NUTRIENT AND ANTINUTRIENT CONTENTS OF RAW, COOKED, SUNDRIED AND STORED VEGETABLE AMARANTH GROWN IN DAR ES SALAAM, TANZANIA.

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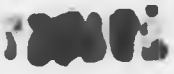

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DECLARATION

I, Mziray Rajabu Salim, hereby declare that this thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to my wife Leila for her love and care, my son Salim, my sisters and brothers for their support and to my late father and mother who passed away some years back.

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ABSTRACT

Amaranthus hybridus was grown for leafy vegetables in four sites in Dar es Salaam, Tanzania. Freshly harvested leaves from each of the growing sites were analyzed for proximate composition, ascorbic acid, beta-carotene, minerals iron, calcium, phosphorous and lead; and the antinutrients oxalates and nitrates.

The leaves from the four sites were then bulked. Samples from the bulk were cooked by boiling in distilled water and drained. The drained vegetables were analyzed for ascorbic acid, beta-carotene, iron, calcium, phosphorous, lead, oxalate and nitrate. Also samples from freshly harvested bulks were blanched and sundried in a shade provision drier. The dried samples were stored in polythene bags at 22°C, 28°C and room temperature (30°C to 32°C). At the beginning and thereafter every month during storage, the vegetables were analyzed for beta-carotene and ascorbic acid and dried samples stored at 22°C were subjected to sensory evaluation in comparison with fresh cooked leaves.

Results indicated that fresh amaranth vegetables from the four sites had comparable high moisture contents ranging between 85.3% to 86.5% and levels of beta-carotene of between 25.2 mg/100g to 37.3 mg/100g, ascorbic acid of between 455 mg/100g to 535 mg/100g, protein of between 28.2% to 31.6%, and the mineral calcium between 2062 mg/100g and 2263 mg/100g, iron between 108 mg/100g and 128 mg/100g and phosphorous between 500 mg/100g and 553 mg/100g on dry weight basis. They also had antinutrients such as nitrates and oxalates at levels of between 501 mg/100g to 560 mg/100g and 3383 mg/100g to 4333 mg/100g dwb respectively.

On cooking of the vegetables there were significant reductions ($P < 0.05$) in the levels of vitamin C of up to 50.4%, minerals of up to 41.4% for phosphorus, nitrate 39.2%, and oxalate 40.2%. Sundrying of the amaranth leaves also resulted in significant reductions

($P < 0.05$) in the levels of ascorbic acid and beta-carotene by 87.4% and 16.3% respectively.

After storage of the dried vegetables for three months, the retention at 22°C, 28°C and room temperature of ascorbic acid and beta-carotene were 42, 40 and 36 mg/100g and 32.4, 29.5 and 27.4 mg/100 g dwb respectively.

Sensoric quality evaluation of amaranth leaves showed that significant differences ($P<0.05$) existed in appearance and colour, flavour, texture and overall acceptability, although the dried vegetables were still acceptable in all the sensory characteristics tested.

The study established that amaranth vegetables grown in four sites of Dar es Salaam were good sources of nutrients, had low nitrates levels but high oxalate contents. Furthermore the cooking losses were not excessive. The leaves could be dried to produce an acceptable product which maintained its eating quality for up to three months in storage.

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CHAPTER ONE

1. INTRODUCTION.

Amaranths (African spinach, Pig weed) are among the important traditional vegetables of Tanzania. They are consumed cooked, often by boiling in large volumes of water which is thereafter discarded, then stewed in onion, tomato, oil or groundnuts and condiments. They are then eaten as side dishes for *ugali* (a paste made from maize meal) (Imungi, 1984). The most common variety used in this form is *Amaranthus hybridus*.

Amaranth is usually grown in small vegetable gardens mainly in towns, but also in rural areas, with or without irrigation. In towns the most common growing sites are the road sides and river valleys. In these places, the vegetables are exposed to exhaust fumes from automobiles, and to effluent from the formal and the cottage industries which often dump their effluent waste into rivers like the Msimbazi river. The fumes from automobiles and effluent from the industries contain the heavy metal lead. Manufacture of products containing lead as well as the number of automobiles are on the increase in developing countries, and therefore the metal is increasingly becoming an important public health problem. The major consequence of environmental contamination by lead and other heavy metals is toxicity to plants, man and animals (Adaudi *et al.*, 1990; Assey, 1995; Isabel and Concepcion, 1997).

The population of Tanzania depends predominantly on plant foods as source of micronutrients. Amaranth leaves are a good source of fibre, protein, vitamin C and carotene (pro-vitamin A). The vegetables are particularly rich in minerals such as calcium, iron, and phosphorous (Ifon and Bassir, 1979; Graham, 1983; FAO, 1988; Dhan and Pal, 1991, 1992; Ricardo, 1993; Feliciano and Harold, 1998). Nevertheless, the main constraint to their nutritional exploitation is presence of some anti-nutritional and toxic principles such as nitrate, oxalate and saponin (Dhan and Pal, 1991).

Cooking methods for leafy vegetables in developing countries are variable. The most common method is boiling the vegetables in large volumes of water which are thereafter discarded (Imungi, 1984; Samuel, 1985; Imungi, 1990). This results in large losses of water-soluble nutrients but also anti-nutrients.

Green leafy vegetables are available within short season periods after which they are no longer available or when available, are quite expensive in the market. It would therefore be beneficial to carry out some form of preservation to increase availability during the time of scarcity. In Tanzania, limited preservation by sundrying is being practised by some communities around Tanga and Dodoma. The methods are, however, not standardized. Moreover, the extent of nutrient loss or retention of antinutrients during the process has not been established, neither has the extent of loss of the nutrients during storage of the dried vegetables. It has, however, been reported (Imungi, 1984; Beltiz, 1987) that losses of vitamin C and beta-carotene are heavy during storage of dehydrated leafy vegetables.

In spite of all this, however, sundrying of amaranth leaves would serve to preserve the vegetable and improve its availability during periods of scarcity.

This study was therefore designed to study nutrient and antinutrient contents of raw, cooked and sundried and stored vegetable amaranth grown in urban Dar es Salaam. The objective was achieved by addressing the following specific objectives.

1. To determine the proximate composition of raw vegetable amaranth.
2. To determine the nutrient and antinutrient contents of raw amaranth leaves.
3. To determine the nutrient and antinutrient contents of cooked vegetable amaranth
4. To determine the retention of ascorbic acid and beta-carotene in sundried amaranth leaves during storage.
5. To determine the organoleptic properties of the sundried and stored vegetable amaranth

CHAPTER TWO

2. LITERATURE REVIEW.

2.1. Nutritional Significance of Green Leaf Vegetables.

Green leafy vegetables are prized for their supply of micronutrients in diets. The traditional vegetables in Africa have been found to be nutritionally superior to the European type vegetables which are still consumed in some communities to satisfy a desire to be sophisticated (Imungi, 1984). The traditional vegetables are widely available and consumed as the principle source of protein, vitamins and minerals (George, 1985; Belitz, 1987; FAO, 1995). They are particularly rich in calcium, iron, zinc, potassium, carotenes (pro-vitamin A), vitamin C, riboflavin and niacin (Ifon and Bassir, 1979). The protein from the vegetables has been reported to have good levels of most essential amino acids (Graham, 1983; FAO, 1988; Dhan and Pal, 1991, 1992; Ricardo 1993; Feliciano and Harold, 1998).

Because they constitute a substantial proportion of the diets of the peasants, leafy vegetables could therefore be effectively used as means of combating specific nutrient deficiencies in such populations (Ifon and Bassir, 1979). Breinholt *et al.* (1995), Pobel *et al.* (1995) and Nijhoff *et al.* (1995) also reported that consumption of the vegetables reduced the risk of stomach and colorectal cancer.

2.2. Production and Utilization of Vegetable Amaranth.

2.2.1. Production and cultivation.

Vegetable Amaranth [*Amaranthus* spp, pigweed, African spinach, *Mchicha* (Swahili)] is a common leafy vegetable, and members of the genus are found throughout the tropics (Ayiecho, 1985; FAO, 1988). According to Grubben (1977) the three *Amaranthus* spp cultivated mainly for leaf vegetables in Africa are *A. cruentus*, *A. caudatus* and *A. hybridus*. Other African species of *Amaranthus* are *A. deflexus* L., *A. tricolar* L., *A. viridis* L., *A. sparqaniocephalus* Thell. and *A. thuberqii* Moq (FAO, 1988; Ricardo, 1993).

In Tanzania vegetable amaranth is grown in home gardens or on small plots using either labour intensive or less labour intensive practices. It requires optimal edaphic environmental and agronomic factors similar to those required by other agricultural crops, although bests yield are obtained on organic and mineral rich soils (Samuel, 1985). However, a wide variety of soils from silt, loam to saline types can be used (FAO, 1988; Dhan & Pal, 1991). The optimal soil pH range is 4.5 to 7.5, but some cultivars will tolerate more alkaline soil conditions. Amaranth grows best in lowland areas up to 800m and requires temperature of 22°C to 30°C and semi arid to humid conditions with rainfall between 400mm to 800mm per annum (George, 1985; FAO, 1988).

2.2.2. Food uses of amaranth in Tanzania

In East Africa like other tropical countries green leafy vegetables are only available during the rainy season (Imungi, 1984). However, irrigation is practised in some areas, even though it is more expensive compared to natural rain (FAO, 1988). Vegetable amaranths in Tanzania are grown mainly for home consumption and local marketing. For local consumption, the leaves are usually cooked and incorporated into a variety of dishes, soups and sauces. The cooking involves boiling the leaves, then stewing with onion, tomato, oil or groundnuts and condiments with or without meat and eaten as relish (Grubben, 1976; Imungi, 1984; FAO, 1988). Vegetable amaranths have also been used as animal fodder (Samuel, 1985). The seeds are also normally roasted, pounded to flour and used in the preparation of breads, pancakes, biscuits and many other dishes (FAO, 1988; Feliciano and Harold, 1998).

2.2.3. Other uses of vegetable amaranth.

In some areas, vegetable amaranths are used as medicine and as a source of dye. For example *A. hybridus* is used as tobacco substitutes and as a source of red dyes; *A. dubius* is used as browse and as a medicinal for stomach complaints; *A. cruentus* as a source of dye used by Indians in USA and Mexico (FAO, 1988). Also Aye *et al.* (1995) reported that aqueous extracts of *Amaranthus spinosus* was used to treat menstrual disorder in women.

2.3. Nutritional Value of Vegetable Amaranths.

Vegetable amaranths provide many important nutrients such as protein (Dhan and Pal, 1991; Feliciano and Harold, 1998), vitamins and minerals (Ifon and Bassir, 1979; Graham, 1983; FAO, 1988; Ricardo, 1993; FAO, 1995). Anti-nutrient factors are also present at levels that are comparable with other common vegetables. The main anti-nutritive factors in amaranth vegetables are oxalates and nitrates (Dhan and Pal, 1991). The usable protein in amaranth has been reported to be approximately 26-30% on dry matter basis (Imbamba 1973; Anon., 1984; Samuel, 1985; Dhan and Pal, 1992; Ricardo, 1993). Vitamin C and pro-vitamin A have been reported as important components of vegetable amaranth (Grubben, 1976; Maeda and Salunhke, 1981; Dhan and Pal, 1991; Ricardo, 1993). Vegetable amaranth contains significant amounts of minerals including iron and calcium that are important in human nutrition (Oke, 1980; Ricardo, 1993; Sean, 1993). The vegetables also contain oxalates which are thought to bind divalent cations particularly Ca^{2+} making them unavailable for metabolism. Studies have, however, shown that the oxalate and the nitrate levels normally found in amaranth do not pose a serious health problem to reasonably healthy individuals if consumption does not exceed 100g of leaf per day (FAO, 1988).

Toxicity problems have been reported in animals fed on vegetable amaranth (Marshall *et al.*, 1967; Spearman and Johnson, 1989; Ferreira *et al.*, 1991; Casteel *et al.*, 1994; Mekonnen, 1994; Binta *et al.*, 1996). However, most researchers have concluded that for

man, there is no danger of feeding on vegetable amaranth since the amount eaten per day is small and does not constitute any problem, given that the vegetables are crushed, boiled and the cooking water discarded, which removes much of nitrates and the soluble oxalates (Samuel, 1985).

2.3.1. Vitamin C (ascorbic acid).

Vitamin C is the anti-scurvy vitamin. Lack of the vitamin in diets causes fragile capillary walls, easy bleeding of the gums, loosening of teeth and bone joint diseases (FAO, 1995). The vitamin is necessary for normal formation of collagen, which is an important constituent of skin and connective tissue. Vitamin C enhances absorption of iron (Imungi, 1984; Latunde-Dada and Neale, 1986; FAO, 1995).

Fruits and vegetables are excellent sources of vitamin C (FAO, 1995; Ana and Lia, 1997). The values reported indicate that the ascorbic acid contents of vegetables vary considerably even within the same species due to variations in season, climate, agronomical and soil conditions (Phillip and Nancy, 1974; Imungi, 1984; Ricardo, 1993). Vitamin C contents of the amaranth leaves on dry matter basis vary from 491 mg/100g (Ricardo, 1993) to as high as 1700mg/100g (Maeda and Salunkhe, 1981).

Ascorbic acid, is easily destroyed in foods depending on a large number of factors such as processing temperature and conditions, and equilibrium relative humidity, oxygen partial

pressure, light, catalysts, package permeability and package configuration in storage (Labuza, 1972; Barth *et al.*, 1990; Ana and Lia, 1997), and it is the vitamin most easily lost during processing, cooking and storage (FAO, 1995). Blanched and dehydrated blanched cabbage showed losses of 20% and 30% ascorbic acid respectively (Thomas, 1968). It should be noted that sometimes, vitamin C losses are due to leaching into the cooking or processing water, enzymic degradation and chemical degradation particularly in the presence of traces of heavy metal ions (Belitz, 1987). Conservative processing and low temperature storage are critical for vitamin C preservation (Barth *et al.*, 1990). Nelson (1972) reports that vitamin C is a nutrient affected by most forms of processing. In view of this, its retention is used as index for retention of other nutrients (Maeda and Salunkhe, 1981).

2.3.2. Vitamin A.

Vitamin A occurs in plants as very closely related orange coloured precursors. (Meyer, 1960). Beta-carotene is the most effective precursor (Wu and Salunkhe, 1974). Carotenoids are also potent antioxidants, scavenging potentially harmful oxy radicals, some of which are commonly associated with inducement of certain cancers (Gareth *et al.*, 1998).

Deficiency of vitamin A is among the most widely spread and serious nutritional disorders that affect mankind, especially in developing countries (Imungi, 1984; Ricardo, 1993). The deficiency leads to night-blindness, failure of normal bone and tooth development in

the young, diseases of the epithelial cells and membrane of the nose, throat and eyes and decreases body's resistance to infection (Imungi, 1984; Belitz, 1987; FAO, 1995).

The levels of beta-carotene in amaranths are as high or even higher than those reported for other green vegetables (Maeda and Salunkhe, 1981; Ricardo, 1993). The values reported by the same authors ranged from 17 mg/100g to 110 mg/100g respectively, expressed as equivalent of carotene on a dry weight basis (dwb).

Beta-carotene is fat-soluble and therefore it is not affected by the washing and blanching steps, but is moderately destroyed (5 to 40%) during retorting in canning (Belitz, 1987). According to Lee *et al.* (1982) the carotene in blanched and canned peas appeared to be relatively unaffected by heat processing. The Beta-carotene content in blanched and canned samples were found to be slightly higher than those in raw samples. This may be due to an apparent gain caused by loss in soluble solids during processing or by more effective extraction from the tissue during analytical procedures facilitated by the heat process (Imungi and Potter, 1983, 1985). Gareth *et al.* (1998) reported total carotenoid losses of 13-20% during fluid bed drying of *Dunaliella salina*. The author also suggested that carotene stability can be improved by storage in reduced oxygen and in the dark. It is well documented that dehydration of leafy vegetables results in substantial destruction of their carotene contents (Gomez, 1981; Maeda and Salunkhe, 1981).

2.3.3. Minerals.

Green leafy vegetables are one of the main sources of minerals in the diets of most people living in the tropics. Iron, phosphorous, calcium, magnesium and zinc are of the minerals of major nutritional importance (FAO, 1995). Amaranth leaves have high levels of iron, calcium, phosphorous, magnesium and potassium (Ricardo, 1993). Since iron has an important role as a constituent of haemoglobin, calcium and phosphorous are important in the functions of the body. Hence, these three minerals are considered in some detail below.

2.3.3.1. Iron.

Iron is found in foods derived from both animals and plants and may be present either as haem or as nonhaem compounds (Chipperfield, 1993). Iron as haem is an essential component of haemoglobin (red pigment in blood), myoglobin (muscle tissue pigment) and active groups in enzyme and electron carriers (cytochrome) (Imungi, 1984; Chipperfield, 1993; FAO, 1995). Although haem proteins are most common in animals, photosynthesis in plants also involves similar haem proteins (Chipperfield, 1993; Sean, 1993).

Iron deficiency has been shown to be the most common cause of anaemia in the World (WHO, 1972). Negative iron balance leads to a reduction in the iron content of all functional components, which results in anaemia When iron availability to support metabolic systems in the tissues is reduced, the main physiological consequences are

impaired oxygen delivery and reduced metabolic rate. Anaemia caused by tissue iron deficiency per se results in mucosal and epithelial abnormalities, deterioration of immunity leading to easy infection, skeletal muscle dysfunction and also behavioural and neurological abnormalities (Sean, 1993). The green leafy vegetables native to many tropical countries have iron levels that are superior to vegetables that are considered good source by European Standards (Imungi, 1984). The concentrations in vegetable amaranths, vary from 0.05 to 0.5g/100g (dwb) (Ifon and Bassir, 1979; Ricardo, 1993).

The main problem with iron is that, not all of it in the food is physiologically available. Bio-availability refers to the amount that is actually absorbable through the gastro-intestinal tract from a meal. It has been established that iron availability is highest in diets rich in animal products than foods of plant origin (Baker and DeMaeyer, 1979). Addition of meat or ascorbic acid to a vegetable diet enhances iron absorption (Monsen *et al.*, 1978). On the other hand the presence of calcium salt, high roughage content and oxalate may negatively influence iron absorption (Imungi, 1984).

2.3.3.2. Calcium.

Calcium is the most abundant cation in the body. This abundance generally reflects the presence of hard tissues containing an inorganic matrix, impregnated with salts in which calcium is the major cation. Calcium is usually found in combination with carbonates or

phosphates as a crystalline extracellular deposit. It has a major structural function in skeletons and teeth (Imungi, 1984; Belitz, 1987).

Deficiency of calcium will result in offset in the balance of all processes requiring calcium (enzyme activation). This can result in dental carries, rickets and osteomalacia (Walker, 1972). The daily requirement of calcium is 0.8 to 1.0g (Belitz, 1987).

Vegetables contain more calcium than fruits. For example, green beans, cabbage and onion contain more than 0.1% of calcium, while most fruits contains less than 0.1% calcium (FAO, 1995). The calcium/phosphorus (Ca/P) ratio is essential for calcium fixation in the human body, this ratio being considered normal at 0.7 for adults and 1.0 for children (Molins, 1991; FAO, 1995). Gomez (1982) reported that the calcium contents of some Kenyan vegetables, ranged from 55 to 618 mg/100g of edible portion. Ifon and Bassir (1979) and Ricardo (1993) reported that levels of calcium in vegetable amaranth ranged from 2 to 3 g/100g (dwb)

Calcium bio-availability from vegetables is dependent on the specific vegetable itself. Vitamin D is required for efficient calcium absorption (Hegsted, 1973). Oxalic acid is the main constituent in some foods that limits calcium utilization. However, this is not of practical importance where the amount of calcium consumed is sufficiently liberal (Imungi, 1984).

2.3.3.3. Phosphorous.

Phosphorous exists in many allotropic forms, which reflect various modes of catenation (Ellinger, 1972; Belitz, 1987; Garg and Weginwar, 1993). The total phosphorous content in an adult human body is 700g, its daily requirement is about 0.8 to 1.2g (Belitz, 1987).

Phosphorous is an essential nutrient, required for many different intracellularly and extracellularly functions in body tissues. This element, which exists in biological systems as phosphates, is used by cells to make structural molecules (John, 1993). It serves as a component of intracellular regulatory molecules and buffering component in both intra and extracellular fluids. It is an important factor in cellular energetics, where it is used to make most of the high -energy bonds needed for cellular activities (John, 1993; Garg and Weginwar, 1993).

The metabolism of inorganic phosphate (Pi) is closely linked to that of calcium, and therefore deal to some extent with calcium and its homeostatic control (John, 1993) Adequate phosphorous and calcium intakes are critical not only for skeletal growth, but also for growth and development of soft tissues (John, 1993; FAO, 1995).

Dietary phosphorous deficiency, although highly unlikely because of the abundance of this element in foods, contributes to low serum phosphate concentration and thereby limits bone mineralisation via osteoblast and the total amount of bone mineral mass deposited in the skeleton. Furthermore, phosphate deficiency increases bone turnover,

which during infancy, can lead to rickets (John 1993). Its deficiency in adults may occur due to excessive use of alcohol, prolonged vomiting, liver diseases and/or hyperparathyroidism (Garg and Weginwar, 1993).

Vegetables are richer in phosphorus than fruits (FAO, 1995), whereby leafy vegetables contain 0.02 to 0.5% (Garg and Weginwar, 1993). The levels of phosphorus in vegetable amaranth reported by Ricardo (1993) and Ifon and Bassir (1979) ranged from 0.45 to 0.7g% (dwb).

2.4. Antinutrients of Vegetable Amaranth.

There is a wide diversity of toxic constituents in plants (Imungi, 1990). These toxicants may occur either naturally, or as contaminants through environmental pollution (Flegal, 1993). The antinutrients in vegetable amaranth commonly consumed by residents of Dar es Salaam include lead, nitrate and oxalate due to increased in automobiles, industrial activity, uncontrolled disposal of industrial influent and excess use of inorganic fertilizers.

2.4.1. Lead.

Lead is widely recognised as a hazardous environmental pollutant due to its toxicity to all organisms (Adaudi *et al.*, 1990; Isabel and Concepcion, 1997) including human beings. Pathways for human exposure include food and food related products, household paint and water (Vather *et al.*, 1992). According to Albert (1993) and David and Craig (1994) up to 70% of the total human exposure to lead is through the diet.

Because lead is a naturally occurring element found in the earth's crust, it enters the diet at low background levels. However, the majority of dietary lead results from environmental pollution and from processing equipment and storage containers using lead in their construction. These could be solders, paints, ceramic, glaze and pipes (Liener, 1969; FAO, 1986; David and Craig, 1994; Dowd *et al.*, 1994). High levels of the lead will also be found in vegetables grown near highways, presumably due to contamination from exhaust fumes of automobiles using leaded gasoline (Liener, 1969; FAO, 1986; Gordon and Wayne, 1993;).

Accumulation of heavy metal differs according to plant species (Vasil *et al.*, 1995; Hooda *et al.*, 1997), the age of plants, the environmental conditions in which the plant is grown and the part of the plant analyzed (Albert, 1993; Isabel and Concepcion, 1997). Seasonal differences in lead concentrations have been reported (Isabel and Concepcion, 1997). This can be related to the biochemical changes in plant during maturation, the older parts having higher concentration of heavy metals than the younger parts.

Leafy crops are most susceptible to contamination from atmospheric deposition of industrial lead. These crops include, wheat, rice, spinach and lettuce whose contemporary baseline lead concentration are 5 to 100 fold above natural concentrations (Flegal, 1993). According to Hooda *et al.* (1997) spinach tissues and leaves grown on the sludge amended soils accumulated substantially higher metal concentration than those grown on the uncontaminated soils. However, the copper and lead accumulation in the plants

grown in sludge amended soils showed only small increases compared to those grown on uncontaminated soil. Albert (1993) pointed out that the lowest lead concentrations are found in plants in which the edible portion is above the ground and shielded from airborne lead deposition.

Metal accumulation in plants is strongly affected by their concentration in soil (Singh and Jeng, 1993; Assey, 1995). Metal availability to plant varies with soil type and plants grown on soil with pH value in the neutral range and clayed texture tend to accumulate less metals than those grown on soil with acidic pH and a coarse texture. (Hooda *et al.*, 1997)

Food processing and preparation can contribute significantly to increased lead contents of food stuffs (Albert, 1993). According to David and Craig (1994) oxygen has been found to accelerate lead leaching into food from lead soldered cans.

Lead is a highly toxic trace element with no recognized biological requirement in organisms (Flegal, 1993). It has toxic effects on several organ systems. Blood-cell-forming system (haematological) is the first to be affected during lead poisoning. Other systems which get affected are:- the central nervous, renal, endocrine, and reproductive (Bryce, 1980; Bodwell and John, 1988; Albert, 1993; Gordon and Wayne, 1993). Other possible toxic effects of lead in man are less well defined and remain unsubstantiated. Some studies showed that, high level lead exposure in rodents can produce kidney

tumours, but there is no conclusive evidence that lead is carcinogenic in man (Albert, 1993). Again, there are experiments indicating that high doses of lead are toxic to the reproductive functions of both male and female laboratory animals but data on humans are limited and inconsistent (Albert, 1993; Gordon and Wayne, 1993). Dowd *et al.* (1994) reported that lead is known to impair some of the functional properties of osteocalcin, a small protein active in some mineralization and resorption. Lead induced inactivation of osteocalcin affect bone mineral dynamics and may be related to the observed inverse correlation between the blood lead level and stature and chest circumference observed in growing children. Lead toxicity in man is not serious as compared to that in animals (Clarke and Clarke, 1975; Albert, 1993). Infants and young children are more sensitive to lead and are subject to greater exposure than most adults, especially in urban areas, because of environmental pollution (Kuhnert *et al.*, 1985; Bodwell and John, 1988; Gordon and Wayne, 1993;).

All living creatures are continually absorbing lead and the toxic dose is that amount necessary to bridge the gap between this normal intake and potential dangerous level (Clarke and Clarke, 1975). Similar arguments have been made by Flegal (1993) that there may be no concentration threshold for lead toxicity in human and other mammals . Nevertheless, the maximum allowable concentration of lead in food recommended by WHO is 0.05 mg/l (Herscholdoefer, 1968). The US Food and Drug Administration

Advisory Panel suggest that no more than 100µg of lead per day be consumed from food (Gordon and Wayne, 1993).

2.4.2. Nitrates and Nitrites.

Natural occurrence of nitrates and nitrites in foods is a consequence of the nitrogen cycle. Nitrates are natural constituents of many soils and are also found in water and most growing plants (Mohri, 1993).

Leafy vegetables are the main contributors of nitrates in diets, usually accounting for more than 75% of the total amount ingested (Imungi, 1984, 1990; Michael, 1990; Mohri, 1993; Ricardo, 1993). The vegetables which contain relatively high levels of nitrate and small quantities of nitrites are cabbage, celery, lettuce, several root vegetables (e.g. carrot, beets), and spinach (Mohri, 1993). Increased nitrate and nitrite levels in these vegetables usually results from the use of nitrogenous fertilizers (Mohri, 1993; Ricardo, 1993). Extreme weather conditions such as prolonged cloudiness or drought can influence the nitrate level (Graham, 1983). The nitrate contents of plant vary considerably within varieties (Imungi, 1990) due to differences in season, growth location and age of the plant (Liener, 1969; Graham, 1983; Dhan and Pal, 1991).

Vegetable amaranths were suggested as potential plant sources for nitrate (Cannor *et al.*, 1980; Oke, 1980; Samuel, 1985; FAO, 1988; Spearman and Johnson, 1989; Dhan and

Pal, 1991). Their nitrate contents range from 0.27% to 0.74% and can reach up to 3.25% on dry weight basis (Graham, 1983; Ricardo, 1993).

Acceptable Daily Intakes (ADI) by WHO for nitrate, and nitrite expressed as NO_3^- and NO_2^-, is 220mg and 8mg respectively, for a person of 60kg body weight. Nevertheless, these levels are not applicable to infants under 6 months of age, who are susceptible to methaemoglobinaemia (Mohri, 1993). Studies have shown that nitrate levels normally found in vegetable amaranths do not present a serious health problem to reasonably healthy individuals, if consumption does not exceed 100g of leaf per day (FAO, 1988).

Nitrates per se are not toxic at the levels normally present in food. The toxic chemical form of these nitrogenous compounds is nitrite. The toxicity of ingested nitrate is due to its *in vivo* reduction to nitrite (Mohri, 1993). It has been reported that a single dose of 30 to 35g of nitrate can be fatal to adults and more potent nitrite can kill if consumed at the level of 20 to 25mg/kg body weight (Imungi, 1990). However, according to Larry and Michael (1990) the lethal dose of nitrite in human beings is 25mg/kg body weight or 2g to 4g in total. Infants and children are more vulnerable to nitrate and nitrite toxicity than adults due to the low pH, in their stomachs. At low pH values, nitrates are easily reduced to nitrites which react with blood causing fatal methaemoglobinaemia (Liener, 1969; Graham, 1983; Imungi, 1990; Mohri, 1993). Methaemoglobinaemia is the most prevalent and potentially the most serious complication, caused by nitrate and nitrite exposure. The condition is characterised by cyanosis, stupor and cerebral anoxia. Nitrite directly oxidises

the haemoglobin ferrous ion state Fe (II) to the ferric state Fe(III) which cannot bind oxygen and results into reduced oxygen transport that produces tissue hypoxia (Mohri, 1993).

Nitrates are not carcinogenic (Mohri, 1993; Pobel *et al.*, 1995). However, nitrates may be reduced to nitrites, which may react with secondary amines or amides to form N-nitroso compounds under conditions similar to those in the human stomach (Sen, 1980; Miloslav, 1983; Wong, 1989; Michael, 1990). Scanlan (1993) pointed out that over 300 N-nitroso compounds including many nitrosamine have been demonstrated to be carcinogens in experimental animals. However, the amount of a given nitrosamine which is required to induce cancer in humans is unknown. Furthermore, Pobel *et al.* (1995) reported that high intakes of pre-formed N-nitrosodimethylamine (NDMA) was associated with increased risk for gastric cancer compared to intakes of nitrate and nitrite. However, amaranth leaves (*Amaranthus* spp) have been associated with several poisoning in animals (Sizelone *et al.*, 1988; Salles *et al.*, 1991; Ferreira *et al.*, 1991; Lemos *et al.*, 1993; Casteel *et al.*, 1994; Binta *et al.*, 1996). According to Spearman and Johnson (1989) cattle were found dead at pasture and the post mortem (PM) examination revealed large amounts of undigested *Amaranthus reproflexus* in the rumen. It was concluded that, death may have been due to the large amount of nitrate in the plant or from oxalate accumulation.

Nitrates and nitrites are water soluble and therefore some degree of leaching is possible during washing or processing in water. Most of the nitrites present are oxidised to nitrate, and upon cooking, they leach out of the vegetable (Samuel, 1985; Barbara and Ken, 1987; Imungi, 1990; Ricardo 1993). Abo Bakr *et al.* (1986) reported that 16% of the nitrate was lost from peas, 34% from beans, 51% from carrot and 34% from spinach, when these vegetable were boiled for an unspecified time in water (1:2 v/v, vegetable : water). Similar findings were reported by Barbara and Ken (1987) who showed nitrate decrease in frozen carrots, and that during boiling of fresh carrots, 57% of the nitrate was leached into the cooking liquid. The degree of leaching depends on water temperature, surface area of the vegetable and ratio of carrot to water (Varoquax *et al.*, 1986).

Most researchers have concluded that there is no danger of feeding on vegetable amaranths as amounts eaten per day are small and could not constitute a health problem, given that vegetables are crushed, boiled and the cooking water is usually discarded, which removes much of the nitrate and nitrite (Samuel, 1985; Imungi, 1990; Ricardo, 1993).

2.4.3. Oxalates.

Oxalates, occur in plant materials at relatively high levels, mainly as soluble sodium and potassium salt or as insoluble calcium oxalate. Oxalate contents are high in some leafy vegetables such as Amaranths (Connor *et al.*, 1980; Oke, 1980; FAO, 1988; Dhan and

Pal, 1991; Lathika *et al.*, 1995). Variation in oxalate contents of plants can occur depending on season, species, variety, age and part of the plant and soil condition during growth (Kasidas and Rose, 1982; Gad *et al.*, 1982).

Oxalates affect calcium metabolism and possibly block renal function by precipitation of insoluble oxalate (oxalate crystals) and death has been reported from this condition (Graham, 1983; Miloslav, 1983; Armesto *et al.*, 1989).

Amaranths leaves contain high levels of oxalate which is thought to bind divalent cations particularly calcium and zinc making them unavailable for metabolism (FAO, 1988; Ricardo, 1993). This is manifested as calcium deficiency even in diets with adequate levels of calcium. This has more significance in growing children than in adults, because of the developing bones and teeth (Imungi, 1990).

On a dry weight basis, the oxalate levels in amaranth range from 1.1% to 7.9%, (0.20 - 1.02 on fresh weight basis (fwb) (Ricardo, 1993) and 1815 mg/100g fwb (Imungi, 1990). Oxalate levels normally found in amaranths do not present a serious health hazard for reasonably healthy individuals if consumption does not exceed 100g of leaf per day (FAO, 1988). Toxic levels for humans have been indicated to be 2 to 5 g of oxalic acid per day for population consuming low levels of calcium (Ricardo, 1993). According to Graham (1983) oxalic acid of 5 g or more can be fatal in man, causing corrosive gastro-enteritis, shock, convulsion and renal damage. Oxalate poisoning can be aggravated when

the calcium intake is low. Oxalate toxicity problems has also been reported in animals fed on vegetable amaranth (Marshall *et al.*, 1967; Armesto *et al.*, 1989; Spearman and Johnson, 1989; Lemos *et al.*, 1993; Kommers *et al.*, 1996; Torres *et al.*, 1997).

Except for leaching, oxalic acid is minimally changed during processing and preparation of food. However, it might leach out into the cooking water bound to calcium, which severely reduces the levels of the element in the cooked materials if the cooking water has to be discarded. This, however, removes most of the soluble oxalate (Gad *et al.*, 1982; Samuel, 1985; Imungi, 1990).

2.5. Processing and Storage of Leafy Vegetables.

Processing techniques, whether canning, freezing or drying, have been improved to an extent where final products are more palatable, nutritious and have substantially longer storage capability as compared to fresh vegetables (Belitz, 1987; FAO, 1990). Sun drying is the most ancient method of drying food and is still in use in many parts of the world. The sun is by far the cheapest source of heat for the removal of water from fruits and vegetables (Salunkhe *et al.*, 1974). Small scale drying of cowpea leaves in direct sunshine or under shade has been done in Africa (Imungi, 1984). Similarly, drying of amaranth leaves in direct sunshine have been reported by FAO (1988).

2.5.1. Dehydration of vegetables.

Vegetable dehydration reduces the natural water content below the level critical for growth of micro-organisms (12-15%) without being detrimental to important nutrients

(Belitz, 1987). Maeda and Salunkhe (1981) pointed out that increasing the rate of water removal (drying) minimises losses of ascorbic acid provided that there is no marked increase in the temperature of the product. This also preserves flavour, aroma and appearance and the ability to regain the original shape and taste, when the vegetables are rehydrated and cooked. Dehydrated cowpeas leaves were rehydrated, cooked and served in accompaniment with *ugali* in acceptability trials with fresh leaf as control. The dehydrated leaves, on a hedonic scale did not differ significantly ($P<0.05$) from the scores of the fresh vegetable (Imungi, 1984).

2.5.2. Nutrient changes during processing, preparation and storage of leafy vegetables.

There is a general recognition that dehydration of leafy vegetable results in substantial destruction of their protein, carotene and vitamin C contents (Imungi, 1984; Belitz, 1987). Gomez (1981) noticed no significant changes ($P<0.05$) in the carotene contents of solar dried cowpea leaves after three months storage. The cowpea leaves dried under shade provision showed excellent carotene retention and storage stability retaining over 80% of carotene after three months. Similarly, Maeda and Salunkhe (1981), dried four vegetables including African spinach (*Amaranthus* sp) in solar drier with or without shade provision, after blanching in boiling water. The study showed maximum ascorbic acid and carotene retentions occurred in vegetables dried in the enclosed drier with shade provision. Direct exposure of the vegetable to sunshine as is often practised in the tropics resulting in only marginal retention of the two vitamins, due to the increased oxidation from exposure to ultra violet radiations.

The effects of cooking, blanching, and canning of leafy vegetables on their carotene contents may be quite variable. Losses, no change, and increases in the carotene contents of vegetables from the above process have been reported (Imungi, 1984). Gomez (1981) found a general increase in the carotene contents of several Kenyan leafy vegetables ranging between 22-58% after the vegetables had been boiled for 15 minutes in 5 volumes of water. This was attributed to loss in water soluble solids during cooking such that when expressed on dry matter basis, a concentration effect occurs.

Heat processing of leafy vegetables results in large losses of their ascorbic acid. Dehydration and storage of vegetables commonly results in appreciable destruction of the vitamin (Addo, 1981). Maeda and Salunkhe (1981) retained less that 25% of vitamin C in each of four leafy vegetables after solar drying with shade provision.

Loss of ascorbic acid during cooking of vegetables depend on the method of cooking, the volume of water used and vegetable species. Most of the ascorbic acid lost from cooked vegetable could be traced to the cooking water (Imungi, 1984). According to Staivok (1977) the vitamin C content declines during storage regardless of storage condition and variety. It has been found that Oxygen is one of the factors influencing the loss of vitamin C (Philip and Manuel, 1991). Similar findings have been demonstrated in dehydrated model food systems (Kirk *et al.*, 1977; Dennison and Kirk, 1978). The extent of ascorbic acid destruction by oxygen depends on the storage temperature as it has been

reported by Kefford *et al.* (1959). Also Smooth and Nagy (1979) indicated that storage of commercial canned single strength grape fruit juice at 10°C-50°C resulted in vitamin C loss ranging from 3-50% within the first four weeks and the magnitude of the losses was high depending on storage temperature and time.

Minerals are lost from foods during processing, mainly through leaching into the processing water (Imungi, 1984). There are metal ions which are derived from food itself or acquired during food processing and storage (David and Craig, 1994). These may interfere with the quality and appearance of food. They can cause discoloration of vegetable products and may catalyze reactions responsible for taste defects (Belitz, 1987)

The study of the effects of processing and storage on the mineral contents of vegetables has been centred mainly on elements of significant nutritional importance like, calcium, iron, phosphorus and zinc. The minerals phosphorous, sodium, calcium, magnesium and potassium were retained in the blanched spinach in values ranging from 44% for potassium to 103% for calcium (Bengtson, 1969). The reason given for the higher retention in the spinach was the high level of oxalate in the vegetable such that most of the calcium was in complex form and was not soluble.

Cooking in large volumes of water which was drained after cooking increased losses of calcium, iron and phosphorous by 19 to 23% in cabbage and 9 to 27% in spinach

(Imungi, 1984). These losses through leaching could be attributed to the high surface area to volume ratio of the vegetables.

Dehydration of vegetable tends to change their colour which reduces the quality as it was pointed out by Steel and Tong (1996). The degree of greenness is important in determining the final quality of processed green vegetables.

CHAPTER THREE

3. MATERIALS AND METHODS.

3.1. Materials.

Amaranthus hybridus seeds were purchased from Kariokoo market in Dar es Salaam and these were planted in three replicates at each of the four locations in Dar es Salaam city. The locations were selected to include two along the roadside (Makumbusho and Mchicha Tazara) and two along the valleys and away from the roads (Msimbazi Valley and Chang'ombe Kibasila) the two are most common sites for productions of amaranth vegetables in Dar es Salaam.

The vegetables were produced as follows: Plots were finely prepared by hand hoe and chicken manure was evenly applied on each plot at the rate of $4kg/m^2$. The very small seeds were mixed with dry sand to ensure uniformity of distribution. They were broadcasted on the plots at an approximately rate of $3\text{-}10g/m^2$ (1 to 2 kg/ha). This gave a plant density of approximately 100 plants/m^2. A grass mulch was used to protect freshly sown seeds from irrigation displacement or heavy rain, but this was removed after seed germination which occurred after 4 to 6 days.

The vegetables were grown as rainfed, however, during dry weather they were watered evenly in the morning and evening daily. The amaranth leaves were harvested 23 days after planting. The whole aerial portion of the plant was cut at 2 cm above the soil surface

and the harvested vegetables from each of the growing sites were quickly transported in closed plastic bag to the Government Chemists Laboratory (GCL) and immediately prepared for analyses of proximate chemical composition, nutrients and antinutrients. For cooking and dehydration, the vegetables from the four growing sites were pooled then sampled.

3.2. Methods.

3.2.1. Preparation methods.

3.2.1.1. Cooking of the vegetable amaranths.

The edible portions of the harvested plant were removed. Lots of about 500g leaves were washed with distilled water, drained and placed in stainless steel pot, then 400ml of distilled water was added and the pot contents heated to boil and allowed to boil for 15 min. They were cooled in a water bath to room temperature, then drained (by careful decantation).

3.2.2. Processing methods.

3.2.2.1. Dehydration.

Four hundred gram lots of the edible portion of the vegetable were blanched in boiling water for 30 sec., as established by Maeda and Salunkhe (1981). They were then dried in an enclosed drier of dimensions 150 cm x 50 cm x 40 cm constructed from local wooden material and provided with shade of transparent plastic covers. The blanched vegetables were spread on trays at the rate of 8 kg/m^2 and the trays inserted into the driers. They were dried for 7 h from 9.00 am to 4.00 pm each day for three days. The dried vegetables

were removed from the drier tray and packed in air-tight polythene bags (16 cm x 28 cm) for moisture contents, beta-carotene, ascorbic acid and sensory evaluation.

3.2.2.2. Storage of dehydrated vegetable amaranth.

Eighteen samples of 500 g each in polythene bags (16 cm to 28 cm) were randomly divided into three equal batches. One batch was stored at 22°C, another at 28°C and the other at room temperature (30-32°C), and away from direct sunshine. All batches were stored for 3 months. From each batch two polythene bags were opened each month and the vegetables analyzed for ascorbic acid and beta-carotene. This was CRD with 3x3 factorial experiment replicated twice with the factors being storage time and temperature each at 3 levels.

3.3. Analytical Methods.

3.3.1. Determination moisture content.

The moisture contents of fresh vegetables were determined by AOAC methods (AOAC, 1984) by weighing 5 g of the sample and drying in an air oven at 105°C to constant weight. The weight of the residue was converted to percent total solids (Dry matter) and the moisture content was calculated as difference. Moisture contents of the dried vegetables were determined by drying 1g sample using Ohaus moisture determination balance model MB 200 at 103°C for 1h. The moisture content was calculated as percent loss in weight.

3.3.2. Determination of total ash.

Total ash was determined by AOAC methods (AOAC, 1984). Two grams of dried vegetables were weighed in porcelain ashing dishes previously dried in an air oven at 98°C-100°C, cooled and tarred. The dishes were held in a muffle furnace at approximately 550°C overnight. They were then cooled to room temperature in a dessicator and weighed. The weight of residue represented the total ash.

3.3.3. Determination of crude fibre.

Crude fibre content was determined following AOAC methods (AOAC, 1984). Crude fibre consists mainly of the cell wall materials, lignin, cellulose and hemicelluloses. Two grams of ground sample was used to determine the crude fibre as loss on ignition of dried residue remaining after boiling of the sample with 1.25% H_2SO_4 and 1.25% NaOH solutions under specific conditions.

3.3.4 Determination of ether extract.

Ether extract (crude lipid) was determined by AOAC methods (AOAC, 1984). Five grams of sample were weighed in cellulose extraction thimbles (Whatman, 22x80 mm) and extracted with analytical grade petroleum ether (Bp 40°C - 60°C) in a Soxhlet extraction unit for 16 hours. The ether extract was transferred to a 250 ml round-bottomed flask which had been previously dried, cooled and tarred. Excess petroleum

ether was evaporated and the residual extract in the flask was dried to constant weight and expressed as percentage of the original dry weight (percent ether extract).

3.3.5. Determination of crude protein.

Crude protein was determined as total nitrogen using the semi-micro Kjeldahl method (AOAC, 1984). The total nitrogen was converted to protein using a factor of 6.25. Samples of 1g were weighed in 100ml Kjeldahl flasks. Sulphuric acid at the rate of 20 ml per sample was added followed by 2 Kjeldahl tablets and 2 ml of Hydrogen peroxide. The mixture was left until frothing ceased. The flasks were heated on Kjeldahl heating assembly until all frothing stopped and clear solution remained. After cooling to room temperature, the white residue was dissolved in 200ml distilled water. The solution was mixed with 20ml of a solution containing 60% sodium hydroxide and 5% sodium thiosulfate, then steam distilled. About 50 ml distillate was collected from each sample in a 125 ml erlenmeyer flask containing 20 ml of 2% boric acid and two drops of a mixed indicator (prepared by dissolving 0.2% methyl red and 0.2% methylene blue in methanol). The quantity of ammonia in the distillate was determined by titration with 0.02 N hydrochloric acid. The total nitrogen and hence percent protein were calculated.

3.3.6. Determination of soluble carbohydrates (Nitrogen free extract).

Soluble carbohydrates was determined by difference.

3.3.7. Determination of specific minerals (Pb^{2+}, Ca^{2+}, Fe^{2+} and P).

These minerals were analysed in fresh vegetable amaranths and cooked vegetable amaranths samples. The elements were analysed in all the samples using Unicam 939/959 Atomic Absorption Spectrophotometer (AAS) equipped with an air acetylene flame and hollow cathode lamp and recorder. The device was operated at standard conditions using wavelengths and slit widths specified for each element.

3.3.7.1. Determination of lead.

Lead was determined following the method described by FAO (FAO, 1986).

Approximately 10g of dry sample was weighed accurately into silica dish and dry ashed at 450 - 500 °C until the ash was grey or white. The ash was cooled to room temperature and moistened with 5 ml of dilute HNO_3/water (1:9), and briefly re-ashed if needed. The final ash was then dispersed in a mixture of 5ml of distilled water and 5ml concentrated HCl and evaporated to dryness.

The residue was dissolved in 6 ml of a mixture of conc. HCl and distilled water (1:5) then filtered through Whatman No. 42 filter paper into 25ml volumetric flasks and made to the mark with distilled water.

Appropriate dilutions were carried out for standard solutions in 25ml volumetric flasks using 0, 0.10, 0.20, 0.50 and 1.00ml of the standard lead solution. To each flask 1.0ml of HCl was added and made up to the mark with distilled water.

To all of the 25 ml of the diluted standard and sample extracts including a blank were each transferred into a separatory funnel into which 0.5 ml of 10% ascorbic acid was added to prevent stannous interference, and inverted to mix the content. Immediately 1.5 ml of 1% Diethylammonium-diethylcarbodithioate (DDCD) solution was added. The mixture in the separatory funnel was shaken for 30 seconds and allowed to stand for 15 minutes to allow phases separation. Then the lower phase was drained into a volumetric flask and the organic layer was drained for lead determination, where DDCD in Methyl isobutyl ketone (MIK) was as convenient alternative to the Spectrophotometer procedure. MIK solvent was aspirated in AAS before and after each sample or standard in order to establish the baseline.

3.3.7.2. Determination of iron, calcium and phosphorus.

The elements were determined by AOAC methods (AOAC, 1990). Accurately weighed 1g of dried and ground sample was placed into 150ml pyrex beaker and 10ml HNO_3 added and allowed to soak thoroughly, followed by addition of 3ml of 60% $HClO_4$ and heated on hot plate slowly at first and until HNO_3 was almost evaporated. Exactly 10ml HNO_3 were added and heating was continued until white fumes of $HClO_4$ were observed. The beaker was cooled at room temperature and the residue was dissolved in 10ml of HCl (1:1) and the content was quantitatively transferred to 50ml volumetric flask. Appropriate dilutions were carried out and the element analyzed against their standards.

Before analyzing the calcium, it was necessary to add 10ml 5% La_3O to the extracts to prevent phosphorous interference.

3.3.8. Determination of oxalates.

The oxalate was determined by calcium oxalate precipitation (AOAC, 1995). The method involved titration of acidic aqueous extracts of the sample with standard solution of potassium permanganate.

3.3.9. Determination of nitrate.

Nitrates were determined by the method described by FAO (FAO, 1980) as follows: Each sample was thoroughly mixed, macerated or homogenized and 5g weighed into 150ml beaker followed by addition of 50ml distilled water and heating at 80°C while stirring gently for 10 minutes. This was followed by addition of 20ml of alumna cream and quantitatively transferred to a 100ml calibrated flask. The mixture was cooled and made to volume with water then filtered through a 15cm Whatman No. 4 filter paper rejecting the first 10ml of filtrate. The filter paper had been washed with 100ml boiling water to remove any traces of nitrate.

Ten millilitres of filtrate were pipetted into a 50ml volumetric flask. Exactly 5ml of buffer (ammonium chloride and ammonia with pH. 9.6) solution and 1g of wet cadmium were added and the flask stoppered and shaken for 5 minutes. The mixture was filtered through Whatman No. 4 filter paper into a 50ml volumetric flask, and the cadmium and the filter paper rinsed with 5ml of water. Two millilitres of arsanilic acid solution were added, and

mixed. After standing for 5 minutes, 2ml of naphythylethylenediamine solution were added and the contents mixed and allowed to stand for 10 min. If the solution was not clear the contents were transferred to a 100ml separatory funnel, saturated with salt and the colour extracted with 20, 15 and then 5ml of n - butanol. The butanol extracts were mixed and passed through a small pledge of cotton wool in a funnel into a dry 50ml volumetric flask and made to volume with n - butanol. The concentration of nitrate was determined by reading the absorbency in a Unicam 8700 series UV/Visible spectrophotometer at 538nm using a 1 cm cell. The nitrate contents were calculated from a standard curve prepared from solution of pure sodium nitrate in distilled water.

3.3.10. Determination of ascorbic acid (vitamin C).

Ascorbic acid was determined by the method of Tomohiro (1990). Standard Indophenol solution was prepared by dissolving 0.042g $NaHCO_3$ in 50ml of distilled water followed by 0.05g of 2, 6- dichloroindophenol and made to 200ml, then stored in a brown bottle after filtration through fluted paper. Ascorbic acid standard solution was prepared by dissolving 0.05g of pure ascorbic acid (Sigma chemical Co., St. Louis Mo,) in 50ml of 10% Trichloroacetic acid (TCA).

a). Standardisation of indophenol solution.

To each of the three the volumetric flasks 5ml of 10% TCA was pipetted, followed by 2ml of standard ascorbic acid solution. The mixture was titrated with indophenol solution until a faint pink colour appeared. To the three flasks 7ml of 10% TCA were added, followed by equal volume of water to that of indophenol used and the mixture was

titrated against indophenol solution until pink colour was formed. This titre represented the blank.

Ascorbic acid content was then computed using the following expression.

$$\text{mg of ascorbic acid equivalent to 1.0ml of indophenol solution} = \frac{\text{mg of ascorbic acid in 2 ml of std solution}}{\text{Titre of indophenol solution}} = \text{factor C} \quad \text{.....(i)}$$

b). Sample determination.

Exactly 5g of sample was macerated completely in a laboratory blender with a small volume of 10% TCA, the mixture was filtered immediately. The sample was quantitatively transferred into 100ml volumetric flask and made to volume with 10% TCA solutions. Ten millilitres of the sample were transferred into conical flasks and titrated with indophenol solution until pink colour appeared. For blank, 10ml of 10% TCA solution was measured into conical flasks followed by equal volume of water to indophenol solution used, was added and titrated against indophenol solution until pink colour appeared.

Calculation:

Ascorbic acid content was computed using the following expression.

Vitamin C content (mg/100g) = (A-B) x C x 100/10 x 1/S x 100 whereby:-

A = Volume in ml of the indophenol solution used for sample.

B = Volume in ml of the indophenol solution used for blank

C = Mass in mg of ascorbic and equivalent to 1 ml of standard indophenol solution from expression (i)

S = Weight of sample taken in gram (exactly 5g).

3.3.11. Determination of beta-carotene (pro-vitamin A).

Beta-carotene was determined as carotene equivalent using acetone-hexane (1:9) as solvent, by AOAC methods (AOAC, 1995). The methods involved extraction and pigment separation. The concentration of carotene was read directly from the Unicam 8700 series UV/Visible spectrophotometer at 436nm after proper calibration of the instrument with standard solutions of pure beta - carotene (Sigma chemical Co., St. Louis, Mo.) in the acetone - hexane mixture. The beta carotene standard concentration ranged from 0 to 50mg per 100ml.

3.4. Sensory Evaluation.

Sensory evaluation was performed on the dried samples which had maximum retention of vitamins as shown in fig. 1 and 2 for lots stored at 22°C at the end of storage period. Organoleptic quality characteristics such as appearance/colour, flavour, texture and overall acceptability was assessed by the taste panel consisting of 10 Tanzanian adults familiar with the cooked vegetable amaranth. The test compared fresh cooked with dried cooked vegetable amaranth.

For presentation to panel members the vegetable amaranths were prepared as follows: Ten grams of finely chopped onions were weighed into an aluminium pot with 10g

shortening (Kimbo). The pot was then heated on an electric plate at medium heat setting until the onions were just beginning to turn golden yellow in colour. Then 0.5g curry powder (Knorr Naehrmittel A.G., CPC Kenya Limited) was added followed by 100g drained leaves and 1.2g salt (Iodised Safi salt Nish Enterprise Dar. Tanzania) and the ingredients thoroughly mixed. The pot was covered and heating continued for 5 minutes with occasional mixing of the vegetables. The heating setting was changed to low and vegetables simmered for another 5 minutes. The samples were then presented to the panel members as coded randomised duplicates such that each taster had 4 samples (2 products in duplicate) for testing.

The panellists were asked to score the sensory attributes of the samples on a nine point hedonic rating scale with 1=dislike extremely and 9=like extremely on the score chart presented in appendix 17.

3.5. Data Analysis.

Data for proximate composition and raw nutrients and antinutrients were subjected to analysis of variance (ANOVA) RCBD-ANOVA and means were separated by Duncan Multiple Range Test. While the data for raw and cooked vegetables were subjected to unpaired t-test after pooling the variances and tested at an appropriate number of degree of freedom and probability levels. Data for storage tests were subjected to CRD in factorial experiment and means were separated by Duncan Multiple Range Test. All data were analyzed using the SAS - C. Statistical package.

CHAPTER FOUR

4. RESULTS AND DISCUSSION.

4.1. Proximate Chemical Composition of Raw Amaranth Leaves.

The proximate chemical compositions of raw *Amaranthus hybridus* leaves from different production sites are shown in Table 1. As Table 1 shows, location of production had significant effect ($P<0.05$) on these parameters except for moisture and lipid contents.

The dry matter contents of the amaranth vegetables produced in the four different sites ranged between 13.5 (Msimbazi Valley) to 14.7% (Mchicha Tazara). The value for Msimbazi valley was significantly different ($P<0.05$) from the value for Mchicha Tazara and Changombe Kibasila which were not significantly different from each other, but was not significantly different from the value for Makumbusho. All the values, however, compare favourably with reports from other studies (Dhan and Pal, 1991, 1992; Ricardo, 1993).

The crude protein contents varied between 28.4% (Msimbazi valley) to 31.6% (Changombe Kibasila). These two values were significantly different ($P<0.05$) from each other. The value for Makumbusho was not significantly different ($P<0.05$) from that of Msimbazi valley but was significantly different from that of Mchicha Tazara the latter of which was not significantly different from that of Changombe Kibasila.

Table 1: Proximate chemical composition of raw *Amaranthus hybridus* leaves[m] expressed as percent of component on dry matter basis.

Location of growth	Moisture content (%)	Dry matter (%)	Crude protein (Nx6.25)	Total Ash	Crude fibre	Lipid	soluble carbohydrates
Makumbusho	86.5[a]	13.5[a]	28.2[a]	17.2[a]	15.2[b]	4.1[a]	18.6[a]
Msimbazi valley	86.3[a]	13.7[a]	28.4[a]	18.8[b]	16.4[a]	4.00[a]	16 1[b]
Mchicha Tazara	85.4[a]	14.6[b]	31.5[b]	17.5[a]	16.8[a]	3.8[a]	12.4[c]
Chang'ombe Kibasila	85.3[a]	14.7[b]	31.6[b]	17.0[a]	15.4[b]	3.9[a]	14.2[d]
SE	±0.4	±0.2	±0.3	±0.3	±0.1	±0.1	±0.7

[m]Mean ± SE (n=3)

Means within columns superscripted by similar letter are not significantly different from each other ($P<0.05$)

The total ash contents ranged between 18.8% (Msimbazi valley) to 17% (Changombe Kibasila). The value for Msimbazi valley was significantly different ($P<0.05$) from the values for Makumbusho, Mchicha Tazara and Changombe Kibasila which were not significantly different among each other.

The crude fibre contents of the vegetables varied between 15.2g/100g (Makumbusho) to 16.8g/100g (Mchicha Tazara). These two values were significantly different ($P<0.05$)

from each other. The value for Makumbusho was not significantly different ($P<0.05$) from that of Changombe Kibasila, but was significantly different from the value for Msimbazi valley and Mchicha Tazara.

The values for crude lipid were not significantly different ($P<0.05$) among each other. The value for soluble carbohydrates ranged between 12.4% (Mchicha Tazara) to 18.6% (Makumbusho). Significant differences ($P<0.05$) existed among all the soluble carbohydrate contents of the vegetables amaranth from the four locations.

Although the proximate compositions of raw *Amaranthus hybridus* were within reported limits (Dhan and Pal, 1991, 1992; Ricardo, 1993), there were slight variation within the vegetables from the growing sites, especially in dry matter, crude protein, total solid, total ash, crude fibre, and soluble carbohydrates. These differences possibly could be attributed to the nature of soil condition and previous land use practices of the location.

4.2. Levels of Vitamins and Minerals in Amaranth Leaves.

The levels of beta-carotene, ascorbic acid, iron, calcium and phosphorous are shown in Table 2. The levels of beta-carotene ranged between 25.2 mg/100g in vegetables grown in Changombe Kibasila and 37.3 mg/100g in vegetables grown in Mchicha Tazara. The levels of beta-carotene in the vegetables grown in Makumbusho and Msimbazi valley were significantly lower ($P<0.05$) than the levels of beta-carotene in the vegetables grown in Mchicha and significantly higher than the levels in the vegetable grown in Changombe

Kibasila. However, the two levels were not significantly different ($P<0.05$) from each other.

Table 2: Levels of beta-carotene, ascorbic acid, iron, calcium and phosphorus in raw *Amaranthus hybridus* [m] as mg/100g on dry matter basis

	β-Carotene	Ascorbic acid	Iron	Calcium	Phosphorus
Makumbusho	29.5^{b}	509^{b}	108^{a}	2188^{a}	530^{a}
Msimbazi valley	29.1^{b}	535^{a}	110^{a}	2233^{a}	500^{a}
Mchicha Tazara	37.3^{a}	462^{c}	128^{a}	2263^{a}	553^{a}
Chang'ombe Kibasila	25.2^{c}	455^{c}	112^{a}	2062^{a}	518^{a}
SE	±1.20	±8	±11.8	±86.2	±13

[m]Mean ± SE (n=6)

Means within columns superscripted by the same letter are not significantly different from each other ($P<0.05$)

The levels of beta-carotene in this study compare quite well with values reported by Maeda and Salunkhe (1981) and Ricardo (1993). It has been estimated that the availability of vitamin A from leafy vegetables is 5,000 IU per 100g fresh weight (Kays, 1991). It would therefore be expected that the availability of vitamin A from these vegetables would be equivalent to the beta-carotene contents of 3 mg/100g to 37 mg/100g dry weight.

The levels of ascorbic acid varied between 455 mg/100g in Changombe Kibasila to 535 mg/100g in Msimbazi valley. The levels of ascorbic acid in the vegetables grown in Changombe Kibasila and Mchicha Tazara were not significantly different ($P<0.05$) from each other, but were significantly lower than the levels of ascorbic acid in the vegetables grown in Makumbusho and Msimbazi valley. The latter two levels were, however, significantly different ($P<0.05$) from each other. The variation in ascorbic acid contents of the vegetables would possibly be due to the nature of soil condition and previous land use practices of the location. The levels of ascorbic acid contents in the amaranth leaves from four sites compare quite closely with the levels reported by Ricardo (1993). Maeda and Salunkhe (1981) found a considerably higher value for ascorbic acid in the same vegetables.

Insignificant variability ($P>0.05$) was observed in the four sites for the 3 minerals analysed. This could indicate that the mineral content of the soils in the four different sites were nearly the same. The levels of these minerals in *Amaranthus hybridus* leaves compared quite well with the levels reported by Ifon and Bassir (1979) and Ricardo (1993).

4.3. Levels of Lead, Nitrate and Oxalate in the Raw Amaranth Vegetable.

The levels of lead, total nitrate, and total oxalate in raw amaranth leaves are presented in Table 3. As the Table shows, the levels of lead in the vegetables from all growing locations were trace. According to FAO (1986) dust in the garden atmosphere or settled

on the surfaces may contain lead, especially if the garden is near the road carrying a lot of vehicular traffic.

Table 3: Levels of nitrates, oxalates and lead in raw *Amaranthus hybridus*[m] leaves expressed per 100g dry matter of edible portion.

Location	Nitrate (mg)	Oxalate (mg)	Lead (ppm)
Makumbusho	513[a]	3800[b]	Trace[a]
Msimbazi valley	501[a]	3383[c]	Trace[a]
Mchicha Tazara	560[a]	3900[b]	Trace[a]
Chang'ombe Kibasila	541[a]	4333[a]	Trace[a]
SE	±16.2	±212	

[m]Mean ± SE (n=6)

Means within columns superscripted by the same letter are not significantly different from each at ($P<0.05$)

In this study, even the vegetables that had been grown on the road side contained trace amount of lead. It is possible that detectable lead might have accumulated on the surface of those vegetables during the growing period, but this was washed off either by rain or irrigation water.

Also, amaranth leaves were analyzed at three weeks from planting. It is possible that by the time absorption from the soil and translocation to the leaves had not resulted in accumulation of sufficient mineral to be detected by the used analytical method. Isabel and Concepcion (1997) have reported that concentration of an element in the leaves depends on the age of the plants, environmental conditions in which the plants are grown and the specific part of the plant analyzed. Nevertheless, very little data are available to date on the mineral status of soil and plants and the relationship between them (Isabel and Concepcion, 1997). Accumulation of lead in vegetables during growth was therefore such that the levels achieved at harvest were not high enough to warrant any public health concern.

No significant differences ($P>0.05$) existed among the nitrate contents of the vegetables from the four sites. This would indicate that the nitrogen contents of the soils in the four different sites were nearly the same. The levels of nitrate in this study agree quite well with values reported from other studies (Graham, 1983; Dhan and Pal, 1991; Ricardo, 1993).

Significant differences ($P<0.05$) existed among the levels of oxalate in the vegetables. These levels ranged from 3383mg/100g in vegetables from Msimbazi valley to 4333mg/100g in vegetables from Changombe Kibasila. The Oxalate contents of the vegetables from Changombe Kibasila were significantly higher ($P<0.05$) than the contents of the vegetables from all the other growing areas while the value for the vegetables from

Makumbusho were significantly lower than all the others. The oxalate contents of the vegetables from Makumbusho and Mchicha Tazara were not significantly different ($P<0.05$) from each other. The variability in oxalate contents within the same species can be attributed to soil condition during growth (Kasida and Rose, 1982; Gad *et al.*, 1982).

The oxalate contents of *Amaranthus hybridus* were within the values reported by Ricardo (1993) for vegetables grown in Guatemala and Dhan and Pal (1991) for those grown in India. However, the value were much higher than the levels of oxalates in some Kenyan amaranth leaves reported by Imungi (1990).

4.4 Nutrients Retention During Cooking of Amaranth Leaves.

The retention of beta-carotene, ascorbic acid, iron, calcium and phosphorus by amaranth leaves during cooking are presented in Table 4

4.4.1. Effects of cooking on beta-carotene contents.

Cooking of amaranth leaves resulted in a 4% increase in their carotene content. This apparent increase was statistically insignificant ($P>0.05$) in Table 4.

Table 4: Vitamin and mineral contents of raw and cooked amaranth leaves[m] (per 100g dry matter).

	Beta-carotene (mg)	Ascorbic acid (mg)	Iron (mg)	Calcium (mg)	phosphorus (mg)
Raw	29.7[a]	655[a]	140[a]	2500[a]	700[a]
Cooked drained leaf	30.9[a]	325[b]	90[b]	1800[b]	410[b]
SE	±0.9	±5.4	±10	±60	±10

[m]Mean ± SE (n=12)

Means within columns superscripted by the same letter are not significantly different at ($P<0.05$) by unpaired t-test.

These results agree quite well with reports by Lee *et al.* (1982) and Imungi and Potter (1983) that carotenes in blanched and canned leafy vegetables appeared to be relatively unaffected by heat processing. However, carotenes are likely to undergo some conformational change during heat processing with loss in vitamin activity (Imungi, 1984). The increase in beta-carotene contents during cooking could be attributed to losses in soluble solids through leaching into the cooking water, so that when expressed on dry matter basis, The beta-carotene which remains unaltered by cooking shows an apparent increase (Imungi and Potter, 1983). Also, it is possible that the cooking facilitates a more effective extraction from tissues during analysis (Gomez, 1981; Lee *et al.*, 1982; Imungi, 1984).

4.4.2. Effects of cooking on ascorbic acid content.

Amaranth leaves after cooking and draining retained only 49.6% of the ascorbic acid originally present in the raw leaves. This was expected since vitamin C easily undergoes heat degradation and oxidation during cooking. Moreover, being water soluble, considerable amounts leach into the cooking water. Oxidative degradation is facilitated by such factors as temperature, oxygen, light and catalysts. Loss of ascorbic acid during cooking of vegetable also depends on the method of cooking, the volume of water used and the species of vegetable. The kind of loss noted in this study has been reported by Thomas (1968), Imungi and Potter (1983), Imungi (1984), Belitz (1987), Mathooko and Imungi (1994) and FAO (1995) for other leafy vegetables.

4.4.3. Effect of cooking on minerals contents.

Results in Table 4 show that cooking had significant reduction ($P<0.05$) on mineral contents of the vegetables. After cooking the minerals leached into the cooking water in varying proportions, the levels retained being 63.5%, 73.6% and 63.1% for iron, calcium and phosphorous, respectively. The main route of loss of mineral from cooked vegetables would be leaching into the cooking water. The reason for higher calcium retention was probably due to the high levels of oxalate in the vegetable amaranth such that most of the calcium was in bound forms and was therefore not soluble. This has also been reported by Bengtson (1969) and Imungi (1984). Since it is free calcium which is leached out during cooking, it follows that the cooked vegetables become poorer in the available calcium.

4.5. Retention of Nitrates and Oxalates During Cooking of Amaranth Leaves.

The levels of nitrates and oxalates retained by amaranths leaves after cooking are presented in Table 5.

4.5.1. Effects of cooking on nitrate content.

Table 5 shows the levels of nitrates and oxalates in raw and cooked drained amaranth leaves. After cooking the drained vegetable retained 60.8% of the nitrate originally present in the raw leaves. This means that 39.2% of the nitrate had leached into the cooking water. This level of leaching has also been reported by Abo Bakr *et al.* (1986), Varoquax *et al.* (1986) and Barbara and Ken (1987) for other leafy vegetables.

Table 5: Nitrate and total oxalate contents of raw and cooked amaranth leaves[m] expressed per 100g on dry matter basis.

	Nitrate (mg)	Total oxalate (mg)
Raw	657.9[a]	4825[a]
Cooked drained leaf	399.7[b]	2883.3[b]
SE	±11.5	±149.9

[m]Mean ± SE (n=12)

Means within columns superscripted by the same letter are not significantly different at ($P<0.05$) by unpaired t-test.

Cooking of the vegetables was done in four volumes of water for 15 minutes. It is likely that if larger volumes of cooking water for longer periods were used, the percent of

nitrates leaching into the cooking water would have been higher. It has been established by Varoquax *et al.* (1986) that the degree of leaching depends on the cooking temperature, surface area to the volume ratio of the vegetable and ratio of vegetable to water.

The traditional cooking of green leafy vegetables in Tanzania always utilizes large volumes of water and longer times than in this study. Most often, the water is discarded in order to get rid of the bitter and toxic principles that could possibly be present in the leaves. In the preparation of certain dishes, however, the cooking water may not necessarily be discarded. In the latter method, more nitrate per unit weight of the cooked vegetable would be ingested than in the former method of cooking.

From the results the nitrate levels in the cooked amaranth are such that a person of 60 kg body weight consuming more than 50g equivalent of the dry matter would exceed the ADI of 220mg for the antinutrient. However, under normal circumstances, the amounts of cooked vegetables consumed by individuals rarely exceed 30g to 100g of fresh weight by Imungi (1990) and FAO (1988) respectively.

4.5.2. Effects of cooking on total oxalate contents.

Cooking significantly reduced ($P<0.05$) the total oxalate contents of the vegetables. The cooked drained vegetables retained 59.8% of the oxalate originally present in the raw leaves. It is interesting to note that retention of oxalate in vegetable amaranth was accompanied by calcium present which indicates that the most of oxalate retained in the cooked vegetable was in bound forms and was not soluble (see table 4). Similar observations have been reported by Bengtson (1969), FAO (1988) and Ricardo (1993). The oxalate leaching out would mainly be the soluble free oxalic acid. Similar reduction in the oxalate contents during cooking has also been reported by Gad *et al.* (1982) for other leafy vegetables and legumes. The author also noted that the reduction in oxalate contents depended on the original concentration and the processing condition.

The result show that after cooking and draining the oxalate levels are, however, within the range reported to be fatal, which was 2 to 5g per day for population consuming low levels of calcium (Graham, 1983; Ricardo, 1993).

It has been observed that many residents of Dar es Salaam cook amaranth leaves without discarding the cooking water. They would therefore be at great risk of apparent calcium deficiency if they consumed foods containing low calcium levels. However, as has already been mentioned, the general population in Tanzania will cook the vegetable in large

volume of water which is thereafter discarded. In such cases, the risk of apparent calcium deficiency will be greatly reduced even if low calcium foods are generally consumed.

4.6. Effect of Sundrying on Beta-carotene and Ascorbic Acid Contents of Amaranth Leaves.

The beta-carotene and ascorbic acid contents of raw and dehydrated amaranth leaves are shown in Table 6. These results indicated that sun drying of vegetable amaranth under shade provision resulted in significant reduction of both beta-carotene and ascorbic acid. Nevertheless, the destruction would have been more pronounced if the drying were done without shade provision as has been reported by Mudambi (1977) and Maeda and Salunkhe (1981).

Table 6: Beta-carotene and ascorbic acid contents of raw and dehydrated amaranth leaves[m] per 100g dry matter basis.

Vegetable	Beta-carotene (mg)	Ascorbic acid (mg)
Raw	43[a]	508[a]
Dehydrated.	36[b]	64[b]
SE	±0.5	±1.5

[m]Mean ± SE (n=3)

Means within columns superscripted by the same letter are not significantly different at ($P<0.05$) by unpaired t-test.

It is a general recognition that dehydration of leaf vegetables results in losses of their vitamins the extent of loss depending on the type of vegetable (Thomas, 1968; Labuza, 1972; Salunkhe *et al.*, 1974; Addo, 1981; Gomez, 1981; Maeda and Salunkhe, 1981; Belitz 1987; Gareth *et al.*, 1998*)*

4.6.1. Vitamins retention in dried amaranth leaves during storage.

The retention of beta-carotene and ascorbic acid in dried amaranth leaves stored at 22°C, 28°C and room temperature (30°C -32°C) for three months were analysed.

4.6.1.1. Retention of beta-carotene.

The retention of beta-carotene during storage of dried amaranth leaves is presented in Figure 1. In Figure 1, 100% represents 36 mg/100 (dwb) beta-carotene.

Loss in carotene was slightly higher at room temperature (30°C -32°C) and decreased correspondingly with decrease in storage temperature. At the end of three months storage, the retentions were 32.4 mg, 29.5 mg and 27.4 mg/100g (dwb) at 22°C, 28°C and room temperature respectively. This kind of loss of beta-carotene from dried leaf vegetables has been reported by Gomez (1981) while working with Kenyan green leafy vegetables. Losses of beta-carotene in stored dehydrated vegetables are usually due to oxidation mainly by the oxygen retained in the package and catalyzed by light (Labuza, 1972; Gareth *et al.*, 1998). It is therefore usually recommended that dehydrated vegetables be stored away from direct sunlight.

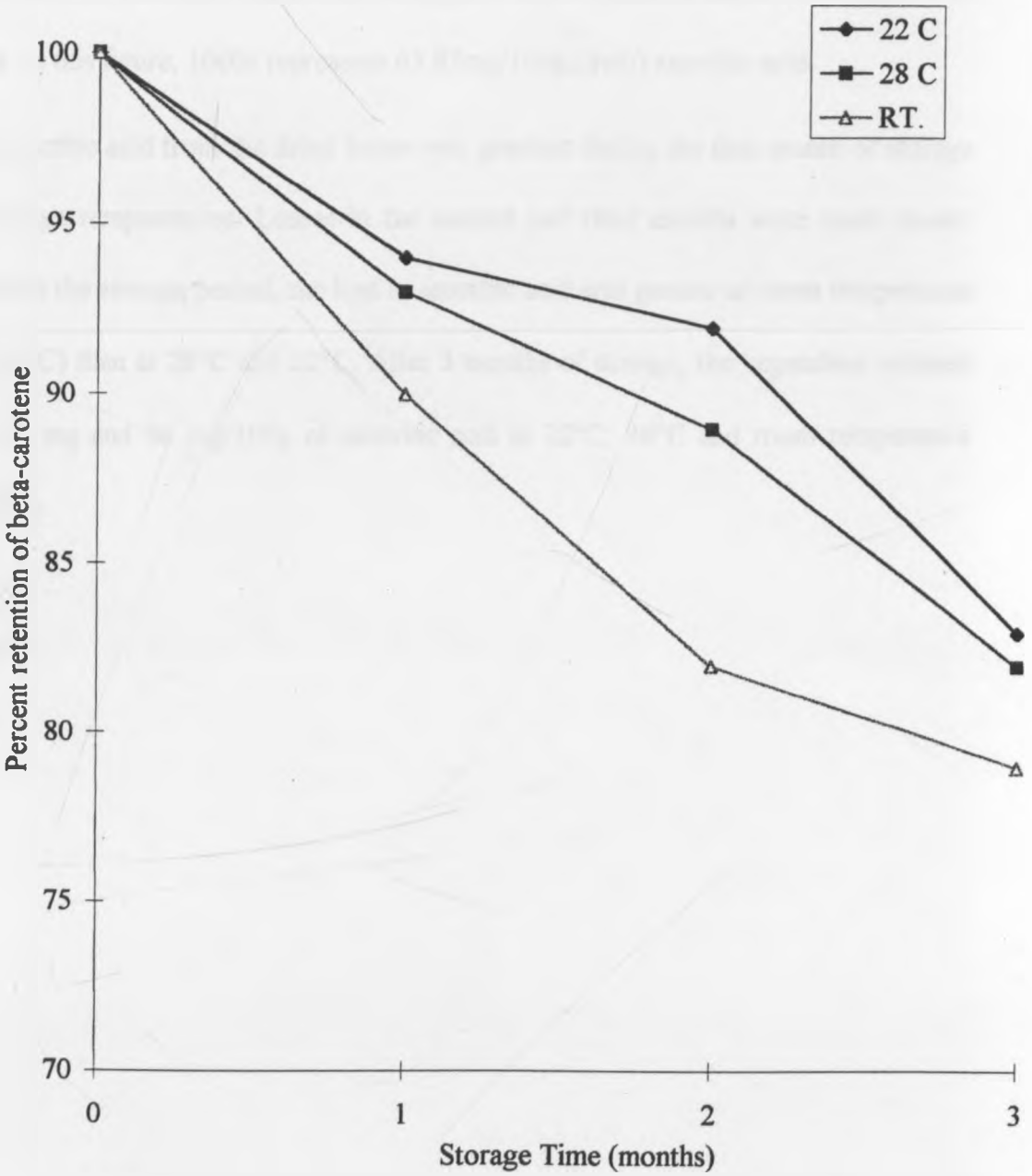

Fig. 1. Retention of Beta-carotene in dried amaranth leaves during storage for three months, 100% = 36mg/100g dwb.

4.6.1.2. Retention of ascorbic acid.

The retention of ascorbic acid during storage of dried amaranth leaves is presented in Figure 2. In this figure, 100% represents 63.87mg/100g (dwb) ascorbic acid.

Loss in ascorbic acid from the dried leaves was greatest during the first month of storage at all storage temperatures. Losses in the second and third months were much lower. Throughout the storage period, the loss in ascorbic acid was greater at room temperature (30°C -32°C) than at 28°C and 22°C. After 3 months of storage, the vegetables retained 42 mg, 40 mg and 36 mg/100g of ascorbic acid at 22°C, 28°C and room temperature respectively.

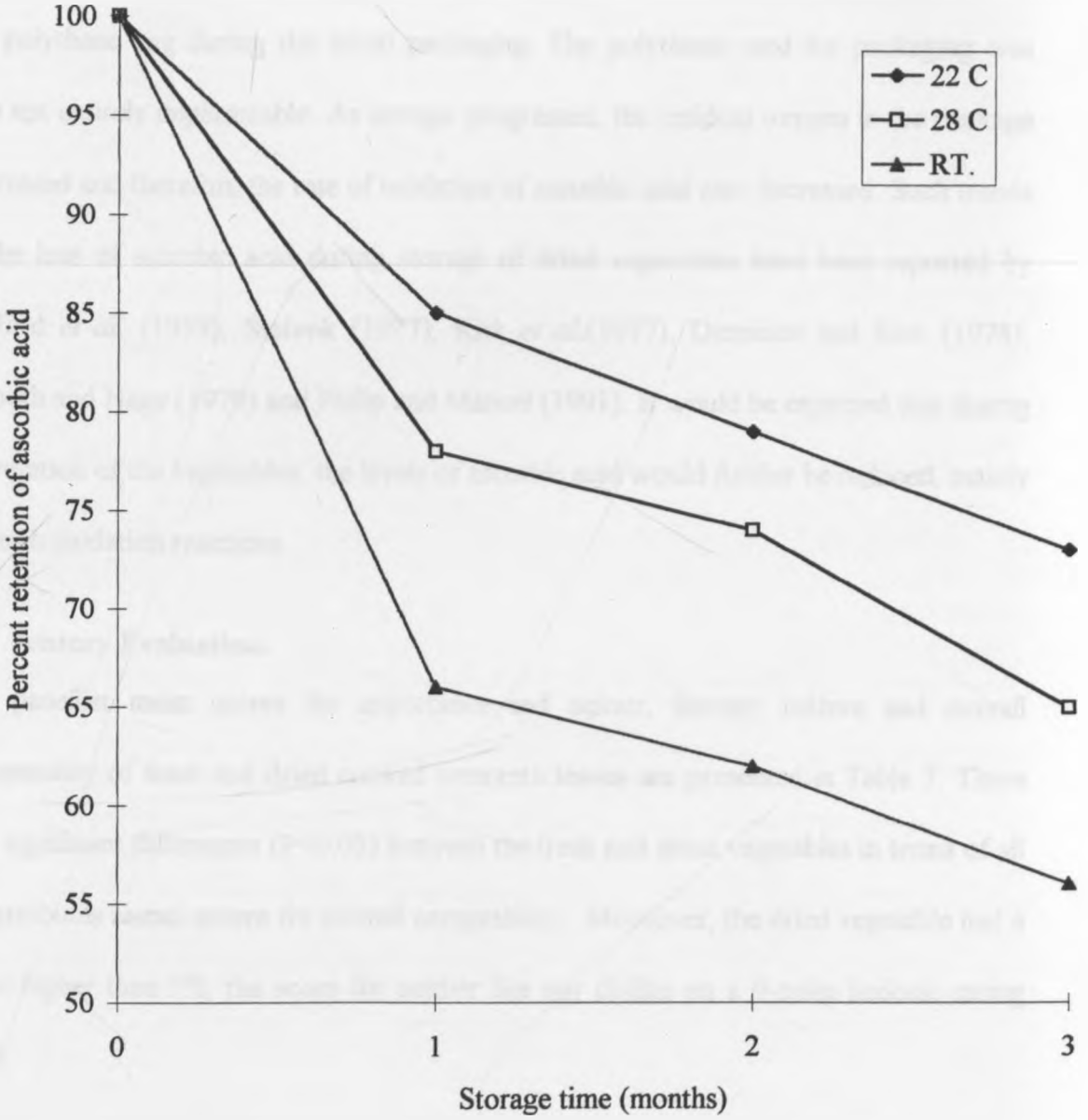

Fig.2. Retention of ascorbic acid in dried amaranth leaves during storage, 100% = 63.87 mg/100g dwb.

The higher rate of ascorbic acid loss during the first month of storage as compared to the second and third months was probably due to the effect of the residual oxygen retained in the polythene bag during the initial packaging. The polythene used for packaging was also not entirely impermeable. As storage progressed, the residual oxygen in the package decreased and therefore the rate of oxidation of ascorbic acid also decreased. Such trends in the loss of ascorbic acid during storage of dried vegetables have been reported by Kefford *et al.* (1959), Staivok (1977), Kirk *et al.*(1977), Dennison and Kirk (1978), Smooth and Nagy (1979) and Philip and Manuel (1991). It would be expected that during rehydration of the vegetables, the levels of ascorbic acid would further be reduced, mainly through oxidation reactions.

4.7. Sensory Evaluation.

The panellist mean scores for appearance and colour, flavour, texture and overall acceptability of fresh and dried cooked amaranth leaves are presented in Table 7. There was significant differences ($P<0.05$) between the fresh and dried vegetables in terms of all the attributes tested except for overall acceptability. Moreover, the dried vegetable had a score higher than 5.0, the score for neither like nor dislike on a 9-point hedonic rating scale.

Table 7: Mean sensory scores for fresh and dried cooked amaranth[a].

	Colour and Appearance	flavour	Texture	overall acceptability
Fresh cooked	7.2[a]	7.9[a]	7.0[a]	7.9[a]
Dried reconstituted Cooked	5.6[b]	6.4[b]	6.0[b]	7.5[a]
SE	±0.2	±0.3	±0.2	±0.2

[a]Mean ±SE (n=20)

Means within columns superscripted by the same letter are not significantly different from each other at ($P<0.05$).

During dehydration of vegetables there is usually a loss in colour appeal due to the alteration of the colour of chlorophyll by pheophytinization during blanching and oxidation of the same during the drying process (Steel and Tong, 1996). Oxidation reactions also produce substances which might alter the flavour and texture of the vegetables. Moreover, during rehydration cooking the water removed during dehydration cannot be replaced in exactly the same manner as it existed in the fresh vegetables. All these contribute to the lowering of the consumer acceptability of the dried vegetables as compared to their fresh counterparts.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION.

5.1 Conclusion.

This study showed that fresh *Amaranth hybridus* vegetables grown in the four sites in Dar es Salaam had high levels of protein, vitamin A (beta-carotene), vitamin C and the minerals calcium, iron, and phosphorous. The vegetable had only trace amounts of lead but the levels of the antinutrients nitrates and oxalates were high.

There were no differences in the levels of lipid, iron, calcium, phosphorous, lead and nitrate in vegetable grown in four locations.

Cooking of the vegetables resulted in retentions of nutrients. The levels of total oxalate and nitrate in cooked drained vegetables were decreased.

The study finally showed that amaranth leaves could be solar dried to produce an acceptable and shelf stable product with sufficient levels of beta-carotene and minerals, and moderate levels of ascorbic acid. These products would serve to provide the vegetables in the absence of fresh ones.

5.2. Recommendations.

In view of the results of this study, the following recommendations are made:

This study was done during a rainy season. Similar study should be carried out during the dry season to determine whether the levels of lead, nitrate and oxalate would change.

A study should be carried out to store the dried vegetable in other packages, especially those that are conventionally used by communities to store dry foods.

Storage of the dried vegetables should be carried out for longer periods than the three months in this study.

This study utilized simple, low cost technology which should be transferred to the communities for preservation of these foods.

Promotion for increased acceptability and consumption of the dehydrated vegetables should be done among the rural communities, where the deficiency of vitamin A and iron is likely to be rampant during the period of drought.

6. REFERENCES:

Abo. Bakr, T. M., El-Iraqui, S. M and Hussein, M. H. (1986). Nitrate and nitrite contents of some fresh and processed Egyptian vegetable. *Food chem.* 19:265 - 268.

Adaudi, A. O., Gbodi, T. A and Aliu, Y. O (1990). The lead content of plant and animals as indicator of environmental contamination. *J. Veterinary and Human Toxicology* 32:5, 454 - 456.

Addo, A. A (1981). Ascorbic acid retention of stored dehydrated Nigerian vegetables. *Nutr. Rep. Int.* 24:44, 769 - 771.

Albert, L. E (1993). Lead toxicology. In *Encyclopaedia of Food science Food Technology and Nutrition. Vol. 4* (eds.) Macrea, R., Robinson, R. K and Sadler, M. J. Academic press, London. pp. 2670 - 2673.

Ana, M. R and Lia, N. G (1997). Influence of system composition on ascorbic acid destruction at processing temperatures. *Journal of science of Food Agric.* 74:369 - 378.

Anon (1984). Amaranth: *Modern prospects for an ancient crop*. The Board on Science and Technology for International Development (BOSTID). Natural Academy press, Washington D.C.

AOAC (1984). Association of official Analytical chemists. *Official method of Analysis* 14th edition, Washington, D.C.

AOAC (1990). Association of official Analytical chemists. *Official method of Analysis* 15th edition, Washington, D.C.

AOAC (1995). Association of official Analytical chemists. *Official method of Analysis* 16th edition, Washington, D.C.

Armesto, R. R., Grande, H. A. Baroni, A.C, Baron, E.E., Bogglio, J. and De-Baroni, A.C. (1989). *Amaranthus quintensis* intoxication in Holando Agentina heifers. *Veterinaria Argentina* **6**:60, 692 - 700.

Assey, J.P. (1995). The Concentrations of Lead in Industrial Effluent, Soil, lead Absorption by Soil and its uptake by Maize (*Zea Mays*) *MSc, Thesis*, SUA, Morogoro.

Aye, T., Win, M., Mu-mu, S., Tin, M. and Mya, B. (1995). The effect of some medicinal plants on uterine smooth muscle. *Journal of Myanmar-Health Sciences Research* 7:2, 70 - 74.

Ayiecho, P.O. (1985). Quantitative studies in two Grain amaranth population using two selection method. *PhD Thesis*, University of Nairobi.

Baker, S.J., and DeMaeyer, E.M. (1979). Nutritional anaemia: its understanding and control with special reference to the work of the World Health Organisation. *Amer. J. Clin., Nutr.* **32**:365.

Barbara, E.S. and Ken Lee (1987). Nitrate and nitrite method of analysis and levels in raw carrots, processed carrots and in selected vegetables and grain products. *J. of Food Sci.* **52**:6, 1632 - 1636.

Barth, M.M., Perry, A. K, Schmidt, S.J. and Klein, B.P (1990). Misting effects on ascorbic acid retention in broccoli during cabinet display. *J. of Food Sci.* **55**:4, 1187 - 1188.

Belitz, H.D. (1987). *Food chemistry, Springer* - verlag. Berlin.

Bengtson, B.L. (1969). Effect of blanching on nutrients and oxalate content of spinach. *Journal of Food* Tech. **4**:14 - 16.

Binta, M.G., Adom, E.K., Diteko. T., and Mushi E. Z (1996). *Plant poisoning cases among livestock in Botswana. Bulletin of Animal Health and Production in Africa* **44**:1,39 - 44.

Bodwell, C.E and John, W.E. Jr. (1988). *Nutrient Introduction.* Marcel Dekker, Inc., New York.

Breinholt, V., Schimerlik, M. Dahswood, R. and Bailey, G. (1995). Mechanism of chlorophyllin anticarcinogenesis against aflatoxin B1. *Chemical Research in Toxicology* **8**:4,506 - 574.

Bryce, S.D. (1980). Lead and brain function in food and health. In *Applied science and Technology.* (ed.) Brich. P. Applied Science Publishers Ltd. London pp 511 - 520.

Casteel, S.W., Johnson, G.C., Muller, M. A., Chudomelka, H.S, Cupps, D.E. and Haskins, H.E. (1994). *Amaranthus retroflexus* (red root pig weed) poisoning in cattle. *Journal of the American veterinary medical Association* **204**:7,1068 - 1070.

Chipperfield, J.R. (1993). Iron properties and determination. In *Encyclopaedia of Food Science Food Technology and Nutrition Vol. 4* (eds.) Macrae, R., Robinson, R.K and Sadler, M.J. Academic press, London. pp. 2573 - 2577.

Clarke, E.G.C and Clarke, M.L (1975). *Veterinary Toxicology*. Biiliere Tiridald, London.

Connor, J.K., Gartner, R.J.W., Brownyn, M.R and Amos R.N. (1980). *Amaranthus edulus*. An ancient food sources. *Aust Journal Expt. Agricultural Anim. Husb.* **20**: 150 - 160.

David, E.S. and Craig, L.M (1994). Lead content in grapefruit juice and its uptake upon storage in open containers. *Journal of Science Food Agric.* **66**: 405 - 410.

Dennison, D.B and Kirk, J.R. (1978). Oxygen effect on the degradation of ascorbic acid in dehydrated food systems. *J. of Food sci.* **43**:2,609.

Dhan, P. And Pal, M. (1991). Nutritional and antinutritional composition of vegetable and grain amaranth leaves. *Journal Science Food Agric.* **57**: 573 - 583.

Dhan, P. And Pal, M. (1992). Seed Protein, fat and fatty acid profile of amaranths species. *Journal Science Food Agric.* **58**: 145 - 147.

Dowd, T.L., Rosen, J.F., Gundberg, C.M. and Gupta, R.K. (1994). The displacement of calcium from osteocalcin at submicromolar concentration of free lead. *Biochimica et Biophysica Acta-Molecular Basis of Disease* **1226**: 2, 131 - 137.

Ellinger, R.H. (1972). P*hosphates as food ingredients*. CRC Press, Florida.

FAO, (1980). *Manual of food quality control*. Additive, contaminants technique. Food and Agricultural Organisation of the United Nations, Rome, Italy.

FAO, (1986). *Manual of food quality control.* Food analysis, general technique. additive, contaminants and compounds. Food and Agricultural Organisation of the United Nations, Rome, Italy.

FAO, (1988). *Traditional food plants*. Food and agricultural organisation of the United Nations. Vol. 42, Rome, Italy.

FAO, (1990). *Manual of food quality control*. Food for export. Food and Agricultural Organisation of the United Nations, Rome, Italy.

FAO, (1995). *Fruits and vegetables processing* FAO Agricultural services Bulletin 119 Food and Agricultural Organisation of the United Nations, Rome, Italy.

Feliciano, P.B and Harold, C. (1998). Protein quality evaluation of Amaranthus wholemeal flour and protein concentrates. *Journal science Food Agric.* **76**:100 - 106.

Ferreira, J.L.M., Riet, C.F., Schild ,A.L and Mendez M..D.C (1991). Poisoning of cattle by *Amaranthus* spp (*Amaranthaceae*) in Rio Grande de. *Pesquisa veterinaria Brasileira* **11**:3 - 4,49 - 54.

Flegal, A.R. (1993). Lead. Properties and determination. In *Encyclopaedia of Food Science Food Technology and Nutrition. Vol. 4* (eds.) Macrae, R., Robinson, R.K and Sadler, M.J. Academic Press, London. pp. 2665 - 2670.

Gad, S.S., El-Zalaki, M.E., Mohamed, M.S and Mohasseb, S.Z. (1982). Oxalate content of some leafy vegetables and dry legumes consumed widely in Egypt. *Food chemistry* **8**:169 - 177.

Gareth, L., Gisela, O. and Rui, M. (1998). Productions of a carotenoid - rich product by alginate entrapment and fluid-bed drying of *Dunaliella salina Journal Science Food Agric.* **76**: 298 - 302.

Garg, A.N and Weginwar, R.G. (1993). Phosphorus properties and determination. In *Encyclopaedia of Food Science Food Technology and Nutrition.Vol. 6* (eds.) Macrae, R., Robinson, R.K and Sadler, M.J. Academic Press, London. pp.3567 - 3571.

George, R.A.T. (1985). *Vegetables seed production*. Longman, London.

Gomez, M.I. (1981). Carotene content of some green leafy vegetable of Kenya and effects of dehydration and storage on carotene retention. *Journal of plant Foods* **3**:231 - 234.

Gomez, M.I. (1982). *The evaluation of fruits and vegetables resources in the machakos district*, in relation to seasonal deficits and micronutrient deficiencies. IDRC/KIFST/DFST Project, Nairobi, Kenya.

Gordon, L.K and Wayne, R.S. (1993). Heavy metal toxicology. In *Encyclopaedia of Food Science Food Technology and Nutrition.Vol. 4* (eds.) Macrae, R. , Robinson, R.K. and Sadler, M.S. Academic press, London. pp 2318 - 2324.

Graham, T. (1983). *Leaf Protein Concentrates* AVI publishing Co. Inc., Westport, Conn.

Grubben, G.J.H. (1976). *The cultivation of amaranth as a tropical leafy vegetable*. Royal Tropical Institute, Amsterdam.

Grubben, G.J.H. (1977). Tropical vegetable and their genetic resources. In *International Board for plant Genetic resource* (IBPGR). (eds.) Tindal, H.D and Williams, J.T. FAO, Rome, Italy.

Hegsted, D. M (1973). Calcium and Phosphorous. In *Modern Nutrition in Health and Disease,* 5th edition (eds.) Goodhart, R.S. and Shils, M.E., p. 268. Lea and Febiger, Philadelphia, Pa.

Herschodoerfer, S.M, (1968). *Quality control on food industry* Vol.2 Academic Press, London.

Hooda, P.S., McNutty, D., Alloway, B.J. and Aitken, M.N. (1997). Plant availability of heavy metals in soils previously amended with heavy applications of sewage sludge. *Journal science Food Agric.* **73**:446 - 454.

Ifon, E.T. and Bassir, O. (1979). The nutritive value of some Nigerian leafy green vegetables. Part 1: vitamin and mineral contents. *Food chemistry* **79**:263 - 262.

Imbamba, S.K. (1973). Leaf protein content of some Kenyan vegetables. *East Africa Agric. and forestry Journal* **38**:248 - 251.

Imungi, J. K. and Potter, N. N. (1983). Nutrient contents of raw and cooked leaves. *J. Food Sci.* **48:**4, 1252 - 1254.

Imungi, J. K. and Potter, N. N. (1983). Nutritional and sensory properties of canned and stored cowpea leaves. *Nutritional Reports International* **31:**1, 21 - 34.

Imungi, J.K. (1984). Nutritional and sensory properties of cooked, canned and stored cowpea leaves. *PhD Thesis*. Cornell University.

Imungi, J.K. (1990). *Some undesirable constituents in Kenyan plant Foods presented at the FAO/ANP seminar*, Dept. of Food Tech. and Nutrition. University of Nairobi, June 25 - 27. Kenya.

Isabel, A. and Concepcion, G. O. (1997). Mineral composition of soils and vegetation form six mountain Grassland communities in Northern Spain. *Journal Science Food Agric.* **73**:200 - 206.

John, B. A. (1993). Phosphorus physiology. In *Encyclopaedia of Food Science Food Technology and Nutrition. Vol.6* (eds.) Macrae, R., Robinson, R.K. and Sadler, M.S. Academic press, London. pp 3271 - 3577.

Kasidas, E.P. and Rose, G.A. (1982). Oxalate content of some common foods determination by an enzymatic method. *Journal of Human Nutrition*, **31**:225 - 226.

Kays, S. L. (1991). Postharvest physiology of perishable plant products. Van Nostrand Reinhold, New York.

Kefford, J.K., Mckanzie, H.A and Thompson, P.C.O. (1959). Effects of oxygen on quality and ascorbic acid retention in canned and frozen orange juices. *Journal of science Food and Agric.* **10**:51 - 53.

Kirk, T., Dennison, D., Kokoczka, P. and Heldman, D. (1977). Degradation of ascorbic acid and in dehydrated food system. *J. of Food sci.* **42**:5,1274 - 1277.

Komners, G.D. Torres, M.B., Dantas, A.F. and Barros, C.S.L. (1996). Experimental poisoning of pigs with species of *Amranthus* in Rio Grade do Sul State, Brazil. *Pesquisa Veterinaria Brasileira* **16**:4,121 - 125.

Kuhnert, M.P., Kuhnert, R.B., Ernhart, C.B., Brittenham, G.M. and Erhard, P. (1985). Blood levels of bead, protoporphyrin and ferritin in two year old urban children. In *Trace elements in man and animal.* (ed.) Mills, C.F, Bremner, I. and Chesters, J. K. CAB, London.

Labuza, T.P. (1972). Nutrient losses during drying and storage of dehydrated foods. CRC-critical Reviews. *Journal of Food Technology* **3**:217 - 219.

Larry, A. B. and Michael, P. D. and Seppo, S. (1990). *Food Additives*. Marcel Dekker Inc., New York.

Lathika, K.M. Sharma, S. Inamdar, K. V. and Raghavan, K.G. (1995). Oxalate depletion form leafy vegetables using alginate entrapped banana oxalate oxidase. *Biotechnology letters* **17**:4, 407 - 410.

Latunde-Dada, G.O. and Neale, R.J. (1986).. Availability of Iron from foods. Review *Journal of Food Technology* **21**:255 - 268.

Lee, C. Y., Massey, L. M., Jr. and Van Buren, J.P. (1982). Effect of Post-harvest Handling and processing on vitamin contents of Peas. *J. of Food sci.* **42**:961 - 964.

Lemos, R.A., Barros, C.S.L., Salles, M.S., Barros, S. S. and Peixoto, P.V. (1993). Naturally occurring *Amaranthus spinosus* (*Amaranthaceae*) poisoning in cattle. *Pesquisa veterinaria Brasileira* **13**:1 - 2, 25 - 34.

Liener, I. E. (1969). *Toxic constituents of plant food stuffs*. Academic press, New York.

Maeda, E. E. and Salunkhe, D. K. (1981). Retention of ascorbic acid and total carotene in solar dried vegetables. *J. of Food sci.* **46**:1288 - 1290.

Marshall, V. L., Buck, W.B. and Bell, G. L. (1967). Pig weed (*Amaranthus retroflexus*) an oxalate containing plant. *American Journal Veterinary Res.* **28**:888 - 889.

Mathooko, F. M. and Imungi, J. K. (1994). Ascorbic acid change in three indigenous Kenyan leaf vegetables during traditional cooking. *Ecology of Food and Nutrition* **32**:1, 239 - 245

Mekonnen, Y. (1994). A survey of plant (potentially) toxic to livestock in the Ethiopian flora. Sinet, *Ethiopian Journal of science* **17**:1, 9 - 35.

Meyer, L. H. (1960). *Food chemistry*. AVI publishing Co. Inc., Westport Conn.

Michael, H.G.(1990). *Principles and Application of GLC in Food Analysis*. Ellis Horwood, England.

Miloslav, R. Jr. (1983). *CRC Handbook of Naturally occuring Food Toxicants*. CRC press Inc., Florida.

Mohri, T. (1993). Nitrates and Nitrites. In *Encyclopaedia of Food Science Food Technology and Nutrition Vol.5.* (eds.) Macrae, R., Robinson, R. K. and Sadler, M. J. Academic press, London. pp. 3240 - 3244.

Molins, R.A. (1991). *Phosphates in food* CRC press, Inc. Florida.

Monsen, E.R., Hallberg, L., Layrisse, M., Hegsted, D.M., Cook, D.J., Mertz, W. and Finch, C.A. (1978) Estimation of available dietary iron. *Amer. J. Clin. Nutr.* **31**: 134.

Mudambi, S. R. (1977). Effects of storage on ascorbic acid content of orange. *Journal of food Technology* **12**:6, 639 - 641.

Nelson, P. E. (1972). Processing effects on nutritional components of horticultural crops. *Horticultural Science* 7:151 - 153.

Nijhoff, W.A., Grubben, M.J.A.L., Nagengast, F.M., Jansen, J.B.M.L., Verhanen, H.I. Vanpoppel, G. and Petter, W.H.M. (1995). Effect of consumption of brussels sprouts on intestinal and lymphocytic glutathiones-transferases in humans. *Carcinogenesis*: **16**:9,2125 - 2128.

Oke, O.L. (1980). *Amaranth in Nigeria*. Proc of the 2nd. Amaranth Conference. Rodale Press Inc., Emmaus.

Philip, E.S. and Manuel, G.M. (1991). Ascorbic acid retention on Orange Juice stored under simulated consumer home conditions. *J. of Food Sci.* **56**:3,867 - 868.

Phillip, L.W. and Nancy, S.R.D. (1974). *Nutritional qualities of fresh fruits and vegetables*. Futura publishers Co. Inc., New York.

Pobel, D., Riboli, E., Cornee, J., Henon, B. and Guyader, M. (1995). Nitrosamine, nitrate and nitrite in relation to gastric cancer. A case control study in Marseille, France. *European Journal of Epidemiology* **11**:1,67 - 73.

Ricardo, B. (1993). Amaranth. In *Encyclopaedia of Food Science Food Technology and Nutrition. Vol.1* (eds.) Macrae, R., Robinson, R.K. and Sadler, M.J. Academic press, London. pp. 135 - 140.

Salles, M.S., Lombardo-de-Barros, C.S., Lemos, R.A. and Pilati, C. (1991). Perirenal edema associated with *Amaranthus* spp poisoning in Brazilian Swine. *Veterinary and Human Toxicology* **33**:6, 616 - 617.

Salunkhe, D.K., Do, J.K. and Bolin, H.R. (1974). Developments in technology and nutritional value of dehydrated fruits, vegetables, and their product. In *Storage, processing, and Nutritional quality of Fruits and vegetables.* (ed.) Salunkhe, D.K. CRC press Inc., Ohio.

Samuel, G. (1985). Electrophoresis as an Aid for Identification of Various Species and cultivars of grain Amaranthus. *M.Sc. Thesis*. University of Nairobi.

Scanlan, R.A. (1993). Nitrosamines. In *Encyclopaedia of Food Science Food Technology and Nutrition. Vol.5* (eds.) Macrae, R. Robinson, R. K. and Sadler, M. J. Academic press, London. pp. 3245 - 3249.

Sean, R.L. (1993). Iron physiology. In *Encyclopaedia of Food Science Food Technology and Nutrition Vol.4* (eds.) Macrae, R. Robinson, R. K. and Sadler, M. J. Academic press, London. pp. 2577 - 2583.

Sen, N. P (1980). *Nitrosamine.* In *Safety of foods*. 2nd edition (ed.) Graham H.. AVI publishing Con Inc., Westport conn.

Singh, B. R. and Jeng, A. S (1993). Uptake of Zinc, cadmium, mercury, lead, chromium and nikel by rye grass grown in a sandy soil. Norwegian *Journal of Agriculatural Sciences* 7:147 - 157.

Sizolone, W. Hay, T., Johnson, B.J. and Burows, G.E. (1988). Perirenal edema in a calf. F (Amaranth poisoning). *Journal of veterinary and Human Toxicology* **30**:3, 265 - 266.

Smooth, J.M., and Nagy, S.N. (1979). Effects of storage temperature on total vitamin C content of canned single strength grape fruit juice. *Food Science Technology Abstract*. **10**:1, 1 H 70.

Spearman, G. and Johnson, K. (1989). Redroot pigweed toxicosis in cattle. *Canadian veterinary Journal* **30**:3,255 - 256.

Staivok, G. (1977). Changes in the chemical composition of some potato variety tubers during storage. *Chemical abstract* **89**: 174382 K.

Steel, J.A. and Tong, C.H. (1996). Degradation kinetics of green colour and calorimetry and HPLC. *J. of Food Sci.* **61**:5, 924 - 927.

Thomas. E.F. (1968). *CRC Handbook of Food additives*. The Chemical Rubber Co. Cleverland, Ohio.

Tomohiro, S. (1990). *Laboratory Manual for Food analysis,* Technical Co-operation Project of Jomo Kenyatta University of Agriculture and Technology.

Torres, M.B., Kommers, G.D., Dantas, A.F.M. Lombardo-de-Barros, C.S.L (1997) . Redroot pigweed (*Amaranthus retroflexus*) poisoning of cattle in Southern Brazil. *Journal of Veterinary and Human Toxicology* **39**:2,94 - 96.

Varoquax, P., Varoquax, F. and Tichit, L. (1986). Technical note: Loss of nitrate from carrots during blanching. *Journal of Food Technology* **21**:401 - 407.

Vasil, A.V., Ratnikor, A.N., Aledsakhin, R.M. Sirotkin, A.N. Krasnora, Kudryavtsev, E.G. and Grudina, V.N. (1995). Characteristics of radionuclide and heavy metal

transfer within the soil plant, animal product system in livestock farming. *Khimiya-v-sel'skom-Khozyaistve* No 4,16 - 18

Vather, M., Johansson, G., Akesson, A and Rahnster, B. (1972). Faecal elimination of lead and cadmium in subjects on a mixed and four lactovegetarian diet. *Food chemist* **30**:4, 281 - 287.

Walker , R.R.P. (1972). The human requirements of calcium. Should low intake be supplemented. *Amer. J. Clin. Nutr.* **25**:518.

WHO. (1972). *Nutritional anemias*. WHO. Tech. Rep. Ser. 503. World Health Organisation, Geneva.

Wong, D.W.S. (1989). *Mechanism and theory in food chemistry*. Van Nostrand Reinhold, New York.

Wu, M.T. and Salunkhe, D.K. (1974). The use of certain chemicals to increase nutritional value and to extend quality in economic plants. In *Storage, processing and Nutritional quality of Fruits and Vegetables.* (ed.) Salunkhe, D.K. CRC press Inc., Cleveland, Ohio.

7. APPENDICES

Appendix 1: Analysis of variance for moisture content

Source	DF	sum of square	Mean square	F-value	Pr
Rep	2	2.6545	1.3273	2.67	0.148
Loc	3	3.3712	1.1237	2.26	0.1816
Error	6	2.9815	0.4969		
Total	11	9.0072			

CV = 0.8017%

Appendix 2: Analysis of variance for total solids

Source	DF	SS	MS	F-Value	Pr>F
Rep	2	0.5400	0.2700	0.87	0.464
Loc	3	6.3292	2.1097	6.83	0.023
Error	6	1.8533	0.3088		
Total	11	8.7225			

CV = 3.22%

Appendix 3: Analysis of variance for crude protein.

Source	DF	SS	MS	F-Value	Pr>F
Rep	2	1.3294	0.6647	1.81	0.242
Loc	3	32.3050	10.7683	29.33	0.006
Error	6	2.2027	0.3671		
Total	11	35.8372			

CV = 2.02%

Appendix 4: Analysis of variance for ash

Source	DF	SS	MS	F-Value	Pr>F
Rep	2	2.0529	1.0265	3.28	0.1088
Loc	3	5.8139	1.9379	6.20	0.0287
Error	6	1.8753	0.3125		
Total	11	9.7422	3.125		

C.V. = 3.18

Appendix 5: Analysis of variance for crude fiber

Source	DF	SS	MS	F-Value	Pr>F
Rep	2	1.5245	0.7622	15.12	0.0045
Loc	3	5.5180	1.8393	36.49	0.0003
Error	6	0.3025	0.0504		
Total	11	7.3450			

C.V. =1.41%

Appendix 6: Analysis of variance for lipid (Ether extract)

Source	DF	SS	MS	F-Value	Pr>F
Rep	2	0.0613	0.0306	0.83	0.4813
Loc	3	0.1405	0.0468	1.27	0.3672
Error	6	0.2219	0.0369		
Total	11	0.4236			

C.V.= 4.88%

Appendix 7: Analysis of variance for nitrate.

Source	DF	SS	MS	F-Value	Pr>F
TRT	1	400140.298	400140.298	254.10	0.0001
Loc	3	12583.581	4194.527	2.66	0.0773
Error	19	29919.653	1574.719		
Total	23	442643.531			

C.V. = 7.50%

Appendix 8: Analysis of variance for oxalate

Source	DF	SS	MS	F-Value	Pr>F
TRT	1	222620416.7	22620416.7	83.92	0.0001
Loc	3	2737916.7	912638.9	3.39	0.0395
Error	19	5121250.0	269539.5		
Total	23	30479583.3			

C.V. = 13.47%

Appendix 9: Analysis of variance for total carotene

Source	DF	SS	MS	F-Value	Pr>F
TRT	1	8.4491	8.4491	0.97	0.3365
Loc	3	464.0848	154.6949	17.80	0.0001
Error	19	165.078900	8.6884		
Total	23	637.612735			

C.V. = 9.74%

Appendix 10: Analysis of variance for ascorbic acid

Source	DF	SS	MS	F-Value	Pr>F
TRT	1	654076.675	654076.675	1867.37	0.0004
Loc	3	26569.375	8856.458	25.28	0.6096
Error	19	6655.051	350.266		
Total	23	687301.100			

C.V. = 3.82%

Appendix 11: Analysis of variance for iron.

Source	DF	SS	MS	F-Value	Pr>F
TRT	1	0.0155	0.0155	18.71	0.0004
Loc	3	0.0155	0.0005	0.62	0.6096
Error	19	0.0155	0.0008		
Total	23	0.0328			

C.V. = 25.12%

Appendix 12: Analysis of variance for calcium

Source	DF	SS	MS	F-Value	Pr>F
TRT	1	2.9260	2.9260	65.64	0.0001
Loc	3	0.1369	0.0457	1.02	0.4041
Error	19	0.8470	0.0446		
Total	23	3.9099			

C.V. = 9.67%

Appendix 13: Analysis of variance for phosphorus

Source	DF	SS	MS	F-Value	Pr>F
TRT	1	0.3480	0.3480	311.71	0.001
Loc	3	0.0090	0.0030	2.68	0.0760
Error	19	0.0212	0.0011		
Total	23	0.3782			

C.V. = 6.36%

Appendix 14: Analysis of variance for β-carotene (storage test).

Source	DF	SS	MS	F-Value	Pr>F
Treatment	8	63.4444	7.9306	23.79	0.0001
Temp.	2	16.7778	8.3889	25.17	0.0002
Time	2	44.111	22.0556	66.17	0.0001
Temp. Time	4	2.5556	0.6389	1.92	0.1918
Error	9	3.0000	0.3333		
Total	17	66.4444			

C.V = 1.84%

Appendix 15: Analysis of variance for ascorbic acid (storage test)

Source	DF	SS	MS	F-Value	Pr>F
Treatment	8	571.3480	71.4185	30.62	0.0001
Temp.	2	401.6890	200.8447	86.11	0.0001
Time	2	165.8490	82.9247	35.55	0.0001
Temp. Time	4	3.8090	0.9523	0.41	0.7985
Error	9	20.9915	2.3324		
Total	17	592.3395			

C.V = 3.37%

Appendix 16: Analysis for cooked amaranth quality score.

Quality attributed	SV	DF	SS	MS	F-value	Tab
Appearance and Colour					Cal	95%
	Product	1	26.37	26.37	37.67	4.38
	Panelists	19	9.87	0.53	0.74	2.16
	Error	19	13.13	0.7		
	Total	39	49.37			
Flavour:						
	Product	1	22.50	22.50	31.69	4.38
	Panelists	19	13.10	0.69	0.97	2.16
	Error	19	13.50	0.71		
	Total	39	49.10			
Texture:						
	Product	1	10	10	31.25	4.38
	Panelists	19	6	0.32	1.00	2.16
	Error	19	6	0.32		
	Total	39	22			
Overall acceptability						
	Product	1	2.02	22.50	31.69	4.38
	Panelists	19	8.27	0.44	1.39	2.16
	Error	19	6.48	0.34		
	Total	39	16.77			

Appendix 17. Score sheet for cooked amaranth leaves sensory evaluation.

Name..... Date.....

Please look at and taste the cooked amaranth samples presented to you and indicate your degree of liking for Appearance and Colour, Flavour, Texture and overall acceptability of each sample on the basis of the following scale:

9 = like extremely

8 = like very much

7 = like moderately

6 = like slightly

5 = neither like nor dislike

4 = dislike slightly

3 = dislike moderately

2 = dislike very much

1 = dislike extremely

Place the number matching your degree of liking for each quality attribute in the corresponding box for each sample.

Sample Code				
Appearance and colour				
Flavour				
Texture				
Overall acceptability				