06b	1.31 ±0.19b
FB60H2	0.67 ± 0.01bc	0.30± 0.27ab	0.39 ± 0.02b	0.12 ± 0.01b	1.12 ± 0.09a	1.00 ±0.08b	1.21±0.11bc
FB60H3	0.61 ± 0.10bc	0.18 ±0.10ab	0.39 ± 0.02b	0.14 ± 0.01b	1.17 ±0.02a	0.98 ± 0.04b	1.25±0.10bc
FB60H4	0.61 ±0.02bc	0.56 ± 0.03a	0.38 ± 0.03b	0.12 ± 0.01b	1.11 ± 0.06a	0.96 ±0.03b	1.10±0.04bc
FB60H5	0.53 ±0.04c	0.31 ± 0.18ab	0.39 ± 0.02b	0.08 ± 0.03b	1.00±0.07ab	0.90 ± 0.04b	1.06±0.07bc

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of at least three independent measurements.

The changes that happen in the dough during mixing are still not completely understood, but an increase in dough stiffness occurs that is generally considered to result from ‘optimization’ of protein-protein interactions within the gluten network (Shewry et al., 2002). While in gluten-free blends, both rice and banana flours are low in proteins and fail to form a viscoelastic network that is usual of gluten-containing flours in general and wheat flour in particular. During this stage, the addition of hydrocolloids had little impact on the mechanical behaviour of the blends resulting in low torque values for all the gluten-free blends.

At point C3, which a measure of starch gelatinization which can be linked to peak viscosity, there were no significant differences ($p<0.05$) between the treatments showing that the dosage of the hydrocolloids did not influence the results. At this stage, an increase in torque was observed in the 5 treatments compared to the untreated sample. The torque in the different blends was within the range of 1.00 - 1.17 for FB60H5 and FB60H3 respectively. The addition of hydrocolloid play an important role in the increase of torque due to firstly their interaction and further complex formation between the leached amylose and/or amylopectin: xanthan gum solutions behaved as weak gels, whereas guar gum solutions form a viscoelastic, gel-like structure (Martín-Alfonso et al., 2018). Since it is a new equipment, the information related to its utilization on different flour quality and the effects of hydrocolloids is quite limited. In a study by Rosell et al (2007) in which different hydrocolloids were used individually or in combination, it was found that xanthan gum at a level of 0.5% significantly increased the torque. It should be noted that the effect of hydrocolloids in a system and starch gelatinization depend greatly on water availability. Taking into account the fact that water addition was limited during the experiment, the effect of the presence of hydrocolloids could not be clearly observed as the water is used during starch gelatinisation (Rosell et al., 2007).

Stage 4 measures hot paste stability. The cooking stability is related to the stability of the already broken starch granules (due to mechanical stress coupled with gelatinisation) when maintained at a constant heating temperature (Rojas et al., 1999). Overall there was a decrease in torque for all gluten-free blends which can be explained by the fact that banana flour is not less resistant to high mechanical shear as well as the effect of the addition of guar gum and xanthan. It was reported by (Rosell et al., 2001), that the presence of guar gum in wheat blend resulted in a

significant depletion of the cooking stability. This decrease of starch resistance to thermal treatment and mechanical shearing has been attributed to dissociations between guar and starch through a structural shrinkage of this polymer rather than to the decrease in mechanical resistance of the starch granules (Funami et al., 2005). According to Collar, (2003), the viscosity during pasting and holding can be a good indication of bread quality during storage. Low cooking stabilities would be related with extended shelf-life of bread.

During the last stage, a major increase of the torque was recorded on all the blends with blends containing hydrocolloids have a lower increase than the control gluten-free blend. The addition of guar gum at a level above 0.5% (FB60H1, FB60H2 and FB60H3) resulted in a major increase in torque compared to other blends (FB60H4 and FB60H5). Similar results were obtained when guar gum was added to wheat flour (Rosell et al., 2007) and corn starch (Funami et al., 2005) due to this gum promoted the retrogradation of starch at a very early stage of storage. There is a highly significant correlation between the staling kinetics of bread and the pasting behaviour of the dough. In order to delay bread staling, a flour needs high pasting temperature, high viscosity during pasting (peak viscosity) and low viscosities after the heating period (Collar, 2003).

4.3.3.2 Baking performance of the freshly baked bread

4.3.3.2.1 Crust and crumb colour characteristics

The colour characteristics of the crust and crumb of the baked bread are found in Table 4.4.

Table 4.4 Crust and crumb colour of bread containing hydrocolloids compared to the control

	Crust colour			**Crumb colour**		
	L*	a*	b*	L*	a*	b*
Standard	59.83±0.16a	10.81±0.07c	28.13±0.24a	69.24±1.98a	3.09±0.12e	21.99±0.29a
FB60W4	42.85±0.61c	15.23±0.04b	25.76±0.46bc	53.69±0.24cd	8.25±0.33a	13.51±0.65b
FB60H1	53.75±1.92b	6.75±0.70e	19.54±0.8e	52.22±0.92d	6.58±0.17d	11.2±0.34e
FB60H2	57.1±1.25a	7.78±0.33d	25.43±0.85bc	55.1±1.3c	6.89±0.12c	12.07±0.09d
FB60H3	39.61±2.7c	15.27±0.43b	21.01±2.59de	57.58±1.08b	7.13±0.11bc	12.45±0.12cd
FB60H4	42.15±2.42cd	15.33±0.35b	23.52±2.63cd	57.42±1.13b	7.24±0.11b	12.35±0.13cd
FB60H5	43.09±1.56d	16.75±0.47a	24.99±1.61c	58.4±1.19b	7.33±0.12b	12.86±0.23c

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of at least three independent measurements.

The crust lightness (L* values) of the gluten-free bread (ranging from 39.61 to 57.1 for FB60H3 and FB60H2, respectively) was darker than that of standard wheat (59.83). It was also less yellow (b* value) (19.54 to 25.76 for FB60H1 and FB60W4, respectively) and redder (a* value) (ranging from 6.75 to 16.75 for FB60H1 and FB60H5, respectively), compared to the standard wheat (a*= 10.81, and b*=28.13). A similar trend was observed with the crumb colour where the gluten-free sample was darker, redder and less yellow than the standard wheat.

The difference in colour could be explained by the fact that the banana flour is generally darker, redder and less yellow compared to wheat flour (section 3.3.1.4). However, there was no specific trend observed with the addition of hydrocolloids, implying that hydrocolloids play a minimal role in the colour of the baked bread. The colour of the banana flour is associated with different factors such as the variety of banana used, pigments that are present in the fruit at the time of harvest and the processing parameters (such as the use of sodium metabisulphite) used to prepare the flour (Bhatt & McMaster, 1976).

4.3.3.2.2 Baking characteristics

The addition of guar gum and xanthan gum in the gluten-free formulations, improved the overall quality characteristic of the gluten-free bread, compared to the gluten-free control, with regards to bread volume (Figure 4.6, Table 4.5), with regards to bread volume, and crumb (Figure 4.7).

Table 4.5 Baking performance of the baked bread containing hydrocolloids

	Moisture content (%)	Height (cm)	Volume (cm^3)	Specific volume (cm^3/g)
Standard	35.32 ± 2.54a	4.53 ± 0.06a	197.0 ± 3.32a	3.59 ± 0.14a
FB60W4	54.55 ± 0.38b	2.20 ± 0.17d	81.49 ± 9.6e	1.37 ± 0.16e
FB60H1	55.01 ± 2.01b	2.90 ± 0.01b	121.2 ± 2.18b	2.18 ± 0.14b
FB60H2	53.84 ± 2.01b	2.67 ± 0.06c	106.1 ± 7.69c	1.75 ± 0.13cd
FB60H3	56.66 ±0.74b	2.57 ± 0.06c	96.39 ± 3.45d	1.62 ± 0.05d
FB60H4	54.33 ± 1.34b	2.63 ± 0.06c	107.0 ± 3.24c	1.81 ± 0.06cd
FB60H5	55.48 ± 2.2b	2.90 ± 00.1b	114.4 ± 0.74bc	1.94 ± 0.04c

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of at least three independent measurements.

The volume of the gluten-free breads increased significantly with the addition of hydrocolloids, in the following order: FB60H3 < FB60H2 < FB60H4 < FB60H5 < FB60H1. The addition of Guar gum at 2% (FB60H1), resulted in the bread with the highest volume increase (± 40% compared to FB60W4) (114.4 cm^3). However, when compared to the standard wheat bread, the volume was significantly lower (in percentage) (197 cm^3). The height of the gluten-free bread containing hydrocolloids, ranged from 2.57 cm to 2.90 cm for FB60H3 and FB60H1 - FB60H5, respectively. These results were slightly higher than that of gluten-free control (FB60W4, 2.20 cm) and significantly lower than that of standard wheat (4.53 cm). The specific volume (which is calculated by dividing the volume of the bread by the mass of the bread), gives an indication of the density of the baked bread. As expected, the gluten-free bread with the highest volume (FB60H1, 121.2 cm^3), also had the highest specific volume (2.18 cm^3/g), which was also higher than the gluten-free control (FB60W4) (1.37 cm^3/g). These results are in accordance with previous studies on gluten-free breads containing hydrocolloids, in which it was observed that the addition of xanthan and/or guar gum significantly improve the baking properties of the samples (Hager & Arendt, 2013, Gadallah et al., 2016, Hejrani et al., 2017, Ahmed & Thomas, 2018, Encina-Zelada et al., 2018, Jafari et al., 2018, Kang et al., 2018).

The main reason for the improved baking performance and crumb structure of yeast fermented gluten-free bread, containing hydrocolloids, is the improved viscosity of the dough. During the proofing step, the yeast generates carbon dioxide gas cells (bubbles), expanding the dough volume. The gas cells are trapped, stabilized and retained in the viscous dough due to the presence of the hydrocolloids. The hydrocolloids imitate the visco-elastic properties of wheat gluten proteins in wheat flour, increasing the loaf volume and hence increases the loaf specific volume of gluten-free bread (Matuda et al., 2008, Hager & Arendt, 2013).

The moisture content of bread, including gluten-free bread, is very important for eating quality (mouthfeel and texture), as well as shelf life (Rogers et al., 1988). For the gluten-free bread, the moisture content (Table 4.5) of the different formulations ranged from 53.84% – 56.66%, for FB60H2 and FB60H3, respectively. These

values were significantly higher than standard wheat bread (35.32%). The higher moisture content of the gluten-free bread can be explained by the fact that the main ingredient, banana flour, has a tendency to hold more water compared to wheat flour (section 3.4.2.2). In addition, according to Kang et al, (2018), the addition of hydrocolloids in gluten-free bread formulation has a tendency to increase the residual moisture content of the baked product. This is due to the hydrophilic nature of the different hydrocolloids (Kang et al., 2018).

Digital images of the crumb structure showed visual differences among the different formulations and are illustrated in Figure 4.8 and the texture analysis results are found in Figure 4.7. The control gluten-free sample (FB60W4) presented fissuring in the crumb and larger uneven distributed gas cell caused by the structure that collapsed during baking. When compared to FB60W4, the addition of hydrocolloids improve the structure of the crumb, preventing fissuring and better gas distribution. Overall all gluten-free formulations resulted in bread texture which is firmer compared to the standard wheat. This could be because banana flour when in solution form is more viscous than wheat flour (section 3.4.3.2). FB60H1 looked more compacted and had the highest firmness compared to other formulations because guar gum is known to give highly viscous solutions which influence the texture of the bread. (Martín-Alfonso et al., 2018).

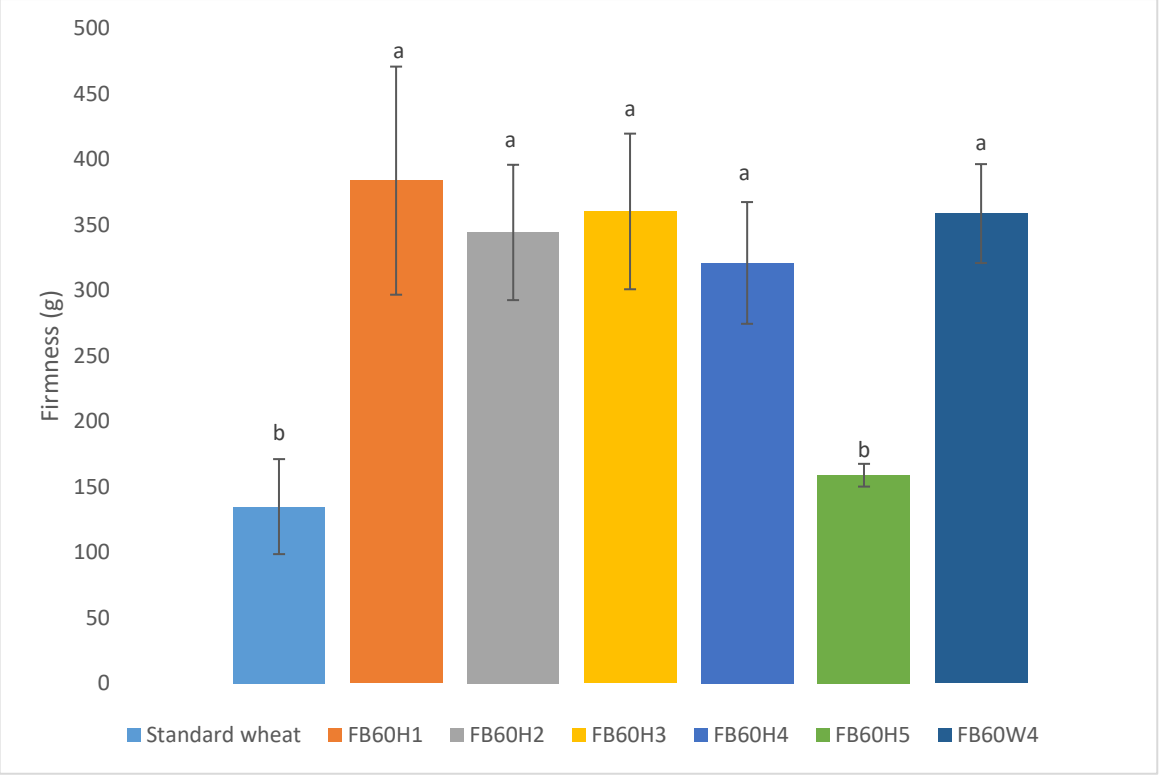

Figure 4.7 Firmness of bread samples containing hydrocolloids compared to standard wheat and control gluten-free (FB60W4)
Columns with any same letter in a given cluster are not significantly ($p>0.05$) different. Values reported are means of at least three independent measurements.

The sample containing xanthan gum at 2% (FB60H5) obtained the lower value for firmness which was not significantly different from that of the standard wheat sample. This is in contradiction with results from a previous study on gluten-free bread from rice flour in which it was found that xanthan gum at 1% gave the highest firmness compared to other hydrocolloids (Demirkesen et al., 2010a).

In resume, overall the addition of hydrocolloids significantly improved the quality characteristics of the baked bread. FB60H1 presented the best improvements in terms of sample height, volume and specific volume while FB60H5 had the softness closed to the standard wheat. On the other hand, FB60H3 was reported to have the least improvement showing that it is not ideal to use the two hydrocolloids in equal amount.

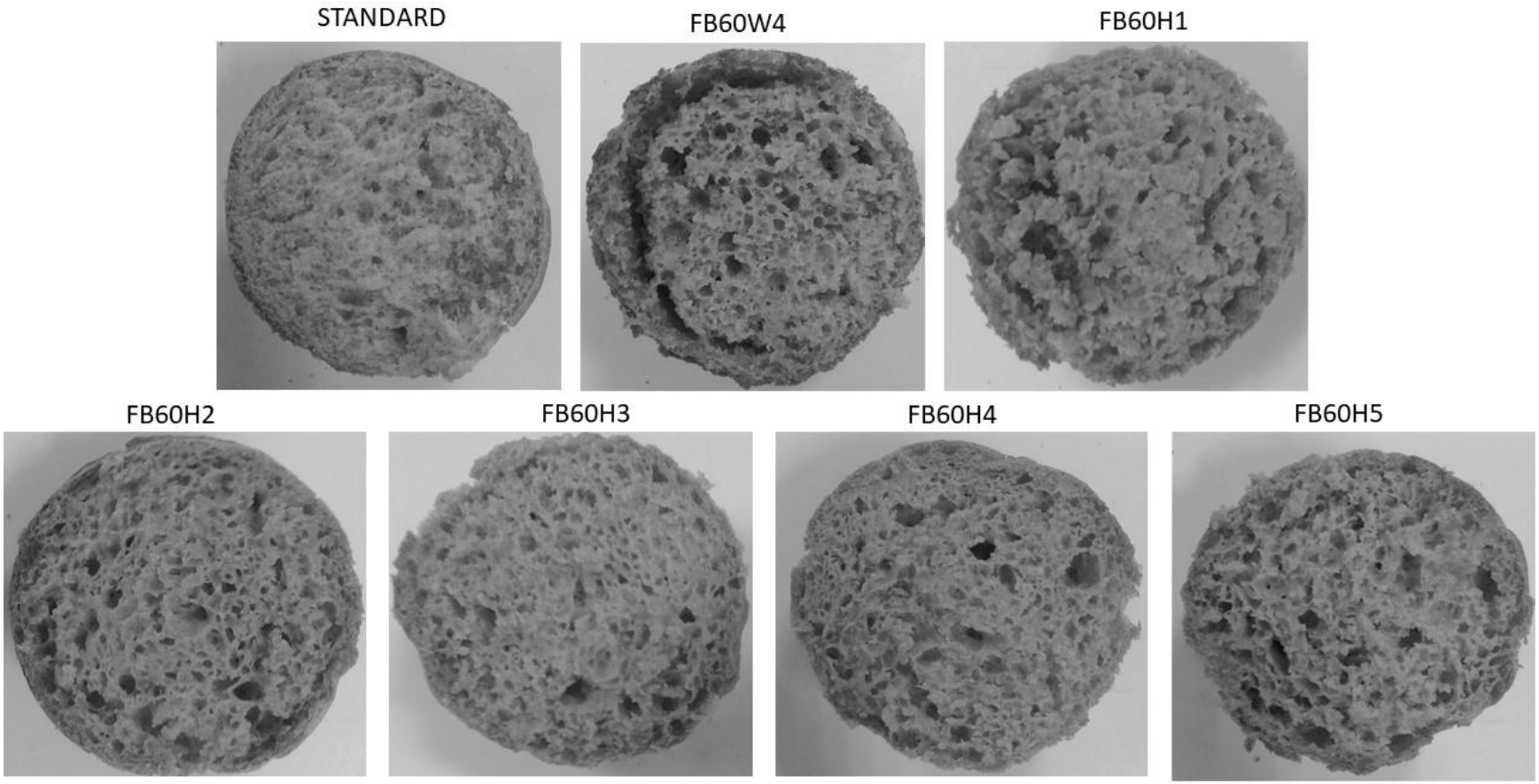

Figure 4.8 Crumb structure of baked bread containing hydrocolloids

There were no significant variations between most properties for FB60H2 and FB60H4, with both formulations giving bread with better quality than the control sample (FB60W4). Due to the fact that the bread is destined to human consumption, the sensory evaluation will provide further insight into the bread acceptability by panellists.

4.3.3.3 Sensory evaluation

The sensory evaluation of the freshly baked bread was performed by untrained panellists using a 9-point hedonic scale for appearance, aroma, texture, taste and Overall acceptability. Hedonic ratings are presented in Figure 4.9.

For appearance, there was significant variation ($p < 0.05$) in the appearance of the freshly baked gluten-free bread compared to the gluten control (FB60W4) and standard wheat. The bread containing hydrocolloids scored from 3.7 (dislike slightly) for FB60H1 to 7.0 (like moderately) for FB60H4, respectively. The control gluten-free scored 3.2 (dislike moderately) which was lower than all the bread containing hydrocolloids. The standard wheat sample scored 8.2 (like very much) which was higher than all gluten-free bread. Comments on the appearance of the bread were mainly related to the colour of the bread. All gluten-free bread was found to be visually darker than the wheat sample. These comments are in accordance with measurements obtained for the colour analysis of the samples (Table 4.5).

For the texture, there were significant ($p < 0.05$) differences between the bread containing hydrocolloids compared to control gluten-free bread (FB60W4) and standard wheat. FB60H4 scored 7.3 (like moderately) was not significantly different ($p > 0.05$) than standard wheat (8, like very much). This shows that the addition of hydrocolloids results in bread with textural properties that are similar to those of wheat bread. For the aroma of the bread, there were significant differences ($p < 0.05$) between the different bread samples. The gluten-free bread ranged from 3.3 (dislike moderately) to 7.2 (like moderately) for FB60H1 and FB60H4 respectively.

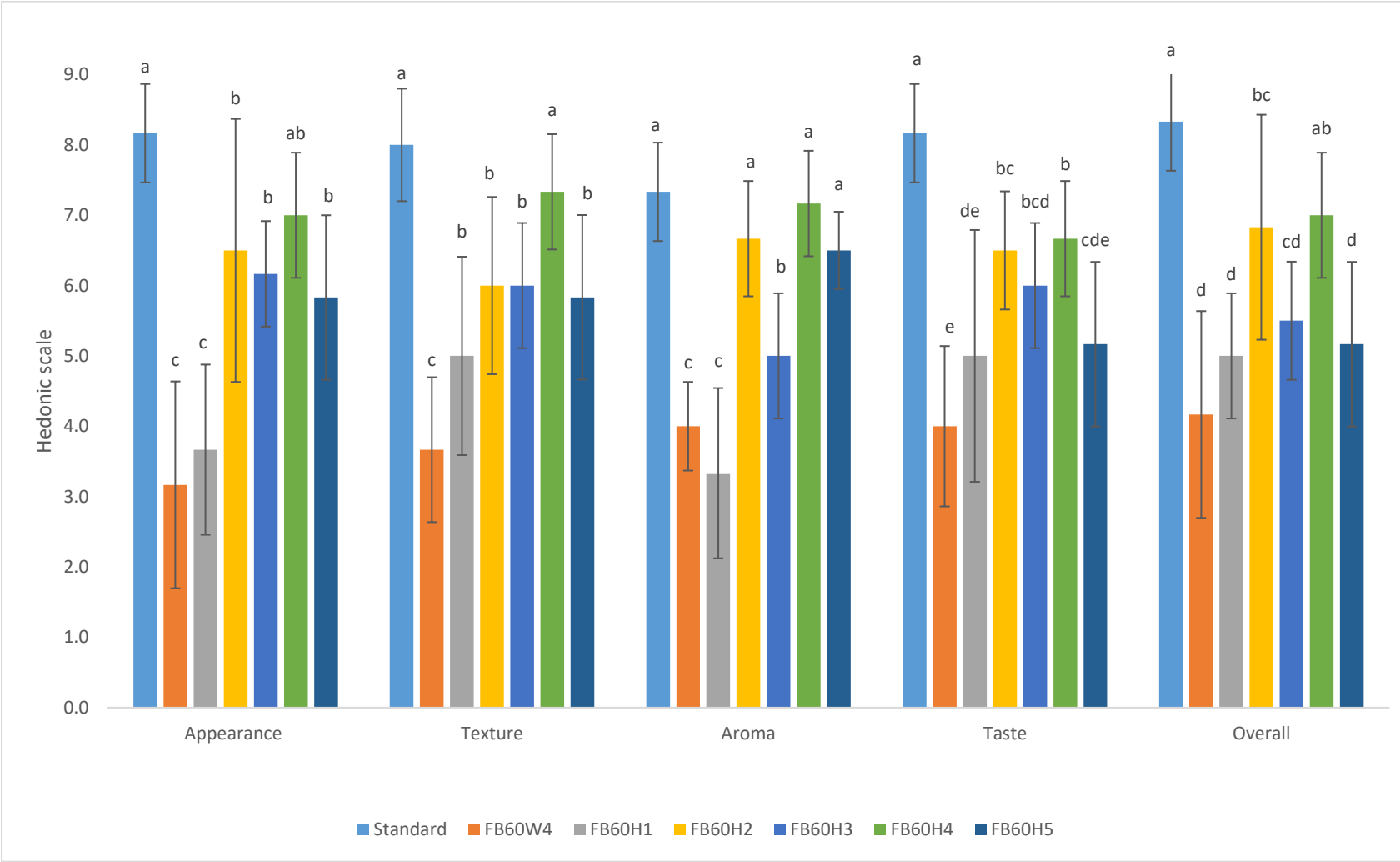

Figure 4.9 Sensory evaluation of bread made with hydrocolloids
Columns with any same letter in a given cluster are not significantly ($p>0.05$) different.
Values reported are means of at least three independent measurements.

The score obtained for FB60H1 was comparable to that of FB60W4. This suggests that guar gum at a high concentration impart properties to the bread that are not desirable on the aroma of the bread. For the taste of the bread, there were significant differences ($p \leq 0.05$) between the gluten-free bread containing hydrocolloids compared to the control gluten-free and standard wheat. Overall, there were significant differences between the likings of all gluten-free treatments ($p \leq 0.05$). FB60H2 and FB60H4 scored 6.8 and 7.0 respectively for overall acceptability. However, from the two formulations, only FB60H4 was comparable to the control wheat for overall acceptability. Although they did not present the best baking performance, these two formulations were selected to be used in combination with sourdough.

4.3.4 Effect of sourdough addition

4.3.4.1 Characterization of sourdough

The sourdough produced was characterized in term of colour and pH. The sourdough had a pH of 3.89 with an L* value of 66.11, a* value of 1.083 and b* value of 7.128.

4.3.4.2 Effect of sourdough addition on the rheological properties of the dough

The rheological properties of dough with sourdough addition is shown in Table 4.6.

4.3.4.2.1 Stage 1 (C1): The initial consistency and amplitude of the dough.

The initial consistency of the dough was determined at C1 (Torque). There was no significant difference in the torque value of the different treatments containing sourdough (ranging from 1.25 to 1.38 Nm for SD3 and SD1 respectively) as well as the gluten-free control (FB60W4, 1.02 Nm). This indicates that sourdough addition does not have an effect on the hydration rate of the flour. In contrast, the standard wheat flour had a lower torque value (0.67 Nm) mainly due to the fact that wheat flour requires less water in comparison to banana flour, to obtain the required viscosity of 1.1 Nm. This value assists in determining the amount of water required in dough formulations (Schmiele et al., 2017).

Table 4.6 Rheological properties of formulations containing sourdough

Treatment	C1			C2	C3	C4	C5
	Torque (Nm)	Amp(Nm)	Stability (min)	Torque (Nm)	Torque (Nm)	Torque (Nm)	Torque (Nm)
Standard	0.67±0.12c	0.28±0.17b	0.39±0.01b	0.11±0.05c	0.31±0.06c	0.17±0.01b	1.95±0.20a
FB60W4	1.02±0.17b	0.24±0.34b	7.23±6.26a	0.55±0.16a	1.78±0.69a	1.17±0.68a	1.98±0.14a
SD1	1.38±0.04a	1.1±0.12a	0.38±0.04b	0.56±0.07a	0.88±0.10bc	0.32±0.01b	1.94±0.18a
SD2	1.28±0.19a	0.76±0.53ab	0.37±0.03b	0.41±0.11ab	1.17±0.69ab	0.66±0.56ab	2.05±0.04a
SD3	1.25±0.03ab	0.9±0.45ab	0.34±0.04b	0.36±0.05b	0.69±0.03bc	0.27±0.02b	1.88±0.06a

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of at least three independent measurements

The amplitude was also determined at C1 (Amp) and gives an indication of the elasticity of the dough. The amplitude of the different gluten-free formulations ranged from 0.76 to 1.1 Nm for SD2 and SD1, respectively, which was higher than the gluten-free control (FB60W4, 0.24 Nm). Hence, the addition of sourdough increased the elasticity (amplitude) of the gluten-free doughs. The main reason is due to the presence and nature of acids that are produced by *L. Plantarum* in the sourdough (Moore et al., 2008). The produced acids in sourdough, reduces the pH of the dough, causing partial hydrolysis of the polysaccharides in the dough, resulting in more elastic dough consistency (Moore et al., 2008). When compared to the gluten-free formulations, wheat flour had a lower torque value indicating that it is less visco-elastic than the gluten-free formulations. Although its torque value is lower, wheat flour has the consistency to give to right properties to the baked bread due to the presence of gluten protein as discussed in section 2.3.3.

4.3.4.2.2 Stage 2 (C2): Behaviour of proteins in the system.

It was observed that the torque value reduced (ranging from 0.36 to 0.56 Nm for SD3 and SD1, respectively) with increasing sourdough concentration. The reduction in torque can be interpreted as denaturation and partial hydrolysis of proteins present in the dough (Di Cagno et al., 2002). This is a result of the addition of the sourdough containing acids (having a low pH (pH 3.89)). However, the mechanisms by which proteolysis takes place in the dough is still unclear due to the various endogenous microbial and proteolytic enzymes that are present in the sourdough (Zotta et al., 2006). According to Moroni et al.,(2009), LAB plays a minor role in protein hydrolysis as they lack extracellular proteinase activities. Thus the proteolysis observed is caused by endogenous enzymes present in the flour which are activated by low pH (Moroni et al., 2009). Similar trends were observed for wheat flour where the torque decrease (0.11 Nm) giving an indication of the onset of protein denaturation.

4.3.4.2.3 Stage 3 (C3): Starch gelatinization.

During stage 3, the temperature of the dough is increased resulting in starch gelatinization. No significant differences were observed between the different

gluten-free treatments that contained sourdough (with torque values ranged 0.69 to 1.17 Nm for SD3 and SD2, respectively). However, the addition of sourdough significantly lowered the torque, compared to the control (FB60W4, 1.78 Nm). The reduced torque of the doughs containing sourdough can be explained by partial hydrolysis of polysaccharides and modification of starch gelatinization due to the presence of enzymes from the sourdough (Wu et al., 2012). In contrast, there was an increase in the torque of the wheat (0.31 Nm) indicating a typical behaviour of starch when subjected to heating.

4.3.4.2.4 Stage 4 (C4): Stability of hot paste

At stage 4, the temperature of the dough remains constant with continuous mixing. This stage gives an indication of the resistance of starch to mechanical stress under constant temperature. During stage 4, the torque values of doughs containing sourdough decreased (ranging from 0.27 to 0.66 Nm for SD3 and SD2 respectively), compared to the control, FB60W4 (1.17 Nm). The lower torque value can be explained by the fact that banana flour is less resistant to shear as discussed in section 3.3.3.1. However, these values were slightly higher than that of wheat (0.17 Nm). The difference can be explained by the higher viscosities of banana flour compared to wheat as discussed in section 3.3.3.1.

4.3.4.2.5 Stage 5 (C5): Retrogradation of starch

At stage 5, which gives a measure of starch retrogradation during cooling, there was no significant difference between the formulations containing sourdough (SD1, SD2, and SD3) compared to FB60W4 and Standard wheat.

In a study by Edema et al.,(2013) on fonio, it was reported that sourdough improved significantly the rheological properties of gluten-free formulations allowing to obtain bread with better quality properties.

These similarities in rheological properties show that the addition of sourdough improved dough properties and allow to obtain formulations which are comparable to those of wheat-based doughs.

4.3.4.3 Baking performance

4.3.4.3.1 Crust and crumb colour

The colour characteristics (L, a, b values) of the crust and crumb, of the baked bread, are shown in Table 4.7.

Overall, the addition of sourdough resulted in a crust which was lighter (ranging from 49.92 - 56.56 for SD1 and SD2, respectively), less red (ranging from 7.52 - 12.69 for SD2 and SD1, respectively) and less yellow (ranging from 20.89 - 29.52 for SD2 and SD1, respectively) compared to the control gluten free bread (42.85, 15.23 and 25.76 for L*, a* and b*, respectively). A similar trend was observed with the colour characteristics of the crumb of the baked bread, the addition of sourdough resulted in a crumb which was lighter (ranging from 60.06 – 60.92 for SD3 and SD2, respectively), less red (ranging from 4.87 – 4.99 for SD3 and SD1, respectively) and less yellow (ranging from 10.95 – 11.45 for SD2 and SD1, respectively) compared to the control gluten free bread (53.69, 8.25 and 13.51 for L*, a* and b*, respectively). The difference in colour between the bread containing sourdough and the gluten-free control is due to the fact that the sourdough was lighter (value) in colour, which increased the lightness of the dough when added. In addition, it was found that acid treatment of banana prevents browning which leads to undesirable colour characteristics of the flour (Anyasi et al., 2015).

Table 4.7 Colour characteristics of bread baked with formulations supplemented with sourdough

	Crust colour			**Crumb colour**		
	L*	a*	b*	L*	a*	b*
Standard	59.83 ±0.16a	10.81 ± 0.07c	28.13 ± 0.24a	69.24 ± 1.98a	3.09±0.12c	21.99±0.29a
FB60W4	42.85 ± 0.61d	15.23 ± 0.04a	25.76 ± 0.46b	53.69 ± 0.24c	8.25±0.33a	13.51±0.65b
SD1	49.95 ± 2.27c	12.69 ± 1.07b	26.63 ± 0.45b	60.19 ± 0.51b	4.99±0.03b	11.45±0.10c
SD2	56.56 ± 1.06b	7.52 ± 0.83d	20.89 ± 1.4c	60.92 ± 0.06b	4.95±0.04b	10.95±0.21c
SD3	55.13 ± 1.28b	8.11 ± 0.59d	22.28 ± 0.76c	60.06 ± 0.44b	4.87±0.05b	11.02±0.09c

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of at least three independent measurements.

4.3.4.3.2 Baking characteristics

The formulations used for the preparation of the dough containing sourdough are presented in Table 4.2 (page 90). Images of the dough and bread crumb structure is presented in Figure 4.10.

There was no noticeable difference in the dough supplemented with sourdough compared to control gluten-free (FB60W4). The dough appeared sticky, difficult to mould, with a lot of gas bubbles and elastic compared to wheat flour dough which is extensible, with fewer gas bubbles and mouldable. After baking, the addition of sourdough resulted in an improvement of the crust colour and structure of the different treatments (SD1, SD2 and SD3) resulting in a more uniform crust with low fissuring due to the decrease in dough resistance to extension caused by lactic acidification (Di Cagno et al., 2002).

The baking performance of formulations containing sourdough is found in Table 4.8. Overall the addition of sourdough resulted in the improvement of the quality characteristics of the gluten-free bread resulting in bread with increased height, volume, specific volume as well as crumb texture and structure.

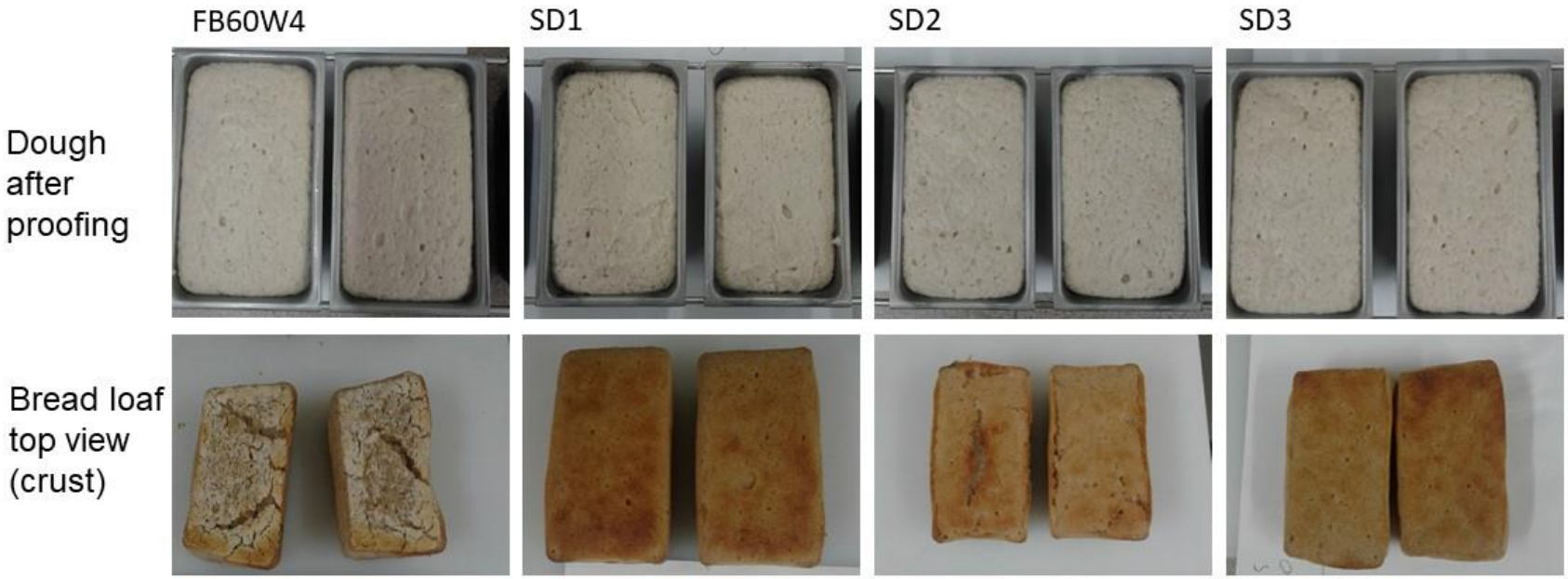

Figure 4.10 Structure of the dough and baked bread containing sourdough

Table 4.8 Baking performance of formulation containing sourdough

	Moisture content	Mass after baking	Height	Volume	Specific volume
	%	G	Cm	cm^3	cm^3/g
Standard	35.32 ± 2.54a	59.97 ± 1.52a	4.53±0.06a	196.99±3.32a	3.59±0.14a
FB60W4	54.55 ± 0.38b	59.40 ± 0.59a	2.20±0.17d	81.49±9.60d	1.37±0.16
SD1	56.33 ± 0.22bc	58.63 ± 0.61a	2.77±0.21b	130.9±7.77b	2.23±0.12b
SD2	56.81 ± 0.16c	60.29 ± 0.62a	2.63±0.06bc	116.29±3.5c	1.93±0.04c
SD3	56.27 ± 0.44bc	59.35 ± 1.16a	2.40±0.10cd	106.67±2.27c	1.80±0.07c

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of at least three independent measurements.

The moisture content of the gluten-free bread (ranging from 56.27 - 56.81 for SD3 and SD2, respectively) were not significantly different than the control sample (FB60W4).

The volume of the bread significantly increased with the addition of sourdough from 81.49 cm^3 for FB60W4 to a range of 106.67 – 130.9 for SD3 and SD1 respectively. The more sourdough (50% for SD3) was added, the less increased was observed (30%). A similar trend was observed for the height (ranging from 2.40 – 2.77 cm for SD3 and SD1, respectively) as well as the specific volume (ranging 1.80 – 2.23 cm^3/g for SD3 and SD1, respectively). This could be explained by the fact during sourdough fermentation, due to enzymatic reactions taking place, pH drops gradually allowing amylolytic, proteolytic, lipoxygenases, peroxidases, catalases and polyphenol oxidases to modify dough improving the rheological behaviour of the dough and consequently the baking characteristics(Galle et al., 2012, Naqash et al., 2017).

Images of the bread crumb structure are presented in Figure 4.11. The addition of sourdough resulted in an improvement of the crumb structure compared to the gluten-free control. The bread containing sourdough (SD1, SD2 and SD3) appeared with no fissuring and uniform small gas cells distribution compared to FB60W4. The more sourdough was added, the better the gas distribution and the denser the bread looked. The improvement observed in the structure has been associated with the presence of exopolysaccharides produced by *L. Plantarum (Moore et al., 2008)*. However, the bread containing sourdough looked harder compared to the control gluten free. This observation was confirmed by the measurement of bread firmness that is reported in Figure 4.12. The bread firmness ranged from 1439.97g to 1567.51g for SD1 and SD2 respectively. These results were significantly higher than that of gluten-free control (FB60W4) which was 383.77g (Figure 4.7). The difference could be attributed to shrinkage that occurs in sourdough bread due to the presence of acid. According to Crowley et al., (2002), the addition of sourdough at a level of 40% in a wheat formulation resulted in an increase of bread firmness and chewiness, showing that there is a maximum level to not exceed in order to obtain bread with the best characteristics. There is also evidence that crumb cell structure contributes to the texture and eating quality (Crowley et al., 2002).

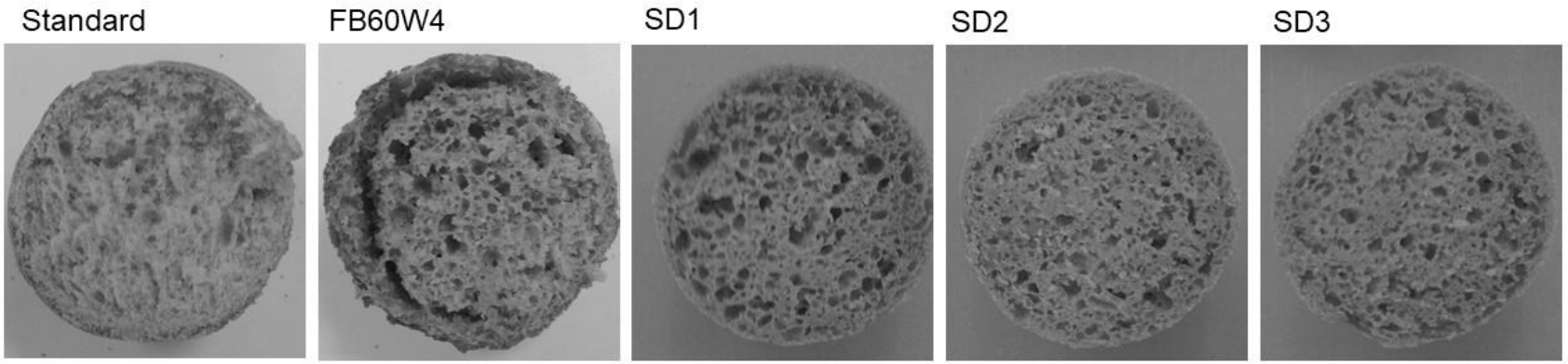

Figure 4.11 Crumb structure of bread containing sourdough (SD1, SD2 and SD3) compared to control gluten-free (FB60W4) and standard wheat

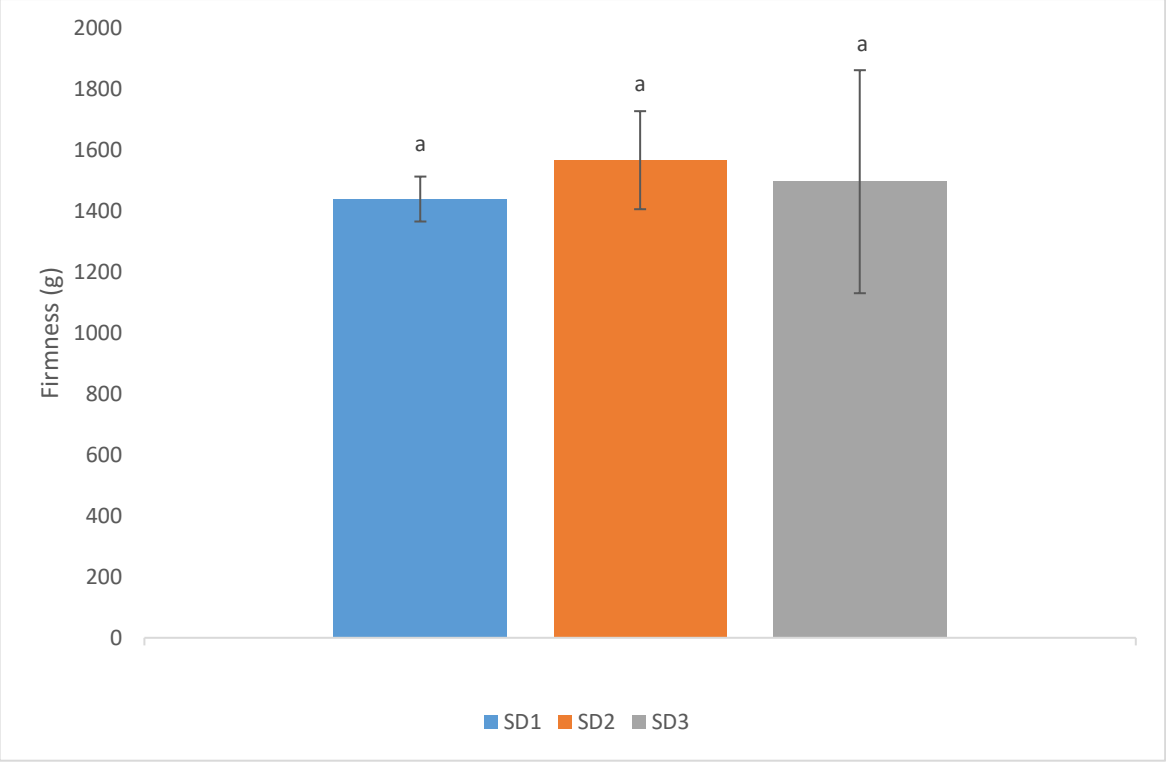

Figure 4.12 Bread firmness of formulations containing sourdough
Columns with any same letter are not significantly (p>0.05) different.
Values reported are means of at least three independent measurements

4.3.4.4 Sensory evaluation

The results of the sensory evaluation of gluten-free formulations containing sourdough using a 9 point hedonic scale are illustrated in Figure 4.13. Overall, there was no significant different between the results of appearance (ranging from 6.00 to 6.50 for SD3 and SD1, respectively), aroma (ranging from 4.00 to 6.00 for SD3 and SD1, respectively) and texture (ranging from 5.67 to 7.17 for SD3 and SD1, respectively) of different samples containing sourdough. It was noticed that the more sourdough was added to a formulation the less the formulation was appreciated. SD3 particularly scored less than 5 for most of the attributes except for appearance. Comments of panellists for SD3 was that it had a strong fermented smell, a strong acidic taste which affected the overall acceptability. This can be explained by the fact most panellists are not familiar with sourdough fermented bread. Significant differences were observed for taste and overall acceptability of samples with SD1 being the most preferred sample scoring 7.50.

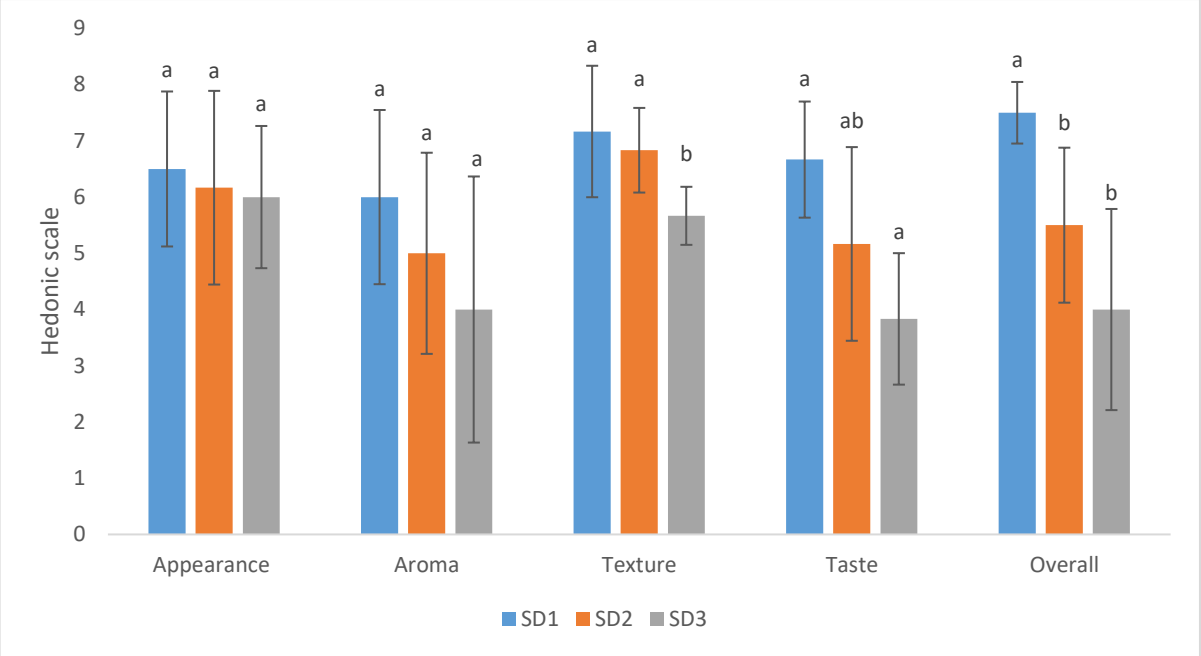

Figure 4.13 Sensory evaluation of sourdough bread
Columns with any same letter in a given cluster are not significantly (p>0.05) different.
Values reported are means of at least three independent measurements.

4.3.5 Effect of sourdough with hydrocolloid addition

4.3.5.1 Mixolab measurements

The rheological properties of the formulations containing sourdough and hydrocolloids (guar gum and xanthan gum) are presented in Table 4.9.

Table 4.9 Rheological properties of formulations containing sourdough and hydrocolloids

Treatment	C1		C2	C3	C4	C5
	Torque (Nm)	Amp(Nm)	Torque (Nm)	Torque (Nm)	Torque (Nm)	Torque (Nm)
Standard	0.67±0.12c	0.28±0.17b	0.11±0.05c	0.31±0.06b	0.17±0.01a	1.95±0.2b
FB60W4	1.02±0.17b	0.24±0.34b	0.55±0.16ab	1.78±0.69a	1.17±0.68a	1.98±0.14b
SD1	1.38±0.04a	1.1±0.12a	0.56±0.07ab	0.88±0.10ab	0.32±0.01a	1.94±0.18b
SD1H2	1.05±0.07b	0.49±0.14b	0.69±0.17a	1.49±0.78a	0.94±0.74a	2.38±0.08a
SD1H4	0.93±0.06b	0.49±0.09b	0.48±0.04b	1.46±0.91a	0.89±0.73a	2.14±0.12ab

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of at least three independent measurements.

4.3.5.1.1 Stage 1 (C1): The initial consistency and amplitude of the dough.

At C1, the torque ranged from 0.93 to 1.38 Nm for SD1H4 and SD1, respectively. There was no significant difference between the formulations containing both hydrocolloids and sourdough, as well as the control formulation (FB60W4). This would indicate that the addition sourdough, in combination with hydrocolloids did not influence the rheological behaviour of the batter, at stage 1. However, the addition of the hydrocolloids decreased the amplitude of the batter SD1H2 (0.49 Nm) and SD1H4 (0.49 Nm) compared to SD1 (1.1Nm), resulting in a consistency similar to that of the standard (contain wheat flour) (0.28 Nm). Amplitude is an indication of dough elasticity, which is responsible for trapping of carbon dioxide produced during fermentation and proofing, which ultimately leads to the crumb and crust structure of the baked bread. The hydrocolloids act in gluten-free formulations by retaining water and controlling moisture mobility thus acting as texture improvers (Hejrani et al., 2017).

4.3.5.1.2 Stage 2 (C2): Behaviour of proteins in the system.

There was no significant difference at C2 between the different gluten-free formulations. The torque ranged from 0.48 Nm – 0.69 Nm for SD1H4 and SD1H2, respectively. This could be explained by the fact that banana flour is low in protein, containing between 2.6 and 4.5 % per 100 g flour as presented in Table 2.3. In addition, the hydrocolloids used (which is primarily composed of polysaccharides) did not contribute to the protein content of the formulations (as was previously discussed in 4.3.3.1). In contrast, standard wheat had the lowest torque (0.11 Nm) recorded, due to the fact that wheat used in the study contained 13% protein per 100 g flour, and the gluten protein present in the flour has a major effect on the properties of the dough. As previously discussed, gluten protein due to its structure plays a critical role in the formation of the viscoelastic network which allows for the entrapment of gas during proofing (Wieser, 2007). However, although lacking proteins, the high torque values obtained in gluten-free formulations can be explained by the presence of non-starch polysaccharides which influence the viscosity of the solution (Tester & Sommerville, 2003).

4.3.5.1.3 Stage 3 (C3): Starch gelatinization.

At C3 there were significant differences in the torque values for the sourdough formulations containing the hydrocolloids (ranging from 1.46 to 1.49 Nm for SD1H4 and SD1H2, respectively) compared to the sourdough without hydrocolloids (SD1 0.88 Nm). This difference can be explained by the fact that the addition of hydrocolloids increased the peak viscosity of the batters, and consequently the rheological behaviour of the batters. From previous studies, the addition of guar gum and xanthan gum in combination in a solution resulted in a higher viscosity than that obtained for each separately (Casas et al., 2000, Chaisawang & Suphantharika, 2006, Martín-Alfonso et al., 2018). However, the torque value for the control (FB60W4, 1.78 Nm), was higher, compared to the sourdough formulations. In the formulations containing sourdough, the lower torque is due to partial hydrolysis of native polysaccharide, found in the banana flour, caused by the presence of lactic acid fermentation enzymes present in the sourdough (as previously explained in 4.3.4.2.3) (Lynch et al., 2018).

4.3.5.1.4 Stage 4 (C4): Stability of hot paste.

Similar trends to C3, were observations at C4, were the sourdough containing hydrocolloids (SD1H2 (0.94 Nm) and SD1H4 (0.89 Nm)) has significantly higher torque, compared to SD1 (0.34 Nm). This increase can mainly be attributed to the hydrocolloids, which bind the available water in the batter, increasing the viscosity of the system (Lazaridou et al., 2007, Torbica et al., 2010, Mir et al., 2016).

4.3.5.1.5 Stage 5 (C5): Retrogradation of starch

At C5, the final torque of the sourdough containing hydrocolloids (SD1H2 (2.38 Nm) and SD1H4 (2.14 Nm)) was significantly higher than sourdough without hydrocolloids SD1 (1.94 Nm). The increase in torque is attributed to the increased viscosity of the batter due to the presence of the hydrocolloids, as previously discussed in section 4.3.3.1. In addition, during lactic acid fermentation of the sourdough, the fermenting organisms hydrolyse the starch into a variety of exopolysaccharides, which can also increase the viscosity of the batter, by bind available water (Galle and Arendt, 2014, Lynch et al., 2018).

Overall, the presence of sourdough and hydrocolloids will improve the rheological properties of the batter due to the increased water binding capacity and low pH which results in the activation of endogenous enzymes responsible for proteolysis and starch degradation. However, from all formulations containing sourdough, SD1 should be expected to produce bread of better quality because and stale slower because, a flour need high pasting temperature, high viscosity during pasting (peak viscosity) and low viscosities after the heating period to delay retrogradation (Collar, 2003).

4.3.5.2 Baking performance

4.3.5.2.1 Crust and crumb colour

The colour characteristics of the crust and crumb of the different bread containing sourdough and sourdough with hydrocolloids are shown in Table 4.10. The crust lightness (L*) of the bread containing hydrocolloids and/or sourdough were similar, ranging from 47.56 to 51.92 for SD1H4 and SD1H2, respectively. However, they were slightly lighter than the gluten-free control (FB60W4, 42.85). In addition, the bread appeared less red (ranging from 11.27 to 14.58 for SD1H2 and SD1H4, respectively) and more yellow (ranging from 26.63 to 29.42 for SD1 and SD1H4, respectively) than the control FB60W4 (a*=15.23, b*=25.76). Similar trends were observed for the crumb colour for the bread containing sourdough, and sourdough with hydrocolloids, these bread have lighter crumb (ranging from 60.19 to 64.06 for SD1 and SD1H4, respectively), and were less red (ranging from 3.69 to 4.99 for SD1H4 and SD1, respectively) compared to the control, FB60W4 (L*= 53.69, a*= 8.25). However the samples appeared less yellow (ranging from 10.28 to 11.45 for SD1H2 and SD1, respectively) compared to FB60W4 (b*=13.51).

Table 4.10 Colour characteristics of formulations containing hydrocolloids and sourdough

	Crust colour			**Crumb colour**		
	L*	a*	b*	L*	a*	b*
Standard	59.83 ± 0.16a	10.81 ± 0.07c	28.13 ± 0.24b	69.24 ± 1.98a	3.09 ± 0.12d	21.99 ± 0.29a
FB60W4	42.85 ± 0.61d	15.23 ± 0.04a	25.76 ± 0.46c	53.69 ± 0.24d	8.25 ± 0.33a	13.51 ± 0.65b
SD1	49.95 ±2.27bc	12.69 ± 1.07b	26.63 ± 0.45c	60.19 ± 0.51c	4.99 ± 0.03b	11.45 ± 0.1c
SD1H2	51.92 ± 2.35b	11.27 ± 1.49bc	28.48 ± 1.10ab	60.50 ± 0.08c	3.9 ± 0.03c	10.28 ± 0.12d
SD1H4	47.56 ± 0.12c	14.58 ± 0.46a	29.42 ± 0.34a	64.06 ± 0.38b	3.68 ± 0.05c	10.76 ± 0.04d

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of at least three independent measurements.

The improved colour characteristics may be explained by the fact that the addition of the sourdough (containing lactic acid), may have reduced the development of browning pigments during the baking process and affect the native pigments in the banana flour. Various studies have shown that organic acid prevents enzymatic browning to some extends (Ponting, 1960, Martinez & Whitaker, 1995, Chaisakdanugull et al., 2007, Purlis, 2010, Anyasi et al., 2017). Many different factors can affect the characteristics of baked bread. In this case, the addition of the sourdough which was slightly lighter improved the colour of the baked bread as previously discussed in 4.3.4.

4.3.5.2.2 Baking characteristics of the bread

The baking characteristics of the gluten-free bread are found in Table 4.11.

The moisture content of the different gluten-free bread, containing sourdough, and sourdough with hydrocolloids ranged from 56.33% – 60.64%, for SD1 and SD1H4, respectively. These values were significantly higher than standard wheat bread (35.32%). The higher moisture content of the gluten-free bread can be attributed to the presence of hydrocolloids. Hydrocolloids are hydrophilic in nature and have a tendency to bind available moisture during baking, which increases the overall moisture content of the bread (Rosell et al., 2001, Lazaridou et al., 2007, Torbica et al., 2010, Mir et al., 2016). In addition, the banana flour also has a higher water holding capacity compared to wheat flour (as previously discussed in section 3.3.2.2).

The height of the gluten-free bread containing hydrocolloids, ranged from 2.77 cm to 3.97 cm for SD1 and SD1H4, respectively. The height of the bread was slightly higher than that of gluten-free control (FB60W4, 2.20 cm), but significantly lower than that of standard wheat (4.53 cm). The main factors for the low height of the gluten-free bread include low proofing height and slight collapsing during the baking step.

Table 4.11 Baking characteristics of formulations containing sourdough and hydrocolloids

	Moisture content	Height	Volume	Specific volume
	%	Cm	cm^3	g/cm^3
Standard	35.32±2.54d	4.53±0.06a	196.99±3.32a	3.59±0.14a
FB60W4	54.55±0.38c	2.20±0.17d	81.49±9.60d	1.37±0.16d
SD1	56.33±0.22bc	2.77±0.21c	130.9±7.77c	2.23±0.12c
SD1H2	60.64±0.40a	3.78±0.03b	168.9±2.22b	2.93±0.06b
SD1H4	58.43±0.33b	3.97±0.15b	180.12±9.41b	3.07±0.18b

Means with any same letter in a given column are not significantly ($p>0.05$) different.
Values reported are means of at least three independent measurements.

The volume of the gluten-free bread (containing hydrocolloids and/or sourdough) increased significantly (SD1 < SD1H2 < SD1H4), compared to the gluten-free control 81.49 cm^3. Obviously, the gluten-free bread had lower volume compared to the standard wheat bread, but the bread containing xanthan gum with sourdough, was slightly lower (8.5% lower for SD1H4, than the Standard). The increase observed was similar to that observed for the bread containing only hydrocolloids (Section 4.3.3.2.2), however, the addition of the sourdough improved the bread volume significantly in all cases (Table 4.8). As expected, the gluten-free bread with the highest volume (SD1H4, 180.12 cm^3), also had the highest specific volume (3.07 cm^3/g), which was also higher than the gluten-free control (FB60W4) (1.37 cm^3/g). Specific volume is an indication of the density of the bread.

These results can be explained by the combined positive effect of hydrocolloids and sourdough to the formulations. Although there are no studies available on the use of sourdough in combination with hydrocolloids, there is a large number of studies on sourdough and hydrocolloids use separately that confirm their positive effect on the dough (Corsetti et al., 2000, Moore et al., 2008, Coda et al., 2011b, Hager & Arendt, 2013, Campo et al., 2016, Gadallah et al., 2016, Rizzello et al., 2016a, Hejrani et al., 2017, Rinaldi et al., 2017, Ahmed & Thomas, 2018, Encina-Zelada et al., 2018, Jafari et al., 2018, Kang et al., 2018). The mechanism by which sourdough improves the properties of bread is that the low pH obtained activate enzymes that are present and ease biochemical modifications of the starch and protein fractions of the flour, resulting in the increase of water binding ability and dough elasticity (Di Cagno et al., 2002, Zotta et al., 2006, Moore et al., 2008, Galle et al., 2011, Galle & Arendt, 2014, Lynch et al., 2018).

Digital images of the crumb structure are presented in Figure 4.14. From the images, it is clear that bread containing both sourdough and hydrocolloids (SD1H2 and SD1H4), had improved crumb structure compared to the bread containing only sourdough (SD1). These bread were also better than gluten-free control.

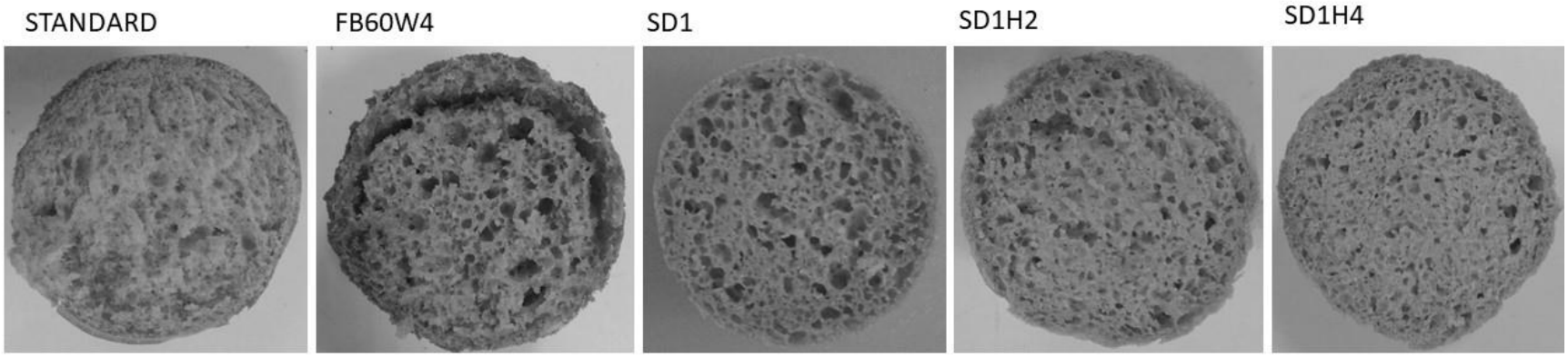

Figure 4.14 Crumb structure of bread containing hydrocolloids and sourdough

The addition of sourdough and hydrocolloids resulted in a sample without fissuring or cracking. SD1H4 appeared to have the best gas cell distribution with small bubbles uniformly distributed throughout the surface. The improvement comes from the fact that hydrocolloids hold water allowing a visco-elastic network to form coupled with the low pH of the sourdough addition which further improves the network. (Encina-Zelada et al., 2018, Lynch et al., 2018).

The firmness of the bread ranged from 1257 to 1486 g for SD1H2 and SD1H4, respectively (Figure 4.15). The addition of guar gum resulted in a bread (SD1H2) with lower firmness compared to bread containing sourdough (SD1) only. In contrast, the addition of xanthan gum resulted in an increase of firmness indicating the synergic effect with polysaccharides present in sourdough which have the ability to form highly viscous solutions (Corsetti et al., 2000, Arendt et al., 2007).

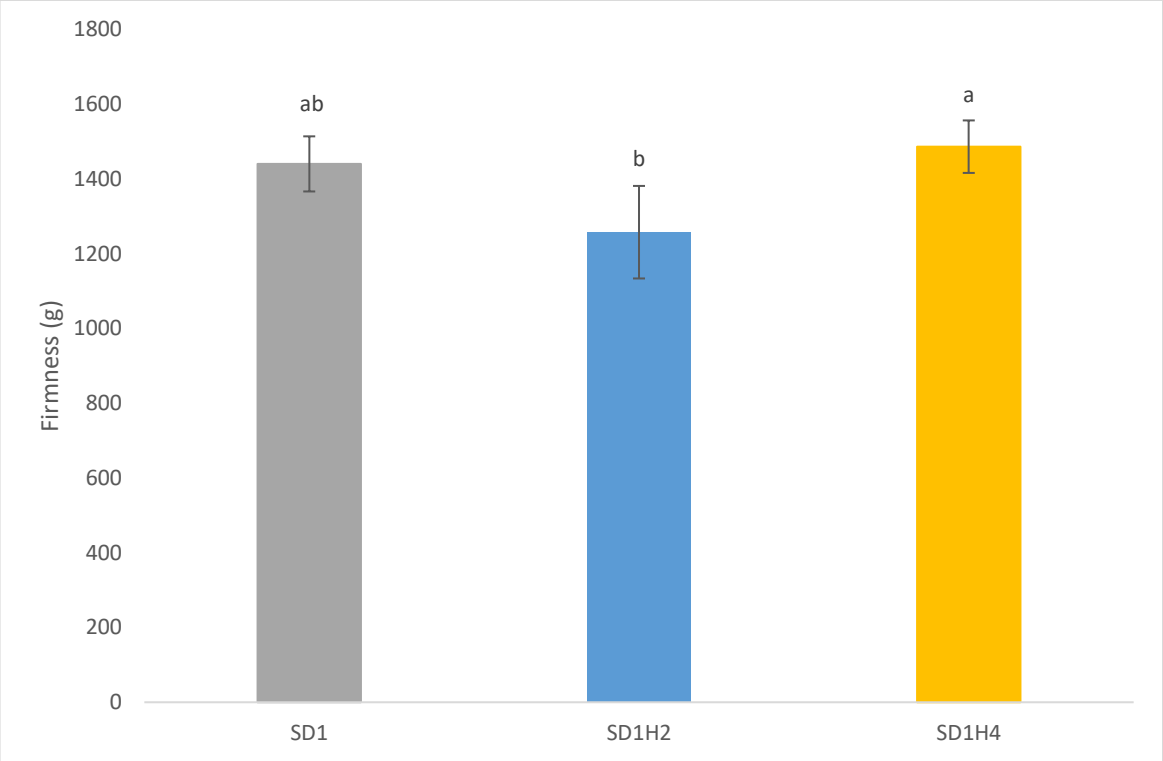

Figure 4.15 Bread firmness of different formulations sourdough and hydrocolloids (SD1H2 and SD1H4) compared to sourdough only (SD1)
Columns with any same letter are not significantly ($p>0.05$) different.
Values reported are means of at least three independent measurements.

However, when compared to previous data obtained in the studies (Figure 4.7, page 114), the bread containing sourdough had higher firmness values compared to

bread containing hydrocolloids. This could be explained by the fact that during sourdough fermentation, starch is partially hydrolysed to sugars increasing the sugars content of the bread (Corsetti et al., 2000). The elevated level of sugars will cause moisture to migrate from starch/proteins network causing stiffness and consequently firmer texture of the bread(Mohamed et al., 2010).

4.3.5.3 Sensory evaluation

The results of the sensory evaluation of bread containing hydrocolloids and sourdough are presented in Figure 4.16. The sensory was performed on SD1H2 and SD1H2 as other samples were previously subjected to sensory evaluation (Section 4.3.3.3 and section 4.3.4.4)

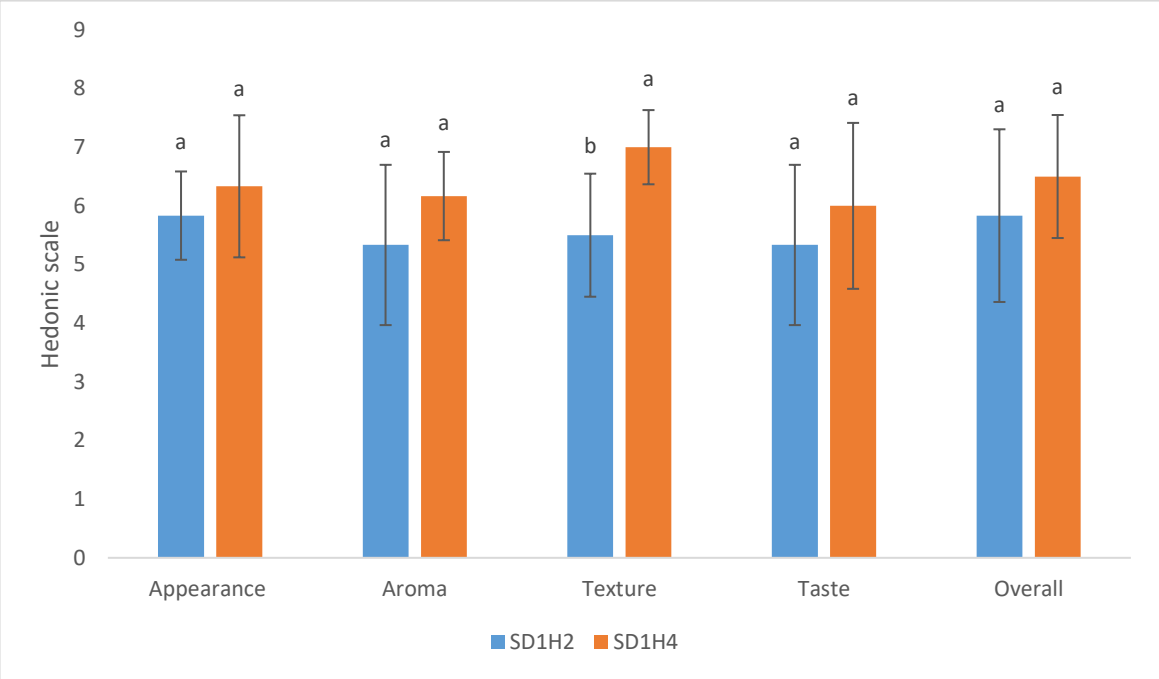

Figure 4.16 Sensory evaluation of formulations containing a combination of hydrocolloids and sourdough
Columns with any same letter in a given cluster are not significantly (p>0.05) different. Values reported are means of at least three independent measurements.

There were no significant differences (p>0.05) between the two samples in term of appearance (5.8 for SD1H2 and 6.3 for SD1H4), aroma (5.3 for SD1H2 and 6.2 for SD1H4), taste (5.3 for SD1H2 and 6.0 for SD1H4), and overall liking (5.8 for SD1H2 and 6.6 for SD1H4).

There was significant differences ($p \leq 0.05$) and texture (5.5 for SD1H2 and 7.0 for SD1H4). This preference can be explained by the fact that SD1H4 had a more uniform crumb structure which could have been appealing. Taking that into consideration, SD1H4 was selected to be submitted to consumer acceptability test.

4.3.6 Sensory evaluation and consumer acceptability

The results of sensory evaluation of the most preferred samples (FB60H4, SD1 and SD1H4) that were subjected for consumer acceptability testing, using 26 untrained panellists, are presented in Figure 4.17. These samples were selected based on results from previous bench top sensory evaluation (Section 4.3.3.3, section 4.3.4.4 and section 4.3.5.3). Each attribute will be discussed separately.

For the appearance of the bread, there was a significant difference ($p \leq 0.05$) between the bread containing sourdough (SD1) and the bread containing a mixture of hydrocolloids and sourdough (SD1H4). The bread containing sourdough, without hydrocolloids (SD1) was the most preferred, with a mean score of 7.0, compared to other samples, with mean scores ranging from 5.6 to 6.0 for FB60H4 and SD1H4, respectively.

For the texture of the crumb during eating (mouthfeel), similar trends were observed. The bread containing sourdough, without hydrocolloids (SD1) was the most liked, with a mean score of 6.9. These results correlate the firmness of the bread, as well as the crumb structure, as previously discussed. Briefly, in which it was found SD1 resulted in bread with the highest specific volume (section 4.3.4.2).

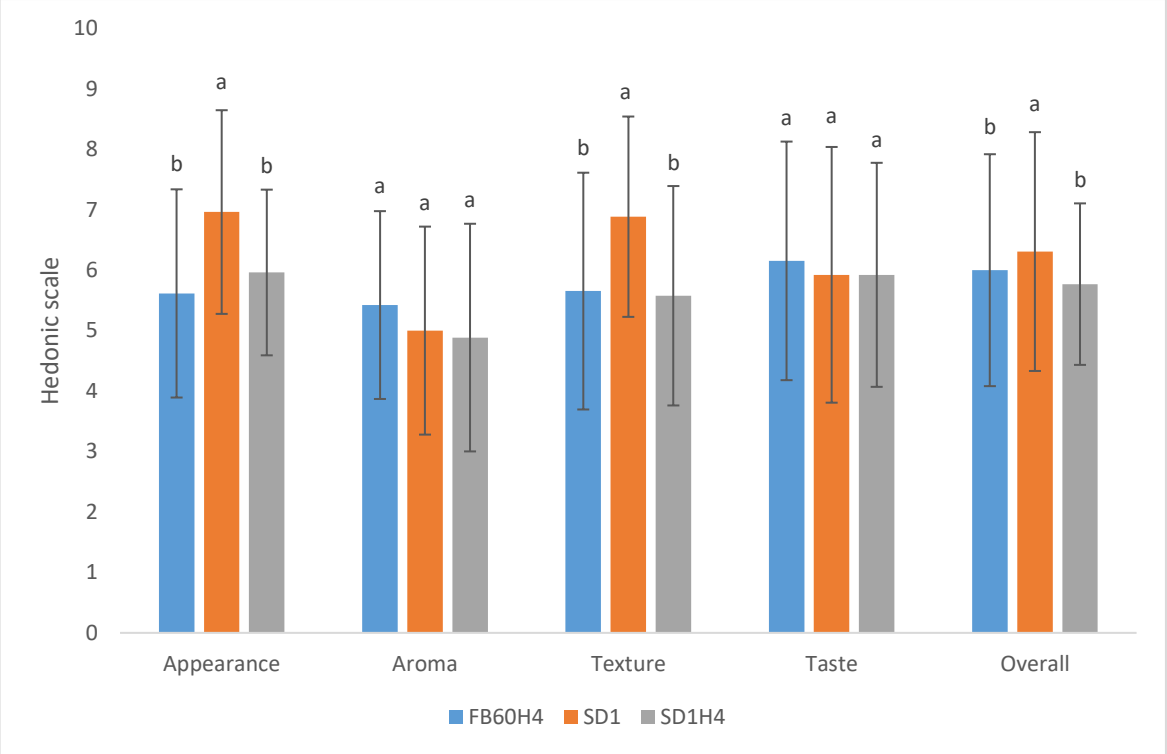

Figure 4.17 Consumer acceptability sensory evaluation results of selected formulations

Columns with any same letter in a given cluster are not significantly (p>0.05) different.

Values reported are means of at least three independent measurements

For the aroma and the taste, no significant differences (p>0.05), was observed between the samples. The breads were neither like nor dislike for aroma (score 5) and like slightly for taste (score 6). However, the overall sensory quality of the bread evaluated ranged from 5.8 to 6.3, for SD1H4 and SD1 respectively, indicating consumers liked the bread slightly. Of these samples, sourdough bread, without the addition of hydrocolloids (SD1) preferred (p=0.000), obtaining a mean score of 6.3. Based on these results, SD1 was selected for further shelf life testing.

4.3.7 Shelf life study

The microbiological quality and firmness of the final gluten-free bread (SD1) was performed over a period of 3 days, stored at ambient temperature, to determine the shelf stability of the baked bread.

The microbiological quality, total plate count and yeasts and mould, of the baked bread, are presented in Figure 4.18.

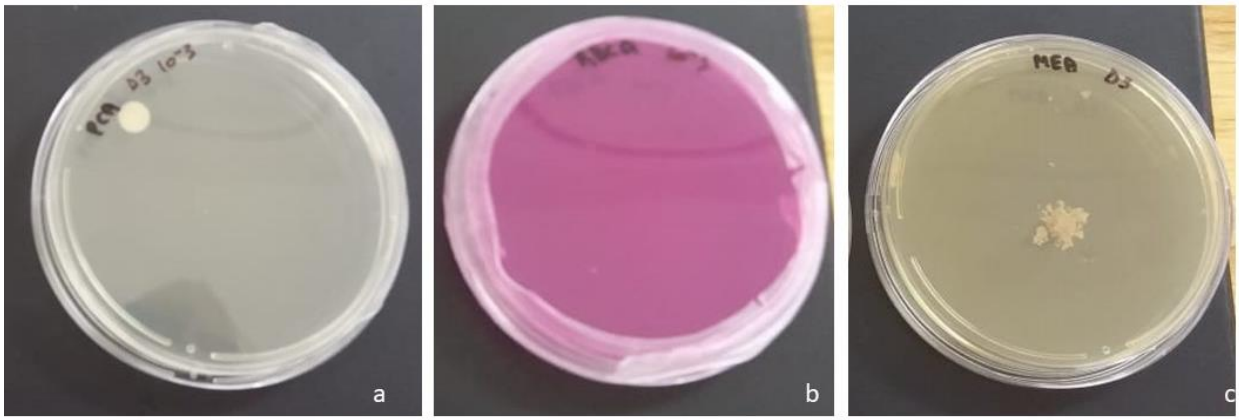

Figure 4.18 Microbiological analysis of bread on day 3 after baking: on PCA (a), RBCA (b) and MEA (c)

For total plate count, which gives an indication of the total microbial population present on the bread, no growth was observed over a period of three days of storage. In addition, no growth was observed for the yeasts and mould (for both MEA and RBCA respectively). A number of factors could be responsible for the absence of spoilage microorganisms in the baked bread, namely: The sodium metabisulphite used during the production of the green banana flour (as previously discussed), could have inhibited the growth of spoilage organisms on the baked bread. The high heat during baking killed all microorganisms present in the dough, and sourdough containing the *L. Plantarum* is known to produce various antifungal compounds, which could have inhibited the spoilage organisms in the bread (Moore et al., 2008, Coda et al., 2011a). The addition of sourdough to gluten-free bread formulations was reported to inhibit fungal growth in the bread, extending its microbial shelf life (Axel et al., 2016).

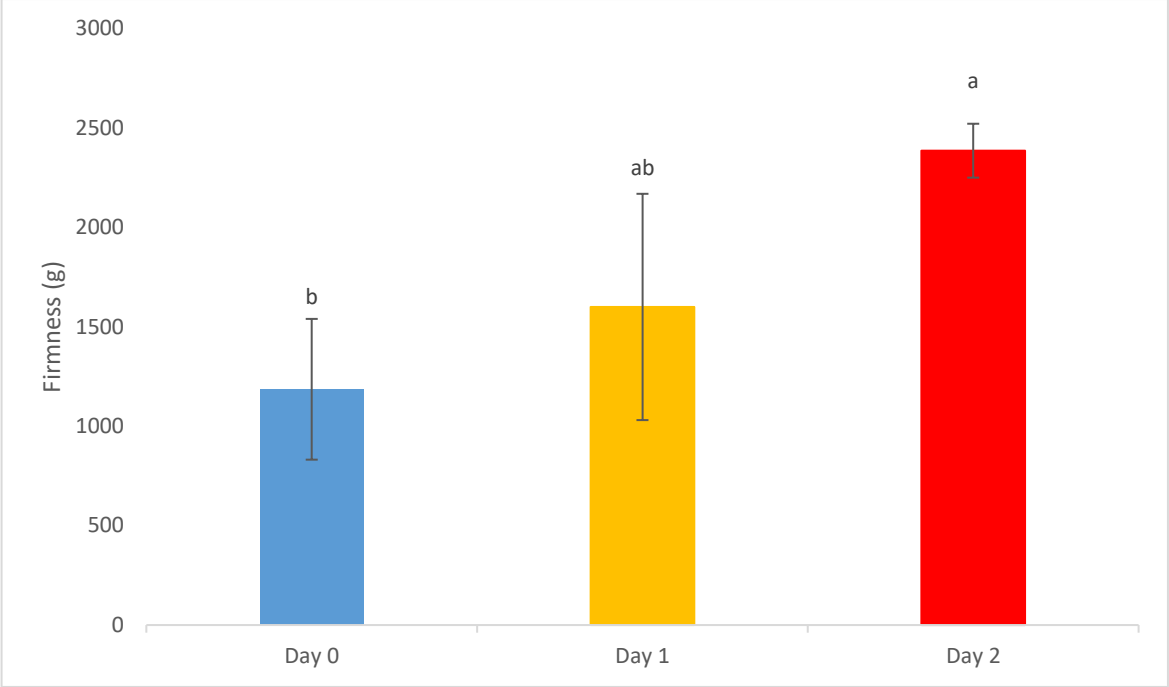

Figure 4.19 Firmness of bread (SD1) over a period of 3 days
Columns with any same letter are not significantly (p>0.05) different.
Values reported are means of at least three independent measurements.

The results of the crumb firmness, over a period of 3 days, are presented in Figure 4.19. Over the three days of storage, the crumb texture of the bread became significantly firmer, ranging from 1185.46 g on day 1 to 2385.03 g. By day three the bread was hard and brittle, making it unacceptable for consumption. However, the moisture content of the bread did not change significantly (results), during storage, indicating no moisture loss. Due to this, the increased firmness of the crumb can be attributed to a high staling rate of the banana starch present in the bread. Unripe banana flour typically has a high retrograde rate, as previously discussed (section 3.4.3.1). In addition, during sourdough fermentation, starch is partially hydrolysed to sugars which have a direct effect on water migration from other bread components (protein and gelatinized starch) causing bread stiffness (Mohamed et al., 2010).

In a study, by Mohamed et al.,(2010) conducted on wheat-based bread, containing unripe banana flour (30%), it was found that the higher the banana flour content, the higher the firmness of the bread, resulting in a faster staling rate.
Staling describes the changes that take place in breads during storage. It does not include microbial contamination and spoilage (Ahlborn et al., 2005, Ronda & Roos,

2011). Changes which occur in the texture of the crumb include: the crumb becomes harder, tougher, as well as more crumbly and opaque. Although a complex series of events occurs during staling including changes in the crystallinity of the starch during storage, bread staling is mainly associated with the firming of crumb (Arendt et al., 2007, Paciulli et al., 2016) Crust staling is generally caused by moisture migration from the crumb to the crust resulting in a soft, leathery texture and is generally less objectionable than crumb staling.

4.4 CONCLUSION

The aim of this chapter was to assess the effect of the addition of hydrocolloid and/or sourdough on the quality characteristics of gluten-free bread, containing unripe banana flour as the main ingredient.

Compared to the gluten-free control the addition of hydrocolloids, and/or sourdough, improved the overall baking properties of the bread, by increasing the bread volume. The additive also had a positive effect on the crumb structure, being less dense with uniform gas cell formation, as wells as improving the crumb texture. Sensory panels also indicated that they preferred these bread compare to the control. However, the shelf life of gluten-free bread, containing unripe green banana flour, is short, primarily due to a high staling rate. Hence, reducing the staling rate of the gluten-free bread required further investigation.

CHAPTER 5 : CONCLUSIONS AND RECOMMENDATIONS

5.1 ACHIEVEMENTS OF OBJECTIVES

The present study aimed to characterize the physico-chemical properties of unripe banana flours, obtained from 10 banana cultivars grown in South Africa. Important functional properties studied included the water and oil holding capacity, foaming and emulsifying capacity and stability of different flours. The thermal and rheological (pasting) properties of the flour, including the set gel texture of the gels, were investigated. In addition, a major aim of the study was to develop a gluten-free bread with good quality characteristics using one of the flours. For this part of the study, the effect of hydrocolloids and sourdough on the rheological properties, baking performance and overall quality of the gluten-free bread was investigated. The objectives set out in section 1.4 were achieved using standard methods. Where necessary methods were modified to accommodate the specific characteristics of the unripe banana flour.

The general properties of unripe banana flour indicate that it is composed primarily of starch, including high amounts of resistant starch, higher than that of maize and wheat. The high resistant starch makes banana flour suitable as an ingredient in food products where increased levels of dietary fibre are required. Of interest, microscopy studies showed that the starch granules of banana flour are generally elongated/oval and bigger than that of maize and wheat. Compared to wheat and maize, unripe banana flour is generally darker, redder and more yellow, due to the presence of polyphenols in the fruit, as well as products produced by enzymatic browning during processing. The colour of the flour negatively affected the colour of the final baked product.

Of the functional properties studied, unripe banana flour has good water and oil holding capacity, indicating that within a food application banana flour can absorb more water and oil. Thus in an application where product viscosity is important, small amounts of banana flour could be used as an additive to achieve a product with good viscosity. In addition, it was clear that unripe banana flour could also be considered a good emulsifying agent.

Under heating conditions, in the presence of water, unripe banana flour can form a thick paste at a lower temperature, compared to wheat. This would indicate that less energy and lower temperatures could be used during the processing of various foods. However, variations in pasting properties and gelatinization temperatures were observed between the different banana cultivars, meaning it would be important to select the correct cultivar for a specific food application. Upon gelling, the unripe banana flour also resulted in stronger gels, compared to wheat and maize.

The principle component analysis showed that the unripe banana flours, irrespective of dessert banana variety, were distinguishable from both wheat and maize flour by breakdown viscosity, resistant starch content, and peak paste viscosity. For unripe banana flours, the different varieties were distinguishable from one another based on pasting temperature and gel adhesiveness. Linear regression assessment indicated (R^2 = 0.898), that the unripe/green desert banana flour resistant starch content could be predicted from the flour pasting breakdown viscosity.

During the development of gluten-free bread containing unripe banana flour, the formulation required the addition of more water compared to the wheat formulation. The addition of hydrocolloids resulted in the improvement of rheological properties of the formulations. This in turn, after baking, resulted in bread with improved volume and better crumb with the more uniform gas distribution. The addition of hydrocolloids improved the overall acceptability of bread when subjected to sensory evaluation. However, one set back is the fact that being additives, there is a cost increase in the formulations.

The addition of sourdough to improve the quality characteristics of gluten free-bread containing unripe banana flour. The baked bread resulted in improved properties mainly due to the effect of acidification of dough rheological properties (Moroni et al., 2009, Naqash et al., 2017). However, it was noticed that a high amount of sourdough addition resulted in less acceptable bread particularly in terms of aroma and taste. The sourdough used in combination with hydrocolloids resulted in the

improvement of rheological properties of formulations as well as the best baking performance of the bread. However, when subjected to consumer acceptability test, a clear preference for gluten free-bread containing sourdough only was observed. One of the advantages is that on an industrial scale it can be cost effective as the starter is purchased once and subcultured. Yet, the sourdough bread had a short shelf life mainly due to a high staling rate typical of most gluten-free breads. Hence, there is a need to investigate ways to improve the shelf life of the bread containing sourdough.

5.2 CONTRIBUTION OF THE STUDY

This is the first study that investigated the characteristics of unripe banana flour from cultivars grown in South Africa, from the same orchard plot. These cultivars were subjected to identical environmental conditions, thus reducing variabilities based on environmental factors. This study provides new knowledge that banana cultivars from the same genomic group can be different in terms of the banana flours general characteristic, such as functional and thermal properties. Thus it would be important to select the banana cultivar based on the application for the flour.

In addition, it was shown that unripe banana flour (Gros Michel) can be used successfully as the main ingredient for the development of gluten-free bread, with or without the use of hydrocolloids. This study also investigated the use of sourdough, not previously reported in the literature.

This results of this study have been presented to the scientific community at the following proceedings:

- Kongolo, J.I., Da Silva, L.S., Wokadala, O.C., Du Plessis, B., Husselman, J., Ngcobo, M.E., Emmambux, N.M. & Daneel, M. 2017. *Pasting, thermal, gel texture, resistant starch and colour properties of unripe banana flour from 10 desert banana varieties cultivated in South Africa*. Journal of Food Measurement and Characterization, 1-9.
- Kongolo, J.I., Da Silva, L.S., Wokadala, O.C., Du Plessis, B., & Daneel, M. *Effect of different banana varieties on the physico- chemical properties of the flour and the quality characteristics of selected gluten-free bread.* Oral

presentation at the SAAFoST 22nd Biennial International Congress and Exhibition, 3-6 September 2017, Cape Town, South Africa.

- Kongolo, J.I., Da Silva, L.S., Wokadala, O.C., Du Plessis, B., & Daneel, M. *Development and characterization of gluten-free bread made from unripe banana flour using sourdough fermentation.* Poster presentation at the IUFoST 19th World Congress of Food Science and Technology, 23-27 October 2018, Mumbai, India.

5.3 SHORTCOMINGS OF THE STUDY

Although all objectives were achieved, minor shortcomings were identified:

- Taking into account that a chemical treatment (sodium metabisulphite) was needed to prevent enzymatic browning of the raw banana before the drying step, phenolic compounds present in raw banana should be quantified, to determine which cultivars are more prone to enzymatic browning, as this has a negative effect on the colour of the final flour. This too would have a negative effect on the colour of the final product.
- Non-starch polysaccharides appear to play a big role in the functional and sensory properties of the resulting product (Rao et al., 2007, Sim et al., 2015). Characterisation of non-starch polysaccharides in the banana flours was not studied and requires further investigation.
- The ratio of amylose to amylopectin in the starch component of the flour was not determined. The ratio of these starch components plays a role in understanding the thermal and pasting properties of the starch. Hence this should be further investigated.
- For the development of the gluten-free bread, only one cultivar was selected. Due to the differences observed in the functional and thermal properties of the flours, the effect of cultivar differences on the quality of gluten-free bread should be further investigated.

5.4 RECOMMENDATIONS AND FURTHER WORK

From the finding of this study, the following recommendations can be made for further research:

- Determining the physico-chemical, thermal and structural characteristics of starch isolated from different banana cultivars grown in South Africa.
- Characterisation of non-starch polysaccharides present in unripe banana flour.
- Investigating the effect of different unripe banana flours on the quality characteristics of gluten-free bread.
- Investigating additional applications for the use of banana flour in the food industry.
- The synergistic effect of the addition of sourdough and hydrocolloids on gluten-free bread containing UBF is being investigated to determine whether or not the improvement observed is dose-dependent.

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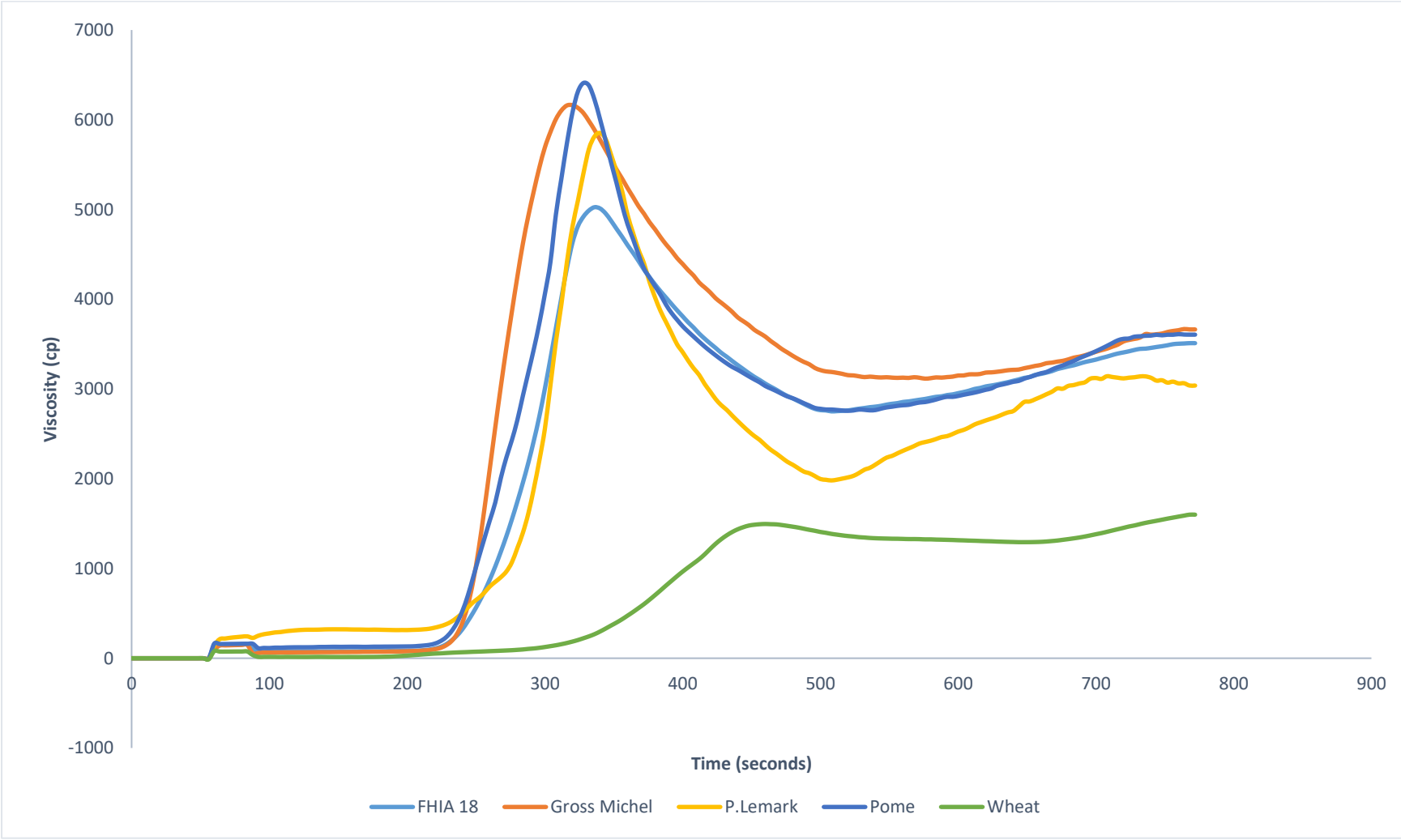
APPENDICES

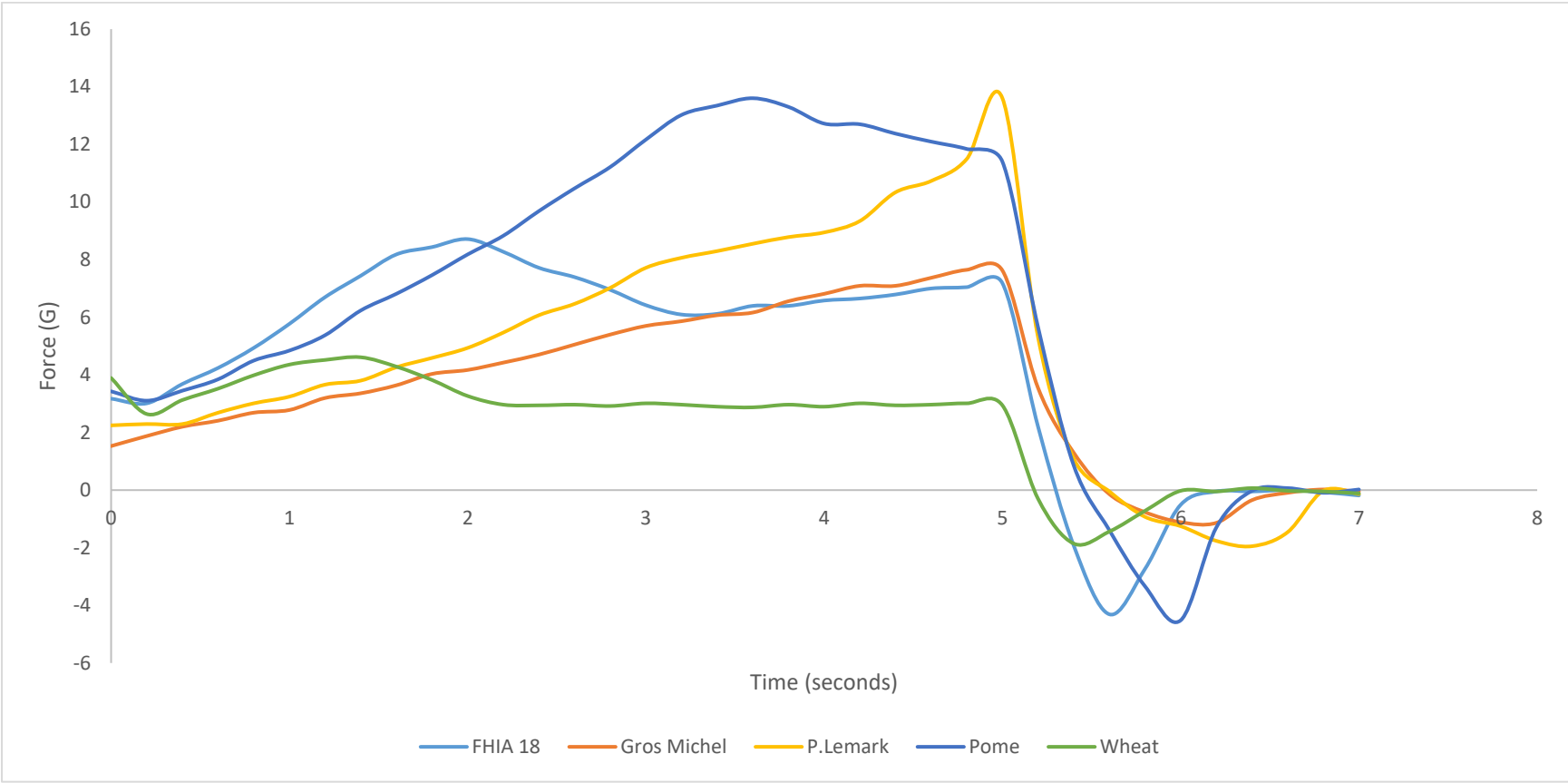
Appendix 1: Preparation method of chemicals used in starch determination analysis

Solutions	Preparation
Sodium hydroxide (4 M)	Sodium hydroxide (160 g) was dissolved in 1 L of deionized water to make the solution
Sodium maleate buffer (100 mM, pH 6.0) plus 5 mM calcium chloride dihydrate and sodium azide (0.02% w/v)	Maleic acid (23.2 g) was dissolved in 1600 mL of deionized water and the pH adjusted to 6.0 with 4 M sodium hydroxide. Calcium chloride dihydrates (1.47 g) and sodium azide (0.4 g) was then added to the solution and dissolved. The volume was then adjusted to make 2 L.
Sodium acetate buffer (1.2 M, pH 3.8).	A volume of 69.6 mL of glacial acetic acid (1.05 g/mL) was added to 800 mL of deionized water and adjusted to pH 3.8 using 4 M sodium hydroxide. The volume was then adjusted to 1 L with deionized water.
Sodium acetate buffer (100 mM, pH 4.5)	A volume of 5.8 mL of glacial acetic acid was added to 900 mL of deionized water and adjusted to pH 4.5 using 4 M sodium hydroxide. The volume was then adjusted to 1 L with deionized water.
Potassium hydroxide solution (2 M)	A mass of 112.2 g KOH was dissolved into 900 mL of deionized water. The volume was then adjusted to 1 L.
Aqueous ethanol (approx. 50% v/v)	A volume of 500 mL of ethanol (99% v/v) was mixed with 500 mL of H_2O.
Solution 1: Dilute Amyloglucosidase (AMG)	Concentrated AMG (bottle 1, Megazyme resistant starch kit) solution (2 mL) was diluted to 22 mL with 0.1 M sodium maleate buffer (0.1 M, pH 6.0).
Solution 2	Immediately before use, pancreatic α-amylase (bottle 2, Megazyme resistant starch kit) (1 gram) was dissolved in 100 mL of sodium maleate buffer (100 mM, pH 6.0) and stirred for 5 min. Dilute AMG (1 mL) was added and mixed into the solution. The solution was then centrifuged at 1057 rcf for 10 minutes and the supernatant was decanted and used on the day of preparation.
Solution 4: GOPOD reagent	GOPOD Reagent Buffer (bottle 3, Megazyme resistant starch kit) was diluted to 1 L with deionized water (this is solution 3). GOPOD reagent enzymes (bottle 4, Mregazyme resistant starch kit) was dissolved in 20 mL of Solution 3 and quantitatively transfer to the bottle containing the remainder of Solution 3. The bottle was covered with aluminium foil to protect the enclosed reagent from light.

Appendix 2: Typical graph of pasting properties of selected UBFs compared to wheat



Appendix 3: Typical graph of texture of set gels of selected UBFs compared to wheat



Appendix 4: Sensory evaluation consent form

Panelist Number: _____

SENSORY EVALUATION: GLUTEN FREE BREAD MADE WITH UNRIPE BANANA FLOUR

Dear Panelist, thank you for showing interest in this sensory study. The study will be looking at the sensory quality and consumer acceptability of three different gluten-free bread made with unripe banana flour. Water will be used as a palate cleanser. If you're willing to take part in this study, please sign the consent form below.

I, _____ is willing to take part in this sensory study.

_____

(Signature)

Please complete your personal information

Gender:

Age Group: 16-25 25-35 Older than 35

Are you familiar with gluten-free bread? Please gluten free bread you are familiar with and use regularly:

Instructions for the Sensory Evaluation: You have been presented with three coded bread samples, and a cup of water to be used as a mouth cleanser, before tasting, as well as between different samples. Please rinse your mouth with the water provided and evaluate the first sample. Rinse your mouth with water before tasting the next sample. Please evaluate the following sensory attributes: ***appearance, aroma, texture, taste and overall acceptance,*** according to the 9-point hedonic scale indicating how much you like or dislike each bread sample. Please motivate your response with a comment in the space provided.

Sample code	Attributes	HEDONIC SCALE								
		Dislike extremely	**Dislike very much**	**Dislike moderately**	**Dislike slightly**	**Neither like or dislike**	**Like slightly**	**Like moderately**	**Like very much**	**Like extremely**
	Appearance	1	2	3	4	5	6	7	8	9
		Comment:								
	Aroma/ Odour	1	2	3	4	5	6	7	8	9
		Comment:								
	Texture	1	2	3	4	5	6	7	8	9
		Comment:								
	Taste	1	2	3	4	5	6	7	8	9
		Comment:								
	Overall	1	2	3	4	5	6	7	8	9
		Comment:								
	Appearance	1	2	3	4	5	6	7	8	9
		Comment:								
	Aroma/ Odour	1	2	3	4	5	6	7	8	9
		Comment:								
	Texture	1	2	3	4	5	6	7	8	9
		Comment:								
	Taste	1	2	3	4	5	6	7	8	9
		Comment:								
	Overall	1	2	3	4	5	6	7	8	9
		Comment:								

Appendix 6: Published article

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Pasting, thermal, gel texture, resistant starch and colour properties of unripe banana flour from 10 desert banana varieties cultivated in South Africa

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