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Why sorghum and wheat biscuits have similar texture: The roles of protein, starch and sugar

by

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DECLARATION

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

Olumide Ayomide Adedara
February 2017

DEDICATION

This dissertation is dedicated to the hungry African child who has little or nothing to eat. The journey to a food secure African continent continues with you.

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ABSTRACT

Why sorghum and wheat biscuits have similar texture: The roles of protein, starch and sugar

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To help improve food security in sub-Saharan Africa, producing products such as biscuits from locally grown sorghum represents a viable alternative to using imported wheat. Sorghum biscuits are also valuable option for coeliac patients due to their gluten-free nature. Unlike the inferior quality that characterizes many gluten-free products, sorghum biscuits have similar texture to wheat biscuits. Although gluten is present as part of the wheat biscuit matrix, there are debates about its actual state and role in the texture of wheat biscuits. The objective of this work was to determine why sorghum biscuits have similar texture to wheat biscuits despite the absence of gluten.

Biscuits were made from sorghum flour and their level of hardness and brittleness were compared with laboratory made sugar-snap and commercial Marie wheat biscuits. The roles of protein, starch and sugar in biscuit texture were investigated. SEM and TEM showed that in sorghum biscuits, the kafirins (prolamin storage proteins) remained encapsulated in their protein bodies and were unlikely to contribute to the texture of sorghum biscuits due to their confined state. However, a continuous gluten sheet was observed to envelope intact starch granules in sugar-snap and Marie wheat biscuits. Starch granules were observed to be intact in both sorghum and wheat biscuits.

Stereomicroscopy indicated that starch granules were embedded in the sorghum biscuit matrix with visible strands of starch gel suggesting some starch granules, although minute were gelatinized, while vast majority of the starch granules remained intact and few swollen. Polarizing light microscopy (PLM) confirmed that most of the starch granules were ungelatinized as they remained birefringent, while some were non-birefringent. Increasing dough water level increased the hardness and brittleness of sorghum biscuits and the texture

was similar to that of Marie wheat biscuit at both 35% and 40% water on a flour basis, while it was similar to sugar-snap wheat biscuit at 30% water on a flour basis. Addition of different levels of pre-cooked sorghum flour reduced the hardness and brittleness of sorghum biscuits. As the level of pre-cooked sorghum flour was increased, biscuits became softer, less cohesive and more crumbly. This indicates that gelatinization resulted in weakening of the biscuit structure and could not have been responsible for the increase in the hardness of sorghum biscuit when water level was increased.

PLM also indicated that the starch granules were embedded in the sorghum biscuit matrix through the formation of networks with a sugar glass.. Increasing sugar level also increased the hardness and brittleness of sorghum biscuits with biscuits containing 20% sugar on a flour basis having a similar texture to sugar-snap and Marie wheat biscuits, while biscuits containing no sugar were extremely soft and crumbly. This indicated that the sugar was responsible for holding other components of the biscuit together. DSC and XRD confirmed that the sugar in sorghum biscuit was present as a glassy amorphous matrix, as was also the case in wheat biscuits. It appears the sugar glass becomes more continuous as sugar level is increased, thereby allowing the formation of stronger networks with the starch granules which caused an increase in biscuit hardness.

This study shows that even though gluten is not present in sorghum biscuits, sorghum biscuits of similar texture to wheat biscuits can be produced by optimizing the level of sugar and water used in dough formation. On the other hand, the texture and role of gluten in wheat biscuits depends on the type of wheat biscuit being investigated as they differ in formulation. It appears due to the inability of kafirins to form a continuous network in sorghum biscuits, an oil emulsified sugar glass infiltrates the biscuit matrix embedding starch granules, protein matrix and cell wall materials. The structure formed through the interaction of the sugar glass with other components of the sorghum biscuit, especially starch seems to produce a functionality that compensates for the absence of gluten and this is responsible for the similar texture of sorghum to wheat biscuits.

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1 INTRODUCTION

Sorghum is well adapted to the harsh climatic conditions in Africa (Belton and Taylor, 2004). This is unlike wheat which is expensive in Africa, as it is mostly imported due to low availability (FAOSTAT, 2015). This has necessitated the need for cheaper and locally available alternative raw materials for the production of products such as bread and biscuits. Biscuits are widely consumed at all levels of the society as a result of their ready to eat nature, good nutritional quality, availability in different varieties and affordable cost (Sudha *et al*., 2007). They also have a long shelf life due to their low moisture content 1-5% (Wade, 1988). Although biscuits are commonly made with wheat flour (Wade, 1988), research efforts have identified other cereals such as sorghum as an alternative for making biscuits (Taylor *et al.*, 2006). Sorghum is widely cultivated in Africa (FAOSTAT, 2015), and its increased utilization can help Africa reduce wheat importation, while also increasing the income of local farmers.

Although the importance of the viscoelastic properties of gluten (formed from wheat prolamin) in baked products such as biscuits has been emphasized in the literature (reviewed by Gallagher *et al*., 2004), biscuits of comparable textural qualities to wheat biscuits have been made from sorghum despite the absence of gluten (Serrem *et al.*, 2011; Omoba *et al.*, 2015). Descriptive sensory profiling of these sorghum biscuits revealed that they were indistinguishable from whole wheat biscuits in terms of hardness, roughness and coarseness, although they were less dry and less crispy (Serrem *et al.*, 2011; Omoba *et al.*, 2015).

Sorghum biscuits also represent a gluten-free alternative for coeliac patients. Furthermore, compositing the sorghum flour with legume such as soya improves the protein quality of the biscuits (Serrem *et al.*, 2011). This makes the composite biscuits a good option for alleviating macronutrient malnutrition in Africa. While the reasons for improved nutritional qualities of these sorghum biscuits have been well established, questions about their textural semblance to wheat biscuits remain unanswered.

The success of non-wheat cereals such as sorghum in the production of biscuits is particularly interesting due to the lack of consensus on the actual state and role of gluten in wheat biscuits. Donelson (1988) stated that gluten, even though a major group of wheat flour proteins, is not essential to the baking performance of biscuits. While some authors have also stated that gluten is undeveloped in biscuits (Slade *et al.*, 1993), others have argued that the gluten precursor proteins, gliadin and glutenin, are not spectator components especially during biscuit baking

(Gaines, 1990). Gaines (1990) suggested that during the later stage of baking, considerable association of these proteins probably occurs as a result of expansion of gas cells forcing the hydrated proteins into sufficiently close proximity for aggregation. Manley (2000) also described hard sweet wheat biscuits as containing a gluten network. In terms of the role of gluten in biscuits, Gaines (1990) stated that it affects texture (hardness), while Doescher *et al.* (1987) and Maache-Rezzoug *et al*. (1998) stated that it contributes to the spread of biscuits due to the influence it has on dough viscosity. It appears the perception of the role of gluten in wheat biscuits is dependent on the type of biscuit being investigated. However, it is important to note that the wheat proteins are part of the biscuit matrix and their importance to the structural integrity of wheat biscuits cannot be underestimated.

Thus, the objective of this work was therefore to determine the scientific reasons why sorghum biscuits have similar texture to wheat biscuits despite the absence of gluten in sorghum biscuits.

2 LITERATURE REVIEW

This literature review looks at the structure and chemical composition of wheat and sorghum grains in relation to the biscuit making properties of their flours. The science and technology of biscuit making will also be discussed in order to gain insight into the science of sorghum biscuit making. The roles of the different chemical components of biscuits will also be reviewed to understand how they impact biscuit quality. Specialised analytical techniques for analysing the contributions of the different components of biscuits to their texture will also be reviewed.

2.1 Structure of wheat and sorghum kernels

The wheat kernel like other cereal grains, is divided into three parts; the germ (embryo), pericarp (outer layer) and endosperm which composed 3%, 14% and 83% of the grain, respectively (Fig. 2.1) (Bushuk and Scalon, 1993). Similar to the wheat kernel, the sorghum kernel is made up of the pericarp (outer layer, 6.5%), germ (embryo, 9.4%) and endosperm (storage tissue, 84.2%) (Fig. 2.2) (Serna-Saldivar and Rooney, 1995).

To produce flour from these grains, a combination of milling and refining is used to separate the endosperm from other components of the grain (Evers and Millar, 2002). Both wheat and sorghum grains are usually conditioned (by adding water and tempering) prior to milling in order facilitate good separation of bran and germ from the endosperm to achieve optimal sieving (Munck 1995; Posner, 2009). Soft wheat types which produce flour with a low level of starch damage are preferred for biscuit making (Donelson and Gaines, 1998). Hard sorghum kernels are generally considered best for milling (Munck, 1995).

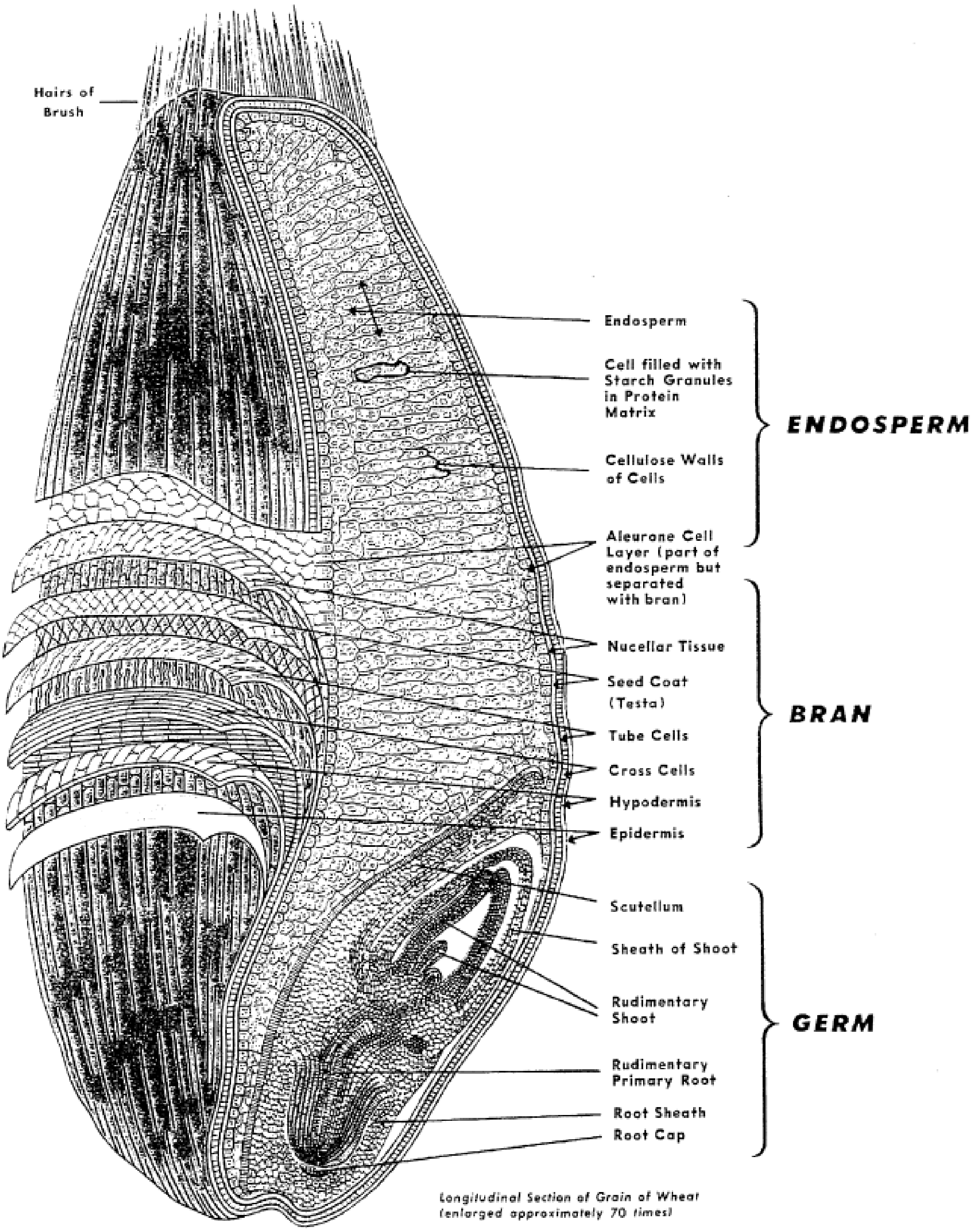

Figure 2.1: Longitudinal section of wheat grain (Slavin *et al*., 2000).

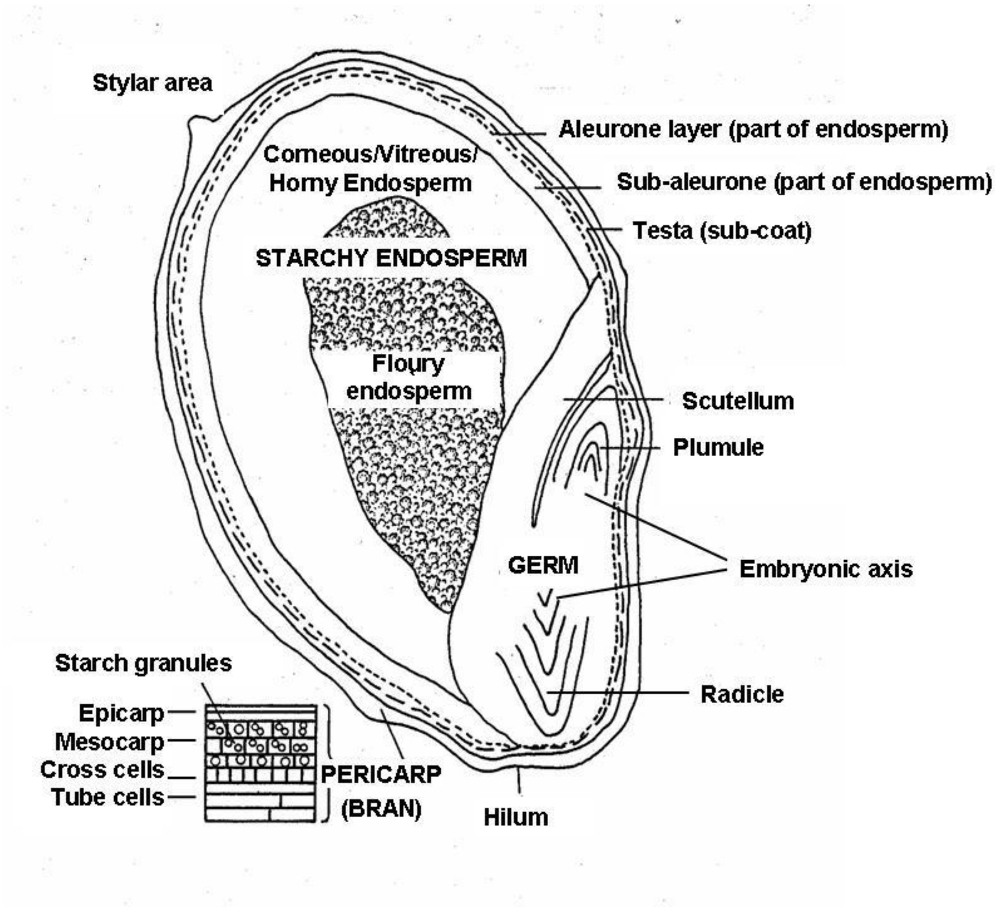

Figure 2.2: Cross section of sorghum grain (Taylor and Belton, 2002).

2.2 Chemical composition of wheat and sorghum grains

Like other cereal grains, wheat and sorghum grains are composed primarily of starch, protein, fat and non-starch polysaccharides (NSP). The properties of these individual components which affect the utilization and functionality of these grains in biscuit making are briefly reviewed.

2.2.1 Starch

Starch is deposited in the endosperm tissue at levels between 60 and 75% of the weight of the cereal grain (Delcour and Hoseney, 2010). It is composed of two major components, amylose and amylopectin, which together represent approximately 98-99% of its dry weight (Tester *et al.*, 2004). However, the ratio of these two components differs between starches (Delcour and Hoseney, 2010). Yasui *et al*. (1996) found amylose contents of 26-28% and 1.2-2.0% in non-waxy and waxy wheat starch, respectively. High-amylose wheat starch contains up to 37.5% amylose (Hung *et al.*, 2005). Similarly, Sang *et al.* (2008) found an amylose content of 24% and 0% in normal and waxy sorghum starch, respectively. Hill *et al*. (2012) found an amylose content of up to 56% in high-amylose sorghum starch.

The granules of wheat starch are present in a bimodal distribution consisting of the large "A" and small "B" type granules (D'Appolonia and Rayas-Duarte, 1994). The larger "A" granules have a lenticular shape and a diameter of 15-30 µm, while the "B" granules which are usually spherical have a diameter of less than 10 µm (Fig. 2.3A) (Stone and Morell, 2009). Type "A" and "B" granules constitute 51.6% and 45% of the total starch content, respectively (Betchel *et al*., 1990). The damage to these granules during milling is important for the functionality of starch in biscuits, as will be discussed later (section 2.4.1). Also important in the functionality of wheat starch is its gelatinization and pasting behaviour when heated in water. Wootton *et al.* (1998) reported a peak gelatinization temperature of 63-66°C for a range of non-mutant wheat starches. D'Appolonia and Rayas-Duarte (1994) summarized the functionality of wheat starch in baking to include diluting of gluten to the desired consistency, setting of structure, and serving as surface for gluten interaction.

Sorghum starch is classified as type-B crystalline type because of its moderate swelling property compared to type-A starches (potato, cassava) which are high swelling starches (Beta and Corke, 2001). The starch granules are essentially spherical in shape (Tester *et al*., 2004), and have an average diameter of about 20 µm (Fig. 2.3B) (Delcour and Hoseney, 2010). Beta and Corke (2001) reported a gelatinization peak temperature of 65-73°C for sorghums grown in southern Africa, while Akingbala *et al*. (1981) reported a temperature range of 71-83°C for sorghums grown in India. Importantly, this is considerably higher than for wheat starch.

In addition to the known functionality of starch in baked products, the hydrophobic nature of sorghum proteins means that the starch can be expected to play a major role in influencing the property of sorghum dough. Kulamarva (2005) found that boiling water affected the cohesiveness, gumminess, and hardness of sorghum dough. It was stated that this could be due to starch gelatinization. Elhassan *et al*. (2015) also found that sorghum cultivars with combined waxy (high amylopectin) and high protein digestibility traits produced a softer paste, higher flour solubility and pasting viscosity than normal non-waxy, normal protein digestibility sorghums. This indicates that amylose-amylopectin ratio also influences the quality of end products. These authors stated that the properties of these combined waxy and high protein digestibility sorghum cultivars suggest that their flour may have better properties for making dough-based sorghum products.

The higher level of water used in sorghum biscuit dough (Serrem *et al*., 2011), higher gelatinization temperature of sorghum starch (Akingbala *et al*., 1981), and probably non-gelatinization of starch during baking of biscuits (Delcour and Hoseney, 2010) indicate that the contribution of sorghum starch to the texture of sorghum biscuits is an important focus.

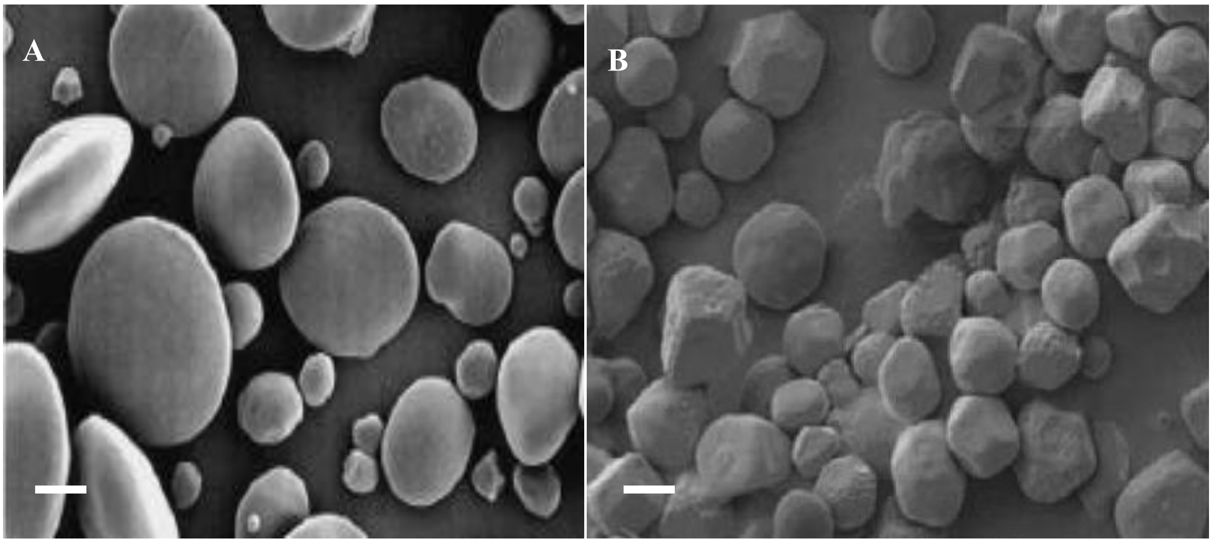

Figure 2.3: Scanning electron micrographs showing bimodal distribution of wheat starch (A) (Yoo and Jane, 2002) and unimodal sorghum starch granules (B) (Sun *et al.*, 2014). Bar is 10 µm.

2.2.2 Proteins

Wheat proteins can be classified into gluten and non-gluten proteins based on their functionality (Goesaert *et al*., 2005). The gluten proteins constitute the major storage proteins (80-85%) that are present in the starchy endosperm cells, while the non-gluten proteins occur mainly in the outer layers of the wheat kernel with lower concentration in the endosperm. The gluten proteins comprise the prolamins, glutenin and gliadin (Shewry and Tatham, 1997a), which form a continuous proteinaceous matrix in the cells of the mature dry grain (Fig. 2.4A), and are aggregated into a viscoelastic network when wheat flour is hydrated and worked to form a dough (Shewry *et al.* 2002). While the gliadins contribute mainly to the viscosity and extensibility of the dough, the glutenins are responsible for the strength and elasticity of the dough (Wieser, 2007). The structure and properties of the gluten proteins are influenced by disulphide bonding (Shewry and Tatham, 1997a). The gliadins are soluble in aqueous alcohols and are present as monomeric proteins, which either lack disulphide bonds or have only intra-chain disulphide bonds. On the other hand, the glutenins are insoluble in aqueous alcohols and consist of protein subunits present in polymers stabilised by inter-chain disulphide bonds (Shewry and Tatham, 1997b). These proteins are largely responsible for the viscoelastic properties that allow doughs to be processed into bread and various other food products including cakes, pasta, noodles and biscuits. High gluten elasticity is desired in products such as pasta and noodles, whereas more extensible gluten is desired in products such as cakes and biscuits (Shewry and Tatham, 1997b).

Sorghum proteins can be broadly classified into prolamin and non-prolamin proteins (Mesa-Stonestreet *et al.*, 2010). Approximately 80%, 16% and 3% of sorghum proteins are located in the endosperm, germ and pericarp, respectively (Taylor and Schüssler, 1986). Kafirins, the prolamin proteins of sorghum are located in protein bodies in the starchy endosperm (Fig. 2.4B). They are classified as either α-, β-, γ-, or δ-kafirins based on their amino acid composition and sequence (Belton *et al.*, 2006). Unlike the viscoelastic gluten formed when wheat is hydrated and worked, kafirin does not normally exhibit viscoelastic properties. It has been suggested that the non-participation of kafirins in viscoelastic dough formation could be due to their hydrophobic nature which limits hydration and prevents plasticization (Oom *et al.*, 2008). This hydrophobic nature of kafirin may also be related to its mainly α-helical structure (reviewed by Belton *et al*., 2006), in contrast to gluten which has a high proportion of β-sheet and β-turn structure (Belton, 1999). The sharp contrast between the properties of gluten and kafirin proteins makes the contribution of the latter to the quality of biscuits another interesting focus of research.

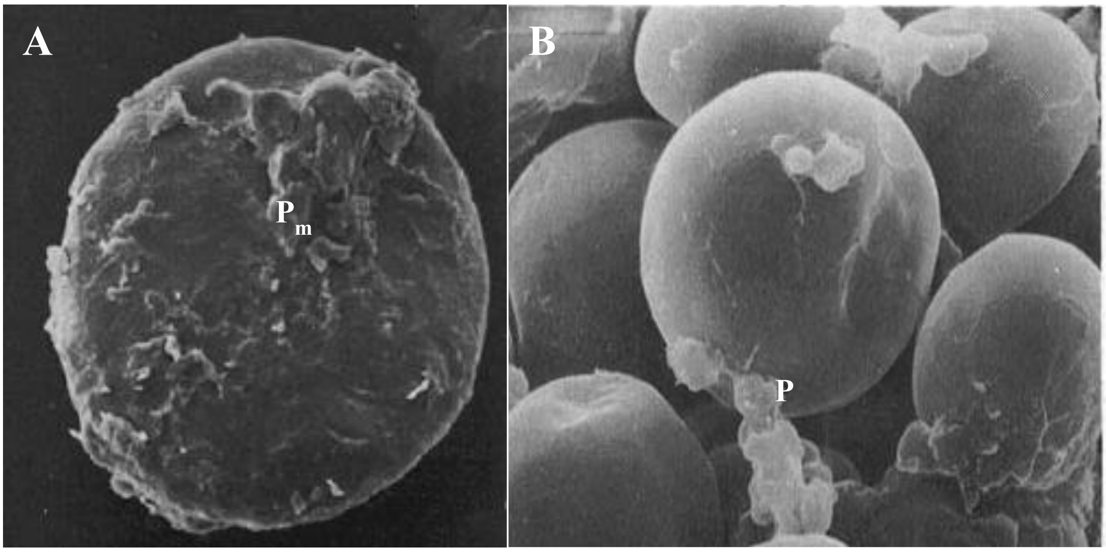

Figure 2.4: Scanning electron micrographs showing attachment of protein matrix (P_m) and protein bodies (P) to wheat starch (A) (Barlow *et al*., 1973), and sorghum starch (B) (Hoseney *et al*., 1974).

2.2.3 Lipids

Lipids are distributed throughout the wheat kernel with the germ having the highest concentration (Delcour and Hoseney, 2010). They can be classified based on their association with the starchy endosperm into starch lipids and non-starch lipids (Hoseney, 1994). Non-starch lipids comprise about 75% of total flour lipids and consist predominantly of triacylglycerols, as well as other non-polar lipids, and digalactosyl diacylglycerols. Starch lipids are generally polar with the major constituents being lysophospholipids especially lysophosphatidylcholine (lysolecithin) (Van der Borght *et al*., 2005). Although generally regarded as minor constituent of wheat flour, they significantly affect the quality of bakery products such as bread and biscuits. This can be traced to the effect they have on dough properties. Papantoniou *et al.* (2003) reported that biscuit dough made from defatted wheat flour had high viscoelasticity which was characterized by high storage (G') and loss (G") moduli. They further reported that biscuits made from such flour were flatter, denser, harder, and showed collapse of gas cells during baking when compared to biscuits from non-defatted wheat flour. MacRitchie (1977) stated that flour lipids are able to stabilize or destabilize gas cells during expansion by acting as a surfactant.

Sorghum contains between 2.1 and 5.0% lipids (Delcour and Hoseney, 2010). The total lipids are 90% non-polar lipids, 6% glycolipids and 4% phospholipids. About 75% of the lipids are in the germ, with the remainder split almost evenly between the bran and the endosperm. The importance of wheat lipids in the quality of wheat products appear to suggest an important role for sorghum lipids in baked sorghum products. However, previous studies report otherwise. Badi and Hoseney (1976) found that replacing wheat lipids with sorghum lipids in wheat biscuits through fractionation and reconstitution resulted in biscuits of poor quality. They also found that adding wheat lipids to non-defatted sorghum flour gave similar results to when wheat lipids was added to defatted sorghum flour. This suggest that the sorghum lipids were not functional in sorghum biscuits. They stated that the difference observed was due to wheat lipids containing certain components that are missing in sorghum lipids. Glover *et al*. (1986) also found that sorghum lipids did not display functionality in cakes and suggested it was due to their lower concentration of glycolipids in comparison to wheat lipids. Delcour and Hoseney (2010) reported glycolipids content of 20% and 6% for wheat and sorghum, respectively. It could therefore be important to choose the right lipid source to compensate for the non-functionality of endogenous sorghum lipids in order to improve the quality of sorghum biscuits.

2.2.4 Non-starch polysaccharides (NSP)

Wheat NSP are composed of arabinoxylans (AX) (Fig. 2.5), cellulose and arabinogalactan-peptides (AGP) which are present in the cell walls of the endosperm and bran tissues (Van der Borght *et al*., 2005). The endosperm NSP are predominantly AX, which are the most important of the wheat NSP (Pareyt and Delcour, 2008). AX constitute about 1.5-2.5% of the NSP present in wheat flour, and are either water extractable (WE-AX, 0.5%) or water unextractable (WU-AX, 1.5%) (Van der Borght *et al*., 2005). AX is made up of β-1,4-linked D-xylopyranosyl residues, substituted at the C(O)-3 and/or the C(O)-2 position with monomeric α-L-arabinofuranoside (Delcour and Hoseney, 2010). AX have the capacity to greatly influence the properties of wheat dough (Courtin and Delcour, 2002) and as such will affect the quality of wheat biscuit. Although the WU-AX are unable to directly affect viscosity by forming part of the aqueous phase of the dough, they influence the distribution and entrapment of water (Courtin *et al*., 1999). On the other hand, WE-AX are able to increase dough viscosity. Pareyt and Delcour (2008) were of the opinion that neither the WU-AX nor the WE-AX are beneficial in biscuit making due to their effect of increasing dough viscosity and reducing the spread of biscuit dough. This was in agreement with the findings of Kaldy *et al*. (1991) that higher levels of NSP were associated with smaller biscuit diameter.

Verbruggen *et al.* (1993) found that in sorghum, NSP constitute 5% of whole grain flour. The NSP are mostly water-insoluble glucuronoarabinoxylans (GAX) (Fig. 2.5c) which constitute more than 50% of NSP and β–D glucans (Verbruggen *et al.*, 1995). GAX has a backbone of 1,4 β-D xylopyranose substituted with single unit of arabinofuranose at C(O)-3 or at both C(O)-2 and C(O)-3 of certain xylose units (Verbruggen *et al.*, 1998). However, unlike wheat arabinoxylan, sorghum GAX is highly substituted with glucuronic acid residue (Verbruggen *et al.*, 1995). The insolubility of GAX suggests it may also be unable to affect viscosity of sorghum dough, similar to the WU-AX in wheat dough. Taylor and Emmambux (2010) suggested that they are unlikely to act as hydrocolloids since they are insoluble.

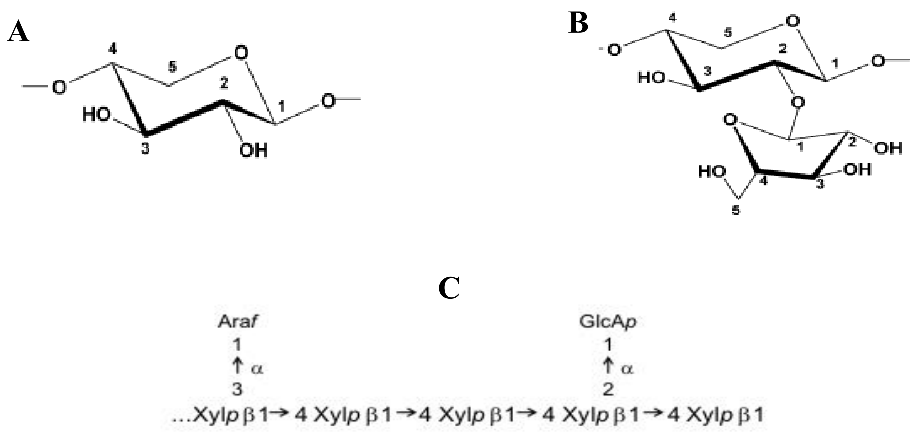

Figure 2.5: Structures of arabinoxylans. (A) non-substituted D-xylopyranosyl residue, (B) D-xylopyranosyl residue substituted at C(O)-2 with an L-arabinofuranosyl residue (Goesaert et al., 2005), (C) Structure of glucuronoarabinoxylan.

2.3 Science and technology of biscuit making

Biscuits are made primarily from flour, sugar, fat, salt, leavening agent, and water (Maache-Rezzoug *et al.*, 1998). Differences in the proportion of these ingredients allow for the production of products with varied textures and shapes. The production of biscuits is usually accompanied by a series of biochemical and physicochemical reactions which include almost complete water evaporation, protein denaturation, starch damage, browning inducing Maillard reactions, and dough expansion through gas production (Chevallier *et al.*, 2000b).

2.3.1 Classification of different types of biscuits

Biscuits have been classified in several ways using various criteria. Manley (2000) grouped biscuits according to their texture and hardness, method of forming the dough and dough piece, and also enrichment of the formulation with fat and sugar. Based on the method of forming the dough, biscuits were classified as fermented, developed, cut, laminated, moulded and extruded among others. However, from a food technologist's perspective, Manley (2000) stated that it is useful to categorize biscuits based on their external and internal appearance in order to have a good grasp of their recipe formulation and baking properties. For example, in wheat biscuits where gluten has developed due to sufficient hydration, a continuous matrix is observed, while those containing higher fat and sugar are more crumbly with an open structure. Although numerous types of biscuit have been described based on their composition, in this review, a simplified classification based on gluten development is followed.

2.3.1.1 Short dough biscuits

Manley (2000) described this type of biscuits as made from dough that lacks extensibility and elasticity. Their formulation consists of high quantities of fat and sugar which makes the biscuit dough plastic and cohesive but with minimal formation of a gluten network. Examples of this type of biscuit are Shortcake and Digestive biscuits (Lawson, 1994). Manley (2000) stated that the overall structure of this type of biscuit is a mixture of protein, starch and sugar glass with the protein existing as a non-continuous matrix and the fat present in form of large globules between the starch-protein matrixes.

2.3.1.2 Semi-sweet and hard-sweet biscuits

This type of biscuits contain a gluten network, but as the amount of sugar and fat are increased, the gluten network becomes less elastic and more extensible (Manley, 2000). The hard-sweet biscuit type include Cabin biscuits, Rich Tea and Marie biscuits. While one may think the presence of gluten in this type of biscuit is solely responsible for their hardness, there appears to be more to the hardness of wheat biscuits than just the presence or absence of gluten. With the semi-sweet biscuits, a low sugar level and high flour protein content produce biscuits with a harder texture. However, when the sugar level was increased, the texture became more tender and fragile (Manley, 2000). While this classification may further highlight the contribution of protein to the hard texture of biscuits, it further deepens the argument as to the role of sugar in biscuits. Manley (2000) stated that biscuits rich in sugar may be hard due to the effect of super-cooled sugar glass. Manohar and Rao (1997) found that increasing sugar level reduced the development of gluten by reducing elastic recovery. Despite this, these authors found an increase in biscuit breaking strength when sugar level was increased, thus suggesting a harder biscuit. It therefore appears the contribution of gluten to the hardness of wheat biscuits depends on its level of development which in turn is dependent on the level of fat and sugar present. More specifically, the hardness of wheat biscuits appears to be variously dependent on protein interactions or gluten development (influenced by level of fat) and the level of sugar used.

2.3.2 The biscuit making process

As stated, biscuits are made primarily from flour, sugar, fat, salt, leavening agent and water. Usually, these components are all first mixed together into a dough (Manohar and Rao, 2002). Mixing is the initial step in the biscuit making process and it allows for the aggregation of flour, water and other ingredients so they form a coherent mass (dough) with the application of mechanical energy (Maache-Rezzoug *et al.*, 1998).

During dough mixing, the levels of each ingredient and mixing conditions are carefully chosen and tailored to meet the desired dough consistency. As stated, gluten is developed in certain doughs while its development is minimized in others. The gluten network causes the dough to be hard and stiff, while with minimal gluten development the dough is short and soft (Wade, 1988). After forming the dough, it is processed into smaller dough pieces using a combination of sheeting, gauging and cutting (Manley, 2000). Dough pieces are then baked in an oven at temperature of about 200°C to drive off moisture (Wade, 1988) and transform the dough into a solid matrix which is cooled and then develops a final characteristic texture (Pareyt and Delcour, 2008). Curley and Hoseney (1984) attributed the development of texture and crispness in biscuit to the slow crystallization of sucrose from the concentrated sugar syrup formed during the earlier stage of baking. Texture development is, however, accompanied by a series of other reactions such as Maillard reaction and gas expansion which all combine to make biscuit the acceptable and palatable product (Manley, 2000).

2.3.3 Changes in dough during biscuit production

Most of the physicochemical changes occurring during biscuit making take place during baking. However, the changes occurring during dough formation cannot be underestimated.

Dough mixing allows for the production of a uniform product and also incorporates air into the dough (Delcour and Hoseney, 2010). The composition of the dough also influences the extent to which certain reactions occur. For instance, Delcour and Hoseney (2010) stated that biscuit formulations with low sugar and/or high water content may have some starch gelatinization.

The level of fat used also affects gluten development (Manley, 2000). Similarly, overworking the wheat dough during mixing leads to strong gluten formation and hard biscuit texture. Manohar and Rao (2002) stated that the rheology of wheat dough influences the machinability and quality of biscuits. Manley (2000) added that the consistency of the dough influences the quality of biscuits. Water absorption by starch, partial hydration of proteins and partial dissolution of sugar are some of the reactions that occur during dough formation (Pareyt and Delcour, 2008).

Manley (2000) categorized the changes occurring during biscuit baking into three groups: development of structure, reduction of moisture and change of surface coloration. It is important to note that these changes do not occur in sequence but overlap during the baking process (Wade, 1988). The development of structure is characterized by changes such as starch granule swelling and gelatinization, protein denaturation, setting, production of gases by the leavening agent, expansion of gas bubbles, rupture and coalescence of some of the bubbles (Manley, 2000). Wade (1988) explained that increase in the volume of the dough during baking is due to the action of the leavening agent and steam produced from the dough moisture.

As the biscuit temperature rises in the baking oven (Fig 2.6), a moisture gradient is created as moisture is continually lost from the surface of the dough, while the centre remains moist. Moisture from the centre then migrates to the surface through capillary action and diffusion, and is subsequently evaporated (Manley, 2000). The initial moisture content of the dough ranges from about 11-30%, while for the freshly-baked biscuit, it is within the range of 1-5%, depending on the type of product (Wade, 1988). A combination of Maillard reactions and caramelization of sugar are responsible for the colour changes that take place during the baking of biscuits (Manley, 2000).

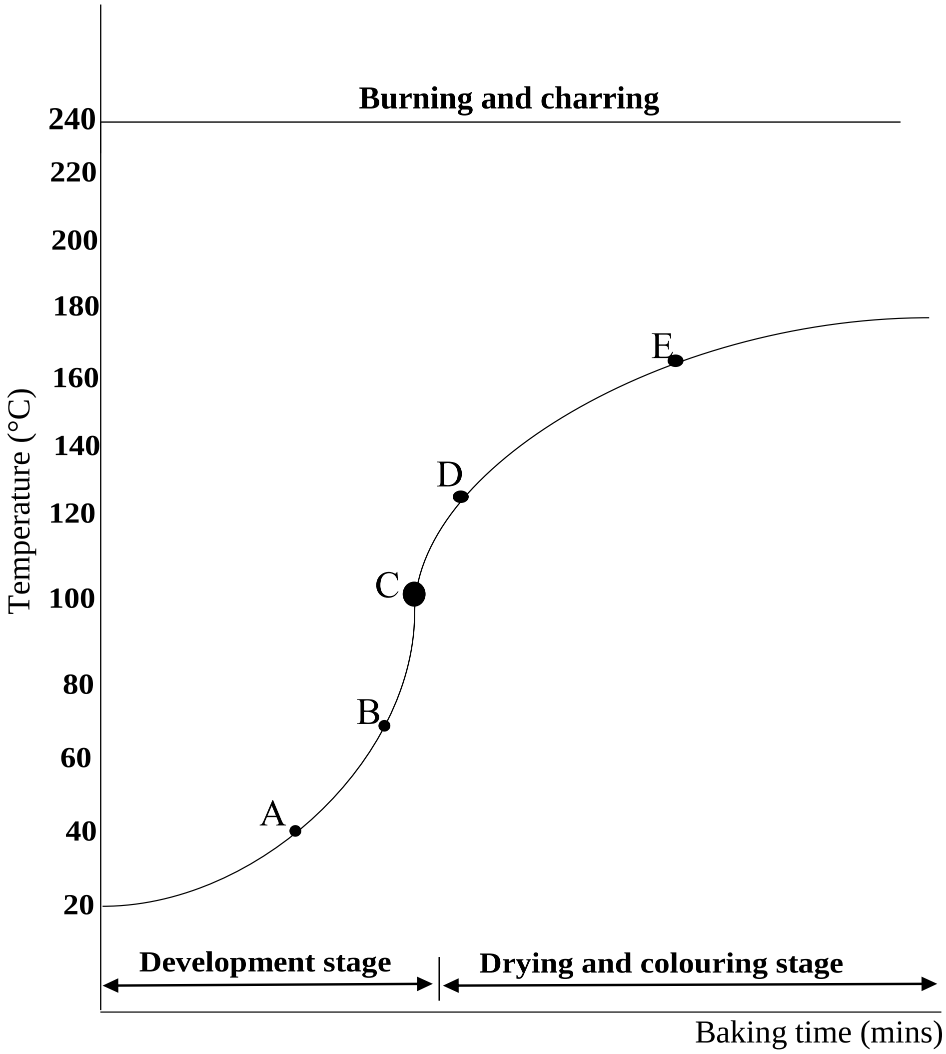

Figure 2.6: Changes occurring during baking of biscuits (adapted from Manley, 2000). (A) Fat melting, (B) Gluten coagulation and starch gelatinization, (C) Drying starts and gas production stops, (D) Caramelization starts, (E) Formation of brown dextrin.

2.4 Role of the different chemical components in biscuits

2.4.1 Starch

As stated, the starch content of wheat biscuit flour and the nature of the starch granules (damaged or undamaged) affects their functionality in biscuit making (Wade, 1988). The starch granules are generally regarded as fillers because they are not disrupted to a point where they form a continuous starch gel. Hoseney and Rogers (1994) stated that the lower level of damaged starch in soft wheat flour when compared to hard wheat flour is one of the reasons why biscuits are preferentially made from soft wheat flour. These authors added that for reasons unclear, soft wheat flour produces more tender biscuits than hard wheat flour. They, however, stated that due to the high level of water absorption of damaged starch in hard wheat flours, the spread of the biscuit dough is reduced during baking. It could be suggested that due to the higher level of water absorption due to more damaged starch in hard wheat flours, their doughs have to be baked for a longer time, thus rendering the biscuit harder and less palatable. In addition, the higher level of water absorption of hard wheat flour suggests that more water will be required to achieve the desired dough consistency. This would also likely increase the tendency of high gluten formation during dough mixing. Gluten formation is known to impact on the hardness of wheat biscuits (Manley, 2000). The higher level of starch damage in hard wheat flour is due to the higher force required to crush the kernel due to its hardness (Delcour *et al.* 2010). The hardness of the kernel itself depends on the strength of the starch-protein interactions in the wheat endosperm. Miller and Hoseney (1997) reported that damaged starch increases dough viscosity by absorbing larger amount of water, while soluble starch increases the viscosity of the aqueous phase.

Pareyt and Delcour (2008) who reviewed the role of starch in biscuits instead maintained that most of the starch granules do not gelatinize. Wade (1988) found that starch granules that swell to the point where loss of birefringence occurred maintained their integrity and none of the biscuits studied contained starch granules disrupted to a point where they formed a continuous starch gel. The non-gelatinization of starch in biscuits has been attributed to the high levels of sugar normally used (Chevallier *et al.*, 2000a). Delcour and Hoseney (2010) also stated that it is due to insufficient water. However, they maintained that biscuits containing high water levels and low sugar levels may show some starch gelatinization. This suggests that sorghum biscuits may have some starch gelatinization since sorghum requires more water to form a dough than wheat (Serrem *et al*., 2011).

As already noted, biscuit dough spread is affected by the level of starch damage. Although no specific mechanism has been given for this difference, it is expected that the difference in the level of water absorption arising from different levels of starch damage would play a major role, possibly via viscosity. Hoseney and Rogers (1994) stated that undamaged starch granules absorb about 30% of their weight, while damaged granules absorb 10 times that amount. However, because starch is not the only hydrophilic component in biscuits, increased starch hydration may not be solely responsible for the difference in spread.

Abboud *et al.* (1985), although emphasising proteins, related the spread of wheat biscuit dough to its viscosity. Gaines and Finney (1989) stated that soft wheat dough spreads faster than hard wheat dough because the former is less viscous during baking. Miller and Hoseney (1997) reported that a combination of increased dough viscosity due to damaged starch and an increase in viscosity of the aqueous phase due to soluble starch may explain the difference between the spread of biscuit dough from hard and soft wheat. However, this did not explain differences in the spread of biscuit doughs made from various hard and soft wheat flours, respectively. They stated that the protein content of the flour also appears to affect the spread of the biscuit dough. Donelson and Gaines (1998) could not find a correlation between biscuit dough spread and starch damage. It would therefore appear that biscuit dough spread is related to an interplay of factors contributing to dough viscosity such as starch, protein, NSP, water and temperature.

2.4.2 Protein

The quantity and quality of proteins present in wheat flour have a major influence on the rheological properties of biscuit dough (Maache-Rezzoug *et al.*, 1998). Pareyt and Delcour (2008) reviewed that proteins have a major influence on biscuit quality, particularly diameter. Souza *et al.* (1994) stated that the quality of wheat biscuits is affected more by the level of proteins in the flour than the composition of the proteins. This was later corroborated by Igrejas *et al*. (2002). They found that alleles encoding different glutenin and gliadin loci in 150 lines of a soft wheat variety did not result in any significant difference in the spread of biscuit dough. They concluded that total protein content is more important for biscuit quality than the composition of the protein. Typical soft wheat biscuit flour has a protein content in the range 7-9% (N × 5.7) (Wade, 1988). Flour from hard wheat has a higher protein content, 10-14% (Manley, 2000). This further relates to why soft and hard wheat flours produce biscuits of different quality, in addition to their different levels of starch damage.

While insufficient water has been reported to prevent starch gelatinization (Delcour and Hoseney, 2010), it also prevents an extensive gluten network from forming (Gaines, 1990). The presence of interfering components such as sugar and fat also prevents aggregation of the proteins. However, the actual nature of the gluten in biscuits remains a subject of debate. This is as a consequence of differences in formulations and subsequent microstructure of the different types of biscuits studied. While Gaines (1990) found an extensive gluten network in sugar-snap biscuits, Slade *et al*. (1993) stated that these biscuits contain undeveloped gluten. Hoseney and Rogers (1994) stated that protein is the continuous phase in wheat biscuits. In fact, they attributed the hardness of biscuits to the rigid protein-starch structure. Doescher *et al.* (1987) proposed that when gluten undergoes a glass transition during baking, it expands to form a continuous matrix. Gaines (1990) considered that expansion of gas cells during baking probably allows the protein molecules to come within distances sufficiently small enough for them to associate. The research of these authors appears to provide a strong argument for the formation of certain level of gluten in biscuits.

There are two theories as to why biscuit dough stops spreading during baking. Doescher *et al.* (1987) and Miller *et al.* (1996) stated that the setting of the biscuit is determined by the gluten protein undergoing an apparent glass transition during baking. Doescher *et al.* (1987) stated that the mobility gained by the gluten during heating allows it to swell and form a continuous network which reduces water mobility and increases dough viscosity, thus allowing the biscuit to set. Further, Miller *et al.* (1996) stated that the viscosity of the continuous gluten network is sufficient to stop the spreading of the biscuit dough. A common theme is viscosity. On the other hand, Slade *et al.* (1989) suggested that biscuit dough does not set but that the proteins exhibit viscous expansion (expansion controlled by dough viscosity) and creep followed by a structural collapse. Viscosity also features in this alternative mechanism. It thus appears the main factor controlling the spread of biscuit dough is its viscosity, which is in turn influenced by dough composition. Pareyt and Delcour (2008) in their review harmonized the two theories and stated that the "apparent" glass transition the gluten undergoes allows for physical entanglement of gluten molecules in the final product, while structural collapse, spread or creep can be regarded as macroscopic observations. Pareyt *et al.* (2008) using different gluten-starch blends concluded that dough setting was not determined by an "apparent" glass transition because set time and temperature did not depend on the level of gluten. In fact, they found that the greatest dough spread was obtained when no gluten was added, although the structure was unacceptable. As expected, the diameter of the biscuits reduced as the level of gluten was increased. This is because the dough becomes stronger as gluten level is increased thereby resulting in a lesser spread of the biscuit dough. Their results also showed that the final diameter of biscuits is dependent on the spread onset time which itself depends on the amount of water available to the non-gluten component of the biscuit.

Furthermore, the work of Kaldy *et al*. (1993) and later Maache-Rezzoug *et al.* (1998) which revealed that an increase in gluten level reduced the spread of wheat biscuit dough further indicates that the role of proteins in biscuits is dependent on other factors such as component interaction. One would expect an increase in gluten level to increase the spread of biscuit dough if indeed the gluten was the component responsible for the final diameter of biscuit. Donelson (1988) also omitted the gluten fraction from the biscuit formulation using a reconstitution technique and reported that gluten was not important for the baking performance of biscuits.

However, because sensory profiling was not done to determine the acceptability of these types of biscuits, the question still remains as to whether biscuits of acceptable eating quality can be made in the absence of gluten? The use of sorghum flour which does not contain proteins that form gluten like wheat should help provide some answers to this question as clearly there is still much to learn and understand about the role of gluten in wheat biscuits.

2.4.3 Sugar

The functionality of sugar in biscuits is also of interest due to its numerous functions, as reviewed by Pareyt and Delcour (2008). They stated the important properties of sugar includes: solubility, hygroscopicity, particle size distribution and viscosity. Sugar (sucrose) also delivers sweetness and influences the structural and textural properties of biscuits (Pareyt *et al*., 2009). Chan (2006) stated that it acts as a tenderizing ingredient contributing to crispiness and providing crust colour, while Pareyt and Delcour (2008) proposed that it contributes to biscuit dough spread. Chevallier *et al.* (2000a) stated that sugar is responsible for the cohesiveness of biscuit structure, while Doescher and Hoseney (1985) stated that the ability of sucrose to recrystallize during baking appears to be responsible for the surface cracking of biscuits.

The contribution of sugar to biscuit structure commences at the dough making stage where it affects viscosity. Macche-Rezzoug *et al.* (1998) found that an increase in sugar content decreased the viscosity of wheat dough with the texture going from firm to extremely soft. This was probably due to sugar dissolving and creating additional liquid volume which lowers viscosity. Hoseney and Rogers (1994) stated that each gram of sucrose dissolves in water to create an additional liquid volume of 0.66 ml. Although there is insufficient water to dissolve all the added sugar during dough making (Manley, 2000), the undissolved sugar subsequently dissolves during baking and the dough spreads (Hoseney and Rogers, 1994). Lai and Lin (2006) related sugar dissolving during baking to the fat surrounding creamed sugar melting, thereby allowing available water to migrate to the sugar and dissolving it. Presumably, this would occur before all the water evaporates from the biscuit. However, this theory may need to be extended to accommodate possible cases of non-solubilisation of all the sugar before the end of baking. Raemy and Schweizer (1983) found onset and peak melting temperatures of 160°C and 185°C for sucrose, respectively. It is possible undissolved sucrose will melt during baking if a high enough temperature (190°C) is reached. A question is therefore if the presence of any undissolved/un-melted sugar will affect the final quality of the biscuit? Hoseney and Rogers

(1994) described undissolved sugar crystals as free flowing and not having any properties that suggest they would contribute to the hardness or crispness of biscuits.

Unlike the case of gluten, there appears to be a consensus on the state of sugar in biscuits. Manley (2000) described the structure of biscuits as comprising of sugar glass and Slade *et al.* (1993) described it as comprising of a continuous glassy sucrose matrix. Chevallier *et al.* (2000a) stated that sugar melts during baking and becomes glassy in biscuits on cooling. The sugar glass could form a link between starch and protein (Chevallier *et al.* 2000a) or even a continuous phase embedding starch granules, protein and fat (Slade *et al*. 1993; Chevallier *et al*. 2000a). The importance of glass transition in biscuits underlines why they lose crispness and become flexible when left under moist condition. Delcour and Hoseney (2010) stated that a material left at constant temperature with increased moisture content can change from a glassy to leathery or rubbery state.

Sugar has also been reported to contribute to the non-gelatinization of starch in biscuits especially at high sugar levels (Chevallier *et al.*, 2000a). Related to this, it was reported to increase the gelatinization temperature of starch (Spies and Hoseney, 1982). These authors found that increasing the sucrose concentration in sugar-flour water suspensions increased the gelatinization temperature of starch. Different mechanisms have been used to explain how sugar increases the gelatinization temperature of starch. Spies and Hoseney (1982) proposed that sugar lowers the water activity in a starch-water system and thus decreases the chemical potential of water. Reactions involving the water then require more energy than they would in pure water and this results in a higher starch gelatinization temperature. They proposed that sugar interacts with the starch chain, thereby stabilizing its amorphous region and thus, higher energy is required for gelatinization. Assifaoui *et al.* (2006) using ^{1}H NMR to study water mobility in biscuit dough concluded that the preference of water for sugar in a sugar-starch mixture causes a reduction in the level of intra-granular water in starch granules.

Slade and Levine (1988) used a polymer science approach to explain the effect of sugar on starch gelatinization. They stated that because starch gelatinization occurs at a higher temperature in sugar solution than in water alone suggests anti-plasticization by the sugar-water solution relative to plasticization by water alone. Johnson *et al.* (1990) hypothesized that the increase in gelatinization temperature is not simply about preferential hydration of either starch or sugar. They suggested that dissolution of sugar in water creates a solution with solvent properties different to those of ordinary water. They also showed that even though sucrose increases the gelatinization temperature of starch, the endotherm also became narrower as sugar level was increased. This is in agreement with the work of Ghiasi *et al*. (1983). These authors concluded that even though sugar may bind water at ambient temperature, it gradually loses this binding ability as temperature is increased and water then become available for starch gelatinization. This reasoning was used to explain the narrowing of the gelatinization endotherm at higher sugar level which is similar to the effect of additional water in calorimetric study.

Chiotelli *et al*. (2000) summarized these various mechanisms and stated that the effect of sugar on starch gelatinization is due to an interplay of factors such as lowering of water activity, increase in the volume of sugar-water co-solvent, anti-plasticization by sugar, stabilization of the starch granular structure due to starch-sucrose interactions. Pareyt and Delcour (2008) in their review also added that displacement of water by sugar increases the gelatinization temperature of starch. Clearly, sugar is far more than just a sweetener in biscuits and its role in their texture cannot be underestimated.

2.4.4 Fat

Fats and oils are generally regarded as the third largest component after flour and sugar (Manley, 2000). Fats (lipids that are solid at ambient temperature) are largely used in biscuit making, while oil (liquid at ambient temperature) is used to a lesser extent. Wheat biscuit doughs made using oil have inferior structure during baking (Manley, 2000). Lai and Lin (2006) stated that when the melted fat solidifies, it takes one of the three main crystal forms: alpha (α), beta (β) or beta prime (β'). The β form is the most stable but the β' is generally preferred due to its smoothness and better creaming properties. Selecting the right fat and quantity is therefore important for the quality of biscuits.

The functions of fat in biscuits as reviewed by Pareyt and Delcour (2008) can be briefly summarized to include imparting shortening, improving flavour and mouthfeel. Shortening refers to the ability of fat to lubricate, weaken or shorten the structure of food components so they function in specific manner capable of imparting desirable textural properties (Ghotra *et al.*, 2002). In the absence of fat, the proteins will hydrate and an extensive gluten network will develop. The dough is therefore tougher and the biscuits harder (Manley, 2000). Fat contributes to the spread of biscuit dough during baking (Maache-Rezzoug *et al*., 1998), and influences the structural integrity and shelf-life of the biscuits (Ghotra *et al.*, 2002). The contribution of fat to dough spread is also likely to be due to reduction in viscosity, as earlier noted with respect to protein and sugar. Pareyt *et al.* (2009) reported that the diameter of wheat biscuits increased as the level of fat in the formulation was increased. They stated this was likely to be due to increased mobility in the system when fat melted as there was more oil phase in the biscuit. Given (1994) stated that fat also serves as solvent and vehicle for hydrophobic flavour and aroma compounds released during baking or added during formulation. This author further stated that it has the ability to stabilize air cells and contribute to desirable textural qualities.

Pareyt and Delcour (2008) reviewed that fat competes with water (aqueous phase) for the flour particles surface during dough mixing and it is the main ingredient binding the other ingredients in the biscuit dough. This is logical considering the low quantity of water normally used in forming a biscuit dough and the ability of fat molecules to aggregate. Ghotra *et al*. (2002) stated that fat surrounds the protein molecules and starch granules, isolating them, thereby breaking the continuity of the protein-starch structure. The dough produced is therefore less elastic and hence desirable for biscuit making (Maache-Rezzoug *et al*., 1998). This phenomenon is said to result in biscuits which are less hard, shorter and easily melt in the mouth (Manley, 2000). Wade (1988) stated that doughs containing up to 20% fat have a developed gluten structure and are usually extensible, while at higher fat levels, little or no gluten network is formed. Manohar and Rao (1997) found that the hardness of biscuits decreased as the level of fat was increased. The decrease in hardness was accompanied by a lower level of gluten development, as indicated by a decrease in elastic recovery from 0.52 mm to 0.38 mm. This further strengthens the concept that the level of gluten development influences the hardness of biscuits. Manley (2000) stated that if the quantity of fat used is high, there is sufficient lubrication of the dough such that little water, if any, is required to achieve the desirable dough consistency.

2.4.5 Water

Water is added to make biscuit dough formable and change the characteristics of other ingredients but it is almost completely removed during baking (Manley, 2000). As stated, the moisture content of a biscuit dough ranges from 11-30% prior to baking, while the freshly baked biscuit has a final moisture within the range of 1-5% (Wade, 1988). Water is essential for the solubilisation of some ingredients, in particular sugar, salt and various leavening agents (Maache-Rezzoug *et al*., 1998). It is also important for the hydration of proteins, starch and NSP. Manley (2000) stated that water aids the dispersion of fat and other ingredients through the dough. Furthermore, it provides lubrication to the biscuit dough and also affects texture of biscuits (Wade, 1988).

Another attribute usually affected by moisture is dough viscosity. Hoseney and Rogers (1994) stated that the dough viscosity is determined by competition for available water. They stated that as more water is held by the insoluble components, less is available to dissolve the sugar, which would result in higher dough viscosity and slower spread rate of the biscuit dough. This further strengthens the proposition that biscuit dough spread is mainly controlled by dough viscosity. Moisture also affects the surface cracking pattern of biscuits (Doescher and Hoseney, 1985). These authors found that a high flour moisture content increased the degree of symmetry in wheat biscuits. They also found that the surface cracking of biscuits reduced with increasing flour moisture content.

Water also affects texture by plasticizing and softening the starch-protein matrix, thereby altering mechanical properties (Katz and Labuza, 1981). Plasticization of hydrophilic food materials by water decreases the T_g (Levine and Slade, 1989), which in turn determines the conformation and state of the biopolymers in food. It is also reported that water could have anti-plasticizing effect such as hardening and toughening on food matrix, as reviewed by Pittia and Sacchetti (2008). Even though water is used in small quantity in biscuit dough preparation, it clearly plays an important role in the final quality of biscuit.

2.4.6 Other ingredients

The other ingredients used in biscuit making are generally considered as minor ingredients. Although used in small quantities (< 2% on flour basis), they have marked effects on the properties of the dough and the biscuits (Wade, 1988). The other ingredients include: leavening agents, salt (sodium chloride), dairy products, flavours and colours, enzymes and emulsifiers (Manley, 2000).

Leavening agents such as sodium bicarbonate ($NaHCO_3$) and ammonium bicarbonate (NH_4HCO_3) are the most important. They produce gases, CO_2 and NH_3, (Fig. 2.7) which form the nuclei for the textural development within a biscuit (Manley, 2000). While NH_4HCO_3 decomposes when heated, $NaHCO_3$ will react with any acidic material in the presence moisture. Apart from acting as leavening agents, they also help control the pH of dough and biscuit

(Wade, 1988). Salt is added for its flavour and in recipes where appreciable gluten development occurs, it is added to toughen the gluten and make dough less sticky (Manley, 2000).

$$NaHCO3 \xrightarrow{heat} NaHCO_3 + H_2O + CO_2$$

$$NH_3 + H_2O + CO_2 \xrightarrow{heat} NH_4HCO_3$$

Figure 2.7: Production of carbon dioxide from leavening agents (Wade, 1988).

Other ingredients such as emulsifiers are also used to enhance the quality of biscuits. Emulsifiers help reduce the hardness of biscuits by interrupting the gluten structure in the dough (Manley, 2000). They allow for uniform spreading of the fat phase such that it forms interface with the hydrophilic component of the dough. When emulsifiers are used, the amount of fat required can be reduced. Manohar and Rao (1999a) working with different emulsifiers concluded that the use of emulsifiers in biscuits has beneficial effects as they produce softer dough with improved machinability and biscuits with better textural characteristics.

2.5 Similarities between the texture of wheat and sorghum biscuits

Serrem *et al*. (2011) using descriptive sensory panel found that sorghum biscuits were associated with a crispy and dry texture. Omoba *et al*. (2015) using the same test found that such sorghum biscuits were indistinguishable from whole wheat biscuits in terms of hardness, roughness and coarseness. However, they were denser, less dry and less crispy than the wheat biscuits. While crispness was suggested to be due to the absence of gluten, dry texture was said could be due to the poor water absorption of sorghum dough. As explained, the endosperm kafirin proteins of sorghum are hydrophobic (Duodu *et al*., 2003). While the impact of hydrophobic kafirins on the dry texture of sorghum biscuit seems logical, the absence of gluten appears insufficient to solely explain the crispness of sorghum biscuits. The contribution of sugar to crispiness is well noted (Maache-Rezzoug *et al.*, 1998). The starch-protein matrix is also reported to contribute to biscuit crispness (Hoseney and Rogers, 1994).

Dovi (2013) found that sorghum biscuits made from white sorghum had similar hardness to standard wheat biscuits when baking margarine was replaced with sunflower oil. Sorghum biscuits made with baking margarine were extremely soft with an open structure. The weakness of the biscuit structure was attributed to the margarine entrapping air bubbles during creaming thereby giving sorghum biscuit an open structure and soft texture. This further indicates that just like in wheat biscuits, ingredient selection also influences the texture of sorghum biscuits. How the different components of sorghum flour and dough actually contribute to the development of biscuit texture is not understood.

2.6 Analytical techniques for determining the causes of biscuit texture

The texture of biscuits, as noted, is dependent on the state and interaction between the various components of the biscuit. To understand how these components influence texture, a combination of instrumental and microscopic techniques have been used. Other techniques such as thermal analysis, digital image analysis and time lapse photography have also been used. The principles of some of the more important analytical techniques and their previous application is discussed.

2.6.1 Polarized Light Microscopy

A polarizing light microscope uses two polarizers (one between light source and specimen, and the other between the objective and the observer) to produce and transmit plane-polarized light which is impinged upon the specimen (Kaláb *et al*., 1995). Rotating the second polarizer such that transmitted vibration is at right angle to the vibration of the incident light causes crystalline or ordered region in a specimen to appear very bright, while the amorphous region will appear dark. Due to the birefringence of native cereal starches, a characteristic birefringent figure known as a “Maltese cross” is observed when starch is studied (Faridi and Faubion, 1994). This phenomenon makes it possible to determine if starch gelatinized during biscuit baking. As gelatinization proceeds, the starch granules lose their native structure and hence their birefringence (Ghiasi *et al*., 1982). Mamat *et al*. (2010) used polarizing light microscopy to determine the nature of starch granules in semi-sweet biscuits and reported that some of the starch granules in the biscuit were birefringent. The crystalline nature of sucrose (Roos, 1993), and amorphous nature of sugar glass (Chevallier *et al*., 2002a) make it possible to also analyse and distinguish them using this technique. Polarizing light microscopy will therefore be used to investigate the interaction of sugar with starch granules in sorghum biscuits.

2.6.2 Differential Scanning Calorimetry (DSC)

DSC is a technique used to study thermally induced transitions and conformational transitions in macromolecules (Bruylant *et al.*, 2005). It measures how the physical properties of a sample change with temperature over time (reviewed by Gill *et al.,* 2010). The DSC measures the quantity of heat gained or lost by a sample on the basis of a temperature difference between the sample and a reference material. The sample is enclosed in a pan alongside an empty reference pan with both placed on a thermoelectric disk which is electrically heated. Due to the heat capacity (C_p) of the sample, a temperature differential is created between the sample pan and the empty reference pan.

As reviewed by Slade and Levine (1994), DSC has been used to study biscuit dough with respect to baking performance. They reported its use to investigate fat melting, water evaporation during baking, and the extent of sucrose dissolution, melting or recrystallization. Abboud and Hoseney (1984) have also used DSC to determine starch gelatinization in wheat biscuits. DSC will therefore prove beneficial in understudying the functionality of sorghum biscuits with respect to the thermal properties of its components.

2.6.3 Scanning and Transmission Electron Microscopy (SEM and TEM)

Both the SEM and TEM use an electron beam in place of visible light to analyse and obtain information about the microstructure of samples (Aguilera and Stanley, 1990). SEM uses low energy electron beam to scan a specimen and secondary electrons which are backscattered from the surface of the sample are collected by a detector (Faridi and Faubion, 1994). This is then processed to form an enlarged image (Kaláb et al. 1995). Prior to SEM, sample surfaces are coated with a very thin, electron-conductive layer of carbon (Faridi and Faubion, 1994). This is to prevent the charging phenomenon in which electrical charges build-up on the surface of the sample leading to deflection of the electron beam and distortion of image (Aguilera and Stanley, 1990). SEM has been used to investigate the nature of starch granules and the presence of a continuous protein network in wheat biscuits (Doescher *et al.*, 1987; Pareyt *et al.*, 2010).

Just like SEM, TEM uses a beam of electrons emitted from heating an electron gun (tungsten filament) to obtain structural information about a sample. But unlike in SEM where the electrons are backscattered, in TEM they pass through the sample (Aguilera and Stanley, 1990). For samples to withstand the high vacuum environment and strong electron beam, it must be dry, physically strong and extremely thin (Faridi and Faubion, 1994). Samples are prepared for analysis using a combination of fixing, washing, dehydration and embedding in resin before sectioning and cutting into thin slices (Aguilera and Stanley, 1990). TEM has, for example, been used for the analysis of protein structure in raw and cooked sorghum flour (Oria *et al*., 1995), while it appears it is not commonly used in the study of wheat flour and products. However, Bechtel *et al*. (1978) used TEM to study structural changes that take place during the different stages of breadmaking. Here, SEM and TEM will be used to investigate how starch and proteins contribute to the texture of biscuits.

2.7 Conclusions

Although sorghum has clear potential as an alternative to wheat in biscuit making, replicating the desirable eating qualities of wheat biscuit using non-wheat cereals like sorghum remain a challenge for food scientists. While there are uncertainties about what exactly is responsible for the texture of wheat biscuits, it is clear that gluten is an important component of the wheat biscuit structure and its role depends on the type of wheat biscuit being investigated. Even if not formed during dough making, there is evidence that some gluten is formed through protein molecular association during biscuit baking. It is also clear that in addition to gluten, other components of wheat flour such as lipids contribute to the desirable qualities of wheat biscuits. Sorghum endogenous lipids appear to be non-functional in sorghum biscuits, while the NSP are also not expected to play any role due to their highly branched nature which renders them insoluble.

The probable inability of sorghum kafirins to participate in dough formation due to their encapsulation in protein bodies and hydrophobicity suggests other components such as starch and sugar may be critical to development of texture in sorghum biscuits. Although starch is widely regarded as ungelatinized in wheat biscuits due to insufficient water being present, the higher amount of water required to form sorghum dough suggests that starch granules maybe gelatinized in sorghum biscuits. Also, because of the important contributions of sugar to the texture of wheat biscuits, it is thought that sugar will contribute greatly to the texture of sorghum biscuits in the absence of gluten. An understanding of how these various components interact to compensate for the absence of gluten in sorghum biscuits is therefore critical to understanding why they have similar texture to wheat biscuits, while also laying a foundation for future work on the development of high quality gluten-free biscuits.

3 HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

Hypothesis 1

Kafirins will remain entrapped in their protein bodies and will not contribute to the structure and hardness of sorghum biscuits.

Taylor *et al.* (1984) found that kafirins formed in the protein bodies of sorghum. Belton *et al.* (2006) reviewed that the kafirins are hydrophobic and exhibit extensive disulphide cross-linking. Oom *et al*. (2008) found that they do not participate in dough formation. These authors suggested that kafirins may be too hydrophobic and as such could not be hydrated by water to participate in dough formation. Furthermore, Hamaker and Bugusu (2003) stated that sorghum proteins must form structures with themselves or other constituents during processing in order to directly impact on the quality of sorghum-based foods. It is therefore unlikely these proteins will affect the texture of sorghum biscuits since they do not participate in dough formation.

Hypothesis 2

Increasing the level of water in making sorghum biscuits will lead to the gelatinization of sorghum starch during baking and consequently increase the hardness of sorghum biscuits.

Mamat *et al*. (2010) found that some starch granules remained intact in wheat biscuits, while others were either partially birefringent, non-birefringent with the spherical shape intact or disrupted with loss of granular integrity. They stated that starch granules were only partially gelatinized in the biscuits due to a lack of sufficient water as well as the presence of excess sugar. Similarly, Abboud and Hoseney (1984) found the presence of a starch gelatinization endotherm in both wheat biscuit dough and baked wheat biscuits when analysed by DSC. They reported that a comparison of the gelatinization endotherm of wheat dough and biscuits indicated that only a small portion of the starch was gelatinized during baking, possibly due to considerable loss of water during baking. Manley (2000) stated that biscuits containing ungelatinized starch are softer eating. However, there does not seem to be any direct experimental evidence on the effect of high water levels on starch gelatinization in biscuits and its resultant effect on biscuit hardness.

Hypothesis 3

Sugar (sucrose) will melt during baking of sorghum biscuits and on cooling form a continuous matrix of sugar glass, which will form networks with protein, starch and non-starch polysaccharides and subsequently increase the hardness and cohesiveness of sorghum biscuits.

Maache-Rezzoug *et al*. (1998) found that sugar increased the cohesiveness and crunchiness of wheat biscuits, while also acting as a hardener. Furthermore, Chevallier *et al*. (2000a) found that X-ray diffraction peaks of crystalline sugar were absent in the centre of wheat biscuit. They proposed that this was due to the presence of sugar as an amorphous glassy matrix resulting from the melting and cooling of crystalline sucrose. They further added that the cohesiveness of the biscuit structure could be due to the ability of sugar melt to form a bridge between starch and protein molecules or even a continuous phase of molten sugar embedding protein, starch granules and lipids. However, there seems to be no experimental evidence that sugar indeed form networks with starch or protein in wheat biscuits even though its occurrence as an amorphous glassy matrix appears to be true.

3.2 Objectives

Objective 1

To determine the state and role of sorghum proteins in the hardness of sorghum biscuits with the aim of understanding how sorghum kafirins affect the hardness of sorghum biscuits.

Objective 2

To determine the effect of different water levels on the hardness of sorghum biscuits with the aim of understanding the effect of starch gelatinization on the hardness of sorghum biscuits.

Objective 3

To determine the role of sugar in the hardness and cohesiveness of sorghum biscuits with the aim of understanding how the formation of sugar glass affects the hardness of sorghum biscuits.

4 RESEARCH

Roles of protein, starch and sugar in the texture of sorghum biscuits

ABSTRACT

Sorghum has the potential to replace wheat in biscuit making especially in African countries where wheat is mostly imported due to low availability arising from unfavourable climatic conditions. This study showed that sorghum biscuits of similar texture to wheat biscuits can be made despite the absence of gluten in sorghum biscuits. SEM and TEM revealed that sorghum kafirins remained isolated in their protein bodies and are unlikely to contribute to the structure and texture of sorghum biscuits. An increase in water level increased the hardness and brittleness of sorghum biscuits and the texture was similar to that of Marie wheat biscuits at 35% and 40% water on flour basis. Increasing the level of pre-cooked sorghum flour reduced the hardness of sorghum biscuits which suggested that starch gelatinization weakened the biscuit structure. Similarly, an increase in sugar level also increased the hardness and brittleness of sorghum biscuits. With 20% sugar on flour basis, sorghum biscuits had similar hardness and brittleness to both sugar-snap and Marie wheat biscuits. Stereomicroscopy revealed that starch granules were embedded in the biscuit matrix, while polarizing light microscopy revealed that the starch granules formed networks with a sugar glass with the granules becoming entrapped in the sugar glass matrix as the level of sugar was increased. The sugar glass holds other components of the biscuit together and its interaction with starch granules appears to be responsible for its similar texture with wheat biscuits.

4.1 INTRODUCTION

Unlike in breads where development of a gluten network is critical (Gallagher *et al*., 2004), the gluten network in biscuits needs to be only slightly developed for the dough to be cohesive and not too elastic (Contamine *et al*. 1995). However, while some authors have stated that gluten is not developed in wheat biscuits (Slade *et al*., 1993), others have proposed mechanisms to explain how it could develop, especially during baking (Gaines, 1990). The perception of gluten development in wheat biscuits seems to depend on the type of biscuit being studied. Hard biscuit doughs have a well-developed gluten network, while the level of hydration in short biscuit dough is insufficient to allow the formation of a gluten network (Manley, 2000).

Due to the seemingly unimportance of gluten in some types of wheat biscuit, it is therefore not surprising that considerable headway has been made in the development of quality gluten-free biscuits. Schober *et al*. (2003) used a combination of rice, maize, potato, and soya flours with high-fat powders to produce sheetable biscuit doughs and biscuit quality was similar to wheat biscuits. Sorghum biscuits of similar texture to wheat biscuits have also been developed (Omoba *et al*., 2015). Descriptive sensory profiling of these sorghum biscuits revealed that they were indistinguishable from whole wheat biscuits in terms of hardness, roughness and coarseness, although they were less dry and crispy.

Other components of biscuit such as starch and sugar have been identified to play a major role in the final quality of biscuits. Gallagher (2011) stated that due to the minimal formation of a gluten network in wheat biscuits, the texture can be attributed to starch gelatinization and super-cooled sugar rather than a protein-starch network. This is contrary to the view of Hoseney and Rogers (1994) that a rigid protein-starch structure is responsible for the hardness of wheat biscuits. Chevallier *et al*. (2000a) attributed the cohesiveness of wheat biscuit structure to sugar that melts during baking and cools to form a bridge between starch-protein particles. Maache-Rezzoug *et al*. (1998) also reported that wheat biscuits rich in sugar have a highly cohesive structure and a crisp texture.

Therefore in the absence of a gluten network in sorghum biscuits, a study of the role and effects of major components of sorghum biscuits, protein, starch and sugar should help understand why sorghum biscuits can have a similar texture to wheat biscuits.

4.2 MATERIALS AND METHODS

4.2.1 Materials

All ingredients used were purchased from retail outlet in Hatfield, Pretoria, South Africa. These were: sorghum meal Monati Super Mabela (made from red non-tannin sorghum at an extraction rate of approximately 90%) (RCL Foods, Westville, South Africa), “Snowflake” wheat cake flour (Premier, Waterfall City, South Africa), “Stork” baking margarine (Unilever, La Lucia, South Africa), “Royal” baking powder (Kraft foods, Sandton, South Africa), “P n P” white sugar and sunflower oil (Pick n Pay retailers, Kensington, South Africa).

4.2.2 Methods

4.2.2.1 Biscuit making

Wheat biscuits

Sugar-snap wheat biscuits were made according to AACCI approved method: 10-50D (AACC International, 1999).

Sorghum biscuits

Sorghum meal was further milled into a flour using a hammer mill (Drotsky, Alberton, South Africa) fitted with a 500 µm opening screen. Sorghum dough was prepared by mixing all dry ingredients (sorghum flour, sugar, baking powder) and wet ingredients (water and oil) in separate stainless bowls. Both dry and wet ingredients were then combined, thoroughly mixed by hand and pressed into a ball. The dough was then rolled out evenly on a flat surface using a wooden rolling pin. After flattening, the dough surface was made uniform by hand. A rectangular cutter with dimensions 50 mm × 25 mm was then used to cut dough into biscuit sized pieces of 4 mm thick. Dough pieces were then transferred using a stainless steel egg-lifter onto a baking tray on which a little flour had been evenly sprinkled to prevent the dough from sticking. Baking tray containing dough pieces was then placed in a convection oven (Unox, Padova, Italy) and baked at 190°C for 18 ± 2 minutes. The typical baked aroma of baked products was used as indication that the biscuits are properly baked. After baking, the biscuits were cooled for 30 minutes at room temperature (25°C) before they were packaged in polyethylene zip-lock bags and stored at -20°C. Photography and texture analysis of the biscuits were conducted before storage. To perform other analyses of the biscuits, they were crushed into a powder (< 500 µm) using a mortar and pestle. Un-defatted biscuits were used for analysis except where otherwise stated.

Figure 4.1 shows a flow diagram of the experimental design. All sorghum biscuits were essentially made using the above procedure but with modifications and variations in dough formulation. Tables 4.1–4.3 show the various biscuit dough formulations and where applicable, modification to the general procedure is described.

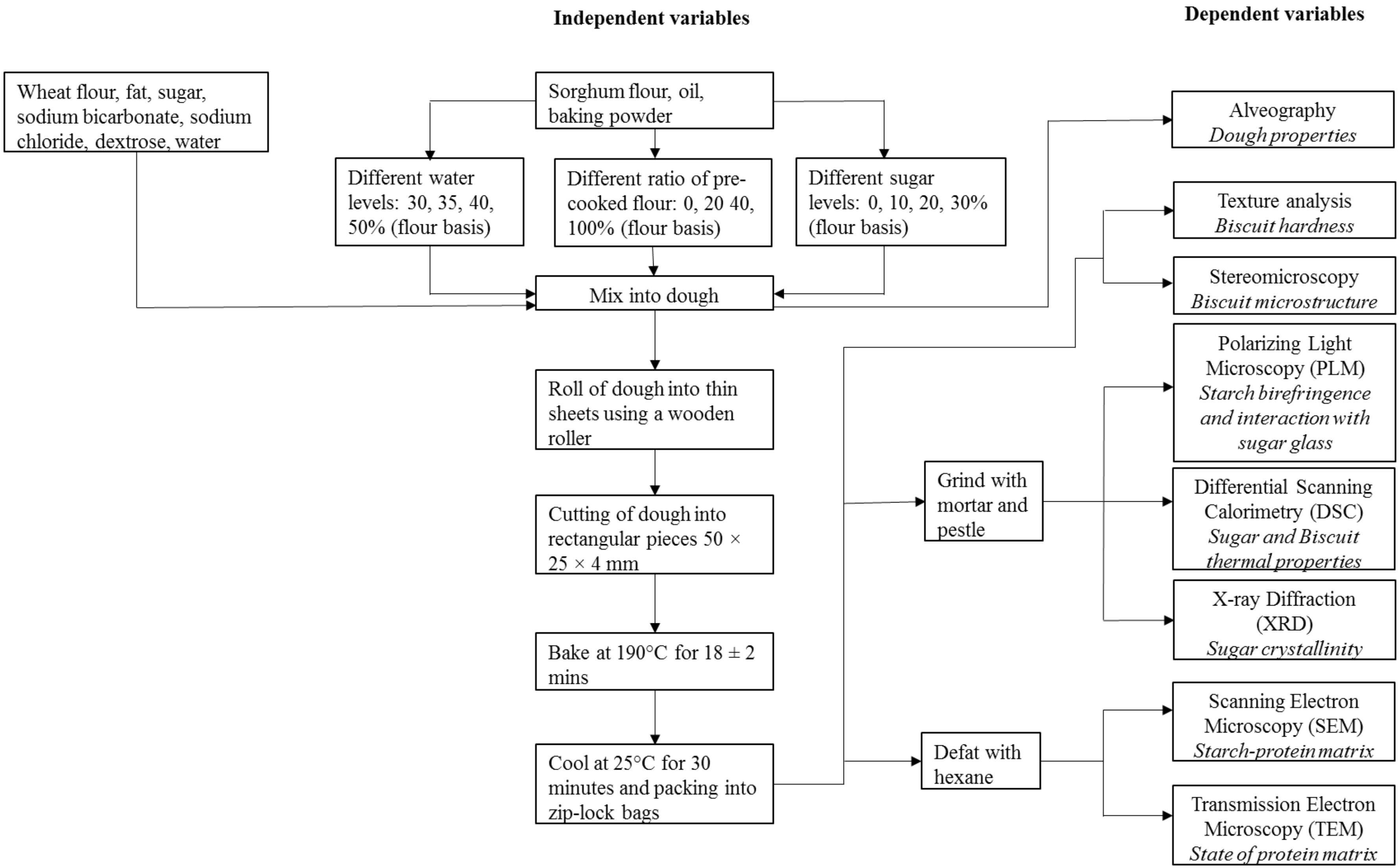

Figure 4.1: Flow diagram of experimental design.

Different water levels

Table 4.1: Composition of sorghum biscuits made using different water levels

	Water level			
Ingredient	**30%**	**35%**	**40%**	**50%**
Sorghum flour	150 g (100)[1]	150 g (100)	150 g (100)	150 g (100)
Sugar	37.5 g (25)	37.5 g (25)	37.5 g (25)	37.5 g (25)
Sunflower oil	43.5 g (29)	43.5 g (29)	43.5 g (29)	43.5 g (29)
Baking powder	1 g (0.67)	1 g (0.67)	1 g (0.67)	1 g (0.67)
Water	45 g (30)	52.5 g (35)	60 g (40)	75 g (50)

[1]Values in parentheses represent the amount of ingredient expressed in baker's percentage

Different ratios of pre-cooked sorghum flour

Sorghum doughs were prepared according to the composition in Table 4.2. The prescribed portion of sorghum flour was weighed into a stainless bowl and mixed with 60 g water. The mixture was weighed before cooking at 90°C for 3 min. After cooking, the cooked flour was cooled to ambient temperature (25°C) and then reweighed to estimate water loss due to evaporation during cooking. The pre-cooked flour was then added to the raw portion of the sorghum flour with other dry ingredients (sugar and baking powder). Water lost during cooking of the sorghum flour was then mixed with oil and added to the mixture to form a dough.

Table 4.2: Composition of sorghum biscuits made using different levels of pre-cooked sorghum flour

	Raw flour : Pre-cooked flour ratio			
Ingredient	**100:0**	**80:20**	**60:40**	**0:100**
Raw flour	150 g (100)[1]	120 g (80)	90 g (60)	0 g
Pre-cooked flour	0 g	30 g (20)	60 g (40)	150 g (100)
Cooking water	0 g	60 g (40)	60 g (40)	60 g (40)
Added water	60 g (40)	a	b	c
Sugar	37.5 g (25)	37.5 g (25)	37.5 g (25)	37.5 g (25)
Sunflower oil	43.5 g (29)	43.5 g (29)	43.5 g (29)	43.5 g (29)
Baking powder	1 g (0.67)	1 g (0.67)	1 g (0.67)	1 g (0.67)

[1]Values in parentheses represent the amount of ingredient expressed in baker's percentage
abc = Amount of water re-added to sorghum dough in order to make up for losses due to evaporation during pre-cooking.

Different sugar levels (undissolved and pre-dissolved)

Table 4.3: Composition of sorghum biscuits made using different levels of sugar (undissolved and pre-dissolved)

	Sugar level			
Ingredient	**No sugar**	**10%**	**20%**	**30%**
Raw flour	150 g (100)[1]	150 g (100)	150 g (100)	150 g (100)
Water	52.5 g (35)	52.5 g (35)	52.5 g (35)	52.5 g (35)
Sugar	0 g (0)	15 g (10)	30 g (20)	45 g (30)
Sunflower oil	43.5 g (29)	43.5 g (29)	43.5 g (29)	43.5 g (29)
Baking powder	1 g (0.67)	1 g (0.67)	1 g (0.67)	1 g (0.67)

[1]Values in parentheses represents the amount of ingredient expressed in baker's percentage

4.2.2.2 Analyses

4.2.2.2.1 Moisture and protein contents

Moisture and crude protein contents of sorghum (N × 6.25) and wheat (N × 5.7) flours were determined according to AACCI approved methods: 44-15A and 46-19, respectively (AACCI, 2000).

4.2.2.2.2 Alveography

An Alveograph (Chopin NG Consistograph, Paris, France) was used to determine the rheological properties of dough according to AACCI approved method: 54-30A (AACCI, 1999) and in combination with the Alveograph NG Consistograph instructional manual (Chopin, 2010). Alveogram values: tenacity or resistance to extension (P, mm H_2O), extensibility (L, mm), deformation energy (W, J x 10^{-4}), and curve configuration ratio (P/L) of the dough were obtained.

4.2.2.2.3 Texture analyses

The hardness of biscuits was determined by measuring the force required to break biscuit pieces on a texture analyser (TA.XT2, Stable Microsystems, Godalming, UK). The maximum peak force during first compression was measured using a three point bend rig (HDP/3PB) attachment, comprising an upper blade and one rig base plate with two adjustable supports adjusted to 30 mm. A vertical force was applied using the upper blade on a biscuit placed horizontally like a bridge over the two supports at a cross-head pre-test speed of 1.0 mm/sec, test speed of 3.0 mm/sec, post-test speed of 10.0 mm/sec and distance of 10 mm. The maximum peak force required to break a mean of 12 biscuits was obtained. The fracture properties of biscuits were analysed using the following formula:

$$\sigma = \frac{3FL}{2bh^2} \qquad \varepsilon = \frac{6h}{L^2}$$ (Baltsavias *et al.*, 1997)

Where σ is the Stress at midpoint (MPa), ε is the Strain, F is the force at the beam centre (N), L is the distance between the supports (mm), b is the biscuit width (mm) and h is the biscuit thickness (mm). The texture of sorghum biscuits was compared to that of sugar-snap and commercial Marie wheat biscuit standards.

4.2.2.2.4 Defatting of biscuits

Biscuit crumbs were defatted using hexane in a shaking water bath at 25°C. Glass beaker containing hexane and biscuit crumbs was covered with an aluminium foil and left in the shaking water bath for 120 min. This procedure was repeated once more and biscuit crumbs were dried overnight through a stream of air in a fume hood.

4.2.2.2.5 Scanning Electron Microscopy (SEM)

Defatted biscuit crumbs were mounted on aluminium stubs using carbon paint and a cross section of the biscuits was coated with carbon using an Emitech K950X carbon coater (Ashford, England). Samples were viewed using a Zeiss 540 Crossbeam SEM (Oberkochen, Germany) at a voltage of 1 kV.

4.2.2.2.6 Transmission Electron Microscopy (TEM)

Defatted biscuit crumbs were prepared for TEM by fixing in 2.5% glutaraldehyde in 0.075 M phosphate buffer overnight at 5°C. Fixed samples were then rinsed with the phosphate buffer 3x at 10 min intervals each before dehydrating in an ethanol series (50, 70, 90 and 100% ethanol) for 15 min each. Samples were further post-fixed with aqueous osmium tetroxide under a fume hood for 1 h before rinsing again 3x with the phosphate buffer. Fixed samples were transferred into plastic tubes and infiltrated with 33 and 66% ethanol-epoxyresin mixture for 1 h each before infiltration with 100% epoxyresin for another 1 h. Samples were finally infiltrated with fresh 100% epoxyresin for 6 h before polymerizing in the oven at 50°C for 3 days. Thin slices of 100 nm were obtained from polymerized samples using a Diatome diamond knife with a Reichert Ultracut R (Vienna, Austria) and were stained in uranyl acetate for 15 min before rinsing with triple distilled water and gently dried with a filter paper. Samples were examined with a Philips CM10 TEM (Eindhoven, The Netherlands) operated at 80kV.

4.2.2.2.7 Stereomicroscopy

The microstructure of biscuit crumbs was analysed using a stereomicroscope (Zeiss Discovery V20, Jena, Germany). Biscuit samples were fractured and viewed through the cross section with a field of view 3.5 mm, 1.8 µm resolution and 64 µm depth of field. All images were taken at a magnification of 65x.

4.2.2.2.8 Polarizing light microscopy

Polarizing light microscope (Zeiss Axio Imager.Alm, Jena, Germany) was used to analyse starch and sugar in sorghum and wheat biscuits. For starch analysis, 50 mg of milled biscuit samples were weighed into Eppendorf tubes and 0.5 ml of 30% glycerol solution was added. The tubes were vortexed at high speed for about 15 seconds to disperse and suspend the biscuit particles. Two drops of the vortexed sample was then placed on a glass slide using a Mohr pipette and then covered with a cover slip. Glass slides containing the samples were placed on the microscope stage and analysed with visual magnification at 200x. The same procedure was repeated for sugar analysis but by replacing glycerol with ethanol to prevent the sugar glass from dissolving.

4.2.2.2.9 Differential Scanning Calorimetry (DSC)

Thermal analysis of biscuit samples was conducted using a DSC (HP DSC827^{e}, Mettler Toledo, Greifensee, Switzerland). Milled biscuit samples (10-15 mg) was weighed into DSC pans and hermetically sealed. Samples were scanned at 10°C/min from 25°C to 220°C. The procedure was repeated by adding 30% crystalline sucrose to biscuit samples.

4.2.2.2.10 X-ray Diffraction (XRD)

Milled biscuit samples were dried over silica gel in a desiccator for about 120 hours. Samples were analyzed using a PANalytical X'Pert Pro powder diffractometer (Almelo, The Netherlands) in θ–θ configuration with a X'Celerator detector and variable divergence- and fixed receiving slits with Fe filtered Co-Kα radiation (λ=1.789Å). The diffraction phases were identified using X'Pert Highscore plus software.

4.2.2.2.11 Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA). A minimum of 12 biscuit pieces were evaluated for texture analysis and means were compared at $p = 0.05$ using the Tukey Least Significant Difference Test (LSD).

4.3 RESULTS AND DISCUSSION

4.3.1 Moisture and protein composition

The moisture and protein content of the sorghum and wheat flour (Table 4.4) were significantly different ($p < 0.05$) with wheat flour having higher values. The protein content of both the sorghum and wheat flours were higher than 7-9% stated for wheat biscuit flour by Wade (1988) but similar to the 8-11% reviewed by Pareyt and Delcour (2008). However, the moisture content of both sorghum and wheat flours were lower than 14% stated by both set of authors. The protein content of the wheat flour was high because in South Africa, low extraction bread wheat flour and not biscuit wheat flour is used for biscuit making. The protein content of sorghum flour was higher than 8.43% and 9.53% reported for whole and refined sorghum flour, respectively (USDA, 2016).

Table 4.4: Moisture and protein composition* of sorghum and wheat flours

Flour type	Moisture content (g/100 g)	Protein content[2] (g/100 g)
Sorghum flour	$11.19^{a} \pm 0.03$[1]	$10.26^{a} \pm 0.06$
Wheat flour	$11.86^{b} \pm 0.01$	$11.45^{b} \pm 0.02$

[1]Mean ± Standard deviation (n = 3)
[2]Calculated using N × 6.25 for sorghum and N × 5.70 for wheat
[ab]Values within a column with different superscript differ significantly ($p < 0.05$)
*Composition as is basis

4.3.2 Alveography

As expected, unlike the wheat dough, the sorghum dough could not hold air (Fig. 4.2). The ability of wheat dough to hold air was due to the formation of viscoelastic gluten when wheat flour is hydrated (Gallagher *et al*., 2004), while a gluten-like network is not formed when sorghum is hydrated (Taylor *et al*., 2006). The properties of wheat flour (Table 4.5) were expectedly different from those reported for different American soft wheat biscuit flour by Yamamoto *et al*. (1996). As already stated, the South African bread wheat flour has a high protein content, while protein content in soft wheat flours was less than 9% (Yamamoto *et al*., 1996). However, the properties of the wheat flour were similar to those reported for durum wheat flour with up to 15% protein (Boyacioğlu and D'Appolonia, 1994). Yamamoto *et al.* (1996) reported 21 to 56 mm, 69 to 188 mm, and 43 to 173 ($\times 10^{-4}$ J) for P, L and W, respectively, for different cultivars of soft wheat flour, while Boyacioğlu and D'Appolonia

(1994) reported 118 mm, 53 mm and 220 (× 10^{-4} J) for P, L, and W, respectively, for durum wheat flour.

Table 4.5: Dough properties of sorghum and wheat doughs as measured using an alveograph

Flour type	Stability (P, mm H_2O)	Extensibility (L, mm)	Curve configuration ratio (P/L)	Deformation energy (W, J × 10^{-4})
Sorghum	Dough cracked and could not hold air			
Wheat	151.0[1] ± 7.0	37.0 ± 2.6	4.1 ± 0.5	237 ± 3.6

[1]Mean ± Standard deviation (n =3)

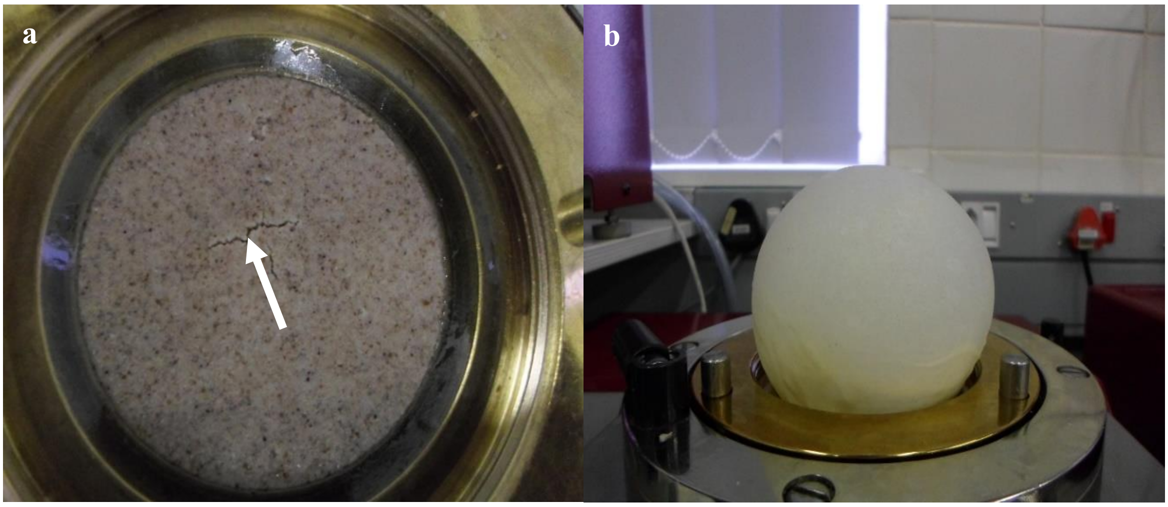

Figure 4.2: Alveography of sorghum and wheat doughs. (a) Sorghum dough, (b) Wheat doughs. Arrow shows crack in dough.

4.3.3 Investigations concerning the role of proteins in the texture of biscuits

4.3.3.1 Effect of dough composition and flour types on dough properties

Figure 4.3 compares the physical properties of the wheat and sorghum biscuit doughs. The wheat dough was cohesive after formation, while the sorghum dough was not but could hold together by manual kneading as indicated by the circle (Fig. 4.3b). The cohesiveness of the wheat biscuit dough can be attributed to the hydration of the wheat proteins. In addition, fat is also known to bind other ingredients in the wheat biscuit dough together (Pareyt and Delcour, 2008). Oil was used in making sorghum biscuits because fat resulted in extremely soft biscuits, while oil produced biscuits of similar hardness to wheat biscuits (Dovi, 2013).

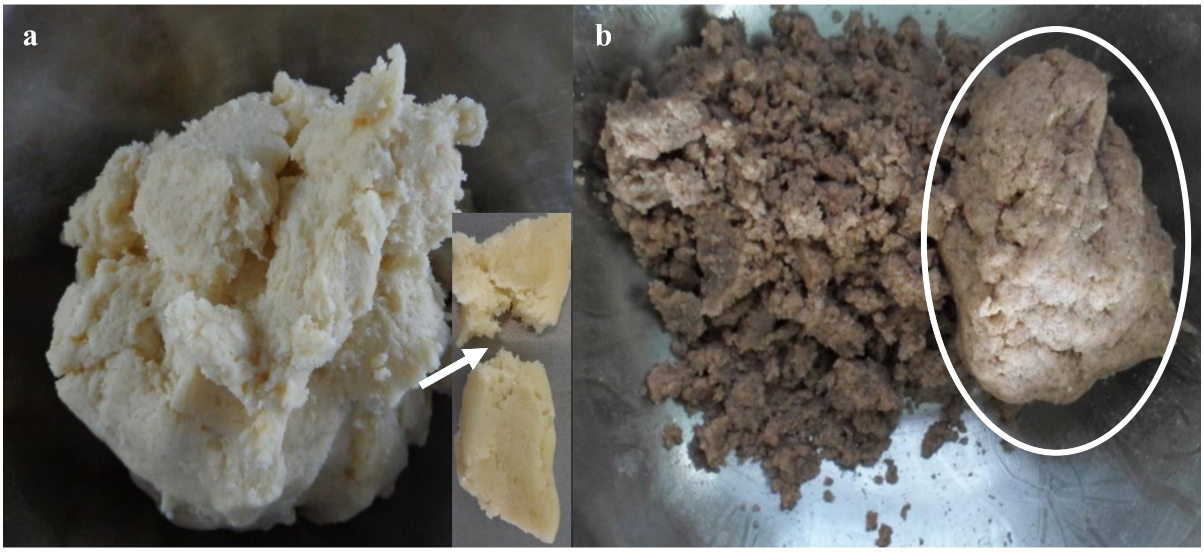

Figure 4.3: Effect of flour types on the appearance of wheat and sorghum biscuit doughs. (a) Sugar-snap wheat dough, (b) Sorghum dough. Arrow shows wheat dough is short and inextensible when stretched, circle shows sorghum dough holding together after manual kneading.

The wheat biscuit dough was also observed to be short and inextensible when stretched as indicated by the arrow (Fig. 4.3a), suggesting gluten was undeveloped. The alveograph of the wheat flour (Fig. 4.2b) showed that gluten could be developed. The inability of gluten to develop in the wheat biscuit dough can be attributed to fat which prevented aggregation of the wheat proteins, and subsequently gluten development. Ghotra *et al*. (2002) stated that fat surrounds wheat proteins thereby breaking their molecular continuity. In addition, the small amount of water used to form the wheat biscuit dough, and non-working of the wheat dough may also have contributed to the inextensible nature of the dough. Manley (2000) stated that a small amount of water, if any, is required to achieve the desired consistency of wheat biscuit dough due to the lubricating effect of fat. The wheat dough was not worked because of the undesirable effect high gluten formation has on the quality of wheat biscuits (Wade, 1988). Belton (1999) stated that working of wheat dough favours the formation of disulphide bonds which increases the number of protein-protein interactions and subsequently the formation of glutenin polymers from the high molecular weight (HMW) glutenin subunits. The HMW subunits present in the glutenin polymers (Shewry and Halford, 2002), are responsible for the strength and elasticity of wheat dough (Wieser, 2007).

4.3.3.2 Effect of wheat and sorghum flours on the size and crumb structure of their biscuits

Wheat biscuit dough rose and spread during baking unlike the sorghum biscuit which remained the same (Fig. 4.4). Wheat biscuit dough increased from 50 mm × 25 mm × 4 mm to 60 mm × 35 mm × 5 mm in the baked biscuit (Fig. 4.4a). The increase in the volume of the biscuit was probably due to the ability of gluten to trap air produced by the leavening agent. Gluten matrix is recognized for its gas holding property (Gallagher *et al.*, 2004). This was further reflected in the far more open crumb structure of the wheat biscuit compared to the sorghum biscuit which was compact and dense (Fig. 4.5). There are arguments about what is responsible for the spread of wheat biscuit dough but it is certain that dough viscosity influences the spread of wheat biscuit dough. It was earlier noted that the more viscous a dough, the less it spreads during baking. Gaines and Finney (1989) found that dough from soft wheat flour was less viscous than dough from hard wheat flour and spreads at a faster rate. Delcour and Hoseney (2010) stated that because starch is not gelatinized in biscuit, viscosity is presumably a property of the flour proteins. Gluten, as one of the components contributing to dough viscosity influences the spread of wheat biscuit dough. The absence of a gluten network in sorghum biscuit may explain why the dough did not spread during baking and could also account for its dense crumb structure (Fig. 4.5b).

Two mechanisms involving the behaviour of gluten have been used to explain its contribution to the spread of wheat biscuit dough. Miller *et al.* (1996) stated that the setting of wheat biscuit is determined by the gluten protein undergoing an apparent glass transition during baking. They further added that the viscosity of the gluten network is sufficient to stop the spreading of biscuit dough. This is similar to an earlier view by Abboud *et al*. (1985) that wheat biscuit dough spreads to a point where a sudden increase in viscosity occurs. Concerning the second mechanism, Slade *et al.* (1989) stated that the spread of biscuit dough is due to the proteins exhibiting viscous expansion and creep followed by a structural collapse. Both mechanisms recognize viscosity as a factor and as such, the effect of gluten proteins on dough viscosity will also affect the spread of wheat biscuit dough.

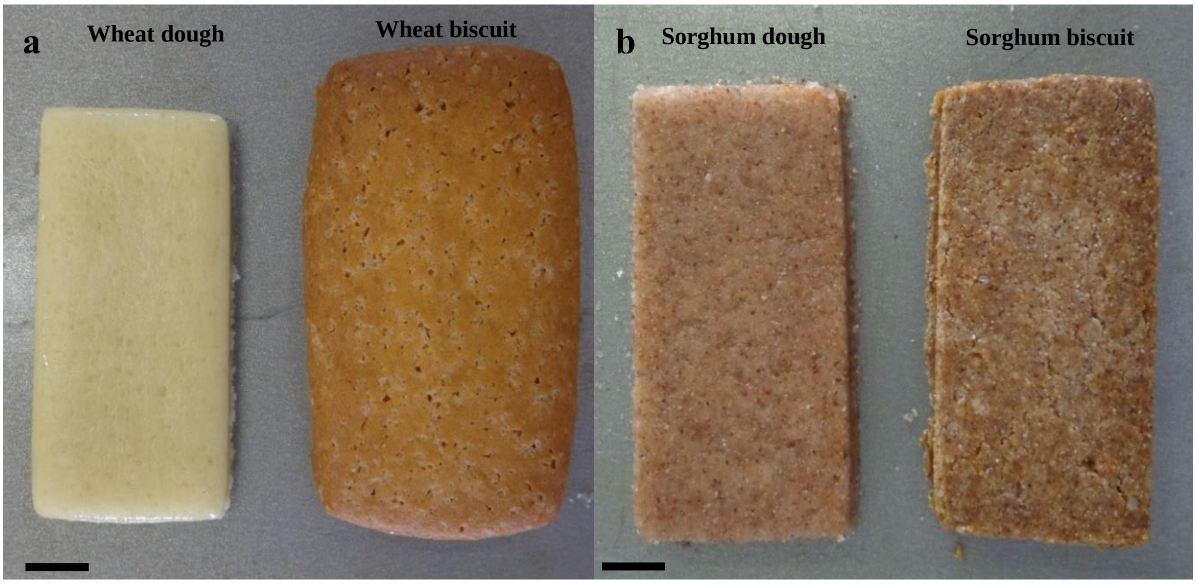

Figure 4.4: Effect of flour type on dough spread and size of wheat and sorghum biscuits. (a) Sugar-snap wheat biscuit, (b) Sorghum biscuit. Bar is 10 mm.

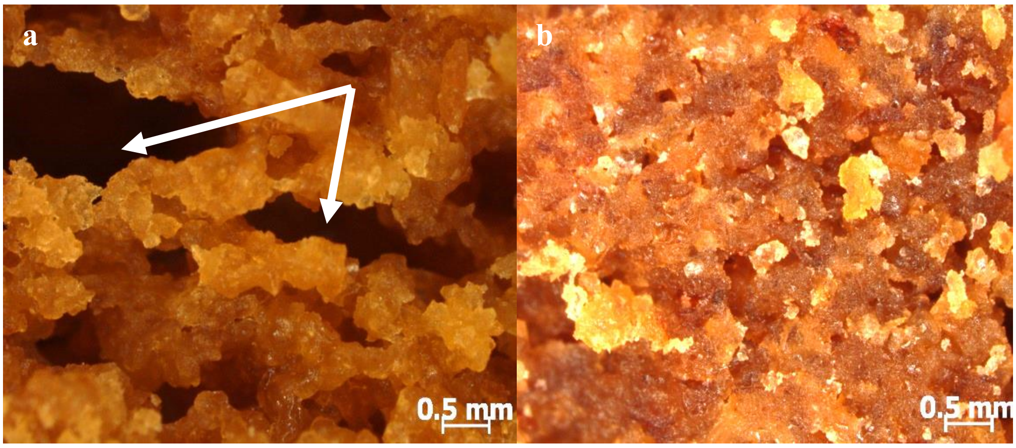

Figure 4.5: Stereomicroscopy of the crumb structure of the biscuits. (a) Sugar-snap wheat biscuit, (b) Sorghum biscuit. Arrows indicate open crumb structure.

4.3.3.3 Textural properties of wheat and sorghum biscuits

Table 4.6 shows that sorghum biscuit was harder (higher stress value) than both the sugar-snap and Marie wheat biscuits, which were not significantly different ($p \geq 0.05$). However, the brittleness of sorghum biscuit was not significantly different from that of Marie wheat biscuit, while sugar-snap wheat biscuit was the least brittle. Szczesniak (1963) defined hardness as the force necessary to attain a given deformation, while brittleness was defined as the force with which a material fractures. Brittleness is considered a pleasant sensory property of biscuit if it does not become extremely great (Zoulias *et al*., 2002).

Szczesniak (1963) stated that the hardness of a brittle material can vary from low to high. Brittle materials with certain degree of hardness often produce a sound effect during mastication. This indicates that a hard material can also be brittle and as such, it was not unusual for a harder biscuit (sorghum) to have same level of brittleness as a softer biscuit (Marie wheat). The question is what was responsible for this phenomenon? Likewise, why did the two types of wheat biscuits have different level of brittleness despite having the same level of hardness? The levels and interaction of components in the biscuits were presumably responsible.

Table 4.6: Effect of flour type on biscuit hardness

Biscuit type	Force (N)	Stress (kPa)	Strain (%)	Brittleness (Stress/Strain)
Sugar-snap wheat	$24.57^b \pm 2.30^1$	$1.28^a \pm 0.12$	$3.33^c \pm 0.00$	$0.38^a \pm 0.04$
Sorghum	$16.15^a \pm 1.78$	$1.71^b \pm 0.30$	$2.78^b \pm 0.26$	$0.63^b \pm 0.14$
Marie wheat	$16.50^a \pm 2.25$	$1.38^a \pm 0.19$	$2.00^a \pm 0.00$	$0.66^b \pm 0.09$

[1]Mean ± Standard deviation (n = 12)

[ab]Values in a column with different superscripts differ significantly ($p < 0.05$)

The difference in brittleness between the sugar-snap and Marie wheat biscuit could be due to their difference in sugar level. Although the level of sugar in the commercial Marie wheat biscuit was unknown, Wade (1988) and Manley (2000) stated sugar levels of 21% and 19% for Marie wheat biscuit on flour weight basis. This is much lower than the level of sugar (58% on flour basis) in the sugar-snap wheat biscuit. Maache-Rezzoug *et al.* (1998) found that wheat biscuits rich in sugar have a highly cohesive structure and crispy texture. However, despite the probable higher level of sugar in sugar-snap wheat biscuit, it had similar hardness to the Marie wheat biscuit. It is possible the protein-starch structure in Marie wheat biscuit was more rigid than that of sugar-snap wheat biscuit and this masked the effect that the difference in sugar content could have had on the hardness of the two types of wheat biscuits. Hoseney and Rogers (1994) stated that the rigid protein-starch structure in wheat biscuit is responsible for its hardness.

Sorghum biscuits which contained only 25% sugar on flour basis was also harder than the sugar-snap wheat biscuit which contained more sugar (58%). As noted, this suggests that apart from the level of ingredients used, component interaction may also influence the texture of biscuits. With sorghum kafirins being suggested to be non-functional in sorghum biscuits, determining the roles of both sorghum starch and sugar seems critical in further understanding the development of texture in sorghum biscuits.

4.3.3.4 Microstructure of wheat and sorghum biscuits

The microstructure of sorghum and wheat biscuits indicated component interaction in biscuit crumbs (Fig. 4.6). Notably, most of the starch granules in sorghum biscuit appeared intact and embedded as visible white specs (arrows) in the sorghum biscuit matrix, while the swollen granules existed in a discrete form (Fig. 4.6b). The largely intact nature of starch granules in the sorghum biscuit could be due to poor starch hydration. The hydrophobic protein bodies in sorghum are suggested to act as a barrier to starch gelatinization (granule hydration) (Chandrashekar and Kirlies, 1988). It was also observed that the starch granules in Marie wheat biscuit appeared more swollen than those of the sugar-snap wheat biscuit. This could be due to the Marie biscuit containing less sugar than the sugar-snap wheat biscuit as suggested earlier. The effect of sugar level on starch gelatinization has been earlier summarized. The observed difference in the extent of starch swelling in the two types of wheat biscuits appears to further support the suggestion that a difference in sugar level impacted on their brittleness. Starch granules are referred to as swollen because they apparently are not gelatinized as noted earlier. However, this will be further investigated when considering the role of starch in the biscuit texture (section 4.3.4).

Furthermore, it appears that the swollen starch granules were more tightly bound to a protein network in Marie wheat biscuits (Fig. 4.6c) than in the sugar-snap wheat biscuits (Fig. 4.6a). Instead, the sugar-snap wheat biscuit appears to have its starch, protein and fat molecules dispersed in the biscuit matrix. The difference in the microstructure of the two types of wheat biscuits also suggests a more rigid protein-starch structure is present in Marie wheat biscuits. This seems to further support the concept that the rigidity of the protein-starch structure in Marie wheat biscuits masked the effect a difference in sugar level would have had on the hardness of the two types of wheat biscuits.

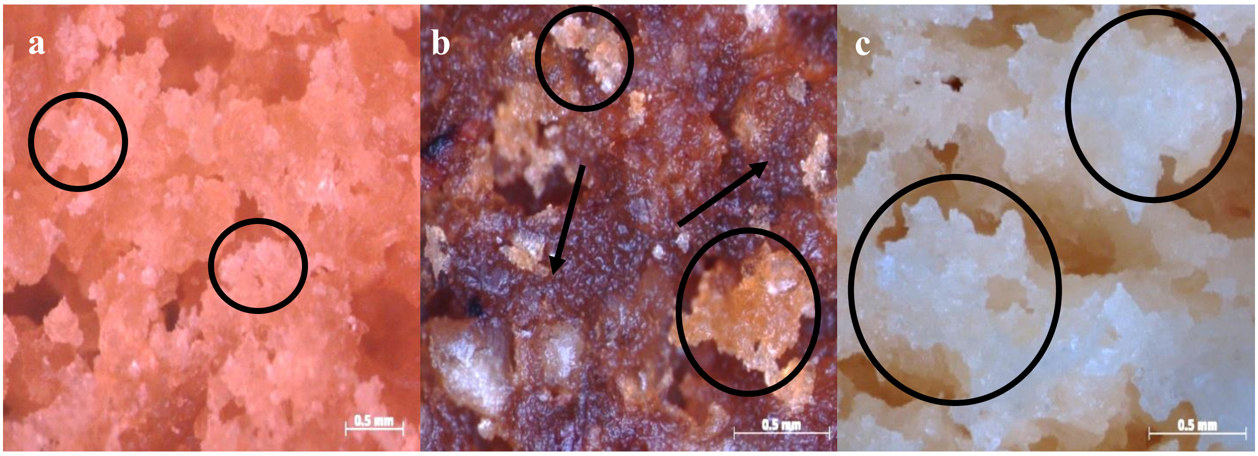

Figure 4.6: Stereomicroscopy of wheat and sorghum biscuits. (a) Sugar-snap wheat biscuit, (b) Sorghum biscuit, (c) Marie wheat biscuit. Circles show swollen/disrupted starch granules, arrows indicate intact starch granules embedded in sorghum biscuit matrix.

4.3.3.5 SEM and TEM of wheat and sorghum biscuits

SEM of sorghum and wheat biscuits (Fig. 4.7) shows that the starch granules were intact in all the biscuits. However, a continuous network appeared to envelope the starch granules in both the sugar-snap and Marie wheat biscuits, while in the sorghum biscuit, there was mainly an aggregation of starch granules with visible protein bodies (P) (Fig. 4.7b). The continuous network in wheat biscuits was probably a gluten sheet. Marie wheat biscuit have a developed gluten network (Manley, 2000), while the presence of a gluten network in sugar-snap wheat biscuit has also been reported (Pareyt *et al.*, 2010). The inelastic nature of sugar-snap wheat dough (Fig. 4.3a) and the presence of a gluten sheet in its biscuit (Fig. 4.7a) suggests the gluten was developed later during baking.

However, Marie wheat biscuits appeared to have a denser protein-starch network than the sugar-snap wheat biscuits due to the extent the gluten sheet enveloped the starch granules. This is similar to what was observed by stereomicroscopy (Fig. 4.6). Furthermore, the intact appearance of the starch granules in the biscuits also suggests that they were not gelatinized during baking. The intact protein bodies in sorghum biscuit also indicates that the kafirin proteins did not participate in dough formation and did not influence the biscuit texture.

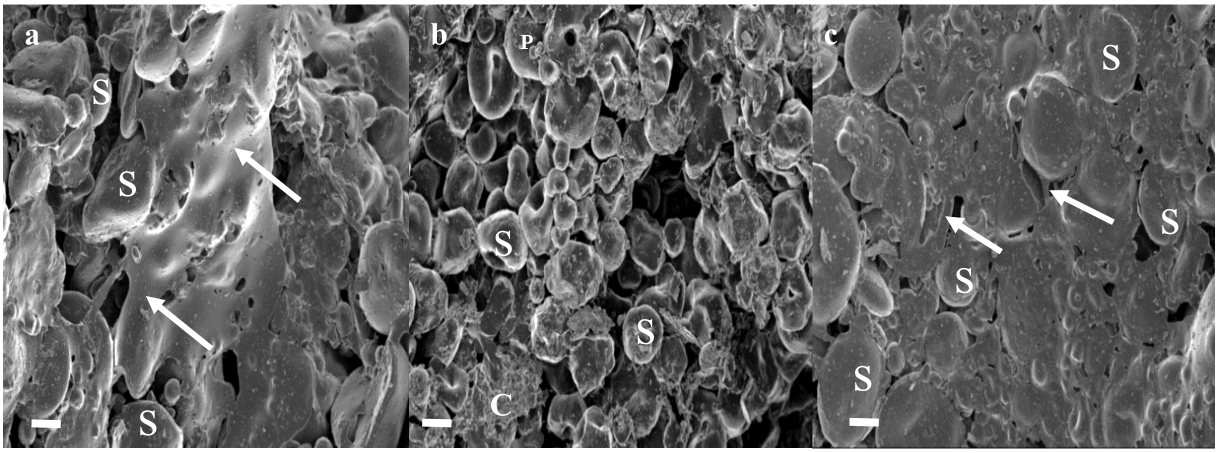

Figure 4.7: SEM of wheat and sorghum biscuit crumbs. (a) Sugar-snap wheat biscuit, (b) Sorghum biscuit, (c) Marie wheat biscuit. Bar is 10 µm. S = Intact starch granules, P = Protein body, C = Cell wall materials. Arrows indicate gluten sheets.

TEM further revealed the state and nature of the protein and starch granules in the sorghum and wheat biscuits (Fig. 4.8). Sorghum protein bodies were intact and packed around starch granules (Fig 4.8b), while there was a continuous network of gluten around the starch granules in both the sugar-snap and Marie wheat biscuits. The intact nature of the sorghum protein bodies showed by TEM provides further evidence that sorghum kafirins did not participate in dough formation and could not have contributed to the texture of sorghum biscuits. TEM also shows that the gluten sheet in Marie wheat biscuits was broader and more tightly associated with the starch granules than in the sugar-snap wheat biscuits, as was observed by stereomicroscopy and SEM.

The presence of a gluten network in sugar-snap wheat biscuits is supported by the work of Gaines (1990). This author stated that the gluten precursor proteins (glutenin and gliadin) cannot be described as non-functional in wheat biscuit dough especially during baking. It was suggested that even though gluten is not developed during dough mixing, some molecular protein association occurs during the mixing process. This author further stated that more association of the proteins probably occurs when the expansion of gas cells force the hydrated proteins into sufficiently close proximity. Doescher *et al*. (1987) also found that even though wheat biscuit dough has a discontinuous structure, the baked biscuit has a continuous structure suggested to be gluten.

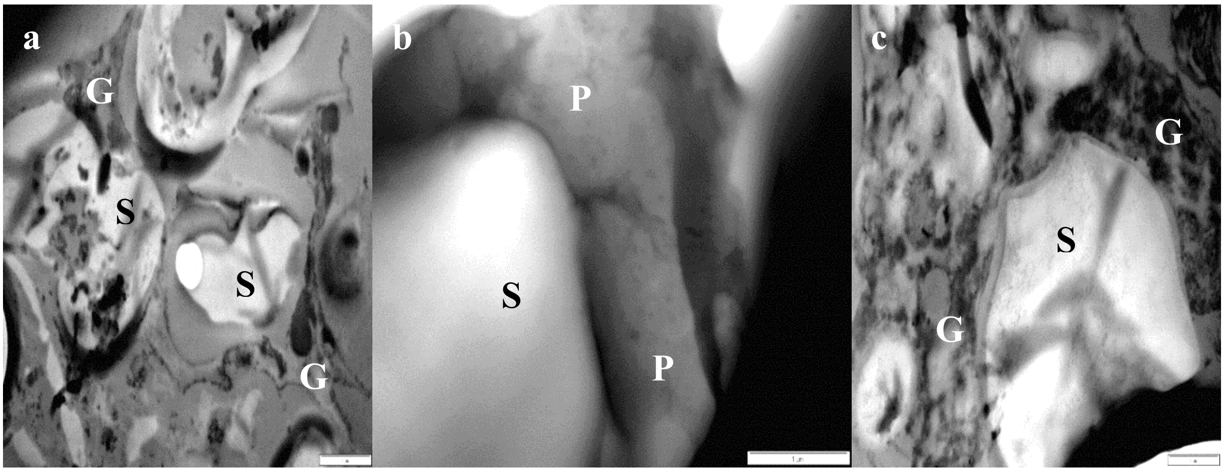

Figure 4.8: TEM of protein matrix in wheat and sorghum biscuit crumbs. (a) Sugar-snap wheat biscuit, (b) Sorghum biscuit, (c) Marie wheat biscuit. Bar is 1 µm. S = Starch granules, P = Protein bodies, G = Gluten network.

4.3.4 Investigations concerning the role of starch in the texture of biscuits

4.3.4.1 Effect of different water levels on the appearance of sorghum dough

As noted earlier, the sorghum doughs were non-cohesive but held together when manually kneaded (Fig. 4.2b). It was observed that sorghum doughs became softer as water level increased (Fig. 4.9). At 50% water, the dough formed a batter (Fig. 4.9d). Taylor *et al*. (2006) stated that addition of low levels of water to sorghum flour produces firm doughs that lack elasticity, while high water addition results in liquid, batter-like system. The softening of sorghum dough as water level increases is presumably related to increased hydration of non-soluble components and solubilisation of water-soluble components in the sorghum flour and other baking ingredients, especially sugar. Since not all the sugar is dissolved during biscuit dough making (Abboud and Hoseney, 1984), an increase in water level would increase the dissolution of sugar in sorghum dough, thereby resulting in softer doughs and reduced dough viscosity. Manohar and Rao (1999b) also found that an increase in water level resulted in reduced visco-elasticity and softening of wheat biscuit dough.

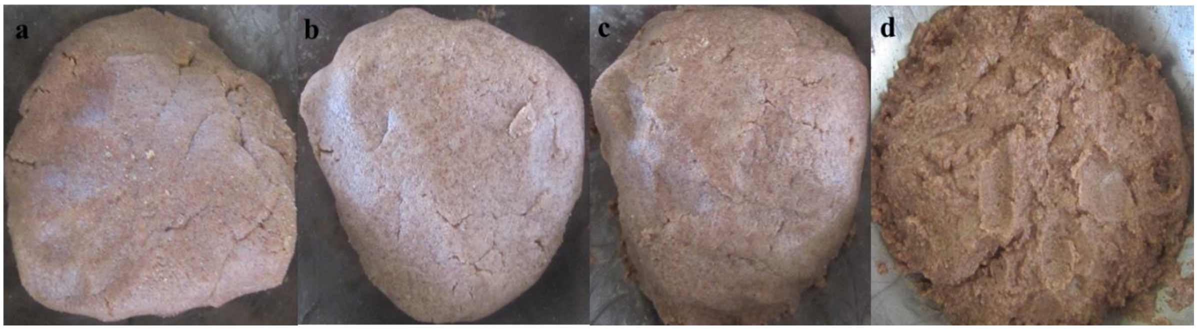

Figure 4.9: Effect of different water levels on the appearance and firmness of sorghum dough. (a) 30%, (b) 35%, (c) 40%, (d) 50%.

4.3.4.2 Effect of different water levels on the appearance and hardness of sorghum biscuits

Visual observation of the sorghum biscuits indicated that an increase in water level of sorghum dough increased the intensity of the brown colour of the biscuits with biscuits becoming darker (Fig. 4.10). This is evidence of the effect of water level on the interaction of biscuit components and overall quality of biscuits. The development of the brown colour in biscuit (browning) resulted from non-enzymatic reactions such as Maillard reaction and caramelization of sugar. Maillard reaction occurs when reducing sugar is heated with amino acid, while caramelization occurs when carbohydrates such as sucrose and reducing sugars are directly heated (BeMiller and Whistler, 1996). Capuano *et al.* (2008) stated that during baking, both sucrose and starch can be hydrolysed into reducing sugars that can simultaneously participate in both Maillard reactions and caramelization. Manohar and Rao (1999b) also reported that the intensity of the brown colour of wheat biscuits increased with increasing water level when determined both instrumentally and by sensory evaluation.

The hardness of sorghum biscuits increased with increasing water level (Table 4.7). The hardness of sorghum biscuits with 35% and 40% water was not significantly different ($p \geq 0.05$) from that of Marie wheat biscuit, while the hardness of sorghum biscuits with 30% water was not significantly different ($p \geq 0.05$) from both the sugar-snap and Marie wheat biscuits. Similarly, the brittleness of sorghum biscuits containing 35% and 40% water was similar to Marie wheat biscuits, while the brittleness of sorghum biscuits containing 30% water was similar to the sugar-snap wheat biscuits. The similarities between the textures of sorghum biscuits with different water levels and different types of wheat biscuits further reveals that dough composition influences the final quality of biscuits.

Similar to sorghum biscuits, Manohar and Rao (1999b) also found that an increase in water level increased the hardness of wheat biscuits. Gaines (1990) also stated that panellists informally reported an increase in the hardness of some wheat biscuits when water level was increased. In the absence of a gluten network in sorghum biscuits, the increase in the hardness of sorghum biscuits as water level increased could be due to starch gelatinization. Although there does not seem to be any experimental evidence confirming that starch gelatinization increases the hardness of biscuits, Manley (2000) stated that biscuits containing ungelatinized starch are softer eating. This statement seems to be based on the assumption that the hardness of wheat biscuits made from doughs containing a high amount of water and low quantity of sugar was due to starch gelatinization rather than protein association or gluten development. However, it remains to be seen if the amount of sugar in sorghum biscuits was high enough to inhibit starch gelatinization (section 4.3.5) or if the water level was high enough to gelatinize the starch (section 4.3.4.4 and 4.3.4.5).

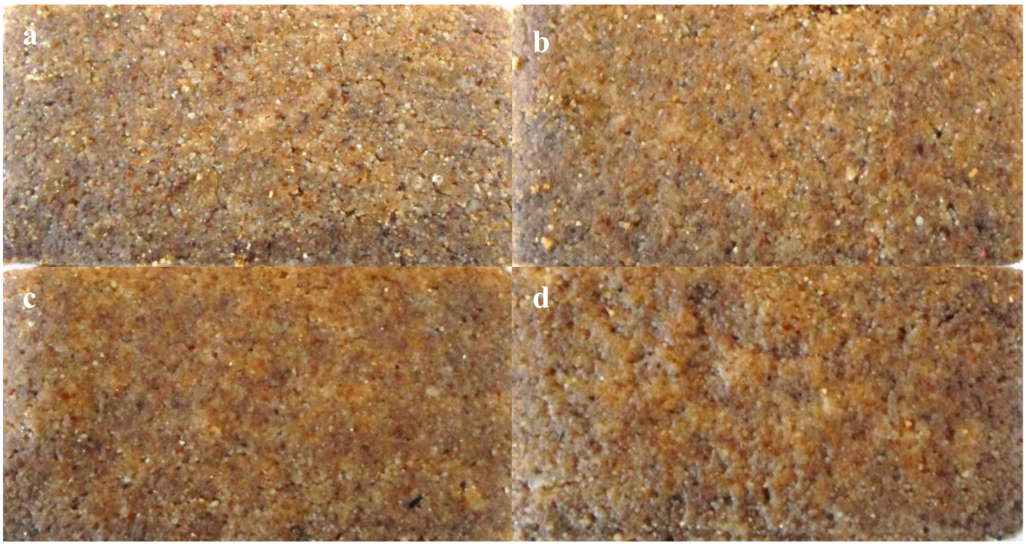

Figure 4.10: Effect of different water levels on the appearance of sorghum biscuits. (a) 30%, (b) 35%, (c) 40%, (d) 50%.

Table 4.7: Effect of different water levels on sorghum biscuit hardness

Water level on flour basis (%)	Force (N)	Stress (kPa)	Strain (%)	Brittleness (Stress/Strain)
30	11.19[a] ± 1.58[1]	1.16[a] ± 0.28	2.83[b] ± 0.30	0.42[a] ± 0.13
35	16.14[b] ± 1.78	1.82[b] ± 0.20	2.78[b] ± 0.26	0.63[b] ± 0.14
40	15.74[b] ± 1.29	1.77[b] ± 0.15	2.78[b] ± 0.26	0.62[b] ± 0.14
50	26.45[c] ± 2.11	2.75[c] ± 0.59	2.83[b] ± 0.30	1.00[c] ± 0.25
Marie wheat	16.50[b] ± 2.25	1.38[ab] ± 0.19	2.00[a] ± 0.00	0.66[b] ± 0.09
Sugar-snap wheat	24.57[c] ± 2.30	1.28[a] ± 0.12	3.33[c] ± 0.00	0.38[a] ± 0.04

[1]Mean ± Standard deviation of twelve biscuit samples (n = 12)
[a]Values in a column with different superscripts differ significantly ($p < 0.05$)

4.3.4.3 Effect of different water levels on the microstructure of sorghum biscuits

Sorghum biscuits prepared from doughs at all water levels contained strands of what appeared to be starch gels, while starch gel was not observed in the two types of wheat biscuits (Fig. 4.11). The state of the starch granules in the sorghum biscuits can be categorized as: intact, swollen/disrupted and gel. The intact granules which are embedded as white specs in the biscuit matrix constituted a vast majority of the total starch population, while few are swollen and a minute portion exist as gel. The high proportion of intact granules in the biscuit suggests gelatinization was largely inhibited in sorghum biscuits. As noted earlier, this could be due to the hydrophobic protein bodies acting as a barrier to starch gelatinization (Chandrashekar and Kirlies, 1988), or insufficient water level (Delcour and Hoseney, 2010). The undesirable batter-like nature of sorghum dough at 50% water (Fig. 4.9d) and the starch gel strand in the biscuit at this water level indicates that even above optimum water level, there was insufficient water to gelatinize most of the starch in sorghum biscuits.

The formation of the free starch gels in sorghum biscuits could be due to the sorghum dough requiring a higher level of water to form than wheat dough (< 25% on flour basis). The absence of a starch gel in the wheat biscuits was similar to the findings of Wade (1988). This author reported that none of the wheat biscuits investigated contained starch granules disrupted to the point that they formed a continuous starch gel. The granules were regarded as fillers in the matrix provided by the other components of the biscuit. On the contrary, Abboud and Hoseney (1984) compared the gelatinization endotherm of raw wheat dough and baked wheat biscuits and reported that a small portion of the starch was gelatinized during baking.

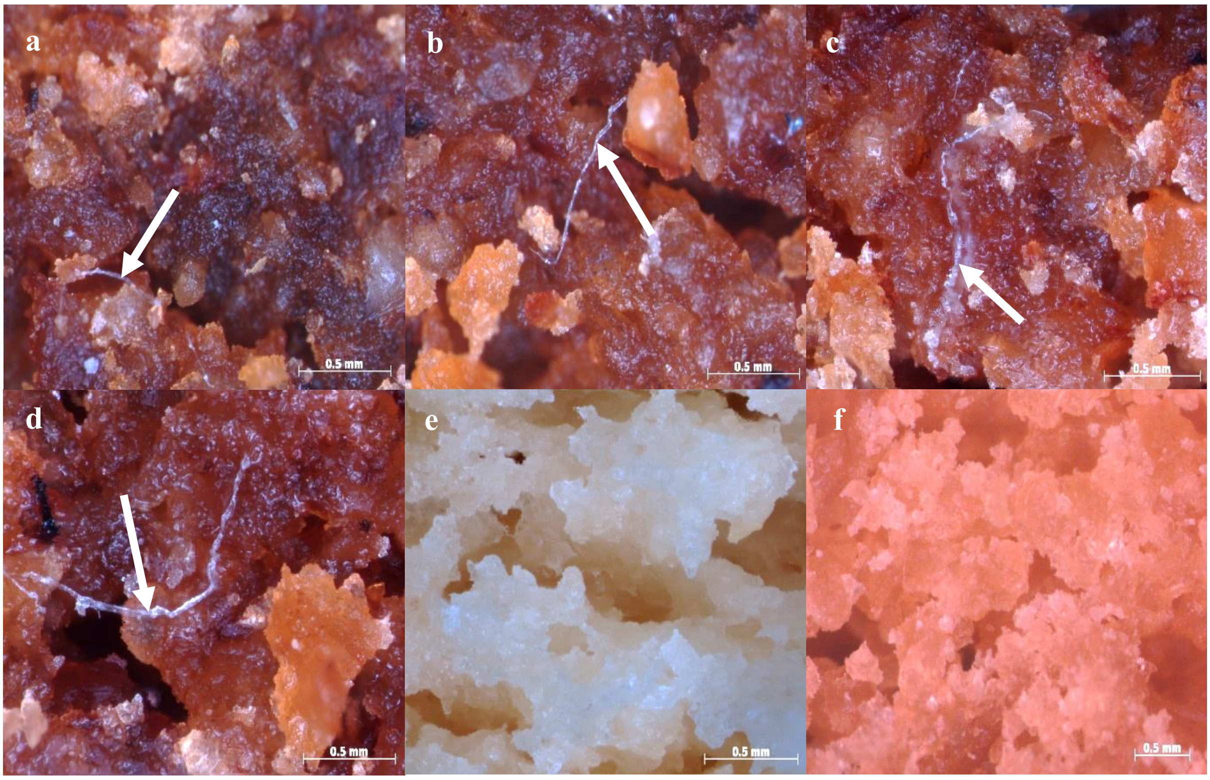

Figure 4.11: Effects of different water levels on the microstructure of sorghum biscuits when compared to wheat biscuit standards. (a) 30%, (b) 35%, (c) 40%, (d) 50%, (e) Marie wheat biscuit, (f) Sugar-snap wheat biscuit. Arrows indicate possible continuous starch gels.

4.3.4.4 Effect of different water levels on the birefringence of starch in sorghum biscuits

Starch granules were observed to be birefringent, partially birefringent and non-birefringent in both the sorghum and wheat biscuits (Fig. 4.12). Presumably, the intact granules were birefringent, the swollen/disrupted granules partially birefringent and the gel non-birefringent. The loss of birefringence in some of the starch granules provides further evidence that some starch granules were indeed gelatinized during baking. Mamat *et al.* (2010) also analysed the morphology of starch granules in wheat biscuits by polarizing light microscopy and stated that starch granules were partially gelatinized due to insufficient water and presence of excess sugar.

The comparison of the birefringence of starch granules in sorghum biscuits at different water levels does not appear to suggest that there were differences in the degree of starch gelatinization. Although, Abboud and Hoseney (1984) reported an increase in the peak temperature of the starch gelatinization endotherms of wheat biscuit doughs when water level was increased, the experiment was conducted in sealed DSC pans so that there is no water loss during heating. These authors attributed the low degree of starch gelatinization in biscuits to considerable water loss during baking. Therefore it is extremely difficult to determine how the rate of water loss during baking affected starch gelatinization in biscuits baked from doughs with different water levels. Since there was no evidence to suggest that an increase in starch gelatinization was responsible for the increase in the hardness of sorghum biscuits as water level increased, sorghum biscuits were then prepared using different levels of pre-cooked sorghum flour to determine the effect of starch gelatinization on biscuit texture.

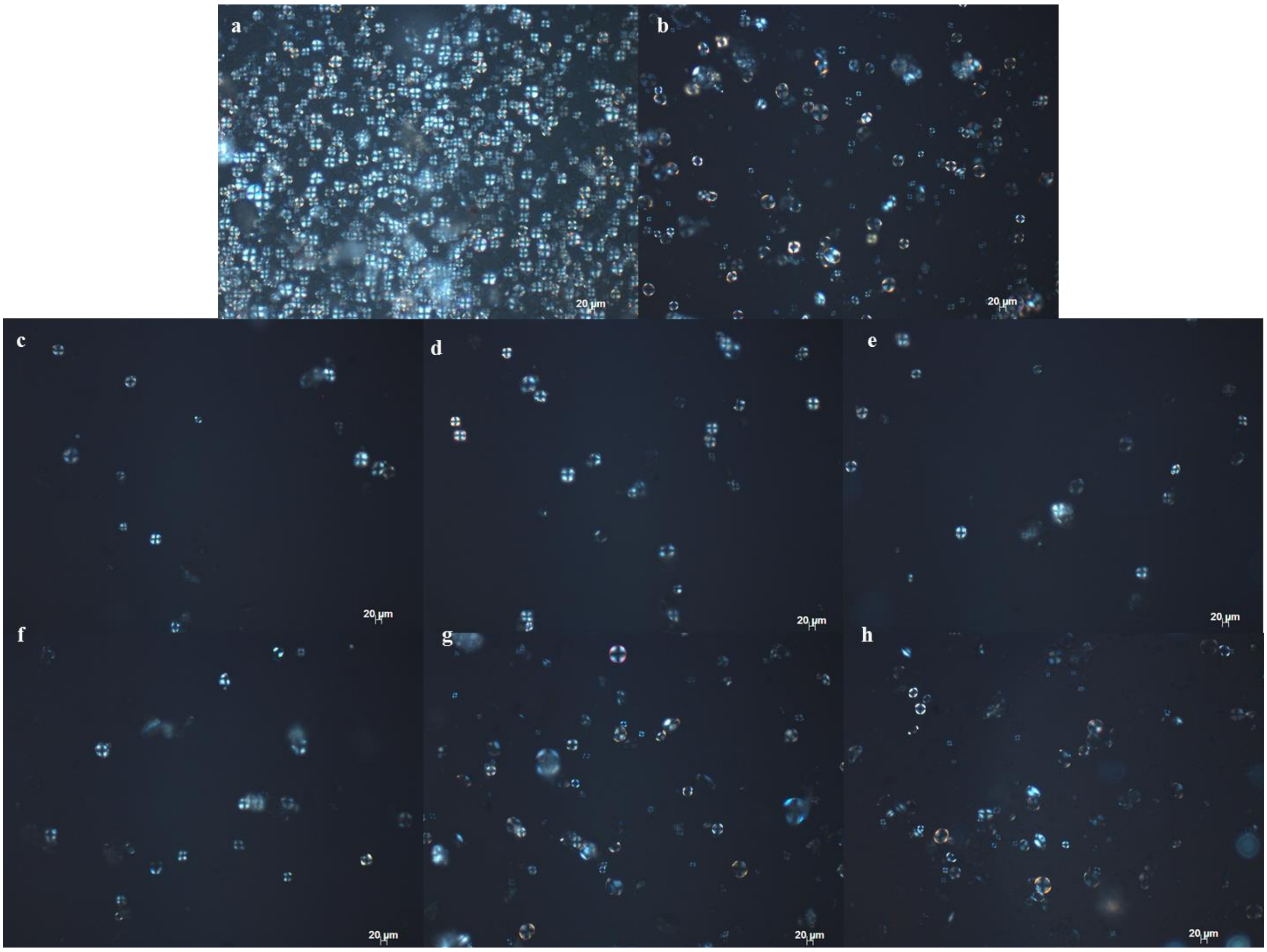

Figure 4.12: Effect of different water levels on the birefringence of starch in sorghum biscuits when compared to the raw flour and wheat biscuit standards. a) Raw sorghum flour, (b) Raw wheat flour, (c) 30%, (d) 35%, (e) 40%, (f) 50%, (g) Marie wheat biscuit, (h) Sugar-snap wheat biscuit.

4.3.4.5 Effect of different ratios of raw to pre-cooked sorghum flour on the appearance of sorghum dough

Addition of different levels of pre-cooked sorghum flour to sorghum flour resulted in doughs with a gritty and coarse texture (Fig 4.13). The coarse nature of the doughs containing pre-cooked sorghum flour appears to be due to undissolved sugar crystals which could be seen as white specs on the dough surface. These doughs also became progressively more dry, more viscous and coarse as the amount of pre-cooked sorghum flour was increased. This could be due to a reduction in the solubilisation of sugar in the doughs. As stated, Hoseney and Rogers (1994) found that when sucrose dissolves in water, each gram creates an additional volume of 0.6 cm^3. This suggests that as more sugar crystals remain undissolved, the fluidity of the liquid phase of the dough becomes reduced, and there is less lubrication of flour particles. Also, the increase in gelatinization of starch granules as a result of cooking may also have contributed to increased viscosity of the doughs. When starch is heated in the presence of sufficient moisture, the granules absorb water and swell (Biliaderis, 1991). As granular swelling continues, amylose leaches out into the intergranular spaces with a resultant increase in viscosity.

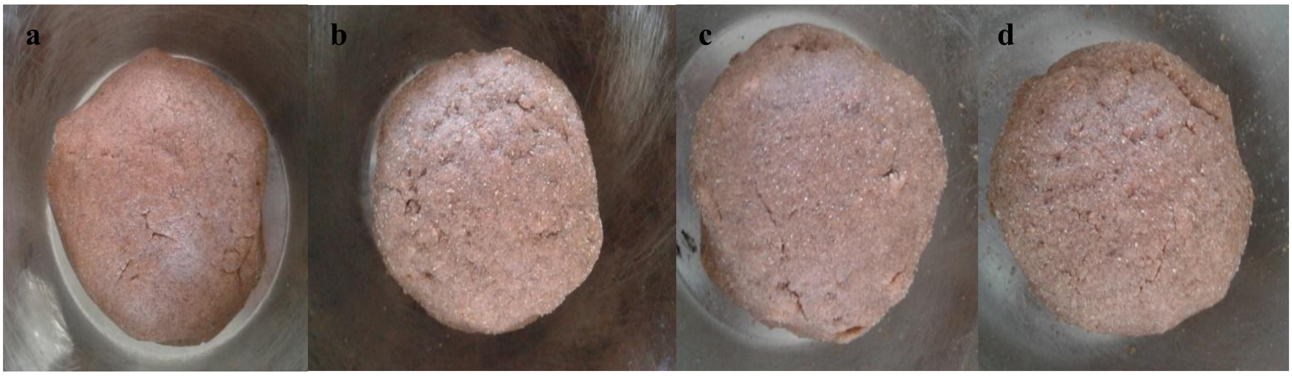

Figure 4.13: Effect of different ratios of raw to pre-cooked sorghum flour on dough appearance. (a) 100:0, (b) 80:20, (c) 60:40, (d) 0:100.

4.3.4.6 Effect of different ratios of raw to pre-cooked sorghum flour on biscuit appearance and texture

Distinct cracks were observed in sorghum biscuits containing pre-cooked sorghum flour (Fig 4.14). This was unlike the biscuits made without pre-cooked sorghum flour which had a smooth and compact surface. It appears the biscuit matrixes became less cohesive as the level of pre-cooked flour increased. Szczesniak (1963) defined cohesiveness as the strength of the internal bonds present in a product. It was earlier observed that swollen starch granules and free starch gels existed in discrete forms within sorghum biscuit matrix (Fig. 4.11). Thus, it was expected that as more of the swollen/gelatinized starch structure was present in the biscuit, fewer starch granules became embedded in the biscuit matrix with a resultant weakening of the internal bonding of the biscuits.

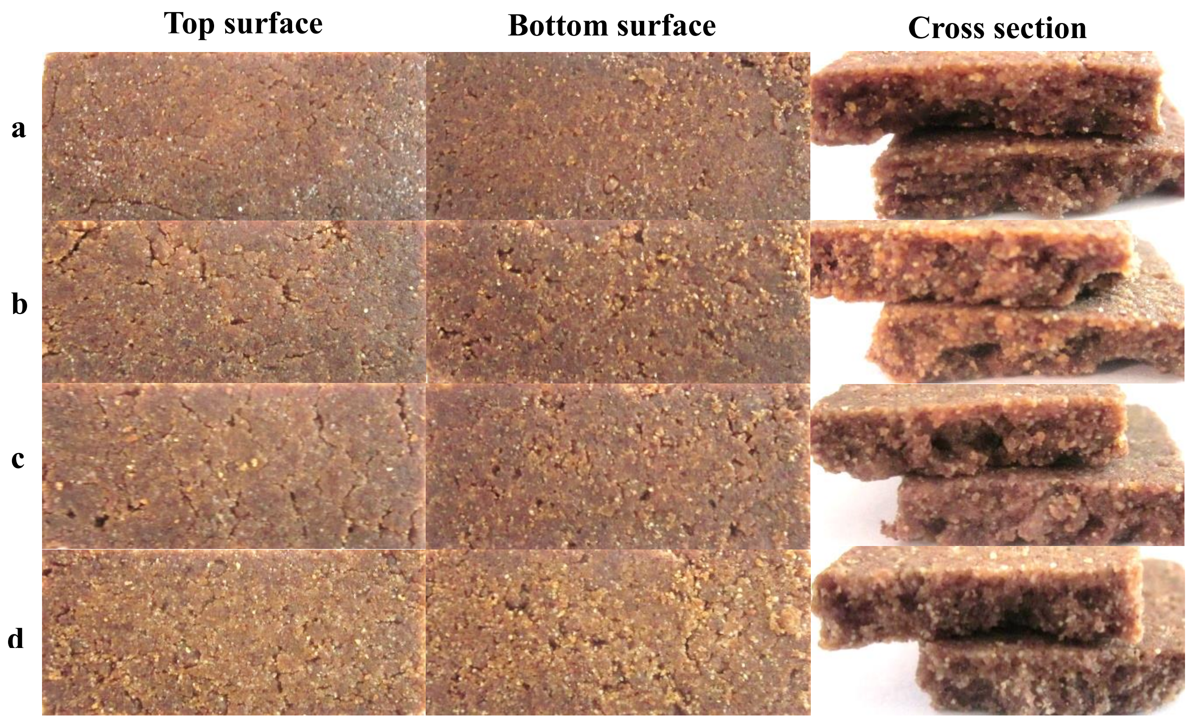

Figure 4.14: Effect of different ratios of raw to pre-cooked sorghum flour on the biscuit appearance. (a) 100:0, (b) 80:20, (c) 60:40, (d) 0:100.

An increase in the level of pre-cooked sorghum flour also reduced the hardness and brittleness of the biscuits (Table 4.8). This was probably due to weakening of the internal bonding in the biscuits as suggested earlier. This is contrary to the statement of Manley (2000) that biscuits with ungelatinized starch are softer to eat. However, this author emphasized that high sugar level could result in hard biscuits due to the effect of super-cooled sugar glass that may be formed when the biscuit is cooled. But since these sorghum biscuit treatments contained the same amount of sugar, sugar level could not be responsible for the difference in texture.

It was stated earlier that sugar dissolved to different extents in sorghum doughs containing different levels of pre-cooked sorghum flour (Fig. 4.13). Could the difference in the extent of sugar dissolution in sorghum doughs be responsible for the difference in texture of sorghum biscuits? This was investigated by microscopic study (section 4.3.4.7).

Table 4.8: Effect of different ratios of raw to pre-cooked sorghum flour on sorghum biscuit hardness

Ratio[2]	Force (N)	Stress (kPa)	Strain (%)	Brittleness (Stress/Strain)
100:0	$15.74^{d} \pm 1.29$[1]	$1.77^{d} \pm 0.15$	$2.46^{b} \pm 0.33$	$0.73^{d} \pm 0.13$
80:20	$12.00^{c} \pm 1.73$	$1.41^{c} \pm 0.21$	$2.78^{c} \pm 0.26$	$0.52^{c} \pm 0.10$
60:40	$8.54^{b} \pm 1.18$	$0.99^{b} \pm 0.24$	$2.83^{c} \pm 0.30$	$0.36^{b} \pm 0.11$
0:100	$3.09^{a} \pm 0.92$	$0.38^{a} \pm 0.14$	$2.78^{c} \pm 0.26$	$0.14^{a} \pm 0.06$
Marie wheat	$16.50^{d} \pm 2.25$	$1.38^{c} \pm 0.19$	$2.00^{a} \pm 0.00$	$0.66^{d} \pm 0.09$
Sugar-snap wheat	$24.57^{e} \pm 2.30$	$1.28^{c} \pm 0.12$	$3.33^{d} \pm 0.00$	$0.38^{b} \pm 0.04$

[1]Mean ± Standard deviation of twelve biscuits (n = 12)
[2]Raw to cooked sorghum flour
[a]Values in a column with different superscripts differ significantly ($p < 0.05$)

4.3.4.7 Effect of different ratios of raw to pre-cooked sorghum flour on the biscuit microstructure

The microstructure of sorghum biscuits showed a reduction in the extent to which intact starch granules were embedded in the biscuit matrix (Fig. 4.15). While intact starch granules could be seen as white specs within the biscuit matrix, swollen granules in their discrete form interrupted the continuity of the starch granules-embedded biscuit matrix. Biscuit microstructure consisted of predominantly swollen granules which were poorly embedded in the biscuit matrix when all the sorghum flour was pre-cooked (Fig. 4.15d). Possibly, the presence of the swollen granules reduced the strength of the intermolecular hydrogen bonding within the biscuit matrix, especially between starch and sugar. Also, the microstructure of biscuits became less homogenous as the level of pre-cooked sorghum flour increase. Saleem *et al.* (2005) showed that large inhomogeneity reduced the stress and strain values of semi-sweet wheat biscuits which is consistent with the reduction in the hardness of sorghum biscuits as the level of pre-cooked sorghum flour increased.

On the other hand, undissolved sugar particles which were present in the sorghum dough were, however, not observed after baking the biscuits. This was presumably due to the sugar melting during baking. Roos (1993) reported an onset melting temperature of 173°C for anhydrous sucrose. The biscuits were baked at 190°C, a temperature sufficient to melt the sugar crystals during baking. This also suggests that the reduction in the hardness of the sorghum biscuits was not due to difference in sugar dissolution during dough making but more likely related to starch gelatinization.

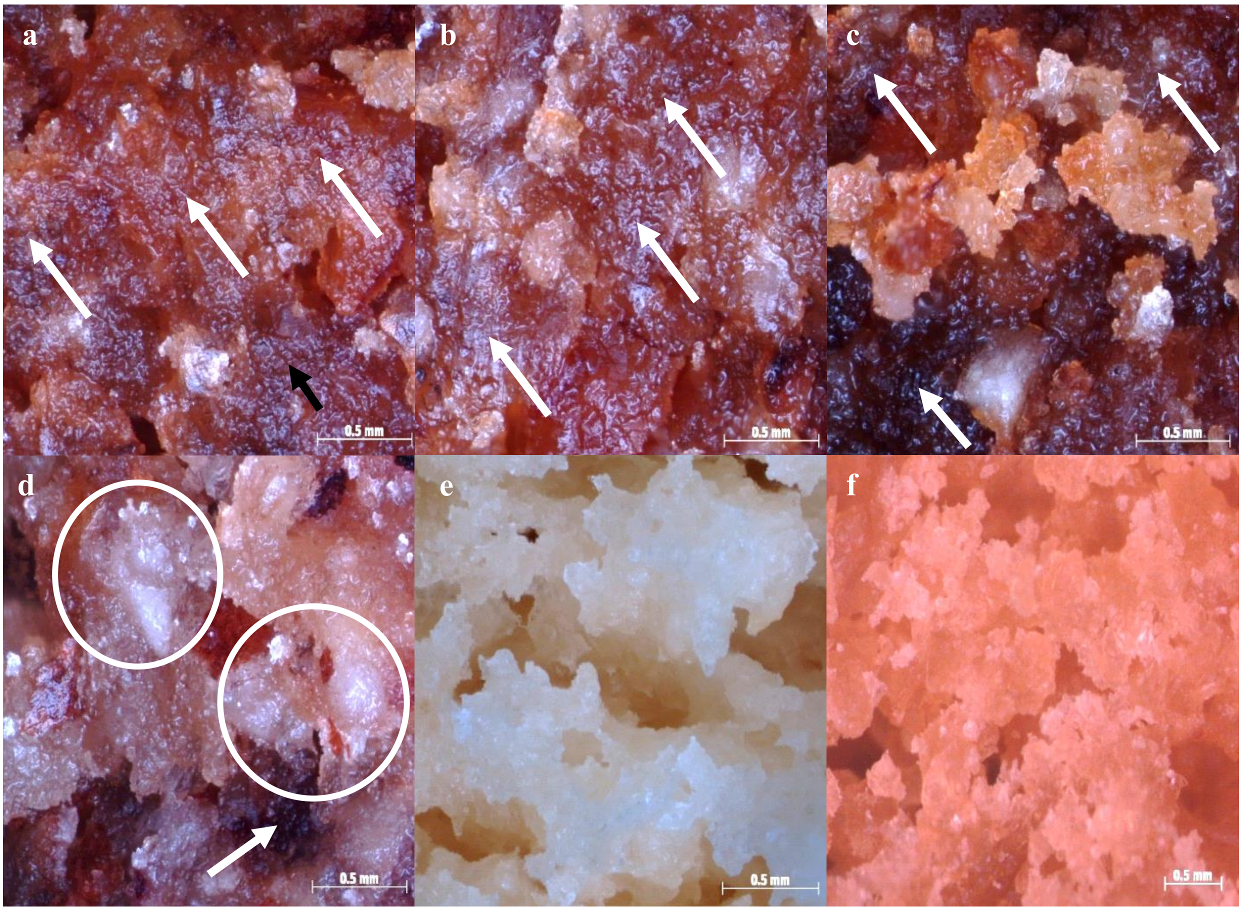

Figure 4.15: Effect of different ratios of raw to precooked sorghum flour on the microstructure of sorghum biscuits when compared to wheat biscuit standards. (a) 100:0, (b) 80:20, (c) 60:40, (d) 0:100, (e) Marie wheat biscuit, (f) Sugar-snap wheat biscuit. Arrows show embedded intact starch granules, circles show poorly embedded swollen starch granules.

4.3.4.8 Effect of different ratios of raw to precooked sorghum flour on biscuit starch birefringence

Notably, the proportion of partially birefringent and non-birefringent starch granules increased as the level of pre-cooked sorghum flour increased (Fig. 4.16). This indicates that, as expected, more starch granules became disrupted/gelatinized as the level of pre-cooked sorghum flour increased. Similar to the birefringence of the starch granules in sorghum biscuits at different water levels, the starch granules were either birefringent, partially birefringent or non-birefringent. This observation provides evidence that an increase in starch gelatinization reduced the hardness of sorghum biscuits, and could not have been responsible for the increase in the hardness of sorghum biscuits when water level was increased.

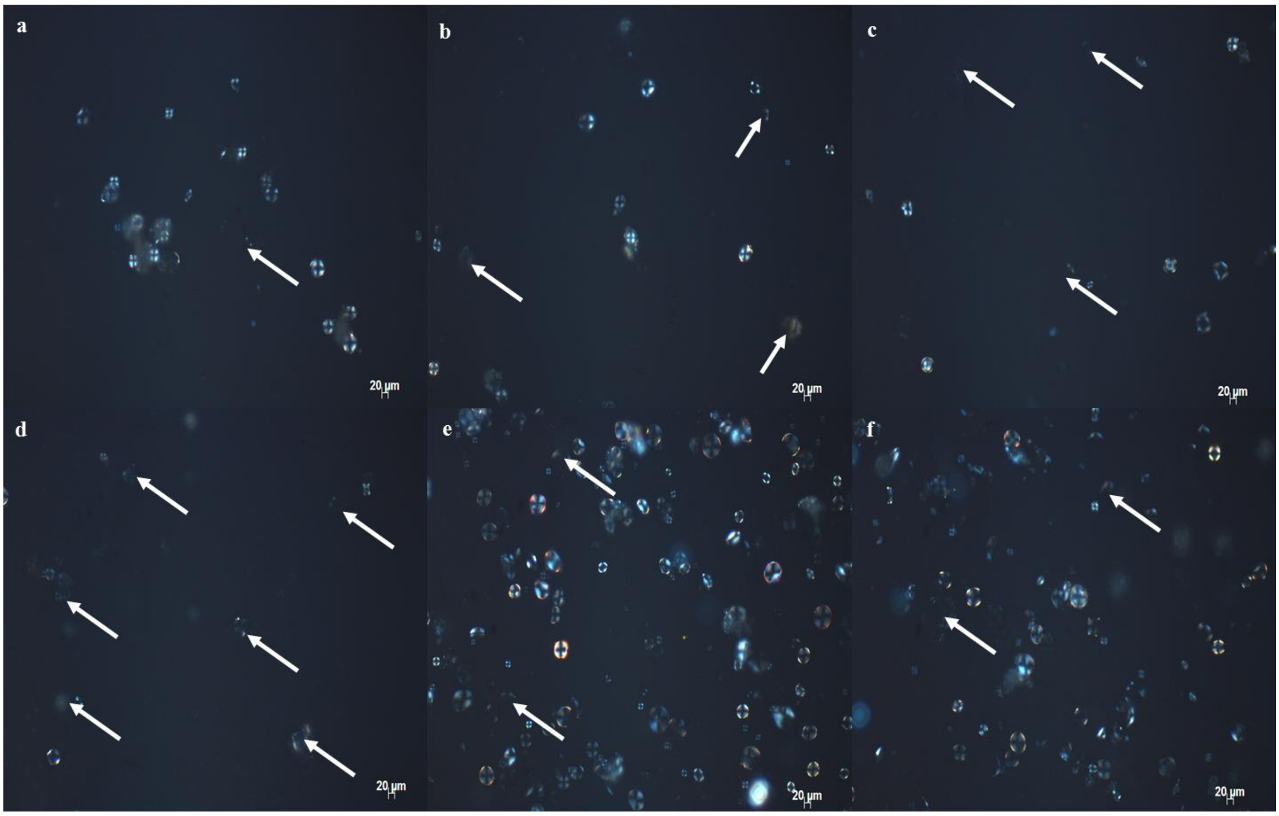

Figure 4.16: Effect of different ratios of raw to pre-cooked sorghum flour on the starch birefringence of sorghum biscuits when compared to the wheat biscuit standards. (a) 100:0, (b) 80:20, (c) 60:40, (d) 0:100, (e) Marie wheat biscuit, (f) Sugar-snap wheat biscuit. Arrows show partial or complete loss of birefringence.

4.3.5 Investigations concerning the role of sugar in the texture of biscuits

4.3.5.1 Effect of different sugar levels (undissolved and pre-dissolved) on sorghum dough

An increase in sugar level increased the softness of sorghum doughs (Fig. 4.17). It was also observed that sorghum doughs with pre-dissolved sugar were softer than doughs with the same level of undissolved sugar. The increase in dough softness can be explained by the increase in liquid volume theory of Hoseney and Rogers (1994). As explained, these authors stated that sugar creates additional liquid volume when it dissolves in water. This suggests that as sugar level is increased, the fluidity of the dough is also increased with a resultant softening of the dough. Manohar and Rao (1997) also found an increase in wheat dough softness as sugar level was increased.

The sorghum doughs prepared by pre-dissolving sugar were softer than the doughs containing the same level of undissolved sugar. This suggests a difference in the level of sugar solubilisation and subsequent increase in liquid volume. Manley (2000) stated that the low level of water used in dough formation prevents total solubilisation of sugar. Furthermore, Abboud and Hoseney (1984) found sugar dissolving endotherm when wheat biscuit dough was analysed by DSC and they concluded that not all the sugar in the dough dissolved during mixing. Also, flour particles compete with sugar for water during mixing (Pareyt and Delcour, 2008), thereby limiting the amount of water available for sugar solubilisation. Therefore separating these two processes (hydration and solubilisation) by pre-dissolving the sugar would permit better solubilisation of sugar. The separation of the two processes would also permit more uniform distribution of the sugar solution during dough mixing, while also enabling the sugar to interact more readily with other components of the dough.

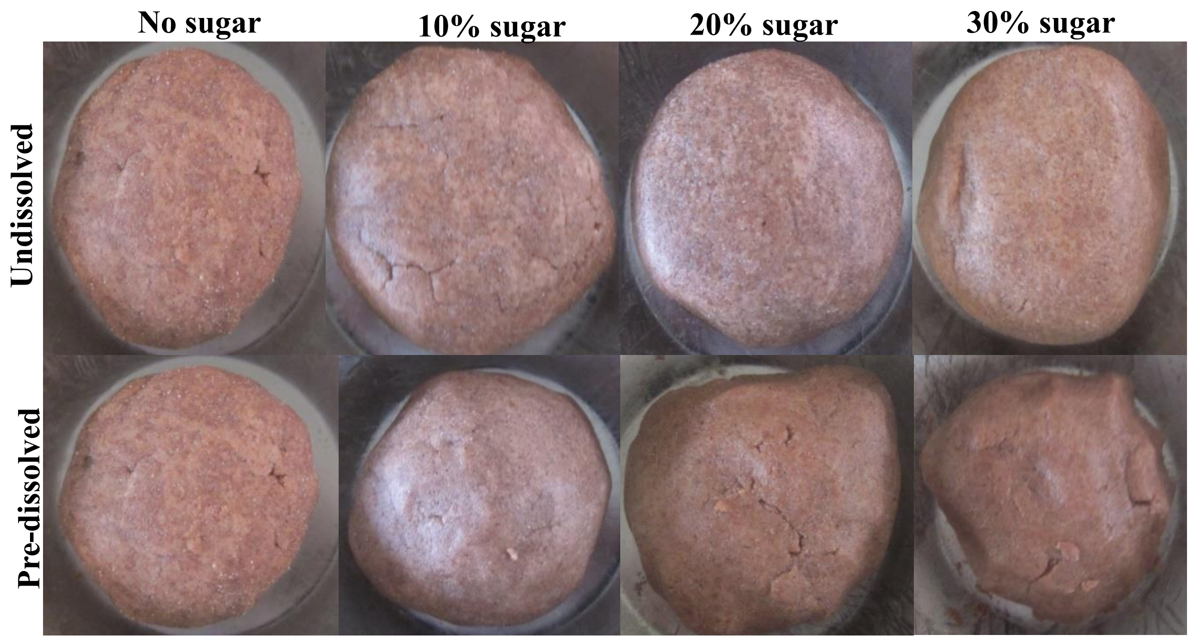

Figure 4.17: Effect of different levels of sugar [undissolved (top) and pre-dissolved (bottom)] on sorghum biscuit dough appearance.

4.3.5.2 Effect of different sugar levels (undissolved and pre-dissolved) on the sorghum biscuits

Visual observation of the sorghum biscuits showed that there was a transition from a light brown colour in biscuits without sugar to a dark brown colour for biscuits containing 30% sugar (Fig. 4.18). However, the colour of the sorghum biscuits made with the same level of pre-dissolved and undissolved sugar was essentially the same. Manohar and Rao (1997) also found that increase in sugar level increased the intensity of the brown colour of wheat biscuits.

Sorghum biscuits made with pre-dissolved sugar seemed to have a smoother appearance when compared to those made with the same level of undissolved sugar. While the differences in the colour of biscuits containing different sugar levels can be related to different levels of caramelization, the difference in surface cracking can be related to the state of the sugar and consequently sugar particle size during dough mixing. Pareyt and Delcour (2008) stated that sugar particle size can influence the surface appearance of biscuits. Lai and Lin (2006) stated that coarse sugar dissolves less readily than the fine sugar and would result in more surface cracking of the baked biscuits. The statement of both set of authors appears to explain the difference in the surface characteristics of sorghum biscuits made with sugar in the liquid or crystalline state.

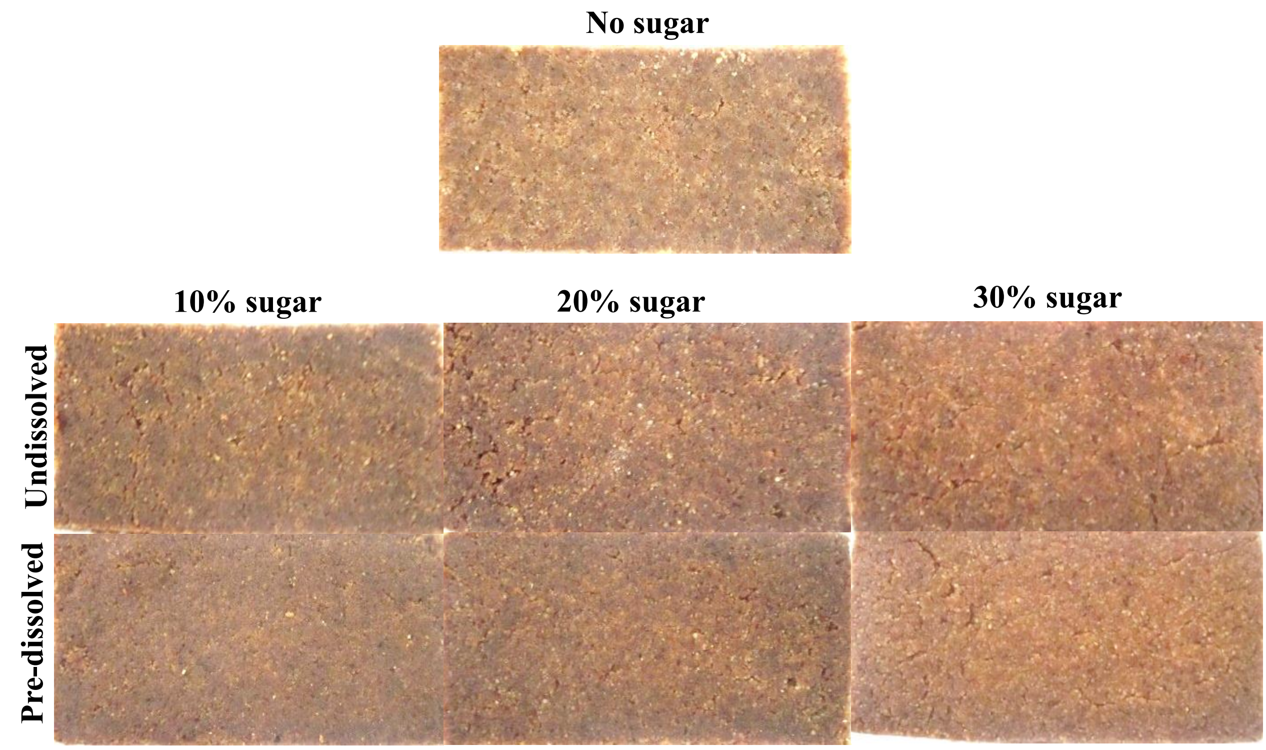

Figure 4.18: Effect of different sugar levels on sorghum biscuits appearance.

4.3.5.3 Effect of sucrose crystallization on the surface characteristics and state of sugar in biscuits

There were crystalline sucrose on the surface of sugar-snap wheat biscuit, while there were no crystals on the sorghum biscuits (Fig. 4.19). The crystallization could be due to sugar that remained un-melted after baking since the wheat dough contained a much higher level of sugar (Sugar-snap wheat 58%, sorghum 30%). Similarly, the crystallization may have been due to sugar-snap wheat dough having a higher sucrose to water ratio (2:1) than sorghum dough (3:4). The higher amount of water used in forming the sorghum dough may have caused dissolution of most or all the sucrose during dough mixing, thereby preventing or limiting sucrose crystallization in the biscuits. Crystallization of sucrose is affected by water content and presence of other ingredients (Bhandari and Hartel, 2002).

Chevalier *et al.* (2000a) found that sucrose crystallized at the surface of wheat shortbread biscuits. These authors proposed that as temperature rises in the oven, the biscuit surface dries and hence, insufficiently hydrated sucrose remains crystalline in the baked biscuits. They also suggested that the temperature at the surface of the biscuit during baking was lower than the melting point of sugar. Although the baking temperature of the biscuit was not specified by these authors, Raemy and Schweizer (1983) found onset and peak melting temperatures of 160°C and 185°C, respectively, for sucrose.

Doescher and Hoseney (1985) linked the surface cracking pattern of biscuits to the ability of sucrose to recrystallize. These authors replaced 10% sucrose with different syrups and found that surface cracking in the biscuits did not occur. It is important to state that these authors referred to the formation of these surface cracks as "islanding", highlighting how intense, deep and numerous these cracks were. The cracks in sorghum biscuits were not intense and not likely due to sucrose recrystallization. With baking at a temperature (190°C) higher than the peak melting temperature of sucrose (185°C) (Raemy and Schweizer, 1983), it is unlikely that sucrose would crystallize from the sugar melt. However, even if sugar crystals were present, they would unlikely affect biscuit texture. Hoseney and Rogers (1994) stated that sugar crystals are free flowing in the wheat biscuit matrix and their presence does not suggest they influence hardness.

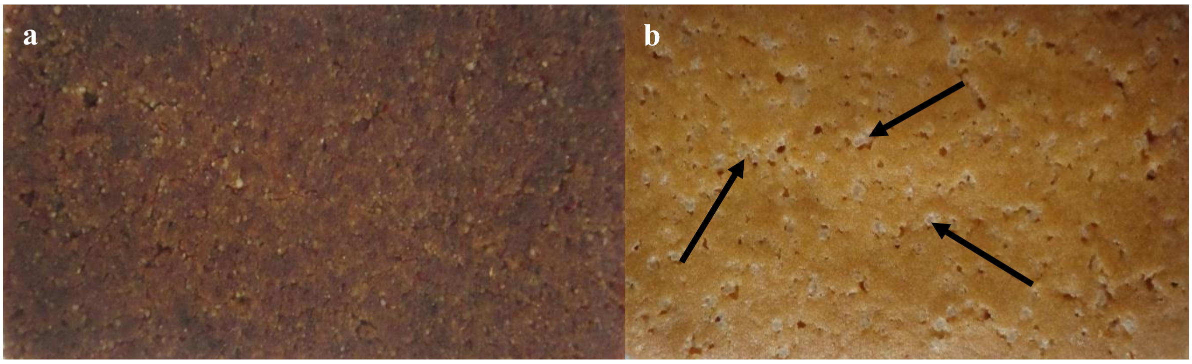

Figure 4.19: Effect of sucrose crystallization on the surface appearance of biscuits. (a) Sorghum biscuit* (b) sugar-snap wheat biscuit. Arrows indicate sucrose crystals. *Sorghum biscuits prepared with 30% sugar during dough formulation as sucrose has a high tendency to crystallize at this concentration.

DSC thermal analysis of sugar and biscuits (Figs. 4.20 and 4.21) confirmed the presence of sucrose as a sugar glass matrix in both sorghum and wheat biscuits. The absence of starch gelatinization endotherms was due to the absence of water in the DSC pans. While crystalline sucrose was observed to have melting peak of 193°C, sugar glass did not melt (Fig. 4.20). Raemy and Schweizer (1983) reported onset and peak melting temperatures of 160°C and 185°C for sucrose, respectively. Roos (1993) found onset and peak melting temperatures of 173°C and 190°C, respectively. Similarly, only biscuits containing 25% added crystalline sucrose showed a melting endotherm during analysis (Fig. 4.21). This is evidence that the sucrose in the biscuits was present as a glassy amorphous matrix. However, unlike pure crystalline sucrose, melting peaks of sucrose added to biscuits occurred between 183°C and 186°C. This lower melting temperature was probably due to the presence of other components of the biscuit as impurities. Roos and Karel (1991) found that traces of moisture decreased the onset melting temperature and heat of fusion of different sugars. However, contrary to the suggestion of Hoseney and Rogers (1994) that amorphous sucrose develops mobility and could crystallize during DSC analysis of sugar-snap biscuits, no recrystallization exotherm for sucrose was found in any of the biscuits analysed.

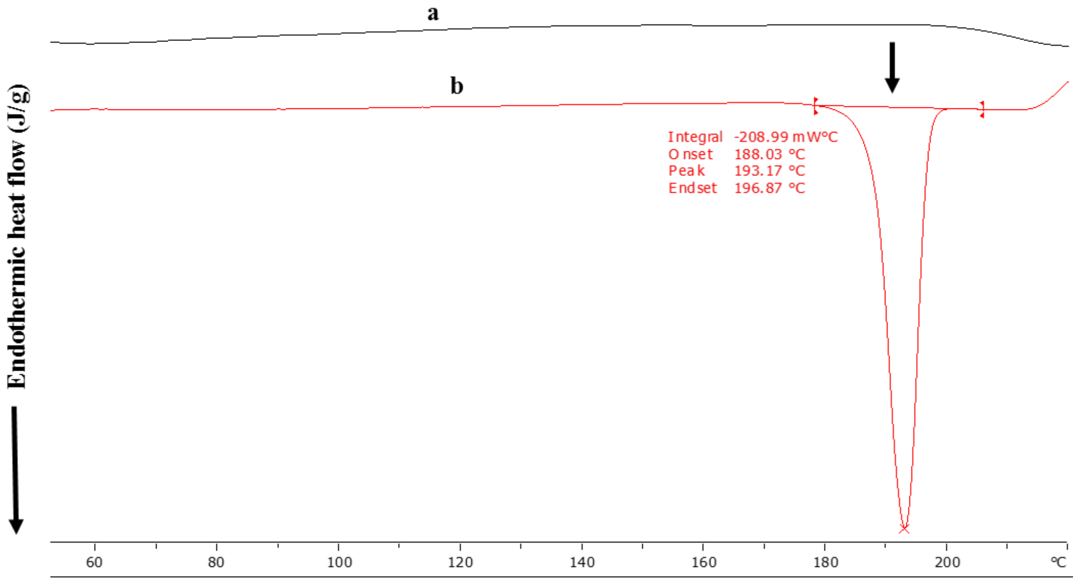

Figure 4.20: DSC thermal analysis of sucrose. (a) Sugar glass, (b) crystalline sucrose. Arrow indicates melting endotherm of crystalline sucrose.

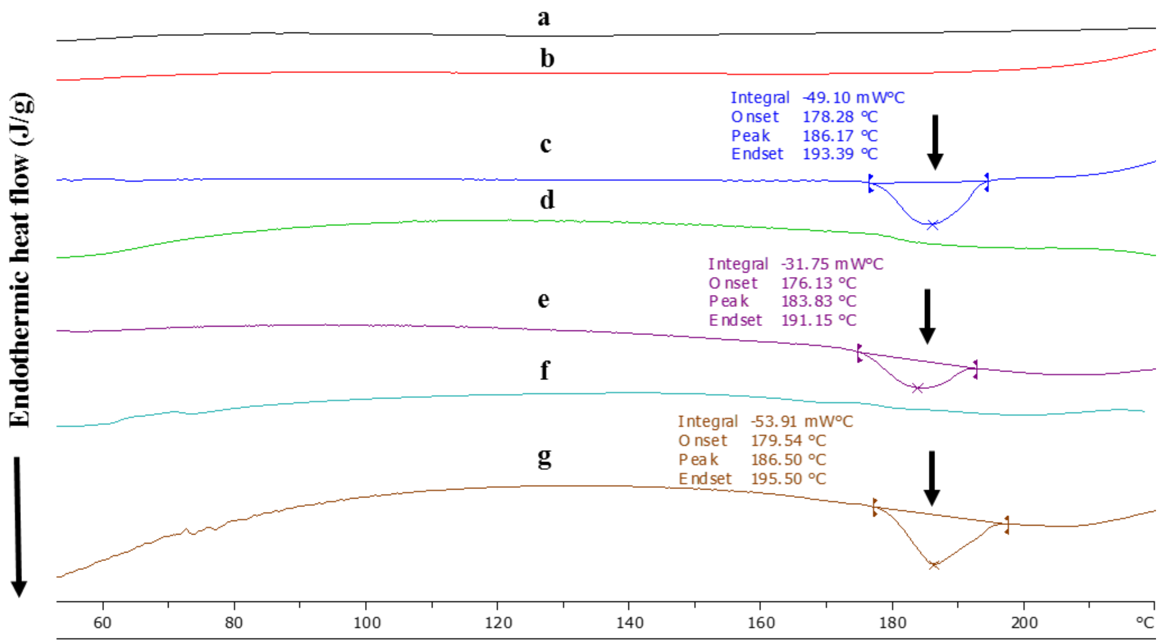

Figure 4.21: DSC thermal analysis of sorghum and wheat biscuits. (a) Sorghum biscuit without sugar, (b) Sorghum biscuit*, (c) Sorghum biscuit* with 25% crystalline sucrose, (d) Marie wheat biscuit, (e) Marie wheat biscuit and 25% crystalline sucrose, (f) Sugar-snap wheat biscuit, (g) Sugar-snap wheat biscuit and 25% crystalline sucrose. *Sorghum biscuit prepared with 30% sugar during dough formulation. Arrows indicate melting endotherm of crystalline sucrose.

Similar to DSC findings, XRD showed difference between the diffraction patterns of crystalline sucrose and sugar glass (Fig. 4.22a, b). However, unlike in sorghum and Marie biscuits which did not exhibit crystalline peaks, a singular peak similar to that of crystalline sucrose occurred with portion from the centre of sugar-snap wheat biscuits (4.22f), while more similar peaks were observed from the surface of the biscuit (Fig 4.22i). The diffraction patterns of the biscuits were mostly similar to that of sugar glass and to starch granules which were present in raw flour. The detection of crystalline sucrose peaks from the surface of sugar-snap wheat biscuit was expected since sucrose crystals were visible on the surface (Fig. 4.19). Sugar crystals in the centre of sugar-snap wheat biscuits has been reported (Hoseney and Rogers, 1994). Similarly, Chevallier *et al*. (2000a) also observed sucrose diffraction peaks on the surface of wheat shortbread biscuits, but no diffraction peaks were detected in the biscuit centre.

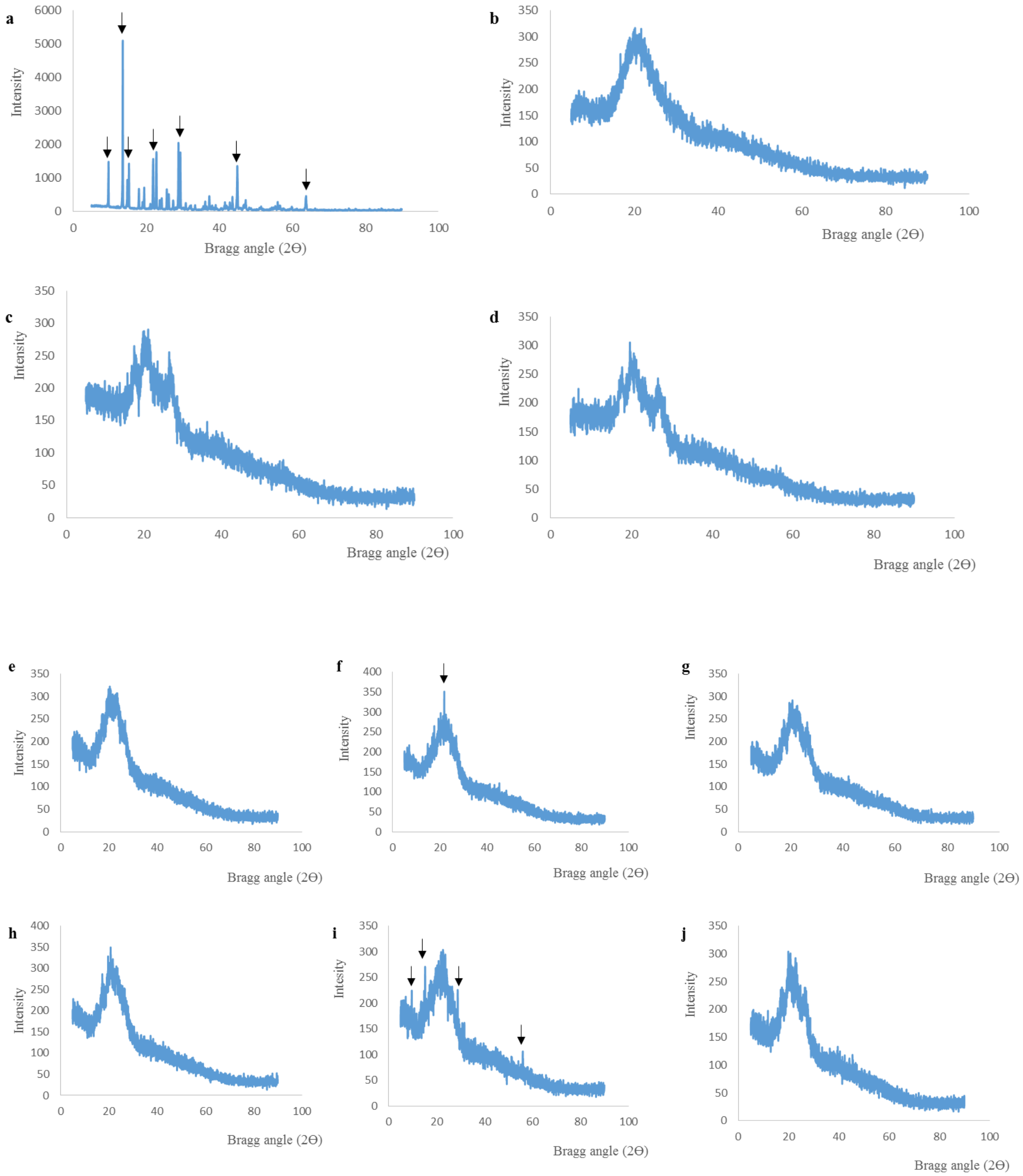

Figure 4.22: Diffraction patterns of sugar, flour and biscuits. (a) Crystalline sucrose, (b) sugar glass, (c) sorghum flour, (d) wheat flour, (e) sorghum biscuit* (centre), (f) Sugar-snap wheat biscuit (centre), (g) Marie wheat biscuit (centre), (h) sorghum biscuit* (top), (i) sugar-snap wheat biscuit (top), (j) Marie wheat biscuit (top). Arrows show detected diffraction sucrose peaks. *Sorghum biscuit prepared with 30% sugar during dough formulation.

4.3.5.4 Effects of different sugar levels and state (undissolved and pre-dissolved) on the hardness and brittleness of sorghum biscuits

An increase in sugar level increased the hardness of sorghum biscuits (Table 4.9). However, the state of sugar during dough mixing had no significant effect ($p \geq 0.05$) on biscuit hardness and brittleness. This is further evidence that differences in the extent of sugar dissolution did not affect biscuit hardness as was observed with different ratios or raw to pre-cooked sorghum flour (section 4.3.4.7). Manohar and Rao (1997) similarly found that increasing sugar level increased the hardness of wheat biscuits. In the absence of sugar, sorghum biscuits were extremely soft and crumbly. Both DSC and XRD indicated that the sugar in sorghum and wheat biscuit was an amorphous glass.,This suggests that an amorphoussugar glass in the biscuit matrix was responsible for holding the biscuit structure together. Perhaps, an increase in sugar level increased the continuity of this sugar glass, thereby allowing it to form a stronger network within the sorghum biscuit.

Slade *et al.* (1993) described the structure of sugar-snap wheat biscuits as comprising undeveloped gluten, fat and a continuous glassy sucrose matrix within which ungelatinized starch granules are embedded. Similarly, Chevallier *et al.* (2000a) described the structure of shortbread wheat biscuit as a continuous phase of molten sugar embedding proteins, starch granules and lipids. With sorghum kafirins remaining intact in their protein bodies, and sugar indicated to be present as a glassy matrix in sorghum biscuits, it is likely that the starch granule-embedded matrix observed in sorghum biscuits was starch granules embedded in a sugar glass. Possibly, stronger interaction between the sugar glass and starch granules was responsible for the increase in sorghum biscuit hardness when sugar level was increased.

Table 4.9: Effects of different sugar levels and state (undissolved and pre-dissolved) on sorghum biscuit hardness

Sugar level on flour basis (%)	State of Sugar	Force (N)	Stress (kPa)	Strain (%)	Brittleness (Stress/Strain)
0	No sugar	$3.74^{a} \pm 0.37^{1}$	$0.40^{a} \pm 0.07$	$2.83^{b} \pm 0.30$	$0.14^{a} \pm 0.04$
10	Undissolved	$7.17^{b} \pm 0.70$	$0.79^{b} \pm 0.17$	$2.83^{b} \pm 0.30$	$0.29^{abc} \pm 0.08$
	Pre-dissolved	$6.74^{b} \pm 0.44$	$0.75^{ab} \pm 0.12$	$2.78^{b} \pm 0.26$	$0.27^{ab} \pm 0.06$
20	Undissolved	$11.96^{c} \pm 1.73$	$1.26^{c} \pm 0.20$	$2.83^{b} \pm 0.30$	$0.46^{cd} \pm 0.10$
	Pre-dissolved	$12.70^{c} \pm 1.93$	$1.38^{c} \pm 0.38$	$2.83^{b} \pm 0.30$	$0.50^{de} \pm 0.16$
30	Undissolved	$24.24^{e} \pm 2.16$	$2.61^{d} \pm 0.54$	$2.83^{b} \pm 0.30$	$0.95^{f} \pm 0.26$
	Pre-dissolved	$22.39^{e} \pm 1.80$	$2.41^{d} \pm 0.52$	$2.83^{b} \pm 0.30$	$0.87^{f} \pm 0.24$
Marie wheat	NA	$16.50^{d} \pm 2.25$	$1.38^{c} \pm 0.19$	$2.00^{a} \pm 0.00$	$0.66^{e} \pm 0.09$
Sugar-snap wheat	NA	$24.57^{e} \pm 2.30$	$1.28^{c} \pm 0.12$	$3.33^{c} \pm 0.00$	$0.38^{bcd} \pm 0.04$

[1]Mean ± Standard deviation of twelve biscuits (n = 12)
[a]Values in a column with different superscripts differ significantly ($p < 0.05$)
NA = Not applicable

4.3.5.5 Effect of different sugar levels on sorghum biscuit microstructure

As previously noted, the structure of wheat biscuits contrasted with that of sorghum biscuits. In addition to the continuous starch granules-embedded matrix of sorghum biscuits, swollen starch granules were predominant in sorghum biscuits without sugar (Fig 4.23a). These swollen starch granules became less prominent as the level of sugar was increased, while at the highest sugar level, starch granules were observed to form clusters at different points in the biscuit matrix (white arrows) (Fig. 4.23d). The clustering of starch granules at 30% sugar was probably due to supersaturation of the sugar glass matrix by the flour particles which allowed the sugar glass form a stronger network with starch granules. The presence of more swollen starch granules in sorghum biscuits without sugar is understandable because of the influence of sugar on starch gelatinization. Several authors including Ghiasi *et al*. (1983) and Aboud and Hoseney (1984) found that sugar increased starch gelatinization temperature.

The presence of sugar increases the gelatinization temperature of starch and renders the granules mostly ungelatinized in biscuits. Abboud and Hoseney (1984) found that small portion of the starch granules in wheat biscuit dough gelatinized at 100°C, while gelatinization temperature rose up to 135°C at higher sugar concentration. Chiotelli *et al.* (2000) considered this phenomenon to be due to an interplay of factors such as the anti-plasticizing properties of sugar relative to water, lowered water activity of the system and stabilization of the granular structure due to starch-sugar interactions. Since an increase in sugar level reduces starch gelatinization and also increases biscuit hardness, it is improbable that an increase in starch gelatinization increased biscuit hardness when water level was increased (Section 4.3.4.2 and Table 4.7). Furthermore, since swollen starch granules are usually not embedded in the biscuit matrix, increased sugar level would reduce the population of swollen granules since less water is available to the starch granules. This would result in more intact starch granules becoming embedded in the sorghum biscuit matrix, thereby creating a stronger starch network in the biscuit.

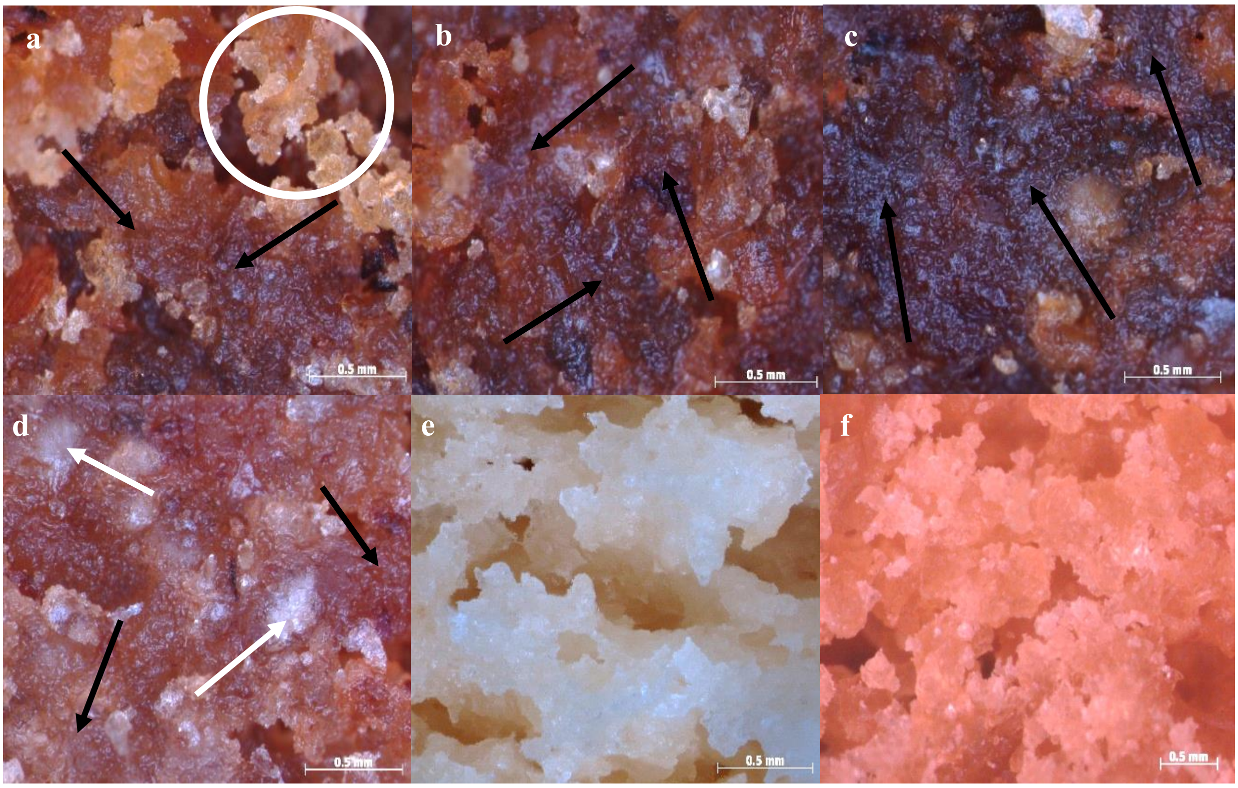

Figure 4.23: Effect of different sugar levels on sorghum biscuit microstructure when compared to wheat biscuit standards. (a) 0% sugar, (b) 10% sugar, (c) 20% sugar, (d) 30% sugar, (e) Marie wheat biscuit, (f) Sugar-snap wheat biscuit. Circle indicates swollen starch granules, black arrows indicate starch granules embedded in biscuit matrix, and white arrows indicate clusters of starch granules.

4.3.5.6 Effect of different sugar levels on the interaction of starch and sugar in biscuits

As was suggested, polarizing light microscopy indicated that starch granules were embedded in the biscuit matrix through interactions with sugar glass (Fig. 4.24). In the absence of sugar in the sorghum biscuits (Fig. 4.24c), the structure was an aggregate of intact and disrupted starch granules, while in the presence of sugar, the starch granules appeared to form networks with the sugar glass. At the highest sugar level (30%), the sugar glass surrounded the starch granules (Fig. 4.24f), suggesting that the sugar glass was more continuous and formed stronger sugar glass-starch networks within the biscuit matrix. The result of this network was an extremely hard sorghum biscuit (Table 4.9).

Similarly, the starch granules in wheat biscuits were embedded in the sugar glass. With electron microscopy (SEM and TEM) indicating interaction between starch and gluten in wheat biscuits (Figs. 4.7 and 4.8), it appears the sugar glass is the bridge between starch and gluten as suggested by Chevallier *et al.* (2000a). These authors stated that sugar melt could form a bridge between starch and protein particles in wheat biscuits. They added that sugar could also form a continuous phase of molten sugar embedding starch, protein and lipids.

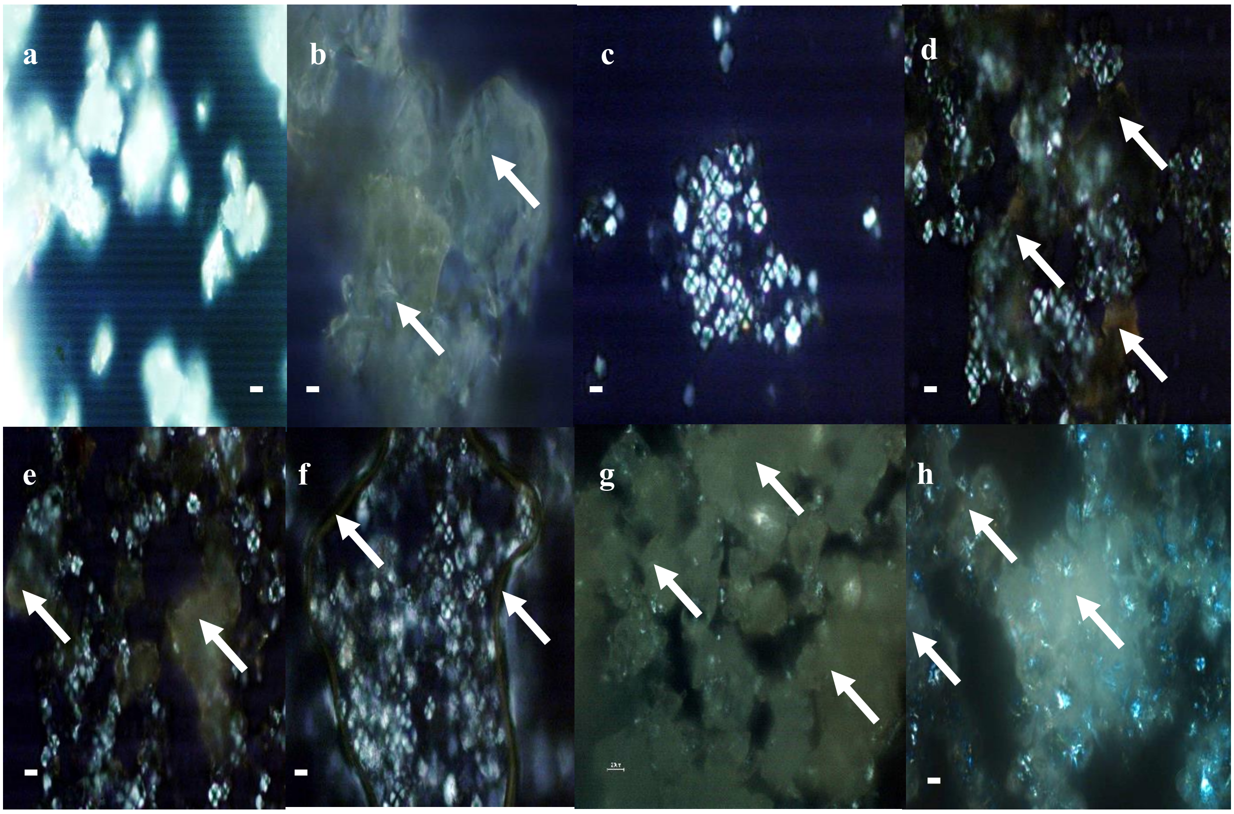

Figure 4.24: Effect of different sugar levels on the interaction between starch and sugar glass when compared to wheat biscuit standards in ethanol. (a) Crystalline sugar, (b) sugar glass, (c) sorghum biscuit with no sugar, (d) sorghum biscuit with 10% sugar, (e) sorghum biscuit with 20% sugar, (f) sorghum biscuit with 30% sugar, (g) Marie wheat biscuit, (h) Sugar-snap wheat biscuit. Bar is 20 µm. Arrows indicate sugar glass.

4.3.6 Effects of oil on the structure and texture of sorghum biscuits

A comparison of the microstructure of un-defatted and defatted sorghum biscuits showed that oil also formed a continuous matrix with the sugar glass (Fig. 4.25). A question is then whether both the sugar glass and oil are responsible for holding the biscuit structure together? The sorghum biscuit structure was still intact after defatting with the only difference being in the loss of the oily gloss in the microstructure of the defatted biscuit (Fig. 4.25b). This provides further evidence that the sugar glass is responsible for holding the biscuit matrix together.

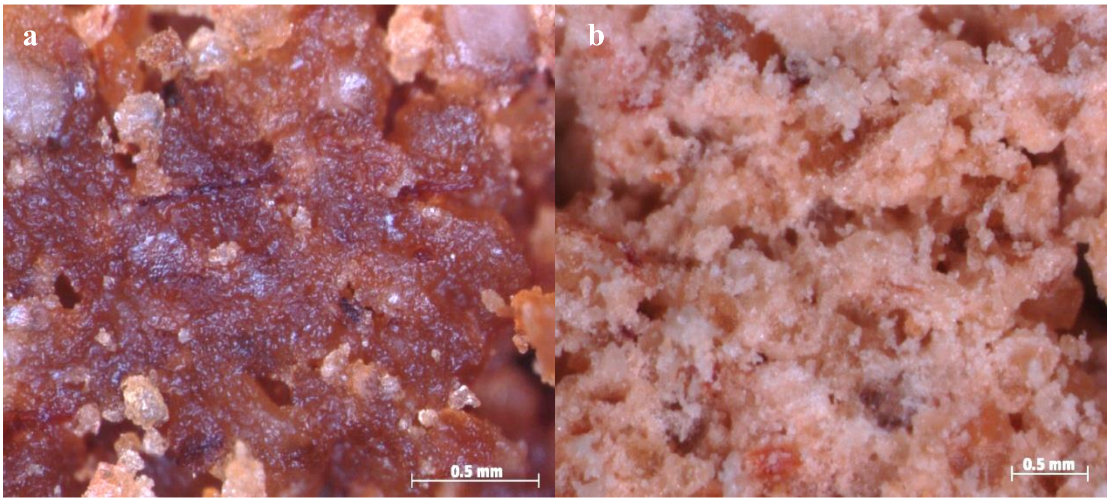

Figure 4.25: Effect of defatting on the microstructure of sorghum biscuit. (a) Un-defatted sorghum biscuit, (b) defatted sorghum biscuit.

4.4 CONCLUSIONS

This work shows that the science of the texture of sorghum biscuits lies in the optimization of the level of water and sugar used in dough formulation and also how the different components of the biscuit interact in order to compensate for the absence of gluten. A sugar glass matrix is responsible for the cohesiveness of the biscuit and also holds the biscuit matrix together. The sugar glass appears to form networks with the starch granules and the strength of this network determines the hardness of sorghum biscuits.

5 GENERAL DISCUSSION

This discussion critically reviews the strengths and weaknesses of the major methodologies applied in this research work. It then discusses how the components of both sorghum and wheat biscuits contribute to their textures based on the research findings. Lastly, a model for the crumb structure of sorghum biscuits is proposed.

5.1 Methodological appraisal

A major technological challenge in baking the biscuits was the type of oven used. Industrially, biscuits are mostly baked in travelling ovens (Manley, 2000), whereas a static oven was used in this work. Travelling ovens have a moving steel band which conveys biscuit dough pieces through the baking tunnel (Manley, 2000). Lawson (1994) stated that the oven is in sections, with each section having its own independent heat source and control systems. Manley (2000) further explained that in travelling ovens, baking conditions such as temperature, humidity and moving speed can be adjusted during baking to improve overall biscuit quality. The author added that because it is not normally possible to quickly change the temperature of a static oven, the resulting biscuits are normally different from those of travelling oven. With the oven used in this work, although it was a small-scale commercial bakery type oven, it was difficult to ensure specific oven conditions at different stages of baking. Furthermore, oven temperature fluctuated and rose up to about 10°C above the set temperature of 190°C before decreasing to the set temperature. A different oven with a stable thermostatic control could have been used.

While hand-feel was used to describe the softness of sorghum doughs, dough textural analyses could also have been done instrumentally to better understand how changes in dough properties influenced biscuit texture. For instance, the texture analyser used to determine biscuit strength could be used to determine the hardness/softness and stickiness of sorghum dough through a compression test (Pareyt *et al.*, 2008). Likewise, sorghum dough extensibility could be determined using a Kieffier rig. Kulamarva (2005) used a texture analyser to determine the hardness, gumminess and cohesiveness of sorghum doughs. In addition, thermal analysis of sorghum dough could also help explain the changes that occurred during baking. For example, thermo-analysis of sorghum dough by DSC could provide evidence of possible starch gelatinization and sugar dissolving or melting.

Sorghum biscuits were made with different water levels to determine the effect of starch gelatinization on biscuit texture (Table 4.1). Although the chosen water levels (30%, 35%, 40%, and 50%) were used to ensure that a workable dough was formed, the range of water levels was probably too narrow. Perhaps, a wider and lower range such as 10%, 20%, 30%, and 40% could have been used, especially since it was observed that the formulation resulted in a batter with 50% water (Fig. 4.9d). It would also have been logical to make biscuits from sorghum dough without water. This would have helped to better understand the effect of water level on starch gelatinization and its subsequent effect on biscuit texture. In this regard, it is pertinent that wheat doughs can be made without adding any water in the presence of very high level of fat (Manley, 2000).

To investigate the effect of possible starch gelatinization on sorghum biscuit texture, different levels of pre-cooked sorghum flour were added to raw sorghum flour. Pre-cooked sorghum flour portions were prepared by adding the same water level to different quantities of sorghum flour (Table 4.2). While the idea was to ensure progressive starch gelatinization as flour level was decreased, another possibly better approach would have been to maintain a constant flour to water ratio in the pre-cooked sorghum flour portions so that there was an equal degree of starch gelatinization. As such, the difference in texture observed in sorghum biscuits prepared with different ratios of pre-cooked sorghum flour could then be attributed to differences in degree of starch gelatinization during baking. Alternatively, different ratios of powdered commercial pre-gelatinized starches, such as pre-gelatinized maize starch, could be used to replace some of the sorghum flour during dough making.

While sugar-snap wheat biscuits were made according to a standard method, another type of wheat biscuit could have been made using the same recipe as used for the sorghum biscuits, but with less water to reduce the tendency of gluten formation. This would have enabled a more direct comparison between the functionality of protein, starch, oil and sugar in both sorghum and wheat biscuits. It was proposed that the high sugar to water ratio of sugar-snap wheat dough (2:1) was responsible for sucrose crystallization in sugar-snap wheat dough, while the low sugar to water ratio of sorghum dough (3:4) could not allow for sucrose crystallization in sorghum biscuits. This suggestion could have been directly tested by making wheat biscuits from a wheat dough with the same sugar to water ratio as the sorghum dough, and also by making sorghum biscuits from a dough containing the same sugar to water ratio as the sugar-snap wheat dough. Also, another type of wheat biscuits could have been made without sugar to better understand the influence of sugar on the structure and texture of wheat biscuits.

In the research, defatted sorghum and wheat biscuits were used for SEM and TEM crumb structural analysis. This was done to prevent interference from the oil. For SEM, the presence of oil in the biscuit crumbs could prevent proper coating of the biscuit surface by the carbon layer. This is because the oil could adsorb onto the coated carbon (Ahmad *et al*., 2005) and interfere with or prevent backscattering of secondary electrons. This could lead to the charging phenomenon in which charges build up on the surface of sample and lead to distortion of images (Aguilera and Stanley, 1990). Likewise, in TEM the osmium tetroxide fixative that was used is suitable for fixation of lipids (Aguilera and Stanley, 1990) and could produce image artefacts. Conversely, defatted biscuits could also have been used for the analyses of starch and sugar by polarizing microscopy. In fact, it was observed that some starch granules and the sugar glass were covered by a layer of oil (Figure not included). Regions where starch granules and sugar glass were clear and not covered by oil had to be found in order to avoid any possible interference of the oil.

5.2 How the components of sorghum and wheat biscuits contribute to their texture

The state and role of protein, starch and sugar in sorghum and wheat biscuits is summarized (Tables 5.1–5.3). SEM and TEM revealed that the sorghum kafirins protein did not participate in dough formation as they remained apparently unchanged in their protein bodies and hence, were unlikely to influence the structure and texture of sorghum biscuits. In contrast, a continuous gluten network appeared to be developed in the sugar-snap wheat biscuits even though it may not have been present during dough mixing. Similarly, the observed presence of a gluten sheet in the Marie wheat biscuits as revealed by SEM and TEM was expected as it is a hard-sweet biscuit type, which are reported to contain a gluten network (Manley, 2000).

The gluten partially enveloped the ungelatinized starch granules in the sugar-snap wheat biscuits, as observed by SEM (Fig. 4.7). This is similar to the findings of Pareyt *et al*. (2010). These authors described the gluten as a clay-like substance, similar to the sheet of gluten observed in this current work. SEM also revealed that the Marie wheat biscuit had sheets of gluten enveloping its starch granules, but it had denser protein-starch granules matrix than the sugar-snap wheat biscuits. This difference in density of protein-starch granules matrix between the two types of wheat biscuits was further indicated by TEM (Fig. 4.8). Pareyt *et al.* (2010) stated that due to conformational changes, gluten proteins are able to crosslink through a sulphydryl-disulphide (SH-SS) interchange leading to the polymerization of glutenins and gliadins into a gluten network. Lagrain *et al*. (2008) found that at temperatures exceeding 90°C, free SH-groups of glutenin can induce covalent linkage with gliadin through a heat-induced SH-SS exchange mechanism. Furthermore, Pareyt *et al*. (2010) also found that the addition of an oxidizing agent increased the crosslinking of the gluten proteins and this increased the envelopment of starch granules by the clay-like gluten network. These mechanisms support the concept of gluten formation in wheat biscuits.

It was observed that sorghum doughs generally became softer as water and sugar levels were increased. Softer sorghum doughs produced harder biscuits. Manohar and Rao (1999b) similarly found an increase in the hardness of wheat biscuits as dough became softer with increasing water level. The increase in sorghum biscuit hardness as dough became softer could be due to increased lubrication of the flour particles by the continuous phase of the dough. The sorghum dough structure can be described as a continuous phase of emulsified sugar solution (oil and sugar solution) enveloping flour particles and undissolved sugar. It seems that as the fluidity of the continuous phase of sorghum dough increases, flour particles became more immersed in it (continuous phase), thereby creating more interactions between the emulsified sugar solution and flour particles, especially with the starch granules. It is proposed that the resultant effect of this interaction is the observed increase sorghum biscuits hardness (Tables 4.7 and 4.9).

The starch granules in sorghum biscuits were seen to exist in three forms: intact (ungelatinized) granules, swollen/disrupted granules, and a continuous gel (Section 4.3.4.3). The intact granules constituted the vast majority of the granule population, while few granules were swollen and single strands of starch gel were observed. These different forms were further reflected in the difference in their birefringence when viewed by polarising light microscopy. Starch granules were either fully birefringent, partially birefringent or non-birefringent (Fig. 4.12). The presence of a starch gel in the sorghum biscuit and complete loss of birefringence in some of the starch granules is evidence that some starch granules were indeed gelatinized during biscuit baking. However, the high proportion of intact starch granules in the sorghum biscuits as indicated by the polarizing light microscope suggests that only small portion of the starch gelatinized during baking. This is similar to the DSC findings of Abboud and Hoseney (1984) in sugar wheat biscuits, and was also observed in both sugar-snap and Marie wheat biscuits in this work.

However, unlike in wheat biscuits where swollen starch granules appeared to form networks with protein (gluten), the swollen granules in sorghum biscuits existed in a discrete form. The regions around the swollen granules were generally porous which suggests a weaker biscuit crumb network. But what was the overall impact of these swollen granules on the hardness of sorghum biscuits? Theoretically, one would expect more starch granule swelling and increased porosity as water level increased. This should result in less intact starch granules becoming embedded in the biscuit matrix thereby resulting in softer sorghum biscuits. On the contrary, the biscuits became harder as water level increased, while they became softer when the proportion of swollen/gelatinized starch granules were increased by pre-cooking part of the sorghum flour. This led to consideration of the effect of water level on the mechanical properties of sorghum biscuits.

It is possible that the hardening of sorghum biscuits as water level increased was due to an increase in intermolecular hydrogen bonding within the biscuit matrix. The sugar molecular surface area would increase when it dissolves, thereby allowing for more interaction with flour particles. Perhaps, an increase in water level allowed for more hydrogen bonding between the hydrophilic components of sorghum dough, starch and sugar, with a resultant increase in sorghum biscuit hardness. This is similar to the stated effect that increased water level had on wheat biscuit hardness (Manohar and Rao, 1999b). The increase in the cohesiveness of wheat dough as water level increases, as reported by these authors, indicated there was an increase in protein association with a resultant increase in wheat biscuit hardness. It thus appears that the effect of water on starch gelatinization in biscuits is not as important as the effect it has on the degree or extent of interaction among dough components. These interactions could be protein-protein or protein-carbohydrate. This may also explain why in the literature there seems to be more focus on the functionality of sugar, fat and proteins in wheat biscuits than there is on starch. However, it suggests that food scientists should pay attention to the functionality of starch in future research on gluten-free biscuits.

The increase in the hardness of sorghum biscuits as sugar level increased (Table 4.9) is further evidence of the role of sugar in the hardness of biscuits. An increase in sugar level was also found to increase the hardness of wheat biscuits (Manohar and Rao, 1997b). Sugar was also found to affect the swelling/gelatinization of starch granules in sorghum biscuits. Chevallier *et al*. (2000a) stated that the high level of sugar used in wheat biscuits contributes to the non-gelatinization of starch. Sorghum biscuits containing no sugar had more swollen starch granules than those containing sugar (Fig. 4.23). However, even in the absence of sugar, most of the starch granules were still intact with only few of them appearing swollen. This indicates that the relatively high amount of water used in making the sorghum biscuits was still not sufficient to cause starch gelatinization even in the absence of sugar. This observation seems to support the view of Abboud and Hoseney (1984) that water loss during baking is also responsible for the non-gelatinization of starch during biscuit baking. Therefore it appears the non-gelatinization of starch during the baking of biscuits is primarily due to insufficient water level, while high sugar level can be considered secondary.

DSC and XRD analysis revealed that sugar is present as a glassy amorphous matrix in both sorghum and wheat biscuits. This was further confirmed by polarizing light microscopy. The polarizing light microscopy also revealed that sugar glass formed networks with starch granules. The sugar glass is responsible for the cohesiveness of sorghum biscuits and also holds other components of the biscuit together. Chevallier *et al*. (2000a) stated that sugar melts during baking and could form a bridge between starch and protein molecules or a continuous matrix embedding protein, starch granules and lipids. The observed presence of starch Maltese crosses around and within sugar glass (Fig. 4.24) indicated that there was a sugar bridge between the protein and starch in wheat biscuits. On the other hand, starch granules and protein bodies were embedded in the sugar glass in sorghum biscuits.

Table 5.1: Summary of the state and role of protein in the texture of sorghum and wheat biscuits

Biscuit type	State	Role and effects	Scientific explanation
Sorghum	Kafirins remained in protein bodies	Inert, does not appear to have any effect on the biscuit texture	Biscuit making process could not disrupt the intact protein bodies
Marie wheat	Well-developed gluten network	Forms a crosslink with swollen starch granules. Contributes to biscuit hardness	Low fat level allows gluten to develop during dough making. Additional gluten development during baking resulted in a dense gluten network
Sugar-snap wheat	Partially developed gluten network	Poorly crosslinked with swollen starch granules. Contributes to biscuit hardness	High level of fat prevented gluten formation during dough making. Gluten development mainly resulted from protein associations during baking

Table 5.2: Summary of the state and role of starch in the texture of sorghum and wheat biscuits

Biscuit type	State	Role and effects	Scientific explanation
Sorghum	Granules mostly intact (ungelatinized), few swollen (disrupted), strands of starch gel (gelatinized)	Intact granules are embedded in the biscuit matrix, while swollen granules and starch gels exist as discrete components. Some granules form networks with sugar glass and contributes to biscuit hardness	Amount of water used in dough making is insufficient to cause complete starch gelatinization. Hydrophobic protein bodies also limit starch granule hydration and swelling
Marie wheat	Swollen ungelatinized granules, few disrupted granules	Forms a dense network with protein (gluten), which contributes to biscuit hardness	Level of water used in dough making not sufficient to completely gelatinize starch
Sugar-snap wheat	Swollen ungelatinized granules, few disrupted granules	Partially enveloped by gluten sheet, structure formed also contributes to biscuit hardness	Level of water used in dough making not sufficient to completely gelatinize starch

Table 5.3: Summary of the state and role of sugar in the texture of sorghum and wheat biscuits

Biscuit type	State	Role and effects	Scientific explanation
Sorghum	Amorphous glass	Infiltrates the biscuit matrix and holds the biscuit structure together. Also contributes to biscuit hardness	Sugar melts during baking and forms an amorphous sugar glass matrix on cooling
Marie wheat	Glassy, no evidence of crystallinity	Appears to form a bridge between starch granules and gluten. Also contributes to biscuit hardness	Sugar also melts and form an amorphous sugar glass on cooling. All the sucrose were amorphous glass
Sugar-snap wheat	Glassy, with crystalline sucrose on the surface	Appears to form a bridge between starch granules and gluten. Also contributes to biscuit hardness	Sugar melts during baking and forms an amorphous sugar glass matrix on cooling. Crystals that were insufficiently hydrated during dough making remain crystalline on the biscuit surface

5.3 Proposed structure of sorghum biscuits

Slade *et al*. (1993) described the structure of wheat biscuits as composed of a continuous glassy sucrose matrix embedding ungelatinized starch granules, undeveloped gluten and fat. Baltsavias *et al*. (1999) stated that low-fat biscuits are fat-dispersed systems, while high-fat biscuits are bicontinuous systems. Chevallier *et al.* (2000a) described high-fat wheat biscuit structure as comprising a composite matrix of protein aggregates, lipids, and sugar, embedding both intact and damaged starch granules. They further stated that the sugar could form a continuous phase of molten sugar embedding protein, starch granules and lipids. The cohesiveness of the biscuit structure was attributed to sugar that melts during baking and become glassy during cooling.

A comparison of the crumb structure of defatted and un-defatted sorghum biscuits (Fig. 4.25) suggests that oil is an integral part of the biscuit matrix. The loss of the oily gloss in defatted sorghum biscuit suggests a continuous oil phase rather than a dispersed system as described by Baltsavias *et al*. (1999). The oily gloss also suggest separation of oil from the system due to creaming effect. However, it is important to note that the structure of defatted sorghum biscuits was still intact. This is in contrast to the crumbly and fragile nature of sorghum biscuits containing no sugar. The continuous nature of sugar glass in the sorghum biscuit matrix as revealed by polarizing light microscopy suggests that the sugar glass holds the biscuit structure together.

In summary, the structure of sorghum biscuits can be described as a bicontinuous system of oil and sugar glass in which ungelatinized starch granules, protein matrix and cell wall materials are embedded (Fig. 5.2). The cohesiveness and hardness of the biscuit structure can be attributed to the degree of the networks formed between the continuous sugar glass phase and embedded intact starch granules.

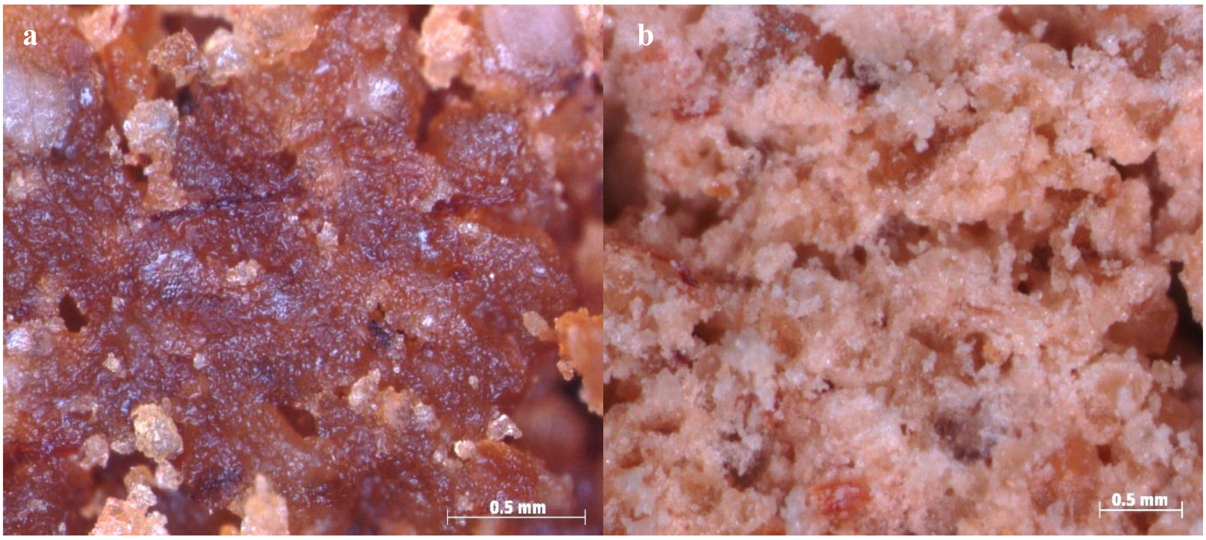

Figure 4.25: Effect of defatting on the microstructure of sorghum biscuits. (a) Un-defatted sorghum biscuit, (b) defatted sorghum biscuit.

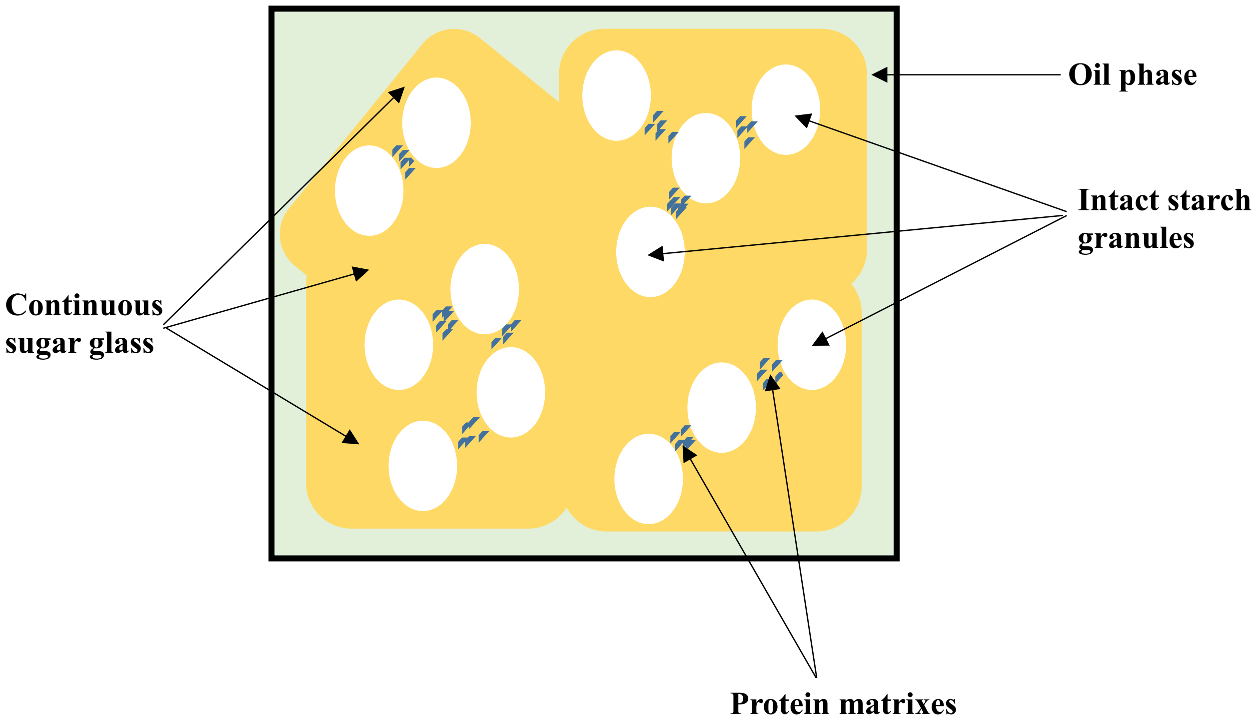

Figure 5.1: Schematic diagram of the proposed microstructure of sorghum biscuits.

6 CONCLUSIONS AND RECOMMENDATIONS

Sorghum biscuits of similar texture to wheat biscuits can be made despite the absence of gluten in sorghum biscuits. Sorghum kafirin proteins remain isolated in their protein bodies indicating they do not participate in dough formation and hence, they are unlikely to influence the structure and texture of sorghum biscuits. On the other hand, the extent of gluten development in wheat biscuits depends on the type of biscuits as they differ in formulation.

An increase in the hardness of sorghum biscuits with increasing water level appears to be due to an increase in the level of the intermolecular hydrogen bonding between starch and sugar glass. This is because increasing the level of swollen/gelatinized starch by pre-cooking an increasing portion of the sorghum flour reduced sorghum biscuit hardness. Similar to wheat biscuits, some level of starch gelatinization occurs during the baking of sorghum biscuits. However, the gelatinized starch granules constitute a minute fraction of the total starch granules population, with the vast majority of the granules remaining intact and a few are swollen. In wheat biscuits, the starch granules are swollen and connected to the gluten network through a sugar glass, while intact starch granules are embedded in an oil emulsified sugar glass in sorghum biscuits.

The crumbly nature of sorghum biscuits in the absence of sugar suggest a sugar glass is responsible for the cohesiveness of the biscuit structure with the sugar glass holding other components in the biscuit matrix together. The sugar glass becomes more continuous in the biscuit matrix as sugar level increases. Since sugar glass on its own is hard, increasing sugar content means more sugar glass and thus higher force.

Although sorghum biscuits can have similar hardness and brittleness to wheat biscuits, they have negative sensory characteristics due to their dense and gritty texture. It appears the absence of a continuous protein network and the inability of the sorghum dough to spread during baking are responsible for the dense nature of the sorghum biscuits. The poor hydration and predominantly intact nature of the starch granules in sorghum biscuits may account for their grittiness, while the swollen nature of starch granules in wheat biscuits due to water absorption may account for their non-gritty texture.

Future works on sorghum biscuits should focus on how to reduce their grittiness for improved sensory properties since there seems to be some level of understanding on why it is as hard and brittle as wheat biscuits. It is also recommended that the role of oil/fat in sorghum biscuits should be further investigated to understand how it influences the structure and texture of the biscuits. Two major questions warrant further investigation. Would it be possible to form sorghum dough without adding water if oil is increased to a level similar to high-fat wheat biscuit dough, and what would be the effect of increasing oil levels on sorghum biscuit texture?

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8 RESEARCH OUTPUT FROM THIS WORK

Adedara, O. A (2017). Making good quality gluten-free biscuits. Postgraduate showcase, *FST Magazine*, in press.