

A COMPARATIVE STUDY OF ANTIOXIDANT POTENTIALS OF SOME
LEAFY VEGETABLES: EMPHASIS ON AFRICAN LEAFY VEGETABLE
AND EXOTIC VEGETABLES

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DECLARATION

I, **Sakhile Mathe**, declare that the research reported in this thesis, except where otherwise indicated is my original work. This thesis has not been submitted for any degree or examination at any other university.

Sakhile Mathe

November 2014

I certify that the above statement is correct.

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ABSTRACT

.Due to malnutrition and food insecurity problem around the globe, mainly in developing countries, cheap nutritional food sources are required. In South Africa, a large proportion of the population is considered “poor” and with limited resources. However, South Africa as a whole is rich in indigenous leafy vegetables which have the capacity to help mitigate the problem of malnutrition and food insecurity.

Amaranthus hybridus, an African indigenous leafy vegetable was tested for seed quality and potential essential antioxidants. Exotic (to Africa) leafy vegetables (*Brassica oleracea* and *Brassica oleracea* var. *capitata* f. *rubra*) were used as references for potential antioxidants.

Amaranthus hybridus seed quality was tested using two different coloured seeds, red and white gold. Seed viability and vigor were tested using germination, electrolyte leakage and antioxidant content. Data recorded indicated better seed quality for red seeds than white gold seeds; therefore red coloured seeds were planted along with purchased *Brassica oleracea* and *Brassica oleracea* var. *capitata* f. *rubra* for quantifying antioxidant content.

Selected antioxidant types were measured on weekly harvests of the studied vegetables. From the results it was evident that the indigenous leafy vegetable amaranth with total antioxidants [FRAP(3174.91 mmol Fe₂ SO₄ 100g⁻¹ DW) and DPPH(8.3 mmol trolox 100g⁻¹), proteins (6.88 mg.g-1 DW), total phenols (345 mg 100g⁻¹ DW), flavonoids (79 mg 100g⁻¹ DW), Chlorophyll and carotenoids (2.8 mg 100g⁻¹ DW), ascorbic acid (86 mg 100g⁻¹ DW) and soluble sugars (1.07 Brix %), could be used in conjunction with available commercial leafy vegetables to combat malnutrition and food security problems.

Further, these results indicate that in resource limited regions this vegetable can act as a main source of nutrients and a supplement in resource abundant regions of the country and/or continent. Further, analysis of selected enzymatic antioxidants was carried-out on leaf material of the studied vegetable to evaluate the capability of indigenous leafy vegetables to protect themselves against oxidative damage. Indigenous leafy vegetables exhibited high antioxidant activity against lipid peroxidation at early stages of growth and high antioxidant enzyme activity at similar stages thus high capability of mitigating ROS effect.

Data obtained from the study indicated that indigenous vegetables are a good source of essential antioxidants which are beneficial to human health; therefore the intensity of their use needs to be increased, especially in areas of high prevalence of malnutrition and diseases.

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CHAPTER 1

Nutrient and antioxidant enzyme composition of Leafy Vegetables during Different Growth and Development Stages:

Comparative nutrient and antioxidant enzyme composition of *Amaranthus* (*Amaranthus hybridus*) and Green and Red Cabbage (*Brassica oleracea* and *Brassica oleracea var. capitata f. rubra*): A Review

1.2 Introduction

The world population is estimated to be at 6.7 billion to date, and it is estimated to increase up to 9 billion by 2050. Africa is estimated to have a more rapid increase in population, more than 70% compared to that of Asia (UNEP, 2013). The increase in population induces a further increase in demand for food, thus Africa will need to feed more people in the future. Smith (2013) reported that Africa feeds its population below targeted standard of nutrition and food quantities, resulting in malnutrition (FAO 2012). Malnutrition results from food insecurity; it is a condition that is due to a diet where particular nutrients are lacking, in excess, or in the wrong proportion (Schönfeldt and Pretorius, 2011). It is reported to be more prevalent in children and women from developing countries (UNICEF 1997; Fotso and Barthelemy 2006; Feber *et al.*, 2006; DBSA, 2008; Schönfeldt and Pretorius, 2011; WFP, 2013). Food security is a physical and economic access of food by all people in a society at all times for a culturally and nutritionally appropriate healthy and active lifestyle (FAO, 1996). Under this definition, obesity and hunger are equally considered to be a food insecurity challenge facing most of the developing countries (Chappell and LaValle, 2009).

South Africa is among the developing countries facing food insecurity challenge (Van der Merwe, 2011) despite the wealth of indigenous and underutilized crops suitable for a healthy active diet (Haq 2004; Maundu, 2013). Traditional (indigenous) knowledge about most of these crops is usually lost through evolution of lifestyle and farming cultures. Farming has generally evolved into a largely monoculture orientated model due to increases in demand

and returns of certain crops (Norberg-Hodge, 2010). A negative correlation between agriculture and biodiversity has been reported by a number of authors (Perrings, 2001; Chappell and La Valle, 2009), which they described to commercialization of certain crops. This correlation, however, excluded the traditional and underutilized crop species in South Africa, which account for approximately 10% of the world's higher plants population (Odhav *et al.*, 2005). These traditional crops have a potential to fight food security and malnutrition problems in South Africa and the African continent at large. According to Jansen van Rensburg *et al.*, (2007), the consumption of African Leafy Vegetables (ALVs) in South Africa is determined by level of poverty, urbanization, distance to fresh produce and season (Uusiku *et al.*, 2010).

African Leafy Vegetables (ALVs) are indigenous and traditional, they are mostly underutilized (Odhav *et al.*, 2005, Mibei *et al.*, 2011), and yet they present a rich and cheap nutritious diet where leaves, shoots, flowers and seeds may be consumed. They are rich in antioxidants and minerals (Mibei *et al.*, 2011) and used for other necessities either than consumption, like medicinal purposes (Odhav *et al.*, 2005). African Leafy Vegetables (ALVs) are plentiful throughout Africa and they are easily accessible to the predominately 'poor' rural communities, thus serve as an answer to the malnutrition and food security problem in Africa (de Sherbinin, 2008; Fotso and Barthelemy, 2006; Weller *et al.*, 2014; Zere and McIntyre, 2003).

There is a range of Amaranth species widely used in Africa for consumption, *Amaranthus thunbergii* (L), *A. greazicans*, (L), *A. spinosus* (L), *A. deflexus* (L), *A. hypochondriacus* (L), *A. viridus* (L) and *A. hybridus* (L). *Amaranthus hybridus* is one of the indigenous ALVs that has a potential to be used to eradicate food insecurity and malnutrition problems. It is nutritious and easily accessible to rural "poor" communities who are the most affected by food insecurity and malnutrition problem (Europa, 2009). However, it is underutilized and comparably under researched than exotic leafy vegetables. Exotic vegetables have high but comparable antioxidant and nutritional contents to the indigenous leafy vegetables (Amin *et al.*, 2004; Odhav *et al.*, 2005; Singh, 2009). There are numerous reports on anti-oxidative and nutritional properties of the commercial exotic vegetables and their ability to combat free radicals (.....2-3 authors....).

Free radicals are unstable and very reactive molecules having an unpaired electron in the exterior orbit (Fang *et al.*, 2002; Blokhina *et al.*, 2002; Bhattacharjee, 2005). Examples of

oxygen free radicals are superoxide, peroxy (RO_2^*), alkoxy (RO^*), hydroxyl (OH^*), hydroperoxy (HO_2^*), organic hydroperoxide ($ROOH$), etc. (Bhattacharjee, 2005), while Nitric oxide and nitrogen dioxide (*NO_2) are two nitrogen free radicals (Fang *et al.*, 2002; Valko *et al.*, 2007). These can be converted to non-radical molecules like hydrogen peroxide, hypochlorous acid ($HOCl$), hypobromous acid ($HObR$), and peroxyxynitrite ($ONOO^*$). They are not all bad as they have a role in origin and evolution of life

Adverse effects of these radicals are reduced or minimized by antioxidants Vitamin A, Vitamin C, Vitamin E, polyphenols, glutathione, lycopenes etc. (Amin *et al.*, 2004), which are reducing agents, while enzymatic antioxidants superoxides dismutase (SOD), catalase (CAT) and peroxidase (POX) catalyze the reaction of reducing free radicals (Blokhina *et al.*, 2002; Fang *et al.*, 2002). These antioxidants are present in both leaves and seeds; therefore we need to consider both for human consumption. Seeds are a source of life, thus they have to be alive and vigorous to produce sound living plants.

However these commodities are under-utilized and less researched and thus cannot contribute to reducing food insecurity and malnutrition (Schönfeldt and Pretorius, 2012) and chronic vascular diseases caused by free radicals. Research findings would contribute to scientific knowledge about indigenous leafy vegetables and prompt awareness to the scientific and ordinary worlds about the indigenous leafy vegetables nutritional contents, thus commercialization potential.

1.3 Leafy Vegetable Antioxidants

African Leafy Vegetables (ALVs) are a rich source of nutrients and antioxidants thus can be used for medical and nutritional purposes (Amin *et al.*, 2004; Odhav *et al.*, 2005; Singh, 2009). Their daily intake could help in lowering the incidence of degenerative diseases, cancer and cardiovascular diseases (Amin *et al.*, 2004; Ferreira *et al.*, 2005; Khanam *et al.*, 2012). The antioxidant activity is primarily due to their redox properties which enable them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelator, thus mitigating the presence of free radicals (Singh *et al.*, 2004; Torres, 2005).

An antioxidant may be defined as “any substance that when present at relatively low concentrations compared with those of the oxidisable substrate; it significantly delays or

inhibits oxidation of that substrate” (Apak *et al.*, 2013). Reducing ability of antioxidants does not depend on the quantity rather on quality (Chitintingu *et al.*, 2013).

Antioxidants in plants are present as enzymatic and non-enzymatic, these antioxidants aid in survival of plants in the field but when their sources are harvested and consumed by animals specifically humans they contribute to human health improvement. Enzymatic antioxidants include *Superoxide dismutase (SOD)*, *Catalase (CAT)* *Peroxidase (POX)*. These antioxidants work in conjunction to each other as to effectively mitigate cell damage from ROS. Some of the individual non-enzymatic antioxidants are carbohydrates (sugar-alcohols), carotenoids, vitamin C, phenolic compounds and proteins.

1.3.1 Superoxide dismutase (SOD)

Superoxide dismutase (SOD) is an important enzymatic antioxidant believed to be in the primary defence mechanism in plants cell toxicity (Jithesh *et al.*, 2006; Tesfay, 2009). SODs cause dismutation of superoxide to produce a ROS - H_2O_2 which is a signalling second messenger molecule for abiotic and biotic stress responses in plants (Bhattacharjee, 2005; Jithesh *et al.*, 2006; Tesfay 2009; Filaire and Toumi, 2012). Scavenging of singlet oxygen ($O_2^{\cdot-}$) radicals is achieved by SODs activity of dismutation (Jithesh *et al.*, 2006; Tesfay 2009). Excess H_2O_2 is problematic to plant cell membranes as it is a ROS, thus reduction of H_2O_2 to oxygen (O_2) and water (H_2O) by glutathione peroxidase enzyme is required.

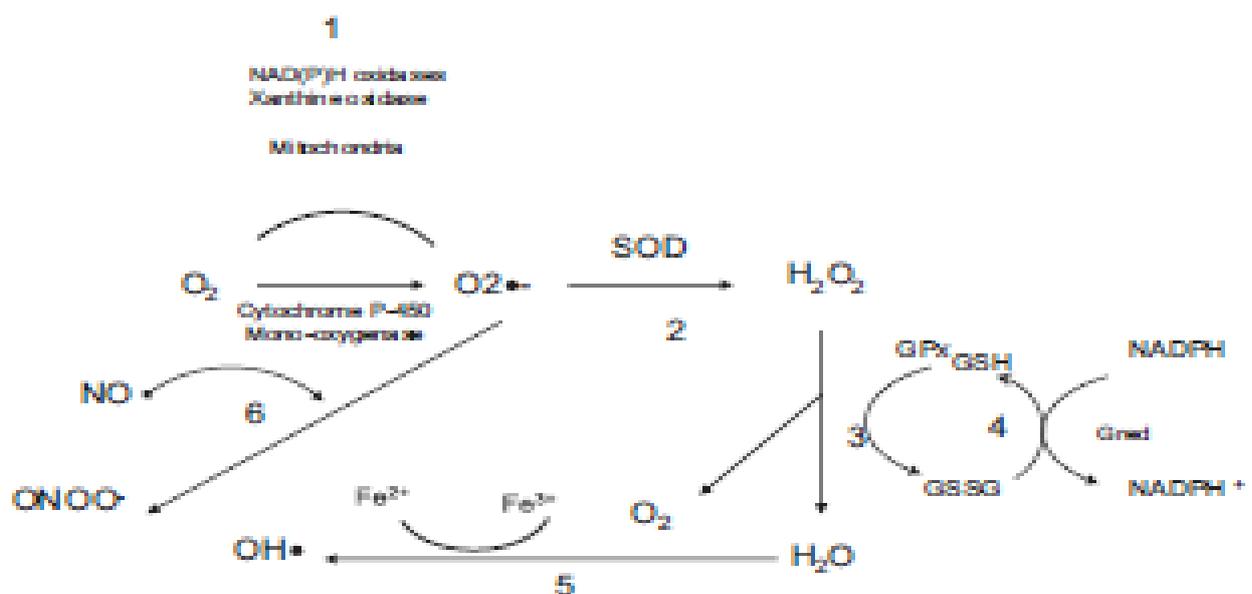


Figure 1.1: Pathways of ROS formation. Reaction 1: the superoxide anion radical (O_2^-) is formed by the process of reduction of molecular oxygen mediated by NAD(P)H oxidases, xanthine oxidase. Reaction 2: superoxide radical is dismuted by the superoxide dismutase (SOD) to hydrogen peroxide (H_2O_2). Reaction 3: Hydrogen peroxide is most efficiently scavenged by the enzyme Glutathione Peroxidase (GPx) which requires glutathione (GSH) as the electron donor. Reaction 4: the oxidized glutathione (GSSG) is reduced back to GSH by the enzyme glutathione peroxidase (Gred) which uses NADPH as the electron donor. Reaction 5: some transition metals (e.g. Fe^{2+}) can breakdown hydrogen peroxide to reactive hydroxyl radical (Fenton reaction). Reaction 6: the superoxide anion radical can combine with nitric oxide (NO) to produce peroxynitrite ($ONOO^-$) (Filaire and Toumi, 2012).

1.3.2 Catalase (CAT) and peroxidases (POX)

Catalase (CAT) and *peroxidase* (POX) catalyze the reduction of H_2O_2 to molecular O_2 and H_2O which are used by plants in metabolic reaction and photosynthesis (Penter, 1996; Tesfay, 2009; Torres, 2005). Many authors have reported these enzymes as being involved in many stress regulations caused by light, heat, drought, salinity, chemicals, chilling temperature and ozone (Torres, 2005). When plants are able to withstand/respond to harsh environmental conditions they become available for human health consumption and are able to preserve plant reserves for human health and postharvest.

1.3.3. Peroxidase (POD)

Peroxidases are a large family of enzymes that catalyze an electron donor reaction (oxidation reaction), they mainly peroxidase enzymes work on hydrogen peroxide substrate, which is a harmful by-product of biochemical reactions (Azevedo *et al.*, 2003). However, other peroxidases are more active with organic hydroperoxides such as lipid peroxides, there are plenty of these enzymes in plants and animals, humans included (Rusha *et al.*, 1985). They are well known for their important defensive role against plant pathogens (Karthikeyan *et al.*, 2005; Atamna and Boyle, 2006).

1.3.4 Phenolic compounds

Plant phenols are bioactive substances which form a significant part of our daily diet. They are mainly high in oily fruits and vegetables. They have high healing power due to their antioxidant, anti-tumoral, antiviral and antibiotic properties (Dai and Mumper, 2010). Phenolic compounds represent a large group of 'secondary plant metabolites' which play a critical role in plant environmental adaptation (Podsdek, 2005; Chitindingu *et al.*, 2006; Silva *et al.*, 2006; Apak *et al.*, 2007). Plant phenols help in plant defense against pest, diseases and predators (herbivores). Their metabolic origin is via the pentose phosphate, shikimate pathway and phenylpropanoid metabolism (Eghadami and Sadeghi, 2010), following the phosphoenolpyruvate → phenylalanine → cinnamate → 4-coumarate course, leading to chalcone, flavanone, dihydroflavonol, and anthocyanin (Apak *et al.*, 2007) (Figure 1.2).

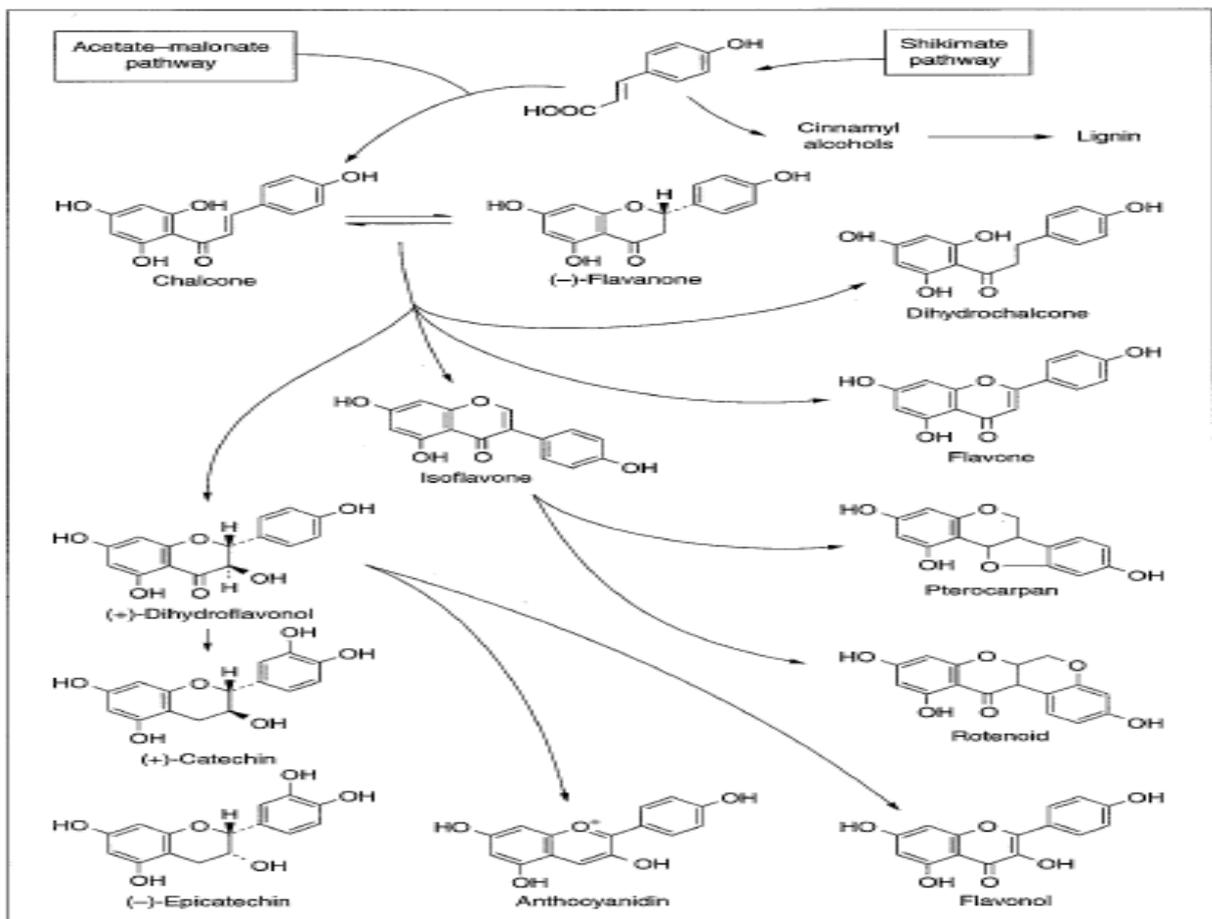


Figure 1.2: The shikimate and acetate-malonate pathways. The inter-relationship between flavonoid classes and principal biosynthesis pathways of antioxidant property compounds. (Rice-Evans *et al.*, 1997).

Phenolics are hydroxylated compounds with at least one aromatic ring with one or more attached –OH groups, both in free and membrane bound state (fig 1.3). Free phenolics are more desirable as they increase the antioxidant property of the phenols in a host (Apak *et al.*, 2007; Tesfay, 2009).

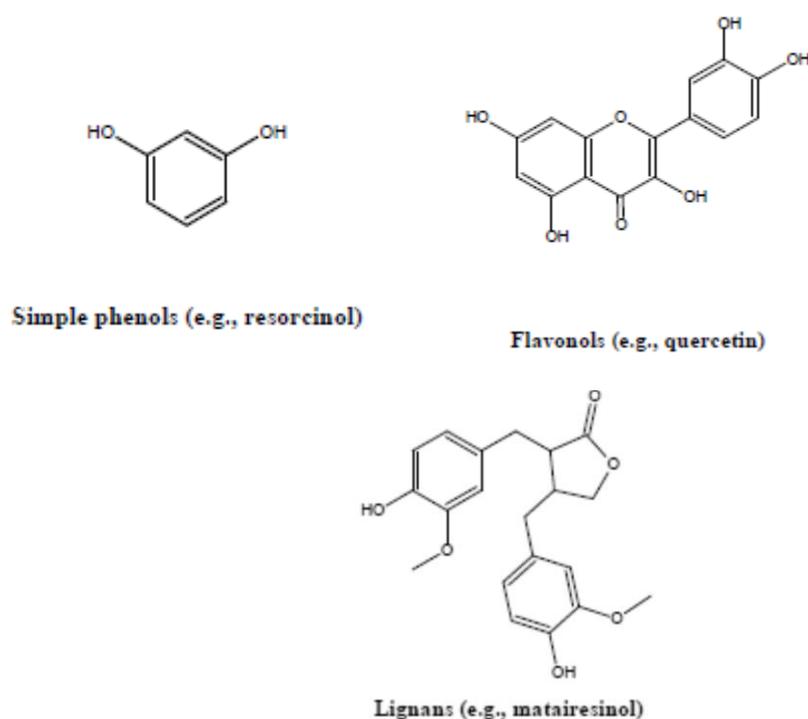


Figure 1.3: Basic phenolic compound structures - resorcinol; quercetin; matairesinol Apak *et al.*, (2007).

1.3.5 Ascorbic acid (Vitamin C)

Ascorbic acid (l-ascorbic acid; AsA), extensively known as Vitamin C, is a plant metabolite of multiple functions in both plants and animals (Pastori *et al.*, 2003), reduces ROS cell and membrane damage - regenerates Vitamin E – (α - tocopherol) from tocopheroxyl radical (Filaire and Toumi 2012; Heaney *et al.*, 2008). It is the main ROS detoxifying compound in aqueous phase as it can donate electrons to both enzymatic and non-enzymatic reactions. Fruits and vegetables provide approximately 85% of Vitamin C to human diet (Podsdek, 2005)

Plants synthesize Vitamin C as a result of response to environmental stress including photooxidation (Torres, 2005) where it acts as a reductant of plant tissue free radicals thus reducing oxidative cell membrane damage (Fuchs 1998; Torres, 2005).

1.3.6 Carotenoids

Carotenoids are organic plant pigments that are found in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms, including some bacteria and some fungi (Mortensen, 2006). Carotenoids beings are hydrocarbons, beta-carotene, alpha-carotene andlycopene and the xanthophylls, or oxygen-containing carotenoids, beta-cryptoxanthin, lutein and zeaxanthin (Krinsky and Johnson, 2005). Animals are unable to synthesise these metabolites thus they are only accessible through consumption of plant material (Fraser and Bramley, 2004). Sources are a range of fruits and vegetables, however mostly its tomato and tomato products that contain high quantities of carotenoids mostly lycopene (Johnson, 2002). Fruits and vegetables that contain high quantities of carotenoids have been associated with decreasing the risk of various age related diseases mostly cancer and site (eye) diseases (Krinsky and Johnson, 2005).

Carotenoids possess antioxidant and prooxidant properties. Carotenoids are found to increase with abiotic and biotic stress, to help protect cells against bruising and mechanical damage of cells from ROS that arise from plant stress, this mechanism of plant cell protection is assumed to be the same with animal's cells when the plants have been consumed (Atkinson *et al.*, 2011).

1.4 Carbohydrates

Carbohydrates are plant metabolites that act as an energy source; for structural components and other functions for plant growth and metabolism (White 1973). They are large biological molecules consisting of carbon (C), hydrogen (H), and oxygen (O) atoms, usually with hydrogen: oxygen atom ratio of 2:1. Carbohydrates containing six carbon molecules are referred to as monosaccharide which are simple sugars, glucose (C₆H₁₂O₈) being a common monosaccharide (Tharanathan, 2002).

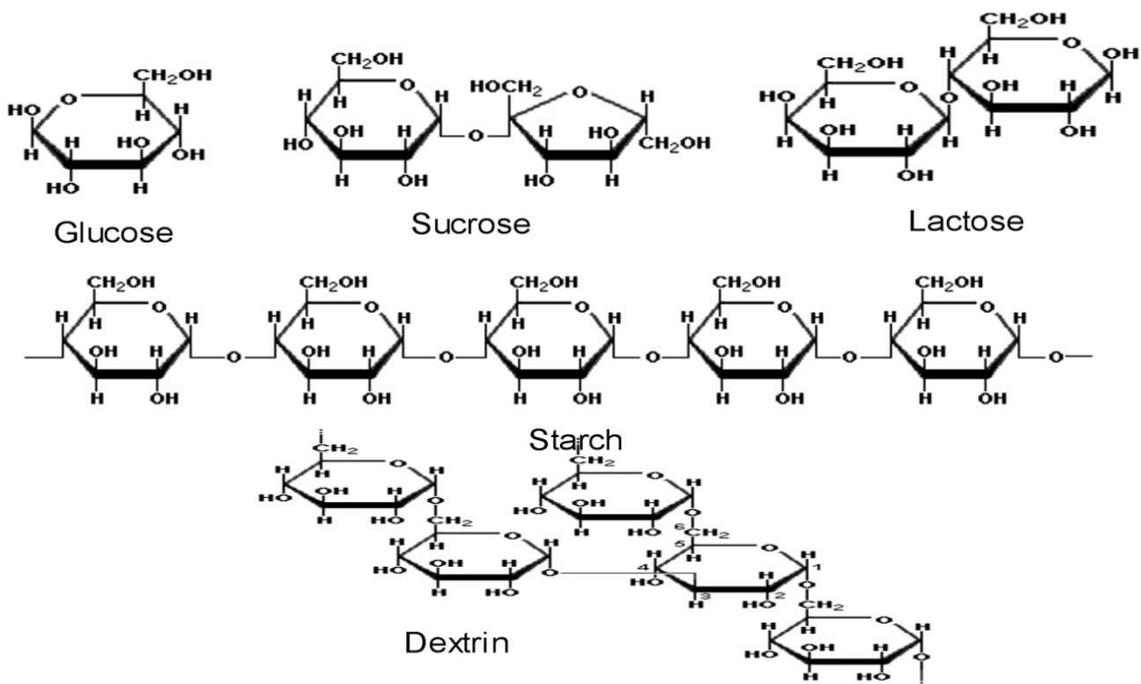


Figure 1.4: Carbohydrates molecules, from simple sugars (glucose, sucrose, lactose) to complex sugars (starch). R-ose is a suffix used to refer to simple sugars when naming sugars according to their chemical and structural makeup (FAO 1997).

Polysaccharides mainly act as storage and structure molecules while simple sugars act as energy suppliers. Grains mainly contain starch while fruits and vegetables contain sugars. Carbohydrates also determine the antioxidants production capacity of the plant. High antioxidant production capacity results in increased cell and cell membrane protection ability. Carbohydrates have been found to increase with increases in abiotic stress. Stress thus ensuring higher membrane protection and enzyme activity as a result of the increased sugar production. This process enables plant survival under harsh environmental conditions and continuous supply of carbohydrates to humans. This cell protection mechanism and energy supply by a plant are assumed to be similar for humans after consuming such plants thus leading to a healthier diet for humans (Morsy *et al.*, 2005; Rosa *et al.*, 2009; Palafox-Carlos *et al.*, 2011).

1.5 Information on nutritional components of the studied vegetables

1.5.1 Green cabbage

Green cabbage (*Brassica oleracea* or variants) is a green leafy biennial vegetable, but grown as an annual vegetable due to its high density leaves. It is a major crop in South Africa where most of it is produced in Kwa-Zulu Natal. It can grow in diverse climatic regions of diverse climatic conditions and in most of adaptable regions it can be grown throughout the year. The optimum temperatures for growth and development are between 180 and 200 °C. It has ability to withstand frost and cold temperatures up to - 30 °C. It can grow in a variety of soil types ranging from sandy to clayey soils but best prefers well drained, moisture-retentive fertile loamy soils, with pH ranging between 5.5 and 6.5. Soils with pH greater than 6.5 causes leaf margin burn and dark coloured leaves while below 4.5 causes restricted growth and development (DAFF, 2013). Like many other crops consumed today, cabbage was thought of being a weed for many years, but today it is highly valued by consumers and farmers as it is a rich source of nutrients for consumers and high value crop for farmers. A change in nutrient content of raw cabbage against cooked cabbage was reported by Rouzaud et al., (2004), which supports other studies done by Miccozi *et al.* (1985); Amin *et al.*, (2004); Kala & Prakash (2004).

The cabbage is rich source of beta-carotene, Vitamin C and fibre and also shows good characteristics in preventing cancer due to presence of glucosinolates (Steinmetz and Potter 2001; Song and Thornalley 2006). While rich in the mentioned nutrients it also contains several other nutrients like Vitamin E, proteins, iron, and sodium (Nolte, 2013).

1.5.2 Red cabbage

Red cabbage (*Brassica oleracea var. capitata f. rubra*) is of high nutritional value as it is rich in minerals, vitamins, oligosaccharides, and a number of bioactive polyphenols, such as anthocyanins, flavonols, and glucosinolates, which have a positive impact on human health (Volden *et al.*, 2008; Wiczowski *et al.*, 2012). Red cabbage is also valued by consumers for its taste and for being a source of an intensive red colour which increases esthetic value of the food. For these reasons, red cabbage is a frequently consumed vegetable in the form of fresh-cut salads. In addition, red cabbage is characterized by high shelf-life; therefore it can be easily stored and accessible in a fresh form for a long period (Wiczowski *et al.*, 2012).

Although it is called red cabbage and known for having purple to red leaves, its colour is mainly depended on the acidity of the soil, when the soil is acidic the leaves appear dark red to purple, while in a neutral solution they become blue and yellowish green in basic (alkaline) solution (Halmenstine, 2013; Nolte, 2013). Red cabbage also has a longer shelf life than green cabbage (Nolte, 2013). Leaf colour is usually associated with anthocyanin content which is a group of flavonoids mainly related to shelf life and ROS scavenging from their antioxidant properties (Wiczowski *et al.*, 2012). Anthocyanin are composed of anthocyanidins and sugars, and sugars act as nutrient reserve for the crop postharvest, therefore higher anthocyanin content may result in longer storage life (He and Giusti, 2010). Other than these benefits, cabbages of red leaf colour have a variety of phytochemicals contents which are beneficial to human health.

Red cabbage has a rich variety of nutrients which are positive to human health, e.g. vitamin A and C, Iron, fiber, phenols and carotenoids but low in sodium (Podsędek, 2007, Wiczowski *et al.*, 2012, Nolte, 2013). The most abundantly available compound anthocyanins are often associated with the red colour of the crop. The crop requires air temperatures of approximately 25 °C for optimum growth and 18 °C to 25 °C of soil temperature for vigorous seed germination. Growth ceases at temperatures below 18 °C.

Due to its richness in nutrients it is widely used and it is of high value (nutritionally and monetary), but it is somewhat expensive for the poor, thus inaccessible, therefore does not address food and nutrition insecurity. Easily accessible, cheap, readily available and nutritious food products are required. *Amaranthus* is discussed as an ALV that can be used in malnutrition mitigation.

1.5.3 *Amaranthus*

Amaranth (*Amaranthus hybridus*) is known as *misbredie*, *hanekam*, *varkbossie* in Afrikaans, pigweed, cockscomb and hell's curse in English, *unomdlomboyi*, *imbuya*, *umifino umtyuthu* in isiXhosa, *imbuya*, *isheke*, *indwabaza* in isiZulu, *thepe*, *theepe* in IsiPedi, Sesotho and Setswana, *umbuya*, *isheke* in siSwati, *vowa*, *theebe* in Tshivenda, *theyke*, *cheke* in Xitsonga, *mohwa* in Shona and *imbuya*, *tyutu* in Pondo (Van Wyk and Gericke, 2000; Vorster *et al.*, 2002; Jansen van Rensburg *et al.*, 2007).

Amaranthus thunbergii (L), *A. greazicans*, (L), *A. spinosus* (L), *A. deflexus* (L), *A. hypochondriacus* (L), *A. viridus* (L) and *A. hybridus* (L) are among the most widely used

amaranth species in South Africa (Schippers, 2000; Van Wyk and Gericke, 2000; Vorster *et al.*, 2002; Hart and Vorster, 2006; Jansen van Rensburg *et al.*, 2007), Amaranth belongs to the Amaranthaceae family (Jansen van Rensburg *et al.*, 2007). The young leaves, growth points and *seedlings* are harvested and cooked for human consumption.

Cooked/processed amaranth leaves are a good source of vitamin A, vitamin C, and folate. They are a supplementary source of vitamins such as thiamine, niacin, and riboflavin plus dietary minerals such as calcium, iron, potassium, zinc, copper, and manganese. Cooked amaranth grains are also a complementing source of thiamine, niacin, riboflavin, and folate, and dietary minerals comprising of calcium, iron, magnesium, phosphorus, zinc, copper, and manganese - comparable to common grains such as wheat, oats and others (USDA, 2011).

Amaranth may be a promising source of protein as its protein does not contain gluten like protein found in grains such as wheat and rye (Gruss, 2014). Amaranth nutrient content is comparable with that of gluten-free vegetables and grains such as buckwheat, corn, millet, wild rice, oats and quinoa (Gallagher *et al.*, 2004).

Amaranth seeds contain lysine, an essential amino acid, which is limited in other grains; however, amaranth is also limited in some essential amino acids, such as leucine and threonine (Bressani *et al.*, 1989). Amaranth seeds are therefore a complementary source of essential amino acids which are abundant in other grains such as wheat germ, oats, and corn (Písaíková *et al.*, 2006).

Several studies have shown that like oats, amaranth seed oil may be of benefit for those suffering from hypertension and cardiovascular disease; regular consumption reduces blood pressure and cholesterol levels, while improving antioxidant prominence and some immune parameters (Czerwiński *et al.*, 2004; Martirosyan *et al.*, 2007). While the active ingredient in oats appears to be water-soluble fiber, amaranth appears to lower cholesterol via its content of plant stanols and squalene (Czerwiński *et al.*, 2004).

Amaranth is known as an extremely variable, erect spreading herb with a height at maturity varying between 0.3 m and 2 m, depending on the species, growth habit and environment (Jansen van Rensburg *et al.*, 2007; DAFF, 2010), while its colour is mostly green but they all have a maroon or crimson colour in their stem and leaves (DAFF, 2010). Amaranth seeds need soil temperatures of between 18 °C and 25 °C to germinate and an air temperature above 25 °C for optimum growth. The growth stops at temperatures below 18 °C. It grows well under warm conditions, with drought and adverse condition tolerance however these adverse condition and drought situations induce flowering and low yield (Jansen van Rensburg *et al.*, 2007).

Amaranth is seldomly planted in South Africa, as it is believed that it will grow naturally, although some parts of the country do plant and harvest amaranth, (Jansen van Rensburg *et al*, 2007). This crop has potential of commercialization as seen to have minimum management requirements and that it is under-utilized, this opens a window as a ‘niche’ market for farmers. Table 1.1 shows potential planting and harvesting dates published by the South African Department of Agriculture Forestry and Fisheries (2010).

Table 1.1: Amaranth production schedule for a year

Months	Jan	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Planting												
Harvesting												

Adapted: DAFF, 2010

This schedule can be manipulated in commercial agriculture by use of greenhouses, and genetic breeding for other cultivars.

Amaranth remains an active area of scientific research for both human nutritional needs and foraging applications therefore this study aims to explore and contrast the seed and plant nutritional content of red cabbage and amaranth plant.

The study objectives were:

- ❖ To explore the seed quality status of *Amaranthus hybridus spp.* with a preliminary seed study
- ❖ To compare and contrast the phytonutrient contents of *A. hybridus* with *Brassica oleracea* and *Brassica oleracea var. capitata f. rubra* at different growth stages
- ❖ To compare and contrast the enzymatic activity based on protein content of the studied vegetables at different growth stages

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CHAPTER 2

Amaranth (*Amaranthus hybridus*) seed characterization and Seed quality

2.1 Abstract

The aim was to investigate the effect of Amaranth (*Amaranthus hybridus*) seed colour on quality status of the seeds, including standard germination percentage, seed weight, germination velocity index (GVI), mean germination time (MGT) and electrolyte leakages (EC) and antioxidant content. The dark red seeds showed higher germination percentage (79.3%), lower seed weight (0.104 g after 6 d), higher MGT (5.87 days), higher GVI (91.89), higher EC (13949.15 $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$ in 24 h), higher total antioxidant content (2.75 mmol $\text{Fe(3)SO(4)100g}^{-1}$) and radical scavenging percentage (73.20%). while white gold coloured seeds recorded lower germination percentage (50.67%), higher seed weight (0.313 g after 6 d), Lower MGT (4.82 days), lower GVI (53.83), lower EC (9170.83 $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$ in 24 h), lower total antioxidant content (0.962 mmol $\text{Fe(3)SO(4)100g}^{-1}$) and radical scavenging percentage (46.30%). Overall, based on the results red colour seeds are of higher quality than white gold coloured seeds.

2.2 Introduction

The need to increase and improve food production and accurate supply remain an immense issue around the world; this is usually referred to as “food security” (Basra, 2011). Increasing food production through use of high quality seed among other inputs is crucial for providing adequate food for rising population throughout the world (Basra, 2006). Use of quality seeds is very important in crop production as seeds are a primary agricultural input which all other agricultural inputs are dependent on for success (Basra, 2011). The higher the seed quality the better food quality and quantity, thus minimized/eradicated food insecurity (Basra, 2011). Seed quality main characteristics are genetical quality, healthy (free from seed borne diseases), purity, seed weed content, moisture, grain volume and weight volume (Kjaer, 1961). According to Ellias (2006), seed quality is a collective term for genetic

homogeneity, physical appearance, viability, vigour and uniform seed conditions. To a farmer seed quality translates to high and uniform yield capacity under a wide range of environmental field conditions. Good quality seeds result in low sowing rate, high crop emergence, reduced replanting, vigorous early growth which gives a competitive advantage towards harsh early growing conditions like weeds, insects and drought (FAO, 2003; Department of Agriculture, Government of Puducherry, 2012).

In general seed quality characteristics are mainly vigour and viability. Seed viability is the ability of the embryo to germinate. Viability test is used to determine which seed tissues are alive and have a potential to germinate under optimum conditions (Angelovici *et al.*, 2010; Maile, 2013). The test uses a chemical called tetrazolium (TZ), a colourless chemical that reacts with cells and paint them red, it only detects tissues that are alive or viable (Maile, 2013). Seed vigour is the ability of a seed to perform under different environmental conditions mostly focusing on unfavorable conditions (Ferguson and Keys, 1914; Tekrony and Egli 1991; Maile, 2013), and it is classified as the closest test/measure of seed potential field performance opposed to laboratory seed germination, where percentage germination is performed under seed optimal condition (Ferguson and Keys, 1914; Maile, 2013). Vigour test is species specific a wide range of seed tests are available, but most species use the rapid tetrazolium seed viability and vigour test (Maile, 2013). However, traditional leafy vegetables are mostly landraces with no hybrids while amaranth (*Amaranthus hybridus*) is believed to be a natural hybrid with no pure line breed. Most traditional leafy vegetables are also believed to have dormant seeds. Etèka *et al.*, (2010) found seeds of African traditional leafy vegetables, *S. radiatum* and *C. sesamoides* to have as low as 15% germination rate. Rathi *et al.*, (2011) used Quantitative Trait Loci (QTL) to measure dormancy in indigenous rice of Assam, India. Traditional African leafy vegetables have dearth of information on the seed quality which is one of the key information required in plant production. Amaranthus is one of the most widely growing traditional leafy vegetable at household level throughout Africa and South Africa inclusive (Jansen van Rensburg *et al.*, 2004; Modi, 2007). Modi (2007) reported on *Amaranthus spp.* seed germination under different temperatures, where temperature and seed age influenced germination significantly depending on the sub-specie.

Phenotypic make-up of the seed which is affected by genetic make-up also influences seed quality, for example seed coat colour has been found to influence Bambara seed

germination by Sinefu (2011) where brown seeds coat colour exhibited better germination capacity than white coloured seed. Odindo (2007) found that varying only seed coat colour in cowpea had significant effect on seed quality, germination inclusive. Phenotypic traits like seed size, seed coat thickness and mass have a significant effect on seed quality.

However, report on seed quality of traditional leafy vegetables is scarce. The study was then conducted to add to scientific and general knowledge of seed quality of *Amaranthus spp.* This would also add seed quality information of *Amaranthus hybridus* available for farming, breeding and other scientific and/or general needs.

2.3 Materials and Methods

2.3.1 Seed material

The *Amaranthus hybridus* seeds were collected from Ukulinga, University of Kwa-Zulu Natal (UKZN) research farm located 30° 24' S, 29° 24' E and were also donated by the University of Free State, South Africa.

2.3.2 Chemicals

Chemicals used for antioxidant analysis were purchased either from Sigma-Aldrich®, Saarchem® or Fluka®.

2.3.3 Germination

Red and white gold coloured *Amaranthus hybridus* seeds were used for germination capability test. A seed testing water bath (Copenhagen Seed-Testing Bath, coupled with Grant FH60 circulation system) was used to germinate *Amaratnus hybridus* seeds where 100 seeds weighing 0.027 g were placed on a whatman paper no. 1 on the germination table (CT/FH60). Temperature fluctuations on the table were in the range of 17.3 to 26.5°C. A destructive sampling of germinating seeds was used to measure germinated seeds, daily

(24hrs) count and weighing, to check the germination percentage (number) and imbibition process. There were three replicates (100 seeds per germination paper) per time treatment.

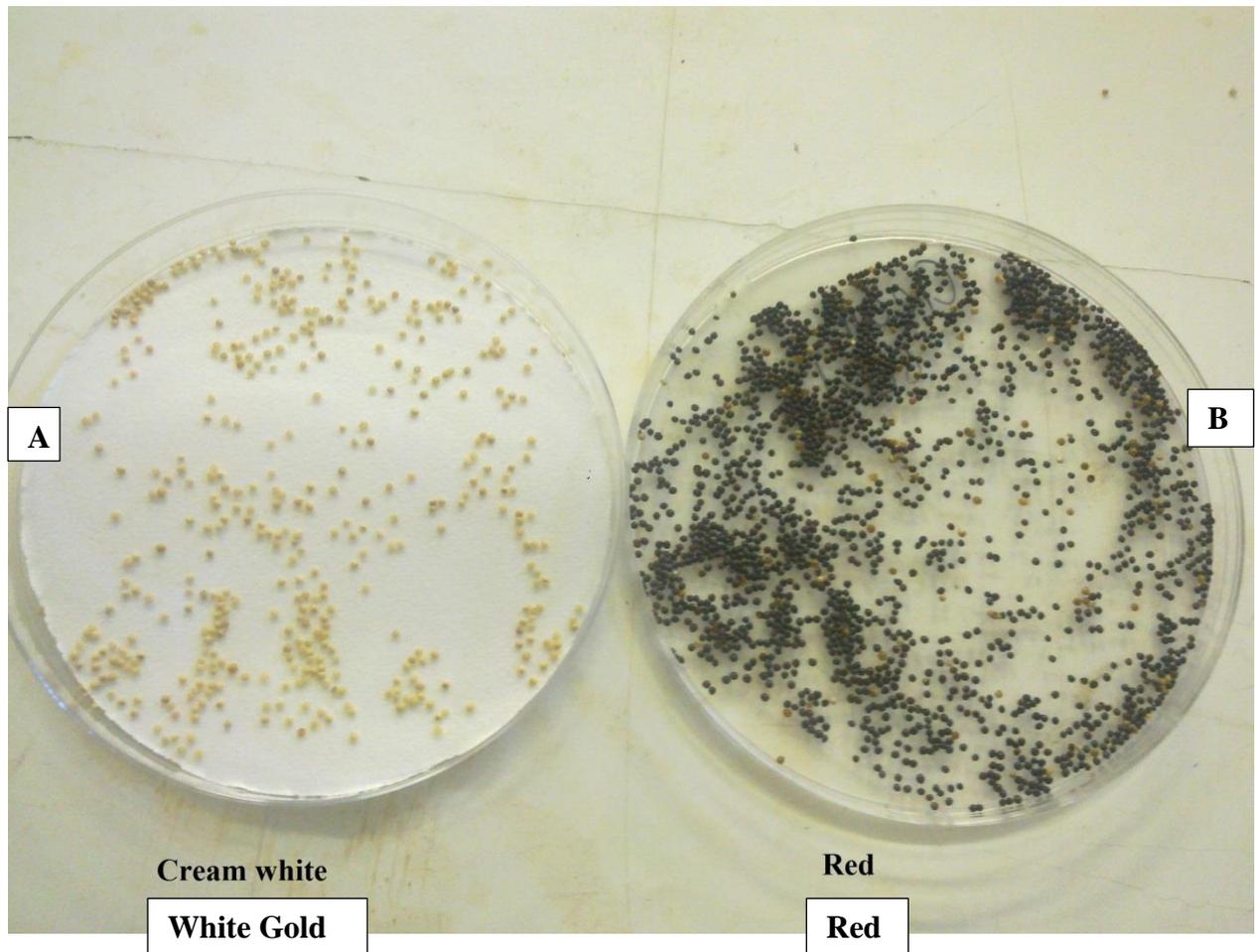


FIGURE 2.6: Seed of *A. hybridus* different coloured seeds A- White Gold and B- Red

Germination velocity index (GVI) was also measured and it indicates the speed of germination and was calculated using the formulae by Maguire (1962).

$$\text{GVI} = \text{G1}/\text{N1} + \text{G2}/\text{N2} + \dots + \text{Gn} / \text{Nn} \quad \text{Equation 2. 1}$$

Where:

GVI = germination velocity index

G1, G2...Gn = number of germinated seeds in first, second... last count.

N1, N2...Nn = number of sowing days at the first, second... last count.

For further analysis Mean Time to Germination (MGT) was also calculated according using the formulae by Ellis and Roberts (1981):

$$\text{MGT} = \frac{\sum Dn}{\sum n}$$

Equation 2. 2

Where:

MGT = mean germination time,

n = the number of seed which were germinated on day D, and

D = number of days counted from the beginning of germination.

2.3.4. Electrolyte leakage (EC)

Bulk EC was measured using CM 100-2 conductivity meter (Reid Associates CC, South Africa), 10 dark red seeds weighing 2.8 mg and cream white seeds weighing 6.3 mg were placed in CM 100 tray wells and 1 ml of distilled water was added. After 24 hours readings were taken and analysed (EC reading).



FIGURE 2.2: Seed analysis EC meter (CM 100-2)

2.3.5 Antioxidants assays

Anti-oxidant levels were first determined as “total anti-oxidant capacity” (TAOC) using the FRAP (ferric reducing ability of plasma) assay (Benzie and Strain, 1996) and expressed as $\mu\text{mol FeSO}_4\cdot 7\text{H}_2\text{O g}^{-1}$ DW equivalents. Second, total anti-oxidant activity (TAOA) was also determined using the DPPH assay according to Leong and Shui (2002), in order to gauge the presence of hydrophilic as well as lipophilic anti-oxidants. Briefly, an aliquot (40 μl) of an extract was added to 3 ml of methanolic DPPH solution. The change in absorbance at 515 nm was measured at 30 min. The antioxidant capacity based on the DPPH free radical scavenging ability of the extract was expressed as $\mu\text{mol Trolox}$ equivalents per gram of plant material on dry basis.

2.3.6 Statistical analysis

Statistical Analysis was performed using GenStat (version 17.0; VSN International, Hemel Hempstead, UK). Standard deviation values were calculated and differences among treatments were separated by the least significant difference (LSD) at $P= 0.05$.

2.4 Result and Discussion

2.4.1 Standard Germination

Seeds usually germinate due to being exposed to moisture and a warm environment. Therefore, amaranth seeds begin germination like any other seed, by swelling due to water uptake during imbibition, followed by radicle emergence from moistened and ruptured seed coat, which is related to seed germination.

There were significant differences ($P<0.001$) in seed germination of the two seed colours, with the red coloured seed coat having higher germination percentage than white gold coloured seeds throughout the germination period. The germination percentage of red and white gold colour seeds of *Amaranthus hybridus* showed increasing trend overtime. Red

colour seeds had a final average germination percentage of 79.3%, while white gold coloured seeds had a final germination percentage of 52.3%. Both *Amaranthus hybridus* seeds types (colour) showed expansion after 24 hours, which is a sign of imbibition and radicle emergence potential, then on the second day that is when seed germination (radicle emergence) was noticed which was at an average of 40.0% on red seeds and 13.3 % for the white gold seeds. Germination increased rapidly during the early hours of seed imbibition period for both seed types. Red coloured seeds showed higher germination percentage throughout the germination period, they both started germination after 48 hours. Results are in agreement with Odindo (2007) and Mavi (2010) who both reported that dark coloured seeds of cow pea and watermelon crimson sweet, have higher germination percentage than light coloured seeds respectively. The germination percentage can also be correlated with seed weight, as seed needs to imbibe water to germinate (produce radicles). The germination results obtained in this study show that *Amaranthus hybridus* has high vigour under normal laboratory conditions as radicle emergence can be associated with seed vigour and viability.

Amaranth of dark red coloured seeds had a final germination velocity index (GVI) of 91.89 and mean germination time (MGT) of 5.87 whereas the white coloured seeds had GVI of 53.83 and MGT of 4.82. These results show that red coloured seeds have higher GVI than white coloured seeds, high GVI suggests that quicker root:shoot ration, this can be associated with a study by Sinefu (2011). The low MGT shows that *Amaranthus hybridus* white coloured seeds can take fewer days to reach their full germination potential than red coloured seeds. It was also observed that there was a strong correlation ($r=0.72$) among GVI, MGT and germination percentage.

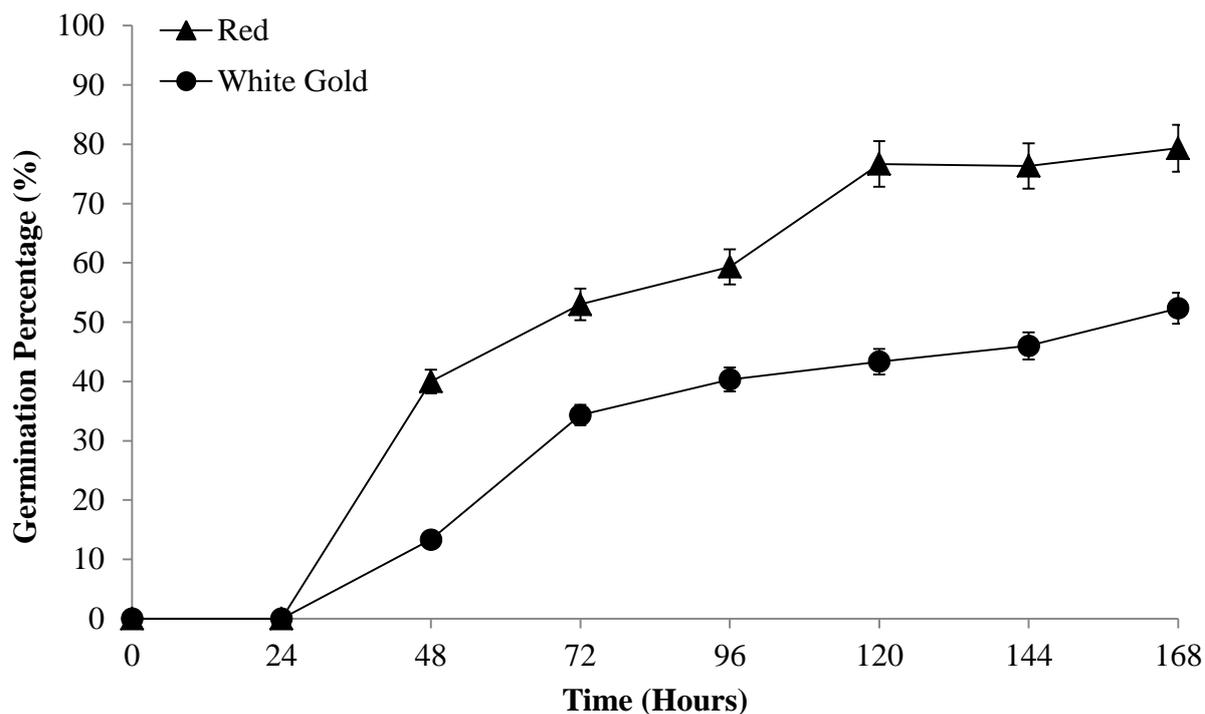


FIGURE 2.7: Daily germination percentage of Amaranthus (*Amaranthus hybridus* spp.) over 168 hours. Vertical bars represent \pm SE. (n=5).

2.4.2 Seed weight

Seed weight increased over time as the seed imbibed water through its semi-permeable membrane. The increased seed weight linearly correlated with the germination percentage as this showed radicle emergence. There were significant differences ($P < 0.001$) in seed weight between the two coloured seeds, result in different stages of radicle emergence. The weight gain of the seeds is due to radicle emergence and water imbibition. The higher seed weight of white gold coloured seeds might be due to higher seed moisture content, but this seems not to affect germination as both colour seeds started germination on the same day. The study further included electrolyte leakage which also showed differences in seed vigour.

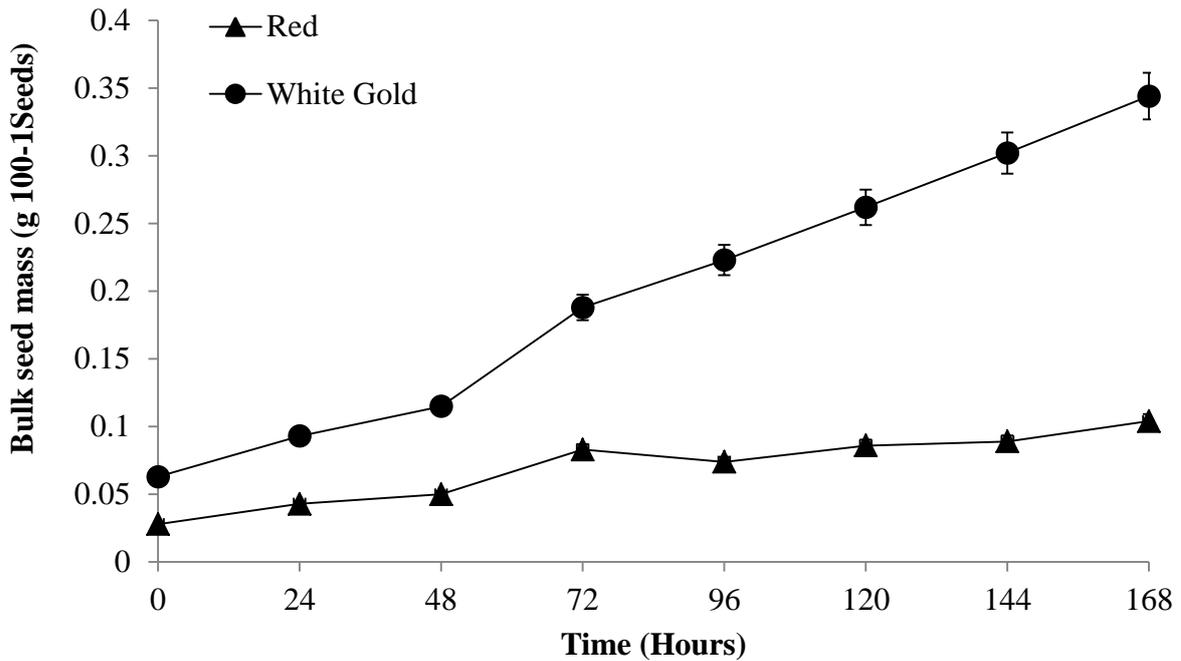


FIGURE 2.8: Daily seed weight of red and white gold *Amaranthus* (*Amaranthus hybridus* spp.) over 168 hours. Vertical bars represent \pm SE. (n=5).

2.4.3 Electrolyte leakage

Electrolyte leakage (EC reading) measures the amount of solutes released by the seeds. High EC shows low seed vigour and low EC shows high vigour (Ramos *et al.*, 2012). According to EC readings red coloured seeds had low vigour and white had high vigour. Electrolyte leakages increase with membrane deterioration, mitochondrial changes, chromosomal aberrations and free radicals in seed lots. As seed deterioration increases, cellular membranes lose their selective permeability, permitting the cytoplasmic metabolites to leach into the intercellular spaces. Membrane degradation occurs from both hydrolysis of phospholipids by phospholipase and phospholipids oxidation (Mavi, 2010).

There were significant differences ($P < 0.001$) in electrolyte leakage of the two coloured seeds, red coloured seeds had a significantly higher EC than light coloured seeds throughout the study period. This result is supported by Odindo's (2007) findings on cowpea seeds; dark coloured seeds had highest solute leakage compared to light coloured seeds and

on contrary findings were reported by Mavi (2010) on watermelon crimson sweet, when brown seeds showed lower EC readings than light yellow seeds.

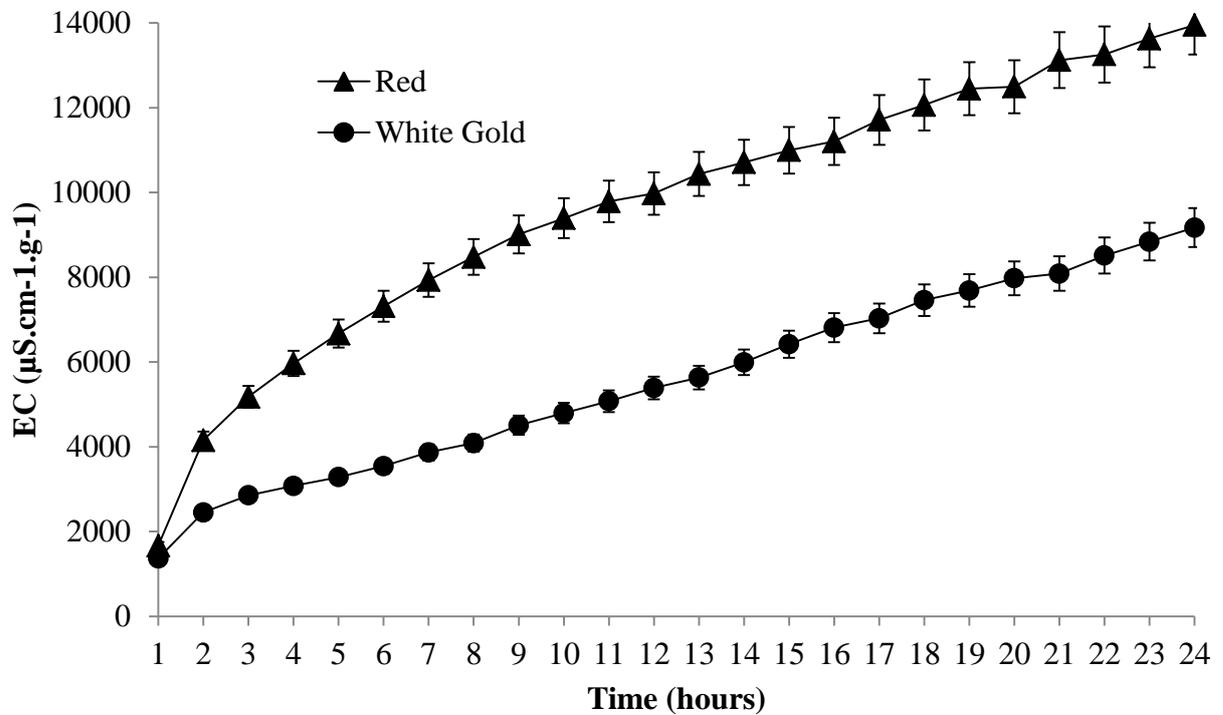


FIGURE 2.9: Electrolyte leakage (EC) of Amaranthus (*Amaranthus hybridus* spp.) over 24 hours. Vertical bars represent \pm SE. (n=5).

Electrolyte leakages increase when seeds are subjected to deterioration and seed maturation (Ramos *et al.*, 2012). However, maturation can also be associated with increased seed solute contents (Odindo, 2007; Salinas *et al.*, 2010). Therefore, higher electrolyte leakage from red coloured seeds may be due to higher seed solute contents than white gold coloured seeds.

2.4.4 Total antioxidants

There were significant differences ($P < 0.001$) in the total antioxidant content of the two different coloured seeds. Red coloured seeds showed higher total antioxidant content;

this could be due to higher anthocyanin content on the red coloured seeds. This supports EC readings on these seeds, where red coloured seeds showed higher electrolyte leakages which were assumed to be from higher total soluble solutes contents on red coloured seeds.

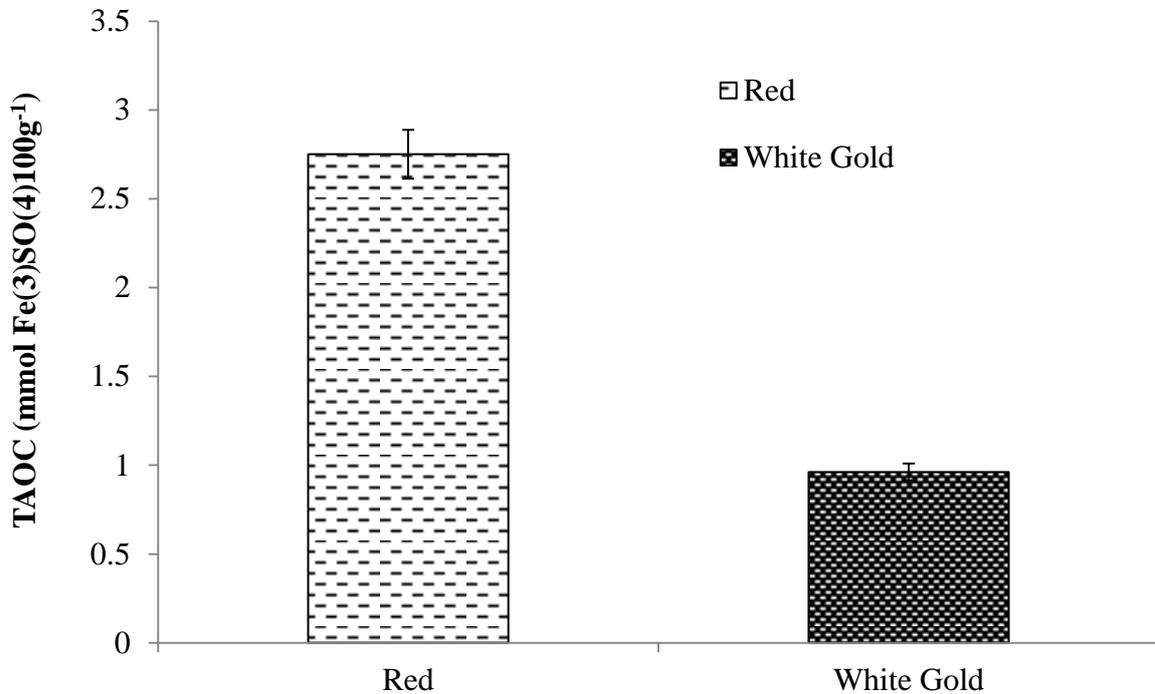


FIGURE 2.6: Total Antioxidants content of different coloured Amaranthus (*Amaranthus hybridus spp.*) seeds. Vertical bars represent \pm SE. (n=5).

There were also significant differences ($P < 0.001$) in radical scavenging percentages of the differently coloured seeds. Red coloured seeds showed higher radical scavenging percentage which is in line with the expectation as red coloured seeds had higher total antioxidant content. These findings are in agreement with the findings of Thaipong et al., (2006) on different guava fruits genotypes, in which high antioxidant content resulted in high antioxidant activity/ radical scavenging ability.

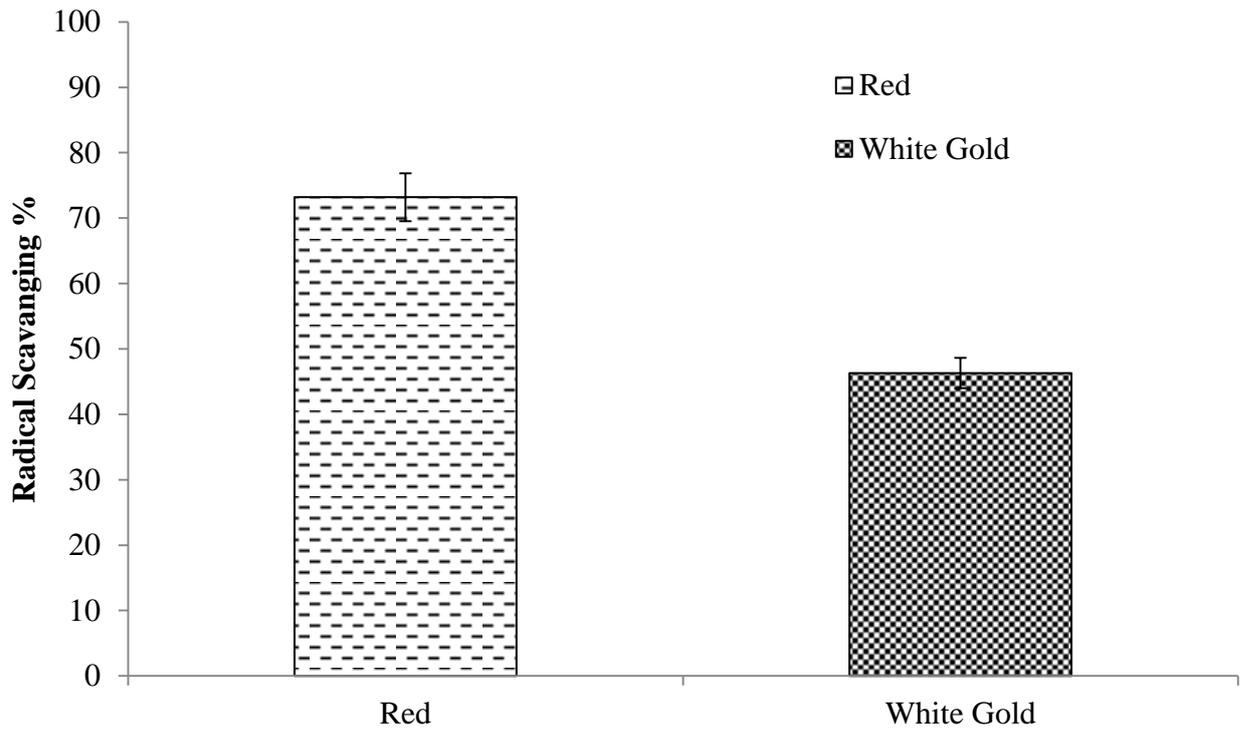


FIGURE 2.7: Radical scavenging activity of different coloured *Amaranthus (Amaranthus hybridus spp.)* seed. Vertical bars represent \pm SE. (n=5).

2.5 Conclusion

Electrolyte leakage and antioxidant content show that red seeds are of higher quality in terms of total solute contents which are required during seed germination; this could also be used for better seed germination, emergence and plant stand of high quality and nutrition.

This investigation demonstrated that red coloured seeds have higher seed quality than white gold coloured seeds. Higher seed viability and low vigour was showed by red coloured seeds while white coloured seeds have high vigour and low viability. Red coloured seeds were preferred for post studies as they showed better quality traits.

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CHAPTER 3

Comparative chemical composition of *Amaranthus* (*Amaranthus hybridus*) and Green and Red Cabbage (*Brassica oleracea* and *Brassica oleracea* var. *capitata f. rubra*)

3.1 Abstract

Fruits and vegetables are considered a health promoting food source due to their phytonutrient constituents. However, commercialized fruits and vegetables are mostly inaccessible to “poor” rural communities therefore there is a need for alternative indigenous food sources especially for alleviating nutrient deficiency problems. *Amaranthus Hybridus* leafy vegetable was tested for selected phytonutrient content during different growth stages and compared with *Brassica oleracea* and *Brassica oleracea* var. *capitata f. rubra*. Antioxidants (DPPH and FRAP assays), phenols, flavonoids, carotenoids and chlorophyll, protein, vitamin C and total soluble sugars were measured. Results obtained showed that *A.hybridus* contained comparable high phytonutrient contents. In conclusion, amaranth can be beneficial in mitigating reactive oxygen species (ROSs) and supplement the nutrient deficiency problems, while also aiding in mitigating neurotic chronic diseases faced by people of old age and those who have poor nutrition standards. A variety of nutrient sources/vegetables is preferred to singular as different sources/vegetables contain different types and levels of phytonutrients, thus balancing the daily nutrients requirement.

3.2 Introduction

Vegetables are important protective foods and highly beneficial in disease prevention and health maintenance. Therefore, their importance has gained increasing consumers’ attention due to the biochemical constitute of fruits and vegetables in association with human health and prevention or mitigating neurotic diseases. Several studies have indicated that a high intake of certain fruits and vegetables can be associated with a reduced risk of degenerative chronic diseases, such as cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration, and diabetes (Fang *et al.*, 2002; Nautiyal *et al.*, 2008; Nnamani *et al.*, 2009). However, antioxidants from fruits and vegetables are proven to work effectively when

in conjunction with other nutrients to reduce reactive oxygen species (ROS) (Trombino *et al.*, 2004), due to effective synergistic interaction than individual action (Nautiyal *et al.*, 2008).

Vitamin C and E are among the potent antioxidants which contribute significantly to protective effects of fruits and vegetables (Nautiyal *et al.*, 2008). Other than these constituents, many other phytochemicals are available for health benefits such as phenols, carotenoids, proteins and carbohydrates. However, these phytochemicals possess different antioxidant activity, but are collectively beneficial, for example glucosinolates possess low antioxidant activity than antioxidant vitamins, carotenoids and polyphenols but their hydrolysis product can protect against cancer incidences (Plumb *et al.*, 1996; Nautiyal *et al.*, 2006). Different vegetables have different levels of phytochemical constituents.

The phytochemical antioxidant system in plants is very complex, with different interactions and factors affecting their content (Palafox-Carlos, 2011). Variation in biochemical/ phytochemical contents can be due to a variety of factors: variety, growing condition, harvest maturity and postharvest handling (Finau, 2011).

Therefore this experiment was designed to assess comparative levels of plant phytochemicals that can be produced during different plant developmental stages. Accordingly different analytical assays were performed to determine plant phytochemical contents.

3.3 Materials and methods

3.3.1 Plant material

Amaranthus hybridus seeds were harvested from the University of Kwa-Zulu Natal farm, while some were donated by University of Free State, South Africa.

Red coloured *A.Hybridus* seeds were planted in seedling trays along with purchased *Brassica oleracea* and *Brassica oleracea var. capitata f. rubra* seeds (HYGROTECH®). Seedlings were transplanted after four weeks; the experimental design was: total of 80 seedlings per vegetable, 4 replications, 20 seedlings per replication; 80 pots containing a premixed growth medium were used. Samples were collected fortnightly for seven successive times, and then the samples ground into powder for further analysis.

3.3.2 Nutrient analysis

Leaf nutrients were analyzed by an independent laboratory at Cedara College of Agriculture in Hilton, just outside Pietermaritzburg town.

3.3.3 Antioxidants determination

Antioxidants were determined using two assays FRAP and DPPH.

Anti-oxidant levels were first determined as “total anti-oxidant capacity” (TAOC) using the FRAP (ferric reducing ability of plasma) assay (Benzie and Strain, 1996) and expressed as $\mu\text{mol FeSO}_4\cdot 7\text{H}_2\text{O g}^{-1}$ DW equivalents. Second, total anti-oxidant activity (TAOA) was also determined using the DPPH assay according to Leong and Shui (2002), in order to gauge the presence of hydrophilic as well as lipophilic anti-oxidants. Briefly, an aliquot (40 μl) of an extract was added to 3 ml of methanolic DPPH solution. The change in absorbance at 515 nm was measured at 30 min. The antioxidant capacity based on the DPPH free radical scavenging ability of the extract was expressed as $\mu\text{mol Trolox equivalents per gram}$ of plant material on dry basis.

3.3.7 Phenols

The total phenolic content of the extracts was determined according to Eghadami and Sadeghi, (2010) using the Folin- Ciocalteu reagent. The reaction mixture contained: 200 μl of sample extract, 800 μl of Folin-Ciocalteu’s phenol reagent and 2 ml of 7.5% sodium carbonate. The final mixture was diluted to 7 ml with deionized water. The reaction mixtures were kept in dark at ambient conditions for 2 h to complete the reaction. Absorbance at 765 nm was measured. Gallic acid was used as a standard for drawing a calibration curve and the results were expressed as mg gallic acid equivalent (GAE)/g sample dry weight.

3.3.8 Flavonoids

Total flavonoid content was determined according to eghadami and Sadeghi, (2010) using aluminium chloride (AlCl_3), using quercetin as a standard. The plant extract (0.1 ml)

was added to 0.3 ml distilled water followed by 5% NaNO₂ (0.03 ml). After 5 min at 25°C, AlCl₃ (0.03 ml, 10%) was added. After a further 5 min, the reaction mixture was treated with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with distilled water and absorbance was measured at 510 nm. The results were expressed as mg gallic acid equivalent (GAE)/g sample dry weight.

3.3.9 Carotenoids and chlorophyll concentration

Carotenoids and chlorophyll, the major pigments in the plant kingdom, were extracted and determined according to Lichtenthaler (1987). Freeze-dried, ground material (0.10 g d.wt) was placed into centrifuge tubes, 10ml of extracting solvent (100% Methanol) added, given 10 minutes stand time on ice covered with aluminum foil, homogenized with Ultra-Turrax using 1 minute burst twice and centrifuged at 2500 g for 5 minutes. The supernatant was decanted into 2-4 ml cuvettes and absorbance was read at various wavelengths (663.2 nm, 646.68 nm and 470 nm) as this is a simultaneous extraction of carotenoids and chlorophyll. Necessary calculations and conversions were made accordingly (Table 3.1).

Table 3.2: Formulas used to calculate chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids.

	$C_a = (12.25 \times A_{663.2}) - (2.79 \times A_{646.8})$
80% Acetone	$c_b = (21.50 \times A_{646.8}) - (5.10 \times A_{663.2})$
	$C_{a+b} = (7.15 \times A_{663.2}) - (8.71 \times A_{646.8})$
	$C_{x+c} = (1000 \times A_{470}) - (1.82 \times C_a) - (85.02 \times C_b)/198$
<hr/>	
$C_a = \text{Chlorophyll a}, C_b = \text{Chlorophyll b}, C_{a+b} = \text{Total Chlorophyll}, C_{x+c} = \text{Total carotenoids}$	

The experiment was replicated three times and absorbance reading triplicated.

3.3.11 Vitamin C

The ascorbic acid (Vitamin C) concentration was determined according to Böhm et al. (2006) with slight modifications. Briefly, 100-1000 of sample was mixed with 5 mL of 0.56 M metaphosphoric acid, vigorously shaken, centrifuged at 2988 g and the supernatant transferred into a volumetric flask. This procedure was repeated twice and the combined

extracts made up to 20 mL using 0.56 M meta-phosphoric acid. Subsequently, 200 μ L of the combined extract were mixed with 300 μ L of 0.3M trichloroacetic acid, centrifuged at 17212 g for 10 min. Subsamples of the supernatant (300 μ L aliquots) were mixed with 100 μ L of 2,4-dinitrophenylhydrazine reagent (0.013 M in 30 % perchloric acid), and heated to 60 °C for 1 h and subsequently cooled in an ice bath for 5 min. Thereafter, 400 μ L of 15.75 M sulphuric acid were added to the sample and the absorbance read at 520 nm after 20 min. The ascorbic acid concentration was calculated by comparison of the values obtained with an L-ascorbic acid standard curve.

3.3.12 Soluble sugars

Soluble sugars were extracted and determined according to Liu et al. (1999). Freeze-dried, ground material (0.10 g d.wt) was mixed with 10 mL of 80 % (v/v) ethanol and homogenized for 60 s. Thereafter, the mixture was incubated in an 80 °C water bath for 60 min and kept at 4 °C overnight. After centrifugation at 12 000 x g for 15 min at 4 °C, the supernatant was filtered through glass wool and taken to dryness in a Savant Vacuum Concentrator (SpeedVac, Savant, NY, USA). Dried samples were re-suspended in 2 mL of ultra-pure water, filtered through 0.45 μ m nylon filters and analyzed using refractometer to give Brix % reading of total soluble solutes. The concentration of individual sugars was determined by comparison with authentic sugar standards.

3.3.13 Statistical analysis

Statistical Analysis was performed using GenStat (version 17.0; VSN International, Hemel Hempstead, UK). Standard deviation values were calculated and differences among treatments were separated by the least significant difference (LSD) at P= 0.05.

3.4 Results and discussion

3.4.1 Total antioxidants

The TAOC capacity of the ALVs evaluated by FRAP and DPPH assays is presented in Fig 3.1. There were significant differences at in TAOC among all vegetables. Almost all the vegetable types showed increasing trend in TAOC from 2 WAT towards maturity. However, amaranth was dominating in its total antioxidant capacity during 4 to 6

WAT, with total antioxidants content (TOAC) of 3174.91; 2245.56; 2381.56 mmol Fe₂ SO₄ 100g⁻¹ DW, *Amaranthus hybridus*, Green cabbage and Red cabbage, respectively. These findings are in agreement with studies by Tesfay and his team (unpublished work, 2014), which showed increasing antioxidant content of indigenous leafy vegetables over time. This has also been confirmed by correlation analysis among different studied parameters (data not presented). The increasing total antioxidant content of ALVs could be from various environmental status and cellular mechanisms.

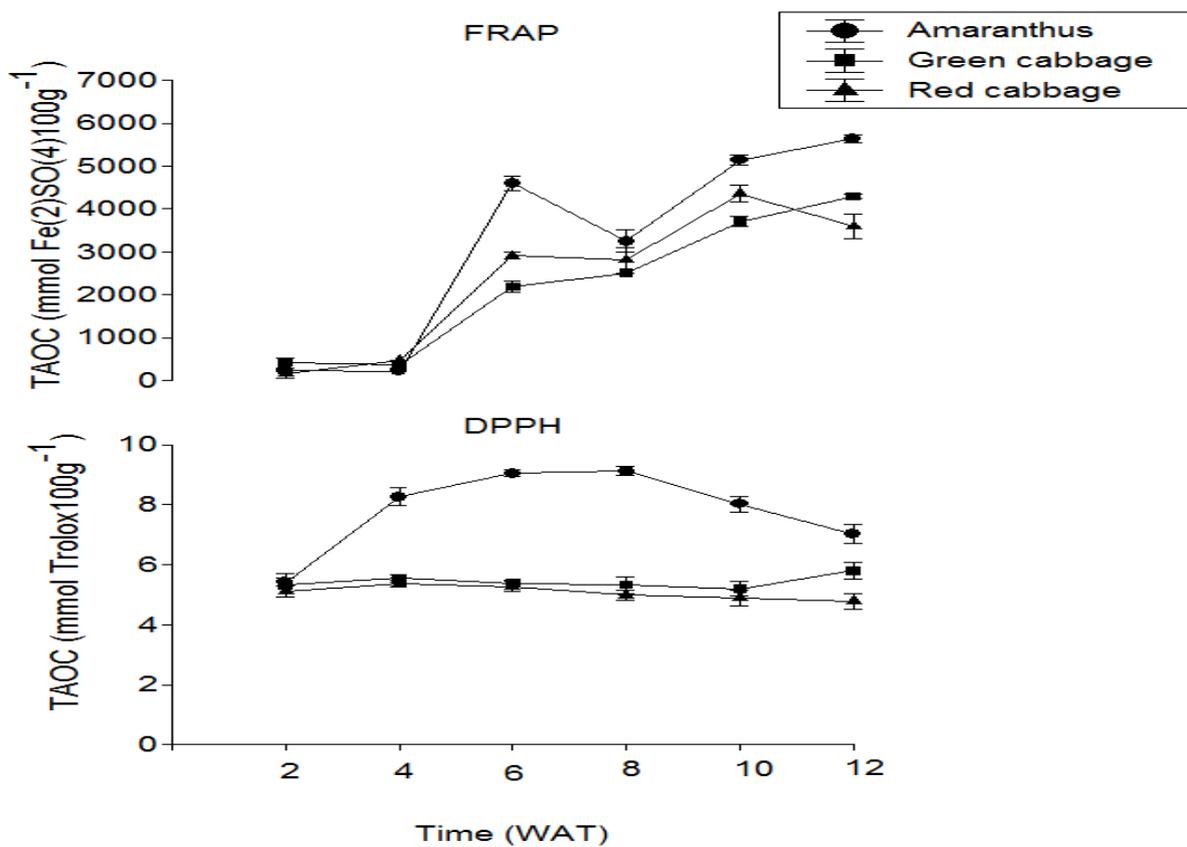


FIGURE 3.1: TAO contents for *Amaranthus hybridus*, *Brassica oleracea*, *brassica oleracea var. capitata f. rubra* over different growing period. Vertical bars represent \pm SE. (n=5). WAT – Weeks After Transplanting

Antioxidants are substances that are capable of inhibiting the oxidation of other substances; they are nature’s defense against the damaging effects of free radicals or Reactive

Oxygen Species (ROS), guarding cellular and DNA structures against oxidation (Kerchev and Ivanov, 2008, Murugan *et al.*, 2013).

Free radicals are unstable, highly reactive molecules that damage the protein, DNA, lipid and leading to side- effects associated with plant necrosis and wilting and cell death (Rahmnan, 2007; Arora *et al.*, 2002; Hatamzadeh *et al.*, 2011; Sharma *et al.*, 2012). When chain reaction is formed from metabolic activities with induced stress, antioxidant activity is required to break the chain reaction.

The antioxidant activity of fruits and vegetables depend on a variety of factors like lipid composition, antioxidants concentration, temperature, oxygen pressure and presence of other anti-oxidative components like phenols, carotenoids, etc. and food components such as water and nutrients (Rahmana, 2007). Antioxidants can directly scavenge ROS or indirectly by protecting the cells, these activities are referred to as primary and secondary defense mechanism.

3.4.2 Total phenols and Flavonoids

Plant phenolics were significantly different for all vegetables during plant developmental stages (Fig 3.2). Amaranth total phenols increased during early stage of plant development from 2 WAT, accumulated to maximum at 6 WAT (360 mg/100 g DW) and then showed a sharp decrease towards maturity. Exotic vegetables produced these phenols at a relatively decreasing trend towards maturity. Dumas *et al.*, (2003) have also reported the same trend for commercial vegetable phenols. Phenol production, although affected by growth conditions, their biosynthesis is normally stimulated by abiotic factors, such as UV light etc. The greater accumulation of flavonoids in the presence of UV light suggests a protective role against UV damage. It has been reported that these compounds accumulate in leaves of higher plants to screen out harmful UV radiation (Mazza *et al.*, 2000). Increases in flavonoids after exposure to UV light have also been reported in *Brassica napus* by Olsson *et al.*, (1998). Ryan *et al.*, (1998) reported greater accumulation of flavonoids in response to UV radiation. Furthermore, Krizek *et al.*, (1998) also reported high rates of accumulation of UV-absorbing compounds in lettuce grown in the presence of radiation. Biosynthesis and concentration of phenolic compounds in plants depends on genetic and environmental factors. Plant phenols are known for their anti-oxidative properties; depending on dominant type, they

govern plant taste, which impacts consumers' perception. It has been reported that plant phenols might have contributed for taste of amaranthus and also suspected to black jack if they are eaten uncooked. Rahman, et al., (2007) reported phenolic compounds influence seed bitterness and astringency as well as the overall quality of plant. Although ALVs produce high concentration of phenols, in rural communities people still prefer using them as vegetables for household consumption. As this plant (*Amaranthus*) produces high concentration of carbohydrates, mainly fructose; it was thought that plant bitterness as well as astringency could be improved, in the future. While the plant flavonoids were more 2 WAT and 4 WAT (117 and 124 mg/100g DW); there was a linear decrease for all vegetables towards maturity. Plant flavonoids reported to have mutagenic and fatal effect after consumption, therefore noticing its decreasing trend, it has been suggested that, either prepare these vegetables in raw or cooked meal.

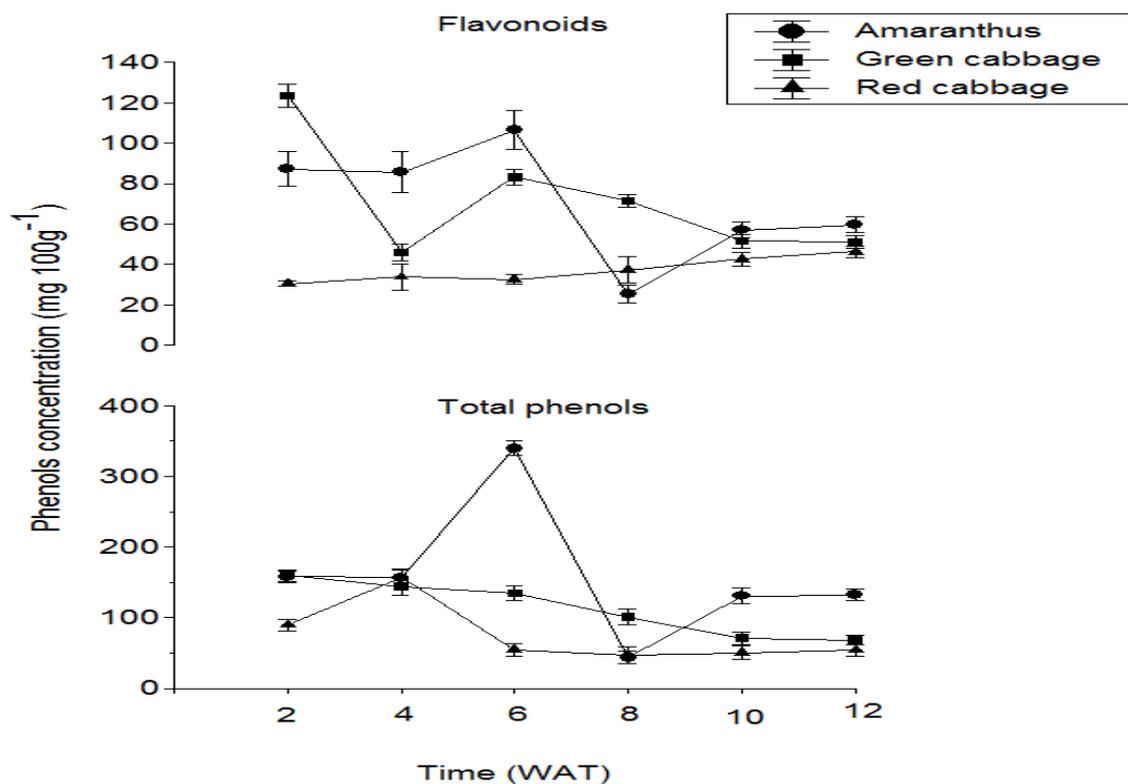


FIGURE 3.2: Phenolic and Flavonoids contents of *Amaranthus hybridus*, *Brassica oleracea*, *brassica oleracea* var. *capitata* f. *rubra*. Vertical bars represent \pm SE. (n=5). WAT – Weeks After Transplanting.

Phenolic compounds play an important role in plant defense or resistance against microbial infections, which are related to reactive oxygen species (ROS). These compounds contribute significantly to the overall healthiness of the plant especially flavonoids, which possess different biological activities, but most important are antioxidant activity (Re *et al.*, 1999, Nautiyal *et al.*, 2008). Many in-vitro studies demonstrate phenolic compounds to have higher antioxidant properties than vitamins and carotenoids antioxidants (Vinson, *et al.*, 1995, Podsedek, 2007)

3.4.5 Carotenoids and chlorophyll

The total carotenoids of the studied vegetables were significantly different (Fig 3.3). Amaranthus displayed the highest accumulation of total carotenoids (2.8mg/100g DW) at early stage of plant development and slightly fluctuating over maturity. Most leafy vegetables respond to different types of light having supra-optimal light intensities, they accumulate high concentration of carotenoids during early developmental stages. Having accumulated total carotenoids during early plant development the plant would probably increase its nutritional value. Whereas, the green and red cabbages had reasonable pigment accumulation and unlike amaranthus, pigment accumulation in exotic vegetables exhibited increasing trend towards maturity. Plant carotenoid production pattern at different development stages is assumed to be cultivar specific, for example the high content at early development stages by ALVs while commercial vegetables increasing with maturity. Similar observations were also reported by Reif (2012) carotenoids production is more likely cultivar specific than growing season.

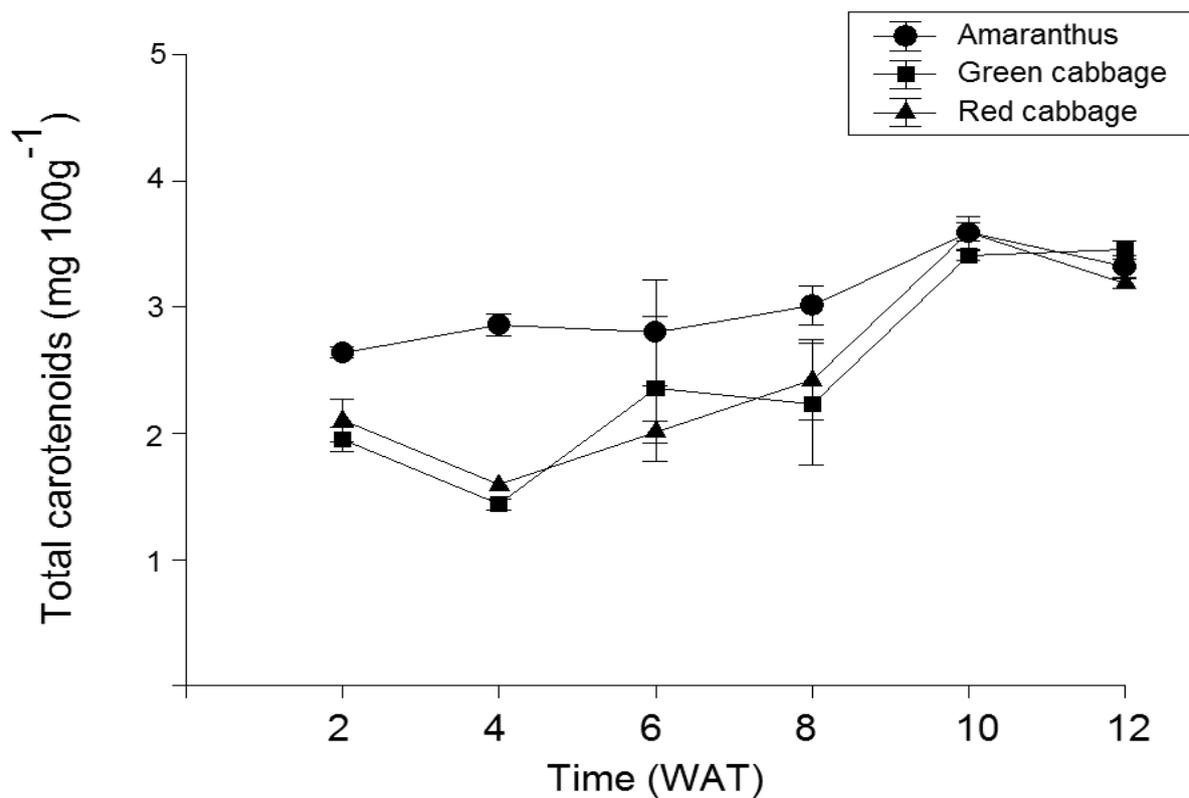


FIGURE 3.3: Total carotenoids content of *Amaranthus hybridus*, *Brassica oleracea*, *brassica oleracea* var. *capitata* f. *rubra* over different growing period. Vertical bars represent \pm SE. (n=5). WAT – Weeks After Transplanting.

Chlorophyll ‘a’ content shown in Fig 3.4 was similar among the vegetable types, but amaranthus had the highest chlorophyll ‘a’ content throughout the observed period. Green and red cabbage had lower chlorophyll ‘a’ content, but they showed an increasing trend with plant growth, their chlorophyll ‘a’ amounts were following same trend although they were slight fluctuations with green cabbage having higher contents in the 6th week, but red cabbage later regained the second position in chlorophyll content.

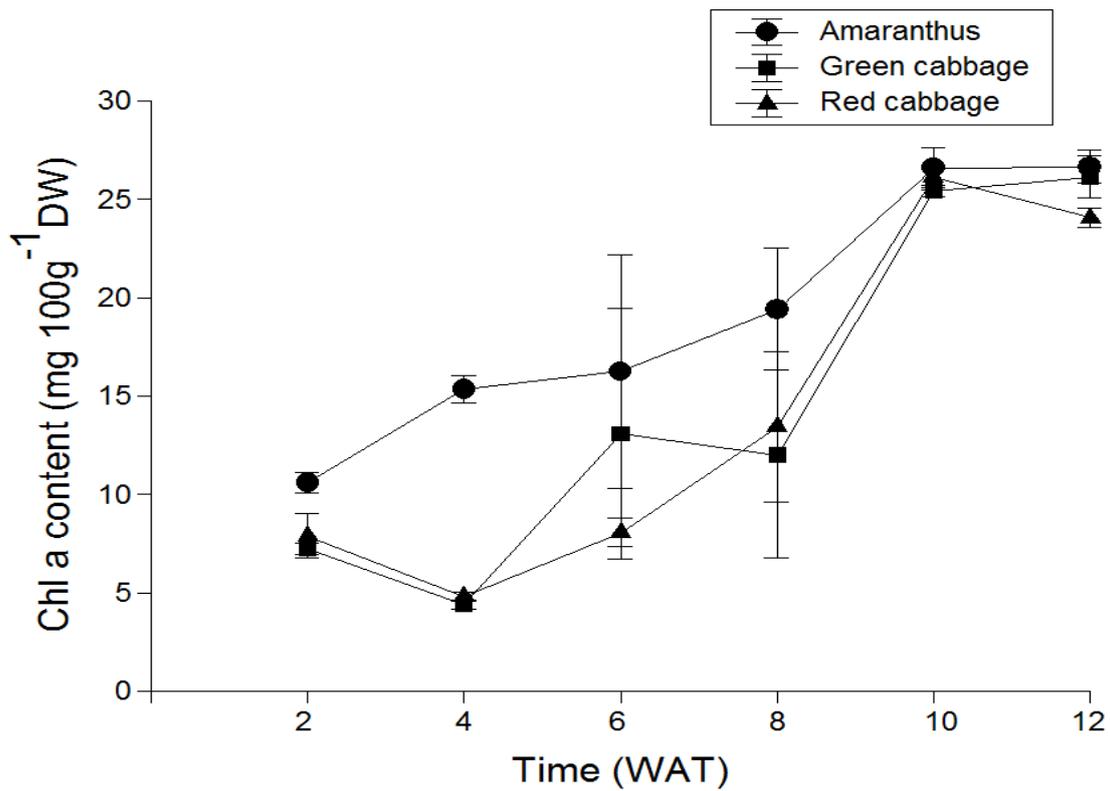


FIGURE 3.4: Chlorophyll a content of *Amaranthus hybridus*, *Brassica oleracea*, *brassica oleracea* var. *capitata* f. *rubra* over different growing period. Vertical bars represent \pm SE. (n=5). WAT – Weeks After Transplanting.

Chlorophyll ‘b’ content shown in Fig 3.5 was fluctuating in all the vegetables; with growth *Amaranthus* had higher contents for most weeks: 2nd, 4th, and 8th week and green cabbage alternated with red cabbage during weeks 6 and 10, respectively. A decreasing trend was observed after the 10th week for all vegetables, at the end of the trial green cabbage had the highest and red cabbage had the lowest chlorophyll ‘b’ content.

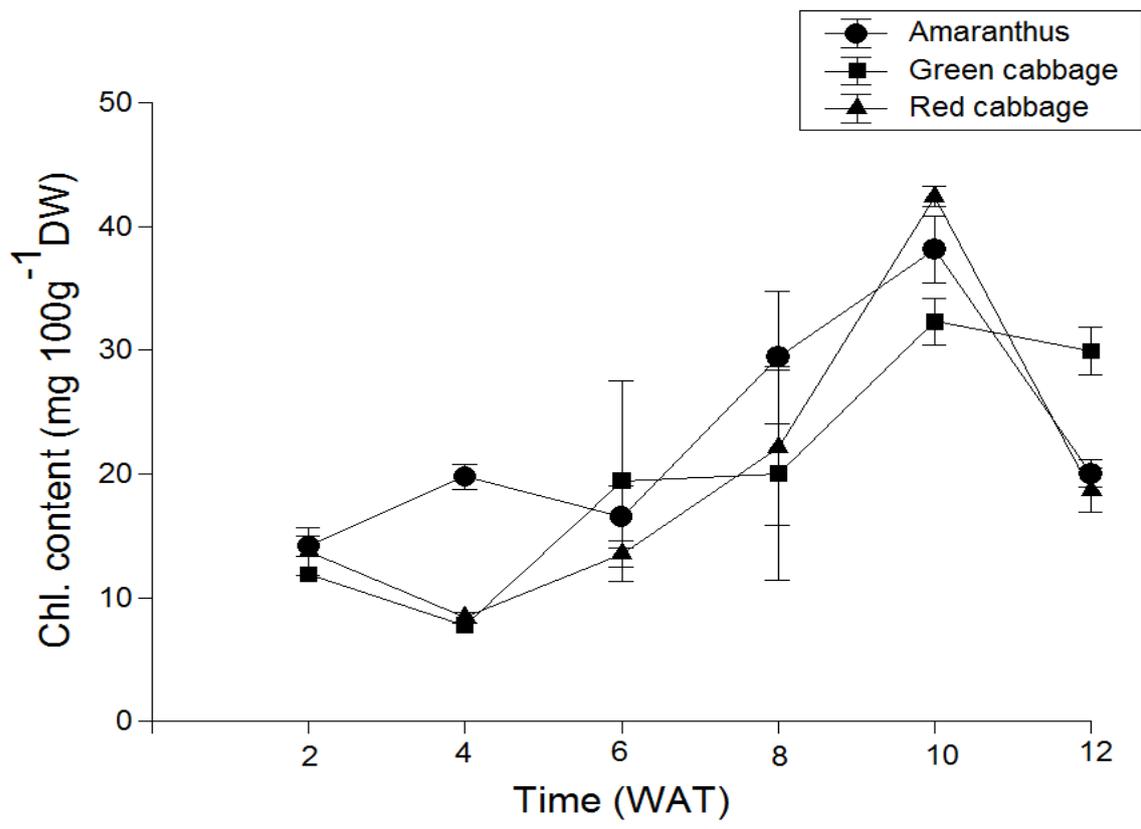


FIGURE 3.5: Chlorophyll b content of *Amaranthus hybridus*, *Brassica oleracea*, *brassica oleracea* var. *capitata f. rubra* over different growing period. Vertical bars represent \pm SE. (n=5). WAT – Weeks After Transplanting.

Chlorophyll (a+b) in Fig 3.6 showed that amaranthus had higher chlorophyll content, followed by the green cabbage and then red cabbage, respectively. Whereas with green cabbage, chlorophyll content increased as it grew to maturity, followed by amaranthus and red Cabbage.

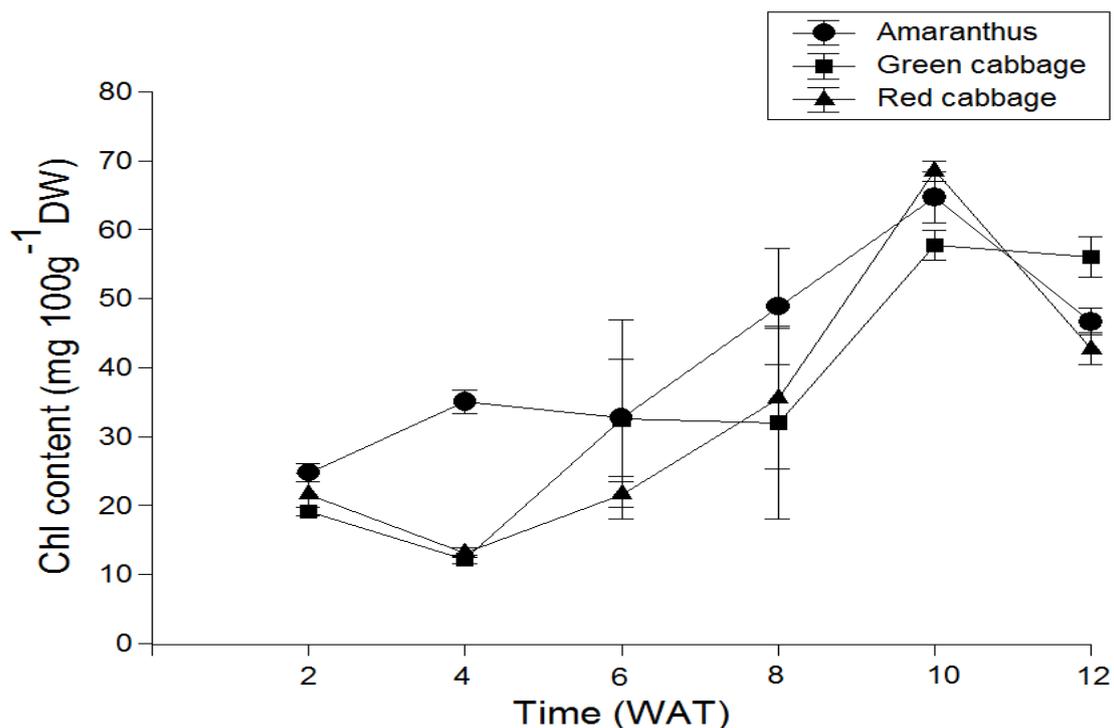


FIGURE 3.6: Chlorophyll (a+b) content of *Amaranthus hybridus*, *Brassica oleracea*, *brassica oleracea* var. *capitata* f. *rubra* over different growing period. Vertical bars represent \pm SE. (n=5). WAT – Weeks After Transplanting.

3.4.6 Ascorbic acid (Vitamin C)

Plant ascorbic acid (Vitamin C) was significantly different for the vegetables. Its accumulation was also affected by plant developmental stages, amaranth was found dominating in producing ascorbic acid during the first 6 WAT (Fig 3.7) and started to decline towards the end of plant growth. On the contrary, the other vegetables, red and green cabbages, showed increasing trend of total ascorbic acid accumulation over time. ALVs ascorbic acid content showed significant increase during early vegetative development. The higher level of ascorbic acid content in young leaves of ALVs may be attributed to higher concentration of glucose, which is the main precursor in the biosynthesis of L-ascorbic acid. This argument is in agreement with Dumas et al., (2003) who reported leaves of tomato plant produced high concentration of ascorbic acid, which seemed strongly correlated to high concentration of sugar and had direct impact on the biosynthesis of ascorbic acid.

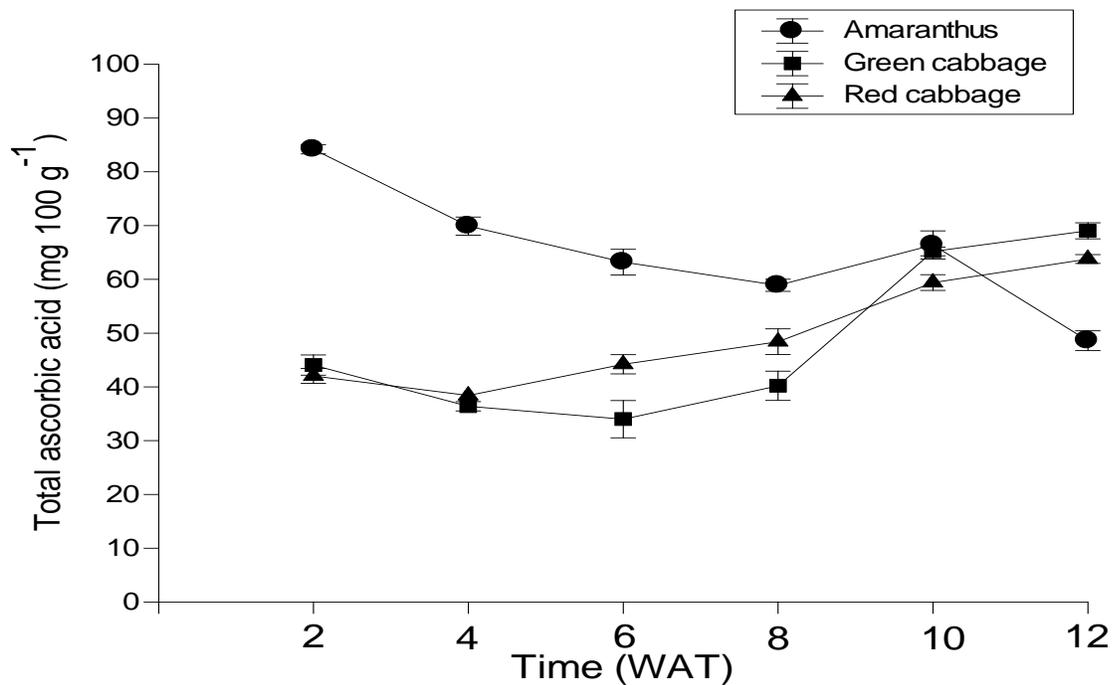


FIGURE 3.7: Ascorbic acid (Vit C) contents of *Amaranthus hybridus*, *Brassica oleracea*, *brassica oleracea* var. *capitata* f. *rubra* over different growing period. Vertical bars represent \pm SE. (n=5). WAT – Weeks After Transplanting.

3.4.7 Total soluble sugars (TSS)

Total soluble sugars contents of *Amaranthus hybridus* (Amaranthus), *Brassica oleracea* (Green cabbage) and *Brassica oleracea* var. *capitata* f. *rubra* (Red Cabbage) are shown in Fig 3.8. There were significant differences ($p < 0.001$) between the total soluble content of the studied vegetables. The exotic leafy vegetables had fluctuating but increasing total soluble sugars towards maturity while the indigenous *A.hybridus* had stably decreasing contents towards

maturity. Green cabbage showed the highest TSS content followed by red cabbage and lastly *amaranthus hybridus*, respectively. Although ALVs produce high concentration of phenols causing bitterness at early stages they have high concentration of soluble sugars. Therefore, rural communities still prefer using them as vegetables for household consumption. Because this plant produces high concentration of carbohydrates, mainly fructose, it was thought in future the plants' bitterness and astringency could be improved.

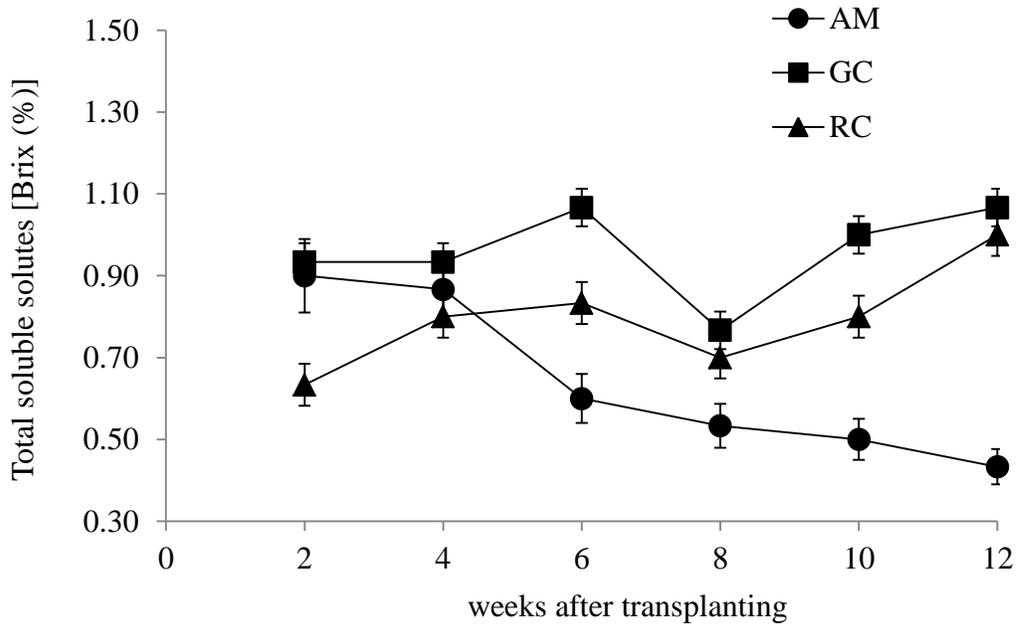


FIGURE 3.8: Total soluble sugars contents of *Amaranthus hybridus*, *Brassica oleracea*, *brassica oleracea var. capitata f. rubra* over different growing period. Vertical bars represent \pm SE. (n=5). WAT – Weeks After Transplanting.

3.5 Conclusion

The key findings of this study are that the African leafy vegetable, *Amaranthus*, had different antioxidant production trends compared to exotic vegetables. Amaranth produced higher content of carbohydrates, carotenoids, ascorbic acid, phenols during 4th and 6th week after transplanting, whereas the exotic vegetables produce these compounds on approaching to harvesting stage. Consumers of African Leafy Vegetables (ALVs) prefer young tender leaves than older leaves. The sequential harvest nature of ALVs results in increases food access for an extended period. The plant biomass per plant also increases with the number harvests.

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CHAPTER 4

Assessment of specific antioxidant enzymes of traditional leafy vegetable *Amaranthus hybridus* compared to exotic leafy vegetables

4.1 Abstract

Reactive oxygen species (ROS) are dangerous to plant and human cell membranes. They are produced during metabolic reactions in living organisms, however when they are in excess they are harmful to cells. Leafy vegetables contain enzymatic and non-enzymatic antioxidants that are used to prevent chain reaction that cause harm to plant/human cells. Specific enzyme activity of two exotic leafy vegetables (*Brassica oleracea* and *Brassica oleracea* var. *capitata* f. *rubra*) and indigenous (*Amaranthus hybridus*) leafy vegetables were compared over different growing stages. *Amaranthus* showed dominantly higher enzyme activity over the entire growing period mainly towards maturity beginning at the 4th week after transplant to the 6th week after transplant. In conclusion, this indigenous leafy vegetable could potentially be utilized as a medicinal plant.

4.2 Introduction

Fruits and vegetables are an important source of protective foods, which are largely beneficial in human health maintenance and disease prevention (Nnamani *et al.*, 2009). Indigenous leafy vegetables are also recognized as protective foods due to their richness in nutritive antioxidants. They are vegetables that are locally/indigenously grown, mainly under cultivated; cultivated at subsistence or household level (Ekasa *et al.*, 2009). They are also mainly referred to as weeds in other parts of the world. However, they may or may not be confined to that region of locality/ traditional practice (Nnamani *et al.*, 2009). These indigenous leafy vegetables are able to withstand harsh environmental conditions (Ekasa *et al.*, 2009) by

balancing the chemical and metabolic reaction which produces reactive oxygen species (ROS) and antioxidants.

Enzymatic antioxidants catalyze the defense reaction against the damaging effect of free radicals. As with chemical (non-enzymatic) antioxidants, cells are protected by an interacting network of anti-oxidative enzymes (Rahman, 2007, Lobo *et al.*, 2010). Fig 4.1 shows an interactive effect of antioxidants in protecting cells against ROS damage.

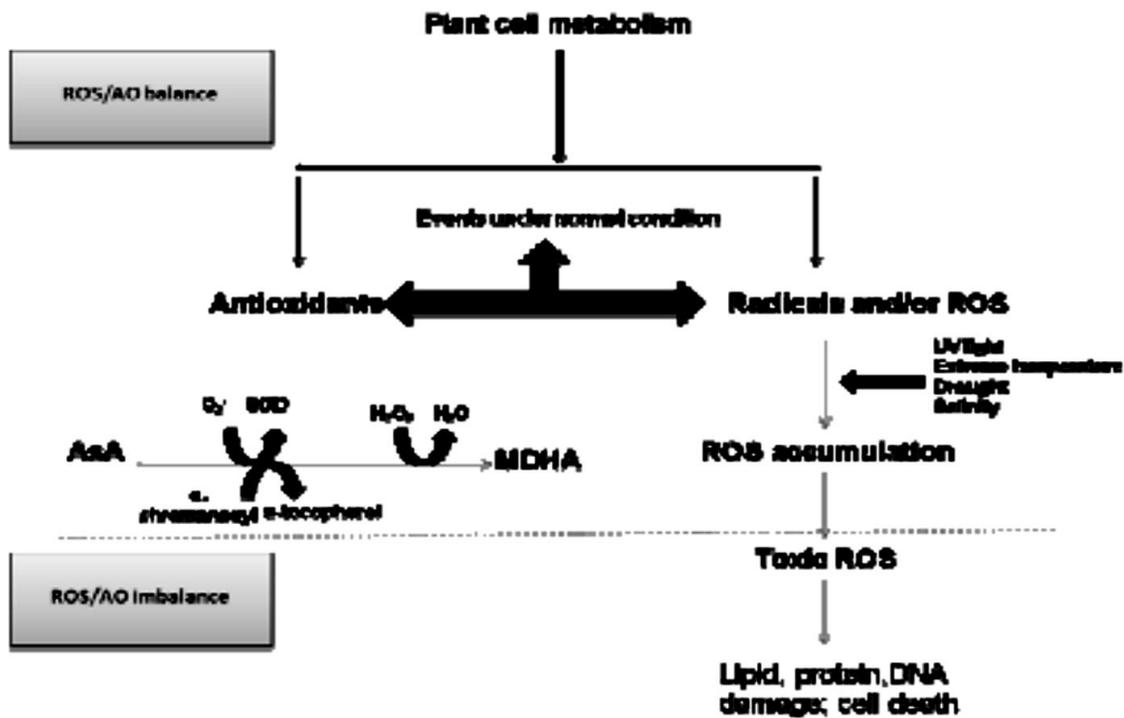


Figure 4.1: A schematic diagram of cellular production of anti-oxidants and reactive oxygen species (ROS) and their state of metabolic equilibrium affected by different stress factors which may result in cell death (Foyer and Noctor, 2005).

However, their activity as anti-oxidative enzymes largely depends on adequate availability of trace minerals such as selenium, iron, copper, zinc and manganese. It has been reported that inadequate availability of these micronutrient cofactors may impend the activity of

anti-oxidative enzymes. Fig 4.2 shows different enzymes and molecules at different stages of mitigating oxidative stress.

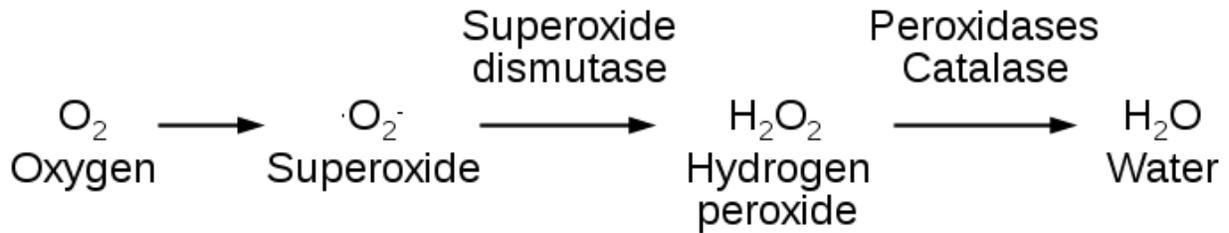


Figure 4.2: A schematic diagram showing different enzymes required at different stages of free radical production and transforming to an acceptable product (H_2O), adapted from Sharma *et al.*, (2012).

Therefore, the presence and activity of enzymes in vegetables is important for plant protection after against ROS. However, indigenous vegetables lack such documented information over growing stage. The aim was to investigate the presence of different enzymes and their activities at different stages of plant growth.

4.3 Materials and methods

4.3.1 Plant material

Amaranth (*Amaramthus hybridus*) was collected from the University of Kwa-Zulu Natal farm (UKULINGA research farm). Brassica oleracea and Brassica oleracea var. rubra captiva were purchased from local seed supplier HYGROTECH®. The plants were grown in the tunnels and sampled weekly for lab analysis

4.3.2 Analyses

4.3.2.1 Total soluble protein extraction

Total soluble proteins were extracted according to Kanellis and Kalaitzis (1992) with slight modifications. Frozen mesocarp tissue powder (1 g DW) was extracted in 5 mL 50 mM Tris-HCl buffer (pH 7.4 containing 0.2 M NaCl, 20 mM MgSO₄, 1 mM EDTA, 5 mM β-mercaptoethanol, 0.5 mM PMSF, 10 mM leupeptin, and 10 % (v/v) glycerol). The mixture was allowed to stand on ice for 15 min and then centrifuged at 20000 g for 20 min. The supernatant was used for enzyme assays after being passed through Miracloth®.

4.3.2.2 Total protein assay for determination of enzyme activity

The Bradford micro-assay was used to determine the protein content of the samples (Bradford 1976). Bradford dye reagent was prepared by diluting the dye concentrate with distilled water 1:4. The dye (1 mL) was added to test tubes containing 20 μL sample extract, mixed and incubated at room temperature for 5 min. Samples were then read spectrophotometrically at 595 nm and the protein concentration determined by comparing results with a standard curve constructed using bovine serum albumin (BSA).

4.3.2.3 Superoxide dismutase (SOD)

SOD (E.C. 1.15.1.1) activity was determined by measuring the ability of the enzyme to inhibit the photochemical reduction of nitroblue tetrazolium chloride (NBT), as described by Giannopolitis and Ries (1977). The reaction solution (3 mL) contained 1.5 mM NBT, 0.12 mM riboflavin, 13 mM methionine, 0.1 M EDTA, 67 mM phosphate buffer (pH 7.8), and contained 10 to 100 μL enzyme extract. Riboflavin was added last and tubes were shaken and placed under fluorescent lighting with an intensity 8.42 μmol m⁻² s⁻¹. Blanks and controls were determined by withholding illumination and without addition of enzyme, respectively. The absorbance's of

the illuminated and non-illuminated solutions were determined spectrophotometrically at 560 nm. One unit of SOD activity was defined as the amount of enzyme inhibiting 50% NBT photoreduction. The results were expressed as Units x (mg protein)⁻¹ = 1000 x (μg enzyme extract resulting in ½ max. inhibition)⁻¹. Where ½ max. inhibition was determined using pure enzyme dilution 0, 20, 40, 60, 80, 100 factor of BHT (Butylated hydroxytoluene).

4.3.2.4 Catalase (CAT) activity assay

A method originally described by Beers and Sizer (1952) was used with slight modifications to determine CAT (E.C.1.11.1.6) activity. The reaction solution (3 mL) contained 0.05 M potassium phosphate (pH 7.0), 0.059 M hydrogen peroxide, 0.1 mL enzyme extract and 1.9 mL distilled water. To start the reaction, the mixture, in absence of enzyme extract was incubated for 4 to 5 min to achieve temperature equilibration (25 °C) and to establish a blank rate. To this mixture 0.1 mL diluted enzyme extract was added and the disappearance of H₂O₂ was followed spectrophotometrically every 20 s for 3 min via the decrease in absorbance at 240 nm. The change in absorbance (Δ240 nm/min) from the initial (20 s) in the linear portion of the curve was calculated. One unit of CAT activity was defined as the amount that decomposes one μmol H₂O₂. Enzyme activity was reported as Units/mg protein using the following equation: Units/mg protein = (Δ240/min x 1000) x (43.6 x mg enzyme/mL of reaction mixture)⁻¹.

4.3.2.5 Peroxide activity (POX) assay

Peroxidase (E.C. 1.11.1.7) activity was assayed according to Reuveni et al., (1992) by measuring the oxidation of guaiacol as the increase in absorbance at 470 nm from 0 to 3 min. The reaction mixture contained 15 mM of phosphate buffer buffer (pH 6.0) and 100 μL enzyme extract in a total volume of 1 mL. The reaction was initiated by adding 500 μL substrate, which consisted of a 30 mM H₂O₂ and 80.7 mM guaiacol solution (o-methoxy-phenol). The peroxidase activity was calculated as the change in absorbance over a 3 min period and expressed as units

per milligram of protein: Units/mg protein = $(\Delta 470/\text{min} \times 1000) \times (26.6 \times \text{mg enzyme/mL of reaction mixture})^{-1}$.

4.3.2.6 Lipid Peroxidation

Lipid peroxidation MDA (mmol g⁻¹d wt) was measured according to Hodges et al., (1999). Two milliliters of extraction solution and 3 mL 0.5% TBA including 5% TCA were mixed vigorously. The mixture was heated at 95°C in a constant temperature water bath for 30 min and then cooled in ice to room temperature. After centrifuging at 5000 g for 15 min, the supernatant was detected at 450, 532 and 600 nm. The concentration of MDA was determined using the formula CMDA ($\mu\text{mol mL}^{-1}$) = $6.45 \times (D_{532} - D_{600}) - 0.56 \times D_{450}$, where D₄₅₀, D₅₃₂ and D₆₀₀ are the absorbencies at 450, 532 and 600 nm, respectively.

All the results obtained for enzyme activity were from the same sample extract of proteins.

4.3.2.7 Statistical analysis

Analyses of variance between tissues and growing months, and correlation analyses among tissue parameters were performed using GenStat version 17. Standard deviation values were calculated where a significant variance was found at $P < 0.05$ between individual values.

4.4 Results and Discussion

4.4.1 Protein

There were no significant differences ($P > 0.001$) in the protein amounts of studied vegetables. However, all vegetables showed an increasing trend towards maturity where all

studied vegetables reached their maximum protein content at 5 weeks after transplant, red cabbage (7.42 mg/g of DW), green cabbage (6.88 mg/g DW) and amaranth (6.16 mg/g), while the protein content of exotic vegetables decreased significantly after the 5th harvest while amaranth had slower decrease, Fig 4.3.

The protein increase towards maturity may be due to high metabolic rate which results in accumulation of plant metabolites required by the plant at a later stage for quality maintenance during storage. The increase in protein would also aid in enzyme activity as enzymes function well with high protein content, substrate concentrations(s), at optimum pH, ionic strength and nature of salts present, and temperature (Scopes, 2002).

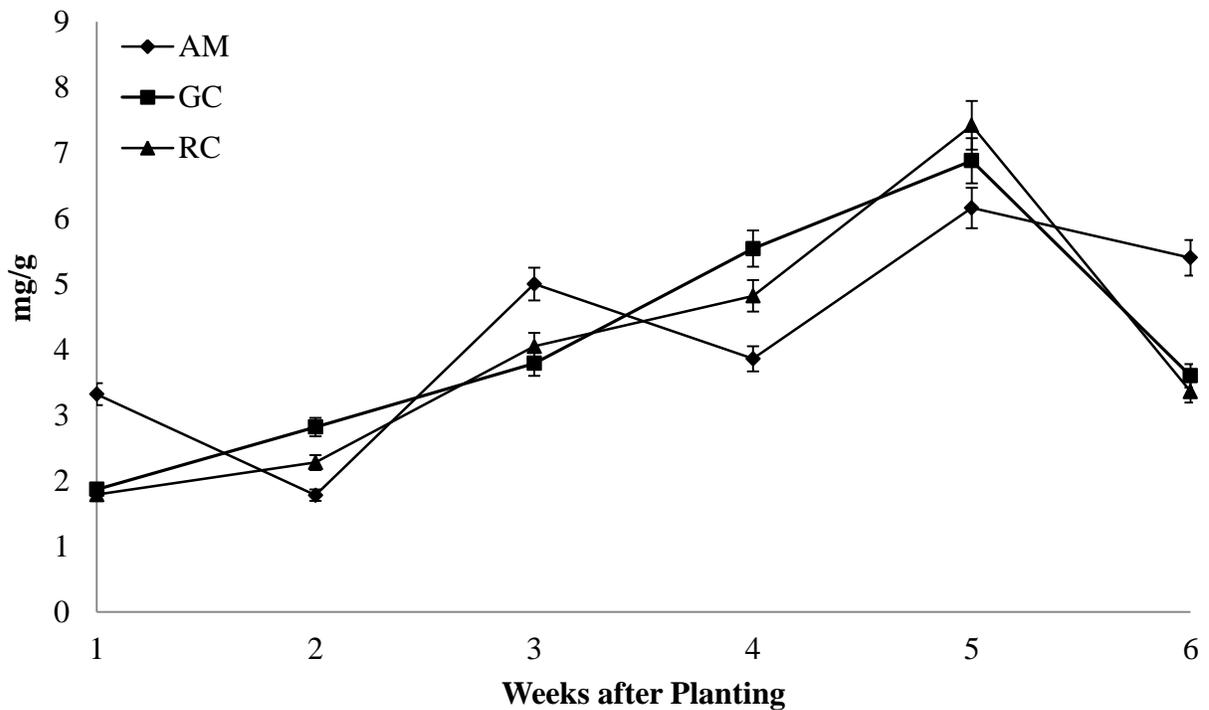


FIGURE 4.3: Protein content of different studied leafy vegetables AM (*Amaranthus hybridus*), GC (Green Cabbage), RC (Red Cabbage) over 6 weeks harvest period. Vertical bars represent \pm SE. (n=5).

4.4.2 Lipid peroxidation

Lipid peroxidation is an oxidative degradation of lipids resulting in cell membrane damage due to chain reaction of ROS. There were no significant differences in lipid peroxidation across all the studied vegetables. Red cabbage seemed to be fluctuating more steadily than green cabbage and *A.hybridus*, green cabbage had the highest lipid peroxidation up to 4WAT and decreased onwards while amaranth increased from 5 WAT. The high lipid peroxidation at the early stages of plant growth are due to cell immaturity, thus more susceptible to ROS by products of high metabolic rate and later at maturity stage especially for amaranth with a short growing period are due to cell ageing, thus also more susceptible to ROS.

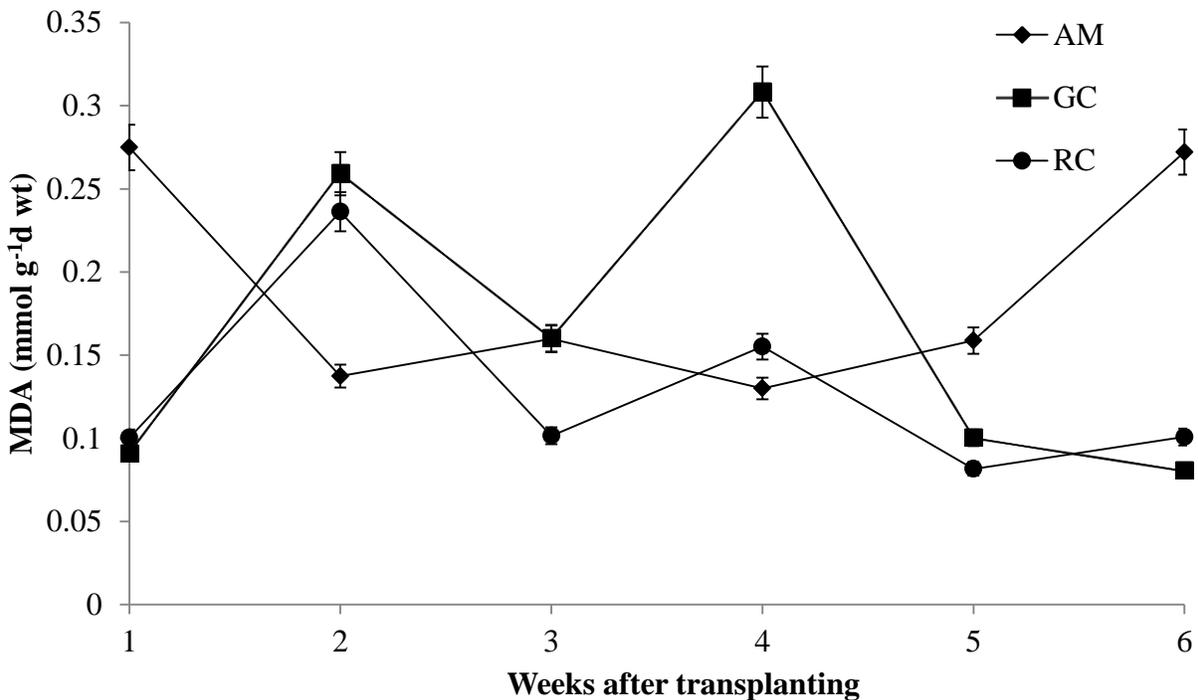


FIGURE 4.4: Lipid Peroxidation of different studied leafy vegetables AM (*Amaranthus hybridus*), GC (Green Cabbage), RC (Red Cabbage) over 6 weeks harvest period. Vertical bars represent \pm SE. (n=5).

4.4.3 Enzymatic antioxidants (POD, CAT, SOD)

The plant enzymatic antioxidants had significant differences over maturity. Plant SOD was dominant for amaranth compared to the commercial vegetables (Fig 4.2). Their activity increased the first few weeks after transplanting and declined when approaching the final phase (120-160U/mg/min). CAT and POD responded in a similar way, but the ALV appeared dominating. It was also noticed that activity of these enzymes was increasing, CAT (> 80160U/mg/min) and POD (>49160U/mg/min) during 4 WAT towards 6 WAT. The increase in enzyme activity during the first weeks of plant development could be associated with the plants high metabolic rate, resulting in the accumulation of oxidants and the plant counter this by producing antioxidants to create a redox balance within the cell. The SOD may have dismutase activity which converts singlet oxygen radicals into hydrogen peroxide, and the CAT activity can then possibly increase as regulated by accumulation of H₂O₂. H₂O₂ accumulation in early to middle developmental stages possibly coincided with increased SOD, CAT and POD activities, which suggests that H₂O₂ acts as a signaling molecule. Thereafter, the gradually decreasing SOD, CAT and POD activities presumably led to an increase in OH levels in later developmental stages. It is suspected that, in the later stages, more H₂O₂ is likely to transform to OH so as to breakdown polysaccharide and mediated the lignification of old fully matured leaf.

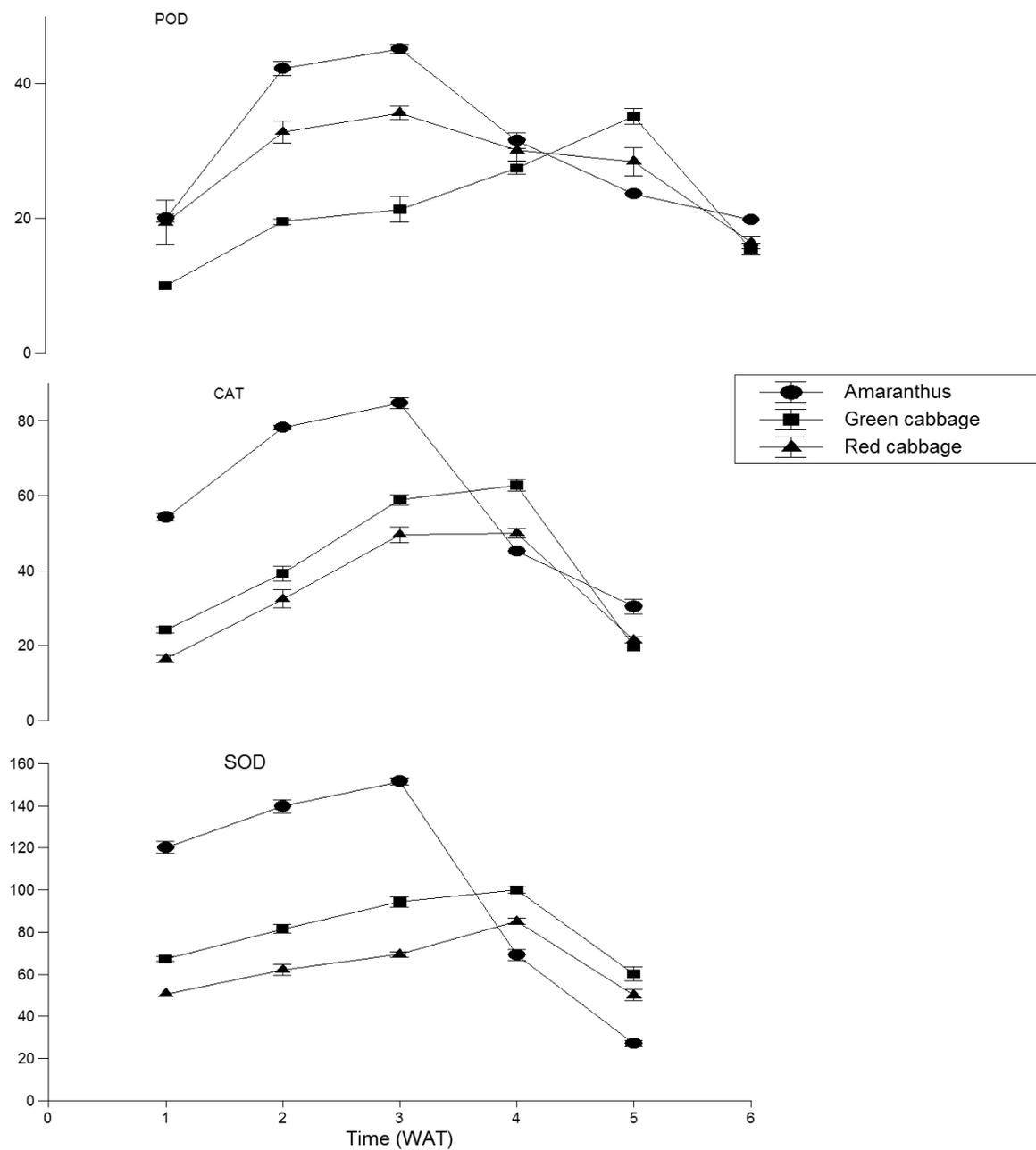


Figure 4.5: Ezymic antioxidants (CAT, POD, SOD) of different studied leafy vegetables AM (*Amaranthus hybridus*), GC (Green Cabbage), RC (Red Cabbage) over 6 weeks harvest period. Vertical bars represent \pm SE. (n=5). WAT – Weeks After Transplanting.

4.4 Conclusions

Plants have different metabolic reaction rates at different growth stages, during reactions ROS which are harmful to cells are released shown by lipid peroxidation (Fig 4.4), therefore mechanisms of mitigating effect of these ROS are required. High metabolic rates are mainly experienced in early stages of plant growth (Fig 4.4) therefore high mitigating activity is mainly required at these stages of plant growth (Fig 4.5), also at the early stages of plant growth, plants are more susceptible to different harsh environmental effects.

Diverse environmental stresses differentially affect plant processes that lead to loss of cellular homeostasis accompanied by the formation of reactive oxygen species (ROS), which causes oxidative damage to membrane, lipids, proteins and nucleic acids (Srivalli *et al.*, 2003). The fluctuating lipid peroxidation results (Fig 4.4) are not fully understood, however in the early stages it might be due to premature leaf abscission, and late growth stages might be due to leaf senescence shown by Dhindsa *et al.*, 1981 on *Nicotiana tabacum* L. leaves and increased peroxidation has been reported on ageing animals by Packer *et al.*, (1967). The coordinate function of antioxidant enzymes such as SOD, CAT, POX (Fig 4.3) and GPx assist in processing of ROS and regeneration of redox ascorbate and glutathione metabolites (Halliwell, 1974; Wise, 1995; Foyer and Nector, 2000). The oxidative damage to cellular components is limited under normal growing conditions due to efficient processing of ROS through a well-coordinated and rapidly responsive antioxidant system consisting of several enzymes and redox metabolites.

Lipid peroxidation (Fig 4.4) requires active O₂ uptake (Bor, *et al.*, 2002) this process results in production of superoxide radical (O₂⁻) (Fridovich, 1975). The O₂⁻ is produced by a process of phosphorylation; this O₂⁻ is then converted to hydrogen peroxide by SOD, than further reduced catalase to peroxidase (Mao *et al.*, 1992). Conjugated action of enzymes is much more effective than sole action by an enzyme this is shown by transgenic studies by Ho *et al.*, (1998) which showed the importance of enzyme activity in protecting animals against injuries. Ismail *et al.*, (2010) showed the importance of SOD, CAT and GPx on hepatic tissue from children with chronic cholestatic liver disease. The relative high activity of *A.hybridus* CAT, POD, POX enzymes can aid in some medical conditions associated with active oxygen reactions.

SOD, CAT and POX (Fig 4.5) are the major antioxidant enzymes in pant systems, which exert their effect in different pathways shown by different scientific works (Yücel *et al.*, 2014).

The most famous pathway is the phosphorylation process (Fuhler *et al.*, 2011) that releases superoxide, which is first converted into hydrogen peroxide and further converted to peroxide. The plant system constitutes, enzymes and redox metabolites work synergic to mitigate the effect of ROS on plant cells. In this study, it is meritable enough to show that the horticultural plants of interest possess systems to protect themselves from oxidative damages during their different stages of development.

Both indigenous and exotic leafy vegetables showed similar trends in enzyme activity through different growth stages although indigenous had higher activity.

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GENERAL DISCUSSION AND CONCLUSION

This study was undertaken based on the hypothesis that indigenous African leafy vegetables can be used to substitute or in conjunction with available exotic leafy vegetables and other food types to aid combat the problem of malnutrition and food security.

A seed quality analysis of indigenous *Amaranthus hybridus* was done on different coloured seeds red and white gold. Germination viability was tested under controlled laboratory conditions with a temperature variation of 17.3/26.5 °C for 7 days. Red coloured seeds were found to have higher germination percentage than white coloured seeds, to further test seed quality electrolyte leakage (EC), total antioxidants capacity and activity were measured. Red coloured seeds had higher electrolyte leakage which suggested red coloured seeds to be of lower vigor than white gold seeds, antioxidant capacity and activity was also higher in red coloured seeds which is in contradiction with Mavi, (2010) findings, that brown seed lot had lower electrolyte leakage than light coloured seed lot. Therefore, in the current study high electrolyte leakage was subsequently associated with high total soluble solutes content of red coloured seeds. Red coloured seeds were then germinated on seedling trays and transplanted to growth pots, along with comparable brassica leafy vegetables. These vegetables were harvested weekly, freeze dried, ground and stored under -72 °C for biochemical/antioxidants analysis.

From this study it was shown that *A.hybridus* leafy vegetable had high and comparable (to exotic leafy vegetables) measured phytonutrients. The studied vegetables exhibited antioxidant properties, due to both enzymatic and non-enzymatic antioxidant contents. Non-enzymatic antioxidants were significantly different within and between the studied leafy vegetables, with indigenous *A.hybridus* having higher contents than the exotic leafy vegetables. Enzymatic antioxidant type and activity fluctuated between and within the compared leafy vegetables. The maximum contents and activity of enzymes were mainly reached between the 4th and 6th week after transplant, therefore this is the ideal time of harvest for indigenous leafy vegetable studied, this results supports the growth pattern of *A.hybridus* which is continuous young leaves harvest (Daff, 2010). Both enzymatic and non-enzymatic antioxidant activity were maximal at the optimum harvest time.

Different types of antioxidants, food species along with food types have been proven to work better in synergetic rather than individually (Yang, *et al.*, 2009; dos Santos *et al.*, 2010; Jain *et al.*, 2011). Therefore, quantifying and comparing different types of antioxidants of different food species, is considered more significant than quantifying single type and specie with no comparison to other types or species.

High antioxidant content in fruits and vegetables is beneficial to human health (Bjelakovic and Gluud, 2007; Rao and Rao, 2007; Heiting, 2014), Therefore the study proved that *A.hybridus* leafy vegetable can be used for human health benefits, as a supplement to current spectrum of antioxidant sources or as a main source for “poor” communities which have limited resources, and as such are unable to access commercial leafy vegetables and other antioxidant sources. Other indigenous leafy vegetables can also be used in conjunction i.e. Black jack which has been studied by Mbokazi *et al.*, (unreported work).

FUTURE RESEARCH AND COMMERCIAL APPLICATION

Studies on the growth requirements and parameters are required to increase the scientific knowledge regarding indigenous plants:

- ❖ Seed quality attributes regarding the hybridization of all off springs of *A.hybridus* and how the seeds performs under different environmental conditions.
- ❖ Plants stand performance under different environmental conditions, stress-full conditions; i.e. time taken to flower under water scarce conditions, high temperatures, etc.
- ❖ Postharvest requirements relating to packaging material and storage requirements with primarily measuring primary and secondary metabolites and their specifics i.e. C7 sugar contents which have been found to be associated storability and postharvest quality (Bertling and Bower, 2005; Bertling and Tesfay 2011).
- ❖ More intense studies are required on indigenous leafy vegetables quality attributes regarding nutritional and non-nutritional attributes.

Laboratory research alone will not effectively help in alleviating food security issues, therefore community outreach studies are also required for community awareness and preferences on the indigenous leafy vegetables studied.

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