Nutritional composition, antioxidant activity and bioaccessibility of phenolic compounds in selected wild cereal and pseudo-cereal grains found in Zimbabwe

By

Kudakwashe Chitindingu

Thesis submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy in Biochemistry

November 2014

Natural Products Group
Department of Biochemistry
Faculty of Science
University of Zimbabwe
P.O. Box MP167
Mt Pleasant
Harare
Zimbabwe
Abstract

The nutritional composition of five wild and two domesticated cereal grains was determined using standard analytical methods. It was hypothesised that wild cereal and pseudo cereal grains found in Zimbabwe contained macro and micronutrients which are beneficial to humans. The wild cereal grains that were used in the study were *Brachiaria brizantha*, *Panicum maximum*, *Rottboellia cochinchinensis*, *Sorghum arundinaceum* and *Amaranthus hybridus*, a pseudo cereal was studied. The domesticated cereal grains used were *Eleusine corocana* and a red variety of *Sorghum bicolor*. Samples were collected from fields in Harare and some in Buhera, a district in Manicaland province of Zimbabwe. Phenolic compounds were extracted from the cereal grains and were quantified. The phenolics were characterised, the antioxidant properties studied and the bioaccessibility of the phenolic compounds was determined using a gastrointestinal model system assay.

Macronutrients determined were proteins, carbohydrates and fats. Minerals were determined as well. *A. hybridus*, a wild pseudo-cereal, had the highest protein and fat content of 21.44 ± 0.05 % and 11.50 ± 0.03 % respectively, compared to all the other cereal grains. *B. brizantha* had the highest fibre content of 30.43 ± 0.01 % while the red variety of *S. bicolor* had the least fibre content of 2.51 ± 0.07 %. The values were comparable to those reported elsewhere for traditional cereal grains. Phosphorus was detected in all cereal grains studied. Calcium was detected in all cereal grains except in red variety of *S. bicolor*. The mineral values obtained were lower than those reported elsewhere for traditional cereal grains like wheat.

*E. corocana* and *S. arundinaceum* had significantly higher total phenolic compounds than all other cereal grains studied. *S. arundinaceum* had the highest concentration of total flavanoids while *A. hybridus* had the lowest. The highest amounts of proanthocyanidins were determined in *S. arundinaceum* with 12.2 ± 0.08 % followed by *S. bicolor* with 4.6 ± 0.03 % proanthocyanidins content. The HPLC method was used to tentatively identify the constituent phenolic compounds in the cereal grain extracts. Ferulic acid and p-coumaric acid were detected in all cereal samples. Caffeic acid, catechin, gallic acid, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, protocatechuic acid, quercetin, syringic acid and vanillic acid were detected but were not common to all the samples.

The ability of the cereal grains to quench the DPPH radical was assayed. *E. corocana* and *S. arundinaceum* had the highest ability and statistically, there was a positive correlation between the concentration of phenolic compounds and the ability to scavenge for DPPH radicals. The ability of extracts to reduce ferrous ions increased as the amount of extract added was increased. Cereal grain extracts were found to delay/halt lipid peroxidation and the extracts of all the cereal grains were also found to prevent the bleaching of β-carotene to varying extents.

The bioaccessibility of phenolic compounds was generally high for all cereal grains. *A. hybridus* had the highest intestinal bioaccessibility percentage of 95.4 ± 0.01 % while the cereal with the lowest intestinal bioaccessibility was *R. cochinchinensis* with 81.85 ± 0.03 %.

The research work demonstrated the importance of wild cereal and pseudo cereal grains as a potential source of nutrition and industrially utilisable natural products.
ACKNOWLEDGEMENTS

It would not have been possible to write this thesis without the help and support of the kind people around me, to only some of whom it is possible to give particular mention here. Foremost I would like to express my deepest gratitude to my supervisor and advisor Prof. M. Muchuweti for the continuous support of my Ph.D study and research, for her patience, motivation, enthusiasm, and immense knowledge. Her guidance helped me during the research process and writing of this thesis. I could not have imagined having a better advisor and mentor for my Ph.D study. Thank you Amai!!! You are a true example indeed.

The good advice and support of my second supervisor, Prof. M.A.N Benhura has been invaluable on both an academic and a personal level, for which I am extremely grateful. With his enthusiasm, his inspiration, and his great efforts to explain things clearly and simply, he helped to make research fun for me. His support, guidance, advice throughout the research, as well as his pain-staking effort in proof-reading the drafts, are greatly appreciated. Thank you Mukanya, Vhuzijena, mahomu homu anopona nekuba!!!!

Every result described in this thesis was accomplished with the help and support of fellow labmates Michael Bhebhe, Batsirai Chipurura and Mr Kasiyamhuru. Thank you for your constructive contributions, the laughs and the criticisms that have made me grow over the years. I salute you guys.

I am happy to have worked with Dr Ashwell Ndhlala, Chipo Mupure and Tapiwa Chinaka. Thank you guys for the time we spent together in the lab. I will always remember you.

To the entire staff in the Biochemistry department, my deepest thanks for the support. Keep up the good work!!!

I would also like to thank my family for the support they provided me through my entire life. I would not have contemplated this road were it not for my parents, Isaac and Pheona, who instilled within me a love of creative pursuits, science and language, all of which find a place in this thesis. To my parents, thank you. My siblings, Ethel, Tinashe, Stany and Tawonga have also been the best of friends along this journey. To my sister Ethel, may God bless you for your unwavering support through thick and thin. To my brothers Tinashe, Stany and Tawonga, thank you for your encouragement and support.
This research would not have been possible without the financial assistance from the University of Zimbabwe Research Board, WF Kellog’s Foundation, DFID and The German Academic Exchange programme (DAAD).

Most importantly I would like to thank God for the wisdom and perseverance that he has bestowed upon me during this research period, and indeed, throughout my life: "I can do everything through him who gives me strength." (Philippians 4: 13).
Contents

Chapter 1 .................................................................................................................................................. 1
  1.1 Introduction....................................................................................................................................... 1
  1.2 Research question .......................................................................................................................... 5
    1.2.1 Hypotheses .................................................................................................................................. 5
  1.3 Aims and objectives ....................................................................................................................... 5
    1.3.1 Specific objectives .................................................................................................................... 6
  2.1 Oxidative stress ............................................................................................................................... 7
    2.2.1 Classification of antioxidants .................................................................................................. 15
    2.2.2 Uses and potential applications of antioxidants .................................................................. 17
    2.3.1 Classification of phenolic compounds .................................................................................... 21
    2.3.2 Biosynthesis of polyphenols .................................................................................................. 37
    2.3.3 Mechanism of antioxidant activity of polyphenols ............................................................... 40
    2.3.4 Commercially available antioxidants ..................................................................................... 42
  2.4 Bioaccessibility .................................................................................................................................. 42
    2.4.1 Phenolic compounds bioaccessibility and bioavailability ...................................................... 44
    2.4.2 Antioxidant release and bioaccessibility ................................................................................ 48
  2.5 Cereals ............................................................................................................................................... 49
    2.5.1 Cereals as natural sources of antioxidants ............................................................................ 50
    2.5.2 Phenolic compounds from cereal grains .................................................................................. 51
    2.5.3 Distribution of phenolics in cereal grains ................................................................................ 55
    2.5.4 Cereals found in Zimbabwe ..................................................................................................... 56
  2.6 Methods in assaying for phenolic compounds ............................................................................... 68
    2.6.1 Methods of extracting phenolic compounds ......................................................................... 69
    2.6.2 High Performance Liquid Chromatography (HPLC) ............................................................ 70
    2.6.3 Classification of antioxidant methods ...................................................................................... 70

Chapter 3 .................................................................................................................................................. 78
  3.0 Materials and methods ................................................................................................................... 78
  3.1 Collection of samples ..................................................................................................................... 78
  3.2 Determination of moisture ............................................................................................................. 81
3.3 Extraction of total phenolic compounds ........................................... 81
3.4 Extraction of simple phenolic acids for HPLC analysis .................. 81
    3.4.1 Analysis of phenolic compounds by HPLC .......................... 82
3.5 Determination of phenolic compounds using the Folin Ciocalteu assay ................................................................. 83
3.6 Vanillin assay for flavanoids ....................................................... 83
3.7 Determination of proanthocyanidin using the butanol-HCl assay ...... 83
3.8 Antioxidant activity assays.......................................................... 84
    3.8.1 Determination of the ability to scavenge DPPH radicals .......... 84
    3.8.2 Reducing power ............................................................... 85
    3.8.3 Ability to chelate metal ions .............................................. 85
3.9 Antioxidant capacity in model systems ......................................... 86
    3.9.1 Ability to inhibit phospholipid peroxidation ....................... 86
    3.9.2 Ability to prevent oxidation of β-carotene .......................... 86
3.10 Nutritional analysis ..................................................................... 87
    3.10.1 Determination of ash ......................................................... 87
    3.10.2 Determination of crude protein ......................................... 87
    3.10.3 Determination of crude fibre ............................................. 89
    3.10.5 Determination of calcium and phosphorus ......................... 90
3.11 Bioaccessibility assays ............................................................... 92
    3.11.1 In vitro physiological approach ......................................... 93
        3.11.1.1 Determination of indigestible fraction ....................... 93
        3.11.1.2 In vitro colonic fermentation .................................... 95
    3.11.2 Chemical approach: Determination of polyphenol content .... 98
    3.11.4 Extractable polyphenols ................................................... 98
        3.11.5. Non-extractable polyphenols ................................. 99
Chapter 4 ......................................................................................... 101
4.0 Results and discussion .............................................................. 101
4.1 Moisture content ....................................................................... 101
4.2 Nutritional composition ............................................................. 104
    4.2.1 Protein ........................................................................... 106
    4.2.2 Fibre ............................................................................. 107
4.2.3 Fat................................................................................................................................. 108
4.2.4 Minerals: ................................................................................................................... 109
  4.2.4.1 Calcium and Phosphorus .................................................................................... 109
4.3 Total phenolic content ............................................................................................... 113
4.4 Tentative identification of Phenolic compounds using HPLC ..................... 116
4.5 Vanillin assay for flavanoids ..................................................................................... 122
4.6 Butanol-HCL assay for condensed tannins (proanthocyanidins) ................. 124
4.7 Scavenging activity on the DPPH radical ................................................................. 127
4.8 Reducing power of extracts of cereal grains ......................................................... 129
4.9 Chelating abilities of plant extracts on ferrous ions .............................................. 133
4.10: Inhibition of phospholipids peroxidation assay .................................................. 136
4.11 Antioxidant activity in the β-carotene bleaching assay ........................................ 140
4.12: Bioaccessibility of phenolic compounds from cereal grains ...................... 146
  4.12.1: Effect of digestive enzymes on the bioaccessibility of phenolic compounds from selected cereal grains........................................................ 146
  4.12.2: Bioaccessibility in the small intestines ............................................................ 154
  4.13.4: Total Intestinal Bioaccessibility ...................................................................... 160
  4.13.5: Non-extractable polyphenols ........................................................................... 161
Chapter 5 ............................................................................................................................. 164
5.0 Conclusions .................................................................................................................. 164
6.0 References ....................................................................................................................... 167
7.0 Appendices ..................................................................................................................... 196
Appendix 1: HPLC chromatograms of Brachiaria brizantha, Rottboellia cochinchinensis, Panicum maximum, Amaranthus hybridus, Sorghum arundinaceum, Sorghum bicolor and Eleusine corocana........................................... 196
Appendix 2: Publications arising from this thesis ......................................................... 200
List of figures

**Figure 2.1**: Some of the disorders caused by oxidative stress to the human health............................................................... 8

**Figure 2.1.1.1**: The defense network *in vivo* against oxidative stress................. 9

**Figure 2.3.1.1**: Chemical structures of a) salicylic alcohol, b) gentisinic alcohol, c) coniferyl alcohol and d) sinapic alcohol................................................................. 23

**Figure 2.3.1.2**: Chemical structures of compounds from which phenolic acids are derived.................................................................................................................. 24

**Figure 2.3.1.3.1**: General chemical structure of condensed tannins............... 25

**Figure 2.3.1.3.2**: The chemical structure of Penta galloyl-D-glucose........ 26

**Figure 2.3.1.4**: Chemical structure of resveratrol........................................ 27

**Figure 2.3.1.5.1**: General chemical structure of flavonols.......................... 29

**Figure 2.3.1.5.2**: Structure of flavones.................................................. 30

**Figure 2.3.1.5.3**: Chemical structure of flavanones.................................. 31

**Figure 2.3.1.5.4**: Structure of flavan-3-ols............................................. 32

**Figure 2.3.1.5.5**: Chemical structure of anthocyanidins............................. 33

**Figure 2.3.1.5.1.6**: The general chemical structure of isoflavones............. 34

**Figure 2.3.1.5.7**: The chemical structures of the chalcones; phloretin and xanthohumol.................................................................................................................. 35

**Figure 2.3.1.5.8**: The general chemical structure of a dihydrochalcone..... 36

**Figure 2.3.1.5.9**: General chemical structure of dihydroflavonols.......... 37

**Figure 2.3.2**: Biosynthesis of hydroxycinnamic acids, hydroxybenzoic acids and flavonoids................................................................................................................ 39

**Figure 2.3.3** Antioxidant activity to structure relationship of flavonoids...... 41

**Figure 2.4.1.1**: General human bioabsorption of phenolic compounds contained in beverages or similar foods poor in fiber................................................................. 47
Figure 2.4.1.2: General human bio-absorption of phenolic compounds contained in foods rich in dietary fiber ................................................................. 48

Figure 2.5.4.1.1: *Amaranthus hybridus* ......................................................................................................................... 57

Figure 2.5.4.1.2: *Rottboellia cochinchinensis* .................................................................................................................. 60

Figure 2.5.4.1.3: *Panicum maximum* .......................................................................................................................... 62

Figure 2.5.4.1.4: *Brachiaria brizantha* .......................................................................................................................... 63

Figure 2.5.4.1.5: *Sorghum arundiceum* .......................................................................................................................... 64

Figure 2.5.4.2.1: *Sorghum bicolor* (red variety) .............................................................................................................. 66

Figure 2.5.4.2.2: *Eleusine corocana* ............................................................................................................................. 68

Figure 3.11.1: Schematic diagram of the method used to determine the intestinal bioaccessibility of dietary polyphenols ......................................................................................................................... 97

Figure 3.11.2: Schematic diagram of the method used to determine the intestinal bioaccessibility of cereal grain polyphenols ......................................................................................................................... 98

Figure 4.4.1: HPLC chromatogram of phenolic compound standards ..................................................................................... 117

Figure 4.4.2: HPLC chromatogram of *Panicum Maximum* ................................................................................................. 119

Fig 4.7.1: Antioxidant scavenging effects of cereal methanolic extracts .................................................................................. 129

Figure 4.8.1: The reducing power of the cereal methanolic extracts ....................................................................................... 131

Figure 4.8.2: The reducing power of the cereal methanolic extracts ....................................................................................... 132

Figure 4.10.1: Inhibition of phospholipid peroxidation ......................................................................................................... 137

Figure 4.10.2: Inhibition of phospholipid peroxidation ......................................................................................................... 138

Figure 4.11.1: The rate of bleaching of β-carotene by extracts of cereal grains ........................................................................ 141

Figure 4.11.2: Antioxidant activity of extracts of cereal extracts .......................................................................................... 142

Fig 4.12.1.1: The absorbances of phenolic compounds released from the grain matrix as a result of action by pepsin ................. 147
Figure 4.12.1.2: The absorbances of phenolic compounds released from the grain matrix as a result of action by pancreatin.......................................................... 149

Figure 4.12.1.3: The absorbances of phenolic compounds released from the grain matrix as a result of action by lipase.......................................................... 151

Figure 4.12.1.4: The absorbances of phenolic compounds released from the grain matrix as a result of action by α – amylase.......................................................... 152

Figure 4.12.1.5: The absorbances of phenolic compounds released from the grain matrix as a result of action by amyloglucosidase.................................................. 153

Figure 4.12.2.1: Estimated percentage bioaccessibility of phenolic compounds in the small intestine........................................................................................................ 156

Figure 4.13.3.1: Estimated percentage bioaccessibility of phenolic compounds in the colon.................................................................................................................. 158

Figure 4.13.4.1: Estimated total percentage bioaccessibility of phenolic compounds in the intestines........................................................................................................ 160
LIST OF TABLES

Table 2.1.1: Some Reactive Oxygen and Nitrogen species of biological interest................................................................. 11

Table 2.5.2.1: Some phenolic acids reported in cereal grains................................................................. 53

Table 2.5.2.2: Flavonoids reported in cereal grains........................................................................... 54

Table 3.1: Cereal grains used in the study........................................................................ 80

Table 4.1.1: The moisture content in the cereal grains......................................................... 101

Table 4.2.1: Results of proximate composition analyses on samples of selected cereal........................................................................ 105

Table 4.2.2: The Ca/P ratio of selected wild and domesticated cereal grains........................................................................ 112

Table 4.3.1: The amount of total phenolic compounds in selected wild and domesticated cereal grains........................................................................ 114

Table 4.4.1: Phenolic compounds detected in selected cereal grains using HPLC........................................................................ 118

Table 4.5.1: Levels of flavanoid content in selected wild and domesticated cereal grains........................................................................ 123

Table 4.6.1: Levels of condensed tannins in selected wild and domesticated cereal grains........................................................................ 125

Table 4.9.1: Ability of extracts to chelate ferrous ions......................................................... 134

Table 4.11.1: Antioxidant activity of extracts of wild and domesticated cereal grains........................................................................ 144

Table 4.12.2.1: (A) The total phenolic compounds determined from the undigested samples and (B) the total phenolic compounds determined in digested sample........................................................................ 155

Table 4.13.3.1: A) Total phenolics in unfermented sample and B) Total phenolics in fermented sample........................................................................ 159

Table 4.13.5.1: The percentage of condensed tannins determined in the residue obtained after colonic........................................................................ 162
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAPH</td>
<td>2,2'-Azo-bis (2-amidinopropane) dihydrochloride</td>
</tr>
<tr>
<td>ABAPH</td>
<td>(2,2'-Azo-bis (2-amidino-propane) hydrochloride</td>
</tr>
<tr>
<td>ABTS</td>
<td>2, 2-Azino-bis(3-ethyl Benzothiazoline-6-sulfonic acid) diamonium Salt</td>
</tr>
<tr>
<td>BHA</td>
<td>Butylated Hydroxyanisole</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylated Hydroxytoluene</td>
</tr>
<tr>
<td>CAT</td>
<td>Catalase</td>
</tr>
<tr>
<td>CHI</td>
<td>Flavanone Isomerase</td>
</tr>
<tr>
<td>CHS</td>
<td>Chalcone Synthase</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclo-Oxygenase</td>
</tr>
<tr>
<td>Cyt C</td>
<td>Cytochrome C</td>
</tr>
<tr>
<td>DF-PC</td>
<td>Dietary Fibre-Phenolic Compounds</td>
</tr>
<tr>
<td>DPPH</td>
<td>1,1-Diphenyl 2,2 Picryl Hydrazyl</td>
</tr>
<tr>
<td>EBT</td>
<td>Eriochrome-Black-T indicator</td>
</tr>
<tr>
<td>ET</td>
<td>Electron Transfer</td>
</tr>
<tr>
<td>FRAP</td>
<td>Ferric ion Reducing Antioxidant Power</td>
</tr>
<tr>
<td>GSH</td>
<td>Glutathione</td>
</tr>
<tr>
<td>HAT</td>
<td>Hydrogen Atom Transfer</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>IFS</td>
<td>Isoflavone Synthase</td>
</tr>
<tr>
<td>LA</td>
<td>Linoleic Acid</td>
</tr>
<tr>
<td>LOX</td>
<td>Lipo-oxidynase</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>MSPD</td>
<td>Matrix Solid Phase Dispersion</td>
</tr>
<tr>
<td>mtDNA</td>
<td>mitochondrial DNA</td>
</tr>
<tr>
<td>NBT</td>
<td>Nitro Blue Tetrazolium</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>ORAC</td>
<td>Oxygen Radical Absorbance Capacity</td>
</tr>
<tr>
<td>PAL</td>
<td>Phenylalanine Ammonia Lyase</td>
</tr>
<tr>
<td>PE</td>
<td>beta-Phycoerythrin</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive Nitrogen Species</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen species</td>
</tr>
<tr>
<td>SE</td>
<td>Soxhlet Extraction</td>
</tr>
<tr>
<td>SFE</td>
<td>Supercritical Fluid Extraction</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide Dismutase</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid Phase Extraction</td>
</tr>
<tr>
<td>SPME</td>
<td>Solid Phase Micro-Extration</td>
</tr>
<tr>
<td>TBA</td>
<td>Thiobarbituric Acid</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>TCA</td>
<td>Trichloroacetic Acid</td>
</tr>
<tr>
<td>TEAC</td>
<td>Trolox Equivalence Antioxidant Capacity</td>
</tr>
<tr>
<td>TPTZ</td>
<td>2,4,6-Tri (2 – pyridyl) – s – triazine</td>
</tr>
<tr>
<td>TRAP</td>
<td>Total Radical Trapping Antioxidant Parameter</td>
</tr>
</tbody>
</table>
Chapter 1

1.1 Introduction

A large number of deaths around the world, has been attributed to non-communicable diseases, most of them associated or linked to oxidative stress (Haplin et al., 2010). Thirty-six million deaths, representing 63% of the 57 million global deaths in 2008 were due to non-communicable diseases (World Health Organization, 2008). The main killer diseases worldwide include cardio-vascular complications, cancers, chronic lung ailments and diabetes which account for 17 million, 7.6 million, 4.2 million and 1.3 million deaths respectively each year. Nearly eighty percent of the deaths caused by non-communicable diseases occur in low and medium income countries mainly in Africa (World Health Organization, 2008). In 2010 alone, cancer caused the death of 8 million people; heart diseases and strokes caused the death of 12.9 million people, while diabetes claimed 1.3 million lives worldwide (Rodin and de Ferranti, 2012).

Oxidative stress is defined as an imbalance between production and elimination of reactive oxygen and nitrogen species (ROS/RNS) leading to undesirable oxidative modifications of basic and regulatory processes. ROS/RNS are second messengers in tissue-specific oxygen-sensing cascades that are essential for energy supply, detoxification, chemical signalling and immune functions (Acker, 2005). ROS/RNS are continuously produced in the human body by electron transport chains and by the first group of enzymes that includes xanthine oxidase, aldehyde oxidase and cytochrome-P450 monooxygenase. When there is excess production of ROS/RNS, another group of endogenous enzymes that includes superoxide dismutase, glutathione peroxidase, and catalase remove the excess ROS/RNS (Devasagayam et al., 2004).
An increase in the production of ROS may be promoted by exogenous factors which include temperature variation, radioactivity, ultraviolet radiation and xenobiotics (Blokhina et al., 2003). Metabolic disorders or inherited diseases disturbing the electron transport chain may increase production of ROS. Lack or low production of antioxidant enzymes or impaired metabolism of antioxidants can lead to increase in ROS concentration over steady-state levels (Kirkinezosa and Moraes, 2001). ROS may lead to cell death when their targets are proteins or lipids.

Oxidative stress is stimulated in cells by an increase in oxidant generation, a failure to repair oxidative damage or an exposure to external oxidant substances (Aruoma, 1998; Saha and Tamrakar, 2011). The harm caused by ROS on biomolecules in the body has been linked to an increased risk of cardiovascular disease, cancer and other chronic diseases (Blokhina et al., 2003). The oxidative damage on the body could be reduced by antioxidants (Saha and Tamrakar, 2011).

Antioxidants are components that deactivate ROS/RNS. They include free radicals that intervene before ROS/RNS can attack cell proteins, lipids and/or carbohydrates. Compounds with antioxidant activity inhibit or delay oxidative processes. Antioxidants neutralize free radicals through binding the lone electrons on the radical thereby rendering the molecule harmless (Beris, 1991). Antioxidants play an important role in neutralising the reactive oxygen and nitrogen species that enhance the progression of diseases such as cancer and diabetes.

Synthetic antioxidants have been used in foods for the purpose of negating the effects of ROS and RNS. Some of the synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiarybutylhydroquinone. Synthetic antioxidants
have, however, been reported to contribute to toxicity and carcinogenicity on the human body (Ito et al., 1986). Natural antioxidants, mainly polyphenolic compounds, found in plants are advocated for by scientists in the food industry, to replace synthetic antioxidants, hence our study on the antioxidant composition of plant natural antioxidants.

In 1995, the Food and Agricultural Organisation reported that cereals supply 46 percent of the energy requirements; roots as well as tubers supply 20 percent, whilst animal products cater for 7 percent of the energy requirements in an average African diet (Alexandratos, 2006). The dependence of developing countries on starch-based foods as a protein source accounts in part, for protein deficiency which prevails among the populations as recognized by Food and Agricultural Organization (Akubugwo, 2007). In Africa, where the population is predominantly poor, the daily diet is dominated by starchy staple foods such as cereals and vegetables. Cereals are relatively cheaper, more readily available sources of proteins, vitamins, minerals and essential amino acids when compared to other sources like animal products (Akubugwo, 2007).

Cereal grains are grown in greater quantities worldwide than any other type of crop, providing more food energy to the human race than any other crops (Food and Agricultural Organisation, 1995). In the poorest families in Zimbabwe, cereal food is almost entirely the source of nutrition since other sources of nutrition such as meat are very expensive (Pinchuck, 1996). Production of traditional cereal grains such as maize and wheat has been lowered by poor rainfall patterns. Due to the poor production of traditional cereals it is important to find other alternatives that are drought-tolerant to replace or supplement the traditional cereals. In this study amongst other objectives the focus was on the macro-nutritional composition of selected drought-resistant wild cereal grains found and
consumed in Zimbabwe during times of famine. The cereals include; *Amaranthus hybridus, Panicum maximum, Brachiaria brizantha, Sorghum arundinaceum* and *Rottboellia cochinchinensis*.

Amongst other nutrients, cereal grains contain phenolic compounds that have antioxidant properties (Chitindingu *et al.*, 2007). Phenolic compounds are the most abundant antioxidants found in plants (Manach *et al.*, 2004). In addition to their antioxidant properties, phenolic compounds have biological activities which could be useful in prevention of cancer as well as coronary heart diseases (Yuan *et al.*, 2005).

Due to their protective properties, natural antioxidants could replace the synthetic antioxidants like butylated hydroxyanisole (BHA), which have cancer-causing properties (Ito *et al.*, 1983). Natural antioxidants constitute a broad range of compounds including phenolic compounds, nitrogen compounds and carotenoids. Consumption of foods containing phenolic compounds is also associated with lowering the incidence and mortality rate due to degenerative diseases (Chun *et al.*, 2005).

In order to exert their biological properties, phenolic compounds have to be available in the target tissue. The biological properties of dietary phenolic compounds may depend on their absorption in the gut and their bioaccessibility (Saura-Calixto *et al.*, 2000). Bioaccessibility of a food constituent is the amount of that food constituent particularly present in the gut, as a consequence of the release of the constituent from the solid food matrix, and the released constituent should be able to pass through the intestinal barrier (DEFRA and Environment Agency, 2002c).
Only phenolic compounds released from the food matrix, by the action of digestive enzymes in the small intestine and bacterial microflora in the large intestine, are bioaccessible in the gut and therefore potentially bioavailable. In studies on the bioaccessibility of phenolic compounds, pure single molecules have been used although their bioaccessibility from whole foods may be substantially different (Saura-Calixto and Goñi, 2004). The bioaccessibility of phenolic compounds in the cereal grains was investigated in order to clearly ascertain the actual amount of the phenolic compounds released from the solid food matrix and are potentially absorbed in the gut.

1.2 Research question

Were there wild or pseudo-cereal grains in Zimbabwe with potential to be alternate sources of beneficial macro and micro-nutrients?

1.2.1 Hypotheses

H₀: Wild cereal and pseudo-cereal grains found in Zimbabwe did not contain any macro and micronutrients with potential benefit to humans.

H₁: Wild cereal and pseudo-cereal grains found in Zimbabwe contained macro and micronutrients that are potentially beneficial to humans.

1.3 Aims and objectives

In this study, the aim was to determine both the macro and micro nutritional composition, bioaccessibility of phenolic compounds as well as to characterise the phenolic compounds in randomly selected wild and domesticated cereal grains.
1.3.1 Specific objectives

The specific objectives of the study were to quantify phenolic compounds, their bioaccessibility, antioxidant capacity and nutritional composition of wild and domesticated cereal grains commonly consumed in areas affected by drought in Zimbabwe as listed below. The objectives were to:

1. Determine the proximate nutritional composition of selected wild and domesticated cereal grains.
2. Determine the total phenolic compounds, proanthocyanidins and flavonoids in selected wild and domesticated cereal grains using colorimetric methods.
3. Tentatively identify simple phenolic acids in selected wild and domesticated cereal grains using the HPLC method.
4. Assess radical scavenging activity of extracts from selected wild and domesticated cereal grain using the DPPH’ radical scavenging assay.
5. Determine the potential of cereal grain extracts to reduce ferric ions using the reducing power assay.
6. Determine the ability of cereal grain extracts to chelate metal ions.
7. Evaluate the ability of cereal grain extracts to halt or delay lipid peroxidation.
8. Determine the ability of cereal grain extracts to delay or prevent the oxidation of β-carotene in solution.
9. Determine bioaccessibility of phenolic compounds in selected wild cereal grains.
Chapter 2: Literature Review

2.1 Oxidative stress

The health of the cells in the body is constantly threatened by free radicals which are capable of damaging cells and DNA. The body generates free radicals as by-products, when turning food into energy. Other radicals are found in the food and the air we breathe. Some are generated by sunlight's action on the skin and eyes (Sies and Jones, 2007).

Free radicals comprise many chemical configurations but they all have an appetite for electrons which they acquire from any nearby substances that will yield to them. The extraction of an electron by a free radical can alter the losing compound’s structure or function. The loss in electrons results in oxidative stress which is an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage (Chauhan et al., 2010).

Oxidative stress can be defined as a large rise in the cellular reduction potential, or a large decrease in the reducing capacity of the cellular redox couples. The effects of oxidative stress depend upon the extent of the rise in the cellular reduction potential. A cell is able to overcome small perturbations and regain its original state but a more severe oxidative stress can cause cell death. Even moderate oxidation can trigger cell death, while more intense stresses may cause tissue death (Chauhan et al., 2010).

A particularly destructive aspect of oxidative stress is the production of reactive oxygen species, which include free singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. Some of the less reactive of the reactive oxygen species, such as the superoxide anion, can be converted by oxido-reduction reactions with transition metals into
more aggressive radical species that can cause extensive cellular damage (Sies and Jones, 2007).

Free radical damage can alter the instructions coded in a strand of DNA and can cause a circulating low-density lipoprotein (LDL) molecule more likely to get trapped in an artery wall. Radical damage can alter a cell's membrane by interfering with the bilipid layer leading to a change in the flow of what enters the cell and what leaves it (Chauhan et al., 2010). Oxidative damage can lead to disorders in the vital organs such as the heart and liver. Some of the disorders that may be caused by oxidative stress are shown in figure 2.1.

![Diagram of oxidative stress and its effects](image)

(Adapted from: NIST, National Institute of Standards and Technology, 2000).

**Figure 2.1:** Some of the disorders caused to the human health by oxidative stress.
Although the body's internal production of antioxidants is not enough to neutralize excess free radicals, the body responds to free radical attack by making molecules that quench free radicals and by extracting free-radical fighters from food. Radical quenchers from food are often lumped together and termed antioxidants. Antioxidants work by giving electrons to free radicals without turning into electron-scavenging substances themselves (Chauhan et al., 2010).

2.1.1 Oxidation reactions leading to disease

Normally the body maintains a balance between oxidation species and antioxidants. The lines of defence through which the body regulates the balance of oxidation species and antioxidants is shown in figure 2.1.1.1.

Figure 2.1.1.1: The defense network in vivo against oxidative stress (Source: Niki, 2010).
As part of the normal metabolism of the body it is estimated that 2% of the oxygen consumed by humans produces reactive oxygen species (Acworth and Bailey, 1996). Disease occurs when the production of reactive oxygen species exceeds the ability of the natural defences of the body to handle them and this state is called oxidative stress (Devasagayam et al., 2004).

Devasagayam et al., (2004) reported on the reactive species in biological systems that have caused or have led to disease. Some of the ROS/RNS that are crucial to understanding the oxidative reactions leading to disease are shown in table 2.1.1.
### Table 2.1.1: Some Reactive Oxygen and Nitrogen species of biological interest; adapted from Devasagayam et al. (2004).

<table>
<thead>
<tr>
<th>Species</th>
<th>Symbol</th>
<th>Reactivity/ Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide</td>
<td>(O_2^\cdot)</td>
<td>Generated in mitochondria, in cardiovascular system.</td>
</tr>
<tr>
<td>Hydroxyl radical</td>
<td>(\cdot\text{OH})</td>
<td>Very highly reactive, generated during iron overload in our body.</td>
</tr>
<tr>
<td>Peroxyl radical</td>
<td>(\text{ROO}^\cdot)</td>
<td>Formed from lipids, proteins, DNA and sugars during oxidative damage.</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>(\text{NO}^\cdot)</td>
<td>Neurotransmitter and blood pressure regulator can yield potent oxidants during pathological states.</td>
</tr>
<tr>
<td>Peroxynitrite</td>
<td>(\text{ONOO}^-)</td>
<td>Formed from NO and superoxide, highly reactive.</td>
</tr>
</tbody>
</table>
Reactive oxygen radicals may be caused by an increase in intracellular iron or copper (Perron and Brumaghim 2009). The following reactions indicate the possible ROS generation cells:

1. \( \text{Fe}^{2+} \text{ or Cu}^{+} + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{Fe}^{3+} \text{ or Cu}^{2+} + \cdot\text{OH} + \text{H}_2\text{O} \)

2. \( 2\text{O}_2^{-} + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \)

3. \( [2\text{Fe}^{2+} \ 2\text{Fe}^{3+} - 4\text{S}] + \text{O}_2^{-} + 2\text{H}^+ \rightarrow [\text{Fe}^{2+}3\text{Fe}^{3+} - 4\text{S}] + \text{Fe}^{2+} + \text{H}_2\text{O}_2 \)

4. \( [\text{Fe}^{2+}3\text{Fe}^{3+} - 4\text{S}] \rightarrow [3\text{Fe}^{3+} - 4\text{S}] + \text{Fe}^{2+} \)

5. \( \text{O}_2^{-} + \text{Fe}^{3+} \text{ or Cu}^{2+} \rightarrow \text{O}_2 + \text{Fe}^{2+} \text{ or Cu}^{+} \)

6. \( \text{O}_2^{-} + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{O}_2 + \cdot\text{OH} + \text{H}_2\text{O} \)

Reactive oxygen species cause a chain reaction leading to oxidation. A chain reaction results in the propagation of free radicals: the molecules destabilized by a single electron in turn become free radicals that remove an electron from another molecule and will themselves become radicals, and so on. These radicals attack molecules like fat, protein, DNA and sugar (Perron and Brumaghim, 2009). Saha and Tamrakar (2011) identified five ways through which oxidative stress lead to disease, and these include:

a) DNA damage

Cancer is associated with damage to DNA. Cancer cells are under continuous oxidative stress (Pervaiz and Clement, 2004; Schumacker, 2006; Kryston et al., 2011). ROS affect mitochondrial cells, which are the major source of reactive oxygen species, and alter their normal respiration. The mitochondrial dysfunction may induce a low coupling efficiency of the mitochondrial electron chain, increasing electron leakage thus leading to enhanced ROS formation. The resulting oxidative stress may cause further damage to both mitochondrial DNA (mtDNA) and the respiratory chain, amplifying ROS generation (Zorov et al., 2006).
b) Glutathione (GSH) depletion

The depletion of glutathione (GSH) has been linked to hypertension in research experiments where rats have been used as models (Nosratola et al., 2000). Impaired liver function when GSH is depleted may occur as GSH is needed in the clearing of oxidants produced during the liver detoxification. The depletion of glutathione has been associated with elevated incidences of hypersensitivity and increased susceptibility to toxicity (Nosratola et al., 2000).

c) Direct damage to proteins

Antioxidants cause harm to proteins in the body. ROS, free radicals as well as nitric oxide (NO) participate in the pathogenesis of acute central nervous system (CNS) injury by forming peroxynitrite, which promotes oxidative damage and tyrosine nitration (Acarin et al., 2005). In the aging human eye, oxidative damage plus accumulation of pro-oxidant lysosomal lipofuscin cause functional decline of the retinal pigment epithelium (RPE), which contributes to age-related macular degeneration (Acarin et al., 2005).

d) Rises in intracellular free iron – Membrane peroxidation and destruction – Injury to adjacent cells.

Iron is essential for several metabolic pathways including formation of haemoglobin, but its concentration inside cells has to be tightly regulated because the iron can catalyze the formation of free radicals. Iron overload is associated with increased cardiovascular morbidity and mortality. Elevated iron levels in the myocardium lead to impaired systolic and diastolic function and elevated oxidative stress (Oudit et al., 2004). The harmful action of heavy metals can be divided according to the chemical and physical properties including: i) generation of ROS by auto-oxidation; ii) blocking of essential functional
groups in biomolecules. In proteins, heavy metals block the functional groups by the inactivation of the SH-groups in enzymes active centers and polynucleotides (Baranowska-Morek, 2003; Mithöfer et al., 2004); and iii) substitution of essential metal ions by other incorrect ones (Rai et al., 2004).

c) Increased lipid peroxidation – increased damage to DNA, proteins, lipids

Lipids are the major constituents, by mass, of cellular membranes. Peroxidation of the membrane lipid seriously impairs membrane function. Lipid peroxidation occurs as a result of oxidative stress in intact cells and is a radical-initiated chain reaction that is self-propagating in cellular membranes. As a result, isolated oxidative events may have profound effects on membrane functioning.

Reactive oxygen species play a pivotal role in the development of diabetes complication (Ferdinando and Brownlee, 2010). Increased intracellular reactive oxygen species may cause defective angiogenesis in response to ischemia, activate a number of pro-inflammatory pathways, plus initiate long-lasting epigenetic changes that drive persistent expression of pro-inflammatory genes after glycemia is normalized. Atherosclerosis and cardiomyopathy in type 2 diabetes may be caused in part by pathway-selective insulin resistance, which increases mitochondrial ROS production from free fatty acids. Over-expression of superoxide dismutase in transgenic diabetic mice prevents diabetic retinopathy, nephropathy and cardiomyopathy (Ferdinando and Brownlee, 2010).

2.2 Antioxidants

Living tissues have control mechanisms to keep ROS in balance (Erenel et al., 1993). When ROS are generated in vivo, many antioxidants come into play. The relative
importance of antioxidants depends upon which ROS are generated, how and where the ROS are generated, as well as which target of damage is considered (Halliwell and Gutteridge, 2007). The human body defends itself from ROS via endogenous antioxidants (Halliwell and Gutteridge, 2007; Chaudiere and Ferrari-Iliou, 1999). When endogenous antioxidants become insufficient to quench free radicals or imbalanced in defence against oxidants, exogenous antioxidants may help restore the balance. Antioxidants inhibit the production of ROS by directly scavenging free radicals, decreasing the amount of oxidants in and around our cells, preventing ROS from reaching their biological targets thereby limiting the propagation of oxidants such as the ones occurring during lipid peroxidation thereby preventing the onset of degenerative diseases and ageing.

2.2.1 Classification of antioxidants

Antioxidants can be classified as endogenous and exogenous antioxidants.

2.2.1.1 Endogenous antioxidants

Endogenous antioxidants are mainly enzymes that catalytically remove oxidants. Endogenous antioxidants are superoxide dismutase, superoxide reductase, catalase and glutathione peroxidase. Endogenous enzymes play a key role in decreasing the content of oxidants as well as preventing oxidative damage. Other endogenous antioxidant molecules, such as heme oxygenase, minimize the availability of oxidants. The heme oxygenase enzyme is strongly induced by oxidative stress and removes heme, an oxidant, while generating bilirubin, a putative antioxidant that is sensitive to $^{1}\text{O}_2$ and iron, a pro-oxidant. In addition, high ferritin levels result in an increased iron scavenging capacity that may confer increased resistance to oxidative stress (Vile et al., 1994).
The levels and composition of endogenous antioxidant molecules differ from tissue to tissue and by cell type depending on the role of the cells and the tissue. For example, embryonic and adult stem cells express high levels of antioxidant enzymes, which decrease as cells differentiate (Dernbach et al., 2004). The endogenous antioxidant molecules are increased after exposure to oxidants (Halliwell and Gutteridge, 2007). However, this “antioxidant pool” is gradually consumed as oxidant exposure increases over time, triggering the need for exogenous sources of antioxidants.

2.2.1.2 Exogenous antioxidants
Exogenous antioxidants are antioxidants that cannot be synthesized by the human body. They comprise vitamins, trace elements, and antioxidants from plants. Vitamin E is a powerful lipid-soluble antioxidant and it inhibits the peroxidation of membrane lipids. Vitamin E (tocopherol) reacts with free radicals to form the radical tocopheryl, a stable substance that stops the chain reaction of the membrane lipids. Tocopherol has the ability to break the chain reaction by membrane lipids. It works in conjunction with other antioxidants such as vitamin C and selenium.

Vitamin C is a water-soluble vitamin and has a strong antioxidant activity that protects cells against damage by free radicals. Vitamin C reacts with the tocopheryl radical to regenerate and restore vitamin E while it becomes the ascorbyl radical, which is relatively more stable (Halliwell and Gutteridge, 2007). Trace elements such as selenium are important cofactors of the activity of antioxidant enzymes.

Phenolic compounds are another group of exogenous antioxidants, mainly found in plants. Phenolic compounds are found in most plants (Halliwell and Gutteridge, 2007). The
benzene ring on the phenolic compounds gives the molecules stability when the compounds donate electrons to unstable radicals generated within the body.

2.2.2 Uses and potential applications of antioxidants

The biological roles that antioxidants have of scavenging for free reactive oxygen species in living systems is useful and many antioxidants have been isolated for this purpose (Niki, 2010). Increased levels of free radicals in the body have been linked to cancer, diabetes and coronary heart disease (Devasagayam et al., 2004). Some of the applications of antioxidants by the body for protection against damage are:

a) antioxidants such as β-carotene and other carotenoids, vitamin C, vitamin A, zinc and selenium help in boosting the immune system and protect the blood vessels against hardening (Bernardini et al., 2008). Blood vessels can become clogged with plaque and begin to harden, a condition known as arteriosclerosis. A diet rich in antioxidants assists in clearing and purifying the blood of oxidants and thereby preventing the onset of various cardiovascular diseases.

b) antioxidants play an important role in protecting membrane integrity (Devasagayam et al., 2003). Antioxidants, in addition, protect cell wall integrity and supportive collagen tissue from damage by reactive oxygen species (Hamid et al., 2010). Antioxidants therefore play a major role in protecting membranous structures such as the skin against aging.

c) Oxidative stress may contribute to male infertility. Excessive production of free radicals or reactive oxygen species (ROS) can damage spermatozoa (Agarwal et al., 2005).
Antioxidants may reverse and prevent the effects of oxidative stress on spermatozoa, thereby contributing to the maintenance of male fertility.

d) antioxidants contribute to the regulation of metabolism (Hu et al., 2010). An example of how antioxidants contribute to the regulation of metabolism is that they support phase 1 of the detoxification process of toxins that the liver does, and glutathione is required for both phases of the detoxification processes. The liver uses a two-step enzymatic process to neutralize toxic chemical compounds such as drugs, pesticides, toxins and waste substances from metabolic processes (Chan et al., 2008). These chemical compounds can become extremely toxic if they accumulate in the body. The toxins are therefore transformed during Phase I of the liver detoxification process, producing free radicals as by-products. Insufficient free radical defences result in damages to the liver each time the liver neutralizes a toxin. Antioxidants, such as reduced glutathione, vitamins C and E, carotenoids, phenolic compounds and selenium reduce the damage caused by these free radicals.

e) antioxidants may reduce the progression of allergies (Dharajiya et al., 2007). A decreased intake of dietary antioxidants increases risk of allergic reaction onset and manifestation. For example, quecetin, a flavonoid helps the control of the histamine release mechanism and is, therefore, useful for treating allergies (Chirumbolo, 2010).

f) antioxidants, mainly flavonoids, have positive application in the prevention of rheumatoid arthritis (Abdolhossein et al., 2011). Flavonoids and vitamins C and E may be used to assist in the treatment of arthritis and pain of the joints. Antioxidants maintain elasticity of the veins and help to enhance blood circulation (Abdolhossein et al., 2011).
g) Antioxidants are used as stamina enhancers and as antimicrobials. Antioxidants, mainly polyphenols have a potential application in the monitoring of stress in plants. In addition, phenolic compounds such as tocopherols and tocotrienols, collectively known as tocols, which are widely distributed in plant tissues, are used as food additives (Shahidi, 2003). Carotenoids form another group of antioxidant nutrients that act mainly as secondary antioxidants in foods by quenching singlet oxygen. Carotenoids are a good synergist with tocopherols. β-Carotene, lutein, lycopene and isozeaxanthin are typical carotenoids that are used commercially to retard oxidation in foods (Mikova, 2001).

### 2.3 Phenolic compounds

Phenolic compounds are the biggest group of phytochemicals with antioxidant activity, and many have been found in plant-based foods (Tsao, 2010). Phenolic compounds have one or more hydroxyl substituent groups bonded onto an aromatic ring (Maestri et al., 2006). Phenolic compounds may have several or many phenolic hydroxyl-substituents and are often referred to as polyphenols. Phenolic compounds are secondary plant metabolites with structures ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins (Maestri et al., 2006).

Phenolic compounds are broadly distributed in the plant kingdom with more than 8,000 phenolic structures known (Dimitrios, 2006). Plant phenolics are produced by plants as a way to defend the plant against ultraviolet radiation or aggression by pathogens, parasites and/or predators. Phenolics are an important indication of fruit quality since they contribute to the taste, colour and nutritional properties of fruits (Cheynier, 2005).
Plant phenols have not been adequately studied because of the complexity of their chemical nature and varied occurrence in plants (Dimitrios, 2006). Polyhydroxy flavones, flavanones, flavanols, isoflavones, chalcones and many other polyphenols are natural substances that have a high degree of antioxidant activity, and phenolic compounds are found to be widespread in plant material (Dimitrios, 2006).

There is increasing interest in plant phenols since phenolic compounds have been reported to have numerous health benefits (Beecher, 2003). Work done has been focused on bioavailability, exploring different sources for antioxidants and organising the existing data of polyphenols in databases (Manach et al., 2004; USDA, 2003). More work has been going on polyphenols to enhance the content of phenolics in plants, produce phenolics with improved pharmacological characteristics, explore novel effects of phenolic compounds and elucidating the quantitative structure-activity relationships of various phenol classes (Wilhelm et al., 2000; Kontogianni et al., 2003; Nenadis et al., 2003; Kontogiorgis et al., 2005).

Phenolic compounds have important beneficial qualities that include acting as metal chelators, antimutagens or anticarcinogens, antimicrobial agents and clarifying agents (Proestos et al., 2005). Some phenolic compounds can chelate transition metals, preventing metal-induced free radical formation. Phenolic compounds with catecholate and gallate groups can inhibit metal-induced oxygen-radical formation either by coordination with Fe$^{2+}$ and enhancing autoxidation of Fe$^{2+}$, or through formation of an inactive complex with Cu$^{2+}$, Fe$^{2+}$ or Cu$^{+}$ with relatively weaker interaction (Yoshino and Murakami, 1998). The beneficial effects of polyphenols are attributed to their antioxidant properties, since they can act as radical chain breakers or radical scavengers depending on their chemical
structures (Rice-Evans, 2001). Polyphenols have been reported to activate changes in gene-signalling pathways and subsequent gene expression (Chen et al., 2002; Pfeilschifter et al., 2003). It is possible that the distinct chemical and receptor-mediated activities of polyphenols might result in similar outcomes via pathways different to the gene-signalling pathways (Weiss and Landauer, 2003).

Catechin is a phenolic compound that has been used as a natural antioxidant in oils and fats against lipid oxidation, supplement for animal feeds both to improve animal health and to protect animal products, as antimicrobial agent in foods and as functional health ingredient in various foods and dietary supplements (Yilmaz, 2006).

The antimicrobial properties of polyphenols are exerted by the ability of the polyphenolic compounds to alter microbial cell permeability and transport macromolecules (Fung et al., 1977; Ikigai et al., 1994; Tamba et al., 2007). Flavonoids, a class of phenolic compounds, are responsible for the antioxidant, anticarcinogenic and anti-arteriosclerotic actions of teas (Wang and Lin, 2000). Chalcones (trans-1,3-diaryl-2-propen-1-ones) are biosynthetic products of the shikimate pathway, belonging to flavanoid family and are precursors of open chain flavonoids and isoflavonoids, which are abundant in edible plants. Chalcones exhibit multiple biological activities, including antinflammatory, antioxidant and anticancer properties (Szliszka et al., 2010).

### 2.3.1 Classification of phenolic compounds

Polyphenolic substances are divided into several classes according to the number of phenol rings that they contain and to the structural elements that bind these rings to one another.
The main groups of polyphenols are flavonoids, phenolic acids, phenolic alcohols, stilbenes and tannins (D’Archivio et al., 2007).

### 2.3.1.1 Phenolic alcohols

Phenolic alcohols (C₆-C₁) have hydroxyl groups and may be differentiated by the number of hydroxyl groups that may be free or methylated. These compounds are rare in higher plants. Among the phenolic alcohols, the most abundant are salicylic, hentisinic, coniferylic and sinapic alcohols. Salicylic alcohol (saligenin) is an aglycone of salicin, found in a bark of Salix spp., possessing anti-inflammatory action. Coniferylic alcohol plays an important role as biological precursor of lignin. Sinapic alcohol (sirenginin) is one of the main compounds involved in biosynthesis of lignin in Angiosperms and is an aglycone of sigengin. Some examples of phenolic alcohols are shown in figure 2.3.1.1.
Figure 2.3.1.1: Chemical structures of a) salicylic alcohol, b) gentisinic alcohol, c) coniferylic alcohol and d) sinapic alcohol. (Source: ChemSynthesis chemical database, 2015).

2.3.1.2 Phenolic acids

Phenolic acids, derivatives of benzoic acid and cinnamic acid, are present in all cereals (Kim et al., 2006). Derivatives of benzoic acid include gallic acid; derivatives of cinnamic acid include p-coumaric, caffeic and ferulic acid. Caffeic acid is the most abundant phenolic acid in many fruits and vegetables, most often esterified with quinic acid as in chlorogenic acid, which is the major phenolic compound in coffee. Another common phenolic acid is ferulic acid, which is present in cereals and is esterified to hemicelluloses in the cell wall (Kim et al., 2006). Some examples of phenolic acids are shown in figure 2.3.1.2.
2.3.1.3 Tannins

Tannins are complex polyphenolic compounds found in a wide range of plant species. Tannins are subdivided into hydrolysable tannins and condensed tannins. Condensed tannins are oligomers or polymers of flavan-3-ol linked through an interflavan carbon bond. Condensed tannins are referred to as proanthocyanidins since they are decomposed to anthocyanidins through acid-catalyzed oxidation reaction upon heating in acidic alcohol solutions. The structure diversity of tannins is a result of the variation in hydroxylation pattern and the location and type of interflavan linkages, as well as the degree and pattern of methoxylation, glycosylation and galloylation (Hemingway et al., 1999). The general structure of condensed tannins is shown in figure 2.3.1.3.1.

Hydrolysable tannins are compounds containing a central core of glucose or another polyol esterified with gallic acid and an example of the hydrolysable tannins is shown in figure 2.3.1.3.2. Hydrolysable tannins that are esterified with gallic acid are called gallotannins while tannins with hexahydroxydiphenic acid are called ellagitannins (Yoshida et al., 1997). The great variety in the structure of these compounds is due to the many possibilities in forming oxidative linkages. Intermolecular oxidation reactions give rise to
many oligomeric compounds having a molecular weight between 2,000 and 5,000 Daltons (Hagerman et al., 1998).

Figure 2.3.1.3.1: The structure of condensed tannins where n is any number that makes up the polymer. (Source: ChemSynthesis chemical database, 2015).
Figure 2.3.1.3.2: The chemical structure of Penta galloyl-D-glucose, a hydrolysable tannin.
(Source: ChemSynthesis chemical database, 2015).

2.3.1.4 Stilbenes

Stilbenes are a class of phenolic compounds with a C6- C2- C6 skeleton that consists of two aromatic rings joined by a two-carbon bond. Stilbenes exist as two possible isomers. They may exist as trans-1,2-diphenylethylene, called trans-stilbene or as cis-1,2-diphenylethylene, called (Z)-stilbene or cis-stilbene, and are sterically hindered and less stable because the steric interactions force the aromatic rings out-of-plane and prevent conjugation. Stilbenes are found in only low quantities in food plants (Vitrac et al., 2002). One of these, resveratrol shown in figure 2.3.1.4, for which anticarcinogenic effects have been shown during screening of medicinal plants and which has been extensively studied, is found in low quantities in wine (Bertelli et al., 1998; Bhat and Pezzuto, 2002). Since
resveratrol is found in such small quantities in the diet, any protective effect of this molecule is unlikely at normal nutritional intakes.

Figure 2.3.1.4.: Chemical structure of resveratol, a stilbene. (Source: ChemSynthesis chemical database, 2015).

2.3.1.5 Flavonoids

Flavonoids are compounds with a C6-C3-C6 skeleton that consists of two aromatic rings joined by a three-carbon bond. Flavonoids are the most abundant polyphenols in the human diet and can be classified into 9 classes which are chalcones, dihydrochalcones, dihydroflavonols, flavanols, flavanones, flavones, isoflavonoids and anthocyanins (Neveu et al., 2010; Beecher, 2003). Structural variation in each subgroup is partly due to the degree and pattern of hydroxylation, methoxylation, prenylation or glycosylation. Some of the most common flavonoids include a) quercetin, a flavonol abundant in onion, broccoli, and apple; b) catechin, a flavanol found in tea and several fruits. Naringenin is the main flavanone in grapefruit, cyanidin-glycoside, is an anthocyanin abundant in berry fruits (black currant, raspberry and blackberry) and daidzein, genistein and glycine, the main
isoflavones in soybeans. Flavonoids, according to Taylor and Grotewold (2005), have important developmental and physiological functions in plants and are involved in control of auxin transport, allelopathy, pollen function in some species, signalling with symbiotic micro-organisms and somatic embryogenesis (Buer et al., 2007).

Caution must be exercised in the extraction of flavonoids from plants since they can be degraded by enzyme action when collected material is fresh or non-dried. It is thus advisable to use dried, lyophilized, or frozen samples when extracting flavonoids.

### 2.3.1.5.1 Flavonols

Flavanols exist in both the monomer catechin form and the polymer proanthocyanidin form. Catechins are found in many types of fruit (Manach et al., 2005). Catechins are also present in red wine, but green tea and chocolate are by far the richest sources (Manach et al., 2005). The chemical structures of kaempferol, quercetin and myricetin, all of which are flavonols, are shown in figure 2.3.1.5.1.
### 2.3.1.5.2 Flavones

Flavones are comprised chiefly of glycosides of luteolin and apigenin. The structures of luteolin and apigenin are shown in figure 2.3.1.5.2. Some edible sources of flavones identified are parsley and celery (Manach et al., 2005). Cereals such as millet and wheat contain glycosides of flavones (Boyle et al., 2000; Graefe et al., 2001). Large quantities of polymethoxylated flavones: tangeretin, nobiletin, and sinensetin (up to 6.5 g / L) of essential oil of mandarin, have been identified on the skin of citrus fruit (Nielsen et al., 2003). These polymethoxylated flavones are the most hydrophobic flavonoids.

---

**Figure 2.3.1.5.1**: The flavonols quercetin, kaempferol and myricetin and variant R – groups specific each flavonol. (Source: ChemSynthesis chemical database, 2015).
Figure 2.3.1.5.2: Structure of flavone and variant R – groups specific for apigenin and luteolin. (Source: ChemSynthesis chemical database, 2015).

2.3.1.5.3 Flavanones

Flavanones are found in tomatoes and certain aromatic plants such as mint, but they are present in high concentrations only in citrus fruit. The main non-sugar component that results from hydrolysis of glycoside flavanones is naringenin in grapefruit, hesperetin in oranges, and eriodictyol in lemons (Erlund et al., 2000). Flavanones are glycosylated by disaccharide at position seven: either a neohesperidose, which imparts a bitter taste such as to naringin in grapefruit, or a rutinose, which is flavourless. The structures of some flavanones are shown in figure 2.3.1.5.3.
Figure 2.3.1.5.3: Chemical structure of flavanones and the variant R – groups specific for hesperetin and naringenin. Source: ChemSynthesis chemical database (2015)

<table>
<thead>
<tr>
<th></th>
<th>R₁</th>
<th>R₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Narigenin</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>Hesperetin</td>
<td>OH</td>
<td>OCH₃</td>
</tr>
</tbody>
</table>

2.3.1.5.4 Flavan-3-ols

Flavan-3-ols exist in both the monomer catechins form and the polymer proanthocyanidin form. Sources of catechins include many fruit types of fruit such as apricots, green tea, red wine and chocolates (Manach et al., 2005; Day et al., 2001). Some examples of flavan-3-ols are shown in figure 2.3.1.5.4.
Figure 2.3.1.5.4: Structure of a) catechins and b) epicatechins and the variant R – groups specific for each flavan-3-ol. (Source: ChemSynthesis chemical database, 2015).

2.3.1.5.5 Anthocynadins

Anthocyanidins are a type of flavonoids found mostly in teas, honey, wines, fruits, vegetables, nuts, olive oil, cocoa, and cereals. Anthocyanins are derivatives of anthocyanidins, which include pendant sugars. Anthocynadins are odourless and nearly
flavourless. The most common anthocyanidins are cyanidin, delphinidin and pelargonidin (Jakobek et al., 2009). Examples of antocyanidins are shown in figure 2.3.1.5.5.

<table>
<thead>
<tr>
<th>Anthocyanidin</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanidin</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>Delphinidin</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>Perlagonidin</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Malvinidin</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>Peonidin</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>H</td>
</tr>
<tr>
<td>Petunidin</td>
<td>OH</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

**Figure 2.3.1.5.5:** The anthocyanidins cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin and variant R – groups specific for each anthocyanidin. (Source: ChemSynthesis chemical database, 2015).

Anthocyanins are the major polyphenol pigments in plants. Anthocyanins are highly reactive compounds and contribute to the antioxidant activity of all fruits (~90%) more than flavonols, flavan-3-ols and phenolic acids (~10%) (Jakobek et al., 2009).

### 2.3.1.5.6 Isoflavones

Isoflavones are a class of organic compounds that act as phytoestrogens in mammals. Some act as antioxidants because of their ability to trap singlet oxygen (Kaufman, 1997). Isoflavones are found almost entirely in leguminous plants particularly in soy beans as aglycones or glycosides, depending on the soy preparation (Ryan-Borchers et al., 2006).
Isoflavonoids have antioxidant, antimutagenic, anticarcinogenic and antiproliferative properties (Birt et al., 2001; Iwasaki et al., 2008). Isoflavonoids are derived from the flavonoid biosynthesis pathway via liquiritigenin or naringenin.

![Isoflavone structure](image)

<table>
<thead>
<tr>
<th>Isoflavones</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daidzein</td>
<td>H</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>Genistein</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
</tr>
</tbody>
</table>

**Figure 2.3.1.5.1.6:** The isoflavones daidzein and genistein and variant R – groups specific for each isoflavone. (Source: ChemSynthesis chemical database, 2015).

### 2.3.1.5.7 Chalcones

Chalcones are a type of antioxidant that has attracted attention of both researchers and industrialists because of its numerous applications (Batovska and Todorova, 2010). Chalcones (1,3-diaryl-2-propen-1-ones) are open chain flavonoids that are widely biosynthesized in plants. Structural examples of chalcones are shown in figure 2.3.1.5.7. Chalcones are important for the pigmentation of flowers and, hence, act as attractants to the pollinators. Chalcones have a defensive role of protecting plants against pathogens and insects. These compounds also exhibit numerous health benefits such as antioxidant, cytotoxic, anticancer, antimicrobial, antiprotozoal, antiulcer, antihistaminic and anti-inflammatory activities. Some lead compounds with various pharmacological properties
have been developed based on the chalcone skeleton. Clinical trials have shown that these compounds reached reasonable plasma concentrations and did not cause toxicity.

**Figure 2.3.1.5.7:** The chalcones; a) phloretin and b) xanthohumol. (Source: ChemSynthesis chemical database, 2015).

### 2.3.1.5.8 Dihydrochalcones

Dihydrochalcones are a family of bicyclic flavonoids, defined by the presence of two benzene rings joined by a saturated three carbon bridge (Nakamura et al., 2003). Dihydrochalcone is a chemical compound related to chalcone. The general chemical structure of dihydrochalcones is shown in figure 2.3.1.5.8. Examples of these polyphenols are phloretin, phloridzin, phloretin 2'-O-xylosyl-glucoside, 3-hydroxyphloretin 2'-O-xylosyl-glucoside and 3-hydroxyphloretin 2'-O-glucoside.
Figure 2.3.1.5.8: The general chemical structure of a dihydrochalcone. (Source: ChemSynthesis chemical database, 2015).

2.3.1.5.9 Dihydroflavonols

Dihydroflavonols are synthesised from tetrahydroxychalcone by chalcone isomerase. Tetrahydroxychalcone is a result of the action of chalcone synthase on 4-coumaroyl CoA and malonyl-CoA, both products of the phenylpropanoid pathway. Examples of dihydroflavonols include dihydroquercetin 3-O-rhamnoside, dihydroquercetin and dihydromyricetin 3-O-rhamnoside. The general chemical structure of dihydroflavonols is shown in figure 2.3.1.5.9.
2.3.2 Biosynthesis of polyphenols

Polyphenols are naturally synthesised in plants only through the shikimate pathway. Biosynthesis of complex polyphenols such as flavonoids is through primary metabolism of plastid and mitochondrial-derived intermediates, each requiring export to the cytoplasm where it is incorporated into separate parts of the molecule. The biosynthetic pathways of phenolic compounds in plants are well-documented (Macheix et al., 1990; Dixon and Paiva, 1995; Strack, 1997) and a general scheme is outlined in Figure 2.3.2.

The aromatic ring B and the chromane ring shown in figure 2.3.2 are considered to originate from the amino acid phenylalanine, itself a product of the shikimate pathway, whereas ring A, from three units of malonyl-CoA (Tsao and McCallum 2009). These three malonyl-CoA units are added through sequential decarboxylation condensation reactions which initiate flavonoid biosynthesis.
Phenylalanine ammonia lyase (PAL) is an enzyme of the phenylpropanoid pathway. PAL catalyzes the conversion of phenylalanine to cinnamate leading to the formation of C6–C3 structures. The final intermediate 4-coumaroyl-CoA and three molecules of malonyl-CoA are then condensed to yield the first flavonoid structure naringenin chalcone by the enzyme chalcone synthase (CHS) (Fatland et al., 2004). Chalcone is isomerized by chalcone flavanone isomerase (CHI) to a flavanone intermediate which is pivotal because it is essentially from where all classes of flavonoids including their subgroups branch out. Chalcone is also where isoflavones and coumestrols branch out from through different enzymes including CHI and isoflavone synthase (IFS). Glucosylation of flavonoids is catalyzed by glucosyltransferase (Bohm, 1998). Understanding the biosynthetic pathways of polyphenols can help to design feed with enhanced polyphenol content and health benefits (Tsao et al., 2006).
Figure 2.3.2: Biosynthesis of hydroxycinnamic acids, hydroxybenzoic acids and flavonoids. Solid arrows represent well-characterised reactions catalysed by single enzymes. Dashed lines represent transformations that require multiple enzymes that are less characterised, or vary among plant species. Enzymes: CA4H, cinnamic acid 4-hydroxylase; CHS, chalcone synthase; 4CL, 4-coumarate coenzyme A ligase; PAL, phenylalanine ammonialyase. (Source: Häkinnen, 2000).
2.3.3 Mechanism of antioxidant activity of polyphenols

The antioxidant properties that enable polyphenols to act as free radical scavengers are related to their chemical structure. The factors determining the antioxidant activity of polyphenols include the type of compound, the degree of methoxylation and the number of hydroxyl groups attached to the molecule. The inhibition of oxidation by phenolic acids is related to the chelation of metal ions via the ortho-dihydroxyphenolic structure, the scavenging of alkoxy and peroxyl radicals, and the regeneration of α-tocopherol through reduction of the tocopheryl radical (Bors et al., 1990). Phenolic compounds possess hydroxyl and carboxyl groups that are able to bind mainly iron and copper (Lambert and Elias, 2010).

The structural features that have been associated with antioxidant activity are the catechol group on the B-ring, which confers high stability to the radical formed after the capture reaction of the free radical, the 2,3-double bond in conjugation with a 4-oxofunction of a carbonyl group in the C-ring and the presence of hydroxyl groups at the 3 and 5 position (Figure 2.2.3.3b) (Bourne and Rice-Evans, 1998).
Figure 2.3.3 Antioxidant activity of flavonoids. (a) a catechol moiety of the B-ring, (b) presence of hydroxyl groups at the 3 and 5 position, (c) the 2,3-double bond in conjugation with a 4-oxofunction of a carbonyl group in the C-ring.

Flavonoids in plant cells are located in the membrane at the interface lipid/water, being the first to react with the ROS formed in these areas. The type of conjugation of the flavanoids during biotransformation and the location of the flavanoids in the body, determines the ability of enzyme inhibition and antioxidant capacity. Flavanoids can act directly as "scavengers" of free radicals, by hydrogen or electron donation, leading to more stable compounds, or compounds that can stabilize compounds obtained from free radicals or may have an additive effect on the endogenous antioxidant defence system by increasing or maintaining this antioxidant defence (Lambert and Elias, 2010). The mechanism of scavenging for free radicals is the same for phenolic acids. However, for the phenolic compounds to be able to exert their function on the body, they have to be bioaccessible and bioavailable to the body.
2.3.4 Commercially available antioxidants

Most commercially available antioxidants are available as food supplements. Bilirubin, ferritin, glutamine reductase, glutaminine peroxidase and superoxide dismutase are some examples of antioxidants that can be found commercially (Chen et al., 2004; Harder et al., 2004). Mangosteen® (XanGo) and MonaVile® are examples of commercially available antioxidants. Mangosteen® is a product that contains xanthones which are antioxidants and anti-inflammatory agents. MonaVile® is a brand name for the antioxidants from the Accai berry.

2.4 Bioaccessibility

Bioaccessibility of a food constituent is the amount of that food constituent particularly present in the gut, as a consequence of the release of the constituent from the solid food matrix, and the released constituent should be able to pass through the intestinal barrier (DEFRA and Environment Agency, 2002c). The relationship between food intake and health has been the focal point of much scientific investigation, in attempts to identify the specific phytochemicals that are beneficial to the health of the body. Cereals, fruits and vegetables, apart from being sources of vitamins, minerals, and fiber, are rich sources of potentially bioactive compounds known as phytochemicals. Antioxidants are phytochemicals that prevent some of the processes involved in the development of cancer and cardiovascular disease. Evidence for their role in the prevention of other diverse degenerative diseases is continuously emerging (Denny and Buttriss, 2007).

The bioaccessibility and bioavailability of each antioxidant varies greatly, and the most abundant antioxidants in ingested fruit may not necessarily be the ones leading to the
highest concentrations of active metabolites in target tissues (Manach et al., 2005). A number of factors interfere with the bioaccessibility and bioavailability of antioxidants. The food source and the chemical interactions with other phytochemicals and biomolecules present in the food affect the bioaccessibility of the antioxidants (Parada and Aguilera, 2007). Cereal antioxidants are mixed with different macromolecules such as carbohydrates, lipids, and proteins to form the food matrix. In plant tissue, carbohydrates are the major compounds found and phenolic compounds are found free or conjugated to the carbohydrates (Manach et al., 2004).

Dietary fibre, the indigestible cell wall component of plant material, plays a positive role in human diet and health (Adiotomre et al., 1990; Prosky, 2000; Montagne et al., 2003; DeVries, 2004). However, these complex carbohydrates may directly interact with the food antioxidants and interfere with the adequate assimilation of the antioxidants into the body (Faulks and Southon, 2005; Parada and Aguilera, 2007; Porrini and Riso, 2008; Del Rio et al., 2009; Pérez et al., 2009).

Most studies on antioxidant bioaccessibility are focused on foods and beverages from which antioxidants are easily released (Furr and Clark, 1997; Ferguson et al., 2004; Lafay et al., 2006; Lafay and Gil-Izquierdo, 2008). Research concerning the bioaccessibility of phenolic compounds and other antioxidants from solid matrices have not been done and are important. The studies are important because only the compounds released from the food matrix in the small intestine are bioaccessible and potentially bioavailable and in a condition to exert their beneficial effects (Lafay and Gil-Izquierdo, 2008).
2.4.1 Phenolic compounds bioaccessibility and bioavailability

The properties of antioxidants in the humans depend not only on their concentrations in fruits, vegetables and cereals but also on their bioaccessibility and bioavailability after ingestion (Parada and Aguilera, 2007). In many studies, the focus has been on the bioavailability of antioxidant compounds after their ingestion (Manach et al., 2005; P’erez et al., 2009). Understanding bioaccessibility and bioavailability is essential to all individuals involved in food production and nutritional evaluation in determining diet and health relationships (Manach et al., 2004; Manach et al., 2005).

Bioavailability of an antioxidant is the fraction of that antioxidant that is absorbed by the body and reaches the normal systemic circulation intact. Measurement of bioavailability relies greatly upon estimates of amounts of antioxidant absorbed. Bioaccessibility is the amount of an ingested nutrient that is available for absorption in the gut after release from the food matrix during digestion (Hedren et al., 2002). The bioavailability of a food substance depends on the bioaccessibility of that food substance.

Originally, the absorption of polyphenols from the diet was believed to be insignificant, given that the majority of food flavonoids are bound to glycosides. It was believed that only the aglycones could pass without restraint into the blood flow from the stomach wall, since no enzymes are secreted in the intestines that can cleave glycosidic bonds (Manach et al., 2005). However, the bioavailability and bioaccessibility of specific flavonoids and phenolic compounds is way higher than formerly believed (Robles-Sardin et al., 2010).

The metabolism of phenolic compounds from beverages and food lacking dietary fiber starts in the small intestines and post-absorption modifications occur in the liver and other organs (Manach et al., 2005). The majority of polyphenols are absorbed in some form from
the intestine after consumption and can pass through the gut wall into the blood stream (Lafay and Gil-Izquierdo, 2008) as illustrated in figures 2.4.1.1 and 2.4.1.2.

In order for the uptake of flavonoids from the small intestines to take place, the sugars that are conjugated to the flavonoid skeleton must be cleaved (P’erez et al., 2009). The cleavage of the conjugated sugar is controlled by the action of enzymes such as mammalian β-glucosidase produced in the small intestines, resulting in the release of the flavonoid skeleton called the aglycone (Denny and Buttriss, 2007). A considerable portion of ingested dietary flavonoids and related compounds is not taken up in the small intestines but rather proceeds to the large intestines. In the large intestines, flavonoids are degraded by the colonic micro-flora to simple phenolic acids, which can be absorbed into the circulatory system or exert antioxidant activity in the intestinal surroundings (Del Rio et al., 2009).

The nature of the sugar attached to the flavonoid skeleton determines the extent of absorption of glycosylated flavonoids (Donovan et al., 2006). The position at which the sugar is attached to the flavonoid skeleton affects the method by which glycosylated flavonoids are absorbed (Donovan et al., 2006). Once the sugars have been disconnected from the skeleton for absorption, flavonoids are further metabolized in the gut and consequently in the liver and kidneys to generate a vast number of flavonoid secondary metabolites. The metabolism of flavanoids in the gut involves additional conjugation of the flavonoid through the joining of glucuronate, sulfate, or methyl groups. In the intestines, the metabolism of phenolic compounds controlled by enzymes produced by the gut bacteria that play a vital role in the metabolism of plant bioactive compounds (Denny and Buttriss, 2007).
Flavonoid secondary metabolites can be detected in human blood and urine following the consumption of flavonoid-containing fruits and beverages, but only very small quantities of non-conjugated flavonoids in their original form can be found in the same specimens (Kroon et al., 2004). The flavonoid secondary metabolites enter the circulation and evidence suggests that it is the secondary metabolites of the flavanoids, rather than the native flavonoids found in fruits and vegetables, that exert biological effects in the body (Kroon et al., 2004; Denny and Buttriss, 2007).
Figure 2.4.1.1: The human intestinal digestion and absorption of phenolic compounds (■) and sugars (●) contained in beverages or similar foods poor in fiber.

(Adapted from: Palafox-Carlos et al., 2010)
Figure 2.4.1.2: The human intestinal digestion and absorption of phenolic compounds (■), single sugar compounds (●) and polysaccharides/fibre (---) contained in foods rich in dietary fiber, such as cereals and vegetables.

2.4.2 Antioxidant release and bioaccessibility

The initial physical conversion of food matrices during eating occurs in the mouth, and mastication is the primary step in the digestion of foods. Mastication is characterised by
grinding food into small pieces and mixing the food pieces with saliva to form a bolus that can be swallowed. Decreasing the particle size of food enlarges the surface area available for action by digestive enzymes, thus increasing overall efficiency of digestion and the potential gastrointestinal absorption of antioxidants (Kulp et al., 2003). The next stage would be the digestion of the food particles by enzymes released in the mouth, stomach and small intestines thereby releasing nutrients for potential absorption.

Only antioxidants released from the cereal and vegetable environment by the action of digestive enzymes in the small intestines and by action of bacterial micro-flora in the large intestines, are bioaccessible in the stomach and therefore potentially bioavailable (Saura-Calixto et al., 2007).

Bioaccessibility is not taken into account in studies regarding the bioavailability of polyphenols. Most studies on bioavailability of polyphenols use mainly pure single molecules isolated from food or chemically synthesized some beverages, and single foods; however, the bioavailability from whole foods may be substantially different (Manach et al., 2005).

### 2.5 Cereals

Cereals belong to the grass family (Poaceae) which has five sub-families with approximately 10,000 species according to the Missouri botanical garden classification (Chapman, 1996). A cereal is grown for its edible seed, kernel or grain called caryopsis and is usually utilized as a staple food (Chapman and Carter, 1976). Cereals are annual plants, and must be reseeded for each growing season. Cereals are the most accessible crops to mankind (Chapman, 1996).
2.5.1 Cereals as natural sources of antioxidants.

Cereal grains are a staple in most countries worldwide and an important natural source of antioxidants that are beneficial to human health. Six cereal grains wheat, maize, rice, barley, sorghum and millet provide 56 % of food energy and 50 % of the proteins consumed (Stoskopf, 1985). Wheat, maize and rice together comprise at least 75 % of the world’s grain production (Cordain, 1999).

The cereal grain is the fruit of the cereal and consists of the bran, endosperm and the germ. The bran is the outer layer of the grain. It contains fibre, omega-3 fatty acids, vitamins and dietary minerals. The fibre has phenolics bound to it which can be released when acted upon by enzymes. Grains can be consumed as whole grains or refined grains where the germ and bran are removed. The bran which is removed during refining contains many useful nutrients including phytochemicals (Harold et al., 2000). The endosperm is a part of the grain which is mainly starch, whereas the germ, amongst other nutrients, contains vitamin E, folate, thiamine, phosphorus and magnesium.

Whole grains provide a broad range of nutrients and phytochemicals that work synergistically to enhance human health (Harold et al., 2000). Consuming whole grains has the potential to reduce the risk of cancers whereas incidences of cancers have been associated to refined foods (American Cancer Society, 2011). Use of underutilised food sources such as wild cereal grains and evaluation of ethnic and traditional foods could offer many advantages in the promotion of human health (Dimitrios, 2006). Both domesticated and wild cereals are possible food sources that contain phytochemicals with potential action against non-communicable diseases.
2.5.2 Phenolic compounds from cereal grains

The most common phenolic compounds found in whole grains are phenolic acids and flavonoids. A number of phenolics that include flavanoids, have been isolated from fruits, vegetables and tea (Quideau et al., 2011; Naczk and Shahidi, 2006; Slimestad and Verheul, 2009; Rusak et al., 2008) and many phenolic compounds found in fruits and vegetables have been reported in cereals.

Whole-grain cereals contain phytochemicals not found in fruits and vegetables. Various classes of phenolic compounds such as phenolic acids, anthocyanidins, quinones, flavonols, chalcones, flavones, flavanones, and amino-phenolic compounds have been reported in cereal grains (Lloyd et al., 2000; Maillard and Berset, 1995; Shahidi and Naczk, 1995; Thompson, 1994). Ferulic acid and diferulates are examples of phenolic compounds found predominantly in grains but which are not present in significant quantities in fruits and vegetables (Bunzel et al., 2001; Shahidi and Naczk, 1995).

Phenolic acids in cereals occur in both free and bound form. Free phenolic acids are found in the outer layer of the pericarp (Jacobs et al., 2002; Hahn et al., 1983; Sosulski et al., 1982; Subba and Muralikrishna, 2002). The bound forms of phenolic acids are esterified to cell walls and acid or base hydrolysis is required to release these bound compounds from the cell matrix (Hahn et al., 1983; Kim et al., 2006; Robbins, 2003). The most widely distributed phenolic acids in cereals are ferulic acids and \( p \)-coumaric acid (Gani et al., 2012). Phenolic acids mainly exist as glycosides linked to various sugar moieties or as other complexes linked to organic acids, amines, lipids, carbohydrates and other phenols.

Tannins are a class of phenolic compounds found in cereal grains. Tannins in barley are monomers, dimers and trimers whereas those found in sorghums are polymers. Tannins
bind to proteins, carbohydrates and minerals. The binding decreases digestibility of nutrients and reduces feed efficiency of ruminants and monogastric animals during feeding. However, one form of tannins called condensed tannins, has high oxidant activity in vitro compared to monomeric phenolic compounds (Hagerman et al., 1998). In addition, condensed tannins may have anticarcinogenic, cardiovascular, gastroprotective, anti-ulcerogenic and cholesterol-lowering properties whereas they have also been documented to promote urinary tract health (Hagerman et al., 1998). Dykes and Rooney (2007), reported that tannin sorghum and black rice had the highest antioxidant activity whereas white rice, wheat, and waxy barley had the lowest activity levels.

Wild varieties of cereals have the highest polyphenol levels that have been attributed to plant species exposed to stress conditions (Cruz de Carvalho, 2008). Wild varieties of grains are likely to experience a lot of stresses such as water shortages compared to domesticated varieties which can be irrigated when there is poor rainfall, hence the trend is that wild cereals are likely to have higher quantities of polyphenols when compared to domestic cereals (Manach et al., 2004). Researchers have suggested that plants under stress potentially have high phenolic levels. This suggestion is important as it helps in the exploration of new polyphenols that would otherwise go unnoticed in most domesticated plants due to their production in low concentrations. Some of the phenolic acids found in cereal grains are shown in table 2.5.2.1.
Table 2.5.2.1: Some phenolic acids reported in cereal grains. (Adapted from: Dykes and Rooney, 2007).

<table>
<thead>
<tr>
<th>Phenolic Acid</th>
<th>Grain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxybenzoic acids</td>
<td></td>
</tr>
<tr>
<td>Gallic</td>
<td>Millet, rice, sorghum</td>
</tr>
<tr>
<td>Protocatechuic</td>
<td>barley, maize, millet, oat, rice, rye, sorghum, wheat</td>
</tr>
<tr>
<td>Vanillic</td>
<td>barley, maize, millet, oat, rice, rye, sorghum, wheat</td>
</tr>
<tr>
<td>Hydroxycinnamic acids</td>
<td></td>
</tr>
<tr>
<td>Ferulic</td>
<td>barley, maize, millet, oat, rice, rye, sorghum, wheat</td>
</tr>
<tr>
<td>Caffeic</td>
<td>maize, millet, oat, rice, rye, sorghum, wheat</td>
</tr>
<tr>
<td>p-Coumaric</td>
<td>barley, maize, millet, oat, rice, rye, sorghum, wheat</td>
</tr>
<tr>
<td>Cinnamic</td>
<td>maize, millet, sorghum</td>
</tr>
<tr>
<td>Sinapic</td>
<td>barley, millet, oat, rice, rye, sorghum</td>
</tr>
</tbody>
</table>

Hydroxycinnamic acids are the most abundant phenolic compounds in cereals. The major hydroxycinnamic acid is ferulic acid followed by diferulic acid then sinapic acid, p-coumaric acid and caffeic acid (Adom and Liu, 2002; Adom et al., 2003; Andreasen et al., 2000; Garcia-Conesa et al., 1997; Hatcher and Kruger, 1997; Zhou et al., 2004).

The amount of ferulic acid found in cereals is highly variable both in whole-grain and in bran. The final amount of the phenolic acid in food is dependent on cereal variety and on milling procedure (Adom et al., 2003). Ferulic acid is found chiefly in the outer parts of the grain. The aleurone layer and the pericarp of wheat grain contain 98% of the total ferulic.
acids (Garcia-Conesa et al., 1997). Some of the flavonoids that have been reported to be found in cereal grains are shown in table 2.5.2.2

**Table 2.5.2.2:** Flavonoids reported in cereal grains. (Adapted from Dyke and Rooney, 2007).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Grains</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthocyanins</strong></td>
<td></td>
</tr>
<tr>
<td>Apigeninidin</td>
<td>Sorghum</td>
</tr>
<tr>
<td>Cyanidin 3- glucoside</td>
<td>Barley, maize, rice, rye, wheat</td>
</tr>
<tr>
<td>Cyanidin 3- rutinoside</td>
<td>Maize, rice, wheat</td>
</tr>
<tr>
<td>Luteolinidin</td>
<td>Sorghum</td>
</tr>
<tr>
<td><strong>Flavones</strong></td>
<td></td>
</tr>
<tr>
<td>Apegenin</td>
<td>millet, oat, sorghum</td>
</tr>
<tr>
<td>Luteolin</td>
<td>millet, oat, sorghum</td>
</tr>
<tr>
<td><strong>Flavanones</strong></td>
<td></td>
</tr>
<tr>
<td>Naringenin</td>
<td>Sorghum</td>
</tr>
<tr>
<td><strong>Flavonols</strong></td>
<td></td>
</tr>
<tr>
<td>Kaempferol</td>
<td>Maize, oat</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Maize, oat</td>
</tr>
<tr>
<td><strong>Dihydroflavonols</strong></td>
<td></td>
</tr>
<tr>
<td>Taxifolin</td>
<td>Sorghum</td>
</tr>
<tr>
<td><strong>Flavanols (monomeric/ dimers)</strong></td>
<td></td>
</tr>
<tr>
<td>Catechin</td>
<td>Barley, sorghum</td>
</tr>
<tr>
<td>Leucocyanidin</td>
<td>Barley, maize</td>
</tr>
<tr>
<td>Procyanidin B-1</td>
<td>Sorghum</td>
</tr>
</tbody>
</table>
2.5.3 Distribution of phenolics in cereal grains

Polyphenols are widely distributed in plants since they have many roles such as protection against predators and contribution to taste, colour and appearance. Polyphenols are located in the outer layers of the plants such as the peels, shells and hulls (Erkkilä, et al., 2005; Jacobs et al., 2000; Jensen et al., 2004; Koh-Banerjee et al., 2004). The location of phenolics in the outer layers is probably to offer a protective role to the inner materials of the grain.

Polyphenols contribute to the mechanical strength of cell walls. Phenolic compounds play an additional regulatory role in plant growth and in the cell response to stress and pathogens (Wallace, 1994; Baucher, 1998). Ferulic and \textit{p}-coumaric acids may be esterified to pectins and arabinoxylans, or cross-linked to cell wall polysaccharides in the form of dimers such as dehydroferulates and truxillic acid (Brett et al., 1999; Renger and Steinhart, 2000). According to Dykes and Rooney (2007), flavonoids in the grains are located in the pericarp. About 95% of all grain phenolic compounds are linked to cell wall polysaccharides and are referred to as dietary fibre-phenolic compounds (DF-PC) (Hatfield et al., 1999).

The location of the DF-PC polyphenols and their binding to the cell wall polysacharride affects efficiency of extracting the polyphenols. The polyphenols are contained in the bran which has thick walls and a cellular structure that makes the extraction process difficult (Harold et al., 2000). A number of phenolic acids are linked to various cell wall components such as arabinoxylans and proteins (Harris and Hartley, 1976). The polyphenols have different solubility ranges from water-soluble to lipid-soluble which affects the extraction process.
2.5.4 Cereals found in Zimbabwe

Some of the staple cereals that are traditionally cultivated and consumed in Zimbabwe are maize, sorghum, wheat, rice, barley and millet. Cereal production in-country is highly rainfall-dependent. Drought in Zimbabwe is the most common climate-related factor to impact negatively on agricultural production, with related effects on livelihoods, income and food security. Drought is a chronic threat in parts of Matabeleland North and South, Masvingo and Midlands provinces, and other drier parts of the country (Moyo, 2000). In times of drought or irregular rains, agricultural production, especially maize, is negatively impacted leading to Zimbabwe relying on regional maize imports to make up this cereal deficit.

Cereal yield in Zimbabwe was last measured at 751.50 kilograms per hectare in 2010, according to the World Bank for wheat, rice, maize, barley, oats, rye, millet, sorghum, buckwheat, and mixed grains (Dirk, 2011). Maize production estimate for the 2011/12 market year was 1.4 million MT on an area of 1.6 million hectares However, Zimbabwe had to import more than 100,000 tons of maize to meet local demand. Preliminary estimates were that only about 6,000 hectares of wheat had been planted for the 2011/12 market year, compared to 12,000 hectares in the 2010/11 market year (Dirk, 2011). Zimbabwe had to import at least 280,000 tons of wheat to meet local demand. Increased production of more drought-tolerant small grains (sorghum and millet) would help mitigate the dependence on regular rainfall, especially in the more marginally productive areas of the country.
2.5.4.1 Some wild cereal grains found in Zimbabwe

In Zimbabwe, some wild cereal and pseudo-cereal grains which include: *Amaranthus hybridus*, *Brachiaria brizantha*, *Panicum maximum*, *Rottboellia cochinchinensis* and *Sorghum arundnaceum* have been consumed in times when the domesticated cereals have failed due to drought (Shava, 2003). The wild cereal grains have been found to be drought tolerant and they take shorter periods to mature (Shava, 2003). Below are detailed descriptions of the wild cereals including *Amaranthus hybridus*, a pseudo-cereal.

2.5.4.1.1 *Amaranthus hybridus* (pseudo-cereal)

![Amaranthus hybridus](image)

*Figure 2.5.4.1.1: A. hybridus* a) leaves and reddish purple fluorescence and b) seeds.

*Amaranthus hybridus*, commonly known in English as the green amaranth, green pigweed, hybrid amaranth, prince's feather, slim amaranth, smooth amaranth or smooth pigweed, is a monoecious annual herb that belongs to the *Amaranthaceae* family. The *Amaranthaceae* family consists of hardy, weedy, herbaceous, fast-growing plants (Oke, 1980). *A. hybridus*
named “mowa dhongi or mowa guru” in Zimbabwe is a wild plant that is found in disturbed areas such as dumpsites. The plant is found in Zimbabwe and the other countries including India, China and the United States (He et al., 2002).

Amaranth leaves are eaten as spinach or as green vegetables (Shava, 2003). In Mozambique, leaves are boiled and mixed with groundnut sauce and eaten (Oliveira, 1975), or pureed into a sauce and served over vegetables in West Africa (Martin and Telek, 1979).

*A. hybridus* produces tiny black, sometimes shiny, compressed seeds that are 0.8-1.3 mm long and faintly reticulate near the margins (Wagner et al., 1999). Each seed averages between 1.0-1.5 mm in diameter and 1000 seeds weigh between 0.6-1.2 g (Saunders and Becker, 1984).

*A. hybridus* is a C₄ plant and it is one of the few dicotyledons in which the first product of photosynthesis is a four carbon compound. The production of a four carbon compound means that photosynthesis is particularly efficient in conditions of drought, extreme heat and great solar intensity (Wagner *et al.*, 1999). The combination of the broad leaves in amaranth and C₄ metabolism, results in increased efficiency in the use of CO₂ under a wide range of both temperature and moisture stress environments. The characteristics of the plant contribute to the plant species’ wide geographic adaptability to diverse environmental conditions. The metabolic pathway of producing a four carbon compound first during photosynthesis allows the plants that undergo the pathway to convert twice the amount of solar energy for growth than plants that use the C3 system and for the same amount of water absorbed by the plant.
A. hybridus can withstand some harsh conditions such as droughts, which cultivated grains like maize may not due to irrigation. The amaranthus plant is not cultivated in Zimbabwe but in the disturbed areas where it grows, most people find the plant important for its leaves rather than for its seed. It is therefore part of the aims of this research to establish the macro and micro-nutritional potential that the grain has to humans. It is important to analyze the macro and micro-nutritional composition of the grain in order to work on complementing the traditionally consumed grains that fail when droughts occur.

2.5.4.1.2 Rottboellia cochininchensis (Itchgrass)

Rottboellia cochininchensis, known in English as itchgrass is a member of the grass tribe Andropogoneae. Itchgrass is characterized by the inflorescence disarticulating into floral units consisting of a sessile spikelet, pedicellate spikelet, and internode. Diagnostic features of R. cochininchensis include the racemose inflorescence, a pedicel fused to the swollen floral internode, and heterotropic and awnless spikelets.

R. cochininchensis is an annual grass that invades distressed lands and farming areas throughout Zimbabwe. Grass stems and leaves are covered with stiff, irritating hairs that give rise to the name “itchgrass”. The plant is unpalatable to livestock and wildlife. R cochininchensis stems can reach a height of 0.3-3 m (Thomas, 1970). Under favourable environmental conditions, itchgrass starts producing seeds 6-7 weeks after emergence. Production of seeds continues throughout the growing period. Its leaf blades are 15-45 cm long, 5-20 mm wide and flat.

R. cochininchensis is an aggressive weed under various ecological conditions. In some areas, the grass seems to prefer wet, coarse-textured soils, sometimes even growing in shallow water. In other areas, it is dominant in open, well-drained places at higher
altitudes. In Africa and Malaysia it causes severe crop damage to corn, cotton, peanut, soybean, and sugarcane. Thomas (1970) reported the species in Rhodesia only in heavier soils that were clay to sandy. In the Americas *R. cochinchinensis* is an aggressive weed of sugarcane fields and this characteristic may render it an important grass to study since it has a potential to outcompete other weeds when cultivated as a useful crop. The itchgrass is shown in figure 2.5.4.1.2.

![Image](image_url)

a) plant leaf and inflorescence, and b) caryopsis in ventral view (left) and dorsal view (right).

(Source: Scher and Walters, 2010).

**Figure 2.5.4.1.2**: *Rottboellia cochinchinensis* a) plant leaf and inflorescence, and b) caryopsis in ventral view (left) and dorsal view (right).

### 2.5.4.1.3 *Panicum maximum* (Guinea grass)

*Panicum maximum* commonly known as Guinea grass is a perennial, tufted grass with a short, creeping rhizome. *Panicum* is derived from the Latin name for millet which is used in bread-making; *maximum* may refer to the great height that this plant attains (Gibbs Russell *et al.*, 1991). The stems of this robust grass can reach a height of up to 2 m. As the stems bend and nodes touch the ground, roots and new plants are formed. The leaf sheaths
of *P. maximum* run at the bases of the stems and are covered in fine hairs. The leaf blades are up to 35 mm wide and taper to a long fine point. The inflorescence is a large multi-branched, open panicle with loose, flexuous branches. The lower branches of the inflorescence are arranged in a whorl. The lower floret is usually male with a well-developed palea (upper bract enclosing flower) (Gibbs Russell *et al.*, 1991). The fertile (female) upper lemma is pale. Spikelets are green to purple and flowering occurs from November to July.

*P. maximum* is an ideal forage plant since it grows well on a wide variety of soils and even under light shade of trees and bushes and thus can be grown with other crops. The plant remains green until late into winter. It can survive long dry spells and quick-moving fires which donot harm the underground roots (Holm *et al.*, 1977). Panicum responds quickly to fertiliser and watering and this characteristic make the grass a good candidate of study for potential nutritional advantage to humans. *P. maximum* grows from sea level up to 1,200 m and its seeds are dispersed by birds (Motooka *et al.*, 2003). The leaf blade and large multi-branched, open panicle and grains of *P. maximum* are shown in figure 2.5.4.1.3.
2.5.4.1.3: *Panicum maximum* (a) leaf blade and large multi-branched, open panicle with loose, flexuous branches, and (b) grains.

2.5.4.1.4 *Brachiaria brizantha* (bread grass).

*Brachiaria brizantha*, also known in English as bread grass, is a tufted perennial grass that is 60 to 120 cm high, with roots that go down up to 2 m deep and short rhizomes. The grass has stout erect or slightly decumbent culms and bright green leaves. The inflorescence of *B. brizantha*, form a panicle consisting of 2 to 16 racemes that are 4 to 20 cm long. The plant has spikelets that are usually on a single row, elliptical and are between 4 to 6 mm long with a sub-apical fringe of long purplish hairs. *B. brizantha* is very variable in habit and therefore can be cultivated in diverse areas of Zimbabwe. For this reason, the grass can be studied for nutritional potential. The grass also varies in leafiness, hairiness and yield of the seed (McGregor *et al.*, 1988). The grass and the seeds of *B. brizantha* are shown in figure 2.5.4.1.4.
Figure 2.5.4.1.4: *Brachiaria brizantha* a) plant and b) the edible grain seeds

2.5.4.1.5 *Sorghum arundinaceum* (common wild sorghum).

*Sorghum arundinaceum*, known in English as common wild sorghum, is an annual or short-lived perennial plant without rhizomes. The grass has culms that grow 0.3 to 4 m high and are often robust. The odes of *S. arundinaceum* are glabrous or pubescent. Its leaf-blades are variable and are often large ranging from 5 to 75 cm long and 5 to 70 mm wide. The panicle is linear to broadly spreading ranging from 10 to 60 cm long. The primary branches of *S. arundinaceum* are compound, ultimately bearing racemes of 2 to 7 spikelet pairs. The plant has sessile spikelets that are lanceolate to narrowly ovate ranging from 4 to 9 mm in length. It has glabrescent to white pubescent that is sometimes tomentose or fulvously pubescent, sometimes awnless or more often with an awn that is 5 to 30 mm long. *S. arundinaceum* has pedicelled spikelets which are linear to lanceolate and usually are male or barren. The grass is common in most places around Zimbabwe and the diversity in its
habitat can render it a useful candidate for study as a potential nutritional source to humans in Zimbabwe. *S. arundinaceum* plant leaf blades, inflorescence and seeds are shown in figure 2.5.4.1.5.

![Figure 2.5.4.1.5: *Sorghum arundinaceum* a) leaf blades and inflorescence b) caryopsis.](image)

(Source: Gann et al., 2007).

**Figure 2.5.4.1.5**: *Sorghum arundinaceum* a) leaf blades and inflorescence b) caryopsis.

### 2.5.4.2 Domesticated cereals

A comparison of the wild cereal grains and the domesticated ones was made. The domesticated cereal grains chosen for this purpose were *Sorghum bicolor* (red variety) and *Eleusine corocana* to compare and contrast the characteristics of wild and domestic ones.
2.5.4.2.1 *Sorghum bicolor* (red variety)

*Sorghum bicolor* (L.) Moench, commonly known in English as sorghum, is an annual or short-term perennial plant that has culms that grow up to 4 m or more in height. The panicle is 8-40 cm long and the plant has loose or contracted and sessile spikelets that are 4-6 mm long. Mature glumes of sessile spikelets are red, reddish brown, straw-coloured or yellowish, sometimes flushed with dark red or reddish-brown. The grain is predominantly red or reddish-brown. Mature, sessile spikelets of *S. bicolor* are persistent and are less than twice as long as they are wide. The bicolor sorghums are characterized by long, clasping glumes that are at least three-fourths as long as the broadly elliptical grain (Whiteman and Wilson, 1965).

One advantage of sorghum is that the seed can become dormant under adverse conditions and can resume growth after a relatively severe drought. Drought in the early stages of the plant growth stops growth before floral initiation and the plant remains vegetative. Sorghum will resume leaf production and will flower when conditions become favourable for growth again. Occurrence of drought at the later stages of plant growth stops leaf development but not floral initiation (Whiteman and Wilson, 1965).

Bukantis (1980) reported that though sorghum was used largely for forage in the US, it was used worldwide in human diets, with over 300 million people dependent on it. Sorghum is grown for grain, forage, syrup, sugar and industrial uses of stems and fibers. Sorghum is a staple cereal in the hot dry tropics where the threshed grain is ground into wholesome flour. All sorghums contain phenolic acids and most contain flavonoids as well. Only varieties with a pigmented testa have condensed tannins. The sorghum plant and seeds are shown in figure 2.5.4.2.1.
2.5.4.2.1: *Sorghum bicolor* (red variety) a) plant and b) the edible seed

2.5.4.2.2 *Eleusine corocana* L. Gaertn. (finger millet)

*Eleusine corocana* L. Gaertn., known in English as African finger millet, is a tufted, annual grass that grows to a height of 21 to 62 cm tall. The leaf blades are shiny, strongly keeled and difficult to break and are 220 to 500 mm long and 6 to 10 mm wide. The leaves and culms of the plant are typically green in colour. *E. coracana* has an exceptionally strong root system and it is difficult to pull the plant out of the ground by hand (Van Wyk and Van Oudtshoorn 1999). The culms and the leaf sheaths are prominently flattened. The ligule of *E. coracana* is a fringed membrane. The inflorescence consists of spike-like main branches that are open or contracted and are digitate or sub-digitate. The spikelets are 5 to 8 mm long and 3 to 4 mm wide. The spikelets do not disarticulate (break apart at the joints) at maturity. The grains are globose. The grains of *Eleusine* are unusual in that the outer layer
called the pericarp is not fused and can be easily removed from the seed coat (Van Wyk and Van Oudtshoorn 1999).

There are two subspecies of African finger millet, the wild form (*E. coracana* subsp. *africana*) and a cultivated form derived from it (*E. coracana* subsp. *coracana*). Wild African finger millet (*E. coracana* subsp. *africana*) is similar to Indian goose grass (*E. indica*) and may be confused with it, but the latter has smaller spikelets and oblong, not rounded grains (Van Wyk and Gericke 2000).

*E. coracana* is found in warm temperate regions of the world from Africa to Japan and in Australia (National research Council, 1996). *E. coracana* is an important staple crop in many parts of Africa and has been cultivated in eastern and Southern Africa since the beginning of the iron-age. Before maize was introduced in Southern Africa, *E. coracana* was the staple crop of the region (National research Council, 1996). *E. coracana* plant and its seeds are shown in figure 2.5.4.2.2.
2.6 Methods in assaying for phenolic compounds

Methods that are commonly used in the analysis of phenolic compounds include sample preparation, extraction, separation and analysis of the phenolic compounds. Samples must be prepared in a way that does not lead to the degradation of the phenolic compounds by agents such as direct sunlight and excessive heat. Solid samples are milled, ground or homogenised before freeze- or air-drying (Mattila et al., 2005). Centrifugation or filtration can be used for liquid samples followed by rotary evaporation. The variation in the polarity, acidity, number of hydroxyl groups and aromatic rings, concentration levels and complexity of the matrix of phenolic compounds, determine the optimal procedures (Stalikas, 2007).
2.6.1 Methods of extracting phenolic compounds

The most common methods of extraction for phenolic compounds are liquid-liquid and solid-liquid extractions. Extraction using a solvent is used to separate free phenolic acids that are located in the outer layer of the pericarp (Hahn et al., 1983; Mattila et al., 2005). Alcohol-based solvents are used for the majority of extraction procedures (Subba and Muralikrishna, 2002). Alcohol based solvents include methanol and ethanol. Other solvents such as acetone, diethyl ether, and ethyl acetate are used for extraction of phenolic compounds as well. Very polar solvents which include; benzoic acid, isoform, hexane and benzene, are appropriate for the extraction of non-polar compounds like waxes, oils, sterols and chlorophyll from the plant matrix. pH, temperature, sample-to-solvent volume ratio, the number and time intervals of individual extraction steps must be monitored since the parameters affect the extraction method (Hahn et al., 1983).

Some extraction methods used for polyphenols include: supercritical fluid extraction (SFE), soxhlet extraction (SE), solid phase micro-extraction (SPME), matrix solid phase dispersion (MSPD) and solid phase extraction (SPE).

Vortexing and centrifugation are often used in extraction procedures to enhance the efficiency of the extraction methods. Acid extraction or saponification is used to extract phenolic acids which are found esterified to cell walls (McDonough et al., 2000; Zhou et al., 2004). Enzymes like pectinases, cellulases and amylases are used as biological extraction agents to degrade carbohydrate connections (Stalikas, 2007).
2.6.2 High Performance Liquid Chromatography (HPLC)

HPLC employs the use of a stationary phase, a pump that moves the mobile phase(s) and analyte through the column, and a detector to identify the retention time for the analyte. The detector, which is usually a diode array detector, may provide additional information related to the analyte. For example, the detector may provide UV/Vis spectroscopic data for the analyte if so equipped. A pump provides the high pressure required to move the mobile phase and analyte through the densely packed column (Knox et al., 1978). The elevated density in the column arises from smaller particle sizes and the high density allows for better separation on columns of shorter length and ensures higher velocity of the analyte through the column.

HPLC can either be performed in normal or in reversed phase. Normal phase HPLC uses a polar stationary phase and a non-polar, non-aqueous mobile phase, and works effectively for separating analytes that are readily soluble in non-polar solvents. Reversed phase HPLC has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase, which we used in this research, is silica which has been treated with RMe₂SiCl, where R is a straight chain alkyl group such as C₁₈H₃₇ or C₈H₁₇. With the C₁₈H₃₇ or C₈H₁₇ stationary phases, the retention time is longer for molecules which are less polar, while polar molecules elute more readily (Stalmach et al., 2006)

2.6.3 Classification of antioxidant methods

Based on the reaction between the antioxidant and the free radicals, the methods for determining antioxidant capacity are classified as Hydrogen Atom Transfer (HAT) or Electron Transfer (ET) assays (Huang, 2005).
2.6.3.1 Assays based on transfer of hydrogen atoms

These assays are used to measure or quantify the ability of the antioxidant compounds to donate hydrogen atoms by a proton-coupled electron transfer reaction, where it measures the chain-breaking antioxidant capacity. HAT assays are based on the reaction between a synthetic generator of free radicals, oxidisable molecular probe and an oxidant where reaction kinetics is derived from the kinetic curve. The oxygen radical absorbance capacity, total radical trapping antioxidant parameter assay, β-carotene–linoleic acid (linoleate) assay, hydroxyl radical scavenging assay, lipid peroxidation inhibition capacity assay, nitric oxide radical inhibition activity assay, scavenging of H₂O₂ radicals, ABTS radical scavenging method and scavenging of super oxide radical are examples of assays that are based on the HAT reaction.

2.6.3.1.1 The hydroxyl radical scavenging activity method

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. The hydroxyl radical scavenging activity assay involves the generation of hydroxyl radicals in-vitro using a Fe³⁺/ascorbate/EDTA/H₂O₂ system. The extent to which the generated hydroxyl radical is scavenged by the antioxidant is measured. In one of the methods, the hydroxyl radicals formed by the oxidation are made to react with dimethyl sulphoxide to produce formaldehyde. The formaldehyde formed produces an intense yellow colour with Nash reagent (2M ammonium acetate with 0.05M acetic acid and 0.02M acetyl acetone in distilled water). The intensity of yellow colour formed is measured at 412 nm spectrophotometrically against reagent blank. The activity is expressed as % hydroxyl radical scavenging (Babu et al., 2001).
2.6.3.1.2 The (2, 2-Azinobis(3-ethyl Benzothiazoline-6-sulfonic acid) diamonium Salt (ABTS) method

The ABTS assay is a measure of antioxidant activity as opposed to antioxidant concentration which might include a proportion of biologically inactive antioxidants. The method permits the measurement of antioxidant activity of mixtures of substances and helps to distinguish between additive and synergistic effects. The assay is based on interaction between antioxidant and ABTS$^+$ radical cation which has a characteristic colour showing maxima at 645, 734 and 815 nm (Rice-Evans and Miller, 1994; Simonetti et al., 1997).

2.6.3.1.3 The Total Radical Trapping Antioxidant Parameter (TRAP) method

The TRAP method measures the fluorescence of R-Phycoerythrin that is quenched by ABAP (2,2’-azo–bis (2-amidino-propane) hydrochloride), a radical generator. The antioxidative potential is evaluated by measuring the delay in decoloration (Ghiselli et al., 1995).

2.6.3.1.4 The Oxygen Radical Absorbance Capacity (ORAC) method

The ORAC assay is based on the use of a reaction induced by peroxyl radical oxidation to measure the antioxidants chain breaking ability of the test sample. The assay uses beta-phycoerythrin (PE) as an oxidizable protein substrate and 2,2’-azobis (2-amidinopropane) dihydrochloride (AAPH) as a peroxyl radical generator or Cu$^{2+}$, H$_2$O$_2$ as a hydroxyl radical generator. The ORAC assay is the only method that takes free radical action to completion and uses an area under curve technique for quantitation. The assay combines both
inhibition percentage and the length of inhibition time of the free radical action by antioxidants into a single quantity (Cao et al., 1993).

2.6.3.1.5 β-carotene linoleate model system method

The β-carotene-linoleate method depends on the oxidation of linoleic acid induced by reactive oxygen species (Joseph et al., 1994). The products of the oxidation of linoleic acid oxidise β-carotene leading to discoloration. In the presence of antioxidants, the extent of discoloration of β-carotene is reduced and the reaction is measured using a spectrophotometer at 434 nm (Joseph et al., 1994).

2.6.3.1.6 Thiobarbituric Acid (TBA) Method

The thiobarbituric acid test is used for measuring the peroxidation of lipids. The method involves the isolation of microsomes from the liver of a rat and induction of lipid peroxides with ferric ions leading to the production of small amount of Malonaldehyde (MDA). The TBA reacts with MDA to form a pink chromogen, which can be detected at 532 nm (Kimura et al., 1984).

2.6.3.2 Electron transfer-based assays

The Electron transfer-based assays measure the reducing capacity of the antioxidant compounds. The assays are based on the redox reaction where antioxidant compounds reduce the free radicals (Huang, 2005). The reduction by antioxidant compounds of the free radical results in the colour change of the reagent, which correlates with the antioxidant
capacity and is measured by the change in absorbance. Some of the electron transfer based assays include; The total phenols assay by Folin-Ciocalteu reagent, Trolox equivalence antioxidant capacity (TEAC), ABTS method, ferric ion reducing antioxidant power (FRAP), total antioxidant potential” assay using a Cu (II) complex as an oxidant, (CUPRAC)-Copper (II) reduction capacity, DPPH free radical scavenging assay, superoxide anion radical scavenging assay (Huang, 2005).

2.6.3.2.1 Folin-Ciocalteu or Total Phenolics Assay.

The Folin C assay has for several years been utilised as a measure of total phenolics in natural products, but the basic mechanism is an oxidation/ reduction reaction (Singleton and Rossi 1999). Developed in 1927, the Folin C method is based on the procedure used in the analysis of proteins that contain the phenolic amino acid tyrosine in which oxidation of phenols by a molybdotungstate reagent yields a colored product with absorbance max at 745-750 nm. The reactions that the method is based on are shown in the equations 1 and 2:

1. \( \text{Na}_2\text{WO}_4/\text{Na}_2\text{MoO}_4 \rightarrow (\text{phenol} – \text{MoW}_{11}\text{O}_{40})^{-4} \)

2. \( \text{Mo(VI)} \) (yellow) + e\(^-\) \( \rightarrow \) \( \text{Mo(V)} \) (blue)

The method is sensitive but it is slow at acid pH, and the method lacks specificity. The Folin C method is simple and can be useful in characterizing and standardizing botanical samples. The disadvantages of the Folin C method are that it suffers from a number of interfering substances particularly sugars, aromatic amines, sulphur dioxide, ascorbic acid and other enediols and reductones, organic acids, and Fe (II), and correction for interfering substances should be made (Singleton and Rossi 1999). Additional nonphenolic organic
substances that react with the Folin C reagent including adenine, adenosine, alanine, aniline, aminobenzoic acid, ascorbic acid, benzaldehyde, creatinine, cysteine, cytidine, cytosine, dimethylaniline, diphenylamine, EDTA, fructose, guanine, guanosine, glycine, histamine, histidine, indole, methylamine, nitriloacetic acid, oleic acid, phenylthiourea, proteins, pyridoxine, sucrose, sulfanilic acid, thiourea, thymine, thymidine, trimethylamine, tryptophan, uracil, uric acid, and xanthine. Singleton and Rossi in 1965 enhanced the method through molybdatungstophosphoric heteropolyanion reagents 3 and 4.

3. \( H_2O \cdot P_2O_5 \cdot 13WO_3 \cdot 5MoO_3 \cdot 10H_2O \) and

4. \( 3H_2O \cdot P_2O_5 \cdot 14WO_3 \cdot 4MoO_3 \cdot 10H_2O \)

The reagents reduced phenols more specifically; the absorbance max for the product is 765 nm. Singleton and Rossi (1965) imposed mandatory steps and conditions to obtain reliable and predictable data. The conditions were: (1) proper volume ratio of alkali and Folin C reagent; (2) optimal reaction time and temperature for color development; (3) monitoring of optical density at 765 nm; and (4) use of gallic acid as the reference standard phenol. If the steps by Singleton and Rossi are followed, a uniformly acceptable method of total phenolics analysis could be established, so that results can be compared rationally.

### 2.6.3.2.2 1,1- Diphenyl-2-Picryl Hydrazyl (DPPH) method

The DPPH assay method is based on the reduction of methanolic solution containing the purple free radical DPPH by a free radical scavenger (Sanchez-Moreno et al., 1999). The procedure involves measurement of decrease in absorbance of DPPH at its absorption peak of 516 nm, which is proportional to concentration of free radical scavenger added to reaction solution. The activity is expressed as a percentage of the final concentration of
DPPH in relation to original concentration of the DPPH solution (Vani et al., 1997; Navarro et al., 1993).

### 2.6.3.2.3 Superoxide radical scavenging activity method

*In vitro* superoxide radical scavenging activity is measured by the reduction of riboflavin by light or Nitro blue tetrazolium (NBT) of which the reduction of NBT is the most popular method. The super oxide radical reduces NBT to a blue coloured formazan complex whose colour intensity can be measured at 560 nm. The capacity of extracts to inhibit the colour to 50 % is termed the EC$_{50}$ value. Antioxidant activity of extracts has been reported in terms of superoxide radical scavenging activity (Robak and Gryglewski 1998). The superoxide radical can be detected by oxidation of hydroxylamine, yielding nitrite which is measured using a colourimetric reaction (Babu et al., 2001).

### 2.6.3.2.4 Reducing power method

In the reducing power assay the increase in the absorbance of the reaction mixture is measured. Upon addition of greater volumes of sample containing the antioxidant, the increase in the absorbance is an indicator of the increase in the antioxidant activity. In the reducing power method, antioxidant compounds form a coloured compound with potassium ferricyanide, trichloroacetic acid (TCA) and ferric chloride and the absorbance is measured at 700 nm. Increase in absorbance of the reaction mixture is an indicator of an increase in the reducing power of the samples (Jayaprakash et al., 2001).
2.6.3.2.5 Ferric Reducing Ability of Plasma (FRAP) method

The ferric reducing ability of plasma method, is a rapid test and the test is useful for routine analysis. The antioxidative activity is estimated by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent containing TPTZ 2,4,6-tri (2 – pyridyl) – s – triazine and FeCl₃·6H₂O. The absorbance is measured spectrophotometrically at 595 nm (Benzie and Strain, 1996).

2.6.3.2.6 The Trolox Equivalent Antioxidant Capacity (TEAC) method

The TEAC assay is a decolourisation assay that can be applied to both lipophilic and hydrophilic antioxidants. The TEAC assay is based on the inhibition of the absorbance of the radical cation of 2,2’-azinobis (3-ethylbenzothiazoline 6-sulfonate) (ABTS) by antioxidants. The ABTS free radical has an absorption spectrum with maxima at 660, 734 and 820 nm. The assays are carried out using a decolourisation assay, which involves the generation of the ABTS chromophore by the oxidation of ABTS with potassium persulphate (Vinson and Hontz, 1995).

There is limited information on the antioxidant activities, the nutritional content and bioaccessibility of phenolic compounds from wild cereal grains that are consumed in times of drought in Zimbabwe. The phenolic compound profiles, phenolic acid content, antioxidant activities, nutritional content and bioaccessibility of selected wild and domesticated cereal grains found in Zimbabwe are documented in this study.
Chapter 3

3.0 Materials and methods

The chemical reagents used for all assays were of analytical grade. Phenolic acid standards were obtained from Sigma–Aldrich Chemie (Steinheim, Germany) and were of high purity grade. Tannic acid, gallic acid, catechin, vanillic acid, caffeic acid, $p$-coumaric acid, protocatechuic acid, ferulic acid, $p$-hydroxy-benzoic acid, $p$-hydroxybenzaldehyde, Folin-Ciocalteau, Nitroblue tetrazolium salt (NBT), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•), butylated hydroxy anisol (BHA), phenazine methosulphate (PMS), $\beta$-carotene, ascorbic acid, FeCl$_3$, Tween 80, trichloroacetic acid (TCA), potassium ferricyanide and ferrozine were obtained from Sigma–Aldrich Chemie (Steinheim, Germany). Reduced nicotinamide adenine dinucleotide (NADH) was obtained from Boehringer, Manheim, Germany. Sodium carbonate, methanol (Analytical and HPLC grade), ascorbic acid, butanol, HCl, ethyl acetate, diethyl ether, anhydrous Na$_2$SO$_4$, acetonitrile, acetic acid and ferric ammonium sulphate were obtained from local suppliers.

3.1 Collection of samples

The cereal grains that were studied include: *Sorghum arundinaceum*; *Eleusine corocana*; *Sorghum bicolor* (red variety); *Amaranthus hybridus*; *Rotboellia cochinchinensis*; *Panicum maximum* and *Brachiaria brizantha*. Samples were collected using a purposive sampling method and were authenticated by Mr Christopher Chapano, a botanist at the National Herbarium of Zimbabwe. Samples were collected from surrounding farms in Harare and from the wild in Buhera district of Zimbabwe. The exact sites of collection of samples, English and Shona names of the cereal grains are shown in table 3.1.
Cereal grains were weighed and dried outdoors in the shade for 20 – 30 days (depending on the grain type), where there was free circulation of air until moisture was constant. Dried samples were threshed in sacks to release the seeds. Seeds were ground to a powder using a Siebtechnik G.M.B.H. Mülheim-Ruhr type TS. 100 A pulveriser, at the Institute of Mining Research (IMR), University of Zimbabwe, and stored in brown bottles away from sunlight for further use.
Table 3.1 Cereal grains used in the study. All the cereal grains except for *Amaranthus hybridus* (which belongs to the Amaranthaceae family) belong to the Poaceae family.

<table>
<thead>
<tr>
<th>Latin</th>
<th>English</th>
<th>Shona</th>
<th>Site of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus hybridus</em> L.</td>
<td>Smooth amaranth, Smooth pigweed, Red amaranth</td>
<td><em>Bonongwe, Mowa dhongi,</em>  <em>Mowa guru</em></td>
<td>Murambinda (19° 16’ 12” S; 31° 39’ 00” E)</td>
</tr>
<tr>
<td><em>Brachiaria brizantha</em> (Hochst. ex A. Rich.) Stapf.</td>
<td>Beard grass, Palisade grass, Palisade signal grass, Signal grass</td>
<td><em>Zinyaruzoka</em></td>
<td>Highlands (17° 48’ 27” S; 31° 05’ 17”E)</td>
</tr>
<tr>
<td><em>Panicum maximum</em> Jacq.</td>
<td>Guinea grass, Buffalograss</td>
<td><em>Chitseretsere, Chivavane</em></td>
<td>Cleveland Dam ( 17° 48’ 44” S; 31° 08’ 52” E)</td>
</tr>
<tr>
<td><em>Rottboellia cochinchinensis</em> (Lour.) Clayton</td>
<td>Itchgrass, Guinea fowl grass</td>
<td><em>Mulungwa</em> (Tonga)</td>
<td>Hatcliffe (17° 41’ 18” S; 31° 06’ 35” E)</td>
</tr>
<tr>
<td><em>Sorghum arundinaceum</em> * (Desv.*) Stapf.</td>
<td>Common wild sorghum</td>
<td><em>Mapfunde emusango</em></td>
<td>University of Zimbabwe (17° 44’ 35” S; 31° 13’ 42” E)</td>
</tr>
<tr>
<td><em>Eleusine corocana</em> (L.) Gaertn.</td>
<td>Finger Millet</td>
<td><em>Rukweza, Zviyo</em></td>
<td>Buhera, Birchenough (19° 58’ 03” S; 32° 22’ 01” E)</td>
</tr>
<tr>
<td><em>Sorghum bicolor</em> (Red variety) (L.) Moench</td>
<td>Sorghum</td>
<td><em>Mapfunde matsvuku</em></td>
<td>Buhera, Murambinda (19° 16’ 12” S; 31° 39’ 05” E)</td>
</tr>
</tbody>
</table>
3.2 Determination of moisture

Samples (2 g) of ground cereal grains were weighed into a crucible and placed in an oven until a constant weight at 110 °C was reached. Moisture content was determined and reported as percentage dry mass using the equation below:

\[
\text{Moisture content (\%)} = \left[\frac{\text{(Mass of sample before oven drying} - \text{Mass of dried sample)}}{\text{Mass of sample before oven drying}}}\right] \times 100
\]

3.3 Extraction of total phenolic compounds

To extract total phenolic compounds, each finely ground cereal sample (2 g) was extracted twice with cold 50 % aqueous methanol (10 mL, 1:1 v/v) in a 50 mL test tube suspended in ice and subjected to ultrasonication in a Westwood Ultrasonicator (Model 90s) for 20 minutes (Makkar, 1999). The two extracts were combined, made up to 20 mL with 50 % aqueous methanol, centrifuged at 3000 rpm for 10 min and the supernatant transferred into small sample bottles ready for analysis. All analyses were done using freshly extracted samples. All antioxidant assays were done using samples extracted by this method.

3.4 Extraction of simple phenolic acids for HPLC analysis

To extract simple phenolic acids for HPLC analyses, 5 g of ground sample was extracted 3 times in 10 mL diethyl ether and 3 times with ethyl acetate (10 mL) to obtain a total 60 mL of extract after the sequential extractions (Pena-Neira et al., 2000). After 30 min of drying with anhydrous Na$_2$SO$_4$, the extract was evaporated to dryness under vacuum. The residue
was re-dissolved in 2 mL of 50 % methanol (1:1 v/v with water) and the 2 mL of sample transferred into vials ready for HPLC analysis.

3.4.1 Analysis of phenolic compounds by HPLC
A Shimadzu™ HPLC system with a SCL-6B Shimadzu system controller, C-R AX Shimadzu Chromatopac, Shimadzu SPD-10 AV UV-Vis detector equipped with a Dynamax 60 Å C18 column was used for analysis of phenolic compounds. Samples, which were in duplicate, were filtered through a 0.22 μm filter unit (Millex®-GV, Molsheim, France) before injection and the mobile phase solvents were filtered through a 0.45 μm filter (Whatman, Maidstone, England). Mobile phase A, containing water-acetic acid (98:2 v/v), and mobile phase B, containing water-acetonitrile-acetic acid (78:20:2 v/v/v), were used for eluting the sample. The gradient profile used had 100 % solvent A and 0 % Solvent B at time 0 min. Solvent A fell to 20 % while solvent B rose to 80 % within 55 min. The mobile phases remained at 20 % and 80 % for solvent A and B respectively up to 70 min of the run time. Solvent A, rose back to 100 % while solvent B was fell back to 0 % at between 70 min and 80 min of the run time. Detection was carried out at an absorbance of 280 nm. The flow rate was set at 1 mL/min. The sample volume injected was 5 µL. After each run, the system was reconditioned by running 100 % solvent A for 15 minutes before analysis of the next sample. Tentative identification of phenolic compounds was done by comparing the retention times of the cereal extracts to those of known standards obtained from Sigma–Aldrich Chemie (Steinheim, Germany). The method followed was according to Pena-Neira and colleagues (2000).
3.5 Determination of phenolic compounds using the Folin Ciocalteu assay

Total phenolic compounds were determined following the method described by Makkar (1999). To 50 μL of sample, 950 μL of distilled water was added to make up 1 mL, followed by 500 μL of 1 N Folin C reagent and 500 μL of 2 % sodium carbonate. After 40 min of incubation at room temperature, absorbance at 725 nm was read on a Spectronic 20® Genesys™ spectrophotometer (SIGMA-RBI, Switzerland) against a blank that contained methanol instead of sample. Total phenolics were expressed in terms of equivalent amounts of gallic acid. The gallic acid standard concentration used was 1 % in distilled water.

3.6 Vanillin assay for flavanoids

Cereal grain samples (5 μL) were made up to 1 mL with distilled water after which 2.5 mL of methanol-HCl (5:1 v/v) and 2.5 mL of vanillin reagent (1 g/100 mL distilled water) were added. After 20 minutes of incubation at room temperature, absorbance at 500 nm was read using a Spectronic 20® Genesys™ spectrophotometer (SIGMA-RBI, Switzerland) against a blank that contained methanol instead of the sample. The concentration of flavonoids was expressed as catechin equivalents, following the method by Porter and coworkers (1986). The concentration of the catechin standard used was 4 mg/mL in distilled water.

3.7 Determination of proanthocyanidin using the butanol-HCl assay

To 500 μL of the samples, 3 mL of the butanol-HCl reagent (95:5 v/v) was added, followed by 100 μL of the ferric reagent (2 % ferric ammonium sulphate in 2 M HCl). After mixing,
the tubes were placed in a boiling water bath for 60 min after which absorbance at 550 nm was measured using a Spectronic 20® Genesys™ spectrophotometer (SIGMA-RBI, Switzerland) against a blank. The blank was prepared by mixing 500 µL of the tannin containing extract, 3 mL of the butanol reagent and 100 µL of the ferric reagent with the mixture not being heated. The percentage of condensed tannin in dry matter was calculated using the formula: \( \frac{A_{550nm} \times 78.26 \times \text{Dilution factor}}{\% \text{ dry matter}} \) as described by Makkar (1999) and expressed as leucocyanidin equivalent.

3.8 Antioxidant activity assays

3.8.1 Determination of the ability to scavenge DPPH radicals

The radical scavenging activity of cereal grain extracts was determined following the method described by Kuda and colleagues (2005) with modifications. A 2.920 µL methanolic solution of 1 mM DPPH was prepared and the absorbance of the solution was measured on a Spectronic 20® Genesys™ Spectrophotometer (SIGMA-RBI, Switzerland) at 517 nm. The absorbance value obtained was treated as the initial value where the concentration of the unreduced DPPH was 100 %. Sample volumes of 80 µL were added to the DPPH solution and the decrease in the absorbance was recorded at 2 min intervals for 40 min for each sample. BHA with a concentration of 0.5 %, in distilled water, was used as a positive control. The percentage DPPH remaining in the solution was calculated as follows: DPPH (%) = \( \left[ \frac{A_{t=x}}{A_{t=0}} \right] \times 100 \% \); where \( A_{t=x} \) was the absorbance at 517 nm of the sample at each 2 min interval mark and \( A_{t=0} \) was the initial absorbance of DPPH before adding the sample.
3.8.2 Reducing power

The reducing power effects of the cereal extracts were determined following the method by Kuda and colleagues (2005). The samples (80 μL, at concentrations of 20 mg/mL, 40 mg/mL, 80 mg/mL and 100 mg/mL) and 80 μL of 0.5 % ascorbic acid for the control solution were mixed with 0.2 mL phosphate buffer (0.2 M, pH 7.2) and 0.2 mL of 1 % potassium ferricyanide. The mixtures were incubated at 50°C for 20 min, after which 0.2 mL of 10 % trichloroacetic acid (TCA) was added to each of the reaction mixtures. After transferring a 0.125 mL aliquot of the mixture into a microtitre plate, 0.125 mL distilled water and 0.02 mL of 0.1 % FeCl₃ was added and the absorbances at 655 nm were measured on a Spectra MAX 340 microtitre plate spectrophotometer (Molecular Devices Corp, California, USA).

3.8.3 Ability to chelate metal ions

The ability of the cereal grain extracts to chelate metal ions was estimated following the method by Dinis et al. (1994) with minor modifications. Samples of 100 μL volumes, at concentrations of 20 mg/mL, 40 mg/mL, 80 mg/mL and 100 mg/mL were added to 0.1 mL distilled water and 0.025 mL FeCl₂ (0.5 mM). After initial absorbance at 550 nm was measured (A550nm1), 0.025 mL of 2.5 mM ferrozine was added and the absorbance (A550nm2) was read after 20 min of incubation at room temperature. The control was treated the same as the test runs with samples but had distilled water added instead of samples. The activity was calculated as shown below.

\[
\text{Ferrous ion chelating activity} \% = \left[ \frac{(1 - (A_{550nm2} - A_{550nm1}))}{(\text{control } A_{550nm2} - \text{Control } A_{550nm1})} \right] \times 100 \text{ where } A_{550nm} \text{ is absorbance of sample at 550 nm.}
\]
3.9 Antioxidant capacity in model systems

3.9.1 Ability to inhibit phospholipid peroxidation

The ability of cereal extracts to inhibit phospholipid peroxidation was determined following the method by Aruoma et al. (1996). Female Sprague Dawley rats (*Rattus norvegicus*) were obtained from the Animal House at the University of Zimbabwe and dissected to obtain the brain. The rat brains were stored at -85°C until used. Rat brain (2 g) was homogenised in chloroform: methanol mixture (2:1, v/v) followed by centrifugation at 3000 x g for 5 minutes. The supernatant obtained was used as the source of phospholipids. The test run contained the 50 μL phospholipids solution, 0.5 mL of the sample extract, 0.2 mL of 50 % methanol and 0.5 mL of FeSO₄. The blank contained 50 μL of the phospholipid solution mixed with 0.5 mL distilled water instead of the phenolic compound containing sample and 0.2 mL of 50 % methanol. BHA with a concentration of 0.5 % was used as the positive control. Incubation of the reaction mixture at 37°C for one hour was followed by the addition of 0.5 mL thiobarbituric acid (TBA) and 4 mL of trichloroacetic acid (TCA) and the solution was then heated in a boiling water bath for 15 min. After cooling the sample on ice, absorbance was read at 532 nm on a Shimadzu UV-1601 uv-visible spectrophotometer (Shimadzu Corporation, Australia).

3.9.2 Ability to prevent oxidation of β-carotene

The β-carotene-linoleic acid assay was done following the method by Shon and coworkers (2003). β-Carotene (2 mg) was dissolved in 10 mL of chloroform. A 1 mL aliquot of the β-carotene solution was taken and chloroform was removed by vacuum on a rotary vapor. Linoleic acid (40 mg) was added to the almost dry material followed by 400 mg of Tween 80 and 100 mL of distilled water with rigorous shaking. Aliquots (3 mL) of the mixture
were combined with samples (100 μL, at a concentration of 80 mg/mL). The reaction mixture was shaken until an emulsion was formed and the absorbance at zero time was measured at 470 nm using a Shimadzu UV-1601 uv-visible spectrophotometer. Measurement of absorbance was continued at 5 min intervals for 2 h. A blank without β-carotene was prepared for background correction. Butylated hydroxyl anisol (BHA), with a concentration of 0.5 % in distilled water, was used as a positive control.

3.10 Nutritional analysis

Proximate analyses were conducted using standard AOAC methods (1990) for dry matter.

3.10.1 Determination of ash

A clean labeled crucible was placed in a muffle furnace (Model MRBZ-017-8) at 600 °C for 1 h. The crucible was transferred to a desiccator using metal tongs and allowed to cool before measuring its weight. The sample (2 g) was transferred into the weighed crucible and placed back into the muffle furnace at 600 °C for 24 h. The crucible and its contents were moved into a desiccator to cool to room temperature. The crucible and its contents were weighed and percentage ash was calculated as shown below:

\[
\text{Ash (\%)} = \left(\frac{\text{weight of Ash}}{\text{weight of sample}}\right) \times 100
\]

3.10.2 Determination of crude protein

The sample (2 g) was transferred into an 800 mL Kjeldahl flask containing 25 mL of sulphuric acid, 10 g of catalyst mixture and some porcelain boiling chips. The flask and its
contents were placed on a digestion rack of the Kjeldahl apparatus and the heat was turned on as well as the exhaust fan. Low heat was applied until frothing of the solution ceased, and then the flask was subjected to strong heating until the solution was clear. Heating of the solution was continued for 30 min. The content of the flask was then allowed to cool. Before the contents solidified, 300 mL of cold, distilled water was added with stirring while cooling the flask and its content under running water. In a separate 250 mL Erlenmeyer flask, 50 mL of boric acid and three drops of bromocresol green indicator was added forming the indicator solution and the flask placed such that the tip of the delivering tube from the condenser of the distillation unit dipped into the solution. To the Kjeldahl flask, a few pieces of mossy zinc and 100 mL of 40 % NaOH were added with the flask tilted so that the reagents ran down the side to the bottom of the flask forming a separate layer. With the stopper on, the contents were gently mixed and fitted on the Kjeldahl apparatus. Heat was applied until there was an accumulation of 200 mL of solution on the receiving flask with boric acid. The collected solution was titrated with 0.1 M HCl. The end point was reached when the indicator solution turned purple from green. The blank contained reagents only and the titre value was subtracted from the sample value. The crude protein was calculated as follows:

\[
\text{Nitrogen (\% of sample)} = \left( (\text{volume of acid titrated} - \text{volume of blank titrated}) \times (\text{Acid M} \times 0.14 \times 100) \right) / \text{weight of sample (g)}
\]

\[
\text{Crude protein (\%)} = \text{N \%} \times 6.25
\]
3.10.3 Determination of crude fibre

The ground sample (3 g) was defatted by washing with portions of 25 mL petroleum ether. The defatted dried sample was placed in a long-necked 500 mL digestion flask. Two drops of octyl alcohol were added and 200 mL of 1.25 % sulphuric acid were siphoned into the same flask. The flask was attached to a finger condenser and boiled gently for 30 min rotating the flask at 5 min intervals to prevent the sample from sticking to the sides of the flask. At the end of the acid digestion, the contents of the flask were filtered through a Whatman No. 1 filter paper and the residue was washed with 200 mL of hot 1.25 % NaOH. The solution was boiled gently for 30 min as before and the second solution (25 mL) was filtered through to a sintered glass crucible with zero porosity and attached to a vacuum pump apparatus using gentle suction. The sample was washed with boiling water, 1 % HCl and again with boiling water and finally 3 times with petroleum ether. The sample was dried in an oven at 105°C overnight. The sample was cooled to room temperature in a desiccator and the weight of the contents was recorded. The contents of the crucible were ignited in a muffle furnace (model MRBZ-017-8) at 550°C to 600°C for 6 h. The crucible was cooled in a desiccator to room temperature and weighed. The loss in weight was recorded as crude fibre and the percentage was calculated as below:

\[
\text{Crude fibre (\%) = \left( \frac{\text{loss in weight on ignition}}{\text{weight of sample}} \right) \times 100}
\]

The weight of sample is the weight before drying and ether extraction.
3.10.4 Determination of crude fat

In duplicate, 2 g of oven-dried cereal sample were weighed into a cellulose extract thimble. The thimble was plugged with glass wool and then placed into a Soxhlet extraction chamber. Sixty – seventy five mL of anhydrous ethyl ether (CAUTION) was added to a round bottomed flask. The round bottomed flask was then attached to the extraction chamber and condenser. Heat was applied gently to the flask on a heating mantle until the solution was boiling. Ether was condensed and run back into the extraction chamber. The flask containing ether and fat was removed. The ether was carefully evaporated using a rotary evaporator. After evaporating the ether, the flask was heated in a 100˚C oven for 1 hour, then cooled in a desiccators. The flask was weighed until a constant weight was obtained. The flask was rinsed with ether and then several times with distilled water. The flask was dried in the oven until a constant weight is obtained. The % fat was determined using the following formula:

\[
\% \text{ Crude Fat} = \frac{\text{Weight of ether extract}}{\text{Weight of Sample}} \times 100
\]

3.10.5 Determination of calcium and phosphorus

A sample weighing 3 g was ashed in a muffle furnace at 600˚C for 5 h. To the ash, 10 mL of HCl was added followed by 15 mL of distilled water. The mixture was evaporated to 10 mL on a hot plate at 100˚C. The resultant concentrate was cooled and filtered through Whatman filter paper number 40 (Hardened ashless) into volumetric flasks. The ash was used for the determination of calcium and phosphorus.
3.10.5.1 Determination of calcium

A 5 mL aliquot of the ash solution was transferred into centrifuge tubes containing saturated ammonium oxalate. A drop of methyl red indicator was added and pH was adjusted to 5 (faint pink colour of the indicator) using dilute HCl and NH₄OH. The contents were thoroughly mixed and let to stand for 1 h followed by centrifugation at 3000 rpm for 5 minutes. The supernatant was discarded and the residue was resuspended as above and centrifuged again, discarding the supernatant. The final residue was dissolved in 10 mL of HCl and transferred into a 100 mL conical flask before adding 30 mL of distilled water. Magnesium sulphate standard solution (5 mL) was added followed by 5 drops of Eriochrome-Black-T indicator (EBT) and 10 mL buffer solution (ammonium chloride/ammonium hydroxide). The mixture was titrated with EDTA standard and calcium concentration was calculated as below:

\[ 1 \text{ mL } \text{Ca}^{2+} = 1 \text{ mg } \text{Ca}^{2+} = y \text{ ml of EDTA (concentration of } \text{Ca}^{2+} \text{ standard is } 1 \text{ mg/mL}) \]

\[ \text{Ca (\%)} = \left[ \frac{(\text{EDTA equiv to } 5 \text{ mL Ash } \times 20 \text{ (d.f.)})}{\text{mass of sample}} \right] \times 100 \]

3.10.5.2 Determination of phosphorus

A 1 mL aliquot of the ash sample was placed into a 50 mL volumetric flask containing 25 mL of distilled water and 5 mL of ammonium molybdate reagent. The contents were mixed thoroughly in 2 mL of 2-naphthol-4-sulphonic acid (ANSA reagent). The absorbance of the solution was measured using a Spectronic 20® Genesys™ spectrophotometer (Spectronic Instruments, USA) at 630 nm after 20 min of adding ANSA reagent. The blank contained
regents only and no sample. The concentration of phosphorus was calculated by extrapolation from a standard curve and percentage values were obtained as follows:

\[
\text{Phosphorus (\%)} = \left( \frac{P \text{ mg in aliquot} \times \text{total mL of Ash solution}}{\text{volume of aliquot} \times \text{weight of sample}} \right) \times 100
\]

3.11 Bioaccessibility assays

There were three steps that were taken in the methodology applied to estimate the bioaccessibility of dietary polyphenols (Saura-Calixto et al., 2000). The steps included: (a) the incubation of each enzyme with one domesticated and one wild cereal grain to estimate the contribution of each individual enzyme to the bioaccessibility of the phenolic compounds. The cereal grains were incubated following the conditions described in section 3.11.1 for each enzyme, however, the time of incubation varied depending on the time it took to reach a maximum absorbance value at 725 nm read on a Spectronic 20® Genesys™ spectrophotometer (Spectronic Instruments, USA), which in turn indicated the maximum phenolic compounds that could be released from the grain matrix as a result of that particular enzyme; (b) isolation of the indigestible fraction to estimate small intestine bioaccessibility; and (c) colonic fermentation of the indigestible fraction to estimate large intestine (colon) bioaccessibility (Figure. 3.11.1). The indigestible fraction is the part of the cereal grains that is not digested or absorbed in the small intestine and reaches the colon, where it serves as a substrate for fermentative microflora (Saura-Calixto et al., 2000). The indigestible fraction is made up of dietary fibre and other compounds of proven resistance to the actions of enzymes, such as indigestible protein, resistant starch, polyphenols and other bioactive compounds (Saura-Calixto and Goñi, 2004). Analytical conditions for
indigestible fraction determination were close to physiological conditions of pH, temperature, and incubation times. The indigestible fraction was composed of two fractions: a soluble fraction (supernatant of enzymatic digestion) and an insoluble fraction (residue of enzymatic digestion) (Figure 3.1.1). The estimation of the bioaccessibility of polyphenols in the small intestine was calculated by the difference between polyphenols content in the original sample and in the residues of enzymatic digestion.

In the in vitro colonic fermentation model, the total indigestible fraction which consisted of soluble and insoluble fibre was fermented in strict anaerobic conditions, using rat caecal matter as inoculum. Several compounds are released from the food matrix by the action of bacterial enzymes, while other compounds remain in the food matrix as a part of the residue, after fermentation. The residue after fermentation contained compounds of proven resistance to enzymatic and colonic bacterial degradation, which probably would be excreted in the faeces. The difference in polyphenol content between the total indigestible fraction and the residue after the fermentation process is the estimate value of bioaccessibility of polyphenols in the large intestine.

3.11.1 In vitro physiological approach

3.11.1.1 Determination of indigestible fraction

To determine the indigestible fraction, samples of each cereal grain were successively incubated with digestive enzymes to simulate digestion in the small intestines (Saura-Calixto et al., 2000). Briefly, 300 mg of each cereal sample were incubated with pepsin (EC 3.4.23.1, 0.2 mL of a 300 mg/mL solution in HCl-KCl 0.2 M buffer, pH 1.5, 40 °C, 1
h, Merck 7190) followed by 1 mL of 5 mg/mL pancreatin solution in 0.1M phosphate buffer (pH 7.5, 37 °C, 6 h, Sigma P-1750). Incubation in lipase followed (EC 3.1.1.3, 2 mL of a 7 mg/mL solution in phosphate buffer 0.1 M; pH 7.5, 37 °C, 6 h, Sigma L-3126) and bile extract from porcine (2 mL of a 17.5 mg/mL solution in phosphate buffer 0.1 M; pH 7.5, 37 °C, 6 h, Sigma B-8631) was added at the same time as the lipase enzyme. Finally, α-amylase (EC 3.2.1.1, 1 mL of a 120 mg/mL solution in tris-maleate buffer 0.1 M; pH 6.9, 37 °C, 16 h, Sigma A-3176) was added and then samples were centrifuged for 15 min at 25 °C with a centrifugal speed of 3000 g and supernatants removed. Residues were washed twice with 5 mL of distilled water and all supernatants combined. Half the residues were stored at -18°C for colonic fermentation (fraction A) and the other half for analysis of polyphenols associated with the insoluble indigestible fraction (fraction B).

Each supernatant was incubated with 100 µL of amylglucosidase (EC 3.2.1.3, Roche, 102 857) for 45 min at 60 °C, transferred into dialysis tubes (12000–14000 molecular weight cut-off; Dialysis Tubing Visking, Medicell International Ltd., London, UK), and dialyzed against water for 48 h at 25 °C (water flow 7 L/h). Retentates contained soluble dietary fibre and other associate compounds, such as polyphenols and carotenoids. Dialysis retentates were stored mixed with fraction B for the analysis of polyphenols associated with the soluble indigestible fraction, and the other dialysis retentates were concentrated to 5 mL in a R-114 Büchi vacuum rotatory evaporator and then added to their corresponding residue (fraction A) and stored at -18 °C in a freezer for colonic fermentation.
3.11.1.2 *In vitro* colonic fermentation

The *in vitro* fermentation method used was described by Barry *et al.*, (1995) and standardised by Goñi and Martin- Carrón (2000) with minor modifications. Male Sprague Dawley (*Rattus norvegicus*) rats fed with standard maintenance diets adjusted to rat dietary requirements were obtained from the Animal House at the University of Zimbabwe. Rats were killed in a carbon dioxide chamber and fresh rat caecal contents were obtained for use as the inoculum. Caeca were removed and rat caecal contents were scraped, weighed and added to a flask containing sterile anaerobic medium to give a 100 g/L inoculum.

The anaerobic medium adapted from Goering and Van Soest (1970), contained trypticase, micromineral and macromineral solutions and resazurin as anaerobic redox indicator. The inoculum was mixed for 10 min in a Stomacher 80 Lab Blender (Seward Medical, London, UK) and filtered (1 mm mesh) before use. Total indigestible fractions from enzymatic treatments were mixed with 8 mL of fermentation medium at 4 °C for 16 h. Tubes were placed in a GasPak™ EZ Anaerobe Container System for fermentation. Two millilitres of inoculum were added and the GasPak™ EZ Anaerobe Container system containing the tubes was placed in a shaking water bath that was set at 37 °C for 24 h. Blanks containing no substrate and lactulose (Sigma L-7877) were included in the experiment as zero and completely fermentable substrate, respectively. All the steps were carried out in a GasPak™ EZ Anaerobe Container system that provided an oxygen-free CO₂ saturated atmosphere.

After the incubation time, 1 M NaOH was used to stop the fermentation process. Samples were centrifuged at 2500 g for 10 min at a temperature of 25 °C and the supernatants as well as the residue were collected and stored at - 80 °C for polyphenols analysis. The
residue was analysed for polyphenols associated with the residue after fermentation while the supernatants were analysed for polyphenols available after colonic fermentation. The polyphenol content of supernatants and residues were corrected with blanks of fermentation.
In vitro physiological approach

Wild cereal grains

In vitro enzymatic digestion
- Pepsin (pH 1.5)
- Pancreatin (pH 7.5)
- Lipase + bile salts (pH 7.5)
- α-amylase (pH 6.9)

Centrifugation

Supernatant

Dialysis

Soluble indigestible fraction

Total Polyphenol content

In vitro colonic fermentation: anaerobic conditions

Fermentation supernatant: Total phenolics content

Dialysis

Residue

Insoluble Indigestible fraction

Total indigestible fraction (soluble + insoluble)

Determination of: Condensed tannins

Residue after fermentation: Condensed tannins

Figure 3.11.1: Schematic diagram of the method used to determine the intestinal bioaccessibility of dietary polyphenols. Source: Saura-Calixto et al., (2007)
3.11.2 Chemical approach: Determination of polyphenol content

A chemical approach was employed to determine the total content and intake of polyphenols in the diet (Figure 3.11.2).

**Figure 3.11.2:** Schematic diagram of the method used to determine the intestinal bioaccessibility of cereal grain polyphenols. Source: Saura-Calixto et al., (2007)

3.11.4 Extractable polyphenols

Original sample was extracted by shaking at room temperature with methanol–water (50:50 v/v, 50 mL/g sample, 60 min, room temperature; with constant shaking) and acetone–water (70:30 v/v, 50 mL/g sample, 60 min, room temperature; with constant shaking). After centrifugation (15 min, 25 ℃, 3000g) supernatants were combined and used to determine extractable polyphenols content in the original samples by the Folin–Ciocalteau procedure (Singleton et al., 1965). The test sample (0.5 mL) was mixed with 0.5 mL of Folin–Ciocalteu reagent and swirled. After 3 min, 10 mL of sodium carbonate solution (75 g/L)
was added and mixed. Additional distilled water was added (14 mL) and mixed thoroughly by inverting the tubes several times. After 1 h, the absorbance at 750 nm was recorded. The results were expressed as gallic acid equivalents.

3.11.5. Non-extractable polyphenols

In the residue of the methanol/acetone/water extraction of polyphenols, condensed tannins-proanthocyanidins were determined separately.

3.11.5.1 Condensed tannins-proanthocyanidins

Residues from the methanol/acetone/water extraction were treated with 5 mL/L HCl–butanol (3 h, 100 °C) for condensed tannins determination following the method of Reed et al., 1982. Condensed tannins were calculated from the absorbance at 550 nm of the anthocyanidin solutions. Condensed tannins from Mediterranean carob pod (Ceratonia siliqua L) supplied by Nestle´ S.A. were treated under the same conditions to obtain standard curves.

3.11.6 Calculations

To determine the polyphenols bioaccessibility the following calculations were used:

- Polyphenols accessible in the small intestine: difference between polyphenols content in the original sample and polyphenols associated with the indigestible fraction.
• Polyphenols accessible in the large intestine: differences between polyphenols associated to the indigestible fraction and polyphenols content in the residue after fermentation.

3.12 Statistical analysis of data

All samples were analysed in triplicate and the results were given as means ± standard deviation. Oneway ANOVA packaged in the GraphPad Prism for Windows Version 6.0 was used for the statistical evaluation, with P<0.05 being considered statistically significant. The Turkey’s honestly significant difference (HSD) post hoc test was used to confirm where differences occurred between groups.
4.0 Results and discussion

4.1 Moisture content

The percentage moisture content in all the cereal samples is shown in table 4.1.1

Table 4.1.1 The moisture content in the cereal grains determined after drying for 14 days in the sun.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus hybridus</em></td>
<td>a9.43 ± 0.07</td>
</tr>
<tr>
<td><em>Brachiaria brizantha</em></td>
<td>b7.82 ± 0.03</td>
</tr>
<tr>
<td><em>Panicum maximum</em></td>
<td>c6.58 ± 0.09</td>
</tr>
<tr>
<td><em>Rottboellia cochinchinensis</em></td>
<td>e8.84 ± 0.04</td>
</tr>
<tr>
<td><em>Sorghum arundinaceum</em></td>
<td>e8.92 ± 0.02</td>
</tr>
<tr>
<td><em>Eleusine corocana</em></td>
<td>a9.64 ± 0.01</td>
</tr>
<tr>
<td><em>Sorghum bicolor</em> (red variety)*</td>
<td>f13.24 ± 0.03</td>
</tr>
</tbody>
</table>

*In the same column, means with different superscript letters are significantly different. Values are means ± standard deviation of triplicate determinations. *D* was used to indicate domesticated cereal grains.
The percentage of moisture content ranged from 6.58 % in \textit{P. maximum} which had the lowest to 13.24 % in \textit{S. bicolor} (red variety) which was the highest. Although all samples were dried under the same conditions, there were significant (p<0.05) differences in moisture content among several of the grains as indicated using different superscript letters in table 4.1.1. Factors like fat content, grain size and grain structure contribute to variations in moisture content. \textit{A. hybridus} had significantly higher moisture content of 9.43 % (p<0.05) than \textit{P. maximum}, \textit{B. Brizantha}, \textit{S. arundinaceum} and \textit{R. cochinchinensis} with 6.58 %, 7.82 %, 8.92 % and 8.84 % respectively. The reason may be that the outer seed coat for \textit{A. hybridus} is tougher and offers greater resistance to moisture loss of the grain (Rao \textit{et al.}, 2006).

Generally larger seeds dry slower than smaller ones because moisture has greater distance to diffuse to the boundary layer and the difference in moisture content between \textit{A. hybridus} (9.43 %) and \textit{S. bicolor} (13.24 %) can be explained by this characteristic. The average size of the \textit{A. hybridus} grain is 0.7-1 mm in diameter while that of \textit{S. bicolor} averages 5-6 mm in diameter. \textit{S. bicolor} seeds are more than five times larger than \textit{A. hybridus} seeds, hence the slower loss in moisture over a given period of time of \textit{S. bicolor} compared to \textit{A. hybridus}.

Another factor that may have contributed to the differences in moisture content may be the fat content in the grains. Fatty seeds will dry to lower moisture content than non-fatty seeds because fatty seeds do not allow water molecules to bind whereas dry matter in starchy seeds attract and hold water (Rao \textit{et al.}, 2006). As shown in table 4.2.1, the fat content in \textit{A. hybridus} of 11.5 % is significantly higher (P<0.05) than the fat content in \textit{S. bicolor} (red
variety) of 3.32 % hence *A. hybridus* will repel more water molecules than *S. bicolor* resulting in a lower moisture content in *A. hybridus*.

The moisture content obtained for all but one cereal grain were all below the recommended 12.5 % (GMB dura, 2013) and the low moisture levels may reduce the likelihood of spoilage due to development of moulds or seed germination. Moist conditions promote the growth of microbes and controlling the moisture content of the grains to the required levels for the purposes of storage reduces post harvest losses (Binod *et al.*, 2007). *S. bicolor* (red variety) had a moisture content of 13.24 %, which was higher than the recommended maximum of 12.5 % and this means that the grain would be rejected on delivery to the GMB in Zimbabwe which recommends delivery moisture content of 12.5 % or less (GMB dura, 2013).

Very low moisture content below 4 % may damage seeds due to extreme desiccation or cause hardness of the seed coat in some crops. Moisture content which is too low, below the recommended 12.5 %, may reduce profit income due to less weight (Dorn, 2009). Loss of moisture means loss of weight, and in turn meaning loss in profit, if grain is sold too dry. For the purposes of marketing the grain, it is recommended to stop the drying process when the moisture content is still as high as 12.5 % to improve on the selling weight (Dorn, 2009).

For the purposes of this research, the moisture content in the cereal was determined only for the purposes of accurately estimating the phytochemical composition of the grain on dry matter basis. The percentage moisture content of each cereal grain was taken into consideration and all results were expressed as a fraction of the sample dry matter.
4.2 Nutritional composition

The results of the analyses done to determine the macro-nutritional and selected mineral content of the cereal grains are shown in table 4.2.1. The crude protein, crude fat, fibre, ash, calcium and phosphorus were determined and are reported in this section.
Table 4.2.1: Results of proximate composition analyses on samples of selected cereal grains expressed as a percentage of the dry weight.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Calcium</th>
<th>Phosphorus</th>
<th>Ash</th>
<th>Ether Extract</th>
<th>Crude Protein</th>
<th>Crude Fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus hybridus</em></td>
<td>a0.68 ± 0.01</td>
<td>a0.86 ± 0.02</td>
<td>f2.16 ± 0.02</td>
<td>a11.50 ± 0.03</td>
<td>a21.44 ± 0.05</td>
<td>e5.78 ± 0.01</td>
</tr>
<tr>
<td><em>Brachiaria brizantha</em></td>
<td>c0.06 ± 0.02</td>
<td>b0.19 ± 0.04</td>
<td>b8.94 ± 0.01</td>
<td>b4.13 ± 0.03</td>
<td>e8.24 ± 0.01</td>
<td>a30.43 ± 0.01</td>
</tr>
<tr>
<td><em>Panicum maximum</em></td>
<td>b0.14 ± 0.05</td>
<td>b0.31 ± 0.03</td>
<td>a13.16 ± 0.09</td>
<td>b4.65 ± 0.05</td>
<td>b12.82 ± 0.03</td>
<td>b26.03 ± 0.06</td>
</tr>
<tr>
<td><em>Rottboellia cochinchinensis</em></td>
<td>b0.20 ± 0.03</td>
<td>b0.20 ± 0.02</td>
<td>b8.37 ± 0.05</td>
<td>b4.70 ± 0.01</td>
<td>g6.83 ± 0.03</td>
<td>b24.89 ± 0.09</td>
</tr>
<tr>
<td><em>Sorghum arundinaceum</em></td>
<td>b0.19 ± 0.07</td>
<td>b0.26 ± 0.01</td>
<td>c7.38 ± 0.06</td>
<td>c3.04 ± 0.01</td>
<td>c10.66 ± 0.04</td>
<td>c12.40 ± 0.05</td>
</tr>
<tr>
<td><em>Eleusine corocana</em>^P^</td>
<td>b0.21 ± 0.02</td>
<td>b0.19 ± 0.01</td>
<td>c3.05 ± 0.07</td>
<td>c2.04 ± 0.03</td>
<td>h4.57 ± 0.06</td>
<td>f3.52 ± 0.04</td>
</tr>
<tr>
<td><em>Sorghum bicolor</em> (red variety)^P^</td>
<td>ND</td>
<td>b0.24 ± 0.04</td>
<td>f2.28 ± 0.01</td>
<td>c3.32 ± 0.02</td>
<td>f7.84 ± 0.02</td>
<td>f2.51 ± 0.07</td>
</tr>
</tbody>
</table>

*In the same column, means with different superscript letters are significantly different (P<0.05). Values are mean ± standard deviation of triplicate determinations. ^P was used to indicate domesticated cereal grain. ND indicates that the component investigated was not detected.
The nutritional and mineral content varied significantly (p<0.05) among the cereal grains. In order to determine the importance of the nutrient data obtained from the nutritional analyses of selected wild cereal grains used as plant foods, a comparison of the nutritional data was done with that of domesticated grains that are consumed traditionally by the inhabitants of the semi-arid regions of Zimbabwe during times of severe droughts (Shava, 2003). The domesticated grains used for the comparisons are *E. corocana* and the red variety of *S. bicolor*.

4.2.1 Protein

The protein content of five (5) wild cereal grains, two (2) commercial domesticated grains is shown in table 4.2.1. The order starting with the cereal grain with the most protein percentage content is as follows: *A. hybridus > P. maximum > S. arundinaceum > B. brizantha > S. bicolor (red variety) > R. cochinchinensis > E. corocana*.

*A. hybridus*, a wild cereal grain, was shown to contain a protein concentration of 21.44 % which is higher than most traditionally consumed grains with the exception of soya beans (Dhellot *et al.*, 2006). The average protein composition of some traditional cereal grains like wheat is 14 %, soybeans 37 %, rice 7 % and maize with 9 % according to O’Brien and Price (2008). The percentage of protein content in other relatives of *Amaranthus hybridus* like *Amaranthus cruentus* was reported to be about 16.2 % (Mendoza and Bressani, 1987) and 17.9 % in *Amaranthus hypochondriacus* seeds (Cai *et al.*, 2004).

Cereal grains are the most abundant and available source of nutrition throughout the world (Akubugwo *et al.*, 2007). In third world countries like Zimbabwe, cereals are almost
entirely the source of nutrition for the poorest families. The over dependence on cereals, of the poor families, accounts in part, for the protein deficiency which dominates amongst the general population as recognized by Food and Agricultural Organization (Ladeji et al., 1995). The considerably high protein content in wild cereals which include, *A. hybridus* (21.44 %), *S. arundinaceum* (10.66 %) and *P. maximum* (12.82 %), compared to domesticated varieties, *E. corocana* (4.57 %) and red *S. bicolor* (7.84 %) (p<0.05), is a result that provides a lead to potentially cheaper protein sources.

The protein content in *A. hybridus*, of 21.44 %, which is higher than that of commercial wheat with 12-16 % protein (Shewry, 2000), makes the amaranthus flour potentially utilizable in the food industry as an alternative to wheat flour. However, further research into the protein quality is of importance in order to be able to safely recommend the effective use of the cereal grains.

4.2.2 Fibre

Measurements of dietary fibre are vital to the assessment of potential beneficial and disease preventive effects of fiber intake. *B. brizantha* was found to contain significantly (p<0.05) higher content of crude fibre (30.44 %), followed by *P. maximum* (26.03 %) and *R. cochinchinesis* (24.89 %). *S. bicolor* (2.51 %), *E. corocana* (3.52 %) and *A. hybridus* (5.78 %) and *S. arundinaceum* (12.40 %) had low fibre content. The fibre content amongst the cereal grains differed markedly when the high fibre containing grains were compared to the low fibre containing grains. The high fibre content in *B. brizantha, P. maximum* and *R. cochinchinesis* can be taken advantage of to fortify food products with low fiber composition in order to enhance digestion in the humans. A high fibre diet can help lower
cholesterol, control blood sugar mainly if soluble fibre, prevent constipation as well as help in bulking excreta if fibre is insoluble (Johnson, 2004).

All of the wild cereal grains had significantly high fiber content than the selected domesticated cereal grains (p<0.05). The red variety of *S. bicolor* had the least crude fiber content of 2.51 % (p<0.05) followed by *E. corocana* (3.52 %) and *A. hybridus* (5.78 %) respectively in increasing order. The recommended daily allowance (RDA) of total fibre intake for the Americans is 25-35 % (Spiller, 1993). The Committee on Medical Aspects of Food Policy (COMA) in the United Kingdom, recommended that an intake of 18 g dietary fibre was needed to result in a healthy bowel habit (i.e. a daily faecal weight of at least 100 g/day), with identical levels for men and women, since there is no sex difference in what is considered a healthy faecal weight and intake is independent of energy intake (Department of Health, 1991). The 5.78 % crude fibre content determined for *A. hybridus* effectively meant that for every 100 g of amaranthus sample, 5.78 g was crude fibre. So for an individual to consume fibre that is within the RDA range, one would need to consume at least 500 g of the *A. hybridus* daily.

### 4.2.3 Fat

The fat content of wild and domesticated cereal grains are shown in table 4.2.1. The fat content in *A. hybridus* of 11.5 %, was significantly higher (p<0.05) than the other grains studied which had values ranging from 2.04 % - 4.7 %. The fat content found in *A. hybridus* was higher than in some cereals reported by Sinclair and O’Dea in 1990, who used a similar method to the one we used to determine crude fat. Sinclair and O’Dea (1990) reported that cereals were low in fats averaging 3.6 % fat for their total caloric
content. The fat content found in *P. maximum* (4.65 %) and *R. cochinchinensis* (4.70 %) was not significantly different. *S. arundinaceum* (3.04 %), *B. brizantha* (4.13 %), *S. bicolor* (red variety) (3.32 %) and *E. corocana* (2.04 %) had low fat content. The variation in the fat content amongst the cereal grains may have been due to the variation in the genetic make up and habitat of the cereal grains. For traditionally consumed grains, Belitz and colleagues (2009) reported that wheat contained 1.8 % fat, maize had 3.8 %, rice had 2.2 % and millet was reported to contain 3.9 % fat. The values compare well with the ones we obtained for the wild cereal grains. However, *A. hybridus*, had more fat when compared to the other cereal grains.

Plant fat contains essential fatty acids that humans cannot synthesise, and must be acquired through diet. Essential fatty acids are long chain polyunsaturated fatty acids derived from linolenic, linoleic, as well as oleic acids and are necessary for the formation of healthy cell membranes, the proper development and functioning of the brain and nervous system (Ercisli and Orhan, 2007). A predominantly cereal- plus plant-based diet could contribute 5–10 g per person per day of linoleic acid (LA), the major Ω-6 polyunsaturated fatty acid found in grains (Sinclair and O’Dea, 1990).

### 4.2.4 Minerals:

#### 4.2.4.1 Calcium and Phosphorus

The levels of calcium and phosphorus in the selected wild and domesticated cereal grains are shown in Table 4.2.1. Calcium content was significantly higher in *A. hybridus* (0.68 %) when compared to all the cereal grains studied. Statistically, there was no difference in the calcium content in *P. maximum* (0.14 %), *S. arundinaceum* (0.19 %), *R. cochinchinensis*
(0.20 %) and *E. corocana* (0.21 %). The lowest calcium content of 0.06 % was detected in *B. brizantha*. The element was not detected in the *S. bicolor* (red variety) grain sample used for this research. This result was unusual and inconsistent with values reported by other researchers for the same grain. The sorghum seeds were collected during the rainy season where humidity levels were high, so the calcium content may have been affected. Salinity build-up might have also caused calcium deficiency because it decreases the water uptake by the plant, but however, further investigations may be needed to ascertain the cause of calcium deficiency in the sorghum seeds. Zaparrart and Salgado, in 1994, reported 0.03 % calcium content in sorghum flour.

Calcium is the most abundant element in the human body by mass and 99 % of the element is found in the bones and teeth. Some of the calcium is essential for the contraction of heart, other muscles of nerves and enzyme functions as well as for blood clotting. Calcium uptake by the plant is passive and does not require energy input. The movement of calcium in the plant takes places mainly in the xylem, together with water. Therefore, uptake of calcium is directly related to the transpiration rate of the plant, so conditions of high humidity, cold and low transpiration rates may result in calcium deficiency (de Freitas and Mitcham, 2012). One other factor that may have affected the calcium levels is the soil pH. Usually soils that have a lower pH level contain less available calcium (de Freitas and Mitcham, 2012).

Phosphorus was detected in all the cereal grains with variations that were insignificant among several of the grains. *A. hybridus* had a significantly higher phosphorus content of 0.86 % when compared to all the other grains studied. In the other cereal grains the
phosphorus content ranged from 0.19 % in *B. brizantha* to 0.31 % in *P. maximum* but however, the difference amongst the grains in this range was statistically insignificant.

Phosphorus is after calcium, the most abundant mineral element in the body. It is found in bones and teeth and in cellular fluids. The mineral plays an essential function in the liberation and utilization of energy in animal and vegetable tissue, therefore is widely distributed in all foods. The mineral values reported here were lower for both calcium and phosphorus compared to what was reported by Milton and co-workers (1999) who determined mineral concentrations in 16 species of wild and 4 species of cultivated fruits in American Samoa. The availability of calcium and phosphorus in most of the cereal grains studied is a positive attribute that could render the cereals useful as healthy sources of minerals in the diet.

### 4.2.4.2 Ca/P ratio

The Ca/P ratio determined for the cereal grains is shown in Table 4.2.2.
Table 4.2.2: The Ca/P ratio of selected wild and domesticated cereal grains. D was used to indicate domesticated cereal grains.

<table>
<thead>
<tr>
<th>Cereal grain</th>
<th>Ca/P ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amaranthus hybridus</td>
<td>0.79</td>
</tr>
<tr>
<td>Brachiaria brizantha</td>
<td>0.32</td>
</tr>
<tr>
<td>Panicum maximum</td>
<td>0.45</td>
</tr>
<tr>
<td>Rottboellia cochinchinensis</td>
<td>1.00</td>
</tr>
<tr>
<td>Sorghum arundinaceum</td>
<td>0.73</td>
</tr>
<tr>
<td>Eleusine corocana D</td>
<td>1.11</td>
</tr>
<tr>
<td>Sorghum bicolor (red variety) D</td>
<td>0 calcium</td>
</tr>
</tbody>
</table>

A low Ca/P ratio can reduce the growth of bones and metabolism of the animal consuming the cereal. The recommended, ideal Ca/P ratio for humans and animals when they consume foods is 1:1. In the United States where a study was carried out by Calvo in 1993, the Ca/P ratio averages 0.64 for women and 0.62 for men. S. bicolor, B. brizantha and P. maximum had significantly lower (p<0.05) ratios when compared with the other cereal grains studied. Consumption of excess dietary phosphorus, when calcium intake is adequate or low, leads to secondary hyperparathyroidism and progressive bone loss (Calvo, 1993). Therefore, one would need to supplement the intake of calcium if Ca/P ratio is low as is the case in some of the cereal grains reported.
The net effect of low calcium content and a low Ca/P ratio is the induction of bone mineral pathologies in populations like the poor in Zimbabwe, reliant entirely upon cereal grains as their main source of nutrition (Berlyne et al., 1973; Ford et al., 1972; Robertson et al., 1981).

### 4.3 Total phenolic content

In table 4.3.1, the total phenolic composition of wild and domesticated cereal samples are shown. The wild cereal grains studied were: *A. hybridus, S. arundinaceum, P. maximum, R. cochinchinensis, B. brizantha* while the domesticated ones were: the red variety of *S. bicolor* and *E. corocana*. The phenolic composition was expressed as mg of gallic acid in 100 mg of sample.
Table 4.3.1: The amount of total phenolic compounds in selected wild and domesticated cereal grains determined by Folin Ciocalteu assay, expressed as equivalence of gallic acid.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total Phenolic compounds (mgGA/100 mg sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amaranthus hybridus</td>
<td>$^{f}1.13 \pm 0.01$</td>
</tr>
<tr>
<td>Brachiaria brizantha</td>
<td>$^{c}3.18 \pm 0.07$</td>
</tr>
<tr>
<td>Panicum maximum</td>
<td>$^{c}2.58 \pm 0.02$</td>
</tr>
<tr>
<td>Rottboellia cochinchinensis</td>
<td>$^{c}3.31 \pm 0.04$</td>
</tr>
<tr>
<td>Sorghum arundinaceum</td>
<td>$^{a}6.18 \pm 0.03$</td>
</tr>
<tr>
<td>Eleusine corocana</td>
<td>$^{a}6.31 \pm 0.03$</td>
</tr>
<tr>
<td>Sorghum bicolor (red variety)</td>
<td>$^{b}3.98 \pm 0.02$</td>
</tr>
</tbody>
</table>

*In the same column, means with different superscript letters are significantly different (P<0.05). Values are mean ± Standard deviation of triplicate determinations. \(^{D}\) was used to indicate domesticated cereal grains.

The total phenolic content varied considerably (p<0.05) between cereal samples, with the highest total phenolic content being determined in *E. corocana* (6.31 mg GA/100 mg sample) and *S. arundinaceum* (6.18 mg GA/100 mg sample), while the least amount was determined in *A. hybridus*, a wild cereal grain.
There was significant variation within cereals of the same sub-family. *S. arundinaceum* and the red variety of *S. bicolor* belong to the same sub-family called *andropogoneae* and their phenolic contents were determined to be 6.18 and 3.98 mg GA/100 mg sample respectively. The variation in the phenolic content may be due to the different environmental conditions the cereal grains were exposed to and their genetic makeup which will code for different amounts and types of phenolics compounds in plants that differ genetically. Environmental conditions like temperature, humidity, rainfall amount, soil type, disease and exposure to predators influence the amount of phenolic compounds produced by a plant (Vinson *et al*., 2001).

Harsh environmental conditions including droughts and high temperatures, lead to an increased production of phenolic compounds. The differences in phenolic compounds were more apparent when the domesticated and wild cereal grains were compared. The assumption was that the wild conditions were harsher than domestic conditions. The wild cereal, *S. arundinaceum*, contained more phenolic compounds, with a concentration of 6.18 ± 0.03 mg GA/100 mg when compared to the domestic sorghum which had a phenolic content of 3.98 ± 0.02 mg GA/100 mg.

*E. corocana* and *S. arundinaceum* contained higher phenolic content than all the other cereal grains studied. The phenolic content in all the cereal grains was higher than the values reported by Bendelow and LaBerge in 1979 who reported that barley contained 0.2 to 0.4 mg/100 mg sample of phenolics by weight of grain. David and colleagues in 2007 reported a total phenolic composition of 3.34 mg/100 mg in Mexican white corn.
The high phenolic composition in the grains is of importance because of the potential applications of the phenolic compounds as natural antioxidants in the pharmaceutical as well as the food industries. Phenolic compounds prevent degenerative diseases such as coronary heart disease and cancer. The phenolic compounds facilitate delay or inhibit lipid peroxidation and when added to foods, the phenolic compounds minimize rancidity, retard the development of toxic oxidation products, and help preserve the nutritional quality thereby enhancing the shelf life of the foods (Fukumoto and Mazza, 2000). The grains in this study, therefore, could be used as potential sources of antioxidants with benefit to the food industry as food preservatives and the human health in preventing diseases such as cancer.

Not all phenolic compounds exhibit the same extent of antioxidant activity. Whether the quantity or quality of phenolics determined the antioxidant capacity of the cereal samples, is a question that was addressed in the results of antioxidant assays.

### 4.4 Tentative identification of Phenolic compounds using HPLC

The antioxidants in the wild cereal grains studied were determined using High Performance Liquid Chromatography (HPLC) analysis. Five hydroxybenzoic acids: gallic acid, \( p \)-hydroxybenzoic acid, \( p \)-hydroxybenzaldehyde, syringic acid and vanillic acid; three hydroxycinnamic acids: ferulic acid, caffeic acid, \( p \)-coumaric acid, one flavonol: quercetin and one flavanol: catechin, were successfully separated within 70 min with the order of elution as follows; starting with the standard which took the least elution period to the one that took the longest time to be eluted: gallic acid, protocatechuic acid, quercetin, \( p \)-hydroxybenzoic acid, \( p \)-hydroxybenzaldehyde, caffeic acid, vanillic acid, \( p \)-coumaric acid,
syringic acid and ferulic acid respectively. The HPLC chromatogram for the mixture of standard phenolic compounds is shown in figure 4.4.1.

**Figure 4.4.1:** HPLC chromatogram of standard phenolic compounds (5.0 mg/L each). The abbreviations below represent: Caffeic acid (CA), Catechin (Cat), p-coumaric acid (pC), Gallic acid (GA), ferulic acid (FA), p-hydroxybenzoic acid (pHB), p-hydroxybenzaldehyde (pH), protocatechuic acid (Pr), Quercetin (Qu), Syringic acid (S) and vanillic acid (V).
Table 4.4.1: Phenolic compounds detected in selected cereal grains using HPLC.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Phenolic compounds detected</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus hybridus</em></td>
<td>CA, FA, pC, pHB</td>
</tr>
<tr>
<td><em>Brachiaria brizantha</em></td>
<td>CA, FA, pC, Pr, S</td>
</tr>
<tr>
<td><em>Eleusine corocana</em></td>
<td>GA, FA, pC, Pr, pH, S, V.</td>
</tr>
<tr>
<td><em>Panicum maximum</em></td>
<td>CA, Cat, FA, pC, pHB</td>
</tr>
<tr>
<td><em>Rottboellia cochinchinensis</em></td>
<td>Cat, FA, Pr, pC</td>
</tr>
<tr>
<td><em>Sorghum arundinaceum</em></td>
<td>Cat, FA, GA, pC, pHB, pH, Qu, S, V</td>
</tr>
<tr>
<td><em>Sorghum bicolor</em> (Red variety)</td>
<td>GA, FA, pHB, pC, Qu.</td>
</tr>
</tbody>
</table>

The abbreviations in the table represent: Caffeic acid (CA), *p*-coumaric acid (pC), Gallic acid (GA), ferulic acid (FA), *p*-hydroxybenzoic acid (pHB), *p*-hydroxybenzaldehyde (pH), protocatechuic acid (Pr), Quercetin (Qu), Syringic acid (S) and vanillic acid (V).

Phenolic compounds identified in the cereal grain samples analyzed are shown in table 4.4.1. There were several unidentified peaks in all the sample profiles due to lack of suitable standards (see Appendix 1 for the other chromatograms) and in figure 4.4.2 which is the chromatogram for *P. maximum*, the unlabelled peaks indicate the unidentified peaks.
Figure 4.4.2: HPLC chromatogram of Panicum Maximum. The abbreviations represent the following phenolic compounds; CA: caffeic acid, Cat; Catechin, Pr: protocatechuic acid, pHB: p-hydrobenzoic acid, FA: ferulic acid and P-Co: p-coumaric acid.

Ferulic acid and p-coumaric acid were detected in all the cereal samples while Caffeic acid, catechin, gallic acid, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, protocatechuic acid, quercetin, syringic acid and vanillic acid were not common to all the samples. Ferulic acid is the most abundant phenolic acid found in cereal grains hence its presence in all the studied cereal grains (Lempereur et al., 1997). Wheat was reported to contain about 0.8-2 g/kg dry weight of ferulic acid, which may represent up to 90 % of total polyphenols (Sosulski et al., 1997; Lempereur et al., 1997). Ferulic acid is a potent antioxidant that is
capable of inhibiting lipid peroxidation in membranes (Trombino et al., 2004). The acid was reported to be a powerful scavenger of alkylperoxyl radicals in the β carotene – linoleic acid system (Terpinc and Abramovic, 2010). \( p \)-Coumaric acid has antioxidant properties and it reduces the risk of stomach cancer by preventing the formation of carcinogenic nitrosamines (Kikugawa et al., 1983). The characteristics of ferulic acid and \( p \)-coumaric acid, give the cereal extracts that were studied, relevance in the health and food industry as disease preventive agents and preservatives respectively.

Nine phenolic compounds among the available standards were identified in \( S. \) arundinaceum. The phenolic compounds identified were: \( p \)-coumaric acid, gallic acid, ferulic acid, \( p \)-hydroxybenzoic acid, \( p \)-hydroxybenzaldehyde, quercetin, syringic acid and vanillic acid as shown in table 4.4.1. The greater number of phenolic compounds identified in \( S. \) arundinaceum than in all the other cereal grains evaluated, may have been due to the abundance in proanthocyanidins which gave it a deep red colour. Darker or deeply pigmented cereal grain samples have higher content of phenolic compounds than non-pigmented samples (Wojdylo and Oszmainski, 2007). The cereal sample with the least number of identified phenolic acids and flavonols was \( R. \) cochinchinensis. However, the presence of less phenolic compounds does not necessarily mean less antioxidant activity and this is shown later in this document.

The hydroxycinnamic acids, \( p \)-coumaric acid, caffeic acid and ferulic acid have been reported to be prevalent among plants though their concentrations differ among species (Vickery and Vickery, 1981). The differences in the constitution of hydroxycinnamic acids in the cereal grains in table 4.4.1 are in agreement with the findings of Vickery and Vickery
(1981) who found out that there was variation in the composition of hydrocinnamic acids among grains from different families.

Ferulic and p-coumaric acids may be esterified to pectins and arabinoxylans or cross-linked to cell wall polysaccharides in the form of dimers such as dehydroferulates and truxillic acid (Bunzel et al., 2000). In studies that have been done, it was indicated that hydroxycinnamates had strong antioxidant activity, but the antioxidant effects of hydroxycinnamates depended on their bioaccessibility and absorption in the gut (Kroon and Williamson 1999).

From table 4.4.1, *A. hybridus, P. maximum, R. cochinchinensis* contained mostly hydroxycinnamic acids while *B. brizantha* and *E. corocana* contained mainly a mixture of hydroxycinnamic acids and hydroxybenzoic acids. *S. arundinaceum* and the red variety of *S. bicolor* contained mostly hydroxycinnamic acids, hydroxybenzoic acids, flavanols and flavonols. The wide range of phenolic classes found in the cereals, impart *S. bicolor* (red variety) and *S. arundinaceum* with a quality of exhibiting higher antioxidant activity than other cereal grains.

Caffeic acid was detected in *A. hybridus, P. maximum, B. brizantha*. Caffeic acid is not a desirable phenolic acid in high concentrations in foods because it complexes and precipitates, Ca$^{2+}$ which is essential for bone formation. The acid additionally complexes and precipitates casein, a phospho-protein found in mammalian milk which is a major component of cheese used as a food additive. Caffeic acid complexes and precipitates pectin, a structural hetero-polysaccharide that is used as a filling in medicines, sweets and as a stabilizer in fruit juices as well as in milk drinks. Pectin is a source of dietary fiber and
flavanol gallates such as the aflavin gallate and some thearubigins (tea creams) which are valuable antioxidants are found complexed to the hetero-polysaccharide (Tomás-Barberan and Robins, 1997). The presence of caffeic acid in the cereal grains means that there may be need to process the cereal grains before consumption in order to get rid of the acid, thereby preventing the negative effects of the phenolic acid to the human body.

4.5 Vanillin assay for flavanoids

The flavanoid content in all the six cereal grains included in the study is shown in table 4.5.1.
Table 4.5.1: Levels of flavanoid content as determined by the vanillin-HCl method in wild and domestic cereal grains and expressed as catechin equivalent.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Flavanoid concentration [mg/g on dry weight basis (DW)]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus hybridus</em></td>
<td>f0.035 ± 0.001</td>
</tr>
<tr>
<td><em>Panicum maximum</em></td>
<td>e0.500 ± 0.003</td>
</tr>
<tr>
<td><em>Brachiaria brizantha</em></td>
<td>e0.058 ± 0.002</td>
</tr>
<tr>
<td><em>Sorghum arundinaceum</em></td>
<td>a4.840 ± 0.001</td>
</tr>
<tr>
<td><em>Rottboellia cochinchinensis</em></td>
<td>g0.020 ± 0.004</td>
</tr>
<tr>
<td><em>Eleusine corocana</em>D</td>
<td>b1.210 ± 0.005</td>
</tr>
<tr>
<td><em>Sorghum bicolor</em> (Red variety)D</td>
<td>c0.880 ± 0.003</td>
</tr>
</tbody>
</table>

*In the same column, means with different superscript letters are significantly different (P<0.05). Values are means ± standard deviation of triplicate determinations. D was used to indicate domesticated cereal grains.

All the cereal grains evaluated, wild and domesticated contained flavanoids of varying concentrations. *S. arundinaceum*, a wild cereal grain contained the largest amounts of flavanoids, while *E. corocana*, a domestic cereal grain followed. When compared to other cereal grains, this higher level of flavanoids in the two cereals may be due to the presence of proanthocyanidins which impart the deep red color of the cereal grains. *S. arundinaceum*
grains are reddish in colour, while those of *E. corocana* are reddish-brown and the colours indicate an abundance of proanthocyanidins (Du Toit *et al.*, 2007). *R. cochinchinensis*, a wild grain, contained the least levels of flavanoid.

Higher levels of flavonoids in the plants could be explained by the fact that the cereal development occurs in warmer, drier as well as sunnier environmental conditions (Mlambo *et al.*, 2007). Such weather conditions trigger elevated synthesis of phenolic compounds (Veberic *et al.*, 2008).

Masa and colleagues (2007) reported that variation in the composition of flavonoids in plants is primarily determined by genetic factors, seasonal conditions, physical as well as chemical characteristics of the soil. These may be the contributory factors to the variations in flavanoid concentration among the cereals. Knowledge of the factors that increase flavanoid content in the cereal grains enables us to enhance the yield of this class of phenolic compounds for the benefit of the food and pharmaceutical industries.

### 4.6 Butanol-HCL assay for condensed tannins (proanthocyanidins)

The percentages of condensed tannins in wild cereal grains are shown in table 4.6.1.
Table 4.6.1: Levels of condensed tannins in selected wild and domesticated cereal grains as determined by the Butanol-HCL assay for condensed tannins (proanthocyanidins) and expressed as % leucocyanidin equivalence.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Leucocyanidin equivalence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus hybridus</em></td>
<td>c2.5 ± 0.06</td>
</tr>
<tr>
<td><em>Panicum maximum</em></td>
<td>c2.2 ± 0.01</td>
</tr>
<tr>
<td><em>Brachiaria brizantha</em></td>
<td>g0.7 ± 0.02</td>
</tr>
<tr>
<td><em>Sorghum arundinaceum</em></td>
<td>a12.2 ± 0.08</td>
</tr>
<tr>
<td><em>Rotthoellia cochinchinensis</em></td>
<td>f0.9 ± 0.09</td>
</tr>
<tr>
<td><em>Eleusine coracana</em></td>
<td>d3.3 ± 0.06</td>
</tr>
<tr>
<td><em>Sorghum bicolor</em> (red variety)</td>
<td>b4.6 ± 0.03</td>
</tr>
</tbody>
</table>

*In the same column, means with different superscript letters are significantly different (P<0.05). Values are mean ± standard deviation of triplicate determinations. D was used to indicate domesticated cereal grains.

*S. arundinaceum* had a significantly higher (p<0.05) proanthocyanidin content (approx 12.2 %) compared to all the other cereal grains. *S. arundinaceum*, a wild cereal grain, had a higher content of proanthocyanidins than its domestic relative *S. bicolor*. Proanthocyanidins are not widely distributed in plants (Haslam, 1989), hence, the low
percentages that were obtained and are shown in the table 4.6.1. There was a wide variation in the proanthocyanidins content, ranging from 0.9 in *R. cochinchinensis* to 12.2 % in *S. arundinaceum*, among the selected cereal grains.

The condensed tannins are responsible for the astringent quality of fruit and beverages such as wine, cider, tea, beer including the bitterness of chocolate (Rasmussen *et al*., 2005). Proanthocyanidin levels in plants are affected by a range of environmental factors which include drought, type of soil including its structure, ecology, disease status and damage by herbivores (Oszmianski *et al*., 2007; Alonso-Amelot., *et al* 2007). All the plants were collected from different locations in and around Harare, as well as in Buhera where conditions of soil, rainfall, ecology including exposure to herbivores differed markedly. The variations in the conditions of growth of the cereals may have led to the variation in the proanthocyanidins content among the cereal grains.

The antinutritional effects of tannins include diminished growth rate (Jambunathan and Mertz 1973), protein digestibility (Maxson *et al*., 1973a, 1973b; Cousins *et al*., 1981) as well as feed efficiency in rats, hamsters, swine, poultry and ruminants (Muindi and Thomke 1981; Mehansho *et al*., 1987a, 1987b; Knabe 1990). The high tannins content in the *S. arundinaceum*, *S. bicolor* (red variety) and *E. corocana* may reduce digestibility and efficiency of utilization of absorbed nutrients from 30 % to 15 % (Hagerman *et al*., 1998).

The presence of high levels of phenolic compounds such as proanthocyanidins has adverse effects on mineral composition of cereals. Higher molecular weight phenolic compounds bind to form complexes with divalent ions (Wallace *et al*., 1994). The high content of phenolic compounds in all the cereal grains studied may have contributed to lowering the
concentrations of minerals. For sorghum which was collected during the rainy season, the high rainfall may have leached the calcium and the leaching may have contributed to the lack of calcium (Wall, 2006).

There are several approaches to assist in detoxification of cereals containing tannins. The most commonly used ways of reducing tannin levels in plant foods are by decortication and malting. Decortication is the removal of the pericarp and testa, where most tannins are located in the grains (Reichert et al., 1988). High-tannin sorghum varieties, however, are preferred by some industrial sorghum companies that deal with malting as the grain is more resistant to mould infection during the moist, warm conditions (95-100 % RH, 25-30°C) used for malting (Reichert et al., 1988). For the grains with relatively high condensed tannins in this study, it would be necessary to process them before consumption in order to lessen the adverse effects of the compounds.

4.7 Scavenging activity on the DPPH radical

The extent, to which the methanolic extracts of selected cereal grains were able to quench the DPPH radical, is shown in table 4.7.1. *E. corocana* and *S. arundinaceum* took the least time to deplete the most DPPH radical from the solution. The fast rate of depletion of DPPH radical over time indicated that the antioxidants in the two cereal grains were more efficient in quenching the DPPH radical than in all the other grains tested. Statistically, there was a strong positive correlation between the concentration of phenolic compounds and the ability to scavenge for the DPPH radicals for *E. corocana*, *S.arundinaceum* and *S. bicolor* (r = 0.97, 0.95 and 0.90 respectively). The same could not be said for *R. cochinchinensis* and *A. hybridus*. *R. cochinchinensis* had more than double the amount of
total phenolic compounds in A. hybridus, but the scavenging activity of A. hybridus was significantly greater (p<0.05) than of R. cochinchinensis. The lack of correlation between the total phenolic content and the radical scavenging activity of R. cochinchinensis and A. hybridus may have been due to the type of phenolic compounds in R. cochinchinensis which exhibited lower activity than in A. hybridus.

The rate of decrease in the percentage of the DPPH radical remaining in solution indicated the scavenging power of the phenolic compounds in the cereal extracts. The graphs, relative to each other, with the steeper slopes in figure 4.7.1, were those of the cereals with the extracts that had the fastest scavenging activity. There was variation in the rate of depletion of the DPPH radical from the solution and the extent to which the radical was depleted differed among the extracts from the different cereal grains because the extracts were from different species of cereal plants.

Katalinic et al., in 2005, reported a strong relationship between the amount of phenolic compounds and scavenging capabilities of 70 medicinal plants. Chahardehi and coworkers (2009), in contrast, did not find any relationship between the content of phenolic compounds and the radical scavenging capabilities of plant extracts they studied. Our findings and the contrasting reports by other researchers indicate that antioxidant activities may depend on the types of the phenolic compounds rather than the amount of the phenolic compounds in a particular plant.
Fig 4.7.1: Antioxidant scavenging effects of methanolic extracts of *Sorghum arundinaceum* (Δ), *Sorghum bicolor* (red variety) (▲), *Rottboellia cochinchinensis* (○), *Eleusine corocana* (○), *Brachiaria brizantha* (□), *Amaranthus hybridus* (■), *Panicum maximum* (▼) and BHA (●) which was used as the standard, on discoloration of the DPPH radical. Values are mean ± standard deviation of triplicate determinations.

4.8 Reducing power of extracts of cereal grains

The reducing power of seven cereal grains are shown in figures 4.8.1 and 4.8.2. The presence of antioxidants with reducing capabilities in the cereal samples resulted in the reduction of Fe$^{3+}$ (ferric) complex to the Fe$^{2+}$ (ferrous) form. All the cereal extracts had the
ability to reduce the Fe$^{3+}$ to its ferrous form and the ability of the extracts to reduce the ion solution increased with the increase in the concentration of the sample extract. The reducing powers of the cereal grain extracts may have been due to the electron donating capacity of the compounds (Shon et al., 2007).

*E. corocana*, a domesticated cereal and *S. arundinaceum*, a wild cereal grain, had the highest reducing power at 100 mg sample equivalent per µL. The higher reducing power, than all other cereal grains, exhibited by *E. corocana* and *S. arundinaceum* may have been due to the higher phenolic composition in the cereal grains than in other grains.

Zhenbao and colleagues (2007) reported that reducing power increased as they increased the concentration of the extract of *Cassia tora* L., a legume which is well known to have diuretic, anti diarrheal and anti hypertensive activity. Bae and Suh (2007) reported that reducing power of five different mulberry cultivars increased as the concentration of extract increased. The trend of increasing reducing power as sample concentration increased was consistent with our findings in this research.

The reducing power of cereal extracts might be due to their hydrogen-donating ability (Shimada et al., 1992) meaning that the cereal grains might contain higher amounts of Fe$^{3+}$ reducing compounds which could react with free radicals to stabilise and block radical chain reactions. In the food industry, the reducing power may be used to assist in preservation of food stuffs while in the medical industry, the ability of the samples to stop radical chain reactions is an important property that could be used to stop or reduce progression of degenerative diseases like cancer.
**Figure 4.8.1:** The reducing power of the methanolic extracts of *Eleusine corocana* (○), *Brachiaria brizantha* (□), *Amaranthus hybridus* (■), *Panicum maximum* (▼) and the standard used was Butylated hydroxyanisole (BHA) (●). An increase in the absorbance of the reaction mixture indicated the increased reducing power. Values are mean ± standard deviation of triplicate determinations.
**Figure 4.8.2:** The reducing power of the methanolic extracts of *Sorghum arundinaceum* (Δ), *Sorghum bicolor* (red variety) (▲), *Rottboellia cochinchinensis* (◊) and the standard used was Butylated hydroxyanisole (BHA) (●). An increase in the absorbance of the reaction mixture indicated the increased reducing power. Values are mean ± standard deviation of triplicate determinations.
4.9 Chelating abilities of plant extracts on ferrous ions

The chelating effect of extracts from selected wild and domesticated cereal grains found in Zimbabwe, on ferrous ions is presented in table 4.9.1.
Table 4.9.1: Ability of extracts from selected wild and domesticated cereal grains to chelate ferrous ions.

<table>
<thead>
<tr>
<th>Cereal Grain</th>
<th>0.2 mg/mL</th>
<th>0.4 mg/mL</th>
<th>0.6 mg/mL</th>
<th>0.8 mg/mL</th>
<th>1.0 mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus hybridus</em></td>
<td>b7.5 ± 0.5</td>
<td>a16.5 ± 0.1</td>
<td>a36.1 ± 0.8</td>
<td>b56.5 ± 0.1</td>
<td>c66.4 ± 0.3</td>
</tr>
<tr>
<td><em>Panicum maximum</em></td>
<td>c3.2 ± 0.2</td>
<td>c8.7 ± 0.1</td>
<td>c19.6 ± 0.1</td>
<td>c50.9 ± 0.7</td>
<td>c76.3 ± 0.1</td>
</tr>
<tr>
<td><em>Brachiaria brizantha</em></td>
<td>g2.0 ± 0.6</td>
<td>f7.4 ± 0.6</td>
<td>f15.4 ± 0.3</td>
<td>c46.1 ± 0.3</td>
<td>f50.2 ± 0.5</td>
</tr>
<tr>
<td><em>Sorghum arundinaceum</em></td>
<td>g1.6 ± 0.3</td>
<td>g6.1 ± 0.2</td>
<td>c16.7 ± 0.4</td>
<td>a68.2 ± 0.6</td>
<td>a89.4 ± 0.1</td>
</tr>
<tr>
<td><em>Rottboellia cochinchinensis</em></td>
<td>f2.7 ± 0.2</td>
<td>h4.3 ± 0.3</td>
<td>g13.7 ± 0.2</td>
<td>c44.1 ± 0.8</td>
<td>b84.7 ± 0.2</td>
</tr>
<tr>
<td><em>Eleusine corocana</em></td>
<td>c4.6 ± 0.7</td>
<td>c11.7 ± 0.1</td>
<td>b26.1 ± 0.5</td>
<td>c52.4 ± 0.3</td>
<td>c75.6 ± 0.9</td>
</tr>
<tr>
<td><em>Sorghum bicolor</em> (red variety)*</td>
<td>a9.3 ± 0.9</td>
<td>b13.4 ± 0.7</td>
<td>c19.4 ± 0.7</td>
<td>f32.7 ± 0.1</td>
<td>g43.2 ± 0.6</td>
</tr>
</tbody>
</table>

*In the same column, means with different superscript letters are significantly different (P<0.05). Values are mean ± standard deviation of triplicate determinations. D was used to indicate domesticated cereal grains.*
The ability of the cereal extracts from the cereal grains to chelate ferrous ions increased with increasing cereal grain concentration. At a sample concentration of 0.2 mg/mL, the chelating ability of the samples ranged from 1.6 % in *S. arundinaceum* to 9.3 % in *S. bicolor*. The domesticated cereal grain *S. bicolor* had the highest chelating capability while its wild counterpart *S. arundinaceum* exhibited the lowest chelating ability at 0.2 mg/ml of sample concentration. At a concentration of 0.4 mg/mL and 0.6 mg/mL *A. hybridus* had the highest chelating capability but as the concentration increased, *S. arundinaceum* extract became the strongest chelator of ions. The scavenging of radicals depends on the rate of transfer of hydrogen atoms from the phenolic compound (Shon *et al.*, 2007) so the rate of transfer of hydrogen atoms from *S. arundinaceum* was increasing.

The chelating ability was found to be 89.4 % for *S. arundinaceum* at a dose level of 1.0 mg/mL followed by *R. cochinchinensis* with a chelating ability of 84.7 %. The sample extract with the least chelating capability was found to be *S. bicolor* with an average equilibrium chelating value of 43.2 %. The low chelating ability of the grain extract may have been due to the low composition of phenolics compounds that can chelate ions effectively.

A transition metal ion is capable of generating free radicals from peroxides by Fenton reactions. Since Fe$^{2+}$ has been shown to cause production of oxyradicals and lipid peroxidation, minimization of Fe$^{2+}$ concentrations in the Fenton reaction affords protection against oxidative damage. All extracts from both domesticated and wild cereal grains had the Fe$^{2+}$-chelating effect. In other words, extracts from the cereal grains may be able to afford protection against oxidative damage. Ferrous ions are the most effective pro-oxidants in food systems (Lee *et al.*, 2007). The high ferrous-ion chelating ability of the
extracts from the cereal grains would be of importance in the preservation of food stuffs from the negative effects of ferrous ions.

4.10: Inhibition of phospholipids peroxidation assay

The ability of cereal extracts to prevent lipid peroxidation was followed using a system that contained homogenates of rat brain. From figure 4.10.1, methanolic extracts from *S. arundinaceum*, *R. cochinchinensis* and *S. bicolor* (red variety) were shown to protect phospholipids from peroxidation as shown by the decrease in absorbance as sample concentration was increased. Extracts from *A. hybridus, P. maximum, B. brizantha* and *E. corocana*, led to the protection of phospholipid from peroxidation as indicated in figure 4.10.2. Butylated hydroxyanisole (BHA) was used as the positive control.
Figure 4.10.1: Inhibition of phospholipid peroxidation at different concentrations of methanolic extracts of *Sorghum arundinaceum* (Δ), *Sorghum bicolor* (red variety) (▲), *Rottboellia cochinchinensis* (●) and BHA (●). A lower absorbance reading meant that low amounts of MDA were produced, indicating a higher ability of the extract to inhibit peroxidation of phospholipids. The results are presented as mean ± standard deviation of three independent measurements.
Figure 4.10.2: Inhibition of phospholipid peroxidation at different concentrations of methanolic extracts of *Amaranthus hybridus* (■), *Panicum maximum* (▼), *Brachiaria brizantha* (□), *Eleusine corocana* (○) and BHA (●). A lower absorbance reading meant that low amounts of MDA were produced, indicating a higher ability of the extract to inhibit peroxidation of phospholipids. The results are presented as mean ± standard deviation of three independent measurements.

Malonyldialdehyde (MDA), one of the major products of lipid peroxidation, has been studied widely as an index of lipid peroxidation and as a marker of oxidative stress (Janero, 1990). Addition of cereal grain extracts to the Fe$^{2+}$-K$_3$[Fe(CN)$_6$]-TCA system resulted in a decrease in the formation of tissue MDA levels, meaning that the cereal extracts were scavengers of the Fe$^{2+}$ molecule. The Fe$^{2+}$-scavenging activity observed in this study could
mean potential application in the management of diseases involving free radicals and oxidative damage.

Comparatively the order of inhibition of lipid peroxidation followed the order: *S. arundinaceum* > *S. bicolor* (red variety) = *P. maximum* > *A. hybridus* > *B. brizantha* > *R. cochin chinensis* > *E. corocana* as shown in figures 4.10.1 and 4.10.2. The order of inhibition of phospholipids was obtained by calculating the absorbance of each sample at a concentration of 80 mg/ml as a percentage of its absorbance at 20 mg/mL.

*S. arundinaceum* had the greatest increase of 77 %, in the rate of inhibition of phospholipid when its concentration was increased from 20 mg/mL to 80 mg/mL. The inhibition was dependant on the amount of sample extract added for all samples. The increase in inhibition as concentration of sample increased was significant (P<0.05) for all cereal grain antioxidant extracts. *P. maximum* and the red variety of *S. bicolor* inhibited phospholipid peroxidation to the same extent when dosage of sample was increased. The similarity in the level of inhibition of phospholipid peroxidation between the two cereals may be due to the similarity in the constituent phenolic compounds that were responsible for preventing phospholipid peroxidation. However, this needs further confirmation by other methods to positively identify the constituent phenolic compounds.

*S. arundinaceum* and *S. bicolor* (red variety) belong to the same sub-family. Of the two cereals, *S. arundinaceum* is wild cereal, whereas *S. bicolor* (red variety) has been domesticated. The wild cereal exhibited greater potential to prevent lipid peroxidation than its domestic counterpart. The protective capability may be due to the fact that the *S.
*arundinaceum* has a deeper red colour which is usually indicative of a lot of phenolic compounds, mainly anthocyanidins (Wojdylo and Oszmainski, 2007).

The brain is an aerobic organ that has one of the highest oxygen consumption rates on the basis of its weight and it possess high production rates of free radicals, thus the brain may be relatively more susceptible to oxidative damage (Slater, 1984). The protective effects of the cereal extracts may be useful in the protection of the brain cells against oxidative damage that could lead to degenerative diseases of the brain like dementia. However, further research needs to be done to conclusively determine the effects of the phenolic compounds on the brain.

**4.11 Antioxidant activity in the β-carotene bleaching assay**

In figures 4.11.1 and 4.11.2, the extent of protection of β-carotene over 120 minutes is shown. All the cereal extracts delayed the bleaching of β-carotene. The protection of the β-carotene by the cereal extracts was a positive occurrence that meant that the integrity of the β-carotene would be maintained and in turn be used to make vitamin A in the body. For the negative control, no sample was added to prevent bleaching of β-carotene, and the rate of discolouration was rapid. Catechin was used as the positive control.
Figure 4.11.1: The rate of bleaching of β-carotene by extracts of *Sorghum arundinaceum* (Δ), *Sorghum bicolor* (red variety) (▲), *Rottboellia cochinchinensis* (◊) in β-carotene/linoleic acid system. Catechin (●) was used as the positive control and in the negative control (♦), distilled water was added in place of the sample. Slow decrease in absorbance signified protection of β-carotene. Results are presented as the mean ± standard deviation of three independent measurements.
Figure 4.11.2: Antioxidant activity of extracts of *Amaranthus hybridus* (■), *Panicum maximum* (▼), *Brachiaria brizantha* (□) and *Eleusine corocana* (○) in β-carotene/linoleic acid system. Catechin (●) was used as the positive control and in the negative control (♦), distilled water was added in place of the sample. Slow decrease in absorbance signifies protection of β-carotene. Results are presented as the mean ± Standard deviation of three independent measurements.

Oxidation of carotenoids, which is induced by light, heat or peroxyl radicals (Ursini *et al.*, 1998) results in bleaching of carotenoids (Huang, 2005). Antioxidants that can donate hydrogen atoms to quench radicals can prevent or reduce decolorisation of carotenoids (Burda and Oleszek, 2001). β-carotene is the most abundant form of provitamin A in plants (Ross, 1999) and is an effective source of vitamin A in both conventional foods and supplements. Bleaching of β-carotene in this assay would have meant that the β-carotene
would be damaged and could not be used by the body as a precursor for the formation of vitamin A.

Catechin, a pure standard antioxidant was used as a positive control. The absorbance of β-carotene incubated with catechin at 2 h was assumed to be 100% meaning that bleaching of β-carotene was completely prevented.
Table 4.11.1: Antioxidant activity of extracts of wild and domesticated cereal grains relative to a catechin standard. Assumption is, after 2 hours, catechin inhibited bleaching of β-carotene by 100%.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Relative β-carotene remaining after 2h expressed as a percentage with 100% meaning that bleaching of β-carotene was completely prevented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin</td>
<td>100.0 ± 0.01</td>
</tr>
<tr>
<td>No sample</td>
<td>0.0 ± 0.01</td>
</tr>
<tr>
<td><em>Amaranthus hybridus</em></td>
<td>46.9 ± 0.05</td>
</tr>
<tr>
<td><em>Brachiaria brizantha</em></td>
<td>38.7 ± 0.03</td>
</tr>
<tr>
<td><em>Eleusine corocana</em></td>
<td>62.3 ± 0.02</td>
</tr>
<tr>
<td><em>Panicum maximum</em></td>
<td>40.7 ± 0.07</td>
</tr>
<tr>
<td><em>Rotthoellia cochinchinensis</em></td>
<td>20.1 ± 0.04</td>
</tr>
<tr>
<td><em>Sorghum arundinaceum</em></td>
<td>59.7 ± 0.02</td>
</tr>
<tr>
<td><em>Sorghum bicolor</em> (red variety)</td>
<td>65.5 ± 0.04</td>
</tr>
</tbody>
</table>

Relative to catechin, the order of protection of β-carotene can be shown from table 4.11.1 and it was as follows: *S. bicolor* (red variety) > *E. corocana* > *S. arundinaceum* > *A. hybridus* > *P. maximum* > *B. brizantha* > *R. cochinchinensis*. There were no notable links or trends between the wild and the domestic cereal grains in this investigation but all cereal grains showed ability to protect β-carotene. *E. corocana*, a domesticated cereal grain had
65.5% of the β-carotene remaining unbleached after 2 h, which was the highest percentage of all the cereal grains under study. *R. cochinchinensis*, a wild cereal had the least amount of β-carotene remaining after 2 h. Different extracts of various cereal grains have been reported to have potential inhibiting activity against the oxidation of β-carotene molecules (Cardador-Martínez et al., 2002) and therefore could lead to the protection of vitamin A, thereby making it available for use by the body.

The differences in the ability of the extracts of the cereal grains to protect against the bleaching of β-carotene depended on the phenolic compound constituents in the individual grains. Environmental factors affect the amount of potential antioxidants in the sample.

All the cereal grains contained phenolic compounds. There was no correlation between the amount of phenolic compounds in the cereal grains and the ability to prevent the bleaching of β-carotene (r = 0.134), suggesting that the ability to inhibit bleaching of β-carotene depended more on the type of constituent phenolic compounds rather than amount of total phenolic compounds in the cereal extracts. All cereal grains had the ability to prevent bleaching of β-carotene. All cereals differed (p<0.05) significantly in their ability to prevent the bleaching of β-carotene, the speculation being that they varied in their flavanoid content. Flavonoids have greater ability to delay bleaching of β-carotene than other compounds with similar structures and some cereals contained more flavanoids than others.
4.12: Bioaccessibility of phenolic compounds from cereal grains

4.12.1: Effect of digestive enzymes on the bioaccessibility of phenolic compounds from selected cereal grains.

The individual contribution of the digestive enzymes pepsin, pancreatin, lipase, amyloglucosidase and α-amylase to bioaccessibility of phenolic compounds in *S. bicolor* and *S. arundinaceum* are shown in figures 4.12.1.1 to 4.12.1.5. *S. arundinaceum*, a wild grain and the red variety *S. bicolor*, a domesticated cereal were used to demonstrate the effect of exposing cereal grains to enzymes.
**Fig 4.12.1.1:** The absorbances of phenolic compounds released from the grain matrix incubated in pepsin at pH 1.5 buffer solution. The higher the absorbances the higher the phenolic compounds released. *Sorghum arundinaceum* (○), *Sorghum bicolor* (red variety) (●) and no sample (■). Results are presented as the mean ± standard deviation of three independent measurements.

The effect of exposing cereal grains to the enzyme pepsin was monitored and the results were shown in figure 4.12.1.1. A higher absorbance value indicated a larger amount of phenolic compounds released from the cereal grain matrix because more phenolic compounds reduced the Folin C reagent thereby giving a higher absorbance value. The absorbances read in the control indicated the phenolic compounds that could be released from the food matrix without the involvement of enzymes. For both the *S. arundinaceum* and the red variety of *S. bicolor*, there was an increase in the absorbances in the first 1hr as...
indicated by the absorbances of 0.351 and 0.189 respectively. The increase in the absorbances meant the amount of phenolic compounds being released from the grain was increasing. The increase in the absorbances indicated that there was an increase in the amount of phenolic compounds in the reaction mixture.

After 4 hours, there was a 62 % decrease in absorbance in the solution containing S. arundinaceum. In the solution containing the red variety of S. bicolor, there was a 22 % decrease when we compared the absorbance peak at 1h to that recorded at 4 h. Some phenolic compounds like caffeic, chlorogenic, and gallic acid are unstable at very low values of between pH 1-2 and very high pH values of between 11 and 14 (Hong et al., 2002; Friedman and Jürgens 2000). The instability of phenolic compounds at extreme pH values may account for the sharp decrease in the absorbance values in figure 4.12.1.1 where the prolonged exposure of the phenolic compounds to acidic pH may have degraded the phenolic compounds (Zhu et al., 2002). The graph stabilised and flattened after the sharp decrease at 8hrs. Friedman and Jürgens (2000) reported that chlorogenic acid, (-)-catechin, (-)-epigallocatechin, ferulic acid, rutin, and trans-cinnamic acid are resistant to major pH-induced degradation. The findings by Friedman and Jürgens (2000) may be a good explanation to the flattening of the graph since some phenolic compounds were not affected by low pH conditions.
Figure 4.12.1.2: The absorbances of phenolic compounds released from *Sorghum arundinaceum* (o), *Sorghum bicolor* (red variety) (●) when incubated in the pancreatin solution over time and phenolic compounds released when no enzyme (■) was added are shown. Results are presented as the mean ± standard deviation of three independent measurements.

In figure 4.12.1.2, the rate of increase in absorbance increased rapidly within the first 45 minutes, and then the increase slowed down till the graph flattened at 6 hours. Pancreatin contains the pancreatic enzymes trypsin, amylase and lipase which assist in the digestion of protein, carbohydrate and fats respectively (Zhu *et al.*, 1997). The increase in absorbance supposes an increase in the phenolic compounds that were released into solution from the food matrix. The rapid increase in the amount of phenolic compounds released was due to the synergistic effect of the enzymes in the digestion process. Different enzyme would be
acting on different building components of the cereal matrix, thereby, releasing the bound phenolic compounds at a faster rate than when a single enzyme is used. The slowing down of the release of phenolic compounds that happened after 45 minutes of incubation could be an indicator that the form of the phenolic compounds in the food matrix was more conjugated to molecules of proven resistance to enzyme digestion, hence the longer the time of digestion (Sang et al., 2005). Phenolic compounds are mainly located in the seed coats (hulls) and play an important role in the defence system of seeds that are exposed to oxidative damage by many environmental factors such as light, oxygen, free radicals and metal ions (Okuda 1993; Okuda et al. 1991). Most phenolic compounds have been reported to be stable at pH ranges of 4 – 8.0 (Zhu et al., 1997). The pH at which the phenolic compounds are exposed when incubated with pancreatin is pH 7.5, and most phenolics are stable at this pH (Hong et al., 2002; Sang et al., 2005) so the graph flattened instead of decreasing in gradient.
Figure 4.12.1.3: The absorbances of phenolic compounds released from *Sorghum arundinaceum* (○), *Sorghum bicolor* (red variety) (●) when incubated in the lipase enzyme solution over time and phenolic compounds released when no enzyme (■) was added are shown. Results are presented as the mean ± standard deviation of three independent measurements.

The amount of phenolic compounds released when the samples were incubated with the lipase enzyme solution was significantly lower (p<0.05) than the amount released when the samples were incubated in pancreatin, pepsin, amylase and amyloglucosidase. The lower amount of phenolic compounds released may be due to lower fat content of the cereal grains reported earlier in this study. *S. arundinaceum* and *S. bicolor* (red variety) contained 3.04 % and 3.32 % fat respectively. The effect of digestion of the cereals by a fat digesting enzyme was significantly lower (p<0.05) than those phenolics released when carbohydrate specific enzymes were used. The percentage difference between the phenolics released by carbohydrate specific enzymes and lipase was greater than 70 %.
Figure 4.12.1.4: The absorbances of phenolic compounds released from *Sorghum arundinaceum* (o), *Sorghum bicolor* (red variety) (●) when incubated in α - Amylase enzyme solution over 16 hrs and phenolic compounds released when no enzyme (■) was added are shown. Results are presented as the mean ± standard deviation of three independent measurements.

The slope of the graph in figure 4.12.1.4 increased steadily over a period of more than 12 hours. The steady increase may be an indicator that some phenolic compounds are conjugated mainly to carbohydrates. Because the amylase enzyme digests carbohydrates non-specifically to produce both glucose and maltose, some of the phenolic compounds remained conjugated and hence were not readily bioaccessible until the molecules were further digested and the phenolic compounds became aglycones. Benzoic acids vanillic, *p*-hydroxybenzoic, syringic, protocatechuic, salicylic and gallic acids are some of the phenolic compounds that are usually found conjugated with sugars or organic acids.
Cinnamic acid derivatives are mainly found in several conjugated forms, mainly esters of hydroxyl acids such as tartaric acid and sugar derivatives (Shahidi and Nacsk, 1995).

![Absorbance at 725 nm](image)

**Figure 4.12.1.5**: Phenolic compounds released from *Sorghum arundinaceum* (○), *Sorghum bicolor* (red variety) (●) when incubated in amylglucosidase enzyme solution over time and phenolic compounds released when no enzyme (■) was added are shown. Results are presented as the mean ± standard deviation of three independent measurements.

The rate of increase in absorbance for both, *S. arundinaceum* and *S. bicolor* (red variety), as shown in figure 4.12.1.5, was fast. Within 45 minutes the absorbances had stopped increasing meaning the amount of phenolic compounds that could be released due to the action of amylglucosidase had been released from both cereal samples.
Amyloglucosidase is known as glucan 1,4-α-glucosidase; exo-1,4-α-glucosidase; glucoamylase; lysosomal α-glucosidase or 1,4-α-D-glucan glucohydrolase and will cleave α(1-6) glycosidic linkages, as well as the last α(1-4) glycosidic bonds at the non-reducing end of amylose and amylopectin, to yield glucose. The results obtained after exposing the cereal grains to different enzymes were used to design an intestinal model system that was efficient and would help us in estimating the bioaccessibility of phenolic compounds in the small intestines.

4.12.2: Bioaccessibility in the small intestines

The bioaccessibility of the phenolic compounds was expressed as a percentage. The percentage bioaccessibility was calculated from the concentration of phenolic compounds in the digested sample relative to the undigested sample. The undigested sample contained the total phenolics in the sample that were extracted non-enzymatically to extract as much phenolics as possible from the sample. The phenolics obtained by the non enzymatic method were assumed to be the 100 % value for phenolic compounds. S. arundinaceum had the highest phenolic compound content in both the undigested but fully extracted sample and enzymatically digested with 220.0 and 50.6 mg/100g gallic acid equivalence respectively, followed by P. maximum with 120.2 and 24.1 mg/100g gallic acid equivalence respectively. The concentrations used for the calculations of percentage bioaccessibility in the small and large intestines are shown in tables 4.12.2.1 and 4.13.3.1 respectively.
Table 4.12.2.1: (A) The total phenolic compounds determined from the undigested samples and (B) the total phenolic compounds determined in supernatant of enzymatically digested sample in the small intestine digestion model assay. Results were expressed as gallic acid equivalence in mg/100g of sample.

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus hybridus</em></td>
<td>51.41 ± 0.02</td>
<td>14.06 ± 0.07</td>
</tr>
<tr>
<td><em>Panicum maximum</em></td>
<td>120.19 ± 0.07</td>
<td>24.13 ± 0.04</td>
</tr>
<tr>
<td><em>Brachiaria brizantha</em></td>
<td>88.65 ± 0.06</td>
<td>17.79 ± 0.03</td>
</tr>
<tr>
<td><em>Sorghum arundinaceum</em></td>
<td>220.01 ± 0.06</td>
<td>50.61 ± 0.01</td>
</tr>
<tr>
<td><em>Rottboellia cochinchinensis</em></td>
<td>72.59 ± 0.01</td>
<td>13.62 ± 0.09</td>
</tr>
<tr>
<td><em>Eleusine corocana</em> w</td>
<td>70.52 ± 0.03</td>
<td>23.32 ± 0.08</td>
</tr>
<tr>
<td><em>Sorghum bicolor</em> (red variety)</td>
<td>65.37 ± 0.04</td>
<td>19.64 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of triplicate determinations.  w was used to indicate domesticated cereal grains.
Figure 4.12.2.1: Percentage bioaccessibility of phenolic compounds in the small intestine, as estimated by an *in vitro* small intestine model. SA = *Sorghum arundinaceum*; EC = *Eleusine corocana*; SB = *Sorghum bicolor*; AH = *Amaranthus hybridus*; RC = *Rottboellia cochinchinensis*; PM = *Panicum maximum*; *Brachiaria brizantha* = BB.

The percentage bioaccessibility of phenolic compounds in the small intestines estimated by the *in vitro* intestinal model is shown in figure 4.12.2.1. The cereal grains that had the highest bioaccessibility were *E. corocana* and the red variety of *S. bicolor* (red variety) with 33.06 ± 2.12 % and 30.04 ± 0.83 % respectively. The cereal with the lowest bioaccessible phenolics in this model was *R. cochinchinensis* which had a bioaccessibility percentage value of 18.76 ± 0.53 %. The average bioaccessibility of all the cereal grains when subjected to the small intestine model was 28 %.
Phenolic compounds that were released from the food matrix as a result of human enzymatic action were generally low (p<0.05), averaging only 28 % bioaccessibility. The low bioaccessibility may be as a result of phenolic compounds in plants being found conjugated to other molecules like carbohydrates, cellulose, lignin etc that are resistant to digestion. Plant foods contain indigestible compounds (indigestible fraction) that are neither digested nor absorbed in the small intestine and the results obtained here were consistent with studies reporting that proanthocyanidins are not released and absorbed in the small intestine (Rios et al., 2002).

Cereals consists not only of dietary fibre but also of other compounds of demonstrated resistance to the action of digestive enzymes, such as resistant starch, resistant protein, polyphenols and other associated compounds (Saura-Calixto et al., 2000). The indigestible fraction passes into the colon, where it provides a substrate for fermentative microflora. The residue of the indigestible fraction after the fermentation is a part of the unavailable matter excreted in faeces (Guillon et al., 1995). Colonic digestion will contribute to bioaccessibility by releasing the phenolic compounds bound to the cellulose and fibre.
4.13.3: Bioaccessibility of phenolic compounds in cereal grains after colonic fermentation

Figure 4.13.3.1: Percentage bioaccessibility of phenolic compounds in the colon, as estimated by an *in vitro* colonic fermentation model. SA = *Sorghum arundinaceum*; EC = *Eleusine corocana*; SB = *Sorghum bicolor*; AH = *Amaranthus hybridus*; RC = *Rottboellia cochinchinensis*; PM = *Panicum maximum*; BB = *Brachiaria brizantha*.

The percentage bioaccessibility of phenolic compounds resulting from digestion through *in vitro* colonic fermentation is shown in figure 4.13.3.1. The bioaccessibility estimated for the large intestines using the model assay was significantly (p<0.05) higher than that observed in the small intestine model system. The average bioaccessibility in the colon was
62% with *E. corocana* having the highest percentage bioaccessibility and *P. maximum* having the lowest. For all the cereal grains, the percentage bioaccessibility was significantly higher (p<0.05) in the colon when compared to bioaccessibility in the small intestines. The average bioaccessibility in the colon was found to be 62% while in the small intestines, it was found to be 28%.

Table 4.13.3.1: A) Total phenolics in unfermented sample and B) Total phenolics in fermented sample that were determined after colonic fermentation of cereal samples and expressed as gallic acid equivalence in mg/100g of sample.

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus hybridus</em></td>
<td>51.41 ± 0.02</td>
<td>34.73 ± 0.02</td>
</tr>
<tr>
<td><em>Panicum maximum</em></td>
<td>120.19 ± 0.07</td>
<td>80.31 ± 0.04</td>
</tr>
<tr>
<td><em>Brachiaria brizantha</em></td>
<td>88.65 ± 0.06</td>
<td>56.78 ± 0.09</td>
</tr>
<tr>
<td><em>Sorghum arundinaceum</em></td>
<td>220.01 ± 0.06</td>
<td>149.06 ± 0.03</td>
</tr>
<tr>
<td><em>Rottboellia cochinchinensis</em></td>
<td>72.59 ± 0.01</td>
<td>46.97 ± 0.07</td>
</tr>
<tr>
<td><em>Eleusine corocana</em> <em>D</em></td>
<td>70.52 ± 0.03</td>
<td>42.31 ± 0.01</td>
</tr>
<tr>
<td><em>Sorghum bicolor</em> (Red) <em>D</em></td>
<td>65.37 ± 0.04</td>
<td>39.90 ± 0.05</td>
</tr>
</tbody>
</table>

Values are mean ± Standard deviation of triplicate determinations. *D* was used to indicate domesticated cereal grains.
**4.13.4: Total Intestinal Bioaccessibility**

![Graph showing bioaccessibility of phenolic compounds in cereals](image)

**Figure 4.13.4.1:** Percentage bioaccessibility of phenolic compounds in the intestines, as estimated by an *in vitro* colonic fermentation model. SA = *Sorghum arundinaceum*; EC = *Eleusine corocana*; SB = *Sorghum bicolor*; AH = *Amaranthus hybridus*; RC = *Rottboellia cochinchinensis*; PM = *Panicum maximum*; BB = *Brachiaria brizantha*.

The estimate bioaccessibility of five cereal grains are presented in figure 4.13.4.1 and is expressed as a percentage of the total phenolic compounds in an unfermented sample. The amount of estimated phenolic compounds released in the small intestines using this model system was low. The low bioaccessibility in the small intestine compared to the large intestine bioaccessibility may have been because, phenolic compounds which include vanillic, *p*-hydroxybenzoic, syringic, protocatechuic, salicylic and gallic acids, are found
conjugated to complex compounds such as dietary fibre which cannot be digested by human enzymes in the small intestines (Macheix et al., 1990). The high molecular weight proanthocyanidins and phenolics are associated with dietary fibre and indigestible compounds (Saura-Calixto and Goñi, 2004). The phenolic compounds may have been released in the large intestine model assay as a result of fermentation in the presence of enzymes from colonic bacteria. Enzymes from bacteria are capable of digesting cellulose and lignin while human enzymes are not and that is why bioaccessibility of phenolic compounds was lower in the small intestine model than the large intestine model which contained caecal matter with colonic bacteria from a rat gut.

The fibre content reported earlier for the cereal grains were 12.4 % for S. arundinaceum, 3.52 % for E. corocana, 2.51 % for S. bicolor, 5.78 % for A. hybridus, 24.89 % for R. cochinchinensis and 26.03 % for P. maximum. From the bioaccessibility results we observed that generally, the cereal grains with higher fibre content resulted in less bioaccessibility of phenolic compounds. R. cochinchinensis with 24.89 % fibre, had small intestine bioaccessibility of 20 % while P. maximum with 26.03 % fibre content had 26 % bioaccessibility. Inversely, S. bicolor had 2.51 % fibre and the bioaccessibility was significantly higher (36 %) compared to R. cochinchinensis and P. maximum.

4.13.5: Non-extractable polyphenols

In the residue of the colonic digestion condensed tannins also known as proanthocyanidins were determined and are presented in table 4.13.5.1.
Table 4.13.5.1: The percentage of condensed tannins determined in the residue obtained after colonic fermentation of cereal grain samples and compared with the percentage of condensed tannins in the undigested sample.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Proanthocyanidins (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sorghum arundinaceum</em></td>
<td>4.37 ± 0.07</td>
</tr>
<tr>
<td><em>Eleusine corocana</em></td>
<td>1.03 ± 0.03</td>
</tr>
<tr>
<td><em>Sorghum bicolor (red variety)</em></td>
<td>2.06 ± 0.02</td>
</tr>
<tr>
<td><em>Amaranthus hybridus</em></td>
<td>3.27 ± 0.03</td>
</tr>
<tr>
<td><em>Rotboellia cochinchinensis</em></td>
<td>11.10 ± 0.09</td>
</tr>
<tr>
<td><em>Brachiaria brizantha</em></td>
<td>9.03 ± 0.06</td>
</tr>
<tr>
<td><em>Panicum maximum</em></td>
<td>9.54 ± 0.05</td>
</tr>
</tbody>
</table>

*In the same column, means with different superscript letters are significantly different (P<0.05). Values are mean ± standard deviation of triplicate determinations. D was used to indicate domesticated cereal grains.

The percentages of condensed tannins that remained in cereal grains after digestion are shown in the Table above. *R. cochinchinensis* had a significantly higher (p<0.05) proanthocyanidin content of approximately 11.10%, compared to all the other cereal grains. There was a general trend where the wild cereal grains were shown to have higher percentage contents of proanthocyanidins than the domestic grains in the study. The low percentage in the content of proanthocyanidins obtained in the final residue after digestion
in all the samples may indicate that most proanthocyanidins were bioaccessible in the colon, mainly due to colonic fermentation.
Chapter 5

5.0 Conclusions

The objective of the study was to determine macro and micro nutritional composition of selected wild and domesticated cereal grains found in Zimbabwe. The macro nutrients that were determined included proteins, fats, fibre, and minerals calcium and phosphorus. The micronutrients studied were phenolic compounds that included total phenolic compounds, condensed tannins and flavanoids. HPLC was used to tentatively identify the constituent compounds. The antioxidant activity of the phenolic compounds was determined and the bioaccessibility was determined using model system assays.

All the cereal grains had valuable nutrients. Proteins and fats were found in varying concentrations in all the cereal grains. The protein and fat content in *A. hybridus* was high and this makes amaranthus a good source of protein and fat compared to all the cereal grains that were studied. Fibre was found in varying concentrations in the cereal grains, but the high content of fibre in *B. brizantha* could render it a suitable fortification cereal for foods with low fibre content. The minerals calcium and phosphorus, that are important for human health, were detected in the cereal grains.

Phenolic compounds were detected in all the wild and domesticated cereals in this study. The concentrations of the phenolic compounds were varied among the different grain species and *S. arundinaceum*, a wild cereal, had significantly higher levels of total phenolic compounds. *S. arundinaceum* contained the largest amounts of flavanoids and proanthocyanidins.
p-Coumaric acid and ferulic acid were found in all the cereal grains while caffeic acid, catechin, gallic acid, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, protocatechuic acid, quercetin, syringic acid and vanillic acid varied in their distribution among the cereals.

All the cereal grain extracts were able to quench free radicals though their capabilities differed according to the particular cereal. *S. arundinaceum* and *E. corocana* extracts had the most potent free radical quenching phenolic compounds. All the cereal extracts had the ability to reduce the Fe$^{3+}$ to its ferrous form and the ability of the extracts to reduce the ion solution increased with the increase in the concentration of the sample extract. *E. corocana* and *S. arundinaceum* had the highest reducing power at 100 mg sample equivalent per µl. The ability of the cereal extracts from the cereal grains to chelate ferrous ions increased with increasing cereal grain concentration.

The extracts from the cereals protected phospholipids from peroxidation. Oxidation of carotenoids which is induced by light was prevented by all the extracts from the cereal grains. The red variety of *S. bicolor* and *E. corocana* prevented the bleaching of β-carotene to a greater extent than all the other samples. There was no correlation between the total amount of phenolic compounds and the antioxidant activity. This meant that the antioxidant activity was dependant mainly on the quality of phenolic compounds than the quantity.

The percentage bioaccessibility of phenolic compounds in the small intestines was estimated by the *in vitro* intestinal model and the cereal grains that had the highest bioaccessibility were *E. corocana* and the red variety of *S. bicolor* with 33.06 % and 30.04 % respectively. The bioaccessibility of phenolic compounds in the small intestines was lower than in the large intestines. The lower bioaccessibility in the small intestines meant
that human digestive enzymes could be incapable of digesting most food constituents to which phenolic compounds were conjugated.

It was shown in this study that wild cereal grains are good sources of nutrients and if further research on them is done, wild cereal grains could be an addition to the domesticated cereals thereby reducing hunger in Zimbabwe. The phenolic compounds that were detected in the cereal grains could be used in the food and pharmaceutical industries as natural antioxidants. Further research on the stability and bioavailability of the cereal extracts is needed to clearly ascertain the potential benefits of the phenolic compounds.
Chapter 6

6.0 References


Date accessed: 20 December 2013


Makkar, H.P.S. (1999), Quantification of Tannins in Tree Foliage: A laboratory manual for the FAO/IAEA Co-ordinated Research project on 'Use of nuclear and Related Techniques to Develop Simple Tannin Assay for Predicting and Improving the Safety and Efficiency of Feeding Ruminants on the Tanniniferous Tree Foliage. *Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture*, Vienna, Austria *pp 1-29*.


Scher, J. L. and D. S. Walters. 2010. Federal noxious weed disseminules of the U.S. California Department of Food and Agriculture, and Center for Plant Health Science and Technology, USDA, APHIS, PPQ.


7.0 Appendices

Appendix 1: HPLC chromatograms of *Brachiaria brizantha*, *Rottboellia cochinchinensis*, *Panicum maximum*, *Amaranthus hybridus*, *Sorghum arundinaceum*, *Sorghum bicolor* and *Eleusine corocana*.

HPLC chromatogram of *Brachiaria brizantha*. The abbreviations represent the following phenolic compounds: CA, caffeic acid; FA, ferulic acid; pC, p-coumaric acid; Pr, protocatechuic acid and S; Syringic acid.
HPLC chromatogram of *Rottboellia cochinchinensis*. The abbreviations represent the following phenolic compounds: Cat, Catechin; FA, Ferulic acid; pC, p-Coumaric acid; Pr, Protochatechuic acid.

HPLC chromatogram of *Amaranthus hybridus*. The abbreviations represent the following phenolic compounds: CA, caffeic acid; pC, protocatechuic acid; FA, ferulic acid; pHB, p-hydrobenzoic acid.
HPLC chromatogram of *Sorghum arundinaceum*. The abbreviations represent the following phenolic compounds: FA, ferulic acid; pC, *p*-coumaric acid; S, Syringic acid; Qu, quercetin; GA, gallic acid; pH, *p*-hydroxybenzoic acid; Cat, catechin; pH, *p*-hydroxybenzaldehyde and V, vanillic acid.

HPLC chromatogram of *Sorghum bicolor*. The abbreviations represent the following phenolic compounds: FA, ferulic acid; pC, *p*-coumaric acid; Qu, quercetin; GA, gallic acid; pH, *p*-hydrobenzoic acid.
HPLC chromatogram of *Eleusine corocana*. The abbreviations represent the following phenolic compounds: FA, ferulic acid; pC, p-coumaric acid; Pr, protocatechuic acid; S, Syringic acid; GA, gallic acid; pH, p-hydroxybenzaldehyde and V, vanillic acid.
Appendix 2: Publications arising from this thesis


