EFFECT OF SORGHUM VARIETY ON BATTER RHEOLOGY AND QUALITY OF CASSAVA-SORGHUM-AMARANTH BREAD

By

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Declaration

This thesis is my original work submitted for the degree MSc Food Science and Technology at the University of Nairobi and has not been presented for a degree in any other university.

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Abstract

Bread made from sorghum-based flours has poor textural characteristics. This is because sorghum has certain physico-chemical properties which negatively affect its functionality in gluten-free bread. In this study, the effect of sorghum variety on batter rheology and bread quality of cassava-sorghum-amaranth bread was investigated. The physico-chemical properties of six native and five malted (germinated) sorghum varieties and characteristics of cassava-sorghum-amaranth batters and breads were evaluated. In addition, the impact of storage time on the textural parameters of gluten-free bread was also determined. Native and malt flour characterization included proximate composition, starch, damaged starch, total soluble sugars, reducing sugars, pasting properties, diastatic power, free amino nitrogen and tannin content. Sorghum varieties were malted and the one with the highest diastatic power was used for rheological and textural quality tests of the gluten-free batter and bread, respectively. Composite flour comprising cassava starch, sorghum and amaranth flours and modified with malt was used to formulate gluten-free batter and bread. Sorghum grain colour varied between white and reddish brown while 1000-kernel weight and hardness were 16.62-27.76 g and 1.34-2.64 N·s, respectively. There was variability in native and malt flour characteristics. Malted flour had significantly higher ($P \leq 0.05$) damaged starch, total soluble sugars, reducing sugars, diastatic activity and free amino nitrogen, but significantly lower ($P \leq 0.05$) in tannins and peak viscosity than native flour. The consistencies of the batters ranged between 76.03 and 216.90 N. Crumb hardness,

springiness, resilience, cohesiveness and chewiness were 12.02-47.43 N, 81.27-87.13%, 0.21-0.27, 0.42-0.55 and 5.80-17.20 N after 24 h, respectively. Crumb hardness and chewiness increased with storage, however, resilience and cohesiveness did not change significantly ($P > 0.05$). Sorghum variety with highest amount of damaged starch (10.73%) gave least firm (12.02 N) and least chewy (5.80 N) crumb after 24 h. Genotypic variations were observed in characterisation of native and malted sorghum varieties, rheology and quality of cassava-sorghum-amaranth batter and bread, respectively. There is potential for blending of cassava starch, sorghum and amaranth flours in breadmaking. If institutionalised, it can create a positive impact on production and commercialisation of cassava, sorghum and amaranth as well as reduce wheat import to the country. These crops if developed and produced in Kenya would enhance food diversification and food security by tapping their unexploited potential.

1. INTRODUCTION

Sorghum (*Sorghum bicolor)* (L.)Moench) is indigenous to the semi-arid tropics of Africa and is an important food security crop in sub-Saharan Africa. It is traditionally used to prepare porridge and beer. The increased use of sorghum as a food in this region could alleviate the problem of chronic food insecurity, as sorghum is much better suited to cultivation in the semi-arid region than non-indigenous cereals such as wheat or maize. It can endure hot and dry conditions and also withstand heavy rainfall accompanied by some water logging. The grain can also provide a good basis for industrial production of sorghum bread and thereby contribute to reducing the region's dependence on imported wheat and improving the economic livelihoods of Africans (Frederick, 2009).

In many countries, bread is mainly prepared from wheat flour because it contains gluten which plays a key role in the unique baking quality of wheat by imparting appropriate water absorption capacity, cohesiveness, viscosity, and elasticity to the dough (Wieser, 2007). Gluten-free breads, therefore, require a different technology of production due to the lack of a gluten network. Gluten-free bread can be made from any carbohydrate rich material, including cereals, pseudo-cereals, legumes, and roots or tuber crops (Schober, 2009).

Sorghum-based bread has a coarse, gritty or sandy mouth feel, which is associated with particles from the bran and the vitreous portion of the endosperm. The bran

can be partially or wholly eliminated by decorticating the grains prior to milling. It is also possible to decrease the coarseness associated with sorghum-based bread by malting the grains (Hugo et al., 2000). Malting has been reported to have positive effects on sorghum flour characteristics and to improve its composite breadmaking quality (Hugo et al., 2000). It has been identified as a traditional processing technology that can be used to improve the nutritional quality of sorghum by improving protein quality characteristics, including percentage protein, the nitrogen solubility index and the content of the first limiting amino acid, lysine (Dewar, 2003).

1.1. Problem statement

Sorghum has certain physico-chemical properties which negatively affect its functionality in the manufacture of gluten-free bread. These unfavourable properties include firm encapsulation of protein bodies in the endosperm resulting in coarseness of gluten-free bread (Schober, 2009). Also, the protein prolamins (kafirins) are not able to form cohesive dough when mixed with water (Taylor and Belton, 2002).The batter is not coherent enough to hold the fermentation gases and cannot effectively rise during proofing. Part of the gases escape too early and part are retained to form irregular cells (Cauvain, 1998; Satin, 1988). The resultant breads tend to be rigid with irregular and crumbly texture. The high gelatinization temperature of sorghum may also cause inadequate gelatinization during baking (Schober, 2009; Taylor and Belton, 2002).

Advances have been made in improving the texture of gluten-free bread by addition of substances that mimic the viscoelastic properties of gluten such as native and pre-gelatinised starches, hydrocolloids, emulsifiers, proteins and enzymes (Onyango et al., 2010a; Schober et al., 2005; Hugo et al., 1997; Olatunji et al., 1992b;Onyango et al., 2009b; Hart et al., 1970; Onyango et al., 2009a; Olatunji et al., 1992a; Onyango et al., 2010b). However, the impact of each of these additives varies depending on its type and level of addition. The physico-chemical properties of sorghum can also be modified by fermenting the flour (Schober et al., 2007) or malting the grain (Hugo et al., 2000) prior to use. In a study on wheat-sorghum bread, Hugo et al. (2000) found that malted sorghum decreases the gelatinisation temperature of sorghum and alleviates the coarseness caused by the inclusion of sorghum flour.

1.2. Justification

Cassava and sorghum are important crops in Africa and constitute major sources of dietary energy for many people in the semi-arid zones of Africa (Taylor and Dewar, 2001; Balagopalan, 2002).Africa is the largest producer of sorghum, with Sub-Saharan Africa annually producing about 18 million metric tons of sorghum, representing around 70% of the cereals produced in West Africa, 30% in East Africa, and 10% in Southern Africa (Taylor and Belton, 2002). The cultivation and utilization of cassava, sorghum and amaranth in Africa is derived from their

attribute as drought resistant crops (Rosling, 1987; Dewar, 2003; Bressani, 2003).Also, present interests in amaranth have developed because the grains have a high protein content and quality (Bressani, 2003). In spite of the potential for these crops to address food security, their utilisation is low as many consumers consider them a poor man's food or survival crop to be consumed during hardships. Wider utilisation of cassava, sorghum and amaranth will contribute to the food security status of producing and consuming households in Africa and transform these crops from 'a poor man's food' into commercial commodities.

Coeliac disease, also known as gluten-sensitive enteropathy, is an immune-mediated disorder, affecting genetically susceptible individuals (Cureton and Fasano, 2009; Curic et al., 2007; Rodrigo, 2006). It is the end result of genetic predisposition, environmental factors and immunologically-based inflammation of the small bowel's mucosa and sub-mucosa (Curic et al., 2007; Rodrigo, 2006; Murray, 1999). The disease is characterized by inflammation, villous atrophy and crypt hyperplasia in the small intestine (tiny hair-like projections in the small intestine that absorb nutrients from food are damaged on exposure to these peptides). This interferes with the ability of the body to absorb basic nutrients such as proteins, carbohydrates, fat soluble vitamins, folic acid and minerals (Curic et al., 2007). Individuals affected by the disease have adverse reactions upon ingestion of wheat gluten as well as secalin from rye, hordein from barley and avenin from oat (Sciarini et al., 2010; Curic et al., 2007). Typical symptoms of the

disease include abdominal pain, chronic diarrhoea, steatorrhea (fatty stools), vomiting, weight loss, weakness, iron deficiency anemia and reduced bone density. Atypical symptoms include neurologic problems, abnormalities of blood chemistry, dental enamel defects and infertility (Alaedini and Green, 2005; Wieser and Koehler, 2008; Green, 2009).

Currently, the only certain remedy is a strict lifelong adherence to a gluten-free diet (Gallagher et al., 2004).This means that coeliac patients cannot consume bread made from wheat, rye or barley because they contain gluten. Sorghum is one of the gluten-free cereal grains often recommended as a safe food for coeliac patients because it is more closely related to maize than to wheat, rye, and barley (Kasarda, 2001). Cassava and amaranth are also gluten-free and can be consumed by coeliac patients (Schober, 2009).

1.3. Objectives

1.3.1. Main objective

To establish the effect of sorghum variety on the batter rheology and crumb properties of a cassava-sorghum-amaranth bread.

1.3.2. Specific objectives

1) To study the physico-chemical properties of five sorghum varieties (KARI Mtama II, Serena, Gadam, Seredo and Kaguru) grown in Kenya and a commercial variety (Milomehl).
2) To study the effect of malting on physico-chemical properties of the five local sorghum varieties.
3) To study the influence of sorghum variety on the rheological properties of cassava-sorghum-amaranth batter.
4) To study the influence of sorghum variety on the crumb texture of cassava-sorghum-amaranth bread.

1.4. Hypotheses

1) Different sorghum varieties differ significantly in terms of physico-chemical properties.
2) Malt from different sorghum varieties differ significantly in terms of physico-chemical properties.
3) Different sorghum varieties differ significantly in rheology of cassava-sorghum-amaranth batter.
4) Different sorghum varieties differ significantly in crumb texture of cassava-sorghum-amaranth bread.

2. LITERATURE REVIEW

2.1. Sorghum

2.1.1. Properties and composition

Sorghum is a monocotyledonous plant that belongs to the Gramineae (Poaceae) family, Panicoideae subfamily and Andropogeneae tribe (Morrison and Wrigley, 2004). Two of the best known species are *Sorghum vulgare* and *Sorghum bicolor* (L.) Moench (Palmer et al., 1989). Sorghum has a variety of local names: great millet and guinea corn in West Africa, kafir corn in South Africa, *dura* in Sudan, *mtama* in East Africa, *jowar* in India, *kaoliang* in China, and milo or milo-maize in United States of America (FAO, 1995).

Sorghum kernels are typically round varying in weight from 1.0 to 3.0 g per 100 kernels (Frederick, 2009). The kernel is a naked caryopsis, typically 2-5 mm in length and 2-3 mm thick at the widest point (Taylor and Belton, 2002). Due to genetic diversity, the grains vary widely in colour, shape and size. The colour of the kernel varies from white or yellow to red, whereas the endosperm colour can be yellow or white (Schober and Bean, 2008).

The grain is made up of a pericarp, endosperm or storage tissue and germ or embryo. The pericarp region comprises a pericarp, testa (seed coat) and aleurone layer (Taylor and Belton, 2002). Sorghum is unique in that it is the only cereal grain that has starch granules in the pericarp. The testa separates the pericarp from

the aleurone layer. The testa is thin in low tannin sorghum varieties but thicker and highly pigmented in high tannin sorghums (Taylor and Belton, 2002). The outer edge of the endosperm is composed of the aleurone layer containing lipids, enzymes and protein bodies. Under the aleurone layer is the outer corneous endosperm fraction which is a hard, horny, vitreous layer surrounding an inner floury or soft core (Schober and Bean, 2008; Chandrashekar and Mazhar, 1999). The outer corneous endosperm is tightly packed with starch bodies covered with a continuous protein matrix, whereas the floury endosperm, in the centre of the kernel, is loosely packed with a discontinuous protein matrix and round starch granules (Rooney and Clark, 1968).

The major component of sorghum is starch (50-75%). Starch is located in the endosperm and pericarp of the grain. Starch granules in sorghum range from 2 to 30 μm in diameter (Taylor and Belton, 2002). The granules are often misshaped due to the compressive effects of contact with the protein bodies and as a result take on many complex shapes (Taylor and Belton, 2002). Starch from normal grains contains 23-30% amylose (Taylor and Belton, 2002). Sorghum starch is characterised by a high gelatinisation temperature (71-80°C), but there are considerable differences between cultivars. Starch isolated from the corneous endosperm has a higher gelatinisation temperature and intrinsic viscosity and lower iodine binding activity than starch from the floury endosperm (Cagampang and Kirleis, 1984). The digestibility of sorghum starches may be lower than that of

other starches, probably due to interference from the protein bodies (Wankhede et al., 1989).

Proteins make up 9–14.1% of the grain (Waniska et al., 2004). Most of the protein content of sorghum is located in the endosperm and may be divided into two classes: glutelins and prolamins (Taylor and Belton, 2002). Sorghum prolamins are also known as kafirins (Belitz et al., 2009) and account for 70-90 % of the total grain protein (Hamaker et al., 1995). Kafirins are storage proteins that serve as nitrogen reserve for the next generation of plant (Taylor and Belton, 2002). Glutelin is a structural component (FAO, 1995). A notable feature of sorghum proteins is their low apparent digestibility compared to other cereals (Taylor and Belton, 2002). In sorghum varieties containing tannins, this may be explained by the enzyme inhibition effects of tannins. However, other factors besides tannin content may be involved in controlling protein digestibility (Elkins et al., 1996; Duodu et al., 2003; Hamaker and Bugusu, 2003; Taylorand Belton, 2002).

Minor components of sorghum grain are lipids, phenolic compounds, minerals and vitamins. Although these compounds occur in small amounts, they significantly influence the processing parameters and nutrient quality of the grain (Taylor and Belton, 2002). The lipid content of sorghum varies from 2.1 to 6.6%. The lipids are mainly located in the germ, although there are smaller amounts in the endosperm (Taylor and Belton, 2002). All sorghums contain phenolic compounds

such as anthocyanin and anthocyanidin pigments and phenolic acids, but not all sorghum contain tannins (Dykes and Rooney, 2006; Taylor and Belton, 2002). The main minerals in the grain are calcium (28 mg/100 g), iron (4 mg/100 g) and zinc (2 mg/100 g) (Juliano, 1999).Sorghum contains 0.22, 0.13 and 2.8 mg/100 g thiamine, riboflavin and niacin respectively (Juliano, 1999).

2.1.2. Processing and utilization

Sorghum is the fifth most important cereal in the world after wheat, rice, maize and barley in terms of production (FAO, 2005). It is an important staple food in the arid and semi-arid regions of sub-Saharan Africa because it is drought-tolerant, and can grow where other crops fail (Dewar, 2003). Sorghum is utilized as food, feed and industrial products with more than 35% grown directly for human consumption (Dicko et al., 2006).

There is a wide array of foods prepared from sorghum and they include breads (both fermented and unfermented), porridges and traditional beers. The most popular unfermented flat breads are roti (India) and tortillas (Central America) with injera (Ethiopia), kisra (Sudan) and dosa (India) being staple sorghum fermented flat breads (Taylor and Belton, 2002). Another common sorghum based food is porridge. Porridges are prepared by cooking either fermented or unfermented slurries of sorghum flour in boiling water. They are typically either thick or thin, with the differences in viscosity caused by flour concentration, pH of

the cooking water, flour particle size, and endosperm hardness (Taylor and Dewar, 2001). Flavour of the porridges is determined by the extent of fermentation treatment on the sorghum flour. Traditional beers made from sorghum are opaque and viscous due to the suspension of cereal starch, other grain material and yeast particles. The alcohol content of these beers is low (3% w/w) as compared to the commercial beers, and are not typically pasteurized (Taylor and Belton, 2002). Alcoholic beverages are characterized by a sour, lactic acid flavor, provided either by lactic acid fermentation or by the addition of commercially produced lactic acid (Taylor and Dewar, 2001).

Sorghum grain is also utilized as animal feed and the plant stem and foliage are used for green chop, hay, silage, and pasture after harvesting the grain (Reddy et al., 2010). Livestock feed manufacturers prefer to use grains from white sorghums or low tannin pigmented sorghums due to the negative effect of tannins on protein digestibility. Sorghum has a lower energy density and protein digestibility compared to maize and is therefore not a direct replacement for maize in a livestock ration (NRI, 1998). In developing countries, a major reason for low inclusion of sorghum in livestock feeds is lack or inconsistency of supply in the market.

2.2. Cassava

2.2.1. Properties and composition

Cassava is a dicotyledonous perennial plant that belongs to the Euphorbiaceae family (Falade and Akingbala, 2008). Cassava varieties are often grouped as: bitter or sweet, high or low cyanogenic varieties and early or late maturing (Norman, 1995). The sweet cassava contain low cyanoglucoside content (less than 140 μg/g) while the bitter forms have more than 140 μg/g on dry weight basis. Two of the commonly known species are *Manihot esculenta* Crantz and *Manihot utallisima* Phol. (Falade and Akingbala, 2008). Cassava roots are generally 15–100 cm long and 3–15 cm wide. They are cylindrical, conical, or oval, with a coffee, pink, or cream-colored peel that is covered by a thin brown bark. The parenchyma is generally white, cream or yellow. Cassava plants produce 5-10 roots weighing 0.5–2.5 kg each (Wheatley et al., 2003). Root size, shape and colour depend on the variety and environmental conditions.

The root is composed of three distinct tissues: bark (periderm), peel, and parenchyma. The parenchyma is the edible portion of the fresh root and comprises approximately 85% of the total weight. It consists of xylem vessels radially distributed in a matrix of starch-containing cells. A central fibrous vascular bundle becomes progressively larger as the roots mature. Other fibrous bundles may develop throughout the root. The peel layer comprises sclerenchyma, cortical

parenchyma and phloem and constitutes 12% of the root weight, with the periderm layer comprising another 2% (Wheatley et al., 2003).

The cassava root is essentially a carbohydrate source and its composition depends on factors such as geographical location, variety, age of the plant, and environmental conditions (Tewe and Lutaladio, 2004). Total carbohydrates make up over 90% of parenchyma dry weight and 64-72% is made up of starch containing 30% amylose and 70% amylopectin. The protein content is uniformly low (1-2%), as are fats (0.2–0.6%) and ash content. The fibre content is more variable, and increase with plant age (Wheatley et al., 2003). A great variation in total cyanogen content has been related to environment factors, size of roots and moisture content with parenchyma values of 30–100 mg/kg common for low-cyanogenic cultivars for direct consumption, compared with 1350 mg/kg in industrial varieties used for processing (Wheatley et al., 2003). In cassava, cyanide is synthesized in the leaf and transported to the roots, where it is partitioned between peel and parenchyma. Some 85% of the cyanide occurs as cyanoglucosides, mainly linamarin but also lotaustralin (Wheatley et al., 2003).

2.2.2. Processing and utilization

Cassava is the third most important food source in the tropics after rice and maize (Bradbury and Denton, 2010). Cassava is extensively cultivated throughout the tropics and subtropics regions due to its ability to grow in diverse soil conditions

and under minimal management (Boonnop et al., 2009). The importance of cassava has been realized as a high energy food, animal feed and as an industrial raw material for sweeteners, ethanol and various other chemicals (Balagopalan,2002). It is a major source of dietary energy and its year-round availability, tolerance to extreme stress conditions and suitability to present farming and food systems in Africa makes cassava vital to food security in Africa (Hahn and Keyser, 1985; Hahn et al., 1987).

Traditionally, cassava roots are processed by various methods into numerous products and utilized in various ways. The processing methods include peeling, boiling, steaming, slicing, grating, soaking or steeping, fermenting, pounding, roasting, pressing, drying, and milling (Balagopalan, 2002). Processing techniques and procedures differ with countries and localities within a country according to food cultures, environmental factors such as availability of water and fuel-wood, the varieties used, and the types of processing equipment and technologies available. The dried cassava roots (both fermented and unfermented) are often mixed with sorghum, millet and/or maize and milled into composite flour. Dried cassava roots are used in animal feed formulation (Balagopalan, 2002).

The importance of cassava as an industrial raw material has been realized. Fresh cassava roots or flour from dried cassava can be used to produce ethanol and starch (Balagopalan, 2002). Starches extracted from dried cassava exhibit

differences in functional properties as compared to those from fresh roots even though the quality is still acceptable. Cassava starch is used as a raw material for production of sweeteners like glucose, glucose syrups, high fructose syrups, maltose syrups and maltodextrins for various confectionery and pharmaceutical purposes, liquid adhesives and bio-degradable plastics (Balagopalan, 2002).

2.3. Amaranth

2.3.1. Properties and composition

Amaranth is dicotyledonous plant of the Amaranthaceae family and Amaranthoideae tribe (Morrison and Wrigley, 2004).The genus *Amaranthus* includes about 60 species distributed in many areas of the world (Saunders and Becker, 1984). Most studies have been carried out on *Amaranthus caudatus, Amaranthus hybridus, Amaranthus cruentus* and *Amaranthus hypochondriacus* since these varieties have also been investigated for suitability as a food source (IENICA, 2002). Amaranth has a variety of common names: Inca-wheat and grain amaranth for *Amaranthus caudatus*, purple amaranth and red amaranth for *Amaranthus cruentus* and prince's feather for *Amaranthus hypochondriacus* (Morrison and Wrigley, 2004).

The amaranth inflourescence produces 50,000-100,000 seeds weighing 0.6–1.3 mg each. The seeds are small in size, about 1-1.5 mm in diameter and are pale coloured, reddish or dark brown. Amaranth seeds are unusually high in protein for

a non-legume around 11.8–17.6% crude protein, 4.8–8.1% crude fat, 7.6–16.4% dietary fibre, 2.8-3.8% ash and 54.5–69.7% carbohydrates (Bressani, 2003). Of its carbohydrate, 48–69% is made up of starch which contains 4.8–7.22% amylose. The main minerals in the grain are phosphorus (578 mg/100g), potassium (541 mg/100 g) and magnesium (327 mg/100 g) (Bressani, 2003). Amaranth contains 43.8, 42.5 and 4.47 mg/100 g folic acid, biotin and vitamin C respectively (Bressani, 2003).

2.3.2. Processing and utilization

Amaranth seeds have some desirable functional characteristics, having been processed in popped, flaked, extruded, and ground flour forms (Agong, 2006). Since the food uses are similar to such cereal grain grasses as wheat and oats, amaranth is sometimes called a "pseudocereal". Most of the amaranth in food products starts as ground flour that is blended with wheat or other flours to make cereals, crackers, cookies, bread or other baked products. Studies have shown amaranth can often be blended at 50% or even 75% levels with other flours in baked products without affecting functional properties or taste (Thomas Jefferson Institute, 2002). The seeds are fermented to make alcoholic beverages such as *tella*, a beer in Ethiopia (Agong, 2006).

2.4.Gluten-free bread

2.4.1. Introduction

The ability of wheat proteins to develop a viscoelastic matrix is what makes wheat the most appropriate cereal for bread making. The absence of gluten in other flours often results in breads that are characterized by deficient quality characteristics as compared to wheat breads. Gluten-free sorghum bread is prepared by mixing sorghum, starch, water, sugar, salt, fat and yeast to obtain a batter, which is then proofed before baking (Onyango et al., 2009b).

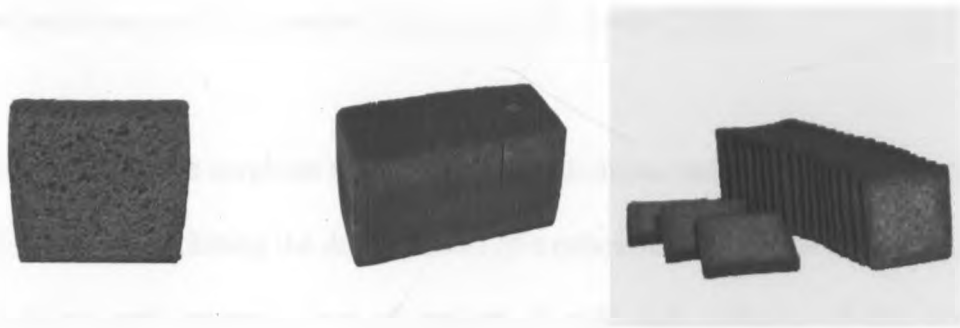

Figure 1. (a) Cross-sectional view, (b) longitudinal view and (c) slices of gluten-free bread prepared from sorghum and cassava starch. Source C. Onyango (unpublished data).

2.4.2. Ingredients and additives used to make gluten-free bread

Starch

A wide range of starch-rich gluten-free materials can be used to make gluten-free bread: cereals (rice, corn, sorghum, millet and teff), pseudocereals (amaranth, buckwheat and quinoa), root or tuber crops (Schober, 2009). The addition of sorghum flour to water produces a slurry that is neither cohesive nor viscous and with no elasticity. The batter is not coherent enough to retain the fermentation

gases and cannot effectively rise during proofing. Native starches from cassava, maize or potato could be added to sorghum flour at replacement levels of 10-30% to modify the batter's rheological properties and texture of the bread. At a constant water level and increasing starch replacement, the batter becomes less viscous (Onyango et al., 2011b). Sorghum batter containing native starch has a thin viscous consistency. Settling of particles and rising of gas bubbles, in this batter, does not occur even in the absence of a gelling agent thus enabling formation of a leavened crumb. Without addition of native starch, leavened is impossible as the bubbles would rise and leave the system and a large dense bottom resulting from settled particles could be expected in the crumb (Schober, 2009).

Replacing part of the sorghum with native starch induces early onset gelatinisation of the mixture, facilitating the development of a cohesive crumb network that traps gas bubbles, and prevents loss of carbon dioxide and collapse of the crust. Gelatinisation of starch in sorghum is limited by its high gelatinisation temperature and entrapment of starch in extended web-, or sheet-like structures by sorghum proteins upon heating (Hamaker and Bugusu, 2003; Lineback, 1984). Early gelatinization causes early increase in crumb consistency during baking, whereas a slower transition from batter to crumb causes the crumb to collapse (Schober et al., 2007).

Starch improves the volume of sorghum bread by diluting the endosperm and bran particles, which interfere with the stability of the sorghum gel and liquid films around the gas cells (Taylor et al., 2006). Acceptable sorghum bread can be made from sorghum flour and starch in ratio of 60:40 to 75:25 and with 80-105% water (Onyango et al., 2011b; Schober et al., 2005; Hugo et al. 1997; Olatunji et al., 1992a). Onyango et al. (2011b) studied the effect of different concentrations of native cassava starch on the rheological and texture properties of sorghum bread. Increasing starch concentration decreased crumb firmness and chewiness; and increased cohesiveness, springiness and resilience. However, it has to be recalled that starches from different origins have different pasting properties and may induce different rheological and texture effects in the sorghum batter and bread, respectively. Onyango et al. (2011b) reported that cassava starch gives gluten-free sorghum bread with better crumb properties than maize, potato or rice starches.

Partially replacing sorghum flour with pregelatinised starch imparts cohesiveness and increases the viscosity of the batter (Onyango et al., 2011a). Pregelatinised starch binds considerable amounts of water and swells. However the quality of the bread is inferior to that made from sorghum and native starch, and crumb quality declines with increasing concentration of pregelatinised starch. A major texture defect is the increase in crumb adhesiveness (increased crumb wetness and stickiness) with increasing concentration of pregelatinised starch (Onyango et al.,

2011a). This is attributed to starch breakdown due to gas pressure and stiffness of the batter.

Other studies have recommended a mixture of native and pregelatinised starch in the sorghum formulation. Olatunji et al., (1992b) and Hugo et al., (1997) found that sorghum, pregelatinised and raw cassava starch mixed in a ratio of 70:20:10 gave good quality bread. It appears that raw and gelatinised starches complement each other in the sorghum-based formulation. The starch gel provides cohesiveness, viscosity and traps air bubbles in the batter (Hugo et al., 1997; Olatunji et al., 1992b) whereas raw starch which is gelatinised during breadmaking serves to increase the elastic strength of the system (Olatunji et al., 1992b). However, this unnecessarily increases the cost of the formulation, and does not significantly improve the quality of the bread when compared to that made from sorghum and native starch. High concentrations of gelatinised cassava starch forms strong structures that do not permit expansion of the gas cells; and as fermentation proceeds, the gas cells break and release gas which is trapped in the crumb (Hugo et al., 1997). The breads develop large holes inside the crumb which weakens the crumb structure. Also, high concentration of gelatinised starch limits the amount of free water available in the batter and thus gelatinisation. Low starch gelatinisation restricts the release of amylose and consequently decreases its ability to act as a binder, causing the fermentation gases to escape easily. This is attributed to starch breakdown due to gas pressure and stiffness of the batter.

Protein

Sorghum protein occurs in a matrix with starch and affects the processing quality. Proteins soluble in the liquid phase of the batter destroy the crumb texture by aggregating during baking and forming lumps or strands (Schober et al., 2007). The aggregated proteins interfere with the starch gel, form points of weakness, press the gel down or reduce extensibility so that the crumb raptures under the gas pressure and collapses leaving a hole in the crust (Schober, 2009). Surface-active soluble proteins in the grains might help in the stabilisation of liquid films around the gas bubbles (Gan et al., 1995; Gan et al., 1990).

Proteins from animal or plant sources can be incorporated in the gluten-free formulations to form a network-like structure resembling that of gluten network and to enhance characteristics of bread crust and crumb (Abdel-Aal, 2009). Examples include soy protein isolates, cotton seed, groundnut, chick pea, horse bean, sesame, high lysine corn flour, high protein fractions from wheat, fish protein concentrate, food grade yeast, eggs, dairy fractions or synthetic amino acids. However use of some of these proteins is impractical, on account of availability, cost, ease of production, absence of inhibitors, allergenic potential, ability to complement the amino acid balance of carbohydrate flours and influence on product characteristics such as flavour of the bread.

Onyango et al. (2009a) compared the effect of egg white, skim milk powder, soy protein isolate and soy protein concentrate on the rheological and textural properties of gluten-free batter and bread prepared from sorghum and pregelatinised cassava starch. Resistance of the batters to deformation, in the order from least resistant to most resistant, was: egg white powder, skim milk powder, soy protein isolate, soy protein concentrate. The staling rates of the breads, from lowest to highest, were: egg white powder (58 g/day), soy isolate (168 g/day), soy concentrate (229 g/day), skim milk powder (260 g/day). Bread containing egg white powder had the lowest crumb firmness at 24, 48, 72 and 96 h; and the highest specific volume. Egg white stabilises the liquid films around the gas cells due to its surface activity and it helps in the setting of the crumb when it coagulates. Denatured egg white in gluten-free bread crumb forms web- or film-like structures resembling gluten (Ahlborn et al., 2005; Moore et al., 2004; Moore et al., 2006). Moore et al. (2006) studied the ultrastructure of gluten-free batters and breads containing soy flour, skim milk powder or whole egg powder. Dispersion of proteins and starch granules were most homogeneous in batters containing egg powder followed by soy flour and skim milk powder. The egg supplemented bread showed a protein network similar to gluten while less evident networks were formed by skim milk powder or soya flour (Moore et al., 2004; Moore et al., 2006). Breads with egg white had higher volume and finer crumb than bread with skim milk powder or soy flour. The protein matrices counteract staling by masking some of the changes originating from starch retrogradation

(Schober, 2009). Schober et al. (2005) used response surface methodology to vary the amount of skim milk (1.2-4.8%) in sorghum bread. The authors found that increasing skim milk powder decreased loaf height, caused crust collapse, increased bake loss and reduced crumb cohesiveness. They concluded that milk proteins and lactose interfere with the starch gel by competing for water or disrupting the uniformity of the starch gel. The only positive effect of milk protein was observed in the improved crust appearance.

Water

The rheological properties of prebaked sorghum-based formulations vary from "batter-like" to "dough-like". The rheological character of the batter/dough is influenced by the water content, the nature (native or pregelatinised starch) and amount of added starch, and degree of damaged starch in formulation (Schober et al., 2005; Onyango et al., 2011a). Formulations with high water levels produce liquid "batter-like" systems whereas low water levels produce firm doughs that lack extensibility and elasticity. Batters with high water levels have been found to give breads with improved volumes (Schober et al., 2005). Generally the acceptable water to flour ratio required to make gluten-free batter is about 1:1 when the composite flour is composed of sorghum and native starch (Schober, 2009).

Hydrocolloids

Hydrocolloids structure the bread crumb, promote retention of carbon dioxide formed during fermentation and temporarily bind water required to gelatinise starch. Water-soluble pentosans are naturally occurring hydrocolloids in sorghum. Water soluble pentosans bind water and thus increase viscosity of batters (Abdel-Aal, 2009). They are also surface active substances that stabilize liquid films around the gas bubbles (Gan et al., 1995; Gan et al., 1990). Schober et al. (2005) studied the water uptake potential of nine sorghum hybrids and found negative correlations between pentosan contents and water levels required to standardise the consistencies of batters. The higher the content of pentosans, the softer the batter consistency and the less water required. Total and soluble pentosans in the nine dehulled and milled sorghum hybrids were below 1.6 and 0.3%, respectively (Schober et al., 2005) showing that sorghum is generally not rich in pentosans (Karim and Rooney, 1972). Sorghum pentosans are located in the pericarp and most are lost during dehulling. Schober et al.(2005) concluded that pentosans do not influence crumb properties of sorghum bread. Pentosans are found in rye ranging from 6-12% of which 1.5-3% are water extractable (Abdel-Aal, 2009). By contrast, rye is rich in pentosans, which are responsible for structuring its bread crumb (Cauvain, 2007). Addition of rye pentosans to sorghum bread can improve the volume and reduce staling rate (Casier et al., 1977). It must be remembered that the use of rye pentosans is not acceptable if the bread is meant for coeliac

patients. This is because rye contains prolamin fractions, known as secalins, which have toxic epitopes (Ciclitira and Ellis, 1987).

Hart et al. (1970) found that most hydrocolloids prevent sorghum loaves from collapsing upon baking. Among the investigated hydrocolloids, bread containing 2% methylcellulose such as Methocel 4000 cps had the best structure. Some results obtained with hydrocolloids are contradictory. For example, Schober et al. (2005) found that xanthan gum reduced loaf volume, whereas Satin (1988) found that xanthan improved bread quality. Onyango et al. (2009b) studied the effect of cellulose-based derivatives (cellulose, carboxymethylcellulose, hydroxypropylmethylcellulose, methylcellulose, microcrystalline cellulose and hydroxypropylcellulose) on the crumb properties of sorghum bread. Increasing concentration of methylcellulose, carboxymethylcellulose or hydroxypropylcellulose increased crumb firmness whereas the opposite effect was noted with microcrystalline cellulose. Increasing HPMC concentration had no effect on crumb firmness. Increasing hydrocolloid concentration decreased the staling rate, except for HPMC, which increased. The ability of hydrocolloids to prevent staling is due to their ability to bind water and possibly inhibit amylopectin retrogradation (Guarda et al., 2004).

Enzymes

Olatunji et al. (1992a) reported that fungal amylase improves the texture of sorghum bread, whereas Hart et al. (1970) found that α-amylase weakened the crumb structure. These studies were limited by the lack of objective measurement of crumb properties. Schober et al. (2007) used maltogenic α-amylase and sourdough fermentation to modify the texture of sorghum bread. Breads treated with α-amylase had a lower staling rate than the control but the differences between the crumb firmness of the two breads were not significant at any given time. The limited efficiency of α-amylase in the sourdough sorghum bread was attributed to inactivation of the enzyme during incubation in the sourdough medium or insufficient dosage of the enzyme. In another study, Onyango et al. (2010a) showed that crumb firmness and chewiness of bread prepared from sorghum and native or pregelatinised cassava starch declined whereas crumb adhesiveness increased with increasing enzyme concentration. Adhesiveness is an undesired crumb attribute whose sensory equivalence is perceived as a wet and sticky crumb. The defect results from enzymatic degradation of pregelatinised and mechanically damaged starch during proofing and in the early stages of baking before the enzyme is inactivated. Also crumb springiness and resilience declined with increasing enzyme concentration.

Transglutaminase is a cross-linking enzyme that is widely accepted as a processing aid in the food industry. Onyango et al. (2010b), Renzetti et al. (2008) and Moore

et al. (2006) have investigated the use of transglutaminase to make gluten-free bread. These authors reported that increasing enzyme concentration increased crumb firmness and chewiness. However, recent research in the molecular mechanism of celiac disease demonstrate that microbial transglutaminase can deamidate gluten proteins that generate gluten peptides that can stimulate gluten-specific T cells from celiac patients' peptides (Dekking et al., 2008).

Protease can be used to break down the protein matrix of the corneous endosperm in order to decrease crumb grittiness. Attempts have also been made to study the effect of proteases on the texture of gluten-free bread. Proteases are unable to degrade kafirins because they are located in the interior of stable protein bodies (Oria et al., 1995). However, proteins in the liquid phase can be degraded by proteases (Schober et al., 2007; Elkhalifa et al., 2006). This, therefore, means that as long as protein matrix from the corneous endosperm is not destroyed crumb grittiness of the bread will remain. Other undesirable changes due to protease action on sorghum bread are increased adhesive property of sorghum batter and weakened crumb structure (Hart et al., 1970).

Fats and emulsifiers

Polar lipids in fermenting batters are surface active compounds that can help to stabilize liquid films around the gas bubbles (Gan et al., 1995; Gan et al., 1990). Sorghum is low in polar lipids, due to the low amounts of glycolipids, and it is

unknown if added polar lipids can improve the quality of sorghum bread (Schober, 2009). Nevertheless, up to 3% fat improves loaf volume and decreases crumb firmness and crumb firming ratio or staling rate of sorghum bread. More than 3% fat decreases loaf volume and increases crumbliness due to decrease in crumb cohesiveness (Hugo et al., 1997). Fat also improves the energy value of the bread.

Emulsifiers or surface-active compounds are compounds that lower the interfacial energy between two immiscible phases, thus facilitating the dispersion of one phase into the other. The level of natural surface active compounds in sorghum is low, so these need to be added in order to stabilize the gas bubbles. Emulsifiers induce mixed performance in sorghum bread. Onyango et al. (2009b) found that increasing emulsifier concentration; whether sodium stearoyl-2-lactylate, diacetyl tartaric acid esters of mono- and diglycerides, calcium stearoyl-2-lactylate or glycerol monostearate from 0.4 to 2.4% decreased crumb firmness and staling rate but increased crumbliness. Hugo et al. (1997) found that increasing concentration of succinylated monoglycerides of glycerol 2% or sodium stearoyl-2-lactylate by up to 5% increased staling rate. The difference between these two studies may be because Onyango et al. (2009b) used pregelatinised cassava starch whereas Hugo et al. (1997) used native cassava starch. Pregelatinised starch is able to bind more water and better prevent it's loss during baking. Also different emulsifiers were used in the two studies. Other findings of Hugo et al. (1997) showed that increasing concentration (0-5%) of succinylated monoglycerides, glycerol or

sodium stearoyl-2-lactylate decreased loaf volume and crumb firmness, while increasing crumb fragility. Furthermore high concentrations of succinylated monoglycerides gave the bread a bitter taste and produced crumbs with larger, fewer and thicker cells. An optimal combination of fat and emulsifier may give acceptable bread. Hugo et al. (1997) found the best sorghum bread from a combination of 1% fat and 1% succinylated monoglyceride. The bread had a fine crumb texture, low crumb firmness and low staling rate. But it also had an off-flavour, which was difficult to account for since fat or succinylated monoglyceride do not have off-flavours. In another study, Olatunji et al. (1992b) found that monoglycerol palmitate up to 0.6% flour-weight-basis improved crumb structure and specific volume of sorghum bread. They argued that the emulsifier counteracts repulsive forces between starch granules in the batter and causes them to adhere to each other. Higher levels of monoglycerol palmitate imparted deleterious effects on taste and flavour. Hart et al. (1970) found that glycerol monostearate, vegetable shortening or mono and diglycerides of fat-forming fatty acids weakened the crumb structure of sorghum breads.

Sourdough

Sourdough is a mixture of flour, water, and other ingredients that is fermented by naturally occurring lactic acid bacteria and yeast (Gobetti et al., 2008). Other than its natural and additive-free image, sourdough has various positive effects in breadmaking. It improves the texture, flavour, nutritional value and shelf-life of

wheat and rye breads (Moroni et al., 2009; Gobetti et al., 2008). The positive effects of sourdough can be exploited in gluten-free bread production since the microbiological and qualitative characterisation of local gluten-free fermented products indicate an overlap with the microbiota of wheat/rye fermentation (Moroni et al., 2009).

Hart et al. (1970) found that sourdough fermentation did not improve texture of sorghum bread relative to bread prepared from unfermented batter but altered the flavour. This study was limited in that there were no objective measurements of the loaf's properties. Schober et al. (2007) prepared sorghum bread from sourdough sorghum. Fermentation of the total amount of sorghum flour was achieved using *Lactobacillus plantarum.* During sour dough fermentation the batter became thinner due to enzymatic degradation of proteins and damaged starch. Rheological evaluation showed that sourdough sorghum formed a starch gel with a higher peak viscosity than the control (unfermented sorghum). The bread had a round top and a continuous cohesive crumb, whereas the control had a collapsed crust and hole in the crumb. Sourdough sorghum bread had a slightly slower staling rate than the control although the differences were not significant at any given time. This is despite the fact that sourdough bread had α-amylase, which is known to have antistaling properties (Morgan et al., 1997). As already mentioned, probably the enzyme concentration was too low or it was inactivated in the sourdough medium. The sourdough breads were more cohesive or less brittle

than the controls. Also, the crumbs were less gritty in the mouth, probably due to degradation of coarse particles during sourdough fermentation. However, sourdough fermentation cannot totally eliminate the gritty mouthfeel because it does not degrade the protein bodies from vitreous endosperm but rather only the proteins in the liquid phase which would aggregate on baking. Crumb analysis using laser scanning confocal microscopy showed the presence of aggregated proteins in the control sorghum bread whereas sourdough sorghum bread had only small isolated patches of proteins bodies embedded in protein matrix. The authors concluded that a strong starch gel without interference of aggregated proteins is desirable for sorghum bread (Schober et al., 2007).

Sourdough bread is a niche product consumed mostly in central and eastern Europe and its acceptability could be problematic to consumers not accustomed to sour-tasting bread (Moroni et al., 2009). It is not known how consumers in sub-Saharan Africa would react to sourdough sorghum bread but it is known that lactic acid fermented sorghum porridge is widely consumed in the region (Taylor and Belton, 2002). To improve consumer acceptance, the sour taste of sorghum bread may be neutralised by adding calcium carbonate (Schober et al., 2007).

Malt

Malting involves germination of the grain in moist air followed by drying the "green" malt to produce a shelf-stable product. Malting induces important

beneficial biochemical changes in sorghum grains. The enzymes produced during germination lead to hydrolysis of starch and proteins with the release of sugars and amino acids. Proteolytic enzymes improve amino acid availability, particularly lysine, methionine and tryptophan that are lacking in cereals (Hounhouigan et al., 2003). The malting process essentially involves steeping, germination and drying.

Steeping involves immersing the grain in water until it has taken a sufficient amount to initiate germination at the optimum steeping temperature of 25-30°C (Dewar et al., 1997). Steeping time varies from 6-24 h and increases grain moisture content to 33-35% on wet-weight-basis (Taylor and Belton, 2002). During steeping the sorghum grains swell and the soluble carbohydrates are degraded. The emergence of the radicle through the pericarp marks the end of steeping. The steeped grain is then transferred to the germination chamber.

The optimal temperature for germinating sorghum to produce malt of the highest quality diastatic power and free amino nitrogen is 24-28°C (Taylor and Belton, 2002). During germination, hydrolytic enzymes progressively degrade the starch and the protein in the endosperm. Much of the nitrogen in the kernel is transferred to the shoots and roots resulting in increased protein and non-protein nitrogen. This is due to the translocation of the products of protein breakdown in the kernel to the shoots and roots (Taylor and Belton, 2002).

Drying involves reducing the moisture content of the green sorghum malt to around 10%. For further processing, the drying temperature should not exceed 50°C (Taylor and Belton, 2002). This is because at high temperatures amylases are inactivated resulting in a malt with dark colour and bitter taste. The enzymatic activity in malt is dominated by α-amylase and proteolytic enzymes.

The protein bodies in sorghum flour are held together by matrix protein originating from the vitreous endosperm (Duodu et al., 2002). These protein bodies contribute to the gritty mouthfeel of sorghum bread (Schober et al., 2007). Protein bodies cannot significantly hydrolyse themselves in ungerminated sorghum. Addition of proteinase extract from germinated sorghum degrades the matrix protein and to a lesser degree the protein bodies (Taylor and Evans, 1989). Malting also leads to starch breakdown. Starch is required for formation of a cohesive crumb network, and its absence would result in a collapsed crust and hole in the bread crumb. There is also increase in dextrins that contribute to a wet and sticky crumb.

There is no evidence that malted sorghum has been used in the production of gluten-free sorghum bread. However malted sorghum has been used in wheat-sorghum bread. Bread made with boiled malt flour (30%) had an improved crumb structure, crumb softness, water-holding capacity and resistance to staling, as well as a fine malt flavour compared with the bread made with unmalted flour (30%).

Consumers preferred the malted sorghum bread over the bread made with unmalted flour (Hugo et al., 2000).

Other ingredients

Sugar is required to improve the flavour, promote yeast activity and mask the smell of sorghum. Replacing glucose with sucrose will lower the gelatinisation temperature of sorghum starch which ensures more complete gelatinisation. Salt is added to improve flavour. Too much salt may affect taste and also inhibit yeast activity. Yeast may added as instant active dry yeast without reconstitution or active dry yeast that requires reconstitution in warm water, compressed yeast or liquid yeast.

3. MATERIALS AND METHODS

3.1. Research design

All experiments were designed as single factor completely randomized designs with three replicates. The treatment factor investigated was sorghum variety. Six varieties were used for characterization of sorghum flour, batter rheology and quality of gluten-free bread. Five varieties were used for characterization of sorghum grain and malt because Milomehl was purchased in the form of flour. The results were subjected to one-way analysis of variance and differences in treatment means identified at $P \leq 0.05$ by Duncan's Multiple-range Test using GenStat Edition 13 software (VSN International Ltd, UK).

3.2. Materials

Five sorghum varieties (KARI Mtama II, Serena, Gadam, Seredo and Kaguru) were purchased from Kenya Agriculture Research Institute. Milomehl red sorghum was purchased from Birlin-Mühle (Rheinfelden-Degerfelden, Germany) and was the control in this study. Cassava starch (11% moisture, 87.6% starch and 0.11% ash) was purchased from Universal Starch Public Company Limited, Bangkok, Thailand. Precooked amaranth flour (*Amaranthus cruentus*) was purchased from Allgrain Company Ltd., Nairobi, Kenya. It had the following composition: 5.5% moisture content, 12.6% crude protein, 5.2% crude fibre, 2.6% total ash, 6.6% crude fat and73% total carbohydrates.

3.3. Characterization of sorghum grain

The grains were classified on the basis of colour, weight and hardness. Colour was evaluated visually. The 1000-kernel weight was determined by weighing 100 grains and thereafter multiplying the weight obtained by 10. Hardness was determined using a craft knife adapter attached to a TA.XT.*plus* Texture Analyser (Stable Micro Systems, Surrey, UK). Ten grains were used for determining grain hardness. Cutting force was measured against time at the following conditions: test speed 1 mm/s, trigger force 50 g, post-test speed 10 mm/s and compression distance 9.5 mm. The area under the curve (total work/energy to cut or toughness)was used as an indicator of grain hardness.

3.4. Characterization of sorghum flour

3.4.1. Particle size distribution

The grains were dehulled using a PRL /IDRC dehuller (KIRDI, Kenya) before determining the extraction rate and milled using a UTL USCH/UZ mill (Bauermeister GmbH, Hamburg, Germany). The particle size distribution of the flours was determined by sieving flour (50 g) for 10 min in a Minor M200 electric sieve shaker (Endecotts Limited, London, England) with sieve apertures of 125, 180, 300 and 500 μm.

3.4.2. Moisture content

Moisture content of the milled sorghum was determined according to AOAC Method 14.004 (AOAC, 1984). Sorghum flour samples (5 g) were dried in an air oven at 105°C to constant weight. Moisture content was calculated as a percentage of the total dry matter in the sample.

3.4.3. Crude protein

Crude protein content (N x 6.25) was determined according to the improved Kjeldahl method (Approved Method 46-12A; AACC, 2000)with slight modifications. About 0.5 g ground sample of known dry-matter content was accurately weighed in a nitrogen free-filter paper, folded carefully and placed in a Kjeldhal flask. One tablet of Kjeldhal catalyst and 5 ml of concentrated sulphuric acid were added to the flask. The mixture was digested in a fume cupboard for about 2h until a clear solution was obtained. A blank sample of only a filter paper, Kjeldhal catalyst and sulphuric acid was also digested. After cooling, enough distilled water was added to increase the volume of the mixture to three-quarters of the flask. The flask was connected to the distillation unit after adding 1 ml phenolphthalein and 10 ml 40% sodium hydroxide solution. Distillation was carried out until a drop of distillate did not react with Nessler's reagent placed in a test tube. The distillate was collected in a 400 ml conical flask containing 50 ml 0.1 mol/l hydrochloric acid solution and 2-3 drops methyl orange indicator. The

excess hydrochloric acid solution in the distillate was back titrated with 0.1 mol/l sodium hydroxide. The percent nitrogen was calculated as follows:

$$\%\,\text{nitrogen} = C_{HCl} \times \frac{(V_{HCl(s)} - V_{HCl(b)}) \times 14.007}{S}$$

Where: C_{HCl} = normality of hydrochloric acid

$V_{HCl(s)}$ = volume of hydrochloric used to titrate the sample in ml

$V_{HCl(b)}$=volume of hydrochloric used to titrate the blank in ml

S = sample weight in g

% protein content was calculated by multiplication of % nitrogen obtained by 6.25.

3.4.4. Crude fibre

Crude fibre was determined according to AOAC Approved Method 985.29 (AOAC, 1985). Approximately 2 g ground sample of known dry-matter content was accurately weighed into a graduated 600 ml beaker and about 100 ml boiling distilled water and 2.04 mol/l sulphuric acid solution added. The volume of the mixture was made up to 200 ml with boiling distilled water and maintained at this volume whilst boiling for 30 min on a hot plate. The mixture was then filtered using a buchner funnel lightly packed with glass wool. The residue was washed three times with boiling distilled water. The residue and the glass wool were transferred quantitatively back to the beaker and about 100 ml of boiling distilled water and 25 ml of 1.73 mol/l potassium hydroxide solution added. The volume was made up to 200 ml with boiling distilled water and this volume maintained

whilst boiling on a hot plate for 30 min. The mixture was filtered again using glass wool and washed three times with boiling distilled water. The residue was further washed three times with small amounts of ethanol. The residue and glass wool were transferred quantitatively to a porcelain dish and dried in an air oven at 105°C for 2 h. The sample was cooled and weighed in the porcelain dish before igniting at 550°C in a muffle furnace to constant weight. The sample was cooled in a dessicator and weighed. The crude fibre content was calculated and expressed as a percentage of the sample dry matter content.

3.4.5. Total ash and mineral profile

The ash content of the flours was measured according to AOAC Approved Method 942.05 (AOAC, 1984). Approximately 2 g of each sample was weighed into a porcelain crucible and placed in a temperature controlled furnace preheated to 550°C. The sample was held at this temperature for 2 h. The crucible was then transferred directly to a desiccator, cooled and weighed. Ash content was reported as a percentage of the whole sample.

For mineral profiling of the samples, the ash was added to 10 ml 50% hydrochloric acid and heated until a yellow colour was observed. This was then made up to 50 ml using distilled water and the concentrations of calcium, iron, zinc, magnesium, manganese, copper, and potassium determined using an AA-6300atomic absorbance spectrophotometer (Shimadzu Scientific Instruments, Columbia,

USA). Ammonium vanadate molybdate solution was added to the extract at a ratio of 4:1.The phosphorus content was determined by reading the absorbance of this solution using a Cary 50 UV-Vis spectrophotometer (Agilent Technologies, California, USA) at 530 nm against standards containing 2, 4, 6, 8 and 10 ppm phosphorus.

3.4.6. Crude fat

Crude fat was determined according to AOAC Approved Method 24.005 (AOAC, 1984) with slight modifications. Approximately 5 g ground sample of known dry matter content was weighed accurately into an extraction thimble and covered with cotton wool. The thimble was placed into the soxhlet extractor and the fat extracted into a tared flask for 6 h using petroleum ether (boiling point 40-60°C). The solvent was then evaporated in a rotary evaporator and the residue dried in an air oven at 105°C for 1 h before weighing. The crude fat content was expressed as percentage of the sample dry-matter content.

3.4.7. Total carbohydrates

Total carbohydrate content was estimated by the difference between 100 and the sum of values for fat, protein, crude fibre and total ash on dry matter basis.

3.4.8. Starch

Total starch content was determined according to the AACC Method 76.13(AACC, 2000), Megazyme Total Starch Assay Procedure (Amyloglucosidase/α-Amylase), K-TSTA 04/2009 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). The sample was ground to less than 0.5 mm and 100 mg weighed into centrifuge tubes. Aqueous ethanol (80% v/v, 5 ml) was added into the tubes which were then incubated at 80-85°C for 5 min. The contents were mixed on a vortex mixer, 5 ml of 80% ethanol added and centrifuged for 10 min at 2,000 x g in a CN-2060 centrifuge (MRS Laboratory Equipment, Holon, Israel). The supernatant was discarded and the pellet resuspended in 10 ml 80% ethanol and mixed in a vortex mixer. This was then centrifuged for 10 min at 2,000 x g and the supernatant poured off. Thermostable α-amylase solution (3 ml) was added to each tube and incubated in a boiling water bath for 6 min while stirring vigorously after 2, 4 and 6 min for 5 s in a vortex mixer. Amyloglucosidase solution (0.1 ml) was added to each tube, stirred and incubated at 50°C for 30 min. The contents of the test tubes were transferred to 100 ml volumetric flasks, filled to the mark with distilled water and mixed thoroughly. Aliquots of these solutions were centrifuged at 2,000 x g for 10 min and 0.1 ml aliquots of the supernatant solution transferred to three test tubes. Glucose determination reagent solution (3 ml) was added to each tube (including glucose standards and reagent blank tubes) and incubated at 50°C for 20 min. The absorbance of the solutions was measured using a Cary 50 UV-Vis

spectrophotometer (Agilent Technologies, California, USA) at 510 nm against a reagent blank and total starch content calculated as follows:

$$\text{Starch, \%} = \Delta A \times \frac{F}{W} \times FV \times 0.9$$

Where:

ΔA = absorbance

F = 100 (μg of glucose)/absorbance for 100 μg of glucose

W = weight in milligrams of sample

FV = final volume

3.4.9. Starch damage

Starch damage was determined according to the AACC Method 76.31 (AACC, 2000), Megazyme Starch Damage Assay Procedure, K-SDAM 05/2008 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland).Samples (100 mg) were weighed into centrifuge tubes and pre-equilibrated at 40°C for 5 min. Pre-equilibrated fungal α-amylase solution (1 ml) was added to each tube, mixed for 5 s in a vortex mixer and incubated at 40°C for 10 min. Dilute sulphuric acid (8 ml)was added to each tube and stirred for 5 s. The tubes were centrifuged at 2,000 x g for 5 min in a CN-2060 centrifuge (MRS Laboratory Equipment, Holon, Israel) and 0.1 ml aliquots of the supernatant solution transferred to two test tubes. Amyloglucosidase solution (1 ml)was added to each tube, stirred and incubated at 40°C for 10 min. Glucose determination reagent solution (4 ml) was added to each

tube (including glucose standards and reagent blank tubes) and incubated at 40°C for 20 min. The absorbance of the solutions was measured using a Cary 50 UV-Vis spectrophotometer(Agilent Technologies, California, USA) at 510 nm against a reagent blank and starch damage calculated as follows:

$$\text{Starch Damage, \%} = \Delta E \times \frac{F}{W} \times 8.1$$

Where:

ΔE = absorbance

F = 150 (μg of glucose)/absorbance of 150 μg of glucose

W = weight in milligrams of sample

3.4.10. Total soluble sugars

The amount of total soluble sugars was estimated by the phenol sulphuric acid method (Dubois et al.,1956). Extraction of the sugars was done by adding 1 g of sample to 1000 ml distilled water. After extraction, 1 ml diluted sample was pipetted into a test tube and 1 ml distilled water added. To each test tube (standard and sample tubes), 0.05 ml 80% phenol was added and mixed in a vortex tube mixer for 5 s. Concentrated sulphuric acid (5 ml) was added and mixed in a vortex mixer. The tubes were left to stand for 10 min and then placed in a water bath at 25°C for 10 min. The tubes were vortexed again and the absorbance of the solutions measured using a Cary 50 UV-Vis spectrophotometer (Agilent Technologies, California, USA) at 490 nm against a reagent blank. The

concentration of total soluble sugars was determined from the glucose standard curve.

3.4.11. Reducing sugars

The reducing sugars were determined using the Nelson-Somogyi alkaline copper reduction method as described by Krishnaveni et al., (1984). Extraction of the sugars was done by adding 0.5 g of sample to 25 ml hot 80% ethanol and mixed vigorously on a vortex mixer for 10 min. The mixture was then decanted and the procedure repeated with the precipitate. The decantate was centrifuged at 3,980 × g for 10 min before the supernatant was collected and the ethanol evaporated in a water bath at 80°C. Distilled water (20 ml) was added to dissolve the sugars. The extract (1 ml) was added to 4 ml distilled water, mixed with 5 ml copper solution (0.185 g sodium sulphate, 23.96 g sodium carbonate, 12.14 g sodium potassium tartrate and 4 g copper sulphate diluted to 1 l with distilled water) and heated in boiling water for 60 min. the mixture was cooled to 25°C and reacted with 5 ml arsenomolybdate solution (49.43 g molybdic acid, 21 ml concentrated sulphuric acid and 5.93 g arsenic acid diluted to 1 l). The reducing sugar content was determined by reading the absorbance using a Cary 50 UV-Vis spectrophotometer(Agilent Technologies, California, USA) at 546 nm against standards containing 0, 0.36, 0.72, 1.08 and 1.44 mg glucose monohydrate in 100 ml distilled water.

3.4.12. Diastatic power

Diastatic power (joint α- and β-amylase activity) was determined following extraction of 5 g sample in 100 ml distilled water. Extraction was done at 30°C for 2.5 h. The filtrate (10 ml) was added to soluble starch solution (2% w/v, 200 ml) at 30°C. The reaction was stopped after 30 minby the addition of 20 ml 0.5 mol/l sodium hydroxide and the volume made up to 250 ml with distilled water. The sugar content of the solution (5 ml) was determined using alkaline ferricyanide procedure according to American Society of Brewing Chemists (ASBC, 1958). The digested sample (5 ml) was added to 10 ml 0.05 mol/l alkaline ferricyanide reagent (16.5 g potassium ferricyanide and 22 g anhydrous sodium carbonate dissolved in distilled water and made up to 1 l) in a 125 ml erlenmeyer flask. After mixing well, the flask was immersed in a vigorously boiling water bath for exactly 20 min and cooled under running water to room temperature. Thereafter, 25 ml acetic acid-salt solution (70 g potassium chloride and 20 g crystallized zinc sulphate dissolved in distilled water, 200 ml of glacial acetic acid added and made up to 1 l with distilled water) and 1 ml of potassium iodide solution were added. This was mixed well and titrated with the 0.05 mol/l sodium thiosulphate solution to the complete disappearance of the blue colour. The diastatic power was calculated as:

$$\text{Diastatic power}^{\circ}\text{(dry basis)} = \frac{(B - A) \times 23 \times 100}{100 - M}$$

Where:

B = ml of sodium thiosulphate used for the blank correction titration

A = ml of sodium thiosulphate used for the direct titration

M = per cent moisture in the sample

3.4.13. Free amino nitrogen

Whole milled malt (1 g) was added to 40 ml 5% trichloroacetic acid at 30°C and extracted for 1 h at 30°C. At 15 min intervals, the extraction tubes were swirled to suspend the contents. The extract (10 ml) was centrifuged at 3,980 x g for 10 min using a CN-2060 centrifuge (MRS Laboratory Equipment, Holon, Israel) and 1 ml of clear supernatant diluted to 25 ml with distilled water. The samples were then subjected to ninhydrin assay according to AOAC Approved Method 10.180(AOAC, 1980). The diluted sample (2 ml) was transferred to each of three 10 x 150 mm test tubes to obtain 1-3 mg FAN/l in diluted solution. A blank of 2 ml distilled water and a standard of 2 ml glycine working solution was also transferred to 10 × 150 mm test tubes. Glycine working solution was prepared by dissolving 107.2 mg glycine in water and diluted to 100 ml for the stock solution and 1 ml of this solution was diluted to 100 ml. Ninhydrincolour reagent was prepared by dissolving 10 g sodium hydrogen phosphate, 6g potassium dihydrogen phosphate, 0.5 g 1,2,3- indantrione.H_2O and 0.3 g fructose in water and diluted to 100 ml. The ninhydrin colour reagent (1 ml) was added to the sample, blank and standard test tubes and heated exactly for 16 min in boiling water bath. This was then cooled for 20 min in water bath at 20±1°C and 5 ml dilution solution (2 g

potassium iodate dissolved in 600 ml water and 400 ml alcohol added) was added. After mixing thoroughly the absorbance was read using a Cary 50 UV-Vis spectrophotometer (Agilent Technologies, California, USA) at 570 nm against water within 30 min. Free amino nitrogen in the sample was calculated by:

$$\text{mg free amino}\frac{\text{nitrogen}}{\text{l}} = \frac{(A_s - A_b) \times 2 \times \text{dilution}}{[(A]_g - A_b)}$$

Where:

A_s= absorbance of the sample

A_b = absorbance of the blank

A_g = absorbance of the glycine standard

3.4.14. Pasting properties

Viscograph-E (Brabender GmbH and Co. KG, Duisburg, Germany) was used to characterize the flours. Moisture content of samples was determined as described in 3.4.2. Samples (40 g) were transferred into a canister and approximately 420 ± 0.1 ml of water was added (corrected to compensate for 14% moisture basis). The temperature-time conditions were: heating from 30 to 95°C at the rate of 1.5°C/min and holding at 95°C for 10 min. The parameters measured were: paste temperature (°C), peak viscosity (Brabender units) and time to peak viscosity (min).

3.4.15. Tannins

Tannin content was determined using the modified vanillin-hydrochloric acid assay (Price et al., 1978). Tannins were extracted by shaking 1 g sample in 10 ml acidified methanol (1 ml concentrated hydrochloric acid/100 ml methanol) in centrifuge tubes at 25°C for 20 min. The sample was centrifuged at 3,980 x g for 15 min in a CN-2060 centrifuge (MRS Laboratory Equipment, Holon, Israel) before pipetting 1 ml into a test-tube. Vanillin-hydrochloric acid reagent was prepared by mixing equal portions of vanillin solution (4 g vanillin/100 ml methanol) and acidified methanol (8 ml concentrated hydrochloric acid/100 ml methanol). The vanillin-hydrochloric acid reagent (5 ml) was added to the sample and absorbance read in 1 cm cuvettes using a Cary 50 UV-Vis spectrophotometer (Agilent Technologies, California, USA) at 500 nm after 20 min against vanillin-hydrochloric acid reagent as blank. To correct for interference of natural pigments, sample blanks were prepared by subjecting the original extract to the conditions of the reaction but without the vanillin-hydrochloric acid reagent. A standard curve was prepared by adding 1 g tannic acid (FlukaChemie GmbH, Buchs, Switzerland) to 100 ml acidified methanol and the stock solution used at various dilutions from 1:10 to 1:50.

3.5. Characterization of malted sorghum

3.5.1. Preparation of sorghum malt

Each sorghum variety was washed using tap water then steeped for 24 h in tap water at 24°C and the steep water changed after 4 and 8 h. After steeping, excess water was drained and the grains were placed in perforated nylon bags and allowed to germinate for 72 h at 24°C. Twice daily the bags were immersed for 10 min in tap water after which they were gently turned to avoid meshing of the roots and shoots. At the end of germination, the green malt was sun-dried for 72 h. The dried malt (together with external roots and shoots) was milled using a disc mill and the flours stored in moisture-proof containers at 24°C prior to use.

3.5.2.Physico-chemical composition of sorghum malt

Moisture content, crude protein, crude fibre, total ash, crude fat, total carbohydrates, starch, starch damage, total soluble sugars, reducing sugars, diastatic power, free amino nitrogen, pasting properties and tannin content of malted sorghum flours were determined as described in section 3.4.2 to 3.4.15 respectively.

3.6. Effect of sorghum variety on rheology of gluten-free batter

3.6.1. Development of a recipe

Milomehl was used to develop a bread recipe for evaluation of batter rheology and crumb texture. There was one formulation made from 50% cassava starch and

50% sorghum flour (Onyango et al., 2011b) used to serve as the control whereas the other three were prepared by varying levels of cassava starch and sorghum flour while amaranth flour remained constant. The different ratios of cassava starch to sorghum flour to amaranth flour were 60:30:10, 50:40:10, 40:50:10. The other ingredients, weighed on flour-weight-basis, water (75%), sugar (6.3%), baker's fat (2.5%), salt (2.1%) and malt flour (1%). Malt flour having the highest diastatic power (as determined in 3.4.12) was used in the formulation for batter and bread. The recipe with the best results was used for measuring consistency of batter containing each sorghum variety.

3.6.2. Batter rheology

Six batter formulations were prepared containing the different sorghum varieties. The ingredients were mixed using a Kenwood 900 Watts KM264kitchen mixer (Kenwood Limited, Hampshire, UK) at slow speed for 2 min to obtain homogenous batter. The batters were incubated at 30°C for 1 h before measuring their consistencies using an HDP/FE forward extrusion cell of a TA.XT.*plus* Texture Analyzer equipped with a 50 kg load cell(Stable Micro Systems, Surrey, UK). Prior to measurements, the pastes were manually stirred to guarantee homogeneity and 100 g loaded into the cell. Compression force was measured at the following conditions: pre-test speed 10 mm/s, test speed 1 mm/s, trigger force 50 g, post-test speed 10 mm/s, compression distance 20 mm, outlet diameter of

diameter of extrusion cell 3 mm. The average force after reaching a plateau (at 12-18 s) was used as an indicator of paste consistency.

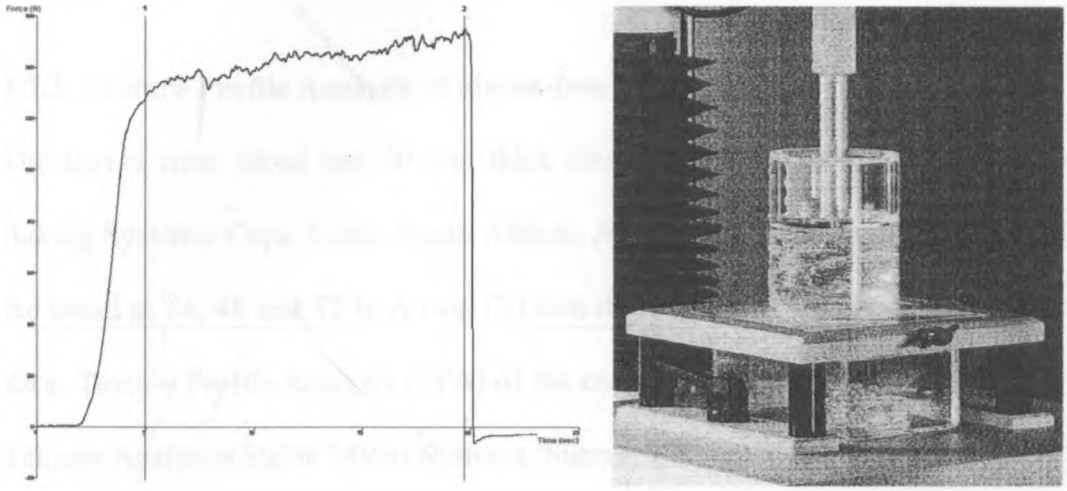

Figure 2.(a) Region in which the average force (N) was determined, (b)front view of TA.XT.*plus* Texture Analyser used to measure consistency of gluten-free batter.

3.7. Effect of sorghum variety on crumb texture of gluten-free bread

3.7.1. Preparation of gluten-free bread

Six bread formulations were prepared containing the different sorghum varieties.The batter formulations were prepared as described in 3.6, but also with the addition of instant active dry yeast (3.8% flour-weight-basis). The dry ingredients were manually mixed in a wide bowl then added to the mixing bowl containing water and fat. The components were mixed for 3 min using a Kenwood 900 Watts KM264 kitchen mixer (Kenwood Limited, Hampshire, UK). Batter (400 g) were weighed into baking tins and proofed for 10 min at 32°C and 85% relative humidity. After baking, the loaves were depanned and placed on cooling

racks for 2 h then packed in moisture-permeable polythene bags and closed with a twist tie and stored for 22 h at 25°C.

3.7.2. Texture Profile Analysis of gluten-free bread

The loaves were sliced into 20 mm thick slices using a bread slicer(MacAdams Baking Systems, Cape Town, South Africa). A slice was taken from the centre of the bread at 24, 48 and 72 h. A ring (30 mm diameter) was punched out from the slice. Texture Profile Analysis (TPA) of the crumbs was done using a TA.XT.*plus* Texture Analyzer(Stable Micro Systems, Surrey, UK) equipped with a 50 kg load cell.

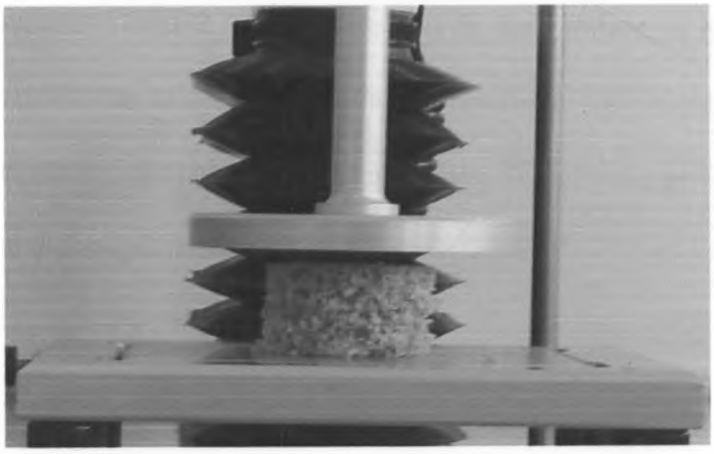

Figure 3. Front view of TA.XT.*plus* Texture Analyser showing the mechanism of compression.

The instrument settings were: pre-test speed 1.0 mm/s; test speed 5.0 mm/s; post-test speed 5.0 mm/s; distance 10 mm (i.e. 50% compression), trigger type auto force 5 g; data acquisition rate 200 pps, 75 mm diameter aluminium probe. The waiting time between the first and second compression cycle was 5 s. Hardness,

cohesiveness, springiness, resilience, adhesiveness and chewiness were calculated from the Texture profile analysis graph (Figure 4). Hardness (N) is the peak force that occurs during the first compression cycle. Cohesiveness (dimensionless) is the ratio of the positive force area during the second compression to that during the first compression i.e. area between anchor 4 and 6/area between anchor 1 and 3. Resilience (dimensionless) is the ratio of the area between anchor 2 and 3 to the area between anchor 1 and 2. Adhesiveness (g.s) is the area between anchor 3 and 4. Springiness or elasticity (%) is defined as the height that the food recovers during the time that elapses between the end of the first bite and the start of the second. It is the ratio of the time between anchor 4 and 5 to the time between anchor 1 and 2. Gumminess (N) is the product of hardness x cohesiveness. It applies to semisolid materials. Chewiness (N) is the product of gumminess x elasticity. It applies to solid materials. Therefore, a product cannot be both gummy and chewy simultaneously (Bourne, 2002).

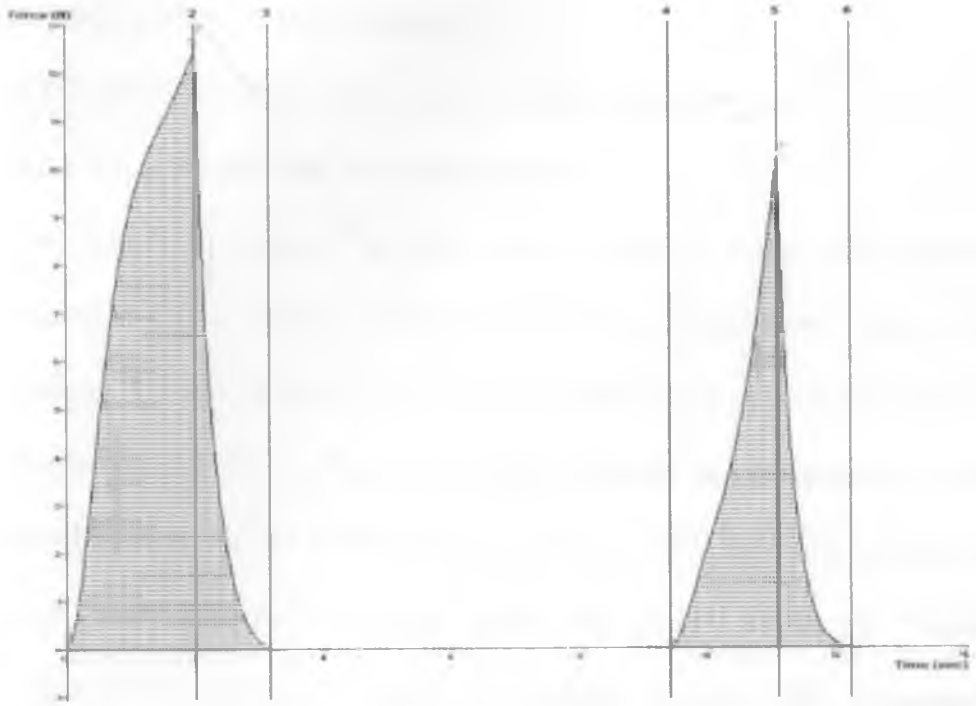

Figure 4.An example of a Texture Profile Analysis graph.

4. RESULTS AND DISCUSSION

4.1. Physico-chemical properties of sorghum varieties

4.1.1. Characterization of sorghum grain

The kernels of sorghum varieties varied widely in colour with Gadam being lightest in colour (white), whereas KARI Mtama II and Seredo were the darkest (reddish brown). Kaguru and Serena varieties had pink and brown kernels, respectively (Table 1, Figure 5). These findings are comparable to those of Schober et al. (2005) and Subramanian et al. (1992) in which sorghum kernels have been described as being white, red, yellow or brown. Tannins are polyphenolic compounds found in sorghum varieties with pigmented testa (Schober and Bean, 2008). Sorghum varieties with pigmented testa can have any pericarp colour including white meaning that the presence of tannins is not linked to kernel colour in sorghum (Schober and Bean, 2008). The presence of pigment is a genetic character controlled by the B1/B2 genes (Waniska, 2000).

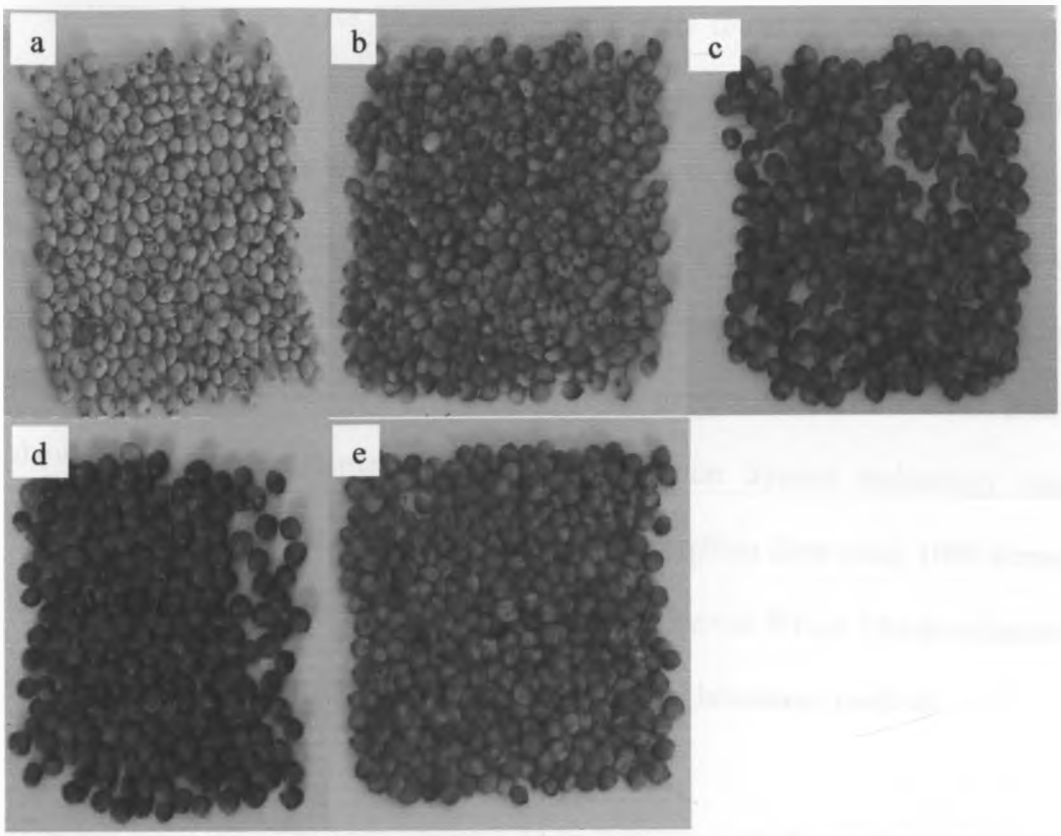

Figure 5. Sorghum grain colour Gadam(a), Kaguru(b), KARI Mtama IIc), Seredo (d) and Serena(e).

Table 1. Characterization of sorghum grain

Variety	Colour	1000-kernel weight (g)*	Grain hardness(N.sec)
Gadam	White	19.86 ± 0.18^{b}	1.34 ± 0.32^{a}
Kaguru	Pink	16.62 ± 0.14^{a}	2.64 ± 0.70^{b}
KARI Mtama II	Reddish brown	24.20 ± 0.12^{c}	1.70 ± 0.42^{a}
Seredo	Reddish brown	27.76 ± 0.08^{e}	1.67 ± 0.50^{a}
Serena	Brown	24.85 ± 0.07^{d}	1.76 ± 0.53^{a}

Values followed by the same superscript letter in the same column are not significantly different at $P > 0.05$.
*Values are given on dry-matter basis.
Milomehl was not included because it was purchased in the form of flour.

Significant differences for 1000-kernel weight were found ($P \leq 0.05$) among the various sorghum varieties (Table 1). Values ranged from 16.62 g for Kaguru to 27.76 g for Seredo. These results are comparable to those of Pederson et al. (1996) who studied the application of Single Kernel Wheat Characterization System to sorghum and compared this method to the traditional laboratory methods of determining grain hardness, diameter, weight and density. Results from their study showed that Single Kernel Wheat Characterization System technology was successfully applied to sorghum seed. For the 16 sorghum lines used, 1000-kernel weight values ranged from 15.5-38.0 g for Single Kernel Wheat Characterization System technology and 14.4-42.9 g for the traditional laboratory method.

Sorghum grain hardness varied from 1.34 to 2.64 N·s with Kaguru variety being significantly harder ($P \leq 0.05$) than the other varieties (Table 1). Grain hardness or endosperm texture (grain strength) is an important physical grain quality attribute that plays a role in plant defense against infestation (Chandrashekar and Mazhar, 1999); and in the processing of cereal grains and end-use quality of cereal-based products such as breads and snack foods (Bettge and Morris, 2000; Cagampang and Kirleis, 1984). The relative proportion of corneous to floury endosperm varies widely in sorghum and overall grain hardness is well correlated to percent vitreosity of the kernel (Hallgren and Murty, 1983). The endosperm hardness of sorghum has also been positively correlated with both protein content and prolamin composition. Hard grains tend to deposit α- and γ-prolamins and proteins

in greater amounts than soft grains. These prolamins are particularly higher in the translucent, outer portions of endosperm (Chandrashekar and Mazhar, 1999). Grain hardness is a key component governing the end-use quality of sorghum based foods that impacts starch damage of the flour. Amount of damaged starch in turn plays a large role in the quality of gluten-free bread.

4.1.2. Characterization of sorghum flour

The extraction rate after decortication and milling of the grains was 79.4% for Gadam, 76.7% for Kaguru, 78.4% for KARI Mtama II, 64.0% for Seredo and 77.1% for Serena. Extraction rate for Milomehl was not determined because it was purchased in the form of flour. Lower extraction rates of sorghum flour increases the light colour of the bread and crumb fineness but also increases the milling loss. On the other hand, a higher extraction rate yields bread whose crumb is darker and richer in bran particles. The latter bread has a coarse texture and small volume (Hart et al., 1970).

Flour particle size is an indication of the degree of fineness of a flour sample (Pratt, 1971). Table 2 shows the percentages of sorghum flour retained on sieves with different pore diameters. Less than 5% of the flour passed through the 125 μm sieve for all sorghum varieties. The amount of flour that passed through the sieve of aperture size 500 μm ranged from 58% for Seredo to 86% for KARI Mtama II. The granularity of sorghum flour depends on the milling technique and

kernel properties such as hardness (Schober, 2009). The considerably large particle sizes of milled sorghum flour are because a considerable portion of the starch is still embedded in the protein matrix (Schober et al., 2007; Duodu et al., 2003). Granularity influences the speed of swelling and the speed with which soluble components are extracted from the particles into the surrounding liquid phase (Schober, 2009).

Table 2. Particle size distribution of sorghum flour

Sorghum variety	% Retained on sieve*				%Passed through 125 µm sieve
	500 µm	300 µm	180 µm	125 µm	
Gadam	19.47±3.00^{ab}	41.63±4.05^{ab}	26.95±6.96^{bc}	7.40±1.06^{b}	2.74±0.11^{ab}
Kaguru	18.32±1.93^{a}	40.19±2.72^{ab}	31.14±1.88^{bc}	4.56±1.04^{a}	2.94±1.04^{b}
KARI Mtama II	13.72±2.09^{a}	44.52±0.22^{b}	35.40±2.50^{c}	4.22±0.16^{a}	1.71±0.16^{ab}
Seredo	42.26±1.26^{d}	39.59±1.53^{ab}	12.32±0.59^{a}	3.33±0.22^{a}	1.57±0.11^{ab}
Serena	32.37±1.05^{c}	38.90±0.24^{ab}	18.82±0.27^{ab}	4.81±0.37^{a}	3.03±0.53^{b}
Milomehl	26.87±2.97^{bc}	35.18±1.79^{a}	32.88±3.55^{c}	2.79±0.30^{a}	0.88±0.25^{a}

Values followed by the same superscript letter in the same column are not significantly different at $P > 0.05$.

Table 3 shows the proximate composition, on dry-weight-basis, of the sorghum flours. The composition was 9.76-12.30% moisture; 8.16-13.19% crude protein (N x 6.25); 1.71-2.61% fibre; 1.30-2.02% ash; 2.77-3.69% fat and 79.63-84.76% total carbohydrates. Gadam had the highest protein content though this was not significantly different ($P > 0.05$) from Seredo. KARI Mtama II had the highest fibre content and Milomehl the lowest, but there no significant differences ($P > 0.05$) among the sorghum varieties. KARI Mtama II was significantly higher ($P \leq$

0.05) in ash. Fat was highest in Milomehl although this was not significantly different (P > 0.05) from Gadam, Seredo and Serena. Serena had the highest total carbohydrates but this was not significantly different (P > 0.05) from Milomehl, Kaguru and KARI Mtama II. Generally, these values are in agreement with those reported for other sorghum varieties by Dicko et al. (2006), Liu et al. (2012) and Schober et al. (2005). The nutrient content of dehulled grains depends on the extent to which the nutrients in the bran are removed during processing. The most affected components are fibre, vitamins and minerals, which are concentrated in the outer bran and aleurone layers of the grain (Mckevith, 2004; Badi et al., 1976).

Table 3. Proximate composition of sorghum flour*

Variety	Moisture content (%)	Crude protein	Crude fibre	Total ash	Crude fat	Total carbohydrates
Gadam	10.02±0.09^{a}	13.19±0.75^{d}	2.00±0.36^{a}	1.56±0.05^{bc}	3.62±0.36^{b}	79.63±0.99^{a}
Kaguru	9.76±0.03^{a}	10.51±0.54^{bc}	2.22±0.70^{a}	1.46±0.13^{ab}	2.77±0.09^{a}	83.05±1.31^{bc}
KARI Mtama II	12.30±0.73	9.48±2.28^{ab}	2.61±0.51^{a}	2.02±0.24^{d}	2.77±0.25^{a}	83.12±2.54^{bc}
Seredo	12.05±0.12^{c}	11.46±0.67^{cd}	2.49±0.39^{a}	1.71±0.08^{c}	3.37±0.42^{b}	80.97±0.64^{ab}
Serena	10.11±0.08^{a}	8.16±0.26^{a}	2.24±0.57^{a}	1.61±0.08^{bc}	3.23±0.23^{ab}	84.76±0.23^{c}
Milomehl	10.85±0.02^{b}	9.34±0.38^{ab}	1.71±0.17^{a}	1.30±0.03^{a}	3.69±0.21^{b}	83.95±0.58^{c}

*Values are given on dry-matter basis (g /100 g) except for moisture content.
Values followed by the same superscript letter in the same column are not significantly different at P > 0.05.

The major minerals (>5 mg/100 g) in sorghum were potassium followed by phosphorus, magnesium and iron (Table 4). The contents of calcium, copper, zinc and manganese were less than 5 mg/100 g dry-matter basis. Calcium and manganese were significantly higher ($P \leq 0.05$) in Gadam while copper and phosphorus were significantly ($P \leq 0.05$) higher in Kaguru and KARI Mtama II

respectively. Milomehl had the highest magnesium levels, though there were no significant differences ($P > 0.05$) among all the varieties. Iron content was highest in Seredo but not significantly different ($P > 0.05$) from Gadam and Kaguru. KARI Mtama II had the highest potassium content but this did not differ significantly ($P > 0.05$) from Gadam, Kaguru, Serena and Milomehl. Zinc content was lowest in Serena while there were no significant ($P > 0.05$) differences among Gadam, Kaguru, KARI Mtama II, Seredo and Milomehl. These values are 10-15% lower than those of Dicko et al. (2006). Minerals are concentrated in the pericarp, aleurone layer and germ and are removed by decortication resulting in deficiency in the endosperm flour (O'Kennedy et al., 2006).

Table 4. Mineral composition of sorghum flour (mg/100 g dry-matter basis)

Variety	Calcium	Magnesium	Iron	Potassium	Zinc	Copper	Manganese	Phosphorus
Gadam	1.11±0.06^{d}	8.14±0.18^{a}	5.57±1.19^{b}	284.00±6.04^{ab}	2.50±0.20^{b}	0.17±0.03^{b}	4.14±0.14^{e}	24.99±1.38^{bc}
Kaguru	0.90±0.08^{c}	8.04±0.14^{a}	5.47±0.44^{ab}	273.90±8.75^{ab}	2.85±0.33^{b}	0.31±0.07^{d}	1.77±0.18^{c}	10.25±1.05^{a}
KARI Mtama II	0.55±0.03^{ab}	8.05±0.16^{a}	5.39±0.46^{a}	289.61±28.23^{b}	2.51±0.27^{b}	0.16±0.02^{b}	2.73±0.59^{d}	99.06±11.75^{d}
Seredo	0.49±0.10^{a}	8.09±0.05^{a}	7.12±1.71^{b}	261.73±9.83^{a}	2.48±0.19^{b}	0.25±0.02^{c}	1.21±0.23^{ab}	21.82±0.48^{bc}
Serena	0.61±0.02^{b}	8.13±0.06^{a}	4.08±0.10^{a}	271.38±4.40^{ab}	1.72±0.07^{a}	0.07±0.01^{a}	1.33±0.03^{bc}	16.15±0.92^{ab}
Milomehl	0.51±0.01^{ab}	8.17±0.22^{a}	4.27±0.18^{a}	268.15±4.41^{ab}	2.64±0.08^{b}	0.15±0.01^{b}	0.79±0.04^{a}	29.14±0.73^{c}

Values followed by the same superscript letter in the same column are not significantly different at $P > 0.05$.

Table 5. Characterization of sorghum flour

Variety	Starch (g/100 g)	Total soluble sugars (g/100 g)	Reducing sugars (g/100 g)	Damaged starch (g/100 g)	Diastatic power (°)	FAN (mg/l)	Tannins (mg/100 g)
Gadam	64.41±4.41^{ab}	4.06±0.22^{a}	0.029±2.22^{c}	2.64±0.36^{c}	5.96±1.48^{a}	39.74±5.65^{c}	1023.56±126.69^{a}
Kaguru	60.69±2.99^{a}	7.28±0.65^{b}	0.018±0.00^{a}	1.69±0.16^{ab}	5.95±1.47^{a}	26.07±1.95^{b}	1412.64±158.43^{b}
KARI Mtama II	61.81±1.28^{a}	8.38±0.76^{c}	0.028±1.32^{c}	2.15±0.11^{bc}	6.12±1.51^{a}	26.32±4.51^{b}	2433.44±331.59^{c}
Seredo	58.77±3.55^{a}	4.19±0.27^{a}	0.020±6.95^{ab}	2.61±0.08^{c}	5.23±0.00^{a}	22.42±0.93^{ab}	1729.12±148.58^{b}
Serena	63.49±2.50^{ab}	8.88±0.68^{c}	0.026±1.28^{bc}	1.23±0.06^{a}	4.26±1.48^{a}	22.13±3.75^{ab}	1533.63±69.94^{b}
Milomehl	68.21±3.77^{b}	8.64±0.76^{c}	0.020±3.89^{ab}	10.73±0.61^{d}	4.30±1.49^{a}	16.43±2.13^{a}	1031.38±101.25^{a}

Values followed by the same superscript letter in the same column are not significantly different at $P > 0.05$.
FAN: free amino nitrogen.

Starch is the main reserve polysaccaharide in sorghum and the principal source of carbohydrates. The starch content of the different sorghum varieties ranged between 58.77–68.21 g/100 g (Table 5) and was within the range that has been reported for other sorghum varieties (Dicko et al., 2006). Milomehl had the highest starch content, although this was not significantly different ($P > 0.05$) from Gadam and Serena. Total soluble sugars were highest in Serena (8.88 g/100 g) but not significantly different ($P > 0.05$) from Milomehl and KARI Mtama II. Gadam (4.06 g/100 g) and Seredo (4.19 g/100 g) had significantly lower values ($P \leq 0.05$). Reducing sugars ranged between 0.018 g/100 g and 0.029 g/100 g. Gadam was highest in reducing sugars content although this was not significantly different ($P > 0.05$) from KARI Mtama II and Serena varieties.

The amount of damaged starch of sorghum flour was 1.23-10.73%. The local varieties were significantly lower ($P \leq 0.05$) in starch damage compared to Milomehl (Table 5). Nonetheless, these values are much lower than those reported by other authors (Schober et al., 2005; Aboubacar and Hamaker, 1999). The difference between Milomehl and the other varieties may be due to the milling method used (Frederick, 2009). The milling technique and kernel properties affect the amount of mechanically damaged starch in flour, which in turn has an effect on water-binding capacity and susceptibility to enzyme activity (Schober, 2009). During milling, intact starch granules become damaged leaving starch granules that have been fractured, shattered or chopped (Chen and D'Appolonia, 1986).

Flour from the floury endosperm pulverizes easily and less of its starch is damaged. Starch granules are tightly packed into a rigid protein matrix in the horny endosperm of sorghum, and fine milling breaks the whole matrix apart, together with the embedded starch, resulting in the production of large quantities of mechanically damaged starch (Schober, 2009; Hallgren et al., 1992). It, therefore, appears that the lower amount of damaged starch in the local varieties meant that they were not subjected to severe impact milling as compared to Milomehl. Hydrolysis of damaged starch by α-amylase releases maltose, which is fermented by yeast to form carbon dioxide that causes the dough to rise. Thus, flours with higher damaged starch content will have more gas production, smaller cells and consequently softer crumb (Schober et al., 2005). For wheat, the optimum damaged starch level is considered to be between 14.1% and 16.5% (Rao et al., 1989).

Free amino nitrogen (FAN) is composed of amino acids and small peptides and is a product of proteolytic breakdown of endosperm proteins (Dewar et al., 1997). The FAN content ranged between 16.43 mg/l and 39.74 mg/l. Gadam flour had significantly higher ($P \leq 0.05$) FAN than the other varieties (Table 5). This was followed by KARI Mtama II, Kaguru, Seredo and Serena. Milomehl had the lowest FAN content. Diastatic power is a measure of the joint activity of α- and ß-amylase (Dewar, 2003). Diastatic power ranged between 4.26° and 6.12° (Table 5). There were no significant differences ($P > 0.05$) among the sorghum varieties.

Tannin content for the sorghum varieties varied between 1023.56 mg/100 g for Gadam and 2433.44 mg/100 g for KARI Mtama II (Table 5). KARI Mtama II had significantly higher ($P \leq 0.05$) tannin content than the other varieties. Sorghum grains are commonly classified based on the presence or absence of tannins but the colour of the grain should not be used as an indicator of the tannin content (Taylor and Belton, 2002). Tannins are secondary metabolites in the grain and are of major interest in sorghum because of their influence on the grain before and after harvest. Tannins occur mainly in the testa and to a limited extent in the pericarp (Taylor and Belton, 2002). Tannins are agronomically advantageous because they act as defense chemicals that protect the plant from predatory attacks of birds, herbivores, pathogenic fungi, parasitic weeds and insects (Schober, 2009; Dykes and Rooney, 2006). In storage, tannins prevent grain losses by premature germination and damage due to mould. Tannin-rich sorghum varieties have positive health-promoting properties and can be used as nutraceuticals and functional foods (Dykes and Rooney, 2006; Rooney and Awika, 2005). On the negative side, tannins also have antinutritional properties because they bind enzymes of the digestive tract, adversely affecting the utilisation of proteins and carbohydrates and availability of minerals (FAO, 1995).

Table 6. Pasting properties of sorghum flour

Sorghum variety	Pasting temperature (°C)	Peak viscosity (BU)	Time to peak viscosity (min)
Gadam	85.65 ± 0.21^{a}	252.00 ± 5.66^{bc}	43.34 ± 0.03^{ab}
Kaguru	87.85 ± 0.21^{ab}	249.00 ± 22.63^{bc}	43.25 ± 0.35^{ab}
KARI Mtama II	89.70 ± 0.14^{b}	189.50 ± 0.71^{a}	43.31 ± 0.07^{ab}
Seredo	89.80 ± 0.00^{b}	176.00 ± 2.83^{a}	43.48 ± 0.03^{b}
Serena	87.45 ± 0.92^{ab}	232.00 ± 18.32^{b}	43.13 ± 0.07^{ab}
Milomehl	86.60 ± 2.12^{a}	276.00 ± 1.41^{c}	42.79 ± 0.38^{a}

Values followed by the same superscript letter in the same column are not significantly different at $P > 0.05$.

Table 6 shows the pasting temperature, peak viscosity or maximum hot paste viscosity and time to peak viscosity or time required to achieve peak viscosity of the sorghum flours. Pasting temperature ranged between 85.65°C and 89.80°C. Seredo and KARI Mtama II had the highest pasting temperature, though this was not significantly different ($P > 0.05$) from Kaguru and Serena. Peak viscosity varied between 176 BU and 276 BU. Milomehl exhibited the highest peak viscosity, although this was not significantly different ($P > 0.05$) from Gadam and Kaguru. KARI Mtama II and Seredo exhibited the lowest peak viscosity. Time to peak viscosity ranged between 42.79 min and 43.48 min. Seredo had the highest time to peak viscosity, although this was not significantly different ($P > 0.05$) from Gadam, Kaguru, KARI Mtama II and Serena. Ragaee and Abdel-Aal (2006) studied pasting properties of starch in selected cereals and quality on their food properties. They reported higher pasting temperature values (94.9°C) than those in this study. This difference may result from genetic variations of sorghum used.

Pasting properties are important in determining the cooking and baking qualities of flours (PBIP, 1995). Starch when heated increases in viscosity as a result of the swelling of the starch granules. This may be attributed to the removal of water from the exuded amylose by the granules as they swell (Adenji et al., 2010). The onset gelatinization temperature indicates water absorption and swelling of the granules resulting in increased viscosity. Pasting temperature is the temperature at which the paste viscosity starts to increase. This gives an indication of the minimum temperature required to cook a sample and also influence energy cost. Lower pasting temperatures suggest low cost implication regarding processing. Peak viscosities attained during the heating portion indicates the water binding capacity of starch mixtures. This often correlates with final product qualities (Osungbaro et al., 2010).

4.2. Effect of malting on physico-chemical properties of sorghum varieties

Table 7 shows the proximate composition of sorghum malt, on dry-weight-basis, for the different sorghum varieties. The composition was 9.59-12.14% moisture; 7.97-12.57% protein (N x 6.25); 2.43-3.58% fibre; 1.38-1.66% ash; 2.74-3.94% fat; and 78.74-84.80% total carbohydrates. Gadam had the highest protein content, although this was not significantly different ($P > 0.05$) from Kaguru, KARI Mtama II and Seredo. Fibre was highest in KARI Mtama II, though this was not significantly different ($P > 0.05$) from Gadam, Kaguru and Seredo. There were no significant differences ($P > 0.05$) among the varieties in ash content. Crude fat was

significantly higher ($P \leq 0.05$) in Gadam. Serena had the highest carbohydrate content, although this was not significantly different ($P > 0.05$) from Kaguru, KARI Mtama II and Seredo. There were no significant differences ($P > 0.05$) between the compositions of the flours and malts, except for fiber, ash and fat in Gadam, Seredo, and Kaguru varieties, respectively (Appendix 1). The lack of significant differences ($P > 0.05$) in some nutrients, when flour was compared to malt, implies that the extent of loss of compounds by decortication was similar to the loss by malting. The biochemical and physiological changes that take place during soaking and germination lead to reduction in the levels of protein, fiber, fat, ash and carbohydrates (Elmaki et al., 1999). Results for protein, fibre and fat are comparable to those of Elmaki et al. (1999) who found 8.68-10.1%, 2.5-3.5% and about 2.5% respectively. During seed germination, part of the protein is utilized for growth and development of the embryo resulting in decreased protein content after malting. Also, some is lost as water-soluble nitrogen during steeping of seeds (Wu and Wall, 1980). These authors also observed a decrease in fibre content which they attributed to enzymatic solubilization of part of the seed fibre during seed germination. The decrease in fat observed by Elmaki et al. (1999) was attributed to the fact that part of seed fat is utilized for the production of energy required to support the biochemical and physiological changes occurring during germination.

Table 7. Proximate composition of sorghum malt*

Variety	Moisture content (%)	Crude protein	Crude fibre	Total ash	Crude fat	Total carbohydrates
Gadam	11.90±0.21^{c}	12.57±2.18^{b}	3.38±0.53^{ab}	1.38±0.12^{a}	3.94±0.08^{c}	78.74±2.82^{a}
Kaguru	9.59±0.31^{a}	11.02±1.90^{ab}	2.90±0.33^{ab}	1.39±0.16^{a}	3.39±0.25^{b}	81.30±1.70^{bc}
KARI Mtama II	12.14±0.10^{c}	9.77±0.56^{ab}	3.58±0.87^{b}	1.64±0.19^{a}	2.74±0.23^{a}	82.28±1.00^{bc}
Seredo	11.45±0.10^{b}	10.77±0.88^{ab}	3.05±0.25^{ab}	1.46±0.08^{a}	3.12±0.04^{b}	81.60±1.01^{abc}
Serena	9.73±0.24^{a}	7.97±1.75^{a}	2.43±0.26^{a}	1.66±0.13^{a}	3.15±0.12^{b}	84.80±1.73^{c}

*Values are given on dry matter basis (g /100 g) except for moisture content.
Values followed by the same superscript letter in the same column are not significantly different at $P > 0.05$.

There were no significant differences ($P > 0.05$) in ash content among the sorghum varieties. The major minerals in malt were potassium followed by magnesium, iron and phosphorus (Table 8). The contents of calcium, copper, zinc and manganese were less than 5 mg/100 g dry-matter basis. Calcium content was highest in KARI Mtama II, however, this was not significantly different ($P > 0.05$) from Kaguru and Serena. Magnesium and copper was lowest in Gadam. Iron, zinc and phosphorus were significantly higher ($P \leq 0.05$) in KARI Mtama II, Seredo and Serena, respectively than in the other sorghum varieties. There were no significant differences ($P > 0.05$) for potassium among the varieties. Manganese was highest in Kaguru, although this was not significantly different ($P > 0.05$) from Seredo.

Table 8. Mineral composition of sorghum malt (mg/100 g dry-matter basis)

Variety	Calcium	Magnesium	Iron	Potassium	Zinc	Copper	Manganese	Phosphorus
Gadam	0.98±0.13^{a}	7.98±0.11^{a}	3.29±0.32^{a}	198.15±10.19^{a}	2.16±0.27^{ab}	0.02±0.00^{a}	1.08±0.38^{a}	3.23±0.34^{a}
Kaguru	1.46±0.52^{ab}	9.59±0.24^{b}	6.14±0.17^{c}	197.45±6.79^{a}	2.53±0.07^{b}	1.02±0.39^{b}	2.19±0.18^{c}	5.21±0.82^{b}
KARI Mtama II	1.69±0.22^{b}	9.57±0.15^{b}	8.58±0.91^{d}	185.87±10.51^{a}	2.45±0.06^{b}	1.08±0.03^{b}	1.43±0.25^{ab}	2.96±0.15^{a}
Seredo	0.94±0.26^{a}	9.71±0.28^{b}	4.64±0.69^{b}	187.39±18.97^{a}	3.18±0.35^{c}	1.13±0.29^{b}	1.93±0.51^{bc}	5.62±0.49^{b}
Serena	1.21±0.17^{ab}	9.63±0.24^{b}	5.91±0.72^{c}	206.51±7.48^{a}	1.84±0.27^{a}	0.81±0.06^{b}	0.92±0.16^{a}	8.43±0.86^{c}

Values followed by the same superscript letter in the same column are not significantly different at $P > 0.05$.

Table 9. Characterization of sorghum malt

Variety	Starch (g/100 g)	Total soluble sugars (g/100 g)	Reducing sugars (g/100 g)	Damaged starch (%)	Diastatic power (°)	FAN (mg/l)	Tannins (mg/100 g)
Gadam	57.69±0.32^{b}	10.65±0.33^{a}	0.16±18.49^{d}	3.37±0.09^{bc}	11.31±1.51^{ab}	109.38±6.81^{b}	774.51±67.14^{a}
Kaguru	56.47±2.04^{b}	12.05±1.30^{a}	0.10±16.31^{b}	3.42±0.31^{c}	10.18±0.00^{a}	125.70±9.68^{c}	851.14±122.26^{a}
KARI Mtama II	59.75±1.58^{b}	16.29±0.82^{b}	0.13±7.99^{c}	3.04±0.03^{ab}	17.45±1.51^{c}	102.69±11.76^{b}	2095.82±280.71^{c}
Seredo	51.62±0.80^{a}	9.08±1.06^{a}	0.06±13.74^{a}	3.43±0.22^{c}	10.39±0.00^{a}	60.00±3.44^{a}	1189.09±209.33^{b}
Serena	51.91±2.88^{a}	12.43±3.76^{a}	0.10±8.39^{b}	2.82±0.17^{a}	12.74±0.00^{b}	129.39±1.07^{c}	969.83±113.67^{ab}

Values followed by the same superscript letter in the same column are not significantly different at $P > 0.05$.
FAN: free amino nitrogen.

Starch is the major constituent in sorghum grain and is converted to maltose and other sugars during malting, although, not all the starch present in the grain undergoes modification (Novellie, 1977). Starch content varied from 51.62 to 59.75 g/100 g for all the varieties (Table 9). KARI Mtama II had the highest starch content, but this was not significantly different ($P > 0.05$) from Gadam and Kaguru. These values were 3-18% lower in the malts as compared to the flours (Appendix 3). Total soluble sugars and reducing sugars contents varied between 9.08 and 16.29 g/100 g and 0.06 and 0.16 g/100 g respectively (Table 9). KARI Mtama II and Gadam had significantly higher ($P \leq 0.05$) total soluble sugars and reducing sugars, respectively, as compared to the other sorghum varieties. Significant differences were found ($P \leq 0.05$) between the malt and flour for all the varieties (Appendix 3). In all the varieties, malt had significantly higher ($P \leq 0.05$) total soluble sugars and reducing sugars than the native flours. Chavan et al. (1981) and Elmaki et al. (1999) observed similar results for high-tannin sorghum cultivars germinated for 72 h. They reported starch contents of about 55%. Subramanian et al. (1992) studied the chemical changes and diastatic activity in sorghum grains during germination and found a reduction in starch content in sorghum malt as compared with the values for ungerminated grain. The decrease in total starch content was attributed to starch reserves being degraded to soluble sugars in order to meet the seedling requirements during germination. Damaged starch values ranged between 2.82 and 3.43 g/100 g (Table 9). Seredo had the highest damaged starch, although this was not significantly different ($P > 0.05$)

from Gadam and Kaguru. These values were significantly higher ($P \leq 0.05$) than for the flours, except for Gadam (Appendix 3).

Sorghum malt quality is assessed primarily in terms of diastatic activity and free amino nitrogen (Dewar et al., 1997). Diastatic power ranged between 10.18° and 17.45° (Table 9). KARI Mtama II had the highest diastatic power. For all varieties, results indicated that diastatic power was significantly higher ($P \leq 0.05$) in the malt flours than the native flours (Appendix 4). The production of fermentable mono- and disaccharides in the malting process is dependent upon the activity of α-and β-amylases that develop in sorghum seeds during germination (Hulse et al.,1980). Bureng and Worgan (1982) noted that this activity of amylases increased appreciably during malting.

Free amino nitrogen (FAN) values ranged between 60.00mg/l for Seredo and 129.39 mg/l for Serena(Table 9). Kaguru and Serena had significantly higher ($P \leq 0.05$) FAN content than the other sorghum varieties. For all the sorghum varieties, FAN was significantly higher ($P \leq 0.05$) in malt compared with the flour (Appendix 4). FAN is produced during malting by the action of endogenous proteinase and peptidase enzymes on the protein reserves of the grain (Evans and Taylor, 1990). A similar trend was reported by Dewar (2003), who investigated the effect of malting on sorghum protein quality. In this study, FAN content increased from 28 mg/100 g in ungerminated grain to 230 mg/100 g after malting.

The tannin content of malt from the different sorghum varieties ranged between 774.51 for Gadam and 2095.82 mg/100 g for KARI Mtama II (Table 9). The malt flours had significantly lower ($P \leq 0.05$) tannin contents than the native flour with the exception of Gadam and KARI Mtama II (Appendix 4). This loss of tannins can be attributed to leaching of tannins into the water during soaking and germination of the sorghum (Elmaki et al., 1999). Also, part of the tannins may enter into the endosperm along with the imbibed water and are likely to form complexes with reserve seed protein and enzymes (Price et al., 1978). Tannins present in sorghum seeds have been implicated in inhibiting protein and starch degradation possibly by inactivating hydrolytic enzymes during germination. This also retards seedling growth (Chavan et al., 1981). Elmaki et al. (1999) found that the protein content of malted grains decreased by 8.2% in the high-tannin cultivar and 24.4% in the low-tannin cultivar after malting whereas starch decreased by 41.3% in the high-tannin cultivar and 50.7% in the low-tannin cultivar after malting.

Table 10. Pasting properties of sorghum malt

Sorghum variety	Pasting temperature (°C)	Peak viscosity (BU)	Time to peak viscosity (min)
Gadam	83.00 ± 0.57^{b}	52.50 ± 4.95^{a}	42.19 ± 2.84^{b}
Kaguru	76.20 ± 0.85^{a}	42.50 ± 2.12^{a}	36.60 ± 0.58^{a}
KARI Mtama II	85.60 ± 0.42^{c}	228.50 ± 14.85^{c}	42.79 ± 0.41^{b}
Seredo	85.85 ± 1.34^{c}	136.50 ± 4.95^{b}	43.24 ± 0.28^{b}
Serena	85.60 ± 0.85^{c}	199.00 ± 19.80^{c}	42.57 ± 0.01^{b}

Values followed by the same superscript letter in the same column are not significantly different at $P > 0.05$.

Table 10 shows the pasting properties of sorghum malt. Pasting temperature ranged between 76.20°C and 85.85°C. Seredo had the highest pasting temperature, though this was not significantly different ($P > 0.05$) from KARI Mtama II and Serena. Peak viscosity varied between 42.50 BU and 228.50 BU. KARI Mtama II exhibited the highest peak viscosity, although this was not significantly different ($P > 0.05$) from Serena. Time to peak viscosity ranged between 36.60 min and 43.24 min. Seredo had the highest time to peak viscosity, although this was not significantly different ($P > 0.05$) from Gadam, KARI Mtama II and Serena. The malt flours had significantly lower ($P \leq 0.05$) pasting temperature than the native flour with the exception of Gadam, Seredo and Serena (Appendix 5). Peak viscosity was significantly lower ($P \leq 0.05$) in malt flour than the native flours except for KARI Mtama II and Serena. Time to peak viscosity was significantly lower ($P \leq 0.05$) in malt flour than the native flours except for Gadam, KARI Mtama II and Seredo. The decrease in pasting temperature of sorghum malt is due to the biochemical changes occurring in the grain during malting. During germination, α-amylases hydrolyze starch into dextrins and glucose which renders the starch in the malt easier to gelatinize (Hugo et al., 2000). Consequently, the pasting temperatures and paste viscosity decreases.

4.3. Effect of malted sorghum on rheology of gluten-free batter

4.3.1. Development of a recipe

Preliminary tests carried out to formulate a recipe indicated that gluten-free breads made from cassava starch, sorghum and amaranth flours at a ratio of 50:40:10 produced the best breads in terms of low crumb hardness, chewiness and adhesiveness after 24 h. There were no significant differences ($P > 0.05$) in crumb hardness and crumb chewiness between 60:30:10 and 50:40:10 flour blends (Table 11). However, the 60:30:10 blend had significantly higher ($P \leq 0.05$) adhesiveness resulting in a sticky crumb. This made it unsuitable for breadmaking.

Table 11. Crumb properties of cassava-sorghum-amaranth bread from different formulations

Ratio of cassava starch to sorghum to amaranth	Crumb property		
	Hardness (N)	Chewiness (N)	Adhesiveness (g.s)
50:50:0	29.45^{b}	14.46^{b}	-0.36^{a}
60:30:10	10.09^{a}	4.71^{a}	-5.87^{b}
50:40:10	12.02^{a}	5.80^{a}	-0.87^{a}
40:60:10	45.38^{c}	16.06^{b}	-0.30^{a}

Values followed by the same superscript letter in the same column are not significantly different at $P > 0.05$.

KARI Mtama II malt flour had significantly higher ($P \leq 0.05$) diastatic power than the other varieties (Table 9), and therefore, it was used to formulate the batters for rheology and baking studies. Preliminary tests carried out indicated better bread quality when 1% malt was used. Malt addition higher than this resulted in breads with higher firmness and chewiness, and lower springiness, cohesiveness, and

resilience. Moreover, there was a tendency of the bread crust to collapse and a sticky crumb developed when malt higher than 1% was used.

4.3.2. Batter rheology

Cassava starch, sorghum and amaranth flours were blended in a ratio of 50:40:10. The consistencies of gluten-free batters prepared from cassava starch, amaranth and different sorghum varieties are shown in Figure 6. The extrusion forces required to pass the batter through a die with 3 mm diameter ranged from 76.03 N for Seredo to 216.39 N for Serena. Gluten-free batter consistencies from Gadam, Kaguru, KARI Mtama II and Milomehl showed no significant differences ($P > 0.05$) between the samples.

The amount of damaged starch in sorghum flour-starch mixture plays an important role in the rheological properties of the batter and crumb structure of sorghum bread (Schober et al., 2005). High amounts of damaged starch results in a thick batter where the swollen damaged starch granules and other large particles loosely stick together, form clusters and prevent each other from settling and gas bubbles from rising by steric hindrance (Schober, 2009). Nevertheless, the amount of damaged starch and water content are not significantly correlated, implying that other factors, such as absorption by intact starch granules, may also contribute to water binding (Schober et al., 2005). However in this study, Milomehl had five

times the amount of damaged starch than the other flours yet the extrusion force was not significantly higher ($P > 0.05$).

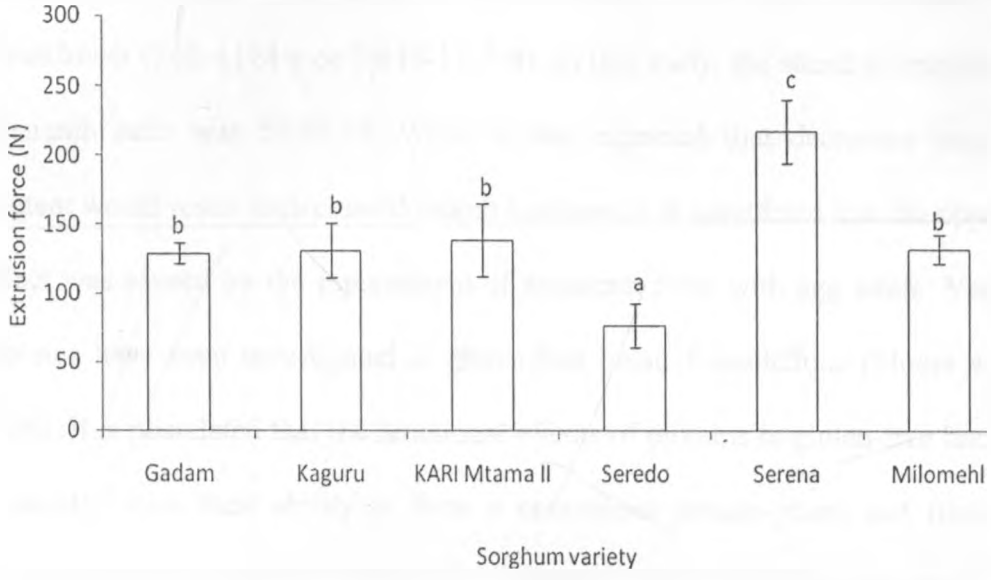

Figure 6. Consistency of gluten-free batter prepared from cassava starch, different sorghum varieties and amaranth. Error bars represent standard deviation. Bars with the same letter are not significantly different at $P > 0.05$.

4.4. Effect of malted sorghum on crumb texture of gluten-free bread

Figure 7 shows the crumb hardness of gluten-free breads prepared from cassava starch and different sorghum varieties. The analysis of hardness indicated that the gluten-free breads made with local sorghum varieties were considerable harder in comparison with the control. Gluten-free bread prepared from Milomehl had the least crumb firmness (12.02-26.06 N). In all treatments, crumb hardness tended to increase with increasing storage time. However, in the case of bread from Kaguru, crumb hardness did not change significantly ($P > 0.05$) after 48 h storage.

Onyango et al. (2011b) investigated changes in crumb firmness of gluten-free bread prepared from 50 parts sorghum and 50 parts cassava, and 7% (flour-weight-basis) egg white and found that crumb firmness increases with storage time, and decreases with increasing starch content. However, their values were about five times lower (760–1174 g or 7.6 N–11.7 N). In this study, the starch to sorghum to amaranth ratio was 50:40:10. While it was expected that decreased sorghum content would result in decreased crumb hardness, it is speculated that the opposite effect was caused by the replacement of amaranth flour with egg white. Various proteins have been investigated in gluten-free bread formulations (Moore et al., 2004). It is postulated that the functional effects of proteins in gluten-free bread is associated with their ability to form a continuous protein-phase and film like structures similar to gluten (Moore et al., 2004). However it appears that different proteins exert different effects on crumb firmness. Onyango et al. (2009a) have shown that gluten-free bread made with egg white tends to be softer than that made with other proteins.

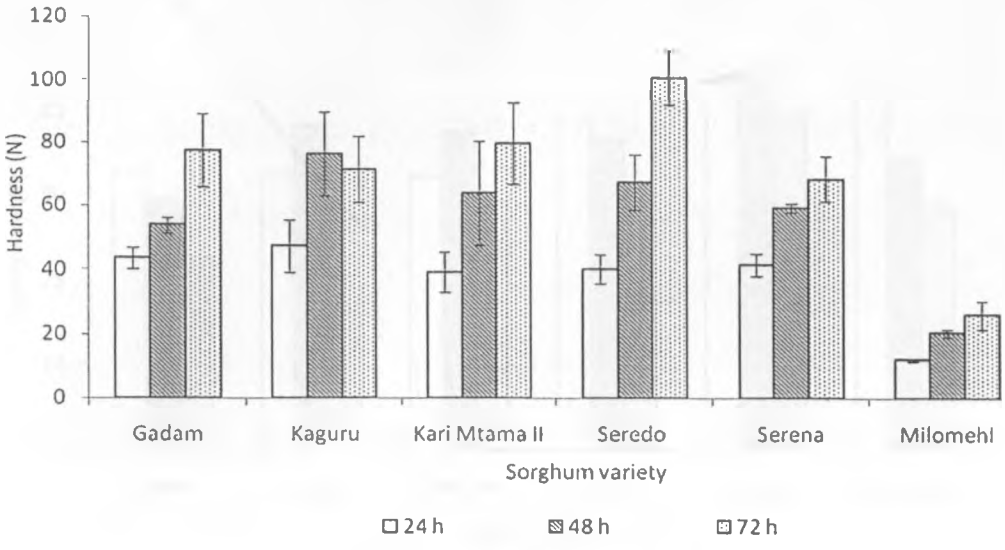

Figure 7.Crumb hardness of gluten-free bread prepared from cassava starch, different sorghum varieties and amaranth flour.

Crumb elasticity is described by both springiness and resilience (Bourne, 2002). A reduction in resilience or springiness characterizes loss of elasticity. Crumb springiness was 81.27-87.13%, 79.79-87.38% and 74.26-85.15% after 24, 48 and 72 h storage, respectively. Springiness declined significantly ($P \leq 0.05$) with increasing storage time for gluten-free bread made from Gadam, Seredo, Serena and Milomehl (Figure 8). For Kaguru, KARI Mtama II and Serena, springiness initially increased between 24 and 48 h and thereafter decreased. Crumb resilience was 0.21-0.27, 0.19-0.22 and 0.18-0.31 after 24, 48 and 72 h storage, respectively. However, crumb resilience of gluten-free bread prepared from cassava starch and different sorghum varieties did not show any definite pattern (Figure 9). It is only the resilience of gluten-free bread containing Kaguru that increased significantly ($P \leq 0.05$) on storage.

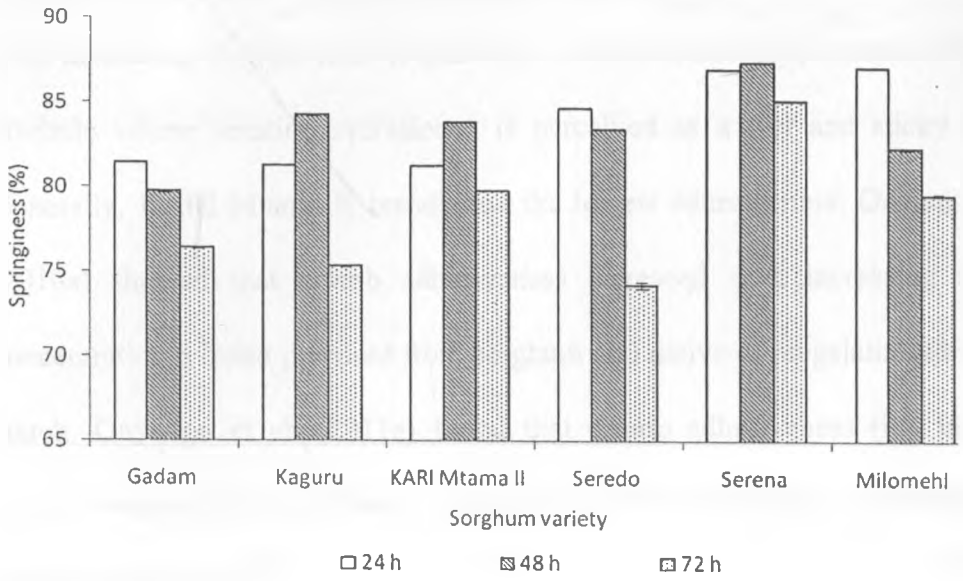

Figure 8. Crumb springiness of gluten-free bread prepared from cassava starch, different sorghum varieties and amaranth flour.

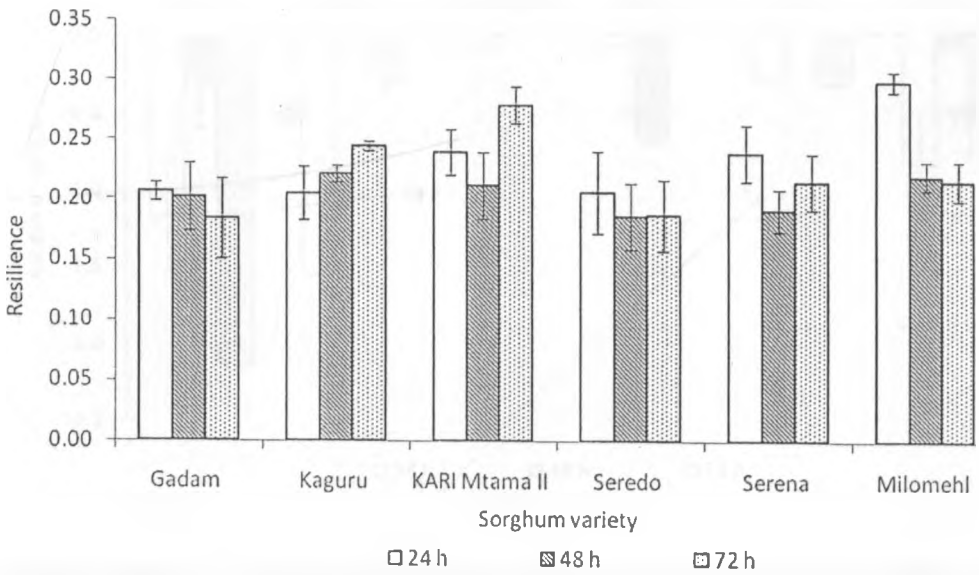

Figure 9. Crumb resilience of gluten-free bread prepared from cassava starch, different sorghum varieties and amaranth flour.

Crumb adhesiveness did not show a clear pattern among the sorghum varieties and with increasing storage time (Figure 10). Adhesiveness is an undesired crumb attribute whose sensory equivalence is perceived as a wet and sticky crumb. Generally, KARI Mtama II breads had the lowest adhesiveness. Onyango et al. (2010a) showed that crumb adhesiveness increased with increasing enzyme concentrationin bread prepared from sorghum and native or pregelatinised cassava starch. Onyango et al. (2011a) found that crumb adhesiveness (i.e. increased crumb wetness and stickiness) increased in with increasing concentration of pregelatinised starch.

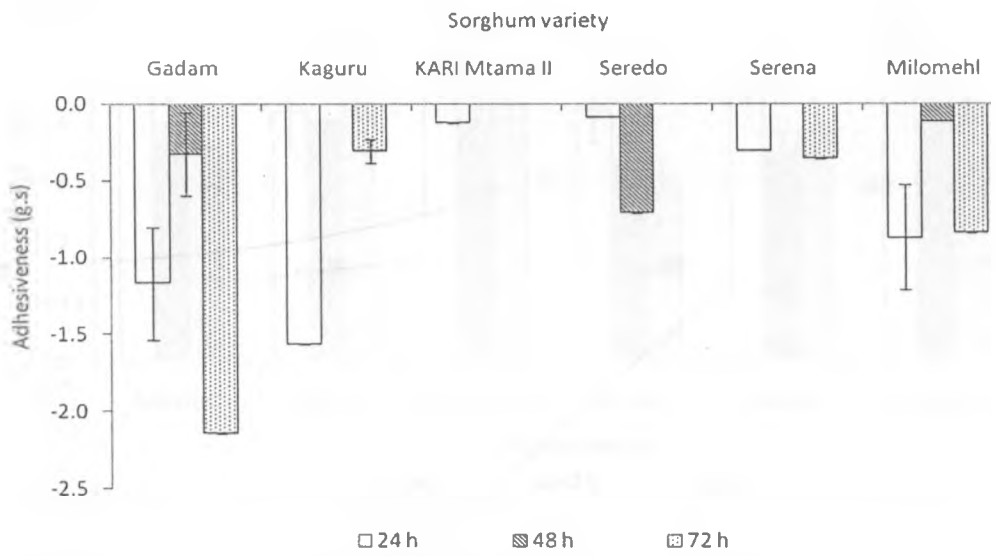

Figure 10. Crumb adhesiveness of gluten-free bread prepared from cassava starch, different sorghum varieties and amaranth flour.

Generally, crumb cohesiveness of the breads did not change significantly ($P > 0.05$) with increasing storage time (Figure 11). Crumb cohesiveness was 0.42-0.55, 0.33-0.40 and 0.36-0.52 after 24, 48 and 72 h storage, respectively. Gluten-free bread prepared from Milomehl had the highest crumb cohesiveness (0.55) after 24 h. Crumb cohesiveness is related to how the bread holds together as it is masticated (Marco and Rosell, 2008). Bread with high cohesiveness is desirable because it can form a bolus, rather than disintegrate, during mastication (Onyango et al., 2010b).

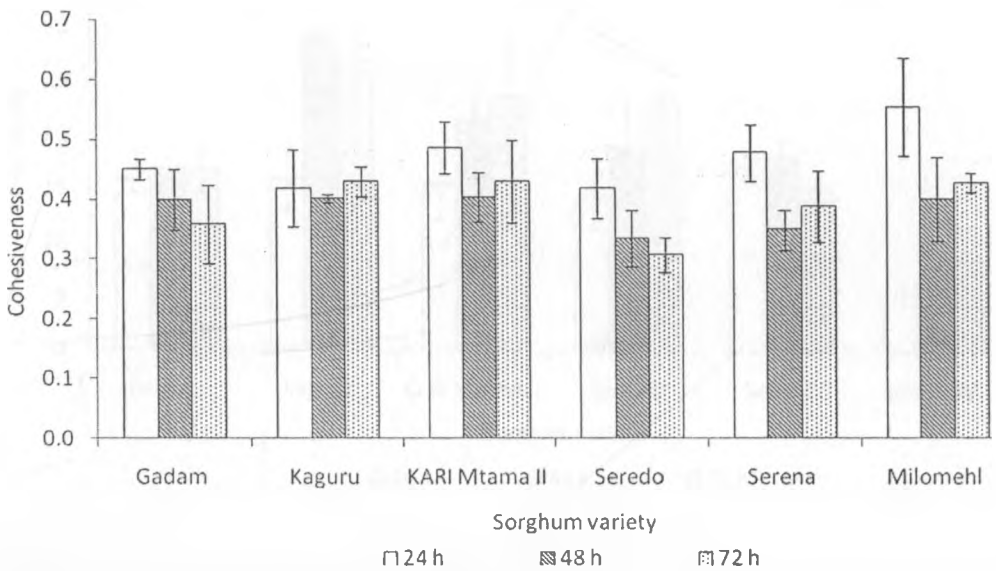

Figure 11. Crumb cohesiveness of gluten-free bread prepared from cassava starch, different sorghum varieties and amaranth flour.

Crumb chewiness increased with increasing storage time for all the sorghum varieties (Table 12). Crumb chewiness ranged from 5.80-17.20 N, 6.71-26.10 N

and 8.76-27.24 N after 24, 48 and 72 h storage, respectively. Gluten-free bread containing Milomehl varieties had the lowest crumb chewiness. Changes in crumb chewiness followed the same trend as crumb hardness. Chewy foods tend to remain in the mouth without rapidly breaking up or dissolving (Bourne, 2002). Chewiness is the product of hardness, cohesiveness and springiness (Bourne, 2002) and this is therefore influenced by the change in any one of these parameters. It therefore appears that hardness had a more dominant effect on crumb chewiness than the other two parameters.

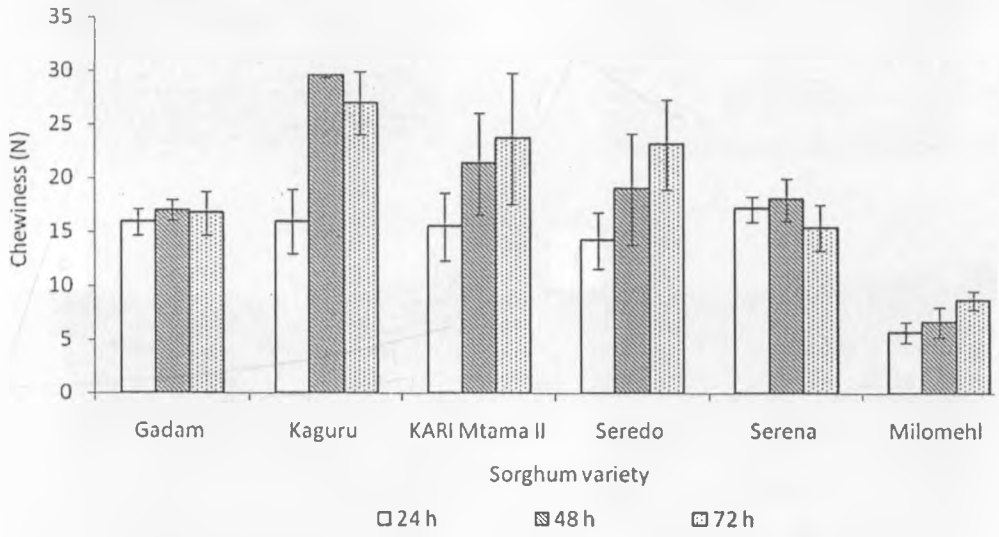

Figure 12. Crumb chewiness of gluten-free bread prepared from cassava starch, different sorghum varieties and amaranth flour.

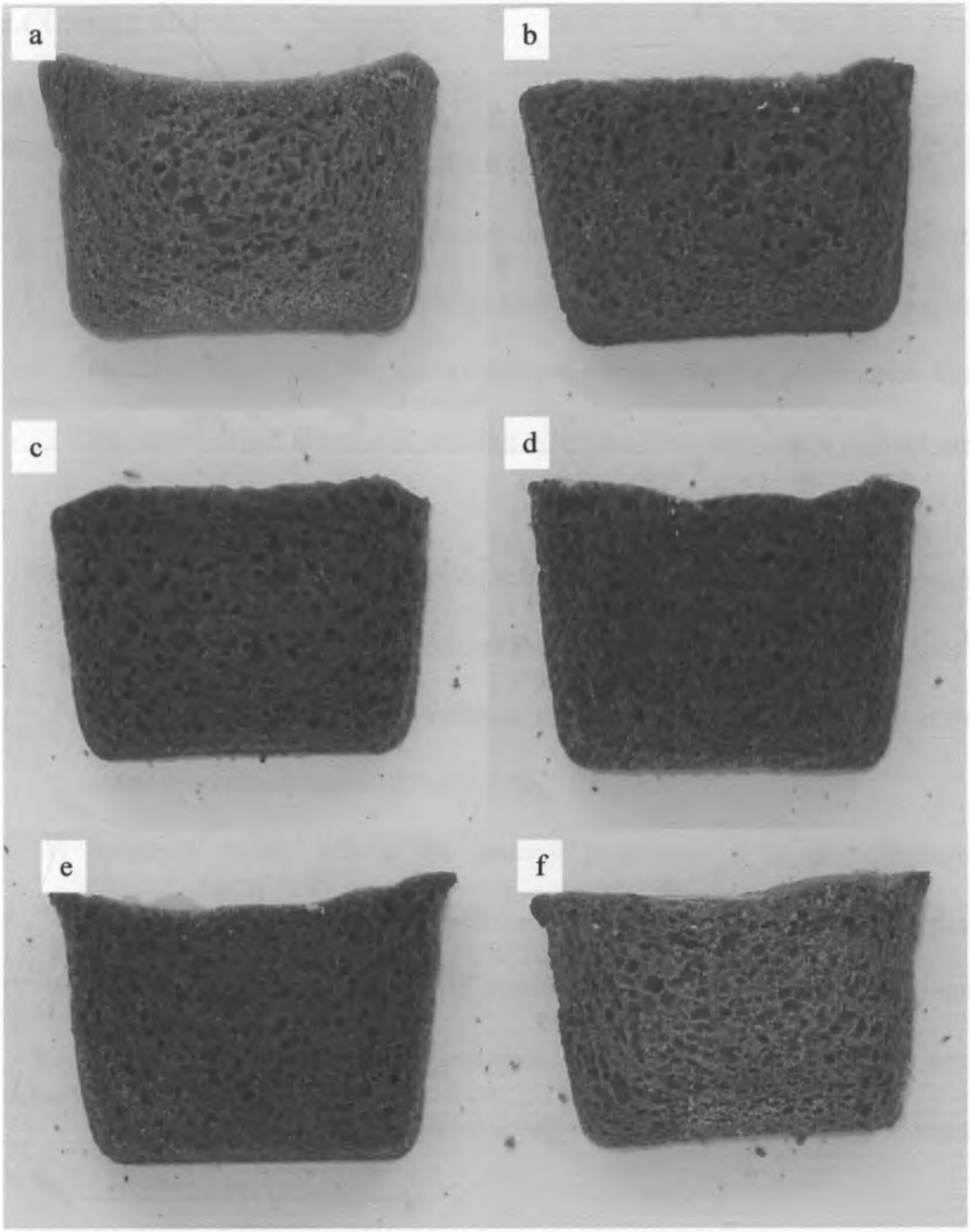

Figure 13.Cross-sectional view of cassava-sorghum-amaranth bread made from Gadam (a), Kaguru (b), KARI Mtama II (c), Seredo (d), Serena (e) and Milomehl (f).

5.CONCLUSIONS

1) Genotypic variations affect grain characterisation and physico-chemical properties of the different sorghum flours.
2) Genotypic variations affect physico-chemical properties of malted sorghum varieties. Malt flour has higher diastatic power, total soluble sugars, reducing sugars and free amino nitrogen released during germination than the native flour. Generally, malting also decreases the tannin content and peak viscosity of malt.
3) Sorghum variety has an effect on the consistency of the cassava-sorghum-amaranth batter. Although Milomehl had five times more damaged starch than the other varieties, this difference did not affect the consistency of the cassava-sorghum-amaranth batter.
4) Sorghum variety affects the textural properties of cassava-sorghum-amaranth breads. Generally, Milomehl had better crumb properties than breads from the local sorghum varieties. Milomehl had the least crumb firmness and least crumb chewiness. Also, storage was associated with bread quality parameters. Gluten-free breads showed increasing firmness and chewiness with storage.

6. RECOMMENDATIONS

Milomehl produced better quality gluten-free bread. The reason for such a better performance is most likely due to higher damaged starch. Further research should include the five local sorghum varieties milled to meet Milomehl flour standards. This would allow for appropriate testing among sorghum varieties to find the best sorghum variety for gluten-free bread production.

Industrialisation of cassava, sorghum and amaranth may serve as an intervention programme to reduce importation of wheat in Kenya. Development through processing and product development will activate the emergence and growth of small and medium scale agro enterprises, which could significantly improve the socio-economic status of Kenya.

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Appendix 1.

Proximate composition of native (N) and malted (M) sorghum flours*

Variety	Protein (N x 6.25)		Fibre		Ash		Fat		Carbohydrate	
	N	M	N	M	N	M	N	M	N	M
Gadam	13.19_x	12.57_x	2.00_x	3.38_y	1.56_x	1.38_x	3.62_x	3.94_x	79.63_x	78.74_x
Kaguru	10.51_x	11.02_x	2.22_x	2.90_x	1.46_x	1.39_x	2.77_x	3.39_y	83.05_x	81.30_x
KARI Mtama II	9.48_x	9.77_x	2.61_x	3.58_x	2.02_x	1.64_x	2.77_x	2.74_x	83.12_x	82.28_x
Seredo	11.46_x	10.77_x	2.49_x	3.05_x	1.71_x	1.46_y	3.37_x	3.12_x	80.97_x	81.60_x
Serena	8.16_x	7.97_x	2.24_x	2.43_x	1.61_x	1.66_x	3.23_x	3.15_x	84.76_x	84.80_x
Milomehl**	9.34	nd	1.71	nd	1.30	nd	3.69	nd	83.95	nd

*g /100 g dry-matter-basis.

Values followed by the same subscript letter in the same row, for each measured parameter, are not significantly different at $P > 0.05$.

**Trade name for an unidentified sorghum variety.

nd: not determined because Milomehl was purchased in the form of flour and thus was not malted.

Appendix 2.

Mineral composition of native (N) and malted (M) sorghum flours*

Variety	Ca		Mg		Fe		K		Zn		Cu		Mn		P	
	N	M	N	M	N	M	N	M	N	M	N	M	N	M	N	M
Gadam	1.11_x	0.98_x	8.14_x	7.98_x	5.57_x	3.29_x	284.00_x	198.15_y	2.50_x	2.16_x	0.17_x	0.02_y	4.14_x	1.08_y	24.99_x	3.23_y
Kaguru	0.90_x	1.46_x	8.04_x	9.59_y	5.47_x	6.14_x	273.90_x	197.45_y	2.85_x	2.53_x	0.31_x	1.02_x	1.77_x	2.19_y	10.25_x	5.21_y
KARI Mtama II	0.55_x	1.69_y	8.05_x	9.57_y	5.39_x	8.58_y	289.61_x	185.87_y	2.51_x	2.45_x	0.16_x	1.08_y	2.73_x	1.43_x	99.06_x	2.96_y
Seredo	0.49_x	0.94_x	8.09_x	9.71_y	7.12_x	4.64_x	261.73_x	187.39_y	2.48_x	3.18_x	0.25_x	1.13_y	1.21_x	1.93_x	21.82_x	5.62_y
Serena	0.61_x	1.21_y	8.13_x	9.63_y	4.08_x	5.91_x	271.38_x	206.51_y	1.72_x	1.84_x	0.07_x	0.81_y	1.33_x	0.92_y	16.15_x	8.43_y
Milomehl**	0.51	nd	8.17	nd	4.27	nd	268.15	nd	2.64	nd	0.15	nd	0.79	nd	29.14^c	nd

*mg/100 g dry-matter-basis.

Ca: calcium; Mg: magnesium; Fe: iron; K: potassium; Zn: zinc; Cu: copper; Mn: manganese; P: phosphorous

Values followed by the same subscript letter in the same row, for each measured parameter, are not significantly different at $P > 0.05$.

**Trade name for an unidentified sorghum variety.

nd: not determined because Milomehl was purchased in the form of flour and thus was not malted.

Appendix 3.

Starch, sugars and damaged starch contents in native (N) and malted (M) sorghum flours*

Variety	Starch		Total soluble sugars		Reducing sugars		Damaged starch	
	N	M	N	M	N	M	N	M
Gadam	64.41_x	57.69_x	4.06_x	10.65_y	0.029_x	0.155_y	2.64_x	3.37_x
Kaguru	60.69_x	56.47_x	7.28_x	12.05_y	0.018_x	0.098_y	1.69_x	3.42_y
KARI Mtama II	61.81_x	59.75_x	8.38_x	16.29_y	0.028_x	0.126_y	2.15_x	3.04_y
Seredo	58.77_x	51.62_x	4.19_x	9.08_y	0.020_x	0.063_y	2.61_x	3.43_y
Serena	63.49_x	51.91_y	8.88_x	12.43_y	0.026_x	0.096_y	1.23_x	2.82_y
Milomehl**	68.21	nd	8.64	nd	0.020	nd	10.73	nd

*g /100 g dry-matter-basis.

Values followed by the same subscript letter in the same row, for each measured parameter, are not significantly different at $P > 0.05$.

**Trade name for an unidentified sorghum variety.

nd: not determined because Milomehl was purchased in the form of flour and thus was not malted.

Appendix 4.

Diastatic power, and free amino nitrogen and tannin contents in native (N) and malted (M) sorghum flours

Variety	Diastatic power (°)		Free amino nitrogen (mg/l)		Tannins (mg/100g)	
	N	M	N	M	N	M
Gadam	5.96_x	11.31_y	39.74_x	109.38_y	1023.56_x	774.51_x
Kaguru	5.95_x	10.18_y	26.07_x	125.70_y	1412.64_x	851.14_y
KARI Mtama II	6.12_x	17.45_y	26.32_x	102.69_y	2433.44_x	2095.82_x
Seredo	5.23_x	10.39_y	22.42_x	60.00_y	1729.12_x	1189.09_y
Serena	4.26_x	12.74_y	22.13_x	129.39_y	1533.63_x	969.83_y
Milomehl*	4.30	nd	16.43	nd	1031.38	nd

Values followed by the same subscript letter in the same row, for each measured parameter, are not significantly different at $P > 0.05$.

*Trade name for an unidentified sorghum variety.

nd: not determined because Milomehl was purchased in the form of flour and thus was not malted.

Appendix 5.

Pasting properties of sorghum native (N) and malt (M) flour

Sorghum variety	Pasting temperature (C)		Peak viscosity (BU)		Time to peak viscosity (min)	
	N	M	N	M	N	M
Gadam	85.65_x	83.00_x	252.00_y	52.50_x	43.34_x	42.19_x
Kaguru	87.85_y	76.20_x	249.00_y	42.50_x	43.25_y	36.60_x
KARI Mtama II	89.70_y	85.60_x	189.50_x	228.50_x	43.31_x	42.79_x
Seredo	89.80_x	85.85_x	176.00_y	136.50_x	43.48_x	43.24_x
Serena	87.45_x	85.60_x	232.00_x	199.00_x	43.13_y	42.57_x
Milomehl*	86.60	nd	276.00	nd	42.79	nd

Values followed by the same subscript letter in the same row, for each measured parameter, are not significantly different at $P > 0.05$.

*Trade name for an unidentified sorghum variety.

nd: not determined because Milomehl was purchased in the form of flour and thus was not malted.