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Phenolic content and antioxidant activity of South African sorghums and of flours and cookies made from them

By

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Submitted in partial fulfilment of the requirements for the degree

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DECLARATION

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

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DEDICATION

This dissertation is dedicated to my late father for inspiring me to achieve my goals and that the sky is the limit. To my late brother Makhosi, my sisters and their families, my beloved mother and daughter Thandekile, thank you for your support and love.

ABSTRACT

Phenolic content and antioxidant activity of South African sorghums and of flours and cookies made from them

By

Constance Chiremba

Supervisor: Dr K G Duodu

Co-supervisor: Prof J R N Taylor

Amongst cereals, sorghum is one of the major sources of phenolic compounds. South African cultivars have not been profiled for their phenolic content and antioxidant activity to highlight their potential benefits. Thus, South African sorghum cultivars representing different sorghum types were evaluated for total phenolic content, condensed tannin content and antioxidant activity and the effect of cultivar on their antioxidant activity. The presence of phenolic antioxidants in the different sorghum cultivars, created an opportunity to develop a sorghum product as a vector of the antioxidants. Cookies were a product of choice due to their shelf stability and high nutrient density. Sorghum cookies were produced from 70%, 90% and 100% extraction rate flours. The effects of flour extraction rates and cultivar on the total phenolic content, condensed tannin content and antioxidant activity of the cookies were determined. Consumer sensory evaluation was used to evaluate sorghum cookie acceptability against a wheat flour cookie.

Total phenolic content of the cultivars, determined by the Folin-Ciocalteu method was 0.20 to 1.42 g catechin equivalents (CE)/100 g. The total phenolic content was 3 to 7 times higher in condensed tannin cultivars than in tannin-free cultivars. Using the modified Vanillin-HCl method, condensed tannins were only measurable in the condensed tannin cultivars. They ranged between 5.16 and 8.39 g CE/100 g. Subsequently, the antioxidant activity of the condensed tannin cultivars measured by the ABTS radical scavenging assay was up to 4 times higher than in the tannin-free cultivars. The high phenolic content and antioxidant activity of condensed tannin cultivars was attributed to the contribution of condensed tannins. Therefore,

condensed tannin cultivars are a major source of antioxidants compared to tannin-free cultivars.

For each sorghum cultivar, cookies of 100% extraction rate flours had 2 to 3 times higher total phenolics compared to those of 70% extraction rate flours, while antioxidant activity was 2 to 10 times higher. Cookies of the condensed tannin sorghum had 2 to 5 times more phenolics compared to those of condensed tannin-free sorghum. Antioxidant activity was 145 to 227 µMol Trolox equivalents (TE)/g in cookies of condensed tannin sorghum compared to 10 to 102 µMol TE/g in those of condensed tannin-free sorghum. Processing sorghum flours to cookies seemed to reduce phenolic and antioxidant activity, but considering the flour component in the formula, cookie antioxidant activity was slightly higher than that of flours.

The texture of all sorghum cookies was less acceptable compared to that of wheat cookies. The consumers showed a slight overall liking of the condensed tannin-free sorghum and wheat flour cookies. The cookies from condensed tannin flours were neither liked nor disliked. Since generally wheat flour is used for making cookies, the similarities in the overall liking of the condensed tannin-free sorghum cookies and the wheat flour cookies indicate strong potential of sorghum flour for cookie making. Therefore, sorghum cookies have a potential as a functional ready-to-eat snack, with target consumers such as school children in feeding schemes to improve their health and nutrition status.

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Above all, thanks be to God, through Him, all things are possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

1.1 Statement of the problem

Sorghum (*Sorghum bicolor* (L) Moench) is an indigenous African cereal, well adapted to the semi-arid and arid sub-tropical conditions prevailing over most of the African continent (Doggett, 1988). Sorghum is important to food security in Africa and is used in a variety of traditional foods in the semi-arid tropics (Rooney, Waniska and Subramanian, 1997). In South Africa, benefits of sorghum have been recognized largely in malt quality. The country produces more than 200 000 tonnes of sorghum annually mainly for the production of commercial malt for opaque beer production and a small portion for malt breakfast cereal (Taylor and Dewar, 2001). However, demand for malt is declining as peoples' preferences are changing to lager beer. This means that there is a decline in the sorghum malt industry and an excess of sorghum is available in the market. In order to save industry from collapsing, alternative uses of the crop need to be identified.

South Africa has a high rate of malnutrition amongst children below the age of 5 years due to a number of issues such as food insecurity, poverty and most importantly compromised-immune systems. Human Immune Deficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS) have become a major threat in Sub-Saharan Africa with many households unable to afford antiretroviral drugs and primary health care. The infection rate in South Africa is one of the highest in the world and according to the World Health Organization report in 2006, it was estimated that the under 5 mortality rate is 67 per 1000 live births (UNAIDS, 2006). Research has shown that sorghum contains high levels of phenolics (Hahn, Rooney & Earp 1984). Phenolic classes found in sorghum are phenolic acids, condensed tannins and flavonoids (Hahn *et al.,* 1984). The structure of phenolic compounds allows them to donate hydrogen atoms or electrons for free radical scavenging, hence their antioxidant activity (Rice-Evans, Miller and Paganga, 1997). Studies have indicated that phenolic compounds are a major source of natural antioxidants in foods of plant origin (Hagerman, Riedl, Jones, Sovik Ritchard, Hartzfeld and Riechel, 1998) and amongst other properties exhibit antiviral activity. There is evidence that tannins inhibit the infection of human T-cells by inhibiting reverse transcriptase and therefore

may slow the progression to full blown AIDS (Nonaka, Nishioka, Nishizawa, Yamagishi, Kashiwada, Dutschman, Bodner, Kilkuskie, Cheng and Lee, 1990). Despite this evidence, researchers in South Africa have not profiled phenolic content and antioxidant activity of sorghum cultivars. The intervention of sorghum phenolics may be pertinent to addressing the issues around HIV/AIDS such as improving children's health and reducing mortality rates. There is also evidence that sorghum consumption in South Africa and other parts of the world is associated with reduced incidences of esophageal cancer when compared to other grains (Van Rensburg, 1981; Chen, Cole, Mi and Xing, 1993).

Phenolics need to be transferred to target consumers through consumption of convenient and affordable products and yet there are limited sorghum products in the market. Modern technologies such as extrusion cooking, micronisation, puffing, and baking may offer opportunities to produce a wide range of ready-to-eat foods and snacks of high value (Rooney and Waniska, 2000). These processes also permit blending of sorghum with other ingredients to make products with improved sensory properties similar to maize and rice (Gomez, Rooney, Waniska and Lusas, 1988). Such alternatives have not been explored for sorghum in South Africa. Thus, unless a major shift from traditional usage of the crop is embarked on, the benefits of phenolics may not be fully realized.

Sorghum products such as cookies may be used as vectors of phenolics and have a potential for school feeding schemes due to their high nutrient density. School feeding programmes are widespread in the developing world, including South Africa, to address the health and nutrition needs of school children. Nutrition and health status of children is compromised by factors discussed above, as well as hunger (Del Rosso, 1994). This is due to the long distances children have to travel to school, cultural meal practices that include no or small breakfasts or a lack of family time or resources to provide adequate meals to children before and/or during the school day (Del Rosso, 1994). Sorghum cookies have the potential of constantly supplying school children with a functional and ready-to-eat snack to address the nutrition and health problems affecting children.

1.2 Literature review

Phenolic compounds in sorghum fall into three broad classes, namely phenolic acids, flavonoids and tannins (Hahn *et al*., 1984). An important aspect of sorghum phenolic compounds is their potential to impact positively on human health because of their antioxidant and antiradical properties (Awika and Rooney, 2004). Innovative ways of incorporating sorghum into the mainstream diet may improve sorghum utilization (Awika and Rooney, 2004). However, sorghum grain and its products have been investigated only to a limited extent for their potential antioxidant properties (Awika, Rooney, Wu, Prior and Zevallos, 2003b). Therefore, this section will discuss phenolic compounds found in different types of sorghum and their mechanisms of antioxidant activity. The potential of sorghum use in ready-to-eat products as well as the effects of milling and baking on sorghum phenolic content and antioxidant activity will also be discussed.

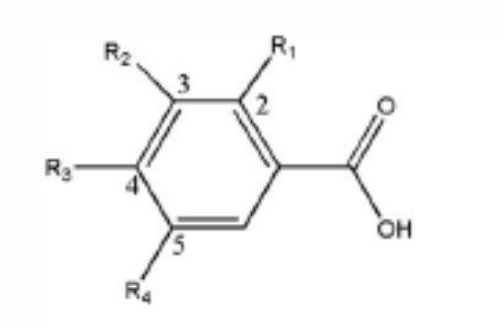
1.2.1 Chemistry of sorghum phenolic compounds

1.2.1.1 Phenolic acids

Phenolic acids are found in all sorghum types and exist as benzoic acid or cinnamic acid derivatives to form hydroxybenzoic and hydroxycinnamic acids, respectively (Dykes and Rooney, 2006). Hydroxybenzoic acids include gallic, *p*-hydroxybenzoic, vanillic, syringic and protocatechuic acids (Figure 1.2.1). Hydroxycinnamic acids include coumaric, caffeic, ferulic and sinapic acids. Phenolic acids exist either in a bound or free form. In the bound form they are mostly associated with cell walls. Ferulic acid is the most abundant bound phenolic acid in sorghum (Hahn, Rooney and Faubion, 1983; Hahn *et al.,* 1984). Free phenolic acids are located in the pericarp, testa and aleurone layers of the kernel. Cinnamic acid is only found in the free form (Hahn *et al.,* 1984). Chromatographic analysis has shown that tannin sorghum has the highest amount of free phenolic acids (Hahn *et al.,* 1983) and white cultivars without a pigmented testa contain the lowest amount of phenolic acids (Waniska, Poe and Bandyopadhyay, 1989). In white sorghum varieties or cereals with low levels of flavonoids, bound phenolic acids are a major source of antioxidant activity and a strong correlation has been found between antioxidant activity and bound ferulic acid

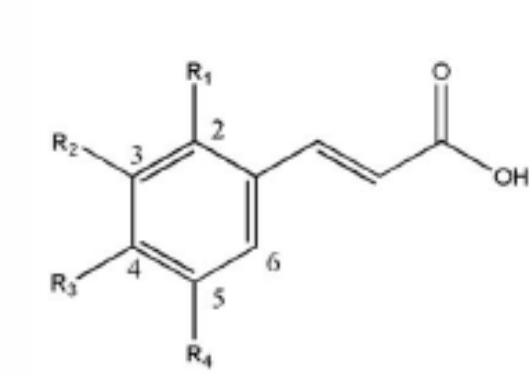
in wheat, maize, rice and oats (Adom and Liu, 2002). However, the composition of phenolic acids and ratio of bound to free phenolic acids is influenced by cultivar differences (Awika and Rooney, 2004).

Benzoic acid derivatives



	Functional group			
	R1	R2	R3	R4
Gallic acid	H	OH	OH	OH
Protocatechuic acid	H	OH	OH	H
P-hydroxybenzoic acid	H	H	OH	H

Cinnamic acid derivatives



	Functional group			
	R1	R2	R3	R4
Caffeic acid	H	OH	OH	H
Ferulic acid	H	OCH_3	OH	H
Sinapic acid	H	OCH_3	H	OCH_3

Figure 1.2.1 Structures of some phenolic acids identified in sorghum (Awika and Rooney, 2004).

1.2.1.2 Flavonoids

Flavonoids consist of three rings i.e., A, B (phenolics) and C (pyran) (Figure 1.2.2) with various levels of hydroxylation and methylation and are present in nature as glycosides (Cook and Samman, 1996). Classes of flavonoids include flavanols, flavones, flavanols, flavonones and anthocyanins. Their structure has a characteristic C_6-C_3-C_6 carbon skeleton (Kong, Chia, Goha, Chia and Brouillard, 2003). The most common anthocyanins in sorghum are the 3-deoxyanthocyanidins (Gous, 1989). These anthocyanins are distinct in that they lack a hydroxyl group at the 3 position (Figure 1.2.3) of the C ring. This feature increases their stability at high pH compared to other common anthocyanins. The two most common 3-deoxyanthocyanidins are apigeninidin and luteolinidin, which confer yellow and red colours, respectively. Other 3-deoxyanthocyanidins reported in sorghum include apigeninidin-5-glucoside, luteolinidin-5-glucoside, 5-methoxyluteolinidin and 5-methoxyapigeninidin among others (Dykes and Rooney, 2006). Luteolinidin and apigeninidin represent 36 to 50% of total anthocyanin content in black and tannin sorghums and 19% apigeninidin in red sorghums (Awika, Rooney and Waniska, 2004). Other flavonoids reported in red sorghums are flavan-4-ols such as luteoforol and apiforol. Flavones such as apigenin and luteolin have also been isolated and identified in sorghum and are predominant in tan plant sorghums (Awika *et al.,* 2004).

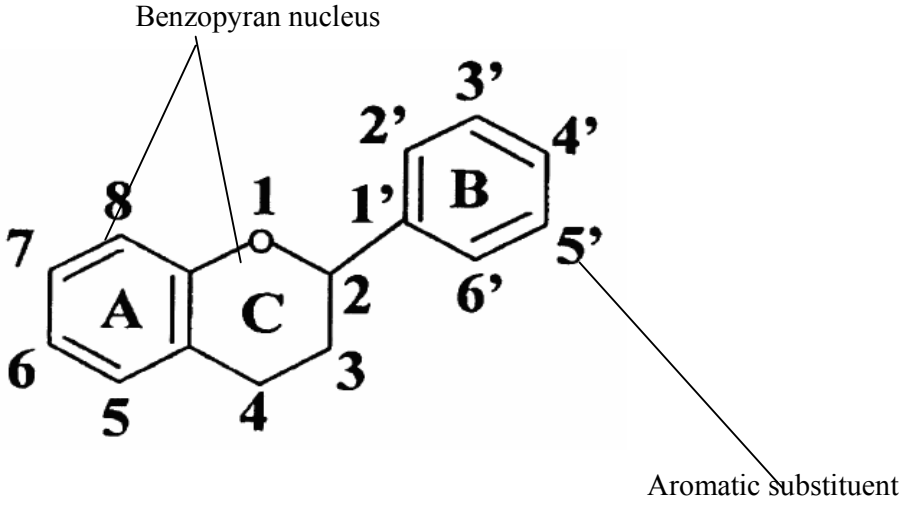
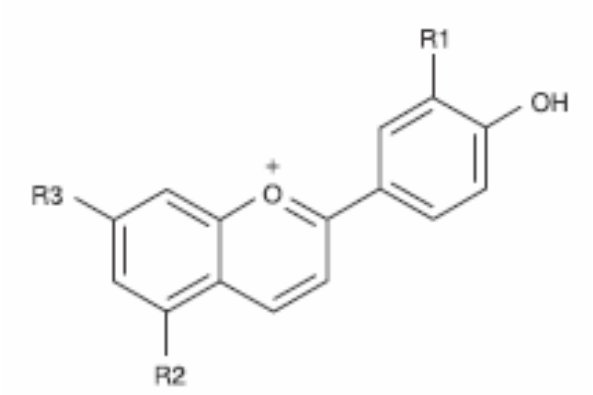


Figure 1.2.2 Basic flavonoid ring structure (Cook and Samman, 1996)



	Functional group		
	R1	R2	R3
Apigeninidin	H	OH	OH
Luteolinidin	OH	OH	OH
5-methoxyapigeninidin	H	OCH_3	OH
5-methoxyluteolinidin	OH	OCH_3	OH

Figure 1.2.3 Structures of 3-deoxyanthocyanidins found in sorghum (Dykes and Rooney, 2006)

1.2.1.3 Condensed tannins

Tannins of sorghum are of the condensed type (proanthocyanidins). Proanthocyanidins have also been reported in wheat (McCallum and Walker, 1990) and barley (Gupta and Haslam, 1978). Condensed tannins are polymeric flavonoids consisting of flavan-3-ol and/ or flavan-3, 4-diol units linked by C4→C8 interflavan bonds and are called B-type proanthocyanidins (Dykes and Rooney, 2006) (Figure 1.2.4). The A-type proanthocyanidin consists of flavan-3-ol units linked by C4→C8 interflavan bonds and by an additional C2→C7 link (Gu, Kelm, Hammerstone, Beecher, Holden, Haytowitz and Prior, 2003). Sorghum proanthocyanidins are of the B-type with (-)-epicatechin as extension units and catechin as terminal units (Gu *et al.,* 2003; Gupta and Haslam, 1978). Proanthocyanidins with both A- and B- type interflavan bonds have also been identified in sorghum. These include

prodelphinidin, and heteroflavan-3-ols consisting of procyanidin or prodelphinidin as extension and terminal units (Gujer, Magnolata and Self, 1986).

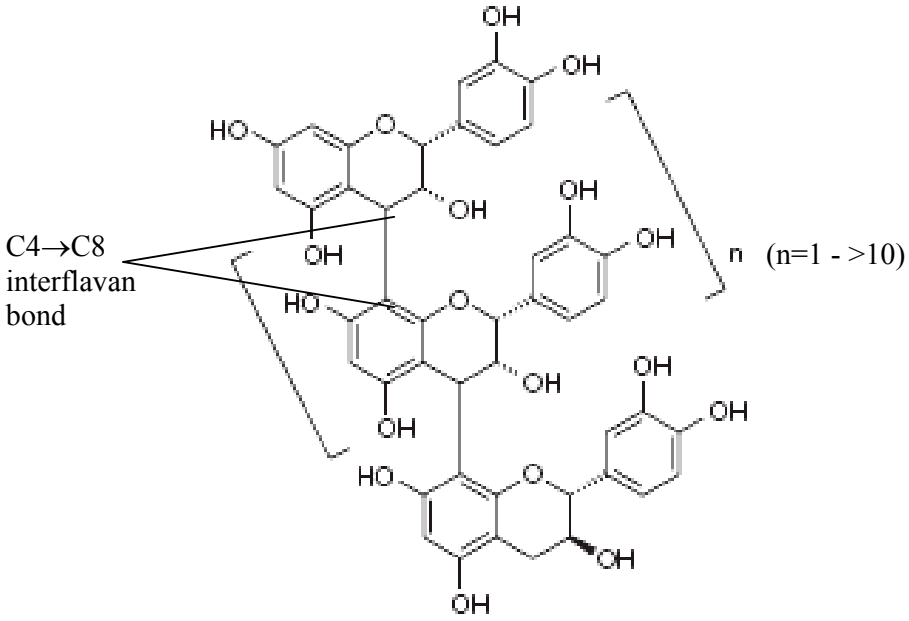


Figure 1.2.4 Structure of proanthocyanidin (condensed tannin) found in sorghum, a polyflavan-3-ol with a B-type linkage (Dykes and Rooney, 2006)

1.2.2 Occurrence of phenolic compounds in the anatomical parts of the sorghum grain

The sorghum kernel (caryopsis) is composed of three main parts: the outer covering layer (pericarp), the storage tissue (endosperm) and the embryo (germ) (Figure 1.2.5) (Earp, McDonough and Rooney, 2004). Sorghum tannins occur only in the pericarp and testa layers of sorghums with dominant B_1 and B_2 genes (Hahn and Rooney, 1986). Tannin sorghums are classified as type III or type II based on extractable quantities. Earp *et al*. (2004) explained the differences in tannin levels in terms of pigment deposition during development. The authors observed that pigments in type II sorghum were deposited in vesicles, while in type III they were deposited along the cell walls of the integuments. The entrenchment of pigments in type II sorghum can explain reduced extractability of tannins than freely deposited pigments along the cell walls of type III sorghum (Earp *et al*., 2004).

Phenolic acids are present in sorghum mainly in the bound form (Hahn *et al.,* 1983). Bound phenolic acids such as ferulic acid are the most abundant and are associated with cell walls (Hahn *et al.,* 1984). The endosperm contains much lower levels of phenolics than are found in the pericarp, about 10 to 100 fold less (Waniska and Rooney, 2000). Pericarp colour is due to a combination of anthocyanin and anthocyanidin pigments as well as other flavonoid compounds (Hahn, 1984). Sorghums with a black pericarp (black sorghum) have the highest levels of anthocyanins in the form of 3-deoxyanthocyanidins, which are concentrated in the bran (Awika *et al.,* 2004).

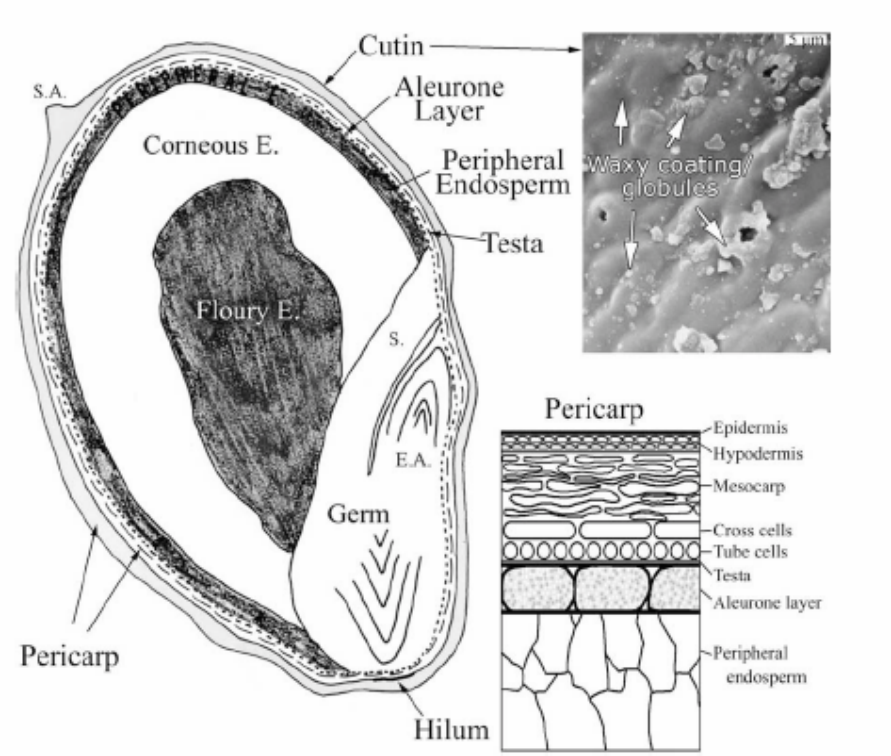
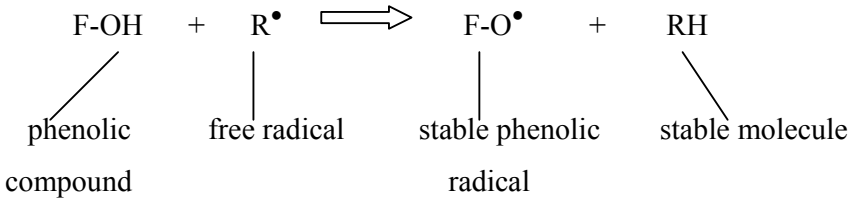


Figure 1.2.5 Schematic section through the sorghum grain with details of the pericarp layers. (Earp *et al*., 2004)

1.2.3 Mechanisms and structure activity relationship of phenolics as antioxidants

Studies have suggested the role of phenolic compounds as the major natural antioxidants in foods of plant origin (Hagerman *et al.,* 1998). Free radicals, which include the hydroxyl radical ($^{\bullet}OH$), superoxide radical ($^{\bullet}O_2$), hydrogen peroxide and

lipid peroxide are termed reactive oxygen species (ROS) and are produced as a result of oxidative stress (Rice-Evans, Miller and Paganga, 1996). All classes of phenolics exert individual antioxidant activity against ROS but generally the antioxidant activity of phenolics and their metabolites depends upon the spatial arrangement of functional groups about their nuclear structure (Rice-Evans *et al.*, 1996). Specific structural components play a role in radical scavenging, chelating and antioxidant activity (Heim, Tagliaferro and Bobily, 2002). The total number of hydroxyl groups substantially influences several mechanisms of antioxidant activity. Free radical scavenging capacity is primarily attributed to the high reactivities of hydroxyl substituents that participate in the following reaction:



The antioxidant activity of phenolic acids depends on the number of hydroxyl groups. Between the two classes of phenolic acids, hydroxycinnamates are more effective antioxidants than hydroxybenzoates (Rice-Evans *et al.*, 1996). The carboxylic group ($-CO_2H$) in benzoic acid has electron-withdrawing properties, which have a negative effect on the hydrogen donating abilities of hydroxybenzoates, whilst the presence of $–CH=CH-CO_2$ in hydroxycinnamates ensure greater hydrogen donating ability and radical stabilisation (Rice-Evans *et al.*, 1996). Therefore, hydroxycinnamates such as caffeic, ferulic, sinapic and *p*-coumaric acids are generally more potent antioxidants than the hydroxybenzoates such as syringic, vanillic, protocatechuic and *p*-hydroxybenzoate notably in lipid peroxidation (Cuvelier, Richard and Berset, 1992). Hydroxycinnamates are more effective when the hydroxyl group is in the *para* position such as *p*-coumaric acid.

In flavonoids the B ring hydroxyl configuration is the most significant determinant of scavenging of ROS (Burda and Oleszek, 2001). Hydroxyl groups on the B ring donate hydrogen and an electron to the hydroxyl, peroxyl and peroxynitrite radicals stabilising them and giving rise to a relatively stable flavonoid radical, which in turn reacts with free radicals and terminate the radical chain reaction (Cook and Samman,

1996). Among structurally homologous flavones and flavonones, peroxyl and hydroxyl scavenging increases linearly and curvilinearly, respectively with an increase in the number of hydroxyl groups (Cao, Sofic and Prior**,** 1997).

Tannin antioxidant activity is dependent on *ortho*-phenolic hydroxyl groups located in the B ring (Schofield, Mbugua and Pell, 2001). High molecular weight tannins have the greatest antioxidant activity on a molar basis among natural antioxidants due to the proximity of many aromatic rings and hydroxyl groups (Hagerman *et al.,* 1998).

Besides free radical scavenging activities, phenolics possess metal chelating properties. Polyvalent phenolics in the flavonoid molecular structure allow some flavonoids to chelate metal ions (Yilmaz and Toledo, 2004), which are involved in the formation of metal-catalysed free radicals, particularly iron and copper (Rice-Evans *et al.,* 1997). The flavonoid structure is essential for metal chelation and imparting greatest antioxidant activity (Rice-Evans *et al.,* 1997). There are two points in the flavonoid molecule for the attachment of transition metal ions for metal chelation. These are the *o*-diphenolic groups in the 3'4' hydroxy positions in the B ring and the ketol structures 4 *keto,* 3-hydroxy or 4 *keto* and 5-hydroxy in the C ring of the flavonols (Figure 1.2.2) (Rice-Evans *et al*., 1997).

Currently *in vitro* antioxidant activity is the most common parameter used to assess or predict the potential health benefits of plant phytochemical compounds (Awika and Rooney, 2004). In sorghum, phenolic content correlates strongly with antioxidant activity measured by various methods, indicating that phenolics are largely responsible for the activity (Awika *et al*., 2003b). Table 1.2.1 gives some of the data on the antioxidant activity of different sorghum types and products. The data show that high tannin grain and in particular its bran have higher levels of antioxidant levels than non-tannin cultivars. However, processing such as decortication and baking reduces phenolic content and antioxidant activity compared to unprocessed whole grain and bran.

Table 1.2.1 Levels of total phenolics and antioxidant activity reported in sorghum whole grain and fractions.

Sample	Total Phenolics [a]	Standard	Antioxidant activity (ABTS) [b]	Reference
White grain (non tannin)	1	Gallic acid	6	Awika *et al*. (2003b)
White bran (non tannin)	28	Gallic acid	5	Awika *et al* (2003b)
Red grain (non tannin)	5	Gallic acid	53	Awika *et al*. (2003b)
Red bran (non tannin)	20	Gallic acid	230	Awika *et al*. (2003b)
Sumac grain (high tannin)	23	Gallic acid	226	Awika *et al.* (2003b)
Sumac bran (high tannin)	66	Gallic acid	768	Awika *et al*. (2003b)
Sumac bread	6	Gallic acid	108	Awika *et al*. (2003b)
Sumac cookies	14	Gallic acid	130	Awika *et al*. (2003b)
SC103 (high tannin)	13.5	Gallic acid	114	Awika *et al*. (2005)
Sorghum whole grain (non tannin)	4.1	Catechin	52	Ragaee *et al*. (2006)
Macia (tan plant non-tannin)	2.7	Catechin	22	Dlamini *et al*. (2007)
Macia decorticated	2.2	Catechin	6	Dlamini *et al*. (2007)
NS5511 grain (high tannin)	22.4	Catechin	384	Dlamini *et al*. (2007)
NS5511 decorticated	4.7	Catechin	49	Dlamini *et al*. (2007)

[a]Values expressed as mg/g dry weight basis, Folin-Ciocalteu method.

[b]Values expressed µMol TE/g dry weight basis, Trolox equivalent antioxidant capacity.

1.2.4 Sorghum milling technologies

Milling involves the partial separation and/or modification of the three major constituents of the cereal grain - the germ, endosperm and the pericarp (Eastman, 1980). Milling not only separates anatomical parts of the grain but also for the purpose of obtaining maximum yield of clean endosperm. Contamination of the endosperm flour by the bran is undesirable as the bran discolours the flour and the germ contains oil, which make the flour rancid (Taylor and Dewar, 2001).

Mechanisms employed in milling include the removal of bran and germ and then reduction of the endosperm as is generally practised in maize milling (Taylor and Dewar, 2001). Degermination as a practice in maize milling is unsatisfactory for sorghum milling, as the germ cannot be wholly removed. Moreover, fracture through the endosperm results in contamination of the flour with bran, causing it to be specky. Alternatively, the roller milling principle can be applied where the kernel is initially squeezed and abraded through shearing forces of metal rollers at slightly different speeds (Munck, 1995). The exposed endosperm is then scraped off the bran by passing through corrugated rollers. Roller milling is commonly used for wheat milling (Taylor and Dewar, 2001), on account of the deep crease in the wheat kernel (Munck, 1995). Plain roller milling is also unsuitable for sorghum because of high processing costs, low extraction, and reduced quality affecting consumer acceptability. However, in the light of Kebakile, Rooney and Taylor (2007), roller milling gave finer meals and highest extraction rates compared to hand pounding and abrasive decortication, followed by hammer milling. Due to the round shape of sorghum, dehulling is recommended for sorghum milling (Munck, 1995), although the process actually is decortication since sorghum is a naked caryopsis and does not have a hull (Taylor and Dewar, 2001).

The decortication principle involves the separation of bran and germ from the endosperm, which can then be milled separately (Munck, 1995). Attrition and abrasion type decortications may be used for sorghum milling. Attrition decortication uses pressurised cylinders, which have grinding effects due to metal-kernel interaction in addition to grain surfaces rubbing against each other (Munck, 1995). Grain is normally conditioned before milling and bran and embryo separated as coarse bran.

Although attrition is effective for sorghum milling it is still not as commonly used as abrasive decortication (Taylor and Dewar, 2001).

Abrasive decortication, followed generally by hammer milling of the endosperm material into meal or flour is the most common way of milling sorghum (Taylor and Dewar, 2001). However, one drawback is that abrasive decortication, does not remove all the germ and thus the endosperm meal still has a high fat content, between 2 and 4% (Taylor and Dewar, 2001).

Probably the most popular type of decorticating machine is the PRL (Prairie Research Laboratory) dehuller (Figure 1.2.6) (Schmidt, 1992). A PRL-type dehuller comprises a horizontal barrel containing 13 evenly spaced carborundum disks (25 cm diameter, 2.1 cm wide) that rotate clockwise against the grains at approximately 2,000 rpm (Taylor and Dewar, 2001). An electric motor, diesel or petrol engine provides power. The barrel may be lined with rubber to provide greater abrasion and reduce noise levels. Dry sorghum (5 to 25 kg) is fed into the barrel by means of a hopper fitted with a flow regulator. The bran and germ are progressively abraded off and removed by means of a cyclone fan. Decortication may be carried out either in a batch with a single machine or continuous basis using several decorticators in series (Taylor and Dewar, 2001). There are more than 200 PRL-type dehullers in southern Africa and their success is owed to their simplicity and robustness making them ideal for use in developing countries (Taylor and Dewar, 2001).

A hammer mill is usually used to reduce the decorticated particles in size (FAO, 1995). It consists of blunt blades rotating rapidly in an enclosed cylinder with an outlet covered by a screen. The size of the holes in the screen determines the size of the particles of flour. However, small holes will reduce the throughput of the mill, and if they are too small overheating may result. Although hammer milling is energy efficient it produces grainy, sandy flours that are less acceptable to consumers (Rooney and Waniska, 2000). Further processing such as fermentation or porridge production may reduce sandiness.

Figure 1.2.6 Prairie Research Laboratory (PRL) type dehuller (Rural Industries Innovation Centre, Kanye, Botswana).

1.2.5 Sorghum use in ready-to-eat products

Sorghum and sorghum-wheat flour blends are commonly used to produce products such as flatbreads, cakes, muffins and cookies (Rooney and Waniska, 2000). Loaf type breads of sorghum can be made from composite flours with wheat. The level of sorghum substituted for wheat flour depends on the strength and quality of gluten in the wheat flour, baking procedure, colour, particle size and aroma of sorghum flour, the use of gums, additives and emulsifiers. Bread quality deteriorates as the percentage of non-wheat flour increases but cakes and biscuits can be made using flour with much higher levels of non-wheat flour. Besides composite bread, 100% sorghum can be used to produce gluten-free bread (Hart, Graham, Gee and Morgan,

1970; Schober, Bean and Boyle, 2007). Schober *et al.* (2007) investigated baking quality of sorghum bread with hydroxypropyl methylcellulose and potato starch. Potato starch was found to produce better quality bread than maize starch, attributed to its lower gelatinisation temperature, hence increased batter/crumb structure consistency during baking. Addition of 2% hydroxypropyl methylcellulose has been found to improve bread quality by increasing gas retention and preventing loaves from collapsing (Hart *et al.,* 1970). However, these treatments did not eliminate the problem of flat tops and holes in the crumb structure. Sour dough fermentation was found to eliminate these shortcomings (Schober *et al.*, 2007). Fermentation degraded dough proteins into peptides smaller than kafirin monomers, which caused a significantly higher resistance to deformation after gelatinisation compared to non-sour dough (Schober *et al.,* 2007).

Sorghum also finds use in cakes and cookies and is more appropriate for these products, since gluten formation is not necessary for dough rise (reviewed by Taylor, Schober and Bean, 2006). However, the quality of cakes and cookies was reported to be inferior as they dried and crumbed off easily, and developed more off-flavour than wheat products. Investigations by Glover, Walker and Mattern (1986) using fractionation-reconstruction techniques showed that sorghum lipids and starch were responsible for reduced cake volume, brittle crumb structure and inferior crust appearance. Sorghum lipids, glyco- and phospholipids were shown to have no functionality in improving volume and crumb structure. High starch gelatinization temperature was also found to be responsible for lower volumes and inferior texture due to the absence of starch gelatinisation in the centre of the cake (reviewed by Taylor *et al.,* 2006).

Badi and Hoseney (1976) produced cookies from 100% sorghum, which were fragile, tough and had a gritty and mealy texture. The cookies did not spread and had no top surface cracks, attributes the authors considered desirable for cookies. The inferiority of sorghum cookies was also attributed to sorghum lipids as substituting wheat lipids for sorghum lipids in defatted flour improved cookie quality. Wheat lipids resulted in cookies with improved top grain and spread. Other dough improvements included a combination of hydrated dry flour and unrefined soy lecithin, which improved cookie surface character and greatly increased cookie spread. Increasing the pH of the dough

by using sodium carbonate instead of sodium bicarbonate reduced grittiness. However there were no conditions found to improve fragility of cookies. Rooney and Waniska, (2000) recommended the addition of 5% pregelatinised waxy maize starch to reduce breakage during handling attributed to the continuous film of starch holding the crumb more effectively.

Cruz Y Celis, Rooney and McDonough (1996) studied the potential use of waxy and non-waxy micronised white tan plant sorghum in ready-to-eat breakfast cereals. Whole and decorticated micronised grain flakes were prepared into a granola. Micronised whole grain flakes of waxy sorghum had the lowest density, best flavour, texture and most gelatinisation. They produced the most acceptable whole grain fibre rich flakes with a puffed texture that resulted in acceptable granola type ready-to-eat breakfast cereal and granola bars. Decorticated non-waxy granola had the hardest texture although the flakes expanded more than whole grain flakes.

1.2. 6 Effects of milling and baking on phenolic content and antioxidant activity

Decortication removes the pericarp and testa and therefore most phenolics (Serna-Saldivar and Rooney, 1995). Youssef, Bolling, Moustafa and Moharram (1988) observed a marked reduction in sorghum phenolic content after decortication and extraction to 70%. Phenolic reductions ranged from 68 to 97% indicating that most sorghum phenolics are concentrated in the pericarp of the grain. The highest losses were in a tannin-containing cultivar and slight variations in phenolic reduction were noticed in the different solvents used for extraction. Dlamini *et al.* (2007) also did similar work and reported the highest phenolic loss due to decortication in tannin sorghum compared to non-tannin sorghum. Decortication also reduced tannin content by 79 to 92% compared to whole grain. Antioxidant activity was reduced by 73 to 87% in both tannin and non-tannin sorghum. The results are evidence that decortication results in a significant loss of phenolics, hence antioxidant activity.

Awika, Dykes, Gu, Rooney and Prior (2003a) processed condensed tannin sorghum bran into bread and cookies and reported tannin losses of up to 82% and 52%, respectively relative to raw bran. The more pronounced tannin losses in bread were attributed to the bread formulation and process conditions, which required longer

mixing time and moisture. Handling in bread making allows interactions of tannins with food macromolecules increasing the degree of polymerisation of tannins. Polymerised tannins are difficult to extract (Hagerman and Butler, 1989). Tannins were better retained in cookies because of limited moisture and the degree of interaction with other dough components (Awika *et al.*, 2003a).

1.2.7 Determination of phenolics and antioxidant activity

1.2.7.1 Analyses of phenolics

The quantification of phenolics and tannins in plant materials can be done using numerous spectrophotometric methods (Naczk and Shahidi, 2004). The assays are based on different principles and are used to determine various structural groups present in phenolic compounds (Naczk and Shahidi, 2004). Methods such as Folin-Ciocalteu (Singleton and Ross, 1965) and the Prussian Blue assay (Graham, 1992) are popular for quantifying total phenolics. Vanillin-HCl (Price, Van Scoyoc and Butler, 1978) and butanol-HCl (Porter, Hrtstich and Chan, 1986) assays are more specific to tannins. Quantities and recovery of phenolics vary with sorghum type, extractant and method of determination (Youssef *et al*., 1988). This is because solubility of phenolic compounds is governed by the type of solvent used, degree of polymerization of phenolics, as well as interaction of phenolics with other food constituents and formation of insoluble complexes (Naczk and Shahidi, 2004). Besides solvent polarity, solvent-to-sample ratio, extraction time, pH and temperature may also affect quantification of phenolics (Makkar and Becker, 1996). However there is no uniform or completely satisfactory procedure that is suitable for extraction of all phenolics or a specific class of phenolic substances in plant materials (Naczk and Shahidi, 2004). Commonly used extractants for phenolics are methanol, ethanol, acetone, water, ethyl acetate and, to a lesser extent, propanol, dimethylformamide, and their combinations are frequently used (Antolovich, Prenzler, Robards and Ryan, 2000). Maximum recovery of phenolics is necessary, as recovery will also affect antioxidant activity. The general procedure for phenol and antioxidant determination involves sampling, sample extraction, detection and quantification (Awika *et al.,* 2003b).

1.2.7.1.1 Condensed tannin assays

1.2.7.1.1.1 Acid-butanol assay

The butanol-HCl reaction (Porter *et al.,* 1986) uses an acid catalyst to oxidatively depolymerise condensed tannins to yield red anthocyanidins (Schofield *et al*., 2001). The reaction is diagnostic for the polyflavan structure, which is the greatest strength for the method (Schofield *et al.,* 2001). The reaction involves a solution of butanol and concentrated hydrochloric acid (95:5, v/v) in which condensed tannins are converted to anthocyanidins in the presence of this acidic solution (Porter *et al.*, 1986). The conversion occurs through autoxidation of carbocations formed by cleavage of interflavanoid bonds. The yield of this reaction depends on the concentration of HCl and water, temperature and reaction time, the presence of transition metals, as well as the degree of polymerization of proanthocyanidins. The presence of transition metals enhances both the reproducibility and the yield of conversion of proanthocyanidins to anthocyanidins hence colour development (Hagerman *et al.,* 1998). Ferrous and ferric ions are the most effective catalysts in the formation of anthocyanidins (Porter *et al.,* 1986).

1.2.7.1.1.2 Vanillin-HCl assay

The Vanillin-HCl assay (Price et *al*., 1978) depends on the reaction of vanillin with condensed tannins to form coloured complexes (Schofield *et al*., 2001). The assay is specific for flavan-3-ols, dihydrochalcones and proanthocyanidins, which have a single bond at the 2, 3-position and possess free meta-hydroxy groups on the B ring (Gupta and Haslam, 1978). The method uses HCl as a catalyst and acidified methanol, when used as an extractant gives higher absorbance values than methanol, indicating that more tannins are extracted in an acidic environment (Price *et al*., 1978). Extraction time is critical to quantifying tannins because absorbance decreases slowly with extraction time, presumably due to destruction of tannins by HCl. Tannins have been observed to be unstable for long periods of time in acidic environment even at room temperature, giving 40 to 70% lower changes in absorbance (ΔA_{500}) when extraction was carried out for 24 h (Price *et al.,* 1978). The authors recommended 20 min extraction time; as such several analyses can be run in a

day making the assay rapid. The Vanillin-HCl assay has the drawback of interference by coloured solvent extract material, which absorbs at the same wavelength as tannins. Tannins are overestimated but can be corrected by the subtraction of a blank for each sample (Price and Butler, 1977). Catechin is the commonly used standard. However, when its reaction kinetics was compared with that of partially purified tannin standard, the kinetics showed that catechin overestimated tannin content (Price *et al.,* 1978). Despite these limitations, the assay is widely recognized for detection and quantification of tannins because of its sensitivity and specificity to tannins and simplicity (Naczk and Shahidi, 2004).

1.2.7.1.2 Total phenolic assays

1.2.7.1.2.1 Folin-Ciocalteu method

Folin-Ciocalteu method (Singleton and Rossi, 1965) is commonly used for determining total phenolics. The assay is non-specific for a particular group of phenolic compounds and quantifies the total concentration of phenolic hydroxyl groups and their ability to reduce the phenolic reagent to form chromogens that can be detected spectrophotometrically (Waterman and Mole 1994). The method is an oxidation-reduction reaction in which the phenolate ion is oxidised while the phosphotungstic-phosphomolybdic compounds are reduced (Waterman and Mole 1994) to a blue complex in alkaline solution (Naczk and Shahidi, 2004). The non-specificity of the Folin-Ciocalteu reagent makes it a suitable reagent for total phenol analysis (McGrath, Kaluza, Daiber, Van der Riet and Glennie, 1982) despite facing a shortfall of detecting phenolic groups in extractable proteins and interference by reducing substances such as ascorbic acid (Naczk and Shahidi, 2004).

1.2.7.1.2.2 Prussian Blue assay

The Prussian Blue procedure measures total phenolics and is also not specific for a particular group of phenolics. The reaction is an oxidation-reduction where the phenolate ion is oxidized while the ferricyanide ion is reduced (Graham, 1992). The reaction occurs as follows:

$$\text{Polyphenol} + 2Fe(CN)_6^{3-} \longrightarrow \text{oxidised polyphenols} + 2Fe(CN)_6^{4-}$$

(ferricyanide ion) (ferrocyanide ion)

$$\text{then } 3Fe(CN)_6^{4-} + 3Fe^{3+} \longrightarrow Fe_4[Fe(CN)_6]_3$$

(Prussian Blue)

The reactivity of phenolic hydroxyl groups is strongly affected by reaction time reagent and concentration (Schofield *et al.,* 2001). The major shortcomings of the assay are precipitate formation after short incubation periods and increased colour intensity with time. However, the method remains popular owing to its simplicity, rapidity and low interference by non-phenolic compounds (Graham, 1992).

1.2.7.2 Analysis of antioxidant activity

Antioxidant activity is predicted by the ability of phenolics to donate hydrogen or electrons for free radical scavenging activity (Rice-Evans *et al.,* 1997). Assays employed measure the ability of antioxidants to scavenge free radicals such as Azinobis (3 ethyl-benzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) and 2, 2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) (Awika *et al.,* 2003b).

1.2.7.2.1 ABTS radical scavenging assay

This assay measures the ability of antioxidant to scavenge the $ABTS^{\bullet+}$ radical cation generated in aqueous phase as compared with Trolox (a water soluble Vitamin E analogue) standard (Awika *et al.,* 2003b) and is expressed as the Trolox equivalent antioxidant capacity. The reduction of the blue-green $ABTS^{\bullet+}$ radical by hydrogen-donating antioxidant is measured by the suppression of its characteristic wave absorption spectrum (Miller and Rice-Evans, 1997). A drawback of the method is that the $ABTS^{\bullet+}$ assay only indicates the presence of antioxidants but cannot infer structure-activity relationship (Nenadis, Wang, Tsimidou, and Zhang, 2004). Moreover, $ABTS^{\bullet+}$ is not very stable and a fresh working solution needs to be prepared every 2 h. However, the method is popular because it is applicable to both lipophilic and polar phenolics and it is simple and rapid (Nenadis *et al.,* 2004).

1.2.7.2.2 DPPH radical scavenging assay

This method uses DPPH$^{\bullet}$, which is a stable free radical with a maximum absorption at 515 nm (Awika *et al.*, 2003b). However, DPPH$^{\bullet}$ reacts slowly with phenolic compounds, up to 6h or longer (Awika *et al.*, 2003b). The assay is also prone to interference by colour in samples that contain anthocyanins, which leads to an underestimation of antioxidant activity (Arnao, 2000). However, it is still widely used because of its repeatability (Awika *et al.*, 2003b).

1.3 Conclusions

South Africa is the largest industrial processor of sorghum in Africa mainly for meal, malt and breakfast cereals. Although sorghum is a source of phenolic compounds that could have potential health benefits, there seem to be no data on the phenolic content and antioxidant activity of South African sorghum cultivars and possible application. There is need to investigate the levels of phenolic contents and antioxidant activity of different sorghum types grown in South Africa as well as process sorghum into products that would be vectors of phenolics.

1.4 Hypotheses and Objectives

1.4 1 Hypotheses

1. Condensed tannin sorghum grain, its flour and cookies will have higher total phenolic content, tannin content and antioxidant activity than those of tannin-free sorghum. Condensed tannins contain more multiple phenolic hydroxyl groups compared to simple phenolics on a molar basis and would therefore give higher contents of total phenolics (Hagerman *et al.,* 1998). Phenolic compounds with multiple hydroxyl groups (such as condensed tannins) possess a large number of sites for radical scavenging; therefore have high antioxidant activity.

2. Sorghum flours and cookies from flours with high extraction rate will have higher total phenol content, tannin content and antioxidant activity than flours and cookies from flours with low extraction rate. Flours with high extraction rate have less removal of pericarp where phenolic compounds are concentrated (Hahn and Rooney, 1986; Dykes and Rooney, 2006) and so will have higher levels of total phenolics than flours with low extraction rate. Flours and cookies with high levels of total phenolics will contain more phenolic hydroxyl groups, which provide more sites for radical scavenging activity and therefore will have high antioxidant activity.

3. Phenolic content will affect sensory attributes of cookies, namely, colour, flavour and taste. Pigmented sorghums, due to the presence of flavonoid-type compounds (e.g. anthocyanins, which produce colour) will produce darker coloured cookies compared to tan plant sorghum. The presence of condensed tannins in sorghum cookies will affect their taste and flavour due to sensations such as astringency (Lu and Bennick, 1998).

1.4.2 Objectives

The objectives of the project were,

1. To determine the effect of cultivar on the total phenolic content, condensed tannin content and antioxidant activity of different sorghums, flours and cookies.

2. To determine the effects of flour extraction rates on total phenolic content, condensed tannin content and antioxidant activity of sorghum cookies.

3. To determine the effect of phenolics on consumer acceptability of sorghum cookies.

CHAPTER 2: RESEARCH

2.1. Phenolic content and antioxidant activity of South African sorghums

2.1.1. Abstract

South African sorghum cultivars were investigated for their potential as sources of phenolic antioxidants. Phenolic content and antioxidant activity varied among the sorghum types. Condensed tannin sorghums had the highest total phenolic content, which was 3 to 7 times higher than that of condensed tannin-free cultivars, while antioxidant activity was 2 to 4 times higher. Condensed tannins were only measurable in the condensed tannin cultivars. The correlations between total phenolics and condensed tannins with antioxidant activity were highly significant, (r = 0.97, $p < 0.001$) and (r = 0.98, $p < 0.001$) respectively, indicating the contribution of phenolic compounds to antioxidant activity. The research findings demonstrate that South African sorghums of the condensed tannin type have higher phenolic content, hence antioxidant activity than the condensed tannin-free types and can be exploited for potential health benefits.

2.1.2. Introduction

Sorghum (*Sorghum bicolor* L. Moench) represents a large portion of the total energy intake in many parts of Africa, in the semi-arid tropics where it is largely grown (FAO, 1995). Research suggests that sorghum is a source of phenolic compounds, which have antioxidant activity similar to that of well-studied fruits and vegetables (Awika and Rooney, 2004) and other cereals (Ragaee *et al.,* 2006). Therefore, there is growing interest to highlight the potential antioxidant activity of sorghum phenolic compounds and exploit them for their health benefits.

South Africa is the largest commercial processor of sorghum grain in Africa (Rohrbach, 1990). Notwithstanding this, there are a number of constraints to sorghum utilization in South Africa such as the 14% value added tax (VAT), which sorghum meal carries whereas maize meal does not. As such maize meal would be preferred to sorghum because of cheaper price. Moreover, the South African sorghum industry has failed to develop new markets for sorghum in the time that the market for opaque beer has been in severe decline, due to consumers turning to Western products such as lager beer and carbonated soft drinks. However, as sorghum is a rich source of phenolic antioxidants, it may be processed into various products that could be vectors of antioxidants and diversify sorghum use in South Africa. Studies have been conducted elsewhere to determine the levels of phenolics and antioxidant activity in different sorghum types (Dykes, Rooney, Waniska and Rooney, 2005). The findings showed varying levels of phenolic content and antioxidant activity, which differed with genotype. In South Africa, there have been reports of antioxidant activity of phenolic extracts from sources such wines and herbs (De Beer, Joubert, Gelderblom and Manley, 2005; Steenkamp, Grimmer, Semano and Gulumian, 2005) among others. However, there are no detailed reports on sorghum antioxidant levels of the various commercial cultivars although sorghum would be a more ideal source as it also forms a large part of energy intake. The objective of the study was to determine the effects of cultivar on the phenolic content and to evaluate the potential of commercial South African sorghum phenolics as antioxidants.

2.1.3. Materials and methods

2.1.3.1. Materials

2.1.3.1.1. Sorghum samples

Cultivars comprised of three condensed tannin and eighteen condensed tannin-free (consisting of fifteen red and two white tan plant) sorghum types. The sorghums represented widely cultivated cultivars and new cultivars being evaluated. The cultivars were grown in two localities namely, Potchefstroom and Klerksdorp. Table 2.1.1 summarises the environmental conditions at the localities during the sorghum-growing season covering the period from November 2005 to February 2006. The differences in temperature and rainfall between the two localities were not substantially different. Grain was mechanically threshed and thoroughly mixed to obtain a representative sample and further hand-cleaned to remove broken kernels and foreign material. Cleaned grain was milled through a 1 mm screen with a Cyclotec mill (Foss Tecator, Höganäs, Sweden) to obtain a fine powder. Samples were sealed in polyethylene bags and stored at 4°C before use.

Table 2.1.1 Average daily maximum temperature and total rainfall recorded during the sorghum-growing season (November 2005 to February 2006)

Location	Temperature (°C)	Rainfall (mm)
Potchefstroom	27.3	527
Klerksdorp	25.8	501
Mean	26.5	514

South Africa weather services, http://www.weathersa.co.za

2.1.3.2. Methods

2.1.3.2.1. Grain colour

Colour of whole sorghum grain was measured using a HunterLab ColorFlex (Hunter Associates Laboratory, Reston, Virginia). The measurements were based on *L, a, b* Hunter tristimulus scale, (*L*= lightness; +*a* = red, -*a* = green, +*b* = yellow, -*b* = blue).

2.1.3.2.2. Determination of total phenolics

A modified Folin-Ciocalteu method was used (Waterman and Mole, 1994). This method is based on the reducing power of the phenolic hydroxyl groups (Hahn *et al.*, 1984), which react with the Folin-Ciocalteu phenol reagent to form chromogens that can be detected spectrophotometrically. In brief, phenolic extracts were prepared in 15 ml acidified methanol (1% conc. HCl in methanol, v/v) from 1 g sorghum flour. Centrifuged extracts were mixed with Folin Ciocalteu phenol reagent and then sodium carbonate (20%, w/v) solution within 8 min from the addition of the phenolic reagent. The contents were left to stand for 2 h, after which absorbance was read at 760 nm. Catechin was used as a standard.

2.1.3.2.3. Determination of condensed tannins

Tannins were determined using the modified Vanillin-HCl method (Price *et al.*, 1978) with and without blank subtraction. Milled sorghum samples (1 g) were extracted in 50 ml absolute methanol at 30°C before reacting with 5 ml Vanillin reagent (1% Vanillin in methanol and 8% HCl in methanol). Reactants were incubated at 30°C in a water bath for exactly 20 min and absorbance read at 500 nm. Sample blanks were prepared by adding 4% HCl in methanol instead of Vanillin reagent. Catechin was used as a standard.

2.1.3.2.4. Determination of antioxidant activity

Antioxidant activity of samples was determined using the ABTS radical assay according to Awika *et al*. (2003b). The ABTS radical ($ABTS^{\bullet+}$) was generated in the

dark for at least 12 h by reacting equal volumes of 3 mM potassium persulphate ($K_2S_2O_8$) with 8 mM ABTS (2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) salt in distilled water. The buffered radical solution (pH 7.4) was added to sample extracts prepared in acidified methanol as for total phenolic assays. Reactants were allowed to stand for exactly 30 min and their absorbance measured at 734 nm. Trolox was used as standard.

2.1.3.3. Statistical analysis

Experiments were repeated three times and duplicate samples were analysed to obtain a total of six observations. The effect of sorghum cultivar on total phenolic content, condensed tannin content and antioxidant activity was determined using one way analysis of variance (ANOVA) and Fischer's least significant difference test at $p < 0.05$. Pearson's correlation coefficient was used to analyse linear relationships between total phenolic content, condensed tannin content and antioxidant activity.

2.1.4. Results and discussion

In both localities, the condensed tannin and red tannin-free cultivars had lower Hunter *L* and higher Hunter *a* values than the white tannin-free cultivars (Table 2.1.2). This was because of the pigmentation, which occurs in the pericarp of condensed tannin and red tannin-free cultivars (Rooney and Miller, 1982). The results indicate variations in grain colour with sorghum type. In sorghum, a combination of anthocyanin and anthocyanidin pigments as well as other flavonoid compounds affect grain colour (Hahn *et al.,* 1984). Condensed tannin sorghums, which have a pigmented testa, contain substantial levels of condensed tannins with varying degrees of pericarp pigmentation (Awika and Rooney, 2004). Hence condensed tannins also affect grain colour.

Table 2.1.2 Effect of cultivar on Hunter *L*, *a* and *b* values of South African sorghums

Cultivar	*L*		*a*		*b*	
	Potchefstroom	Klerksdorp	Potchefstroom	Klerksdorp	Potchefstroom	Klerksdorp
Condensed tannin cultivars						
NS 5511	32.4 ab (0.3)	32.1 bcd (0.4)	10.3 gh (0.4)	10.4 f (0.1)	12.3 ab (0.4)	12.2 g-j (0.6)
PAN 8229	32.2 a (0.0)	32.6 cd (0.4)	9.6 e-h (0.5)	8.7 d (0.2)	12.0 a (0.2)	10.7 b-e (0.4)
PAN 8625	34.4 cd (0.3)	31.1 ab (1.1)	9.9 e-h (0.1)	9.7 ef (0.3)	13.2 cd (0.3)	10.7 bcd (0.5)
Red tannin free cultivars						
PAN 8043	39.4) j (0.6	32.9 def (0.2)	9.7 e-h (0.2)	9.7 ef (0.2)	14.4 ef (0.0)	11.5 d-h (0.2)
PAN 8346	38.8 ij (0.0)	32.7 cde (0.4)	9.4 c-f (0.4)	9.9 f (0.4)	14.5 fg (0.4)	12.0 g-j (0.5)
PAN 8141	38.8 ij (0.4)	34.2 efg (2.1)	10.1 fgh (0.2)	9.1 de (0.9)	14.7 fg (0.0)	12.0 f-j (0.5)
PAN 8609	37.8 hi (0.8)	34.1 efg (0.9)	9.6 d-g (0.4)	8.8 d (0.4)	14.3 ef (0.1)	11.6 d-h (0.4)
PAN 8123	37.3 gh (0.7)	36.1 h (0.4)	9.9 e-h (0.2)	9.7ef (0.2)	14.2 ef (0.3)	12.3 hij (0.7)
PAN 8420	36.8 fgh (0.3)	34.2 fg (0.7)	9.4 c-f (0.3)	9.1 de (0.2)	13.4 cd (0.3)	11.8 f-j (0.4)
PAN 8564	36.6 e-h (0.7)	36.2 h (0.3)	9.2 cde (0.1)	8.7 d (0.6)	13.8 de (0.1)	12.6 ij (0.1)
PAN 8806	36.5 e-h (0.8)	33.0 def (0.6)	9.7 e-h (0.8)	9.1 de (0.3)	13.2 cd (0.1)	11.7 e-i (0.8)
PAN 8738	36.0 efg (0.8)	31.3 abc (0.2)	10.1 fgh (0.0)	8.9 d (0.3)	13.1 c (0.3)	10.3 bc (0.3)
PAN 8446	35.4 de (1.1)	30.3 a (0.3)	10.3 gh (0.3)	7.6 c (0.6)	12.9 bc (0.6)	9.3 a (0.2)
PAN 8534	35.9 efg (0.6)	35.0 gh (0.1)	8.8 cd (0.2)	7.4 c (0.3)	13.2 cd (0.1)	11.7 d-i (0.6)
PAN 8247	35.7 def (0.1)	33.4 def (0.1)	10.0 fgh (0.1)	9.1 de (0.1)	13.3 cd (0.3)	11.3 c-g (0.1)
Banjo	33.6 bc (1.0)	30.3 a (0.8)	10.3 i (0.4)	10.2 f (0.3)	12.4 ab (0.4)	10.2 ab (0.5)
NS 5655	40.0 j (0.5)	36.4 h (0.7)	10.3 gh (0.5)	9.7 ef (0.1)	15.1 gh (0.1)	12.8 j (0.7)
Overflow	36.3 efg (1.3)	32.3 bcd (0.6)	8.8 c (0.6)	10.1 f (0.3)	13.3 cd (0.5)	11.0 b-f (0.5)
White tannin free cultivars						
PAN 8706	44.7 k (0.4)	47.2 i (0.5)	6.6 b (0.2)	4.8 b (0.2)	16.4 i (0.2)	15.3 k (0.1)
PAN 8648	46.9 l (0.4)	47.8 i (0.1)	5.0 a (0.3)	3.6 a (0.0)	15.4 h (0.4)	12.4 hij (0.5)
Mean	37.3 (3.6)	34.7 (4.7)	9.3 (1.3)	8.7 (1.7)	13.7 (1.1)	11.7 (1.3)

Values for Hunter *L*, *a* and *b* with different letters in the same column are significantly different ($p<0.05$).

Standard deviations are given in parentheses.

Condensed tannin cultivars had 3 to 7 times more phenolics than tannin-free cultivars (Table 2.1.3), which means that genetically, testa-containing cultivars have the highest phenolics. Values and trends of total phenolic content of different sorghum types recorded in literature were somewhat similar to the findings of this study. Dykes *et al.* (2005) reported similar trends in phenolic content when they investigated cultivars of varying genotypes. Awika *et al*. (2005) found the total phenolic content of condensed tannin sorghums ranging from 1.35 to 2.25 g gallic acid equivalents (GAE) /100 g sample. Red condensed tannin-free sorghums had 0.5 g GAE/100 g (Awika *et al*., 2003b) and 0.41 g GAE/100 g (Ragaee *et al.,* 2006). Values of 0.1 g GAE/100 g were reported for white tan plant sorghum by Awika *et al.* (2003b). The trends in values between condensed tannin and tannin-free sorghums are similar to those of this study.

Table 2.1.3 Effect of cultivar on the total phenolic content of South African sorghums

Cultivar	Potchefstroom	Klerksdorp
Condensed tannin cultivars		
NS 5511	1.00 i (0.01)	1.20 h (0.04)
PAN 8229	0.72 h (0.00)	1.16 h (0.02)
PAN 8625	1.20 j (0.04)	1.42 i (0.01)
Red tannin free cultivars		
PAN 8043	0.35 cd (0.02)	0.31 d-g (0.02)
PAN 8346	0.42 fg (0.07)	0.31 efg (0.07)
PAN 8141	0.36 c-f (0.00)	0.25 a-d (0.06)
PAN 8609	0.35 cde (0.06)	0.33 g (0.02)
PAN 8123	0.41 d-g (0.01)	0.30 c-g (0.02)
PAN 8420	0.35 cd (0.01)	0.33 g (0.01)
PAN 8564	0.35 cd (0.02)	0.32 fg (0.01)
PAN 8806	0.32 c (0.06)	0.29 b-g (0.03)
PAN 8738	0.41 d-g (0.01)	0.26 a-f (0.02)
PAN 8446	0.31 bc (0.02)	0.28 b-g (0.02)
PAN 8534	0.43 g (0.02)	0.31 d-g (0.04)
PAN 8247	0.36 c-f (0.02)	0.26 a-e (0.02)
NS 5655	0.35 cd (0.00)	0.24 abc (0.04)
Banjo	0.47 g (0.06)	0.29 b-f (0.02)
Overflow	0.32 efg (0.02)	0.30 c-g (0.01)
White tannin free cultivar		
PAN 8706	0.25 ab (0.01)	0.23 ab (0.02)
PAN 8648	0.20 a (0.02)	0.21 a (0.02)
Mean	0.45(0.03)	0.43(0.03)

Values with different letters in the same column are significantly different ($p<0.05$).

Standard deviations are given in parentheses.

Values expressed as g catechin equivalents/100 g dry basis

Condensed tannin cultivars had substantial levels of condensed tannins, as measured with and without a blank correction (Table 2.1.4). "Condensed tannins" were only measurable in tannin-free cultivars when reported without a blank correction. The apparent presence of condensed tannins is an artifact due to interference by coloured, non-tannin pigments such as flavonoids (Price and Butler, 1977). Reported condensed tannin levels vary considerably among condensed tannin cultivars. These range from 0.9 to 6.7 g CE/100 g

with blank correction (reviewed by Awika and Rooney 2004); 5.01 g CE/100 g (Awika *et al*., 2005); 4.91 g CE/ 100 g, (Dlamini *et al.,* 2007) and 5.48 g CE/100 g (Beta, Rooney, Marovatsanga and Taylor, 1999). Generally, the condensed tannin cultivars in this study had higher condensed tannin values compared to those reported in literature, except for NS 5511 which had average condensed tannin content of 5.32 g CE/100 g with blank correction.

Table 2.1.4 Effect of cultivar on the condensed tannin content of South African sorghums

Cultivar	Potchefstroom		Klerksdorp	
	B-	B+	B-	B+
Condensed tannin cultivars				
NS 5511	7.82 b (0.10)	8.64 g (1.05)	8.39 b (0.14)	9.49 e (0.29)
PAN 8229	5.16 a (0.34)	7.33 f (0.30)	5.28 a (0.27)	7.40 d (0.84)
PAN 8625	7.87 b (0.18)	10.01 h (0.76)	8.39 b (0.29)	9.65 e (0.43)
Red tannin free cultivars				
PAN 8043	ND	1.45 b-e (0.04)	ND	0.36 bc (0.02)
PAN 8346	ND	1.83 de (0.24)	ND	0.37 bc (0.04)
PAN 8141	ND	1.55 b-e (0.17)	ND	0.33 abc (0.02)
PAN 8609	ND	1.27 bcd (0.08)	ND	0.57 c (0.04)
PAN 8123	ND	1.56 b-e (0.20)	ND	0.55 c (0.09)
PAN 8420	ND	1.50 b-e (0.07)	ND	0.44 bc (0.04)
PAN 8564	ND	1.50 b-e (0.29)	ND	0.23 abc (0.02)
PAN 8806	ND	1.98 e (0.03)	ND	0.51 c (0.03)
PAN 8738	ND	1.43 b-e (0.16)	ND	0.13 abc (0.06)
PAN 8446	ND	1.21 bcd (0.11)	ND	0.52 c (0.04)
PAN 8534	ND	1.14 bcd (0.13)	ND	0.38 bc (0.07)
PAN 8247	ND	1.08 ab (0.12)	ND	0.22 abc (0.07)
NS 5655	ND	1.12 bc (0.13)	ND	0.19 abc (0.04)
Banjo	ND	1.82 cde (0.24)	ND	0.59 c (0.07)
Overflow	ND	1.76 b-e (0.28)	ND	0.45 bc (0.04)
White tannin free cultivars				
PAN 8706	ND	0.40 a (0.06)	ND	-0.13 a (0.04)
PAN 8648	ND	1.10 ab (0.13)	ND	0.01 ab (0.02)
Mean	6.95 (0.20)	2.43 (0.20)	7.35 (0.23)	1.64 (0.10)

Values with different letters in the same column are significantly different ($p<0.05$).

Standard deviations are given in parentheses.

B- values reported with blank subtraction, B+ values reported without blank subtraction.

Values expressed as g catechin equivalents/100 g dry basis

ND, Not detected.

Concerning antioxidant activity, condensed tannin cultivars had the highest antioxidant activity, followed by the red tannin-free cultivars and lastly white tannin-free cultivars

(Table 2.1.5). Antioxidant activity of condensed tannin cultivars was 2 to 4 times higher than in condensed tannin-free cultivars. This is similar to the situation with phenolic content (Table 2.1.3), which indicates a dependence of antioxidant activity on phenolic content. The higher phenolic content in condensed tannin cultivars was due to the contribution of condensed tannins, which resulted in more phenolic hydroxyl groups for radical scavenging than in condensed tannin-free cultivars. Condensed tannins have more phenolic hydroxyl groups on a molar basis or per unit mass of sample compared to simple phenolics (Hagerman *et al.*, 1998). Results for condensed tannin cultivars were similar to those obtained by Awika *et al.* (2005) of 240 µMol TE/ g, although values reported by Dlamini *et al.* (2007) were slightly higher than the findings of this study. Values reported for red condensed tannin-free (Awika *et al.,* 2003b; Ragaee *et al.,* 2006) and white tan plant cultivars (Awika *et al.,* 2003b; Dlamini *et al.*, 2007) were slightly lower than those obtained in this study.

Table 2.1.5 Effect of cultivar on the antioxidant activity of South African sorghums

Cultivar	Potchefstroom	Klerksdorp
Condensed tannin cultivars		
NS 5511	232.0 f (8.4)	243.1 e (0.4)
PAN 8229	206.4 e (7.6)	239.8 e (4.0)
PAN 8625	250.5 g (10.2)	278.0 f (1.4)
Red tannin free cultivars		
PAN 8043	87.9 abc (4.8)	85.7 cd (6.9)
PAN 8346	90.1 abc (7.6)	77.4 bc (5.9)
PAN 8141	88.7 abc (4.7)	78.2 bc (14.0)
PAN 8609	88.0 abc (0.4)	97.6 d (1.3)
PAN 8123	92.4 bcd (1.6)	80.8 bc (9.5)
PAN 8420	96.2 cd (0.1)	85.7 cd (8.8)
PAN 8564	94.4 cd (2.7)	87.3 cd (0.4)
PAN 8806	89.6 abc (0.3)	66.2 ab (2.9)
PAN 8738	94.2 cd (6.0)	83.9 cd (10.8)
PAN 8446	94.0 bcd (1.2)	83.4 cd (4.3)
PAN 8534	100.1 d (5.3)	81.8 c (9.8)
PAN 8247	91.7 a-d (2.1)	66.9 ab (8.0)
NS 5655	92.8 bcd (3.2)	80.6 bc (11.8)
Banjo	97.0 cd (1.4)	75.4 abc (4.6)
Overflow	94.2 cd (4.3)	83.2 cd (7.0)
White tannin free cultivars		
PAN 8706	81.7 a (3.2)	61.7 a (2.7)
PAN 8648	84.0 ab (0.4)	61.5 a (3.1)
Mean	112.3 (3.8)	104.9 (5.9)

Values with different letters in the same column are significantly different ($p < 0.05$).

Standard deviations are given in parentheses.

Values expressed as µMol Trolox equivalents/g of sample dry basis

The correlations in Table 2.1.6 indicate strong positive relationships between total phenolic content and condensed tannin content with antioxidant activity ($p < 0.001$). There was also a highly significant positive correlation between total phenolic content and condensed tannin content ($p < 0.001$). These correlations indicate that antioxidant activity is dependent on phenolic content of which condensed tannins are the major contributor. These findings are in agreement with those reported by several authors for

different sorghum types (Awika *et al.*, 2003b; Awika *et al.*, 2005; Dlamini *et al.*, 2007; Dykes *et al.*, 2005). Strong positive correlations between phenolic content and antioxidant activity have also been reported in other cereals such as wheat ($p < 0.01$), (Adom, Sorrells and Liu, 2003). In finger millet, total phenolics and condensed tannins also correlated strongly with antioxidant activity ($p < 0.001$), respectively (Siwela, Taylor, De Milliano and Duodu, 2007).

Table 2.1.6 Pearson's correlation coefficients between condensed tannin content, total phenolic content and antioxidant activity.

	TPC	CT	AA
TPC	1.00		
CT	0.96***	1.00	
AA	0.97***	0.98***	1.00

Pearson correlation coefficient (r), *** indicates significance at $p < 0.001$.
CT, condensed tannin content
TPC, total phenolic content
AA, antioxidant activity

2.1.5. Conclusions

Condensed tannin sorghum cultivars exhibit higher levels of total phenolics and antioxidant activity than condensed tannin-free cultivars. The highly significant correlations between total phenolic content with antioxidant activity indicate the high contribution of phenolics to antioxidant activity. Condensed tannins are major contributors to sorghum phenolics, hence the much higher antioxidant activity in the condensed tannin cultivars. Therefore, tannin sorghums have the potential to make health benefiting foods.

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2.2 Phenolic content, antioxidant activity and consumer acceptability of sorghum cookies

2.2.1 Abstract

Sorghum cookies were produced to determine their potential as vectors of antioxidants. Different sorghum cultivars and their flour extraction rates were evaluated for their effects on phenolic content and antioxidant activity of sorghum cookies. Consumer acceptance of the sorghum cookies was compared to that of wheat flour cookies. For each sorghum cultivar, cookies of 100% extraction rate flours had 2 to 3 times more total phenolics compared to those of 70% extraction rate flours, while antioxidant activity was 22 to 90% higher. Cookies of the condensed tannin sorghum had 2 to 5 times more phenolics compared to those of condensed tannin-free sorghum. Antioxidant activity was 145 to 227 µMol TE/g in cookies of condensed tannin sorghum compared to 10 to 102 µMol TE/g in those of condensed tannin-free sorghum. The sorghum flours had slightly higher phenolic content and antioxidant activity values than their corresponding cookies. However, on a flour basis, cookies contained slightly higher phenolics and antioxidants. The overall liking of the condensed tannin-free sorghum cookies was similar to that of wheat flour cookies. However, cookies of condensed tannin sorghum were least accepted compared to wheat flour cookies. Condensed tannin sorghum cookies appear ideal as vectors for antioxidants that can be exploited for their health benefits although their sensory properties may require improvement.

2.2.2 Introduction

Sorghum (*Sorghum bicolor* (L) Moench) is an indigenous African cereal, well adapted to the semi-arid and arid sub-tropical conditions prevailing over most of the African continent (Doggett, 1988). It is second in importance after maize in Africa south of the Sahara. Sorghum is important for food security in Africa with human consumption accounting for almost three-quarters of total utilisation and represents a large portion of the total energy intake in many African countries (FAO, 1995). However, despite its relative importance little is processed commercially (Dendy, 1995).

In southern Africa, the highest usage of sorghum is that of malting for opaque beer production and a small amount for malt breakfast cereal as well as sorghum meal and instant porridge. There may be opportunities to develop sorghum into other value-added products such as cookies, snacks and breads. Such value-added products may appeal to many consumers for convenience and may have acceptable taste, texture and shelf stability. Sorghum is considered a rich source of phenolics (Chapter 2.1), with antioxidant activity superior to other cereals such as wheat, barley, millet and rye (Raggae *et al.,* 2006). The antioxidant activity of phenolic compounds may offer potential health benefits such as protection against oxidative and radical damage of tissues, which is responsible for health complications such as cancers, cardiovascular disease, ageing among others (Kamath, Chandreshekar and Rajini, 2004; Kroon and Williamson, 2005). For this reason, sorghum products may act as vectors for phenolic compounds to provide these potential health benefits.

South Africa has a high rate of malnutrition amongst children below the age of 5 years due to several factors such as food insecurity, poverty and most importantly, immune-compromised systems. Human Immune Deficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS) have become a major threat with Sub-Saharan Africa being the most affected in the region (UNAIDS, 2006). The infection rate in South Africa continues to rise especially among pregnant women. According to the World Health Organization report in 2006, it was estimated that in South Africa the under 5 mortality

rate is 67 per 1000 live births (UNAIDS, 2006). The antiviral activity of phenolic compounds may be pertinent as an intervention in the control of HIV and AIDS in addition to the high-energy component of sorghum in the diet. There is evidence that tannins inhibit the infection of human T-cells by inhibiting reverse transcriptase and therefore may slow the progression to full blown AIDS (Nonaka *et al.*, 1990).

Since South African sorghums contain substantial amounts of phenolic antioxidants, (Chapter 2.1), this creates an opportunity to transfer phenolics to target consumers through consumption of convenient and affordable products developed from sorghum. Technologies such as baking may offer an opportunity to produce ready-to-eat foods and snacks of high value (Rooney and Waniska, 2000). Such alternatives have not been explored for sorghum in South Africa and unless a major shift from traditional usage of the crop is embarked on, the benefits of phenolics may not be fully realized. The objective of this work was to produce cookies from sorghum flour and determine how cultivars and flour extraction rates affect cookie antioxidant properties and to establish consumer acceptance of the cookies. Cookies were chosen as a vector for antioxidants due to their shelf-stability, high nutrient density and could be eaten as a convenient ready-to-eat functional food.

2.2.3 Materials and Methods

2.2.3.1 Sorghum grain

Two sorghum types were obtained from Free State Maltsters, South Africa, consisting of PAN 3860 (a red condensed tannin cultivar), and PAN 8564 and PAN 8446 (two condensed tannin-free red sorghums). The latter were mixed in equal proportions. Orbit (a white tan plant cultivar) was obtained from the Agricultural Research Council, South Africa, Potchefstroom.

2.2.3.2 Preparation of flours of different extraction rates

Representative sorghum grain was first decorticated using a Prairie Research Laboratory (PRL) type dehuller (Rural Industries Innovation Centre, Kanye, Botswana). The time taken during which the kernels stayed in the decorticator was regulated to obtain extraction rates of 70% and 90%. The decorticated grain and fines were collected and sieved manually through a 1 mm opening sieve to recover bran. Sieved fractions were weighed to determine the percentage bran removed. Decorticated grain was then successively hammer milled (Augsburg, Germany) through a 2 mm screen and finally through a 250 µm screen to obtain sorghum flour. Whole grain was simply hammer milled to obtain flour of 100% extraction rate. The flours were stored in airtight polyethylene containers and kept at 4^{o}C until used for making cookies and for analysis.

2.2.3.3 Preparation of sorghum and wheat cookies

Cookies were produced according to the sugar snap cookie AACC Method 10-50D, (American Association of Cereal Chemists International, 2000) with modifications. The formula comprised 75 g golden syrup, 90 g white margarine, 8 g sodium bicarbonate and 225 g sorghum flour or wheat flour (10% protein). White margarine, golden syrup and sodium bicarbonate were creamed at low speed for 3 min using an electric mixer. The creamed ingredients were then mixed with flour to obtain homogenous dough. Dough was rolled to thickness of 6 mm on a lightly greased aluminium cookie sheet and cut with a circular cookie cutter of 50 mm diameter, excess dough was removed. Cookies were baked in a pre-heated oven set at 180^{o}C for 10 min. Cookies were then cooled to room temperature and stored at 4^{o}C before analyses.

2.2.3.4 Analyses

2.2.3.4.1 Colour

Colour of sorghum grain, flours of different extraction rates and cookies was measured using a HunterLab ColorFlex (Hunter Associate Laboratories, Reston, VA, USA). The measurements were based on *L, a, b* Hunter scale, (L= lightness; +a = red, -a = green, +b = yellow, -b = blue).

2.2.3.4.2 Endosperm texture

The endosperm texture of sorghum grain was evaluated visually based on the observed proportion of corneous to floury endosperm on a scale of 1 (corneous endosperm) to 5 (soft, floury endosperm texture), (Rooney & Miller, 1982). Six kernels were each held firmly and cut longitudinally. The proportion of floury to corneous endosperm was observed and scored.

2.2.3.4.3 Pericarp thickness

Sorghum kernels were scraped with a scalpel to observe pericarp thickness. Pericarp thickness was rated as thin, intermediate or thick. A thick pericarp came off in flakes, while a thin one scraped off in small fragments or as a powder (Rooney & Miller, 1982).

2.2.3.4.4 Determination of total phenolic content, condensed tannin content and antioxidant activity.

Total phenolic content, condensed tannin content and antioxidant activity of flours and cookies were analysed as described in Chapter 2.1. Cookies were crushed into powder using a pestle and mortar and passed through a 1 mm test sieve to ensure uniform particle size before analysis.

2.2.3.4.5 Cookie measurements

Cookie baking character was determined by cookie width (W), thickness/diameter (T), and spread factor. The cookie width was measured across one axis then the cookies were rotated 90° and re-measured to obtain average width. Cookies were stacked randomly to determine average thickness. Spread factor was determined by dividing width by thickness (W/T).

2.2.3.4 6 Consumer sensory evaluation

Fifty-nine consumer panellists were recruited randomly (24 males and 35 females). Panellists were introduced to the sensory evaluation software (Compusense ® five 4.8 (1986-2007) Ontario, Canada) and signed consent forms, which stated the nature and composition of the cookies. Three sorghum cookie samples were presented to panellists in polyethylene zip-lock bags labelled with a randomised 3-digit code. A wheat flour cookie prepared in the same way as sorghum cookies was included as a control. Water was used to rinse the mouth before tasting every new sample. Panellists were requested to rate cookies for appearance, flavour/taste, aroma, texture and overall liking by assigning a score based on a nine-point hedonic scale (Table 2.2.1). Evaluation was carried out in three sessions and each panellist tasted samples once.

Table 2.2.1 Nine-point hedonic scale used to evaluate sensory properties of the cookies

Attribute	Rating
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

2.2.3.5 Statistical analysis

Experiments were repeated three times and duplicate samples were analysed to obtain a total of six observations. The effects of sorghum cultivar and flour extraction rate on total phenolic content, condensed tannin content and antioxidant activity on flour and cookies were determined using two-way analysis of variance (ANOVA) and Fischer's least significant difference test ($p < 0.05$). Pearson's correlation coefficient was used to analyse linear relationships between total phenolic content, condensed tannin content and antioxidant activity of the flours and cookies.

2.2.4 Results and Discussion

Table 2.2.2 shows that Orbit had a lighter coloured pericarp than the other sorghums, which were more pigmented. In agreement with this, the Hunter *L* values were significantly higher in Orbit than in the pigmented cultivars. In contrast, Hunter *a* values were significantly lower in Orbit because of the less red pigmentation than in the pigmented cultivars. Hunter *b* values were similar between the pigmented cultivars but slightly lower than in Orbit, which had more yellow pigmentation. PAN 3860 had a thick pericarp and a somewhat floury endosperm, while the tannin free-cultivars had thin

pericarp and somewhat corneous endosperm. Generally condensed tannin-containing cultivars are more floury and softer than condensed tannin-free cultivars (Rooney and Miller, 1982), hence the observed floury endosperm in PAN 3860.

Table 2.2.2 Physical characteristics of the sorghum types used for making cookies

Cultivar	Visual kernel colour	Pericarp thickness	Endosperm texture[a]	Kernel colour (Hunter)		
				L	*a*	*b*
Orbit	Creamy-white	Thin	2.2 a (0.4)	42.6 c (0.8)	6.05 a (0.2)	13.6 b (0.3)
PAN 8564/8446	Red	Thin	2.9 b (0.2)	38.0 b (0.6)	8.36 b (0.2)	10.8 a (0.4)
PAN 3860	Red	Thick	3.5 c (0.4)	34.6 a (0.6)	9.86 (0.5) c	10.7 a (0.4)

[a] Endosperm texture (1 = corneous, 5 = floury).

Values with different letters in the same column are significantly different ($p < 0.05$).

Standard deviations are given in parentheses.

The total phenolic contents of flours and cookies of the condensed tannin sorghum PAN 3860 were significantly higher at each extraction rate than in cookies and flours of PAN 8564/ 8446 and Orbit (Table 2.2.3). For each sorghum, flours of 100% extraction rate and their cookies had the highest total phenolic content, and the least with 70% extraction rate. Decortication to 70% reduced flour phenolic content by 43% to 66% with the highest reduction in PAN 3860 flour. Decortication successively removes the bran, which has a high concentration of phenolic compounds, hence the reduction in phenolic content at lower extraction rates. Dlamini *et al.,* (2007) similarly reported phenolic reductions of 33% to 77% in sorghums of 70% to 81% extraction rates. However, Youssef *et al.,* (1988) reported phenolic reductions of 70% to 85% due to decortication to 70%, higher than findings of this study.

Total phenolic content of the cookies was generally lower than that of flours and it would seem cookie making resulted in changes in total phenolic content by reducing assayable phenolics. However, when the flour component in the cookie is considered (57% sorghum flour in formulation), the phenolic content of cookies becomes higher than that of flours. This is based on the assumption that the phenolics in the cookies are contributed essentially by the sorghum component in the formulation. The dough preparation process is not likely to have resulted in an increase in the phenolic content as this was essentially a dilution. This is demonstrated by the following calculation using the dough prepared with 70% extraction rate Orbit flour as an example.

The dough formulation comprised 225 g flour, 75 g golden syrup, 90 g white margarine and 8 g sodium bicarbonate (total weight of 398 g dough).
TPC of 100 g Orbit flour (dry basis) is 0.13 g CE (at 12.2% moisture content)
On a wet basis, this becomes (0.13 x 100) ÷ (100+12.2) = 0.116 g CE
Therefore, 225 g Orbit flour (in dough) will have (225 x 0.116) ÷ 100 = 0.261 g CE
The assumption is made that the phenolic content of the dough is from the sorghum flour component (225 g).

This then means that all the 398 g of the dough contains 0.261 g CE. Also moisture content of the dough is assumed to be essentially the same as the Orbit flour (12.2%) since no water was added during dough preparation.

TPC of the dough on a dry basis then becomes:

{[(100 x 0.261) ÷ 398] x 100} ÷ (100-12.2) = 0.075 g CE/100 g db

Concerning cookies, the reported total phenolic content of 0.12 g CE (Table 2.2.3) is again assumed to be from the sorghum component, which was 57% of the cookie dough formulation. An equivalent 57% of the flour would give a total phenolic content of 0.074 g CE which is essentially the same as the total phenolic content of the dough but less than that of the cookies. This is an indication that on a basis of sorghum flour component, the cookies had higher phenolic content than both flours and doughs. Besides sorghum phenolics, there is a possibility that some newly formed compounds in the cookies may also reduce the Folin-Ciocalteu reagent.

Table 2.2.3 Effects of sorghum cultivar and flour extraction rate on the total phenolic content of sorghum flour and cookies

Cultivar	Flour extraction rate (%)	TPC	
		Flours	Cookies
Orbit	70	0.13 a (0.01)	0.12 a (0.00)
	90	0.20 bc (0.02)	0.20 b (0.01)
	100	0.30 d (0.04)	0.26 c (0.01)
PAN 8564/8446	70	0.17 ab (0.02)	0.16 ab (0.04)
	90	0.26 cd (0.05)	0.27 c (0.00)
	100	0.39 e (0.00)	0.30 c (0.02)
PAN 3860	70	0.42 e (0.02)	0.20 b (0.02)
	90	0.87 f (0.03)	0.61 d (0.01)
	100	1.25 g (0.04)	0.65 e (0.02)
Mean		0.44 (0.36)	0.31 (0.19)

Values with different letters in the same column are significantly different ($p<0.05$).
Standard deviations are given in parentheses.
TPC, total phenolic content (g catechin equivalents /100 g dry basis)

Condensed tannins were only measurable in PAN 3860 flours of 90% and 100% extraction rates and their cookies (Table 2.2.4). There was 12% reduction in condensed tannin content due to decortication to 90% extraction rate but at 70% extraction rate, condensed tannins could not be measured. Probably at 70% extraction rate, decortication had resulted in the removal of all the testa where the condensed tannins are located (Hahn and Rooney, 1986).

Cookie making caused substantial reductions of 95% and 96% in assayable condensed tannins in 100% and 90% extraction rate flours, respectively. Condensed tannin content of the cookies remained substantially reduced even when expressed on a flour basis (by calculations similar to that above for total phenolics). Condensed tannins form irreversible complexes with sorghum storage protein, kafirin (Emmambux and Taylor, 2003). Sorghum proteins are likely to have complexed condensed tannins during dough

preparation and baking, hence condensed tannins may be present in the cookies as insoluble complexes, which cannot be extracted and measured.

Table 2.2.4 Effects of sorghum cultivar and flour extraction rate on the condensed tannin content of sorghum cookies

Cultivar	Flour extraction rate (%)	CT	
		Flours	Cookies
Orbit	70	ND	ND
	90	ND	ND
	100	ND	ND
PAN 8564/8446	70	ND	ND
	90	ND	ND
	100	ND	ND
PAN 3860	70	ND	ND
	90	6.73 a (0.15)	0.28 a (0.01)
	100	7.78 b (0.02)	0.48 b (0.00)
Mean		7.25 (0.74)	0.38 (0.4)

Values with different letters in the same column are significantly different ($p<0.05$).
Standard deviations are given in parentheses.
CT, condensed tannin content (g catechin equivalents/100 g dry basis)
ND, Not detected

Table 2.2.5 shows that the antioxidant activity of flours and cookies from the condensed tannin sorghum PAN 3860 was at least twice that of PAN 8564/8446 and Orbit. Generally, the condensed tannin cultivar had higher antioxidant activity compared to condensed tannin free cultivars, a similar situation to that of total phenolic content (Table 2.2.3). Condensed tannins have a higher contribution to antioxidant activity because they have more hydroxyl groups on molar or unit mass basis for radical scavenging than simple phenolics (Hagerman *et al*., 1998). For all the cultivars, the 100% extraction rate flour had the highest antioxidant activity, which decreased with decortication, as with total phenolic content (Table 2.2.3). Greatest reductions in antioxidant activity occurred

in decorticated Orbit grain where decortication to 90% and 70% extraction rates drastically reduced antioxidant activity by 7 to 10 times with respect to whole grain flour. Dlamini *et al.* (2007) also reported substantial reductions of 73% to 87% in antioxidant activity due to decortication of both condensed tannin and tannin-free cultivars.

Flours had higher antioxidant activities than their corresponding cookies. However, considering the flour component in the formula, cookie antioxidant activity slightly exceeded that of flours, as was the same with total phenolic content. This finding may indicate the presence of other contributors to antioxidant activity apart from the sorghum flour phenolics. Factors believed to have contributed to excess antioxidant activity included Maillard reaction products formed during baking that exhibited antioxidant activity (Michalska, Amigo-Benavent, Zielinski and del Castillo 2008) and the release of bound phenolic acids from cell walls during baking (Dewanto, Wu and Liu, 2002). Despite their high antioxidant activity, tannin sorghum cookies may have potential negative implications on nutrition among children. Condensed tannins are linked to reduced protein availability by forming insoluble complexes with proteins (Emmambux and Taylor, 2003) and mineral deficiency as they implicated in chelation of metals such as iron and copper (Santos-Buelga and Scalbert, 2000).

Table 2.2.5 Effects of sorghum cultivar and extraction rate on the antioxidant activity of sorghum flour and cookies

Cultivar	Flour extraction rate (%)	Antioxidant activity[1]	
		Flour	Cookies
Orbit	70	13 a (1)	10 a (1)
	90	16 a (1)	15 a (2)
	100	123 c (12)	102 c (6)
PAN 8564/8446	70	98 b (6)	78 b (6)
	90	107 b (2)	94 c (6)
	100	130 c (5)	100 c (5)
PAN 3860	70	178 d (10)	145 d (3)
	90	322 e (3)	202 e (2)
	100	373 f (2)	227 f (2)
Mean		151 (120)	108 (72)

Values with different letters in the same column are significantly different ($p<0.05$). Standard deviations are given in parentheses.

[1]Values expressed as µMol Trolox equivalents/g dry basis

The total phenolics and condensed tannin contents of flours were highly correlated ($p < 0.001$) with antioxidant activity (Table 2.2.6), indicating the dependence of antioxidant activity on phenolic compounds. There was also a highly significant correlation ($p < 0.001$) between phenolic content and antioxidant activity of the cookies (Table 2.2.7) further confirming the contribution of sorghum phenolics to antioxidant activity.

Table 2.2.6 Pearson correlation coefficients between total phenolic content, condensed tannin content and antioxidant activity of sorghum flour

	TPC	CT	AA
TPC	1.00		
CT	0.95***	1.00	
AA	0.96***	0.90***	1.00

Pearson correlation coefficient (r), *** indicates significance at p< 0.001.

TPC, total phenolic content.

CT, condensed tannin content.

AA, antioxidant activity.

Table 2.2.7 Pearson correlation coefficients between total phenolic content, condensed tannin content and antioxidant activity of sorghum cookies

	TPC	CT	AA
TPC	1.00		
CT	0.93***	1.00	
AA	0.87***	0.80***	1.00

Pearson correlation coefficient (r), ***indicates significance at p< 0.001.

TPC, total phenolic content.

CT, condensed tannin content.

AA, antioxidant activity.

Table 2.2.8 shows that milling resulted in higher Hunter *L* values in the flours, which were almost twice that of whole grain (Table 2.2.2). The Hunter *L* values of flours increased slightly as extraction rate decreased to 70%. For each extraction rate, Orbit flours had the highest Hunter *L* values compared to those of pigmented cultivars. Hunter *a* values of flours of pigmented cultivars decreased with extraction and the values were negative for the Orbit flours, which indicates the absence of red pigmentation. Grain pigments are mainly concentrated in the pericarp and also in the testa of condensed tannin sorghums (Rooney and Miller, 1982). During decortication these pigments are reduced since decortication involves the removal of outer layers, resulting in the lighter endosperm. When cookies were made from the flours, they had lower Hunter *L* values coupled with an increase in red and yellow pigmentation (higher Hunter *a* and *b* values). Figure 2.2.1 shows photographs of the sorghum cookies, clearly showing the effect of cultivar and flour extraction rate on their colour. As can be seen, cookies of Orbit were lighter than those of pigmented sorghum.

Table 2.2.8 Hunter *L*, *a* and *b* values of the sorghum flours and cookies

Cultivar	Flour extraction rate (%)	*L*		*a*		*b*	
		Flours	Cookies	Flours	Cookies	Flours	Cookies
Orbit	70	84.7 h (0.3)	55.8 h (0.5)	-1.10 a (0.11)	5.61 a (0.35)	12.1 b (0.0)	20.5 f (0.4)
	90	81.8 g (0.2)	52.2 g (0.1)	-0.89 ab (0.03)	9.59 f (0.11)	15.2 d (0.1)	19.5 e (0.3)
	100	81.2 f (0.1)	43.4 d (0.3)	-0.69 b (0.08)	7.14 c (0.13)	15.5 d (0.2)	21.4 g (0.1)
PAN 8564/8446	70	81.5 fg (0.0)	48.2 f (0.6)	0.90 c (0.11)	8.37 d (0.44)	11.0 a (0.1)	20.5 f (0.6)
	90	76.9 e (0.1)	47.2 e (0.1)	1.85 d (0.17)	9.09 e (0.21)	12.9 c (0.3)	18.3 c (0.0)
	100	68.8 c (0.2)	40.5 c (0.1)	3.19 f (0.06)	6.40 b (0.03)	12.7 c (0.1)	18.0 cd (0.1)
PAN 3860	70	73.7 d (0.4)	46.9 e (0.2)	2.66 e (0.25)	7.32 c (0.10)	12.7 c (0.5)	17.4 c (0.1)
	90	65.1 b (0.2)	31.0 b (0.1)	3.95 g (0.05)	7.52 c (0.11)	11.7 b (0.1)	11.9 b (0.1)
	100	62.0 a (0.3)	30.2 a (0.1)	4.23 g (0.16)	7.19 c (0.02)	11.7 b (0.3)	11.2 a (0.1)
Mean		75.1 (7.9)	43.9 (8.5)	1.56 (2.04)	7.58 (1.23)	12.8 (1.5)	17.6 (3.6)

Values with different letters in the same column are significantly different ($p<0.05$).

Standard deviations are given in parentheses.

Figure 2.2.1 Cookies made from the sorghum flours a, Orbit; b, PAN 8564/8446, and c, PAN 3860 and wheat flour cookies.

Cookie width did not show a consistent trend, while thickness increased with flour extraction rate (Table 2.2.9). Generally, all cookies were gritty, fragile and did not have surface cracks. Similar sorghum cookie characteristics were observed by Badi and Hoseney (1976). However, cookies made with 100% extraction rate flours were the grittiest and most fragile. The reason for these observed textural properties in 100% extraction rate cookies could be the higher levels of bran, which may have resulted in coarse flour particles causing discontinuities in the dough and cookie matrix. For this reason, cookie structure was weakened. This is in addition to the fact that fragility is as a consequence of the absence of gluten. The fragility of the sorghum cookies is in agreement with findings by Schober, O'Brien, McCarthy, Darnedde and Arendt (2003). The authors determined the effect of gluten-free millet flour on the quality of cookies. They showed that coarse particles such as millet flakes disturbed the uniformity of cookie structure, as they were not homogeneously dispersed in the dough and cookies.

Table 2.2.9 Physical characteristics of the sorghum cookies

Cultivar	Flour extraction rate (%)	W[1] (mm)	T[2] (mm)	Spread factor[3]	Fragility	Grittiness
Orbit	70	59.2 ab (0.1)	6.1 a (0.1)	9.7	less fragile	least gritty
	90	59.4 b (0.6)	6.6 bc (0.0)	9.0	fragile	gritty
	100	58.4 ab (0.0)	6.8 c (0.1)	8.6	fragile	gritty
PAN 8564/8446	70	59.0 ab (0.3)	6.3 ab (0.1)	9.4	fragile	gritty
	90	58.1 a (0.1)	6.4 abc (0.0)	9.1	less fragile	gritty
	100	58.5 ab (0.1)	6.4 abc (0.1)	9.1	fragile	gritty
PAN 3860	70	58.3 ab (0.1)	6.3 ab (0.2)	9.3	less fragile	gritty
	90	58.9 ab (0.1)	6.8 c (0.1)	8.7	less fragile	gritty
	100	58.7 ab (0.4)	6.6 bc (0.2)	8.9	fragile	grittiest
Mean		58.7 (0.5)	6.5 (0.3)			

Values with different letters in the same column are significantly different ($p<0.05$).

Standard deviations are given in parentheses.

[1] Cookie width

[2] Cookie thickness (height)

[3] Width / thickness (W/T)

The sensory properties of the sorghum cookies compared to those of wheat flour cookies are shown in Table 2.2.10. Cookies made with 90% extraction rate flours were selected for consumer sensory evaluation. Ideally, wholemeal sorghum flours (100% extraction rate) would have been used to make healthy cookies as they were expected to exhibit the highest antioxidant activity. However, as stated, cookies made from whole meal flours were very fragile and difficult to handle. The sorghum flours with the next highest extraction level were the 90% extraction rate flours. Their cookies were much less fragile and easier to handle and hence suitable for consumer sensory evaluation. Amongst the sorghum cookies, the sensory attributes of PAN 8564/8446 and Orbit cookies were more acceptable than those of PAN 3860, the condensed tannin sorghum. PAN 3860 cookie attributes were generally inferior probably due to the more pronounced grittiness, dark colour and hard texture compared to those from condensed tannin-free sorghums.

In comparison with wheat flour cookies, the texture of all sorghum cookies was significantly less acceptable. This may mean that texture had a greater influence on the quality and acceptability of the cookies than other sensory attributes. Despite the differences between the sorghum cookies and wheat flour cookies, the consumers showed a slight overall liking for the condensed tannin-free sorghum and wheat flour cookies. The cookies from condensed tannin sorghum flours were neither liked nor disliked. Since wheat flour is generally used for making cookies, the similarities in the overall liking of the condensed tannin-free sorghum cookies and the wheat flour cookies indicate strong potential of sorghum flour for cookie making. Although refined wheat flour was used, it would have been ideal to compare sorghum cookies to whole wheat or bran-fortified wheat cookies as the sorghum cookies at 90% extraction rate have much of the bran still attached.

Table 2.2.10 Sensory properties of the sorghum and wheat flour cookies determined using a consumer sensory panel

Cookie	Sensory attributes[1]				
	Appearance	Smell/aroma	Flavour/taste	Texture	Overall liking
Orbit	6.37 ab (1.79)	6.37 ab (1.68)	6.64 b (1.95)	6.36 b (1.95)	6.41 b (1.95)
PAN 8564/8446	6.64 bc (1.68)	6.53 b (1.77)	6.58 b (1.87)	6.63 b (1.81)	6.54 b (1.87)
PAN 3860	5.98 a (2.00)	5.86 a (1.71)	5.51 a (1.92)	5.59 a (1.87)	5.39 a (2.03)
Wheat	7.10 c (1.75)	6.69 b (1.80)	6.98 b (1.70)	7.10 c (1.54)	6.86 b (1.84)
Mean	6.52 (1.84)	6.36 (1.76)	6.43 (1.94)	6.42 (1.87)	6.30 (1.99)

Values with different letters in the same column are significantly different ($p<0.05$).

Standard deviations are given in parentheses.

[1]Ratings based on 9-point hedonic scale

2.2.5 Conclusions

Cookies made from condensed tannin sorghum flours have substantial higher antioxidant activity than those of the tannin-free sorghum. Therefore, condensed tannin sorghum cookies appear ideal as a source of antioxidants that can be exploited for their health benefits. Despite the high antioxidant activity of condensed tannin cookies, consumers prefer them least compared to those of tannin-free sorghum and wheat flour because of grittiness. Improvements to sensory properties may be required such as using finer particle size flour produced by roller milling.

2.2.6 References

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CHAPTER 3: DISCUSSION

This chapter will be in three parts, which will first critically review the important methods used in the study. The second section will attempt to explain the observed trends in terms of the mechanisms of antioxidant activity in sorghum cultivars and cookies. The last part will discuss possible merits of sorghum cookie production and application to school feeding programmes in South Africa.

3.1 Methodologies

Decortication is applied to sorghum to remove bran (Dlamini *et al*., 2007) by progressively removing grain outer parts with minimum kernel breakage (Munck, 1995). However, floury endosperm cultivars of the condensed tannin type require less decortication time to minimise endosperm fragmentation compared to cultivars with a corneous endosperm (Dlamini *et al.,* 2007). Table 3.1 shows the decortication behaviour of the sorghum cultivars in terms of time required to obtain extraction rates of 70 and 90% using the PRL dehuller. The condensed tannin cultivar, PAN 3860 decorticated faster than condensed tannin free cultivars. Hammer milling was found to be somewhat challenging as the mill constantly heated up as the screen size became smaller. To minimise heating up, grain was successively milled from 2 mm screen and finally to 250 µm. This creates potential problems with the method of sorghum flour production and infrastructure.

Table 3.1 Time taken (min) to decorticate sorghum grains to 70% and 90% extraction rates

Cultivar	Extraction rate (%)	
	90	70
Orbit	2	8
PAN 8564/8446	2	10
PAN 3860	1	5

As stated, sorghum cookies of the different extraction rate flours were produced using the AACC Method 10-50D, (American Association of Cereal Chemists International, 2000) with modifications. The original method recommends a baking temperature of 205°C but was adjusted to 180°C as the cookie edges burnt before the whole cookie was adequately baked. The procedure is quick and allows for a substantial number of cookies to be made in a single day as dough preparation and baking take approximately 25 min. Although cookie production was done on a small scale, scaling up seems feasible. However, it may require the use of industrial ovens and dough mixers. The cookie recipe is simple and can be easily adopted. Ingredients used are few and locally available and affordable.

A negative aspect to the sorghum cookie procedure is the dough stickiness, which makes rolling uneasy. Schober *et al*. (2003) investigated the effect of gluten-free flours on the quality of gluten-free biscuits and also found the doughs to be sticky. The authors found that the doughs stuck strongly to the rollers with the consequence of extending the dough unaxially resulting in oval cookies. Binders such as xanthan gum and pregelatinised starch may be used to enhance dough binding and improve rolling properties. However, these will substantially increase costs.

The effects of sorghum cultivar and flour extraction rate on the total phenolic content of cultivars, flours and cookies were determined using the Folin-Ciocalteu method (Waterman and Mole 1994). The Folin-Ciocalteu assay is a widely used colorimetric assay for total phenolics in sorghum (Awika *et al.,* 2005; Dykes *et al.,* 2005; Dlamini *et al.*, 2007). An advantage of the procedure is that it is simple and does not require expensive equipment. However, the Folin-Ciocalteu reagent is non-specific and able to detect any phenolic hydroxyl groups (McGrath *et al.,* 1982). The non-specificity of the Folin-Ciocalteu may be a drawback in that phenolic groups from extractable proteins such as the amino acid tyrosine are also detected (Naczk and Shahidi, 2004) and may be involved in the oxidation-reduction reactions that interfere with phenolic absorbance readings. However, phenolic quantification using the Folin-Ciocalteu method allowed identification of meaningful trends between sorghum cultivars.

As expected, decortication to 70% extraction rate reduced the phenolic content of the flours, as the process removes the pericarp and testa where phenolic compounds are mostly concentrated (Hahn *et al*., 1984). With regard to cookies, the total phenolic values were slightly lower than that of flours. However, when the values were expressed as a function of the sorghum flour in the formula, the quantified phenolics were slightly higher than that of flours. This finding agrees with the non-specificity of the phenolic reagent and in this case, it can be concluded that oxidisable groups other than the sorghum phenolic hydroxyl groups may have reduced the Folin-Ciocalteu reagent. An explanation given by Michalska *et al*. (2008) in relation to rye bread is that some melanoidins (Maillard reaction products) formed due to browning of the bread are powerful antioxidants, which might react with the Folin-Ciocalteu reagent. Furthermore, other phenolics and phenolic by-products with antioxidant activity might also form which probably interfere with the assay. Hunter *L* values of the cookies (Chapter 2.1) were much lower than those of flours, which may partly be attributed to browning due to Maillard reactions during baking. The resultant products are likely to have reacted with the reagent as presumed for rye bread. The cookie formula was ideal for these reactions considering that it contained golden syrup, which contains the reducing sugars glucose and fructose **(**Laroque, Inisan, Berger, Vouland, Dufossé and Guérard, 2008). These probably reacted with the sorghum amino acids to bring about the browning reactions. Therefore, a combination of sorghum phenolic compounds and new antioxidant compounds formed during baking are likely to have resulted in an unexpectedly high level of phenolic content in the cookies, on a flour basis. These findings suggest that sorghum cookie making concentrates antioxidant compounds, which may be of benefit with respect to their health promoting action.

Sorghum cultivars, flours and cookies were evaluated for the presence of condensed tannins using the modified Vanillin-HCl assay of Price *et al*. (1978). An advantage of this method is the 20 min extraction time, which allows several analyses to be carried out in a single day making it rapid and relevant to a large number of samples as was the case with sorghum cultivars (Chapter 2.1). Although the assay is presumed to be specific for flavan-3-ols, dihydrochalcones and proanthocyanidins, which have a single bond at the

2,3-position and possess free *meta*-hydroxy groups on the B ring (Gupta and Haslam, 1978), it is subject to interference by non-tannin pigments such as anthocyanins which absorb at the same wavelength (Awika *et al.,* 2005). However, the modified Vanillin-HCl method allows for correction of background colour by using a blank, which is useful to correct discrepancies caused by non-tannin pigments.

Catechin was used as a standard in the study, although it is criticised for overestimating condensed tannin content making quantification of condensed tannins difficult (Price *et al.,* 1978). The findings of the study showed that condensed tannin content, measured only in condensed tannin cultivars and flours (Chapter 2.1 and 2.2) was generally higher than most values reported in literature (Awika *et al.,* 2005; Awika and Rooney 2004; Beta *et al.,* 1999 and Dlamini *et al.,* 2007). Condensed tannin content of cultivars and the different extraction rate flours ranged from 5.16 to 8.29 g CE/100 g and 7.75 to 8.83 g CE/100 g, respectively. Dykes and Rooney (2006) recommended that such high values of condensed tannin content be viewed as relative indices. The authors argued that tannins are located in the testa, which is only a portion of the outer covering that comprises approximately 5% to 6% (dry weight) of the kernel. Thus, tannin values exceeding 5% to 6% are too high because such a small portion of the kernel (the testa) may not contribute such high levels of tannins. Notwithstanding this limitation of over-estimation of condensed tannin content, catechin is widely used as a standard for Vanillin-HCl assay as there is a lack of appropriate standards for condensed tannins due to their structural complexity (Schofield *et al.,* 2001).

From the results of condensed tannin content of flours and cookies (Chapter 2.2), it is evident that decortication and cookie production had a considerable effect on measurable condensed tannins. As mentioned earlier, with phenolic content, decortication removed the pericarp and testa layers, where condensed tannins occur. In contrast to phenolic content of cookies, which was somewhat higher on a flour basis than corresponding flour, condensed tannin content was substantially less than that of the flour. The reduced extractability of condensed tannins in the cookies may in part be attributed to the condensed tannins forming complexes with sorghum proteins during dough formation

and baking. The major sorghum protein component that binds with condensed tannins is kafirin (Emmambux and Taylor, 2003). Kafirin accounts for 68 to 73% total protein in whole grain flours and the proportions of the various kafirin species in opaque endosperm, typical of condensed tannin sorghum are; α, 66 to 71%, β, 10 to 13% and γ, 19 to 20% (Belton, Delgadillo, Halford and Shewry, 2006). The interaction between condensed tannins and kafirin is irreversible (Emmambux and Taylor, 2003). Hence, insoluble complexes are formed which are difficult to extract. However, phenolic compounds from condensed tannin-free sorghums do not bind significantly to kafirin as they probably have low affinity to complex with the protein (Emmambux and Taylor, 2003). There have been attempts to elucidate the condensed tannin-protein interaction. Factors that affect condensed tannin complexation with protein include molecular size (Spencer, Cai, Martin, Gaffney, Goulding, Magnolato, Lilley and Haslam, 1988; Hagerman and Butler, 1981; Emmambux and Taylor, 2003). Condensed tannins have a high affinity for high molecular weight proteins (Emmambux and Taylor, 2003). The efficacy of condensed tannins in complexation derives from the fact that they function as polydentate ligands with multiple potential binding sites provided by numerous phenolic groups, with the protein, which is potentially a multi site acceptor molecule (Spencer *et al.*, 1988).

It can be inferred that during dough processing and baking, kafirin interacted with condensed tannins forming insoluble complexes, which were not extracted, hence the substantial decrease in condensed tannin content in the cookies with respect to flour. The mode of action for this interaction is presumed to be through hydrophobic interactions and hydrogen bonding (Spencer *et al.*, 1988). Initially, the condensed tannins seek preferred sites on the protein where aromatic rings 'dock' by hydrophobic interactions. The resultant changes in the polypeptide conformation bring aromatic groups into close juxtaposition to form a hydrophobic pocket, which may be implicated in solvent exclusion. Lastly, the hydrogen bonding between the phenolic residues and polar groups on the protein surface reinforce the condensed tannin-protein association. The condensed tannin-kafirin complexes may imply that sorghum protein may not be readily available for utilisation and has implications on the digestibility and availability of protein in

condensed tannin sorghum (Emmambux and Taylor, 2003). However, further studies to determine cookie protein digestibility and the amino acid content will have to be done.

To evaluate the antioxidant activity of sorghum cultivars, flours and cookies, the ABTS radical scavenging assay was used. The assay measures the relative ability of antioxidants to scavenge the $ABTS^{\bullet+}$ radical cation compared to that of Trolox, a water soluble vitamin E analogue. The assay is rapid and can be used over a wide range of pH values (Arnao, Cano and Acosta, 1999) in both aqueous and organic solvent systems. Its major drawback is the free radical generation period of 12 h and the instability of the radical solution after 16 h. This step makes the procedure long and analyses cannot be repeated in a single day. Alternatively the ORAC and DPPH assays may be used. The ORAC assay, although highly automated and standardized, uses expensive equipment and is sensitive to pH (Awika *et al*., 2003b). DPPH radical reacts slowly with phenolic compounds, up to 6 h or longer compared to 20 min in the case of $ABTS^{\bullet+}$ radical (Awika *et al.*, 2003b). DPPH is also prone to interference by colour in samples that contain pigments such as anthocyanins absorbing at the same wavelength (515 nm), which leads to an underestimation of antioxidant activity (Arnao, 2000). $ABTS^{\bullet+}$ radical scavenging activity was measured at 734 nm, outside the absorption range of most plant pigments thus, minimizing interference. Therefore, it is the preferred method as it gives the most consistent results among diverse sorghum varieties and can be easily used to measure total antioxidant activity (Awika *et al.*, 2003b). From the results of determination of the antioxidant activity of cultivars in the sections reported here, there were significant trends between the sorghum types. The condensed tannin sorghum cultivars had substantially high antioxidant activities followed by red and lastly white condensed tannin-free cultivars.

Regarding flours, it is evident that decortication had a great effect on their antioxidant activities. As mentioned, for total phenolic content, the removal of grain outer layers results in a loss of phenolic compounds, which are presumably responsible for radical scavenging. Antioxidant activity of cookies was somewhat higher on a flour basis. This corresponds to the phenolic content of the cookies already discussed above. There are

probably numerous antioxidant compounds formed in addition to those contributed by the flour, which reacted with the $ABTS^{\bullet+}$ radical.

Consumer sensory evaluation was used to determine the acceptability of sorghum cookies. Evaluation was conducted through a laboratory test and panellists were in separated tasting booths to avoid influence from each other and minimise distractions from noise. However, sensory laboratory test may be viewed as presenting unnatural product environment and probably not conveniently located for specific target groups. In this case, a laboratory represented a central point and accessible area for most consumers. There was no training for the consumers except introducing them to the session, which saved a lot of time. This resulted in a number of test sessions being done in a single day. Approximately 80% of the consumers were regular sorghum consumers and likely to represent perceptions of target groups.

Ideally, all cookies of the different extraction rates should have been evaluated but it would not have been feasible to have panellists evaluating nine samples at the same time. The 100% extraction rate cookies were the most fragile hence difficult to handle. The cookies of 90% extraction rate flours had better physical characteristics and were selected for evaluation. A wheat cookie had to be included as a control as wheat flour is generally used for cookie production. The four samples were a smaller number to handle and easier to distinguish unlike when a large number is involved. The results of the panel demonstrated that there were differences in the sensory properties of the condensed tannin cookies compared to those of condensed tannin-free sorghum and wheat flour cookies (Chapter 2.2). Condensed tannin cookies were judged as slightly inferior to tannin-free sorghum and wheat flour cookies.

3.2 Mechanisms of antioxidant activity in cultivars and cookies

For the sorghum cultivars and cookies, the highly significant correlations between phenolic content and antioxidant activity show that antioxidant activity is largely dependent on phenolics and phenolic content can be used as an indicator of antioxidant

activity. The mechanisms of antioxidant activity of phenolics can be explained in terms of structure-activity relationships and depends on the arrangement of functional groups about their nuclear structure (Rice-Evans *et al*., 1996). The total number of hydroxyl (OH) groups substantially influences mechanisms of phenolic antioxidant activity (Heim *et al*., 2002) by donating hydrogen and an electron to the hydroxyl, peroxyl and peroxynitrite radicals (Schofield *et al*., 2001). Considering the structure and size of condensed tannins compared to that of simple phenolics (phenolic acids and flavonoids) found in sorghum, it is evident that the numerous hydroxyl groups in condensed tannins were more effective in quenching the $ABTS^{\bullet+}$ radicals.

The sorghum cookies had slightly lower antioxidant activity than flours. In an attempt to determine the actual antioxidant activity in the cookies, antioxidant activity values were expressed on a flour basis and seemed somewhat higher than that of flours. Cookie antioxidant activity was presumed to be a function of sorghum flour and therefore, expected to be lower than that of flour. The increased antioxidant activity on a flour basis means that, as stated in Chapter 2.2 there were other antioxidants besides those of flour, which contributed substantially.

There is a possibility that thermal processing (baking) resulted in the observed increase in antioxidant activity, on a flour basis. The baking temperature of 180°C probably resulted in the disruption of cell walls releasing bound phenolic acids due to the breakdown of cellular constituents. The release of these phenolic compounds would have led to improved phenolic acid extractability in the cookies than in the flour. Phenolic acids in sorghum are present mostly in bound form associated with cell walls and ferulic acid is the most dominant (Dykes and Rooney, 2006). It is reasonable to assume that the release of ferulic acid and other bound phenolics contributed to increased total phenolic content and antioxidant activity. Dewanto *et al*. (2002) investigated the effect of thermal processing on the phenolic content and antioxidant activity of maize (sweet corn). The authors reported a substantial increase in total phenolic content and antioxidant activity attributed to enhanced release of bound phenolics from the breakdown of cellular constituents. Similar observations were made in roasted dry beans (*Phaseolus* spp.)

(Boateng, Verghese, Walker and Ogutu, 2008). Since ferulic acid is a major bound phenolic acid, it may be used as a marker to demonstrate the effect of heat treatment on the release of bound phenolic acids hence the effect on total phenolic content and antioxidant activity. Gallic acid may also be used to determine the release of bound phenolic acids into the free form, as it only exists in the bound form in sorghum (Dykes and Rooney, 2006).

As stated, Maillard reaction products (MRPs) have also been implicated in radical scavenging activity (Morales and Jiménez-Peréz, 2001; Michalska *et al*., 2008). Advanced (MRPs) such as melanoidins are thought to be responsible for increased antioxidant activity. As mentioned in section 3.1, the conditions for sorghum cookie production favoured Maillard reactions hence the formation of antioxidant products. Studies have revealed that the protein-bound 2,4-dihydroxy-2,5-dimethyl-1-(5-acetamino-5-methoxycarbonyl-pentyl)-3-oxo-2*H*-pyrrol (pronyl-$_L$-lysine) is a powerful antioxidant found in bakery products (Lindenmeier and Hofmann, 2004). In wheat and rye breads, antioxidant activity due to pronyl-$_L$-lysine was found in higher amounts in the crust (up to six fold) than in the crumb (Lindenmeier and Hofmann, 2004; Michalska *et al.*, 2008). Figure 3.1 shows the reaction scheme for the formation of (pronyl-$_L$-lysine). The reaction occurs during baking when glucose is thermally liberated from starch to produce the antioxidant by Maillard reactions via the transient intermediate and penultimate precursor acetylformoin (II in Figure 3.1) and lysine side chains or the N-terminus of the flour proteins (Lindenmeier and Hofmann, 2004). A similar reaction is presumed to have occurred in sorghum cookies resulting in the production of Maillard reaction antioxidants. The reaction was probably faster on the cookie surface as it was more exposed to heat and the reduced water activity on the surface favoured colour development (browning). The resulting MRPs were then involved in the reduction reaction with the $ABTS^{\bullet+}$ radical resulting in reduced absorbance at 734 nm similar to that of the natural antioxidants in sorghum flour. However, further investigations would be required to elucidate the structures of all MRPs formed and the functional groups responsible for antioxidant activity in the sorghum cookies.

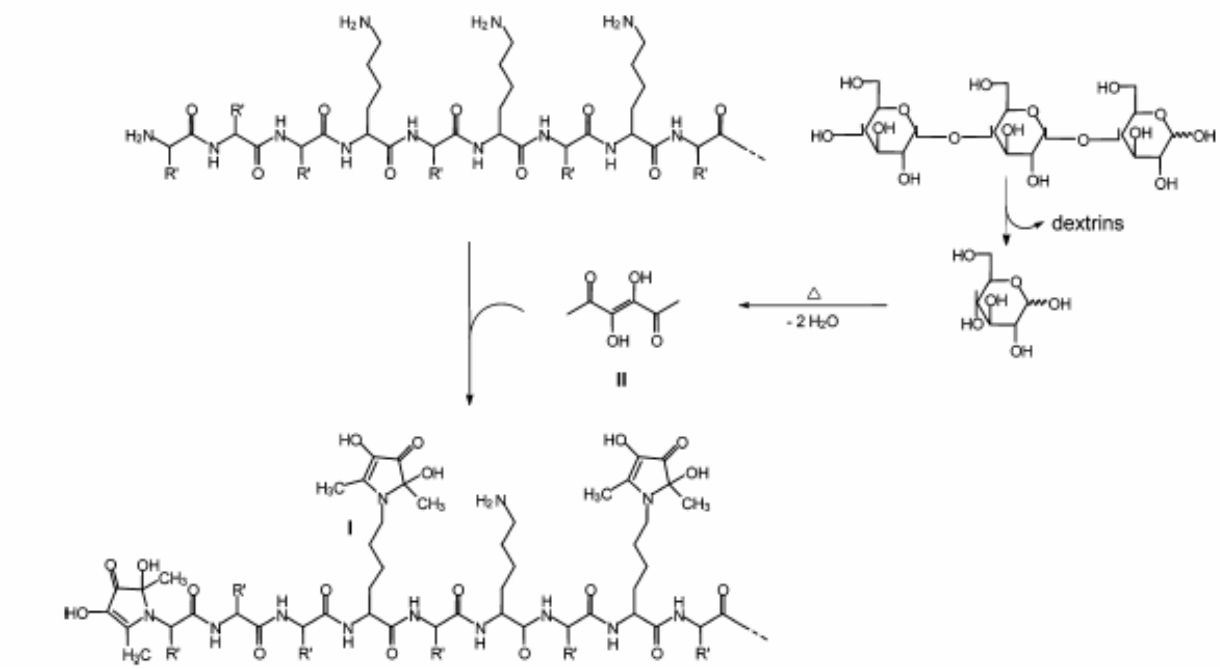

Figure 3.1 Reaction scheme for the formation of protein bound pronyl-$_L$-lysine (I) from proteins and starch via the key intermediate acetylformoin (II).

3.3 Merits of sorghum cookie production

The production of sorghum cookies somewhat increases measurable phenolic content hence, measurable antioxidant activity. Awika *et al.* (2003a) attributed higher antioxidant activity in cookies than in bread to the effect of the different processing conditions. Cookie dough making and baking take a shorter time than bread making, limiting the interaction between dough protein and phenolic components particularly condensed tannins. Therefore, the phenolic compounds are better retained in their original form in the cookies than in bread. Hence, sorghum cookies seem to have more potential as sources of antioxidants with possible health benefits than other baked products.

Sorghum cookie production could constantly supply school children with a functional and ready-to-eat snack. In South Africa, local women's clubs are actively involved in community-based activities such as feeding schemes (personal observation) and they can

be contracted to produce cookies on a large scale. Such measures will facilitate the provision of the cookies at stipulated times to avoid disruption of learning as well as ensuring a consistent ration. The method of cookie production is simple and uses inexpensive equipment although industrial ovens would be required for large-scale production. As cookies are shelf stable, they can be prepared in advance and stored unlike porridge making on site. A problem with preparing porridge may be the availability of constant labour, as it has to be freshly prepared before consumption. However, initial capital expenditure will be required to build facilities for cookie production and storage. This will be a once off cost and other costs incurred thereafter will be for operations.

The cost of producing sorghum cookies can be compared to that of wheat cookies since wheat flour is commonly used for cookie production. Table 3.2 compares the current prices of wheat and sorghum flour in South Africa and the cost of producing cookies. Sorghum flour is approximately 26% cheaper than wheat flour hence, production costs will be lower than using wheat flour. In South Africa, sorghum is charged 14% value added tax (VAT) and cookie production would be cheaper if the tax was scrapped as in the case with brown bread flour. Small-scale millers can produce sorghum flour locally and grain can be procured from local suppliers under contract to ensure a constant supply. This will drastically reduce overhead expenses incurred during the chain of supply such as transport costs.

Table 3.2 Estimation of sorghum flour costs per kg of cookies produced compared to wheat flour cookies (R9.80 = 1 US $)

	Sorghum (VAT incl.)	**Wheat (VAT incl.)**
Cost of flour/kg	R7.00	R9.50
Flour portion/kg formula	0.57 kg	0.57 kg
Cost of flour/kg cookies	R7.00 x 0.57 = R3.99	R9.50 x 0.57 = R5.42
	Sorghum (VAT excl.)	
Cost of flour/kg	R6.02	
Cost of flour/kg cookies	R6.02 x 0.57 = R3.43	

CHAPTER 4: CONCLUSIONS AND RECOMMENDATIONS

The condensed tannin sorghum cultivars have exceptionally higher phenolic content and antioxidant activity than tannin-free cultivars. Condensed tannins contribute significantly to phenolic content hence antioxidant activity. This is confirmed by the highly significant correlations between condensed tannin content and phenolic content with antioxidant activity. Condensed tannin sorghum cultivars would be ideal sources of antioxidants, which can be processed into various products with potential health benefits. Therefore, the sorghum cookies produced have the potential as vectors of phenolics to address nutrition and health needs of children through feeding schemes.

Decortication appears to have a detrimental effect on the phenolic content and antioxidant activity of sorghum flours and cookies, which implies that phenolics are concentrated on the grain outer layers. The sorghum cookies produced from low extraction rate flours have lower antioxidant activity than those of whole grain flour. Therefore, whole grain sorghum cookies would offer more benefits of antioxidants than those produced from low extraction rate flours. It is recommended that whole grain flour be used for producing sorghum products if the benefits of antioxidants are to be fully realised.

The antioxidant activity of cookies made from the different extraction rate flours seems somewhat lower than that of corresponding flours giving an impression that cookie production reduced antioxidant activity. However, the antioxidant activity of the cookies was slightly higher on a flour basis, which implies that antioxidants are concentrated in the cookies. The slight increase is thought to be due to the formation of antioxidants from Maillard reactions and the release of bound phenolic acids from cell walls during baking.

Although condensed tannin sorghum may seem ideal as sources of antioxidants, the sensory properties of their products may be slightly inferior to those of tannin-free sorghum and wheat flour due to grittiness attributed to high levels of bran in the flour. Grittiness may be difficult to overcome due to the coarseness of sorghum flour particles.

However, binders such as xanthan gum and pregelatinised starch may be used to improve dough binding properties, hence fragility. Besides hammer milling, alternative mills such as pin and roller mills could be used to produce finer particle size in sorghum flour that could possibly reduce grittiness. Since the sensory properties of tannin-free and wheat flour cookies are similar, sorghum flour has a strong potential for cookie production. The method of cookie production is simple and requires inexpensive equipment and feasible to scale up, hence cookies can be produced on a large scale.

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6: APPENDIX

Poster presentation from research:

Chiremba, C., Taylor, J. R. N., and Duodu, K. G. 2007. Phenolic content and antioxidant activity of South African commercial sorghum cultivars. 19th SAAFoST Biennial Congress and Exhibition, Durban, South Africa.