Sorghum grain: Development of methodologies for end-use quality evaluation

By

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DECLARATION

I declare that the thesis which I hereby submit for the degree PhD (Food Science) at the University of Pretoria is my own work and has not previously been submitted by me for a degree at another University or institution of higher education.

Doreen Mwiita Hikeezi

Date

DEDICATION

This thesis is dedicated to my late parents Mr Japhet Mwiita Mudenda and Mrs Janet Chatiwa Mudenda who made it possible for me to take that first little step way back in Wankie into a long educational journey which has reached this far at the University of Pretoria.

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ABSTRACT

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Degree: PhD (Food Science)

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Sorghum [*Sorghum bicolor* (L) Moench] is the second major cereal crop in Southern Africa after maize. Despite its importance as a cereal crop it is underutilised in Southern Africa because of insufficient characterisation and development of end-use quality evaluation methods for available cultivars, and the unattractive colour of some products from sorghum. This research aimed at developing simple methodologies for determination of important parameters of sorghum end-use quality, namely kernel hardness and kernel colour. The current research also set out to determine the cause for darkening of porridges made from white tan-plant sorghums and how this may relate to polyphenol oxidase activity.

Sixteen Zambian sorghum cultivars grown during the 2008 and 2009 seasons of predominantly medium size, and ranging in colour from white to brown and red, with or without a pigmented testa were used. Endosperm texture of the sorghums determined by visual endosperm examination ranged from soft to hard. Abrasive hardness index values ranged from 6.28 to 19.64 and percentage water absorption ranged from 8.43 to 26.56%. Percent water absorption was significantly and positively correlated ($r= 0.85$, $p <0.001$) with endosperm texture and negatively correlated with abrasive hardness index ($r=-0.89$, $p<0.01$). The percent water absorption method could separate soft grains from hard grains just as well as endosperm texture and abrasive hardness index. The simplicity of the percent water absorption method

makes it potentially usable by farmers and traders in remote areas where it can contribute to meaningful end-use quality assessment.

The relatively dark colour of food products from white tan-plant (food-grade) sorghums can compromise their acceptability. The relationship between white tan-plant sorghum polyphenol oxidase activity (PPO) and porridge colour was investigated. Sorghums (including 28 white tan-plant samples grown in Zambia over two seasons), wheat and white maize were studied. Sorghum grain was intermediate in PPO between wheat and maize. When white tan plant sorghum and maize flours were cooked into porridges, they became darker with lower L* values. More importantly, the transition from white tan-plant sorghum flour to porridge caused a much larger reduction in mean L* value (27.9) than that with white maize (16.9). There were significantly negative correlations between all white tan-plant sorghum PPO activity and porridge L* values ($r = -0.657$, $p < 0.01$) and between Zambian white tan-plant sorghum PPO activity and porridge L* values ($r = -0.710$, $p < 0.001$). It is apparent that PPO activity in white tan-plant sorghums is an important determinant of the relatively dark colour of food products made from them, as is the case in wheat.

Grain colour is an important quality indicator in sorghum-based foods and rural sorghum farmers in sub-Saharan Africa need simple, more accessible methods for its determination. The development of a simple quantitative method for sorghum grain colour assessment by analysis of the grains and their sodium hydroxide (NaOH) extracts was studied. Sixteen Zambian sorghums (white, red and brown types which were either tannin or non-tannin) from the 2008 and 2009 seasons and a Sudanese white tannin sorghum type were assessed for surface colour using Tristimulus colorimetry before and after treatment with NaOH. The NaOH extracts were also analysed using UV-visible spectrophotometry and reverse phase HPLC. Tristimulus colorimetry of the grain surface was able to distinguish white from coloured sorghums (brown and red) but was unable to separate tannin from non-tannin sorghum. UV-visible spectrophotometry and reverse phase HPLC of NaOH extracts from a representative set of the grains consisting of five sorghum types (red tannin, brown tannin, white tannin, red non-tannin and white non-tannin) separated the tannin from non-tannin sorghums regardless of grain surface colour. However, UV-visible absorption of NaOH extracts from the sorghum grains could not be related to grain surface colour as determined using Tristimulus colorimetry.

Eleven phenolic compounds consisting of two flavan-3-ols, five anthocyanins and four 3-deoxyanthocyanins were identified in the NaOH extracts using UPLC/PDA/MS. NaOH extract from Framida *SDS[3845]23-2-1 (red tannin) contained all of the eleven compounds identified. The flavan-3-ols (catechin and +-catechin-3-O-gallate) were present in NaOH extracts of all the five sorghums. While some anthocyanins could be identified in NaOH extracts from all five sorghums, only Framida *SDS[3845]23-2-1 (red tannin), Sima (white non-tannin) and MMSH625 (red non-tannin) contained any 3-deoxyanthocyanins. Total peak area due to anthocyanins and 3-deoxyanthocyanins was higher for NaOH extracts from tannin sorghums compared to non-tannin. This was in agreement with the UV-visible spectrophotometry of the NaOH extracts which also separated the tannin from non-tannin sorghums. This shows that with NaOH treatment, it is possible to separate tannin from non-tannin sorghums. Colorimetry of NaOH extracts from sorghum may therefore be considered as a potentially simple and cheap alternative method for distinguishing tannin from non-tannin sorghums.

The findings of this research provide a platform for the development of a system which promotes an integrated and inclusive approach in using the methodologies developed for sorghum end-use quality evaluation. The methodologies will form an integral part of the system which can be applied along the sorghum value chain in sub-Saharan Africa and involve various stakeholders such as the gene bank, sorghum breeders, cereal scientists and researchers, sorghum food processors, traders, rural farmers and consumers. The efficient application of this system could lead to increased sorghum production and utilisation and contribute significantly to food and nutrition security.

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1. INTRODUCTION

1.1 STATEMENT OF THE PROBLEM

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop in the world after wheat, rice, maize and barley in terms of production (FAO 2011). It is primarily used as a food crop in Asia and Africa. In Africa south of the Sahara, it is the second major crop produced after maize. It remains an important crop in Southern Africa and other semi-arid regions of the world because of its agronomical advantages. It is drought-tolerant, it can withstand water-logging and unlike maize, it requires minimum agricultural inputs for its cultivation (Belton and Taylor 2004)**.** Furthermore, it is mostly cultivated and consumed by the small-scale farmers in the semi-arid regions of the world and thus contributes to food and nutritional security because it is an important supplier of carbohydrates and proteins to the diets (Dendy 1995; Rohrbach and Obilana 2003).

In Africa, sorghum is consumed as porridge (thin and thick, fermented and un-fermented), breads (leavened and unleavened), non-alcoholic beverages, beer (opaque and clear) and malt (Murty and Kumar 1995). In other parts of the world, sorghum consumption is gaining popularity for reasons different from those of Africa. In the USA for instance, sorghum is gaining popularity as a substitute for wheat in bakery products and beer for people allergic to wheat gluten (Awika and Rooney 2004). In Japan, sorghum is used to prepare speciality products like snacks, cookies and ethnic foods.

In Zambia, however, sorghum as a food crop is underutilized because government policies tend to support the cultivation of maize rather than other crops. Maize is regarded as the major cereal for the preparation of the main staple food Nsima, a thick porridge. According to Jayne, Govereh, Chilonda, Mason, Chapoto and Hantuba (2007), the government after ushering a free market economy in 1991, continued to maintain fertilizer subsidies for maize. In the last four years the government has distributed roughly 45,000 tons of fertilizer a year at 50 % subsidy under its fertilizer subsidy support programme for small scale farmers. Post-harvest service data of 2005 indicate that roughly 90 % of the fertilizer used by small-scale farmers in the last decade has been on maize.

Sorghum is underutilized in Zambia because it is perceived as a poor man's crop. Utilization has also been affected by the unattractive colour of some products made from sorghum. Jagwer (1998) for instance, in sensory evaluation studies on sorghum porridges in Botswana reported the unacceptability of dark porridge made using white sorghum compared to that made from white maize. The low utilization of sorghum is also due to limited collaboration between sorghum breeders and food scientists, even though they are all involved in sorghum improvement. The lack of a common language as to what constitutes quality in the value chain has also affected the utilization of sorghum compared to maize in Zambia. To improve the utilization of sorghum it is therefore necessary to develop simple methodologies for sorghum end-use quality evaluation in areas where sorghum is grown and consumed.

The development of methodologies for end-use quality evaluation is necessary for the following reasons:-

- To capture pertinent information concerning the quality attributes of sorghum cultivars in terms of kernel hardness and colour because these are the kernel attributes associated with sorghum processing for consumption.
- To broaden the end–use quality information in the production of acceptable sorghum-based foods.
- To develop systematic sharing and dissemination of useful end–use quality information on sorghum-based foods in the agricultural activities of national agriculture research stations and the southern africa development community (SADC) region's developmental programmes.
- To document expected product end–use quality of sorghum foods, since product quality prediction for sorghum-based foods is not as developed as that of wheat. For example, whole grain colour cannot be used to predict the colour of the resulting products (Taylor and Dewar 2000). This is because grain colour depends on many underlying factors like endosperm colour or pericarp colour, presence and absence of pigmented testa pH, and milling quality (Taylor and Duodu 2009).

1.2 LITERATURE REVIEW

This review discusses what is known in the literature about sorghum, its physico-chemical properties, how these impact end-use quality and methods for determining end-use quality. The literature on enzymes responsible for darkening of processed plant-based foods, primarily polyphenol oxidase and their mechanisms of action is discussed. A review of sorghum processing methods and technologies is also presented.

1.2.1 Sorghum kernel structure

The sorghum kernel is described as a naked caryopsis (Rooney and Miller 1982) which varies widely in size and shape among sorghum varieties. The sorghum kernel is spherical in shape measuring 4 mm long, 2 mm wide and 2.5 mm thick. The kernel weight ranges from 25 to 35 mg, test weight ranges from 55 to 61 kg/hL and density ranges from 1.28 to 1.36 g/cm^3 (Rooney and Miller 1982; Serna-Saldivar and Rooney 1995).

The structure of the sorghum kernel (Figure 1.2.1) has an important bearing on the processing and end-use quality of foods. The endosperm for instance plays a major role in determining the quality of traditional sorghum foods (Rooney and Murty 1982) and milling yield (Maxson, Fryar, Rooney and Krishinaprasad 1971). Pericarp thickness has an impact on milling properties of sorghum (Scheuring, Sidibe, Rooney and Earp 1983). The sorghum kernel structurally has three main parts; the pericarp which is the outer covering of the sorghum kernel, the endosperm which is the storage tissue and the embryo which is also known as the germ (Rooney and Miller 1982) (Figure 1.2.1).

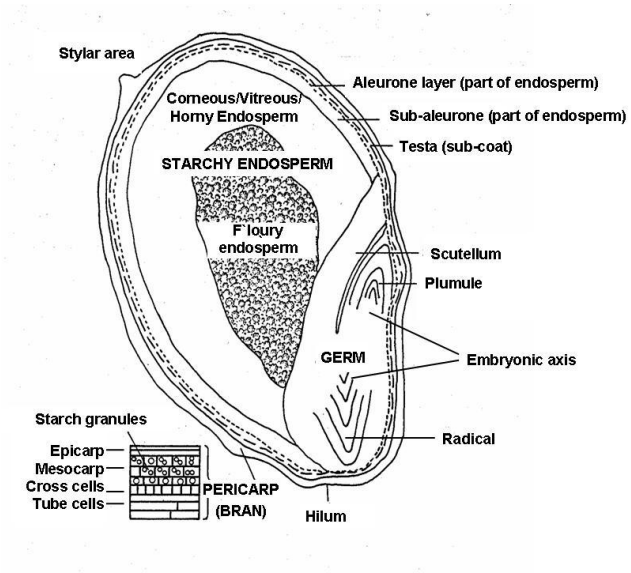


Figure 1.2.1. Longitudinal section of sorghum grain (Taylor and Belton 2004)

The pericarp originates from the ovary wall (Glennie, Liebenberg and Van Tonder 1983). The pericarp thickness, which is controlled by the Z gene, ranges from 8 µm to 16 µm (Earp and Rooney 1982) and varies within an individual mature sorghum kernel. The pericarp is further divided into three tissues: the epicarp, mesocarp and endocarp. The relative proportions of these components vary among sorghum types, influenced by genetic and environmental factors (Serna-Saldivar and Rooney 1995). However, on average these components constitute about 6%, 84% and 10% of the pericarp, respectively (Rooney and Sern-Saldivar 1993).

The epicarp is the outermost part of the three tissues and it is generally covered with a thin layer of wax (Waniska 2000). The epicarp is two or three layers thick. It consists of rectangular cells that often contain pigmented material. In sorghum unlike other cereal grains a thick pericarp usually contains three or four mesocarp layers, which are filled with small starch granules (Hoseney 1994). The inner pericarp tissue, the endocarp, consists of cross and tube cells. The inner tube cells conduct water during the germination of the plant while the outer cross cells form a layer that prevents water loss (Rooney and Miller 1982). A thick, starchy mesocarp may mask a testa layer or a dark endosperm.

A thin translucent pericarp permits the colour of the testa and or endosperm to affect the visual colour of the kernel (Rooney and Miller 1982). Pericarp thickness affects dehulling loss and milling yield (Gomez, Obilana, Martin, Madzvamuse and Monyo 1997). A thin pericarp tends to adhere tightly to the kernel, while a thick pericarp attaches loosely (Bassey and Schmidt 1989). The thickness of the pericarp is an important grain property because sorghum types with a thick pericarp decorticate easier by hand pounding than sorghums with a thin pericarp, and the latter types perform better under mechanical decortication (Maxson et al 1971; Scheuring et al 1983). Pericarp thickness is also important in that sorghums with thick pericarps are prone to weathering (Serna-Saldivar and Rooney 1995). These types of sorghums were shown to consistently contain higher amounts of phenolic compounds (Beta, Rooney, Marovatsanga and Taylor 1999), which if incorporated into food products could cause astringent taste (Drewnowski and Gomez-Carneros 2000).

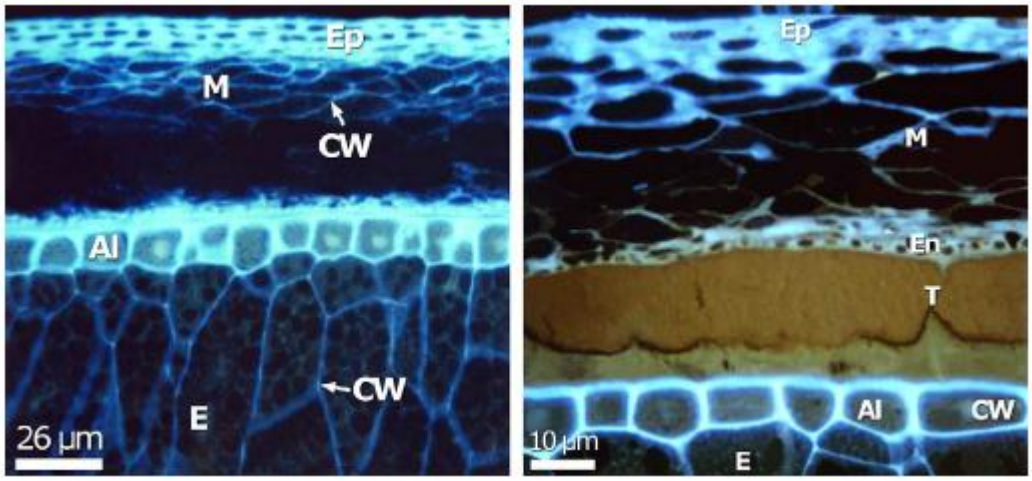


Figure 1.2.2 Fluorescence photomicrographs of sorghum bran cross-section, showing structural differences between a non-tannin sorghum without a testa (left) and a tannin sorghum with a pigmented testa (right). Al, aleurone layer; CW, cell wall; E, endosperm; En, endocarp; Ep, epicarp; M, mesocarp; T, pigmented testa (Awika and Rooney 2004).

The testa (Figure 1.2.2) is the seed coat of the sorghum kernel. It is derived from the ovule integuments (Hoseney 1994**)**. The seed coat or testa is inherently present in all mature sorghum kernels. In some sorghum varieties however the testa or seed coat is highly pigmented. Testa thickness ranges from 8 µm to 40 µm and varies within individual kernels (Earp and Rooney 1982). It lies just beneath the cross and tube cell layers. The presence or absence of the testa is controlled by complimentary B_1 and B_2 genes. The pigmented testa is present when both B_1 and B_2 are dominant (B_1-B_2) together with the spreader gene (S) (Rooney and Miller 1982).

The endosperm (Figure 1.2.2) is triploid resulting from a fusion of a male gamete with two female polar cells. It is composed of the aleurone, peripheral, corneous and floury layers (Earp and Rooney 1982). The aleurone layer is the outermost layer of the endosperm. It consists of a single layer of rectangular cells adjacent to the testa and tube cells. The cells in the aleurone layer have thick cell walls and are rich in proteins, in the form of protein bodies and enzymes. The cells also have phytin bodies which constitute ash and pheromones which contain oil (Hoseney 1994). The peripheral, translucent or vitreous area of the endosperm is composed of several layers of dense cells containing more protein bodies and small starch granules than the floury area. The protein matrix has embedded protein bodies (Serna-Saldivar and Rooney 1995) which gives the corneous endosperm a translucent appearance. The opaque and floury endosperm has a discontinuous protein phase, air voids and loosely packed round starch

granules (Hoseney 1994). The opaque or chalky appearance is due to the presence of air voids which diffract incoming light.

1.2.1.1 Sorghum grain endosperm texture (Kernel hardness)

Endosperm texture is the proportion of corneous (vitreous or hard) fraction of the endosperm with respect to the floury or soft endosperm fraction (Hallgren and Murty 1983). These proportions determine endosperm texture. Kernels with more corneous than floury endosperm are designated as hard or corneous while those with more floury than corneous endosperm are termed soft (Rooney and Miller 1982). The relative proportions of the corneous and floury endosperm vary among sorghum types. This variation is influenced by genetic and environmental factors (Serna-Saldivar and Rooney 1995) and factors such as moisture, proteins, lipids and pentosan content (Stenvert and Kingswood 1997; Glennie, et al 1983). In their review on kernel hardness, Chandrashekar and Mazhar (1999) concluded that grain hardness is due to a combination of factors which include cell wall structure, the types and concentration of prolamins present in the endosperm. They further state that cell wall polymers in the hard grains are more rigid and the protein bodies are more and evenly distributed and contain mostly gamma prolamins which seem to be cross linked by disulphide bonds than in soft grains. According to Shull, Watterson and Kirleis (1991), the amounts of alpha and gamma prolamins appear to be essential for corneous texture than for soft or floury texture.

Kernel hardness is one of the most important grain quality criteria in sorghum grain. In sorghum utilisation, endosperm texture affects both the decortication of the grain and the reduction of the decorticated grain into flour. Sorghum grain milling (decortication and size reduction) also affect the palatability and cooking quality of sorghum. Endosperm texture is also important in grain storage, milling and food processing. In milling the endosperm texture has been shown to be the main component of the grain that influences milling performance (Kirleis and Crosby 1982). Grains with high proportion of corneous endosperm tend to be more resistant to breakage during decortication and milling than grains with a high proportion of floury endosperm (Rooney and Miller 1982). Furthermore sorghum grain endosperm texture is of importance in milling as hard grains tend to yield proportionally cleaner endosperm of larger particle size during milling operations than soft grains. Hard grains are also more resistant to insect and mould damage than soft grains (Chandrashekar and Mazhar 1999). In sorghum with a higher percentage of corneous endosperm, the pericarp is more readily separated from the intact starchy endosperm, giving a higher yield in milling (Norris 1971; Rooney and Miller

1982). Endosperm texture is also vital to the storage of grain. Insects more readily attack soft floury endosperm. Therefore, grain hardness is an important economic and end-use quality trait in cereal grains.

1.2.1.2 Methods for assessment of sorghum kernel hardness

Several methods are used to assess sorghum kernel hardness. These methods may be classified into four main groups: methods based on abrasive decortication and milling yield, methods based on subjective examination of the cut kernel, methods based on density of the kernel and methods based on water absorption.

(a) Methods based on abrasive decortication and milling yield

In dry flour production dehulling is followed by milling (grinding). In the process of decorticating some part of grain is lost due to breakage. Dehulling or decorticating loss gives an indication of hardness. The harder the grain is the lower the dehulling loss, and the higher the dehulling loss, the lower the milling yield (Gomez et al 1997). In principle, the ease with which the outer layers of the kernel can be removed during decortication and milling determines the dehulling loss or milling yield which are directly related to how hard the kernel is. Various pieces of equipment have been used based on this principle to determine sorghum kernel hardness. These include the tangential abrasive dehulling device (TADD) (Reichert, Youngs and Oomah 1981; Reichert, Tyler, York, Schwab, Tatarynovich and Mwasaru 1986; Taylor and Duodu 2009), the Strong-Scott laboratory pearler (Maxson et al 1971) and the Udy Cyclo-Tech grinding mill (Gomez et al 1997; Reichert et al 1981).

Tangential abrasive dehulling device (TADD)

The TADD has a resinoid disk mounted horizontally beneath eight to twelve sample cups. Grain is placed in the cups and the disk is set to rotate using a motor (Oomah, Reichert and Youngs 1981). The grain pericarp is rubbed off by the disk and the fines that are generated are swept out of the machine into the fines collection bag by means of an air flow produced by a fan. The harder the grain, the slower the material is abraded off. Kernel hardness is calculated in terms of the time taken to remove a certain weight percentage of the grain, or percentage of the grain removed over a fixed time (Taylor and Duodu 2009).

The TADD is a reliable piece of equipment with high repeatability and reproducibility. The popularity of the TADD for estimating sorghum kernel hardness may be related to the essentially spherical nature of the grain. The TADD is also used to measure Abrasive hardness

index defined as time in seconds required for abrading 1% by weight of the kernel (Gomez et al 1997). It is also used to measure the extraction rate, a measure of the percent by weight of the kernel that can be recovered as acceptable flour. However, the TADD may be expensive for small scale farmers and the abrasive disks may wear out over time, and these have to be imported.

Strong-Scott laboratory pearler

Pearling is the removal of the bran layers from cereal grains. The parts of the bran removed by pearling include the cuticle, epidermis, hypodermis and the major part of the mesocarp. To estimate kernel hardness using the Strong-Scott laboratory pearler, the weighed grain is fed into the pearler which is equipped with a grit stone, and a system of mesh screens of different sizes, a motor and a timer. The grains pass through the system of sieves as the pearler is operated. The tailings are weighed and expressed as percentage of the unpearled grain to give a parameter known as the pearling value (Rooney and Sullins 1969). The pearling value indicates hardness of the grain; the smaller the value, the harder the grain (Maxson et al 1971).

Assessment of sorghum kernel hardness using the Strong-Scott laboratory pearler is simple and rapid and the pearling value correlates well with density, milling yield and endosperm texture (Maxson et al 1971). However, the equipment has to be imported which could make it inaccessible for rural farmers. The stones wear and tear over time and need replacement and the pearler can only handle small samples.

Udy Cyclo-Tech grinding mill

The Udy cyclone mill is used to determine percentage milling yield, which is calculated by expressing the final weight of the decorticated sample as a percentage of the initial weight of the grain. A high percent milling yield is an indicator of less breakage of the grain. Generally hard grains have high milling yields due to less breakage during decorticating and more flour yield when ground. The test gives an indication of whether the sorghum grain gives a good yield of flour after milling (Gomez et al 1997; Shepherd 1979). Shepherd (1982) using a modified Udy cyclone mill demonstrated that Feterita, a soft sorghum cultivar gave the poorest milling results among the 25 samples studied. Being a soft sorghum, it had a large amount of broken kernels, further reinforcing the fact that soft sorghums have low milling yields. The mill grinds samples by means of high speed impact action provided by a rapidly rotating impeller rubbing the grain against the circular wall of the mill chamber which is coated with an abrasive lining.

The Udy cyclone mill is simple to use and rapid and is suitable for routine analysis of sorghum in breeding programmes, mills and service laboratories. However, it cannot handle large batches of sorghum grain and can only decorticate 5-30 g of sorghum at a given time.

(b) Methods based on subjective visual examination

In this method, sorghum endosperm texture is defined in terms of the proportion of corneous endosperm relative to floury endosperm in the grain (Rooney and Miller 1982). Grains with a high proportion of corneous endosperm are considered to be harder and tend to be more resistant to breakage during decorticating and milling than grains with a high proportion of floury endosperm (Lawton and Faubion 1989). The endosperm texture is measured by visual examination of the grain. Sorghum grains are cut into halves longitudinally. One half is viewed with the naked eye and the relative proportion of corneous to floury endosperm is determined. Grains are classified into: corneous, intermediate and floury in terms of texture and given a score (ICC 2008). This method is easy to use and does not require extensive and sophisticated knowledge and so has wide applicability. It is however subjective and depends on the person estimating kernel hardness.

(c) Methods based on density of the kernel

Grain density is an important factor influencing processing quality and end-use quality mainly in milling and malting (Gomez et al 1997). These indirect methods that measure kernel density as an indicator of kernel hardness are based on density differences between floury and vitreous parts of the endosperm (Kirleis and Crosby 1982) which determine floatation of kernels in a solution of known density. Kernels with a lower density will float and these would be small and soft kernels while those with greater density and heavy will sink and these are the hard kernels. In general heavier or denser sorghums tend to be hard or more vitreous while lighter or less dense sorghums tend to be soft or more floury. In this method, a solution of specific gravity 1.3 is used which was found to be approximately equal to the average density of a wide range of sorghum kernels. In this solution, kernels of lower density than average will float, while those with greater density will sink. Kirleis and Crosby (1982) used tetrachloroethylene and kerosene while Hallgren and Murty (1983) used sodium nitrate solution to determine the density of sorghum kernels respectively.

This method is simple and rapid and can be used in research farms and warehouses to classify a large number of grain samples on the basis of endosperm texture. Chemicals used may however be expensive and may have to be imported in certain instances.

(d) Methods based on water absorption

This method determines the amount of water absorbed by a sample in a given time. It gives an indication of the hardness of the endosperm (Murty, Patilb and House 1982; Gomez et al 1997; Buffo, Weller, and Parkhurst 1998). This is important when one wishes to condition grain before dehulling and milling. Grain with lower percent water absorption require longer conditioning and these are the hard grains whereas soft or floury grains require a shorter time to reach saturation. In other words, hard kernels absorb more water in a given time than soft grains because they take longer to reach optimum conditioning, while soft grains need a shorter time to reach saturation or to reach optimum conditioning or water uptake (Murty et al 1982a; Gomez et al 1997; Buffo et al 1998).

The corneous endosperm (hard endosperm) contains a protein matrix which exists in a continuous interface with starch granules (Hoseney 1994).This structural arrangement is compact or cement-like and it gives the corneous endosperm a translucent appearance. The floury endosperm (soft endosperm) also contains a protein matrix and starch granules but has the starch granules loosely packed in a discontinuous manner and has air voids or spaces which confer chalky appearance to the soft endosperm (Hoseney 1994). The voids in the soft endosperm could be responsible for entry of water into the kernel at a faster rate compared to the corneous endosperm which lack voids. A review on kernel hardness in sorghum and maize by Chandrashekar and Mazhar (1999) concluded that hardness results from a combination of factors such as cell wall structure and the type and concentration of prolamins present in the endosperm. These could also affect the way water penetrates hard and soft grains.

In hard sorghum grains (in contrast to soft grains), it is hypothesised that the cell wall polymers such as arabinoxylans, cellulose and other hemicelluloses are in close association with the protein matrix. Additionally, hard sorghum grains tend to have higher levels of kafirin protein which participate in disulphide bonding with matrix proteins resulting in entrapping of starch polymers and the formation of a rigid structure in the process (Glennie et al 1983; Glennie 1984; Chandrashekar and Kirleis 1988; Ioerger, Bean, Tuinstra, Pedersen, Erpelding, Lee and Herrman 2007). The endosperm cell walls of sorghum grain (Verbruggen, Beldman, Voragen

and Hollemns 1993; Verbruggen 1996) and maize grain (Huisman, Schols and Voragen 2000) are rich in water inextractable (insoluble) glucuronoarabinoxylan type pentosans. They are also rich in phenolic acids such as ferulic acid and *p*-coumaric acid. Phenolic acids, just like cell wall polymers are also said to contribute to the strength of cell walls of plants and cereals (Barriere, Riboulet, Mechin, Maltese, Pichon, Cardinal, Lapierre, Lubberstedt and Matiriant 2007).

Investigating differences in protein cross-linking between vitreous and floury endosperm, Ioerger et al (2007) found vitreous endosperm to have higher levels of total protein and kafirins than floury endosperm. Floury endosperm was found to have higher levels of sodium dodecyl sulphate (SDS)-soluble proteins than SDS-insoluble proteins extracted using sonication. Conversely, vitreous endosperm had a greater proportion of the insoluble proteins. According to Ioerger et al (2007) vitreous endosperm had a higher ratio of disulphide to total sulphydryls than floury endosperm, showing that the proteins in vitreous endosperm have a higher degree of cross-linking than those found in floury endosperm. The amounts of α and γ-prolamins appear to be essential for corneous texture. The γ-prolamins seem to be more cross-linked by disulphide bonds in hard grains than in soft grains (Hoseney 1994), and they are usually higher in hard grains than in soft grains (Chandrashekar and Mazhar 1999; Shull, Chandrashekar, Kirleis and Ejeta 1990). On the other hand, soft or floury sorghum contains a protein called friablin which confers softness to it. This protein is not present in the hard endosperm (Bechtel, Wilson and Martin 1996).

Water absorption is used in industry, plant breeding, dry and wet milling and malting to evaluate the water uptake of grains such as sorghum (Kashiri, Kashaninejad and Aghajani 2010), wheat (Rathejeni, Strounina and Mares 2009), maize (Verma and Prasad 1999) and rice (Bello, Tolaba and Suarez 2004). It has given results that distinguish hard kernels from soft ones in terms of water uptake or absorption in sorghum (Murty et al 1982b; Buffo et al 1998). However it is not yet established as an official method for determining kernel hardness. It however, has been used as a method for measuring kernel hardness through water uptake (Murty et al 1982a; Murty et al 1982b; Buffo et al 1998; Gomez et al 1997), whose effectiveness is based on the endosperm differences just like in other methods of estimating kernel hardness of the grains. This provides a strong motivation for its inclusion in the methods used to determine kernel hardness. Its attractiveness is that it can be used in the rural areas where expensive equipment is unaffordable and where the principle of water absorption is

already being applied in traditional dehulling, milling and in food preparations such as soaking of cereals and pulses to shorten their cooking time. The method can be used in laboratories and breeding programs together with other methods used for kernel hardness. Regular use of this proposed method for kernel hardness estimation can lead to its validation and inclusion in methods for kernel hardness estimation.

1.2.2 Sorghum grain colour

The kernel colours often seen in sorghum are red, lemon yellow, brown or white. Grain colour is mainly due to the polyphenolic pigments anthocyanins and flavan-4-ols, which are located mainly in the pericarp (Taylor and Duodu 2009). Kernel colour is genetically controlled and should be described in terms of the genetics of the factors affecting it (Rooney and Miller 1982). Some of the white sorghum kernels are spotted with red, purple, or brown pigments that leach from the glumes (Bennett, Tucker and Maunder 1990). A number of other factors affect sorghum kernel colour and overall appearance. For instance it is affected by pericarp colour, thickness, presence of pigmented testa and endosperm colour (Rooney and Miller 1982). Sorghum grain colour also affects the colour of the resulting food, especially foods made with alkali, like tortilla and alkaline tô (Rooney and Miller 1982; Dicko, Hilhorst, Grupen, Traore, Laane, van Berkel and Voragen 2002a). Sorghum kernel colour is also affected by weathering as a result of grain on the head in the field being exposed to sunshine, insect attack and mould (Bennett et al 1990; Taylor and Duodu 2009).

1.2.2.1 Genetics of sorghum grain colour

The colour of the sorghum kernel is influenced by the genetics of pericarp, testa and glumes (Rooney and Miller 1982; Waniska 2000). Pericarp colour is genetically controlled by the R and Y genes. The combination of these genes can produce white or colourless (R_YY or rrYY), lemon yellow (rrY_), or red (R_Y_) colour. The presence of the intensifier gene (S) increases the brightness of the pericarp. The presence of the homozygous recessive genes (zz) confers pericarp thickness in some sorghums making them appear chalky (Rooney and Miller 1982), by masking the colour of the endosperm and testa (Earp and Rooney 1986).

Sorghum kernel colour is also influenced by a pigmented testa whose presence is genetically controlled in tannin sorghums. It is present when the B_1 and B_2 genes are dominant. The pigmentation of the testa is controlled by the Tp gene when the testa is brown (Blakely, Rooney, Sullins and Miller, 1979) and tp tp when the testa is purple (Casady 1975). Blakely et

al (1979) reported that the testa was the most pigmented part of the sorghum kernel. Glume colour is associated with plant colour. There are three main plant colours namely red (P-$q^r q^r$), tan (ppqq), and purple (P-Q- or Pqq) (Rooney and Miller 1982). Glumes with intense red and purple colour have a tendency to stain the sorghum kernel because their polyphenolic pigments leach into the pericarp (Dicko et al 2002a).

1.2.2.2 Sorghum kernel colour pigments

Sorghum kernel colour is due to a combination of anthocyanin and anthocyanidin pigments as well as other flavonoid compounds (Hahn and Rooney 1986; Taylor and Duodu 2009). Sorghum grains with varying degrees of pigmentation and their location have been reported (Nip and Burns 1969; Nip and Burns 1971; Blakely et al 1979). The most abundant anthocyanins in sorghum grain are the 3-deoxyanthocyanins of which the most common are apigeninidin and luteolinidin (Bate-Smith 1969; Nip and Burns 1969; Nip and Burns 1971; Gous 1989; Awika et al 2004a). Cyanidin and pelargonidin have also been reported in sorghum (Yasumatsu, Nakayama and Chichester 1965). Other flavonoids isolated and identified in sorghum grains include the flavones apigenin and luteolin, which are predominant in tan-plant sorghums (Seitz 2004; Dykes and Rooney 2005). The flavanones eriodictyol and its 5-glucoside (Kambal and Bate-Smith 1976; Yasumatsu et al 1965; Gujer, Magnolato and Self 1986), and naringenin (Gujer et al 1986; Awika et al 2003) have also been reported in sorghum.

1.2.2.3 Colour measurement

Colour and the perception of colour results from the interaction of the light source, object being viewed, eye and the brain. Colour is composed of two chromatic attributes (hue and purity) and one luminous factor, brightness. Hue refers to the spectral composition of light leaving the object. Purity or saturation is the amount of colour present. The higher the saturation, the lower the greyness. The brightness or (lightness) refers to the capacity of an object to reflect or transmit light. Colorimetric methods that have been used to measure colour involve the use of UV-visible spectrophotometers and tristimulus colorimeters (Awika et al 2003). The UV-visible spectrophotometer uses absorbance or transmittance to determine sample colour at a selected wavelength (Feillet, Autran and Icard-Verniere 2000).

The tristimulus colorimeter gives three readings X (red value), Y (green value) and Z (blue value). These are easily combined to give the value a, which indicates redness when it is

positive, the value b, which indicates yellowness when positive and the value L, which indicates brightness ranging from 0 to 100. The units within L* a* b* give equal perception of colour differences to the human eye.

The importance of grain colour in influencing end-use quality of foods cannot be overemphasized. In Africa white or light coloured sorghums are generally preferred for porridge making while red coloured sorghums are generally preferred for brewing traditional beer (Taylor and Duodu 2009). For industrial production of lager beer, white sorghums are generally selected (Belton and Taylor 2004). In the USA**,** one of the bases of sorghum classification is grain colour. According to the United States Department of Agriculture Grain Inspection, Packers and Stockyards Administration (GIPSA 2007), sorghum is divided into four classes, namely: Sorghum (the pericarp may be any colour), Tannin Sorghum (the grain has a pigmented testa), White Sorghum (the pericarp must be white) and Mixed Sorghum (grain that does not meet the requirements of the other classes (GIPSA 2007). Grain colour is also determined visually (Taylor and Dewar 2001).

1.2.3 Chemical composition of sorghum

1.2.3.1 Starch

Starch is accumulated in granules in the endosperm, it is deposited in layers with various amylose and amylopectin content (Smith et al 1997). Starch granular size distribution and shape have a bearing on the functional properties such as thickening, gelling, stabilising, bulking, texturizing, fat replacement, film, foam stabilising anti-staling and moisture and flavour binding applications (Bultosa 2003). Other non-food industrial applications where starch functional properties are important include paper making, adhesives, plastic and pharmaceuticals (Bultosa 2003). Variation in granule size distribution among cultivars is attributed to genetic control and to some extent to environmental factors such as temperature (Peterson and Fulchner 2001). Starch organization in the granule of cereals has been reviewed by Buléon et al (1998) and Donald (2001).

The starch granule consists of alternating semi-crystalline and amorphous layers (Figure 1.2.3). The semi-crystalline layer is believed to consist of alternating 9 nm crystalline layers of double-helical-glucans extending from intermittent branches of amylopectin, and the amorphous layers of amylopectin branch points. Starch granules of sorghum and maize are similar in size and shape. Their average diameter is about 20 µm. The shape of starch granules

varies from polygonal to almost spherical (Hoseney 1994). The starch granules in cells near the outside of the kernel in the vitreous endosperm tend to be polygonal while those in the centre of the kernel in the opaque endosperm tend to be spherical.Native starch in the granule is stabilized by hydrogen bonding making them resistant to water entry and enzyme hydrolysis without application of heat. The organisation of granules cause plane polarised light to exhibit a characteristic shadow or Maltese cross also known as birefringence (Whistler et al 1984; Manners, 1985).

Sorghum varieties, as shown by Dicko, Hilhorst, Gruppen, Traore, Alphons, Voragen and van Berkel (2006) have different contents of starch, amylose and amylopectin. The mean values of starch, amylose and amylopectin were 63.0%, 13.4% and 49.6%, respectively, with respect to the sorghum kernel. Native starch granules are essentially insoluble in cold water, but through gelatinisation, swelling and hydration of these granules occurs (Zobel 1984). Gelatinisation is the disruption of molecular order within the starch granule which leads to irreversible changes in properties of starch such as granule swelling, native starch melting, loss of birefringence and starch solubilisation (Hoseney 1994). These irreversible changes contribute to food properties like texture, viscosity and moisture retention (Whistler and BeMiller 1997). It appears sorghum starch has higher gelatinization temperature compared to starches from other cereals. Since sorghum is an important cereal in Sub-Saharan Africa, a knowledge of the thermal properties of sorghum starches and flours is cardinal in understanding why some sorghum cultivars' porridges are of superior quality while others are not (Akingbala, Rooney, Palecios and Sweat 1982) The organisation and birefringence of the starch granules is lost during gelatinization (Zobel 1984). Gelatinization temperatures of 68-78^{o}C (Taylor and Belton 2004) and 71-81^{o}C (Taylor and Dewar 2001) have been reported for sorghum compared to 62-72^{o}C and 51-61^{o}C for maize and barley starches respectively. The swelling and solubility properties of sorghum starches and starch cooking properties are good indicators of textural properties of foods made from sorghum (Akingbala et al 1982). These properties however can change depending on the environment where the sorghum cultivars are grown (Akingbala et al 1982).

Starch gelatinisation temperature is influenced mainly by the lengths of the various chains in the amylopectin polymer (Taylor and Dewar 2001). The longer the chain length, the higher the gelatinisation temperature (Dufour, Melotte and Srebrnik 1992). The high gelatinization temperature of sorghum starch affects the processing of sorghum as is the case in sorghum adjunct cooking for sorghum beer brewing, because it has to be thermally processed longer.

This has negative implications for processing of sorghum because of the high energy cost involved (Dufour et al 1992).

When cooled, starch molecules are prone to retrogradation. Retrogradation is the reassociation of starch molecules separated during gelatinisation resulting in the formation of a paste or gel and the liberation of bound water. This leads to the formation of enzyme-resistant starch (Englyst and MacFarlane 1986). Apart from high gelatinisation temperatures of sorghum which attects its industrial and domestic utilization, low sorghum starch digestibility also affects its full utilisation. Factors affecting starch digestibility include the composition of starch, the starch protein interactions, integrity of the endosperm, antinutritional factors and processing history of the product (Rooney and Pflugfelder 1986).

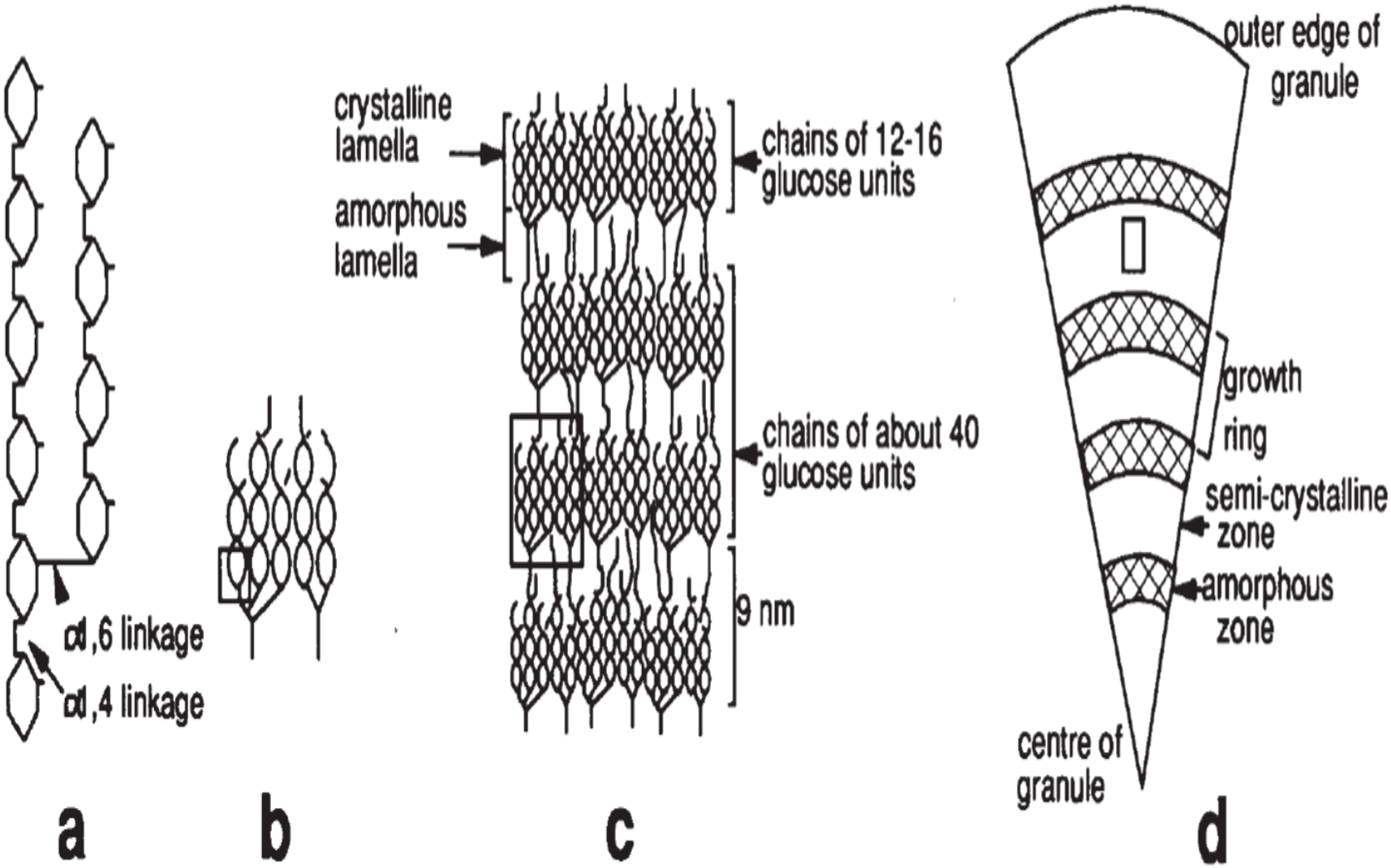


Figure 1.2.3 Schematic representation of levels of organization within the starch granule. The boxes within the diagrams in panels *b*, *c*, and *d* represent the area occupied by the structure in the preceding panel. (*a*) Structure of two branches of an amylopectin molecule, showing individual glucose units. (*b*) A single cluster within an amylopectin molecule, showing association of adjacent branches to form double helices. (*c*) Arrangement of clusters to form alternating crystalline and amorphous lamellae. (*d*) Slice through a granule, showing alternating zones of semicrystalline material, consisting of crystalline and amorphous lamellae, and amorphous material (Smith, Deyner and Martin 1997).

1.2.3.2 Proteins

The protein content of sorghum generally ranges from 7 to 14% (Taylor, Schüssler and Van der Walt 1984b). The sorghum germ contains about 16% of the protein where the nitrogen occurs mostly as low molecular weight nitrogen, albumin and globulin proteins (Taylor and Schussler 1986). Albumins and globulins are richer in most of the essential amino acids than other sorghum protein fractions (Hoseney 1994; Youssef 1998).

The matrix protein around the protein bodies is mainly glutelin (Taylor, Novellie, and Liebenberg 1984a). The prolamins, which are storage proteins in cereals, are called kafirin in sorghum (Hulse, Laing and Pearson 1980). Kafirins are further classified into α-kafirins, β-kafirins and γ-kafirins (Shull, Watterson and Kirleis 1991). They are poor in lysine, which is an essential limiting amino acid. The aleurone layer and the germ contain more proteins that are rich in lysine. Maclean and Graham (1980) found that protein and energy digestibility for whole sorghum was much lower than for wheat, maize and rice. The biological value of sorghum was only 55.9% due to its low lysine content and poor digestibility (Maclean and Graham 1980). The bioavailability of protein in sorghum may be affected by anti-nutritional factors like condensed tannins (Butler 1989; Butler and Rogler 1992; Butler 1990; Hagerman, Reidl, Jones, Sovik, Richard, Hartzfield and Riechel 1998; Guiragossian, Chibber, Van Scoyc, Jamhunathan, Mertz and Axtell 1978) cyanogenic glycosides Butler (1995) and enzyme inhibitors like protease inhibitors (Gomes, Oliva, Lopes and Salas 2011) and α-amylase inhibitors (Bloch and Richardson 1991).

1.2.3.3 Lipids

The major lipids of sorghum like those of other cereals are located mainly in the germ. Smaller amounts are found in the endosperm (Belton and Taylor 2004). The storage organs of fat in cereals are the spheresomes, which are lipid-containing organelles in the germ and aleurone. Sorghum lipids are classified into non-polar lipids, glycolipids, and phospholipids (Belton and Taylor 2004). The total lipids content of sorghum ranges from 0.5 to 5.2 % (Serna–Saldivar and Rooney 1995). Their presence in milled products where the germ was broken to pieces during milling can trigger the oxidation process affecting end-use quality due to rancidity and development of off flavours in processed products (Hoseney 1994).

1.2.4 Sorghum phenolic compounds

Phenolic compounds are the most widely distributed compounds in nature and are ubiquitously present throughout the plant kingdom (Hummer and Schreier 2008). Over 8000 phenolic compounds with highly diverse structures are known to exist) Hummer and Schreier 2008). Their molecular mass ranges from < 100 Da for simple phenolic compounds to >30,000 Da for highly polymerised structures. Classification and subdivision is based on oxidation level of the C-ring (Matus-Cadiz, Daskalchuk, Vema, Puttick, Chibbar, Gray, Peron, Tylerand and Hucl 2008). Stereochemistry, position and nature of the substitutions (hydroxyl, methyl, galloyl, glycosyl, acetyl), combination, degree of polymerisation and linkages between the basic units have made it possible to identify many phenolic compounds found in plants (Matus-Cadiz et al 2008). Classes of plant phenolic compounds include the following: phenolic acids, flavanones, flavones and flavonols, anthocyanidins, flavan-3-ols and proanthocyanidins (PAC) (Hummer and Schreier 2008). They constitute a variety of plant secondary metabolites. Flavonoids for instance are stored mainly in the vacuoles which offer a larger storage space than cell walls. In the vacuoles they can reach concentrations that offer protection against predators and pathogens or as UV light sunscreen or attractants (Matus-Cadiz et al 2008).

1.2.4.1 Sorghum anthocyanidins

The major groups of flavonoids in sorghum are the flavans that is flav-3-en-ols with double bonds between C3 and C4. Those hydroxylated at C3 are the anthocyanidins (Figure 4) (Haslam 1998). The most abundant anthocyanidins in sorghum are the 3-deoxyanthocyanidins (apigeninidin and luteolinidin) (Figure1.2.4) (Bate-Smith 1969; Sweeny and Iacobucci 1981; Gous 1989). They are derived from the flavan-4-ols pro-apigeninidin or leuco-apigeninidin and luteoforol (proluteolinidin or leuco-luteolinidin) (Dicko et al 2005a). They are called 3-deoxyanthocyanins because they do not have a hydroxyl group at the C-3 position of the C-ring (Figure 1.2.4) (Awika et al 2004b). The 3-deoxyanthocyanidins constitute the red colour of the grain pericarp (Bate-Smith 1969). Black pericarp sorghum types have been reported to contain higher levels of flavan -4-ols and 3-deoxyanthocyanidins than other varieties of sorghum (Dykes, Waniska and Rooney 2005). The 3-deoxyanthocyanidins are considered to have good thermal colour stability (Iacobucu and Sweeney 1983:Sweeney and Iacobucu 1981, Awika et al 2003) and have been reported to be more stable in organic solvents as well as acidic solutions than anthocyanidins found in fruits,

vegetables and other cereals (Awika et al 2004b). Identifying the actual 3-deoxyanthocyanidins involved in colour expression in sorghum can add value to sorghum end-use quality. Limited data is available on the types and levels of anthocyanins in sorghum **(**Awika et al 2004b).

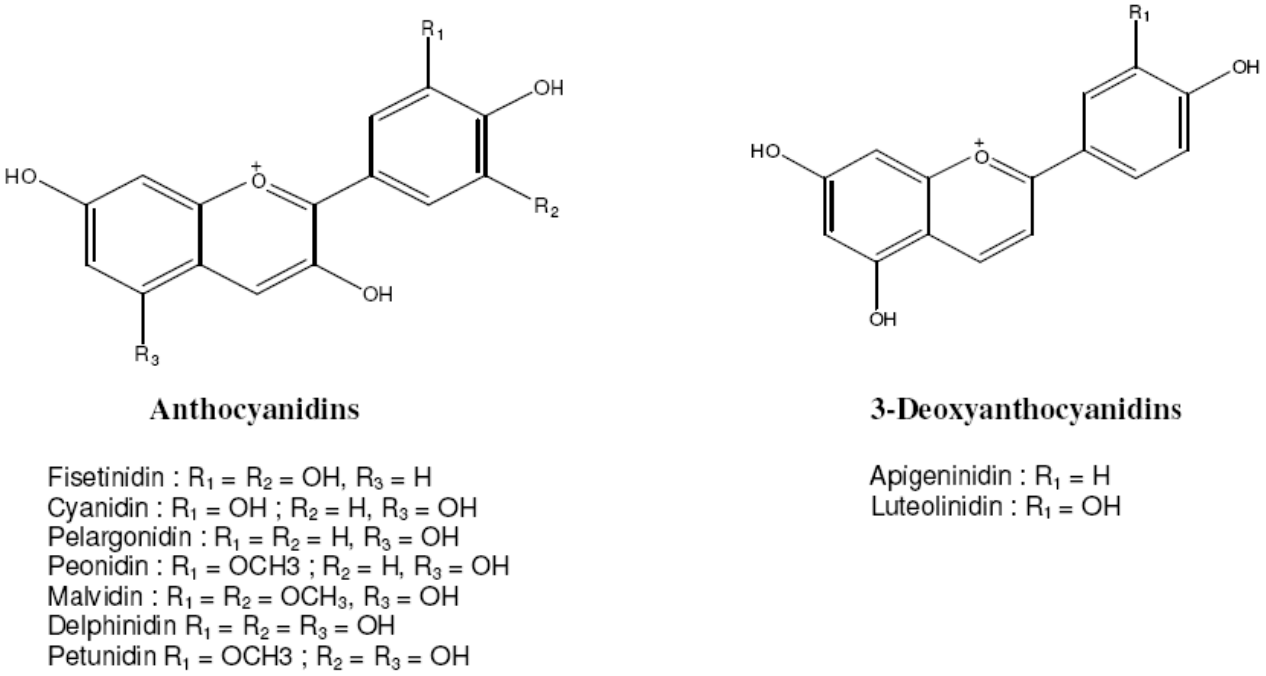


Figure 1.2.4. Structures of anthocyanidins and 3-deoxyanthocyanidins (Kouda-Bonafos et al. 1996; Pale et al 1997; Awika et al 2004b; Dicko et al 2006)

1.2.4.2 Sorghum tannins

The term tannin refers to substances of plant origin capable of transforming fresh hides into leather (Santos–Buelga and Scalbert 2000) and they can precipitate gelatine and other proteins (Porter 1986). Tannins (Figure 1.2.5) are water soluble phenolic compounds with molecular weights ranging from >500 to 3000. The structure of tannins varies in the nature of constitutive units, degree of polymerization i.e. chain length and linkage (Butler 1989).

Sorghum tannins are polymers of flavan-3-ols (catechin or epicatechin) units (Gupta and Haslam 1978). They are the condensed tannins also known as proanthocyanidins (Price, Van Scoyoc and Butler 1978). Different classes of proanthocyanidins exist depending on the substitution pattern of the monomeric flavan-3-ol units. The flavan-3-ol subunits may carry acyl or glycosyl substituents. The most common subsituent bound as an ester is gallic acid to form 3-*O*-gallate. The proanthocyanidins that consist of epicatechin units are designated procyanidins; they are the most abundant type in plants (Hummer and Schreier 2008). The less common proanthocyanidins are the ones containing epiafzelechin or epigallocatechin and are known as properlargonidin or prodelphinidin, respectively (Dicko et al 2006).

Based on their extractable tannin content, sorghums may be classified as type I (no significant levels of tannin extracted by 1% acidified methanol), type II (tannin extractable in 1% acidified methanol but not methanol alone), and type III (tannin extractable in both acidified methanol and methanol alone) (Cunnings and Axtell, 1973; Price et al 1978). This classification according to Awika and Rooney (2004a) has limitations in that it does not specify the varying levels of other major phenolic constituents like anthocyanins or the actual levels and intensity of colour pigments (Cheng, Sun, and Halgreen 2009). Furthermore, Awika and Rooney (2004a) state that sorghum can also be broadly classified based on appearance and total extractable phenols. Classified in this manner the groups are as follows:

(a) Sorghum which is essentially white with no traceable tannins or anthocynins and insignificant levels of total extractable phenol levels (also termed food grade).
(b) Red sorghum which have no tannins but have a red pericarp with significant levels of extractable phenolic compounds.
(c) Black sorghum with a black pericarp and high content of anthocyanins and the brown sorghum which has pigmented testa and contains significant levels of tannins with varying degrees of pericarp pigmentation.

Sorghum with a pigmented testa classified as type II and type III sorghums contain tannins. Additionally they have dominant B_1 and B_2 genes. The B_1 and B_2 genes determine the presence or absence of the pigmented testa (Hahn and Rooney 1986). The dominant presence of the S gene (spreader gene) together with the B_1 and B_2 genes results in pericarp colour becoming phenotypically brown (Earp, Doherty and Rooney 1983) According to Hahn and Rooney (1986), sorghums with dominant S_ gene generally contain easily extractable tannins than the ones with the recessive genes. Sorghums with dominant S_gene also have adverse anti-nutritional effects in animals. Breeding for different combinations of pericarp colour and plant colour without the pigmented testa and spreader gene is possible since pericarp colour and plant colour are genetically controlled. The development of tan-plant sorghums is one such example (Peterson 2011). The presence of tannins in sorghum and other foods affect their nutrient content and acceptability in general (Hammerstone, Lazarus, Schitz, 2000). Current studies which highlight tannin contributions to human health have made it possible for them to be viewed less negatively (Awika and Rooney 2004).

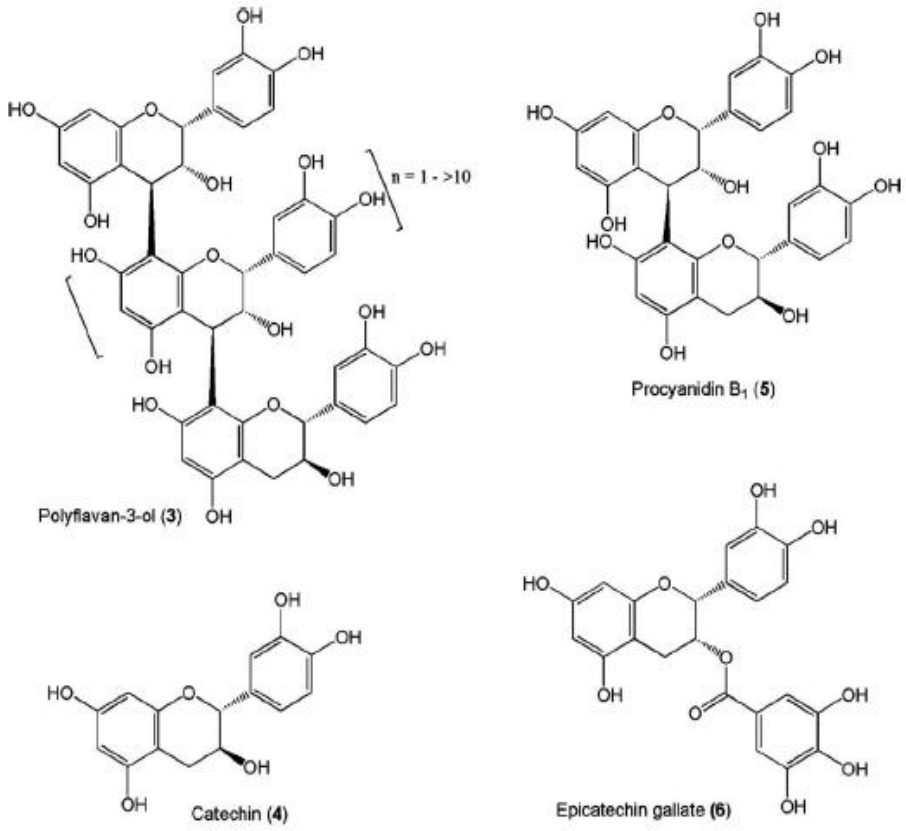


Figure 1.2.5 Structures of proanthocyanidins, catechin and epicatechin gallate (Gupta and Haslam 1978; Gu et al 2002; Awika 2003; Awika and Rooney 2004).

1.2.5 Polyphenol oxidase (PPO)

Utilization of sorghum as a food is affected by the unattractive colour of some sorghum-based foods particularly those made using white sorghum (Jagwer 1998). The unattractive colour of some wheat products like noodles has been associated with polyphenol oxidase activity (Singh and Sheoran 1972; Anderson and Morris 2001; Bettege 2004). In wheat the role of PPO in the darkening of wheat-based products is well established. Polyphenol oxidase is responsible for the discolouration of noodles, (Kruger, Hatcher and DePauw, 1994); chapattis, (Morris, Jeffers and Engle 2000); Middle Eastern flat breads, (Martin, Berg, Fischer, Jukanti, Kephart, Kushnak, Nash and Brucker 2005) and European pasta products (Feillet et al 2000).

Genetic studies by Jimenez and Dubcovsky (1999) indicated that homologous group 2 chromosomes influences PPO activity. This genetic information has resulted in breeding for wheat with low PPO activity, better acceptance of the wheat products and better market value (Raman, Raman, Johnstone, Lisle, Smith, Martin and Allen 2005; McCaig, Fenn, Knox, Depaw, Clarke and Mcleod 1999).

In sorghum the relationship between polyphenol oxidase activity and darkening of sorghum based foods has not been established. This is because research on the role of polyphenol oxidase in the darkening of sorghum based foods is limited unlike the case with wheat. Reported research work acknowledges the dark and unattractive colour of some sorghum foods but with no association with the polyphenol oxidase enzyme. For instance, Jagwer (1998) in sensory evaluation studies in Botswana reported the low acceptability of dark sorghum porridge made using white sorghum compared to maize porridge made from white maize. In their work on important sensory attributes affecting consumer acceptance of sorghum porridge in West Africa as related to quality tests, Aboubacar, Kirleis and Oumarou (1999) observed and reported that near white sorghum flour turned dark upon being used to make porridge. This led to the rejection of these dark appearing porridges compared to the lighter appearing ones (Aboubacar et al 1999). Suhendro, Kunetz, McDonough, Rooney and Waniska (2000) reported darker noodles made from sorghum than those made from rice. Dicko et al (2002a) in work on sorghum generally described PPO and peroxidase (PO) enzymes as determinants of sorghum end-use quality due to their role in oxidation mechanisms in foods. Specific foods affected by the action of these enzymes were not mentioned.

Glennie (1981) in studies on sorghum polyphenol oxidase demonstrated that there was an inverse relationship between PPO activity and tannin development. According to Glennie (1981) from a protective point of view, as tannins increased in the kernels as they matured, the PPO enzyme activity reduced thus increasing the role of tannins in protecting sorghum against moulds pathogens and predators**.** This is true for tannin containing sorghuim.

1.2.5.1 Distribution and mechanism of action of polyphenol oxidase

Polyphenol oxidase (EC 1.14.18) is a widely distributed enzyme among microorganisms, plant and animals. In plants a large portion of polyphenol oxidase activity is located in the endosperm of immature seeds (Demeke, Chang and Morris 2001; Glennie 1981).The level of polyphenol oxidase activity in the endosperm reduces when the kernel ripens, but the level of polyphenol oxidase activity in the outer layers and embryo increases (Marsh and Galliard 1996). Polyphenol oxidase inhibition after physiological maturity can lead to pre-germination of the seeds (Kruger et al 1994). Mechanical damage to seeds might cause variation in kernel polyphenol oxidase since about 50% of the polyphenol oxidase leaches out of seeds into solution (Kruger et al 1994). Most polyphenol oxidase activity in mature kernels is associated with the bran (Hatcher and Kruger 1993). Kernel size also affects the level of activity; the larger the kernel, the more the enzyme activity (Baik, Czuchajowska and Pomeranz 1994). Polyphenol oxidase has one unusual characteristic; it has the ability to exist in an inactive or latent state (Jimínez and Garcícia-Carmona 1999; Jukanti, Bruckner, Habernicht, Foster, Martin and Fischer 2003). For the polyphenol oxidase to be extracted in its latent form the extraction method must be very mild to prevent it from being modified or oxidised.

Polyphenol oxidase can be activated by proteolytic attack, changes in pH and anionic detergents like sodium dodecyl sulphate (SDS). The enzyme is activated by high sodium dodecyl sulphate (SDS) concentrations that would normally denature many other enzymes (Jukanti et al 2003). In nature, the resting state of the enzyme usually consists of 85 to 90% of a met form whereas 10-15 % is in the oxyform (Solomon, Sundaran and Machonkin 1996). Both met and oxy-states of the enzyme can catalyse the two electron oxidation of ortho-diphenols to ortho-quinones, whereas the hydroxylation reaction involved in the monophenolase reaction cycle requires the oxy-form of the active site (Solomon et al 1996; Matoba, Kumagai, Yamamoto, Yoshitsu and Sugiyama 2006).

Monophenols can also bind to the met form of the enzyme but without being oxidised. When monophenols are oxidised by polyphenol oxidase, a lag period is usually detected which is related to the state of the active site of the enzyme. During the lag period the oxy-form of polyphenol oxidase is generated from the met form and the rate of oxidation accelerates to reach the maximum, since the oxy-form can perform catalysis with both monophenols and diphenols (Land, Ramsden and Riley 2004). During oxidation of tyrosine, the diphenolic dihydroxyphenylalanine (DOPA) accumulates in the reaction. DOPA is formed due to the non-enzymatic reactions in the catalysis. By oxidising the DOPA, the polyphenol oxidase enzyme is transformed from the met to the oxy-form within the lag period.

Polyphenol oxidase is a copper containing enzyme also known as metallo-protein (Selinheimo 2008) which catalyses two distinct reactions involving molecular oxygen (Figures 1.2.6). The first one involves the insertion of oxygen in a position *ortho* to an existing hydroxyl group usually followed by diphenol oxidation to the corresponding quinone often referred to as cresolase activity (reviewed by Mayer and Harel 1979). The reaction is also known as the *o*–hydroxylation of mono phenols to *o*-diphenols.The second reaction involves oxidation with hydrogen abstraction of o-diphenol, often referred to as catecholase activity (Mayer and Harel 1979). In the second part of the reaction, the enzyme catalyses the oxidation of *o*-diphenols to *o*-quinones (catecholase activity) also known as the diphenolase reaction.

The quinones undergo secondary non-enzymic reactions, such as auto-oxidation and polymerisation with amino acid groups of cellular protein (Anderson and Morris 2001) to produce melanoidin products. This is because of their high susceptibility to non-enzymatic reactions and their electrophilic nature (Anderson and Morris 2001). This reaction of quinones is likely responsible for the black and brown pigmentation associated with reduced noodle, chapattis and Middle Eastern flat breads quality (Kruger et al 1994; Morris et al 2000).

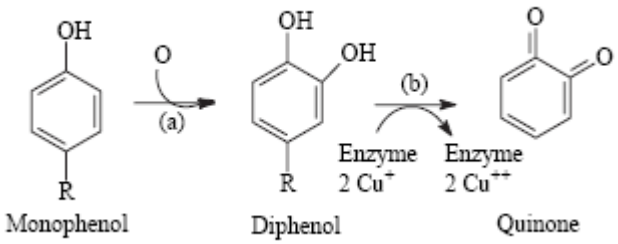


Figure 1.2.6 Reactions of (a) hydroxylation and (b) oxidation catalysed by PPO (Queiroz, Lopes, Fialho and Mesquita 2008)

1.2.5.2 Measurement of polyphenol oxidase activity

Methods for measuring polyphenol oxidase activity are based on two main principles:

- measurement of oxygen consumed (oxygen electrode method) (Marsh and Galliard 1986; Baik et al 1994), or
- monitoring of formation of coloured o-quinone adducts using UV-visible spectrophotometry (Espín, Morales, Varon, Garcia-Canovas and Tudela 1995; Espín, Morales, Varon, Garcia-Canovas and Tudela 1997; Kruger et al 1994; Demeke et al 2001).

Either way, a phenolic substrate is brought into contact with the PPO enzyme in the presence of oxygen. During the reaction, oxygen is consumed and/or coloured o-quinone adducts are produced. The amount of oxygen consumed or o-quinone adducts produced is then monitored and used as an indication of PPO activity.

1.2.5.3 Measurement of oxygen consumed in the reaction

Measurement of oxygen consumption is done using specialised equipment such as the YSI model 10053 oxygen monitor (Yellow Spring Instrument Co., Yellow Spring, Ohio). A known weight of sample in the form of whole grain or flour is weighed into an air-saturated cell of the monitor containing buffer. The role of the buffer is to extract the PPO enzyme. A suitable phenolic substrate e.g. catechol is then added and oxygen consumption during the reaction is monitored. PPO activity is determined as the difference between the basal rate of O_2 consumption and the initial rate following addition of the substrate. It is a quantitative method which measures the change in consumption of oxygen by the ground or whole kernels and mono or diphenolic substrate. It has disadvantages in that it is time consuming and it cannot be used in breeding programmes and routine analysis of PPO activity.

1.2.5.4 Measurement of coloured compounds using spectrophotometry

The catalytic action of PPO on phenolic substrates produces *o*-quinones which covalently modify nucleophiles such as amines, thiols and phenolic compounds forming complex coloured (brown, red or black) polymeric melanins (Whitaker and Lee 1995; Fuerst, Anderson, and Morris 2006). In this assay, the o-quinones formed are reacted with a reagent to produce coloured adducts which are measured spectrophotometrically by monitoring changes in absorbance during the reaction.

Dicko et al (2002) have used this method to determine PPO activity of sorghums. Essentially a known weight of flour is extracted with a buffer to produce the enzyme extract which is then reacted with a phenolic substrate such as L-dihydroxyphenylalanine (L-DOPA). Some 3-methyl-2-benzothiazolinone hydrazone (MBTH) is included in the reaction mixture and its role is to trap the *o*-quinones formed in the reaction to form adducts. The reaction is monitored at 475 nm. PPO activity is expressed in Unit/mg whole grain flour dry basis, where a Unit is the amount of enzyme producing 1 µmol of MBTH-quinone-adducts per minute from the oxidation of L-DOPA (Espín et al.1995; Jime´nez and Garci´a-Carmona 1997). The method has poor specificity for phenolic substrates.

Different substrates have been used to study polyphenol oxidase activity including tyrosine (Dicko et al 2006; Espín et al 1995; Espín et al 1997), catechol (Glennie 1981; Kruger et al 1994), L-DOPA (Morris et al 1998; Anderson and Morris 2001), 4-hydroxyanisole and 3-4 (4HA) dihydroxyphenylpropionic acid (DHPPA) (Dicko et al 2002a,b).

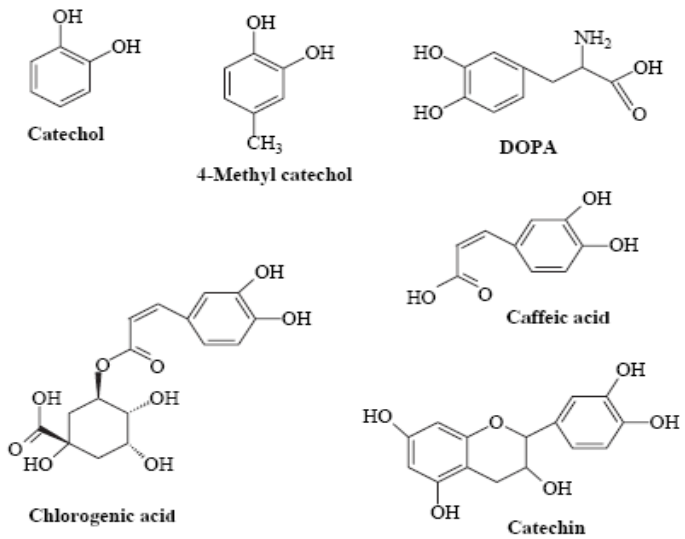


Figure 1.2.7 Structures of some natural substrates of PPO (Queiroz et al 2008).

1.2.6 Sorghum processing in relation to end use quality

1.2.6.1 Sorghum dry milling

Sorghum dry milling consists of two main processes:

- Decortication: Its main objective is to remove the outer, less palatable vitamin-rich (pericarp) and oil-rich (germ) anatomical parts of the grain. This leads to maximum recovery of the endosperm and improved shelf life of the degermed kernel (Munck 1982).
- Size reduction: Its objective is to reduce the endosperm into flour or grits (Munck 1982).Thus milling increases sorghum grain utilisation because different milled products can be produced. A wide variety of milling systems are currently in use including decorticators, stone mills, pin mills, roller mills, hammer mills and traditional pounding using mortar and pestle. To produce acceptable foods of acceptable quality size reduction has to be preceded by decortication. Therefore, milling plays a significant role in determining processing and end–use quality of milled products (Hoseney1994). While wheat, maize and rice milling technologies are highly advanced, sorghum milling technology has not developed to that level. As a result, the milling of sorghum is mostly with milling equipment derived from wheat, maize or rice milling (Reichert et al 1982). For instance, the decortication principle used in sorghum decorticating was earlier used in barley and rice dehullers, polishers and decorticators (Hoseney1994; Lawton and Faubion 1998).

1.2.6.1.1 Decortication and its impact on sorghum grain quality

Decorticating adds value to the sorghum grain by improving its utilization potential and quality (Chibber, Mertz and Axtell, 1978; Mwasaru, Reichert and Mukuru 1988; Eastman 1980; Shepherd 1982). Factors affecting the efficiency of decorticating are shape of the kernel (Mwasaru et al 1988), size (Wills and Ali 1983) and hardness (Desikar 1982; Kirleis and Crosby 1982; Munck 1982; Mukuru et al. 1982; Novillie 1982; Lawton and Fabion 1989). Ease of decorticating and grain hardness are quality criteria that influence the acceptability of new and established cultivars of sorghum for preparation of sorghum-based foods (Chibber 1980; Reichert et al 1982). Removal of the pericarp improves the cooking quality of sorghum. If left in the flour the pericarp swells during cooking and influences

viscosity, though much less than the endosperm. High pericarp content means less starch and a less viscous cooked porridge with a different mouth feel (Novellie 1982).

1.2.6.1.2 Traditional sorghum decortication

In traditional sorghum decortication, water (20-25% by weight) is added to the grain in order to temper it (Eggum, Bach Knudsen, Munck, Axtell and Mukuru 1982). Water penetrates the kernel, causes the pericarp layers to swell and become partially detached from the endosperm. This addition of water toughens the pericarp and softens the endosperm allowing easy separation of these anatomical parts (Mukuru 1990; Kent and Evers 1994). Pounding using mortar and pestle provides the abrasive action necessary to remove the pericarp after tempering. The stamping force of the pestle causes rebounding and shearing effects on the pericarp allowing separation of the endosperm from the pericarp by peeling in large chunks (Scheuring et al 1983).

Studies by Scheuring et al (1983) demonstrated that sorghum types with thick pericarps and hard endosperm were desirable for hand pounding using a mortar and pestle. The amount of water added to temper the grain makes hand pounding a semi-wet milling process. Therefore it creates quality problems for the decorticated grain and the resulting flour, hence the need to dry the final product to about 14% moisture to render it stable (Kent and Evers 1994). The quality of water added impacts on the quality of the flour produced. If water which is not potable is used, the grain is infected with bacteria (Eastman 1980). Hand pounding is laborious requiring about 1 hour to process 2-3 kg of sorghum grain by mostly women (Eastman 1980; Eggum et al 1982). Flour produced using a mortar and pestle has a shelf life of 2-3 days. This method of decortication will however continue to be used, as it is the only affordable processing means within reach of many people residing in the countryside.

1.2.6.1.3 Mechanical sorghum decortication

Mechanical decorticating is usually accomplished by the action of vertically or horizontal mounted disks (Reichert, et al, 1982. It operates on the principle of progressively rubbing off the outer layers of the dry kernel (Oomah, Reichert and Youngs, 1981). The type of abrasive surface used affects significantly the dehulling rate, efficiency and reproducibility (Reichert et al. 1982). The efficiency of decorticating is influenced by the physical characteristics of the sorghum being decorticated hardness being the most important (Shepherd 1982). The most widely used decortication device according to (Schimdt 1992) is the PRL (Prairie Research

Laboratory) dehuller. Its simplicity and robustness makes it ideal for use in developing countries. However, it has limitations in that its abrasive action does not remove all the germ resulting in an endosperm with a high fat content of 2-4% (Gomez 1993) making the resulting flour susceptible to oxidative rancidity (Hoseney 1994) an undesirable food defect.

To simulate the action of large-scale decorticators the tangential abrasive dehulling device (TADD) was constructed (Oomah et al 1982; Reichert et al 1982). The TADD has many advantages, which include multi-sample capacity, highly reproducible results and minimum maintenance requirement. The TADD comprises a motor driving a horizontal carborundum or abrasive paper covered disc. Eight small metal cylinders, which are open at both ends, are mounted in a fixed plate on top of the disc. The sorghum grain samples are put into the cylinders and the motor turned on. The revolving abrasive disc progressively abrades off the outer layers of the grains as they roll around in the cylinders; the harder the grain, the slower material is abraded off.

1.2.6.1.4 Hammer milling (size reduction)

The principle in hammer milling is grinding by impaction. Hammer mills are described in terms of full screen and half screen, full circle or half circle (Hikeezi 1994). In a hammer mill with a half screen, material is introduced directly in the path of the hammers. In a hammer mill with a full screen grain has to be introduced from the side. Hammer size affects the operation of the mill. Three types of hammers are used in hammer milling namely, thick, thin and intermediate. Thin hammers are highly efficient but tend to wear out fast and require constant replacement. The intermediate hammers work more efficiently compared to thin or thick hammers (Kansas State University Milling Practices, 1993; Hikeezi 1994). Hammer mill capacities range from 10 kg to 180 kg per hour with rotor speed varying from 1800 to 3450 rpm and power requirements from 1/2 to 100 Hp. (Kansas State University Milling Practices, 1993). In the 1980s dehullers introduced in Botswana, Zambia and Zimbabwe through the International Development Research Centre (IDRC) were coupled to hammer mills to produce sorghum flour of acceptable quality. These devices had limited impact for sorghum milling in Zambia probably due to limited publicity given to them.

1.2.6.1.5 Roller milling (size reduction)

In roller milling flour is produced by gradually reducing the particle size of the raw material by a series of grinding pairs of counter rotating rolls with intermediate separation of bran, germ and endosperm by sifters and purifiers (Posner and Hibbs 1997). The mode of grinding is shearing when there is differential in roll speed, and crushing when there is no differential in roll speed (Hoseney 1994). In each pair of rolls where there is differential the rolls are separated by a small gap. Gap setting for break rolls is different from reduction rolls as the two types of rolls perform different roles in milling operations. Break rolls have a corrugated surface while the reduction rolls have a smooth surface. Opening of the kernel can be achieved through using the first, second, third, fourth and sometimes fifth breaks. In the break section the aim is to open the kernel for subsequent stages of milling. In the sizing stage the components of the kernel are sized, the germ and bran are flattened and the endosperm is reduced. Sorghum roller milling using conventional roller milling equipment for wheat produced sorghum flour which was sperky (Hikeezi 1994). Using identical processing conditions, Perten (1984) compared the roller milling performance of sorghum, pearl millet and wheat and observed that sorghum and pearl millet milling produced grain products of poor quality and milling performance of sorghum and pearlmillet compared to wheat was lower and the resulting flours were coarse.

1.2.6.2 Porridge making

Porridges in many parts of Africa fall into two major categories; thin and thick (Gomez et al 1997). In the laboratory, procedures for quality evaluation of sorghum and millet (Gomez et al 1997) describe how porridges are evaluated. Elsewhere laboratory procedures and small scale tests are used to develop sorghum cultivars with improved yields, agronomic properties and acceptable porridge making quality.

1.2.6.2.1 Thin porridge

Thin porridges in many parts of Africa are prepared from wet milled pastes or dry milled flour using dehulled or non-dehulled grains (Obilana 1982). Grains may or may not be roasted and may be bleached in tamarind. The thin porridge may be fermented or non-fermented and it is usually eaten as a breakfast food by lactating mothers and young children (Obilana 1982). The texture of thin porridges varies according to flour particle size. The porridge could be smooth or coarse, or can be made of a combination of smooth or coarse particles. Flavour may vary depending on whether the porridge is fermented or non-

fermented. Also flavour variation may be as a result of roasting or germinating the grain before making the porridge. Additives like sugar, salt, fresh milk or sour milk also affect the flavour of the porridge. The colour of the porridge varies according to the colour of the flour used. The colour of the resulting porridge could be white, red, pink or dark brown.

1.2.6.2.2 Thick porridge

This type of porridge is known by many different names in Southern and East Africa, including *nshima* (Zambia), *nsima*, (Malawi, Zambia and Zimbabwe), *buhobe* (Zambia), *bogobe* (Botswana), ubwali (Zambia), *sadza* (Zimbabwe), *pap* (South Africa) *mafo* or *sof* in (Somalia), and *ugali* (East Africa). Stiff porridge is made in a similar way as soft porridge except more flour or sorghum meal is used. Sorghum porridge is affected by both inherent genetic properties and growth, harvest, storage and processing conditions. The combination of moulds, head bugs, and high humidity when the grain is maturing affect sorghum quality for porridge. Factors affecting sorghum porridge quality include amylose content, the interaction between the protein and starch inside the endosperm cells (Bello, Rooney and Waniska 1990).

1.2.6.2.3 Porridge quality evaluation

Acceptable quality characteristics of porridge as described by Aboubacar et al (1999), are keeping quality, texture, colour and taste. Keeping quality of porridge is important because in some parts of Africa the porridge is stored overnight, reheated and consumed the following day. Some varieties of sorghum produce fresh porridge with acceptable eating quality but poor keeping quality. In terms of porridge texture, generally sorghums with intermediate to soft endosperm texture produce porridge with sticky, less firm texture that deteriorates rapidly during storage. In some countries a finely milled flour is used to prepare the thick porridge, in other countries coarsely milled flour or a combination of coarse and fine flour is preferred. Fermented or non-fermented flour may be used to make the thick porridge (Da, Akingbala, Rooney and Miller 1982; Scheuring et al 1982).

Several researchers have studied the characteristics of porridge using instruments and sensory panels. Cagampang, Griffith and Kirleis (1982) in their study found that stickiness of sorghum porridge had a negative correlation with grain hardness. Firmness measurement studies with the Precision Penetrometer (Da et al 1982; Bello, Waniska, Gomez and Rooney, 1995; Aboubacar et al 1999)) have also shown that hard grains produced firmer porridges.

During sorghum porridge evaluation using instruments, Aboubacar et al (1999) reported that the electrometer test (readings ranged from 6.1 to 10.6 mm) and Instron slope measurement were the most reliable methods for predicting consumer response. Aboubacar et al (1999) evaluated sorghum porridge using a taste panel and their results indicate that the most important sorghum porridge sensory attributes that determined consumer acceptance were texture, followed by taste, and aroma. Aboubacar et al (1999) also reported that before cooking the porridge for sensory evaluation, most of the sorghum (the type of sorghums used in the tests) flours appeared light before cooking but on cooking their brightness (L) decreased. They however still reported that the brown and dark appearing porridges were rejected by the panelists. Aboubacar et al (1999) were however able to demonstrate that consumer acceptance of sorghum porridge colour can be predicted by colorimetric methods which use instruments like Hunter lab using the value (L) for lightness.

This is in contrast to the study carried out by Jagwer (1999) in Botswana where he reported that panelists in sensory evaluation tests preferred white maize porridge to dark appearing sorghum porridge made using white sorghum. The ICRISAT/SADC food quality manual (Gomez et al 1997) gives guidelines on how to evaluate thick porridge.

1.2.7 Concluding remarks

Sorghum kernel structure and processing influence end-use quality. In order to utilise sorghum effectively its structure has to be understood in terms of its processing quality, yield and end-use quality. Sorghum grain end-use characterization and evaluation involves a comprehensive analysis of the grain, and the resulting products in order to satisfy end-use requirement. However, end-use quality is not always targeted when initiating sorghum improvement programmes. Methods and procedures for predicting end-use quality in sorghum are not as developed as those of wheat and maize. Sorghum foods that have quality standards are few and where these standards exist, they do not address end-use quality which is an important aspect in sorghum improvement and utilization. The fact that sorghum processing technology is still evolving affects the utilization of sorghum to the full because only a limited number of products can be made using the available limited equipment, majority of which is borrowed from processing other cereals like wheat, rice barley and maize.

Efficient selection of cultivars on the basis of end-use quality relies on the use of instrumental measurements that describe and quantify the attributes of the product in a way that can allow cultivar and product improvements and allow collaboration between sorghum breeders and food scientists. The screening of cultivars for different food applications for the SADC region has stalled since the demise of the Food Science Section of ICRISAT at Matopos, Bulawayo, Zimbabwe.

There is still a knowledge gap as to why sorghum food products like porridge made using white sorghum are dark and hence impact acceptability negatively (Jagwer 1998). In contrast, information on the cause of discolouration in wheat-based foods like noodles, flat breads and chapattis is well documented. The enzyme polyphenol oxidase has been associated with the darkening of the aforementioned wheat foods. In sorghum, the darkening of some sorghum-based foods has been acknowledged. The darkening in porridge made from white sorghum has not been associated with the polyphenol oxidase enzyme. With regard to grain colour measurement, the colour of sorghum kernels has not been studied fully especially in terms of the phenolic compounds associated with colour in all types of sorghums; white, red, brown and black.

This study will be beneficial to farmers, breeders, agricultural extension workers, traders and processors. This is because the study will provide more information on the need to characterise sorghum grain in order to target appropriate and intended use. The information from this study will also help in refocusing research on sorghum cultivars with acceptable end-use colour as a way of making sorghum-based foods more acceptable. The food industry will benefit from the methodologies being proposed as they will also use them in their product development and value addition activities. The development of research methodologies which appeal to rural people will make it possible for them to improve on the quality of their sorghum products through application of sorghum evaluation methods that are easily understood.

1.2.8 Hypotheses and Objectives

Hypothesis 1

The determination of sorghum kernel hardness using percent water absorption will separate sorghum grains into floury and corneous endosperm types in the same way as endosperm texture determination and abrasive hardness index (AHI) using the tangential abrasive dehulling device (TADD). It will therefore be possible to use percent water absorption as a

method to determine sorghum kernel hardness. This is because the extent to which the sorghum kernels will absorb water is related to kernel hardness (Lawton and Fabion 1989; Chandrashekar and Mazhar 1999; Murty et al 1982b; Buffo et al 1999). Hard sorghum kernels contain predominantly corneous endosperm which, (compared to floury endosperm) have more γ-kafirins which participate in disulphide crosslinking with β-kafirins conferring hardness to the endosperm (Mazhar and Chandrashekar1993; Shull et al 1991). The protein matrix in corneous endosperm forms a continuous cement-like structure which holds starch granules and protein bodies together in a tight arrangement devoid of spaces in between (Hoseney1994) making it more difficult for water to penetrate. The protein matrix in the floury endosperm is discontinuous and has voids in between starch granules and protein bodies (Hoseney 1994) making it easy for water to penetrate the soft kernels (Jambunathan et al 1992; Buffo et al 1998). Association between proteins, cell wall polymers and phenolic acids is stronger in corneous endosperm than in floury endosperm and therefore allows less water to penetrate hard sorghum (Verbruggen 1996; Huisman et al 2000; Veerabadhiran and Deepalakshimi 2003).

Hypothesis 2

Porridges made from white tan-plant sorghum flours will be darker than porridges made from white maize flours. The darkening of white tan-plant sorghum porridge is due to the activity of polyphenol oxidase (PPO) which catalyses the reaction between oxygen and phenolic substrates to produce quinones which react further in non-enzymic reactions, such as auto-oxidation and polymerisation with amino acid groups of cellular protein to produce dark melanoidin pigments (Steffens et al 1994; Kruger et al 1994; Anderson and Morris 2001; Feillet et al 2000). White tan-plant sorghums will have higher PPO activity than white maize.

Hypothesis 3

Treatment of sorghum kernels with sodium hydroxide (NaOH) will enhance colour differences between white tan-plant sorghums and coloured (brown and red) sorghums and produce extracts whose absorbance in UV-visible spectrophotometry can be related to the colour of the kernels determined using Tristimulus colorimetry. NaOH treatment of sorghum kernels causes colour pigments to leach out (Matus Cadiz et al 2008; Blakely et al 1979). NaOH extracts from the coloured sorghums (which contain more pigments) will have higher absorbance in UV-visible spectrophotometry and HPLC analysis of NaOH extracts from coloured sorghum than extracts from white tan-plant sorghums.

Objectives

1. To determine sorghum kernel hardness using percent water absorption in comparison with endosperm texture determination and AHI using the TADD.

2. To determine the PPO activity of white tan-plant sorghums and its influence on colour of porridges made from these sorghums.

3. To determine the relationship between the absorbance in UV-visible spectrophotometry and HPLC analysis of NaOH extracts from sorghum kernels and the colour of the kernels as determined using Tristimulus colorimetry.

4. To characterise the sorghum phenolic compounds and pigments extracted using sodium hydroxide.

2. RESEARCH

2.1 Percent water absorption: A simple method for estimation of sorghum grain hardness

2.1.1 Abstract

Sorghum grain endosperm texture (kernel hardness) is defined in terms of the proportion of corneous endosperm to the floury endosperm, which is genetically controlled and also affected by environmental conditions. The use of current kernel hardness methods is limited among end-users such as rural farmers due to various reasons including lack of accessibility to expensive equipment. The objective of this study was to evaluate sorghum kernel hardness using the relatively simple percent water absorption method in comparison with the endosperm texture and abrasive hardness index methods. Sixteen Zambian sorghum cultivars grown during the 2008 and 2009 seasons were characterised for physico-chemical parameters. The sorghums were of predominantly medium size, ranged in colour from white to brown and red with or without a pigmented testa. Test weight (73 to 82 kg/hL), thousand kernel weight (23.44 to 42.08 g) and proximate composition were determined. Endosperm texture of the sorghums determined by visual endosperm examination ranged from soft to hard. Abrasive hardness index values ranged from 6.28 to 19.64 and percentage water absorption ranged from 8.43 to 26.56%. Percent water absorption was significantly and positively correlated ($r = 0.85$, $p < 0.001$) with endosperm texture and negatively correlated with abrasive hardness index ($r = -0.89$, $p < 0.01$). In summary, percent water absorption could separate soft grains from hard grains just as well as endosperm texture and abrasive hardness index. The simplicity of the percent water absorption method makes it potentially usable by farmers and traders in remote areas where it can contribute to meaninful end-use quality assessment.

2.1.2 Introduction

Sorghum grain endosperm texture (hardness) is defined in terms of the proportion of corneous endosperm also variously called horny, glassy, vitreous, or steely, relative to the floury endosperm, also known as mealy, chalky, or opaque (Rooney and Miller 1982). The sorghum grain has both corneous or translucent and opaque endosperm (Hoseney 1994). The relative proportions of the corneous and floury endosperm vary among sorghum types and determine endosperm texture. This variation is influenced by genetic and environmental factors (Serna-Saldivar and Rooney 1995). The translucent appearance of the corneous endosperm is due to the absence of air spaces in the cells (Serna-Saldivar and Rooney 1995). The opaque and floury endosperm has a discontinuous protein phase, air voids and loosely packed round starch granules (Hoseney 1994). The chalky or opaque appearance is due to the presence of the air voids which diffract incoming light (Hoseney 1994).

Endosperm texture has been shown to be the main component that influences milling performance (Kirleis and Crosby 1982). However the endosperm texture or grain hardness parameter is difficult to measure precisely by any one method. This is because several factors contribute to overall grain hardness, including grain size, and shape, the thickness of the pericarp, the adherence of the pericarp to the endosperm and starch-protein interactions (Greenwell and Schofield 1986).

In maize and sorghum, kernel hardness is a key quality criterion in dry milling (Taylor and Dewar 2001; Taylor and Duodu 2009; Chiremba et al 2011). Corneous grains which are normally hard tend to have higher abrasive decortication and milling yields (Mwasaru et al 1988; Lawton and Faubion 1989). This is because grains with a high proportion of the corneous endosperm tend to be more resistant to breakage during decortication and milling. They tend to yield proportionally cleaner endosperm of large particle size during milling operations than soft grains as the pericarp is more readily separated from intact starchy endosperm (Norris 1971; Rooney and Miller 1982).

Endosperm hardness is also important in storage and processing of foods. In storage, hard grains are less susceptible to insect and mould attack (Rooney and Miller 1982; Jambunathan et al 1992). Decorticating behaviour of sorghum and milling influences palatability and cooking quality. The textural quality of cooked sorghum grain determines acceptability to consumers (Cagampang et al 1982). For porridges, kernel hardness, according to Rooney et al

(1986) is the most important and consistent characteristic that affects end-use quality. Akingbala Rooney and Miller (1982) and Bello, Waniska, Gomez and Rooney (1995) have shown that hard grains produced firmer porridges. In brewing coarse grits are preferred for use as cereal adjuncts. Methods for estimating sorghum kernel hardness are limited and are still evolving. The ones that are in current use were originally meant for other grains. Maxon et al (1971) used a Strong Scott laboratory barley pearler to determine sorghum hardness. Kirleis et al (1986) developed a simple technique for sectioning and quantitatively measuring the corneous and floury endosperm areas of the sorghum grain. The Tangential Abrasive Dehulling Device (TADD) which works on the principle of removing successive layers from cereal grains by tangential abrasion is used to measure grain hardness (Oomah et al 1981; Lawton and Faubion 1989; Gomez et al 1997). An abrasive hardness index is derived from TADD data and used as an indication of kernel hardness (Reichert et al 1986). Shepherd (1979) modified an Udy Cylo-Tec grinding mill to develop a method for evaluating the abrasive decorticating milling properties for small samples of sorghum. These laboratory equipment are expensive and not easily available in rural areas. They have also not been used together with other hardness estimation methods such as endosperm texture assessment and percent water absorption to estimate kernel hardness.

Percent water absorption as a method for estimating sorghum kernel hardness, is simple and does not require the use of sophisticated equipment. It is also likely to be easily understood by local people in that they already apply the concept of water absorption by using water in dehulling sorghum and other cereals. Percent water absorption has been used to determine kernel hardness of sorghum by Murty et al (1982a,b), Buffo et al (1998) and Kashiri, Kashaninejad, and Aghajalani (2010). However, a systematic study of percent water absorption as a measure of kernel hardness in comparison with the more established methods has not been carried out.

This was therefore the main objective of this research which involved determination of sorghum kernel hardness using percent water absorption in comparison with the endosperm texture and TADD methods. This will provide some insight into the suitability of percent water absorption for determining sorghum kernel hardness.

ELT1-16 (White, non-tannin)

ELT1-17 (White, non-tannin)

Kuyuma (White, non-tannin)

MMSH1257 (White, non-tannin)

MMSH13440 (White, non-tannin)

MMSH1040 (White non-tannin)

SDS 1958]1-3-2 (White, non-tannin)

SDS 5006* WSV187] 23-2-1 (White, non-tannin)

Sima (White, non-tannin)

ZSV-15 (White, non-tannin)

MMSH375 (Red, tannin)

MMSH413 (Brown, tannin)

MMSH625 (Red, non-tannin)

MMSH740 (Brown, tannin)

Framida*SDS3843] F6-5 (Red, tannin)

Framida*SDS*3843] 16-2-2 (Red tannin)

Figure 2.1.1 Appearance of sixteen Zambian sorghum cultivars used in this research

2.1.3 Materials and methods

2.1.3.1 Grain samples

Sixteen sorghum cultivars (Figure 2.1.1) grown at the Golden Valley Research Station, Chisamba, Zambia in the 2008 and 2009 growing seasons were characterised for grain colour, absence and presence of pigmented testa. Some of the cultivars were open pollinating varieties (OPV) (ZSV-15, Kuyuma and Sima). Others were hybrids (ELT1-17, ELT1-16, MMSH1040, MMSH1340, SDS1958]-3-35, SDS500* WSV-187]23-2-1. MMSH 413, MMSH740, MMSH375, Framida*SDS3845] F-6-5, Framida*SDS3845] 23-2-1 and MMSH625).

2.1.3.2 Sorghum kernel characterization

Kernel size was determined by sieving 100 g samples of clean grain through two test sieves with 4.0 mm and 2.6 openings as described by Gomez et al (2007). Colour of the whole grains was assessed visually (Taylor 2007) and was also measured using a tristimulus colour meter (Minolta Chroma Meter CR-400, Konica Minolta, Sensing, Japan). The presence or absence of a pigmented testa was ascertained using the Bleach Test as described by Taylor (2007). Test weight was assessed as described in the USDA standard for Test weight (2007). Thousand Kernel weight (TKW) was determined by weighing 1000 randomly counted unbroken kernels.

Moisture content was determined by oven drying (AACC Method 44-15A) (AACC 2000). Total ash was determined using AACC Method 08-01 (AACC 2000). Oil content was determined using Soxhlet petroleum ether extraction as described in AACC Method 3025 (AACC 2000). Protein (N x 6.25) was determined by a Kjeldahl nitrogen determination as described in AACC Method 46-39 (AACC 1969).

2.1.3.3 Endosperm texture

Endosperm texture was determined using a subjective method (Taylor 2007; Gomez et al 2007). From each cultivar, 20 mature grains were randomly selected. A small piece of rubbery gum approximately the same size as a sorghum kernel was pressed onto the cutting surface. A sound sorghum grain, germ side up was pushed into the side of the piece of the rubbery gum to hold it in place. The grain was held with forceps and cut into two longitudinally even size halves, so that each half contained an equal portion of the germ. The

process was repeated until 20 grains were cut. One half of each grain was compared against a standard and classified as follows:

Corneous: The endosperm is totally corneous or translucent, or most (>50%) of the endosperm is translucent. This was given a score of 1.

Intermediate: The outer, corneous endosperm is continuous, but comprises less than 50 % of the total endosperm; the inner part of the endosperm appears floury or chalky. This was given a score of 2.

Floury: The endosperm is totally floury or chalky or the outer, corneous endosperm is very narrow and incomplete. This was given a score of 3.

2.1.3.4 Abrasive hardness index

The Tangential Abrasive Dehulling Device (TADD), a mechanical multi-sample abrasion device was used to determine the abrasive hardness index (Reichert et al 1982). Twenty grams of clean sorghum was put into each TADD cup (20 x 8) and decorticated for 1, 2 and 4 min. The TADD used to decorticate the grains was fitted with a 60 grit sand paper (Norton R284 metalite, Saint-Goban Abrasives, Isando, South Africa). Percent kernel removal at each of the 3 decorticating times, based on the weight of the decorticated sample recovered by the vacuum collector was calculated according to Reichert et al (1982). The Abrasive Hardness Index was calculated as the time in seconds required to remove 1% by weight of the kernel fines.

2.1.3.5 Percent water absorption

Percent water absorption was determined according to Gomez et al (1997) with modifications. Twenty grams of whole clean unbroken sorghum grain was weighed into a 100 ml beaker and enough deionised water (at 25^{o}C) to cover the grains (approximately 25 ml) was added. The grains were left to stand for 60 min after which excess water was decanted off. The grains were removed from the beakers and placed on tissue paper. Excess water was blotted using Whatman No 24 filter paper (Whatman GE Healthcare, Amesham Place, UK). The grains were left to dry at room temperature (22^{o}C) for 5 min. After drying each sample was placed in a dry weighing boat and weighed. Percent water absorption was calculated using the following equation:

% WA = [(B-A) ÷ A] x 100

Where WA = Water Absorption; B = the final weight of the grain; A = the initial weight of the grain

2.1.3.6 Statistical analysis

All the experiments were done in duplicate. Data were analysed by multifactor analysis of variance. Fisher's least significance difference (LSD) test used to compare means.

2.1.4 Results and discussion

2.1.4.1 Physical characterization

The white sorghums had higher L* values ranging from 56.56 to 68.14 than the coloured sorghums with L* values ranging from 44.14 to 49.07) (Table 2.1.1). The a* values for all the sorghum cultivars ranged from 2.84 to 8.08 while the b* values ranged from 1.06 to 13.37. A pigmented testa was absent in the ten white sorghum cultivars and in one red non-tannin cultivar, but present in both brown and three red sorghums cultivars. The presence of a pigmented testa is an indication of the presence of tannins (Price and Butler1977; Waniska et al 1992).

Kernel test weight ranged from 73.08 kg/hL - 82.06 kg/hL. Kuyuma and Sima sorghum cultivars had test weight ≥ 80 kg/hL. All the other sorghum cultivars had test weight < 80 kg/hL (Table 2.1.1). Kernel shape, kernel uniformity, size and shape are important factors affecting test weight. Test weight is also a measure of flour yield (Cagampang and Kerleis 1984.Reichert et al 1987). High test weight indicates well matured seed (Helm and Spide 1990). Low test weight may be due to weathering, sprouting disease and shrivelled kernels in a given batch **(**Helm and Spide 1990). Thousand kernel weight (TKW) ranged from 23.08 - 42.08 (Table 2.1.1). The Sima sorghum cultivar was the only one with TKW > 40 g. SDS5006*WSV87]23-2-1, Framida*SDS[3845]F-6-5 and Framida*SDS[3845]23-2-1 had TKW within the 30-40 g range. The rest of the sorghums had lower TKW within the 20-30 g range. Thousand kernel weight is a function of kernel size and kernel density with dense kernels having a higher ratio of endosperm components than small ones. The thousand kernel weight influences crop yield milling and malting quality (Gomez et al 1997).

Sorghum kernel size was grouped into large (>4.00 mm), medium (4.0-2.6 mm) and small (< 2.6 mm). For all the sorghums, medium size kernels constituted the highest proportion

ranging from 69.26% - 99.56% (Table 2.1.1). This agrees with the grain quality evaluation results of Gomez et al (1997) which categorised the sorghum kernels for the SADC region as medium. The proportion of large size sorghums ranged from 0.00 - 32.54% while small kernels ranged from 0.00 % - 5.65 %. All the cultivars had uniform kernel size. Uniform grains are easy to process as they minimise sorting. The cultivar with the highest number of small kernels was ELT1-17 (5.5%). Small kernel size could be a genetic trait but it can also be due to environmental factors and management. Small grains affect milling yield. This is because they tend to be blown away during cleaning procedures and during pericarp (bran) removal (Gomez et al 1997). From an agronomical point of view, small seeds produce weak seedling and affect crop yield if they form a large proportion of the crop (Helm and Spide1990).

Table 2.1.1 Physical characteristics of 16 Zambian sorghum cultivars from 2008 and 2009 seasons

Cultivar	Season	Colour	Kernel colour			Testa	TW (kg/hL)	TKW (g)	Kernel size (%)		
			L*	a*	b*				Large (>4.0 mm)	Medium (4.0-2.6 mm)	Small (< 2.6 mm)
ELT1-16	2008	White	63.60 i (0.09)	3.78 f (0.04)	13.14 n (0.21)	Absent	78.07 l (0.03)	29.13 l (0.01)	2.25 k (0.03)	96.48 k (0.04)	1.52 k (0.02)
	2009		65.08 jk (0.08)	3.77 f (0.05)	13.09 n (0.05)		78.08 l (0.02)	29.34 n (0.03)	2.25 k (0.01)	97.47 o (0.02)	1.76 m (0.01)
ELT1-17	2008	White	59.60 g (0.21)	4.44 k (0.05)	9.37 g (0.01)	Absent	75.83 e (0.08)	23.08 a (0.01)	0.27 b (0.01)	95.92 I (0.01)	5.65 u(0.01)
	2009		59.61 g (0.08)	4.65 l (0.07)	9.36 g (0.01)		74.93 c (0.09)	24.08 d (0.02)	0.56 d (0.01)	94.07 g (0.02)	5.35 t(0.00)
Kuyuma	2008	White	66.53 l (0.06)	3.67 e (0.01)	10.16 h (0.01)	Absent	80.08 q (0.02)	27.17 I (0.01)	1.86 I (0.01)	98.45 u (0.07)	0.26 d (0.01)
	2009		66.68 l (0.01)	3.66 e (0.01)	10.13 h (0.01)		80.08 q (0.04)	27.16 I (0.01)	1.66 g (0.01)	97.89 r (0.08)	0.38 e (0.04)

MMSH1257	2008	White	65.44 jk (0.01)	3.98 h (0.01)	12.85 l (0.01)	Absent	77.34 k (0.03)	29.17 m (0.01)	0.57 d (0.01)	97.70 pq (0.00)	1.87 n (0.02)
	2009		65.66 k (0.02)	3.87 g (0.01)	12.86 l (0.01)		77.14 j (0.02)	29.79 o(0.02)	0.47 c (0.01)	98.07 s (0.01)	1.76 m (0.01)
MMSH1340	2008	White	62.05 h (0.03)	4.04 hi (0.01)	11.70 j (0.01)	Absent	75.84 ef (0.03)	24.07 d (0.01)	0.00 a (0.00)	98.26 t (0.01)	1.75 l (0.00)
	2009		62.06 h (0.01)	4.08 ij (0.01)	11.69 j (0.01)		74.86 c (0.07)	24.16 e(0.00)	0.00 a (0.00)	98.57 v (0.03)	1.46 j (0.01)
MMSH1040	2008	White	59.23 g (0.01)	4.11 j (0.00)	10.28 i (0.01)	Absent	76.82 h (0.07)	28.04 j (0.03)	0.63 n (0.01)	97.10 l (0.07)	2.26 o (0.01)
	2009		58.95 g (0.06)	4.13 j (0.00)	10.27 i (0.01)		75.92 f (0.07)	29.03 k (0.02)	0.66 e (0.01)	97.61 p (0.08)	2.37 p (0.03)
SDS 1958]1-3-2	2008	White	56.56 e (0.15)	3.20 c (0.07)	8.55 f (0.02)	Absent	78.06 l (0.05)	29.34 n (0.01)	1.96 j (0.01)	97.15 lm (0.07)	1.08 h (0.02)
	2009		57.11 f (0.46)	3.37 d (0.01)	8.54 f (0.02)		77.05 i (0.04)	29.34 n (0.03)	1.77 h (0.02)	96.47k (0.09)	1.46 j(0.01)
SDS5006*WSV87]23-2-1	2008	White	66.74 l (0.05)	3.63 e (0.06)	13.37 o (0.01)	Absent	79.00 no (0.00)	31.02 p (0.01)	3.36 m (0.02)	95.06 h (0.04)	1.66 l (0.01)

	2009		66.75 l (0.01)	3.64 e (0.08)	13.35 o (0.01)		78.95 n (0.02)	31.08 q (0.02)	2.86 l (0.03)	96.15 j (0.07)	1.85 n(0.02)
Sima	2008	White	68.13 m (0.01)	3.61 e (0.01)	13.06 mn (0.01)	Absent	81.07 r (0.02)	42.04 u (0.01)	25.89 r (0.02)	74.08 c (0.02)	0.14 b(0.02)
	2009		68.14 m (0.01)	3.63 e (0.01)	13.00 n (0.06)		82.06 s (0.03)	42.08 u (0.02)	24.74 q (0.01)	75.74 d (0.03)	0.20 c (0.03)
ZSV-15	2008	White	64.78 j (0.08)	3.86 g (0.01)	12.76 k (0.01)	Absent	77.08 ij (0.02)	27.17 I (0.01)	1.64 g (0.01)	98.07 s (0.03)	1.16 I (0.01)
	2009		64.76 j (0.01)	3.85 g (0.01)	12.75 k (0.01)		76.05 g (0.04)	27.16 I (0.00)	1.57 f (0.01)	98.29 t (0.05)	1.06 h (0.01)
MMSH375	2008	Red	44.14 a (0.01)	2.85 a (0.01)	5.53 d (0.02)	Present	73.08 a (0.02)	24.14 e (0.02)	0.00 a (0.00)	97.36 n (0.05)	2.66 rs (0.01)
	2009		44.16 a (0.01)	2.86 a (0.01)	5.56 d (0.01)		73.08 a (0.01)	26.16 g (0.01)	0.00 a (0.00)	97.43 no (0.04)	2.63 r (0.04)
MMSH413	2008	Brown	47.16 c (0.01)	2.98 b (0.01)	1.06 a (0.02)	Present	75.04 d (0.04)	23.44b (0.02)	0.00 a (0.00)	98.59 vw (0.05)	1.61 l (0.08)
	2009		47.17 c (0.01)	2.99 b (0.01)	1.07a (0.01)		74.06 b (0.04)	23.76 c (0.01)	0.00 a (0.00)	98.67 w (0.04)	1.42 j(0.08)

MMSH625	2008	Red	49.04 d (0.01)	8.08 n (0.01)	1.88 c (0.01)	Absent	74.05b (0.02)	25.28 f (0.01)	0.00 a (0.00)	97.75 q (0.00)	2.69 s (0.02)
	2009		49.07 d (0.01)	8.05 n (0.02)	1.86 c (0.01)		74.05 b (0.04)	26.22 g (0.02)	0.00 a (0.00)	97.22 m (0.02()	2.48 q(0.04)
MMSH740	2008	Brown	47.15 c (0.01)	6.24 m (0.01)	1.18 b (0.01)	Present	75.06 d (0.02)	27.16 I (0.00)	0.00 a (0.00)	99.56 x (0.01)	0.45 f (0.00)
	2009		47.16 c (0.01)	6.24 m (0.01)	1.17 b (0.01)		76.06 g (0.03)	27.15 I (0.01)	0.00 a (0.00)	99.47 x (0.03)	0.56 g (0.01)
Framida*SDS[3845] F-6-5	2008	Red	45.36 b (0.01)	2.84 a (0.01)	5.83 e (0.01)	Present	79.06 op (0.04)	38.79 t (0.06)	32.54 t (0.02)	69.26 a (0.01)	0.00 a (0.00)
	2009		45.35 b (0.01)	2.84 a (0.01)	5.83 e (0.01)		78.07 l (0.03)	38.73 s (0.05)	30.77 s (0.03)	70.55 b (0.03)	0.00 a (0.00)
Framida*SDS[3845]23-2-1	2008	Red	45.14 b (0.02)	2.94 b (0.01)	5.82 e (0.00)	Present	79.14 p (0.02)	32.10 r (0.01)	9.49 p (0.01)	89.63 e (0.04)	1.07 h (0.03)
	2009		45.15 b (0.01)	2.95 b (0.01)	5.81 e (0.01)		78.17m (0.04)	32.06 r (0.01)	9.18 o (0.02)	90.18 f (0.04)	1.08 h (0.02)

Mean	57.16	3.85	8.53	76.98	29.01	5.18	93.88	1.60
Minimum	44.14	2.84	1.06	73.08	23.08	0.0	69.26	0.00
Maximum	68.14	8.08	13.37	82.06	42.08	32.54	99.56	5.65

Values are Means ± SD. Values followed by the same letter in a column are not significantly different at $p \leq 0.05$ as assessed by Fisher's Least Significant Difference Test

TW – Test Weight; TKW – Thousand Kernel Weight; Kernel size (%) represents the percentage of kernels retained on the specified sieve sizes

2.1.4.2 Chemical characterization

As shown in Table 2.1.2, moisture content of the sorghum cultivars ranged from 10.64% - 12.84%. These values are within the range of values (10.12 - 13.20 %) reported by Sehgal, Kawatra, and Singh (2003). Moisture content is important in storage, trade and processing of sorghum grain. The protein content for the cultivars ranged from 11.37% - 14.81 % which is within the range (8.1 - 16.8%) reported in literature (Bach Knudsen and Munck 1983; Rooney et al 1986; Sehgal et al 2003; Kebakile 2008). Protein content is influenced by cultivar and environment, with the environment playing a major role in the variation (House et al 1995).

Oil content ranged from 2.32 - 5.85%.The values for oil content in sorghum are within the range of 1.42 - 6.2 % reported by Bach Knudsen and Munck (1983) and Rooney et al (1986). The differences in oil content among the sorghum cultivars may be attributed to possible differences in germ size as was reported for maize (Hoseney 1994). High fat content in whole sorghum flour can affect its shelf life due to oxidative rancidity of unsaturated fatty acids mainly from the germ during storage. Ash ranged from 1.17% - .85%.These values were within the range of 1.2 - 7.1% reported for sorghum by Bach Knudsen and Munck (1983) and Rooney et al (1986) but did not agree with those reported by Kebakile (2008) (Table 2.1.2) which were much lower. Ash or mineral content in sorghum is largely influenced by the availability of phosphorus in the soil. The differences in ash content could be influenced by lower levels of phosphorus in the soils where the sorghum is grown (FAO 1995; Kebakile 2008). Crude fibre ranged from 1.94 - 3.03%. These values were within the range of 0.4 - 7.3 % reported by Bach Knudsen and Munck (1983) and Rooney et al (1986). Sorghum contains high levels of insoluble fibre with low levels of β-glucans. Most of the crude fibre is present in the pericarp and endosperm cell walls (Sehgal et al 2003).

Table 2.1.2 Proximate composition (g/100 g as is) of Zambian sorghum cultivars from 2008 and 2009 growing seasons

Cultivar	Season	Moisture	Protein (N x 6.25)	Oil	Ash	Crude fibre	Carbohydrates (by difference)
ELT1-16	2008	12.62 i (0.11)	12.39 n (0.31)	4.83 lm (0.01)	1.18 a (0.01)	2.33 e (0.01)	66.68 defg (0.01)
ELT1-16	2009	12.64 j (0.22)	12.37m (0.22)	4.81 j (0.02)	1.17 a (0.01)	2.34 e (0.02)	68.19 hijk (0.02)
ELT1-17	2008	11.04 b (0.34)	12.11 i (0.35)	5.10 p (0.04)	1.49 kl (0.03)	2.69 l (0.03)	67.60 ghij (0.03)
ELT1-17	2009	11.07 b (0.24)	12.09 k (0.42)	5.09 p (0.03)	1.48 jk (0.02)	2.69 l (0.02)	66.51 cdefg (1.53)
Kuyuma	2008	12.04 fg (0.45)	14.39 t (0.53)	3.91 c (0.02)	1.50 l (0.01)	2.76 c (0.04)	65.49 bc (0.08)
Kuyuma	2009	12.05fg (0.33)	14.38 t (0.61)	3.99 d (0.03)	1.49 kl (0.01)	2.76 c (0.04)	66.34 cdef (1.41)
MMSH1257	2008	12.41 i (0.25)	11.44 c (0.54)	4.94 o (0.04)	1.53 m (0.02)	2.56 c (0.03)	67.15 efgh (0.01)
MMSH1257	2009	12.44 j (0.21)	11.46 c (0.46)	4.93 o (0.05)	1.52 m (0.03)	2.57 c (0.02)	67.61 ghij (0.69)
MMSH1340	2008	11.81 e (0.35)	11.39 b (0.22)	4.72 g (0.06)	1.73 q (0.03)	1.95 a (0.02)	68.42 ijlk (0.01)
MMSH1340	2009	11.83e (0.26)	11.37 a (0.32)	4.71 g (0.04)	1.73 q (0.02)	1.94 a (0.03)	69.14 klm 0.00)
MMSH1040	2008	12.01f (0.42)	11.86 g (0.23)	2.32 a (0.03)	1.69 p (0.03)	2.32 fg (0.02)	69.83 m (0.01)
MMSH1040	2009	12.10 gh (0.34)	11.85 g (0.22)	2.32 a (0.02)	1.68 p (0.04)	2.30 c (0.03)	69.76 m (0.00)
SDS 1958] 1-3-2	2008	12.65 j (0.26)	12.68 o (0.31)	4.64 f (0.04)	1.64 o (0.02)	2.52 j (0.03)	65.90 bcd (0.01)
SDS 1958] 1-3-2	2009	12.62 j (0.32)	12.69 o (0.42)	4.63 f (0.05)	1.63 o (0.03)	2.51 j (0.01)	65.94 cd (0.00)
SDS 5006*WSV87]23-2-1	2008	12.18 h (0.37)	12.05 i (0.51)	4.22 e(0.03)	1.33 g (0.03)	2.77 n (0.02)	67.48 fghi (0.01)
SDS 5006*WSV87]23-2-1	2009	12.16 h (0.42)	12.03 h (0.41)	4.23 e (0.02)	1.35 h (0.02)	2.76 n (0.03)	67.49 fghi (0.01)
Sima	2008	12.82 k (0.22)	14.81 u (0.32)	5.79 s (0.04)	1.25 c (0.01)	2.47 j (0.04)	62.89 a (0.01)
Sima	2009	12.84 k (0.36)	14.80 u (0.22)	5.79 s (0.05)	1.24 cd (0.01)	2.46 i (0.03)	62 .89 a (0.01)
ZSV-15	2008	12.62 j (0.32)	13.39 r (0.53)	4.74 h (0.03)	1.61 n (0.03)	2.89 o (0.02)	64.77 b (0.03)

ZSV-15	2009	12.63 j (0.25)	13.38 s (0.63)	4.73 h (0.02)	1.61 n (0.02)	2.91 p (0.03)	64.76 b (0.00)
MMSH375	2008	11.76 e (0.23)	12.39 n (0.25)	5.85 t (0.03)	1.49 kl (0.03)	2.30 c (0.02)	66.24 cde (0.02)
MMSH375	2009	11.77 e (0.35)	12.39 mn (0.32)	5.84 t (0.05)	1.52 m (0.02)	2.31 de (0.03)	66.20 cde (0.01)
MMSH413	2008	12.01 fg (0.25)	11.39 b (0.41)	4.77 i (0.07)	1.28 f (0.04)	3.02 q (0.02)	67.55 ghi (0.02)
MMSH413	2009	12.04 fh (0.32)	11.40 b (0.32)	4.78 i (0.08)	1.27 ef (0.03)	3.03 q (0.03)	67.51 ghi (0.01)
MMSH625	2008	11.41d (0.23)	11.67e (0.43)	4.82 kl (0.06)	1.21 b (0.02)	2.17 b (0.02)	68.74 jklm (0.01)
MMSH625	2009	11.22 c (0.34)	11.66 e (0.42)	4.81 j (0.07)	1.22 b (0.02)	2.17 b (0.01)	68.94 klm (0.25)
MMSH740	2008	12.02 fh (0.13)	11.72 f (0.22)	5.51 q (0.06)	1.26 f (0.03)	2.67 l (0.02)	66.84 defg (0.00)
MMSH740	2009	12.05 gh (0.14)	11.73 f (0.32)	5.54 r (0.03)	1.28 f (0.02)	2.66 l (0.03)	66.76 defg (0.01)
Framida*SDS[3845] F6-5	2008	10.66 a (0.11)	12.08 jk (0.41)	3.85 b (0.04)	1.85 r (0.03)	2.28 c (0.03)	69.31 klm (0.01)
Framida*SDS[3845] F6-5	2009	10.64 a (0.02)	12.07 j (0.32)	3.84 b (0.03)	1.84 r (0.02)	2.27 c (0.04)	69.36 lm (0.02)
Framida*SDS[3845] 23-2-1	2008	11.08 b (0.13)	12.72 p (0.52)	4.86 n (0.02)	1.47 ij (0.01)	2.38 h (0.03)	67.52 m (0.01)
Framida*SDS[3845] 23-2-1	2009	11.05 b (0.12)	12.73 q (0.53)	4.84 m (0.01)	1.46 i (0.02)	2.39 i (0.02)	67.55 m (0.01)
Mean		11.95	12.40	4.68	1.51	2.52	67.14
Minimum		10.64	11.37	2.32	1.17	1.94	62.89
Maximum		12.84	14.81	5.85	1.85	3.03	69.83
Literature values							
Range		10-13	8.1-16.8	1.4-6.2	1.2-7.1	0.4-7.3	55.6-75.2
Mean		12	11.6	3.4	2.2	2.7	71.8

Values are Means ± SD. Values followed by the same letter in a column are not significantly different at $p \leq 0.05$ as assessed by Fisher's Least Significant Difference Test.

Literature values: Knudsen and Munck 1985; Rooney et al 1986; Hamaker et al 1995; Sehgal 2002.

2.1.4.3 Effects of kernel hardness on endosperm texture, abrasive hardness index and percent water absorption

2.1.4.3.1 Endosperm Texture

Results for endosperm texture (Table 2.1.3) show that cultivars Kuyuma, SDS 5006*WSV87]23-2-1, Sima and ZSV-15 were corneous, while MMSH375, MMSH413, MMSH740, Framida*SDS3845] F-6-5 and Framida* SDS3843] 23-2-1 were floury. The rest of the sorghum cultivars had intermediate endosperm texture. Figure 2.1.2 shows three of the sorghum cultivars as examples of samples that were judged as having corneous, intermediate and floury endosperm. As mentioned earlier, in sorghum utilization, endosperm texture affects decorticating and milling yield (Reichert et al 1982). Endosperm texture also has a bearing on storage of grains. Insects more easily attack soft endosperm than hard endosperm (Rooney and Miller 1982; Jambunathan et al 1992). Endosperm texture is also important in brewing and it also affects the cooking and eating quality of sorghum-based foods (Cagampang et al 1982).

Sima (corneous endosperm) | MMSH1257 (intermediate endosperm) | Framida*SDS[3845] 23-2-1 (floury endosperm)

Figure 2.1.2 Three sorghum samples representing corneous, intermediate and floury endosperm textures

2.1.4.3.2 Abrasive hardness index

The corneous sorghum kernels had highest abrasive hardness indices (AHI) ranging from 18.53 to 19.64, followed by the intermediate sorghum kernels with indices ranging from 12.40 to 13.36, and the floury endosperm kernels had hardness indices ranging from 6.28 to 10.50 (Table 2.1.3). These results seem to agree with those of Reichert et al (1982) which showed that sorghum kernels which were hard had higher AHI compared to softer grains with lower AHI.

According to Reichert et al (1982), the AHI is defined as the time in seconds required to remove 1% by weight of the kernel as fines. The sequential removal of the kernel starting from the surface of the kernel will take a longer time for hard grains than soft grains. Soft and floury grains in general have a tendency to disintegrate during decortication (Gomez et al 1997) and have reduced milling yields (Lawton and Faubion 1998). The visual endosperm texture determination method measures texture by subjectively judging how big the corneous endosperm is in relation to the floury one. On the other hand, the TADD is more objective and measures the fines removed in a specified time as a determinant of hardness.

Table 2.1.3 Zambian sorghum grain hardness characteristics for 2008 and 2009 growing seasons

Cultivar	Season	Endosperm texture	Abrasive hardness index (time in sec)	Water absorption (% of grain weight)
ELT1-16	2008	Intermediate	12.62 d (0.35)	18.94 k (0.25)
ELT1-16	2009	Intermediate	12.63 d (0.18)	18.93 k (0.25)
ELT1-17	2008	Intermediate	11.75 e (0.43)	17.67 j (0.32)
ELT1-17	2009	Intermediate	11.75 e (0.45)	17.87 j (0.30)
Kuyuma	2008	Corneous	19.28 hi (0.42)	8.50 ab (0.32)
Kuyuma	2009	Corneous	19.22 hij (0.30)	8.66 abc (0.33)
MMSH1257	2008	Intermediate	12.27 ef (0.30)	16.39 i (0.42)
MMSH1257	2009	Intermediate	12.83 fg (0.26)	16.41 i (0.38)
MMSH1340	2008	Intermediate	13.35 g (0.47)	15.40 hi (0.46)
MMSH1340	2009	Intermediate	13.32g(0.52)	15.79 hi (0.30)
MMSH1040	2008	Intermediate	13.32 g (0.33)	15.37 h (0.32)

MMSH1040	2009	Intermediate	13.36 g (0.43)	15.15 gh (0.16)
SDS 1958] 1-3-2	2008	Intermediate	12.64 fg (0.50)	12.64 e (0.31)
SDS 1958] 1-3-2	2009	Intermediate	12.49 f (0.47)	12.64 e (0.31)
SDS 5006*WSV87]23-2-1	2008	Corneous	18.57 hi (0.45)	9.35 cd (0.42)
SDS 5006*WSV87]23-2-1	2009	Corneous	18.53 h (0.47)	9.72 d (0.39)
Sima	2008	Corneous	19.51 j (0.50)	8.43 a (0.35)
Sima	2009	Corneous	19.64 j (0.40)	8.45 a (0.28)
ZSV-15	2008	Corneous	18.53 h (0.32)	9.55 d (0.42)
ZSV-15	2009	Corneous	18.64 hi (0.30)	9.23b cd (0.32)
MMSH375	2008	Floury	9.25 c (0.27)	19.45 k (0.47)
MMSH375	2009	Floury	9.23 c (0.30)	19.55 k (0.42)
MMSH413	2008	Floury	10.50 d (0.27)	19.63 k (0.25)
MMSH413	2009	Floury	10.50 d (0.28)	19.63 k (0.25)
MMSH625	2008	Floury	10.40 d (0.27)	14.37 f (0.44)
MMSH625	2009	Floury	10.46 d (0.28)	14.52 fg (0.46)

MMSH740	2008	Floury	8.45 b (0.14)	14.52 fg (0.23)
MMSH740	2009	Floury	8.48 b (0.26)	14.62 fg (0.21)
Framida*SDS[3845] F6-5	2008	Floury	6.28 a (0.25)	25.55 l (0.33)
Framida*SDS[3845] F6-5	2009	Floury	6.48 a(0.25)	25.76 lm (0.31)
Framida*SDS[3845] 23-2-1	2008	Floury	6.66 a (0.13)	26.44 mn (0.35)
Framida*SDS[3845] 23-2-1	2009	Floury	6.94 a (0.26)	26.56 n (0.37)
Mean			12.62	16.56
Minimum			6.28	8.43
Maximum			19.64	26.56

Values are Means ± SD. Values followed by the same letter in a column are not significantly different at $p \leq 0.05$ as assessed by Fisher's Least Significant Difference Test

2.1.4.3.3 Percent water absorption as a determinant of kernel hardness

Table 2.1.3 shows that the floury endosperm of sorghums had percent water absorption ranging from 14.52 - 26.56 %, while sorghums with intermediate endosperm had percent water absorption ranging from 12.64 - 18.94 % and for corneous sorghums, water absorption ranged from 8.43 - 9.72%. These results show that the sorghums with highest percent water absorption were mostly the floury types, while the corneous types had lowest percent water absorption.

Table 2.1.4 Significant correlations between kernel hardness estimation methods

Variable	Endosperm texture	Abrasive hardness Index	Percent water absorption
Endosperm Texture	1		
Abrasive Hardness Index	-0.94***	1	
Percent water absorption	0.85**	-0.89***	1

Level of statistical significance at p <0.01 (**), p <0.001 (***).

Table 2.1.4 shows that there were significant correlations between the three kernel hardness estimation methods, endosperm texture, abrasive hardness index and percent water absorption. Endosperm texture showed a significantly negative correlation ($r = -0.94$, $p < 0.001$) with abrasive hardness index and a significantly positive correlation ($r = 0.85$, $p < 0.01$) with percent water absorption. AHI significantly negatively correlated ($r = -0.89$, $p < 0.001$) with percent water absorption. This means that sorghum cultivars with higher endosperm texture score (more floury and softer) generally had lower AHI and absorbed more water and corneous endosperm sorghums with lower endosperm texture score had higher AHI and absorbed less water.

According to Hoseney (1994) and Buffo et al (1998), floury endosperm has voids in between the loosely-packed starch granules that may allow for easier penetration of water. Murty et al 1982 also reported differences in water uptake based on endosperm texture. In agreement with Murty et al (1982) and Buffo et al (1998), differences in water uptake by sorghum kernels can be related to differences in endosperm texture.

2.1.4.4.1 Effects of cultivar and season with their interaction on Abrasive hardness index and percent water absorption

Table 2.1.5 shows that cultivar highly significantly ($p < 0.001$) influenced both abrasive hardness index and percent water absorption in sorghum kernels. Genetic variations in different sorghum cultivars are reflected in terms of different phenotypic traits (Rooney and Miller 1982) and this is shown in the observed effect of cultivar on abrasive hardness index and percent water absorption. Season and season and cultivar interaction also had a significant influence ($p < 0.05$) on abrasive hardness index and percent water absorption. Their influences were however, not as strong as the cultivar influence on abrasive hardness index and percent water absorption. According to Rooney and Miller (1982) the growing environment affects the expression of vitreousness in sorghum kernels.

Table 2.1.5 Analysis of variance for Abrasive Hardness Index and Water absorption (%) for 16 Zambian sorghum cultivars over 2008 and 2009 growing seasons

Cultivar	DF	SS	MS	F	P
Abrasive Hardness Index	15	885.95	59.06	91.34	<0.001
Water absorption (%)	15	1076.14	71.74	162.47	<0.001
Season					
Abrasive Hardness Index	1	0.02	14.79	0.00	<0.05
Water absorption (%)	1	0.01	17.69	0.00	<0.05
Season and cultivar interaction					
Abrasive Hardness Index	15	1.03	0.07	1.45	<0.05
Water absorption (%)	15	53.3	0.20	1.47	<0.05

2.1.5 Conclusions

This study shows that percent water absorption correlates significantly with endosperm texture determination and AHI which are two well-accepted methods of estimating sorghum grain hardness. Endosperm texture determination is simple and relatively inexpensive but it is a subjective method whose effectiveness depends on the analyst viewing the endosperm structure. AHI is a relatively expensive method which cannot be used by stakeholders such as farmers in rural areas who do not have access to the TADD. The percentage water absorption method is simple, does not require the use of sophisticated equipment and therefore can be readily used by farmers and traders in rural areas as a method for estimating kernel hardness for trade or before processing for food or brewing. Increased use of the percentage water absorption method has the potential to improve utilisation of sorghum for intended use based on the differences in kernel hardness and also to improve trade and extension services particularly in rural areas.

2.2 Relationship between polyphenol oxidase activity and sorghum porridge colour

2.2.1 Abstract

The relatively dark colour of food products from white tan-plant (food-grade) sorghums can compromise their acceptability. The relationship between white tan-plant sorghum polyphenol oxidase activity (PPO) and porridge colour, was investigated. Sorghums (including 28 white tan-plant samples which were primarily lines grown in Zambia over two seasons), wheat and white maize were studied. Sorghum was intermediate in PPO between wheat and maize. White tan-plant sorghum and white maize whole grain flours were similar in colour. However, the transition from white tan-plant sorghum flour to porridge caused a much larger reduction in mean L* value (27.9) than that with white maize (16.9). Cultivar effects on PPO activity, flour and porridge L* values and season effects on PPO activity and flour L* values were highly significant ($p < 0.001$) for white tan-plant sorghums. The correlation between all white tan-plant sorghum PPO activity and porridge L* values was significantly negative ($r = -0.657$, $p < 0.01$), while the correlation between PPO activity and flour L*values was much less significant ($r = -0.489$, $p < 0.05$,). The correlation between Zambian white tan-plant sorghum PPO activity and porridge L* values was highly significantly negative ($r = -0.710$, $p < 0.001$) while the correlation between PPO activity and flour L* values was less significant ($r = -0.523$, $p < 0.05$). Thus, it appears that PPO is responsible for the dark colour of porridge made from white tan-plant sorghum. Breeding can potentially be used to reduce PPO activity in white tan-plant sorghum lines, leading to improved aesthetic appeal of food products made from them.

2.2.2 Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal in sub-Saharan Africa, India and South America. In terms of production, it is the fifth most important cereal crop after wheat, rice, maize and barley (FAO 2011) In Africa, south of the Sahara, it is the second major crop after maize and is important in semi-arid regions of the world mainly because of its agronomical advantages such as drought–tolerance (Belton and Taylor 2004). Sorghum is an important supplier of carbohydrates and proteins in the diets of rural communities in sub-Saharan Africa (Dendy 1995; Rohrbach and Obilana 2003) and therefore plays a major role in contributing to nutrition and food security.

White tan-plant sorghums, also referred to as food-grade sorghum (Pontieri et al 2010) have been developed through plant breeding in order to produce sorghums with inherently improved food quality attributes, such as colour, taste and keeping quality (Scheuring et al 1982; Murty and Subramanian 1982; Miller 1986a,b; Rooney and Waniska 2000; Peterson 2011). The white tan-plant sorghums have a white pericarp, tan-plant colour and straw or tan coloured glumes (Peterson 2011). Their endosperm texture ranges from hard to medium (Rooney and Waniska 2000) as reported earlier in research Chapter 2.1 (Table 2.1.3). These sorghums are aimed at producing flour and other products that have light colour and bland flavour.

In East and Southern Africa, white tan-plant sorghums are used in the preparation of the local staple, thick porridge known by different names such a *nshima* (Zambia), *nsima* in (Zambia and Malawi), *ubwali* in (Zambia) *bohobe* in Botswana, *sadza* in Zimbabwe, *mafo* or *sof* in (Somalia), *ugali* in East Africa (Mukuru et al 1982) and *pap* in South Africa. However, utilization of white tan-plant sorghums as a food can be adversely affected by the unattractive colour of some processed foods from them. Jagwer (1998), in sensory evaluation studies in Botswana reported the low acceptability of darker coloured sorghum porridge made using white tan-plant sorghum compared to porridge made from white maize. Suhendro et al (2000) reported darker noodles made from white tan-plant sorghum than those made from white rice. Iruegas et al (1982) and Futrell et al (1982) also reported darker tortillas made from white tan-plant sorghum when compared to white maize tortillas.

In wheat, the role of polyphenol oxidase (PPO) activity in the darkening of wheat-based products has been established (Baik et al 1994; Kruger et al 1994; Anderson and Morris

2001; Bettege 2004). PPO activity has been found to be associated with the discolouration of noodles, chapattis, Middle Eastern flatbreads (Kruger et al 1994; Baik et al 1994; Bhattacharya et al 1999; Anderson and Morris 2001; Bettege 2004) and wheat products (Kobrehel et al 1974; Feillet et al 2000). Compared to sorghum, research on the role of PPO activity and quality loss in wheat is advanced (Demeke et al 2001).

In contrast, research on PPO activity and its role in the discolouration of foods from white tan-plant sorghums has been limited. Dicko et al (2002a) compared phenolic content, PPO and peroxidase (PO) activities of Burkina Faso sorghums and generally described PPO and PO enzymes as determinants of sorghum end-use quality due to their role in oxidation mechanisms in foods. However, specific effects were not mentioned. Polyphenol oxidase is a copper containing enzyme also known as metallo-protein (Seleniheimo 2008). Polyphenol oxidase catalyses the oxygen dependant hydroxylation of monophenols (monophenolase activity; E.C. 1.14.18.1) and their subsequent oxidation to o-quinones (Dicko et al 2002a; Jukanti et al 2003) and the oxidation of *o*-diphenols to *o*-quinones (diphenolase activity; E.C. 1.10.3.2) (Steffens et al 1994). The resulting quinones react with amines or thiol groups or self polymerize to form complex coloured polymerisation products called melanins ((Howard 1948). The polymerization reactions lead to the discoloration of many food products where PPO activity has been implicated (Feillet et al 2000).

Dicko et al. (2002a) investigated sorghum characteristics suitable for various products and concluded that white grain with low PPO activity (among other characteristics) was best for *tô* (gel-like porridge), couscous, industrial brewing and infant porridges. However, the relationships between sorghum grain oxidative enzymic activities and food product colour were not investigated. In view of the fact that sorghum is gaining in importance in commercial food manufacture such as gluten-free flour applications (Rooney and Awika 2002; Awika and Rooney 2004; Pontieri et al 2010) the objective of this study was to investigate the possible relationship between the relatively dark colour of products from white tan-plant sorghum types and grain PPO activity, using whole grain porridge as a simple food model.

2.2.3 Materials and methods

2.2.3.1 Grain samples

Sixteen Zambian sorghum cultivars grown in a controlled field trial at the Golden Valley Research Station, Chisamba, Zambia in two growing seasons, 2008 and 2009 were characterised for grain colour. Ten were white tan-plant types: ELT1-16, ELT1-17, Kuyuma, Sima, ZSV-15, SDS 1958-1-3-2, [SDS5006*WSV187]23-2-1 [open pollinating varieties (OPVs)] and MMSH-1040, MMSH-1257, MMSH-1340 (hybrids). ELT1-16 and ELT1-17 share a common parent and Kuyuma, [SDS5006*WSV187]23-2-1 and ZSV-15 have a common background. The other six were pigmented types. Three were red tannin: MMSH-375 (hybrid), [Framida*SDS3843]F6-5 and [Framida*SDS3843]16-2-2 (OPVs). Two were brown tannin: MMSH-413 and MMSH-740 and one was a red non-tannin MMSH-625 (all hybrids).

Eight other white tan-plant sorghum cultivars were studied, which were cultivated in Botswana: BSH1 (hybrid), Larsvyt, Sefofu and Segao (OPVs); Kenya: KAT 369 (OPV); South Africa: PEX 606/202 a hybrid; USA: NK8828, Orbit (hybrids), and in addition, eight South African white maize hybrids: CRM3508, Panthera, PAN6043, PAN6045, PAN62213, PAN63355, Saffier, and PAN6Q321 grown at the Agricultural Research Council, Grain Crops Institute, Potchefstroom, and cultivated South African red wheat varieties from the Southern African Grain Laboratory, South Africa: CRM826, Kariega, PAN3118, PAN3355, Steenbras and one South African commercial red bread wheat sample were also studied.

Upon receipt, all grain samples were stored at 8^{o}C under dry and dark conditions until analysis. Approximately 300 g of each grain type was ground with a laboratory hammer mill (Falling Number 3100, Huddinge, Sweden) to pass through a 500 µm opening screen. The milled whole flour samples (45 g) were vacuum-packaged in individual polythene sample bags and stored at -18^{o}C in the dark until use.

2.2.3.2 PPO analysis

PPO was extracted according to the method of Dicko et al (2002a) with modifications. In brief, the enzyme extracts were prepared by mixing 2 g sorghum flour with 9.6 mL 50 mM Tris-HCl pH 7.3 buffer containing 0.5 M $CaCl_2$ with 2% (w/v) polyvinylpyrrolidone (PVP) at 4^{o}C for 1 hr. Insoluble PVP was added to the enzyme extraction buffer to prevent the interaction of PPO with endogenous polyphenols. The homogenate was centrifuged (14000g,

4°C, 45 min) and the resulting supernatant was used as enzyme extract. A spectrophotometric assay was performed as described by Dicko et al (2002a). L-dihydroxyphenylalanine (L-DOPA) was used as the phenolic substrate to determine the *o*-diphenolase activity. The enzyme extract (0.08 mL) was incubated with 1.2 mL 50 mM sodium acetate buffer pH 5.5, 0.08 mL 50 mM 3- methyl-2-benzothiazolinone hydrazone (MBTH), at 25°C for 5 min. The MBTH was used to trap the *o*-quinones formed in the oxidation of the substrate L-DOPA by the enzyme (Espin et al 1995). N,N'-dimethylformamide (DMF) was added to the assay medium at 2% to dissolve the MBTH-quinone adducts. The reaction was started by addition of 0.16 mL 100 mM L-DOPA in 0.15 mM phosphoric acid. The reaction was monitored at 475 nm for 25 min. PPO activity was expressed in U/mg whole grain flour dry basis, where a Unit is the amount of enzyme producing 1 µmol of MBTH-quinone-adducts per minute from the oxidation of L-DOPA (Espín et al.1995; Espín et al 1997).

2.2.3.3 Flour and porridge colour

Twenty gram flour was weighed into a 90 mm Petri dish and covered with the Petri dish lid. Flour colour measurements were performed using a Konica Minolta Chrome Meter C R 400 (Sensing IN, Japan), using the L* a* b* scale (Oliver, Blakeney and Allen 1992). For porridge preparation, a flour-to-water ratio of 1:5 was used (Gomez et al 1997). Porridge was prepared in two stages in order to maximise enzyme activity. Flour (45 g) was mixed with 225 g water to make a slurry. The slurry was held at 45°C in a beaker in a water bath for 15 min. Thereafter, the slurry was then transferred to an electric hot plate where it was cooked a further 15 min at 90°C. After cooking, the porridge was poured in the 90 mm Petri dishes and left to cool. Lids were placed on the Petri dishes after cooling. Porridge colour was measured as described for flour

2.2.3.4 Statistical analysis

PPO activity data were analyzed by analysis of variance using Fischer's Least Significant Difference, (LSD) test. Relationships between PPO activity and flour and porridge L* were assessed by linear regression correlation.

2.2.4 Results and discussion

2.2.4.1 Effects of cultivar and season on PPO activity and flour and porridge colour

Whole grain of sorghum, wheat and white maize showed PPO activity (Table 2.2.1). Wheat cultivars had the highest PPO activity ranging from 228.95 to 1447.37 Units/mg. White maize had the lowest PPO activity ranging from 10.53 to 17.11 Units/mg. The sorghum cultivars were intermediate with PPO activity for white tan-plant sorghum cultivars ranging from 106.58 to 192.11Units/mg and for coloured sorghums (red and brown) ranging from 86.84 to 176.32 Units/mg. Overall, there was no significant difference in PPO activity between white tan-plant sorghums and the red and brown types. The findings with regard to sorghum are in agreement with Dicko et al (2002a) who found PPO activity in mature white and red sorghums. The results, however, are in contrast to Glennie (1981) who did not find any PPO activity in mature red sorghum grain. This may be attributed to the differences in ratio of sorghum flour to enzyme extract. Glennie (1981) used 0.2 g ground sorghum grain and 5 mL buffer, while 2 g ground sorghum grain and 9.6 mL buffer was used in this study, an approximately five times higher ratio.

Table 2.2.1 Polyphenol oxidase activity of different sorghum, maize and wheat cultivars

Country	Cereal and cultivar	Season	Grain type	Polyphenol Oxidase Activity (Units/mg)
Zambia	***Sorghum***			
	ELT1-16	2008	White tan-plant	173.68 lm (3.68)
		2009		175.00 lm (1.84)
	ELT1-17	2008	White tan-plant	150.00 jk (7.63)
		2009		147.37 jk (10.26)
	Kuyuma	2008	White tan-plant	118.68 fghi (5.26)
		2009		115.79 fgh (3.68)
	MMSH-1257	2008	White tan-plant	123.68 fghi (6.58)
		2009		121.05 fghi (3.42)
	MMSH-1340	2008	White tan-plant	178.95 m (1.84)
		2009		192.11 m (3.68)
	MMSH-1040	2008	White tan-plant	115.79 fgh (3.68)
		2009		114.47 fgh (1.84)
	SDS 1958-1-3-2	2008	White tan-plant	185.53 m (1.84)
		2009		184.21 m (0.00)
	[SDS5006*WSV187]23-2-1	2008	White tan-plant	113.16 efgh (3.68)
		2009		106.58 bcdef (1.84)
	Sima	2008	White tan-plant	111.84 efgh (1.84)
		2009		113.16 efgh (3.68)
	ZSV-15	2008	White tan-plant	110.53 defg (3.68)
		2009		115.79 fgh (3.68)
	MMSH-375	2008	Red tannin	89.47 bcd (3.68)

		2009		88.16 bc (1.84)
	MMSH-413	2008	Brown tannin	136.84 ijk (3.68)
		2009		140.79 ijk 1.84)
	MMSH-625	2008	Red non-tannin	173.68 lm (3.68)
		2009		176.32 lm (3.68)
	MMSH-740	2008	Brown tannin	86.84 b (3.68)
		2009		92.11 bcde (3.68)
	[Framida*SDS3843]F6-5	2008	Red tannin	123.68 fghi (3.68)
		2009		131.58 ghij (3.68)
	[Framida*SDS3843]16-2-2-	2008	Red tannin	122 .11 fghi (0.37)
		2009		121.05 fghi (3.68)
Botswana	BSH1		White tan-plant	134.21 hijk (3..68)
	Larsvyt		White tan-plant	121.05 fghi (7.37)
	Sefofu		White tan-plant	131.58 kl (5.79)
	Segao		White tan-plant	157.79 kl (10.53)
Kenya	KAT369		White tan-plant	107.89 cdefg (6.32)
South Africa	PEX606/202		White tan-plant	152.63 ejk (9.21)
USA	Orbit		White tan-plant	147.37 jk (1.84)
	NK8828		White tan-plant	118.68 fghi (0.26)
South Africa	***White Maize***			
	PAN6043			11.58 a (0.00)
	PAN6045			11.05 a (0.00)
	PAN63355			15.79 a (0.00)
	PAN6Q321			15.00 a (1.84)
	Panthera			15.79 a (0.00)

	CRM3508	10.53 a (0.26)
	PAN62213	17.11 a (0.00)
	Saffier	12.89 a (0.26)
South Africa	***Wheat***	
	CRM826	276.32 o (5.53)
	South Africa commercial red wheat	228.95 n (1.86)
	Kariega	771.05 r (0.0)
	PAN3118	697.37 q (0.00)
	PAN3355	1447.37 s (7.37)
	Steenbras	563.16 p (1.84)
	Mean	174.47
	Minimum	10.53
	Maximum	1447.37

Values followed by different letters in a column are significantly different ($P < 0.05$). Values in parentheses are standard deviations.

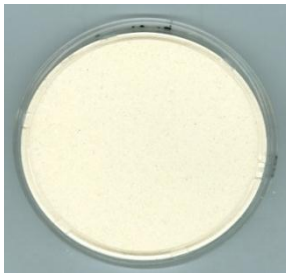
Analysis of effects of cultivar and season with their interactions showed that cultivar had a highly significant effect ($p<0.001$) on PPO activity for all Zambian cultivars (Table 2.2.2). It also had a very highly significant effect ($p<0.001$) on PPO activity for Zambian white tan-plant sorghums. Thus for the Zambian sorghums, cultivar contributed most to the observed variations in the PPO activity. Working with wheat, Park et al (1997) reported that the differences in PPO activity between cultivars could be due to a genetic component. This could also be inferred for sorghum.

Table 2.2.2 Summary statistics of analysis of variance for effects of cultivar, season and their interactions in Zambian sorghums

Source	DF	SS	Mean Square	F Value	P
PPO activity for all Zambian sorghums					
Cultivar	15	0.00	0.00	67.74	<0.001[a]
Season	1	0.00	0.00	0.01	>0.05[c]
Cultivar*Season	15	0.00	0.00	2.29	<0.05[b]
PPO activity for Zambian white tan- plant sorghums					
Cultivar	9	0.00	0.00	445.25	<0.001[a]
Season	1	0.00	0.00	7.93	<0.001[a]
Cultivar *Season	9	0.00	0.00	18.57	<0.001[a]
Colour (L) values for Zambian white tan-plant sorghum flours					
Cultivar	9	65.10	7.23	35.58	<0.001[a]
Season	1	14.68	1.21	72.20	<0.001[a]
Cultivar*Season	9	10.88	1.21	5.95	<0.001[a]
Colour (L) values for Zambian white tan-plant sorghum porridges					
Cultivar	9	210.16	25.35	504.02	<0.001[a]
Season	1	0.07	0.07	1.45	>0.05[c]
Cultivar*Season	9	2.66	0.20	6.38	<0.001[a]

Level of statistical significance (a) $p<0.001$, (b) $p \leq 0.05$ (c) $p \geq 0.05$

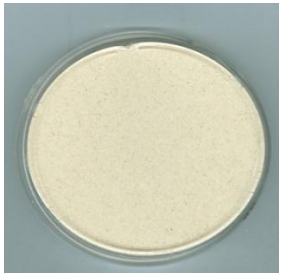
The white tan-plant sorghum whole grain flours were slightly darker (mean L* values of 85.38, Table 2.2.3) than the white maize whole grain flours (mean L* values of 91.57, Table 2.2.4). For both sorghum (Table 2.2.3) and maize (Table 2.2.4), the L* values of the porridges (mean L* values of 57.53 for sorghum porridge and 74.58 for maize porridge) were lower than those of the flours. These results show that cooking sorghum and maize flours into porridge resulted in darkening and a reduction in L* values. This darkening effect is illustrated in Figure 2.2.1 which shows that the porridges of the sorghum and maize samples were darker than the flours. Cultivar also had a highly significant effect ($p<0.001$) on flour L* values and on sorghum porridge L* values of the Zambian white tan-plant sorghums (Table 2.2.2).


PAN 6043 maize flour


PAN 6043 maize porridge


Sima sorghum flour


Sima sorghum porridge


Panthera maize flour


Panthera maize porridge


SDS5006*[WSV 187] 23-2-1 sorghum flour


SDS5006*[WSV 187] 23-2-1 sorghum porridge


KAT 369 sorghum flour


KAT 369 sorghum porridge


SDS 1958 1-3-2 sorghum flour

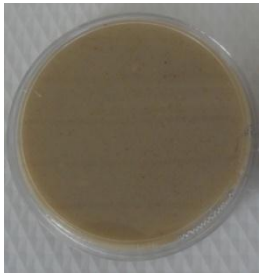

SDS 1958 1-3-2 sorghum porridge

Figure 2.2.1 Effect of cooking into porridge on colour of white maize and white non-tannin sorghum flours

Table 2.2.3 Colour (L *a* b*) values of flours and porridges from white tan-plant sorghum cultivars from different countries

Country	Cultivar	Season	Flour			Porridge		
			L* Value	***a* Value***	***b* Value***	***L* Value***	***a* Value***	***b* Value***
Zambia	ELT1-16	2008	84.54 efg (0.02)	0.75 bc (0.03)	12.12 ijklm (0.10)	55.38 ef (0.24)	1.74 efgh (0.25)	6.36 cde (0.40)
		2009	85.98 hi (0.01)	1.08 b (0.06)	12.55 mnop (0.42)	55.73 fg (0.22)	1.92 ghi (0.06)	6.59 cdefg (1.15)
	ELT-17	2008	84.75 efg (0.01)	1.61 no (0.07)	12.63 nop (0.22)	56.27g (0.21)	1.51 cdefg (0.07)	6.76cdefg (0.42)
		2009	85.25 fgh (0.33)	1.41 ijkl (0.04)	11.70 fghij (0.23)	57.59 h (0.22)	1.52 defg (0.03)	6.61cdefg (0.14)
	Kuyuma	2008	85.81 ef (0.02)	0.78 de (0.02)	12.04 hijkl (0.36)	60.17 jk (0.54)	1.29 bcde (0.46)	6.45 cde (0.20)
		2009	84.41 ef (0.03)	0.87 ef (0.01)	11.26 ef (0.28)	60.24 kl (0.08)	1.52 bcde (0.22)	6.51 cde (0.40)
	MMSH-1257	2008	86.54 ijkl (0.32)	1.03 gh (0.03)	11.36 ef (0.19)	57.75 h (0.30)	2.20 ijkl (0.75)	6.34 bcd (0.50)
		2009	85.98 hi (0.52)	0.70 d (0.04)	12.88 op (0.15)	57.60 h (0.29)	1.82 fghil (0.03)	6.33 bcd (0.54)
	MMSH-1340	2008	84.64 efg (0.51)	0.56 c (0.03)	12.95 p (0.30)	55.36 de (0.21)	1.79 fgh (0.07)	6.31 bcd (0.68)
		2009	84.62 efg (0.47)	0.86 ef (0.01)	12.45 lmno (0.16)	55..36 de (0.61)	1.91 fghil (0.19)	6.34 bcd (0.57)
	MMSH-1040	2008	85.53 ghi (0.28)	1.30 gh (0.04)	11.94 p (0.47)	55.33 de (0.11)	1.63 efg (0.29)	5.67 bc (0.55)
		2009	82.17 ab (0.05)	1.14 h (0.03)	11.87 ghijk (0.46)	56.11 g (0.34)	2.27 jklm (0.04)	7.70 defg (0.81)
	SDS1958]1-3-	2008	82.70 bc (0.61)	1.38ijk (0.60)	12.57 mnop (0.11)	53.94 bc (0.03)	2.13 hijk (0.07)	6.48 cdefg (0.41)
	2	2009	82.82 bc (0.44)	1.53 lmn (0.06)	12.58 mnop (0.33)	53.9 bc (0.05)	2.16 ijk (0.27)	6.52 cdefg (0.40)
	[SDS5006*W	2008	87.39 klm (0.35)	0.72 d (0.03)	11.91 hijk (0.08)	59.63 j 0.35)	1.07 abcd (0.04)	6.68 cdefg (0.09)
	SV187]23-2-1	2009	86.20 klm (0.73)	0.56 c (0.02)	12.01 hijkl (0.66)	57.65 j (0.60)	1.07 abcd (0.16)	6.60 cdefg (0.23)
	Sima	2008	87.50 lm (0.71)	1.46 klm (0.08)	11.41 efg (0.13)	64.4 mn (0.43)	0.96 ab (0.08)	7.03 cdefg (0.09)
		2009	86.17 jklm (0.56)	1.49 klmn (0.04)	11.59 fgh (0.22)	61.65 n (0.43)	0.95 ab (0.05)	7.00 cdefg (0.42)

	ZSV-15	2008	86.37 ijk (0.30)	0.72 d (0.03)	11.23 ef (0.09)	58.58 i (0.27)	0.88 ab (0.04)	5.80 bc (0.16)
		2009	85.25 fgh (0.33)	0.54 c (0.02)	12.27 kllmn (0.09)	58.73 i (0.88)	0.85 a (0.36)	5.60 bc (0.20)
Botswana	BSH1		85.79 hi (0.35)	1.67 o (0.07)	12.57 mnop (0.35)	61.13 mn (0.55)	1.51 cdefg (0.25)	7.07 cdefg (0.25)
	Larsvyt		84.30 ef (0.57)	1.8 p (0.17)	12.77 op (0.35)	57.29 h (0.07	3.28 n (0.17)	7.62 defg (0.25)
	Sefofu		84.49 ef (0.54)	2.9 5 q (0.13)	11.63 fghi (0.84)	60.86 lm (0.16)	5.28 p (0.14)	6.17 bcd (0.20)
	Segao		81.48 a (0.38)	3.53 r (0.06)	10.53 cd (0.28)	52.41 a (0.37)	4.07 o (0.56)	2.26 a (0.07)
Kenya	KAT369		88.17 lm(0.17)	0.94 fg (0.02)	12.00 hijkl (0.40)	62.99 o (0.32)	1.27 abcde (0.09)	7.15 cdefg (0.03)
South Africa	PEX606/202		82.13 ab (0.28)	1.30 i (0.07)	10.94 de (0.03)	54.46 cd (0.10)	2.69 lm (0.07)	6.65 cdefg (0.38)
USA	Orbit		83.97 de (2.69)	1.54 mno (0.13)	12.16 jklmn (0.04)	53.37 b (0.09)	2.52 klm (0.09)	6.08 bcd (0.20)
	NK8828		83.20 cd (0.41)	1.44 jkl (0.04)	10.95 de (0.04)	54.92 de (0.61)	2.76 mn (0.10)	5.62 bc (0.42)
	Mean		85.38	1.97	12.07	57.53	3.23	6.63
	Minimum		81.48	0.54	10.53	52.41	0.84	2.26
	Maximum		88.17	3.53	12.95	62.99	5.28	7.62

Values followed by different letters in a column are significantly different ($p < 0.05$). Values in parentheses are standard deviations

Table 2.2.4 Colour (L* a* b*) values of flours and porridges from South African white maize

Cultivar	Flour			Porridge		
	L* Value	***a* Value***	***b* Value***	***L* Value***	***a* Value***	***b* Value***
PAN6043	91.20 bc (0.37)	-0.90 a (0.08)	10.08 b (0.30)	74.32 d (0.35)	-0.87 b (1.10)	5.30 a (0.08)
PAN6045	91.01 b (0.20)	-0.18 b (0.05)	10.37 b (0.08)	72.94 a (0.75)	-0.79 b (0.01)	5.47 a (0.06)
PAN63355	92.58 e (0.41)	-0.45 a (0.31)	9.25 a (0.18)	75.64 e (0.38)	-1.08 ab (1.08)	5.82 b (0.83)
PAN6Q321	90.40 a (0.27)	-0.04 c (0.02)	10.38 b (0.09)	74.15 c (0.22)	-0.58 d (0.05)	5.36 a (0.03)
Panthera	91.64 d (0.71)	-0.13 b (0.05)	10.20 b (0.21)	75.44 e (0.07)	-0.92 c (0.06)	6.25 c (0.60)
CRM3508	91.49 cd (0.37)	-0.20 ab (0.05)	10.01 b (0.37)	73.82 c (0.28)	-1.07 a (0.04)	6.25 c (0.11)
PAN622313	92.51 e (0.45)	-0.18 b (0.04)	9.26 a (0.27)	75.76 e (0.67)	-1.21 ab (0.02)	5.38 a (0.31)
Saffier	91.72 de (0.64)	0.00 c (0.05)	9.36 a (0.24)	74.60 d (0.06)	-0.93c (0.07)	6.26 c (0.07)
Mean	91.57	-0.15	9.88	74.58	-0.93	5.76
Minimum	90.41	-0.90	9.25	72.94	-1.21	5.30
Maximum	92.58	0.00	10.38	75.55	-0.58	6.26

Values followed by a different letter in a column are significantly different ($p<0.05$). Values in parentheses are standard deviations

For white tan-plant sorghum, the reduction in L* values from flour to porridge (Table 2.2.3) was greater than in white maize (Table 2.2.4). With the transition from flour to porridge for white tan plant sorghums, there was a reduction in mean L* value of 27.85 and a reduction in colour b* values of 4.12, while a* value increased by -1.26. In comparison, the transition from flour to porridge for white maize resulted in a relatively smaller reduction in L* value of 16.99, a reduction in b* value of 4.12 and a decrease in a* value of -0.78. Figure 2.2.1 also shows that the sorghum porridges were relatively darker than the maize. Similar observations on porridge darkening were also made by Jagwer (1988) and Aboubacar et al (1999) in their work with white tan-plant sorghum flours before and after porridge preparation in Botswana and Niger, respectively. These researchers, however, did not attribute the darkening of porridge to PPO activity.

Table 2.2.5 Correlation matrices for sorghum and maize flour PPO activity, flour colour L* values and porridge colour L* values

Variable	PPO	L*Flour
All white tan-plant sorghums n=28		
L* Flour	-0.489*	
L*Porridge	-0.657**	0.749***
Zambian white tan-plant sorghums (n=20		
L* Flour	-0.523*	
L*Porridge	-0.710***	0.890***
South African white maize (n=8)		
L*Flour	-0.492*	
L*Porridge	-0.505*	0.880***

Level of significance *p<0.05,**p<0.01,***p<0.001

2.2.4.2 Relationships between flour and porridge colour and PPO activity

The correlation between PPO activity and flour L* values was significant and negative for all white tan-plant sorghums ($r = -0.489$, $p < 0.05$), the Zambian white tan-plant sorghums ($r = -0.523$, $p < 0.05$), and the South African white maize ($r = -0.492$, $p<0.05$) (Table 2.2.5). These results indicate that PPO activity to an extent influenced flour colour in all white tan-plant sorghums, Zambian white tan-plant sorghums and South African maize. The correlation in the three samples though significant, was weak.

PPO activity was also significantly and negatively correlated with porridge L* values for all white tan-plant sorghums ($r = -0.657$, $p<0.01$), Zambian white tan-plant sorghums ($r = -0.710$, $p<0.001$) and South African white maize ($r = -0.505$, $p<0.05$) (Table 2.2.5). These results indicate that PPO activity was responsible for the porridge darkening in white tan-plant sorghums and in white maize. Thus the effect of PPO activity in white tan-plant sorghum and maize seems to be similar to that in wheat where PPO activity was related to the darkening of wheat-based foods such as Oriental noodles (Kruger et al 1994; Baik et al 1995; Kang et al 2008) pasta products (Kobrehel et al 1974; Feillet et al 2000), chapattis (Singh and Sheoran 1972) and Middle East flat breads (Faridi 1988). The magnitudes of the correlations between PPO activity and porridge L* values and level of significance were higher for the white tan-plant sorghums than the white maize. This may be related to the observed higher levels of PPO activity in white tan-plant sorghum compared to white maize. The slightly higher correlation between PPO activity of Zambian white-tan-plant sorghums and porridge L* values may also be because they were grown in controlled conditions, while the all white tan-plant group consisted of some sorghums whose cultivation history was not known.

Correlations between flour L* values and porridge L* values were highly significant and positive for all white tan-plant sorghums ($r = 0.749$, $p<0.001$), Zambian white tan-plant sorghums ($r = 0.890$, $p<0.01$) and South African white maize ($r = 0.880$, $p<0.001$) (Table 2.2.5). These results indicate that flour colour influences porridge colour. The influence of flour colour on porridge colour probably was stronger in both the white tan-plant sorghum group and in the Zambian white tan-plant sorghum group because of weathering of some grains.Weathering occurs after grain maturity and drying. The extent of damage depends on the amount of rainfall, humidity, temperature and wind (Bennet et al 1990). If the weather is too damp for too long, molds and algae may proliferate and destroy the grain. Excessive moisture for long periods may result in grain sprouting on the head. Heavy rainfall after the

grain has matured can cause discolouration of the grain due to pigments leaching out from the glumes into the kernels (Bennet et al 1990).

Figure 2.2.2 shows that the Zambian and other white tan-plant sorghum PPO activity and porridge L* value data were well distributed and that despite the relatively narrow range of PPO activities and small size of the data set, there was a clear negative relationship between PPO activity and porridge L* value. A summary of PPO activity and porridge L* values for all white tan-plant sorghums and South African maize is given in Figure 2.2.3 and shows that the degree of correlation was even stronger. This is a further indication that PPO activity was responsible for porridge darkening. Other known oxidising enzymes such as laccase and peroxidase could not have played a role in porridge darkening in this research work, mainly because laccase is absent in cereals and the major substrate for peroxidase activity is hydrogen peroxide (Dicko et al 2002a; Feilet et al 2000) which was not one of the substrates used in this research work.

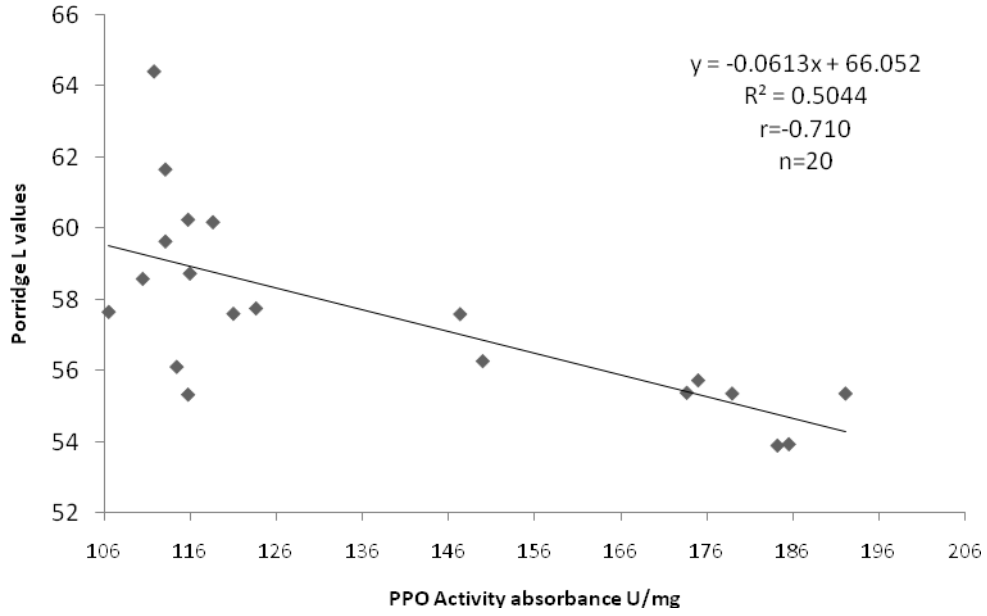


Figure 2.2.2 Relationship between Zambian white tan-plant sorghum porridge L* values and PPO activity. Level of significance p<0.001

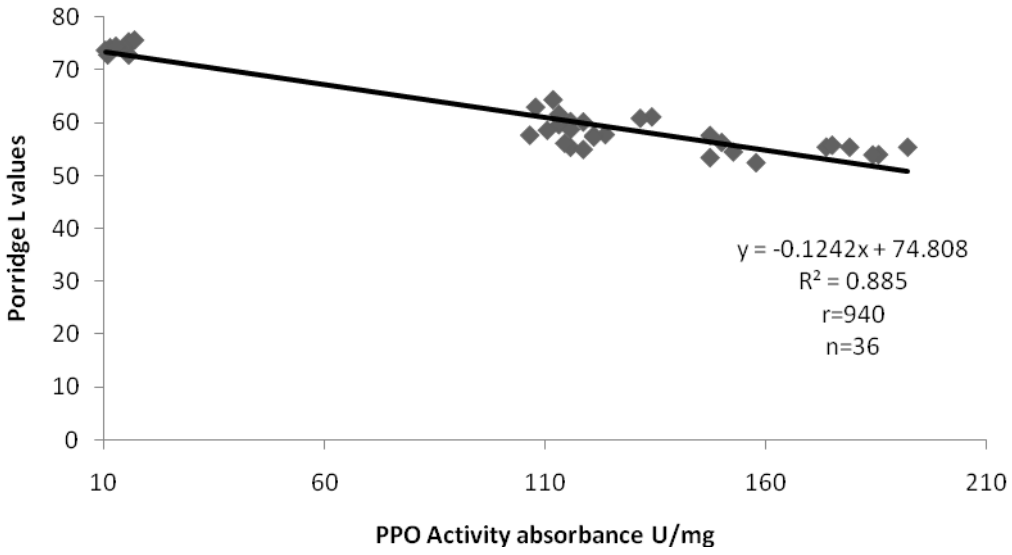


Figure 2.2.3 Relationship between white tan plant sorghum and maize porridge L* values) and PPO activity. Maize =8, Sorghum = 28. Level of significance p <0.001

2.2.5 Conclusions

Due to the relatively high PPO activity of white tan-plant sorghum cultivars in comparison with white maize and the significant negative correlations between white tan-plant sorghum PPO activity and porridge L* value, it is apparent that PPO activity in white tan-plant sorghums is an important determinant of the relatively dark colour of food products made from them, as is the case in wheat. Since PPO activity is genetically controlled, breeding can be potentially used to reduce the levels in white tan-plant sorghum lines, as done with wheat, in order to improve the aesthetic appeal of food products from these sorghums and hence widen their acceptability among consumers.

2.3 Sorghum grain colour assessment using Tristimulus colorimetry and UV-visible spectrophotometry of sodium hydroxide extracts

2.3.1 Abstract

Grain colour is an important quality indicator in sorghum-based foods and is normally measured visually or by the use of Tristimulus colorimetry. For rural sorghum farmers in sub-Saharan Africa, there is a need for simple, more accessible methods for determination of grain colour. The objective of the research was to investigate the development of a simple quantitative method for sorghum grain colour assessment by analysis of the grains and their sodium hydroxide (NaOH) extracts. Sixteen Zambian sorghums (white, red and brown types which were either tannin or non-tannin) from the 2008 and 2009 seasons and a Sudanese white tannin sorghum type were assessed for surface colour using Tristimulus colorimetry before and after treatment with NaOH. The NaOH extracts were also analysed using UV-visible spectrophotometry and reverse phase HPLC. Tristimulus colorimetry of the grain surface was able to distinguish white from coloured sorghums (brown and red) but was unable to separate tannin from non-tannin sorghum. UV-visible spectrophotometry and reverse phase HPLC of NaOH extracts from a representative set of the grains consisting of five sorghum types (red tannin, brown tannin, white tannin, red non-tannin and white non-tannin) separated the tannin from non-tannin sorghums regardless of grain surface colour. However, UV-visible absorption of NaOH extracts from the sorghum grains could not be related to grain surface colour as determined using Tristimulus colorimetry. These results show that with NaOH treatment, it is possible to separate tannin from non-tannin sorghums. Colorimetry of NaOH extracts from sorghum may therefore be considered as a potentially simple and cheap alternative method for distinguishing tannin from non-tannin sorghums.

2.3.2 Introduction

Sorghum grain colour is important in differentiating between sorghum types. Sorghum grain colour can be white, yellow or red (Rooney and Miller 1982) and plays an important role in acceptability of sorghum-based foods (Jagwer 1998). In Africa, white or light sorghums are more generally preferred for porridge making (Taylor and Duodu 2009). Sorghum grain colour is also important in grain trade. In the USA**,** one of the bases of sorghum classification is grain colour and it is measured visually.

Sorghum grain colour is primarily due to flavonoid pigments (Siame, Egeta and Butler 1994), predominantly anthocyanidins. The anthocyanidins in sorghum are unique because they do not contain a hydroxyl group in the 3-position of the C ring and so are called 3-deoxyanthocyanidins (Dykes and Rooney 2006). Apigeninidin and luteolinidin are the two common 3-deoxyanthocyanidins in sorghum and are responsible for the red colour of sorghum grain pericarp (Bate-Smith, 1969). Pigmented sorghums also contain flavan-4-ols such as apiforol (leucoapigeninidin) and luteoforol (leucoluteolinidin) that are believed to be precursors of the 3-deoxyanthocyanidins (Ferreira and Slade 2002; Haslam 1998; Hagerman 2005). Black pericarp sorghum types have been reported to contain higher levels of flavan-4-ols and anthocyanidins than other varieties of sorghum (Dykes et al 2005). The flavones (flav-3-en-ols) are another important group of flavonoids reported in pigmented sorghums. The main flavones in sorghum are apigenin and luteolin and their presence have been reported in red (Dykes et al 2009) and lemon-yellow (Dykes et al 2011) sorghum genotypes.

Colour is a psychological phenomenon resulting from the interaction of the light source, object being viewed by the eye and interpreted by the brain (Ohno 2000). Colour is composed of two chromatic attributes (hue and purity) and luminous factor brightness (Ohno 2000). Hue refers to the spectral composition of light leaving the object. Purity or saturation is the amount of colour present in the object. The higher the saturation, the lower the greyness (Feillet et al 2000). The brightness or (lightness) refers to the capacity of an object to reflect or transmit light.

Colour meters using various tristimulus systems and reflectance spectrophotometers are used in the food and textile industries (Oliver et al 1992). The tristimulus instruments offer various scales which are mathematical derivatives of X, Y and Z. These X, Y and Z values are measurements of proportional contributions from red, green and blue components

(respectively) of the visible spectra that would match the sample colour (Oliver et al 1992; Feillet et al 2000). Available colour measuring methods using the Commission Internationale de l'Eclairage (CIE) L* a* b* system make it possible to investigate colour quality as well as pigment concentration in a systematic way (Wrolstad, Durst, and Jungmin 2005).

There is a need to have an alternative sorghum colour estimation method because visual and Tristimulus estimation, though useful, is limited because it does not consider the pigments that are located in other parts of the kernel like those located in the testa stylar or endosperm. Hence it is only useful for the colour of sorghum grain as seen at the grain surface. An alternative method which is quantitative, simple, cheap and whose results are easy to correlate with other methods can give more information on sorghum grain colour as it will estimate pericarp kernel colour as well as testa colour. A simple method that can assist small scale farmers and traders estimate sorghum grain colour at the local level can increase yield and contribute to food security.

A method for colour determination using sodium hydroxide extracts was reported for wheat by Lamkin and Miller (1980) to distinguish red wheat from white common wheat. Matus-Cadiz et al (2008) also used sodium hydroxide as one of their reagents in their methods to distinguish white wheat from red and brown wheat. The objective of the research study was to investigate the development of a simple, quantitative method for sorghum grain colour estimation by analysis of sodium hydroxide extracts from the grains in comparison with Tristimulus colorimetry.

2.3.3 Materials and Methods

2.3.3.1 Grain samples

Sixteen Zambian sorghum cultivars, grown at the Golden Valley Research Station, Chisamba, Zambia during the 2008 and 2009 growing seasons were used. Some of the cultivars were open pollinating varieties (OPV): ZSV-15, Kuyuma and Sima. Others were hybrids: ELT1-17, ELT1-16, MMSH1040, MMSH1340, SDS1958]-3-3-2, SDS5006* WSV-187]23-2-1, MMSH 413, MMSH740, MMSH375, Framida* SDS3843] F6-5, Framida* SDS3843] 16-2-2 and MMSH625. Feterita, a white tannin sorghum from Sudan was also used. Approximately 200 g of each grain type was cleaned and packaged in polyethylene bags and kept in the cold room at 10^{o}C until use.

2.3.3.2 Grain colour analyses

Ten gram of each grain was weighed into a 90 mm Petri dish and covered with the Petri dish lid. Kernel colour measurements were done using a Konica Chrome meter C R 400 (Sensing IN, Japan) using the L*a*b* scale. The colour of grains treated with sodium hydroxide solution was determined according to Lamkin and Miller (1980). Ten gram of sorghum grain was weighed into a 90 mm Petri dish and 15 mL of 1.25 M NaOH with 1% Triton X-100 was poured over the grains and the Petri dish was covered with the Petri dish lid and left at 25^{o}C for 1 h. The NaOH extract solution was removed by decanting from each Petri dish labelled and kept at 4^{o}C until use. The grains were left overnight at room temperature to dry. Colour for sodium hydroxide-treated grains was measured as described for untreated sorghum grains.

2.3.3.3 UV-visible spectrophotometry of sodium hydroxide extracts of sorghum grains

One mL of each of the NaOH extract solutions obtained above was diluted with 9 mL of distilled water in a test tube. Five mL of the diluted solution was mixed with 5 mL 1.25 M sodium hydroxide in a test tube. One mL of the mixture was placed in a glass cuvette and scanned for colour forming compounds in the wavelength range of 190 to 1100 nm at 5 nm intervals using a T80 + UV/VIS spectrophotometer (PG Instruments, Wibtoft, UK). A baseline correction was done using 1 mL of a 1: 9 diluted (with distilled water) solution of 1.25 M NaOH.

2.3.3.4 High performance liquid chromatography (HPLC) analysis of sodium hydroxide extracts from sorghum grains

HPLC analysis of the NaOH extracts was done using a modification of the method used by Awika et al (2004a). Extracts from five cultivars (Framida*SDS[3843], F6-5 Feterita, MMSH740, MMSH625 and Sima) representing the rest of the cultivars were analysed. In preparation for HPLC analysis, 10 mL of each of the NaOH extracts was diluted with 10 mL methanol. The pH of the mixture was adjusted to 2 using 1 M hydrochloric acid, followed by filtration through 0.45 µm polyvinylidene fluoride (PVDF) membrane filters (Millipore, USA) using a vacuum pump. The filtrates were then passed through Millex LG 0.20 µm polytetrafluoroethylene (PTFE) syringe filters into 2 mL eppendorf tubes to be used for HPLC analysis.

A Waters system (Waters, Milford, MA, USA) consisting of a model 1525 binary pump, a model 2487 dual absorbance detector and a YMC-Pack ODS AM-303 (250 x 4.6 mm i.d., 5 µm particle size) reverse phase column (Waters, Milford, MA, USA) was used. The mobile

phase was (A) 10% formic acid in water and (B) acetonitrile/water/formic acid (5:4:1). The mobile phases were run through the system in a linear gradient as follows: 0-3 min, 12% B isocratic; 3-10 min, 12-30% B; 10-15 min, 30% B isocratic; 15-20 min, 30-40% B; 20-30 min, 40% B isocratic; 30-40 min, 40-100% B; 40-60 min, 100% B isocratic; and 60-63 min, 100-12% B; 63-75 min, 12% B isocratic. Total run time was 75 min. Injection volume was 20 µL and flow rate was 0.5 mL/min. Detection was set at 280 and 480 nm and data acquired by Breeze system software (Waters, Milford, MA, USA).

2.3.3.5 Statistical analysis

Data for treated and untreated sorghums were analysed by multifactor analysis of variance. The means were compared by Fischer's Least Significant Differences (LSD) test. Relationships between colour L* values of dry sorghums and absorbance of NaOH extracts were assessed by linear regression.

2.3.4 Results and discussion

2.3.4.1 Tristimulus colorimetry and the effect of sodium hydroxide treatment on sorghum kernel colour

The L* values for all whole dry sorghum grain before NaOH treatment ranged from 44.14 to 68.14 with a mean of 57.01 (Table 2.3.1). Separating the sorghums according to colour, white sorghum kernel L* values ranged from 56.56 to 68.14, while the L* values for coloured sorghums (brown and red) ranged from 44.14 to 49.07. The L* value for Feterita, a white tannin sorghum was in the same range as the white non-tannin sorghums, an indication that only the grain surface colour was measured by Tristimulus colorimetry and that this method made no distinction between white tannin and white non-tannin sorghum.

The a* values (representing redness) for all sorghums ranged from 2.39 to 8.08 with a mean of 3.97 (Table 2.3.1). White sorghum a* values ranged from 3.20 to 4.65. The coloured sorghum a* values ranged from 2.84 to 8.08. Generally, the a* values for coloured sorghums were higher than for white sorghums, an indication of more colour pigments (red and brown) in the coloured sorghums. The b* values (indicating yellowness) for all grain sorghums ranged from 1.07 to 13.37 with a mean of 6.72. The b* values for white sorghums, ranged from 6.57 to 13.37 and were higher than for coloured sorghums which ranged from 1.07 to 5.85.

Treatment with 1.25 M NaOH led to a general reduction in L values for all sorghums (Table 2.3.1). For all sorghums, there was a mean reduction in L* values by 19.11. The reduction in L* values is illustrated in Figure 2.3.1 which shows that the sorghum grains became darker on treatment with NaOH. There was also a mean reduction in a*values by 2.88 and in b* values by 8.5. The NaOH treatment causes leaching out of pigments from the grain pericarp, testa and stylar that would normally not be measured in untreated grains using Tristimulus colorimetry. For instance, the 3-deoxyanthocyanins which are important compounds responsible for pigmentation in sorghum are located in the pericarp (Nip and Burns 1968; Blakeley et al 1969; Gus 1989; Awika et al 2004a; Dykes et al 2005) and stylar area (Nip and Burns 1968). Condensed tannins, which are the most predominant phenolic compounds in tannin sorghums are located in the testa (Blakeley et al 1969). There may also be some chemical modification of pigments. Beta et al (2000) suggested that NaOH can promote oxidation of phenolic groups. The leaching out of pigments from the grain when treated with NaOH and possibly their oxidation under alkaline conditions may contribute to the observed changes in colour. A similar trend of colour reduction was observed by Matus-Caduz et al (2008) in white and coloured wheat grains after treatment with 1.25 M NaOH. In the NaOH treated sorghums, Feterita (a tannin white sorghum) had the lowest L* values, probably due to oxidation of the tannins.

These results indicate that in untreated sorghum, the Tristimulus colorimetry method is able to separate white tan-plant sorghum from coloured or pigmented sorghum. However, NaOH treatment modifies the colour of these grains and brings the L*, a*, b* values within a narrower range and the method seems to then lose its ability to distinguish between the sorghums based on colour.The effect seems to be different in wheat where it is reported that NaOH treatment enhances colour differences and enables easier separation of red and white wheat types (Lamkin and Miller 1980; Matus-Cadiz, Hucl, Peron and Tyler 2003; Matus-Cadiz et al 2008).

TABLE 2.3.1 Colour (L*, a*, b*) values for dry whole grain of sorghum cultivars and dry whole grain of sorghum cultivars treated with 1.25 M NaOH

Cultivar	**Season**	**Grain Type**	**Dry whole sorghum grain**			**NaOH treated whole sorghum grain**		
			L*value	***a*value***	***b*value***	***L*value***	***a*value***	***b*value***
ELT1-16	2008	White tan-plant	63.60 i (0.09)	3.78 hi (0.04)	13.14 g (0.02)	46.14 fghi (0.01)	3.40 fghij (0.07)	0.87 m (0.01)
	2009		65.08 k (0.08)	3.77 hi (0.05)	13.09 g (0.05)	46.42 ghij (0.01)	3.42 ghij (0.06)	0.85 m (0.01)
ELT1-17	2008	White tan-plant	59.60 g (0.02)	4.44 m (0.05)	9.37 de (0.01)	45.86 efg (0.01)	3.46 ghij (0.05)	0.19 ij (0.01)
	2009		59.61 g (0.08)	4.65 n (0.07)	9.36 de (0.01)	45.78 ef (0.01)	3.47 ghij (0.06)	0.17 i (0.01)
Kuyuma	2008	White tan-plant	66.53 i (0.06)	3.67 g (0.01)	10.16 e (0.01)	47.88 k (0.06)	2.87 def (0.06)	0.71 lm (0.06)
	2009		66.68 i (0.01)	3.66 g (0.01)	10.13 e 0.01)	48.05 k (0.07)	2.86 def (0.07)	0.84 m (0.03)
MMSH1257	2008	White tan-plant	65.44 k (0.01)	3.87 i (0.01)	12.85 g (0.01)	45.78 ef (0.01)	3.77 ij (0.01)	-1.55 h (0.01)
	2009		65.66 k	3.98 j (0.01)	12.86 g	45.79 ef (0.01)	3.78 ij (0.01)	-1.34 h (0.02)

			(0.02)		(0.01)			
MMSH1340	2008	White tan-plant	62.05 h (0.03)	4.04 kl (0.01)	11.70 f (0.01)	45.94 efgh (0.11)	3.63 hij (0.02)	1.13 n (0.01)
	2009		62.06 h (0.01)	4.08 kl (0.01)	11.69 f (0.01)	45.95 efgh (0.13)	3.25 fghi (0.01)	1.13 n (0.01)
MMSH1040	2008	White tan-plant	59.23 g (0.01)	4.11 kl (0.00)	10.28 e (0.01)	45.77ef (0.32)	3.86 j (0.03)	1.21 n (0.01)
	2009		58.95 g (0.06)	4.13 l (0.01)	10.27e (0.01)	45.78 ef (0.50)	3.86 j (0.02)	1.24 n (0.01)
SDS 1958] 1-3-2	2008	White tan-plant	56.56 e (0.05)	3.20 e (0.07)	8.55 d (0.03)	45.69 ef (0.02)	3.00 efg (0.01)	0.56 kl (0.01)
	2009		57.11 ef (0.06)	3.37 f (0.01)	8.54 d (0.02)	45.36 e (0.07)	3.00 efg (0.00)	0.58 kl (0.01)
SDS 5006*wsv 187]23-2-1	2008	White tan-plant	66.74 l (0.05)	3.63 g (0.06)	13.37 g (0.01)	46.61 ij (0.01)	3.35 fghij (0.06)	1.56 o (0.01)
	2009		66.75 l (0.01)	3.64 g (0.08)	13.35 g (0.01)	46.60 ij (0.05)	3.34 fghij (0.05)	1.54 o (0.01)

Sima	2008	White tan-plant	68.13 m (0.01)	3.61 g (0.01)	13.06 g (0.01)	49.03 l (0.07)	3.11 efgh (0.09)	0.42 jk (0.01)
	2009		68.14 m (0.02)	3.63 g (0.01)	13.00 g (0.07)	48.82 l (0.06)	3.10 efgh (0.08)	0.44 k (0.01)
ZSV-15	2008	White tan-plant	64.78 j (0.08)	3.86 i (0.01)	12.76 fg (0.02)	46.77 j (0.01)	3.72 ij (0.02)	0.42 jk (0.01)
	2009		64.76 j (0.01)	3.85 hi (0.01)	12.76 fg (0.01)	46.79 j (0.01)	3.71 ij (0.03)	0.68 lm (0.01)
MMSH375	2008	Red	44.14 a (0.01)	2.85 bc (0.01)	5.53 c (0.02)	42.99 d (0.01)	2.61 cde (0.04)	-5.22 f (0.01)
	2009		44.16 a (0.01)	2.86 cd (0.01)	5.56 c (0.01)	42.98 d (0.01)	2.62 cde (0.04)	-5.23 f (0.01)
MMSH413	2008	Brown	47.16 c (0.01)	2.98 d (0.02)	3.33 b (0.01)	42.06 bc (0.01)	1.79 a (0.01)	-6.47 ab (0.07)
	2009		47.17 c (0.01)	2.99 d (0.01)	1.07 a (0.01)	41.76 b (0.01)	1.91 ab (0.05)	-6.18 cde (0.05)
MMSH625	2008	Red non-tannin	49.04 d (0.01)	8.08 p (0.01)	1.86 a (0.01)	46.49 hij (0.01)	5.20 k (0.02)	-1.42 h (0.01)

	2009		49.07 d (0.01)	8.05 p (0.02)	1.88 a (0.01)	46.48 hij (0.00)	5.14 k (0.07)	-1.84 g (0.01)
MMSH740	2008	Brown tannin	47.15 c (0.01)	6.23 o (0.01)	1.18 a (0.01)	42.68 d (0.08)	2.05 ab (0.08)	-6.32 bc (0.01)
	2009		47.16 c (0.01)	6.24 o (0.01)	1.17 a 0.01)	42.67 cd (0.08)	2.18 abc (0.06)	-6.40 abc (0.04)
Framida*SDS [3843] F6-5	2008	Red tannin	45.36 b (0.01)	2.84 b (0.01)	5.83 c (0.01)	42.57 cd (0.01)	2.31 abc (0.06)	-6.06 de (0.10)
	2009		45.35 b (0.01)	2.84 b (0.01)	5.85 c (0.01)	42.59 cd (0.34)	2.30 abc (0.03)	-6.01 e (0.01)
Framida*SDS[3843] 23-2-1	2008	Red tannin	45.14 b (0.01)	2.94 cd (0.01)	5.83 c (0.00)	41.56 b (0.45)	2.37 bcd (0.09)	-6.34 abc (0.20)
	2009		45.15 b (0.01)	2.95 cd (0.01)	5.84 c (0.01)	41.68 b (0.08)	2.31 abc (0.08)	-6.25 bcd (0.07)
Feterita		White tannin	57.70 e (0.06)	2.39 a (0.03)	6.57 d (0.05)	31.39 a (0.30)	2.26 abc (0.01)	-6.57 a (0.01)
Mean			57.01	3.97	6.72	44.81	3.12	-1.78

Minimum	44.14	2.39	1.07	31.39	1.79	-6.57
Maximum	68.14	8.08	13.37	49.03	5.20	1.56

Values followed by different letters in a column are statistically different at ($p<0.05$) as assed by Fisher's least significant test. Values in parentheses are standard deviations .L* = Lightness where "0" indicates darkness and "100" lightness; a*= Redness; negative a* = greenness;

b* = Yellowness and negative b* = blueness n=33.

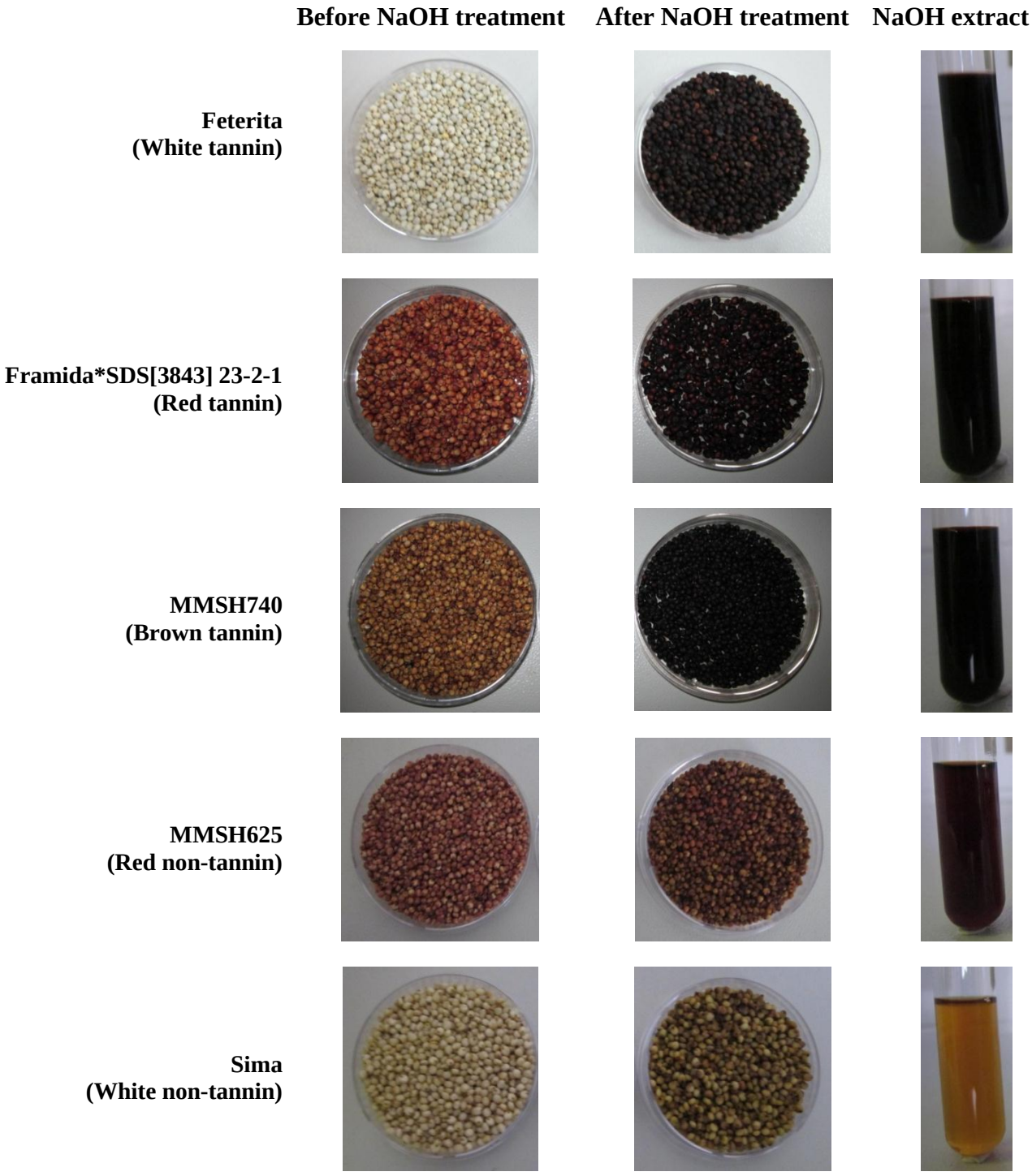


Figure 2.3.1 Appearance of sorghum grains before and after NaOH treatment and NaOH extracts

2.3.4.2 UV-visible spectrophotometry of NaOH extracts from sorghum

Figure 2.3.2 shows the spectra of NaOH extracts from 5 sorghum types which are a representative set of all the sorghum types studied. These 5 sorghum types were, Feterita (white tannin), Framida*SDS[3843] 23-2-1 (red tannin), MMSH740 (brown tannin), MMSH625 (red non-tannin) and Sima (white non-tannin). Sodium hydroxide extracts from the 5 sorghum cultivars (Figure 2.3.1) showed maximum absorbance in the UV region (320-350 nm) (Figure 2.3.2). Phenolic compounds such as anthocyanins, flavan-3-ols, and condensed tannins absorb at 270-280 nm and at 310-370 nm for other flavonoids and some phenolic acids (Markham 1982; Putman and Butler 1988; Velioglu and Mazza 1991). Therefore it is possible that the compounds in the NaOH extracts absorbing at 320 - 350 nm may be phenolic in nature. The absorption maxima of the NaOH extracts observed at 320-350 nm were much higher for the tannin sorghums than the non-tannin ones. Only NaOH extracts from the tannin sorghums showed substantial absorbance in the visible light range of 520-550 nm (Figure 2.3.2). This suggests that the compounds absorbing in this region are tannins as extracts from the non-tannin sorghums showed no absorbance in the visible region.

The absorbance spectra of NaOH extracts of the sorghums do not reflect accurately the colour of the grain as seen by the eye. For example, Feterita, a tannin sorghum which appears white when measured with the tristimulus colorimeter was grouped with the other tannin sorghums (red and brown) which all had high absorbance in the visible region. Overall, the UV-visible spectra show a clear distinction between tannin and non-tannin sorghums (Figure 2.3.2). Extracts from the tannin sorghums had much higher absorbance maxima in the UV region than extracts from non-tannin sorghums. The tannin sorghum NaOH extracts absorbed in the visible region while the extracts from non-tannin sorghums did not. Figure 2.3.1 also shows that the NaOH extracts from the three tannin sorghums appeared much darker than the extracts from non-tannin sorghums. These results show that UV-visible spectrophotometry of NaOH extracts from sorghum could be a potential method of separating tannin sorghum grains from non-tannin grains.

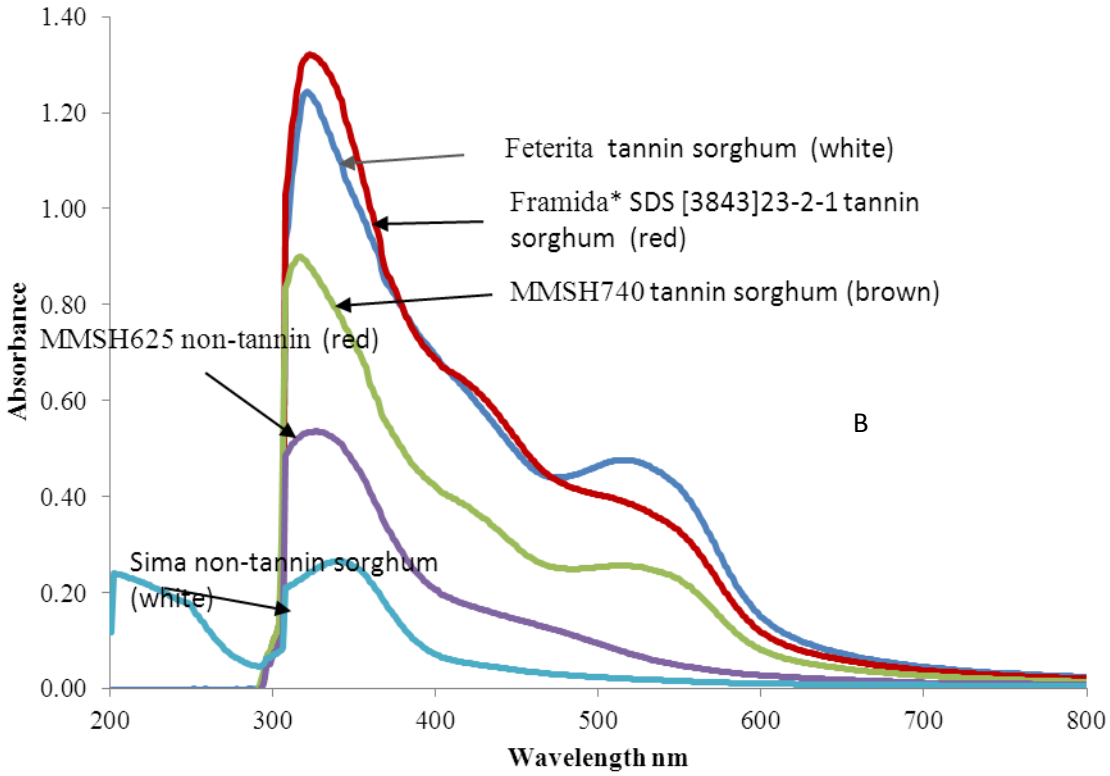


Fig 2.3.2 UV-visible spectra of NaOH extracts prepared from whole grain of five sorghum types

2.3.4.3 Relationship between sorghum grain colour and absorbance of NaOH extracts at 480 nm and 515 nm

In order to investigate a possible relationship between colour of the grain as measured by Tristimulus colorimetry and pigmented compounds within the grain, correlations between L* values and absorbance of the NaOH extracts at 480 and 515 nm were determined. These wavelengths were chosen because 480 nm has been used to detect sorghum 3-deoxyanthocyanidin pigments (Awika et al 2004) and 515 nm was a wavelength of maximum absorbance in the visible region from the UV-visible spectra (Figure 2.3.2). Sorghum grain colour L* values significantly and negatively ($r = -0.79$, $p<0.001$), ($r = -0.81$, $p<0.001$) correlated with absorbance of sorghum grains treated with 1.25 M NaOH at 480 nm (Figure. 2.3.3a) and 515 nm (Figure 2.3.3b), respectively. Generally, the more pigmented grains (lower L* values) produced NaOH extracts with higher absorbance (more pigmented) at the two wavelengths. The lighter coloured grains with higher L* values (mainly the white tan-plant sorghums) produced NaOH extracts with much lower absorbance (less pigmented).

There were however, some exceptions. MMSH625, a pigmented (red, lower L* values) non-tannin sorghum produced an NaOH extract with low absorbance (less pigmented) (Figure 2.3.3a and b). Feterita, a white tannin sorghum (high L* value) was also an exception with its NaOH extract having substantially high absorbance at 480 and 515 nm. Therefore, the absorbance of the NaOH extracts did not always have a consistent relationship with the grain colour. However, the correlations in Figures 2.3.3a and b show that there was a clear separation of non-tannin from tannin sorghums. The NaOH extracts from tannin sorghum always had higher absorbance at 480 nm and 515 nm irrespective of the colour of the grain.

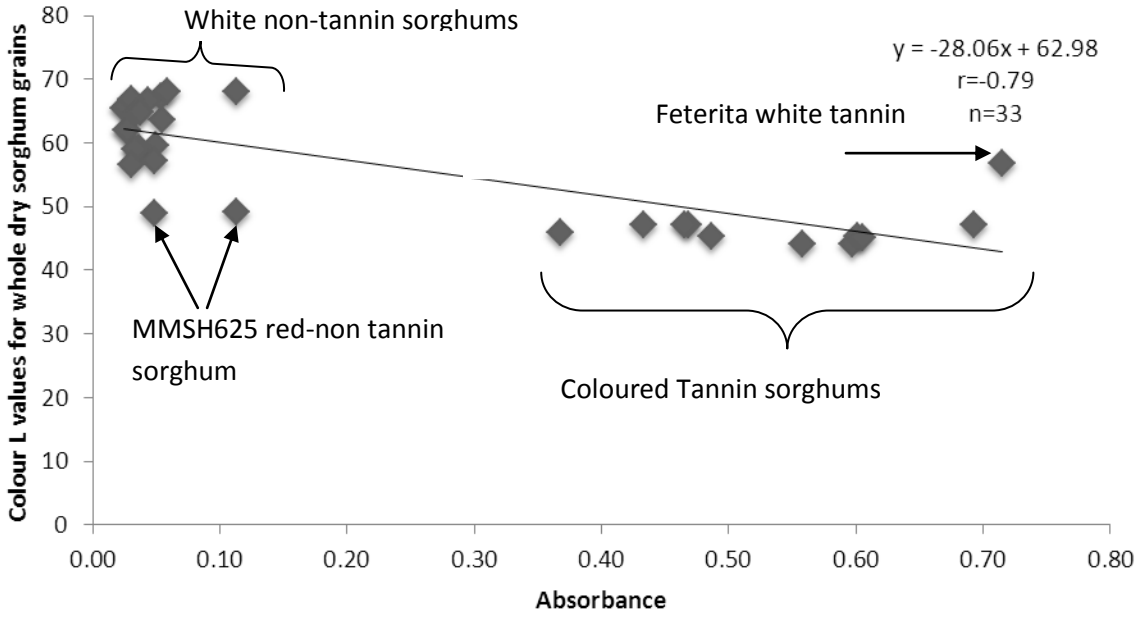


Figure 2.3.3a. Relationship between whole dry sorghum grain L* values and absorbance of their NaOH extracts at 480 nm. n= 33: (White sorghum = 21, Coloured sorghum (brown and red) =12, Level of significance $p<0.001$

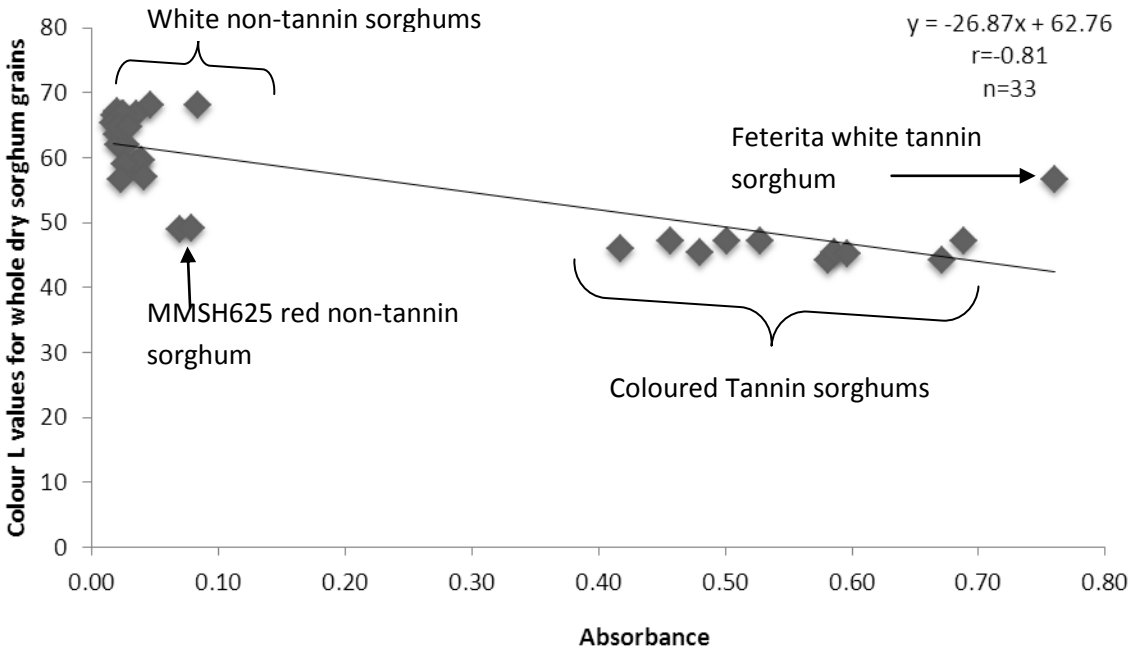


Figure. 2.3.3b Relationship between whole dry sorghum grain L* values and the absorbance of their NaOH extracts at 515 nm n= 33 (White sorghum =21, Coloured sorghum (brown and red) = 12, n= 33: Level of significance $p<0.001$.

2.3.4.4 Effects of cultivar and season with their interactions on whole dry sorghum grains and grains treated with NaOH

Analysis of effects of cultivar and season with their interactions on whole dry sorghum grain and grain treated with NaOH showed that cultivar ($p<0.001$), season ($p<0.05$) and cultivar and season interaction ($p<0.05$) had significant effects on the L*, a* and b* values of the sorghums (Table 2.3.2). A combination of genetics and environmental effects influence the colour of grain sorghum (Waniska, Hugo and Rooney 1992; Waniska 2000; Dicko et al 2002a). Genetics plays a role in sorghum kernel pigmentation (Rooney and Miller 1982). Two genes B_1 B_2 control the presence and absence of a pigmented testa (Rooney and Miller 1981). Grain colour is also mainly due to the flavonoid pigments, anthocyanins and flavan-4-ols, which are located mainly in the pericarp (reviewed by Taylor and Duodu 2009). Regardless of the treatment, cultivar had a strong effect on L*, a* and b* values of sorghum grains. Cultivar and season interactions to a lesser extent also affected the colour of sorghum grains.

TABLE 2.3.2 Summary of statistics for effects of cultivar, season with their interactions on colour L*, a* and b* values of dry whole grains of sorghums without and with 1.25 M sodium hydroxide treatment

Source	DF	SS	Mean Square	F Value	p	DF	SS	Mean Square	F value	p
	Dry whole sorghum grain					NaOH treated whole dry sorghum grain				
Colour (L) values for all Zambian sorghum cultivars										
Cultivar	15	4957.10	330.47	8677.04	<0.001[a]	15	1394.60	92.97	258.82	<0.001
Season	1	1.92	1.93	59.46	<0.05[b]	1	0.02	0.02	0.06	<0.05[b]
Cultivar*Season	15	12.10	0.81	21.16	<0.05[b]	15	10.97	0.73	2.04	<0.05[b]
Colour (a*) for all Zambian cultivars										
Cultivar	15	41.07	2.74	28.36	<0.001[a]	15	112.76	7.52	46.47	<0.001[a]
Season	1	0.10	0.10	1.07	<0.05[b]	1	0.20	0.20	1.24	<0.05[b]
Cultivar*Season	15	1.56	0.10	1.08	<0.05[b]	15	2.59	0.17	1.07	<0.05[b]
Colour (b) values for all Zambian sorghum cultivars										
Cultivar	15	532.38	43.90	7.60	<0.001[a]	15	111 7.72	74.51	233.27	<0.001[a]
Season	1	0.18	0.04	0.04	<0.05[b]	1	0.36	0.36	1.13	<0.05[b]
Cultivar*Season	15	10.3	0.70	0.15	<0.05[b]	15	4.73	0.32	0.99	<0.05[b]

Level of statistical significance (a) p<0.001, (b) p<0.05 n=16.

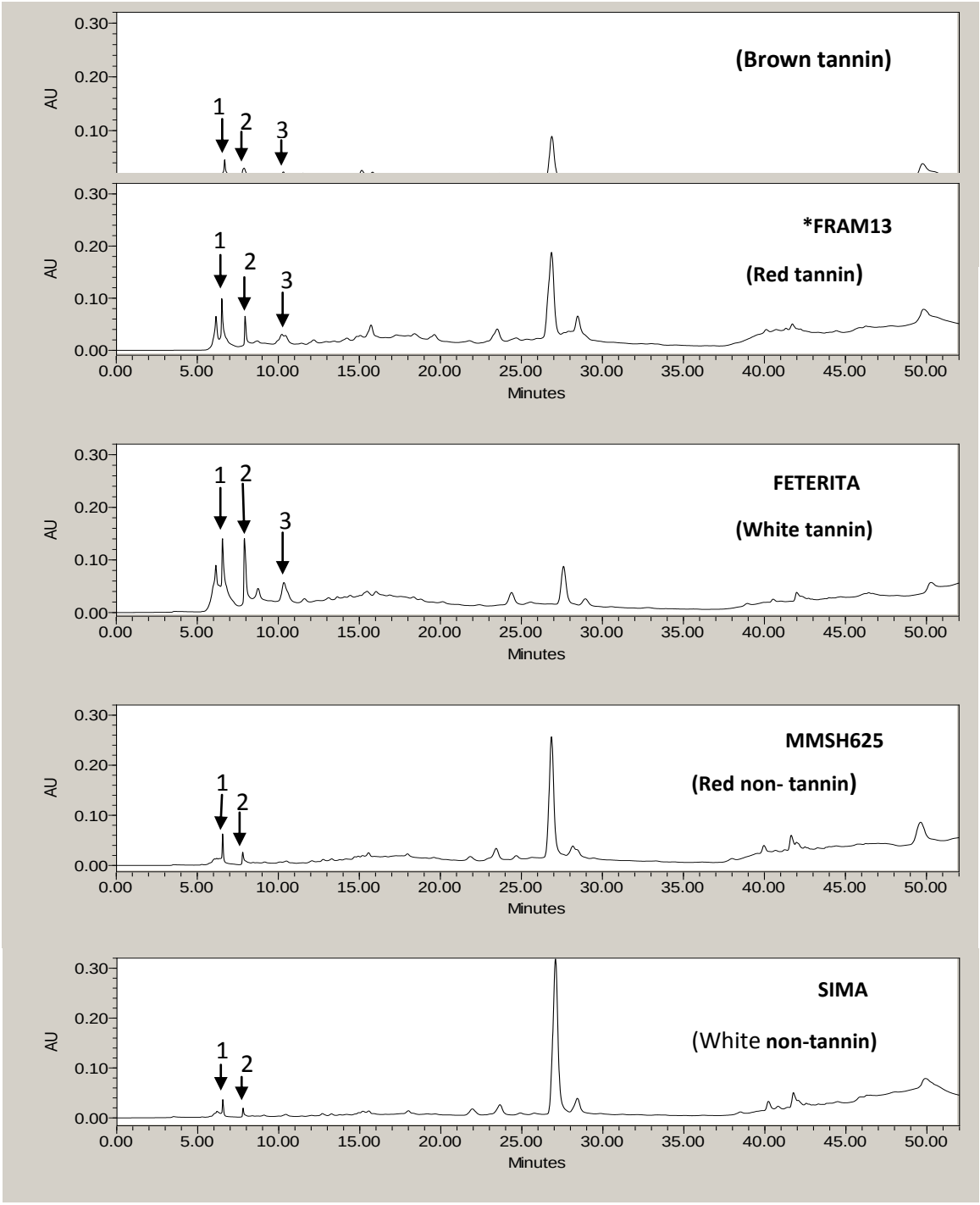


Figure 2.3.4 HPLC chromatograms monitored at 280 nm of NaOH extracts of various sorghum types. Peak 1 – 6.6 min; Peak 2 – 7.9 min; Peak 3 – 10.3 min. *FRAM13 - Framida SDS[3843] 23-2-1

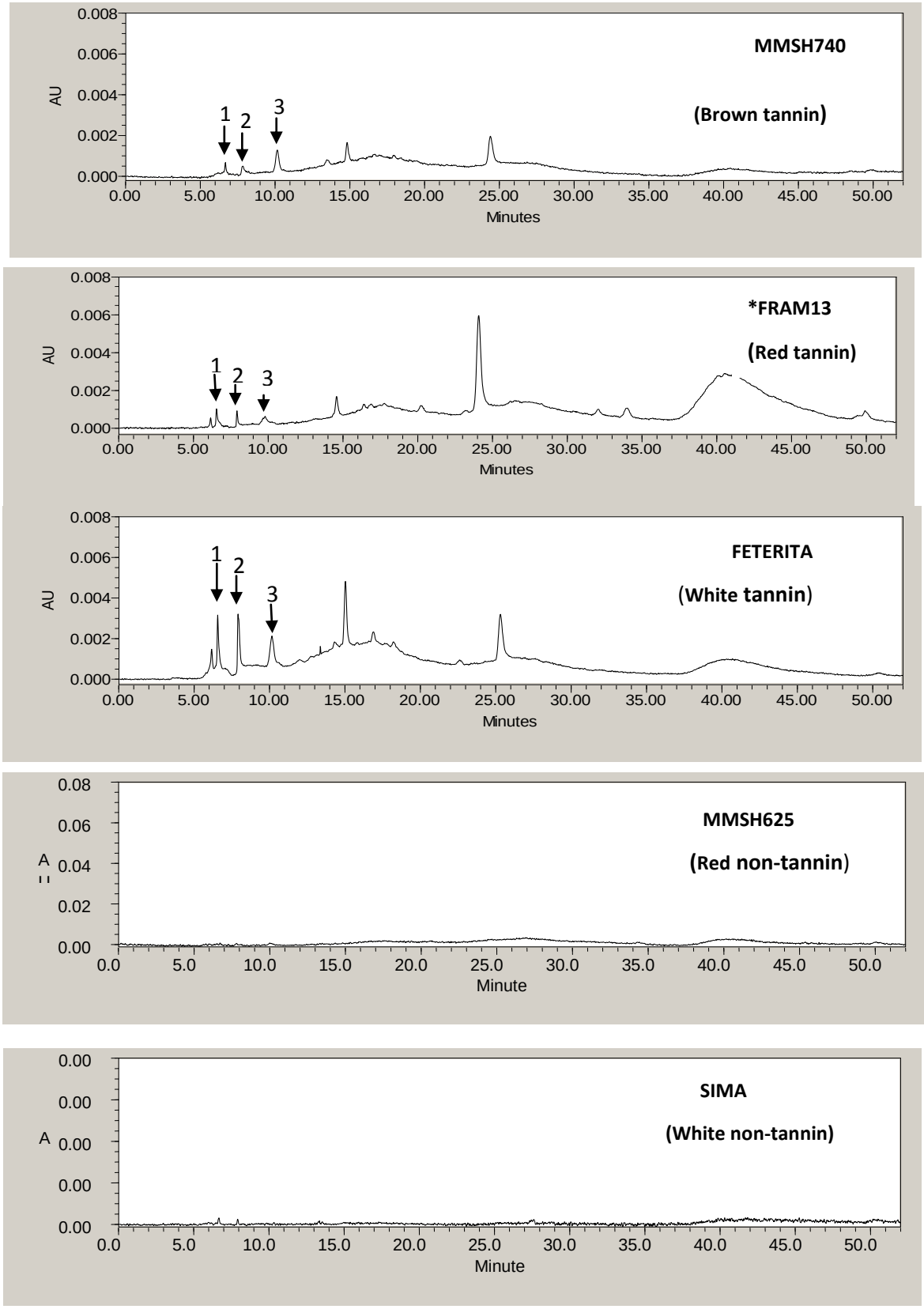


Figure 2.3.5. HPLC chromatograms monitored at 480 nm of NaOH extracts of various sorghum types. Peak 1 – 6.6 min; Peak 2 – 7.9 min; Peak 3 – 10.3 min. *FRAM13 - Framida [3845] 23-2-1

2.3.4.5 HPLC analysis of NaOH extracts from sorghum

The main objective of the HPLC analysis was to determine what types of phenolic compounds were present in the NaOH extracts from sorghum and to relate them to sorghum surface colour. Since phenolic pigment standards were not available, 280 nm and 480 nm were chosen as diagnostic wavelengths. Peaks at the same retention time at 280 nm may be due to phenolic compounds (Velioglu and Mazza 1991). If these peaks are present at the same retention time at 480 nm they could be due to phenolic pigments. Awika et al (2004) detected 3-deoxyanthocyanidin pigments from black and red sorghum at 480 nm during HPLC analysis. The NaOH extracts from tannin sorghums (Feterita, Framida*XDS[3845] F6-5 and MMSH740) showed peaks at retention times of 6.6 min, 7.9 min, and 10.3 min at 280 nm (Figure 2.3.4) and at 480 nm (Figure 2.3.5). On the other hand NaOH extracts from non-tannin sorghums (MMSH625 and Sima) only showed peaks at retention times of 6.9 min and 7.9 min at 280 nm (Figure 2.3.4), but showed no peaks at all, at 480 nm.

The HPLC results are in agreement with the UV-visible spectrophotometry results. The areas of peaks at 6.6 min and 7.9 min in the UV region (280 nm) for the NaOH extracts were higher for tannin sorghums than for non- tannin sorghums (Table 2.3.3). In the visible region (480 nm), NaOH extracts from non-tannin sorghums showed no peaks at all (Figure 2.3.5, Table 2.3.3). The peaks observed at retention time 10.3 min at 280 nm and 480 nm could be due to tannins because they were observed only in NaOH extracts from tannin sorghums.

The HPLC analysis is therefore, able to separate tannin from non-tannin sorghum. However it is not able to separate the grains according to colour as seen by the eye or as measured using Tristimulus colorimetry. This is demonstrated by the fact that both sets of samples (tannin and non-tannin) as distinguished by the HPLC method contained white and pigmented sorghum types. In general, organic solvents are used to extract pigments from sorghum and other cereals. These include acidified methanol (Nip and Burns 1968 and 1971; Awika et al 2003; Awika et al 2004) and aqueous acetone (Kaluza et al 1980; Awika et al 2003). These solvents are recognised as good extractants for pigments and are used regularly to extract colour pigments from sorghum and other cereals. This study shows that NaOH can also be used to extract compounds that may be phenolic pigments.

Table 2.3.3 Areas of peaks with retention times of 6.6 min, 7.9 min, and 10.3 min of HPLC chromatograms monitored at 280 nm and 480 nm of NaOH extracts from five sorghum types

Cultivar	Type	280 nm						480 nm					
		6.6 min		7.9 min		10.3 min		6.6 min		7.9 min		10.3 min	
		Peak area	CV	Peak area	CV	Peak area	CV	Peak area	CV	Peak area	CV	Peak area	CV
MMSH740	Brown tannin	1095440 (48349)[1]	4.41	713422 (10282)[1]	1.44	555012 (24105)[1]	4.34	27280 (1411)[1]	5.17	13712 (274)1	1.99	24733 (1161)[1]	4.70
FRAMxSDS3845] 23-2-1	Red tannin	1145075 (38134)	3.33	734385 (18432)	2.51	611594 (17275)	2.82	22019 (2504)	11.37	15962 (1319)	8.26	15016 (652)	4.34
Feterita	White tannin	2547018 (82228)	3.23	1811456 (75214)	4.15	1360918 (31050)	2.28	48495 (4411)	9.10	33119 (1714)	5.18	31660 (705)	2.23
MMSH 625	Red non-tannin	207866 (5683)	2.73	253176 (75214)	3.16	[2]ND		[2]ND		[2]ND		[2]ND	
Sima	White non-tannin	97829 (7758)	7.93	133977 (2612)	1.96	ND		ND		ND		ND	

[1]Standard deviation in parenthesis, [2]ND - Not detected, CV – Coefficient of variation

2.3.5 Conclusions

Tristimulus colorimetry is able to distinguish white from coloured sorghums (brown and red), but it is unable to separate tannin from non-tannin sorghums. Thus sorghum kernel surface colour does not accurately indicate the presence of tannins in tannin sorghum. Although generally lighter coloured sorghum grains produce less pigmented sodium hydroxide extracts, the extracts from tannin sorghums (irrespective of surface colour of the grain) tend to be more pigmented and absorb strongly in the visible region. Sodium hydroxide treatment therefore makes it possible to distinguish tannin from non-tannin sorghums either by using UV-visible spectrophotometry or HPLC. The HPLC method, while offering similar information as the UV-Visible spectrophotometry, is expensive. Therefore simple colorimetric analysis of sodium hydroxide extracts from sorghum can be considered an alternative to the normally used Chlorox bleach test in distinguishing tannin from non-tannin sorghums as it gives additional information such as the absorption of tannin and non-tannin sorghums as opposed to colour changes only as observed with the former test.

2.4 Characterisation of phenolic compounds and pigments in sodium hydroxide extracts from kernels of different sorghum types using UPLC/PDA/MS

2.4.1 Abstract

Sorghum grain colour is important in differentiating between sorghum types. Sodium hydroxide (NaOH) treatment has been used to differentiate between wheat types according to colour, but not in sorghum. The NaOH treatment causes leaching out of pigments from the grain pericarp that would normally not be measured in untreated grains using tristimulus colorimetry. Since sorghums differ in their content of pigments such as 3-deoxyanthocyanins, it may be possible to use NaOH treatment to differentiate between sorghum types. In this study, phenolic compounds and pigments in NaOH extracts from five sorghum types namely, Framida *SDS[3845]23-2-1 (red tannin), MMSH740 (brown tannin), Feterita (white tannin), Sima (white non-tannin) and MMSH625 (red non-tannin) were characterised using UPLC/PDA/MS. Eleven phenolic compounds in total, consisting of two flavan-3-ols, five anthocyanins and four 3-deoxyanthocyanins were identified. NaOH extract from Framida *SDS[3845]23-2-1 (red tannin) contained all of the eleven compounds identified. The flavan-3-ols (catechin and +-catechin -3-O gallate) were present in NaOH extracts of all the five sorghums. While some anthocyanins could be identified in NaOH extracts from all five sorghums, only Framida *SDS[3845]23-2-1 (red tannin), Sima (white non-tannin) and MMSH625 (red non-tannin) contained any 3-deoxyanthocyanins. Total peak area due to anthocyanins and 3-deoxyanthocyanins was higher for NaOH extracts from tannin sorghums compared to non-tannin. NaOH treatment of sorghum kernels may therefore be used to separate tannin sorghums from non-tannin.

2.4.2 Introduction

Some of the major flavonoids in pigmented sorghum include flavan-3-ols and anthocyanin and 3-deoxyanthocyanin pigments. Flavan-3-ols are monomeric units which are mainly linked through C4 and C8 bonds and sometimes at C4 and C6 bonds. The flavan-3-ol subunits may carry acyl or glycosyl substituents of which the most common substituent bond as an ester is gallic acid to form –*o*-gallatte. The flavan-3-ols monomeric subunits are the building blocks of proanthocyanidins (PA).

Sorghum 3-deoxyanthocyanidins and methylated 3-deoxyanthocyanidins have been found to be the main anthocyanins in sorghum. However, complete information about the 3-deoxyanthocyanin composition of sorghum is lacking (Wrolstad et al 2005). The 3-deoxyanthocyanidins are not common and specifically distributed in certain plants. Sorghum is considered to be the only dietary plant source. The lack of the 3-hydroxyl group makes the 3-deoxyanthocyanidins very different from the other anthocyanins (Dykes and Rooney 2006). Unlike other anthocyanidins with a 3-hydroxyl group, they can occur naturally in the aglycone form. They are also reported to be very stable in acidic solutions compared to other anthocyanidins (Stintzing and Carle. 2004). All of these properties may confer unique biological effects (Wu and Prior 2005). Apigeninidin and luteolinidin are the two common 3-deoxyanthocyanidins in sorghum and are responsible for the red colour of sorghum grain pericarp (Bate-Smith 1969). Awika et al (2003) identified 7-O-methylapigeninidin from *Sorghum caudatum*. The major anthocyanidins reported in sorghum (in decreasing order of natural prevalence) include cyanidin, pelargonidin, peonidin, delphinidin, petunidin (Yasumatsu et al 1965; Wu and Prior 2005; Awika et al 2004b; Koud-Bonafos et al 1996; Pale et al 1997).

Pigmented sorghums also contain flavan-4-ols such as apiforol (leucoapigeninidin) and luteoforol (leucoluteolinidin) that are believed to be precursors of the 3-deoxyanthocyanidins (Ferreira and Slade 2002; Haslam 1998; Hagerman 2005). Black pericarp sorghum types have been reported to contain higher levels of flavan-4-ols and anthocyanidins than other varieties of sorghum (Dykes et al 2005).

The flavones (flav-3-en-ols) are another important group of flavonoids reported in pigmented sorghums. The main flavones in sorghum are apigenin and luteolin and their presence have been reported in red (Dykes et al 2009) and lemon-yellow (Dykes et al 2011) sorghum genotypes. The flavanones eriodictyol and its 5-glucoside (Kambal and Bate-Smith 1976;

Yasumatsu et al 1965; Gujer, Magnolato and Self 1986) and naringenin (Gujer et al 1986; Awika et al 2003) have also been reported in sorghum.

Organic solvents are normally used to extract pigments from sorghum and other cereals. These include acidified methanol (Nip and Burns 1968 and 1971; Awika et al 2003; Awika et al 2004) and aqueous acetone (Kaluza et al 1980; Awika et al 2003). From the existing literature, it appears NaOH has not been used for the primary purpose of extracting phenolic pigments from sorghum. However, it is well known that NaOH does extract pigments and NaOH treatment of wheat kernels has been used to differentiate between wheat types according to colour (Matus-Cadiz et al 2008). The objective of this study was to identify phenolic compounds and pigments in sodium hydroxide extracts of five sorghum types and relate the phenolic profiles to the sorghum kernel colours.

2.4.3 Materials and Methods

2.4.3.1 Grain samples and preparation of NaOH extracts

Five sorghum types namely Framida *SDS[3845]23-2-1 (red tannin sorghum), MMSH740 (brown tannin), Feterita (white tannin), MMSH625 (red non-tannin) and Sima (white non-tannin) were used in this study. Extraction of pigments using sodium hydroxide solution was done according to Lamkin and Miller (1980) with modification. Whole grains (2.5 g) of each sorghum type were weighed separately into Falcon tubes and 3.25 ml of 1.25 M NaOH was poured over the grains in each tube. The tubes were left to stand for 1 h with intermittent mixing. The NaOH extract solutions were then decanted off and filtered through Millex LG 0.20 µm polytetrafluoroethylene (PTFE) syringe filters into amber glass vials to be used for UPLC/PDA/MS analysis.

2.4.3.2 Identification of phenolic compounds using Ultra Performance Liquid Chromatography coupled with diode array detector and a mass spectrometer (UPLC/PDA/MS)

A Waters Acquity UPLC/MS system (Waters Corp., Milford, MA) was used. The UPLC was equipped with a binary solvent manager, sample manager, column heater and photodiode

array detector. The system was interfaced with a tandem quadrupole (TQD) mass spectrometer equipped with an electrospray ionisation (ESI) source.

UPLC/PDA/MS conditions: A Waters UPLC BEH C18 column (100 x 2.1 mm i.d., 1.7 µm particle size) with a column pre-filter was used (Waters Corp., Milford, MA). Mobile phase A was 7.5% formic acid in HPLC grade water while mobile phase B was 7.5% formic acid in acetonitrile. Sample injection volume was 4 µl, auto sampler temperature was set to 12ºC and column temperature was 35ºC. Flow rate was at 0.1 ml/min and total run time was 30 min. Solvents were delivered in a linear gradient as follows: 99% A (0.5 min), 99 to 85% A (14.5 min), 85 to 77% A (5 min), 77 to 72% A (5 min), 72 to 0% A (1 min), 0% A (2 min) and 0 to 99% A (2 min). The Acquity UPLC PDA detector was set to acquire spectra data in the range of 210 to 500 nm. A Synapt HDMS detector with an ESI source was used for the analysis of phenolic compounds and all mass spectra data was acquired in a positive mode. MS spectra were acquired from 50 till 1500 Da with scan duration of 0.4 s. and an inter-scan delay of 0.02 s in the centroid mode. Acquisition in the centroid mode is essential for further data treatment with peak picking programs. Argon was used as a collision gas and leucine enkaphalin for lock mass calibration. The following MS parameters were used: capillary voltage: 3 kV; cone voltage: 15 V; source temperature - 125 °C; desolvation temperature - 275 °C; desolvation gas flow - 650 L/h.; collision energy - 4 eV. For the acquisition of MS/MS spectra, collision energies were set from 10 till 50 eV. Phenolic compounds data was acquired using MassLynx 4.1 instrument software (Waters, Milford, USA).

2.4.3.3 Results and discussion

2.4.3.3.1 Identification of phenolic compounds in NaOH extracts of five sorghum types

Figures 2.4.1 to 2.4.3 show chromatograms extracted at 280 nm of NaOH extracts from the five sorghum types. Table 2.4.1 shows the compounds identified in the extracts and their mass spectral data. The compounds were identified by comparing their elution profile, UV-visible spectra, molecular and fragmentation ions to literature information on phenolic compounds and databases such as Phenol explorer (Neveu et al 2010). Eleven phenolic compounds consisting of two flavan-3-ol compounds, five anthocyanin compounds and four 3-deoxyanthocyanin compounds were identified in the NaOH extracts of the five sorghum types (Table 2.4.1).

The flavan-3-ols identified were catechin (peak 2, m/z 289) and (+)-catechin-3-O-gallate (peak 6, m/z 442) (Table 2.4.1). These two flavan-3-ols were present in the NaOH extracts of all five sorghum types (Table 2.4.1). Flavan-3-ols (catechins) are commonly reported phenolic compounds in sorghum and occur mostly as glycosides with gallic acid as the most common substituent bound as an ester to form 3-O gallate (Hummer and Schreier 2008). In tannin sorghums, the proanthocyanidins (condensed tannins) consist of flavan-3-ol units as monomers linked by C-C (type B proanthocyanidin) and occasionally by C-O-C (type A proanthocyanidin) bonds (Kruger et al 2003; Awika and Rooney 2004b). In summary, catechins and their glycosides may occur as monomeric species in non-tannin and tannin sorghums or as subunits of proanthocyanidins in tannin sorghum types.

Anthocyanins identified were cyanidin (peak 1, m/z 287) and its hexoside (peak 5, m/z 287) and hexosides of delphinidin (peak 4, m/z 465), peonidin (peak 7, m/z 463) and malvidin (peak 8, m/z 493) (Table 2.4.1 and 2.4.2). All anthocyanins identified were present in the NaOH extract from Framida *****SDS[3845]23-2-1 (red tannin sorghum) while the extract from the brown tannin MMSH740 sorghum contained all the anthocyanins except cyanidin-3-O-hexoside. The NaOH extracts from the rest of the sorghum types, Feterita (white tannin), Sima (white non-tannin) and MMSH625 (red non-tannin) each contained only one anthocyanin (cyanidin, peonidin-3-O-hexoside and cyanidin-3-O-hexoside, respectively). Some of the anthocyanins identified in the NaOH extracts have been reported in sorghum. Anthocyanins earlier identified in sorghum include cyanidin, pelargonidin, peonidin, delphinidin and petunidin (Yasumatsu et al 1965; Koud-Bonafos et al 1996; Pale et al 1997).

The 3-deoxyanthocyanins identified were luteolinidin (peak 3, m/z 271), apigeninidin (peak 9, m/z 255), 7-methoxyapigeninidin (peak 10, m/z 269) and 5-methoxyapigeninidin (peak 11, m/z 283). All 3-deoxyanthocyanins identified were present in the NaOH extract of Framida *****SDS[3845]23-2-1 (red tannin sorghum) while the extracts from the brown tannin MMSH740 and white tannin Feterita sorghums did not contain any 3-deoxyanthocyanins. The NaOH extracts from the non-tannin sorghums Sima and MMSH625 each contained one 3-deoxyanthocyanin compound (luteolinidin and 5-methoxyapigeninidin respectively). The 3-deoxyanthocyanins are well known as important and unique types of phenolic pigments in sorghum. Compounds such as apigeninidin and luteolinidin (Awika et al 2004a, b; Guos 1989; Nip and Burns 1989), 7-methoxyapigeninidin (Pale et al 1997; Seitz 2004; Wu and Prior 2005) and 5-methoxyapigeninidin (Seitz 2004) have been reported in sorghum.

The absence of any 3-deoxyanthocyanins in the NaOH extracts of MMSH740 and Feterita was an unexpected result. 3-deoxyanthocyanins have been detected in both coloured and white sorghums (Awika et al 2003; Nip and Burns 1969 and 1971). This may be due to the fact that NaOH was used as an extractant in this work. However, the 3-deoxyanthocyanins are normally extracted with organic solvents such as acetone. This observation suggests that NaOH may not be as effective at extracting 3-deoxyanthocyanins as organic solvents such as acetone.

On examination of the results (Tables 2.4.1 and 2.4.2), there does not seem to be a clear relationship between the profile of phenolic pigments in the NaOH extracts and the colour of the sorghum kernels. The extracts from pigmented tannin sorghums (Framida *SDS[3845]23-2-1 and MMSH740) contained more anthocyanin compounds compared to the white tannin Feterita. However, extracts from MMSH740 and Feterita did not contain any 3-deoxyanthocyanins. The extracts from white (Sima) and red (MMSH625) non tannin sorghums contained one anthocyanin and one 3-deoxyanthocyanin each despite their difference in kernel colour. This apparent lack of association between the profile of phenolic pigments and colour of the grain may be due to the fact that pigments in the NaOH extracts may have come from the pericarp and testa and not simply the surface of the kernel. K ernel colour as seen by the eye and measured using techniques such as Tristimulus colorimetry is a surface measure which may not be influenced by pigments below the grain surface.

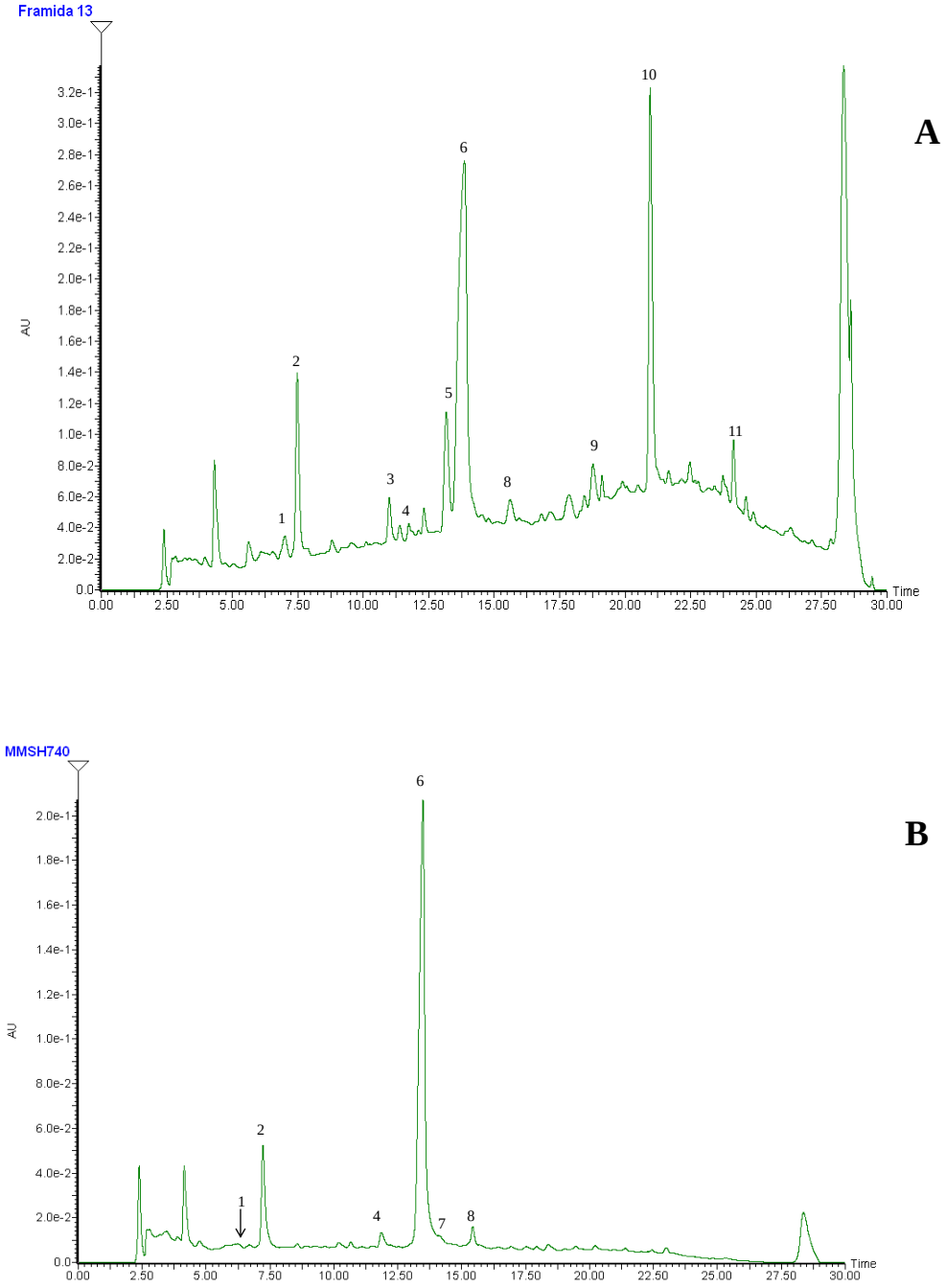


Figure 2.4.1 Chromatograms at 280 nm of NaOH extracts from (A) Framida*SDS[3845] 23-2-1 and (B) MMSH740 sorghums. Key to peak identity; 1: Cyanidin, 2: Catechin, 3: Luteolinidin, 4: Delphinidin-3-O-hexoside, 5: Cyanidin-3-O-hexoside, 6: (+)-Catechin-3-O-gallate, 7: Peonidin-3-O-hexoside, 8: Malvidin-3-O-hexoside, 9: Apigeninidin, 10: 7- Methoxyapigeninidin and 11: 5-Methoxyapegininidin

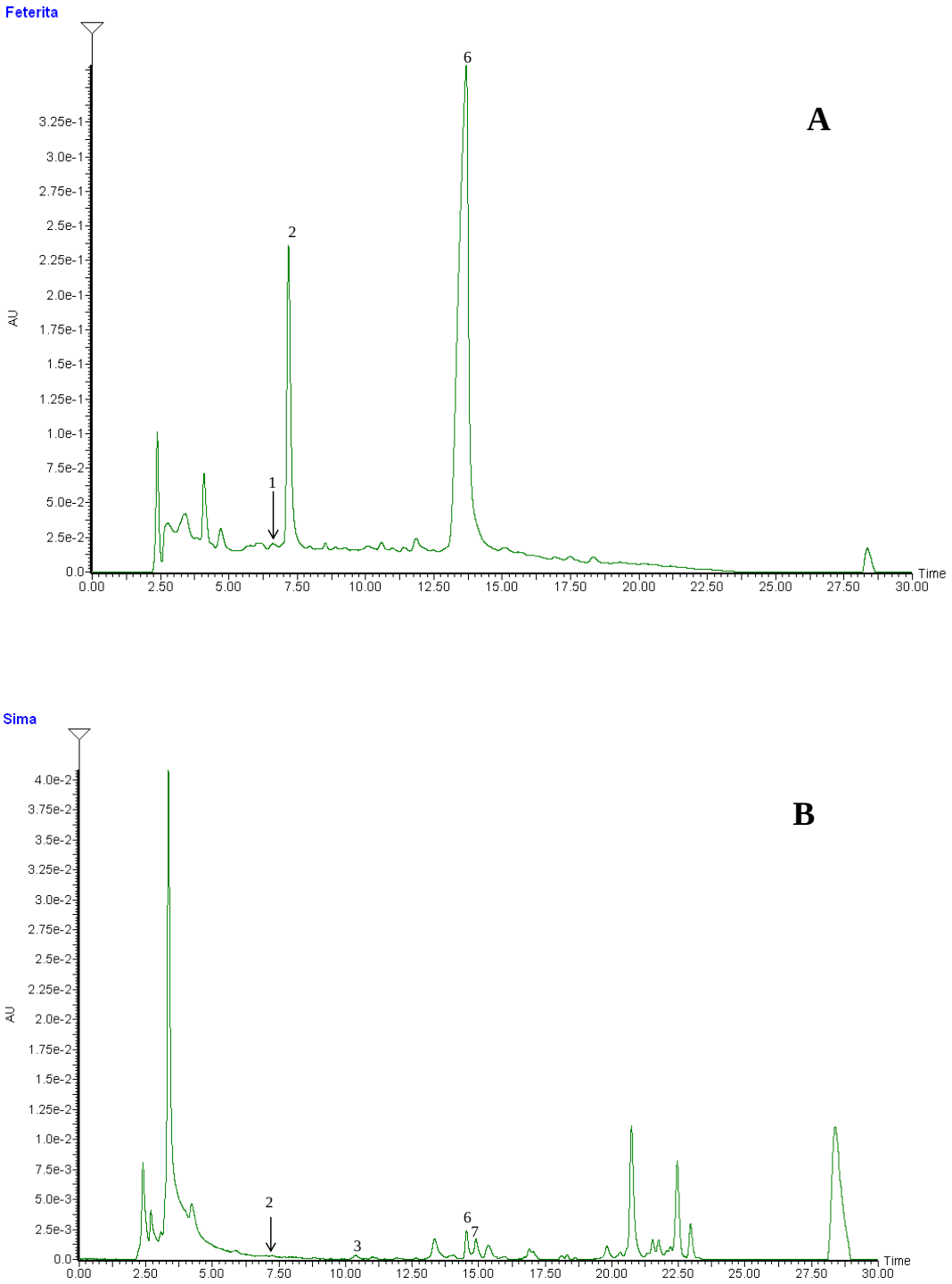


Figure 2.4.2. Chromatograms at 280 nm of NaOH extracts from (A) Feterita and (B) Sima sorghums. Key to peak identity; 1: Cyanidin, 2: Catechin, 3: Luteolinidin, 6: (+)-Catechin-3-O-gallate, 7: Peonidin-3-O-hexoside

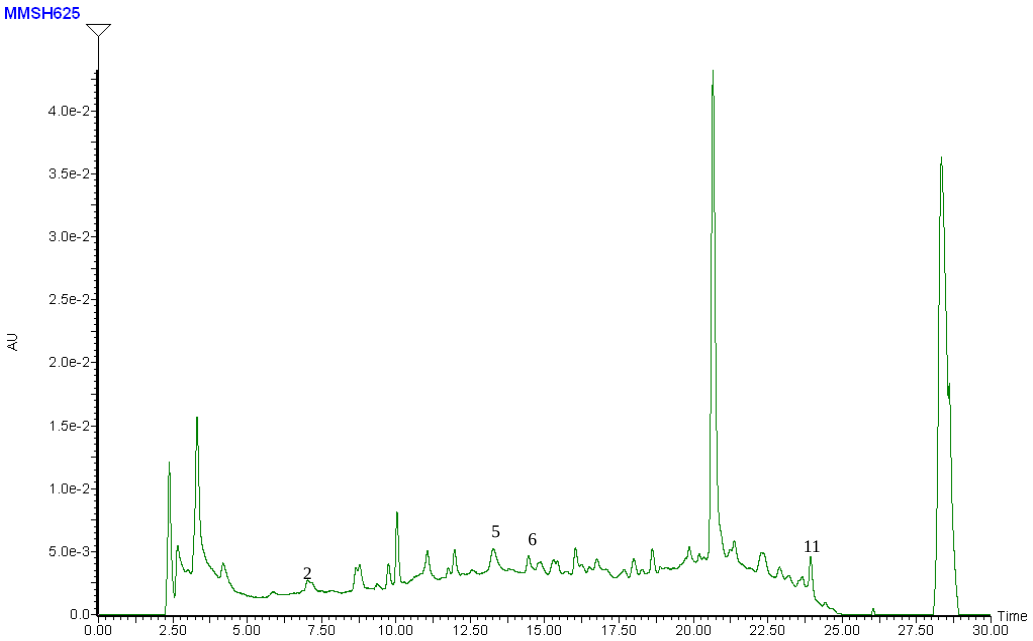


Figure 2.4.3 . Chromatogram at 280 nm of NaOH extract from MMSH625 sorghum. Key to peak identity; 2: Catechin, 5: Cyanidin-3-O-hexoside, 6: (+)-Catechin-3-O-gallate, 11: 5-Methoxyapegininidin

Table 2.4.1 Mass spectra and UV data for phenolic compounds identified in tannin and tannin-free sorghum samples

PK	*Rt	DAD (λ_{max} nm)	Parent ions *m/z* $[M+H]^+$	MS/MS Fragments *m/z*	Proposed compound identification	Tannin sorghum			Non-tannin sorghum	
						FRAM	MMSH740	FETERITA	SIMA	MMSH625
1	6.3	276	287.0	247.1	Cyanidin	✓	✓	✓	ND	ND
2	7.4	276, 411	289.0	187.0	Catechin	✓	✓	✓	✓	✓
3	10.5	277	271.0	257.1, 231.1	Luteolinidin	✓	ND	ND	✓	ND
4	11.7	319	465.0	303.1	Delphinidin-3-O-hexoside	✓	✓	ND	ND	ND
5	13.8	286	449.0	287.1	Cyanidin-3-O-hexoside	✓	ND	ND	ND	✓
6	14.3	321	442.0	194.1	(+)-Catechin 3-O-gallate	✓	✓	✓	✓	✓
7	14.8	342, 366	463.0	318.8, 301.1	Peonidin-3-O-hexoside	✓	✓	ND	✓	ND
8	15.2	298, 318	493.0	331.1	Malvidin-3-O-hexoside	✓	✓	ND	ND	ND
9	18.2	274	255.0	131.9	Apigeninidin	✓	ND	ND	ND	ND
10	21.8	276, 324	269.0	257.1	7-methoxyapigeninidin	✓	ND	ND	ND	ND
11	24.5	286	283.0	257.1, 231.3	5-methoxyapigeninidin	✓	ND	ND	ND	✓

PK: Peak number, *Rt: Retention time, $[M+H]^+$ molecular weight recorded in positive mode, ✓: detected, ND: not detected Framida 13- Framida *SDS[3845]23-2-1

2.4.3.3.2 Peak areas of identified phenolic compounds as an indication of their relative concentrations

Table 2.4.2 shows the peak areas of the phenolic compounds identified in the NaOH extracts. The peak areas give an indication of the concentration of the phenolic compound present in a given sample. Total peak areas for each of the three groups of phenolic compounds (flavan-3-ols, anthocyanins and 3-deoxyanthocyanins) were highest for the red tannin sorghum (Framida *SDS[3845]23-2-1).

Sima (white non-tannin sorghum) had higher total peak areas for each of the three groups of phenolic compounds than MMSH625 (red non-tannin sorghum) (Table 2.4.2). This was an unusual result as one would have expected the pigmented MMSH625 non-tannin sorghum to have a higher total peak area for the phenolic compounds. The apparent relatively high phenolic content of Sima as exhibited by its peak area may be attributed to the activation of the colour pigments in the stylar (a highly pigmented region of the sorghum kernel), by NaOH treatment. Previous research work by Blakely et al (1979) showed that colouration can occur in kernels both with and without a testa regardless of the pericarp colour. This colouration can affect the end use quality of white sorghum with a pigmented stylar. This result further supports observations in Chapter 3 and Chapter 4 that the types and levels of pigments in the NaOH extracts from the five sorghums do not necessarily correspond to the colour of the grain as seen by the eye.

Table 2.4.2 shows that the tannin sorghums (Framida *SDS[3845]23-2-1, MMSH740 and Feterita) had higher total peak area for anthocyanins compared to the non-tannin sorghums (Sima and MMSH625), indicating that the NaOH extracts from tannin sorghums had higher concentrations of the anthocyanins compared to the non-tannin sorghums. In other words, there was a separation of tannin sorghums from non-tannin based on anthocyanin concentration. This observation is in agreement with the findings in Chapter 3 (Section 3.2.4.2) on the UV-visible spectrophotometry of NaOH extracts from these same five sorghum types which showed that NaOH extracts from the tannin sorghums had higher absorption maxima in the visible region (480 and 515 nm) than those from the non-tannin sorghums. This result indicates that although NaOH treatment of sorghum grains may not be useful in separating the grains according to colour as seen by the naked eye, it may be a potentially simple method of separating tannin sorghums from non-tannin which can be easily applied by end users. In general, organic solvents such as acidified methanol (Nip and

Burns 1968 and 1971; Awika et al 2003; Awika et al 2004) and aqueous acetone (Kaluza et al 1980; Awika et al 2003) have been used to extract pigments from sorghum and other cereals. This study shows that NaOH can also be used to extract compounds that may be phenolic pigments from sorghum grains.

Table 2.4.2 Areas of peaks due to flavan-3-ol, anthocyanin and 3-deoxyanthocyanin compounds identified in UPLC chromatograms of NaOH extracts of different sorghum types

Compounds	Framida*	MMSH740	Feterita	Sima	MMSH625
Flavan-3-ols					
Catechin	1034315	378857	267195	432145	324693
+-Catechin-3-O-gallate	197393	85171	86743	121116	70302
Total flavan-3-ols	***1231708***	***464028***	***353938***	***553261***	***394995***
Anthocyanins					
Cyanidin	60908	213016	484587	ND**	ND
Delphinidin-3-O-hexoside	191117	75209	ND	ND	ND
Cyanidin-3-O-hexoside	201571	ND	ND	ND	62120
Peonidin-3-O-hexoside	205232	71625	ND	102776	ND
Malvidin-3-O-hexoside	210176	68266	ND	ND	ND
Total anthocyanins	***869004***	***428116***	***484587***	***102776***	***62120***
3-deoxyanthocyanins					
Luteolinidin	591080	ND	ND	269721	ND
Apigeninidin	212798	ND	ND	ND	ND
7-methoxyapigeninidin	231630	ND	ND	ND	ND
5-methoxyapigeninidin	230560	ND	ND	ND	73082
Total 3-deoxyanthocyanins	***1266068***	***ND***	***ND***	***269721***	***73082***
TOTAL PEAK AREA	**3366780**	**892144**	**838525**	**925758**	**530197**

* Framida – Framida *SDS[3845]23-2-1; **ND – not detected
Sorghum types were: Framida (red tannin), MMSH740 (brown tannin), Feterita (white tannin), Sima (white non-tannin), MMSH625 (red non-tannin)

2.4.5 Conclusions

NaOH treatment of five sorghum types (pigmented and non-pigmented, tannin and non-tannin) extracts three main groups of phenolic compounds namely, flavan-3-ols, anthocyanins and 3-deoxyanthocyanins. The NaOH extracts from the five sorghum types differ in their profiles of anthocyanins and 3-deoxyanthocyanins. There appears to be no relationship between the profile of phenolic pigments and their relative concentrations in the NaOH extracts from these grains and the colour of the grain as seen by the naked eye. The total peak area for anthocyanins in the NaOH extracts can be used to distinguish tannin from non-tannin sorghums. This can be a potentially simple, easy and cheap method of separating tannin and non-tannin sorghums which may be easily applied by various end-users.

3. GENERAL DISCUSSION

The first part of the general discussion is a critical evaluation of the way methods were applied in this study, with the purpose of revealing strengths and weakness in their applications as well as making suggestions for applying the methods better in future. The second section discusses the major findings of the research and their significance in relation to the determination of the end-use quality of sorghum. Finally, some recommendations for further research work are given.

3.1 Methodologies: A critical review

Sixteen sorghum cultivars (consisting of improved, open pollinating and hybrid forms) from two growing seasons 2008 and 2009 were selected and used for this study. These cultivars were grown at Golden Valley Research Station in Chisamba, Zambia under controlled conditions. The cultivars represented a wide variety of physico-chemical characteristics. They included cultivars with corneous, intermediate and floury endosperm texture and tannin and non-tannin sorghums. No indigenous sorghums or sorghums from local farmers were used in this study. It would have been useful to investigate how sorghums from local farmers in different growing regions and indigenous sorghums would have performed in the research activities which involved kernel hardness determination, polyphenol oxidase determination and colour determination. In addition, more insights about the effect of environment on the physico-chemical properties of sorghums could have been generated if the sorghums grown at Golden Valley Research Station were also grown in other geographical regions of Zambia. However, this was not possible due to time limitations.

Nonetheless, selection of the sorghums used was reasonably comprehensive as it covered two growing seasons allowing the effect of season and sorghum type to be studied (Chapter 2.1 Table 2.1.1). The study also included grain types with different pericarp colours and thickness. Sorghum glume colours ranged from tan to red to purple. The bleach test was used to detect the presence of tannin in the sorghums studied. The principle of the test as applied to sorghum grain is that sodium hypochlorite solution (Bleach) containing alkali dissolves away the outer pericarp layer of the grain submerged in the bleach solution revealing the presence of a black pigmented testa layer in the case of tannin sorghums or its absence in the case of non-tannin sorghums (Butler 1977; Waniska et al 1992). The advantage of this method is that it is inexpensive and rapid (Waniska et al 1992; Dykes and Rooney 2002). The limitation of this method is that it is prone to error due to interference from plant pigments in some

sorghum varieties which make it difficult to categorically identify the testa. Also weathered, insect-attacked and mouldy sorghums without a pigmented testa turn black after bleaching (Rooney 2005). The thorough cleaning, selection and removal of insect-damaged and mould-damaged grain removes the incidences of reporting false positives. The bleach test can be done in conjunction with quantitative methods for tannin estimation such as the Vanillin-HCl (Price et al 1979), and Prussian blue assays (Price and Butler 1977).

Kernel hardness estimation for the sixteen cultivars covering 2008 and 2009 seasons was done using three methods, namely endosperm texture, decorticating using the TADD and percent water absorption. The endosperm texture determination and abrasive decortication, which are established as methods of kernel hardness determination, were compared with percent water absorption, a method which was being proposed in this study to be used for determining kernel hardness.

The principle of endosperm texture determination is that sorghum grains are judged based on the relative proportions of corneous and floury endosperm. The grains are cut into halves longitudinally. One half is viewed with the naked eye and the relative proportion of corneous endosperm to floury endosperm is determined by reference to a standard. Endosperm texture is the proportion of corneous (vitreous or hard) fraction of the endosperm with respect to the floury or soft endosperm fraction (Hallgren and Murty 1983). Kernels with more corneous than floury endosperm are designated as hard or corneous, while those with more floury than corneous endosperm are termed soft (Rooney and Miller 1982). Kernels with identical proportions of corneous and floury endosperm are classified as intermediate (ICC 2008). The variation in proportions of corneous and floury endosperm in different sorghums is influenced by genetic and environmental factors (Serna-Saldivar and Rooney 1995). This method is cheap, simple and applicable to all normal (non-waxy) endosperm texture studies, but sample size representing a population is very small and the judgements of texture are subjective. Increasing the sample size and having more than one judge can make the method more effective as the overall hardness score of the same batch would have come from more than one person.

Grain decortication using the TADD works on the principle of progressively rubbing off the outer layers of dry kernel. The grains are decorticated by rubbing against the abrasive surface of the abrasive paper or carborundum disc, against each other and to a lesser extent against

the tube holding the grains. This leads to the separation of the bran (fines) from the kernels. Hard grains have less decortication losses compared to soft grains. The TADD measures the kernel's resistance based on strength of the grain against abrasive action (Anglani 1998; Lawton and Faubion 1989; Chandrashekar and Mazhur 1999). Grains with a high proportion of corneous endosperm tend to resist breakage during decortication and milling operations more than grains with a high proportion of floury endosperm (Rooney and Miller 1982). Grain hardness as determined using the TADD is expressed in terms of percentage extraction rate (Reichert et al 1986). The TADD used in this study was fitted with abrasive paper of 60 grit (Norton type R284 metalite), (Norton Abrasive, Worcester, USA) instead of a caborundum disk. The abrasive paper wears out frequently and a standard check sample needs to be included in each run to monitor wear. Another limitation with the TADD is that it is an expensive piece of equipment which is probably unaffordable and unavailable to subsistence farmers. The TADD is however able to achieve the intended objective of estimating kernel hardness of the sorghum cultivars (Chiremba et al 2011).

The principle of the percent water absorption method as applied to sorghum grains and other cereals is that grains have differing abilities to absorb water based on their relative hardness. Soft grains absorb water faster than hard grains. Water uptake in grains is said to be through portals in the endosperm cell walls and voids in the soft endosperm (Glennie 1984; Hoseney 1994; Buffo et al 1998). Hard grains have lower percent water absorption and require longer conditioning times than soft grains which need a shorter time to reach saturation or optimum saturation. Factors influencing water absorption in hard and soft grains include cell wall structure, type and concentration of prolamins and packing of starch granules within the protein matrix (Murty et al 1982a; Gomez et al 1997; Buffo et al 1998). In hard grains, cell wall polymers such as cellulose, arabinoxylans and other hemicelluloses are more rigid than in soft grains (Glennie 1984; Chandrashekar and Kirleis 1988, Ioerger et al 2007; Vebruggen et al 1997; Vebruggen 1996; Huisman et al 2000).

Ferulic acid and *p*-coumaric acid are believed to be involved in oxidative cross linking of polysaccharides and other cell wall components (Levigne et al 2004). The association of cell wall polymers with the kafirin prolamin storage proteins and phenolic acids such as ferulic acid and *p*-coumaric acid are thought to contribute to the strength of cell walls and hence impact on the way water is absorbed by sorghums with different endosperm textures (Vebruggen 1996). In corneous endosperm the protein matrix comprises a cement-like

continuous interface with starch granules (Hoseney 1994). This gives the corneous endosperm a compact structure with tightly packed protein bodies and therefore more opportunity for cross-linking between protein components (Shull et al 1990). The opposite is the case for the floury endosperm where protein bodies are not tightly packed and the protein matrix is discontinuous thus less opportunity for protein cross-linking (Shull et al 1990). In essence, the floury endosperm consists of a protein matrix and starch granules which are loosely packed in a discontinuous manner with air voids or spaces (Hoseney 1994), which provides an easier and quicker pathway for water absorption in the soft endosperm (Buffo et al 1998).

Sorghum kernel hardness may also be related to prolamin type and degree of crosslinking. Chandrashekar and Mazhar (1999) reported that hard grains generally contain more protein bodies with more crosslinked γ-prolamins. Ioerger et al (2007) reported that although sorghum floury endosperm contains a higher proportion of γ-kafirins, the vitreous (corneous) endosperm contained higher total kafirins and more insoluble polymeric proteins with a higher percentage of disulphide bonds. Therefore the degree of crosslinking was higher in sorghum corneous endosperm than in floury endosperm.

The application of the percent water absorption method to determine kernel hardness is dependent on availability of water within the location where the method is to be used. Another limitation of this research is that the water absorption method was not fully validated as a method for kernel hardness estimation. There is therefore an opportunity for the method to be validated, standardized and included as one of the methods for estimation of sorghum kernel hardness. Validation leading to standardisation of a method requires that the method be used regularly, subjecting it to inter-laboratory schemes in country and international schemes such as East, Central and Southern Africa (ECSA) inter-laboratory proficiency testing schemes and American Association of Cereal Chemists International (AACCI) method development procedures.

The diverse nature of kernel hardness assessment methods currently in use makes it a difficult task to harmonise them (Munck 1982; Anglani 1998). In the application of these methods to estimate grain hardness, different parameters are measured. Perhaps what is missing in all the methods described is local input in the sense that the end-users such as local farmers and traditional processors themselves should also have a say in defining the quality of the grain in

terms of hardness or softness. Information on how or whether in their locality they have different uses for hard or soft grains based on their understanding of their grains is crucial. That way the methods and intended benefits can be more easily understood.

In this research study, flours and porridges were analyzed for colour and PPO activity. In preparation for colour and PPO activity analyses, porridge was prepared according to Gomez et al (1997) with modifications, in that the porridge was prepared in two stages. The first stage for 15 min at 45^{o}C was to activate the PPO enzyme because it is most active at this temperature (Kong et al 2000; Salenheimo 2008). The second stage for another 15 min at 90^{o}C was to cook the porridge and denature the enzyme. The reason for preparing the porridge in two stages as described was in order to maximise enzyme activity. The two stage cooking method attained the desired objective of detecting PPO activity in the sorghum and maize porridges. Only white sorghum and white maize were used for this research because they are the grains of choice for the preparation of porridge, the staple food, which is variously known as *nsima*, *ugali*, *sadza* and *pap* in East and Southern Africa.

The PPO activity test was used to investigate the possible relationship between the relatively dark colour of products from white tan-plant sorghum types and grain PPO activity, using whole grain porridge as a simple food model. The principle is that the sorghum kernel contains phenolic compounds which react with oxygen to produce coloured pigments. Polyphenol oxidase catalyses the reaction of the phenolic compounds with oxygen to produce these complex coloured compounds (melanins) that can be measured at 475 nm (Espín et al 1995; Espínet al 1997; Dicko et al 2002a; Anderson and Morris 2001; Bettege 2004). Experiments measuring the amount of oxygen consumed by the reactants could have been conducted, to shed light on how oxygen, the other reactant in the PPO activity test affects PPO activity in sorghum. Due to limited time these were not conducted. The method used was however able to detect PPO activity in all the three cereals studied (sorghum, wheat and maize).

Sensory evaluation of the porridges could have been done in conjunction with the PPO activity test. Consumer sensory evaluation gives an indication of how the consumers of a product type would accept a particular product. Sensory evaluation of the sorghum porridges could have provided more insights and information about the acceptability of porridges by consumers where the cultivars from which they were made exhibited various levels of PPO activity. The importance of sensory evaluation was shown by observations on porridge

darkening which were made by Jagwer (1988) and Aboubacar et al (1999) in their work with white tan-plant sorghum flours before and after porridge preparation in Botswana and Niger, respectively. The observed darkening of porridge, though acknowledged, was not linked to PPO activity by these researchers. However, in this research, the intended objective was achieved in that the method used for determining PPO activity was able to establish a possible link between PPO activity and porridge darkening.

Flour colour was determined using the L* a* b* scale. L* value is a function of the green spectral contribution and is a measure of the brightness from black (0) to white (100) (Oliver et al 1992). The a* value is a function of the red-green difference with positive a* indicating redness and negative a* indicating greenness. The b* value is a function of the yellow-blue difference with positive b* indicating yellowness and negative b* indicating blueness. The units within the L* a* b* system give equal perception of colour difference to a human observer. According to Chedid and Kokini (1992) a* does not appear to be a good parameter for colour measurement of flour milled from white grain. The measure of greater importance and interest, particularly in this research is L* which measures the brightness of flour milled from white sorghum. All white tan-plant sorghums showed higher L* values compared to brown or red sorghums (Chapter 2, Table 2.1.3). The a* value may be more important when red or purple grains are milled because of their darker pigmentation. Porridge colour was measured as described for flour.

Treatment of the sorghum kernels with NaOH was done in an attempt to develop a simple, quantitative method for sorghum grain colour estimation. Work done by Matus-Cadiz et al (2000) on wheat showed that NaOH enhanced whole grain colour differences among genotypes. Lighter coloured genotypes were lighter and darker coloured genotypes were darker after treatment with NaOH. However, in sorghum, the effect of NaOH treatment was different from what was observed in wheat in that the alkali treatment did not enhance colour differences between lighter and darker coloured sorghums. Instead, NaOH treatment of the sorghum grains caused them to darken and the different sorghum types could not be differentiated based on colour differences. This may be because sorghum has more colour pigments than wheat. For this reason there is increasing interest in extraction of sorghum pigments which can be used as natural colours in food and other industrial applications.

The NaOH treatment caused leaching out of pigments from the sorghum grains. The alkali may cause hydrolysis of glycosylated pigments such as anthocyanins (Brouillard 1977). This releases the aglycones which can then leach out. There was intense colour development in the alkaline extracts which is an indication that anthocyanins have intense colour under alkaline conditions. This is because an increase in pH favours intense colour development such as the quinoidol blue forms which only occur in alkaline conditions (Figure 3.1.1) (Clifford 2000; Ojwang 2006). According to Sosulski and Dabrowski (1984) phenolic extracts at pH 9.3 exhibit significant increases in colour intensity. They attribute this to oxidative changes in the soluble phenolic esters as well as flavonoid and quinone compounds and their interactions under alkaline conditions

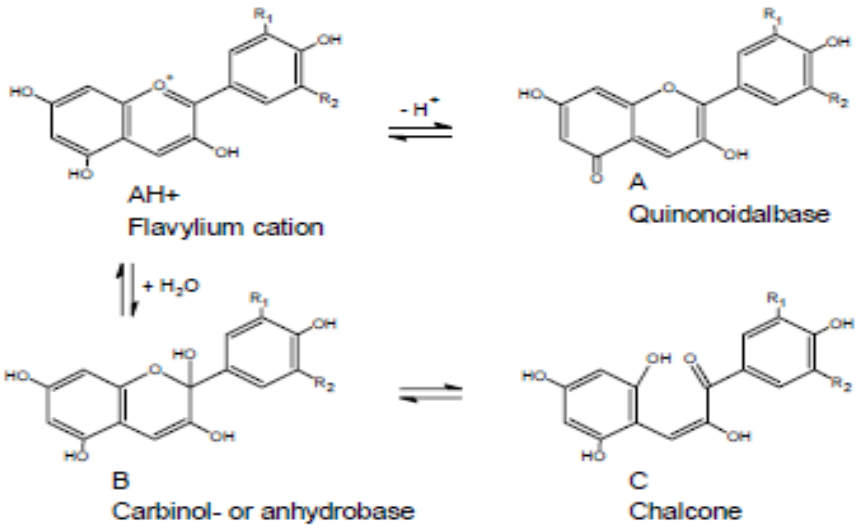


Figure 3.1.1 The four equilibrium forms of anthocyanin existing in aqueous media under acidic and alkaline conditions (Rein 2007).

Reversed phase high performance liquid chromatography (HPLC) analysis of NaOH extracts from the sorghum grains was conducted in order to investigate if there was any relationship between types and levels of phenolic pigments in the extracts and sorghum kernel colour. In the absence of standards, 280 nm and 480 nm were chosen as diagnostic wavelengths. Peaks at the same retention time at 280 nm may be due to phenolic compounds (Velioglu and Mazza 1991). If these peaks are present at the same retention time at 480 nm, it was reasoned that they were due to phenolic pigments. Awika et al (2004) detected 3-deoxyanthocyanidin pigments from black and red sorghum at 480 nm by HPLC.

The major limitation with the HPLC analysis was that it only allowed speculation regarding the identity of the NaOH extract chromatogram peaks. Ultra performance liquid chromatography coupled with photodiode array detection and mass spectrometry (UPLC/PDA/MS) enabled better characterization of phenolic pigments and other phenolic compounds in the extracts. Areas of peaks due to flavan-3-ol, anthocyanin and 3-deoxyanthocyanin compounds identified in the chromatograms of NaOH extracts provided information about the relative concentrations of these compounds in the sorghum cultivars.

LC-MS of the NaOH extracts did not identify and characterise some phenolic compounds that are normally extracted using organic solvents like acidified methanol (Nip and Burns 1968 and 1971; Awika et al 2003; Awika et al 2004) and aqueous acetone (Kaluza et al 1988; Awika et al 2003). This is possibly because the NaOH was unable to extract such compounds from the sorghums. For instance in this research the 3-deoxyanthocyanins were absent in the NaOH extracts of MMSH740 (a brown tannin sorghum) and Feterita (a white type II tannin sorghum), although these compounds have been previously detected in coloured and white sorghums (Awika et al 2003; Nip and Burns 1969 and 1971). However, more fundamentally, the use of NaOH was to investigate what pigments are present when NaOH is used to characterise sorghums in terms of their colour. The use of NaOH also provides an alternative way of extracting phenolic compounds from cereals where known extraction solvents are not easily or readily available.

3.2 Research findings

Sorghum cultivars have a wide range of physico-chemical characteristics which are important in terms of both their agricultural productivity and their end-use quality. This means that particular sorghum cultivars can be utilised in different food products on the basis of the particular characteristics they possess. Physico-chemical information is important in plant breeding as desired traits can be passed on to new cultivars. Thus, germplasm possessing such desirable traits can be stored in gene banks for future use. The information is also important in that it can be used for future characterization of sorghum types not covered in this study such as newly-bred cultivars and landraces (traditional sorghums) grown by local farmers.

3.2.1 Physical characteristics

A summary of some physico-chemical characteristics of sorghum and their related end-use quality evaluation applications is given in Table 3.2.1. Sorghum is traded based on physical

characteristics such as colour, test weight and a thousand kernel weight. In the USA, sorghum grain trade is based on colour (GIPSA 2007). In Zambia, maize and sorghum trade is also based on colour both at commercial and village level (personal experience). Sorghum grain colour plays an important role in processing of products. White coloured sorghums are generally preferred for the preparation of different foodstuffs particularly porridges, and grits for brewing as adjuncts and preparation of non-alcoholic beverages. Coloured grains (brown and red) are used in brewing local opaque beer where they are used as malt grain.

Sorghum kernel size information is important in milling. Uniformity of grains in terms of size makes processing easier as they minimise the need for sorting grains into different sizes. Large to medium grains perform better in milling operations while the small grains tend to be removed with dust and bran due to their low weight. This information is important to millers and other food processors in Sub Saharan Africa and the SADC region in particular.

Test weight (grain bulk density) is important in grain trade. Underweight grain does not fetch a good price because underweight grain contributes to milling and end-use quality loss as they are blown off during grain cleaning operations reducing the quality of the resulting flour or grits in the process. Thousand kernel weight is an important measure of cereals where the weight of 1000 randomly picked kernels which indicate the grain's density is taken. The thousand kernel weight takes into account the smallness of the sorghum grain in a given batch as it represents the average weight of the kernels which are too small to be measured individually. It is also about eliminating shrivelled kernels. In cereal cultivation small grains tend to produce weak seedlings which result to underweight grain if the smallness is not genetic (Helm and Spide1990). Tannin sorghums are traded separately from non-tannin sorghum mainly because of their perceived negative impact on nutrient uptake in humans and livestock (GIPSA 2007).

Table 3.2.1 Physical and chemical characteristics of sorghum grain and their related end-use quality evaluation areas

Physical characteristics	Particular end-use quality evaluation area	Chemical characteristics	Particular end-use quality evaluation area	Typical regional end users
Colour	Trade, food processing, breeding and brewing	Moisture content	Storage, trade, food processing (milling, product preparation and development), nutrition	Grain storage facilities, traders, millers food processors, farmers and consumers
Tannins	Trade, food processing (milling and product preparation and development), breeding and brewing	Protein	Food processing (milling, product preparation and development)	Grain storage facilities, stores, traders, millers, food processors, farmers and consumers
Test weight	Trade, food processing (milling product preparation and development), brewing and breeding	Fat	Storage, food processing (milling, product preparation and development), milling) brewing	Grain storage facilities, traders, millers, food processors, farmers and consumers
Thousand kernel weight	Trade, food processing (milling, product development), brewing and breeding	Ash	Food processing (product preparation and development), milling, nutrition	Grain storage facilities, traders, millers, food processors, farmers and consumers
Kernel size	Trade, food processing (milling) brewing and breeding	Carbohydrates	Food processing (product preparation and development), milling, brewing	Food processors, famers, millers and brewers
Kernel hardness	Food processing (milling, product preparation and development), brewing and breeding	Colour pigments	Food processing (product preparation and development), milling, breeding and brewing	Food industry, farmers, brewers, textile and sorghum breeding programs
Glume colour	Food processing (product preparation and development), brewing and breeding	PPO activity	Food processing (product preparation and development), breeding	Food processors, farmers, consumers and sorghum breeding programmes
Germinative energy	Food processing (product preparation and development (malt foods), breeding and brewing			

Table 3.2.2 Visual kernel colour and endosperm texture characteristics of Zambian non-tannin and tannin sorghum cultivars

Cultivar	**Visual kernel colour**	**Endosperm texture**	**Sorghum type (Tannin / Non-tannin)**
ELT1-16	White	Intermediate	Non-tannin sorghum
ELT1-17	White	Intermediate	Non-tannin sorghum
Kuyuma	White	Corneous	Non-tannin sorghum
MMSH1257	White	Intermediate	Non-tannin sorghum
MMSH1340	White	Intermediate	Non-tannin sorghum
MMSH1040	White	Intermediate	Non-tannin sorghum
SDS 1958] 1.3.2	White	Intermediate	Non-tannin sorghum
SDS 5006WSV87]23-2-1	White	Corneous	Non-tannin sorghum
Sima	White	Corneous	Non-tannin sorghum
ZSV-15	White	Corneous	Non-tannin sorghum
MMSH375	Red	Floury	Tannin sorghum
MMSH413	Brown	Floury	Tannin sorghum
*MMSH625	Red	Floury	Non- tannin sorghum
MMSH740	Brown	Floury	Tannin sorghum
Framida*SDS[3845] F6-5	Red	Floury	Tannin sorghum
Framida*SDS[3845] 23-2-1	Red	Floury	Tannin sorghum

*MMSH625 was the only red sorghum among the coloured cultivars without tannins

Table 3.2.2 gives a summary of the visual kernel colour characteristics and endosperm texture of the Zambian sorghum cultivars used in this research. All white sorghums had intermediate or corneous texture and were non-tannin types. Coloured sorghums (red or brown) all had floury endosperm texture and were tannin types, with the exception of MMSH625 which was a non-tannin type (Table 3.2.2). This observation of white sorghums generally having corneous and intermediate endosperm and coloured sorghums having floury endosperm was also observed by Jambunathan et al (1992) and Chandrashekar and Mazhar (1999). They further suggested that this colour and endosperm difference could be responsible for mould resistance differences between white and coloured sorghums, with white sorghum being more resistant to mould attack than coloured sorghum because white sorghum absorbed less water than coloured grain.

Table 3.2.2 also shows that all tannin sorghums had floury endosperm. This is in agreement with other workers (Glennie, 1983; Hahn and Rooney1984; Dicko et al 2002a; Dicko et al 2005a,b; FAO 1995). From an agronomical pecipective tannins in floury endosperm protect the soft endosperm against abiotic and biotic predators, thus conferring agronomical advantage to these coloured sorghums as they can be grown in regions of the world where sorghum eating birds reduce crop yield (Chandrashekar and Mazhar 1999).

Kernel hardness or endosperm texture is one of the important grain quality criteria in sorghum end-use quality. In sorghum utilisation, endosperm texture affects both the decortication of the grain and the reduction of the decorticated grain into flour, which also affect the palatability and cooking quality of sorghum foods. Endosperm texture is also important in that it affects how grain is stored, milled and processed. In milling the endosperm texture has been shown to be the main property of the grain that influences milling performance (Kirleis and Crosby 1982). Grains with high proportion of corneous endosperm tend to be more resistant to breakage during decortication and milling than grains with a high proportion of floury endosperm (Rooney and Miller 1982). Hard grains store better than soft grains due to less susceptibility to insect attack.

This research showed that cultivar as well as season and cultivar interaction had a significant influence on TADD abrasive hardness index and percentage water absorption. According to Rooney and Miller (1982), in addition to genetic variation, the growing environment affects the expression of vitreousness in sorghum kernels which is related to grain hardness. Through

the use of the water absorption test, information on genetic variation and effect of the environment or seasonal variation on sorghum grain hardness could assist farmers in planning their agricultural activities such as buying of seed and it could also enable them to adapt and mitigate against adverse weather conditions such as droughts, floods, and extreme temperatures which might occur in some seasons. That way good crop yield and appropriate quality of sorghum for required end - uses could be maintained.

Table 3.2.3 shows a summary of the results obtained in this research for kernel hardness estimation using the endosperm texture, TADD and the percent water absorption methods. The cultivars that were identified as hard, intermediate and soft by the endosperm texture and the TADD hardness estimation methods were also identified as such using the percent water absorption method. This indicates that percent water absorption can be used to estimate kernel hardness. The simplicity of the percent water absorption method could make it attractive for use by farmers and traders as a method for estimating kernel hardness in grain trade and food processing. It can also be used together with other methods in agricultural research stations in remote areas to estimate kernel hardness as this attribute plays an important role in storage and food preparation in rural areas. Application of the method can improve extension services delivery, trade and food processing. Information on kernel hardness is important in grain trade and processing because it provides some insights on the status of the grain in terms of endosperm textural differences. The above information on estimation of sorghum grain hardness by grain water absorption will also be useful in studying other sorghum genotypes and landraces not covered under this study.

Table 3.2.3 Summary of results on kernel hardness estimations using endosperm texture, TADD, and percent water absorption

Hardness description	Endosperm texture	TADD	Percent water absorption
Hard	Sima, Kuyuma, ZSV-15, SDS 5006*WSV87] 23-2-1	Sima, Kuyuma, ZSV-15, SDS 5006*WSV87] 23-2-1	Sima, Kuyuma, ZSV-15, SDS 5006*WSV87] 23-2-1
Intermediate	MMSH1-16, MMSH1-17, MMSH1040, MMSH1340, MMSH1257, MMSH1257, MMSH625	MMSH1-16, MMSH1-17, MMSH1040, MMSH1340, MMSH1257, MMSH1257, MMSH625	MMSH1-16, MMSH1-17, MMSH1040, MMSH1340, MMSH1257, MMSH1257, MMSH625
Soft	MMSH375, MMSH413, MMSH740, Framida*SDS[3845] F6-5, Framida*SDS[3845] 23-2-1	MMSH375, MMSH413, MMSH740, Framida*SDS[3845] F6-5, Framida*SDS[3845] 23-2-1	MMSH375, MMSH413, MMSH740, Framida*SDS[3845] F6-5, Framida*SDS[3845] 23-2-1

TADD = Tangential abrasive decorticating device

3.2.2 Chemical characteristics

The findings of this study indicate that PPO activity was responsible for the porridge darkening in white tan-plant sorghums and in white maize. Figure 3.2.1 is a simple reaction scheme that illustrates the involvement of polyphenol oxidase in reactions that lead to production of complex coloured pigments (melanins) and hence darkening of products such as porridge made from white sorghum (Table 2.2.3).

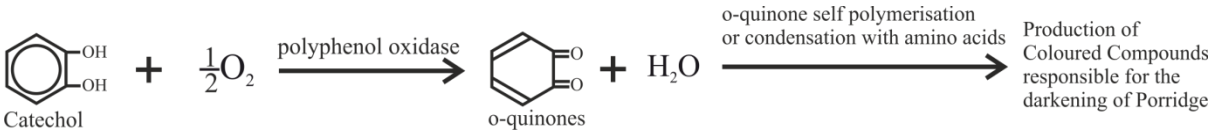


Figure 3.2.1 Proposed simplified reaction sequence for polyphenol oxidase (PPO) catalysed darkening of sorghum porridges

Polyphenol oxidase catalyses the oxidation of a phenolic substrate in the presence of molecular oxygen into quinones which undergo self-polymerization or condensation with amino acids to form complex coloured compounds known as melanins (Feillet et al 2000; Dicko et al 2002a). The addition of water and application of heat during porridge making would likely increase the PPO activity as its latent state, which is normally inactive, is activated by water, chemicals and heat (Feillet et al 2002; Junkanti et al 2003). The darkening was more intense in porridges made from sorghum because sorghum flour had more PPO activity than maize flour. Information on the role of PPO activity in the darkening of porridge will assist in breeding for sorghum cultivars with low PPO activity and hence improved end-use quality in products such as porridge, bread, tortilla, noodles and chapattis whose acceptability is based on aesthetic appeal.

One of the objectives of this study was to develop a simple method for sorghum kernel colour determination. Matus-Cadiz et al (2008) reported that NaOH treatment enhanced whole grain colour differences between wheat genotypes and successfully differentiated between red and white wheat grains. Based on this, it was hypothesized that a similar NaOH treatment of sorghum grains would also enhance colour differences and assist in separating sorghums based on kernel colour. However, NaOH treatment of the sorghum grains did not enhance colour differences but rather caused all the kernels (red or white) to become darkened. The

UV-visible absorption patterns and phenolic pigment composition of the NaOH extracts of the sorghums studied could also not be related to the colour of the sorghum kernels as seen by the naked eye or as determined using Tristimulus colorimetry.

This notwithstanding, the results obtained from UV-visible spectrophotometry of the sorghum NaOH extracts and phenolic pigment composition using chromatographic methods produced a clear separation of tannin type sorghums from non-tannin sorghums. The significance of this finding lies in the fact that it presents an opportunity for development of a simple method for separation of tannin from non-tannin sorghums using an NaOH treatment. Such a test could be used by rural farmers who may not have access to chemicals such as the Chlorox bleach reagent. Sodium hydroxide is readily available in the form of caustic soda. In this regard, it could be suggested that sorghum grains could be treated with 5% (m/v) caustic solution (equivalent to 1.25 M NaOH solution as used in this study). The colour intensity of the NaOH extracts obtained could then be examined possibly against the colour of NaOH extracts of a known tannin and non-tannin sorghum (which would act as a standard). Extracts from the tannin sorghum grains would be more intensely coloured than extracts from the non-tannin sorghums, thus providing a basis for their easy separation.

3.3 Developing a system for integrating end-use quality evaluation in the value chain of sorghum production and utilization

Sorghum is the second most important cereal crop after maize in Sub-Saharan Africa (FAO 2011). Its continued use as a staple cereal in Sub-Saharan Africa is dependent on the importance it plays as a staple food in drought prone parts of the region. It is well known that sorghum performs better than maize in drought prone areas (Bennett et al 1990). This means that there is a strong need for sorghum to continue to be the cereal crop for food and nutrition security in these areas.

Sorghum improvement programmes aimed at increasing production of sorghum were initiated in the SADC region by SADC/ICRISAT in 1983 through the Sorghum and Millet Improvement Program (SMIP) (Mgonja et al 2005). Significant improvements have been made through the introduction of new seed varieties by the SMIP using genotypes suitable for environments across SADC. In spite of this, increasing sorghum cultivation and utilisation still faces many challenges such as limited government support in terms of funding and farm input support, lack of collaboration between breeders and food scientists, low productivity,

lack of quality seed and quality sorghum for production of flour and malt for the opaque beer industry and limited products that can be marketed profitably. A success story worth mentioning is the use of sorghum in lager beer brewing. Crucially, it appears that end-use quality issues are not properly addressed at regional and country level. In addition, it seems that local sorghum landraces and their attributes for the processing of different food products have not been extensively studied. It is envisaged that the findings about end-use quality evaluation of sorghum from this study will provide the baseline information for potential application to sorghum landraces whose end use quality is less known.

The lack of a system integrating end-use quality evaluation in the sorghum value chain in Sub-Saharan Africa has contributed to low agricultural productivity which has led to the production of few quality sorghum-based products. Therefore, there is a need for an organised approach in the form of a system which integrates end-use quality evaluation in all aspects related to agriculture within the sorghum value chain such as sorghum genetic material storage, sorghum breeding, marketing and processing.

Figure 3.3.1 shows a proposed system of collaboration between major stakeholders in sorghum production, research and processing value chain in Sub-Saharan Africa. This system is driven based on the use of sorghum end-use quality evaluation methods such as those used in this research study. An important feature of this system would be the use of the end use quality evaluation methods studied in this research within the various components of the value chain such as the germplasm bank, agricultural research stations and laboratories, marketing institutions, farmers (large, medium and small) and non-governmental organisations that work in agriculture, agro-processing companies and cooperatives and small processors. Within these components of the value chain, it is envisaged that the methods will be used extensively by technical people such as plant breeders, agronomists, food scientists, nutritionists and extension workers with relevant training in agriculture and food science who can reach small scale farmers and processors. Within the system, collaborative work between the various role players and stakeholders is of importance and therefore there is a need for effective communication and message transmission. Such communication can be effected through an efficient agricultural extension service such as field days, demonstrations and farm group meetings in the farm blocks where sorghum is cultivated.

3.3.1 Activities and information flow amongst stakeholders

The major role of the germplasm bank is information storage and retrieval concerning new and improved breeding lines and landraces (Nguni 2011). Information flow from and to the bank is crucial for the success of plant breeding within the sorghum value chain. The role of the plant breeders is to breed new or improved sorghum varieties based on information about desirable qualities and traits received from the germplasm bank and agricultural research laboratories. Apart from desirable quality characteristics such as high yield, early maturing and disease resistant genotypes, other desirable end-use-quality characteristics related to nutritional and processing quality could be more emphasised (Dr Medson Chisi, personal communication, 2008) as targets for sorghum breeders to further improve end-use quality of sorghum. Sorghum plant breeding will also include breeding sorghums with low PPO activity to improve the aesthetic appeal of sorghum foods made using white tan-plant sorghum. The findings from this study could encourage continued study of sorghum types with colour and other end-use quality problems in order to broaden the utilization of sorghum. Agricultural research stations and laboratories will rely on the information about quality traits of newly bred sorghum genotypes from the breeders in conducting trials on the new sorghum types. The end-use evaluation methods should be applied during determination of the nutritional and processing quality of the new sorghums. Information obtained will go back to the sorghum breeders and to farmers who will grow these new and improved varieties (Figure 3.3.1).

Large scale farmers in this system, in addition to their other core farming activities will be encouraged to grow seeds for planting especially for small scale farmers who do not have the expertise to do so. Quality seed supply is a perennial problem for sorghum small scale farmers and as a result, sorghum productivity suffers**.** The Sorghum Improvement Program as mentioned earlier made significant contribution to the release of improved sorghum cultivars in SADC but sustainability in sorghum seed production is still not attained. The large scale farmers will also work with seed companies who have farms for seed for maize and other crops to go into joint ventures for sorghum seed growing. The medium and small scale farmers will also grow improved sorghum varieties with the end-use quality as the guiding factor. Information on the performance of the new cultivars will go back to the research stations for further evaluations. Small scale farmers grow sorghum landraces (traditional sorghum types) in addition to the improved varieties. However, the performance of these traditional sorghums is not fully known. Therefore in this system there will be a way of

evaluating their end-use quality based on the information from this study. The information regarding their performance in preparation of food products from sorghum will be passed to the national laboratories, plant breeders and germplasm bank.

Within the system, national research stations and laboratories will be instrumental in educating farmers about these end-use quality evaluation methods simplified for their use. This can be done through outreach programs involving agricultural extension service workers and non-governmental organisations working in agriculture. They will use visual aids such as sorghums with different kernel colours and will explain the importance of colour in trade and in processing. They could use sorghum NaOH extracts to separate tannin type from non-tannin sorghums. This will be possible because as shown in this research, the colour of 1.25 M NaOH extracts of tannin sorghum is more intense than colour of NaOH extracts of the same strength of non-tannin sorghum. Percent water absorption could be used to explain kernel hardness. This test should be of particular value as water is used in rural processing of grains like pounding using a pestle and motor, whose effectiveness is based on kernel hardness. Approaching sorghum growing and utilisation in this way will enlighten the farmers scientifically as well as visually on the type of sorghum to grow and its intended use. This collaborative approach will also make the importance of growing and utilising sorghum clearer as background information combining agriculture and utilization in extension work would be readily available through the outreach activities and use of common language of end-use quality evaluation in the proposed system.

Traders include large scale organisations such as the grain marketing boards and also large commercial and small scale traders. The grain marketing boards should evaluate sorghum quality for end-use using the methods listed in Figure 3.3.1. This information is important for farmers, research stations laboratories, sorghum breeders as well as the germplasm bank. The use of these end-use quality evaluation methods by traders will yield important and relevant information about sorghum quality within the trade environment, which will be used by other large traders. Small scale traders just like small scale framers could evaluate sorghum grain colour using the NaOH extract method. Thus, for example, adulteration of white tan-plant sorghum (which is in demand for lager beer brewing) with white tannin grains and coloured grains can be minimised. This is unlike what is obtaining currently where grains are mixed without clear separation of tannin from non-tannin sorghum. The traders could assess kernel hardness using percent water absorption, a method which they could easily use as it is not

complicated. Information from the traders could be fed back to large scale farmers growing seed and the medium and small scale farmers who grow sorghum for consumption and other processing concerns.

In this proposed system there will be a continuous assessment of end-use quality of sorghum by all the stakeholders and constant information flow on genetic traits, agriculture, research, marketing and processing within the value chain as depicted in Figure 3.3.1.

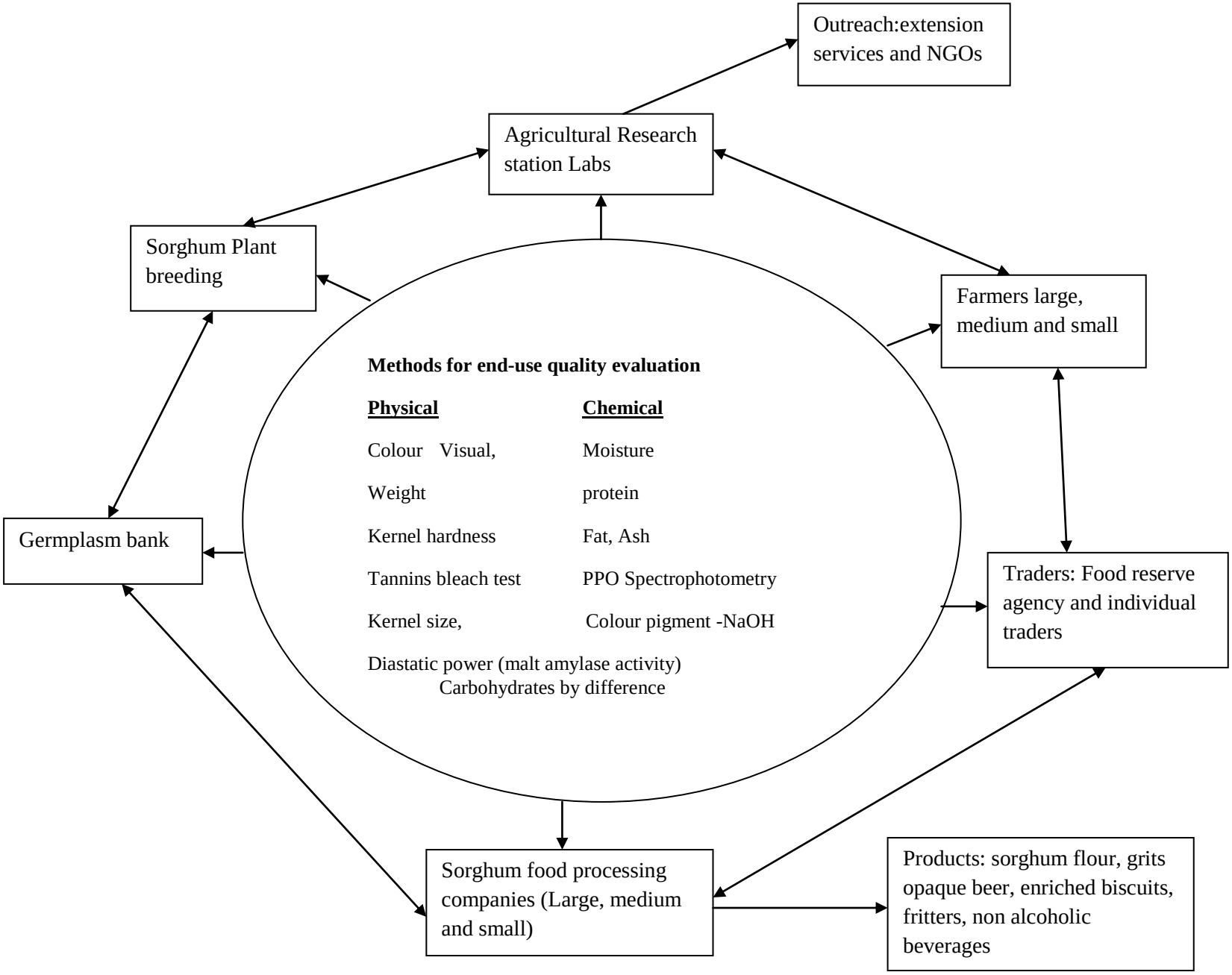


Figure 3.3.1 Proposed system for collaboration between major stakeholders in sorghum production, research and processing in Sub-Saharan Africa

4. CONCLUSIONS AND RECOMMENDATIONS

This study shows that apart from endosperm texture determination and abrasive hardness index using the TADD, percent water absorption could also be used to measure sorghum grain hardness. The percent water absorption method is cheaper for rural communities because it does not require expensive equipment as does the TADD.

PPO activity in white tan-plant sorghum is responsible for the darkening of porridges made using this type of sorghum. PPO catalyses a reaction between phenolic compounds in sorghum flour and oxygen producing quinones which in turn react with surrounding proteins to form melanoidin pigments which give white sorghum porridge a dark appearance.

The study established that the colour of NaOH extracts from sorghum do not correlate with the kernel surface colour as seen by the eye, because the NaOH extracts contain pigments located in other parts of the kernel apart from the surface of the kernel. NaOH treatment of sorghum does not enhance colour differences in the sorghum grain but rather narrows the colour differences making it difficult to differentiate sorghum cultivars based on colour. Therefore NaOH treatment of sorghum is not a good method for differentiating sorghum cultivars based on colour. However, sodium hydroxide treatment can be used to separate tannin from non-tannin sorghum based on differences in absorption maxima in the visible region of the alkaline extracts prepared from the sorghum kernel.

The knowledge of PPO activity in white tan-plant sorghums can be a catalyst for the study of the end-use of other sorghums not covered in this study such as sorghum landraces in countries within the SADC region and sub-Saharan Africa. Furthermore, the information on PPO activity and its role in the darkening of porridges from white tan-plant sorghums can propel the need for more collaborative work between sorghum breeders and food scientists in sorghum research and improvement in the SADC region and sub-Saharan Africa. The collaborative work will be crucial since sorghum in the SADC region suffers the same fate of low agricultural productivity and low acceptability and utilization due to negative perceptions.

It is recommended that plant breeders should study the genetics of PPO activity and identify the loci responsible for PPO activity in sorghum since PPO activity confers darkness in porridge made using white tan-plant sorghum. This will make it possible to select for sorghums with low PPO activity.

Sensory evaluation studies should be included in future studies of porridges made using sorghum with known PPO activity such as descriptive sensory evaluation to gauge people's perception on the colour and taste of the porridges.

There is need for the validation and standardisation of the percent water absorption method for evaluating sorghum kernel hardness in order to increase the number of methods for evaluating end-use quality of sorghum. Currently, methods for evaluating the end-use quality of sorghum are not as many as those for maize and wheat in the SADC region and Sub-Saharan Africa.

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APPENDIX

Publications and presentations from this work

Scientific paper

Doreen M. Hikeezi, Kwaku G. Duodu, Medson Chisi, Lloyd W. Rooney & John R.N. Taylor. 2013. Polyphenol oxidase activity in white tan-plant-type sorghums: An important determinant of the relatively dark colour of their porridges. International Journal of Food Science and Technology 48: 941-946.

Conference Paper

Doreen M. Hikeezi, Medson Chisi, Lloyd W. Rooney, and John R. N. Taylor. 2010. Development of a system for sorghum cultivar end-use quality characterization in: proceedings of the CST-SA-ICC International Grains Symposium on Quality and Safety of Grain Crops and Foods. M. Labuschagne and K. G. Duodu, eds. 3-5 February 2010, Pretoria South Africa. ISBN: 978 0 86886.

Poster

Doreen M. Hikeezi, Medson Chisi, Lloyd W. Rooney, and John R. N. Taylor. 2010. Development of a system for sorghum cultivar end-use quality characterization. Cereal Science and Technology- South Africa (CST-SA) - International Association of Cereal Science and Technology (ICC). International Grains Symposium on Quality and Safety of Grain Crops and Foods. 3-5 February 2010, Pretoria, South Africa.