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Properties of a Complementary Food based on Amaranth Grain (*Amaranthus cruentus*) Grown in Kenya

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ABSTRACT

Amaranth grain (A*maranthus cruentus*) is a pseudo cereal consumed in various parts of the world with potential as a source of dietary nutrients. Amaranth grain is a good source of protein and vitamins and therefore is used largely for feeding children and the elderly. Although it can be used to alleviate malnutrition, its processing and nutritional characteristics are not well established. Development of new products from amaranth will expand utilization of this raw material as cereal-based foods which play an important role in the diets of many people in Kenya. The aim of this study was to determine the nutritional and functional properties of *Amaranthus cruentus* grain grown in Kenya for preparation of a ready-to-eat product that can be recommended for nutritional interventions as infant complementary food. The effect of processing on the physicochemical and nutritional properties of amaranth grain was analysed. The functional properties, acceptability and stability of amaranth grain product were also determined. The treatment structure involved ungelatinized (raw) amaranth grain flour used as the control and pregelatinized amaranth grain flour referred to as the product. The product was well accepted with 20 minutes steaming period considered as the average time required to acquire a ready to eat product. The colour of raw amaranth grain was cream with a lightness (L*) value of 79.4, which slightly reduced after processing to 74.1, giving a slightly dark cream product. A notably high fat, protein and ash content was demonstrated, both in raw and processed grain. The proximate analysis mean values for raw and processed grain were moisture 10.2% and 2.4%; protein 17.2 and 16.7%; fat 7.0%, 7.0% ash 2.7 and 2.6%; crude fiber 3.8 and 3.1%; carbohydrates 59.2 and 68.3%, respectively. Amaranth grain contained good amount of unsaturated fatty acids 76.1%, with predominant ones being oleic 36.3% and linoleic 35.9%. The fatty acid profile associated with good amount of protein makes pregelatinized amaranth grain product a nutritionally balanced food appropriate for infant feeding. Amaranth grain product was rich in potassium, phosphorus, calcium and magnesium, which were not significantly ($P>0.05$) affected by the processing method. The tannins significantly ($P\leq0.05$) decreased during processing while phytates were not affected. The water soluble vitamins reduced during processing which affects the nutritional value of the product. However the product was rich in tocopherols which are essential for infant growth and development. The amino acids composition of processed amaranth grain were not significantly ($P\leq0.05$) affected by the processing method with essential ones identified as were histidine, threonine, valine, methionine, isoleucine, leucine, phenylalanine and lysine. Processing affected the functional properties of amaranth grain with water absorption capacity increasing from 343.9 g/100 g for the raw grain samples to 471.3 g/100 g for the product. However the protein water solubility decreased from 44. 1% to 27.4%. The dilution factor for the amaranth grain product was found to be 15 g/100ml with an acceptable viscosity for infant feeding. Due to moisture reduction in the product the bulk density reduced from 0.7 g/ml for the raw sample to 0.5 g/ml for the product.

This study achieved the objective of developing a complementary product of adequate nutritive value that can be prepared using locally available resources and technology. Steeping and steam pregelatinization of amaranth grain produced a ready nutritious product with improved solubility during reconstitution, suitable for infant feeding.

CHAPTER ONE

1.0. INTRODUCTION

1.1. Background of the study

The major causes of childhood malnutrition in Kenya include low rates of exclusive breastfeeding in the first 6 months of life and limited intake of appropriate foods from 6 to 24 months of age. These combined with high rates of infection and poor feeding practices result in high rates of morbidity (Sandra *et al.,* 2000). Although about 97% of infants in urban Kenya are breastfed at birth and are breastfed for an average for 19 months, the proportions who are exclusively breastfed for six months is minimal.

According to research carried out on effect of work status on exclusive breastfeeding in Nairobi (Alice, 2002) the prevalence of exclusive breastfeeding was found to be 13.3% at three months, with 46.4% of the mothers introducing other foods before one month. The transition from exclusive breastfeeding to family foods, referred to as complementary feeding, should cover the period from 6 to 18-24 months of age, and is a very vulnerable period. It is the time when malnutrition starts in many infants, contributing significantly to the high prevalence of malnutrition in children under five years of age world-wide (WHO, 2005).

It is estimated that worldwide 10.9 million children under five years of age die every year, where two-thirds of these deaths occur during the first year and is related to inappropriate infant feeding practices (WHO, 2005). Out of these under five deaths, 75% occur during infancy due to preventable sickness like diarrhea, pneumonia and neonatal infections and for all these three, exclusive breastfeeding for the fist six months has been found to be the number one evidence based intervention. WHO estimates that 2 out of 5 children are stunted in low-income countries (WHO, 2005). Adequate nutrition and health during the first several years of life is fundamental for child survival and the prevention of malnutrition. Although in comparison with infants never breastfed, those fed a mix of breast milk and customary supplements still benefit immunologically (Wilson *et al.*, 1998; Cesar *et al.*, 1999; Raisler *et al.*, 1999 and Lu and Costello, 2000).

Growth faltering is common among young children in developing countries, affecting as many as 33% of all children under 5 years of age (UN, 2000). Gruels or porridges are a particular

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problem because they are made from staples with a high content of starch which, when cooked with water, gelatinizes and becomes highly viscous. Considerable amounts of water are commonly added to produce low-viscosity porridge, but this dilutes the nutritional value (Ashworth and Draper, 1992). One study conducted in rural Kenya found that toddlers consumed only about 80% of energy requirements, with only 8% of their calories coming from animal products and 13% from fat, compared with 36% and more than 25% in the United States (Murphy *et al*, 1992). Several micronutrients have been shown to be low in children's diets, including zinc, iron, vitamin B12, fat-soluble vitamins (A, D, and E), and calcium (Murphy *et al.,* 1992, Murphy *et al.,* 1995). Iron and zinc are important for the growth and development of infants and young children. Inadequate consumption of these nutrients can have long lasting negative effects on children's learning, behavior and development. Breastfed infants 9 months of age and older get just 10 percent of the iron and zinc they need from breast milk, so they must rely on complementary foods for these nutrients (Krebs *et al.,* 2006).

Protein-Energy Malnutrition (PEM) is a major contributory factor to the high infant mortality rate in most developing countries and is attributed to the poor availability of nutritionally adequate complementary foods (Jansen, 1982). Protein energy malnutrition is a serious problem in Kenya, with the main reason being scarcity and high price of foods of animal origin. Children between the age of 4 months and 2-3 years are suffering from malnutrition because they are neither getting mother's milk nor the supplementary foods. Major problems are generally low incomes, poor environmental conditions and lack of education. Cameron and Hofvender (1983) stress the need to educate families to exploit locally produced foods to produce nutritionally adequate products.

1.2. Statement of the research problem

Grain amaranth offers the prospect of substantially increasing food output in dry land areas of Kenya. Until recently, amaranth was regarded as a vegetable for the poor, but increasingly, the grain is being planted by Kenyan farmers. Despite its multiple values, cultivation of grain amaranth has not received much attention, and processing and utilization of the crop in Kenya is currently low, with its main consumption being at household levels as a leafy vegetable. As more and more people find out about the amaranth crop, the demand will continue to increase, which could eventually make it a sustainable cash crop and help many East African families to rise above the poverty line.

The formulation and development of nutritious complementary foods from local and readily available raw materials has received a lot of attention in many developing countries. The widespread problem of infant malnutrition in the developing world has stimulated efforts in research, development, and extension by both local and international organizations. Extensive research into the process characteristics, nutritional quality, and consumer acceptability of grain amaranth is necessary if these items are to be used effectively to improve the nutrition status of the vulnerable group of the population.

There is a need to expand grain amaranth market by increasing consumption through product development. Research has been done extensively on agronomic, post harvest, chemical and nutritional composition of amaranth grain, but it has not benefited from value added research required for competitiveness on a wider scale, especially in Kenya. There is still need for studies to provide information on the processing and nutritional characteristic of complementary foods based on amaranth grain.

1.3. Justification

Infancy is a time of rapid physical growth as well as physiological, immunological, and mental development. During the first year of life, nutritional requirements are at their highest in the entire life cycle. Deficiency in energy or any of the essential nutrients can have dire consequences, some of which are long-lasting. In the first four to six months of life, the infant's nutritional requirements can be totally satisfied by breast milk. Afterwards, complementary foods need to be introduced to augment energy and nutrient intake. Complementary foods are, therefore, transitional foods consumed between the time when the diet is composed exclusively of mother's milk and the time when it is mostly made up of family foods. Complementary foods are consumed during the relatively short period from around 4 to 6 months to about 12 months of age. During the time they are consumed, complementary foods make up a large proportion of the baby's diet and contribute a significant amount of the nutrients that are necessary for growth and development. The foods, therefore, must contain sufficient amounts of the essential nutrients to complement milk. Infants who do not receive enough complementary foods may be stunted or malnourished or both.

Nowadays, due to the reduced consumption of breast milk, important nutrients such as proteins, zinc, iron and B-vitamins are likely to be deficient in the contemporary diet of the affected infants (LLLI, 2002). If this development is not well handled during this crucial growth period, it can then lead to under-nutrition. As in most other developing countries, the high cost of fortified nutritious proprietary complementary foods is always, if not prohibitive, beyond the reach of most Kenyan families, which often depend on inadequately processed traditional foods consisting mainly of un-supplemented cereal porridges made from maize, sorghum and millet. Due to the fact that grain amaranth has high protein, as well as a high fat content, there is the potential to use it as an energy food. The balance of carbohydrates, fats, and protein, allow amaranth the opportunity to achieve a balanced nutrient uptake with lower amounts of consumption than with other cereals (Morales *et al.,* 1988). It has been noted that high protein rice is the only other cereal which has been cited to satisfy protein and energy needs (Morales *et al.,* 1988).

The amaranth seed is becoming a versatile cash crop in Eastern Africa and could make a good source of income for underprivileged families. It has proved to be a breakthrough in the fight against food insecurity. The challenge remains to incorporate it in the list of Kenyan staple foods. Amaranth grain is 90% digestible and because of its ease of digestion it has traditionally been given to those recovering from illness or fasting period (Morales *et al.,* 1988). Development of new products will expand utilization of this raw material for consumption. The nutritional value of amaranth and environmental adaptability creates an excellent potential for the crop to positively impact on thousands of poor farmers who rely on staple crops that are often neither resilient nor nutritious.

This study will provide information about the processing, nutritional and physicochemical characteristics of a complementary food based on amaranth grain.

1.4. Research Objectives

1.4.1. Overall objective

To develop amaranth grain complementary food product and evaluate its physicochemical and nutritional properties.

1.4.2. Specific Objectives:

a) To formulate amaranth grain complementary food.
b) To identify the appropriate processing method of the amaranth grain product through sensory characteristics.
c) To assess the effect of processing method of amaranth grain on the physicochemical and nutritional properties of amaranth grain product.

d) To determine the antinutrient content and physicochemical properties in the amaranth grain product.

1.5. Hypothesis of the study

Processing amaranth grain has no effect on its physicochemical and nutritional properties.

CHAPTER TWO

2.0. REVIEW OF LITERATURE

2.1. Amaranth grain

The name Amaranth is derived from the Greek word for never-fading flower. Amaranth was a staple in the diets of ancient Aztecs and Incas who believed it had supernatural powers and the grain was part of religious ceremonies and also used in the making of religious statues (Kelly and Martin, 1983). It has been cultivated as a grain for 8,000 years, dating back to the Maya culture of South and Central America (Kelly and Martin, 1983). There are about 60 Amaranth species, several of which are cultivated as leaf vegetables, grains or ornamental plants, while others are weeds (Kauffman and Weber, 1990). The main species grown as vegetables are *A. tricolor, A. dubius, A. lividus, A. creuntu, A. palmeri* and *A. hybridus* while *A. hypochondriacus, A. cruentus* and *A. caudatus* are the main grain species (Teutonico and Knorr, 1985). Amaranth produces a large amount of biomass in a short period of time (Kauffman and Weber, 1990) and therefore has the potential to contribute to a substantial increase in world food production. Grain yield of up to 5,000 kg/ha has been reported (Stallknecht and Schulz-Schaeffer, 1993).

The seed producing Amaranthus is a cereal-like crop, which has an outstanding agronomic performance owing to its resistance to drought, hot climate and pests (Paredes-López *et al.*, 1990). It produces small seeds that constitute an important food resource in areas of Latin America, Africa and Asia (Teutonico and Knorr, 1985), where amaranth seeds and leaves are currently consumed.
The Amaranthaceae family consists of hardy, weedy, herbaceous, fast-growing, cereal-like plants (Opute, 1979), with a seed yield of up to 3 tons/ hectare when grown in monoculture for 3-4 months, and a vegetable yield of 4.5 tons dry matter/hectare after 4 weeks (Grubben and van Sloten, 1981). Amaranth is one of those rare plants whose leaves are eaten as a vegetable while the seeds are used as cereals (Oke, 1983; Saunders and Becker, 1984; Kauffman and Haas, 1983).There is no distinct separation between the vegetable and grain types since the leaves of young plants grown for grain can be eaten as both human and animal food. When the leaves are harvested in moderation, the grain yield is unaltered.

Table 1: Nutritional composition of amaranth grain per 100 g (*Amaranthus cruentus*)

Nutrient	Unit	Composition
Energy	Kcal	345.42-418.9
Moisture	g	6.23 – 6.71
Crude protein	g	13.58-17.6
Total lipids	g	6.3-8.1
Crude fibre	g	3.4-5.3
Crude ash	g	2.3-3.6
Nitrogen-free extracts	g	58.6-68.9
Phosphorous	mg	455-477
Sodium	mg	31.0
Potassium	mg	290-366.0
Calcium	mg	153.0-175
Magnesium	mg	244-266
Iron	mg	7.59-17.4
Zinc	mg	3.18-3.7
Copper	mg	0.777-1.2
Manganese	mg	4.6
Riboflavin	mg	0.19-0.23
Niacin	mg	1.17-1.45
Ascorbic acid	mg	4.2- 4.5
Thiamine	mg	0.07-0.1
Folacin	µg	49.000
Palmitic acid	g	1.284
Oleic acid	g	1.433
Linoleic acid	g	2.834
Phytosterols	mg	24.000
Phytate	mg	0.60-0.50
Tannin	mg	0.043-0.13
Histidine	g	0.389
Isoleucine	g	0.582
Leucine	g	0.879
Lysine	g	0.747
Methionine	g	0.226
Threonine	g	0.558
Tryptophan	g	0.181
Valine	g	0.679
Arginine	g	1.060
Alanine	g	0.799

Teutonico and Knorr (1985)

2.2. Amaranth grain nutritional value

Nutritional composition of *Amaranthus cruentus* grain shown in Table 1. The amaranth grain is rich in carbohydrates (48–69%), protein (12–18%) and fat (5–8%) (Becker *et al.*, 1981; Bressani, 1994). It contains a high concentration of lysine, 0.73% to 0.84%, of the total protein, an essential amino acid lacking in all of the world's main cereal crops (Becker *et al.*, 1981; Bressani, 1994). It is also relatively rich in the sulfur-containing amino acids, which are normally limiting in the pulse crops (Bressani *et al.*, 1987). Several

studies have shown leucine to be the first limiting amino acid in amaranth (Becker *et al.,*1981; Pedersen *et.al.,* 1987; Teutonico and Knorr, 1985; Saunders and Becker, 1984) although some reports indicate that threonine actually may be the amino acid which is more biologically limiting than leucine (Bressani *et al.,* 1987). Amaranth grain has a protein score of 67 to 87 (Kauffman and Weber, 1990). By comparison, wheat (14% protein) scores 47, soybeans (37%) score 68-89, rice (7%) scores 69, maize (9%) scores 35. Although amaranth is theoretically close to the ideal, combining it with another grain increases the quality to the ideal amino acid reference pattern established in 1998 by the FAO/WHO of the United Nations (Kauffman and Weber, 1990).

The potential complimentary nature of amaranth protein has been studied by combining amaranth with wheat (Pant 1985), sorghum (Pedersen *et al.,* 1987) and maize (Tovar and Carpenter, 1982; Sanchez and Maya, 1985). Ordinary maize meal supplemented with as little as 12.7% (by weight) of toasted amaranth flour provides a nutritionally superior source of protein that can satisfy a good portion of the protein requirement of young children, and provide approximately 70% of diet energy (Morales *et al.,* 1988).

The starch component of amaranth is distinctive. The starch granules are polygonal, measure 1 to 3 mm in diameter, and have a high swelling power (Kauffman and Weber, 1990). There is a distinctive gel characteristic to the starch (Yanez *et al.,* 1986). Waxy and non-waxy starch granules have been identified (Kauffman and Weber, 1990). Interest has been expressed in specialized food and industrial applications for amaranth starch as a result of its distinctive characteristics.

Amaranth grain is a good source of iron which is required by a number of enzymes that are required for oxygen metabolism. Iron-deficiency anaemia reduces oxygen-carrying capacity and interferes with aerobic functions (Dallman, 1986). Very severe anaemia is associated with increased mortality during childhood and pregnancy (Van Den Broeck *et al.,* 1993).

2.3. Processing amaranth grain

Grain amaranth can be used as seeds or flour to make products such as cookies, cakes, pancakes, bread muffins, crackers, pasta and other bakery products (Teutonico and Knorr, 1985). Kauffman and Weber (1990) provided a description of the variety of products made from amaranth in different parts of the world. These include soups and stews from whole grain; *alegria*, a confection made from popped amaranth in Mexico; *atolea*, a fermented Mexican drink made from roasted amaranth flour; *chichi*, which is a form of beer made from amaranth in Peru; *sattoo*, a gruel consumed in Nepal, and *chapatti* made in different parts of Asia .

The amaranth processed under conditions that do not damage its protein and its essential amino acids availability, like moist heat cooking and extrusion, presents good protein quality, similar to casein (Mendonza and Bressani, 1987). The digestibility and the protein efficiency ratio are improved if the grain is heat processed (Kauffman and Weber, 1990). The removal of lectins by heat processing has been reported to improve the protein efficiency ratio of the amaranth flour (Singhal and Kulkani, 1988). There are a number of viable methods for processing, including popping, toasting, heat-rolled flakes, extrusion, and wet cooking as gruel. Excessive thermal processing has been shown to reduce the quality of amaranth grain (Bressani and Elias, 1984). The potential for reducing nutritional quality is most evident when amaranth grain is processed using hot dry heat, as in toasting or popping (Bressani and Elias, 1984).

Recently, interest in amaranth has increased because of its nutritional value and functional properties (Gikonyo *et al.,* 2006). Snack foods with good acceptance and high nutritive value have been developed by extrusion cooking of the defatted flour obtained from milling the grain (Chávez-Jáuregui *et al.,* 2000). The extrusion cooking process is based on starch gelatinization and protein denaturation using high pressure and high temperature (Arêas, 1996). Such amaranth snack foods also present characteristics such as cholesterol-lowering effects in hypercholesterolemic rabbits (Plate and Arêas 2002), protein of high biologic value, and high bioavailability of calcium, zinc, and magnesium (Ferreira, 1999).

Processing is important with respect to the protein quality of amaranth grain. According to work carried out by Pederson *et al.,* (1987) on the nutritive value of amaranth grain processed grain were found to increase the protein quality of amaranth grain. However if the processing is carried out under more extreme conditions of time and temperature, it destroys the quality of the product by reducing available Iysine content (Pederson *et al.,* 1987). Of interest is the extrusion process, which for *A. cruentus* and *A. caudatus* yielded cooked flour equal in protein quality to casein (Mendoza and Bressani, 1987).

Cereals are usually the first solid foods given to infants, because they are readily available and culturally acceptable staple foods. In this study, steam pregelatinization of amaranth grain was employed for preparation of ready to eat product. This improved product solubility during reconstitution, resulting in a smooth and light viscous porridge. The use of the whole grain as a food source merits greater attention, particularly as a complementary food, because of its excellent protein quality and relatively high energy content. Similarly, it is important to define better the conditions needed for optimum thermal processing of amaranth grain in view of its nutritive value when eaten raw (Bressani and Elias*,* 1984; Imeri *et al.,* 1987). Although attempts have been made at producing instant foods using drum drying, extrusion or spray drying techniques (Banigo *et al.,* 1974; Cheryan *et al.,* 1979), the commercialization rate of these technologies in developing countries had been very low due to capitalization cost which eventually results in a cost of such manufactured foods exceeding the means of those in need of the products The use of traditional technologies to develop instant and nutritious foods has been encouraged (Malleshi *et al.,* 1986).

2.4. Utilization of amaranth grain in Kenya

In Kenya grain amaranth was registered officially as a crop in 1991 by the Ministry of Agriculture. Since then its spread has been very slow perhaps owing to the low esteem with which it is generally held. But now it is emerging that the crop could be the answer for those areas where finding a suitable cash crop has been a problem. As part of its Rapid Results Initiatives, the Poverty Eradication Commission is promoting amaranth as a cash crop that could help fight poverty (GTZ Sustainet, 2006).

In Kenya, amaranth is sold in some supermarkets in major towns, but in very small quantities. Its consumption is also cited in some important institutions like Kenyatta National Hospital in the private wings and in HIV/AIDS orphaned children's homes, where it is recommended for patients on special diet. Despite its high nutritional value for both human beings and domesticated animals, not many people know about it. There is need to educate people about this important indigenous food and to develop products that can reach a larger population.

Farmers that grow amaranth have marketed their crop in a number of ways. Some sell small bags of the whole grain or flour mail-order to consumers. Many of these purchasers are allergic to wheat products. Other growers sell to local or regional health food stores or restaurants. There are also a few middlemen who buy grain from the farmers and market it to the larger health food companies.

The Sustainable Rural Livelihoods (SRL) program in Uganda, has promoted the use amaranth grain for feeding malnourished children. Amaranth is usually blended with other grains (mainly

maize and millet) and given to children in form of porridge. Amaranth-based porridge has also been adopted for the feeding of normal children as a complementary food, and for feeding the sick including people living with HIV and AIDS (PLWHA). Feedback from the community shows a strong association of amaranth consumption and fast recovery from childhood malnutrition and reduced morbidity of PLWHA. Feedback from the community shows a strong association of amaranth consumption and fast recovery from childhood malnutrition and reduced morbidity of PLWHA (Robertson and Lupien, 2008).

2.5. Complementary feeding

Child malnutrition remains a common problem in developing countries. Early growth retardation is associated with a broad range of adverse functional consequences, including delayed motor development and impaired cognitive function and school performance, and malnourished children have a higher risk of infection, ill-health and death. Indeed, recent analyses indicate that as much as one half of under-five child mortality is associated with malnutrition (WHO 2009). In many countries faulty complementary feeding practices - primarily nutritionally inadequate and frequently contaminated foods that are introduced too early or too late - are a major contributing factor to infant and young child malnutrition, growth failure, and high morbidity and mortality.

The period of complementary feeding refers to the stage of life when foods and/or liquid milks are fed to infants and young children in addition to breast milk; non-breast-milk food items consumed at this time are defined as complementary foods (Kenneth 1997). The adequacy of complementary feeding not only depends on the availability of a variety of foods in the household, but also on the feeding practices of caregivers. Feeding young infants requires active care and stimulation, where the caregiver is responsive to the child clues for hunger and also encourages the child to eat, referred to as active or responsive feeding

WHO recommends that infants start receiving complementary foods at 6 months of age in addition to breast milk, initially 2-3 times a day between 6-8 months, increasing to 3-4 times daily between 9-11 months and 12-24 months with additional nutritious snacks offered 1-2 times per day, as desired (WHO 2009).

Childhood under nutrition remains a major health problem in resource-poor settings. Approximately one-third of children less than five years of age in developing countries are stunted (low height-for-age), and large proportions are also deficient in one or more micronutrients. Recent data shows that just over half of 6-9 month olds are breastfed and given complementary foods and only 39 per cent of 20-23 month olds are provided with continued breastfeeding (UNICEF 2008).

A normal infant experiences a 3-fold increase in weight and a 2-fold increase in length over the first year of life and also experiences dramatic development changes in organ function and body composition. These rapid rates of growth and development impose unique nutritional needs over and above relatively high maintenance needs incident to the higher metabolic and nutritional turnover rate of infants versus adult. In addition, the young infant's lack of teeth and immature digestive and metabolic processes often complicate delivery of the special nutritional needs. (Barbara and Robert, 2001).

The protein requirement of a normal infant also is greater per unit of body weight than that of the adult. In addition the infant is thought to require a higher proportion of essential amino acids than the adult. These include the essential amino acids: leucine, isoleucine, valine, threonine, methionine, phenylalanine, tryptophan, lysine and histidine (Dewey *et al.,* 1996). The required intake of specific protein depends on its quality, which is easily defined as how closely its amino acid pattern resembles that of human milk (Fomon *et al.,* 1973). The amino acid composition of human milk protein is considered ideal and the total protein content of human milk averages only ≈10 g/L. Thus on average, ≈200ml.kg^{-1}.day^{-1} must be ingested to meet the current Recommended Dietary Allowance (RDA) for protein (i.e. 2.0 -2.2 g.kg^{-1}.day^{-1}) (Barbara & Robert 2001).

The higher nutritional value of amaranth compared with maize (Tovar and Carpenter 1982, Mendoza and Bressani 1987) is an important advantage for most of the population in developing countries, where maize is the staple and grain amaranth can be grown. Their combination in home preparations or industrial products for child feeding would significantly enhance the protein and lipid contents of the diet. In the developed world, when abstention from dairy products is necessary, the amaranths represent a uniquely structured protein source with great potential to supplement or complement the common cereals. The high lysine content of amaranth grain makes it particularly attractive for use as a blending food source to increase the biological value of processed foods (Pedersen *et al.,* 1987). This may impart health benefits or desirable physiological effects and can be used to develop new food products with beneficial components. It is therefore evident that amaranth can increase the energy and nutritional density of complementary food to help in combating child malnutrition.

CHAPTER THREE

3.0. MATERIALS AND METHOD

3.1.

3.1. Research design

The research involved processing raw amaranth grain into flour for physicochemical, nutritional and functional properties analysis. The treatment structure involved ungelatinized and pregelatinized amaranth grain flour referred to as the control and the product respectively. Analysis of nutritional and physicochemical properties was done in the laboratory. For each treatment, three sample were analyzed each one in duplicate.

3.2. Materials

Grains of *Amaranthus cruentus* variety were purchased from farmers in Baringo and Nakuru Districts, where the grains were ready for harvest during the period of research and could be easily sampled from the farm during harvesting. The grains were selected randomly, carefully sorted and stored within 48 hours at 15^{0}C before analysis.

Plate 1: *Amaranthus cruentus*

Plate 2: *Amaranthus cruentus* grains

Plate 3: Steamed amaranth grains Plate 4: Amaranth grain product

3.3. Formulation of amaranth grain product

Amaranth ready-to-eat product was prepared from amaranth grains, as shown in Figure 1, by the traditional wet-milling process described by Adeyemi *et al.,* (1988). Pregelatinized amaranth flour was prepared from amaranth grains, where the grains were weighed into a container and steeped overnight by soaking in warm water of initial temperature 70°C (held for 10 minutes). Steeping is important because it improves the cleaning operation, to obtain a higher quality product. During steeping, certain physical and biochemical changes occur, such as swelling of grains, degradation of soluble carbohydrates and removal of some pigments, micro-organisms and bitter substances from the grains (Owuama, 1998). After steeping, the water was decanted and the grains spread on four perforated trays, allowed to drain for 15 minutes, then steamed at atmospheric pressure for 10, 20, 30 and 40 minutes. The steamed grains were dried at 70°C in a cabinet dryer to a moisture content of less than 5%, cooled, conditioned, milled with an attrition mill and sieved to obtain flour fraction of <500 µm (Adeyemi, 1987). Steaming caused softening and cooking of the grains. The grains were dried to acquire desired moisture content and milled aseptically to attain a fine powder in ready-to-eat form.

Ungelatinized amaranth grain flour was also produced from the raw amaranth grains. The clean grains were milled with an attrition mill to obtain flour fraction of <500 µm. These products were packed in sealed plastic bags and stored at temperature below 15^{0}C for further analysis.

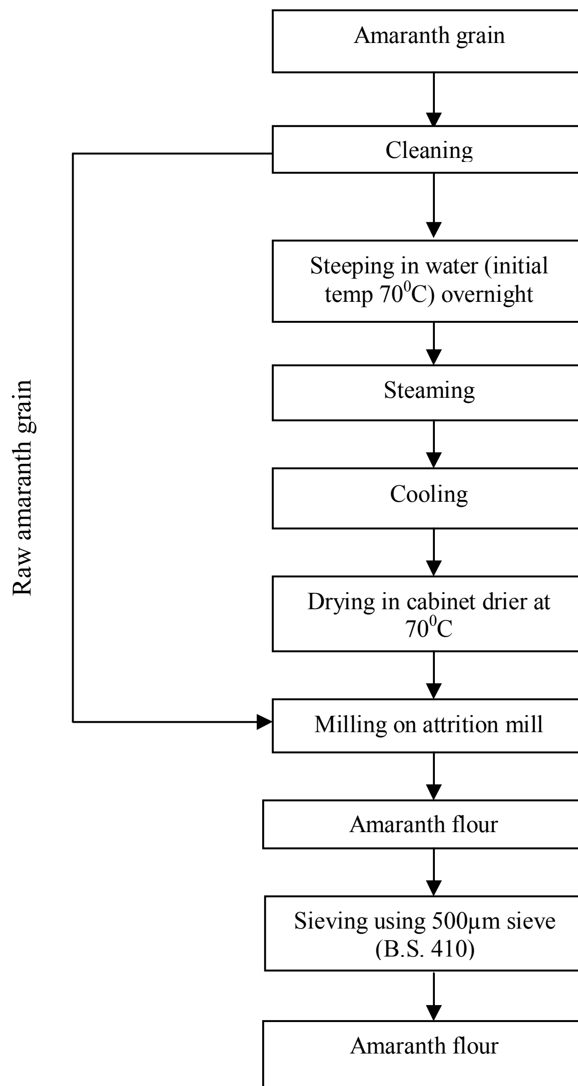

Figure 1: Preparation of ungelatinized and pregelatinized amaranth grain flour samples

3.4. METHODS

3.4.1. Sensory characteristics of pregelatinized amaranth grain product

Pregelatinized amaranth grain product was determined by a panel of ten panelists of mothers selected from the staff and graduate students of the Department of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology. All the panelists were conversant with the factors governing the quality of the products and infant feeding requirements. Each panelist evaluated the following characteristics: a) Color, b) Flavor, c) Texture, and d) Overall acceptability. The samples were prepared by use of clean drinking water boiled for 5 minutes and allowed to cool. The gruels prepared for this purpose were made with a standardized recipe using 15 g of the mix per 100 ml of water to obtain gruel with pouring consistency. This test was used to rate samples on the extent of starch gelatinization.

A 9 point Hedonic scale (Ranganna, 1994) was used to measure the consumer acceptability of the product (Appendix 1). The relative importance of each factor was compared numerically on a scale of 9 to 1 (9 = like extremely, 1 = dislike extremely). Each panelist gave a score. The average score of each sample was then calculated. To ascertain uniformity of judgments among the total score assigned by each of them, the scores were added for the various individual characteristics and the best samples selected on the basis of overall acceptability and least steaming time.

3.4.2. Colour difference

Colour was determined in triplicate with a Minolta Chroma Meter CR-300 (Minolta Camera Co., Osaka Japan). Color was represented by the L* a* b* color notation. This is a 3-D color presentation method in which L* is the lightness of the color, and equals to zero for black and 100 for white. a* is the amount of red (0 to 60) or green (0 to -60) while b* is the amount of yellow (0 to 60) or blue (0 to -60) (Mallikarjunan and Mittal, 1994).

3.4.3. Chemical analysis of amaranth grain product

3.4.3.1. Moisture content

Moisture content was determined by the AOAC (1995) method 930.04

About 2 g of well mixed sample was accurately weighed into a moisture dish and transferred to an air oven previously heated to temperatures of 130°C and drying done for 1 hour. The final weight of the sample was taken after the drying period and cooling in a dessicator. This was reported as the percent weight loss.

3.4.3.2. Crude protein

Protein content (N% x 5.85) was determined using Semi-Micro Kjeldal Method according to the AOAC (1995) procedure 978.04

Digestion: About 1 g of sample was accurately weighed then transferred to a digestion flask together with a catalyst composed of 5 g of potassium sulphate and 0.5 g copper sulphate and 15 ml of concentrated sulphuric acid. The mixture was heated in a fume hood till the digest colour turned blue signifying the end of digestion process. The digest was then cooled and transferred to a 100 ml volumetric flask and topped up to the mark with distilled water. A blank digestion was also prepared.

Distillation and titration: The distilled sample was subjected to distillation and titration using KJELTEC AUTO 1030 analyzer (Tecator AB, Hoganas, Sweden).

Calculations:

Nitrogen % = $(V_1 - V_2) \times N \times 0.014 \times 100/S$

Where;

V_1 = Titer for sample (ml)

V_2 = Titer for blank (ml)

N = Normality of standard HCl solution (0.05)

S = Weight of sample taken (g)

3.4.3.3. Fat content

This was done according to Soxhlet extraction (AOAC 1995) 920.85-32.1.13) method which gives intermittent extraction with excess of fresh diethyl ether. Extraction was done in SOXTEX System HT 1043 Extraction Unit (Tecator AB, Hoganas, Sweden), for six hours.

Calculations: Fat % = (Weight of fat extracted/ Weight of sample) X 100.

3.4.3.4. Ash content

The ash content was done according to the AOAC (1995) method 923.05. 2-5 g sample were weighed in pre-conditioned crucibles. The samples were first charred by flame to eliminate smoking before being incinerated at 550°C to the point of white ash. The residues were then cooled in desiccators and the weights taken.

Crude ash % = (Weight of residue / Weight of sample) * 100

3.4.3.5. Crude fiber

Crude fiber was carried out according to the AOAC (1995) method 920.86.

Sample preparation: Approximately 2 g of sample was weighed and transferred into a 500 ml conical flask. 200 ml of boiled sulphuric acid (1.25%) was then added and boiling continued for 30 min under reflux. Filtration was done under slight vacuum with Pyrex glass filter (crucible type) and the residue washed to completely remove the acid with boiling water. 200 ml of boiling sodium hydroxide (1.25%) was then added to the washed residue and boiling continued under reflux for another 30 min. Filtration was done using the same glass filter previously used with the acid and the residue rinsed with boiling water followed by hydrochloric acid (1%) and again washed with boiling water to rinse the acid from the residue. The residue was then washed twice with alcohol and thrice with ether.

Drying and incineration: The residue was dried with glass filter at 100^0C, cooled to room temperature and weighed to get constant weight. This was incinerated at 450-500^0C for about 1hr then transferred to a dessicator cooled to room temperature then weighed to get constant weight.

Fibre % = [(Weight before incineration – weight after incineration)/ Weight of sample] * 100

3.4.3.6. Nitrogen free extract (NFE)

NFE content was estimated by difference on dry matter basis.

NFE = Total sample weight – (moisture + crude protein + ash + crude fat + crude fibre)

3.4.4. Nutritional evaluation of amaranth grain product

3.4.4.1. Fatty acid composition

Extraction of total lipids: Modified Bligh and Dyer method (1959) was used. 1 g of amaranth grain flour in a 50 ml glass-stoppered centrifuge tube was denatured at 100^0C for 3 minutes. The product was added to 2 ml water and 7.5 ml mixture of methanol-chloroform (2:1, v/v) and the mixture left at room temperature for

several hours with intermittent shaking. After centrifugation, the supernatant extract was decanted into another 50 ml glass-stoppered centrifuge tube and the residue resuspended in 9.5 ml mixture of methanol-chloroform-water (2:1:0.8:, v/v); and the homogenate shaken and centrifuged. The extraction procedure was repeated once and the supernatant combined. To the combined supernatant extracts were added 7.5 ml each of chloroform and water and the mixture centrifuged. The lower chloroform phase was withdrawn, and brought to dryness in a rotary evaporator. The lipid residue in the flask was completely dried under vacuum in a dessicator over fresh KOH pellets (about 1-2 h), and the weight of lipids measured and expressed as percent on dry matter basis. This oil was used for fatty acid analysis by gas liquid chromatography (GLC), and also for vitamin E analysis.

Methyl esterification of lipids for fatty acids test by gas chromatography (GC) was done by refluxing 2-5 mg of oil in 2 ml of 95% methanolic hydrochloric acid (HCl) for 1 hour. Methyl esters formed were extracted thrice using 2 ml of n-hexane. A small amount of anhydrous sodium sulfate was added to the extract, to remove water. The solvent was evaporated to concentrate the extract to 0.3 ml using a stream of nitrogen. This was injected to the GC machine for the fatty acid profile. Identification of fatty acids was done by comparing with known methyl ester standards from Sigma (Code 189-4, 189-17, and M-3378).

Instrumentation: The analyses were performed using a Shimadzu GC-9A (Shimadzu Co., Tokyo, Japan) fitted with a glass column, prepacked and preconditioned by Shimadzu; Shinchrom E-71 5% Shimalite (80-100 Aw), 3.1 m in length by 3.2 mm internal diameter and flame ionization detector. Isothermal column temperature of 200^0C was used and injector/detector temperature of 230^0C.

Flow rate was 8 ml/minute, injection Volume 1µl. Gases used were nitrogen carrier gas at 2.63 $kg/cm^{2,}$ Hydrogen at 0.68 kg/cm^2 and air at 0.35 0.68 kg/cm^2. Shimadzu integrator software was used to calculate the peak areas.

3.4.4.2. Mineral composition

Sample treatment: The ash was dissolved in 15 ml 6 N HCl in a volumetric flask which was then topped up to 100 ml mark with distilled water. This was used for mineral determination according to the AOAC method (1995). Iron, copper, calcium, sodium, magnesium, potassium and zinc were determined by Atomic Absorption Flame Emission Spectrophotometer (Shimadzu Corp., Tokyo Japan, Model AA 6200), using the respective cathode lamps. The individual mineral element composition was calculated from the AAS or UV-Visible spectrophotometer readings obtained for both the blank and the test solution.

Phosphorus was determined with the vanadomolybdate colorimetric method (Pearson, 1976) with potassium phosphate as the standard. 50 ml sample were pippeted into 125 ml Erlenmeyer flask and added a drop of phenolphthalein indicator. 8 ml combined reagent (5 N sulfuric acid, ammonium molybdate solution, 0.1M ascorbic acid) were added and mixed thoroughly. Absorbance was measured after 10 minutes, at 880 nm, using a blank reagent as the reference solution. All determinations were done in triplicate and reported in mg/100g sample.

3.4.4.3. Vitamin E

Vitamin E (α-Tocopherol) was analyzed using Perkin-Elmer (PE) series 400 liquid chromatograph fitted with a UV detector using the method of Zahar and Smith (1990), with some modification.

Saponification and extraction: To a 50 ml stoppered centrifuge tube, 2 ml of oil sample were added followed by 5ml of absolute ethanol containing 1% (w/v) ascorbic acid and 2 ml of 50% (w/v) potassium hydroxide. The tube was sealed and agitated carefully, then placed in a water bath at 80^0C for 20 minutes. The tubes were agitated periodically to ensure complete digestion of fat. After saponification, tubes were cooled with running water and then placed in an ice-water bath. 20 ml of solvent (petroleum ether: diethyl ether 1:1) containing 0.01% (w/v) butylated hydroxytoluene was added. Tubes were again stoppered and mixed vigorously with a vortex for 1 minute, allowed to stand for 2 minutes and again vortexed for 1 minute. 15 ml of cold water (1-2^0C, kept in ice water bath) were added to each tube and tubes inverted at least 10 times, then centrifuged at 1000 rpm for 10 minutes. 10 ml of the upper, organic layer was accurately removed by pipette into a tube and the solvent was evaporated to dryness using rotary evaporator. Immediately the residue was redisolved in 2 ml of HPLC grade methanol.

Standard curve: Standard curve was prepared using known concentrations of α -tocopherol against tocopherol internal standard ration. Linear regression analysis was used to predict concentrations of the unknown.

Instrumentation: The analysis were performed using a HPLC Model LC-10AS, Shimadzu Corp., Kyoto, Japan fitted with UV detector at 205-340 nm wavelength filter, stainless steel column NOVA-PAK C_{18}, 3.9 mmX15 cm column at 35^0C oven temperature. Mobile phase: methanol: water 95:5 (both HPLC grade) at a flow rate of 8 ml/minute and injection volume of 20 µl.

Shimadzu software was used to calculate the peak areas. Peak heights of tocopherol in the sample extracts were measured and compared with those of the standards.

3.4.4.4. Water soluble vitamins

Ascorbic acid, thiamin riboflavin and niacin were determined as described in the method by Ekinci and Kadakal (2005). Deionised water (50ml) was added into 5 g sample, and the mixture homogenized for 1 minute, then centrifuged for 10 minutes at 14 x 10^3 rpm. The stationery phase was flushed with 10 ml methanol and 10ml water then adjusted to pH 4.2 to activate the stationery phase. Acidified water was prepared by adding a 0.05 M HCl solution drop by drop with stirring until the pH reached a predetermined value. The sample was eluted with 5 ml water (pH 4.2) then 10ml methanol at a flow rate of 1ml/minute. The eluent was collected in a bottle and evaporated to dryness. The residue was dissolved in mobile phase, filtered through 0.45 µm micro filters, and injected into the HPLC column.

The column eluate was monitored with a photodiode-array detector at 265 nm for vitamin C, 234 nm for thiamine, 266 nm for riboflavin, 261 nm for niacin, 324 nm for pyridoxine, 282 nm for folic acid, 204 nm for biotin and pantothenic acid.

Standard curve: Standard curves were prepared using known concentrations of vitamin standard (ascorbic acid, niacin, pantothenic acid, pyridoxine, thiamine, folic acid and riboflavin) against vitamin internal standard ration. Linear regression analysis was used to predict concentrations of the unknown water soluble vitamins.

Instrumentation: The analysis were performed using a HPLC (Shimadzu, Japan) PE series 400 liquid chromatograph fitted with a photo-diode detector, a C_{18} column ODS 250 mm x 4.0 mm stainless steel at 35^0C oven temperature. Mobile phase was 0.1 mol/litre di-potassium phosphate (pH 7): methanol 90:10, flow rate 0.7ml/min and injection volume 20 µl. Shimadzu software was used to calculate the peak areas.

3.4.4.5. Amino acids composition

Precolumn derivatization of amino acids with *o*-phthalaldehyde (OPA) followed by reversed-phase HPLC separation with fluorometric detection was used according to method described by

Maria and Federico (2006), with modifications. This technique does not detect amino acids that exist as secondary amines (e.g., proline).
Defatting: Amaranth grain flour 30 g was mixed with 100 ml hexane and let to stand for about 20 minutes, then filtered at 3000 rpm for 10 minutes to remove the supernatant (solvent and the oil).

Protein extraction: This was carried out according to method by Sumner *et al.,* (1980) with modification as described by Aguilera and Garcia (1989).

Amaranth flour 20 g raw and processed was extracted twice with 100 ml H_2O, adjusted to pH 9, while stirring for 20 min. Each extract was separated by centrifuging at 1000 rpm for 20 min. The extracts were combined and the protein precipitated by adjusting to pH 4.5 with 1 N HCl and separated by centrifuging. The precipitate was redispersed in 100 ml H_2O, dissolved at pH 9 and reprecipitated at pH 4.5. After separation of the protein by centrifuging, it was given two 50ml washes at pH 4.5. The flour was slurried and converted to protein extract by adjusting to pH 6.5-7 with 1 N NaOH. The protein isolates were freeze-dried. Each trial was carried out in triplicate.

Protein hydrolysis: Glass Pyrex test tubes were cleaned thoroughly and dried in an oven at 80^0C. About 0.2 g protein isolate were weighed in the test tube and added 6 N HCl containing 5% of phenol. The tubes were held in dry ice-acetone bath where pressure in the tube was reduced by use of a vacuum pump, and sealed off. Hydrolysis was allowed to take place at 110^0C for 24 hours in an oven, allowed to cool and subjected to centrifugation at 2000 rpm for five minutes to force water drops on the inner wall of the test tube to come down to the solution level.

The test tubes were opened and the resultant solution transferred into freeze drying eggplant-shaped flask. The test tubes were rinsed with fresh 6 N HCl and the washing added to the flasks, and then freeze dried for 12 hours.

Sample dilution: Sample diluents was prepared by weighing 9.8 g of sodium citrate dissolved in 400ml distilled water in a 500 ml volumetric flask. 8 ml of perchloric acid and 0.05 of n-caprylic acids were added, and the volume topped to the mark with distilled water. The pH of the solution was adjusted to 2.2 with perchloric acid. The hydrolyzed sample was dissolved in 2 ml of sample diluent.

Standard amino acid solution: Mixed amino acid solution (H-type from Sigma Chemicals) was diluted by taking 0.5 ml of the standard into 10ml sample diluent prepared earlier. The H-type mixed solution available commercially contains 17 amino acids and ammonia each at 2.5µmol/ml. Therefore the concentration in the diluted solution was 0.125µmol/ml. The diluted solution was then mixed with OPA solution at the ratio of 1:1 for HPLC analysis.

Analytical procedure: Chromatography condition was in accordance with the Agilant method (Mengerink *et al.,* 2002). Briefly, the hydrolyzed samples and amino acid standards solutions were automatically derivatized with OPA solution at a ratio of 1:1. OPA solution was prepared by mixing 0.5 mg/ml of OPA to borate diluent (pH 10.4), 0.1% (v/v) 2-mercaptoethanol and 0.1% (v/v) of Brij 35. After derivatization, an amount equivalent to 10 µl of each sample was injected on an ODS column 5µm, 250 x 4.6 mm at 40^0C, with detection at 350 nm excitation wavelength and 450 nm emission wavelength. Mobile phase A was 40mM di-potassium phosphate, adjusted to pH 7.8 with NaOH, while mobile phase B was acetonitrile /methanol/water (45/45/10 v/v/v). The separation was obtained at a flow rate of 1 ml/minute with a gradient program that allowed for 1.9 min at 0% followed by a 16.3 min step that raised eluent B to 53%. The washing at 100% B and equilibration at 0% B was performed in a total of 30 minutes.

Instrumentation: Analysis was performed using a HPLC (Model LC-10AS, Shimadzu Corp., Kyoto, Japan) PE series 400 liquid chromatograph fitted with a binary pump delivery system, column thermostat and a fluorescence detector.

Peak identification and quantification: Amino acids were detected based on the retention time established for the individual amino acid under defined experimental conditions. Calculation was based on the intensity established for a given amino acid of known concentration.

3.4.5. Antinutrient factors in amaranth grain product

3.4.5.1. Phytates

Analysis of phytic acid in amaranth grain products was done by HPLC combining the column/mobile phase conditions established by Tanjendjaja *et al.,* (1980), with modification as detailed by Camire and Clydesdale (1982).

A known amount of amaranth grain flour sample was weighed into a 125 ml Erlenmeyer flask, extracted with 25 ml of 3% sulphuric acid for 30 minutes on a shaker bath at medium speed and at room temperature. The slurry was filtered through fast filter paper (Whatman #41) and rinsed using a fine jet stream from a squeeze bottle, with a small volume of extracting solvent. The filtrate was transferred to a 50 ml screw cap centrifuge tube and placed in a boiling water bath (BWB) for 2-5 minutes (to aid in the precipitation of ferric phytate), before addition of 3 ml of a ferrous chloride solution containing 6mg ferric iron per ml in 3% sulphuric acid. The tubes were heated in a BWB for 45 minutes to allow for complete precipitation of the ferric phytate complex. This was centrifuged at 2,500 rpm for 10 minutes and supernatant discarded, while the precipitate was washed once with 30 ml of distilled water, centrifuged again and supernatant discarded. The remaining content in the tube was added 3 ml of 1.5 N NaOH and 1 ml of distilled water. A glass rod was used to break the precipitate and then sonicated to completely disperse the precipitate, which was topped to 30 ml. The samples were cooled, centrifuged and the supernatant quantitatively transferred to 50ml volumetric flask. The precipitate was rinsed once with approximately 10ml of distilled water, centrifuged and added to the volumetric flask.

Preparation of standard curve: A stock solution containing 10 mg/ml of sodium phytate in distilled water was prepared. Serial dilutions were made to contain from 1 g/100 ml to 100 mg/100 ml. The sample and standard dilutions were injected to the HPLC using a 20 µl sample loop.

Instrumentation: Analysis were performed using a HPLC Model LC-10AS, Shimadzu Corp., Kyoto, Japan equipped with a UV detector at 205-340 nm filter, 250 mm X 4.6 mm ID column containing spherisorb ODS C18 10 µ packing and oven temperature 35^0C. Mobile phase was 0.005M sodium acetate, flow rate 0.5 ml/minute and injection volume 20 µl. Shimadzu software was used to calculate the peak areas.

3.4.5.2. Tannins

Condensed tannins in the amaranth grain were determined using the Vanillin-Hydrochloric acid method (Burns, 1963; Price *et al.,* 1978). About 0.25 g milled sample was weighed into Erlenmeyer flasks, pippeted 10 ml of 4% HCL in methanol and flask closed with parafilm. This was shaken for 20 minutes on a wrist-action shaker. The extract was centrifuged for 10 minutes at 4,500 rpm (3300xg), and the supernatant aliquot transferred to a 25 ml volumetric flask. The residue was rinsed back from the centrifuge

tubes in to the original conical flasks using 5 ml of 1% HCl in methanol. This was covered with parafilm with shaking for 20 minutes then centrifuged again for 10 minutes. The aliquot was combined with the first extract. The extracts were filled to 25 ml volume with methanol and mixed well. 5 ml vanillin-HCl reagent was added to 1ml of the extract and catechin standard solutions (0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml made to 1ml volume with methanol. The blank sample was prepared by adding 5 ml of 4% HCL in methanol to 1 ml aliquot of the extract. Absorbance was read in the spectrophotometer at 500 nm exactly 20 minutes after adding vanillin-HCl reagent. The spectrophotometer was adjusted with methanol as the reference solvent.

Calculation: A standard curve of absorbance against catechin concentration was prepared from the catechin standard solution readings.
Percent catechin equivalents were calculated using the formula;
%CE = (CC x VM / VE x wt) x100
Where
CC= Catechin concentration (mg/ml-1)
VM = volume of the extract (25 ml)
VE = volume of extract taken (1 ml)
Wt = weight of sample (mg)

3.4.6. Physicochemical properties of amaranth grain product

3.4.6.1. Viscosity

Method by Kieran *et al.,* (1999). Pregelatinized amaranth grain flour was reconstituted to porridge for viscometer determination using a Brookfield Viscometer model LVDV-II+P (Brookfield Engineering Laboratories USA).
The flour was mixed with 500 ml water in to a thick paste in different flour rations: 8%, 10%, 15% and 20 %. The samples were held in water bath at 40^0C and analysed for viscosity in millipascal second [mPa·s].

3.4.6.2. Water absorption capacity

The evaluation of the rate of water uptake of flour was carried out by the centrifugation method of Lin *et al.,* (1974), as explained by Mwasaru *et al.,* (1999). One gram of flour was mixed with 50 ml of distilled water and the suspension stirred
using a magnetic stirrer for 5 min. The suspension was transferred into centrifuge tubes and centrifuged at 3,500 rpm for 30 min. The supernatant obtained was measured using a 50 ml measuring cylinder. The density of the water was assumed to be 1 g/ml. The water absorbed was calculated as the difference between the initial water used and the volume of the supernatant obtained after centrifuging. The result was expressed as a percentage of water absorbed by the blends on g/100g basis. At least three measurements were conducted for each sample and the mean was expressed as ml of liquid retained per gram of sample.

3.5.6.4 Bulk density

The bulk density of the flour was determined by placing a sample in a 10 ml graduated cylinder with gentle uniform tapping during filling. The cylinder was filled to the mark and the weight of the flour was measured. The bulk density was calculated as mass by volume in grams per milliliter (g/ml).

3.5.6.5 Protein water solubility

Protein water solubility of amaranth grain flour was determined by the method of Oshodi and Ekperigin (1989), with slight modification as described by Stoscheck (1990). A 0.02 g sample of flour was added to 10
ml of distilled water, mixed with a spatula and centrifuged at 3500 rpm. The protein in the supernatant was determined as described by the Semi-Micro Kjeldal; 20.87 (32.1.22) AOAC, (1995). Solubility was expressed as the percentage ratio between water-soluble protein and total protein content

3.4.7. Data analysis

Data was subjected to the analysis of variance and treatment means that differed significantly (P<0.05) were separated by the least significant difference (LSD) using SAS program (SAS Institute Inc., Cary NC, USA 2002-2003).

CHAPTER FOUR

4.0. RESULTS AND DISCUSSION

4.1. Sensory evaluation of amaranth grain product

The mean sensory evaluation scores for color, flavor, texture and overall acceptability of samples subjected to different heat treatment are presented in Table 2. Raw amaranth grain samples (A) were rated lowest in all characteristics, and were not significantly different (P≤0.05) in colour and flavor from the product steamed for 10 minutes (S^2). The color, flavour, texture and overall acceptability of amaranth grain samples steamed for 20, 30 and 40 minutes were not significantly different (P≤0.05) from each other and showed significant improvement compared to the 10 minutes steaming and the control, and were considered as ready to eat. It was therefore concluded that amaranth grain samples steamed for a minimum of 20 minutes could be used to make a ready to eat complementary product and considering the economic implication of steaming, 20 minutes was taken as the benchmark for further work in this project, with the product obtained referred to as processed amaranth grain flour.

Table 2: Mean sensory scores for amaranth grain flour[1]

Samples	Texture	Colour	Flavour	Overall acceptability
A	4.4±0.4[c]	6.0±0.3[b]	2.6±0.3[b]	5.6±0.4[c]
S^2	5.9±0.4[b]	6.7±0.4[b]	2.3±0.4[b]	7.0±0.3[b]
S^3	8.8±0.1[a]	8.0±0.3[a]	6.8±0.3[a]	8.7±0.2[a]
S^4	8.9±0.1[a]	8.0±0.3[a]	6.7±0.4[a]	8.8±0.1[a]
S^5	8.9±0.1[a]	7.9±0.3[a]	6.8±0.4[a]	8.8±0.1[a]
LSD	0.8	0.9	1.0	0.8

[1]Each score is a mean of 10 panelist ± standard error. In each column means followed by the same letter are not significantly different (P≤0.05).
A is the control with 100% raw amaranth grain flour
S^2 is amaranth grain steamed for 10 minutes
S^3 is amaranth grain steamed for 20 minutes
S^4 is amaranth grain steamed for 30 minutes
S^5 is amaranth grain steamed for 40 minutes.

4.2. Colour description

Table 3 shows colour of amaranth grain flour samples. The colour values L*, a* and b* for raw and processed amaranth grain were significantly different (P≤0.05) in raw and processed grain samples. The colour for the raw grain amaranth flour was light cream turning slightly darker after processing. Processed amaranth flour showed lower L* values and higher a* values than raw amaranth grain flour indicating that processed flour was darker than raw flour due to Maillard reaction (non-enzymatic browning) favoured by the high temperature in steaming and relatively low moisture processing.

Table 3: Colour comparison of amaranth grain flour[1]

	L*	a*	b*
Control[2]	79.4±0.7[a]	1.3±0.2[b]	15.9±0.2[b]
Product[3]	74.1±0.2[b]	2.6±0.1[a]	21.5±0.1[a]
LSD	1.5	0.2	0.5

[1]Means of two samples analysed in triplicate ± standard error. In each column means followed by the same letter are not significantly different (P≤0.05)
[2]Raw amaranth grain flour
[3]Processed amaranth grain flour
L* colour lightness
a* colour redness (0 to 60)
b* colour yellowness (0 to 60)

4.3. Effect of processing on nutritional properties of amaranth grain product

4.3.1. Chemical composition of amaranth grain flour

Average chemical composition of amaranth grain is shown in Table 4. Processing amaranth grain did not have a significant effect (P≤0.05) on its proximate composition; protein, ash, fibre and fat, except moisture reduction in drying, thus raising the nitrogen free extract. The values were in agreement with those reported by other researchers for *A. cruentus* species. Bressani (1994), Williams and Brenner (1995) and Muchová *et al.,* (2000) reported value ranges of crude protein 12-19%, fat 5-8%, crude fibre 4-5% and ash 2-4% on dry matter basis.

Amaranth grain is considered to have a unique composition of protein, carbohydrates, and lipids (Bressani, 1989; Lehman, 1989), thus can achieve a balanced nutrient uptake with lower amounts of consumption than cereals. Grain amaranth has higher protein (12 to 18%) than cereal grains and has a significantly higher lysine content, which makes it particularly attractive for use as a blending food to increase the biological value of processed foods (Pedersen *et al.,* 1987).

Table 4: Chemical composition of amaranth grain flour (grams/100 g) [1]

	Control[3]	Product[4]	LSD
Moisture	10.2±0.1[a]	2.4±0.5[b]	1.1
Protein (db)[2]	17.2±0.4[a]	16.7±0.4[a]	1.2
Fat (db)	7.0±0.1[a]	7.0±0.2[a]	0.5
Ash (db)	2.7±0.1[a]	2.6±0.1[a]	0.2
Fibre (db)	3.8±0.1[a]	3.1±0.3[a]	0.7
Nitrogen free extract(db)	59.2±0.3[b]	68.3±0.4[a]	1.1

[1]Means of two samples analysed in triplicate ± standard error. In each row means followed by the same letter are not significantly different (P≤0.05)
[2]db is dry basis
[3]Raw amaranth grain flour
[4]Processed amaranth grain flour

The protein value of amaranth grains is highlighted when amaranth flour is mixed with cereal grain flours. When amaranth flour is mixed 30:70 with either rice, maize, or wheat flour, the amino acid score (based on casein) rises from 72 to 90, 58 to 81, and 32 to 52, respectively (Bressani, 1989). Amaranth grain protein also differs from cereal grains by the fact that 65% is found in the germ and 35% in the endosperm, as compared to an average of 15% in the germ and 85% in the endosperm for cereals (Bressani, 1989).

4.3.2. Fatty acid composition of amaranth grain flour

The amaranth grain oil fatty acids composition is presented in Table 5. There was an apparent increase in saturated fatty acids due to the decrease of unsaturated fatty acids caused by oxidation during processing. The predominant acids in the oil were linoleic, oleic and palmitic.. Total unsaturated acids ranged from 72.20% to 76.05% and saturated fatty acids 22.28% to 25.05% for the processed and raw amaranth grain, respectively. Linolenic acid was present at low concentration. According to study carried out by Martirosyan *et al.,* (2007) amaranth oil was found to reduce the amount of cholesterol in blood serum, and was recommended as a functional food product for the prevention and treatment of cardiovascular diseases. The inclusion of amaranth oil in the diet contributes to an increase in the concentration of polyunsaturated fatty acids and effective natural antioxidant supplement capable of protecting cellular membranes against oxidative damage (Martirosyan *et al.,* 2007)

The lipid fraction of amaranth grain is similar to cereals, (Lyon and Becker, 1987; Lehman, 1991) being about 77% unsaturated, with linoleic acid being the predominant fatty acid. Also present in the amaranth oil fractions are tocopherol (Table 7), known to effect lower cholesterol levels in mammalian systems (Lyon and Becker, 1987; Becker, 1989; Lehman, 1991) and to protect polyunsaturated fatty acids (PUFAs) from oxidation. Amaranth grain oils in foods not only increase the energy density for infants and children, but also is a transport vehicle for fat soluble vitamins, and provides essential fatty acids like that of omega-6 (PUFA's) needed to ensure proper neural development. Breastfed children take between 40 and 60 % of their energy from fat; therefore it is not advisable to limit the consumption of fat during the first 2 years of life.

Due to the fact that grain amaranth has high protein, as well as a high fat content, it has a potential to use it as an energy food. Furthermore, the presence of high levels of unsaturated fatty acids: oleic and linoleic plus the high protein content makes it a nutritionally balanced grain.

Table 5: Fatty acid composition of amaranth grain oil (%) [1]

	Control[2]	Product[3]	LSD
Lauric	0.5±0.0^{b}	0.6±0.0^{a}	0.1
Myristic	0.1±0.0^{b}	0.2±0.0^{a}	0.1
Palmitic	20.7±0.2^{b}	22.3±0.1^{a}	0.5
Stearic	1.5±0.0^{b}	2.0±0.0^{a}	0.1
Oleic	38.2±0.2^{a}	36.3±0.0^{b}	0.6
Linoleic	37.8±0.2^{a}	35.9±0.0^{b}	0.6
Linolenic	1.7±0.2^{b}	3.4±0.0^{a}	0.5
% Saturated fatty acids*	22.8	25.1	-
% Unsaturated fatty acids*	76.1	72.2	-

[1]Means of two samples analysed in triplicate ± standard error. In each row means followed by the same letter are not significantly different (P≤0.05)
[2]Raw amaranth grain flour
[3]Processed amaranth grain flour
*Calculated

Sample	Ascorbic acid	Pyridoxine	Niacin	Thiamin	Riboflavin	Tocopherol
Control[2]	1.1±0.2^{a}	0.6±0.2^{a}	1.4±0.1^{a}	0.6±0.1^{a}	0.5±0.1^{a}	46.7±1.5^{a}
Product[3]	0.6±0.2^{b}	0.4±0.1^{b}	0.9±0.2^{b}	0.2±0.0^{b}	0.5±0.1^{b}	44.4±1.6^{b}
LSD[4]	0.7	0.4	0.4	0.2	0.3	5.3

4.3.3. Mineral and antinutrient composition of amaranth grain flour

Mineral content, tannin and phytates are presented in Table 6. The processing method did not have a significant effect (P≤0.05) on amaranth grain mineral composition. Amaranth grain product was rich in potassium, phosphorus, calcium and magnesium important for the growth of healthy bones. Processing amaranth grain led to a significant decrease (P≤0.05) in total tannins and no significant effect (P≤0.05) to the phytates level. The phytate and tannin contents were quite low with hydrolysable and condensed tannins being present in negligible amounts.
Phytic acid contents of the samples were found to be in the range reported for other amaranth species (Teutonico and Knorr 1985) and do not seem to be unacceptably high. Phytates constitute 10-20 g kg^{-1} of many cereals and oilseeds, and amounts as high as 60 g kg^{-1} have been found in certain plant foods (Rose 1982). Both tannin and phytate determinations are important because of their alleged interference with mineral absorption (Rose 1982).

Calcium plays an important role in bone and tooth development, blood clotting and maintenance of healthy nerves and muscles. The iron intake of infants is of particular interest because iron deficiency anaemia has been associated with a number of adverse effects in infants and young children, including impaired motor and mental development (Andraca *et al.*, 1997). Iron is needed by infants for the proper growth and formation of healthy blood cells and prevention of iron-deficiency anemia. This mineral is a vital component of hemoglobin, the part of red blood cells that carries oxygen throughout the body; myoglobin, the part of muscle cells that stores oxygen; and many enzymes in the body. A child's growth and development depends on iron, and studies show that inadequate iron intake can have long-term consequences on learning, attention span and behavior. (Krebs *et al.,* 2006). Most babies are born with enough iron stores for the first 6 months of life; after 6 months, infants need a diet rich in iron to meet their needs.
Zinc is essential for growth and development and is a component of many enzymes in the body. It is involved in the formation of protein; creation of DNA and helps the body break down carbohydrates, fats and proteins so they can be used for energy. Zinc boosts immunity and also helps the body heal wounds and maintain normal blood glucose levels. Research suggests that zinc also has a role in improving recall skills, reasoning and attention. (Krebs *et al.,* 2006). The zinc content of breast milk gradually decreases over time, so it's important to introduce foods rich in zinc when infants progress to solid foods.

Amaranth contains 0.3-0.6% phytic acid (Bressani *et al.,* 1987), which is distributed uniformly in seed, and is therefore hardly decreased by abrasive dehuling or extraction with water. Although phytates in cereals have been long considered as antinutrients because of their ability to interact with dietary protein, starch and minerals, it has been suggested that health benefits associated with dietary fibre such as delayed nutrient absorption, decreased cancer risk, increase fecal bulk and lowering of blood lipid, also may be attributed to phytates (Thompson, 1995).

Table 6: Mineral and antinutrient composition of amaranth grain flour on dry matter basis (mg/100 g)[1]

	Control[2]	Product[3]	LSD
Calcium	190.7±2.9^{a}	189.1±1.7^{a}	7.6
Copper	0.6±0.0^{a}	0.6±0.1^{a}	0.2
Manganese	6.3±0.54^{a}	5.9±0.7^{a}	2.0
Magnesium	220.4±2.7^{a}	219.5±0.7^{a}	7.1
Iron	13.9±1.5^{a}	13.0±0.8^{a}	3.7
Zinc	5.2±0.2^{a}	4.8±0.3^{a}	0.7
Potassium	326.8±0.5^{a}	324.4±3.9^{a}	8.9
Sodium	8.1±1.5^{a}	8.0±1.5^{a}	4.7
Phosphorous	323.2±0.4^{a}	322.8±1.0^{a}	2.4
Phytates	0.3±0.0^{a}	0.2±0.0^{a}	0.2
Tannins	0.1±0.0^{a}	0.1±0.0^{b}	0.0

[1]Means of two samples analysed in triplicate ± standard error. In each row means followed by the same letter are not significantly different (P≤0.05)
[2]Raw amaranth grain flour
[3]Processed amaranth grain flour

4.3.4. Vitamin content of amaranth grain flour

Vitamin contents for the amaranth grain flour are presented in Table 7. Water soluble vitamins were assessed as ascorbic acid, pyridoxine, thiamine and riboflavin. There was a significant ($P \leq 0.05$) reduction on the concentration of all the vitamins during processing, which is likely to affect the nutritional value of the product. However, niacin concentration was within the range reported by Afolabi *et al*., (1981) Colmenares and Bressani (1990), Chávez-Jáuregui *et al.,* (2000) in *A. cruentus*, while thiamine, riboflavin and pyridoxine were higher and ascorbic acid was lower. The loss of ascorbic acid during processing was quite high, which may be due to the effect of oxygen and light.

The tocopherol value was quite high in both raw and processed amaranth flour, an important nutrient for infant growth and development.

Niacin is important for proper blood circulation and the healthy functioning of the nervous system. It maintains the normal functions of the gastro-intestinal tract and is essential for the proper metabolism of proteins and carbohydrates. It helps to maintain a healthy skin and dilates the blood capillary system.

Thiamin (vitamin B1), a water-soluble vitamin, is needed by infants to help the body release energy from carbohydrates during metabolism and play a vital role in the normal functioning of the nervous system (FAO/WHO, 2002).

Table 7: Vitamin composition of amaranth grain flour on dry matter basis (mg/100 g)[1]

Sample	Ascorbic acid	Pyridoxine	Niacin	Thiamin	Riboflavin	Tocopherol
Control[2]	1.1±0.2[a]	0.6±0.2[a]	1.4±0.1[a]	0.6±0.1[a]	0.5±0.1[a]	46.7±1.5[a]
Product[3]	0.6±0.2[b]	0.4±0.1[b]	0.9±0.2[b]	0.2±0.0[b]	0.5±0.1[b]	44.4±1.6[b]
LSD[4]	0.7	0.4	0.4	0.2	0.3	5.3

[1]Means of two samples analysed in triplicate ± standard error. In each column means followed by the same letter are not significantly different ($P \leq 0.05$)
[2]Raw amaranth grain flour
[3]Processed amaranth grain flour
LSD[4] is least significant difference

4.3.5. Amino acid composition of amaranth grain flour

The amino acids composition obtained in amaranth grain flour samples prior to and after processing are shown in Table 8. The highest amino acid was glutamic acid followed by aspartic acid and threonine. The essential amino acids of major importance in the amaranth grain product were histidine, threonine, valine, methionine, isoleucine, leucine, phenylalanine and lysine.

The amino acids values were consistent with the reported data (Colmenares and Bressani, 1990; Chávez-Jáuregui *et al.,* 2000; Teutonico and Knorr; 1985, Saunders and Becker, 1984), except for lysine, threonine, and arginine which gave a slight decrease whereas an increase was noted for histidine and methionine. Pant (1985) stated the decrease of lysine level by 36% (from 4.83 to 3.08 g/100g protein) in traditionally popped amaranth grain while in commercially popped grain the lysine level decreased by 25%. Tovar and Carpenter, (1982) found the decrease of lysine, arginine and cysteine levels while using both the method of popping. The levels of amino acids in raw and processed amaranth grain flour confirmed the favourable amino acid composition as reported by Bressani *et al.,* (1987) and Gorinstein *et al.,* (2002).

Some authors (Bressani *et al.,* 1987; Imeri *et al.,* 1987) reported the same or increased nutritional value of amaranth grain after heat treatment in the form of autoclaving, extrusion, atmospheric cooking, toasting, popping, which can be explained by a limited effect of heat-labile anti-nutritive compounds; however, the results of our study showed the higher nutritional value in raw amaranth.

The most studied nutritional aspect concerning the food value of grain amaranth is the identification of the limiting amino acids of the protein component. Amaranth grain is reported to have high levels of lysine, a nutritionally critical amino acid, ranging from 0.7 to 0.8% of the total protein content (Bressani *et al.,* 1987). The limiting amino acid is usually reported to be leucine (Singhal and Kulkarni, 1988), although some reports indicate that threonine actually may be the amino acid which is more biologically limiting than leucine (Bressani *et al.,* 1987).

Table 8: Amino acids contents in amaranth grain (g/100g sample)[1]

Amino Acid	Control[2]	Product[3]	LSD
Lysine	0.5±0.1[a]	0.5±0.0[a]	0.0
Threonine	1.4±0.0[a]	1.4±0.1[a]	0.0
Valine	0.7±0.0[a]	0.7±0.0[a]	0.0
Cysteine	0.9±0.0[a]	0.8±0.0[a]	0.1
Methionine	0.6±0.0[a]	0.5±0.0[a]	0.1
Isoleucine	0.6±0.0[a]	0.6±0.0[a]	0.2
Leucine	0.9±0.0[a]	0.7±0.0[b]	0.1
Phenylalanine	0.4±0.0[a]	0.4±0.0[a]	0.0
Arginine	0.6±0.0[a]	0.5±0.0[a]	0.0
Alanine	0.5±0.0[a]	0.6±0.1[a]	0.1
Histidine	0.6±0.0[a]	0.7±0.0[a]	0.0
Aspartic acid	1.8±0.1[a]	1.6±0.2[a]	0.2
Glutamic acid	7.1±0.2[a]	5.8±1.1[a]	0.2

[1]Means of two samples analysed in triplicate ± standard error. In each row means followed by the same letter are not significantly different ($P \leq 0.05$)
[2]Raw amaranth grain flour
[3]Processed amaranth grain flour

4.4. Physicochemical properties of amaranth grain flour

4.4.1. Viscosity of amaranth grain product

Viscosity of amaranth grain is shown in Figure 2. Different spindle were used for different dilutions depending on porridge thickness. Spindle number 62 was used for the 8% and 10% product reconstitution which were light, giving absolute viscosity of 34.2 and 58.2 respectively. Absolute viscosity was noted to increase with increase in solute content. At 15% concentration the instant amaranth porridge was thick and acceptable, but at 20% the porridge was too thick where a spindle of 64 was used. This result gave the 15% product reconstitution (15 g/100 ml water) appropriate for infant feeding

Viscosity is important in food intake because it contributes to an increase or decrease in the bulk of a cooked cereal product and affects taste intensity. As many of these transitional foods are made from flours or cereals, viscosity has to be taken into account. Low viscosity is needed to make this food easy to consume.

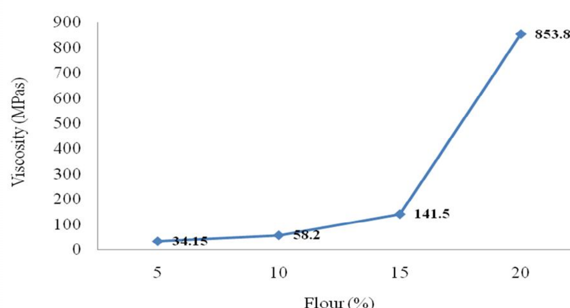

Figure 2: Absolute viscosity of amaranth grain product at 40^0C, for different flour reconstitution

4.4.2. Bulk density, water absorption capacity, protein water solubility

Table 9 gives the bulk density, water absorption capacity and protein water solubility of amaranth grain flour.

The bulk density was significantly affected (P≤0.05) by the processing method and dropped with moisture reduction. The low bulk density is an advantage in the formulation of baby foods where high nutrient density to low bulk is desired. The low bulk density in the product may be due to the smaller particle size as compared to the raw grain flour which had course particles. The bulk density is generally affected by the particle size and the true density of the matter in flour and is important for determining packaging requirements, material handling, and application in wet processing in the food industry.
Processing amaranth grain showed a significant (P≤0.05) effect on water absorption capacity, and protein water solubility. Processed amaranth grain had higher water absorption capacity as compared to the raw grain samples, which increased with moisture reduction. On the other hand processing led to a reduction in protein water solubility. The absorbed negative effect on high steaming temperature on the solubility of proteins may be potentially due to the formation of complexes between soluble proteins and sugars such as is often the case in Maillard reactions.
Water absorption capacity and the protein solubility are important characteristics of flours because physicochemical properties such as viscosity and gelation are dependent on them and give valuable information on the behavior of weaning food products during reconstitution in hot or cold water. Water absorption characteristics are attributed to the protein and starch granules present in the samples as well as their arrangements, and the degree of packing of the granules determine the intermolecular spaces available at the surfaces of the products. Heat treatment of raw materials is known to affect their hydration properties (Phillips *et al.,* 1988). The high water solubility in amaranth grain is probably due to the influence of high levels of proteins in the grain and to a lesser extent the starch and cellulose at room temperature. The gelatinization of starch and the denaturation of proteins that is the result of the application of heat treatment are known to improve the water absorbing capacity. The water holding capacity as an index of the amount of water retained within the protein matrix shows the functional capacity of the product protein in thickening and food formulations.

Table 9: Physicochemical properties of amaranth grain flour[1]

	Control[2]	Product[3]	LSD
Bulk density (g/ml)	0.7±0.0[a]	0.5±0.0[b]	0.0
Water absorption capacity (g/100 g)	343.9±2.8[b]	471.3±2.1[a]	9.6
Protein water solubility %	44.1±1.6[a]	27.4±1.0[b]	5.4

[1]Means of two samples analyzed in triplicate ± standard error. In each row means followed by the same letter are not significantly different (P≤0.05)
[2]Raw amaranth grain flour
[3]Processed amaranth grain flour

4.5. Comparison between amaranth grain product nutritional content with the recommended nutrient intake for infants

A comparison was done for the amaranth grain product with the recommended dietary intake for infants as shown in table 10. The amount of manganese and tocopherol available in the amaranth grain product in one feeding (15%product) met the recommended dietary intake. Considering an infant fed on the product at a reconstitution level of 15% product three times a day, the levels of magnesium, manganese and tocopherol would be far above the recommended intakes, while deficiencies are evident for sodium and copper. Nutrients above the average requirements will be protein 54%, phosphorous 53%, iron 53%, zinc 72%, riboflavin 57% and niacin 59%. Reconstituting the product with milk would enrich the deficient nutrients and improve the complementary product, especially for iron and zinc which are crucial nutrients for infants.

Table 10: Recommended intakes of nutrient for normal infants in comparison with amaranth grain product

Nutrient	Recommended intake per day. 7-12 months[c]	Amaranth grain product composition per 100 g	Amaranth grain product at a reconstitution of 15%
Energy (kcal)[a]	850	402.4	60.4
Protein (g)[a]	14	16.7	2.5
Calcium (mg)[b]	270	189.1	28.4
Phosphorous (mg)[b]	275	322.6	48.4
Magnesium (mg)[b]	75	219.5	32.9
Sodium (mg)[a]	200	8.0	1.2
Potassium (mg)[a]	700	324.4	48.7
Iron (mg)	11	13.0	2.0
Zinc (mg)	3	4.8	0.7
Copper (µg)[b]	220	0.6	0.1
Manganese (µg)[b]	0.6	5.9	0.9
Vitamin E (mg α-tocopherol equivalents)b	6	44.4	6.7
Thiamine (mg)[b]	0.3	0.2	0.1
Riboflavin (mg)[b]	0.4	0.5	0.1
Niacin (mg NE)b	4	0.9	0.1
Pyridoxine (mg)[b]	0.3	0.4	0.1

[a]Recommend Dietary Allowance (for energy and protein) and minimum requirement (for sodium and potassium)
[b]Adequate intake (e.g., mean intake of normal breast-fed infants)
[c]Source: Barbara and Robert, 2001

CHAPTER FIVE

5.0. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusion

From this study, it can be concluded that steeping and steaming pregelatinization of amaranth grain do not significantly affect its nutritional composition, except for the vitamins which drastically reduced with processing.

The sensory characteristics of the amaranth grain product gave a ready to eat product with 20 minutes steaming. The product colour was golden yellow with high flour recovery and good reconstitution at 15% flour concentration.

Amaranth grain product was rich in protein 16.7%, with good amounts of lysine 0.5%, a limiting amino acid in cereals, and methionine 0.5%, limiting in pulses. Other essential amino acids identified were: histidine, threonine, valine, lecine, isoleucine and phenylalanine. This makes the product nutrient rich for infant feeding.

The water soluble vitamins greatly reduced with processing. However the product had good amount of tocopherol important for infant growth and development.

The product was also rich in fat 7.0%, ash 2.6% and fibre 3.1%. The predominant fatty acids were oleic 36.3% and linoleic 35.9%, and some amounts of linolenic acid 3.4% important for infant growth.

The product contained good amounts of minerals of importance being potassium 324.4 mg/100 g, phosphorous 322.8 mg/100 g, calcium 189.1 mg/100 g, magnesium 219.5 mg/100 g, iron 13.0 mg/100 g and zinc 4.8 mg/100 g. On the other hand the antinutrient factors were quite low, phytates 0.2 mg/100 g, and tannins 0.1 mg/100 g, posing no negative effect on the bioavailability of the essential nutrients.

On the physicochemical properties the product gave a low viscosity, high energy density food with high water absorption capacity 471.3 g/100 g that can be used to supplement the already available high viscosity, low energy density, complementary foods. Increasing the nutrient density of complementary foods is a strategy commonly recommended for improving child nutrition. The bulk density was 0.5 g/ml, which reduced with processing, a desirable factor for infant food processing. The protein water solubility also reduced with processing from 44.1% to 27.4%.

Considering amaranth grain product fed to infant three times a day, at a reconstitution of 15% product, the levels of magnesium, manganese and tocopherols were far above the recommended intakes, while protein, phosphorous, iron, zinc, riboflavin and niacin were above the average requirements. Therefore reconstituting the product with milk would enrich the deficient nutrients and improve the complementary product, especially for iron and zinc which are crucial nutrients for infants.

The study proves to be partially true the hypothesis that processing amaranth grain has no effect on its physicochemical and nutritional properties. This is because water soluble vitamins were significantly affected by the processing method, thus affecting the nutritional value of the product.

The processing method is a practical approach aimed at combating problem of malnutrition among infants and young children in Kenya and other developing countries. The technique in this study can be easily adopted at both household and village levels to produce high protein-energy weaning food to help enhance the nutritional status of Kenyans, especially children.

5.2. Recommendations

Based on the results of this study, there is need for improved processing method to preserve nutrients especially the water soluble vitamins, and determine the impact of amaranth grain

product on infants' health and nutritional status and ascertain keeping quality in terms of microbial safety. A cost evaluation for the amaranth grain product should be conducted in comparison to the proprietary formula products. There is also need to incorporate amaranth grain into existing food formulations to modify their functional and nutritional quality and create entirely new products from grain and vegetable amaranth. Since amaranth grain has high nutritional value as compared to cereals, its product development and use can give meaning to agricultural production efforts and create the driving force for a dynamic food chain. There is need to switch from maize as the only staple food to combat the current food crisis in Kenya.

The processing parameters and formulations developed through this study successfully produced a high protein-energy food with acceptable physicochemical and sensory characteristics. The findings can therefore be used to promote amaranth grain production in resource poor areas of Kenya, which can increase the food output and combat infant malnutrition as a complementary food.

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Appendix I: Questionnaire for sensory evaluation of amaranth grain flour

NAME…………………………………………. DATE…………………..

You have been provided with coded reconstituted flour samples. Please evaluate them independently for the following characteristics: a) Color b) Flavor c) Texture and d) Overall acceptability.

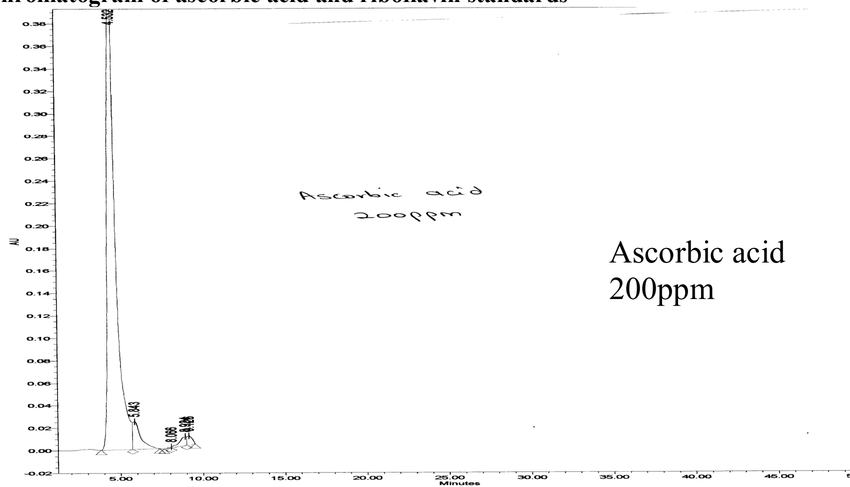
Record your preference by putting a tick on the score that best describes your decision, on the basis of the scale described below. Thank you

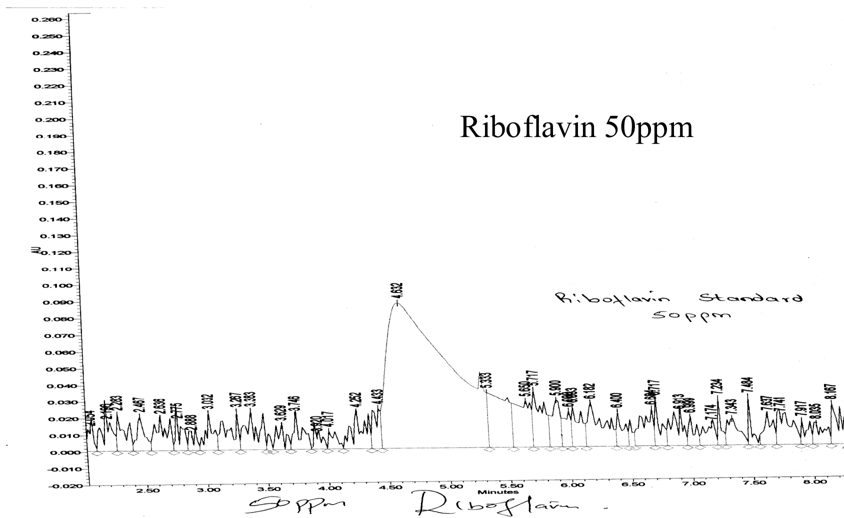
Sample Code Score	Color	Flavour	Texture	Overall acceptability
9 – Like extremely				
8 - Like very much				
7 – Like moderately				
6 – Like slightly				
5 – Neither like nor dislike				
4 – Dislike slightly				
3 – Dislike moderately				
2 – Dislike very much				
1 – Dislike extremely				

A 9 point hedonic scale (Ranganna, 1994)

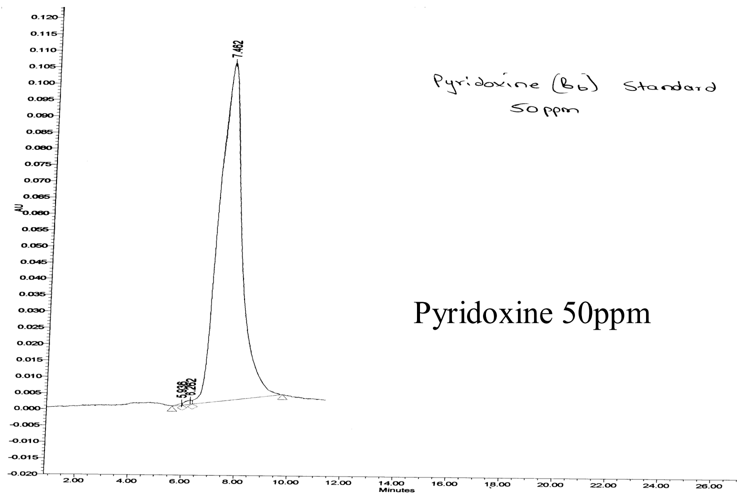
Signature:………………………………………………...

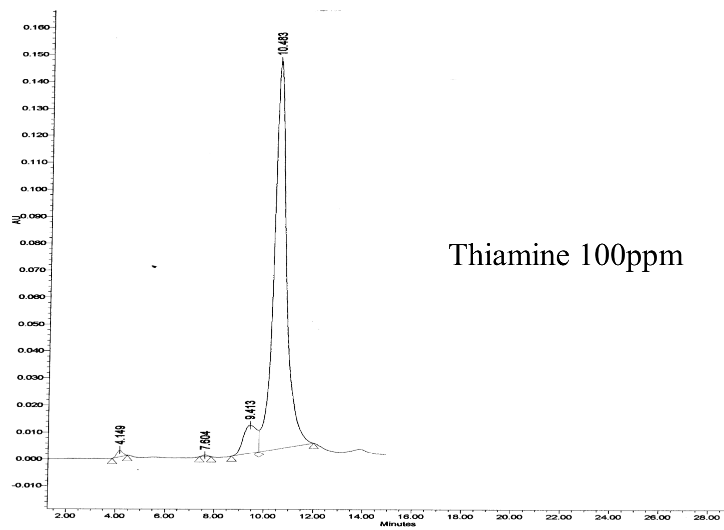
Appendix II: HPLC Chromatogram of ascorbic acid and riboflavin standards



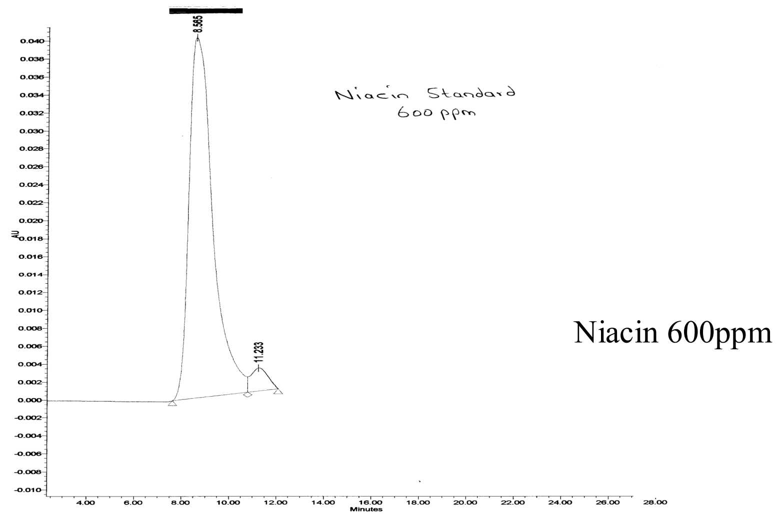


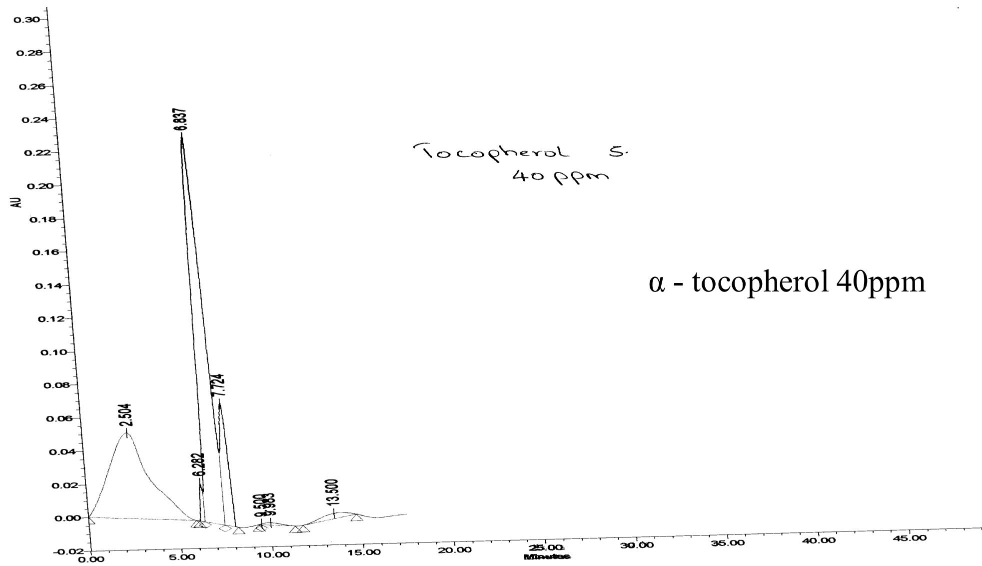
Appendix III: HPLC Chromatogram of pyridoxine and thiamine standards



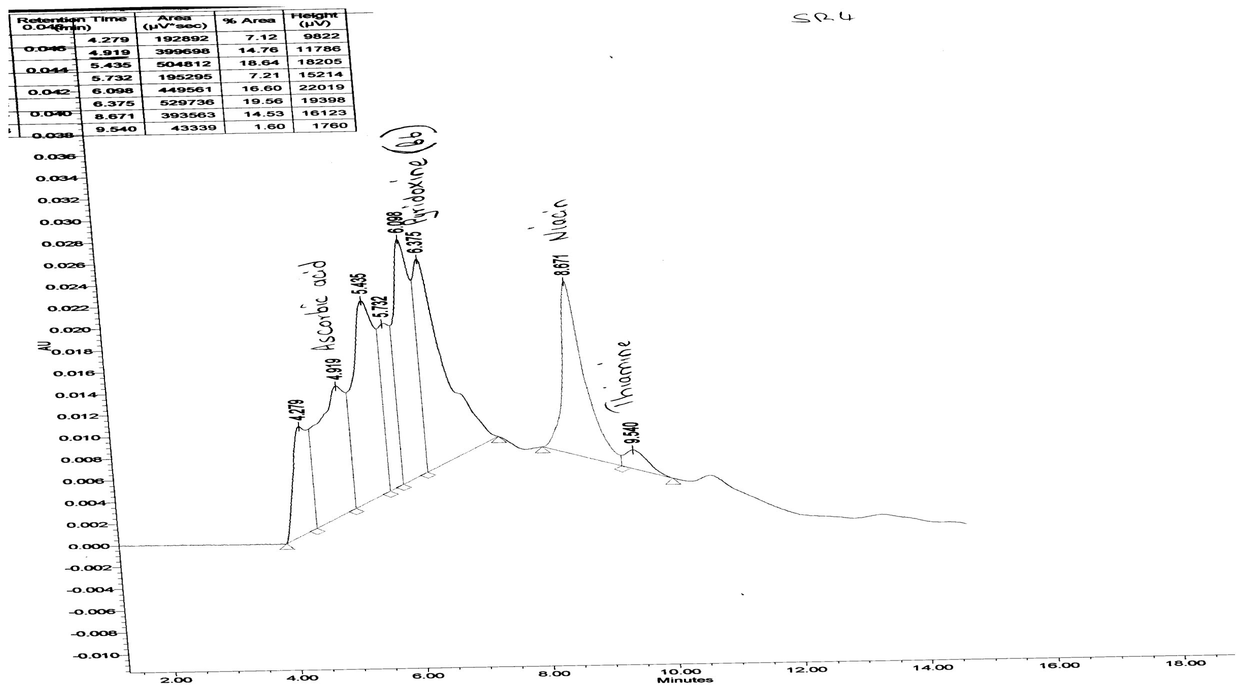


Appendix IV: HPLC Chromatogram of niacin and α- tocopherol standards



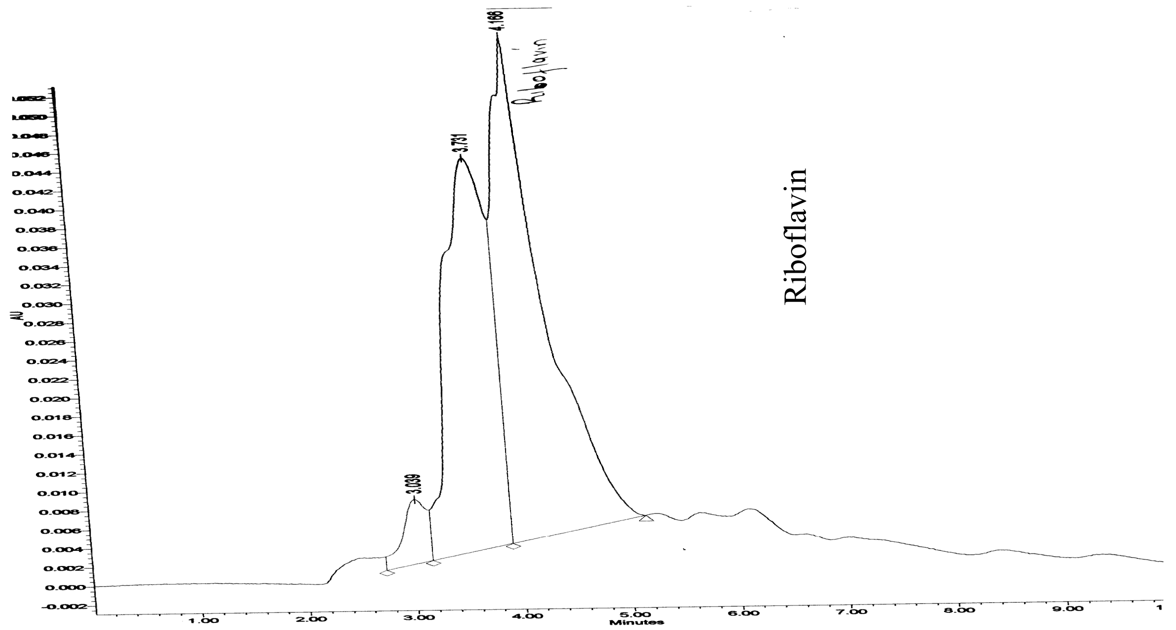


Appendix V: HPLC Chromatogram of water soluble vitamin in amaranth grain product

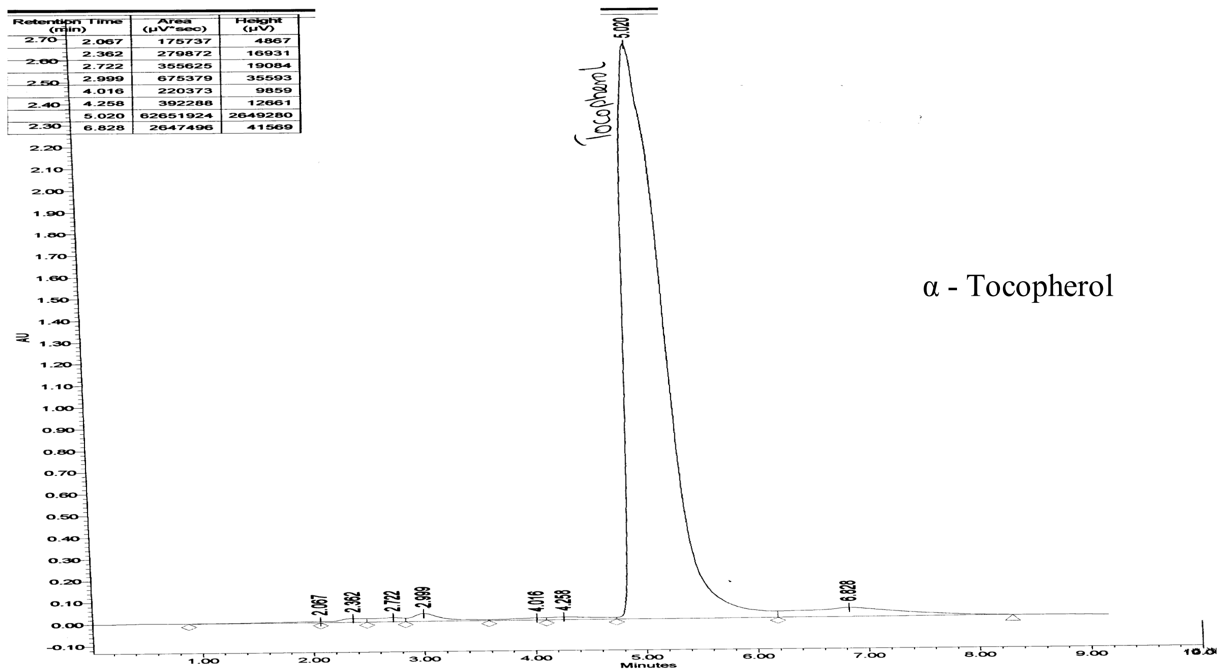


Retention Time (min)	Area (µV*sec)	% Area	Height (µV)
4.279	192892	7.12	9822
4.919	399698	14.76	11786
5.435	504812	18.64	18205
5.732	195295	7.21	15214
6.098	449561	16.60	22019
6.375	529736	19.56	19398
8.671	393563	14.53	16123
9.540	43339	1.60	1760

Appendix VI: HPLC Chromatogram of riboflavin in amaranth grain product

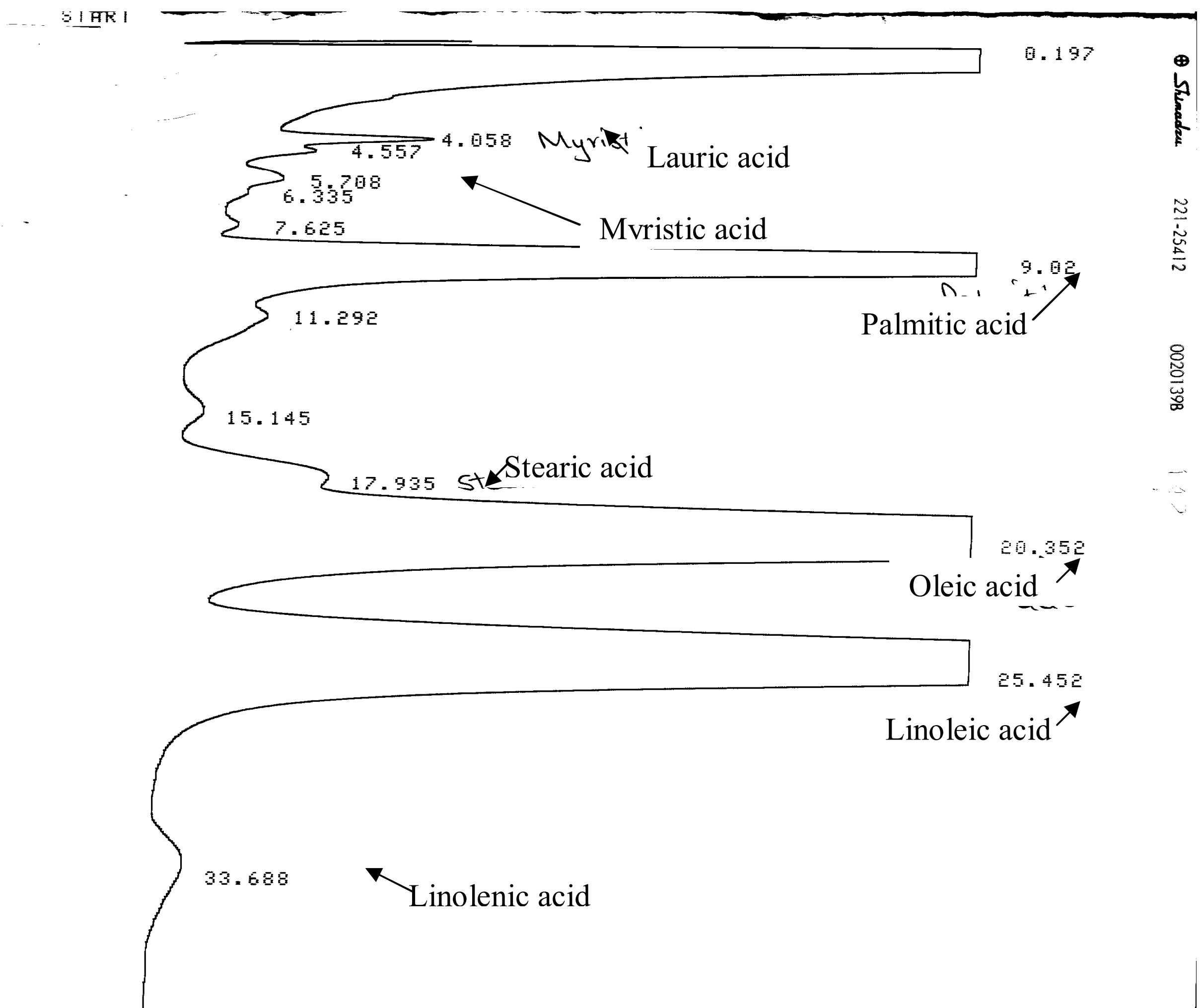


Appendix VII: HPLC Chromatogram of α - tocopherol in amaranth grain product oil



Retention Time (min)	Area (µV*sec)	Height (µV)
2.067	175737	4867
2.362	279872	16931
2.722	355625	19084
2.999	675379	35593
4.016	220373	9859
4.258	392288	12661
5.020	62651924	2649280
6.828	2647496	41569

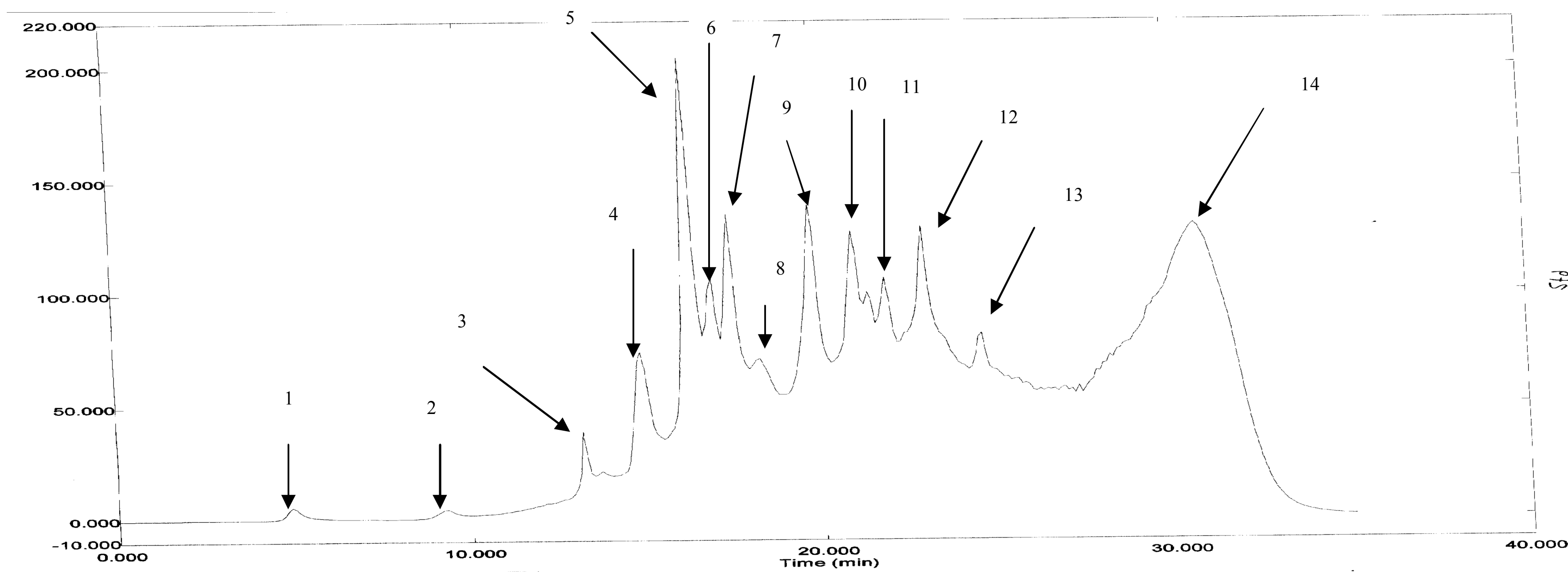
Appendix VIII: GC chromatogram of fatty acids composition in amaranth grain oil



CHROMATOPAC C-R6A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 1414

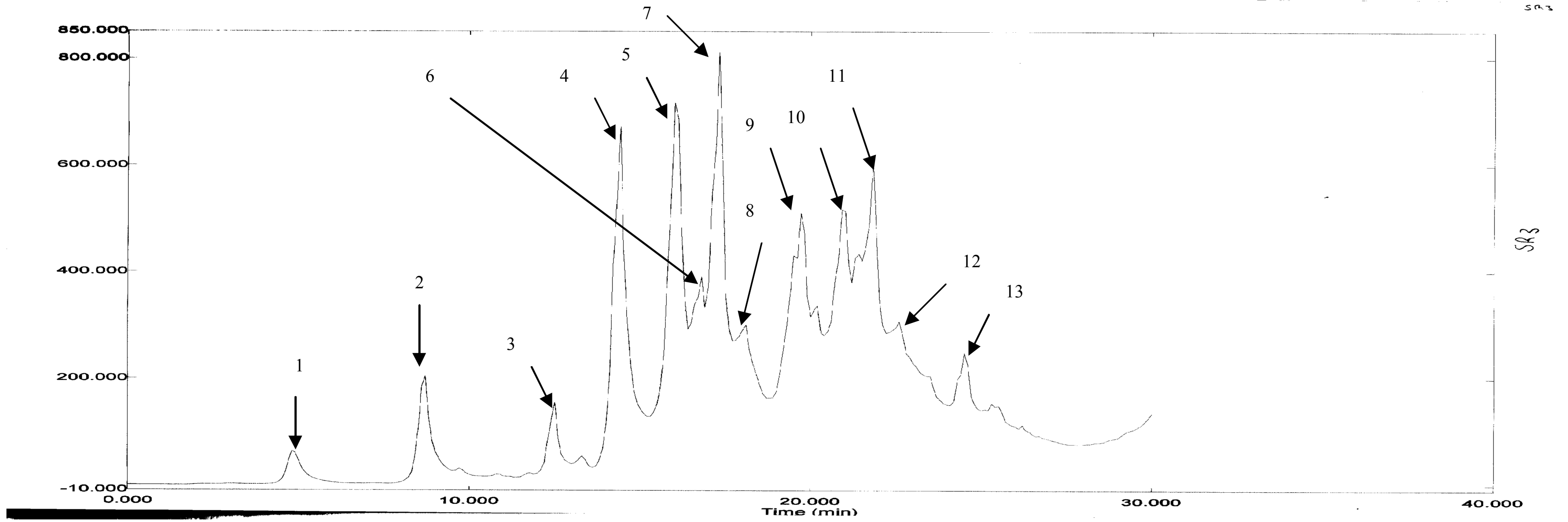
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	0.197	19521150	S E		78.0041	
2	4.058	33298	T		0.1331	
3	4.557	12202	TV		0.0488	
4	5.708	17183	T		0.0687	
5	6.335	3171	TV		0.0127	
6	7.625	5014	T		0.02	
7	9.02	1223047	TV		4.8871	
8	11.292	59376	TV		0.2373	
9	15.145	17092	T		0.0683	
10	17.935	109157	TV		0.4362	
11	20.352	2000000	TV		7.9918	
12	25.452	1971222	TV		7.8768	
13	[illegible]	[illegible]			[illegible]	

Appendix IX: HPLC Chromatogram of amino acids in a standard at a concentration of 0.125µmol/ml



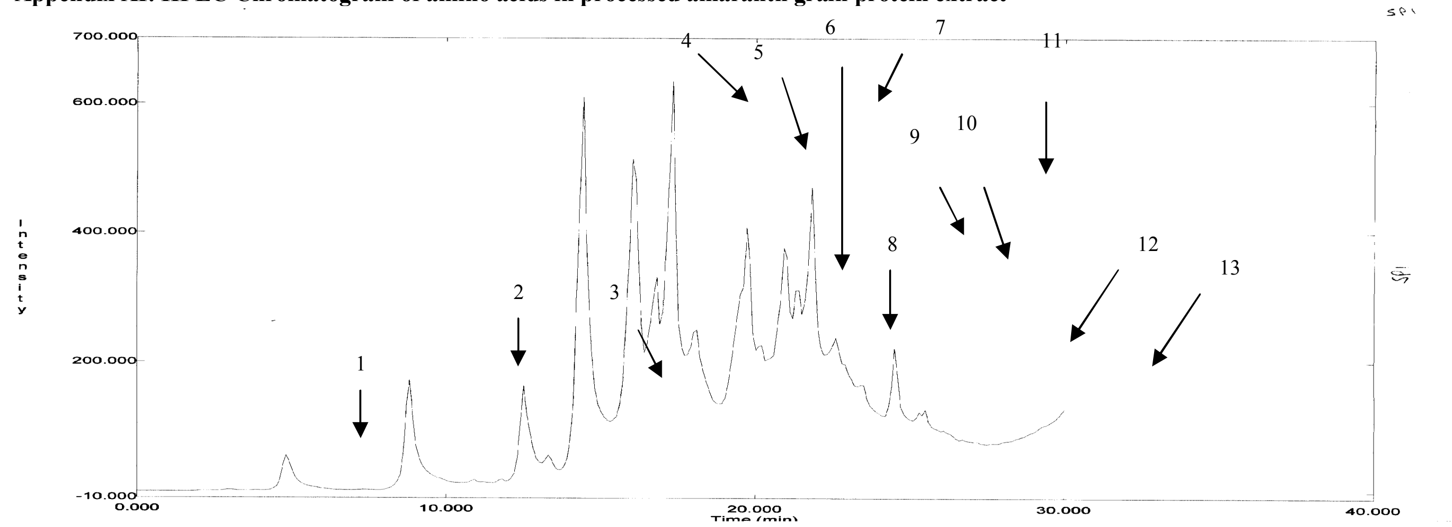
1. Aspartic acid
2. Glutamic acid
3. Histidine
4. Threonine
5. Arginine
6. Alanine
7. Cysteine
8. Valine
9. Methionine
10. Isoleucine
11. Leucine
12. Phenylalanine
13. Lysine
14. Ammonia

Appendix X: HPLC Chromatogram of amino acids in raw amaranth grain protein extract



1. Aspartic acid
2. Glutamic acid
3. Histidine
4. Threonine
5. Arginine
6. Alanine
7. Cysteine
8. Valine
9. Methionine
10. Isoleucine
11. Leucine
12. Phenylalanine
13. Lysine

Appendix XI: HPLC Chromatogram of amino acids in processed amaranth grain protein extract



1. Aspartic acid
2. Glutamic acid
3. Histidine
4. Threonine
5. Arginine
6. Alanine
7. Cysteine
8. Valine
9. Methionine
10. Isoleucine
11. Leucine
12. Phenylalanine
13. Lysine