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

[1] [1]Part of this work has been published: Siwela, M., Taylor, J. R.N., de Milliano, W. A.J., and Duodu, K.G. 2007. Occurrence and location of tannins in finger millet grain and antioxidant activity of different grain types. Cereal Chemistry 84: 169-174

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).

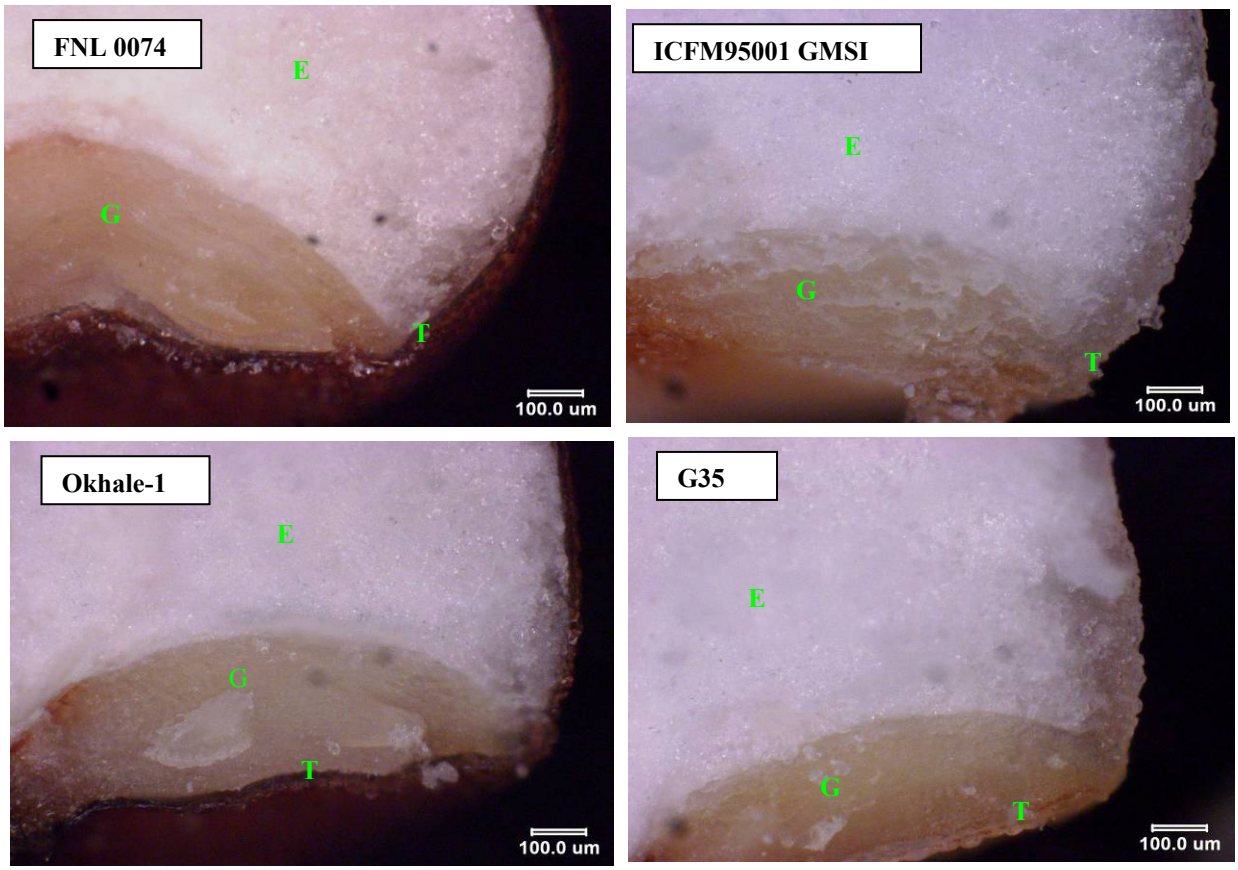

Figure 2.1.1. Light micrographs of finger millet kernels. FNL 0074 and Okhale-1, high-tannin; ICFM95001 GMSI and G35, low-tannin. E = endosperm; G = germ; T = testa.

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.

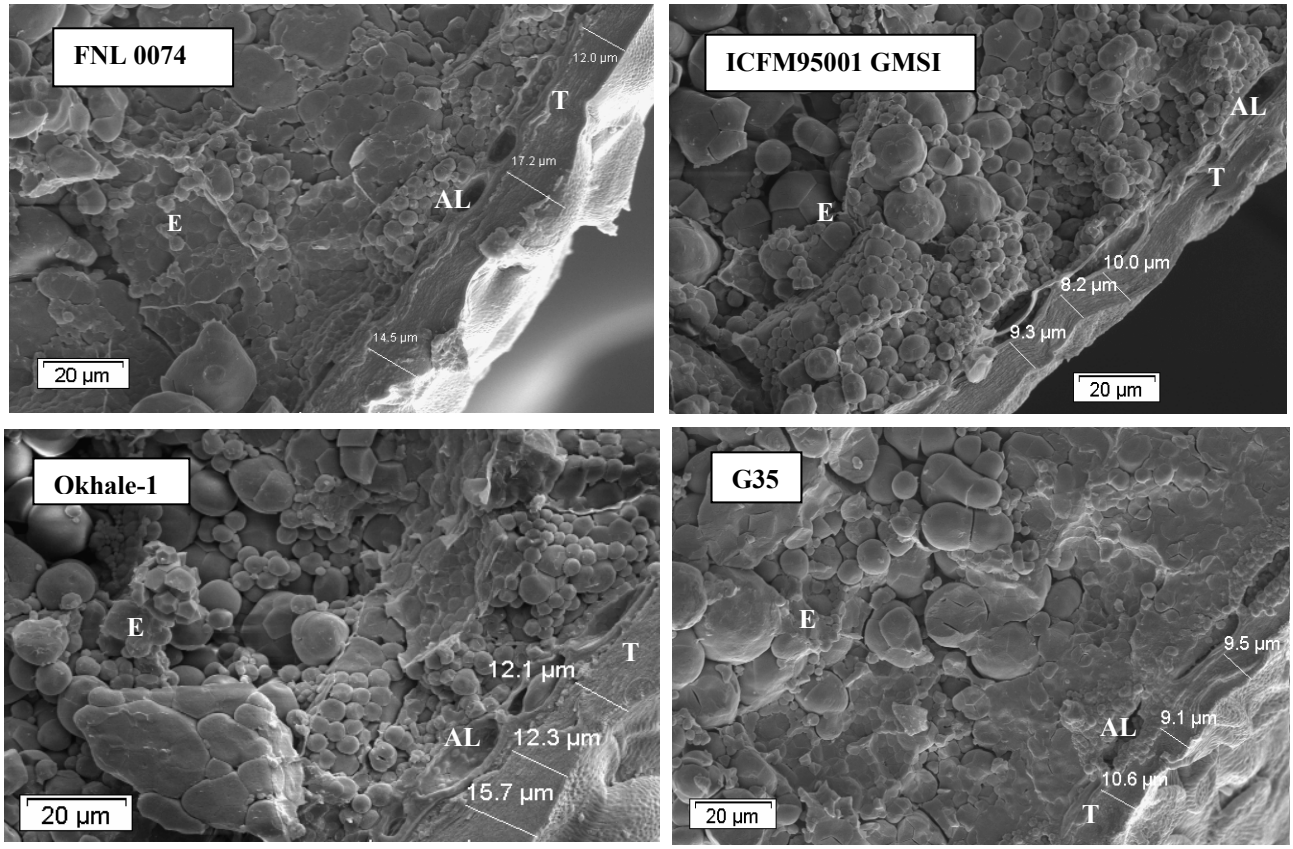

Figure 2.1.2. SEM of testa area of finger millet kernels. FNL 0074 and Okhale-1, high- tannin; ICFM95001 GMSI AND G35, low-tannin. AL = aleurone layer; E = endosperm; T = testa.

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.

2.1.6. References

Angold, R.E. 1979. Cereals and bakery products. Pages 75-138 in: Food Microscopy. R.G. Vaughan, ed. Academic Press: London.

Awika, J.M., and Rooney, L.W. 2004. Sorghum phytochemicals and their potential impact on human health. Phytochemistry 65: 1199-1221.

Awika, J.M., McDonough, C.M., and Rooney, L.W. 2005. Decorticating sorghum to concentrate healthy phytochemicals. Journal of Agricultural and Food Chemistry 53: 6230-6234.

Awika, J.M., Rooney, L.W., and Waniska, R.D. 2004. Properties of 3-Deoxyanthocyanins from sorghum. Journal of Agricultural and Food Chemistry 52: 4388-4394.

Awika, J.M., Rooney, L.W., Wu, X., Prior, R.L., and Cisneros-Zevallos, L. 2003b. Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. Journal of Agricultural and Food Chemistry 51: 6657-6662.

Beta, T., Rooney, L.W., Marovatsanga, L.T., and Taylor, J.R.N. 1999. Phenolic compounds and kernel characteristics of Zimbabwean sorghums. Journal of the Science of Food and Agriculture 79: 1003-1010.

Boren, B., and Waniska, R.D. 1992. Sorghum seed color as an indicator of tannin content. J. Applied Poultry Research 1: 117-121.

Bravo, L. 1998. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews 56:317-333.

Dicko, M.H., Gruppen, H., Traoré, A.S., Van Berkel, W.J.H., and Voragen, A.G.J. 2005. Evaluation of the effect of germination on phenolic compounds and antioxidant activities in sorghum varieties. Journal of Agricultural and Food Chemistry 53: 2581-2588.

Dykes, L., and Rooney, L.W. 2006. Sorghum and millet phenols and antioxidants. Journal of Cereal Science 44: 236-251.

Dykes, L., Rooney, L.W., Waniska, R.D., and Rooney, .W.L. 2005. Phenolic compounds and antioxidant activity of sorghum grains of varying genotypes. Journal of Agricultural and Food Chemistry 53: 6813-6818.

Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., and Riechel, T.L. 1998. High molecular weight plant polyphenols (tannins) as biological antioxidants. Journal of Agricultural and Food Chemistry 46: 1887-1892.

Hahn, D.H., and Rooney, L.W. 1986. Effect of genotype on tannins and phenols of sorghum. Cereal Chemistry 63:4-8.

Harris, H.B., and Burns, R.E. 1973. Relationship between tannin content of sorghum grain and preharvest seed molding. Agronomy Journal 65: 957-959.

Hegde, P.S., and Chandra, T.S. 2005. ESR spectroscopic study reveals higher free radical quenching potential in kodo millet (*Paspalum scrobiculatum*) compared to other millets. Food Chemistry 92: 177-182.

Hoseney, R.C. 1994. Principles of Cereal Science and Technology. 2nd edn. AACC International: St Paul, MN. pp. 1-27.

House, L.R. 1995. Sorghum and millets: History, taxonomy, and distribution. Pages 1-10 in: Sorghum and Millets: Chemistry and Technology. D.A.V. Dendy, ed. AACC International: St Paul, MN.

Hu, M., McClements, D.J., and Decker, E.A. 2004. Antioxidant activity of a proanthocyanidin-rich extract from grape seed in whey protein isolate stabilized algae oil-in-water emulsions. Journal of Agricultural and Food Chemistry 52: 5272-5276.

ICRISAT/FAO. 1996. The World Sorghum and Millet Economies: Facts, Trends and Outlook. ICRISAT/FAO: Patancheru, India/Rome, Italy. pp. 1-2, 31, 40.

Kaur, C., and Kapoor, H.C. 2001. Antioxidants in fruits and vegetables- the millennium's health. International Journal of Food Science and Technology 36: 703-725.

Maxson, E.D., Clark, L.E., Rooney, L.W., and Johnson, J.W. 1972. Factors affecting the tannin content of sorghum grain as determined by two methods of tannin analysis. Crop Science 12: 233-235.

McDonough, C.M., Rooney, L.W., and Earp, C.F. 1986. Structural characteristics of *Eleusine coracana* (finger millet) using scanning electron and fluorescence microscopy. Food Microstructure 5: 247-256.

Menkir, A., Ejeta, G., Butler, L., and Melake-Berhan, A. 1996. Physical and chemical kernel properties associated with resistance to grain mold in sorghum. Cereal Chemistry 73: 613-617.

McMillan, W.W., Wiseman, B.R., Burns, R.E., Harris, H.B., and Greene, L. 1972. Bird resistance in diverse germplasm of sorghum. Agronomy Journal 64: 821-822.

Murty, D.S., and Kumar, K.A. Traditional uses of sorghum and millets. 195. Pages 185-222 in: Sorghum and Millets: Chemistry and Technology. D.A.V. Dendy, ed. AACC International: St Paul, MN.

Obilana, A.B., and Manyasa, E. 2002. Millets. Pages 176-217 in: Pseudocereals and Less Common Cereals: Grain Properties and Utilization Potential. P.S. Belton and J.R.N. Taylor, eds. Springer-Verlag, Berlin.

Price, M.L., and Butler, L.G. 1977. Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. Journal of Agricultural and Food Chemistry 25: 1268-1273.

Price, M.L., Van Scoyoc, S., and Butler, L.G. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. Journal of Agricultural and Food Chemistry 26: 1214-1218.

Ramachandra, G., Virupaksha, T.K., and Shadaksharaswamy, M. 1977. Relationship between tannin levels and in vitro protein digestibility in finger millet (*Eleusine coracana* Gaertn.). Journal of Agricultural and Food Chemistry 25: 1101-1104.

Rice-Evans, C.A., Miller, N.J., and Paganga, G. 1997. Antioxidant properties of phenolic compounds. Trends in Plant Sciences 2: 152-159.

Riedl, K.M., and Hagerman, A.E. 2001. Tannin-protein complexes as radical scavengers and radical sinks. Journal of Agricultural and Food Chemistry 49: 4917-4923.

Scalbert, A., Manach, C., Morand, C., and Remesy, C. 2005. Dietary polyphenols and prevention of diseases. CRC Critical Reviews in Food Science and Nutrition 45: 287-306.

Serna-Saldivar, S., and Rooney, L.W. 1995. Structure and chemistry of sorghum and millets. Pages 69-124 in: Sorghum and Millets: Chemistry and Technology. D.A.V. Dendy, ed. AACC International: St Paul, MN.

Singleton, V.L., and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16: 144-158.

Sripriya, G., Chandrasekharan, K., Murty, V.S., and Chandra, T.S. 1996. ESR spectroscopic studies on free radical quenching action of finger millet (*Eleusine coracana*). Food Chemistry 57: 537-540.

Subba Rao, M.V.S.S.T., and Muralikrishna, G. 2002. Evaluation of the antioxidant properties of free and bound phenolic acids from native and malted finger millet (Ragi, *Eleusine coracana* Indaf-15). Journal of Agricultural and Food Chemistry 50: 889-892.

Taylor, J.R.N. 2001. Methods to be Used to Identify and Specify Characteristics Desired by Industrial Processors that use Sorghum as an Input. Technical Report #2. Task Order No. 4.1. USAID: Gaborone, Botswana.

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

Agarwal, V.K., and Sinclair, J.B. 1987. Principles of Seed Pathology, Vol. II. CRC Press: Boca Raton, FL. pp. 17-34.

Agu, R.C., and Palmer, G.H. 1999. Development of micro-organisms during the malting of sorghum. Journal of the Institute of Brewing 105: 101-106.

Alozie, T.C., Rotimi, C.N., and Oyibo, B.B. 1980. Production of *Aspergillus flavus* in some Nigerian indigenous beverages and foodstuffs. Mycopathologia 70: 125-128.

Arends, A.M., Fox, G.P. Henry, R.J. Marschke, R.J., and Symons, M.H. 1995. Genetic and environmental variation in the diastatic power of Australian barley. Journal of Cereal Science 21: 63-70.

Association of Official Analytical Chemists (AOAC). 2003. Method 923.03 Ash of flour-Direct method (first action 1923); Method 920.85 Fat (crude) or ether extract in flour (first action

1920); Method 968.06 Protein (crude) in animal feed- Dumas method. (First action 1968, final action 1969); Method 2002.04 Amylase-treated neutral detergent fiber in feeds using refluxing in beakers or crucibles (first action 2002). Official Methods of Analysis of the Association of Analytical Chemists International, 17th edn. AOAC International: Gaithersburg, ML.

Audilakshmi, S., Stenhouse, J.W., Reddy, T.P., and Prasad, M.V.R. 1999. Grain mould resistance associated characters of sorghum genotypes. Euphytica 107: 91-103.

Bandyopandhyay, R., Mughogho, L.K., and Prasado Rao, K.E. 1988. Sources of resistance to sorghum grain moulds. Plant Diseases 72: 504-508.

Beta, T., Rooney, L.W., and Waniska, R.D. 1995. Malting characteristics of sorghum cultivars. Cereal Chemistry 72: 533-538.

Beta, T., Rooney, L.W., Marovatsanga, L.T., and Taylor, J.R.N. 1999. Phenolic compounds and kernel characteristics of Zimbabwean sorghums. Journal of the Science of Food and Agriculture 79: 1003-1010.

Bhise, V.J., Chavan, J.K., and Kadam, S.S. 1988. Effects of malting on proximate composition and in vitro protein and starch digestibilities of grain sorghum. Journal of Food Science and Technology 25: 327-329.

Bidlingmeyer, B. A., Cohen, S. A., and Tarvin, T. L. 1984. Rapid analysis of amino acids using pre-column derivatization. Journal of Chromatography 336: 93-104.

Briggs, D.E. 1998. Malts and Malting. Blackie Academic and Professional: London. pp. 1, 165-184, 616-622.

Butler, L.G., Riedl, D.J. Lebryk, D.G., and Blytt, H.J. 1984. Interactions of proteins with sorghum tannin: mechanism, specificity and significance. Journal of the American Oil Chemists' Society 61: 916-920.

Chandrashekar, A., and Satyanarayana, K.V. 2006. Disease and pest resistance in grains of sorghum and millets. Journal of Cereal Science 44: 287-304.

Chavan, J.K., and Kadam, S.S. 1989. Nutritional improvement of cereals by sprouting. CRC Critical Reviews in Food Science and Nutrition 28: 401-437.

Chethan, S., and Malleshi, N.G. 2007. Finger millet polyphenols: optimization of extraction and the effect of pH on their stability. Food Chemistry 105: 862-870.

Chethan, S. Sreerama, Y.N., and Malleshi, N.G. 2008. Mode of inhibition of finger millet malt amylases by the millet phenolics. Food Chemistry (in press).

Christensen, C.M., and Kaufmann, H.H. 1974. Microflora. Pages 161-187 in: Storage of Cereal Grains and Their products. C.M. Christensen, ed. AACC International: St. Paul, MN.

Cole, J.C., and Cox, R.H. 1981. Handbook of Toxic Fungal Metabolites. Academic Press: New York. p.937.

Cowan, M.M. 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews 12:564-582.

Daiber, K.H. 1975a. Enzyme inhibition by polyphenols of sorghum grain and malt. Journal of the Science of Food and Agriculture 26: 1399-1411.

Daiber, K.H. 1975b. Treatment of cereal grain. South African patent 75/4957.

Daiber, K.H., and Taylor, J.R.N. 1995. Opaque beers. Pages 299-308 in: Sorghum and Millets: Chemistry and Technology. D.A.V. Dendy, ed. AACC International: St Paul, MN.

Dicko, M.H., Gruppen, H., Zouzouho, O.C., Traoré, A.S., Van Berkel, W.J.H., and Voragen, A.G.J. 2006. Effects of germination on the activities of amylases and phenolic enzymes in sorghum varieties grouped according to food end-use properties. Journal of the Science of Food and Agriculture 86: 953-963.

D'Mello, J.P.F., and Macdonald, A.M.C. 1997. Mycotoxins. Animal Feed Science and Technology 69: 155-166.

D'Mello, J.P.F., Placinta, C.M., and Macdonald, A.M.C. 1999. Fusarium mycotoxins: review of global implications for animal health, welfare and productivity. Animal Feed Science and Technology 80: 183-205.

Dufour, J.P., Mélotte, L., and Srebrnik, S. 1992. Sorghum malts for the production of a lager beer. Journal of American Association of Brewing Chemists 50: 110-118.

Dewar, J., Taylor, J.R.N., and Joustra, S.M. 1995. Accepted Methods of Sorghum Malting and Brewing Analyses. Methods 1 and 4. CSIR Food Science and Technology: Pretoria.

Domsch, K.H., Gams, W., and Anderson, T-H. 1980. Compendium of Soil Fungi, Vol 1 and 2. Academic Press: London. 859 pp. and 405 pp.

Dykes, L., and Rooney, L.W. 2006. Sorghum and millet phenols and antioxidants. Journal of Cereal Science 44: 236-251.

Glennie, C.W. 1983. Polyphenol changes in sorghum grain during malting. Journal of Agricultural and Food Chemistry 31: 1295-1299.

Gomez, M.I. 1994. Preliminary studies on grain quality evaluation for finger millet as a food and beverage use in the Southern African Region. Pages 289-286 in: Advances in Small Millets.

K.W. Riley, S.C. Gupta, A. Seetharam and J.N. Mushonga, eds. International Science Publisher: New York.

Gyllang, H., and Martinson, E. 1976. Studies on the mycoflora of malt. Journal of the Institute of Brewing 83: 350-352.

Flannigan, B. 1996. The mycoflora of barley and malt. Pages 83-125 in: Brewing Microbiology, 2nd edn. F.G. Priest and I. Campbell, eds. London: Chapman and Hall.

Hahn, D.H., Faubion, J.M., and Rooney, L.W. 1983. Sorghum phenolic acids, their high performance liquid chromatography separation and their relation to fungal resistance. Cereal Chemistry 60: 255-259.

Harborne, J.B. 1994. Do natural plant phenols play a role in ecology? Acta Horticulturae 381: 36-43.

Harris, H.B., and Burns, R.E. 1973. Relationship between tannin content of sorghum grain and preharvest seed moulding. Agronomy Journal 65: 957-959.

Hussein, H.S., and Brasel, J.M. 2001. Toxity, metabolism, and impact of mycotoxins on humans and animals. Toxicology 167: 101-134.

ICRISAT/FAO. 1996. The World Sorghum and Millet Economies: Facts, Trends and Outlook. ICRISAT/FAO: Patancheru, India/Rome, Italy. pp. 1-2, 31, 40.

Jambunathan, R, Butler, L.G., Bandyopandhyay, R., and Mughogho, L.K. 1986. Polyphenol concentrations in grain, leaf, and callus tissues of mold-susceptible and mold-resistant sorghum cultivars. Journal of Agricultural and Food Chemistry 34: 425-429.

Jambunathan, R, Kherdekar, M.S., and Bandyopandhyay, R. 1990. Flavan-4-ols concentration in mold-susceptible and mold-resistant sorghum at different stages of grain development. Journal of Agricultural and Food Chemistry 38: 545-548.

Kambal, A.E., and Bate-Smith, E.C. 1976. A genetic and biochemical study on pericarp pigments in a cross between two cultivars of *Sorghum bicolor*. Heredity 37: 413-417.

Kent, N.L., and Evers, A.D. 1994. Kent's Technology of Cereals, 4th edn. Elsevier Science: Oxford, UK. pp. 279-280.

Kruger, J.E. 1976. Changes in polyphenoloxidases of wheat during kernel growth and maturation. Cereal Chemistry 53: 201-212.

Lefyedi, M.L. Marais, G.J. Dutton, M.F., and Taylor, J.R.N. 2005. The microbial contamination, toxicity and quality of turned and unturned outdoor floor malted sorghum. Journal of the Institute of Brewing 111: 190-196.

Leslie, J.F., and Summerell, B.A. 2006. The Fusarium Laboratory Manual. Blackwell Publishing: Ames, IA.

Lovelace, C.E.A., and Nyathi, C.B. 1977. Estimation of the fungal toxins zearalenone and aflatoxin, contaminating opaque beer in Zambia. Journal of the Science of Food and Agriculture 28: 288-292.

Lorenz, K. 1980. Cereal spouts: Composition, nutritive value, food applications. CRC Critical Reviews in Food Science and Nutrition 13: 353-385.

Mathewson, P.R., and Seabourn, B.W. 1983. A new procedure for specific determination of ß-amylase in cereals. Journal of Agricultural and Food Chemistry 31: 1322-1326.

Malleshi, N.G., and Desikachar, H.S.R. 1986a. Influence of malting conditions on quality of finger millet malt. Journal of the Institute of Brewing 92: 81-83.

Malleshi, N.G., and Desikachar, H.S.R. 1986b. Studies on the comparative malting characteristics of some tropical cereals and millets. Journal of the Institute of Brewing 96: 174-176.

Malleshi, N.G., and Klopfenstein, C.F. 1998. Nutrient composition and amino acid and vitamin contents of malted sorghum, pearl millet, finger millet and their rootlets. International Journal of Food Science and Nutrition 49: 415-422.

Malleshi, N.G., Desikachar, H.S.R., and Tharanathan, R.N. 1986. Free sugars and non-starchy polysacharides of finger millet (*Eleucine coracana*), pearl millet (*Pennisetum typhoideum*), foxtail millet (*Setaria italica*) and their malts. Food Chemistry 20: 253-261.

Mbithi-Mwikya, S., Van Camp, J., Yiru, Y., and Huyghebaert, A. 2000. Nutrient and antinutrient changes in finger millet (*Eleucine coracana*) during sprouting. Lebensmittel-Wissenschaft + I.E. Und Technologie 33: 9-14.

Mbithi-Mwikya, S., Ooghe, W., Van Camp, J., Ngudi, D., and Huyghebaert, A. 2000. Amino acid profiles after sprouting, autoclaving, and lactic acid fermentation of finger millet (*Eleucine coracana*) and kidney beans (*Phaseolus vulgaris* L.). Journal of Agricultural and Food Chemistry 48: 3081-3085.

McCleary, B.V., and Codd, R. 1989. Measurement of ß-amylase in cereal flours and commercial enzyme preparations. Journal of Cereal science 9: 17-33.

McCleary, B.V., and Sheehan, H. 1987. Measurement of cereal α-amylase: A new assay procedure. Journal of Cereal Science 6: 237-251.

McDonough, C.M., Rooney, L.W., and Serna-Saldivar, S.O. 2000. The millets. Pages 177-195 in: Handbook of Cereal Science and Technology, 2nd edn. K, Kulp and J.G., Ponte, Jr., eds. Marcel Dekker: New York.

Melake-Berhan, A., Butler, L.G., Ejeta, G., and Menkir, A. 1996. Grain mold resistance and phenol accumulation in sorghum. Journal of Agricultural and Food Chemistry 44: 2428-2434.

Menkir, A., Ejeta, G., Butler, L., and Melake-Berhan, A. 1996. Physical and chemical kernel properties associated with resistance to grain mold in sorghum. Cereal Chemistry 73: 613-617.

Munar, M.J., and Sebree, B. 1997. Gushing- A maltster's view. Journal of the American Society of Brewing Chemists 55: 119-122.

Murty, D.S., and Kumar, K.A. Traditional uses of sorghum and millets. 195. Pages 185-222 in: Sorghum and Millets: Chemistry and Technology. D.A.V. Dendy, ed. AACC International: St Paul, MN.

Noots, I, Delcour, J.A., and Michiels, C.W. 1999. From field barley to malt: Detection and specification of microbial activity for quality aspects. Critical Reviews in Microbiology 25: 121-153.

Nout, M.J.R., and Davies, B.J. 1982. Malting characteristics of finger millet, sorghum and barley. Journal of the Institute Brewing 88: 157-163.

Novellie, L. 1960. Kaffircorn malting and brewing studies. V. Occurrence of β-amylase in kaffir corn malts. Journal of the Science of Food and Agriculture 8: 457-463.

Odhav, B., and Naicker, V. 2002. Mycotoxins in South African traditionally brewed beers. Food Additives and Contaminants 19: 55-61.

Okoye, Z.S.C. 1987. Stability of zearalenone in naturally contaminated corn during Nigerian traditional brewing. Food Additives and Contaminants 4: 57-59.

O'Sullivan, T.F., Walsh, Y., O'Mahony, A., Fitzgerald, G.F., and Van Sinderen, D. 1999. A comparative study of malthouse and brewhouse microflora. Journal of the Institute of Brewing 105: 55-61.

Pandey, K.N., and Mehrotra, B.S. 1985. Effect of fungal metabolites on germination and sprouting of ragi seeds. Acta Phytopathologica Academiae Scientiarum Hungaricae 20:183-191.

Pitt, J.I. 2001. Toxigenic *Penicillium* species. Pages 467-480 in: M.P., Doyle, L.R., Beuchat and T.J., Montville, eds. Food Microbiology: Fundamentals and Frontiers. ASM Press: Washington, DC.

Rabie, C.J., and Lübben, A. 1984. The mycoflora of sorghum malt. South African Journal of Botany 4: 251-255.

Rabie, C.J., Lübben, A, Marais, G.J., and Van Vuuren, H.J. 1997. Enumeration of fungi in barley. International Journal of Food Microbiology 35: 117-127.

Rabie, C.J., Van Rensburg, S.J. Van Der Watt, J.J., and Lubben, A. 1975. Onyalai- the possible involvement of a mycotoxin produced by *Phoma sorghina* in the aetiology. South African Medical Journal 49: 1647-1650.

Ratnavathi, C.V., and Sashidhar, R.B. 2003. Substrate suitability of different genotypes of sorghum in relation Aspergillus infection and aflatoxin production. Journal of Agricultural and Food Chemistry 51: 3482-3492.

Ravindran, G. 1992. Seed proteins of millets: Amino acid composition, proteinase inhibitors and *in-vitro* protein digestibility. Food Chemistry 44: 13-17.

Rohn, S., Rawel, H.M., and Kroll, J. 2002. Inhibitory effects of plant phenols on the activity of selected enzymes. Journal of Agricultural and Food Chemistry 50: 3566-3571.
Scalbert, A. 1991. Antimicrobial properties of tannins. Photochemistry 30: 3875-3883.

Schapira, F.D, Whitehead, M.P., and Flannigan, B. 1989. Effects of the mycotoxins diacetoxyscirpenol and deoxynivalenol on malting characteristics of barley. Journal of the Institute of Brewing 95: 415-417.

Schwarz, P.B., Beattie, S., and Casper, H.H. 1996. Relationship between *Fusarium* infestation of barley and the gushing potential of malt. Journal of the Institute of Brewing 102: 93-96.

Seetharam, A., and Ravikumar, R.L. 1994. Blast resistance in finger millet- its inheritance and biochemical nature. Pages 449-465 in: Advances in Small Millets. K.W. Riley, S.C. Gupta, A. Seetharam and J.N. Mushonga, eds. International Science Publisher: New York.

Shobana, S., and Malleshi, N.G. 2007. Preparation and functional properties of decorticated finger millet (*Eleusine coracana*). Journal of Food Engineering 79: 529-538.

Shukla, S.S. Gupta, O.P., Swarkar, N.J., Tomar, A.K., and Sharma, Y.K. 1986. Malting quality of ragi varieties: nutrient and mineral composition of their malts. Journal of Food Science and Technology 23: 235-237.

South African Bureau of Standards. 1970. SABS Method 235. Standard test method for the determination of the diastatic power of malts prepared from kaffircorn (sorghum) including bird-proof varieties, and from millet. South African Bureau of Standards: Pretoria.

Sweeney, M.J., and Dobson, A.D.W. 1998. Mycotoxin production by *Apergillus*, *Fusarium* and *Penicillium* specis. International Journal of Food Microbiology 43: 141-158.

Taylor, J.R.N., and Dewar, J. 2000. Fermented products: Beverages and porridges. Pages 751-795 in: Sorghum: Origin, History, Technology and Production: C.W. Smith and R.A. Frederiksen, eds. John Wiley and Sons: New York.

Taylor, J.R.N., and Robbins, D.J. 1993. Factors influencing beta-amylase activity in sorghum malt. Journal of the Institute of Brewing 99: 413-416.

Trinder, D.W. 1988. A survey of aflatoxins in industrially brewed South African sorghum beer and beer strainings. Journal of the Institute of Brewing 95: 307-309.

US National Research Council. 1996. Lost crops of Africa. Volume 1. Grains. National Academy Press: Washington, DC. pp. 39-57.

Veerabhadrappa, P.S., Manjunath, N.H., and Virupaksha, T.K. 1978. Proteinase inhibitors of finger millet. Journal of the Science of Food and Agriculture 29: 353-358.

Viswanath, V., Urooj, A., and Malleshi, N.G. 2009. Evaluation of antioxidant and antimicrobial properties of finger millet polyphenols (Eleusine coracana). Food Chemistry 114: 340-346.

Waniska, R.D., Poe, J.H., and Bandyopandhyay, R. 1989. Effects of weathering on phenolic acids in sorghum caryopses. Journal of Cereal Science 10: 217-225.

Waniska, R.D., Venkatesha, R.T., Chandrashekar, A., Krishvaneni, S., Bejosano, F.P. Jeoung, J., Jayaraj, J., Muthukrishnan, S., and Liang, G.H. 2001. Antifungal proteins and other mechanisms in the control of sorghum stalk rot and grain mold. Journal of Agricultural and Food Chemistry 49: 4732-4742.

World Health Organization. 1985. Energy and Protein Requirements: Report of a Joint FAO/WHO/UNU Expert Consultation (Technical Report Series 724). WHO: Geneva. pp. 120-124.

Ziegler, P. 1999. Cereal beta-amylases. Journal of Cereal Science 29: 195-204.

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).

.

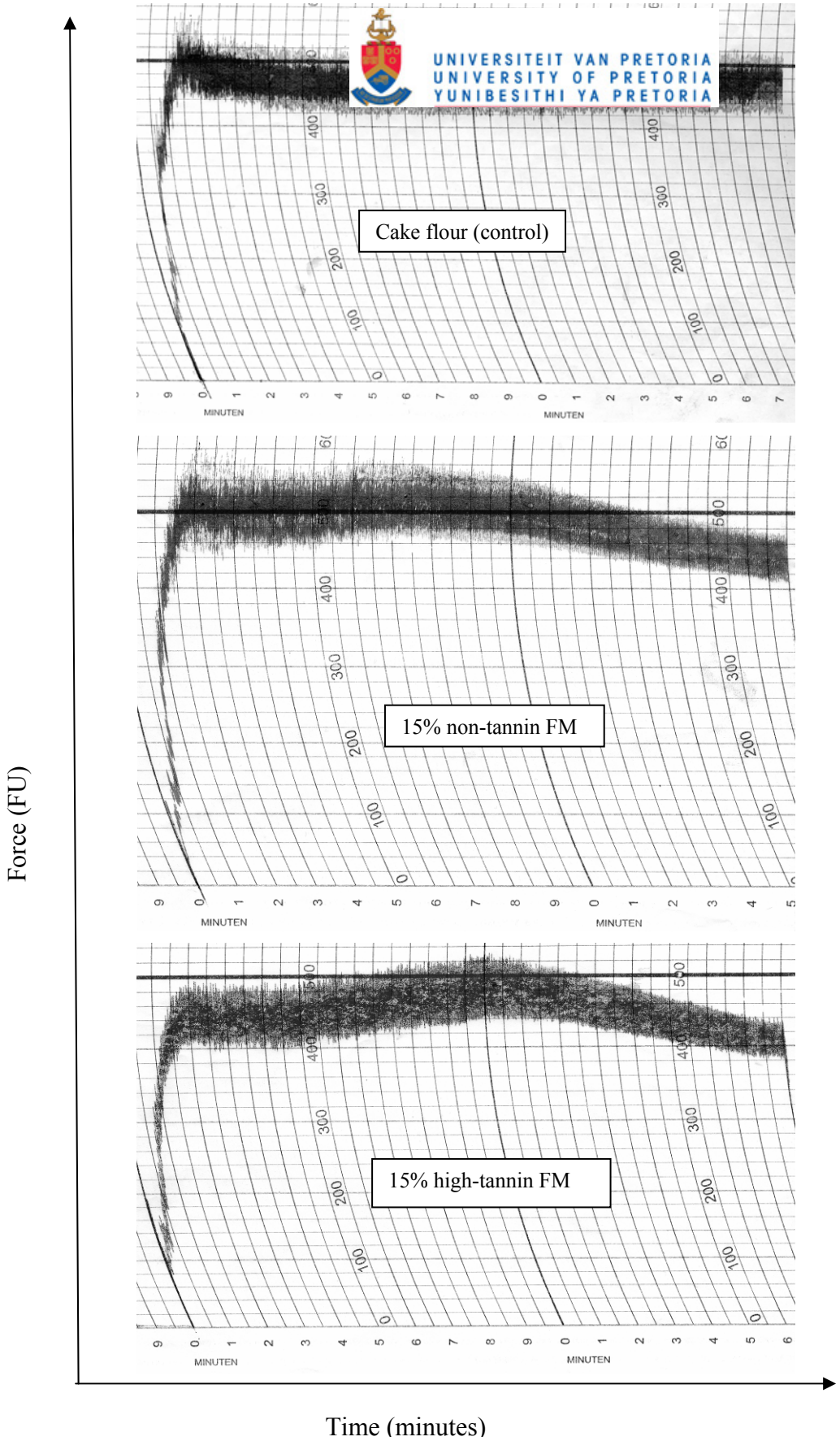

Figure 2.3.1. Effect of finger millet substitution level on the rheological properties of composite wheat-finger millet (FM) doughs. FU, farinograph units.
Non-tannin FM= ICFM 95001 GMSF; high-tannin FM= FMV6.

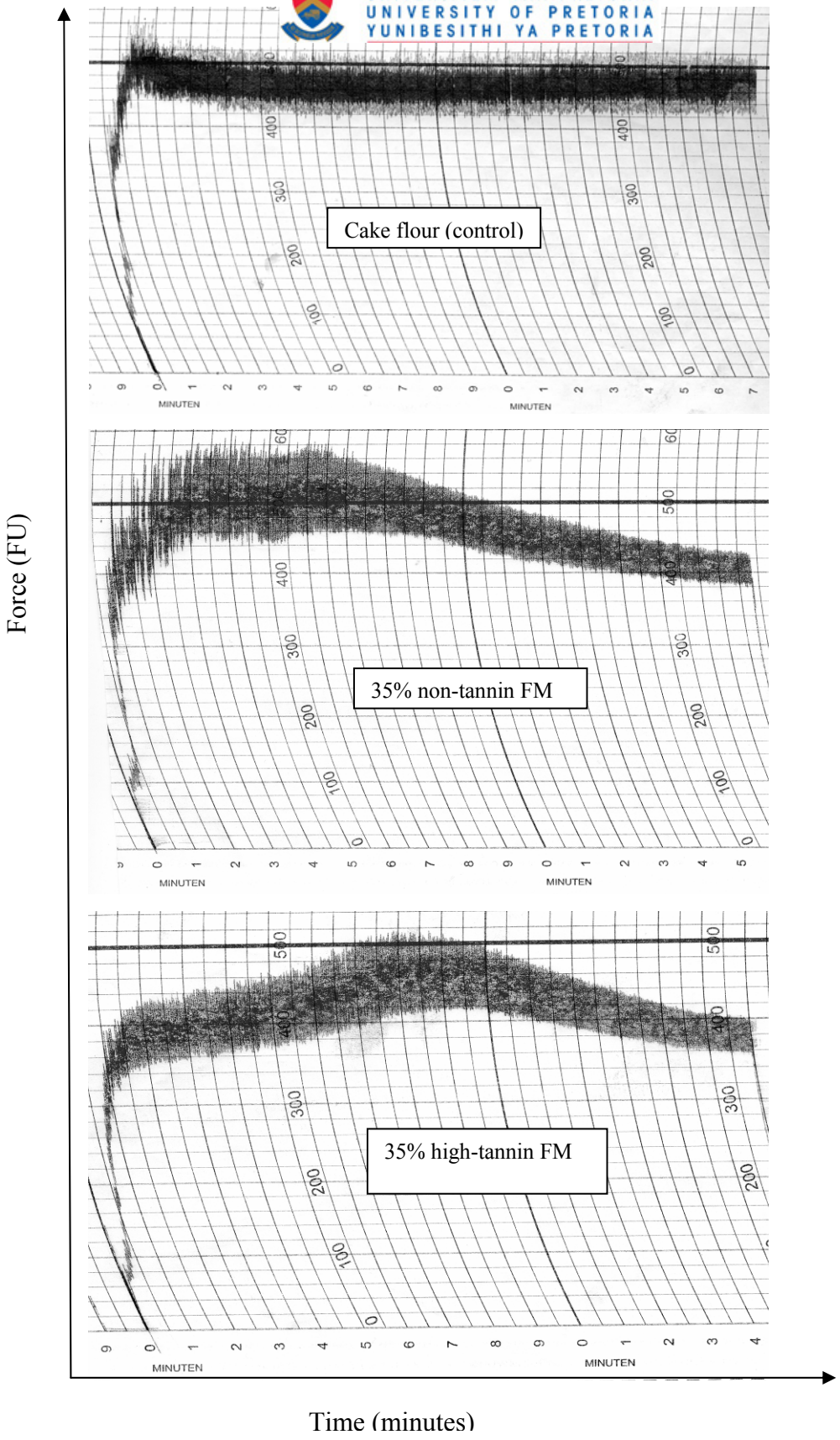

Figure 2.3.1. Continued.

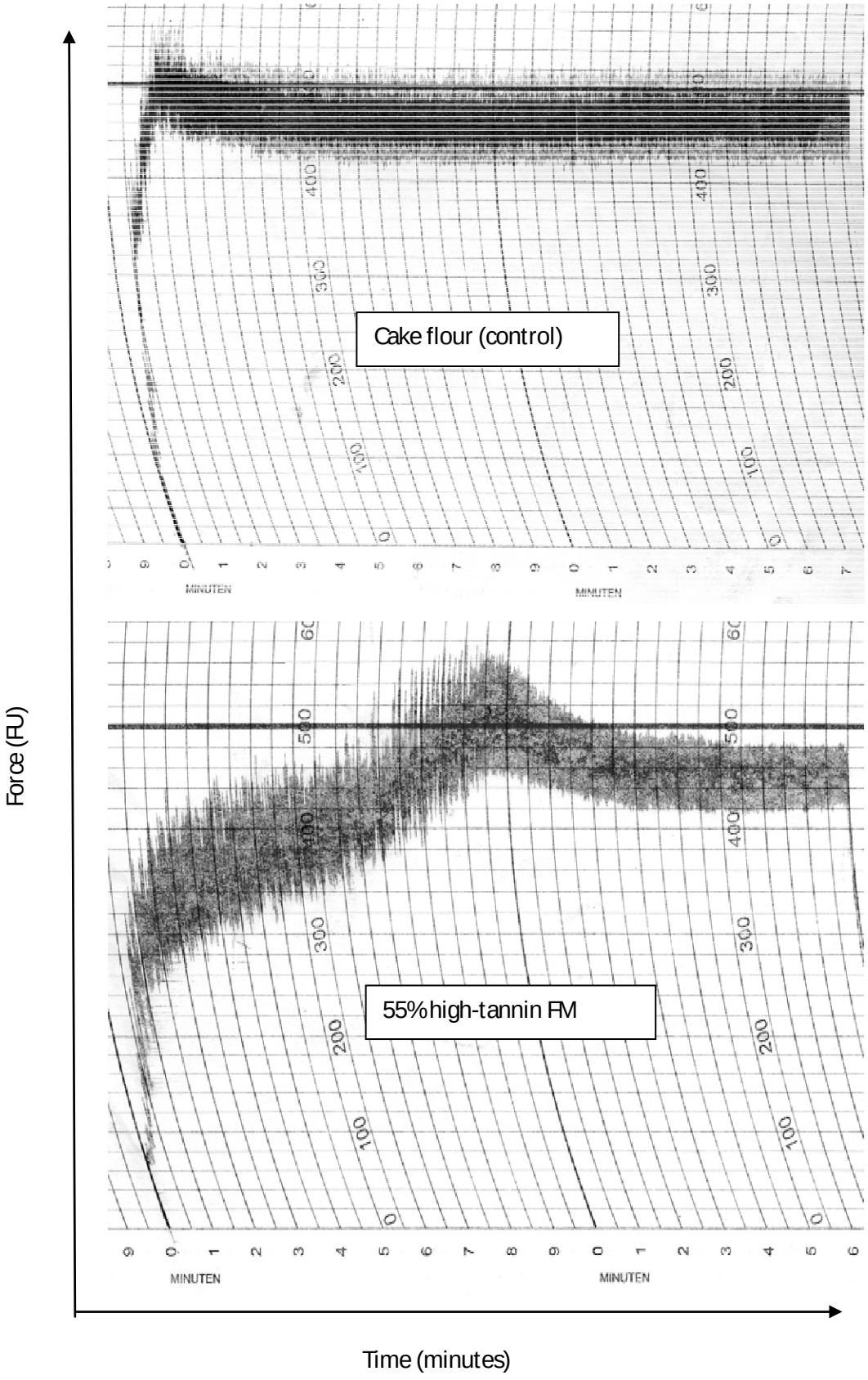

Figure 2.3.1. Further continued.

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

Adom, K.K., Sorrels, M.E., and Liu, R.H. 2005. Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. Journal of Agricultural and Food Chemistry 53: 2297-2306.

American Association of Cereal Chemists. 2000. Approved Methods. 10th edn. AACC method 10-50D Baking quality of cookie flour. AACC International: St Paul, MN.

Anon. 1991. Foods and Cookery, 2nd edn. Department of Education: Pretoria. p 215.

Association of Official Analytical Chemists (AOAC). 2003. Method 923.03 Ash of flour-Direct method (first action 1923); Method 920.85 Fat (crude) or ether extract in flour (first action 1920); Method 968.06 Protein (crude) in animal feed- Dumas method. (First action 1968, final action 1969); Method 2002.04 Amylase-treated neutral detergent fiber in feeds using refluxing in beakers or crucibles (first action 2002). Official Methods of Analysis of the Association of Analytical Chemists International, 17th edn. AOAC International: Gaithersburg, ML.

Awika, J.M., McDonough, C...M., and Rooney, L.W. 2005. Decorticating sorghum to concentrate healthy phytochemicals. Journal of Agricultural and Food Chemistry 53: 6230-6234.

Awika, J.M., Dykes, L., Gu, L., Rooney, L.W., and Prior, R.L. 2003. Processing of sorghum (*Sorghum bicolor*) and sorghum products alters procyanidin oligomer and polymer distribution and content. Journal of Agricultural and Food Chemistry 51: 5516-5521.

Awika, J.M., Rooney, L.W., Wu, X., Prior, R.L., and Cisneros-Zevallos, L. 2003. Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. Journal of Agricultural and Food Chemistry 51: 6657-6662.

Badi, S.M., and Hoseney, R.C. 1976. Use of sorghum and pearl millet flours in cookies. Cereal Chemistry 53: 733-738.

Baublis, A.J., Clydesdale, F.M., and Decker, E.A. 2000. Antioxidants in wheat-based breakfast cereals. Cereal Foods World 45: 71-74.

Bidlingmeyer, B. A., Cohen, S. A., and Tarvin, T. L. 1984. Rapid analysis of amino acids using pre-column derivatization. Journal of Chromatography 336: 93-104.

Biliaderis, C.G., Izydorczyk, M.S., and Rattan, O. 1995. Effect of arabinoxylans on bread-making quality of wheat flours. Food Chemistry 5:165-171.

Bressa, F., Tesson, N., Rosa, M.D., Sensedoni, A., and Tubaro, F. 1996. Antioxidant effect of Maillard reaction products: application to a butter cookie of a competition kinetics analysis. Journal of Agricultural and Food Chemistry 44: 692-695.

Bryngelsson, S., Dimberg, L.H., and Kamal-Eldin, A. 2002. Effects of commercial processing on levels of antioxidants in oats (*Avena sativa* L.). Journal of Agricultural and Food Chemistry 50: 1890-1896.

Dewanto, V., Wu, X., and Liu, R.H. 2002. Processed sweet corn has higher antioxidant activity. Journal of Agricultural and Food Chemistry 50: 4959-4964.

Dlamini, N.R., Taylor, J.R.N., and Rooney, L.W. 2007. The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. Food Chemistry 105: 1412-1419.

Drewnowski, A., and Gomez-Carneros, C. 2000. Bitter taste, phytonutrients, and the consumer: A review. American Journal of Clinical Nutrition 72: 1424-1435.

Duh, P-D., Yen, G-C., Yen, W-J., and Chang, L-W. 2001. Antioxidants effects of water extracts from barley (*Hordeum vulgare L.*) prepared under different roasting temperatures. Journal of Agricultural and Food Chemistry 49: 1455-1463.

Dykes, L., and Rooney, L.W. 2006. Sorghum and millet phenols and antioxidants. Journal of Cereal Science 44: 236-251.

Emmambux, N.M., and Taylor, J.R.N. 2003. Sorghum kafirin interaction with various phenolic compounds. Journal of the Science of Food and Agriculture 83: 402-407.

Goesaert, H., Brijs, K., Veraverbeke, W.S., Courtin, C.M., Grubruers, K., and Delcour, J.A. 2005. Wheat flour constituents: How they impact on bread quality, and how to impact their functionality. Trends in Food Science and Technology 16: 12-30.

Glover, J.M., Walker, C.E., and Mattern, P.J. 1986. Functionality of sorghum flour components in a high ratio cake. Journal of Food Science 51: 1280-1283.

Griffith, T., and Johnson, J. 1957. Relation of the browning reaction to storage stability of sugar cookies. Cereal Chemistry 34: 159-169.

Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., and Riechel, T.L. 1998. High molecular weight plant polyphenols (tannins) as biological antioxidants. Journal of Agricultural and Food Chemistry 46: 1887-1892.

Hamama, A.A., and Nawar, W.W. 1991. Thermal decomposition of some phenolic compounds. Journal of Agricultural and Food Chemistry 39: 1063-1069.

Hoseney, R.C. 1994. Principles of Cereal Science and Technology, 2nd edn. :AACC International: St Paul, MN. pp 41-42, 197-201, 275, 285-290.

International Association for Cereal Science and Technology (ICC). 1972. Method for using the Brabender Farinograph. Standard No. 115/1 (approved 1972; revised 1992), Standard Methods of the ICC. Vienna.

Kent, N.L., and Evers, A.D. 1994. Kent's Technology of Cereals, 4th edn. Elsevier Science: Oxford, UK. pp.3, 177-178, 279-280, 292.

Kigugawa K., Kunugi, A., and Kurechi, T. 1990. Chemistry and implications of degradation of phenolic antioxidants. Pages 65-85 in: Food Antioxidants. B.J.F. Hudson, ed. Elsevier Science: Oxford, UK.

Kissel, L.T., Pomeranz, Y., and Yamazaki, W.T. 1971. Effects of flour lipids on cookie quality. Cereal Chemistry 48: 655-662.

Lesschaeve, I., and Noble, A.C. 2005. Polyphenols: Factors influencing their sensory properties and their effects on food and beverage preferences. American Journal of Clinical Nutrition 81: 330S-335S.

Liyana-Pathirana, C.M., and Shahidi, F. 2007. The antioxidant potential of milling fractions from breadwheat and durum. Journal of Cereal Science 45: 238-247.

McDonough, C.M., Rooney, L.W., and Serna-Saldivar, S.O. 2000. The millets. Pages 177-195 in: K. Kulp and J.G. Ponte, Jr., eds. Hand book of Cereal Science and Technology, 2nd edn. Marcel Dekker: New York.

Miller, H.E., Rigelholf, F., Marquart, L., Prakash, A., and Kanter, M. 2000. Whole-grain products and antioxidants. Cereal Foods World 45: 59-63.

Morad, M.M., Doherty, C.A., and Rooney, L.W. 1984. Effect of sorghum variety on baking properties of U.S. conventional bread, Egyptian pita "balady" bread and cookies. Journal of Food Science 49: 1070-1074.

Nicoli, M.C., Anese, M., and Parpinel, M. 1999. Influence of processing on the antioxidant properties of fruit and vegetables. Trends in Food Science and Technology 10: 94-100.

Obilana, A.B., and Manyasa, E. 2002. Millets. Pages 196-198 in: Pseudocereals and Less Common Cereals: Grain Properties and Utilization Potential. P.S. Belton and J.R.N Taylor, eds. Springer-Verlag: Berlin.

Onyeneho, S.N., and Hettiarachchy, N.S. 1992. Antioxidant activity of durum wheat bran. Journal of Agricultural and Food Chemistry 40: 1496-1500.

Pomeranz, Y. Shogren, M.D. Finney, K.F., and Bechtel D.B. 1977. Fibre in breadmaking-Effects on functional properties. Cereal Chemistry 54: 25-41.

Price, M.L., Van Scoyoc, S., and Butler, L.G. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. Journal of Agricultural and Food Chemistry 26: 1214-1218.

Ramachandra, G., Virupaksha, T.K., and Shadaksharaswamy, M. 1977. Relationship between tannin levels and in vitro protein digestibility in finger millet (*Eleusine coracana* Gaertn.). Journal of Agricultural and Food Chemistry 25: 1101-1104.

Rao, P.U. 1994. Valuation of protein quality of brown and white ragi (*Eleucine coracana*) before and after malting. Food Chemistry 51: 433-436.

Riedl, K.M., and Hagerman, A.E. 2001. Tannin-protein complexes as radical scavengers and radical sinks. Journal of Agricultural and Food Chemistry 49: 4917-4923.

Salukhe, D.K., Jadhav, S.J., Kadam, S.S., and Chavan, J.K. 1982. Chemical, biochemical, and biological significance of polyphenols in cereals and legumes. CRC Critical Reviews in Food Science and Nutrition 17: 277-305.

Scalbert, A., Manach, C., Morand, C., and Remesy, C. 2005. Dietary polyphenols and prevention of diseases. CRC Critical Reviews in Food Science and Nutrition 45: 287-306.

Schober, T.J., O'Brien, C.M., McCarthy, D. Darnedde, A., and Arendt, E. 2003. Influence of gluten-free flour mixes and fat powders on the quality of gluten-free biscuits. European Food Research and Technology 216: 369-376.

Selvaraj, A., Balasubrahmanyam, N., and Rao, P.H. 2002. Packaging and storage studies on biscuits containing finger millet (Ragi) flour. Journal of Food Science and Technology 39: 66-68.

Serna-Saldivar, S., and Rooney, L.W. 1995. Structure and chemistry of sorghum and millets. Pages 69-124 in: Sorghum and Millets: Chemistry and Technology. D.A.V. Dendy, ed. AACC International: St Paul, MN.

Singleton, V.L., and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16: 144-158.

Sripriya, G., Chandrasekharan, K., Murty, V.S., and Chandra, T.S. 1996. ESR spectroscopic studies on free radical quenching action of finger millet (*Eleusine coracana*). Food Chemistry 57: 537-540.

Subba Rao, M.V.S.S.T., and Muralikrishna, G. 2002. Evaluation of the antioxidant properties of free and bound phenolic acids from native and malted finger millet (Ragi, *Eleusine coracana* Indaf-15). Journal of Agricultural and Food Chemistry 50: 889-892.

Sudha, M.L., Vetrimani, R., and Leelavathi, K. 2007. Influence of fibre from different cereals on the rheological characteristics of wheat flour and biscuit quality. Food Chemistry 100: 1365-1370.

Sudha, M.L., Vetrimani, R., and Rahim, A. 1998. Quality of vermicelli from finger millet (*Eleusine coracana*) and its blend with different milled wheat fractions. Food Research International 31: 99-104.

Taylor, J.R.N., Schober, T.J., and Bean, S.R. 2006. Novel food and non-food uses of sorghum and millets. Journal of Cereal Science 44: 252-271.

Thompson, L.U. 1994. Antioxidants and hormone-mediated health benefits of whole grains. Critical Reviews in Food Science and Nutrition 34: 473-497.

Towo, E.E., Svanberg, U., and Ndossi, G.D. 2003. Effect of grain pre-treatment on different extractable phenolic groups in cereals and legumes commonly consumed in Tanzania. Journal of the Science of Food and Agriculture 83: 980-986.

Wang, M., Hamer, R.J., Van Vliet, T., Gruppen, H., Marseille, H., and Weegels, P.L. 2003. Effect of water unextractable solids on gluten formation and properties: mechanistic considerations. Journal of Cereal Science 37: 55-64.

World Health Organization. 1985. Energy and Protein Requirements: Report of a Joint FAO/WHO/UNU Expert Consultation (Technical Report Series 724). WHO: Geneva. pp. 120-124.