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Finger millet grain phenolics and their impact on malt and cookie quality

by

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DECLARATION

I hereby declare that the thesis submitted at the University of Pretoria for the award of PhD degree is my work and has not been submitted by me for a degree at any other university or institution of higher learning.

Muthulisi Siwela

February 2009

ABSTRACT

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Phenolics in finger millet (FM) grain, including tannins, may impact significantly on its antimicrobial properties, functionality and health-promoting potential. Unfortunately, the location of tannins in the grain is unknown and there is limited information on the influence of variety on grain phenolic composition and antioxidant activity (AA). The effect of phenolics in FM grain on its malt fungal load and on the functional quality of its food products, including baked goods, is barely known.

Twenty two FM grain types of varied visual kernel colour were analysed to determine the influence of grain type on phenolic composition, AA, and tannin localisation in the grain. Condensed tannins, anthocyanins and flavan-4-ols were detected. Light coloured grain types had no tannins and had much lower total phenolics (TP) relative to the pigmented types, and types that stained black with the Bleach test had much higher tannin content and much higher AA. The grains that stained black with the Bleach test and had high tannin content (0.60 to 2.08 mg catechin eqivalents/100 mg, db) had a dark coloured testa layer, indicating that the tannins were located in that layer. The results indicate that occurrence of tannins in FM is a varietal property and the tannins are predominantly responsible for the AA of the grain.

Germinative energy (GE), enzymic activity, and total fungal count [TFC], and infection levels of 12 FM grain types of varied phenolic content were measured to determine the impact of phenolics in FM grain on its malt quality. The malt quality of high-phenol FM types was much higher than that of the low-phenol types, with respect to enzymic activity. TFC was negatively correlated with grain total phenolics (TP) and amount of phenolic type (APT) and there were some negative correlations between fungal species infection levels and TP and APT ($p<0.05$). GE and enzymic activity were positively correlated with TP and APT ($p<0.05$) and negatively correlated with TFC ($p<0.01$). The data indicate that phenolics in FM grain impact positively on its malt quality by contributing to its antifungal activity.

Cookies in which wheat cake flour was substituted with 15, 35 and 55% (w/w) of either a non-tannin or a high-tannin FM flour were analysed to assess the impact of FM phenolics on cookie quality and AA (health-promoting potential). FM-substituted cookies, particularly those with high levels of the high-tannin FM, were inferior to cake flour cookies (control), with respect to spread, texture and integrity and their dark colour decreased their acceptance by a consumer panel. However, the acceptability of cookies containing up to 35% of either FM type was similar to that of control cookies. Cookies containing the high-tannin FM had antioxidant activities that were similar to or higher than the antioxidant activities of several plant products on the market. Thus, potentially health-promoting cookies can be made by substituting up to approximately 35% wheat with a high-tannin FM.

The study indicates that high-phenol FM grain types have good malt quality, which is partly due to the antifungal activity of their phenolics. Although FM phenolics, particularly tannins, seem to affect cookie quality negatively, they contribute significantly to their health-promoting potential.

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1. INTRODUCTION

1.1. Statement of the Problem

Finger millet [*Eleucine coracana* (L.) Gaertn.] is a small cereal grain grown in the semi-arid sub-tropical and tropical regions of Africa and Asia where it is one of the cereal staples (ICRISAT/FAO 1996, Obilana and Manyasa 2002). According to the US National Research Council (1996), finger millet has many good qualities. It is adapted to various agro-climatic conditions, its seeds can be stored for several years without insect damage, it is one of the most nutritious cereal grains and it tastes better than most other cereal grains. Finger millet is cultivated mainly in eastern, central and southern Africa, and in Asia, it is produced in India, Nepal and China (House 1995, Obilana and Manyasa 2002). According to Obilana and Manyasa (2002), finger millet annual world production is at least 4.5 million tonnes, of which Africa produces about two million tonnes.

Finger millet grain contains various phenolic compounds, including tannins (Dykes and Rooney 2006) that have been shown to contribute to its antioxidant properties (Sripriya et al 1996). Plant phenolics are currently receiving a lot of attention as they show potential health-promoting effects, which are attributed to their antioxidant activity (Scalbert et al 2005). Tannins have been shown to exhibit higher antioxidant activity than other phenolics (Hagerman et al 1998). Currently, there are limited data on the phenolic composition of finger millet grain; the relationship between grain characteristics (e.g. colour) and phenolic content and composition, antioxidant activity, and the effect of variety on phenolic content and composition are hardly known. Although Ramachandra et al (1977) reported that phenolic compounds, including tannins, were concentrated in the outer layers of the finger millet grain, the location of tannins has not been determined. Knowledge on the above stated issues is required so that the potential of finger millet as a source of antioxidant phenolics can be evaluated.

The prevailing high temperatures and relative humidities in the tropics favour growth of micro-organisms (particularly fungi) on cereal grains (Williams and McDonald 1983). Fungal growth

results in reduction of the quantity and quality of the grain (Christensen and Kaufmann 1974). Additionally, some fungal species can produce mycotoxins, which can be hazardous to humans and animals (D'Mello and MacDonald 1997). Some of the technologies used by the predominantly poor communities in the semi-arid sub-tropical and tropical regions of Africa and Asia to store (McFarlane et al 1995) and process (Murty and Kumar 1995, Daiber and Taylor 1995) sorghum and millet grains may be ineffective and unhygienic and they may thus particularly predispose the grains and their products to contamination by micro-organisms and mycotoxins. Published data indicate that sorghum and its malt are usually contaminated with various fungi and mycotoxins (Rabie and Lübben 1984, McFarlane et al 1995, Bandyopadhyay et al 2002). Although there are barely any published data on fungal and mycotoxin contamination of finger millet and its malt, the trends are probably similar to those reported for sorghum (McFarlane et al 1995). It has been shown that in some sorghum grain types there is a negative correlation between fungal infection levels and total phenolic content and amount of phenolic type, particularly the tannins, the flavonoid 3-deoxyanthocyanins and flavan-4-ols, suggesting that the compounds contribute to resistance to infection (reviewed by Chandrashekar and Satyanarayana 2006). Some finger millet grain types contain tannins as sorghum (Dykes and Rooney 2006) and may contain flavonoid compounds that are similar to those found in sorghum, which may contribute to resistance to fungal infection.

The US National Research Council (1996) states, "Despite its importance, finger millet is grossly neglected both scientifically and internationally". Finger millet is almost entirely a subsistence crop and in Africa, it is used primarily for the production of traditional foods, almost none of which are commercialised (ICRISAT/FAO 1996). While the utilisation of sorghum, another less common cereal, is being increased by using it to produce novel commercial products such as pasta, bread, cookies, and snack foods (Taylor et al 2006), the same is very limited with finger millet. Wheat is the ideal cereal for producing baked goods because it contains gluten proteins, which are essential for the quality of the products, but it grows well in cooler climates and hence countries in hotter regions import part or all of the wheat using the scarce foreign exchange. Partial substitution of wheat with finger millet in bakery products such as cookies may have many advantages including a high nutritional value, saving foreign exchange, and health-promoting (due to the phenolic antioxidants).

1.2. Literature Review

The known chemistry of phenolic compounds and their significance in food quality, and information on their antioxidant activity are reviewed. The structure and composition of finger millet grain are reviewed. Reports on the occurrence and localisation of phenolic compounds in finger millet grain and their contribution to the antioxidant activity of the grain are reviewed. Research on the potential health-promoting effects of sorghum and finger millet phenolics is reviewed. Information on the contamination of sorghum and finger millet grains by fungi and mycotoxins during malting is reviewed. Literature on the effects of malting on the phenolic content of sorghum and millets is reviewed. Information suggesting the contribution of phenolic compounds to the resistance of sorghum and finger millet grains to infection by fungi is evaluated. Research on the effects of thermal processing on phenolic content and antioxidant activity of sorghum and finger millet foods is also reviewed.

1.2.1. Finger millet grain structure and composition

In general, all cereal grains consist of a fruit coat or pericarp, which surrounds the seed and, the seed, consists of an embryo or germ and an endosperm enclosed by a nucellar epidermis and a seed coat or testa (Hoseney 1994). The structure of a cereal grain can be either an utricle or a caryopsis (Angold 1979). In a caryopsis, the pericarp or fruit coat surrounds the seed and adheres tightly to a seed coat (Angold 1979), whereas in an utricle, the pericarp surrounds the seed like a sac but is attached to the seed at only one point. The major cereal grains wheat, maize, barley, sorghum, rice, oats and rye are caryopses (Hoseney 1994), while amongst the millets, finger, proso, and foxtail millets are utricles (McDonough et al 2000).

1.2.1.1. Structure of the finger millet grain

The utricular structure of the finger millet kernel (grain) was reviewed by Angold (1979) and elucidated by McDonough et al (1986). The finger millet kernel is roughly globular to oval and 1-1.5 mm in diameter (Angold 1979) and, the 1 000 kernel weight was found to be 2.64 g

(McDonough et al 1986). The finger millet kernel consists of the outer layers, the germ and the starchy endosperm (Angold, 1979) (Figure 1.2.1).

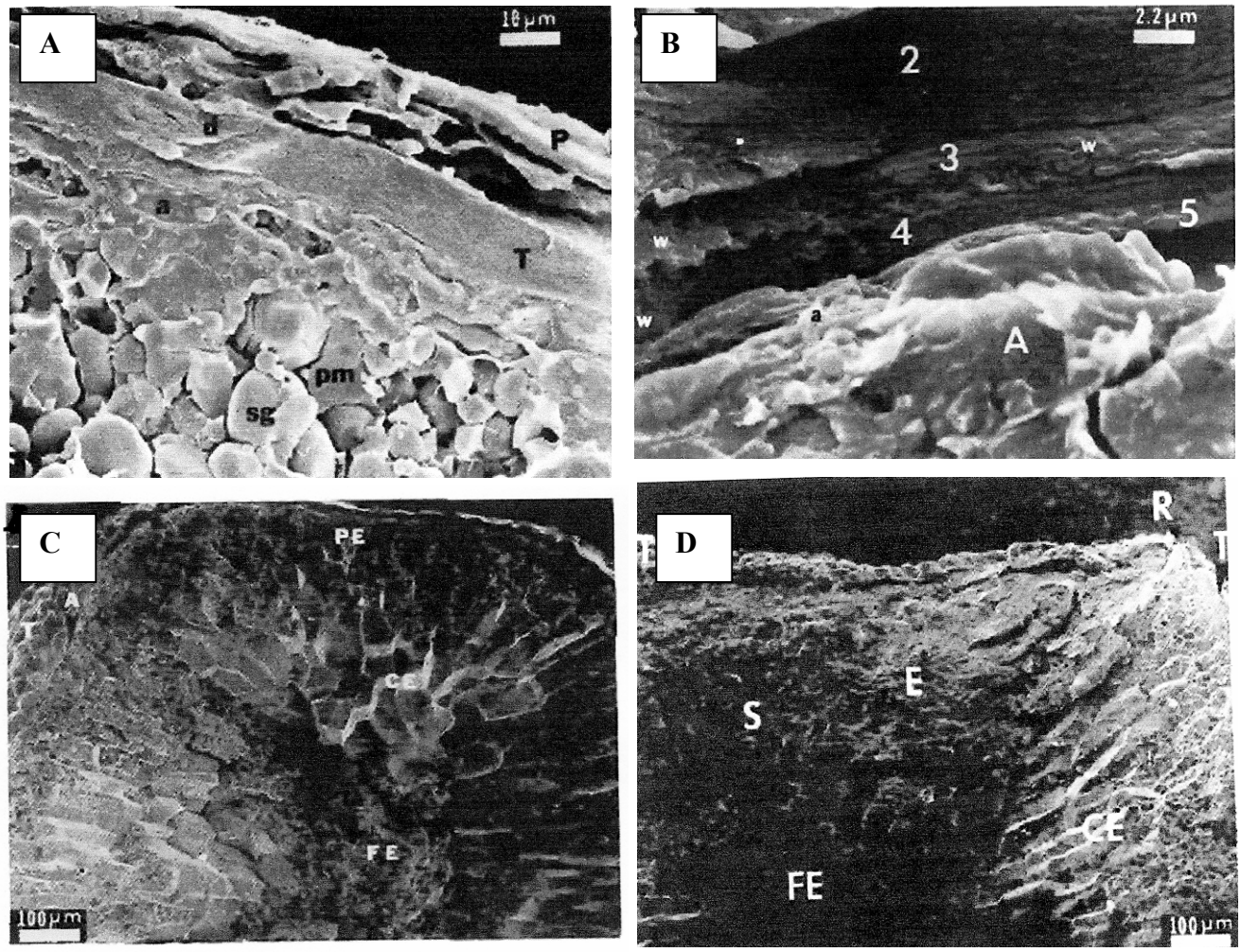


Figure 1.2.1. Structure of anatomical parts of the finger millet grain (McDonough et al 1986)

A- pericarp, testa, aleurone layer and peripheral endosperm layers. P= pericarp; T= testa; a= aleurone; pm= protein matrix; sg= starch granule.

B- Four of the five testa layers, showing wave formations and contour striations. 1-5= testa layers; w= wave formation; A= aleurone cell; a= aleurone cell wall.

C- Three discrete layers of the starchy endosperm. A= aleurone layer; PE= peripheral endosperm; CE= corneous endosperm; FE= floury endosperm.

D- Cross-section of the germ. R= ridge; T= testa; E= embryonic axis; S= scutellum

E- CE= corneous endosperm; FE= floury endosperm.

Outer layers

McDonough et al (1986) showed that the outer layers of the finger millet kernel comprise a membranous pericarp, which is loosely associated with the kernel at maturity, and a testa which overlays an aleurone layer (Figure 1.2.1A). The authors observed that the pericarp is a fragile membranous layer that is not fused to the testa at any particular place and it can easily be removed by rubbing or washing, similar to what had been described earlier by Angold (1979) and Hilu et al (1979). The pericarp appeared to be made up of several layers of tissue (McDonough et al 1986). McDonough et al (1986) also reported that the external appearance of the finger millet testa layer was quite striking and different from other cereals. They observed that the testa of finger millet varied from red to purple in colour and consisted of five distinct layers. The first layer was 1.5 µm thick, and autofluoresced blue, indicating the presence of ferulic acid or lignin. The second layer (beneath the first layer) appeared striated and was made up of sections of "interlocking" tissue that formed mound-like structures (Figure 1.2.1B). Junctions could be seen (Figure 1.2.1B) between the mound-like structures in the outer testa layers when the testa was viewed in cross-section; the junctions were thought to correspond to the interlocking sections seen from the surface. The second layer was the thickest (5.5-17.5 µm) and had darker pigmentation than the lower layers; it probably contained more phenolic compounds than others did. The third and fourth layers were approximately the same thickness (1.4-2.1 µm) and had the same shade in colour. The third layer had distinct wave formations throughout (Figure 1.2.1B), while the fourth layer was largely straight, with some isolated wave patterns. The fifth layer was 1 µm thick and had a distinctly different colour from the previous layers. Although the authors suggested that the bulk of the finger millet grain phenolics, including the tannins, were concentrated in the testa layer, the location of the tannins was not determined.

Endosperm

The endosperm comprises most of the weight of the finger millet kernel (McDonough et al 1986). These authors found that the aleurone layer of finger millet was similar to that of maize, sorghum and pearl millet. It was one cell layer thick and surrounded the entire starchy endosperm. It was packed with aleurone bodies, but starch granules were absent. The aleurone cell walls autofluoresced intensely, suggesting the presence of phenolic compounds. While Angold (1979) documented that the finger millet starchy endosperm had distinct floury and corneous layers,

McDonough et al (1986) identified three distinct types of starchy endosperm, the peripheral, corneous and floury endosperm (Figure 1.2.1C), similar to what was observed in pearl millet, sorghum and maize. The cells of the peripheral endosperm were the smallest of the endosperm cells. The cell contents of the peripheral endosperm were tightly packed. A large number of protein bodies were embedded in a protein matrix and were associated with compound (8.0-16.5 µm in diameter) and simple starch granules. The corneous endosperm formed the largest portion of the endosperm. It had cells of varied size. It had predominantly compound starch granules (3.0-19.0 µm in diameter) and there were simple starch granules in between them. Patches of a protein matrix were associated with the starch granules. The floury endosperm was made up of compound starch granules (11-21 µm in diameter). Protein bodies and a protein matrix were hardly present. The cell walls of the starchy endosperm strongly fluoresced, indicating the presence of phenolics.

Germ

McDonough et al (2000) stated that finger millet has a relatively small germ (270 x 980 µm). McDonough et al (1986) reported that the germ of finger millet was located in a depression surrounded by a characteristic ridge, which ran round the whole germ (Figure 1.2.1D). The hilum was located next to the germ and the stylar was located on the opposite side of the kernel. The scutellum was separated from the floury endosperm by the scutellum epidermis (Figure 1.2.1D). The scutellum contained protein bodies.

It is noteworthy that the finger millet grain is a challenge to process by milling because it is very small and because its testa is bound tightly to the endosperm (McDonough et al 2000).

1.2.1.2. Composition of the finger millet grain

According to the US National Research Council (1996), finger millet grain is more nutritious than most cereal grains with respect to minerals, dietary fibre and amino acids. As stated earlier, finger millet grain contains various phenolic compounds, which, due to their antioxidant properties, are potentially health-promoting (Dykes and Rooney 2006). The chemical composition of finger millet grain is shown in Table 1.2.1. The proximate (chemical)

composition of finger millet grain is affected by both environment and genetics (McDonough et al 2000). Although mean values are given in Table 1.2.1, the amounts of the grain chemical components generally vary widely. The composition and localisation of finger millet grain phenolics are reviewed in section 1.2.4.

Carbohydrates and dietary fibre

According to Obilana and Manyasa (2002), carbohydrates make up 70-76% of the total weight of the finger millet grain and comprise approximately 61.8% starch, 7.9% cellulose, 0.8% reducing sugars, 0.5% dextrins and 4.9% pentosans. As stated earlier, starch is present in the form of either simple or compound granules in the endosperm of the finger millet grain (McDonough et al 1986). Finger millet starch is composed of amylose and amylopectin whose molecular weights are similar to those reported for other cereal starches (Serna-Saldivar and Rooney 1995). The sugars found in finger millet grain include raffinose, sucrose, glucose, fructose and maltose (McDonough et al 2000). Sucrose and glucose constitute 33 and 12.5%, respectively, of the soluble sugars of finger millet grain.

The total dietary fibre (22.0%) of finger millet grain (Table 1.2.1) is relatively higher than that of most of other cereal grains (e.g. 12.6%, 4.6%, 13.4 % and 12.8% wheat, rice, maize and sorghum, respectively [Klopfenstein 2000]). As in other cereal grains, the fibre components of finger millet grain are located in the cell walls (mainly in the cell walls of the pericarp and endosperm) (Serna-Saldivar and Rooney 1995). Kamath and Belavady (1980) reported that dietary fibre made up 18.6% of the finger millet grain and comprised 6.1% non-cellulosic polysaccharides (1.5% water-soluble and 4.7% water-insoluble), 4.6% cellulose and 7.9% lignin. Chethan and Malleshi (2007) reported that finger millet grain contained 15.7% insoluble dietary fibre and 1.4% soluble dietary fibre, while Shobana and Malleshi (2007) reported 22.0% total dietary fibre, 19.7% insoluble dietary fibre and 2.5% dietary fibre. The non-cellulosic polysaccharide components of the dietary fibre of finger millet grain seem to be largely the non-starch polysaccharides arabinoxylans (pentosans), their major sugar constituents are arabinose, galactose, glucose and xylose, and mannose and rhamnose are minor constituents (Nirmala et al 2000, Subba Rao and Muralikrishna 2001).

Table 1.2.1. Chemical composition of finger millet grain

Nutrients								Non-nutrients	
Major nutrients (g/100 g[a])		**Minerals (mg/100 g[c])**		**Amino acids (g/100 g protein[d])**		**Vitamins (mg/100 g[c])**		**Phenolic compounds[d]**	
Moisture	12.0	Calcium	358.0	*Essential amino acids*		Vitamin A (RE)	6.0	*Phenolic assay[f]:*	
Carbohydrate	74.0	Chlorine	84.0	Phe	6.2	Thiamin	0.2	Folin/Ciocalteu	0.55-0.59
Protein	7.3	Copper	0.5	His	2.6	Riboflavin	0.1	Vanillin-HCl	0.17-0.32
Fat	1.3	Iodine (µg)	10.0	Ile	5.1	Niacin	1.0	Phenolic acids[g]:	
Total dietary fibre	22.0[b]	Iron	9.9	Leu	13.5	Vitamin C	1.0	Protocatechuic	23.1
Ash	2.6	Magnesium	140.0	Lys	3.7			Gentisic	61.5
		Manganese	1.9	Met	2.6			p-OH Benzoic	8.9
		Molybdenum (µg)	2.0	Thr	5.1			Vanillic	15.2
		Phosphorus	250.0	Val	7.9			Caffeic	16.6
		Potassium	314.0	*Non-essential amino acids*				Syringic	7.7
		Sodium	49.0	Asp	7.9			Coumaric	56.9
		Zinc	1.5	Glu	27.1			Ferulic	387.0
				Ala	8.0			Cinnamic	35.1
				Arg	5.2				
				Cys[e]	1.6				
				Gly	4.8				
				Pro	6.7				
				Ser	6.9				
				Tyr	3.6				
				Trp[e]	1.3				

[a]Obilana and Manyasa (2002).
[b]Shobana and Malleshi (2007)
[c]US National Research Council (1996).
[d]McDonough et al (2000).
[e]cysteine and tryptophan are not essential amino acids, but they can spare the requirement for methionine and phenylalanine, respectively. [f]mg/100 mg catechin equivalents, dry weight basis; [g]µg/mg, as is.

Protein

The protein content of finger millet grain varies from 4.9 to 11.3% (McDonough et al 2000). Protein content varies due to genotype, water availability, soil fertility, temperatures, and environmental conditions during grain development (Serna-Saldivar and Rooney 1995). White finger millet grain varieties were found to contain more protein than brown varieties (Virupaksha et al 1975, Rao 1994). The mean protein content of 7.3% (Table 1.2.1) is similar to that of rice (7.9%) (Klopfenstein 2000) and either lower or similar to that of other millets, sorghum and wheat (11.0, 9.6, 9.0, 7.9 and 12.6% pearl millet, tef, fonio, sorghum and wheat, respectively [Obilana 2003, Klopfenstein 2000]). Of the Osborne protein fractions (albumins, globulins, prolamins and glutelins), the prolamins constitute the major protein fraction in finger millet grain, followed by glutelins (Serna-Saldivar and Rooney 1995), the same as in sorghum and other millets (pearl, foxtail and proso millets). These fractions (prolamins and glutelins) are located mainly within the protein bodies and protein matrix, respectively, of the starchy endosperm (Serna-Saldivar and Rooney 1995). Albumin, globulin and glutelin fractions are rich in lysine and other essential amino acids (Serna-Saldivar and Rooney 1995). The proteins in finger millet have been found to be apparently nutritionally better balanced than proteins in other millets (Ravindran 1992). Eleusinin, the main protein fraction of finger millet grain, has good amounts of tryptophan, cystine, methionine and total aromatic acids, which are important in human health and growth and deficient in most cereal grains (US National Research Council 1996). Finger millet is particularly high in methionine, ranging around 5% of protein (US National Research Council 1996). However, as with other cereals, lysine is limiting in finger millet grain, but among the millets pearl and finger millets usually have most lysine (McDonough et al 2000). The bioavailability of proteins may be adversely affected by antinutritional factors, which may be present in the finger millet grain, mainly trypsin inhibitors and phenolic compounds, particularly the condensed tannins (Serna-Saldivar and Rooney 1995, McDonough et al 2000).

Lipids

According to Serna-Saldivar and Rooney (1995), sorghum and milllets contain various lipids, including phospholipids, glycolipids, triglycerides, phytosterols, carotenoids and tocopherols, which form a relatively small portion of the proximate composition of the grains. The lipids are mainly located in the scutellum. The lipids in sorghum and millets can be subdivided into polar,

nonpolar, and non-saponifiable lipids and are present as free, bound or structural lipids. The most abundant are the non-polar lipids consisting of the triglycerides (fat/oil). The total lipid content of finger millet grain is approximated to be 5.2%, with palmitic, oleic and linoleic acids being the main constituents (McDonough et al 2000). The fat content (free lipids) (1.3%) (Table 1.2.1) of finger millet grain is relatively lower than that of sorghum and other millets, and similar to that of wheat (4.8, 2.0, 1.8, 2.8 and 1.1% pearl millet, tef, fonio, sorghum and wheat, respectively [Obilana 2003]). Finger millet grain has a low fat (1.3% Table 1.2.1) content probably because it has a relatively small germ (Serna-Saldivar and Rooney 1995). The low fat content of finger millet may be significant in that the grain may have superior storage properties due to a low tendency to become rancid.

Minerals

Finger millet is a rich source of minerals, particularly calcium, which apparently can be 5-30 times more than in most cereals (US National Research Council 1996). Finger millet also has high levels of potassium, iron, magnesium, copper, sodium and phosphorus (Obilana and Manyasa 2002) (Table 1.2.1). The pericarp, aleurone layer, and germ are rich sources of minerals (Serna-Saldivar and Rooney 1995). However, the bioavailability of some of the minerals (e.g. phosphorus and divalent metal ions) may decrease due to their interaction with antinutritional factors, mainly phytic acid, oxalic acid and condensed tannins, which are present in finger millet grain (Serna-Saldivar and Rooney, 1995, McDonough et al 2000).

Vitamins

Finger millet contains both water-soluble and liposoluble-soluble vitamins: thiamin, riboflavin, niacin and apparently vitamin C plus the tocopherols (vitamin E) (Table 1.2.1) (Serna-Saldivar and Rooney 1995, Obilana and Manyasa (2002). Vitamin C is absent in the dried grain (Serna-Saldivar and Rooney 1995). The water-soluble B-vitamins are concentrated in the aleurone layer and germ, while the liposoluble vitamins are mainly located in the germ (Serna-Saldivar and Rooney 1995).

1.2.2. The chemical nature, occurrence and classification of plant phenolics and their significance in food quality

The term 'phenolic' can be defined chemically as a substance that possesses an aromatic ring bearing a hydroxyl substituent, including functional derivatives (esters, glycosides, etc) (Harborne 1989). Plant phenolics, are secondary metabolites that are products of two main pathways, the shikimate pathway and the polyketide (acetate) pathway (Harborne 1994a). Flavonoids, the largest group of phenolic compounds, are biosynthesized by a combination of the shikimate and the polyketide pathways. Phenolic compounds occur throughout the plant kingdom and phenolic type varies considerably with plant group and plant part. Over 8 000 plant phenolics are known. They may be present in the bound or in the free form. Bound phenolics are found in the cell walls where they are chemically linked to cell wall components, mainly polysaccharides. Free phenolics are located in the vacuoles.

Plant phenolics are conveniently classified according to the number of carbon atoms in the basic skeleton (Harborne 1994a). The types of plant phenolics range from simple phenols to complex polymers such as the condensed tannins (Table 1.2.2). Phenolic compounds of different types usually occur together in the same plant tissue or organ. Although p-hydroxybenzoic acid and its derivatives and hydroxycinnamic acid and its derivatives (Figure 1.2.2) are different types in the classical classification scheme in Table 1.2.2, they are often classed together as 'phenolic acids', e.g. in the literature of plant phenolics found in food (King and Young 1999, Scalbert and Williamson 2000). According to King and Young (1999) and Scalbert and Williamson (2000), the main classes of phenolics found in food are phenolic acids (Figure 1.2.2) and flavonoids (including condensed tannins *syn* proanthocyanidins).

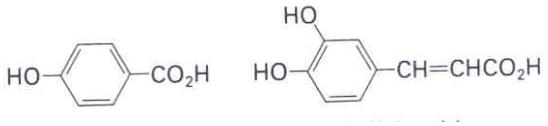
Properties of plant phenolics include that some are lipophilic, but the majority of them are water-soluble; they are usually acidic; they are chemically reactive; unless sterically hindered, they can take part in hydrogen bonding; are susceptible to oxidation; and, as will be reviewed further (section 1.2.3), they can chelate metal ions and have reducing power (Harborne 1994a). The plant phenolics found in foods are of significance as they may contribute to quality, including flavour, colour, nutrient bioavailability and oxidative stability (Salukhe et al 1982, Lule and Xia

2005; Naczk and Shahidi 2006). In addition, as mentioned earlier and as will be discussed further, due to their antioxidant and antimicrobial properties, plant phenolics are potentially health-promoting and may contribute to food safety and to a reduction in microbiological deterioration of food.

Table 1.2.2. The major classes of plant phenolics (Harborne 1994a)

No. of C atoms	Basic skeleton	Class	Example
6	C_6	Simple phenols	Catechol, hydroquinone
		Benzoquinones	2,6-Dimethoxybenzoquinone
7	C_6-C_1	Phenolic acids	p-Hydroxybenzoic, salicylic
8	C_6-C_2	Acetophenones	3-Acetyl-6-methoxybenzaldehyde
		Phenylacetic acids	p-Hydroxyphenylacetic
9	C_6-C_3	Hydroxycinnamic acids	Caffeic, ferulic
		Phenylpropenes	Myristicin, eugenol
		Coumarins	Umbelliferone, aesculetin
		Isocoumarins	Bergenin
		Chromones	Eugenin
10	C_6-C_4	Naphthoquinones	Juglone, plumbagin
13	C_6-C_1-C_6	Xanthones	Mangiferin
14	C_6-C_2-C_6	Stilbenes	Lunularic acid
		Anthraquinones	Emodin
15	C_6-C_3-C_6	Flavonoids	Quercetin, malvin
		Isoflavonoids	Genistein
18	$(C_6$-$C_3)_2$	Lignans	Podophyllotoxin
30	$(C_6$-C_2-$C_6)_2$	Biflavonoids	Amentoflavone
N	$(C_6$-$C_3)_n$	Lignins	-
	$(C_6)_n$	Catechol melanins	-
	$(C_6$-C_2-$C_6)_n$	Flavolans (condensed tannins)	-

- Information not found



A B

Figure 1.2.2. Phenolic acids. A, p-Hydroxybenzoic acid; B, Hydroxycinnamic acid (caffeic acid) (Harborne 1989)

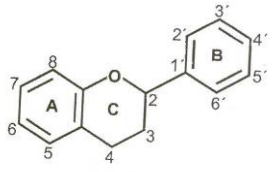


Figure 1.2.3. Basic structure and numbering system of flavonoids (Bravo 1998)

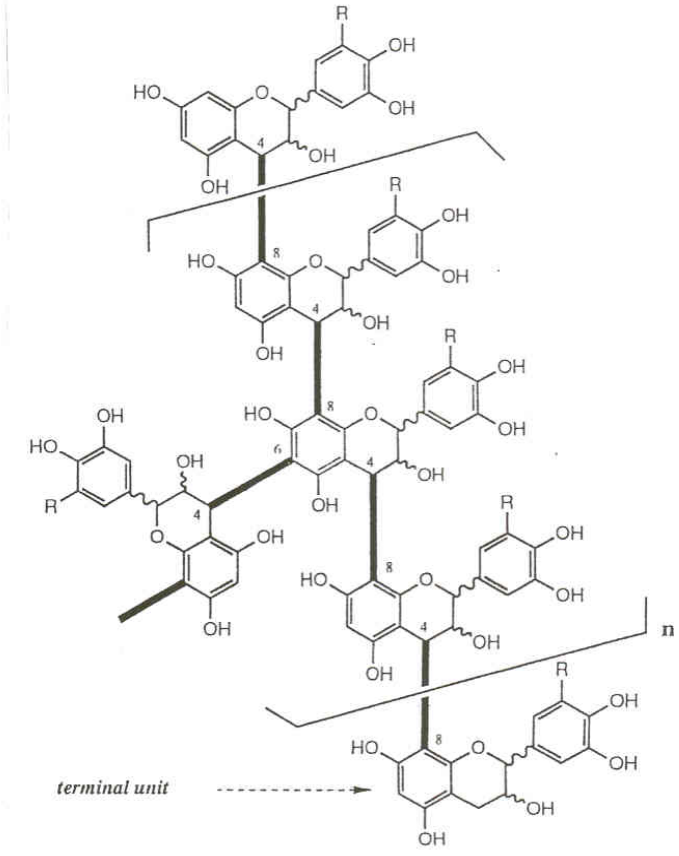


Figure 1.2.4. General structure for proanthocyanindin higher oligomers and polymers (Haslam 1998)

Flavonoids are structurally derived from the parent compound flavone (Harborne 1994a) and their basic structure is a flavan nucleus made up of three rings designated A, B and C (Figure 1.2.3). Flavonoids are classified according to the oxidation state of the central pyran (C) ring into anthocyanins, flavanones, flavones, flavanols, flavonols, chalcones, etc. Flavonoids vary, within each class, according to the number and position of hydroxyl, methoxyl, and other substituents (Harborne 1994a). Isoflavonoids, dimeric flavonoids (biflavonoids), and oligomeric and polymeric flavonoids (flavans [leucoanthocyanins and proanthocyanidins]) can also be included in the flavonoid class (Harborne 1989). Some flavonoids, particularly the anthocyanins, contribute colour to plants (Harborne 1994a) and plant foods (King and Young 1999), and may play a significant role as natural food colourants (Francis 1989). The common natural anthocyanins pelargonin, cyanin, paeonin, delphin, petunin and malvin are glycosides of the principal naturally occurring aglycones (anthocyanidins [flavan-3-en-3-ols]) pelargonidin, cyanidin, paeonidin, delphinidin, petunidin and malvinidin, respectively (Haslam 1998).

The term "tannin'' classically refers to substances of plant origin capable of transforming fresh hide into leather (Santos-Buelga and Scalbert 2000). Tannins are phenolics of relatively high molecular weight, which have the ability to complex strongly with carbohydrates and proteins (Porter 1989) and in the case of condensed tannins, with metal ions (Slabbert 1992). Tannins are divided into two groups, hydrolysable tannins and condensed tannins (*syn* proanthocyanidins (Santos-Buelga and Scalbert 2000). Hydrolysable tannins are esters of phenolic acids and a polyol, usually glucose. The phenolic acids are either gallic acid in gallotannins or other phenolic acids derived from the oxidation of galloyl resides in the case of ellagitannins (Santos-Buelga and Scalbert 2000). As with condensed tannins, hydrolysable tannins can exhibit antinutritional effects (Butler 1992). The most common effects are diminished weight gains and a decrease in nutrient (particularly proteins) utilisation efficiency.

Condensed tannins are oligomers and polymers of flavan-3-ol units (catechin or epicatechin). They are classified into two types, the B and A types. The B type condensed tannins are linked mainly through C4→C8 and/or C4→C6 interflavan bonds (Figure 1.2.4) (Haslam 1998). The A type condensed tannins have, in addition to the C4→C8 and/or C4→C6 interflavan bond, an ether bond between C2→O7. Condensed tannins characteristically yield cyanidin and

delphinidin upon heating in acidic media. Polyhydroxyflavan-3,4 diols (leucoanthocyanidins) are precursors of proanthocyanidins and polyhydroxyflavan-3-ols. They are natural products in their own right and are part of the non-hydrolysable tannin group (Porter 1989). When treated with mineral acid, leucoanthocyanidins produce red anthocyanidins (Haslam 1998). Condensed tannins are well-known for contributing to astrigency in plant foods and for their antinutritional effects, which seem to be mainly due to their interference with the metabolism of absorbed protein and chelation of metal ions (Santos-Buelga and Scalbert 2000).

1.2.3. Antioxidant activity of plant phenolics

Plant phenolics have been shown to possess antioxidant properties. They are hydrogen or electron donating agents and can thus scavenge free radicals. Phenolic compounds can quench reactive oxygen species (ROS) by donating hydrogen or electrons. They can chelate transition metal ions, particularly iron and copper ions. They are able to stabilise and delocalise unpaired electrons on phenoxyl radicals produced by the antioxidative reactions resulting in stable end products. Plant phenolic compounds exhibit higher antioxidant activity than other natural antioxidants; have been shown to be effective antioxidants *in vitro* than vitamins E and C on a molar basis. They exhibit antioxidant activity in both the aqueous and lipophilic phases, though the antioxidant levels may vary with the type of phase for each phenolic type (reviewed by Rice-Evans et al 1997).

According to Rice-Evans et al (1997), the antioxidant activity of plant phenolics has been found to be related to chemical structure. In phenolic acids, an increase in the number of hydroxyl groups and presence of conjugated double bonds tend to increase antioxidant activity (Rice-Evans et al 1996). Conjugated double bonds in the side chain may have a stabilising effect by resonance (delocalisation of electrons) on the phenoxyl radical formed by the antioxidative reaction. The electron-withdrawing property of the carboxylate group in benzoic acids has a negative effect on the H-donating abilities of the hydroxy benzoates. As a result, hydroxylated cinnamates exhibit higher antioxidant activity than their benzoate counterparts (Rice-Evans et al 1996). Some phenolic acids have been found to exhibit prooxidant activity in the presence of Cu^{2+} ions (Natella et al 1999).

In flavonoids, the structural arrangements imparting greatest antioxidant activity have been found to be the ortho 3′,4′-dihydroxy group in the B ring (e.g. in catechin, luteolin and quercetin), the meta 5,7-dihydroxy arrangements in the A ring (e.g. in kaempferol, apigenin and chrysin), the 2,3 double bond in combination with both the 4-keto group and the 3-hydroxy group in the C ring, for electron delocalization (e.g. in quercetin), as long as the O-dihydroxy structure in the B ring is also present (reviewed by Rice-Evans et al 1997). Alterations in the arrangement of the hydroxyl groups and substitution of contributing hydroxyl groups by glycosylation decreases antioxidant activity. The point of attachment of transition metals to the flavonoid molecule are the O-diphenolic groups in the 3′,4′-dihydroxy group in the B ring, and the ketol structures 4-keto, 3-hydroxy or 4-keto and 5-hydroxy in the C ring of flavonols (reviewed by Rice-Evans et al, 1997). Cao et al (1997) found that flavonoids exhibited antioxidant activity against peroxyl and hydroxyl radicals, but exhibited prooxidant activity in the presence of Cu^{2+} ions. Both antioxidant and prooxidant activities were related to flavonoid structure. In general, the more the hydroxyl substitutions were on the flavonoid nucleus, the stronger were the antioxidant and prooxidant activities. Antioxidant activity of flavonoids was found to be pH-dependent (Lemańska et al 2001). Antioxidant activity increased with increasing pH and that was interpreted as being due to deprotonation, which made it easier for the phenolic compounds to donate electrons.

Condensed tannins were found to scavenge $O_2^{\cdot-}$, $OH^{\cdot}$ and 2,2′-azinobis (3-ethyl-benzothiazolline-6-sulphonic acid) ($ABTS^{\cdot+}$) in aqueous solutions often as efficiently as quercetin or butylated hydroxytoluene (BHT). Galloylation increased the scavenging activity of the condensed tannins (reviewed by Santos-Buelga and Scalbert 2000). Condensed tannins were also found to inhibit the radical peroxidation of lipids, but galloylation of the condensed tannins was found to depress their free radical scavenging activity in the lipid phase (reviewed by Santos-Buelga and Scalbert 2000). The B type condensed tannins exhibited higher antioxidant activity than their A type counterparts. As part of their antioxidant activity, condensed tannins may chelate catalytic transition metal ions. On the other hand, condensed tannins may exhibit prooxidant activity in the presence of transition metal ions (reviewed by Santos-Buelga and Scalbert 2000). Complexation of tannins with model proteins such as bovine serum albumin (BSA) and gelatin was found to reduce the free radical scavenging activity of condensed tannins, but the tannin-

protein complexes had substantial free radical scavenging activity (Riedl and Hagerman 2001). Condensed and hydrolysable tannins were found 15-30 times more effective at quenching peroxyl radicals than simple phenolics or Trolox (a water-soluble vitamin E analogue) (Hagerman et al 1998). However, there are contradicting data on the influence of degree of polymerisation on antioxidant activity of condensed tannins (reviewed by Santos-Buelga and Scalbert 2000). In some work, no difference in antioxidant activity was found between monomers, dimers and trimers. In other work, antioxidant activity increased up to the trimers then decreased for larger degrees of polymerisation. The discrepancies could be due to the differences in the antioxidant assay used, to the structure of the condensed tannin tested or to the presence of impurities in the tannin fractions (reviewed by Santos-Buelga and Scalbert 2000).

Phenolic plant antioxidants have been reported to interact with each other, which affects the overall antioxidant activity of the sample. Meyer et al (1998) measured the antioxidant activities of catechin, quercetin, cyanidin, caffeic acid and ellagic acid, singly or in combination of two or three of the phenolic compounds. Acting singly, all the phenolic compounds inhibited the oxidation of low density lipoprotein (LDL), but to different levels. All the antioxidant effects were found to be additive except for combinations including ellagic acid, where the latter exerted a significant antagonistic effect.

1.2.4. Finger millet grain phenolics and their localisation, and their contribution to the antioxidant properties of the grain

Substantial quantities of phenolic compounds have been reported in finger millet grain (reviewed by Dykes and Rooney 2006). Ramachandra et al (1977) reported total phenolics (TP) ranging from 0.06 to 0.10 mg chlorogenic acid equivalents/100 mg in a white finger millet grain variety and 0.37 to 2.44 mg chlorogenic acid equivalents/100 mg in brown grain varieties. Chethan and Malleshi (2007) reported TP ranging from 0.30 to 0.50 mg/100 mg and 1.20 to 2.30 mg/100 mg) in white and brown finger millet grain varieties, respectively. As in sorghum, the phenolic compounds, which have been reported in finger millet, are phenolic acids, flavonoids and condensed tannins (Dykes and Rooney 2006).

The phenolic acids in finger millet grain have been identified using HPLC. McDonough et al (1986) reported moderate levels of gentisic, cinnamic and coumaric acid and high levels of ferulic acid. Although the location of the phenolic acids was not determined, the testa layer and the endosperm autofluoresced suggesting that the phenolic acids and other phenolic compounds were located in these structures. Subba Rao and Muralikrishna (2001, 2002) reported that the major bound phenolic acids in finger millet flour were ferulic acid, caffeic and coumaric acid. Procatechuic acid was found to be the major free phenolic acid in finger millet flour (0.05 mg/100 mg); and small amounts of gallic, caffeic, vanillic, ferulic and coumaric acids were present in the free form (Subba Rao and Muralikrishna 2002). The authors did not determine the location of the phenolic acids. Chethan and Malleshi (2007) showed that 90% of the finger millet grain phenolics were located in the testa. HPLC analysis of methanolic extracts from the testa showed that they contained benzoic acids (gallic acid, proto-catechuic acid, and p-hydroxybenzoic acid) and cinnamic acids (p-coumaric acid, syringic acid, ferulic acid, and trans-cinnamic acid). Similarly, Viswanath et al (2008) reported that the seed coat of finger millet had the highest phenolic content (12.60 mg/100 mg) followed by whole grain flour (7.30 mg/100 mg), whilst other flour fractions had much lower phenolic content (3.30 to 4.30 mg/100 mg) than that of the seed coat. The major phenolic acids identified by these authors using HPLC were diadzene, gallic, coumaric, syringic and vanillic acids.

There seem to be no reports on the detection and identification of flavonoid monomers in finger millet grain. However, Hilu et al (1978) reported eight flavones (orientin, isoorientin, vitexin, isovitexin, saporanarin, violanthin, lucenin-1 and tricin) in finger millet leaves.

Ramachandra et al (1977) reported condensed tannin contents ranging from 0.03 to 0.06 mg catechin equivalents (CE)/100 mg and 0.12 to 3.47 mg CE/100 mg in white finger millet varieties and brown grain varieties, respectively. McDonough et al (1986) reported 0.17 to 0.32 mg CE/100 mg in 10 finger millet grain varieties and noted that red varieties had higher tannin content than white varieties. Although Ramachandra et al (1977) showed that finger millet grain phenolics were concentrated in the outer layers and Chethan and Malleshi (2007) and Viswanath et al (2008) showed that the phenolics were specifically located in the testa layer, the location of tannins has not been specifically determined.

Sripriya et al (1996) reported that methanolic extracts from brown finger millet grain quenched 94% of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, whereas extracts from white finger millet grain quenched only 4% of the radical. The total phenolic content of the brown finger millet grain was 0.10 mg/100 mg, whereas that of the white finger millet grain was 0.03.mg/100 mg. The total phenolic contents of rice, wheat, sorghum and pearl millet (0.00, 0.02, 0.04 and 0.05 mg/ 100 mg) were lower than that of the brown finger millet grain, and they correspondingly had lower DPPH radical quenching capacity. The authors attributed the high DPPH radical quenching activity of the brown type to phytic acid and phenolic compounds, particularly the tannins in the seed coat. However, the authors did not determine the tannin content of the grain and did not show that the tannins were located in the seed coat. Quantitative relationships between total phenolics and tannin content and antioxidant activity were not reported.

Hegde and Chandra (2005) found that brown varieties of finger millet, foxtail millet and great millet and sorghum had higher total phenolics than their white counterparts. In addition, the brown varieties exhibited higher DPPH quenching activity than the white varieties. The two brown finger millet grain varieties studied had substantial condensed tannin levels (0.15 and 0.52 mg catechin equivalents/100 mg, whilst there were no tannins detected in the white finger millet grain variety. However, there was no correlation between total phenolics and tannin content and antioxidant activity of the grain. Subba Rao and Muralikrishna (2002) found that a mixture of free phenolic acids extracted from finger millet exhibited higher antioxidant activity than a mixture of bound phenolic acids extracted from the same grain. The authors suggested that gallic acid, which is known to be a more potent antioxidant compared to other benzoic acids and some cinnamic acids such as coumaric acid, was responsible for the higher antioxidant activity of the free phenolic acids. Viswanath et al (2008) reported that the antioxidant activity, determined as per the ß-carotene Bleaching method, of methanolic extracts from the seed coat of finger millet grain which had higher phenolic content was much higher (86% Bleaching effect) than that (27% Bleaching effect) of extracts from whole grain flour.

1.2.5. Potential health-promoting effects of plant phenolics with particular reference to sorghum and finger millet phenolics

According to Aruoma (1998), free radicals (chemical species that possesses one or more unpaired electrons) and other reactive oxygen species (ROS) are constantly formed in the human tissues. ROS that are of importance in living organisms include free radicals, OH, $O_2^{\cdot -}$, $NO^{\cdot}$, and $RO_2^{\cdot}$ and, non-free radicals, $ONOO^-$, HOCI, H_2O_2, $^1O_2$, and O_3 (Aruoma, 1998). Free radicals and other ROS may, through various reaction mechanisms, including oxidation, cause several human diseases, mainly degenerative conditions such as cancer, cataracts, neurodegenerative diseases and cardiovascular diseases. Transition metal ions in one chemical form (e.g. Fe^{3+} and Cu^{2+}) catalyse free radical reactions. Due to their antioxidant properties (reviewed in section 1.2.3), plant phenolics may scavenge free radicals, quench other ROS and chelate metal ions in human tissues and thereby contribute to limiting the development of degenerative conditions (reviewed by Halliwel et al 1995, Scalbert et al 2005).

Lee and Pan (2003) reported that dietary tannin-sorghum distillery residue inhibited haemoglobin-catalysed oxidation of linoleic acid in cultured mullet fish. The sorghum residue also significantly improved blood-thinning and erythrocyte membrane integrity of the fish blood cells during winter, thus maintaining normal blood fluidity and preventing haemolysis of the erythrocytes induced by H_2O_2. The anti-haemolytic effect of the sorghum residue was attributed to the antioxidant activity of tannins and other sorghum phenolic compounds. The findings suggested that sorghum phenolics may reduce the risk of cardiovascular diseases. An *in vitro* study done by Grimmer et al (1992) indicated that sorghum phenolics, particularly the tannins had antimutagenic properties. Goméz-Cordovéz et al (2001) demonstrated that sorghum tannins had anti-carcinogenic activity against human melanoma cells and contributed to cellular activity against skin damage by UV irradiation.

Hegde et al (2002) reported that 3 mg of methanolic extracts of finger and kodo millet significantly inhibited glycation/glycosylation of collagen at a level similar to that of 125 mg of the antiglycating agent amino-guanidine and 1 mg of the well-known synthetic antioxidant butylated hydroxyanisole (BHA). Glycation/glycosylation is the nonenzymatic, oxidative reaction between the aldehyde group of reducing sugars and the amino group of proteins.

Glycation/glycosylation is associated with the crosslinking of collagen, a major factor responsible for the complications of diabetes and ageing (Monnier 1990). Hegde et al (2002) suggested that glycation was inhibited by phenolic compounds in the methanolic extracts from the millets through their antioxidant activity. Rajasekaran et al (2004) demonstrated that feeding wound healing-impaired diabetic rats with finger millet improved the antioxidant status of the tissues and improved wound healing capacity. The authors suggested that finger millet phenolics could have contributed to these positive effects through their antioxidant activity and other health-promoting effects. In another study, Hegde et al (2005) reported that the levels of antioxidant enzymes (catalase, superoxide dismutase, etc) and non-enzymatic compounds (glutathione, vitamins E and C) were significantly reduced in diabetic rats and restored to normal levels in the diabetic rats fed finger and kodo millets. The millet-fed rats had lower collagen glycation than the control rats. Chethan et al (2008) demonstrated that finger millet phenolics inhibited aldose reductase, an enzyme whose activity is linked to diabetes-induced eye cataract. The inhibition of the enzyme was correlated ($r= 0.99$; $p<0.01$) with the antioxidant activity of the finger millet phenolic constituents, suggesting proton abstracting ability was responsible for the inhibitory effect.

1.2.6. Contamination of sorghum and finger millet grains and malts by fungi and mycotoxins and the potential contribution of grain phenolic compounds to fungal resistance

1.2.6.1. Contamination of sorghum and finger millet grains by fungi and mycotoxins

Based upon their ecology (mainly moisture content requirements), fungi that grow on cereal grains are divided into (1) field fungi and (2) storage fungi (Christensen and Kaufmann, 1974). Field fungi invade grain before harvest or before the grain is threshed, and require a moisture content in equilibrium with relative humidities of 90-100%, which in cereal grains means a moisture content of 22 to 33%, wet-weight basis, or 30 to 33%, dry weight basis (Christensen and Kaufmann, 1974). Storage fungi are primarily saprophytic and are mainly several species of *Aspergillus* and *Penicillium*. They require moisture contents in equilibrium with relative humidities of 70-90% (Williams and McDonald, 1983).

Various fungi (including the potentially toxigenic types) and mycotoxins are known to contaminate the sorghum grain (reviewed by Williams and Rao 1981, Williams and McDonald 1983, McFarlane et al 1995, Bandyopadhyay et al 2002).

In contrast, there is little information on fungi that contaminate finger millet grain and correspondingly there is limited information on mycotoxin contamination of the finger millet grain. McFarlane et al (1995) indeed state, "No specific information is available on the fungal flora and subsequent mycotoxin content of proso millet, *Setaria*, *Eleusine* millet, and teff". However, there are some fungi that are known to infect the finger millet plant causing various diseases (House et al 1995, Esele, 2002). Some of the fungi may or are known to infect the finger millet grain. The most important fungal disease in finger millet is Blast, which is caused by the fungus *Pyricularia grisea*. The fungus *P. grisea* affects finger millet at all stages of plant development, from seedling to grain formation, which may result in seedling death, flower sterility, reduced grain number and grain weight, shrivelled grain and reduced germinability (Ekwamu 1991, Esele 2002). Blast is both economically significant and very destructive, causing over 50% yield losses, especially in wet seasons. Although *P. grisea* is spread primarily by airborne conidia and rain splash, infected grain used as seed may also be a source of innoculum (Esele 2002). Other fungi that are known to cause finger millet diseases (which may include grain infection) include *Helminthosporium nodulosum*, *Sclerotium rolfsii*, *Sclerophthora macrospora*, *Fusarium* spp., *Melano-psichium eleusinis* and *Phyllachora eleusines* (House et al 1995, Esele 2002). *Helminthosporium nodulosum* causes seedling and leaf blight and infects the kernels, which may result in discolouration and death of germinating seeds. The seed is the primary source of innoculum (Esele 2002). *Sclerophthora macrospore* causes mainly a leaf disease called Downy Mildew but the kernels are also infected and the fungus is spread through the seeds. *Melano-psichium eleusinis* affects mainly the finger millet inflorescence at grain formation causing a disease called Smut (the inflorescence and grain are small and shriveled) (Esele 2002). A study of the natural occurrence of *Alternaria* mycotoxins in sorghum and finger millet grains produced by marginal and poor farmers of North Bihar, India, showed that all sorghum and finger millet samples were infected to various extents by *Alternaria alternata* and three out of eight finger millet grain samples were contaminated with one to three *Alternaria* mycotoxins, namely tenuazonic acid (TA), alternariol methyl ether (AME) and altenuene (ALT)

(Ansari and Shrivastava 1990). The other fungi detected in the sorghum and finger millet grains were *Aspergillus*, *Fusarium*, *Penicillium*, *Curvularia* and *Helminthosporium*. The occurrence of fungi in the sorghum and finger millet samples was thought to be largely caused by use of poor storage facilities. The grains were stored in locally made *kothi* (a storage structure made of paddy husk and mud mixture), earthen pots and sacks.

1.2.6.2. Production of sorghum and finger millet malts and their contamination by fungi and mycotoxins

Malting sorghum and finger millet

Malting is the limited germination of cereal grains under controlled conditions (Briggs 1998). The main malting processes are steeping, germination and drying. During malting the cereal, grain undergoes three main types of modification: (1) mobilisation of hydrolytic enzymes; (2) a variety of chemical changes that occur in the grain and; (3) physical changes, which appear as softening and weakening of the grains (Briggs 1998). The modification renders the constituents of the grain more readily soluble, which is significant in different respects including that it results in less viscous food products and it enables biochemical reactions (e.g. mashing reactions in brewing) to occur (Pyler and Thomas 2000).

Malt is viewed as having a better food value than unmalted grain. For example, it has been shown to be nutritionally superior relative to unmalted grain (reviewed by Chavan and Kadam 1989). Its flour has a lower paste viscosity than that of unmalted grain and it has better sensory quality than the unmalted grain (Malleshi and Desikachar 1986b, Pyler and Thomas 2000). Malt is used to make various food products such as porridges, gruels, weaning foods, alcoholic and non-alcoholic beverages and distilled spirits (Pyler and Thomas 2000).

In the semiarid to humid conditions of sub-tropical Africa and some parts of Asia, sorghum and the millets are the cereals of choice for malting (Daiber and Taylor 1995). The malts are used as sources of brewing enzymes, which are primarily the carbohydrases (particularly amylases for hydrolysing starch into fermentable sugars) and proteases (for hydrolysing proteins into soluble short peptides and amino acids that are used by the culture yeast) (Daiber and Taylor 1995). In

Africa, sorghum is the primary cereal for production of traditional opaque beer, but finger millet and other millets are also used. The quality of brewing sorghum malt is generally measured in terms of enzymic activity. The diastatic power, joint α- and β- amylase activity, is probably the single most important measure of malt quality (Taylor and Dewar 2000).

Sorghum and the millets are malted either by floor malting or pneumatic malting (Daiber and Taylor 1995). In Zimbabwe, finger millet and pearl millet are mixed with sorghum and industrially malted together by floor malting. If millets were malted alone, they would pass or block the slotted malting floor. In floor malting of sorghum, the grain is spread in thin layers on outdoor floors and left to germinate under virtually ambient conditions (Taylor and Dewar 2000). The grain may be covered with shade cloth or sacking to reduce evaporation and prevent predation by rodents and birds. The grain is watered at intervals during germination. After germination, the grain is spread in thin layers and dried in the sun with intermittent manual turning. Floor malting is generally inefficient because natural weather conditions are inconsistent. Also, there is uneven aeration as the grains bind together (matting), leading to a build up of local 'hot spots', which favour the proliferation of micro-organisms, including the potentially toxigenic fungi. The quality of the "floor" malt is generally low and inconsistent.

Pneumatic malting is a mechanized, industrial technology whereby the cereal grain is germinated in Saladin box-type maltings under controlled conditions of a forced passage of a strong flow of humidified and attemperated air with mechanical turning. Strict hygiene such as selection of clean grain and use of disinfectants as recommended by Dufour et al (1992) and Daiber and Taylor (1995) is likely to be practised in pneumatic malting. The product is generally of good and consistent quality (Briggs 1998). Unfortunately, in Africa, sorghum and the millets are generally still malted by floor malting, particularly by the predominantly resource-poor communities (Taylor and Dewar 2000) because the technology, though labour-intensive, is cheaper than pneumatic malting (Briggs 1998). However, in Southern Africa, there is a general shift to pneumatic malting in the industrial malting of sorghum (Taylor and Dewar 2000).

Contamination of sorghum and finger millet malts by fungi and effects on malt quality and safety

It has been shown that during malting, micro-organisms can be washed off from some kernels

and be deposited in others. Thus, some micro-organisms may infect fresh kernels. Also, fungal spores in the kernels may germinate and micro-organisms may proliferate (reviewed by Flannigan 1996). Several factors favour the contamination of grain by micro-organisms and their proliferation during malting. These include the presence of micro-organisms in the unmalted grain, conditions of high temperature and moisture, aeration, presence of foreign and broken kernels, the availability of soluble nutrients (reviewed by Flannigan 1996, Noots et al 1999). The inefficiency of the malting technology, e.g. lack of turning leading to grain matting, may also lead to microbial proliferation (Briggs 1998). In floor, malting contamination of the grain by micro-organisms may also be caused by poor hygienic conditions, e.g. contaminated equipment, floors and dust. The unhygienic conditions are highly likely to prevail during the floor malting of sorghum and the millets at the household level, as described by Murty and Kumar (1995). Some of the micro-organisms contaminating malt may be inhibited by the adverse conditions of the drying or kilning process, others survive (Flannigan et al 1982) and on the other hand, some fungi, e.g. *Mucor* spp. have been shown to actually multiply during kilning (Gyllang and Martinson 1976, Douglas and Flannigan 1988).

With regard to malt intended for brewing, micro-organisms may have various effects on the malting process and the quality of the malt and the beer (reviewed by Noots et al 1999). Fungi, particularly the moulds, are known to have the most undesirable effects on the malt. Fungi may cause dormancy, reduce germinative capacity, energy and vigour and seedling growth, and interfere with enzyme synthesis by the germinating grain (reviewed by Noots et al., 1999). Several mechanisms are involved in these effects, including competing for oxygen with the germinating grain (Kelly and Briggs 1992, Doran and Briggs 1993) and production of hormones, enzymes and phytotoxins (including some mycotoxins).

Pandey and Mehrotra (1985) demonstrated that 25 days old culture filtrates of 25 fungal species separately inhibited germination, sprouting and root/shoot elongation of finger millet. Culture filtrate from the fungus *Drechslera rostrata* had the highest inhibitory effect. The inhibition was attributed to the fungal metabolites in the culture filtrates.

Fungal metabolites may upset the hormone balance during germination resulting in an excessive growth of the rootlets and consequently a reduction in malt yield (reviewed by Flannigan 1996). Some of the reported negative effects of fungi on malt quality include high protein modification, resulting in high wort nitrogen; discolouration of the malt and consequently a dark wort; and, although there are conflicting data, low α-amylase activity and diastatic power (Flannigan 1996, Noots et al 1999). Fungal activity may cause the deterioration of the malt, resulting in economic losses and in the production of substances that may adversely affect the quality of the beer, including its sensory properties (reviewed by Noots et al 1999). Some species of *Fusarium* and *Rhizopus* have been associated with the production of extracellular polypeptides that persist through the brewing process thereby affecting beer quality by causing wide foaming (gushing) (reviewed by Noots et al 1999). Some moulds, as explained earlier, are potentially toxigenic, and may thus render the malt, and consequently the beer, unsafe due to contamination with mycotoxins. Mycotoxins (including aflatoxins) may be transmitted from the unmalted grain to malt and through the various stages of the brewing process into the beer where they may occur in significant amounts (reviewed by Scott 1996). In addition, mycotoxins may interfere with the activity of the fermentation yeast resulting in low quality beer (reviewed by Flannigan 1996).

Rabie and Lübben (1984) assessed the occurrence of fungi in South African sorghum malts produced by floor and pneumatic malting. The results indicated that both malt types were contaminated with various fungi. Sixty one species representing 29 genera were detected, including potentially toxigenic types. The most frequently occurring fungi in the samples were yeasts, *Rhizopus rhizopodiformis*, *R.* oryzae, *Aspergillus clavatus*, *A. flavus*, *Fusarium moniliforme*, *F. chlamydosporum* and *Phoma sorghina*. Malt produced by floor malting was infested by a larger variety of fungal species than that produced by pneumatic malting. Rabie and Thiel (1985) reported the occurrence of various toxigenic fungi in South African sorghum malts produced by floor and pneumatic malting. Trinder (1988) showed that although some South African industrial sorghum malt samples contained high levels of aflatoxins, all sorghum beer samples had aflatoxin levels below the legal limit of 20 µg/kg. However, Odhav and Naicker (2002) reported extremely high levels of aflatoxins (200 and 400 µg/kg) in two of the six commercial sorghum beer samples they analysed. Dufour et al (1992) reported a high (80% of the samples) occurrence of *Aspergillus flavus* in malting sorghum grain obtained from several

African countries. They found that 10% of the infected grain samples were contaminated with aflatoxin B_1. The aflatoxin was transmitted to the malt in 52% of the aflatoxin-contaminated germinating grain samples. Odhav and Naicker (2002) reported the occurrence of various moulds in 4 of the 10 South African industrial sorghum malt samples studied and found that 50% of the mould-infected samples contained the mycotoxin zearalenone. Using a simulated commercial outdoor malting process, with or without turning during germination, Lefyedi et al (2005) found that sorghum malt was contaminated by high levels of various micro-organisms (including toxigenic fungi), and the mycotoxins fumonisins, deoxynivalenol and zearalenone were detected.

While mycotoxins generally occur in negligible amounts in the lager type beers and distilled spirits of the Developed World, the situation is different with the African traditional home-brewed opaque beers (Flannigan 1996, Scott 1996). Several reports indicate that traditional home-brewed opaque beers are often contaminated with significant levels of mycotoxins (Lovelace and Nyathi 1977, Alozie et al 1980, Okoye 1987, Odhav and Naicker 2002, Shephard et al 2005). Reports specifically on the contamination of finger millet malt and its products by fungi and mycotoxins could not be found in the literature, but the trends should be similar to those of sorghum malts and its products since the two grains are malted under similar conditions (Murty and Kumar 1995).

1.2.6.3. Effect of malting on the phenolic content of sorghum and finger millet

Reichert et al (1980) reported that germinating sorghum resulted in a 25% weight reduction in assayable tannins. These authors suggested that the reduction of the amounts of assayable tannins in the grain during malting could be partly due to further polymerisation of the tannins in water and/or their complexation with other components of the grain, possibly carbohydrates and proteins. Larger tannin polymers or complexes of tannins with other biopolymers would be insoluble and thus not extractable. These authors suggested that the decrease in the content of assayable tannins could also be due to increased activity of polyphenol oxidase and other catabolic enzymes, as observed by Kruger (1976) in wheat. Glennie (1983) demonstrated that malting resulted in the reduction of assayable total phenolics (TP) of a tannin sorghum grain.

Bvochora et al (1999) reported that malting sorghum caused a decrease in the levels of TP by 25, 45 and 76% in three of the four sorghum cultivars studied. Steeping decreased tannin content of two of the sorghum cultivars studied by 6 and 33% and germination by 56 and 18%. The loss of tannins was attributed to leaching into steep liquor and/or the reaction of the tannins with proteins, which would render them not extractable. Other workers (Chavan 1981, Osuntogun et al 1989, Beta et al 2000) also reported decreases in tannins during the germination of sorghum. Dicko et al (2006) found that, on average, germination did not affect TP, but decreased the content of tannins, 3-deoxyanthocyanidins, and flavan-4-ols.

While many workers reported decreases in the content of assayable tannins and TP during the malting of sorghum, Nwanguma and Eze (1996) reported that germination of sorghum cultivars resulted in an increase in tannin content by between 5 and 11-fold. TP increased by between seven-fold and 14-fold. The increase in phenolic content was thought to be due to *de novo* synthesis and polymerisation of the phenolics. Similarly, Ahmed et al (1996) reported that there was a slight increase in tannin content when sorghum was germinated for different periods. The increase in tannin content was ascribed to the solubilisation of tannins when the grain was soaked in water and migration of tannins to the outer layers as a result of germination, as was indicated by the browning of the germinated grain.

There are indications that phenolic compounds other than tannins may be synthesised during the malting of sorghum, which may result in an increase in the phenolic content of the sorghum malt. McGrath et al (1982) reported the development of a large complement of low molecular weight phenolic compounds in the roots and shoots of the sorghum malt. The roots and shoots were highly coloured, and acid hydrolysis yielded cyanidin as well as the two rarer anthocyanidins luteolinidin and apigenidin. Alkaline hydrolysis released simple phenolic acids from roots and shoots as well as berries (malt after removal of roots and shoots) after malting. Malting caused a change in the characteristics of the grain tannins, probably due to interaction with amino acids and polypeptides/proteins, but the tannins were not detected in the roots and shoots of the sorghum malt. Similarly, Glennie (1983) reported an increase in the anthocyanidin content of kernels, roots and shoots of both tannin and non-tannin sorghum cultivars during malting. The increase in anthocyanidin content was higher in the roots and shoots of the non-tannin cultivars

than in their kernels, while an opposite trend was observed in the tannin cultivar. The increase in the content of anthocyanidins of germinating sorghum grain was likely due to their synthesis by the sorghum seedling as was observed by Stafford (1965) in the seedling of sorghum. Similar to that reported by McGrath et al (1982), tannins were not detected in the roots and shoots of the malt. The tannins were shown to be compartmentalised in the testa of the sorghum grain where they remained during germination.

Singh et al (1988) reported that the tannin contents of finger millet worts increased considerably with increased germination temperature. After 48 h of germination at 35°C, the tannins in the worts were 9.5-15.0 mg tannic acid equivalents (TA eqv.)/100 mL compared to 3.7-5.7 mg TA eqv./100 mL in worts of malts germinated at 20°C. There was a highly significant correlation ($r=0.84$; $p<0.01$) between the degree of modification of the grain and tannin content of the wort, indicating that the extractability of tannins was enhanced with increased modification during malting. Seetharam and Ravikumar (1994) reported that TP and tannin content both decreased when blast-resistant and blast-susceptible finger millet cultivars were germinated. TP decreased from 1.22 to 0.39 mg/100 mg and from 0.88 to 0.31 mg/100 mg in Blast-resistant and Blast-susceptible cultivars, respectively. Tannin content decreased from 1.12 to 0.33 mg/100 mg and from 0.78 to 0.27 mg/100 mg in Blast-resistant and Blast-susceptible cultivars, respectively. Mbithi-Mwikya et al (2000) showed that germination of finger millet for 60 h decreased the assayable tannin content to an undetectable level.

As stated earlier, ferulic, caffeic and coumaric acids were found to be the major bound phenolic acids in finger millet grain (Subba Rao and Muralikrishna 2001). These authors reported a 2-fold decrease in the major bound phenolic acids after 96 h of malting. Subba Rao and Muralikrishna (2002) further reported on changes in free and bound phenolic acids during malting of finger millet grain. As stated previously, in that study protocatechuic, gallic and caffeic acid were found to be the major free phenolic acids, whilst ferulic, caffeic and coumaric acids were found to be the major bound phenolic acids. There was a 3-fold decrease in protocatechuic acid, whereas the decrease was marginal in the case of caffeic acid upon 96 of malting. However, the contents of other free phenolic acids such as coumaric, gallic and ferulic acids were increased by 2-, 4- and 10-fold upon 96 h of malting. Malting finger millet for 96 h resulted in a 2-fold

decrease in the three major bound phenolic acids (ferulic, caffeic and coumaric acids). The decrease in the contents of some phenolic acids and an increase in the contents of others during malting was suggested to have been due to phenolic acid interconversions, *de novo* synthesis and the liberation of bound phenolic acids by the action of esterases on phenolic acid-polysaccharide and/phenolic acid-protein complexes (Subba Rao and Muralikrishna 2001, 2002).

1.2.6.4. Relationship between phenolic content and amount of phenolic type and resistance of sorghum and finger millet grains to fungal infection

Several plant phenolic compounds have been shown to exhibit antimicrobial activity against various micro-organisms (reviewed by Cowan 1999). It is believed that the antimicrobial activity of the phenolic compounds contribute to the plant's defence system against microbial invasion (Harborne 1994b). As stated earlier, sorghum and finger millet grains contain the same type of phenolic compounds, mainly phenolic acids, flavonoids and condensed tannins (reviewed by Dykes and Rooney 2006). These phenolic compounds may play a role in resistance of sorghum and finger millet grain types to fungal infection as suggested by the work reviewed below.

Waniska et al (1989) reported that sorghum cultivars with a pigmented testa contained higher levels of free phenolic compounds and were more resistant to grain moulding than cultivars without a pigmented testa. Mould-susceptibility was related to higher levels of p-coumaric acid. Audilakshmi et al (1999) found that sorghum genotypes with a red pericarp had a relatively higher phenolic content than white and yellow genotypes and were less infected by the fungus *Aspergillus* and had lower amounts of aflatoxin and ergosterol than most of the yellow and white genotypes. The authors also noted that phenolic content of the red sorghum genotypes increased with an increase in fungal infection levels, indicating that the phenolic compounds were part of the immune response to fungal invasion. Similarly, Menkir et al (1996) and Ratnavathi and Sashidhar (2003) reported that darker sorghum grain types had higher amounts of phenolic compounds and were more resistant to mould attack than were the lighter types.

Harris and Burns (1973) demonstrated that there were strong negative correlations (r=-0.89 and r=-0.92 for sorghums grown at two different locations) between tannin content and mould

infection in brown sorghums with a pigmented testa and high tannin contents, indicating that tannins were associated with grain mould resistance. Esele et al (1993) found that in sorghum, a pigmented testa was the single most important trait conferring grain mould resistance. The authors also reported that a red pericarp also contributed to grain mould resistance, and a pigmented testa and a red pericarp had an additive effect on mould resistance. Mould resistance was attributed to the high levels of tannins in the testa and flavan-4-ols in the pericarp. Several other workers (e.g. Kambal and Bate-Smith 1976, McGrath et al 1982, Bandyopandhyay et al 1988) have reported the relationship between tannin content and sorghum grain mould resistance.

Both the dehydroxylated anthocyanins (3-deoxyanthocyanins; e.g. apigeninidin and luteolinidin) (reviewed by Chandrashekar and Satyanarayana 2006) and the flavan-4-ols (reviewed by Waniska et al 2001) have been found to be associated with sorghum grain mould resistance. Menkir et al (1996) studied 231 varied sorghum grain types to determine the physical and chemical kernel properties associated with resistance to grain mould. These authors found that resistance to grain mould was strongly associated with high concentrations of the phenolic compounds apigeninidin, flavan-4-ols and tannins, and kernel hardness and pericarp colour. Jambunathan et al (1986) reported that the levels of flavan-4-ols in mould-resistant sorghum grain cultivars were two- to threefold higher than in mould-susceptible cultivars, which suggest that the compounds play a role in protecting the grain from mould attack. Similarly, Jambunathan et al (1990) found that the concentrations of flavan-4-ols in mould-resistant sorghum grain types were at least 2-fold higher than in mould-susceptible types.

Schutt and Netzly (1991) showed that the anthocyanin apigeninidin inhibited growth of various fungal species, whilst flavan-4-ols had no inhibitory effect on fungi. These authors suggested that the relationship between sorghum grain mould resistance and accumulation of the flavan-4-ol apiforol, which had been reported probably, indicated that apiforol was a biosynthetic precursor of apigeninidin. It has been demonstrated that the phytoalexins (substances released by plants in response to infection [Dixon et al 1983]) of the sorghum plant are the deoxyanthocyanins, though their action has not been directly shown in the sorghum grain (reviewed by Chandrashekar and Satyanarayana 2006).

Hahn et al (1983) identified various phenolic acids in seven sorghum grain varieties and found that there was greater variety and higher amounts of total phenolic acids in the free form in sorghum varieties resistant to fungal attack than in the susceptible ones. The study also showed that some of the varieties that had low phenolic acid contents were resistant to fungal attack. The resistance was attributed to other factors including physical properties of the grain and other chemical factors such as flavonoids in varieties with a red-pericarp and flavonoid polymers (condensed tannins) in varieties with a pigmented testa. Waniska et al (1989) reported the resistance of sorghum grain to mould invasion was related to the phenolic acid composition of the grain. Mould-susceptibility was found to be related to high levels of p-coumaric acid.

Esele (2002) suggested that the incorporation of genetic resistance is probably the best choice of disease (including grain infection) management in finger millet. For this to be achieved, the disease resistance factors have to be identified. Unfortunately, there is little information in the literature on the resistance of finger millet grain to infection by fungi. Although Chandrashekar and Satyanarayana (2006) stated that finger millet grain is resistant to moulds due to the fact that it is rich in phenolic compounds, published data on the relationship between phenolic compounds and grain fungal resistance are scanty. A study by Seetharam and Ravikumar (1994) of 25 Blast-resistant and 23 susceptible finger millet types to determine the inheritance and biochemical nature of resistance factors showed that resistance to the Blast-causing fungus *Pyricularia grisea* was complex and polygenic-multiple biochemical factors were involved. The study showed that there was a relationship between phenolic compounds and resistance of finger millet to infection by *P. grisea*. The workers demonstrated that there was a negative correlation between Blast disease and total phenolic and tannin content in dry and germinating seeds; brown grain types were resistant to Blast and had high total phenolics and tannin content, whilst the white types were susceptible to Blast and had low total phenolics and tannin content. Viswanath et al (2009) reported that methanolic extracts from the seed coat of finger millet, which had higher phenolic content, inhibited the bacterium *Bacillus cereus* and the fungus *Aspergillus flavus* more than extracts from whole grain flour.

1.2.6.5. Proposed mechanisms of antimicrobial activity of phenolic compounds

Several mechanisms have been suggested for the observed antimicrobial activity of phenolic compounds (reviewed by Cowan 1999). Simple phenols and phenolic acids such as cinnamic and benzoic acid derivatives and catechol and pyrogallol, respectively, have been shown to be toxic to microorganisms. The suggested mechanisms of action include enzyme inhibition, possibly through reaction with sulphydryl groups or through non-specific interaction with proteins. Phenolic compounds may be oxidizied to quinones. Quinones are very reactive and possess anti-microbial properties. The antimicrobial activity of quinones may be through free radical activity, e.g. oxidation of microbial membranes and cell components. The quinones may irreversibly complex nucleophilic amino acids in proteins leading to inactivation of protein and function. Surface-exposed adhesins, cell wall polypeptides and membrane-bound enzymes may be affected this way.

Another mechanism of action could be interaction with substrates making them unavailable to microorganisms. As stated earlier, flavonoids such as the deoxyanthocyanins of the sorghum plant are known to be synthesized by plants in response to microbial infection and as such fall into a broad group of substances collectively called phytoalexins. Some of the suggested mechanisms of action of flavonoids against microorganisms are complexation with microbial proteins and cell walls, and disruption of membranes. Tannins may be toxic to microorganisms through several modes of action (reviewed by Scalbert 1991). They may be toxic to the microorganisms through binding of cellular polymers such as enzyme proteins and substrate proteins and carbohydrates. Tannins may complex metal ions in the substrate system making them unavailable for the micro-organism's metabolic processes. Tannins may interact with microbial membranes and thereby negatively affect their function and integrity. McGrath et al (1982) suggested that tannins protect sorghum grain from fungal infection by forming a physical barrier in the testa.

1.2.6.6. Other potential fungi resistance factors of sorghum and finger millet grains

Although there is strong evidence suggesting that phenolic compounds contribute to sorghum grain mould resistance, several authors have also reported weak and/or inconsistent correlations between phenolic content and mould resistance (e.g. Hahn et al 1983, Jambunathan et al 1991, Menkir et al 1996, Audilakshmi et al 1999). Other factors have been found to be associated with sorghum grain mould resistance. These include physical and chemical properties of the grain such as grain hardness (Jambunathan et al 1992, Audilakshmi et al 1999), pericarp thickness (reviewed by Chandrashekar and Satyanarayana 2006), proteins (Kumari et al 1992, 1994, Rodríguez-Herrrera et al 1999, Bueso et al 2000), low fat content (Ratnavathi and Sashidhar 2003) and phytic acid (Ratnavathi and Sashidhar 2003). It has been suggested that a combination of factors contribute to resistance of sorghum grain to fungi (Menkir et al 1996, Bueso et al 2000). Similarly, factors other than phenolic compounds may contribute to resistance of finger millet grain to fungal infection. A non-specific lipid transfer protein, which may have antifungal activity has been reported in finger millet grain (Campos and Richardson 1983). Seetharam and Ravikumar (1994) reported that in dry finger millet grain, total protein was positively correlated with infection by the Blast fungus *P. grisea* ($p<0.01$), whilst in germinating grain, infection was negatively correlated with peroxidase activity ($p<0.05$).

1.2.7. Effects of thermal processing on phenolic content and antioxidant activity of sorghum and finger millet grain foods

Awika et al (2003a) reported that baking sorghum bran into cookies and breads decreased the extractable tannin levels in the bran by 52% and 72%, respectively. There were decreases in all the tannin fractions: procyanidin monomers, oligomers and polymers, but there was a higher decrease in the polymer fractions than the lower molecular weight (MW) fractions. The losses were attributed to interaction of tannins with other bran components, mainly the macromolecular carbohydrates and proteins. The higher tannin loss in bread than in cookies was thought to be due to the fact that bread dough had higher moisture content and was mixed for a longer time, and was baked at higher temperature and for a longer time than the cookies (Awika et al 2003a). Extrusion cooking the sorghum bran caused a pronounced increase in the content of lower MW

procyanidins (DP1-DP4). There was a 478% increase in the extractable monomers. On the other hand, extrusion caused an 85% decrease in procyanidin polymers (DP>10). The increase in the procyanidin monomers was ascribed to the depolymerisation of the higher MW procyanindins, whilst the decrease in the higher MW procyanindins was attributed to their depolymerisation and to their interaction with other bran components, similar to what was suggested in baking. These authors also demonstrated that, when processed into foods, most of the antioxidant activities of the raw sorghums were retained, 57-78% for breads and cookies.

Dlamini et al (2007) reported that conventional and extrusion cooking caused much higher reductions of phenolic content and antioxidant activity of tannin sorghum products than of non-tannin sorghum products. Extrusion cooking caused a much larger reduction of total phenolics, condensed tannin content and antioxidant activity than conventional cooking. The observed effects were thought to be due to the interaction of tannins with other components of sorghum grain, mainly proteins, and due to the polymerisation of tannins with lower MW phenolics, e.g. anthocyanins. Extrusion seemed to promote more interaction of tannins with other sorghum components than conventional cooking. The interaction could have reduced the extractability of the phenolic compounds and masked their antioxidant activity.

Towo et al (2003) reported that boiling sorghum and finger millet grains caused reductions in total phenolics (TP), catechols and resorcinols. In sorghum, TP, catechols and resorcinols decreased from 3 360.0 to 690.0 mg/100 g, 1 350.0 to 270 mg/100 g and 1 100.0 to 130 mg/100 g, respectively. In finger millet, TP, catechols and resorcinols decreased from 420 to 250 mg/100 g, 250 to 150 mg/100g and 150 to 70 mg/100 g, respectively. These authors suggested that reductions in phenolics were due to thermal degradation and leaching of the phenolics into the endosperm where they complexed with macromolecules, mainly starch and proteins.

1.2.8. Conclusions

Although information is limited, the phenolic compounds, which have been reported in finger millet grain, are phenolic acids and condensed tannins. It appears that brown finger millet grain varieties contain higher levels of phenolic compounds, including tannins, than white varieties,

and as such exhibit higher antioxidant activity. The location of tannins in finger millet grain is not known. The limited research suggests that finger millet phenolics may contribute to resistance of the grain to infection by fungi. However, the impact of finger millet phenolics on its malt quality seems not to have been subjected to a scientific study. The effects of thermal processing on the phenolic content and antioxidant activity of finger millet foods are hardly known. These knowledge gaps need to be filled to enable the exploitation of the seemingly huge agronomic, economic, nutritional, and health-promoting potential of finger millet grain.

1.3. Hypotheses

1. As with sorghum grain (Hahn and Rooney 1986), the phenolic composition of finger millet grain will be determined by genetic factors and hence occurrence of specific phenolic types (e.g. tannins) will vary with grain variety.
2. As in sorghum (Dykes and Rooney 2006), tannins are located in the testa layer of the finger millet kernel.
3. Because it has been shown that condensed tannins, probably due to the fact that they are large molecules with many OH groups substituted on their benzene rings (Cao et al 1997), exhibit higher antioxidant activity on a molar basis than other phenolic compounds (Hagerman et al 1998), condensed tannin-containing finger millet grain types will exhibit higher antioxidant activity than the non-tannin types.
4. (a) Because it has been demonstrated that some types of phenolic compounds, through several proposed mechanisms such as binding with microbial enzymes, inhibit fungi (Harborne 1994), resistance of finger millet grain to fungal infection will be positively correlated with phenolic content and contents of phenolic types. Because it has been shown in sorghum grain that tannin content is strongly positively correlated with resistance to fungal infection (Harris and Burns (1973), high-tannin finger millet grain will be less infected by fungi during malting and therefore will have better malting and malt quality than a non-tannin finger millet grain.

   (b) Finger millet malt will have a better nutritional quality (e.g. higher protein and amino acid content) than the unmalted grain due to biochemical modifications and carbohydrate loss by respiration during malting (Chavan and Kadam 1989).

5. Due to differences in the composition (including phenolic composition) of finger millet grain types (Ravindran 1991, Dykes and Rooney 2006) and due to the fact that finger millet does not contain gluten proteins (Hoseney 1994), the quality of cookies containing finger millet will be influenced by finger millet type and finger millet substitution level. Cookies containing a high-tannin finger millet type will be less acceptable than those containing a non-tannin type due to the adverse effect of tannins on the sensory properties of food (Lesschaeve and Noble 2005).
6. Phenolic content and antioxidant activity will decrease during the baking of composite wheat-finger millet doughs due to heat-induced changes such as decomposition of phenolic compounds and their chemical interactions with other components of the dough (Nicoli et al 1999). Cookies containing a high tannin finger millet will exhibit higher antioxidant activity than cookies containing a non-tannin finger millet because tannins have been shown to exhibit higher antioxidant activity on a molar basis than other phenolic compounds (Hagerman et al 1998).

1.4. Objectives

1. To determine the influence of finger millet variety on the phenolic content and composition (particularly occurrence of tannins).
2. To determine the location of tannins in finger millet grain.
3. To determine the influence of grain type (tannin-type or non-tannin type) on the antioxidant activity of finger millet grain.
4. (a)To determine whether there is a relationship between resistance of finger millet grain to fungal infection and phenolic content and amount of phenolic type.
   (b) To determine the influence of phenolic content and amount of phenolic type on the malt quality of finger millet grain.
5. To determine the effect of partial substitution of wheat with finger millet on the quality of composite wheat-finger millet cookies.
6. To determine the effect of cookie making on the phenolic content and antioxidant activity of composite wheat-finger millet flour.

2. RESEARCH

The research, which tested the hypotheses stated in section 1.3, was done in three parts:

2.1. Occurrence and location of tannins in finger millet grain and antioxidant activity of different grain types

2.2. Influence of phenolics in finger millet on its malt quality

2.3. Effect of partial substitution with finger millet on the nutritional and functional quality of cookies, with particular reference to phenolics

2.1. Occurrence and location of tannins in finger millet grain and antioxidant activity of different grain types[1]

2.1.1. Abstract

Grain of 22 finger millet types obtained from Southern and East Africa were analyzed to determine the influence of grain type on tannins, antioxidant properties and tannin localization in the grain. Four grain types were creamy white and 18 were brown. A high proportion (80-100%) of kernels of all but two of the pigmented types stained black with the Bleach test, while the light types did not stain black. There was a wide range of total phenolics, condensed tannin content and antioxidant activity across the grain types. Light coloured grain types had much lower total phenolics and tannins relative to the pigmented types, and types that stained black with the Bleach test had much higher tannin content and much higher antioxidant activity. Light microscopy revealed that kernels that stained black with the Bleach test and had high tannin content had a dark coloured testa layer, indicating that the tannins were located in that layer as in sorghum. This is the first report that the Bleach test can be used to detect tannin finger millet types. The work demonstrates that occurrence of tannins in finger millet grain is a varietal property as in sorghum.

2.1.2. Introduction

Finger millet [*Eleucine coracana* (L.) Gaertn.] is widely grown in the cooler, higher-altitude regions of Africa and Asia (ICRISAT/FAO 1996). In Africa, it is cultivated mainly in eastern, central and southern Africa, and in Asia, it is produced in India, Nepal and China (House 1995, Obilana and Manyasa 2002). Among the millets, finger millet ranks fourth after pearl millet, foxtail millet and proso millet (Obilana and Manyasa 2002). It is the second most important millet in Africa (House 1995) and is estimated to comprise about 8% of the cultivated area and

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11% of production of all millets in the world (Obilana and Manyasa 2002). Finger millet is one of the staple foods of many, predominantly poor, communities in the semi-arid tropics of Africa and Asia (ICRISAT/FAO 1996, Obilana and Manyasa 2002). It is consumed in the form of several food products that are similar to those made from sorghum and other millets. Finger millet products include fermented and non-fermented porridges, pancake-like flatbreads and, fermented alcoholic and non-alcoholic beverages (Murty and Kumar 1995, ICRISAT/FAO 1996).

Finger millet grain contains various phenolic compounds including tannins (Serna-Saldivar and Rooney 1995, Ramachandra et al 1977, McDonough et al 1986). These phenolic compounds have been reported to exhibit antioxidant activity (Sripriya et al 1996, Subba Rao and Muralikrishna 2002, Hegde and Chandra 2005). There is a growing interest in plant phenolic compounds because of their antioxidant and free radical-scavenging activity, which have potential health-beneficial effects (Bravo 1998, Rice-Evans et al 1997). Phenolic antioxidants are potent inhibitors of biological oxidation and because of this, they may reduce the risk of health conditions such as cardiovascular disease and cancer and, may help mitigate the adverse effects related to ageing (Bravo 1998, Rice-Evans et al 1997, Kaur and Kapoor 2001, Scalbert et al 2005).

With regard to sorghum, another cereal that contains tannins, there are well-documented data on phenolic types, variation in tannin content with grain variety, and location of the tannins in the kernel (Awika and Rooney 2004). Similar information on finger millet is either absent or limited. The microstructure of the finger millet kernel was studied by McDonough et al (1986). These workers showed that, as documented earlier by Angold (1979) the finger millet kernel is an utricle, not a caryopsis as is the case with most cereal grains such as sorghum, maize, wheat and barley (Hoseney 1994). They showed that the outer layers of the finger millet kernel comprise a membranous pericarp, which was loosely associated with the kernel at maturity, and a testa which overlays an aleurone layer. The testa varied from red to purple in colour. Three distinct types of starchy endosperm were identified, the peripheral, corneous and floury endosperm. The cell walls of the endosperm strongly fluoresced, indicating the presence of phenolic compounds.

Although McDonough et al (1986) suggested that in finger millet grain, tannins are located in the testa; this has not been shown conclusively.

There are appreciable levels of both total phenolics and tannins in some finger millet grain varieties, particularly the coloured ones (Ramachandra et al 1977, McDonough et al 1986). Sripriya et al (1996) found that brown finger millet grain exhibited higher antioxidant properties than white grain. They suggested that the higher antioxidant activity was due to tannins but this was not evaluated. As with sorghum (Awika et al 2003a, Awika et al 2005), tannin-containing finger millet grain may exhibit higher antioxidant activity than the non-tannin grain. Further, the presence of tannins in some finger millet varieties should be of agronomic significance in that, as shown in sorghum grain, the tannins may confer biotic stress resistance against for example birds (McMillan et al 1972) and moulds (Harris and Burns 1973).

The objectives of this study were to determine how variety affects the presence of tannins in finger millet grain, to establish the location of tannins in the kernel and to evaluate the antioxidant properties of different finger millet grain types.

2.1.3. Materials and Methods

2.1.3.1. Finger millet grain

Twenty two finger millet types from Kenya and Zimbabwe were grown in the 2004/2005 season, on the Experimental Farm of the University of KwaZulu-Natal in Pietermaritzburg, South Africa. The finger millet types were selected as they varied in visual kernel colour from creamy white to dark brown. The finger millet types were selected on the basis that they represented the varied finger millet germplasm found in Southern and East Africa. Table 2.1.1 shows the identities and origins of the finger millet types.

Table 2.1.1. Finger millet types and their origin

Finger millet type	Plant type	Visual kernel colour	Origin
1. G35	Tan	Creamy white	SADC/ICRISAT Zimbabwe
2. ICFM95001 GMSI	Tan	Creamy white	SADC/ICRISAT via DRSS Zimbabwe
3. 95 G 198 W	Tan	Creamy white	SADC/ICRISAT Zimbabwe
4. ICFM 95001GMSF	Tan	Creamy white	SADC/ICRISAT via DRSS Zimbabwe
5. FNL 0069	Tan	Brown	SADC/ICRISAT Zimbabwe
6. P283	Tan	Dark brown	KARI Kenya
7. P224	Purple	Brown	KARI Kenya
8. Gulu Early	Purple	Brown	KARI Kenya
9. FNL 0072	Purple	Brown	SADC/ICRISAT Zimbabwe
10. FNL 0073	Purple	Brown	SADC/ICRISAT Zimbabwe
11. ZIM Mnnursery	Purple	Brown	SADC/ICRISAT Zimbabwe
12. FMV2	Purple	Brown	DRSS Zimbabwe
13. FNL 0071	Purple	Brown	SADC/ICRISAT Zimbabwe
14. ZIM Llobel	Purple	Dark brown	Farmer collection Zimbabwe
15. FMV6	Purple	Brown	DRSS Zimbabwe
16. FNL 0012	Purple	Brown	SADC/ICRISAT Zimbabwe
17. FMV1	Purple	Brown	SADC/ICRISAT Zimbabwe
18. U15	Purple	Brown	KARI Kenya
19. FNL 0051	Purple	Brown	SADC/ICRISAT Zimbabwe
20. Nanjala Brown	Purple	Brown	KARI Kenya
21. Okhale-1	Purple	Brown	KARI Kenya
22. FNL 0074	Purple	Brown	SADC/ICRISAT Zimbabwe

GMSF, Genetic male sterile fertile plant. GMSI, Genetic male sterile infertile plant. DRSS, Department of Research and Specialist Services (Zimbabwe). ICRISAT, International Crops Research Institute for Semi Arid Tropics. KARI, Kenya Agricultural Research Institute. SADC, Southern African Development Community.

Grain was mechanically threshed and then hand cleaned to remove glumes. It was then further cleaned by sieving to remove broken kernels and foreign matter. During cleaning, the pericarps of most of the kernels peeled off as was observed by McDonough et al (1986). The grain was stored at 9-10°C until analysis. It was milled to fine flour with a laboratory hammer mill (Falling Number AB, Huddinge, Sweden) fitted with a 0.8 mm opening screen for chemical analyses.

2.1.3.2. Analyses

Kernel colour

The Hunter L (lightness), a (redness) and b (yellow/green) values of the finger millet kernels were determined using a CR-400 chromameter (Konica Minolta, Sakai Osaka, Japan).

Bleach test

The Bleach test used for detecting sorghum grain with a pigmented testa was applied as described by Taylor (2001). Fifty g/L NaOH in commercial Bleach (3.5% sodium hypochlorite) was added such that it just covered 100 kernels of finger millet in a test tube. Because there were no finger millet grain types that had been tested for presence of condensed tannins for use as standards, a condensed-tannin sorghum and a condensed tannin-free sorghum were used as standards. There was no reaction in any of the 22 finger millet grain types after more than an hour and hence the reaction was left to continue overnight. The reagent was then drained off, the grains dried with paper towel and observed for presence of condensed tannins, which was indicated by the kernels staining black.

Total phenolics

The Folin-Ciocalteu method of Singleton and Rossi (1965) was used to measure total phenolics. Exactly 0.4 g flour was extracted with 20 mL acidified methanol (1% HCl in methanol) for 1 h at room temperature (approx. 25ºC), with vortex mixing at 5-min intervals. The samples were centrifuged for 10 min at 1 200 x g using a temperature-controlled centrifuge set at 25ºC. Three replicate supernatants were obtained. Sample extracts (0.5 mL) were mixed with 2.5 mL Folin-Ciocalteu phenol reagent in a 50 mL volumetric flask, 7.5 mL 20% (w/v) sodium carbonate was added within 8 min after addition of the Folin-Ciocalteu phenol reagent. The contents were mixed and the flask made up to volume with distilled water, stoppered and thoroughly mixed. Sample blanks were included, in which the sample was replaced by distilled water. The flasks were left to stand at room temperature (approx. 25ºC) for 2 h, after which absorbance at 760 nm was measured. Gallic acid was used as a standard.

Condensed tannins

The Vanillin-HCl method of Price et al (1978) with blank subtraction for extract colour was used

to measure condensed tannin content. Extraction was as for determination of total phenolics except that 100% methanol was used in place of acidified methanol. The extracts and the vanillin reagent (4% HCl in methanol and 0.5% [w/v] vanillin in methanol) were maintained at 30ºC in thermostat-controlled water bath before mixing the reactants. Sample extracts (1 mL) were mixed with 5 mL vanillin reagent in test tubes and then maintained at 30ºC in the water bath for 20 min. Sample blanks in which the vanillin reagent was replaced by 4% HCl in methanol were included. Absorbance at 500 nm was measured. Catechin was used as a standard.

Anthocyanins

Anthocyanins were measured as, 3-deoxyanthocyanidin pigments, apigeninidin and luteolinidin, which, as stated earlier, are the form in which anthocyanins occur in sorghum grain (Dykes and Rooney 2006). Analysis was according to the method described by Menkir et al (1996). Exactly 0.25 g of finger millet flour was extracted with 15 mL of absolute ethyl acetate for 30 min and centrifuged for 10 min at 1 200 x g using a temperature-controlled centrifuge set at 25ºC. The residue was extracted further with 15 mL of 0.05% (v/v) methanol in HCl. The extracts were pooled together. Exactly 0.4 g of acid-treated polyvinylpyrrolidone was added to 8 mL of the extract, mixed thoroughly by vortexing, incubated at room temperature (approx. 25°C) for 10 min, and centrifuged. The absorbance of the supernatant was read at 475 nm for apigeninidin and at 495 nm for luteolinidin. Two replicates were analysed.

Flavan-4-ols

Flavan-4-ols were analysed according to Dykes et al (2005). Extraction was as for the determination of total phenolics. Exactly 1 mL of extract was reacted with 5 mL of HCl-butanol reagent (solution of 0.0616 g $FeSO_4.7H_2O$ in 5% [v/v] HCl in *sec*-butanol). The reaction was allowed to stand for 1 h at room temperature and absorbance was then read at 550 nm. Two replicates were analysed.

Antioxidant activity

Antioxidant activity of methanolic extracts was measured using the Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Awika et al (2003b). Equal volumes of 8 mM 2,2′-azinobis [3-ethyl-benzothiazoline-6-sulphonic acid] (ABTS) and 3 mM potassium per

sulphate, both prepared in distilled, deionised water, were mixed and allowed to react for at least 12 h at room temperature (approx. 25ºC) in the dark to obtain a radical cation ($ABTS^{\cdot+}$) solution. The $ABTS^{\cdot+}$ solution was diluted with a pH 7.4 phosphate buffer solution containing 150 mM NaCl to obtain an initial absorbance of about 1.5 at 734 nm. Methanolic extracts were prepared as for determination of total phenolics. Sample extracts (100 µL) and standards (100 µL) were reacted with 2 900 µL $ABTS^{\cdot+}$ solution for 30 min and absorbance at 734 nm was then read. Trolox was used as a standard.

2.1.3.3. Microscopy

Two high-tannin finger millet grain types and two non-tannin types were selected for microscopic analysis. Sound kernels were cut in half longitudinally using a razor blade. The cut kernels were observed using a stereo dissecting light microscope.

Scanning electron microscopy (SEM) was used to study the testa of the kernels. Sound kernels were frozen in liquid nitrogen. The kernels were then freeze-fractured in transverse section with a single-edged razor blade. The fractured kernels were mounted on aluminium stubs using a double-sided carbon tape and then coated with a gold/palladium alloy in a sputter coater in a vacuum. The samples were then viewed in a Philips XL 30 SEM (Philips, Eindhoven, Netherlands) at 15 kV.

2.1.3.4. Statistical analysis

SAS version 8.2 (SAS Institute Inc., Cary, NC) was used to analyze the data. Kernel colour, total phenolics, condensed tannin content and antioxidant activity determinations were replicated three times. Tukey's Studentized Range (Honestly Significant Difference [HSD]) Test was used to compare means and Pearson's Correlation Coefficient (r) was used to analyse for linear relationships.

2.1.4. Results and Discussion

The kernels of the finger millet types were either light (creamy white) or pigmented (brown) (Table 2.1.1). The thousand kernel weight (TKW) ranged from 1.77 to 3.86 g and had a mean of 2.86 g (Table 2.1.2) similar to the 2.5 g documented in the literature (Serna-Saldivar and Rooney 1995). The Hunter L values of the light types ranged from 62.7 to 68.4, while that of the pigmented types ranged from 45.9 to 55.9; the Hunter a values of the light types ranged from 4.3 to 5.0, while that of the pigmented types ranged from 7.1 to 9.4 (Table 2.1.2). The results indicate that grain colour varies with finger millet grain type. In sorghum, phenolic compounds, particularly anthocyanins and condensed tannins (flavonoid polymers), are the major contributors to the colour of the grain (Awika and Rooney 2004). Flavonoids (Table 2.1.2) and condensed tannins (Ramachandra et al 1977, McDonough et al 1986, Table 2.1.2) occur in finger millet grain and presumably may contribute to the colour of the grain.

The Bleach test indicated that the kernels of all four of the light finger millet types did not stain black and two pigmented types (FNL 0069 and P283) essentially did not stain black (0% and 2% kernels stained black, respectively). The other pigmented types had a high proportion of kernels that stained black ranging from 80% to 100% (Table 2.1.2). Grain types that had no pigmented testa were obtained from tan plants and types with a pigmented testa were obtained from purple plants (Tables 2.1.1 and 2.1.2). The four light finger millet types had low levels of total phenolics (in the range not detected to 0.09 mg gallic acid equivalents [GAE]/100 mg, db), whereas the pigmented types had higher levels of total phenolics that varied over a wide range (0.34 to 1.84 mg GAE/100 mg, db). Similarly, light finger millet types had lower levels of anthocyanins and flavan-4-ols than the pigmented types. The vanillin-HCl assay showed the same trend as the Bleach test. The six finger millet types that did not stain black in the Bleach test essentially had no condensed tannins (maximum value 0.07 mg catechin equivalents ([CE]/100 mg, db), whereas the types that stained black had much higher levels of condensed tannins ranging from 0.60 to 2.08 mg CE/100 mg, db.

Table 2.1.2. Kernel characteristics, phenolic content and antioxidant activity of different finger millet grain types

Finger millet type[a]	TKW[b]	Kernel colour (Hunter)[c]		BR[d]	TP[c,e]	CT[c,f]	Anthocyanins[g]		Flvn.[g,j]	AA[c,k]
		L	a				Apig.[h]	Lute.[i]		
1. G35	3.21	67.9 (0.4) ab	4.9 (0.1) c	0	0.05 (0.00) kl	ND k	0.03 (0.00)a	0.01 (0.00)a	0.05 (0.00) a	38.4 (1.6) a
2. ICFM95001 GMSI	2.48	64.6 (0.9) bc	5.0 (0.1) c	0	ND l	ND k	0.03 (0.00)a	0.02 (0.00)a	0.04 (0.01) a	37.9 (1.4) a
3. 95G 198W	2.82	68.4 (0.6) a	4.7 (0.2) c	1	ND l	ND k	0.03 (0.00)a	0.02 (0.00)a	0.04 (0.00) a	40.3 (1.2) a
4. ICFM95001GMSF	2.55	62.7 (0.4) c	4.3 (0.1) c	0	0.09 (0.00) kl	ND k	0.02 (0.00)a	0.00 (0.00)a	0.01 (0.02) a	54.5 (1.6) b
5. FNL 0069	1.77	51.0 (0.3) fg	8.1 (0.3) ab	0	0.34 (0.09) j	0.02 (0.00) jk	0.97 (0.01)hi	0.79 (0.01)jk	2.31 (0.08) bc	75.9 (1.4) c
6. P283	2.43	45.9 (0.9) h	8.0 (0.6) ab	2	0.68 (0.05) jk	0.07 (0.00) j	1.64 (0.01)j	1.43 (0.01)m	2.62 (0.04) cd	69.3 (1.2) c
7. P224	3.30	52.4 (0.4) ef	8.0 (0.5) ab	80	0.79 (0.05) hi	0.60 (0.09 i	0.33 (0.00)b	0.28 (0.00)b	2.42 (0.03) b-d	124.8 (0.9) de
8. Gulu Early	2.77	51.8 (0.4) e-g	8.4 (0.1) ab	86	0.78 (0.06) hi	0.60 (0.09)i	0.89 (0.02)g	0.76 (0.00)ij	1.95 (0.27) bc	117.1 (0.9) d
9. FNL 0072	2.79	55.2 (0.4) de	7.7 (0.1) ab	81	0.92 (0.05) gh	0.72 (0.11) hi	0.94 (0.00)gh	0.81 (0.00)k	1.86 (0.39) bc	125.2 (2.4) de
10. FNL 0073	3.35	55.3 (0.4) de	7.2 (0.4) b	82	1.11(0.05) d-f	0.77 (0.10) hi	0.55 (0.00)d	1.45 (0.01)m	2.27 (0.06) bc	139.3 (1.4) f-h
11. ZIM Mnnursery	3.86	54.9 (0.7) de	8.7 (0.2) ab	94	0.72 (0.05) i	0.77 (0.11) hi	0.92 (0.00)gh	0.76 (0.00)i	4.21 (0.01) f	139.2 (2.1) f-h
12. FMV2	2.60	51.8 (0.2) e-g	8.0 (0.3) ab	80	0.76 (0.06) hi	0.84 (0.15) g-i	0.44 (0.00)c	0.37 (0.00)c	2.62 (0.31) cd	143.4 (4.3) gh
13. FNL 0071	2.53	53.3 (0.3) d-f	8.5 (0.6) ab	97	1.01 (0.05) fg	1.00 (0.09) gh	0.34 (0.00)b	0.27 (0.00)b	2.38 (0.01) b-d	129.5 (1.0) d-f
14. ZIM Llobel	3.31	50.7 (0.5) fg	8.4 (0.2) ab	98	1.75 (0.09) b	1.10 (0.12) fg	0.59 (0.00)d	0.48 (0.00)e	3.47 (0.09) ef	195.4 (2.0) j
15. ZW FMV6	2.17	48.5 (1.1) gh	9.4 (0.6) a	98	1.31 (0.09) c	1.13 (0.12) e-g	1.01 (0.00)i	0.84 (0.00)l	3.13 (0.04) de	123.2 (2.2) de
16. FNL 0012	2.83	55.1 (0.7) de	8.5 (0.2) ab	97	1.21(0.05) c-e	1.34 (0.09) d-f	0.71 (0.00)e	0.60 (0.00)g	1.70 (0.06) b	145.3 (1.4) h
17. FMV1	3.47	52.4 (1.2) ef	7.9 (0.2) ab	98	1.33 (0.05) c	1.35 (0.19) d-f	0.89 (0.00)g	0.73 (0.00)i	2.42 (0.01) b-d	132.8 (1.5) e-g
18. U15	2.66	51.2 (0.6) fg	8.0 (0.2) ab	100	1.25 (0.05) cd	1.41 (0.12) c-e	0.66 (0.00)e	0.54 (0.00)f	4.85 (0.06) g	125.9 (0.9) d-f
19. FNL 0051	3.09	55.9 (0.4) d	8.7 (0.8) ab	98	1.36 (0.05) c	1.65 (0.10) b-d	0.78 (0.04)f	0.64 (0.01)g	2.75 (0.14) c-e	135.7 (2.3) e-h
20. Nanjala Brown	3.78	51.9 (0.7) e-g	7.9 (0.2) ab	99	1.07(0.04) e-g	1.66 (0.09) bc	0.65 (0.00)e	0.56 (0.01)f	5.81 (0.10) h	145.9 (2.0) h
21. Okhale-1	2.38	48.6 (0.2) gh	7.1 (0.1) b	98	1.66 (0.09) b	1.77 (0.15) b	0.71 (0.00)e	0.57 (0.01)f	2.78 (0.02) c-e	174.1 (2.4) i
22. FNL 0074	2.73	50.7 (0.9) fg	7.3 (0.2) b	100	1.84 (0.18) a	2.08 (0.13) a	0.54 (0.00)d	0.44 (0.01)d	3.42 (0.18) e	195.4 (8.3) i
Mean	2.86	54.6 (1.3)	7.5 (0.3)	67.7	0.91 (0.12)	0.86 (0.14)	0.62 (0.28)	0.56 (0.27)	2.41 (1.05)	117.9 (9.8)

GMSI, Genetic male sterile infertile plant; GMSF, Genetic male sterile fertile plant

[a]Listed in order of increasing condensed tannin content (mg catechin equivalents/100 mg, db).

[b]1000 kernel weight (g, db).

[c]Mean of three replicate analyses; standard error in parenthesis. Values within the same column with different letters are significantly different at $p<0.05$

[d]Bleach reaction; % of kernels staining black.

[e]Total phenolics (mg of gallic acid equivalents/100 mg, db).

[f]Condensed tannin content (mg of catechin equivalents/100 mg, db).

[g]Mean of two replicate analyses, standard error in parenthesis. Values within the same column with different letters are significantly different at $p<0.05$.

[h]Apigeninidin (Absorbance at 475 nm/g); [i]Luteolinidin (Absorbance at 495 nm/g); [j]Flavan-4-ols (Absorbance at 550 nm/g).

[k]Antioxidant activity (mM of trolox equivalents/kg sample, db). ND, not detected.

The findings of this study on influence of grain type on total phenolics and tannin content are similar to those of Ramachandra et al (1977). These authors reported total phenolics ranging from 0.06 to 0.10 mg chlorogenic acid equivalents/100 mg in white finger millet, similar to the up to 0.09 mg GAE/100 mg reported in this work. Total phenolics in brown finger millet varieties (Ramachandra et al 1977) ranged from 0.37 to 2.44 mg chlorogenic acid equivalents/100 mg, which is also similar to the 0.34 to 1.84 mg GAE/100 mg reported here. Similar trends have been reported in sorghum grain. For example, Awika et al (2005) obtained a total phenolics of 0.08 mg GAE/100 mg in white sorghum compared to 0.98 to 2.25 mg GAE/100 mg in brown (tannin) sorghums. Similarly, the condensed tannin contents of white and brown finger millet grains reported in this study, in the range not detected to 0.07 mg CE/100 mg and 0.60 to 2.08 mg CE/100 mg in the light coloured grains and pigmented grains, respectively, show the same trend as that reported by Ramachandra et al (1977) (0.03 to 0.06 mg CE/100 mg and 0.12 to 3.47 mg CE/100 mg in the white finger millet varieties and brown varieties, respectively. Information on the occurrence of anthocyanins and flavan-4-ols in finger millet could not be found in the literature. However, similar trends of the relationship between plant type, grain colour and the levels of anthocyanins and flavan-4-ols have been reported in sorghum. Dykes et al (2005) reported low levels of anthocyanins and flavan-4-ols in sorghum grain of tan plant varieties and high levels of these phenolic compounds in purple plant varieties. Dicko et al (2005) found that red sorghum grain varieties contained on average significantly more 3-deoxyanthocyanidins and flavan-4-ols than white varieties.

Finger millet types FNL 0074 and Okhale-1 which stained black in the Bleach test and had high condensed tannin content, and types ICFM95001 GMSI and G35 which did not stain black and had no measurable levels of condensed tannins, were selected for light and SEM analyses. Figure 2.1.1 shows that the kernels of FNL 0074 and Okhale-1 had a dark coloured testa layer, whereas ICFM95001 GMSI and G35 had a light coloured testa layer. In sorghum, the presence of a pigmented testa as indicated by the Bleach test indicates presence of condensed tannins (Price and Butler 1977). Further, correlation analysis (Table 2.1.3) showed that there was a significant negative correlation ($p<0.01$) between the Hunter L values and tannin content of the grain, suggesting that a darker colour was associated with the tannins. Therefore, it can be concluded

that in finger millet, tannins are located in the testa layer, as is the case in sorghum grain (Awika and Rooney 2004).

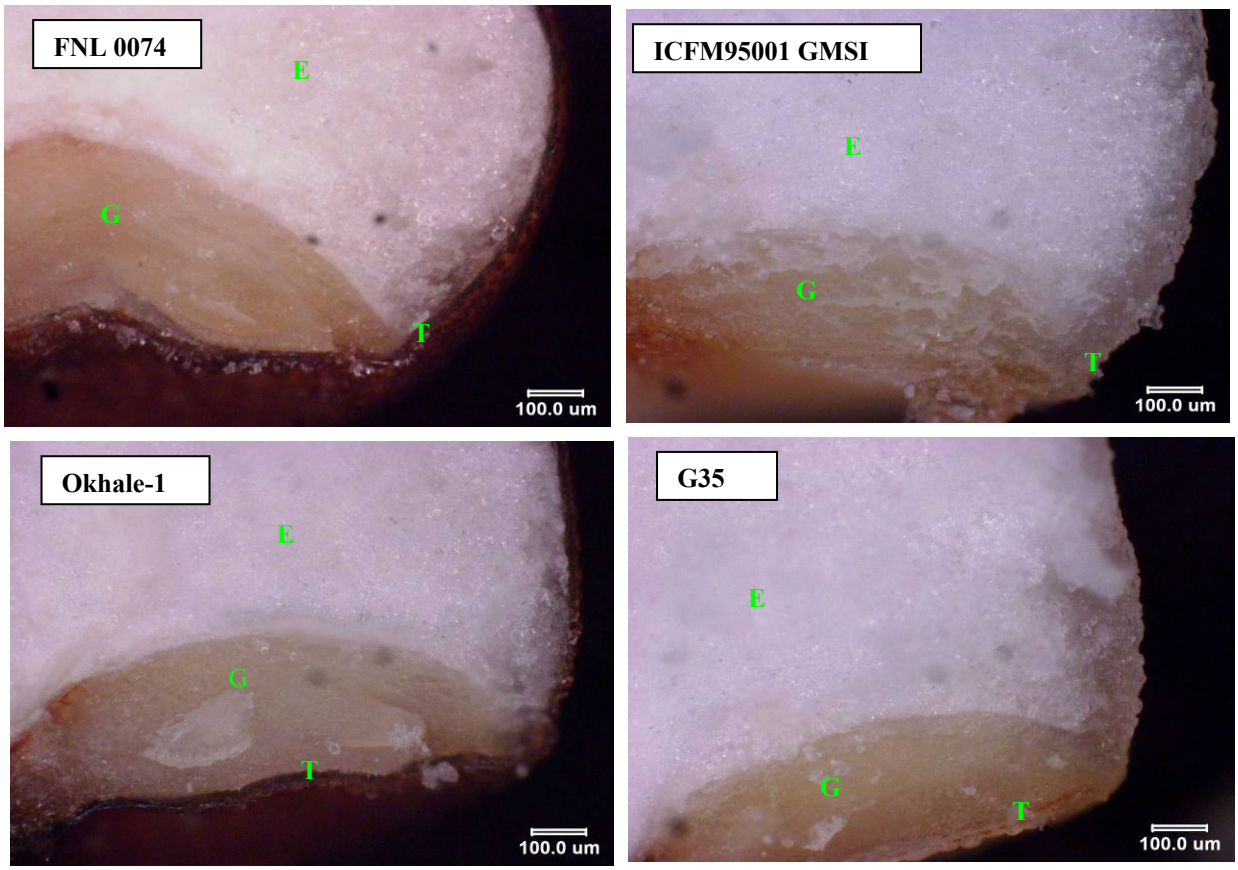

Figure 2.1.1. Light micrographs of finger millet kernels. FNL 0074 and Okhale-1, high-tannin; ICFM95001 GMSI and G35, low-tannin. E = endosperm; G = germ; T = testa.

SEM (Figure 2.1.2) showed that the pericarp was absent from the finger millet kernel, which is characteristic of an utricle (Angold 1979) and also confirmed the observation made in this study that most of the kernels lost their pericarps as the grain was being cleaned. SEM also revealed that the testa layers of the high-tannin types FNL 0074 and Okhale-1 were thicker (mean thickness of the testa layer: 14.6 µm and 13.4 µm, respectively) than the testa layers of the non-tannin types ICFM95001 GMSI and G35 (mean thickness of the testa layer: 9.2 µm and 9.7 µm, respectively) (Figure 2.1.2). The testa is probably thicker in order to accommodate the condensed tannins.

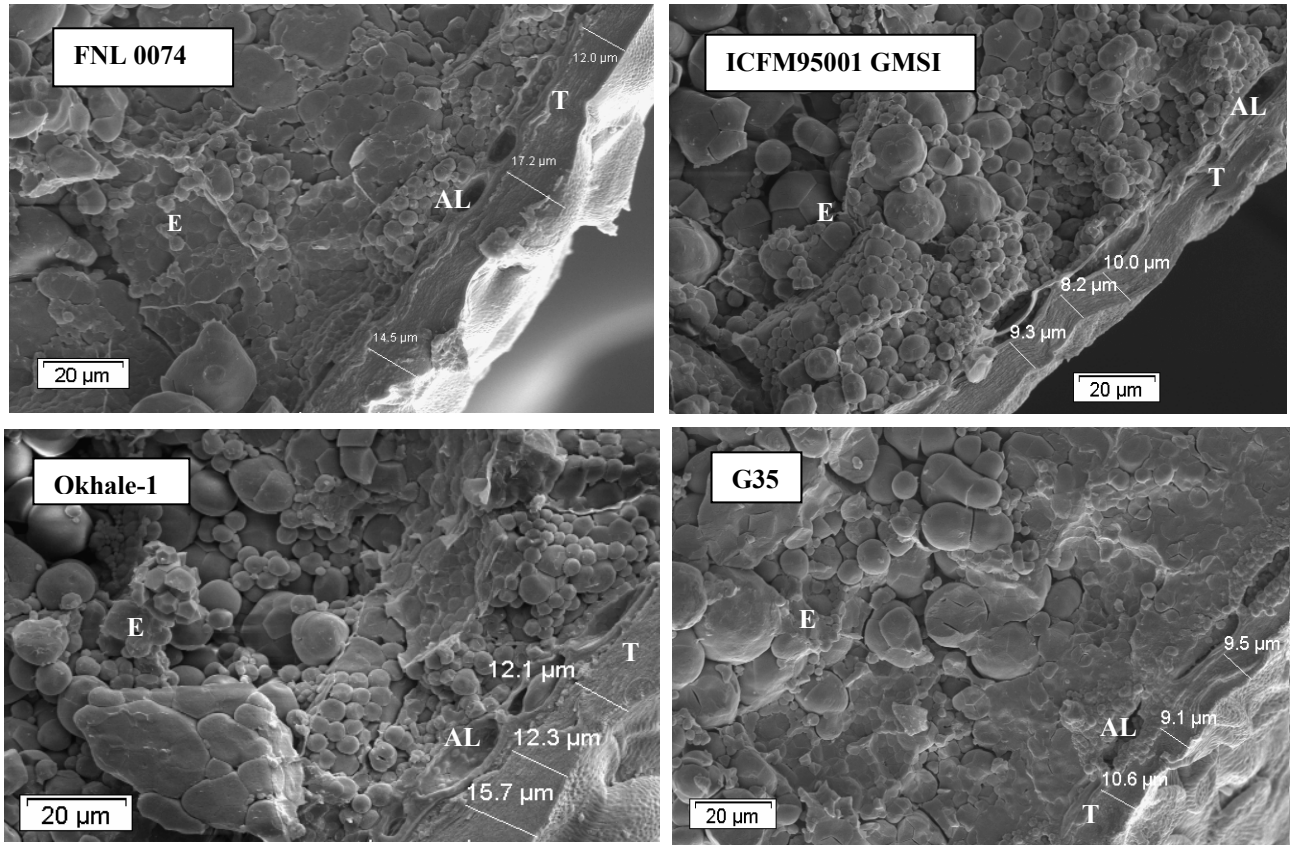

Figure 2.1.2. SEM of testa area of finger millet kernels. FNL 0074 and Okhale-1, high- tannin; ICFM95001 GMSI AND G35, low-tannin. AL = aleurone layer; E = endosperm; T = testa.

Table 2.1.2 shows that methanolic extracts from the grain of all the light finger millet types and two pigmented types (P283 and FNL 0069), which did not stain black, and had low condensed tannins had low antioxidant activity (range 37.9 to 75.9 mM trolox equivalents/kg), whereas the extracts from all the other pigmented types had high antioxidant activity that ranged from 117.1 to 195.4 mM trolox equivalents/kg. Similar results were obtained by Sripriya et al (1996), but on a study of only two finger millet types. Similarly, lower antioxidant activity has been reported in white sorghum grain relative to pigmented grain. Awika et al (2005) reported methanolic extracts from white sorghum had an antioxidant activity of 9.8 mM trolox equivalents/kg, whilst pigmented (black or brown [tannin]) sorghums had antioxidant activity levels ranging from 89 to 240 mM trolox equivalents/kg.

It appears that there were non-phenolic substances in the finger millet grain that also contributed to its antioxidant properties because finger millet types that had no measurable total phenolics

exhibited some antioxidant activity. Sripriya et al (1996) suggested that phytic acid also contributed to the antioxidant properties of finger millet grain. The tocopherols, which are well-known antioxidants, occur in finger millet (Serna-Saldivar and Rooney 1995), and may have also contributed to antioxidant properties of the finger millet grain. There is also the possibility that the antioxidant activity of the grain may have been due to phenolic compounds that were not detected by the Folin-Ciocalteu and the Vanillin-HCl assays.

There was a significant positive correlation ($p<0.01$) (Table 2.1.3) between the Hunter a values (redness) and total phenolic content, suggesting that phenolic compounds contributed to grain colour. As mentioned, flavonoids and condensed tannins (which are also measured in the total phenol assay) may have contributed to the brown colour of the grain. However, other substances in the grain contributed to its colour because the residue of the pigmented grain types, after extraction, was still brown. The highly significant correlations ($p<0.001$) between the Bleach test and the Hunter L and a values suggest that the Hunter L and a values may be used as predictors for presence of a pigmented testa and hence presence of condensed tannins. However, it can been seen in Table 2.1.2 that two pigmented varieties (FNL 0069 and P283) did not test positive for the presence of a pigmented testa. Boren and Waniska (1992) studied 24 sorghum cultivars from different genetic backgrounds and found that grain colour was not an adequate indicator of tannin content in sorghum. Thus, the situation with respect to grain colour and the presence of tannins in finger millet seems to be the same as with sorghum. However, the highly significant positive correlation ($p<0.001$) between the Bleach test and condensed tannin content shows that the Bleach test can be used to predict which batches of finger millet are tannin types, as is the case with sorghum (Maxson et al 1972).

The contents of apigeninidin, luteolinidin and flavan-4-ols were strongly negatively correlated ($r= -0.770$, $p<0.001$; $r= -0.647$, $p<0.01$; $r=0.731$, $p<0.001$, respectively) with the Hunter L values, indicating that anthocyanins and flavan-4-ols contributed to a dark grain colour. Dykes et al (2005) reported a similar strong negative correlation ($r= -0.84$, $p<0.001$) between the Hunter L values and flavan-4-ols, but on the contrary these authors found that anthocyanin content and Hunter L values were weakly correlated ($r= -0.61$, $p<0.05$).

There was a highly significant positive correlation between total phenolics and condensed tannin content ($r= 0.927$, $p<0.001$), indicating that condensed tannins contribute a large proportion of the total phenolic content of tannin-containing finger millet grain. A similar correlation ($r= 0.96$) between total phenolic content and condensed tannin content has also been reported in sorghum (Beta et al 1999). Total phenolics and the contents of apigeninidin and luteolinidin were not correlated, indicating that anthocyanins did not contribute significantly to the phenolic content of the grain. On the other hand, flavan-4-ols and total phenolics were significantly positively correlated ($r= 0.666$, $p<0.01$), suggesting that the flavan-4-ols made significant contribution to the phenolic content of the grain. The highly significant positive correlation ($p<0.001$) between total phenolics and condensed tannin content and antioxidant activity is an indication that condensed tannins are major contributors to the antioxidant properties of finger millet grain. A strong positive correlation between total phenolics and antioxidant activity has also been reported in sorghum grain (Awika et al 2003b, Awika et al 2004) ($r= 0.980$ and $r= 0.990$, respectively). Similarly, it has been shown that high-tannin sorghum varieties exhibit higher antioxidant activity than non-tannin varieties (Awika et al 2003b, Awika et al 2005). It has also been shown that tannins exhibit a relatively higher antioxidant activity than other phenolic compounds (Hagerman et al 1998, Riedl and Hagerman 2001, Hu et al 2004). Apigeninidin and luteolinidin contents were not correlated with the antioxidant activity of the grain, indicating that these compounds did not contribute significantly to the antioxidant activity of the finger millet grain. Similarly, Dicko et al (2005) and Dykes et al (2005) reported either weak or no correlations between anthocyanin content and antioxidant activity of sorghum. Flavan-4-ols content and antioxidant activity of the grain were strongly positively correlated ($r= 0.698$, $p<0.001$). Hence, flavan-4-ols made a large contribution to the antioxidant activity of finger millet grain. These results are different from those reported by Dicko et al (2005) and Dykes et al (2005) who reported either weak or no correlations between flavan-4-ols content and antioxidant activity of sorghum grain.

Table 2.1.3. Correlation coefficients between kernel characteristics, phenolic content, and antioxidant activity of finger millet grain types[a]

	TKW[b]	Hunter L	Hunter a	Bleach[c]	TP[d]	CT[e]	Apig.[f]	Lute.[g]	Flvn.[h]	AA[i]
TKW	1.000									
Hunter L	0.182	1.000								
Hunter a	0.042	-0.820***	1.000							
Bleach	0.379	-0.674***	0.744***	1.000						
TP	0.154	-0.605**	0.567**	0.751***	1.000					
CT	0.246	-0.604**	0.600**	0.795***	0.927***	1.000				
Apig.	-0.107	-0.770***	0.718***	0.282	0.415	0.234	1.000			
Lute.	0.017	-0.647**	0.601**	0.286	0.394	0.176	0.834***	1.000		
Flvn.	0.317	-0.731***	0.717***	0.697***	0.666**	0.666***	0.513*	0.423	1.000	
AA	0.300	-0.675**	0.671***	0.875***	0.905***	0.883***	0.307	0.304	0.698***	1.000

[a]Pearson correlation coefficients (r); *, **, *** indicate significance at $p<0.05$, 0.01, and 0.001, respectively.
[b]TKW, 1000 kernel weight.
[c]% kernels staining black.
[d]TP, total phenolics.
[e]CT, condensed tannin content.
[f]Apig., Apigeninidin.
[g]Lute., Luteolinidin.
[h]Flvn., Flavan-4-ols.
[i]AA, antioxidant activity.

2.1.5. Conclusions

Tannin-containing finger millet types exhibit a similar level of antioxidant activity as tannin sorghums, and as in sorghum, tannins are predominantly responsible for the antioxidant activity. In addition, as in sorghum, the tannins in finger millet grain are located in the testa layer. The Bleach test can be used to detect tannin finger millet types as in sorghum. As in sorghum (Hahn and Rooney 1986), occurrence of tannins in finger millet grain is a varietal property.

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2.2. Influence of phenolics in finger millet on its malt quality

2.2.1. Abstract

The aim of this investigation was to determine the impact of phenolics in finger millet (FM) grain on its malt quality and the influence of the phenolics on the proximate composition of the grain and malt. The germinative energy (GE) of 12 FM types of varied kernel colour and phenolic content was determined and the malt quality of the FM types evaluated in terms of diastatic power (DP), α- and ß-amylase activities and fungal load (total fungal count [TFC] and infection levels). High-phenol FM types had much higher malt quality than the low-phenol types, with respect to all parameters analysed. TFC was negatively correlated with total phenolics (TP) and amount of phenolic type (APT) and there were some negative correlations between infection levels and TP and APT ($p<0.05$). GE was positively correlated with TP and APT ($p<0.05$) and negatively correlated with TFC ($p<0.01$). Low-phenol FM types generally had higher fibre and protein contents than the high-phenol types, but all the 12 FM types had similar fat and mineral contents. Mineral content decreased with malting, whilst protein and fat contents generally increased, irrespective of grain phenolic content. The fibre content of the low-phenol FM types decreased with malting, whilst that of the high-phenol types increased. The data indicate that phenolics in FM grain influence its malt quality positively by contributing to attenuation of the fungal load on the germinating grain.

2.2.2. Introduction

Finger millet ([*Eleucine coracana* (L.) Gaertn.]) is adapted to the semi-arid, tropical and sub-tropical conditions that are prevalent in Africa and parts of Asia, where it is produced for food and in a malted form for the production of weaning foods, opaque beer and other products (Malleshi et al 1986, ICRISAT/FAO 1996, McDonough et al 2000). In the Southern African Development Community (SADC), the primary use of finger millet is as a malting/brewing grain, and it is particularly important in traditional home and village-scale brewing (Gomez 1994).

In malting, the moist and warm conditions and other factors promote the proliferation of micro-organisms on the germinating grain (Noots et al 1999). Micro-organisms contaminating the germinating grain may have negative effects on malt and beer quality, including reduction of enzyme synthesis, malt deterioration and imparting bad quality to beer, such as gushing and off-flavour (Flannigan 1996, Noots et al 1999). Fungi, particularly the moulds, are known to have the most undesirable effects on malt quality (Noots et al 1999). Of more concern is that some of the fungi are potentially toxigenic and as such may produce mycotoxins, which are a health hazard (Hussein and Brasel 2001).

Rabie and Lübben (1984) reported that South African sorghum malts produced by floor and pneumatic malting were infested with various fungi, including the potentially toxigenic types. There is no information on the contamination of finger millet malt by fungi. Although Trinder (1988) reported that, generally, South African commercial sorghum beers had aflatoxin levels below the legal limit, a more recent report by Odhav and Naicker (2002) indicated that South African commercial sorghum beers had significantly high levels of aflatoxins. Several workers have reported significant amounts of mycotoxins in African home-brewed opaque beers, though not specifically in finger millet beer (Lovelace and Nyathi 1977, Alozie et al 1980, Okoye 1987, Odhav and Naicker 2002).

Although knowledge on the factors that affect the quality of finger millet malt quality is limited, it has been shown that the quality is influenced by grain variety (Shukla et al 1986, Gomez 1994) and malting conditions (Malleshi and Desikachar 1986a). Phenolic compounds are known to inhibit the activity of enzymes such as amylases, trypsin and lipases by reacting with them and thereby altering their functional properties such as active sites and solubility (Rohn et al 2002). Tannins inhibit enzyme activity in sorghum malt (Daiber 1975a), and as a result, in Southern Africa, a formaldehyde treatment method has been developed to prevent their inhibition in high-tannin sorghums (Daiber 1975b). Chethan et al (2008) have shown that crude phenolic extracts and individual phenolic compounds from finger millet inhibit starch hydrolysis by finger millet malt amylases. On the other hand, several workers have reported that, in some sorghum grain types, mould resistance is related to phenolic content and composition (Chandrashekar and Satyanarayana 2006). Information on the potential contribution of phenolic compounds to

resistance of finger millet grain to infection by fungi is scanty (Seetharam and Ravikumar 1994, Viswanath et al 2009), but the situation may be similar to that in sorghum grain since the phenolic composition of the two cereals is similar (Dykes and Rooney 2006). Phenolics in finger millet grain may influence positively on its malt quality by contributing to resistance of the grain to infection by fungi during malting. The objectives of the study were: 1) to determine whether there is a relationship between resistance of finger millet grain to fungal infection and phenolic content and amount of phenolic type and some kernel characteristics, and to determine whether finger millet phenolics influence its malt quality; 2) to assess the influence of phenolics in finger millet grain on the proximate composition of the grain and malt; and 3) to determine the effect of malting on the proximate composition of finger millet grain.

2.2.3. Materials and Methods

2.2.3.1.Finger millet grain, sorghum grain standards and control barley malts

The origins and identities of the 22 finger millet grain types studied are given in chapter 2.1, Table 2.1.1. Two sorghum cultivars PAN 8564 (non-tannin) and PAN 8225 (high-tannin), obtained from Agricultural Research Council (ARC)-Grain Crops Institute, Potchefstroom, South Africa were used to make sorghum malt standards. Control barley malts (Megazyme Ceralpha and Betamyl standards, respectively) were obtained from Megazyme International, Ireland.

2.2.3.2. Fungal infection of finger millet grain

Fungi infecting finger millet grains were enumerated, isolated and identified by the direct plating method described by Rabie and Lübben (1984) and Rabie et al (1997). In this method, sample grains are evenly spread over three different growth media to ensure mycelial growth of all possible species present (i.e. those that infected the grain). The growth media serve as nutrients for the fungi, which grow out of the test sample onto the medium. Kernels of each grain type were surface-disinfected by shaking them in a flask of 76% (v/v) ethanol and then rinsing them three times with sterile distilled water. Five kernels were placed on plates (10 each) of Potato Dextrose Agar (PDA), Malt Salt Agar (MSA) and Pentachlorobenzene Agar (PCNB) and

incubated at 25ºC for 2 to 14 days. Kernels with mycelial growth of any fungal type were counted and expressed as a percentage of the total kernels plated. The fungal colonies were isolated and purified on fresh PDA plates and then identified based on morphological features of their fruiting bodies by referring to several standard Mycology books, including Domsch et al (1980) and Leslie and Summerell (2006).

2.2.3.3. Malting

Twelve finger millet grain types that varied in phenolic content were selected from the 22 types and malted. Only twelve finger millet types from the whole set of 22 could be malted because there was little grain of the other finger millet types. Malting was according to CSIR Approved Methods of Sorghum Malting and Brewing Analyses described by Dewar et al (1995). The method involved grain preparation, steeping, germination and drying.

Grain preparation

Finger millet grain was cleaned to remove dust, loose glumes, stalks and light grains by mechanical aspiration in combination with hand winnowing. Undersized and broken kernels were removed by sieving.

Steeping

Exactly 50 g of grain were placed in nylon mesh bags and steeped in 25ºC water in a steeping vessel. The vessel was drained every 3 h and the grain left to stand for 1 h (1 h air-rest period). The grain was steeped for a total of 24 h. The grain was then removed from the steeping vessel and excess surface-held water was removed by leaving the grain covered with absorbent paper for 10 min.

Germination

Germination was done in an incubator set at 25ºC and 100% relative humidity. The steeped grain was placed onto moistened towels on the incubator trays. The grain was removed from the incubator twice a day and on each occasion carefully manipulated by hand to avoid clumping of the roots and shoots. The bags of grain were then submerged in tap water for 10 min. The grain

was allowed to lose excess surface water by placing the malting bags on absorbent paper for 10 min. Germination was done for a total of 5 days.

Drying

The wet malt in the nylon bags was spread out and dried in a forced-draught oven set at 50ºC for 24 h.

The two sorghum cultivars PAN 8564 (non-tannin) and PAN 8225 (high-tannin) were malted in the same way as finger millet to obtain sorghum malt standards.

2.2.3.4. Total fungal count

Total fungal count (TFC) (yeasts and moulds) on the surface of finger millet grain and malt was determined by the standard plate count method. Exactly 10 g of either unmalted or malted finger millet grain types were placed in a 250-mL conical flask and 90 mL peptone water (0.1% [w/v] peptone, 0.85% [w/v] NaCl) was added. The flask was swirled by hand and then shaken for 5 min on a lateral shaker to suspend any micro-organisms on the surface of the kernels in peptone water. Tenfold serial dilutions were prepared in test tubes and then spread plated, in duplicate, on PDA and incubated at 25ºC for 2 to 14 days. Counting was done on plates that had 15-300 colonies. Fungal infection of the unmalted finger millet grain types and their malts were determined as already described.

2.2.3.5. Germinative energy and malt quality

Germinative energy

Germinative energy (GE) is a measure of the proportion (expressed as a percentage) of grain that can be expected to germinate under normal malting conditions. The GE of the finger millet and sorghum grain types was determined according to CSIR Approved Methods of Sorghum Malting and Brewing Analyses (Dewar et al 1995).

Malt quality

Diastatic power

Diastatic power (DP) is a measure of the activity of amylase enzymes, mainly joint α- and ß-amylase activity, in the malt. The DP of the finger millet malts and sorghum malt standards was determined according to the South African Bureau of Standards (SABS) Method 235 (SABS 1970) with modifications according to CSIR Approved Methods of Sorghum Malting and Brewing Analyses (Dewar et al 1995). The principle of the method is that the amylase enzymes of the malt extract act on standard buffered starch solution for 30 min at 30ºC and the reducing sugars that are released are estimated by boiling an aliquot of the solution with ferricyanide and determining the non-reduced ferricyanide by titration against standardised sodium thiosulphate. Finger millet and sorghum malts were milled into flour using a 1093 Cyclotec sample mill (Foss Tecator, Höganäs, Sweden) fitted with a 1 mm opening screen. The modification to the SABS Method 235 for the determination of DP was that 5 g malt was used and the extraction volume was 100 mL, instead of 25 g and 500 mL, and in addition to extraction with 2% peptone as per SABS method 235, extraction was also made with distilled water. The apparatus size and reagent volumes were reduced accordingly. Extraction with peptone prevents inactivation of amylase enzymes by tannins in the high-tannin grain and the peptone has no effect on the determination of the amylases in the non-tannin grain. Water extraction estimates only those amylases, which have not been inactivated by tannins.

α-and ß-amylase activities

Finger millet malts and sorghum malt standards were milled as has been described, whilst the control barley malt were supplied in flour form in the enzyme assay kits.

α-amylase

The α-amylase activity of the finger millet malts, sorghum malt standards and control barley malts was determined by the Alpha Amylase Procedure (Ceralpha Method) following the manufacturer's instructions (Megazyme International, Ireland). The principle of the assay is that α-amylase (e.g. from the test malt extract) cleaves a bond within the blocked p-nitrophenyl maltosaccharide substrate (blocked p-nitrophenyl maltoheptaoside [BPNPG7]) and the non-blocked reaction product containing the p-nitrophenyl substituent is instantly cleaved to glucose

and free p-nitrophenol by excess quantities of thermostable α-glucosidase. The phenolate colour is developed on addition of tri-sodium phosphate and its absorbance measured at 400 nm. The absorbance relates directly to the level of α-amylase in the sample assayed (McCleary and Sheehan 1987).

ß-amylase

The ß-amylase activity of the finger millet malts and sorghum malt standards was determined using the Betamyl Method following the manufacturer's instructions (Megazyme International, Ireland). In this method, which is an improvement by McCleary and Codd (1989) of an earlier method, the total enzymic activity of soluble and insoluble ß-amylase is determined. Cysteine is included in the extracting reagent to extract the insoluble ß-amylase. The substrate reagent of the kit consists of a mixture of two substrates p-nitrophenyl-α-D-maltopentaose (PNPG5) and p-nitrophenyl-α-D-maltohexaose (PNPG6) plus the enzyme α-glucosidase. During the enzyme reaction, ß-amylase (from e.g. test malt extract) hydrolyses PNPG5 to maltose and p-nitrophenyl maltotrioside and the p-nitrophenyl maltotrioside is immediately cleaved by α-glucosidase to glucose and free p-nitrophenol. The rate of release of p-nitrophenol relates directly to the rate of release of maltose by ß-amylase (Mathewson and Seabourn 1983). The phenolate colour is developed on addition of a stopping, reagent Trizma base solution, and its absorbance measured at 410 nm.

2.2.3.6.Nutritional analyses

Ash, fat, protein and fibre

The AOAC Official Methods 923.03, 920.85, 968.06 and 2002.04 (Association of Official Analytical Chemists International 2003) were used to determine the ash, fat, protein and neutral detergent fibre, respectively, of unmalted and malted finger millet grain of eight of the 12 grain types.

Amino acid analysis

The amino acid contents of four unmalted and malted finger millet grain types were determined by a reverse-phase HPLC Pico.Tag Method for rapid analysis of amino acids by pre-column

derivatization (Bidlingmeyer et al 1984). In this method, the proteins are hydrolyzed using HCl and the amino acids are derivatized into phenylthiocarbamyls which are separated due to their differential hydrophobicity. A mixture of amino acid standards (Sigma, Cat no. AAS18) was used to calibrate the HPLC. Sixteen common amino acids were quantified.

2.2.4. Results and Discussion

As shown previously (chapter 2.1, Table 2.1.1), Table 2.2.1 shows that all grain types that had no pigmented testa were obtained from tan plant types and those grain types had no or had much lower condensed tannin contents than the types with a pigmented testa. Grain types with a pigmented testa were obtained from purple plant types. Also, as described previously (chapter 2.1, Table 2.1.2) creamy white grain types (i.e. overall grain colour was creamy white) had much lower levels of total phenolics (TP), condensed tannins (CT), anthocyanins and flavan 4-ols than the pigmented types (i.e. overall grain colour was pigmented [brown]) (Table 2.2.1).

Table 2.2.1 shows that the finger millet grain types had different infection levels of two field fungi *Phoma sorghina* and *Fusarium nygamai* and two storage fungi *Eurotium repens* and *Eurotium rubrum* (the *Eurotium* species belong to the *Aspergillus glaucus* group [Noots et al 1999]). Eight grain types had extremely high levels (72 to 100% kernels infected) of *Phoma sorghina*. The levels of *Fusarium nygamai* in finger millet grain type ICFM 95001GMSF were high (72% of the kernels were infected), whilst its levels in G35, ICFM 95001GMSI and FMV1 were significant but not as high (22 to 226% of the kernels infected). A much higher proportion (3 of the 4) of creamy white grain types had at least 20% of their kernels infected by field and storage fungi than the pigmented types. Few (1 or 2) grain types were also significantly infected by *Fusarium equiseti*, *Penicillium chrysogenum*, *P. griseofulvum*, *P. viridicatum*, *P. islandicum*, *Cladosporium cladosporioides* and *Apergillus restrictus* (results not shown).

Table 2.2.1. Kernel characteristics, total phenolics and amount of phenolic type and fungal infection of finger millet grain

Finger millet type[a]	PC [b]	Visual Kernel Colour [b]	Kernel colour (Hunter)[b,c]		Phenolic content[b,c]					Fungal load (% kernels infected)			
							Anthocyanins						
			L	a	TP	CT	Apig.	Lute.	Flvn.	P.sorg.	F.nyg.	E. rep.	E. rub.
G35	T	W (-)	67.9	4.9	0.05	ND	0.03	0.01	0.05	100	26	0	0
ICFM95001 GMSI	T	W (-)	64.6	5.0	ND	ND	0.03	0.02	0.04	44	26	100	100
95 G 198 W	T	W (-)	68.4	4.7	ND	ND	0.03	0.02	0.04	94	0	100	100
ICFM 95001GMSF	T	W (-)	62.7	4.3	0.09	ND	0.02	0.00	0.01	72	72	100	60
FNL 0069	T	B (-)	51.0	8.1	0.34	0.02	0.97	0.79	2.31	100	0	100	100
P283	T	DB (-)	45.9	8.0	0.68	0.07	1.64	1.43	2.62	2	6	0	0
P224	P	B (+)	52.4	8.0	0.79	0.60	0.33	0.28	2.42	8	0	94	2
Gulu Early	P	B (+)	51.8	8.4	0.78	0.60	0.89	0.76	1.95	0	0	100	0
FNL 0072	P	B (+)	55.2	7.7	0.92	0.72	0.94	0.81	1.86	20	6	22	0
FNL 0073	P	B (+)	55.3	7.2	1.11	0.77	0.55	1.45	2.27	100	0	82	0
ZIM Mnnursery	P	B (+)	54.9	8.7	0.72	0.77	0.92	0.76	4.21	96	18	0	0
FMV2	P	B (+)	51.8	8.0	0.76	0.84	0.44	0.37	2.62	2	0	86	90
FNL 0071	P	B (+)	53.3	8.5	1.01	1.00	0.34	0.27	2.38	2	2	100	64
ZIM Llobel	P	DB (+)	50.7	8.4	1.75	1.10	0.59	0.48	3.47	92	0	20	2
FMV6	P	B (+)	48.5	9.4	1.31	1.13	1.01	0.84	3.13	0	0	4	10
FNL 0012	P	B (+)	55.1	8.5	1.21	1.34	0.71	0.60	1.70	6	0	0	0
FMV1	P	B (+)	52.4	7.9	1.33	1.35	0.89	0.73	2.42	100	22	0	0
U15	P	B (+)	51.2	8.0	1.25	1.41	0.66	0.54	4.85	36	4	0	0
FNL 0051	P	B (+)	55.9	8.7	1.36	1.65	0.78	0.64	2.75	0	0	0	30
Nanjala Brown	P	B (+)	51.9	7.9	1.07	1.66	0.65	0.56	5.81	6	0	0	0
Okhale-1	P	B (+)	48.6	7.1	1.66	1.77	0.71	0.57	2.78	9	0	0	0
FNL 0074	P	B (+)	50.7	7.3	1.84	2.08	0.54	0.44	3.42	2	12	0	0

[a]Listed in order of increasing condensed tannin content (g catechin equivalents/100 g, dry basis [db]). GMSF, Genetic male sterile fertile plant; GMSI, Genetic male sterile infertile plant. PC=plant colour: T= tan; P= purple. W= creamy white; B= brown; DB= dark brown kernel; (+) with and (-) without a pigmented testa. TP, Total phenolics (g gallic acid equivalents/100 g, db); CT, Condensed tannin content (g catechin equivalents/100 g, db). Apig., apigeninidin (Absorbance at 475 nm/g); Lute., luteolinidin (Absorbance at 495 nm/g); Flvn., flavan-4-ols (Absorbance at 550 nm/g).

[b] From chapter 2.1, Table 2.1.2.

[c]Mean of at least two replicate analyses. ND, not detected.

P. sorg., *Phoma sorghina*; F. nyg., *Fusarium nygamai*; E. rep., *Eurotium repens*; E rub., *Eurotium rubrum*

A minimum of 20% infected kernels by any type of fungus is considered significant by the Centre for Applied Mycological Studies (CAMS), University of Pretoria/CSIR, South Africa. This specification will be used in the interpretation of results of infection of finger millet grain by fungi. The field and storage fungi detected in the finger millet grain types of this study also occur in the malting grains of barley (Rabie et al 1997, Noots et al 1999) and sorghum (Williams and Rao 1981). Field and storage fungi may cause grain deterioration, including discolouration, weakening or death of the embryo (resulting in a decrease in germinability), heating, mustiness, shrivelling and rotting (Christensen and Kaufmann 1974, Agarwal and Sinclair 1987). The storage fungi *Eurotium repens* and *Eurotium rubrum*, *Cladosporium cladosporioides*, *Penicillium chrysogenum*, *P. griseofulvum*, *P. viridicatum*, and *P. islandicum, which were detected in the finger millet grain types of this study,* are known to cause grain deterioration (Christensen and Kaufmann 1974, Flannigan 1996, Noots et al 1999). Their occurrence in the grain is therefore undesirable.

Although conditions suitable for fungal growth are not necessarily suitable for mycotoxin production (D'Mello and MacDonald 1997), occurrence of fungal species that are known to produce mycotoxins in the finger millet grain types may be a health risk. *Phoma sorghina* is known to invade the plant while cultivated in the field and eventually end up in the grain (Rabie and Lübben 1984). This fungus is normally associated with wet field condition just before or during the harvesting process. It is also known to produce a mycotoxin, tenuazonic acid, which is responsible for protein synthesis in humans and animals, causing inhibition in growth (Cole and Cox 1981). This fungus is also the causative agent of a disease called *onyalai*, especially among black people living in southern Africa. The fungus produces an unknown mycotoxin that lowers the blood plate count of patients (Rabie et al 1975). This results in the patient developing haemorrhage and thrombocytopenia and can cause death. *Fusarium* species are destructive plant pathogens that produce mycotoxins before or immediately post harvesting (Sweeney and Dobson 1998). *Fusarium equiseti* and *Fusarium nygamai* are among the common *Fusarium* species that produce the trichothecenes, which are a group of chemically diverse mycotoxins that cause various health disorders in mammals (including humans). The disorders include immuno-suppression, hemorrhaging of the intestines and cytotoxic reactions (Cole and Cox 1981, Sweeney and Dobson 1998). Some of the *Penicillium* species detected in the finger millet grain

types produce mycotoxins that Pitt (2001) considered significant in human health. *Penicillium chrysogenum* produces the mycotoxins roquefortine C and cyclopiazonic acid. *P. griseofulvum* produces patulin and roquefortine C and *P. viridicatum* produces cyclopiazonic acid. These mycotoxins either may affect the liver and kidney function or are neurotoxins.

Table 2.2.2 shows that infection by *Phoma sorghina* was significantly positively correlated with the Hunter L values and negatively correlated with the Hunter a values ($p<0.05$). Infection by *Eurotium rubrum* was positively correlated with the Hunter L values. The results suggest that light coloured grain types are more susceptible to fungal infection than the pigmented types. These, results that indicate an association between finger millet grain colour and grain mould resistance, are similar to those reported for sorghum grain. Menkir et al (1996) and Audilakshmi et al (1999) reported that darker sorghums were more resistant to mould infection than were the lighter coloured types. Similarly, Ratnavathi and Sashidhar (2003) found that red sorghum genotypes had lower fungal load and had lower amounts of aflatoxins than the yellow and white genotypes. The resistance may be due to pigments in the grain.

There was a significant negative correlation ($p<0.05$) between *P. sorghina* infection levels and condensed tannin content of the finger millet grain (Table 2.2.2). However, *P. sorghina* infection levels were not significantly correlated with total phenolics (TP), anthocyanin and flavan-4-ol content of the grain. Grain infection by *Fusarium nygamai* was not correlated with the phenolic compounds of the grain. There was a highly significant negative correlation between *Eurotium repens* infection levels and TP and CT content of the grain ($p<0.01$), and the infection levels were negatively correlated with apigeninidin and flavan-4-ol content ($p<0.05$). *Eurotium rubrum* infection levels were negatively correlated with TP, CT, apigeninidin and flavan-4-ol content of the grain ($p<0.01$, $p<0.01$, $p<0.05$, $p<0.05$, respectively) (Table 2.2.2). These correlations suggest that phenolics in finger millet grain are involved in the resistance of finger grain to fungal infection. Seetharam and Ravikumar (1994) similarly reported a negative correlation between Blast disease (caused by the fungus *P. grisea*) and TP and CT content in dry and germinating finger millet grain. Brown grain types were resistant to Blast, had high TP and CT content, while the white types were susceptible to Blast, and had low TP and CT content.

Table 2.2.2. Pearson's correlation coefficients between kernel characteristics, total phenolics and amount of phenolic type and fungal infection of finger millet grain

	Hunter L	Hunter a	TP	CT	Apig.	Lute.	Flvn.	P. sorg.	F. nyg.	E. rep.	E. rub.
Hunter L											
Hunter a	0.600**										
TKW	0.144	0.254									
TP	-0.605**	0.567**									
CT	-0.604**	0.600**	0.927***								
Apig.	-0.770***	0.718***	0.415	0.234							
Lute.	-0.647**	0.601**	0.394	0.176	0.834***						
Flvn.	-0.731***	0.717***	0.666**	0.686***	0.513*	0.423*					
P. sorg.	0.474*	-0.425*	-0.298	-0.470*	-0.268	-0.082	-0.286				
F. nyg.	0.396	-0.358	-0.246	-0.228	-0.170	-0.220	-0.246	0.368			
E. rep.	0.331	-0.335	-0.540**	-0.593**	-0.475*	-0.306	-0.513*	0.136	-0.236		
E. rub.	0.430*	-0.401	-0.541**	-0.536*	-0.445*	-0.466*	-0.476*	0.168	-0.143	0.704***	

TP, Total phenolics; CT, Condensed tannins; Apig., apigeninidin; Lute., luteolinidin; Flvn., flavan-4-ols.
P. sorg., *Phoma sorghina*; F. nyg., *Fusarium nygamai*; E. rep., *Eurotium repens*; E. rub., *Eurotium rubrum*.
*Significant at $p<0.05$; **significant at $p<0.01$; ***significant at $p<0.001$.

The relationship between phenolic content and resistance of finger millet grain to fungal infection shown in this study is similar to what has been found in sorghum grain. Sorghum grain mould resistance has been shown to be related to high levels of TP (Waniska et al 1989, Melake-Berhan et al 1996, Audilakshmi et al 1999), CT (Harris and Burns 1973, Kambal and Bate-Smith 1976, Bandyopandhyay et al 1988), the anthocyanin apigeninidin (Menkir et al 1996), flavan-4-ols (Jambunathan et al 1986, Jambunathan et al 1990, Menkir et al 1996, Waniska et al 2001) and phenolic acids (Hahn et al (1983). The negative correlation between the level of infection by a specific fungal species and amount of a specific phenolic type (CT, anthocyanins or flavan-4-ols) (Table 2.2.2) suggests that specific phenolic types contribute to resistance of finger millet grain to infection by specific fungal species. Genetic differences were probably responsible for the variation in the apparent susceptibility of different fungal species to inhibition by different types of finger millet phenolics. However, condensed tannin content was negatively correlated with infection levels of almost all fungal species detected (Table 2.2.2). Melake-Berhan et al (1996) working with sorghum reported similar results; infection by *Alternaria* spp. was significantly negatively correlated with flavan-4-ols and tannin content (r=-0.78 and r=-0.85, respectively). *Fusarium* spp. and *F. moniliforme* infection levels were negatively correlated with tannin content (r=-0.73 and r=-0.95, respectively) and grain infection by any of the fungal species detected was not significantly correlated with apigeninidin and luteolinidin content (Melake-Berhan et al 1996). However, *Cladosporium* spp. infection levels were significantly positively correlated with the amount of flavan-4-ols (R=0.65).

Several mechanisms have been suggested for the observed inhibitory effect of phenolic compounds on microorganisms. Some of them, as reviewed earlier (chapter 1, subsection 1.2.6.5), are oxidation of microbial membranes and cell components by free radicals formed from the phenolic compounds, irreversible complexation with nucleophilic amino acids in proteins leading to inactivation of proteins and loss of their functionality, and interaction with substrates (e.g. biopolymers such as proteins and polysaccharides) and metal ions making them unavailable to micro-organisms, and interaction with cell membranes, enzyme proteins and protein factors thereby interfering with metabolic processes (Scalbert 1991, Cowan 1999). McGrath et al (1982) suggest that tannins protect sorghum grain from fungal invasion by forming a physical barrier in the testa layer. It is noted that the resistance of finger millet grain types to fungal infection was most likely also due to other factors, such as grain hardness (reviewed in section 1.2.6.6).

Table 2.2.3 shows that finger millet malts had higher total fungal count (TFC) ($1.4x10^5$ to $2.2x10^7$ cfu/g) on the surface of the grains than their respective unmalted grain ($1.2x10^3$ to $3.5x10^4$ cfu/g). TFC of unmalted and malted grain of finger millet types G35, 95G198W and ICFM95001GMSI which had low phenolic levels was higher ($2.1x10^4$ to $2.2x10^7$ cfu/g) than the TFC of unmalted and malted grain of types that had high phenolic levels ($9.1x10^2$ to $7.4x10^5$cfu/g) (Table 3). Finger millet malts had higher TFC than their respective unmalted grain (Table 2.2.3) because the fungi proliferated during malting. Similar results were obtained by O'Sullivan et al (1999) who reported that fungal count increased from 1.5 $x10^2$ cfu/g in unmalted barley to 6.1 $x10^4$ cfu/g in kilned barley malt processed in a conventional malthouse and Lefyedi et al (2005) who found that fungal count increased from $2.7x10^4$ cfu/g in unmalted sorghum up to $5.9x10^6$ cfu/g during a micro-scale floor malting of sorghum without turning. As was stated earlier, fungi proliferated during malting because the malting conditions were favourable to their growth. The favourable conditions included elevated temperature and moisture, aeration and presence of metabolisable components (reviewed by Flannigan 1996, Noots et al 1999). Some of the fungi probably originated from the field and others contaminated the grain in the storage, transport and malting environments as described by Flannigan (1996) and Noots et al (1999).

The presence of a high fungal load on the surface of the finger millet malt is undesirable as the fungi can potentially affect negatively on the quality of the malt and beer as described earlier. There was a lower TFC on the surface of the unmalted and the malted grain of finger millet types that had a higher phenolic content (Table 2.2.3), probably because the phenolic compounds, particularly the tannins, in the outer layers of the grain contributed to a physical barrier to fungal invasion as was suggested for tannins in sorghum grain by McGrath et al (1982). It seems that the fungi would not accumulate on the surface of the grain if there were resistance to their invasion of the grain.

Many *Fusarium* species infected the malts, and all the malt types were infected by *Phoma sorghina*. Ten of the 12 malt types were infected by the *Mucor* species moulds while the mould *Rhizopus oryzae* was detected in only two of the 12 malt types studied (Table 2.2.3). White moulds could be visually observed on the malts from the low-phenol grain types G35, 95G198W and ICFM95001GMSI, but not on the malts from the high-phenol grain types. Levels of

infection by the dominant fungal types were similar across malt types (Table 2.2.3). Infection of the malt by *Fusarium* species (Table 2.2.3) is undesirable as they are implicated in poor malting quality e.g. reduction of seedling growth and α-amylase activity (Schapira et al 1989) and in gushing beer (Schwarz et al 1996, Munar and Sebree 1997). Some of the *Fusarium* species can produce the mycotoxins trichothecenes, zearalenone (ZEN) and fumonisins (Sweeney and Dobson 1998, D'Mello et al 1999). *Fusarium chlamydosporum*, *F. equiseti* and *F. nygamai* detected in the malt (Table 2.2.3) can produce the myocotoxins trichothecenes (Sweeney and Dobson 1998), which cause the various health disorders described earlier. Similarly, infection of the malt by *Phoma sorghina* may be a health risk because the mould is potentially toxigenic and may cause health disorders described earlier. The moulds *Rhizopus oryzae* and *Mucor* species detected in the finger millet malt (Table 2.2.3) are not plant pathogens but may contribute to reduction of grain viability (Gyllang and Martinson 1976). In addition, *Rhizopus oryzae* and *Mucor* species, though not known to be toxigenic, may adversely affect the colour and flavour of beer (Noots et al 1999).

The fungal infection levels across the malt types were similar (Table 2.2.3), probably because changes due to malting resulted in the finger millet grain types having similar susceptibility to fungal infection. The changes may have included damage to the seedling and epidermis of the grain (Daiber and Taylor 1995, Agu and Palmer 1999), and changes in the components of the grain that would be involved in mould resistance such as a partial breakdown of proteins and cell wall non-starch polysaccharides, leading to softening of the grain. (Briggs 1998). Leaching of phenolic compounds (Veerabhadrappa et al 1978, subsection 1.2.6.3), and enzyme-mediated and non-enzyme-mediated interactions of phenolic compounds with other components of the germinated grain such as proteins and polysaccharides (subsection 1.2.6.3, Kruger 1976, Reichert et al 1980, McGrath et al 1982, Glennie 1983, Butler et al 1984, Dicko et al 2006) could have also contributed to the susceptibility of the grain to infection by fungi. The TFC on the surface of the finger millet malt seems not related with the infection levels of the malt (Table 2.2.3).

Table 2.2.3. Fungal load and fungal types on finger millet grain types and their malts[a]

	Fungal load									
	TFC of grain surface		Fungal infection (% malt kernels infected)								
Finger millet type[b]	**Unmalted**	**Malted**	***F.* sp.**	***F. chlam.***	***F. equi.***	***F. nyg.***	***F. scirpi***	***F. vert.***	***P. sorg.***	***Rhizo ory.***	***Mucor sp.***
G35	2.4×10^4	1.3×10^6	100	60	22	30	22	35	100	100	0
ICFM95001GMSI	2.1×10^4	1.7×10^7	100	26	36	36	24	44	88	0	70
95G198W	3.5×10^4	2.2×10^7	100	64	24	28	68	20	98	0	70
FNL 0069	5.5×10^3	5.0×10^5	100	38	22	28	18	40	100	0	80
P283	1.8×10^3	7.4×10^5	100	30	10	16	20	32	22	0	56
Gulu early	3.3×10^3	4.3×10^5	100	6	48	12	16	16	100	0	66
FNL 0072	3.8×10^3	5.0×10^5	98	60	12	18	16	26	44	52	66
Zim Mnnursery	9.1×10^2	6.5×10^5	96	34	20	24	22	32	64	0	66
FMV1	2.4×10^3	1.9×10^5	98	30	24	12	4	30	100	0	70
FNL 0051	4.4×10^3	5.0×10^5	38	14	4	28	38	30	96	0	70
Nanjala Brown	5.3×10^3	1.4×10^5	100	14	32	32	16	48	8	0	32
Okhale-1	1.2×10^3	2.9×10^5	82	10	12	24	0	22	20	0	0

[a]Total phenolics and amount of phenolic type of the grain types are shown in Table 2.2.1.

[b]Listed in order of increasing condensed tannin content. TFC, total fungal count (cfu/g, as is).

F. sp., *Fusarium* species; *F. chlam.*, *Fusarium chlamydosporum*; *F. equi.*, *F. equiseti*.; *F. nyg.*, *F. nygamai*; *F. vert.*, *F. verticillioides*; *P. sorg.*, *Phoma sorghina*; *Rhizo. ory.*, *Rhizopus oryzae*.

Table 2.2.4 shows that the TFC of the finger millet grain and malt was highly negatively correlated with TP ($p<0.01$, $p<0.001$) and negatively correlated with CT, apigeninidin, luteolinidin and flavan-4-ol contents ($p<0.05$). The results indicate that phenolic compounds contribute to resistance of finger millet to contamination by fungi. The possible mechanism of resistance has been stated above.

Levels of infection of the finger millet malt by different fungal species were largely not correlated with the TP, CT, apigeninidin, luteolinidin and flavan-4-ol contents of the unmalted finger millet grain. The exceptions were infection by *Fusarium chlamydosporum* which was negatively correlated with TP and CT content ($p<0.05$), infection by *F. nygamai* was negatively correlated with apigeninidin and luteolinidin contents ($p<0.05$) and infection by *Phoma sorghina* was negatively correlated with flavan-4-ol content ($p<0.05$) (Table 2.2.4). The few negative correlations between levels of infection of the malt by different fungal species and phenolic content and amount of phenolic type in Table 2.2.4 suggest that finger millet phenolics had a limited contribution to resistance of its malt to infection by fungi. If the correlations in Table 2.2.4 are compared with those in Tables 2.2.2, it seems clear that phenolic compounds contributed more to resistance of unmalted finger millet grain to fungal infection than to resistance of the malt. The reason for this has been suggested above, i.e. changes due to malting seem to have made the grain much more susceptible to infection by fungi than the unmalted grain.

Table 2.2.4. Pearson's correlation coefficients between finger millet grain total phenolics and amount of phenolic type and fungal load of finger millet malt

	TP	**CT**	**Apig.**	**Lute.**	**Flvn.**	**RawTF**	**MaltTF**	***F.*** sp.	***F. chl.***	***F. equi.***	***F. nyg.***	***F. sci.***	***F. vert.***	***P. sorg.***	***Muc.*** sp.
TP															
CT	0.927***														
Apig.	0.287	0.307													
Lute.	0.277	0.251	0.847***												
Flvn.	0.611**	0.723***	0.513*	0.427*											
RawTFC	-0.843**	-0.606*	-0.598*	-0.589*	-0.561										
MaltTF	-0.946***	-0.656*	-0.620*	-0.640*	-0.704*	0.663*									
F. sp.	-0.463	-0.549	-0.081	-0.037	-0.146	0.203	0.194								
F. chl.	-0.601*	-0.602*	-0.332	-0.332	-0.537	0.539	0.454	0.368							
F. equi.	-0.336	-0.242	-0.345	-0.319	-0.113	0.339	0.206	0.551	-0.219						
F. nyg.	-0.418	-0.133	-0.600*	-0.668*	-0.114	0.435	0.511	-0.137	0.124	0.004					
F. sci.	-0.430	-0.399	-0.395	-0.428	-0.402	0.651*	0.731**	-0.160	0.459	-0.050	0.365				
F. vert.	-0.263	-0.058	-0.109	-0.113	0.285	0.276	0.023	-0.164	-0.048	00.063	0.625*	-0.164			
P. sorg.	-0.370	-0.399	-0.454	-0.436	-0.616*	0.548	0.341	0.376	0.186	0.285	0.018	0.376	-0.186		
Muc. sp.	-0.067	-0.259	0.226	0.247	-0.059	0.271	0.207	0.341	-0.033	0.424	-0.209	0.341	-0.033	0.424	

TP, Total phenolics; CT, Condensed tannins; Apig., apigeninidin; Lute., luteolinidin; Flvn., flavan-4-ols; rawTF, total fungal count of unmalted finger millet grain surface; MaltTF, total fungal count of finger millet malt grain surface; F. sp., *Fusarium* species; *F. chl.*, *Fusarium chlamydosporum*; *F. equi.*, *F. equiseti*.; *F. nyg.*, *F. nygamai*; *F. sci*., *F. scirpi*, *Fusarium* F. *vert.*, *F. verticillioides; P. sorg.*, *Phoma sorghina*; *Muc.* sp., *Mucor* sp.

* Significant at $p<0.05$ ** $p<0.01$ and *** $p<0.001$.

Table 2.2.5 shows that the GEs of the low-phenol finger millet grain types G35, 95G198W and ICFM95001GMSI were low. A low GE indicates that the grain has a low potential to germinate during malting. This has a negative effect on malt quality, as there would be poor mobilization of enzymes and grain modification. The GEs of the low-phenol finger millet grain types were low, probably because of a high fungal load on the surface of the germinating grain (Table 2.2.3). The fungi on the surface of the germinating grain might have impacted negatively on the GEs of the low-phenol finger millet grain types through various ways, including releasing metabolites that are phytotoxic, e.g. mycotoxins into the grain (Pandey and Mehrotra 1985, Schwarz et al 1996) and their metabolic activities, e.g. respiration, might have contributed to dormancy of water-sensitive grains (Kelly and Briggs 1992). It appears that high-phenol grain types had high GEs because their phenolic compounds contributed to low fungal loads (Table 2.2.3). The positive correlations ($p<0.01$, $p<0.01$ and $p<0.05$) between GE and TP, apigeninidin and CT contents, respectively (Table 2.2.6) indicate that phenolic compounds contributed to the inhibition of the fungi. The highly negative correlation ($p<0.001$) between GE and TFC indicates strongly that fungi on the surface of the grain reduced its germination potential.

Table 2.2.5 shows that, similar to the trends of GEs, the water extract and peptone extract DPs and α- and ß-amylase activities of malts from the low-phenol finger millet types were much lower than the DPs and α- and ß-amylase activities of malts from the high-phenol finger millet types, which was obviously related to poor germination of the low-phenol finger millet types. The DPs of the 12 finger millet types of this study (peptone extract DP: 19.0-73.7 SDU/g) (Table 2.2.5) are similar to those reported for 59 finger millet types by Gomez (1994) (17.2-77.8 SDU/g). The DPs, α- and ß-amylase activities of malts from the high-phenol finger millet types varied randomly across finger millet types, indicating a varietal/cultivar effect, as reported in sorghum (Beta et al 1995), finger millet (Shukla et al 1986, Gomez 1994) and barley (Arends et al 1995). As stated earlier, extraction with peptone prevents inactivation of amylase enzymes by tannins in the high-tannin grain and hence the difference between the peptone extracts DP and the water extract DP indicates the amount of amylase inhibition by the tannins. The differences between the water extract DP and the peptone extract DP of the high-tannin finger millet malt types was large (Table 2.2.5), indicating that tannins inhibit amylase activity as in sorghum (Daiber 1975a). However, the water extract DPs of malts from the high-tannin finger millet types

types were much higher than those of malts from the non-tannin finger millet types, which indicates that malts from the high-tannin finger millet types are of better quality than malts from non-tannin finger millet types.

Table 2.2.6 shows the DP of the finger millet malt was positively correlated with TP ($p<0.05$) and highly positively correlated ($p<0.001$) with apigeninidin and luteolinidin. Alpha-amylase activity was positively correlated with TP, CT, luteolinidin and flavan-4-ol contents ($p<0.001$, $p<0.01$, $p<0.05$ and $p<0.01$, respectively) highly negatively correlated with the TFC ($p<0.001$). These correlations indicate that finger millet phenolics have a positive influence on malt quality, seemingly by contributing to attenuation of the fungal load of the germinating grain. The absence of a significant correlation between α- and ß-amylase activities suggests that the two enzymes play different physiological roles in the germinating grain, as has been suggested in the literature (Ziegler 1999). Other workers have also reported a lack of correlation between α-in sorghum (Beta et al 1995, Dicko et al 2006). It is noted that the exceptionally low GEs of the low-phenol, creamy white grain types (Table 2.2.5) tend to distort the correlations between phenolics and malt quality in Table 2.2.6

It has been reported (Shukla et al 1986) that brown (presumably tannin) finger millet grain varieties/cultivars have better malting quality than the white ones. In addition, according to Murty and Kumar (1995), in rural home-based malting brown/red varieties of finger millet are preferred to the white ones. Since pigmented finger millet grain types have been shown to contain higher levels of phenolic compounds than the light types (Ramachandra et al 1977, chapter 2.1), their superior malting properties may be partly due to the contribution of phenolic compounds (including the tannins) to inhibition of microbial proliferation during malting as is indicated by the results of this study.

Table 2.2.5 shows that the DPs of malts from the high-phenol finger millet types (peptone extract DP: 53.5-73.7 SDU/g) were higher than those of sorghum malt standards (peptone extract DP: 43.0 and 51.5 SDU/g), but lower than those of control barley malts (Megazyme Ceralpha and Betamyl standards, respectively). A comparative study of the malting characteristics of barley, sorghum and finger millet by Nout and Davies (1982), similarly, showed that barley malt had a

much higher DP (peptone extract DP: 439°WK/100 g) than sorghum (103°WK/100 g) and finger millet (87°WK/100 g) malts, but the results were different from those of this study in that sorghum malt had a somewhat higher DP than the finger millet malt.

Table 2.2.5 shows that α-amylase activities of malts from the high-phenol finger millet types were generally higher than the α-amylase activities of the sorghum and barley malt standards. Alpha-amylase is an endo-hydrolase that attacks 1,4-glycosidic bonds of the α-1,4-polyglucan starch chain internally releasing dextrins and some glucose. The enzyme liquefies starch (Briggs 1998). Thus, the starch-liquefying power of malts from the high-phenol finger millet grain types was higher than that of sorghum and barley malt standards as they (finger millet malts) had relatively higher α-amylase activities. The results of this study are different from those reported by Nout and Davies (1982) who showed that, after 6 days of germination, the peptone extract α-amylase activities of finger millet, sorghum and barley were similar (77, 83 and 77°WK/100 g, respectively). However, Malleshi and Desikachar (1986b), similar to this study, reported higher α-amylase activity in finger millet than in sorghum, 200 α-amylase activity units compared to 170 α-amylase activity units, after 96 h of germination.

Table 2.2.5 shows that the ß-amylase activities of malts from the high-phenol finger millet types were much higher than the ß-amylase activities of the sorghum malt standards, but were much lower than that of barley malt. Beta-amylase is an exo-hydrolase, which releases maltose from the non-reducing end of the α-1,4-polyglucan starch chain until the first α-1,6-branching point is encountered. The enzyme saccharifies starch (Briggs 1998). Hence, the saccharifying power of malts from the high-phenol finger millet grain types was much higher than of sorghum malt standards and much lower than that of the barley malt standard, as the ß-amylase activities of finger millet malts were much higher than those of the sorghum malt standards and much lower than that of the barley malt standard. Nout and Davies (1982) found that, different from the results of this study, the ß-amylase activities of sorghum and finger millet malts were similar in magnitude and, similar to the results of this study, they were much lower than that of barley malt. It has been well described that sorghum malt produces much lower amounts of ß-amylase than barley malt (Novellie 1960, Taylor and Robbins 1993, Dufour et al 1992, Beta et al 1995, Dicko et al 2006).

Table 2.2.5 shows that the ß- to α-amylase ratios of finger millet malts (0.4: 1 to 2.1: 1) were higher than that of sorghum malt (0.2: 1), but were lower than that of barley malt standards (4.0: 1). These ratios clearly indicate that the ß-amylase of malts the high-phenol finger millet types contributed a much larger proportion to DP than that of sorghum malt standards, but the ß-amylase of the barley malt standard contributed a much larger proportion to DP than those of malts of the high-phenol finger millet types and sorghum malt standards. In the report of Nout and Davies (1982), after 6 days of germination, the ß- to α-amylase ratios of finger millet, sorghum and barley were 0.1: 1, 0.2: 1 and 4.7: 1, respectively. Novellie (1960) and Beta et al (1995) reported that the ß- to α-amylase ratio of sorghum varied from 0.22: 1 to 0.64: 1 and 0.12: 1 to 0.25: 1, respectively, while the ß- to α-amylase ratio of 11 Australian barley cultivars studied by Arends et al (1995) ranged from 5.2: 1 to 13.4: 1. Barley, wheat and rye belong to the Triticeae tribe while sorghum, finger millet and other cereals are non-Triticeae. The biochemistry of ß-amylase is different in these two groups of cereals. Triticeae cereals produce two types of ß-amylase: the endosperm ß-amylase and the 'ubiquitous' tissue ß-amylase, while the non-Triticeae group of cereals produce only the 'ubiquitous' tissue ß-amylase (Ziegler 1999). This difference may account for the fact that barley has a much higher ß-amylase activity than sorghum and finger millet.

The malts from the high-phenol finger millet types had somewhat higher DPs and much higher ß- and α-amylase activities than sorghum malt standards (Table 2.2.5), probably because they were made from grain types that had a relatively lower tannin content (not detected to 1.77 g catechin equivalents/100 g [Table 2.2.1]) than that often reported in sorghum (e.g. 0.00 to 6.29 g catechin equivalents/100 g [Beta et al 1999]). It is noted that because extraction for the measurement of α- and ß-amylase activities was done in the absence of peptone, the values for the activities of these enzymes for the tannin finger millet malts and the tannin sorghum malt standard could be an underestimate as the tannins might have reduced enzyme activity by their inhibition effect as stated earlier.

Table 2.2.5. Germinative energy (GE) and malt quality of finger millet grain types[1]

		Malt quality				
		Diastatic power (SDU/g, db)		Amylase activities (Units[c]/g, db)[d]		
Finger millet type[a]	**GE (%)**	**Water extract**	**Peptone extract**	**α- amylase**	**ß- amylase**	**ß:α[e]**
G35	70.3 b ± 2.1	26.8 a ± 1.3	24.3 a ± 1.6	114.5 b ± 0.7	80.8 a ± 4.6	0.7: 1
ICFM95001 GMSI	48.3 a ± 12.1	21.6 a ± 1.3	20.8 a ± 0.7	91.0 a ± 7.7	96.5 a ± 2.8	1.2: 1
95G198W	50.0 a ± 2.6	18.9 a ± 0.2	19.0 a ± 1.5	113.4 b ± 4.0	108.6 a ± 6.5	0.9: 1
FNL 0069	85.0 c ± 4.6	51.3 d ± 3.7	54.3 c ± 2.9	380.2 e ± 8.8	543.2e ±38.2	1.4: 1
P283	88.3 cd ± 2.1	67.7 e ± 3.7	71.2 e ± 0.7	296.4 c ± 4.4	608.2 f ± 7.4	2.1: 1
Gulu early	91.3 cdef±1.2	68.7 e ± 5.1	73.7 e ± 3.2	516.4 g ± 7.3	561.9 e ± 7.4	1.1: 1
FNL 0072	100.0 g ± 0.0	50.4 cd ± 5.8	69.0 de ± 3.2	607.8 i ±11.7	318.5 c ± 3.7	0.5: 1
Zim Mnnursery	95.0 defg ±7.0	46.3bcd ± 3.4	56.0 c ± 4.2	608.1 i ± 8.8	231.2 b ± 5.6	0.4: 1
FMV1	94.7defg ±1.5	41.3 b ± 4.3	53.5 c ± 3.9	539.3 h ±10.2	238.1 b ± 3.7	0.4: 1
FNL 0051	91.0 cdef ±5.0	60.7 e ± 0.3	71.6 e ± 2.0	493.3 f ± 7.4	406.2 d ± 4.7	0.8: 1
Nanjala Brown	98.0 fg ± 2.6	48.1bcd ± 5.6	64.7 d ± 2.4	518.2gh ±10.3	294.3 c ± 4.7	0.6: 1
Okhale-1	90.0 cde ± 4.4	49.4bcd ± 4.5	57.0 c ± 3.3	592.0 i ± 8.8	315.0 c ±13.0	0.5: 1
Sorghum malt standards:						
PAN 8564, non-tannin	96.7 efg ± 1.2	42.0 bc ± 1.4	43.0 b ± 2.8	400.1e ± 14.3	77.6 a ± 8.3	0.2: 1
PAN 8225, high-tannin	97.0 efg ± 1.7	41.2 b ± 3.4	51.5 c ± 0.5	483.6 f ± 23.4	91.4 a ± 8.4	0.2: 1
Control barley malts[b]:						
α – amylase assay control malt	ND	165.4 g ± 5.8	172.4 g ± 7.8	332.5 d ± 6.4	ND	
ß- amylase assay control malt	ND	150.1 f ± 3.4	154.4 f ± 0.0	ND	1322.8g ±54.0	4.0: 1

[1]Total phenolics and amount of phenolic type are shown in Table 2.2.1.
[a]Listed in order of increasing grain condensed tannin content. [b]Megazyme Ceralpha and Betamyl standards, respectively.
GE, germinative energy (mean of three triplicate analyses ± standard deviation [SD]); SDU, sorghum diastatic units (mean of duplicate analyses ± SD); [c]Ceralpha units for α- amylase and Betamyl units for ß- amylase. [d]Mean of duplicate analyses ± SD. [e]ß- to α- amylase ratio. Values within the same column with different letters are significantly different at $p<0.05$. ND, not determined.

Table 2.2.6. Pearson's correlation coefficients between finger millet grain total phenolics and amount of phenolic type and germinative energy and malt quality

	TP	CT	Apig.	Lute.	Flvn.	GE	maltTFC	WEDP	PEDP	α-amylase	ß-amylase
TP											
CT	0.927***										
Apig.	0.287	0.307									
Lute.	0.277	0.251	0.847***								
Flvn.	0.611**	0.723***	0.513*	0.427*							
GE	0.755**	0.631*	0.730**	0.740**	0.772**						
maltTFC	-0.946***	-0.656*	-0.620*	-0.640*	-0.704*	-0.946***					
WEDP	0.593*	0.356	0.887***	0.863***	0.557	0.776**	-0714**				
PEDP	0.695*	0.533	0.856***	0.846***	0.689*	0.893***	-0.798**	0.953***			
α- amylase	0.868***	0.748**	0.576	0.581*	0.736**	0.905***	-0.823***	0.647*	0.804**		
ß- amylase	0.398	0.103	0.897***	0.866***	0.440	0.561	-0.521	0.824**	0.749**	0.378	

TP, Total phenolics; CT, Condensed tannins; Apig., apigeninidin; Lute., luteolinidin; Flvn., flavan-4-ols; GE, germinative energy; maltTFC, total fungal count of malt grain surface; WEDP, water extract diastatic power PEDP, peptone extract diastatic power.
*Significant at p<0.05; **significant at p<0.01; ***significant at p<0.001.

Table 2.2.7 shows that the low-phenol finger millet grain types generally had higher fibre, protein and amino acid contents than the high-phenol types, whilst fat and mineral contents were similar across grain types. Unmalted finger millet had significantly higher mineral contents than finger millet malt, whereas the malt had generally significantly higher protein, fat contents and amino acid than the unmalted grain ($p<0.05$), irrespective of the phenolic content of the grain. The fibre content of the low-phenol FM types decreased with malting, whilst that of the high-phenol types increased.

The nutrient composition of these unmalted finger millet grain types is similar to that reported in the literature: neutral detergent fibre/insoluble fibre, 15.7% and 19.7% (Chethan and Malleshi 2007 and Shobana and Malleshi 2007, respectively); protein, 4.9-11.3%; fat, 0.9-7.7% and ash, 2.0-5.0% (McDonough et al 2000). The results of the relationship between finger millet grain phenolic content and protein content of this study is in agreement with that reported previously. As reviewed earlier (chapter 1), white finger millet grain varieties have been found to have lower phenolic content (Ramachandra et al 1977) and higher protein content (Virupaksha et al 1975) than brown varieties. The results of this study indicate that this relationship is similar to that of finger millet grain phenolic content and fibre content. Finger millet grain phenolics seem not to influence its fat and mineral contents.

Several workers have reported an increase in mineral content during malting of cereal grains (reviewed by Lorenz 1980, Chavan and Kadam 1989). In the current study, the mineral content of the malt was, however, lower than that of the unmalted grain (Table 2.2.7) and that was probably due to washing off contaminating dirt. Bhise et al (1988) similarly reported a decrease in ash content from 1.7 to 1% when sorghum was malted for 72 h. Dry matter loss, particularly carbohydrates through respiration and leaching, probably caused the apparent increase in fibre and protein contents on malting (Lorenz 1980, Chavan and Kadam 1989). Several workers have also reported an increase in fibre and protein contents during malting of various cereal grains (reviewed by Lorenz 1980, Chavan and Kadam 1989). Mbithi-Mwikya et al (2000) reported a 29.5% overall increase in protein content when finger millet was malted. Malleshi and Klopfenstein (1998) reported that, on malting, the dietary fibre of sorghum, pearl millet and finger millet increased from 7.8 to 8.6%, 11.0 to 12.0% and 15.2 to 21.7%, respectively. Finger

millet grain phenolics seem to influence changes in its fibre content during malting, whilst changes in protein, fat and minerals during malting seem not to be influenced by the phenolics. High phenolic levels are related to an increase in fibre content during malting (Table 2.2.7). The increase in fat content during malting of the finger millet grain types may be attributed to the synthesis of lipids related to metabolism of the starch (Chavan and Kadam (1989). An increase in fat content as a result of malting has been similarly reported in barley, wheat and oats, but contradictory results have been reported by other workers who found that fat content decreased when sorghum and millets were malted (reviewed by Chavan and Kadam 1989). Malleshi and Klopfenstein (1998) showed that there was a slight increase in fat content when finger millet was malted (1.5 to 1.7%), whilst those of sorghum and pearl millet decreased on malting (2.3 to 2.0% and 5.3 to 4.2%, respectively). An increase in fat content on malting finger millet may be undesirable as that may affect negatively the storage quality of the malt due to rancidity.

Table 2.2.8 shows that the low-phenol finger millet grain types had higher amino acid content than the high-phenol types. There was a general increase in amino acid content with malting of millet grain. The amino acid composition of the unmalted finger millet grain types is in line with that presented in the literature (McDonough et al 2000, chapter 1). The low-phenol finger millet grain types had higher amino acid content than the high-phenol types because they had higher protein content (Table 2.2.7). Several authors have reported a change (increase or decrease) in amino acid content during the malting of cereal grains. There are differences in results across cereal grains and grain types and across amino acids, but an increase in amino acid content, particularly the essential amino acids lysine and tryptophan, has been often reported (reviewed by Chavan and Kadam 1989). It has been suggested that the increase in lysine and tryptophan content during malting is due to transamination (Chavan and Kadam 1989). Malleshi and Klopfenstein (1998) reported a noticeable increase in lysine content of finger millet and a slight increase in pearl millet and no appreciable change in sorghum on malting. Mbithi-Mwikya et al (2000) reported that the essential amino acids methionine, cysteine and lysine increased, arginine decreased and aspartic acid decreased on malting finger millet. A change in amino acid content during malting seems not to be influenced by the finger millet phenolics.

Table 2.2.8 shows that the amino acid scores of the finger millet grain types and their malts, when scored against the pattern of amino acid requirements for an infant (≤12 months) (WHO 1985), were generally less than 1. Lysine had much lower scores (0.3 to 0.5) than other essential amino acids. These findings indicate that finger millet grain and malt would be an inadequate source of essential amino acids, particularly lysine, for infants. The results of this study are in agreement with what is reported in the literature (reviewed in chapter 1), that is, as in other cereal grains (Kent and Evers 1994), lysine is generally limiting in finger millet grain. If finger millet malt were to be used to make, for example weaning foods, other food materials e.g. legumes, would have to be included to ensure an adequate supply of essential amino acids.

Table 2.2.7. Effect of malting on the nutrient content of finger millet grain types (g/100 g, db)[1]

	NDF[a]		Protein[b]		Fat		Ash	
FM type	**Unmalted**	**Malted**	**Unmalted**	**Malted**	**Unmalted**	**Malted**	**Unmalted**	**Malted**
G35	20.5 b ± 0.1	19.1 a ± 0.0	14.2 b ± 0.0	13.2 a ± 0.0	1.9 a ± 0.3	3.4 b ± 0.0	3.9 b ± 0.1	2.9 a ± 0.2
95G198W	22.2 b ± 1.2	18.4 a ± 0.2	14.2 a ± 0.3	16.1 b ± 0.1	2.3 a ± 0.1	3.0 b ± 0.1	4.1 b ± 0.2	3.1 a ± 0.1
FNL 0069	15.0 a ± 0.2	21.8 b ± 0.6	10.4 a ± 0.2	13.7 b ± 0.1	1.4 a ± 0.0	2.4 b ± 0.2	3.3 b ± 0.1	2.8 a ± 0.2
P283	19.0 a ± 0.1	24.9 b ± 0.5	14.0 a ± 0.1	13.8 a ± 0.1	1.5 a ± 0.1	2.4 b ± 0.0	3.5 b ± 0.1	2.8 a ± 0.1
Gulu early	17.3 a ± 0.2	21.2 b ± 0.2	14.0 a ± 0.2	14.3 a ± 0.3	2.2 a ± 0.2	2.2 a ± 0.2	3.9 b ± 0.2	2.6 a ± 0.2
FMV1	21.1 b± 2.4	20.0 a ± 0.2	9.3 a ± 0.2	13.5 a ± 0.1	2.1 a ± 0.0	2.2 a ± 0.0	3.3 b ± 0.1	2.9 a ± 0.2
FNL 0051	18.9 a ± 0.0	21.1 b ± 0.0	10.3 a ± 0.0	15.1 b ± 0.1	1.9 a ± 0.3	2.5 b ± 0.3	4.2 b ± 0.1	2.9 a ± 0.1
Okhale-1	17.2 a ± 0.3	21.9 b ± 0.7	12.6 a ± 0.3	12.8 a ± 0.0	1.1 a ± 0.0	2.6 b ± 0.2	2.9 a ± 0.1	2.8 a ± 0.1
Group mean ± SE	18.9 a ± 0.6	21.0 b ± 0.5	12.4 a ± 0.5	14.1 b ± 0.3	2.0 a ± 0.2	2.4 b ± 0.1	3.6 b ± 0.1	2.9 a ± 0.0

[1]Mean of two replicate analyses ± standard deviation.
[a]Amylase-treated neutral detergent fibre.
[b]Nx6.5.
For each nutrient of each finger millet type, values within the same row with different letters are significantly different at $p<0.05$ according to the t-test for independent samples.

Table 2.2.8. Effect of malting on the amino acid composition of finger millet grain types

Amino acids	G35[a]		FNL 0069		Gulu early		FMV1		
	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	
Essential amino acids									**Pattern of amino acid requirements[e]**
Histidine	0.35[b]	0.21	0.29	0.24	0.24	0.24	0.18	0.23	
	24.65[c]; 0.9[d]	15.90; 0.6	27.88; 1.1	17.52; 0.7	17.10; 0.7	16.78; 0.6	19.35; 0.7	17.00; 0.7	26
Threonine	0.50	0.39	0.42	0.63	0.37	0.41	0.30	0.44	
	35.21; 0.8	29.50; 0.7	40.38; 0.9	45.99; 1.1	26.40; 0.6	28.67; 0.7	32.26; 0.8	32.60; 0.8	43
Valine	0.98	0.66	0.80	0.73	0.73	0.70	0.52	0.79	
	69.01; 1.3	50.00; 0.9	76.92; 1.4	53.28; 1.0	52.10; 0.9	48.95; 0.9	55.91; 1.0	58.50; 1.1	55
Methionine	0.37	0.26	0.43	0.30	0.26	0.26	0.26	0.34	
	26.06; 0.6	19.70; 0.5	41.35; 1.0	21.90; 0.5	18.60; 0.4	18.18; 0.4	27.96; 0.7	25.20; 0.6	42[f]
Isoleucine	0.69	0.44	0.55	0.50	0.51	0.46	0.36	0.52	
	48.59; 1.1	33.30; 0.7	52.88; 1.2	36.50; 0.8	36.40; 0.8	32.17; 0.7	38.71; 0.8	38.50; 0.8	46
Leucine	1.65	0.91	1.37	0.89	1.22	0.89	0.87	1.23	
	116.20; 1.2	68.90; 0.7	131.70; 1.4	64.96; 0.7	87.10; 0.9	62.24; 0.7	93.55; 1.0	91.10; 1.0	93
Phenylalanine	0.82	0.50	0.75	0.51	0.61	0.52	0.46	0.65	
	57.75; 0.8	37.90; 0.5	72.12; 1.0	37.23; 0.5	43.60; 0.6	36.36; 0.5	49.46; 0.7	48.10; 0.7	72
Lysine	0.37	0.33	0.34	0.44	0.24	0.49	0.21	0.42	
	26.06; 0.4	25.00; 0.4	32.69; 0.5	32.12; 0.5	17.10; 0.3	34.27; 0.5	22.58; 0.3	31.10; 0.5	66[g]
Non-essential amino acids									
Aspartic acid[h]	0.56	0.64	0.46	0.75	0.41	0.68	0.22	0.66	
	39.44	48.50	44.23	54.74	29.30	47.55	23.66	48.90	
Glutamic acid[i]	3.67	1.84	2.61	1.62	2.77	1.55	1.53	1.83	
	258.50	139.00	251.00	118.20	198.00	108.40	164.50	136.00	
Serine	0.70	0.44	0.61	0.45	0.52	0.44	0.39	0.47	
	49.30	33.30	58.65	32.85	37.10	30.77	41.94	34.80	
Glycine	0.47	0.37	0.44	0.43	0.52	0.44	0.39	0.47	
	33.10	28.00	42.31	31.39	37.10	30.77	41.94	34.80	
Arginine	0.50	0.36	0.50	0.41	0.35	0.46	0.29	0.42	
	35.21	27.30	48.08	29.93	25.00	32.17	31.18	31.10	
Alanine	0.84	0.62	0.75	0.63	0.61	0.68	0.48	0.68	
	59.15	47.00	72.12	45.99	43.60	47.55	51.61	50.40	
Proline	0.86	0.56	0.84	0.62	0.67	0.59	0.55	0.61	
	60.56	42.40	80.77	45.26	47.90	41.26	59.14	45.20	
Tyrosine	0.59	0.35	0.51	0.38	0.41	0.45	0.33	0.53	
	41.55	26.50	49.04	27.74	29.30	31.47	35.48	39.30	

[a]Finger millet grain type (listed in order of increasing tannin content in the unmalted grain, from left to right).
[b]g/100 g sample, dry basis [db]. [c]mg/g crude protein.
[d]Amino acid score = mg amino acid in 1 g protein of test sample÷ mg amino acid in requirement pattern (WHO, 1985). [e]Pattern of amino acid requirements (mg/g protein; corrected for protein digestibility) for an infant (≤12 months); [f]Methionine + cystine; [g]Phenylalanine + tyrosine (WHO, 1985); Aspartic acid[h] = aspartic acid + asparagine; Glutamic acid[i] = glutamic acid + glutamine.

2.2.5. Conclusions

The several negative correlations between fungal species infection levels and phenolic content and type of the unmalted finger millet grain indicate strongly that the phenolics contribute to its resistance to infection by fungi. High-phenol finger millet types have much higher malt quality than low-phenol types. The negative correlation between the grain fungal load and phenolic content and type, and the positive correlation between GE, DP and α-amylase activity and phenolic content and type indicate that finger millet grain phenolics impact positively on its malt quality by contributing to its resistance to infection by fungi. Malting finger millet grain has the effect of decreasing its mineral content and increasing its protein, fibre and fat contents. The results indicate that finger millet grain and malt are deficient in essential amino acids, particularly lysine.

2.2.6. References

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2.3. Effect of partial substitution with finger millet on the nutritional and functional quality of cookies, with particular reference to phenolics

2.3.1. Abstract

The aim of this investigation was to determine the effect of partial substitution with finger millet (FM) on cookie quality and antioxidant activity (AA). Wheat cake flour was substituted with 15, 35 and 55% (w/w) of either a non-tannin or a high-tannin FM flour to make the composite cookies. The nutrient content, physical quality and sensory acceptability of the cookies were analysed. Total phenolics (TP), condensed tannin (CT) content and AA of the cookies were determined. The composite cookies had higher mineral and fibre contents than wheat cake flour (control) cookies, but had lower protein and fat contents. They were deficient in essential amino acids, particularly lysine. Increasing the amount of FM generally affected cookie quality negatively and the effect was worse with the high-tannin FM than with the non-tannin type. The FM phenolics imparted a dark colour to the cookies, which decreased their acceptance by a consumer panel. However, cookies containing up to 35% of either FM type were as acceptable as control cookies. Cookies made with the high-tannin FM had appreciable TP and CT contents and had antioxidant activities that were similar to or higher than the antioxidant activities of several plant foods on the market. The results indicate that FM phenolics, particularly tannins, affect cookie quality negatively, but sensorially acceptable and potentially health-promoting (due to high AA) cookies can be made by substituting up to approximately 35% wheat with a high-tannin FM.

2.3.2. Introduction

Finger millet [*Eleucine coracana* (L.) Gaertn.] is a cereal staple food in the semi-arid tropical and sub-tropical regions of Africa and Asia (ICRISAT/FAO 1996). It is more nutritious than most of the other cereals (Obilana and Manyasa 2002). Finger millet grain contains various phenolic compounds, including condensed tannins (chapter 2.1, Dykes and Rooney 2006). Phenolic

compounds have been found to contribute to the antioxidant activity of the grain (Sripriya et al 1996, Subba Rao and Muralikrishna 2002, chapter 2.1). Due to their antioxidant activity, phenolic compounds may attenuate cellular oxidation and thereby limit the risk of degenerative conditions such as cancer and cardiovascular diseases (Scalbert et al 2005). Finger millet grain could be used to make phenolic antioxidant-rich cookies. A limitation, however, is that it does not form gluten, which is required for dough quality. On the other hand, wheat is the ideal grain for producing baked goods as it contains gluten proteins (Hoseney 1994). However, wheat grows well in cooler climates (Kent and Evers 1994), and as such, countries in hotter regions import part or all of the wheat, they require using scarce foreign exchange. In the semi-arid tropics, where communities are predominantly poor, composite wheat-finger millet cookies may have many advantages, including having a high nutritional value, saving foreign exchange and being health-promoting.

Badi and Hoseney (1976) reported that cookies in which soft wheat flour had been either wholly or partially substituted with sorghum or pearl millet were of inferior quality to control soft wheat cookies. The cookies containing either sorghum or pearl millet were dense and compact, had a poor top grain character, and were gritty and mealy. However, Morad et al (1984) reported an increase in the spread factor of sugar snap cookies with increasing amounts of sorghum. There is limited information on the quality of cookies containing finger millet. Although Selvaraj et al (2002) reported producing acceptable composite wheat-finger millet cookies, finger millet may, in the same manner as sorghum and pearl millet (Badi and Rooney 1976), impact negatively on cookie quality. In addition, phenolic compounds, particularly the tannins found in some varieties of finger millet (chapter 2.1, Dykes and Rooney 2006), may contribute to undesirable flavours, such as bitterness and astringency (Drewnowski and Gomez-Carneros 2000, Lesschaeve and Noble 2005). Furthermore, phenolic compounds are known to contribute to grain pigmentation (e.g. red, brown and black) in some varieties of sorghum and millets (Dykes and Rooney 2006). Pigmented finger millet grain may impart unusual colours to the composite cookies, which may be objectionable to consumers (Taylor et al 2006).

Awika et al (2003a) reported that baking sorghum bran into cookies and breads caused a reduction of the amounts of extractable tannins. Extrusion cooking the sorghum bran resulted in

a pronounced increase in the content of lower molecular weight tannins, whilst the content of tannin polymers decreased. These authors also reported that most of the antioxidant activities of the raw sorghums were retained when the grain was thermally processed into breads and cookies. Dlamini et al (2007) reported that conventional and extrusion cooking caused much higher reductions in phenolic content and antioxidant activity of tannin sorghum products than of non-tannin sorghum products. Towo et al (2003) reported a decrease in phenolic content when sorghum and finger millet grains were boiled. The objectives of this study were to determine the effect of finger millet type and finger millet substitution level on the quality of cookies especially with reference to phenolics and nutrient composition, and the effect of cookie making on the phenolic content and antioxidant activity of the cookies.

2.3.3. Materials and Methods

2.3.3.1. Wheat and finger millet flours

Two finger millet grain types were used: a creamy white, non-tannin type (ICFM 95001 GMSF) and a brown, high-tannin type (FMV6) (chapter 2.1, Table 2.1.1). The grain was milled to flour with an ultra centrifugal laboratory mill which was fitted with a 0.5 mm screen (ZM1, Retsch, Haan, Germany). Cake flour of the brand "Sasko" and other ingredients were purchased locally.

2.3.3.2. Rheological analysis

A Brabender farinograph (Brabender® OHG, Duisburg, Germany) was used to analyse the rheological properties of the composite wheat-finger millet doughs according to ICC Standard No. 115/1 (International Association for Cereal Science and Technology 1972).

2.3.3.3. Baking

The cookies were made as described by Anon. (1991). The formulation for standard (control) cookies consisted of 250 g margarine, 200 g granulated white sugar, 480 g cake flour, 8.5 g baking powder, 2 beaten eggs (60 mL), 5 mL vanilla essence, 6 g salt and 25 mL water. Composite doughs were made by substituting cake flour with 15, 35 and 55% (w/w) flour of either the non-tannin or the high-tannin finger millet. A portion of each dough type was packaged in a polythene bag and stored in the freezer until analysis. The doughs were put in the same oven and baked together at 200°C for 8 min.

2.3.3.4. Nutritional analyses

Moisture, ash, fat, protein and fibre

The AOAC Official Methods 925.10, 923.03, 920.85, 968.06 and 2002.04 (Association of Official Analytical Chemists International 2003) were used to determine the moisture, ash, fat, protein and neutral detergent fibre, respectively, of the cake and finger millet flours, and cake flour and composite wheat-finger millet cookies.

Amino acid analyses

The amino acid contents of cake and finger millet flours, and the cookies were determined by a reverse-phase HPLC Pico.Tag Method for rapid analysis of amino acids by pre-column derivatization after acid hydrolysis (Bidlingmeyer et al 1984).

2.3.3.5. Spread and thickness

The spread and thickness of the cookies were determined according to AACC Method 10-50D (American Association of Cereal Chemists International 2000).

2.3.3.6. Texture

Cookie texture was measured with a texture analyser (TA~XTPlus, Stable Micro Systems, Godalming, UK) using a method TA~XTPlus Application Study, Reference: BIS1/P2, Revised March 2000. The texture analyser was equipped with a 5 kg load cell and a 2 mm cylinder probe. The settings used were a test speed of 0.5 mm/s with a trigger force of 5 g to penetrate the sample over a distance of 2 mm on a heavy duty platform.

2.3.3.7. Colour

The Hunter L values (lightness) of the cookies were determined with a chromameter (CR-400, Konica Minolta, Sakai Osaka, Japan).

2.3.3.8. Sensory evaluation

Fifty nine panellists, who were consumers of cookies, were selected randomly from the University of KwaZulu-Natal (UKZN) students on the Pietermaritzburg campus. The consumer panel comprised 30 females and 29 males within the 19-24 years age range. Use of human subjects was approved by the UKZN Ethics Committee. Before evaluating the cookies, the panellists signed a consent form, which informed them of the nature of samples that they would analyse, and they agreed that participation was voluntary. Each panellist was served with one cookie of each type and a control cookie on separate white disposable plates, which were covered with a thin transparent polythene sheet. Each sample was labelled with a unique random 3-digit number and the order of sample presentation was determined by random permutations. The panellists were asked to rinse their palates with water before and after testing each sample to reduce any carry over effect. The panellists evaluated the samples for taste, aroma, crispness, texture and appearance and overall acceptability on a 9-point hedonic scale (1= dislike extremely; 9= like extremely). The test was done under white light.

2.3.3.9. Chemical analysis

Total phenolics

The Folin-Ciocalteu method of Singleton and Rossi (1965) was used to measure total phenolics of the cookie doughs and the cookies. Cookies were ground to a fine powder using a pestle and mortar. Exactly 4 g of cookie powder was extracted with 20 mL acidified methanol (1% [v/v] HCl in methanol) for 1 h at room temperature (approx. 25ºC), with vortex mixing at 5-min intervals. Exactly 4 g of cookie dough was dispersed in 20 mL of the acidified methanol in an extraction tube using a glass rod and then extracted in the same way as the cookie powder. The samples were centrifuged for 10 min at 1 200 x g using a temperature-controlled centrifuge set at 25ºC. Three replicate supernatants were obtained. Sample blanks were included, in which the sample was replaced by distilled water. Gallic acid was used as a standard.

Condensed tannins

The Vanillin-HCl method of Price et al (1978) with blank subtraction for extract colour was used to measure condensed tannin content. Extraction was as for determination of total phenolics except that absolute methanol was used in place of acidified methanol. Sample blanks in which the vanillin reagent was replaced by 4% (v/v) HCl in methanol were included. Catechin was used as a standard.

Antioxidant activity

Antioxidant activity of methanolic extracts from the cookie dough and the cookies was measured using the Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Awika et al (2003b). Methanolic extracts were prepared as for determination of total phenolics. Trolox was used as a standard.

2.3.3.10. Statistical analysis

Two way analysis of variance (ANOVA) was used to analyse for the effect of finger millet type and finger millet substitution level on texture, colour and sensory acceptability of the cookies.

Multiple comparisons of the means were made using the Tukey's Studentized Range (Honestly Significant Difference [HSD]) test. The Pearson's Correlation Coefficient (r) was used to analyse for linear relationships.

2.3.4. Results and Discussion

Table 2.3.1. Rheological properties of composite wheat-finger millet doughs

Flour	Subst [a] %	WA (%) (14% mb)	Dev. time (min)	Stability (min)	Consistency (FU)
Cake flour (control)	0	63.5	1.7	17.0	500
Non-tannin finger millet	15	65.6	7.5	11.5	540
	35	66.4	6.0	5.5	540
High-tannin finger millet	15	62.9	10.0	9.0	520
	35	61.1	8.5	3.0	520
	55	59.4	9.5	4.5	580

[a] % (w/w) substitution of wheat flour with finger millet flour; WA, water absorption; Dev., development; FU, farinograph units

Table 2.3.1 shows that as finger millet substitution level was increased, water absorption of flours containing the non-tannin finger millet increased, whilst that of flours containing the high-tannin finger millet decreased. The dough development times of the composite flours were much longer than that of the wheat cake flour (Table 2.3.1; Figure 2.3.1). Dough stability decreased as finger millet substitution level was increased. The composite doughs had higher consistency than the control dough. Fibre is concentrated in the outer layers (pericarp, testa and aleurone) of the finger millet grain and its main components are cellulose and pentosans (Serna-Saldivar and Rooney 1995).

Cake flour had lower fibre content than finger millet flour (Table 2.3.2). Therefore, as finger millet substitution level was increased, the fibre content of the flours increased. The water absorption of flours containing the non-tannin finger millet increased with an increase in finger millet substitution level, probably because of an increase in fibre content. Water-extractable pentosans (Biliaderis et al 1995) and cellulose (Pomeranz et al 1977), and bran (Sudha et al 2007)

have been shown to increase the water absorption of bread flour and cookie flour, respectively. Water absorption decreased as substitution with the high-tannin finger millet was increased, probably because tannins interacted with the wheat gluten proteins and thereby reduced their water-absorption capacity. As stated earlier, tannins are known to form complexes with proteins (Porter 1989, Emmambux and Taylor 2003). Sudha et al (1998), similarly reported that water absorption for vermicelli dough containing finger millet, milled wheat fractions such as whole wheat flour, wheat semolina, wheat flour increased (40-60%, 30-60% and 31-60%, respectively) with increase in finger millet (0-100%) in the composite. Morad et al (1984) also reported an increase in dough development time with increasing levels of sorghum in cookie doughs. Components of finger millet grain appear to be the cause of the much longer dough development times of the composite flours, 6.0 to 10.0 min, compared to 1.7 min of the control flour (Table 2.3.1). Cellulose has been found to cause a considerable increase in the dough development time of bread flour (Pomeranz et al 1977). An increase in fibre content (cellulose content) might have contributed to the longer dough development times of the composite flours. Dough stability decreased as finger millet substitution level was increased, probably because of dilution of gluten proteins, similar to the reports of Pomeranz et al (1977) and Morad et al (1984). Gluten proteins are essential for the formation of a stable visco-elastic gluten network (Goesaert et al 2005). An increase in fibre content with increasing levels of finger millet might also have contributed to a decrease in dough stability. Substituting wheat flour with different amounts of bran caused a decrease in the stability of cookie dough (Sudha et al 2007). Water-unextractable pentosans are thought to interfere with the formation of the gluten network by competing with gluten proteins for water and/or by interacting with the gluten proteins (Wang et al 2003). However, unlike in the current study, Morad et al (1984) reported that dough stability increased with increasing levels of high-tannin sorghum in bread dough. These authors suggested that interaction of tannins with gluten proteins caused the increase in dough stability. The composite doughs had higher consistency than the control dough probably due to an increase in fibre content. Water-extractable pentosans have been shown to increase the consistency of bread dough (Jelaca and Hlynca 1972).

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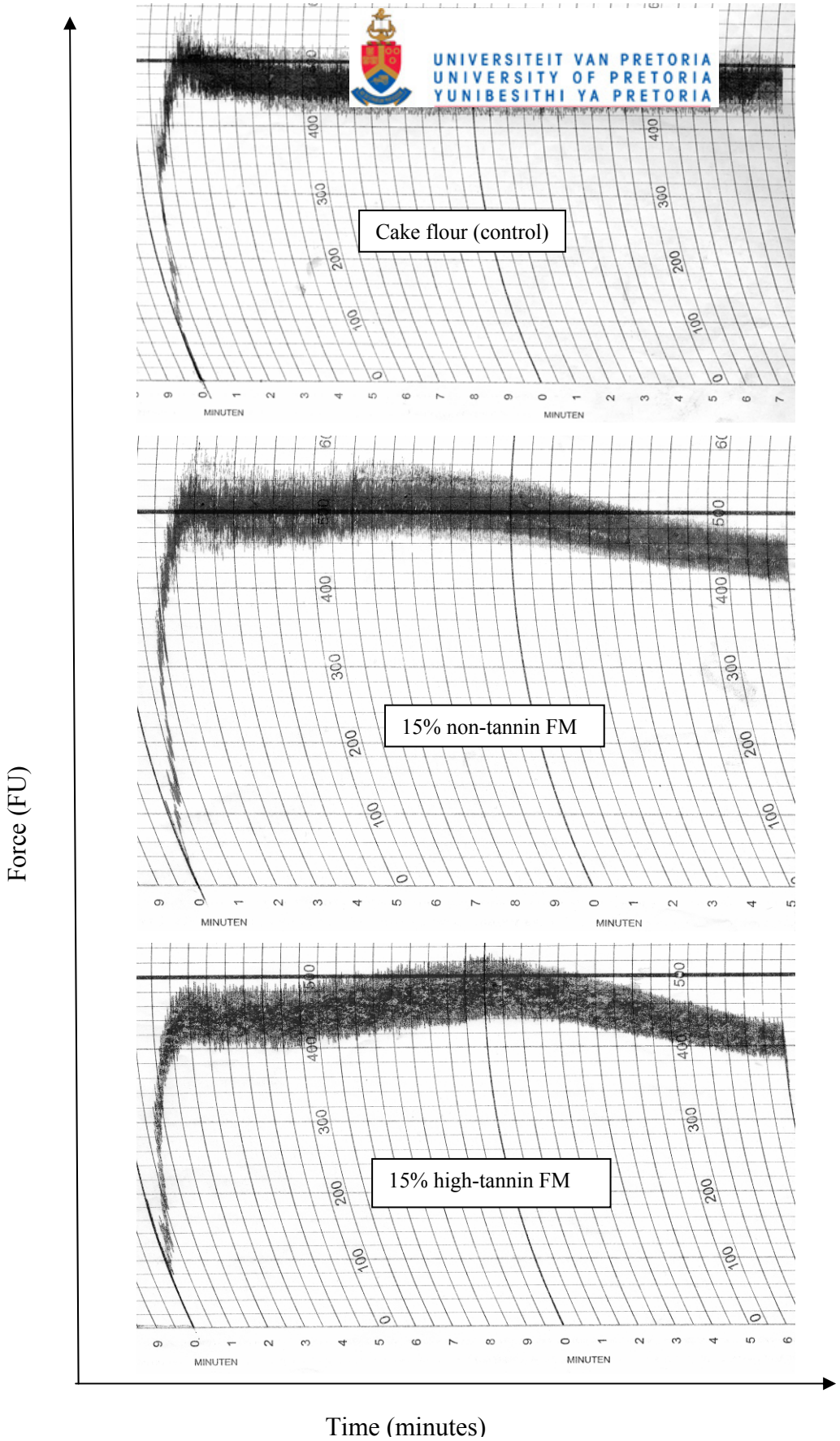

Figure 2.3.1. Effect of finger millet substitution level on the rheological properties of composite wheat-finger millet (FM) doughs. FU, farinograph units.
Non-tannin FM= ICFM 95001 GMSF; high-tannin FM= FMV6.

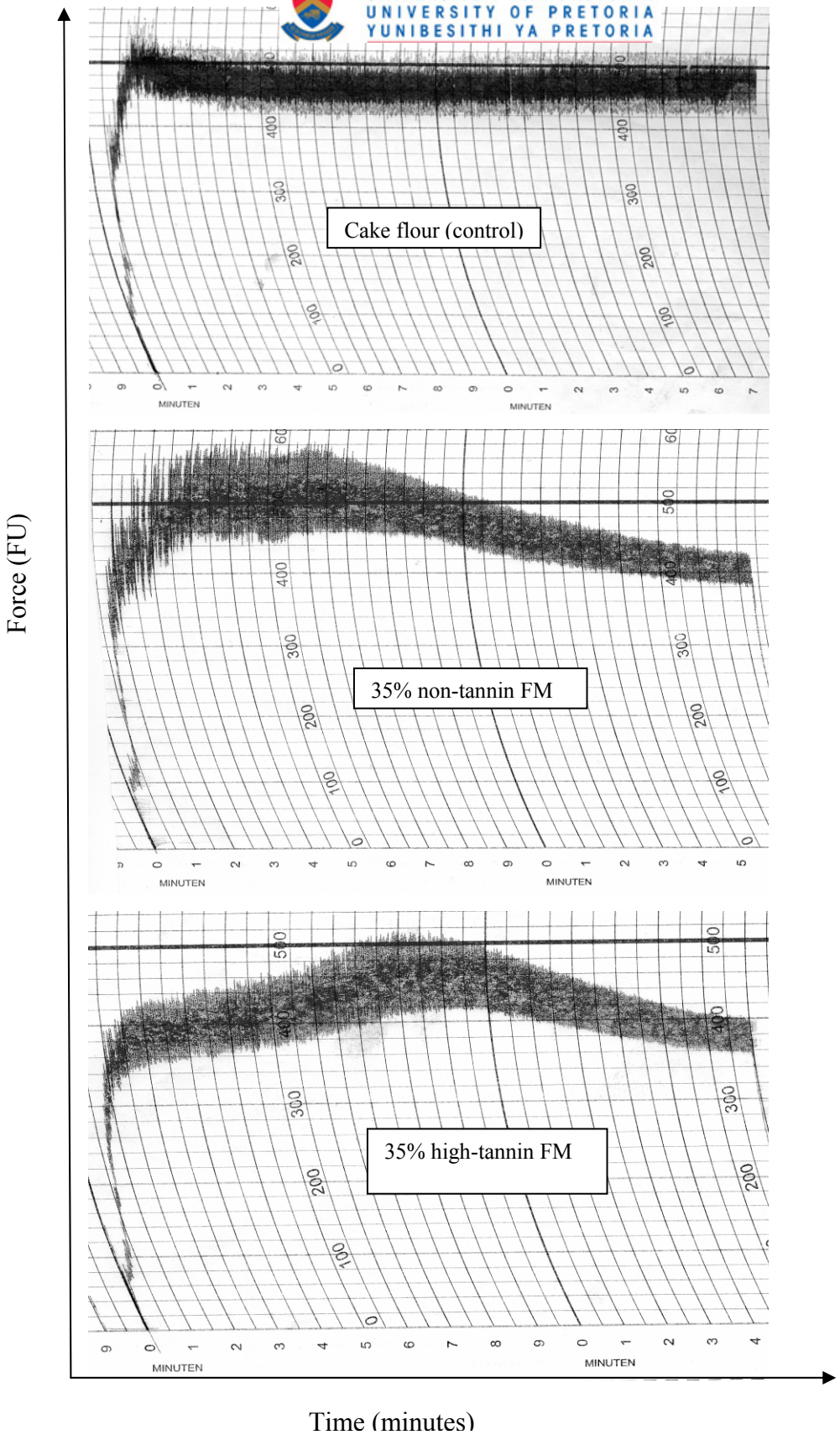

Figure 2.3.1. Continued.

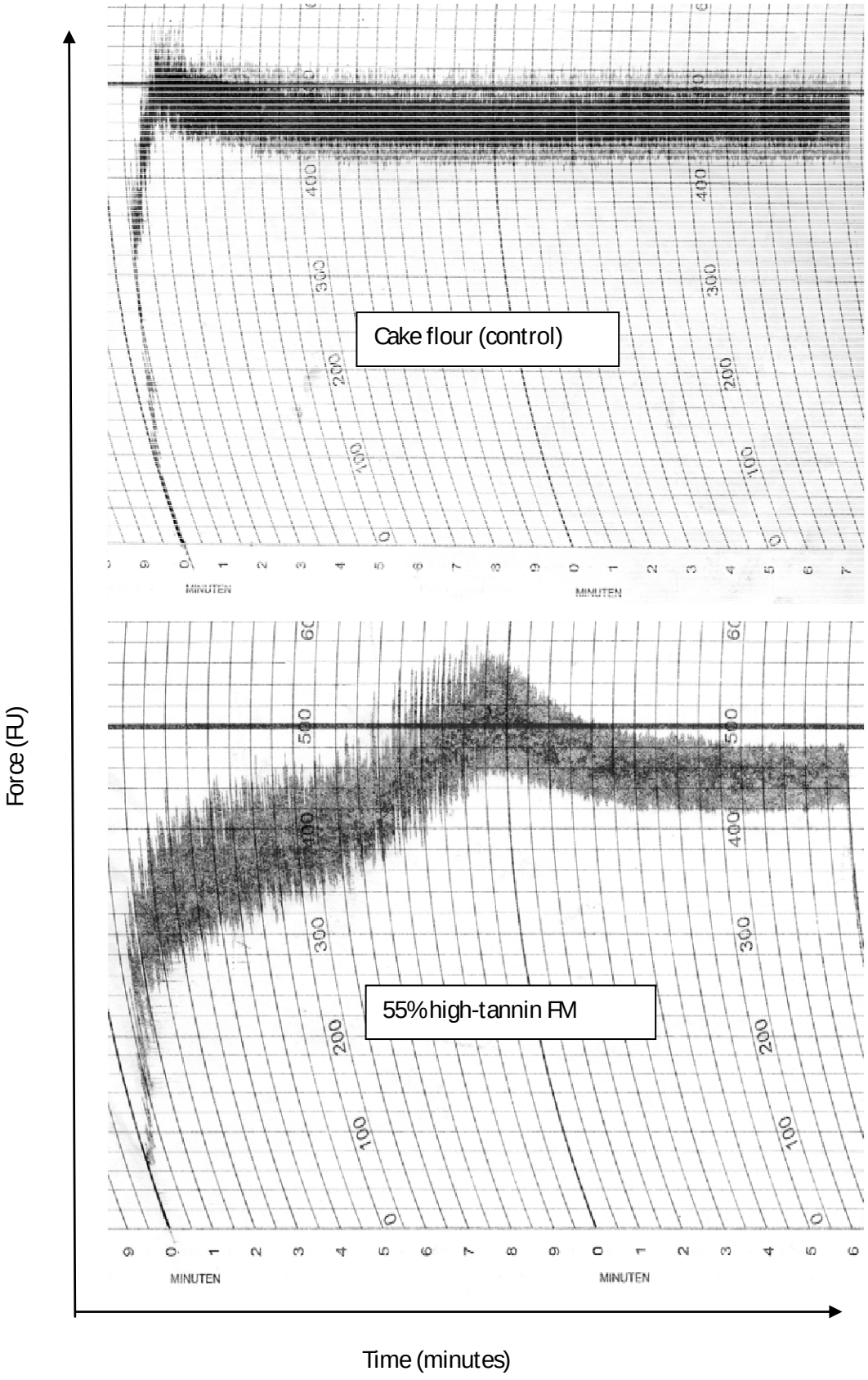

Figure 2.3.1. Further continued.

Table 2.3.2. Proximate composition (g/100 g) of cake and finger millet (FM) flours, and composite wheat-FM cookies

Sample type	Sample	Moisture [a]	Protein [a,b,c]	Fat [a,c]	Fibre [a,c,d]	Ash [a,c]
Flours	Cake flour	13.8 (0.2) f	12.8 (0.1) g	1.2 (0.0) a	1.7 (0.0) a	0.7 (0.1) a
	Non-tannin FM	14.8 (0.2) g	11.1 (0.0) f	1.1 (0.0) a	14.9 (0.1) h	4.3 (0.0) e
	High-tannin FM	15.0 (0.5) g	8.2 (0.0) e	1.1 (0.1) a	12.3 (0.2) g	2.9 (0.0) d
Composite	0% FM (control)	5.7 (0.0) a	8.1 (0.0) d	25.9 (0.2) f	1.4 (0.0) a	1.8 (0.0) b
Cookies	15% non-tannin FM	7.1 (0.0) b	7.5 (0.0) c	23.7 (0.2) cd	3.4 (0.0) b	2.2 (0.1) c
	35% non-tannin FM	7.3 (0.2) b	7.2 (0.0) b	24.2 (0.2) d	4.2 (0.0) d	2.7 (0.1) d
	55% non-tannin FM	8.5 (0.2) c	6.6 (0.0) a	22.5 (0.2) b	6.7 (0.2) f	2.9 (0.0) d
	15% high-tannin FM	8.8 (0.2) cd	7.5 (0.0) c	25.0 (0.5) e	3.8 (0.1) c	2.2 (0.1) c
	35% high-tannin FM	9.1 (0.2) d	7.2 (0.1) b	23.4 (0.1) c	4.9 (0.1) e	2.2 (0.1) c
	55% high-tannin FM	9.8 (0.0) e	6.6 (0.0) a	23.9 (0.6) cd	5.1 (0.2) e	2.8 (0.1) d

[a] Mean of at least two replicate analyses; standard deviation in parenthesis.
[b]Nx5.7 for wheat flour; Nx6.25 for finger millet flour; Nx5.975 for composite cookies.
[c]Values in g/100 g flour or cookies (db)
[d]Amylase-treated neutral detergent fibre.
Values within the same column with different letters are significantly different at $p<0.05$.

Table 2.3.3a. Amino acid composition of cake and finger millet (FM) flours, and composite wheat-FM cookies (g/100 g, db)

	Essential amino acids								Non-essential amino acids							
	His	Thr	Val	Met	Isoleu	Leu	Phe	Lys	Asp	Glu	Ser	Gly	Arg	Ala	Pro	Tyr
Flours																
Cake flour	0.26	0.32	0.53	0.21	0.47	0.98	0.67	0.26	0.33	4.13	0.56	0.44	0.46	0.36	1.47	0.40
non-tannin FM	0.19	0.30	0.55	0.24	0.39	0.94	0.50	0.24	0.38	1.88	0.41	0.30	0.32	0.49	0.51	0.34
high-tannin FM	0.15	0.25	0.43	0.22	0.29	0.72	0.39	0.12	0.22	1.33	0.32	0.22	0.25	0.41	0.47	0.26
Cookies																
0% FM	0.12	0.15	0.24	0.10	0.22	0.45	0.31	0.12	0.15	1.89	0.26	0.20	0.21	0.17	0.67	0.18
15% non-tannin FM	0.12	0.15	0.25	0.10	0.21	0.45	0.30	0.12	0.16	1.77	0.25	0.19	0.20	0.18	0.62	0.18
35% non-tannin FM	0.11	0.15	0.25	0.10	0.21	0.45	0.29	0.12	0.16	1.56	0.24	0.18	0.19	0.19	0.53	0.18
55% non-tannin FM	0.10	0.15	0.26	0.11	0.20	0.45	0.27	0.12	0.17	1.37	0.23	0.17	0.18	0.21	0.45	0.17
15% high-tannin FM	0.12	0.15	0.24	0.10	0.21	0.44	0.30	0.11	0.15	1.76	0.25	0.19	0.20	0.18	0.63	0.18
35% high-tannin FM	0.11	0.14	0.24	0.10	0.19	0.42	0.27	0.10	0.14	1.50	0.23	0.17	0.18	0.18	0.53	0.17
55% high-tannin FM	0.08	0.13	0.23	0.11	0.16	0.39	0.21	0.08	0.14	0.76	0.17	0.12	0.14	0.22	0.24	0.14

Table 2.3.3b. Comparison of essential amino acid concentration in composite wheat-finger millet (FM) cookies with the pattern of essential amino acid requirements

Cookie type	Protein [a]	Histidine	Threonine	Valine	Methionine	Isoleucine	Leucine	Phenylalanine	Lysine
Cake flour (control)	8.1	0.12[b]	0.15	0.24	0.10	0.22	0.45	0.31	0.12
		15[c]	19	30	12	27	56	38	15
15% non-tannin FM	7.5	0.12	0.15	0.25	0.10	0.21	0.45	0.30	0.12
		16	20	33	13	28	60	40	16
35% non-tannin FM	7.2	0.11	0.15	0.25	0.10	0.21	0.45	0.29	0.12
		15	21	35	14	29	63	40	17
55% non-tannin FM	6.6	0.10	0.15	0.26	0.11	0.20	0.45	0.27	0.12
		15	23	39	17	30	68	41	18
15% high-tannin FM	7.5	0.12	0.15	0.24	0.10	0.21	0.44	0.30	0.11
		16	20	32	13	28	59	40	15
35% high-tannin FM	7.2	0.11	0.14	0.24	0.10	0.19	0.42	0.27	0.10
		15	19	33	14	26	58	38	14
55% high-tannin FM	6.6	0.08	0.13	0.23	0.11	0.16	0.39	0.21	0.08
		12	20	35	17	24	59	32	12
Pattern of amino acid requirements (mg/g crude protein)[d]		19[e] (19)[f]	34 (28)	35 (25)	25[g] (22)[g]	28 (28)	66 (44)	63[h] (22)[h]	58 (44)

[a]g/100 g, dry basis (db); [b]Amino acid content (g/100 g, db); [c]Amino acid concentration (mg/g protein; rounded off to a whole number).
[d]WHO (1985); [e]pattern of requirements for pre-school child (2-5 years); [f]Pattern of requirements for a school child (10-12 years); [g]Methionine + cystine; [h]Phenylalanine + tyrosine.

Table 2.3.2 shows that the cake flour cookies had lower moisture content than the composite cookies. Cake flour had higher protein and fat contents than the finger millet flours and the brown, high-tannin finger millet flour had the lowest protein content. Similarly, the cake flour cookies had higher protein and fat contents than composite cookies. Cake flour had lower fibre content than finger millet flours, the non-tannin finger millet flour had the highest fibre content and the cookies showed a similar trend. The trend of ash (mineral) content was similar to that of fibre content.

As discussed earlier, components of the finger millet fibre, particularly the pentosans, are likely to have absorbed and bound water in the cookie doughs. The composite cookies had higher moisture content than the cake flour cookies, probably because their fibre, which was higher than that of the latter, tended to bind more water. The protein content of the cake flour (12.8%) was higher than that reported in the literature (8.5-9.5%) (Kent and Evers 1994) because, in South Africa, cake flour is produced from the same wheat as bread flour. The protein content of the wheat flour used in this study was slightly higher than that of white bread flour found in the literature, 11.5% (Kent and Evers 1994). The protein contents of the whole meal finger millet flours (8.2% and 11.1%) for the high-tannin and non-tannin types, respectively) are within the range of the protein content of finger millet grain found in the literature, (4.9-11.3%) (McDonough et al 2000). Similarly, Rao (1994) reported that white, non-tannin finger millet varieties had higher protein contents than tannin varieties. Increasing the amounts of finger millet, particularly the high-tannin type, would result in a decrease in the nutritional value of the cookies, with respect to protein content. Cake flour had lower fibre and ash (mineral) contents than finger millet flours most likely, because they were lost in the bran during milling. Cake flour was refined, whereas whole meal finger millet flours contained all the grain components. Increasing the amounts of finger millet, particularly the non-tannin type, in the cookie formula would improve the nutritional value of the cookies, in terms of fibre and mineral contents.

Table 2.3.3a shows that finger millet flours had lower amino acid content than cake flour. The high-tannin finger millet flour had the lowest amino acid content. Similarly, composite cookies had lower amino acid content than cake flour cookies. Therefore, increasing the amount finger millet flour, especially the high-tannin type, in the cookie formula would result in a decrease in

the nutritional value of the cookies, with respect to amino acid composition. Table 2.3.3b shows that the concentrations of essential amino acids in the composite cookies were generally lower than the pattern of amino acid requirements for a pre-school child and a school child. The concentration of lysine in the composite cookies (12 to 18 mg/g protein) was much lower than the pattern of lysine requirement for a pre-school child (58 mg/g protein) and a school child (44 mg/g protein) (WHO 1985). If the composite wheat-finger millet cookies were intended for pre-school and school children, they would be an inadequate source of essential amino acids, especially lysine. These results are in agreement with what is reported in the literature (reviewed in chapter 1) and in the previous report (chapter 2.1), that is, like in other cereal grains, lysine is limiting in finger millet grain

Table 2.3.4 shows that as finger millet substitution level was increased, the cookie spread decreased. Cookies containing 55% high-tannin finger millet had the lowest spread factor. Badi and Hoseney (1976) reported similar decreases in cookie spread when soft wheat flour was substituted with either sorghum or pearl millet flour. However, the results of this study are different from those of Morad et al (1984) who when working with sorghum reported an increase in the spread factor of sugar cookies with increasing levels of sorghum. Badi and Hoseney (1976) attributed the decreases in cookie spread when soft wheat flour was substituted with either sorghum or pearl millet flour to a reduction of wheat lipids, whereas Morad et al (1984) ascribed the increase in the spread factor of sugar cookies with increasing levels of sorghum to an increase in the large particles of sorghum flour. Dilution of wheat lipids, which have been shown to be essential for cookie spread (Kissel et al 1971, Badi and Hoseney 1976), could have contributed to reduction of cookie spread factors. An increase in fibre content could have also contributed to the decrease in the cookie spread factor, similar to what was reported on cookies in which wheat flour was substituted with different amounts of wheat, rice, oat and barley brans, respectively (Sudha et al 2007).

At each finger millet substitution level, cookies containing the non-tannin finger millet were harder than those containing the high-tannin finger millet. Increasing the level of either finger millet type above 15% resulted in a decrease in the texture values (hardness) of the cookies. Cookies containing 55% high-tannin finger millet were very brittle (328 gs). All composite

cookies were crumbly and gritty. Badi and Hoseney (1976) also reported that cookies containing either sorghum or pearl millet were crumbly and gritty. Schober et al (2003) similarly reported poor texture and structure in cookies made with non-gluten flour mixes. The crumbliness of the cookies containing finger millet may be ascribed to dilution of gluten by finger millet flour. Gluten is a structural component of dough and cookies. On heating (during baking), gluten is coagulated into a foam in which the fibre-like gluten network is largely responsible for the mechanical structure of the product (Goesaert et al 2005). An increase in fibre content due to substitution of wheat flour with finger millet flour could have disturbed uniformity of the structure of the cookie doughs and their cookies resulting in the crumbliness of the cookies. The grittiness of the cookies may be partly attributed to the presence of ungelatinised starch granules and hard, sharp-edged endosperm particles and/or bran (Taylor et al 2006). A substantial proportion of finger millet starch is likely to have not been gelatinised during baking as the gelatinisation temperature of finger millet (65-69°C [Serna-Saldivar and Rooney 1995]) is higher than that of wheat (58-64°C [Hoseney 1994]). Glover et al (1986) suggested that ungelatinised sorghum starch caused the poor texture in a high ratio cake containing sorghum. Cookies containing 55% high-tannin finger millet flour were very brittle, probably because condensed tannins bound to gluten proteins (Emmambux and Taylor 2003), which resulted in a decrease in the cohesiveness of the dough. Tannins, due to their antioxidant activity, could have also interfered with the interaction of gluten proteins by reducing their disulphide linkages resulting in the formation of sulphydryl groups.

The cookies became darker (Hunter L values decreased) with the increasing amounts of finger millet, irrespective of finger millet type. Cookies containing the high-tannin finger millet, which was brown in colour, were much darker than cookies containing the creamy white coloured, non-tannin finger millet. The cookies containing the high-tannin finger millet flour also became darker as finger millet substitution level was increased, obviously, because the high-tannin finger millet grain was brown and had a dark testa (chapter 2.1). Phenolic compounds (Salukhe et al 1982) could have contributed to the slight dark colour of the cookies containing creamy white, non-tannin finger millet flour. Morad et al (1984) working with sorghum reported similar results, substituting wheat flour with different sorghum types resulted in dark cookies with cookies containing a brown sorghum type being the darkest.

Table 2.3.4. Physical characteristics of composite wheat-finger millet cookies

Flour	Subst [a] (%)	Spread [b] (mm)	Thickness [b] (mm)	Spread factor [c]	Texture [b] ('hardness'[d] [gs])	Colour [b] (Hunter L)
Cake flour (control	0	56.1 (0.8) d	4.2 (0.1) a	13.2	1701 b	78.0 (0.8) d
ICFM 95001 GMSF	15	55.9 (0.6) d	4.9 (0.4) b	11.4	3009 d	70.2 (1.5) c
(non-tannin) finger millet	35	52.7 (0.0) c	5.8 (0.2) c	9.0	2175 c	68.2 (0.5) c
	55	50.5 (0.2) b	5.8 (0.1) c	8.8	1607 b	63.0 (0.5) b
FMV6	15	52.6 (0.1) c	5.0 (0.2) b	10.5	2 265 c	65.0 (0.9) b
(high-tannin) finger millet	35	50.8 (0.6) b	5.8 (0.1) c	8.8	1 585 b	56.8 (0.7) a
	55	48.8 (0.2) a	6.1 (0.1) c	8.0	328 a	54.7 (0.6) a

[a] % (w/w) substitution of wheat flour with finger millet flour.
[b] Mean of at least two replicate analyses; standard deviation in parenthesis.
[c] Spread factor =spread÷thickness.
[d] 'hardness'= area under the Force (g) (Y axis) against Time (s) (X axis) curve.
Values within the same column with different letters are significantly different at $p<0.05$.

Table 2.3.5a. Sensory acceptability of composite wheat-finger millet cookies

Flour	Subst [a] (%)	Acceptability					
		Taste	Aroma	Crispness	Texture	Appearance	Overall acceptability
Cake flour (control)	0	6.60 c	6.31 bc	5.56 a	6.18 abc	6.88 c	6.47 c
non-tannin finger millet	15	6.11 abc	5.71 abc	6.41 bc	6.20 abc	6.45 bc	6.23 abc
	35	6.01 abc	5.56 ab	6.09 ab	6.24 abc	6.07 b	6.10 abc
	55	5.61 a	5.76 abc	5.37 a	5.48 ab	6.05 b	5.73 ab
High-tannin finger millet	15	6.48 bc	5.89 abc	6.37 bc	6.20 abc	6.06 b	6.29 abc
	35	6.30 abc	5.47 ab	7.07 c	6.38 c	5.21 a	6.34 bc
	55	5.85 ab	5.34 ab	6.00 b	5.45 ab	4.84 a	5.52 a

[a]% (w/w) substitution of wheat flour with finger millet flour
Values within the same column with different letters are significantly different at $p<0.05$.

Table 2.3.5b. Effect of finger millet type and finger millet substitution level on sensory acceptability of composite wheat-finger millet cookies

Source of variation	P value					
	Taste	Aroma	Crispness	Texture	Appearance	Overall acceptability
Finger millet type	0.0631	0.4886	0.0026***	0.8225	<0.0001***	0.8555
Substitution level	0.0129*	0.2508	<0.0001***	<0.0001***	0.0001***	0.0010**
Type x substitution	0.953	0.2787	0.0464*	0.917	0.1187	0.4754

*Significant at $p<0.05$; **significant at $p<0.01$; ***significant at $p<0.001$.

Tables 2.3.5a shows that taste and texture acceptability of the cookies decreased with increasing finger millet substitution level, irrespective of finger millet type. The cookies containing 55% non-tannin finger millet or high-tannin finger millet had the least acceptable taste and texture. The results indicate that panellists did not like the taste of finger millet flour in the cookies irrespective of finger millet type (Tables 2.3.5a and b). Taste acceptability was not affected by tannins (in the high-tannin finger millet type), contrary to expectation. The results may be ascribed to that either the tannin levels were too low to be detected or other substances in the cookies had a counter effect on astringency and bitterness, which are associated with tannins. Sugar could have masked bitterness, and proteins and starch could have interfered with the binding of the tannins to the proteins in the taste buds and thereby reduced astringency. Other components of finger millet grain seem to have negatively affected taste acceptability of the composite cookies. Sudha et al (2007) reported a decrease in the taste acceptability of cookies with increasing levels of oat, rice or wheat bran. Similarly, an increase in fibre content with increasing finger millet substitution level could have contributed to the reduction of the taste acceptability of the cookies. It appears that texture acceptability was not influenced by the brittleness of the cookies because there was no difference in texture acceptability between cookies containing 55% non-tannin finger millet and cookies containing 55% high-tannin finger millet, despite the fact that cookies containing 55% high-finger millet were, as measured by a texture analyser, very brittle (Table 2.3.4). Since both types of composite cookies were gritty, texture acceptability could have been negatively affected by grittiness rather than brittleness.

Table 2.3.5b shows that there was no significant ($p<0.05$) difference in the aroma acceptability of the cookies. Finger millet type and substitution level had no influence on the aroma acceptability of the cookies, probably because the composition of aroma substances in the cookie types was similar.

Table 2.3.5a shows the crispness acceptability of the cookies increased with increasing levels of the high-tannin finger millet. The cookies containing 35% of high-tannin finger millet flour had the most acceptable crispness, whilst cake flour cookies and cookies containing 55% of non-tannin finger millet flour had the least acceptable crispness. Interaction of tannins with other flour components, most likely the polymeric carbohydrates and proteins (Awika et al 2003a),

could have contributed to the increased crispness acceptability of cookies containing high levels of high-tannin finger millet.

Table 2.3.5a shows that cake flour cookies had the most acceptable appearance, whilst cookies containing 35% and 55% of a high-tannin finger millet flour, respectively, had the least acceptable appearance. Increasing the levels of either the non-tannin or high-tannin finger millet had a negative effect on the appearance acceptability of the cookies. Increasing the levels of the high-tannin finger millet had a more negative effect on the appearance acceptability of the cookies than increasing the levels of the non-tannin finger millet (Table 2.3.5a). As stated earlier, the cookies became darker as substitution with finger millet was increased, and the high-tannin finger millet had a more darkening effect than the non-tannin finger millet. The panellists seem to have expected lighter cookies. Similarly, Sudha et al (2007) reported that colour acceptance of the cookies decreased as they became darker with increasing levels of oat, rice or wheat bran.

The overall acceptability of cookies was similar to taste and texture acceptability, i.e. the panellists' overall liking of the cookies was affected negatively by an increase in the level of finger millet, irrespective of type (Table 2.3.5a). However, the results indicate that the overall acceptability of cookies containing up to 35% of a non-tannin or a high-tannin finger millet type is similar to that of cake flour cookies.

Table 2.3.6 shows that methanolic extracts from doughs containing the high-tannin finger millet had much higher phenolic content and antioxidant activity than extracts from the control dough and doughs containing the non-tannin finger millet. Phenolic content and antioxidant activity decreased when the doughs were baked. However, the antioxidant activities of the cookies containing the high-tannin finger millet (41.4-55.3 mM of trolox equivalents [TE]/kg) were either higher than or similar to those of a variety of plant foods on the market (0.5-66.0 mM TE/kg) (Table 2.3.7).

Table 2.3.6. Effect of baking on the assayable phenolic content and antioxidant activity of composite wheat-finger millet cookie doughs

Flour	subst[a] (%)	Dough			Cookie			decrease in (%)[c]:		
		TP[b]	CT[b]	AA[b]	TP[b]	CT[b]	AA[b]	TP	CT	AA
Cake flour (control)	0	ND	ND	4.6a (0.2)	ND	ND	3.7a (0.2)	ND	ND	19.3
Non-tannin finger millet	15	ND	ND	4.7a (0.2)	ND	ND	3.7a (0.5)	ND	ND	21.5
	35	0.01a (0.00)	ND	5.4b (0.3)	0.01a (0.00)	ND	3.8a (0.2)	53.8	ND	29.2
	55	0.02b (0.00)	ND	5.8b (0.3)	0.01a (0.00)	ND	3.7 a (0.2)	61.1	ND	37.0
High-tannin finger millet	15	0.13c (0.00)	0.03a (0.00)	56.7c (2.8)	0.09b (0.00)	0.01a (0.00)	41.3b (2.4)	30.8	48.1	27.3
	35	0.17d (0.00)	0.04b (0.01)	61.8c (3.1)	0.12bc (0.00)	0.03b (0.01)	43.0b (2.9)	30.4	31..0	30.5
	55	0.21e (0.00)	0.07c (0.00)	68.7d (3.5)	0.13c (0.00)	0.04b (0.01)	55.3c (3.4)	40.8	42.8	19.4

[a] % (w/w) substitution of wheat flour with finger millet flour.
TP, total phenolics (mg of gallic acid equivalents/100 mg sample, dry basis [db]).
CT, condensed tannin content (mg of catechin equivalents/100 mg sample, db).
AA, antioxidant activity (mM of trolox equivalents/kg sample, db).
[b]Mean of three replicate analyses; standard deviation in parentheses; means within the same column with different letters are significantly different at $p<0.05$.
[c]Reduction in level of assayable total phenolics, condensed tannins and antioxidant activity.
ND, not detected.

Table 2.3.7. Comparison of the antioxidant activity of composite wheat-finger millet cookies with that of some food products on the market

Food products	AA (mM TE[a]/kg)
Standard (control) cookies	3.7
Cookies containing non-tannin finger millet	3.7-3.8
Cookies containing high-tannin finger millet	41.3-55.3
Ready-to-eat- breakfast cereals	13.0-53.0[b]
Fresh vegetables	0.5-14.0[b]
Fresh fruits	1.0-22.0[b]
Berries	19.0-55.0[b]
Dried fruits	58.0-66.0[b]
Juices	3.0-15.0[b]

AA, antioxidant activity. [a]Trolox equivalents. [b]Miller et al (2000).

Wheat grain contains several phenolic compounds and other antioxidant compounds, which are concentrated in the bran (Onyeneho and Hettiarachchy 1992, Adom et al 2005, Liyana-Pathirana and Shahidi 2007). Wheat grain is not known to contain tannins (Serna-Saldivar and Rooney 1995). Most likely, phenolic compounds were not detected in the control wheat dough because they had been lost in the bran during milling. The control dough had a much lower antioxidant activity than that of doughs containing the high-tannin finger millet flour, probably because it had no detectable phenol levels, particularly the tannins, which have been shown to exhibit higher antioxidant activity than other antioxidants (Hagerman et al 1998). Non-phenolic antioxidants could have been responsible for the antioxidant activity of the control dough. The non-phenolic antioxidants probably included wheat grain components such as proteins, polysaccharides and selenium (Thompson 1994, Baublis et al 2000). Doughs containing the high-tannin finger millet flour had a much higher total phenolics (TP), condensed tannin (CT) content and antioxidant activity (AA) than those containing the non-tannin finger millet flour because the high-tannin finger millet grain was shown to have a much higher TP, CT content and AA than the non-tannin finger millet grain (chapter 2.1, Table 2.1.2).

Phenolic content decreased during cookie making, probably because the phenolic compounds were either decomposed by heat (Kikugawa et al 1990, Hamama and Nawar 1991), were

volatilised, and/or interacted with other components of the dough, rendering them not extractable. Phenolic compounds such as the synthetic antioxidants, e.g. BHT, BHA and TBHQ, are known to be degraded by heat (Kikugawa et al 1990). Hamama and Nawar (1991) reported that the synthetic phenolic antioxidants BHT, PG, BHA and TBHQ were lost through evaporation and decomposition when they were heated at 185°C. Thermal decomposition has being suggested to partly cause loss of phenolic compounds in cereal grain foods during thermal processing (Duh et al 2001, Bryngelsson et al 2002, Towo et al 2003). The decrease in tannin levels during cookie making was most probably due to their interaction with other components of the dough. Tannins are known to bind to proteins, carbohydrates and minerals (Dykes and Rooney 2006). Bound tannins are not degraded, but cannot be extracted from the sample and hence there is an apparent decrease in tannin content. The percentage decreases in tannin levels (31.0 to 48.1%) (Table 2.3.6) that occurred when the three cookie dough types containing the high-tannin finger millet flour were baked were of similar magnitude as the 52% decrease in tannin levels which occurred when tannin sorghum bran was baked into cookies (Awika et al 2003a). These authors attributed the loss of the tannins to their interaction with bran components, mainly the polymeric carbohydrates and proteins.

Antioxidant activity decreased when the cookie doughs were baked into cookies, seemingly largely due to the decrease in phenol levels. Other antioxidative components of finger millet and cake flour such as phytic acid, carbohydrates and proteins (Thompson 1994, Sripriya et al 1996, Baublis et al 2000) might also have undergone changes such as thermal decomposition, chemical modification and chemical interaction that resulted in a loss or reduction of their antioxidant activity or formation of pro-oxidants (Nicoli et al 1999). Some components of the dough might have undergone changes that tended to increase the antioxidant activity of the dough, e.g. release of bound antioxidants (Dewanto et al 2002) and formation of antioxidative substances such as the Maillard reaction products (MRPs) (Griffith and Johnson 1957, Bressa et al 1996). However, their antioxidant effect would have been outweighed by changes that resulted in the reduction of the antioxidant activity of the cookies. The composite wheat-finger millet doughs retained 63.0-80.6% of their antioxidant activities when they were baked into cookies, which is similar to that retained by tannin sorghum bran (57-78%) when it was processed into breads and cookies (Awika et al 2003a). However, the antioxidant activities of the composite wheat-finger millet

cookies (3.65-55.34 mM TE/kg) were much lower than those reported for tannin sorghum bran cookies (90-130 mM TE/kg) (Awika et al 2003a). They were lower most likely because whole grain finger millet flour had a lower concentration of phenolic compounds (chapter 2.1, Table 2.1.2) than sorghum bran.

Table 2.3.8 shows that TP and CT content were negatively correlated with appearance acceptability ($p<0.05$), but TP and CT content were not correlated with taste and overall acceptability of the cookies. The results suggest that phenolic compounds had a negative effect on appearance acceptability, but did not affect taste and overall acceptability of the cookies. Overall acceptability was positively correlated with taste and texture acceptability of the cookies ($p<0.05$ and $p<0.01$, respectively), which indicates that the taste and texture of the cookies largely contributed to their overall acceptability. There was a significant and positive correlation between TP, CT content and AA ($p<0.05$) of the cookies. Hence tannins made a large contribution to the antioxidant activity of the cookies, as what was found in different finger millet grain types (chapter 2.1, Table 2.1.3). Although the assayable tannin levels decreased when the doughs were baked, the presumably bound tannins could have also contributed to the antioxidant activity of cookies containing high-tannin finger millet. It has been shown that protein-bound tannins retain up to 50% of their antioxidant activity (Riedl and Hagerman 2001). Texture (hardness) was negatively correlated with condensed tannin content ($p<0.05$), indicating that tannins largely contributed to the brittleness of the cookies containing the high-tannin finger millet. TP, CT content and AA were significantly and negatively correlated ($p<0.05$) with Hunter L values of the cookies suggesting that the lightness of composite wheat-finger millet cookies may be used as a predictor of low phenolic content and antioxidant activity.

Table 2.3.8. Pearson correlation coefficients between phenolic content, antioxidant activity, texture, colour and sensory acceptability of composite wheat-finger millet cookies

	TP	CT	AA	Instr. Text.	Hunter L	Taste	Aroma	Crispness	Sens. Text.	Appear.	OA
TP											
CT	0.892**										
AA	0.835*	0.882**									
Instr. Text.	-0.591	-0.784*	-0.833*								
Hunter L	-0.801*	-0.856*	-0.736*	0.597							
Taste	0.103	-0.207	-0.172	0.298	0.482						
Aroma	-0.539	-0.693	-0.551	0.347	0.849*	0.593					
Crispness	0.569	0.380	0.128	0.223	-0.396	0.348	-0.462				
Sens. Text.	-0.099	-0.355	-0.475	0.645	0.423	0.760*	0.263	0.621			
Appear.	-0.848*	-0.936**	-0.835*	0.689	0.967***	0.393	0.859*	0.462	0.371		
OA	-0.224	-0.496	-0.592	0.633	0.619	0.869*	0.599	0.381	0.916**	0.594	

TP, total phenolics; CT, condensed tannin content; AA, antioxidant activity; Instr. Text., texture measured using a texture analyser; Sens. Text., texture measured using a consumer panel; Appear., appearance; OA, overall acceptability.
*Significant at $p<0.05$; **significant at $p<0.01$; ***significant at $p<0.001$.

2.3.5. Conclusions

The results suggest that potentially health-promoting (due to high antioxidant activity) cookies that are rich in fibre and minerals and are sensorially acceptable can be made by substituting, up to about 35%, wheat with a high-tannin finger millet.

2.3.6. References

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3. GENERAL DISCUSSION

This chapter is in three sections. Firstly, the methodologies applied in this study are critically evaluated. In the second section, the main findings of the investigations of (1) Finger millet grain phenolics and antioxidant activity of different grain types; (2) the Effect of phenolic content and amount of phenolic type on the malt quality of finger millet grain; and (3) the Impact of finger millet grain phenolics on cookie quality, are discussed. Lastly, based on the main research findings, the postulation "finger millet grain is a premium cereal grain for human food" is put forward and critically evaluated.

3.1. Methodologies

This section discusses the strengths and weaknesses of most of the major methods applied in this study. Suggestions of how the methodologies could have been approached differently and/or improved are made.

As stated earlier, the Bleach test was for the first time used to detect the presence of tannins in finger millet grain. The principle of the test, as applied to sorghum grain for which it was first developed, is that sodium hypochlorite solution (Bleach) containing alkali dissolves away the outer pericarp layer of submerged grain, revealing the presence of a black pigmented testa layer in the case of tannin sorghums, or its absence in the case of non-tannin sorghums (Price and Butler 1977, Taylor 2001). The kernels with a pigmented testa stain black in the Bleach reaction. According to the procedure of the Bleach test, when applied to sorghum grain, the reaction should occur in 20 minutes (Waniska et al 1992, Taylor 2001). However, when the Bleach test was applied to finger millet grain, the reaction took a much longer time (incubation had to be left overnight) than in sorghum grain. The reason for this could be that the pericarp of finger millet is thicker than that of sorghum. According to Taylor and Belton (2002), the pericarp of sorghum contains a waxy cuticle layer, a layer of epidermal cells; under these are two or three layers of the hypodermis, beneath which is the mesorcarp. From this review, it seems that the sorghum

pericarp is not made up of many layers. In contrast, microscopic analysis of the outer layers of finger millet grain in this study showed that it was made up of several layers. The observation was similar to that of McDonough et al (1986) who reported that the pericarp of the finger millet grain appeared to be made up of several layers of tissue (subsection 1.2.1.1). The merit of the Bleach test, when applied to sorghum, is that it is simple, rapid and inexpensive (Dykes and Rooney 2006). However, when applied to finger millet, the Bleach test would not be rapid as when applied to sorghum. That would be of disadvantage when used in situations such as in the screening of tannin and non-tannin grains for marketing.

According to Price and Butler (1977), the Bleach test, when applied to sorghum, is subject to error because of interference in some varieties by plant pigments, the colour of which may persist through the test and make identification of the testa ambiguous. Dykes et al (2002) reported that severely weathered, insect damaged or moulded sorghum without a pigmented testa turned dark after Bleaching, which could lead to erroneous, false positive results indicating that a sorghum kernel contained tannins. In the current study, sound finger millet kernels were selected for the Bleach test and sound sorghum standards (tannin and non-tannin sorghum standards) were included. The incidence of false positives caused by the presence of damaged or weathered kernels was therefore unlikely. However, interference by pigments naturally-occurring in the finger millet kernels was possible, but no false positives were found in this study. The Bleach test agreed well with the Vanillin-HCl assay (chapter 2.1, Table 2.1.3). The correlation of the two assays indicates that the Bleach test can be used to test for presence of tannins in finger millet grain.

There may be room for improvement of the Bleach test for use in finger millet grain. Raising the incubation temperature could be one way of reducing the duration of the reaction. The “scratch test” which is an alternative of the Bleach test was deemed inappropriate in finger millet because it would be practically almost impossible to scratch such tiny kernels. The “scratch test” is used to determine the presence of tannins in sorghum grain. In the “scratch test”, the outer pericarp of sorghum grain is scratched away with a sharp knife or scalpel to determine the presence or absence a pigmented testa. The presence of a pigmented testa is a positive result for the presence of tannins in the grain (Waniska et al 1992).

The location of tannins in the finger millet grain was determined by using a combination of methods, *viz*. the Bleach test; the Vanillin-HCl assay and light microscopy (chapter 2.1). The results showed that finger millet grain types that stained black in the Bleach test had condensed tannins, whilst those that did not stain black had no tannins (chapter 2.1, Table 2.1.3). It was thus possible to put the finger millet grain types into two groups, non-tannin types and tannin types, similar to the work of Mason et al (1972) with sorghum. Analysis by light microscopy indicated that the dark colour was concentrated in the testa layer (chapter 2.1, Figure 2.1.1). In contrast, the non-tannin grain types had a light testa layer (Figure 2.1.1.). Because in sorghum, it has been established that tannins are located in a dark testa layer, it could therefore be deduced that in finger millet grain, tannins are located in the testa layer, as in sorghum. Blakely et al (1979) and Moral et al (1981), similarly, used light and electron microscopy to determine the location of tannins in sorghum grain and to analyse the structure of tannin-containing tissues. Blakely et al (1979) observed that the outstanding feature of high tannin sorghums was the presence of a testa, which appeared as a dark strip just external to the aleuronic layer. Their observation is similar to that made in this study (Figure 2.1.1). Because, the methodology used to determine the location of tannins in finger millet grain is similar to the methodologies that have been used to study tannins in sorghum grain, the methodology could have been improved by including non-tannin and high-tannin sorghums as standards.

Acidified methanol was used when extracting for the determination of TP and flavan-4-ols, whilst in the determination of condensed tannins absolute methanol was used. In the determination of anthocyanins, initial extraction was with absolute ethyl acetate and finally with acidified methanol (chapter 2.1). The solubility of phenolic compounds is governed by the polarity of the solvent, degree of polymerisation of the phenolics, as well as interaction of phenolics with other food components and formation of insoluble complexes (Naczk and Shahidi 2004). With regard to the solvents used in this study, methanol and ethyl acetate are polar solvents, and acidified methanol is more polar than absolute methanol and ethyl acetate. Polar solvents are suitable for extracting polar phenolic compounds and non-polar solvents for non-polar phenolic compounds. Therefore, the effectiveness of the solvents used in this study partly depended on the polarity of the phenolic compounds in the samples. While Kaluza et al (1980) found 75% (v/v) aqueous acetone the best solvent for extracting phenolic compounds from

sorghum grain, there are several reports (e.g. Sripriya et al 1996, Dykes et al 2005, Hedge and Chandra 2005, Dlamini et al 2007) in which either absolute or acidified methanol has been used to extract phenolic compounds from sorghum and finger millet. While Waterman and Mole (1994) recommend absolute methanol over acetone and acidified methanol for extracting condensed tannins for the Vanillin-HCl assay, in the Price et al (1978) modification of the Vanillin-HCl method acidified methanol is used to extract the tannins in type II tannin sorghums. It is important to note that in the human being, phenolic compounds are not extracted from food by the solvents used in this study. Instead, the phenolic compounds are extracted into aqueous solutions during mastication and digestion. Therefore, the assayed phenolic levels do not give a direct indication of the phenolic levels that would be extracted in the gut.

Interaction of phenolics, particularly the condensed tannins, with other chemical components of the finger millet grain, and cookie doughs and cookies might have reduced their extractability. The condensed tannins could have interacted with the macromolecular carbohydrates and proteins, and minerals forming insoluble complexes that were not extractable (Porter 1989, Slabbert 1992, Emmambux and Taylor 2003). Phenolic compounds might also have interacted with Maillard reaction products [MRPs] to form insoluble and hence unextractable complexes. The reduction in the levels of assayable total phenolics, condensed tannin content and antioxidant activity during the making of cookies (chapter 2.3, Table 2.3.2) could indeed have been due to a decrease in the extractability of phenolic compounds due to their interaction with other chemical components of the cookie doughs. There is no standard procedure for the extraction of all phenolics or a class of phenolics in plant materials (Naczk and Shahidi 2004). The extraction methodology used in this study could have been improved by employing different extraction procedures in which some of the critical extraction parameters, such as temperature, time and solvent, were varied. The extraction yields could then be compared.

Extraction of cell wall-bound phenolic compounds was not attempted. The extraction of cell wall-bound phenolic compounds involves thie hydrolysis with NaOH (Maillard and Berset 1995). As reviewed earlier, substantial amounts of cell-wall bound phenolic compounds have been reported in finger millet grain (Subba Rao and Muralikrishna 2001, 2002). Therefore, the phenolic contents of the finger millet grain types, and cookie doughs and cookies are actually

assayable phenolic contents. They are highly likely to be an underestimation of the actual phenolic contents of the finger millet grain types, cookie doughs and cookies. It is relevant to note this because in the human being digestion and the action of micro-organisms in the gut may release substantial amounts of cell wall-bound phenolic compounds (Manach et al 2005). Cell-wall bound phenolic compounds have been shown to contribute to the antioxidant properties of finger millet grain (Subba Rao and Muralikrishna 2002).

The Folin Ciocalteu assay was used to estimate the total phenolics (TP) of finger millet grain types, and cookie doughs and cookies chapters 2.1 and 2.3). The Folin Ciocalteu assay is based on the measurement of the reducing power of phenolic hydroxyl groups (Singleton et al 1999). The limitation of the Folin Ciocalteu assay is that it is not specific to a particular class of phenolic compounds (Singleton et al 1999). Interfering compounds, with reducing power, such as unanticipated phenolics or enols (e.g. food additives and microbial metabolites), aromatic amines and aminophenols, purines, tyrosine and tryptophan, proteins, and ascorbic acid can react with the Folin Ciocalteu reagent (Singleton et al 1999). Some of these substances, which may interfere with the Folin Ciocalteu assay, are likely to have been present in the finger millet grain types, and cookie doughs and cookies. These substances would have contributed to TP as measured by the Folin Ciocalteu assay. The shortening (margarine) used in making cookies contained additives, including phenolic antioxidants (shown on the ingredients label of the packaging), which were probably detected by the Folin Ciocalteu assay. In the cookies, Maillard reaction products (MRPs) may have been detected by the Folin Ciocalteu assay, as was reported by Michalska et al (2008) working with bread. Therefore the TP of the finger millet grain types, and cookie doughs and cookies could have been overestimated.

According to Singleton et al (1999), the Folin Ciocalteu assay is usually preferred to other methods in most situations where the objective is to make a direct comparison of the TP of samples that are similar. Other workers, e.g. McDonough et al (1986), Hegde and Chandra (2005), and Chethan and Malleshi (2007) have also used the Folin Ciocalteu assay to measure TP in finger millet grain. In this study, the Folin Ciocalteu assay was similarly deemed appropriate for estimating the relative TP of finger millet grain types, and cookie doughs and cookies, respectively. Inclusion of non-tannin and high tannin and sorghums standards could have

improved the method. Other assays that measure TP, such as the Ferric ammonium citrate assay of the International Organization for Standardization (ISO) (1988) and the Prussian Blue assay (Price and Butler 1977), could also have been employed and the results compared.

The modified Vanillin-HCl assay of Price et al (1978) was used to determine condensed tannins in finger millet grain types, cookie doughs and cookies (chapters 2.1 and 2.3). The Vanillin-HCl assay is based on the reaction of flavanols with the aldehyde vanillin in an acidic medium (Swain and Hills 1959; Porter et al 1986). The Vanillin-HCl assay is not specific for condensed tannins. It detects both polyflavanols (condensed tannins) and flavanol monomers (Scalbert 1992). Thus, the assay can over-estimate condensed tannin content by measuring also the naturally-occurring flavanol monomers. For example, the results of this study indicate the occurrence of flavan-4-ols in the finger millet grain types (chapter 2.1, Table 2.1.2). These compounds could have been detected by the Vanillin-HCl assay. Indeed, the highly significant positive correlation ($r= 0.666$, $p<0.001$) (chapter 2.1, Table 2.1.3) between the flavan-4-ols and condensed tannins suggests that flavan-4-ols contributed to the assayed condensed tannin content of the finger millet grain types.

Sarkar and Howarth (1976) demonstrated that the Vanillin-HCl assay was not specific for flavanols. These authors showed that the requirement for the vanillin reaction is a single bond between C-2 and C-3 of the flavonoid nucleus (chapter 2.1, Figure 1.2.3) and free meta-oriented OH groups on the B ring. Flavonoid compounds such as dihydrochalcones, anthocyanins or anthocyanidins and flavanones may produce a reaction in the Vanillin-HCl assay (Sarkar and Howarth 1976). The flavonoid flavones have been reported in finger millet leaves (section 1.2.4). Flavones have a double bond between C-2 and C-3 (Markham 1989). Thus, they would not be involved in the vanillin reaction, presuming that they were present in the finger millet grain types of this study. However, the results of this study indicate the occurrence of anthocyanins or anthocyanidins (chapter 2.1, Table 2.1.2). These compounds, as already stated, may be involved in the vanillin reaction. Other compounds possessing chemical structures, which are suited to the vanillin reaction, may have been present in the finger millet grain types, and cookie doughs and cookies; and they would have contributed to overestimation of condensed tannins.

In this study, blanks were used in the Vanillin-HCl assay to counteract the possible error due to background colour of other pigments. However, Earp et al (1981) commented that when blanks are subtracted, initial colour is removed, but this still does not eliminate the measurement of non-tannin, vanillin positive compounds. While catechin, a flavanol monomer, was used as a standard in the Vanillin-HCl assay in this study, it has been shown that condensed tannins are less reactive than catechin (Goldstein and Swain 1963, Sun et al 1998). These findings suggest that only some of the internal flavanol units of condensed tannins react with vanillin. Thus, structural variations in the condensed tannins will affect the colour yield with vanillin (reviewed by Schofield et al 2001). Unfortunately, because of the complexity and variability of the condensed tannin structures, it is difficult to obtain an appropriate standard for condensed tannins (Schofield et al 2001).

However, the Vanillin-HCl assay is widely used for quantification of condensed tannins in plant materials, and particularly in grains (Naczk and Shahidi 2004). Other workers (McDonough et al 1986, Sripriya 1996, Hegde and Chandra 2005) have also used the Vanillin-HCl assay to measure tannins in finger millet grain. Earp et al (1981) evaluated seven methods for determining tannins in sorghum, and concluded that, for most of the analytical purposes and food applications, the modified Vanillin-HCl assay of Price et al (1978) when used in combination with either the scratch or Bleach test for determining the presence of a pigmented testa, was the most recommended. These authors commented that determination of absolute values of condensed tannins by the methods they evaluated was not possible; rather, each of those assays was a relative measure of the tannins in sorghum.

As with the measurement of total phenolics, the results of the Vanillin-HCl assay in this study could have been improved by including non-tannin and high tannin sorghums as standards. Other methods for measuring condensed tannins, which, because of their specificity for condensed tannins, could have given better results than the Vanillin-HCl assay, include the optimised Proanthocyanidin (Butanol-HCl) assay (Porter et al 1986), and HPLC techniques (reviewed by Schofield et al 2001, Dykes and Rooney 2006). These methods, however, are not as simple as the Vanillin-HCl assay, and as with the Vanillin-HCl assay, it is difficult to obtain an appropriate standard for condensed tannins (Dykes and Rooney 2006).

The anthocyanin content of the finger millet grain types was determined using a method based on the characteristic behaviour of anthocyanins under acidic conditions (Fuleki and Francis 1968). Under acidic conditions, anthocyanins possess a flavylium ion nucleus, which is responsible for their characteristic red colour. They absorb in the UV-visible light region (Escribano-Bailón et al 2004). In this study, absorbance was measured in the visible region (475 nm and 495 nm [chapter 2.1), which should have been superior to the UV region because interference by UV-absorbing substances such as protein, nucleic acids and amino acids should have been minimal (Naczk and Shahidi 2006). However, other coloured compounds like carotenoids, if they were present in the samples, would have interfered with the determination of anthocyanins.

The spectral properties (including absorbance maxima) of anthocyanins vary according to their chemical nature, such as type of functional groups on the B ring, glycosylation and presence of acyl groups (Escribano-Bailón et al 2004). Although apigeninidin and luteolinidin have been found to absorb maximally at 475 nm and 495 nm, respectively (Menkir et al 1996), other anthocyanins and anthocyanin-related compounds may absorb significantly at these wavelengths. Therefore, if the finger millet grain types of this study contained several molecular types of anthocyanins and other anthocyanin-related compounds, measurement of anthocyanins as 3-deoxyanthocyanins, apigeninidin and luteolinidin, could have resulted in a significant error. The method could have been improved by applying diagnostic techniques, e.g. use of HPLC equipped with a diode array detector and scanning spectrophotometry, to test for the presence of anthocyanin species and their absorption maxima. The data could have been then reported as crude 3-deoxyanthocyanin or anthocyanin content.

The principle of the assay used to measure flavan-4-ols is that, when left to stand at room temperature for about 1 h in acidified alcohol (butanol-HCl), flavan-4-ols convert to pink anthocyanidin pigments, which absorb strongly at 550 nm (Watterson and Butler 1983). Condensed tannins also undergo the same reaction in butanol-HCl, but only after boiling the reaction mixture (Butler 1982). Monomeric flavan-3-ols do not convert to anthocyanidins under these reaction conditions (Butler 1982). The possible drawback of the assay used to measure flavan-4-ols is that naturally-occurring anthocyanidin pigments may also be detected. Hence, the flavan-4-ols contents of the finger millet grain types might have been over-estimated. The

significant and positive correlation (r= 0.513, $p<0.05$) (chapter 2.1, Table 2.1.3) between apigeninidin content and flavan-4-ols content of finger millet grain supports the suggestion that naturally-occurring anthocyanidins could have contributed to the assayed flavan-4-ols contents of the finger millet grain types.

Colorimetric methods could only estimate total phenolics and contents of classes of phenolic compounds in finger millet grain types, cookie doughs and cookies. Molecular types were not identified. Methods such HPLC and NMR techniques could have identified molecular types of the phenolic compounds. HPLC analysis was tried, but unsuccessfully. The HPLC system developed a high pressure when analysis of phenolic acids and flavonoids was attempted in the reverse phase. The peaks were poorly resolved. It was thought that large molecules such as sugars and tannins in the extracts were blocking the column. An attempt was therefore made to purify the samples by separating out the high molecular weight substances through solid phase extraction. Several attempts were made to analyse the purified samples, using new columns and purging the column at intervals. There was no significant improvement in the analysis and the work was abandoned. The HPLC analysis may be successful if sample preparation and analysis procedures are further improved. However, estimation of phenolic contents using colorimetric methods was adequate for determining the relative levels of phenolic compounds in the samples.

The Trolox assay measures the relative ability of an antioxidant to scavenge the free radical chromogen 2,2´-azinobis (3-ethyl-benzothiazolline-6-sulphonic acid) ($ABTS^{\cdot+}$) generated in the aqueous phase, as compared with trolox (Miller and Rice-Evans 1997). Loss of the blue-green $ABTS^{\cdot+}$, due to antioxidant activity, is measured by observing a decrease in absorbance at a specific wavelength. In this study, $ABTS^{\cdot+}$ was generated by reacting an ABTS salt with potassium persulphate and absorbance was measured at 734 nm (Chapter 2.1).

The Trolox assay and the DPPH assay are the most widely used assays that are based on the free radical-scavenging activity of radical chromogens (Arnao 2000). When the Trolox assay was applied in this study, it was found to be rapid (save that $ABTS^{\cdot+}$ generation was overnight), easy to perform and had good repeatability (chapter 2.1, Table 2.1.2 and chapter 2.3, Table 2.3.6). These advantages of the Trolox assay have also been reported by Miller and Rice-Evans (1997)

and Awika et al (2003b). Another advantage of using the Trolox assay in this study was that interferences by coloured compounds, such as anthocyanins and carotenoids (Arnao 2000) and probably also the MRPs of the cookies, would have been minimal as absorbance was measured far away from 415-515 nm range, where significant interferences have been observed (Arnao 2000). Furthermore, because $ABTS^{\cdot+}$ is soluble in both aqueous and organic solvents (Arnao 2000), the antioxidant activity of hydrophilic and lipophilic compounds in extracts from the finger millet grain types, and cookie dough and cookies should have been detected. The two advantages of the Trolox assay, which have been just stated, make it superior to the DPPH assay (Arnao 2000). The Trolox assay was performed in buffered media at pH 7.4, which is relevant as it is the approximate human physiological pH. However, the antioxidant activity of the samples could have been under-estimated due to under-extraction of antioxidant compounds, as discussed earlier.

One of the weaknesses of the Trolox assay is that it is not fully standardized and hence comparison of results across laboratories is problematic (Awika et al 2003b). Another weakness of the assay is the use of the $ABTS^{\cdot+}$, which is foreign to biological systems. Hence, the antioxidant activities of finger millet grain and cookies reported in this study may not be a good indicator of their antioxidant activities *in vivo*. With respect to this, the oxygen radical capacity (ORAC) assay, which measures the ability of antioxidants to protect protein from damage by typical biological systems radicals, $ROO^{\cdot}$, $OH^{\cdot}$ and Cu^{2+} (Cao et al 1993), would have been more appropriate than the Trolox assay. However, the ORAC assay suffers the drawback of using expensive equipment and that data variability can be large across equipment (Awika et al 2003b). On the other hand, Awika et al (2003b) working with sorghum and sorghum products found a very high correlation (r^2= 0.99) between the Trolox assay and the ORAC assay, which indicates that Trolox assay results show antioxidant activity trends that are similar to those of the ORAC assay.

The moulds that infected the unmalted finger millet grain types and their malts were analysed by the direct plating method, while enumeration of all the fungi, i.e. yeasts and moulds (total fungal count [TFC]) that contaminated the surface of the unmalted finger millet grain types and their malts was performed by the standard plate count method (chapter 2.2). In the direct plating

method, the unmalted and malted finger millet kernels were surface sterilized with 76% (v/v) ethanol, and thus the moulds growing on the media after incubation were presumed to be those, which had infected the kernels. Selective media were used to reduce competition amongst the moulds and to supply nutrients to different mould types according to, as far as possible, their requirements. Thus, applying the findings of Rabie et al (1997) working with barley and according to the protocol used by the Centre for Applied Mycological Studies (CAMS) of the University of Pretoria/CSIR, Potato Dextrose Agar (PDA) was used as a non-selective medium; Malt Salt Agar (MSA) for the selective growth of *Aspergillus*, *Eurotium* and *Penicillium* spp.; and Pentachlorobenzene Agar (PCNB) for the selective growth of *Fusarium* spp.

Moulds grow by means of hyphae, which tend to spread rapidly resulting in overgrowth, as opposed to growth by division of individual distinct cells in bacteria (Harrigan 1998). Thus, enumerating moulds by the standard plate count method may be difficult. In this study, counting was done only in plates in which, due to dilution, there were distinct individual mould propagules.

When used in this study, the direct plating method was easy to follow. The mould propagules growing on the plates were distinct, which made their enumeration, purification and subsequent identification possible. Results of the direct plating method (chapter 2.2, Tables 2.2.1 and 2.2.3) showed that finger millet malt grain was infected by more species of moulds than the unmalted grain. These trends seem, as discussed previously, logical as it is expected that changes during malting such as increase in moisture content of the grain would make it more susceptible to fungal infection. Thus, the direct plating method, as used in this study, seems to have been effective at detecting fungal infection levels.

Rabie et al (1997) compared the effectiveness of dilution plating and direct plating methods to enumerate moulds that grew on barley. These authors found that the direct plating method detected a greater diversity of mould species than the dilution plating method. A much larger variety of species of both field and storage fungi were enumerated in the direct plating method. These authors thus recommended the direct plating method over the dilution method. Many workers, e.g. Petters et al (1988), Ackermann (1998), and Lefyedi et al (2005) have used the

direct plating method on the basis that it is one of the best methods for assessing growth of fungi in cereal grains. One drawback of the direct plating method is that, unlike the colony count methods (Harrigan 1998), it does not determine the quantity, as for example cfu/g, of the fungi infecting the kernels. The method only detects the proportion of infected grain in a sample, but the severity (quantity of fungi) of infection is not detected. Another disadvantage of the direct plating method is that it consumes a lot of time, media and plates. Other methods such as microscopic counts, flow cytometry and ATP determination by bioluminescence (Harrigan 1998) could have been tried, but as explained the direct plating method was deemed the most appropriate.

The 9-point hedonic rating test was used, as it is the appropriate method for determining the overall acceptability of a product by consumers (Lawless and Heymann 1998). Although the design of the sensory test followed the principles of randomisation to minimize error, significant "psychological errors" are likely to have occurred. Psychological errors have to do with the peculiarities of human judgment (Stone and Sidel 2004). For instance, psychological errors classified as "contrast and convergence errors" could have occurred. For example, the panellists might have given the light cookies made with cake flour or partially substituted with the non-tannin finger millet an exaggerated high appearance acceptability score because of the contrast in colour between them and the brown cookies containing high levels of the high-tannin finger millet. Whilst the light lightness (Hunter L values) of wheat cake flour cookies was significantly higher than that of composite cookies (chapter 2.3, Table 2.3.2), the appearance acceptability of the cake flour cookies was the same as that of cookies containing 15% non-tannin finger millet (Table 2.3.7). That was probably because the small but significant colour differences between the two types of cookies was, in terms of appearance acceptability, masked or overshadowed (convergence error [Stone and Sidel 2004]) by the large difference between the much lighter cake flour cookies and the much darker cookies containing high levels of the high-tannin finger millet.

The consumer panel comprised university students of different races, gender and ages who were regular consumers of sugar snap cookies. The consumer panel was thought to be appropriate for the test as it represented a wide spectrum of consumers of sugar snap cookies. The drawback in the use of the described panel was that they were not consumers of finger millet. Consumers of

finger millet might have liked the cookies substituted with finger millet more than the panel used in this study. On the other hand, the panel used in this study would be appropriate if the cookies were being targeted at health-conscious, modern urban-based consumers. A second panel of regular consumers of finger millet could have been used to evaluate the market potential of the cookies in a community of regular consumers of finger millet.

Analysis of the acceptability of selected attributes of the cookies gave an indication of their contribution to the overall acceptability of the cookies, but the data did not describe the sensory characteristics of the cookies. The sensory evaluation questionnaire could be improved by including questions eliciting for data on the sensory characteristics of the cookies, and questions eliciting for comments about the cookies could be included. Alternatively, the sensory analysis could be expanded to include analytical tests, e.g. descriptive tests (Lawless and Heymann 1998), to obtain information on the sensory characteristics of the cookies.

3.2. Research findings

This section discusses the main findings of this study, which show that finger millet contains various phenolic compounds that contribute to the antioxidant properties of the grain and its food products, and that the phenolics seem to have a positive influence on finger millet malt quality, but contribute to some of the poor quality attributes of composite wheat-finger millet cookies.

As described earlier (chapter 2.1, Tables 2.1.1 and 2.1.2), this study shows that finger millet grain types produced by tan and purple plant types contain various phenolic compounds, including condensed tannins, flavan-4-ols and anthocyanins. Total phenolics, condensed tannins, flavan-4-ols and anthocyanins vary across grain types. These findings indicate that pigmented grain types contain higher amounts of phenolic compounds than light types. Grain types with a pigmented testa, which are produced by purple plant types, contain condensed tannins, as in sorghum (Dykes et al 2005). These findings clearly indicate that genetic factors (variety) strongly affect the phenolic content and composition of finger millet grain. However, unlike in sorghum (Dykes and Rooney 2006), the actual genotypes that control grain colour, presence of a pigmented testa and tannin content are not known. Although confirmatory analyses are required, this study seems

to be first to report on the presence of the flavonoid monomers anthocyanins (i.e. the 3-deoxyanthocyanidins apigeninidin and luteolinidin) and flavan-4-ols in finger millet grain. Occurrence of these flavonoids in finger millet grain should be significant as they may, together with other phenolic compounds, impact on food quality and safety, as will be discussed further.

Due to its much smaller kernel size, finger millet should have a larger surface area to volume ratio than sorghum. The testa layer of the finger millet grain should therefore have a larger surface area relative to that of the sorghum grain. Since in both sorghum and finger millet tannins are located in the testa layer, it would therefore be expected that the finger millet grain have relatively higher tannin content than sorghum grain. However, the tannin contents (not detected to 2.1 mg of catechin equivalents/100 mg) (chapter 2.1, Table 2.1.2) of the finger millet grain types of this study were generally lower than those often reported for sorghum grain (e.g. 0.0 to 5.5 mg catechin equivalents/100 mg [Beta et al 1999]). The unexpected findings could be due to possible underestimation of tannins in the finger millet as has already been suggested or the difference was due to genetic factors.

Genetic factors seem to strongly affect the phenolic content and composition of finger millet grain. The relationships established in this study between phenotypic attribute (plant type, grain colour and presence of a pigmented testa) and grain phenolic content and composition in finger millet should be significant as they could be used in screening for grain types with the desired phenolic contents and phenolic types in plant breeding, grain marketing and other practical purposes. For example, grain pigmentation may be used as a selection criterion for grain types with high phenolic content as suggested by the significant and positive correlation ($r = 0.567$, $p<0.01$) (Table 2.1.3) between the Hunter a values and TP. The Bleach test may be used to detect tannin finger millet types as discussed earlier.

The findings of this study show that phenolic compounds make a large contribution to the antioxidant properties of finger millet grain (chapter 2.1, Tables 2.1.1 and 2.1.2). Tannin finger millet grain types exhibit much higher antioxidant activity than non-tannin types, which is in agreement with the hypothesis stated in section 1.3. The results indicate that condensed tannins contribute largely to antioxidant activity of finger millet grain. This may be attributed to the

chemistry of condensed tannins. Condensed tannins contain numerous hydroxyl groups (chapter 1, Figure 1.2.4), which can be involved in antioxidant activity reactions by donating hydrogen or electrons and thereby scavenge free radicals and quench reactive oxygen species (Rice-Evans et al 1997, section 1.2.3). An increase in the number of hydroxyl groups in a phenolic compound has been found to correspond with an increase in antioxidant activity (reviewed in section 1.2.3). The findings of this study thus suggest that tannin finger millet grain types/varieties are good sources of phenolic antioxidants, which may have health-promoting effects. These findings are useful as they may be used for the selective breeding of tannin finger millet grain types/varieties, which could be processed into antioxidant-rich ("health") foods as will be discussed further.

The findings of this study indicate that unmalted and malted pigmented finger millet grain types, which had higher contents of phenolic compounds, had lower fungal loads than the light coloured grain types (chapter 2.2, Tables 2.2.1 and 2.2.3). In fact, the fungal loads of unmalted and malted finger millet grain were significantly negatively correlated with phenolic content and type (condensed tannins, anthocyanins or flavan-4-ols) (chapter 2.2, Tables 2.2.2 and 2.2.4). These findings indicate that finger millet grain phenolics contribute to resistance of the grain to fungal invasion. Finger millet grain phenolics thus seem to exhibit antimicrobial activity. These findings are similar to those reported by few (Seetharam and Ravikumar 1994, Viswanath et al 2008) and several authors (reviewed in chapter 1, subsection 1.2.6.4) working with finger millet grain and sorghum grain, respectively.

The positive correlation between finger millet germinative energy and malt quality (enzymic activity) and phenolic content and amount of phenolic type (Table 2.2.6) suggests that phenolics in finger millet grain promote its germination as indicated by high enzymic activity of the malt. The phenolic compounds could have promoted germination by inhibiting fungi. The mechanisms of antimicrobial activity of the phenolic compounds could be the same as those that have been proposed (reviewed in chapter 1, subsection 1.2.6.5). The finger millet tannins might have attenuated invasion of the grain by fungi by forming a physical barrier in the testa, as suggested by McGrath et al (1982) when working with sorghum. The finger millet grain phenolics might have been toxic to the fungi by reacting with their cell walls and membranes and cell

components, such as physiological and structural proteins and carbohydrates, as suggested in the literature (Butler et al 1984, Scalbert 1991, Cowan 1999). Several reaction mechanisms, including hydrogen bonding, oxidation and complexation might have been involved. The integrity and functionality of the fungal cell walls and membranes and cell components would have been affected negatively. For example, the functionality of cell membrane-bound enzymes and protein factors presumably mediating host (grain) penetration by the fungi might have been affected negatively by the finger millet phenolics. The finger millet phenolics could have been toxic to fungi through reacting with their enzymes and thereby inhibiting them from mediating vital metabolic reactions, including those involved in the germination of spores (reviewed by Harborne 1994b). Simple phenols and phenolic acids present in the finger millet grain could have reacted with fungal enzymes through, e.g. oxidation reactions such as the oxidative formation of disulphide bonds or through non-specific interactions (reviewed by Cowan 1999). The finger millet tannins might have complexed with fungal enzymes through hydrogen bonding and hydrophobic interactions (Butler et al 1984, Scalbert 1991). The finger millet grain tannins might have also complexed with metal ions in the grain rendering them unavailable to the fungi, as suggested in the literature (Butler et al 1984, Scalbert 1991). It is noted that whilst this study showed a highly significant positive correlation between finger millet malt quality and total phenolics and amount of phenolic type, the cause and effect hypothesis that phenolics in finger millet grain play a positive role in its malt quality was not tested.

The contribution of finger millet grain phenolics to its antimicrobial activity, as indicated by the findings of this study, should be significant in food quality and safety. Pigmented finger millet grain types with higher contents of phenolic compounds relative to the light grain types could be deliberately selected for, as they would have good storage, malt quality due to the antifungal activity of their phenolics. There would be a low risk that these grain types would be contaminated with mycotoxins. The high-phenol finger millet grain types and their malts would preserve their quality during storage and marketing. The grains of high-phenol finger millet types would be highly suitable for storage as seed. As stated earlier, the prevailing humid and hot conditions in the tropical regions, including much of sub-Saharan Africa, are favourable for the proliferation of micro-organisms, including fungi, which cause grain deterioration and produce mycotoxins. In addition, as stated previously, the communities of these regions are

predominantly poor and thus can not afford modern and effective technologies for storing and processing food. The contribution of finger millet grain phenolics to its resistance to fungi should be, therefore, significant in food security and food safety for these communities.

It is particularly significant to note that the β-amylase activities of the high-phenol finger millet grain types were much higher than those of sorghum malts, although these β-amylase activities were much less than that of barley (chapter 2.2, Table 2.2.5). In order to save foreign exchange, some countries in Africa (e.g. Nigeria, Kenya and Zimbabwe) are now using sorghum to produce lager beers. Sorghum, unfortunately, generally has low levels of β-amylase (Taylor et al 2006). Because finger millet malt has a relatively higher β-amylase activity than that of sorghum malt, it could be a better substitute for barley malt in the brewing of lager beers. However, the economics of replacing barley with finger millet would have to be considered as will be discussed. Furthermore, finger millet has been found to be of superior quality for producing malt-based weaning foods compared to other cereals (pearl, foxtail, proso and barnyard millet, wheat, triticale, maize, rice and sorghum) (Malleshi and Desikachar 1986b). Finger millet was found easy to malt, the malt had high α-amylase activity that contributed to low viscosity, and the malt had desirable flavour and taste. Weaning foods produced from malts of the high-phenol finger millet grain types of this study would also have low viscosities as the malts had high α-amylase activity

The findings of this study suggest that phenolics in finger millet grain, particularly the tannins, impact negatively on the quality of composite wheat-finger millet cookies, with respect to cookie spread, texture and integrity (chapter 2.3). The finger millet phenolics also contribute to an objectionable dark colour in the cookies, especially in cookies substituted with a brown high-tannin finger millet, which appeared to reduce product. However, the finger millet phenolics, particularly the tannins, impact positively on the health-promoting potential of the composite cookies as they make a large contribution to their antioxidant activity. Thus, with respect to health-promoting potential, high-tannin finger millet grain types would be more suitable for making composite wheat-finger millet cookies than non-tannin types. It is noted that, as with the hypothesized positive role played by phenolics in finger millet grain in its malt quality, it was not proven that the presence of finger millet grain phenolics really promoted good health.

The quality of the cookies containing high-tannin finger millet grain could be improved. To improve the appearance acceptability of the dark composite cookies, they could be made in the form of "ginger nut type" cookies. Ginger type cookies are characteristically brown and are acceptable to consumers. The low spread factor of the composite cookies was thought to have been partly due to low levels of plant oils essential for cookie spread (chapter 2.3). Addition of vegetable plant oils could therefore increase cookie spread, as was found by Badi and Rooney (1976) working with sorghum and pearl millet. It was suggested (chapter 2.3) that fibre and large endosperm particles of the whole meal finger millet flour contributed to the grittiness of the composite cookies. The grittiness could be reduced by using refined finger millet flour, i.e. the fibre content and the particle size of the finger millet flour could be reduced by, for example, sieving. The problem with doing this is that the antioxidant activity would be reduced since, as stated earlier, finger millet phenolics are concentrated in the outer layers of the grain. Gums and thickeners, such as locust bean gum and guar gum, and special starches, such as rice, maize and potato starch and modified starches, and vegetable and dairy fat could be added to reduce the crumbliness and brittleness of the composite cookies and improve their surface texture (Badi and Rooney 1976, Schober et al 2003, Gallagher et al 2004). These materials could improve the quality of the cookies through various mechanisms including stabilising the dough and binding the dough components together similar to the action of gluten (Gallagher et al 2004).

Although composite wheat-finger millet cookies would be a suitable carrier of the finger millet grain phenolic antioxidants due to their shelf-stability and high nutrient density, other "health" products could also be made from high-tannin finger millet grain. The other products could include baked goods such as "health" high-tannin finger millet muffins and bread. The pigmented high-tannin finger millet grain would impart a brown colour to the products as with cookies, but similar to what was suggested for "ginger nut type" finger millet cookies above, some consumers might associate the brown products with health (Taylor et al 2006). In addition, as with "ginger nut type" cookies, consumers are familiar with brown muffins and brown bread. Brannan et al (2001) found brown muffins containing brown sorghum hybrids acceptable to a test panel. A large part of Sub-Saharan Africa is plagued by hunger and HIV/AIDS. Phenolic compounds have been reported to exhibit anti-HIV activity (Chen et al 1992, Chang et al 1994). The antioxidant-rich ("health") composite wheat-finger millet cookies of this study and other

"health" high-tannin finger millet products, some of which are suggested above, could be used to feed malnourished and HIV/AIDS-affected children in school feeding programmes. The "health" products could also be consumed by elderly people, as they are susceptible to degenerative diseases, which, as reviewed earlier (Halliwel et al 1995), are largely caused by free radical reactions.

3.3. Finger millet is a premium cereal grain for human food?

This section discusses, based mainly on the findings of this study, the merits and demerits of finger millet grain as a source of human food. The postulation "finger millet is a premium cereal grain for human food" is thus evaluated.

Finger millet grain has a lower fat content (chapters 2.2 and 2.3, Tables 2.2.7 and 2.3.2) relative to that of other cereals, e.g. wheat, barley, sorghum and pearl (Klopfenstein 2000, McDonough et al 2000). As discussed earlier, the low fat content of finger millet grain may be of advantage as its products would be relatively less susceptible to rancidity than products of a high-fat grain such as maize. This is particularly important for the storage quality of its food products because finger millet is generally processed into whole grain or whole grain meal products (US National Research Council 1996).

The findings (chapter 2.2) of this study indicate that finger millet grain phenolics exhibit antifungal activity. Thus, finger millet phenolics should contribute to reduction of grain quantity and quality loss during storage and to the safety of finger millet food products, as discussed earlier (chapter 2.2 and section 3.2).

Although research information could not be found, the literature (US National Research Council 1996, Malleshi 2004) suggests that finger millet grain is resistant to insect attack. The suggested resistance to insect attack may be partly attributed to the tiny size of the finger millet grain. An insect may not be able to complete its life cycle in the tiny grain, and/or tiny grains can pack tightly during storage leaving no spaces for insects (Malleshi 2004). It is also a possibility that phenolic compounds, particularly condensed tannins, contribute to the resistance of finger millet

grain to insect attack, as was found in groundnuts (Grayer et al 1992). The prevailing humid and hot conditions in the tropical regions, including much of sub-Saharan Africa, are also favourable for the proliferation of insects. Resistance to insect attack would, therefore, also contribute significantly to reduction of grain losses and enable storage of grain as seed and ultimately contribute to food security in sub-Saharan Africa.

The findings of this study indicate that finger millet grain is nutritionally superior to other cereal grains, e.g. wheat, rice, maize, barley and sorghum (Klopfenstein 2000), with respect to fibre (Tables 2.2.7 and 2.3.2) and mineral (Tables 2.2.7 and 2.3.2) contents. It was stated earlier that finger millet is generally consumed in the form of whole grain or whole meal products. The minerals and fibre that are concentrated in the outer layers (McDonough et al 2000) are therefore maximally taken in on consumption of finger millet products. The high fibre content of finger millet grain is thought to contribute to the slow digestibility of finger millet foods, which keeps the consumer full for a long period and may contribute to the lowering of glycaemic index (Malleshi 2004). Because cereal grain phenolics are largely associated with the grain fibre, it is currently thought that the fibre plays a significant role in delivering the potentially health-promoting phenolic compounds into the gut (Vitaglione et al 2008). However, it may be a disadvantage that finger millet grain is rich in both dietary fibre and minerals as the fibre may interfere with the absorption of minerals.

However, the findings of this study (chapters 2.2 and 2.3, Tables 2.2.8 and 2.3.3a) indicate that the quality of finger millet protein is poor, in terms of amino acid composition. Similarly, the protein quality, with respect to amino acid composition, of finger millet malts and composite wheat-finger millet cookies of this study was poor (chapters 2.2 and 2.3, Tables 2.2.8 and 2.3.3a). As already stated, lysine is particularly limiting in finger millet, as in other cereal grains. Nonetheless, the protein quality of finger millet grain is still considered better than that of most other cereal grains (subsection 1.2.1.2). However, as discussed earlier, finger millet should not be used as the only source of protein in infant and child foods. Finger millet should be combined with other food materials that have quality protein, such as legumes and dairy products.

The antioxidant activities (38.4-195.4 mM trolox equivalents/kg) (chapter 2.1, Table 2.1.2) of the finger millet grain types of this study are similar to those often reported for sorghums of varied genotype, for example 10.0-175.5 mM trolox equivalents/kg (Dykes et al 2005). Sripriya et al (1996) found the free radical activity of methanolic extracts from brown finger millet higher than methanolic extracts from rice, wheat and other millets (pearl and foxtail millet). The antioxidant property of cookies containing high-tannin finger millet was much superior to that of cake flour cookies and cookies containing a non-tannin finger millet and a variety of plant foods on the market (chapter 2.3). Finger millet grain has been shown, using model animals, to potentially attenuate degenerative diseases, which is thought to be largely due to the phenolic antioxidants (reviewed in section 1.2.5). Therefore, finger millet grain, particularly the high-tannin types, could be a highly suitable material for the production of "health" foods, as discussed previously.

The tiny size of the finger millet grain may be disadvantageous. The appearance of tiny grains may be unacceptable to consumers who are used to larger cereal grains like maize. Handling of the tiny finger millet kernels during quality control and food processing may be difficult. In this study, it was difficult to clean the finger millet grain, as it tended to block the sieve holes. A kernel counter used for counting sorghum kernels could not be used for counting finger millet kernels as more than one kernel passed through the orifice. As stated earlier, the "scratch test" may not be used to detect tannin finger millet types due to the tiny size of the grain. .The tiny size of finger millet grain makes its milling difficult (subsection 1.2.1.2). In industrial floor malting, finger millet and pearl millet are mixed with sorghum and malted together because if the tiny millet grains were malted alone they would pass or block the slotted malting floor (reviewed in subsection 1.2.6.2).

Although the findings of this study indicate that, due to their antifungal and antioxidant activity, finger millet phenolics impact positively on its malt quality and on its health-promoting potential, they may impact negatively on some of the food quality properties of the grain. The phenolics may impart unusual and hence unacceptable colours to some food products, as was the case with the composite wheat-finger millet cookies of this study. Phenolic compounds may impact negatively on the flavour of food. Although it was not the case with the composite wheat-finger millet cookies of this study, the finger millet tannins, in particular, may contribute to bitterness

and astringency. Phenolic compounds are known to inhibit the activity of enzymes by reacting with them and may thus exhibit antinutritional activity. Tannins may interfere with the utilisation of metal ions and proteins and polysaccharides by reacting with them (reviewed in section 1.2.2; Lule and Xia 2005). Finger millet tannins have been shown to reduce the *in vitro* protein digestibility (Ramachandra et al 1977), and crude phenolic extracts and individual phenolic compounds from finger millet have been shown to inhibit finger millet malt amylases (Chethan et al 2008). The results of this study showed a significant difference between the peptone and water extract DP in high-tannin finger millet grain types (chapter 2.2, Table 2.2.5), indicating that the tannins inhibited malt enzymes. This enzymic inhibition effect may be particularly significant if high-tannin finger millet types are malted traditionally at home without treatment with formaldehyde as is done during the industrial malting of sorghum (Daiber and Taylor 1995).

While finger millet can, by virtue of its being adapted to the harsh agro-climatic environments prevalent in sub-Saharan Africa, ensure food security and save foreign exchange, its economic value may be reduced by its low yield and by that, its production is laborious (Obilana and Manyasa 2002). The yield of finger millet, 0.8 mt/ha in 1989 (Obilana and Manyasa 2002) is much lower than that of maize, 1.3 mt/ha (Rohrbach 2003). High-yielding finger millet varieties should be developed. The market price of finger millet is generally higher than that of other cereals, e.g. wheat, maize and sorghum. For example, in Nakuru province, Kenya, the market prices of 1 kg of finger millet, wheat, maize and sorghum, respectively, were 50.00, 31.11, 23.33 and 38.89 Kenyan shillings (Kenya Ministry of Agriculture 2008: http://www.kilimo.go.ke). Thus, commercial products of finger millet may be less profitable than products of other cereals.

As summarised in Table 3.1, finger millet grain has several merits as a human food. The foregoing discussion and Table 3.1 indicate that the merits of finger millet grain outweigh its demerits and thus it is convincing that “finger millet is a premium cereal grain for human food”.

Table 3.1. Merits and demerits of finger millet grain as a human food

Aspect	Merit	Demerit
Grain size	The tiny size of the finger millet grain seems to contribute to its resistance to insect attack.	The appearance of tiny grains may be unacceptable. Tiny grains are hard to handle and process.
Grain colour	Finger millet grain colour, largely due to phenolics, varies from white to dark brown, and hence products of varied colour can be made from finger millet, as preferred by different consumers.	High-phenol, pigmented finger millet grain types may impart unacceptable colour in some foods, e.g. in some normally-light coloured baked goods.
Storage quality and safety	The lower fat content of finger millet grain relative to that of most other cereal grains makes it less susceptible to rancidity. Finger millet phenolics contribute to its antioxidant and antimicrobial properties, which impact positively on storage quality and safety.	Antioxidant and antimicrobial properties are significantly a property of the high-phenol grain types. Low-phenol, light coloured grain types may have lower storage quality and safety.
Health	Finger millet phenolics contribute to its antioxidant activity, which is potentially health-promoting.	Low-phenol, light coloured finger millet types exhibit low antioxidant activity, and hence have low health-promoting potential.
Impact on other functional properties	The high fibre content of finger millet grain may give “body” to its beverage products. Phenolics may contribute to flavour, e.g. bitterness and astringency, which may be traditionally acceptable to some consumers.	Finger millet does not contain gluten, which is required for quality baked goods. The high levels of fibre and phenolics, particularly tannins, may affect the quality negatively, e.g. texture, of finger millet products. Finger millet phenolics may impart objectionable flavour, e.g. bitterness and astringency, to food.
Nutritional quality	Finger millet is nutritionally superior when compared with most other cereal grains. It particularly has relatively higher dietary fibre and mineral contents than most other cereals.	The grain phenolics, particularly the tannins, may exhibit antinutrional activity and thereby reduce the nutritional value of finger millet.

4. CONCLUSIONS AND RECOMMENDATIONS

This study indicates that occurrence of tannins in finger millet grain is a varietal property, as in sorghum. Pigmented grain types have higher levels of phenolics than the light types, and types with a pigmented testa contain condensed tannins, which are located in the testa layer as in sorghum. This study seems to be the first to indicate the occurrence of anthocyanins and flavan-4-ols in finger millet grain. Phenolics in finger millet grain make a large contribution to its antioxidant activity and tannins are predominantly responsible for the antioxidant activity, as in sorghum. Therefore, high-tannin finger millet types have a high health-promoting potential as they exhibit high antioxidant activity.

Phenolics in finger millet grain seem to contribute significantly to its antifungal properties, which should be of importance in grain storage quality and safety. Finger millet types containing high levels of phenolics have considerably superior malt quality to finger millet types with low levels of phenolics, which is seemingly partly due to the antifungal activity of the phenolics.

Phenolics in finger millet grain, particularly the tannins, seem to have a negative effect on the spread, texture and integrity of composite wheat-finger millet cookies, probably by interacting with the wheat gluten proteins and thereby interfering with their functionality. Phenolics in finger millet grain impart a dark colour to the composite cookies, which decreases their acceptance by consumers. Cookies containing a high-tannin finger millet exhibit an appreciable antioxidant activity and therefore could be health-promoting.

The study indicates that finger millet is a valuable food crop. Finger millet is nutritionally superior to most other cereals, although its protein quality is, as that of other cereals, poor. Finger millet grain has a high health-promoting and good storage quality and safety potential. In addition, it is a good malting grain. However, apart from nutritional quality, the good food properties of finger millet grain identified in this study are largely due to its phenolics. Therefore, only the high-phenol grain types would be valuable with respect to these properties.

Further studies should be conducted to confirm the occurrence of 3-deoxyanthocyanin, apigeninidin and luteolinidin, and flavan-4-ols in finger millet grain. The phenolics in finger millet grain should be characterised in order to relate phenolic structure with antioxidant activity. Further work should be done to improve the quality of the composite wheat-finger millet cookies. A wide range of foods containing high-tannin finger millet grain types, particularly those that are normally dark coloured, such as brown bread and muffins (their appearance would be not or less affected by the colour of finger millet phenolics), should be developed to promote the consumption of these grain types, which have a high health-promoting potential. Studies should be done, in a cause effect way, to test the hypothesis that phenolics in finger millet grain play a positive role in finger millet malt quality and in human health.

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6. APPENDIX

Publications from this work

Siwela, M., Taylor, J.R.N., and Duodu, K.G. 2006. Location of tannins in finger millet grain. Microscopy Society of Southern Africa Proceedings 36: 43.

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