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).

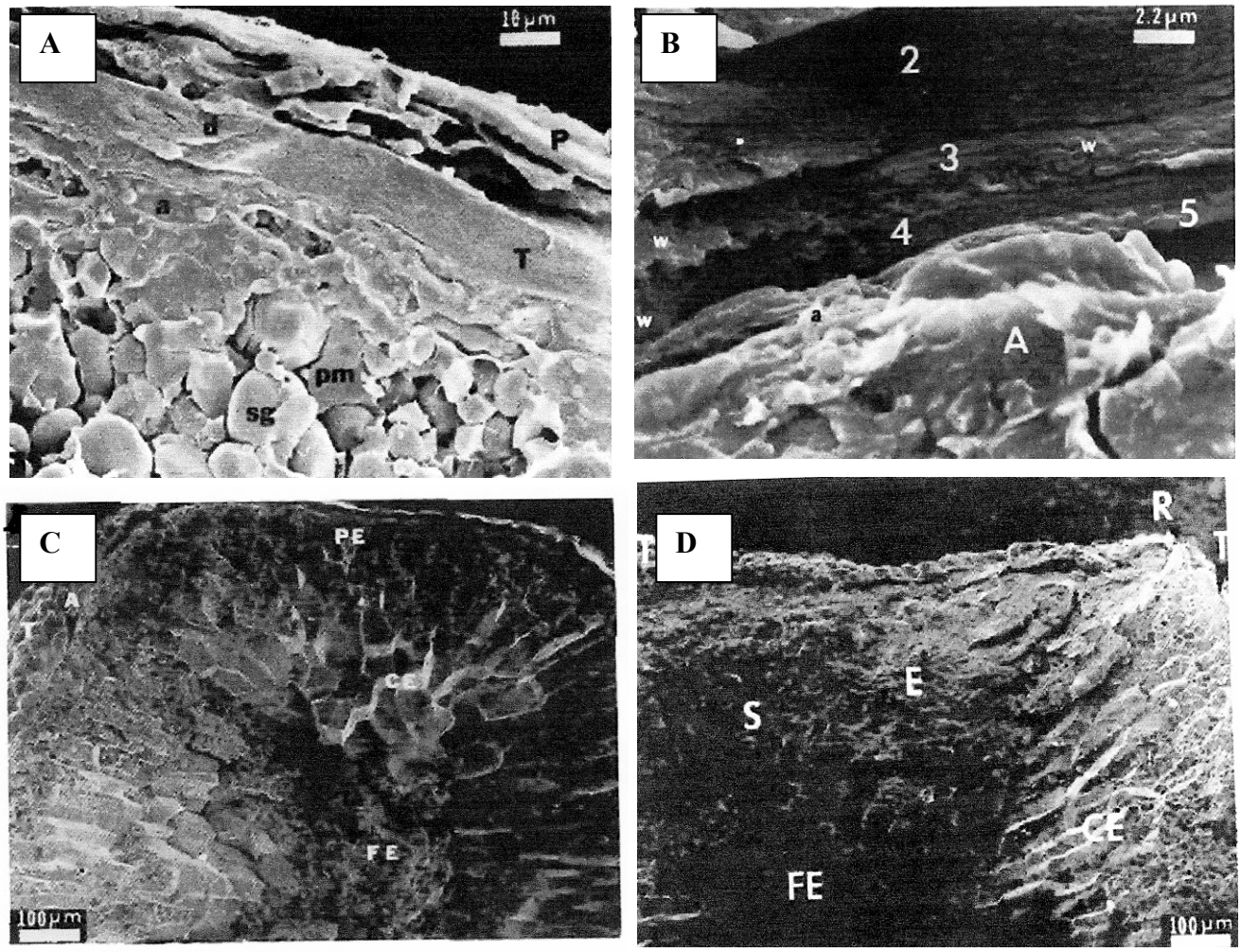

Figure 1.2.1. Structure of anatomical parts of the finger millet grain (McDonough et al 1986)

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)	-

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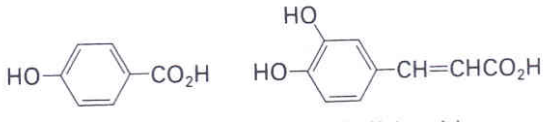

A B

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

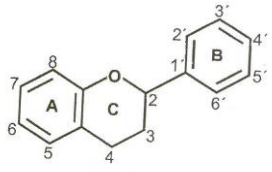

Figure 1.2.3. Basic structure and numbering system of flavonoids (Bravo 1998)

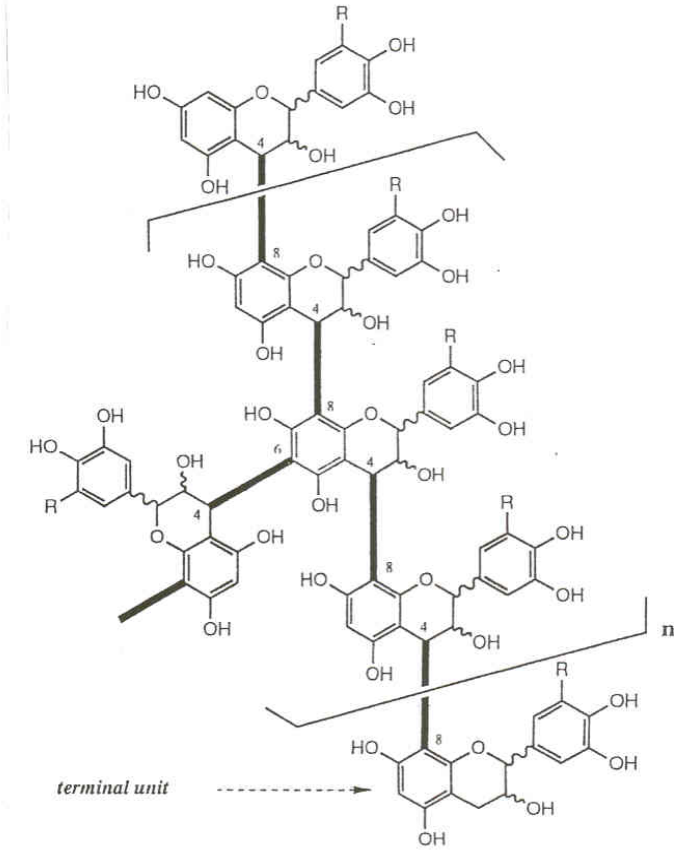

Figure 1.2.4. General structure for proanthocyanindin higher oligomers and polymers (Haslam 1998)

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.