

Effect of Nitrogen Source, Crop Maturity Stage and Storage Conditions on Phenolics and Oxalate Contents in Vegetable Amaranth (*Amaranthus hypochondriacus*)

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Abstract

This study was designed to assess the effect of Diammonium Phosphate (DAP) fertilizer and cattle manure on the accumulation of total phenolics, quercetin and oxalates during growth and storage of vegetable amaranth (var. *Amaranthus hypochondriacus* L). Field trials were set up at the University of Nairobi Field Station-Upper Kabete Campus, Kenya during the long (March –May) rains of 2007 and 2008. Trials were laid out in a randomized complete block design with four fertilization treatments: 20, 40, and 60kg N.ha⁻¹ supplied by DAP (18:46:0), cattle manure (40 kg Nha⁻¹) and an unfertilized control variant. The vegetables were harvested at 6, 7, and 8 weeks after planting and either refrigerated (4+1°C) or stored at ambient temperature (20+3°C) for a period of 4 days. The vegetables were then analyzed for total phenolic, quercetin, total and soluble oxalates content before and after storage. The total phenolic content decreased with increasing N, the extent of decrease depended on the level and source of N. DAP at 20kg Nha⁻¹ had the same effect on the phenolic content as that of cattle manure at 40kg Nha⁻¹. The levels of phenolics and quercetin increased with crop maturity while the converse was true for the oxalate content. During refrigeration, levels of phenolics increased whereas those of oxalates did not change appreciably. There was a decrease in phenolics content during storage at ambient temperatures. The vegetable amaranth accumulates higher levels of total phenolic and quercetin when grown with manure than with DAP for similar levels of N. Total phenolics and quercetin levels increased, while that of oxalates decreased, with maturity of the vegetables. The accumulation of oxalates during the periods considered normal for growth are low enough to maintain their levels within safe limits for human consumption. Storage of the fresh vegetables at 4°C for limited periods improves their quality by increasing total phenolics and quercetin contents that also fulfill beneficial health functions in the human body.

Keywords: vegetable amaranth, fertilizer, phenolics, oxalates, storage

1. Introduction

Vegetable amaranth, members of the genus *Amaranthus*, are widely grown in the tropics. They are a group of fast growing C4 herbs that yield a harvestable product within 4-6 weeks after planting, which makes them attractive to resource-poor farmers. In some countries such as Kenya, they are largely grown by women thus providing them with a degree of financial independence and health nutrition (IPGRI, 2003). The dual purpose amaranth, *Amaranthus hypochondriacus* for instance is an important source of proteins (Carlsson, 1980) and vitamins A and C as well as minerals such as iron, calcium and magnesium (National Research Council, 1984) for a majority of people living in sub-Saharan Africa. In Kenya, for example, the diet of lower socio economic groups consists largely of maize and various traditional leafy vegetables, such as amaranth.

Vegetables deliver health benefits in addition to fulfilling physiological nutritional needs (Hasler, 1998). Their

consumption is associated with protection against major diseases including cancer and cardiovascular diseases (Wargovich 2000; Kaur & Kapoor 2001; Scalbert et al., 2005). The protective action of the vegetables has been attributed to the presence of antioxidants (Prior & Cao, 2000). Studies have shown that the majority of the antioxidant activity may be from secondary metabolites rather than from vitamins C and E, and β -carotene alone (Kähkönen et al., 1999). Moreover, a positive correlation between total phenolics and antioxidant activity in some vegetables such as amaranth and fruits has been reported (Gil et al., 2000; Pyo et al., 2004; Khandaker et al., 2008).

Earlier studies have established the abundance of antioxidants in *Amaranthus* leaves (Sokkanha & Tiratanakul, 2006; Khandaker et al., 2008) and that there was a general trend towards increased antioxidant activity with increased total phenolics content in *Amaranthus tricolor* L. The beneficial effects of phenolics make it important to understand the circumstances they are synthesized and accumulated in these plants. Among the group of flavonoids, quercetin has been reported to be a strong antioxidant (Hollman, 1997). It has been found to chelate metals, scavenge oxygen free radical and prevent oxidation of low density lipoprotein in *in vitro* studies (de Whalley et al., 1990; Kandaswami & Middleton, 1994).

Amaranths in addition to their high nutritional value also accumulate high levels of anti-nutritional factors such as oxalates (Vityakon & Standal, 1989; Gupta et al., 2005). A growing body of evidence indicates that oxalate plays various functional roles in plants, including calcium regulation, plant protection and detoxification of certain metals (Franceschi & Nakata, 2005). For instance, several plants such as buckwheat, taro and rice exude and/or accumulate oxalate *in vivo* to cope with aluminum and lead toxicity (Ma & Miyasaka 1998; Yang et al., 2000). More importantly, oxalates can serve as an antioxidant to quench the oxidative burst during plant response to pathogen attacks (Cessna et al., 2000; Weir et al., 2006). A molecular genetic analysis confirmed a long-held hypothesis that calcium oxalate crystals act as an effective defense against chewing insects (Korth et al., 2006).

Despite their functional roles in plants, excess levels of oxalate in plants have long been a concern to human nutrition and health (Franceschi & Nakata, 2005). The high levels of oxalate in any edible parts of vegetables and food crops significantly lowers their nutritional quality and lead to health concerns. Soluble oxalate decreases calcium bioavailability by forming calcium oxalate crystals (Radek & Savage, 2008) which can also lead to the formation of kidney stones (Massey, 2003). Studies have shown that availability of plant nutrients can be an important factor in determining the activity of secondary metabolism within plants (Kopsell et al., 2003; Aires et al., 2006). Nitrogen is one of the most important factors controlling the yield and quality of vegetables (Juan et al., 2008). In developing countries, such as Kenya, nitrogen is often supplied as manure because of the prohibitive cost of synthetic fertilizers. However information on the effect of manure or inorganic fertilizers on the polyphenols and oxalic acid contents of vegetables is still scanty. Only a few studies (Stout et al., 1998; Juan et al., 2008) have investigated the effect of different levels of nitrogen on the total phenolics and oxalate contents of vegetables.

Secondary metabolites are also known to vary in amount and content depending on the age of the plant (Dumas et al., 2003). Although it has been reported that there exists a large variability in the levels of phenolic compounds at various stages of maturation (Ellnain-Wojtaszek et al., 2001) of some leafy vegetables, the changes that occur in the content of these chemicals at different growth stages of traditional leafy vegetables found in Sub-Saharan Africa is poorly understood. Further, information on the fate of the harvested amaranth vegetables during storage is limited and even more so the fate of their constituents.

The present study was therefore designed to assess the levels of total phenolic, quercetin, and total and soluble oxalates in vegetable amaranth (var. *Amaranthus hypochondriacus*) grown using a mineral fertilizer diammonium phosphate (DAP) and cattle manure, during different stages of maturity. The changes in the levels of these substances during storage at ambient and refrigerated temperatures were also investigated.

2. Materials and Methods

2.1 Site Description

The experiment was conducted at the University of Nairobi field station, Kabete Campus during the long rain seasons (March - May) of 2007 and 2008. Kabete is situated about 15 km to the West of Nairobi city and lies at Latitude 1° 15'S and Longitude 36° 44'E, and at altitude 1930 m above sea level (Sombroek et al., 1982). Kabete has a bimodal distribution of rainfall, with long rains from early March to late May and the short rains from October to December (Taylor & Lawes, 1971). The mean annual temperature is 18°C. The soils in Kabete are characterized as deep, well drained, dark reddish-brown to dark brown, friable clay (Mburu, 1996).

2.2 Experimental Design and Treatments

The trials were laid out in a randomized complete block design with five fertilization treatments replicated three times as follows: one plot with cattle manure at 40kg Nha⁻¹, three plots with DAP at 20, 40, and 60kg Nha⁻¹, and an

unfertilized control plot. The recommended rate of N for vegetable amaranth is 40 kg N ha⁻¹. The different rates were selected to evaluate the N effects on phenolics and oxalates when the amaranth is supplied with either half or one and half the recommended rate of N.

2.3 Agronomic Practices

Amaranth seeds were obtained from Asian Vegetable Research and Development Centre (AVRDC) Tanzania. The seeds were sown in a seedbed fertilized with either manure or diammonium phosphate (DAP; 18:46:0) fertilizer. The plots measured 2 m x 2 m with inter-row and intra-row spacing of 0.15 m x 0.10 m (Palada & Chang, 2003). Care was taken in this trial to use a manure treatment and, where possible, cultivation practices that were comparable to those employed by local small-scale growers. Four seeds were planted in each hole, but two weeks after germination, the seedlings were thinned to one per hole. Irrigation was used whenever rainfall was insufficient to keep the soil moist at all times to avoid moisture stress. Physical weeding was done twice during the growing period using a hand hoe. The vegetables were harvested at 6, 7, and 8 weeks from planting by cutting-off the tender edible stems and leaves.

2.4 Storage Trials on the Vegetables

Bundles of average weight 0.45 kg from each fertilizer treatment harvested at 6, 7 and 8 weeks after planting were stored at ambient (20±3°C) and refrigerated (4±1°C) temperatures for 4 days. Initially and at 2 days interval, the vegetables were analyzed for total phenolics, quercetin, total and soluble oxalates.

2.5 Sample Preparation and Analysis

The edible parts of the vegetables which consisted of leaves and tender stems were separated and freeze-dried. The residue was ground to pass through a 600µm sieve. The powder was stored in tightly closed plastic vials in a freezer (-20°C) to await analyses for total phenolics, quercetin and total and soluble oxalates.

2.6 Determination of Total Phenolics

The total phenolic contents of the filtrate were measured with a slightly modified method of Ragazzi and Veronese (1973). To 1.0 mL of filtrate in a 25mL volumetric flask, 1mL Folin's Reagent (1 N) and 2 ml of 20% Na₂CO₃ were added and mixed thoroughly with a cyclomixer. The mixture was made to 25 mL with distilled water then allowed to stand at room temperature for 30 minutes. The absorbance of the mixture was measured at 720nm on a UV-Visible spectrophotometer (UV mini 1240, spectrum bandwidth 5nm, Shimadzu Corporation, Tokyo, Japan). The concentration of total phenolics in the filtrate was determined using a standard curve prepared from known concentrations of pure gallic acid aqueous solutions subjected to the same analytical procedure. The concentration in the filtrates was used to calculate the total phenolic contents of the vegetables in mg gallic acid equivalent /g on a dry weight basis.

2.7 Determination of Quercetin

Quercetin content was determined using the method of Bovy et al. (2002) with slight modifications. Into a 50ml plastic centrifuge tube, 50mg of the freeze-dried powder was weighed and 4ml of 62.5% aqueous methanol containing 2g/L tert-butylhydroquinone (TBHQ) were added, followed by 1 ml 6N HCL and carefully mixed. The centrifuge tubes were closed and placed in a shaking water bath at 90°C for 2 hours. The extract was cooled, and made to 10ml with methanol and placed in an ultrasonic bath for 5 minutes. This was followed by centrifugation at 1360g for 3 minutes. An aliquot of 1.5 ml was then transferred to an Eppendorf tube and centrifuged at 3600g for 10 min. A 1ml sample of the supernatant was then injected and eluted through HPLC (Column: Waters Nova-pak C18, 4µm, 3.9x150mm; Eluent: 25% acetonitrile in 0.1% trifluoroacetic acid; Detection: Dionex UVD 340S at 370nm; Flow: 0.9 ml/min; Injection: 20µl; Temperature: 30°C). The concentration of quercetin was determined from a standard curve prepared from known concentrations of pure quercetin in methanol.

2.8 Determination of Total and Soluble Oxalates

For determination of total oxalates, 0.1g of powder was weighed and mixed with 30ml of 1M HCl. Each mixture was then shaken in a water bath at 100°C for 30min. To each mixture was added 0.5ml of 5% calcium chloride and thoroughly mixed to precipitate out the calcium oxalate. The suspension was centrifuged at 800g for 15minutes and the supernatant decanted. The pellet was washed twice with 2ml of 0.35M NH₄OH then dissolved in 0.5M H₂SO₄. The solution was titrated with standard solution of 0.1M KMnO₄ with the temperature being maintained at 60°C to a faint violet color that persisted for at least 15 seconds (AOAC, 1999). For soluble oxalates, the same procedure was used except the extraction was done using 30ml distilled water. The total and soluble oxalate contents were calculated as percent of dry weight.

2.9 Statistical Analysis

Data obtained from both fresh and stored samples were subjected to the general analysis of variance (ANOVA), using Genstat statistical software (Payne et al., 2006) to check for both main effects of treatments and their interactions. Fisher's least significant difference (LSD) test was used to identify significant differences among treatment means ($P < 0.05$).

3. Results and Discussion

3.1 Total Phenolic and Quercetin Contents

The levels of total phenolics across all treatments ranged between 50 and 80 mg GAE/g dry weight (Figure 1). These levels are higher than the 45.5 mg/g recorded in leaves of *Larrea tridentata* (D.C) a desert shrub (Hyder et al., 2002), but are less than 98.6 mg/g recorded in leaves of other desert shrubs (Hollechek et al., 1990). The phenolic contents differed significantly ($P < 0.05$) across the sources of N and stage of maturity. The highest content was recorded in the manure and control plots at all maturity stages. The use of DAP led to a decrease in the phenolic contents. The effect of DAP on total phenolics content was increased with increasing levels of N. This could be probably due to the effect that nitrogen has on growth rate of plant. As the carbon/nutrient balance theory predicts, in terms of a high plant photosynthetic activity relative to the nitrogen supply, the excess of carbon is allocated to phenolics, a nitrogen-free defense substance (Yongke et al., 2005). Recent work by Sousa et al (2008) demonstrated an overall trend of higher total phenolics concentration in organically grown tronchuda cabbage, accompanied by lower plant fresh weight, as compared with conventionally fertilized samples. They suggested that the lack of nutrients, particularly insufficient nitrogen supply as a result of a low mineralization rate under organic production, could have boosted synthesis of phenolic compounds while limiting rapid growth of new leaves.

These results are in agreement with those of Li et al. (2008) who found that the total phenolics concentrations of leaf mustard (*Brassica juncea* Coss) were considerably decreased by increasing nitrogen supply. This negative correlation between nitrogen application and total phenolics could be explained by the protein competition model (PCM) (Jones & Hartley, 1999). The PCM hypothesis makes a contingent prediction: when biomass increases in response to elevated nitrogen nutrition, phenolic concentrations will decline because increased protein demand for growth will decrease partitioning of carbon skeletons to phenolics (Jones & Hartley, 1999).

Additionally, water availability, mineral and organic nutrients of the soil have been found to have a marked effect on the phenolic contents of plants (Barberan & Espin, 2001). Under conditions of abundant insulation normally encountered in the tropics, the rate of photosynthesis of carbohydrates may be so high that if the levels of available nitrogen and minerals are low, they get quickly depleted by synthesis of primary metabolites. The excess carbohydrates can then only be shunted to production of nitrogen- and mineral-free molecules such as the phenolic compounds (Waterman et al., 1984). This is probably what happened with the plants in the control and manure plots. It has also been shown that the biosynthesis of phenolic compounds can be induced by sunlight (Harbowy & Balentine, 1997). This could explain the higher levels of phenolics recorded in vegetable amaranth used in this study which was grown under tropical conditions where sunlight is not limiting. The presence of these compounds in green stems and leaves is related to deterring herbivory, with protection of photosynthetic tissues from damage by ultra-violet radiation being a secondary function (Gonzalez-Coloma et al., 1988).

Quercetin: There were significant differences in quercetin content between the two years of study. Significantly low amounts of quercetin were recorded in year 1 than in year 2 (Figure 2). In year 1 there were no clear differences in quercetin content across the different sources of N. In year 2 there were distinct differences with manure 40 kg N ha⁻¹ giving higher contents of quercetin than DAP at the same rate. Just as observed for the total phenolics, the quercetin content decreased with increasing rates of N supplied as DAP (Figure 2). The marked differences in quercetin content between year 1 and 2 may be attributable to sunshine hours the crop was exposed to during the growing season and the cooler air temperatures in year 1 than in year 2 (Table 1). Patil et al (1995) have shown that meteorological factors (including temperature and rainfall patterns) have a stronger influence on quercetin concentration in onion cultivars than soil factors or plant maturity. Thus, the most critical factors for the accumulation of flavonoids like quercetin (e.g. temperature and rainfall) are also the ones that are more difficult to control in open air.

There was a significant difference in content of quercetin between fertilizer treatments and time of harvesting in year 2 (Figure 2). The quercetin contents increased significantly ($P < 0.05$) between weeks 6 and 8 in all treatments except with the 60 kg N ha⁻¹ DAP in year 2. The highest increase occurred at week 8 when 20 kg N ha⁻¹ DAP and 40 kg N ha⁻¹ manure were used. Use of higher levels of N supplied as DAP (40 kg N ha⁻¹) resulted in a slight increase in quercetin content between week 6 and 8. At 60 kg N ha⁻¹, the quercetin content of week 6 harvest was almost equal to that of week 8 harvest. Martirosyan et al. (2003) found that rutin is mostly produced by *Amaranthus* in the

stage of blossoming. According to Kandil et al. (2004), rutin can be changed into quercetin or other compounds during leaf senescence. In this study, the vegetable amaranth started flowering at week 8 and this could explain the increase in quercetin content in all treatments.

Table 1. Weather summary during the growing period of vegetable amaranth

2007					2008				
Month	Week	Rainfall per week (mm)	Temperature per week (°C)	Sunshine per week (Hrs)	Month	Week	Rainfall per week (mm)	Temperature per week (°C)	Sunshine per week (Hrs)
March	1	0.1	19.7	9.5	March	1	3.1	20.2	9.0
	2	13.3	20	10.5		2	1.7	19.9	8.8
	3	23.4	20.5	9.2		3	35.5	21.0	8.0
	4	7.0	18.9	7.0		4	137.6	19.5	3.7
	5(2days)	15.7	20.0	6.4		5(2days)	0.2	18.7	6.3
April	1	3.8	20	5.2	April	1	0	19.0	9.1
	2	124.1	19.7	2.1		2	84.9	18.7	5.9
	3	441.7	19.5	4.4		3	16.7	19.1	8.3
	4	204.1	18.9	6.7		4	102.6	18.1	6.6
	5(2days)	5.4	19.5	8.4		5(2days)	0	19.3	7.4
May	1	1.0	18.8	6.4	May	1	0	18.2	8.2
	2	15.2	18	3.7		2	2.5	19.0	7.0
	3	64.5	18.8	5.8		3	0	17.6	6.4
	4	17.0	18.7	7.3		4	8.0	17.2	4.2
	5(3days)	85.6	18.6	6.1		5(2days)	1.2	17.1	1.0

Source: Kabete Meteorological station, Nairobi, Kenya

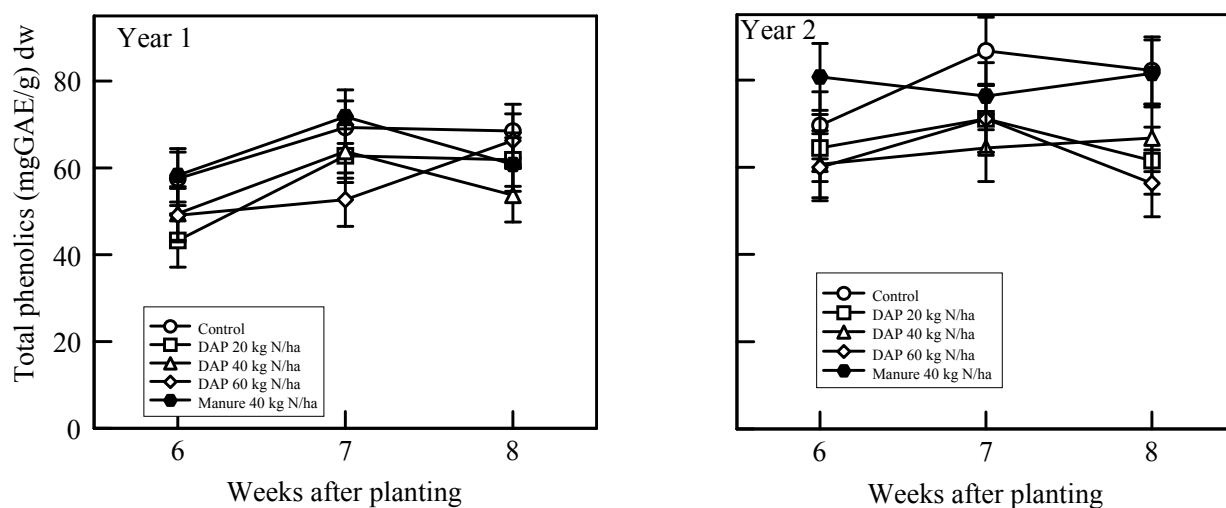


Figure 1. Effects of diammonium phosphate fertilizer, manure and maturity at harvest on total phenolic content of vegetable amaranth (*A. hypochondriacus*) (GAE = Gallic acid Equivalent)

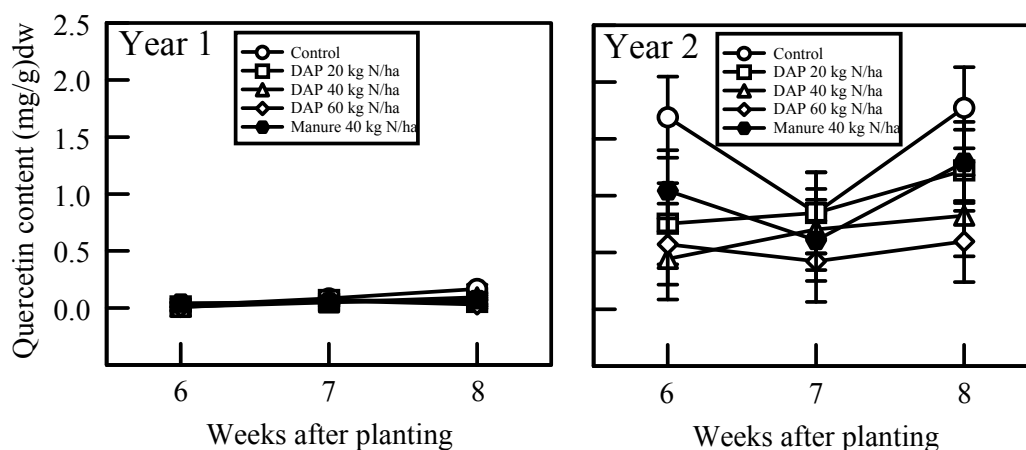


Figure 2. Effects of diammonium phosphate fertilizer, manure and maturity at harvest on quercetin content of vegetable amaranth (*A. hypochondriacus*)

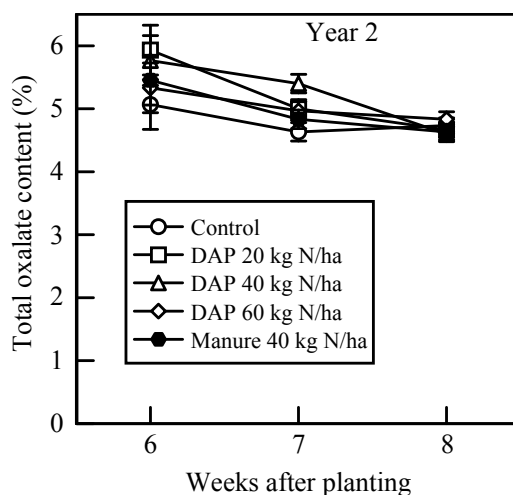


Figure 3. Effects of diammonium phosphate fertilizer, manure and maturity at harvest on total oxalate content of vegetable amaranth (*A. hypochondriacus*)

3.2 Total and Soluble Oxalates

There were no significant differences between treatments in terms of the total and soluble oxalate contents (Figure 3). The total oxalates decreased with stage of maturity of the vegetables for all treatments but this decrease was not significant ($P < 0.05$). These results are comparable with those of Kitchen and Burns (2006) working with Dark Green Bloomsdale spinach who found that total oxalate content was maximum at 32 days after planting and decreased subsequently as the plants developed vegetatively. Some weeds have been known to accumulate compounds such as oxalates to act as a defense against chewing insects (Korth et al., 2006). This could be the case in *Amaranthus hypochondriacus*.

There were no significant differences in soluble oxalates across the fertilizer treatments as well as maturity at harvest (Figure 4). The soluble oxalates remained unchanged through the growing period. The soluble oxalate contents ranged between 3 and 4% dry weight in all treatments (Figure 4). This agrees with results reported by Rahman et al working with napier grass (*Pennisetum purpureum* Schumacher) who found that the soluble oxalate content was not change with increased levels of N fertilization in all cut forages. Schmidt et al (Schmidt et al., 1971) reported 2.9% in *Amaranthus cruentus* L., while Radek and Savage (Radek & Savage, 2008) reported 4.7% soluble oxalates in *Amaranthus viridis* and 4.4% in *Amaranthus cruentus*.

The results (Figure 4) fall between the ranges reported by Schmidt et al (1971) and Radek and Savage (2008). This translates to consumption of between 0.5g and 0.6g of oxalates per 100g of fresh vegetable. The oxalate levels in the vegetables that received manure and DAP were within safe limits as long as consumption does not exceed the equivalent of 800g of fresh leaves per day (Schmidt et al., 1971) which is far above the amounts normally

consumed, for example, in Kenya which are 200g fresh weight per day (Personal observation). Cooking by boiling has been shown to significantly reduce the amount of soluble oxalates in *Colocasia esculenta* (L) Schott, and *Oxalis tuberosa* (Catherwood et al., 2007; Albihn Savage, 2001) through leaching into the cooking water. Since vegetable amaranth is cooked before consumption, it is expected that during cooking, which involves boiling in water which is thereafter discarded the levels of soluble oxalates and therefore the levels of total oxalates in the vegetables as consumed will be substantially reduced.

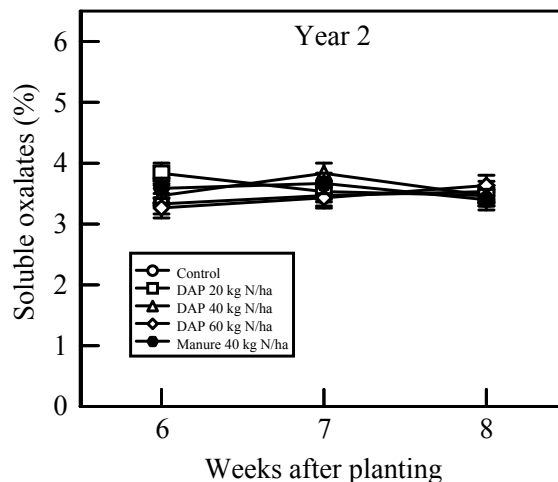


Figure 4. Effects of diammonium phosphate fertilizer, manure and maturity at harvest on total oxalate content of vegetable amaranth (*A. hypochondriacus*)

3.3 Total Phenolics and Quercetin Content during Storage

There were no significant differences in the levels of total phenolic and quercetin contents during storage for both years. The N source and levels did not cause significant changes on total phenolics and quercetin contents during storage. However, maturity at harvest as well as storage temperature caused in significant changes in the phenolics and quercetin contents ($P < 0.05$). There was a gradual decrease in total phenolic throughout the storage period. The decrease in the phenolics was greater when the vegetables were stored at ambient temperatures (Figure 5). For quercetin (Figure 6), there was a slow initial increase after 2 days in storage followed by a gradual decrease after 4 days in storage under refrigeration (4°C). Storage of the vegetable at ambient ($20 \pm 3^{\circ}\text{C}$) temperatures led to an accelerated decrease of the quercetin content.

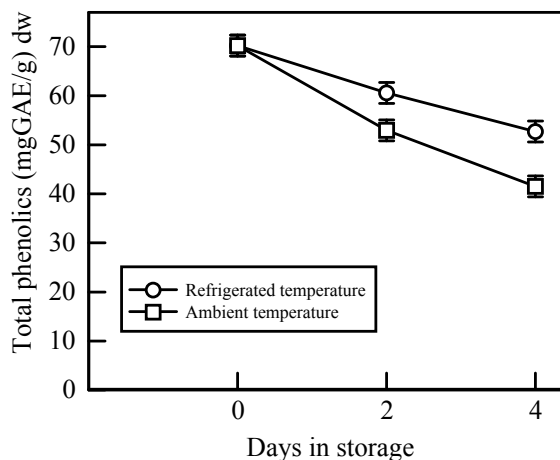


Figure 5. Effect of temperature and duration of storage on total phenolic content of vegetable amaranth

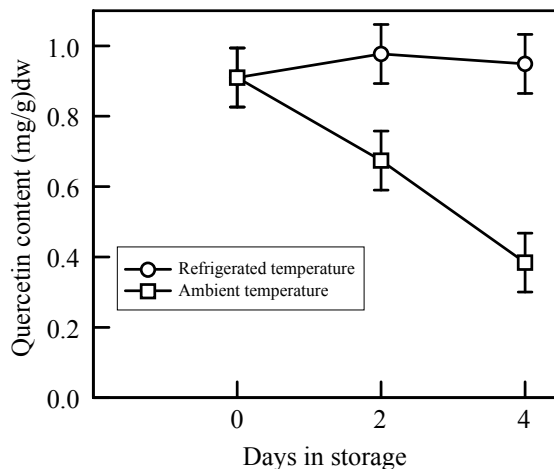


Figure 6. Effect of temperature and duration of storage on quercetin content of vegetable amaranth

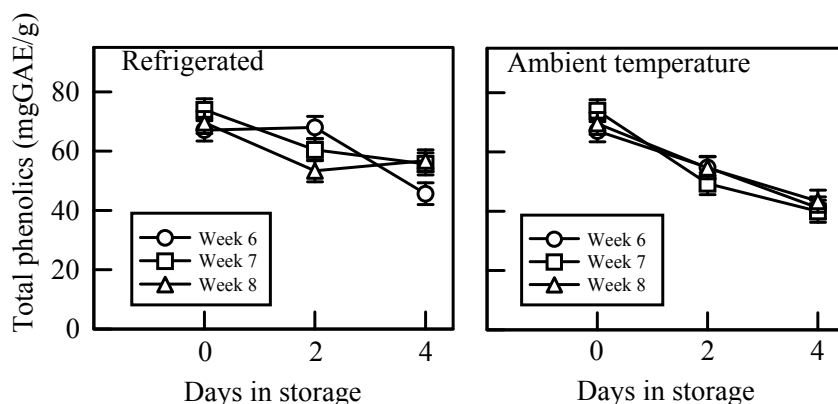


Figure 7. Effect of maturity at harvest, temperature and duration of storage on total phenolic content of vegetable amaranth (*A. hypochondriacus*)

Changes in both total phenolics and quercetin content depended on the maturity at harvest. Vegetables harvested 6 weeks after planting and stored refrigerated had their total phenolics content increase after 2 days followed by a gradual decrease (Figure 7). For vegetables harvested 8 weeks after planting, the increase in total phenolics was recorded after 4 days in refrigeration. Vegetables harvested at 7 weeks after planting had a non-significant increase in total phenolics after 2 days in refrigeration followed by a gradual decrease. Unlike total phenolics, quercetin content of vegetables harvested 7 and 8 weeks after planting increased significantly while that of the 6 weeks harvest decreased when the vegetables were refrigerated (Figure 8).

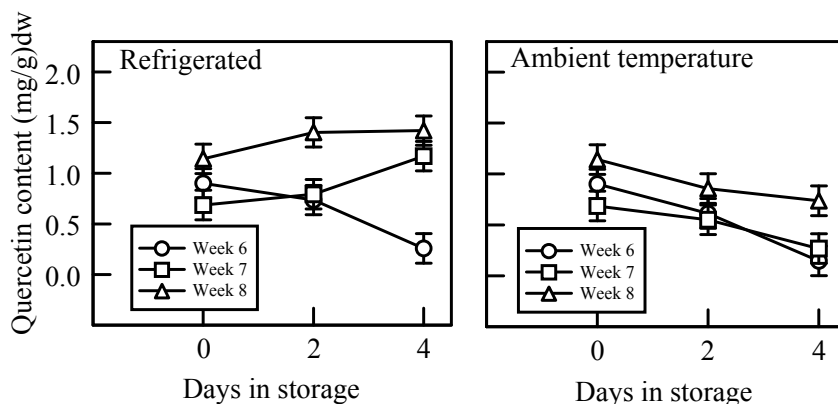


Figure 8. Effect of maturity at harvest, temperature and duration of storage on quercetin content of vegetable amaranth (*A. hypochondriacus*)

Venere et al (2005) storing escarole (*Cichorium endivia* L. *latifolium* group) and radicchio (*Cichorium intybus* L. *rubifolium* group) at 4°C found that in escarole, the most abundant phenolic compound increased markedly during storage after the fifth day. In radicchio, the phenolic content showed marked fluctuations during storage: after an initial increase, it showed a gradual decrease, followed by a sharp increase around the ninth day in storage. The current results are comparable with those of Venere et al (2005) although our storage period was limited to 4 days after which the amaranth becomes unsaleable.

The total phenolics and quercetin contents increased after 2 or 4 days in storage at refrigerated (4±1°C) temperature depending on the stage of maturity at harvest. These observations agree with reports by Souzan and Abd El-aal (2007) and Rivera et al. (2006), both groups working with lettuce. They found that the phenolics content increased during low storage temperature reaching a maximum after 2 and 3 days, then decreased marginally until the end of 8 days in storage. The basis for the increase is not easy to explain, but there is a possibility that depending on the amount and type of stress, the synthesis of phenolic compounds may be induced as a result of increased transcription of genes encoding the enzymes in their biosynthetic pathway (Ferrante et al., 2009); the synthesis of phenolics is known to be up regulated in exposure to light, cold and wounding (Tavarini et al., 2007). While this model is attractive its relevance to *Amaranthus* which is a tropical genus and therefore probably chilling sensitive would need to be confirmed. In storage at high temperatures, most of the phenolics may be lost in the respiration and oxidation processes which results in the decrease observed in this study (Figure 5).

3.4 Total and Soluble Oxalates during Storage

There were no significant changes in both total and soluble oxalates during refrigeration as well as ambient temperatures of storage. The fresh samples had an average total oxalate content of 5.1% which after storage for 4 days in the refrigerator was found to be 5% and while that of the material stored at ambient temperatures was 5.2%. The soluble oxalate content of the fresh samples was 3.4% which increased to 3.5% after storage for 4 days in the refrigerator and to 3.7% when stored at ambient temperatures. A small increase in soluble oxalates during storage has been shown to be related to an overall loss in moisture content in other vegetables (Catherwood et al., 2007). This can explain the small increase shown in vegetable amaranth after 2 and 4 days in storage. These results indicate that minimal changes occurred in soluble oxalates during storage. This is in agreement with those of Kuan (2003) working with soyabeans who found that the levels of soluble oxalates remained constant during 6 months of storage at -20°C, 4°C and 25°C; the temperatures used 4°C and 25°C were similar to those used in this study, albeit considerably shorter storage periods were involved.

4. Conclusion

The vegetable amaranth (var. *Amaranthus hypochondriacus*) accumulates higher levels of total phenolic and quercetin when grown using manure compared to DAP for similar levels of N (40 kg Nha⁻¹). The total phenolics and quercetin levels increase with maturity of the vegetables whereas the converse was true for the oxalates. The accumulation of oxalates during the periods considered normal for amaranth growth were low enough and hence within safe limits for human consumption. Low temperature storage of *A. hypochondriacus* contributes to the maintenance of acceptable levels of both phenolics and quercetin. Both total and soluble oxalates do not change significantly during storage of vegetable amaranth (var. *A. hypochondriacus*) for up to 4 days.

The contents of total oxalates remained unchanged during storage under both ambient and refrigerated storage. It is possible that these conditions do not influence the content of oxalates found in this vegetables. The quantities of soluble oxalates found in *Amaranthus hypochondriacus* used in this study are therefore not high enough to cause public health concerns. From the nutritional point of view therefore, vegetable amaranth consumption offer full beneficial health functions in the human body. Further research on the effect of other types of fertilizers and different modes of application is recommended.

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