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Food Science and Nutrition Program

Effect of Traditional Processing Methods on Nutritional Composition and Anti-nutritional Factors of Anchote (*Coccinia Abyssinica* (lam.) Cogn) Grown in Western Ethiopia

By:

Habtamu Fekadu Gemede

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Science and Nutrition

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List of abbreviations

AAS	Atomic Absorption Spectroscopy
AAU	Addis Ababa University
AgNO3	Silver nitrate
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
Ca	Calcium
CaCl2	Calcium Chloride
cm	Centimeter
CNS	Central Nerve System
Cru	Crude
Cu	Cupper
Cya	Cyanide
DNA	Deoxyribonucleic acid
EHNRI	Ethiopian Health and Nutrition Research Institute
FAO	Food and Agricultural Organization
Fe	Iron
FNB	First National Bank
g	Gram
g/mol	Gram per mole
g/100g	Gram per hundrade gram
H2SO4	Sulfaric acid
Gro	Gross
HCl	Hydrochloric acid
Hrs	Hours
IAR	Investment Advisory Representative
Kcal	Kilocalorie

Kcal/100g	Kilocalorie per hundred gram
KCN	Potassium cyanide
Kg	Kilogram
KH2PO4	Potassium dihydrogen phosphate
Kj	Kilo joule
KMnO4	Potassium per manganate
KOH	Potassium hydro oxide
KSCN	Potassium thiocyanate
M	Morality
mg	Milligram
mg/100g	milligram per hundred gram
Min	Minute
ml	Milliliter
mm	Millimeter
Mn	Manganese
Mol	Molar
N	Normality
NaCN	Sodium cyanide
NaOH	Sodium hydro oxide
nm	Nanometer
Ox	Oxalate
P	Phosphorus
pH	Power of hydrogen
Phy	Phytate
R	Pearson Correlation Coefficient
RDA	Recommended Daily Amount/ Allowance
rpm	Revolution per minute

SE	Standard Error
SPSS	Statistical Product and Service Solutions
Sw	Sweet
Tan	Tannin
Tot	Total
U.S. EPA	United States Environmental Protection Agency
UICC	Universal Integrated Circuit Card
UNESCO	United Nations Educational, Scientific and Cultural Organization
Uti	Utilizable
v/v	volume by volume
w/w	weight by weight
WHO	World Health Organization
Yr	Year
Zn	Zinc
Mg	Magnesium
µg/ml	microgram per milliliter
^{0}C	Degree Celsius

Appendices

Biographical sketch

The author was born in 1985 G.C. Harolago kebele, Jardaga Jarte district, Horro Guduru Wollega zone of Oromia Regional State from his Father Mr. Fekadu Gemede and his Mother Mrs. Buzunesh Bekele. He attended his junior school in Harolago Junior Secondary School from 1991 to 1997 and his secondary and preparatory education in Shambo Secondary and Preparatory School from 1998 to 2004.

After successful pass of Secondary and Preparatory School, he then joined the Food Science and Post Harvest Technology department of Haramaya University. The author pursued his BSc study from September 2005 to June 2007 and graduated with Bachelor of Science degree in Food Science and Post Harvest Technology. Following graduation, he served as a graduate assistant in Food Science and Bioprocess Technology Institute of Wollega University, Ethiopia for about 1 year. Since September 2009, the author has been enrolled in the Graduate program of Food Science and Nutrition at Addis Ababa University, to pursue his Master of Science degree in Food Science and Nutrition. The author is a candidate for the MSc degree with anticipated graduation date in June 2011.

Dedication

This work is dedicated to my family who I love them more than anything.

To: My Wife Ejigayohu Oli

My Mother Buzinesh Bekele

My Father Fekadu Gemede

My Brother Mulgeta Fekadu

Abstract

The raw and traditionally processed Anchote (Coccinia abyssinica (Lam.) Cogn.) tubers *were studied and compared for their nutritional composition: moisture, crude protein, total ash, crude fiber, crude fat, utilized carbohydrate and gross energy; minerals: Ca, Fe, Mg, Zn, and P and antinutritional factors: phytate, oxalate, tannin and cyanide together with respective molar ratios of Ca:phytate, oxalate:Ca, phytate:Zn, Phytate:Fe and [Ca]x[phytate]:Zn. Sensory preference taste of Anchote boiled after peeling and boiled before peeling was also reported. The raw, boiled after peeling and boiled before peeling Anchote tubers had respective contents (g/100g) of moisture 74.93, 81.74, and 76.73; for crude protein contents were 3.25, 2.67 and 3.14; for total ash contents were 2.19, 1.33, and 1.99; for crude fiber contents were 2.58, 3.71, and 2.77; for crude fat contents were 0.19, 0.13, and 0.14; for utilized carbohydrate contents were 16.86, 10.42 and 15.23; for gross energy contents were 82.12, 53.48 and 75.26. The raw, boiled after peeling and boiled before peeling Anchote tubers had respective contents (mg/100g) of Ca 119.50, 115.70, and 118.20; for Fe contents were 5.49, 7.60, and 6.60; for Mg contents were 79.73, 73.50, and 76.47; for Zn contents were 2.23, 2.03, and 2.20; and for P contents were 34.61, 28.12, 25.45. The raw, boiled after peeling and boiled before peeling Anchote tubers had respective contents (mg/100g) of phytate 389.30, 333.63 and 334.74; for oxalate contents were 8.23, 4.23, and 4.66; for tannin contents were 173.55, 102.36 and 121.21; for cyanide contents were 12.67, 8.16 and 11.14. The raw, boiled after peeling and boiled before peeling Anchote tubers have respective molar ratios of Ca: Phy 5.05, 5.66 and 5.78, the respective Ox: Ca molar ratios were 0.03, 0.02, and 0.02, the respective Phy: Zn molar ratios were 17.35, 14.82 and 14.8, the respective Phy: Fe molar ratios were 5.58, 3.92, and 4.28, the respective [ca] [Phy]/ [Zn] millimolar ratios were 0.52, 0.47 and 0.44. This study also revealed that, there was significant ($P<0.05$) taste preference of Anchote boiled before peeling to Anchote tubers boiled after peeling, in which 66% of consumers gave priority of the preference taste for Anchote boiled before peeling.*

Key words: *Anchote, boiled after peeling, boiled before peeling, effect of processing, minerals, Nutritional composition, anti-nutritional factors, molar ratio, sensory preference*

Chapter 1

Introduction

1.1. Background

Plants are the most important source of food for human beings mainly due to their availability and low cost compared to consumption of animal-source foods, which are often unavailable because of economic and/or religious concerns (Rosalind *et al.*, 2006). The importance of plants to man, directly or indirectly, is demonstrated by the useful by-products from both wild and cultivated species, which is related to their numerous uses as sources of food for man and livestock, as sources of energy, medicinal products, structural materials, and many other products. Plants can be classified into different types according to the way they are grown, and the way their produce is used (NRC, 1989). Among plant sources, root and tuber crops occupy a remarkable position in the food security of the developing world due to their high calorific value, and carbohydrate content (Scott *et al.*, 2000). These crops are associated with the human existence, survival, and socio-economic history.

The term root and tuber refers to any growing plant that stores edible material in subterranean root, corm or tuber. Such crops constitute an important source of income in rural and marginal areas; have multiple uses as regular food crops, and cash crops. Also, have long served as the principal source of food, and nutrition for many of the world's resource poor, and undernourished households and are generally valued for their stable yields under conditions in which other crops may fail (Scott *et al.*, 2000).

Therefore, in developing countries, many farmers are highly dependent on root and tuber crops, as supplementary, if not principal, sources of food, nutrition, and cash income (Scott *et al.*, 2000). Sub-Saharan African countries produce about 20% of the world's total production of root, and tuber crops, for consumption of about 10% of the world's total human population (Quin, 2001). These root and tuber crops are usually easy to cultivate, and give high yields per hector. However, they are bulky and expensive to transport, they can rot during storage, because of their high moisture and they are also low in protein, dry matter and supplemented with foods rich in protein, fats, vitamins and minerals (FAO, 1979).

Root and tuber crops are widely cultivated in Ethiopia and which are supporting a considerable portion of the country's population as source of food. Among prominent roots and tuber crops produced in the country are Potato (*Solanum tuberosum* L.), Sweet Potato (*Ipomoea batatas*) (L.), Enset (*Ensete ventricosum* (Welw.) Chees man), Anchote (*Coccinia abyssinica (Lam.) Cogn*), Taro (*Colacasia esculanta* L.), Yam (*Dioscorea* spp.), Ethiopian Dinich (*Coleus parviflorus*), Koteharrie (*Diaspora bulbiferous*), Carrot (*Daucus carota*) and Ginger (*Zingiber officinale*). Among these, Enset, Anchote and some Yams are endemic to Ethiopia (Addis, 2005; Edwards, 1991). These endemic resources contribute considerably to the food, and the overall economy of the country (FAO, 1993).

Tilahun (1989) defines Anchote as a climbing plant with glabrous leaves and twining stems, which coil readily around a stake and perennial through root system but are grown as annual crops and cultivated in home gardens. Anchote is mostly grown in the western parts of the Ethiopian highlands. Though subject to altitude and climate, Anchote can grow, flower and produce fruits within 4-5 months after germination. Anchote forms a local dish and is highly recommended for individuals suffering from bone fractures and displaced joints, as it contains high amount of calcium and protein (Abera, 1995).

Despite the pros and cons of roots and tubers, Walingo (2009) recommended, the importance of proper processing before consumption in order to reduce the effect of antinutritional factors and ther-by improve nutrient availability. In the case of Anchote, however, no published information is available as to which traditional processing methods are optimal to reduce the effects of the inherent antinutritional factors and to increase availability of the contained nutrients. Therefore, it is imperative to investigate which traditional methods are optimal to improve the quality of Anchote for human consumption and decrease of its risk of human health.

1.2. Statement of the Problem

The worsening food crisis and the consequent wide spread prevalence of malnutrition in developing countries have resulted in high mortality and morbidity rates, especially among infants and children in low income groups (Enujuigba and Akanbi, 2005). To acquire the ability to reduce the adverse effect of hunger and/ or starvation, it is pertinent that some of the lesser-known and under-utilized traditionally used food crops should be investigated for their nutritive value, and easy cultivable characteristics. Tubers are widely used as cheap sources of

carbohydrates for man and livestock and they have been adjudged to be of good nutritional value (Nielsen, 1995).

However, nearly all root and tuber crops are rarely fit for direct consumption, and require some prior processing. Anchote, like many other root, and tuber crops, is rarely eaten raw. Traditionally, boiled after peeling or boiled before peeling and/or further cooking are applied before consumption. Such processing can have both detrimental and beneficial effect to the nutrient content of food. Presumed purpose of such processing is to make Anchote more palatable, digestible, to inactivate enzyme inhibitors, and other antinutritional factors to qualify it for human consumption. Even though, boiling may result into improvements of some nutritional values of Anchote, nutrients may be lost during such heat treatment in two ways. First, by degradation of nutrients can take place because of destruction by the applied heat or by other chemical changes such as oxidation. Secondly, nutrient can be leached into the cooking medium (Purcell and Walter, 1982).

Root and tuber crops not only have beneficial nutrients but also contain traces of antinutritional factors, which may have adverse effects on health through inhibition of digestion, absorption, and growth. For example, tannins inhibit the digestibility of protein, while oxalic acid and its salts have been implicated in decreasing calcium absorption, and aiding the formation of kidney stones (Noonan and Savage, 1999). In addition, high oxalate diets can increase the risk of renal calcium oxalate formation in certain groups of people. Phytic acid forms complexes with proteins (protein-phytate complex) and chelates essential dietary minerals such as iron, zinc, calcium, and magnesium, thus decreasing their utilization (Kratzer, 1965). Generally, antinutritional factors are potentially harmful, and give rise to concern for human health in that they prevent digestion, and absorption of vitamins, minerals, and other nutrients.

1.3. Objectives

1.3.1. General objective

The main objective of this research was to determine the effect of traditional processing methods on some nutritional composition, and anti-nutritional factors of Anchote (*Coccinia abyssinica* (Lam.) Cogn.) grown in Western Ethiopia.

1.3.2. Specific objectives

The specific objectives of this study were:

- ✓ To determine the effect of traditional processing (boiled after peeling, and boiled before peeling) on proximate composition (moisture content, total ash, crude protein, crude fiber, crude fat, utilizable carbohydrate, and gross energy), and some minerals (Ca, Fe, Mg, Zn and P) of Anchote tubers.
- ✓ To determine the effect of traditional processing on some antinutritional factors (phytate, oxalate, tannin and cyanide) of Anchote tubers.
- ✓ To identify consumers preference for taste of Anchote boiled after peeling and Anchote boiled before peeling without addition of ingredients.

1.4. Significance of the study

The findings of this study are expected to fill the research gap concerning Anchote, and would benefit the researchers, producers and consumers of Anchote.

- ❖ As far as nutritional contents of Anchote are known, the society used as staple food, and then it involves in reduction of poverty, and ensuring food security in the country.
- ❖ The result of the study will help the society to identify the appropriate processing method, which increase the absorbability of nutrients, and decrease antinutritional factors of Anchote.
- ❖ It can also be used as a base line data for further research in the same area, like to enrich the important nutrient lost during processing, to develop new products etc.
- ❖ It provides necessary information about antinutritional factors (oxalate, tannin and phytate) that would cause health problems through absorption, and digestion.
- ❖ Researchers, teachers, and other concerned bodies may use the outcome of this research as reference materials.

Chapter 2

Literature review

2.1. Overview of Anchote

Anchote is the Afan Oromo name for *Coccinia abyssinica*, which is a tuber crop, belongs to the order *Cucurbitales*, family *Cucurbitaceae* (Asfaw *et al.*, 1992), indigenous to Ethiopia (Addis, 2005; Edwards, 1991). There are about 10 species of Coccinia in Ethiopia; however, only *Coccinia abyssinica* is cultivated for human consumption (Endashaw, 2007). Anchote is a tuberous perennial (Figure 2.1b), with shoots having simple tendrils (Figure 2.1a), and grown principally for its tuberous and as annual crops. The most widely used vernacular name of *Coccinia abyssinica* is Anchote (spelt Ancoote) in Oromo. It is also called: Ushushu in Welayita, Shushe in Dawuro, and Ajjo in Kafigna (Demel *et al.*, 2010). Anchote is found both cultivated and wild (Edwards, 1991). The plant dies after the fruit have matured. The underground tubers produce new shoots at the onset of the wet season. The total yield of Anchote is 150-180 quintals/hectare, which is in the range of the total yield of sweet potato, and potato (IAR, 1986).

Anchote is a valuable food source and according to local farmers, it helps in fast mending of broken/ fracture bones and displaced joints, as it contains high calcium, and proteins than other common and wide spread root and tuber crops (Endashaw, 2007). Traditionally, it is also believed that, Anchote makes lactating mothers healthier and stronger (Abera, 1995). Dawit and Estifanos (1991) reported that the juice prepared from tubers of Anchote has saponin as an active substance and is used to treat Gonorrhoea, Tuberculosis, and Tumor Cancer. Anchote can be safely stored under ground, which thus gives added food security to the population in times of main crop failures. Therefore, the word Anchote, as used by the Oromo, describes the entire plant on one hand, and the tuber on the other. The tubers are the part of most economic concern (Ambecha, 2006).

One of the desirable qualities of Anchote as a tuber crop is its good keeping quality. Anchote tubers usually reach a harvestable stage within four to five months from planning depending on the environment, the plant dies after the tubers have matured. The parts that are harvested are the fruits for seed propagation and leaves, and tubers to be used as a vegetable after being boiled/ cooked. From the single plant one can harvest a tuber only once. The storage of Anchote

is relevant only for tubers, and seeds. The usual storage method is to keep the tubers Underground Pit storage, and to dig them out only when the need arises (Abera, 1995). Anchote tubers kept in open conditions loss their moisture content, and may shrink lose weight, and become more fibrous making Anchote cooking time-consuming. The pit is dug to a size, which can accommodate the amount to be stored. The storage structures of Anchote seed include Baskets, Sacks, and Wooden Boxes (Ambecha, 2006).

Figure: 2.1 a. Anchote (*Coccinia abyssinica)* Plant b. Anchote (*Coccinia abyssinica)* tubers

(Photographs by the Author)

2.2. Taxonomy and name of Anchote species

The scientific classification of Anchote (*Coccinia abyssinica (Lam.) Cogn*) has the following pattern (Candolle, 2007).

Domain: *Eukaryota*

Kingdom: *Plantae*

Subkingdom: *Viridaeplantae*

Phylum: *Tracheophyta*

Subphylum: *Euphyllophytina*

Class: *Magnoliopsida*

Subclass: *Dilleniidae*

Superorder: *Violanae*

Order: *Cucurbitales*

Family: *Cucurbitaceae*

Subfamily: *Cucurbitoideae*

Tribe: *Benincaseae*

Genus: *Coccinia*

Specific epithet: *abyssinica* - Cogn.

Botanical name: *Coccinia abyssinica* (Lam.) Cogn.

2.3. Origin and distribution of Anchote

Anchote is endemic to the Western parts of Ethiopia (Amare, 1973). Though it is a major crop of the area, but this crop is less known to other parts of the world, and their science is almost non-existent (Abera, 1995). Amare (1973) pointed out that there was also an introduction of Anchote to the central, and the eastern provinces. The cultivation of Anchote is mainly in the Western region of Ethiopia highlands in Eestern Wollega, Western Wollega, Kelam Wollega, and Mattu (Westphal, 1974). The Western parts of the Ethiopia/Oromia highland is situated at 1500-2400 meters above sea level, and receives an annual rain fall about 1500mm to over 2000mm per year (Westphal, 1974). Anchote grows at altitudes ranging from 1,300 to 2,800 meters above sea level where the annual rain fall is 762-1016mm. It is also found growing in cooler and higher altitudes of 2820 meters above sea level (Amare, 1973).

2.4. Propagation of Anchote

Anchote can be propagated by both vegetatively, and by seeds methods. The vegetative propagation is achieved by planting either the whole tuber or by slicing it in to two or more pieces, each piece having rootlets, and an external covering. This is usually done to establish mother plants, called Guboo, to serve as a seed source for further plantings. Vegetative propagation is usually practiced on Anchote tubers purchased from the market for consumption. Few tubers are planted, and produce new shoots usually more than one. These newly emerging shoots depend on the reserve food within the tuber making the tuber less suitable for consumption, especially during the early growth. But after the stems are well grown, the shoots start manufacturing their own food, and the tubers again become suitable for consumption. Stem cutting also are effective for propagating Anchote (Ambecha, 2006).

Seed propagation involves taking out of seeds from fully mature red-ripe fruits which are harvested before they start rotting. Such fruits are macerated or sliced to separate the seeds from the fleshy juicy part. The seeds are then mixed with an equal quantity of wood ash, and dried in sun. The moisture content of the seeds, for storage is based on subjective assessment by the Woman of the household. When it has dropped to the desired level, the Woman takes the seeds indoors, and stores them until the next growing season comes. During this storage period the seeds are usually kept in either clay or wooden pots or wrapped in a sheet of cloth (Ambecha, 2006).

2.5. Agronomic requirements of Anchote

Anchote responds strongly to soil fertility, particularly to Wood Ash, and produces large sized tuber of good shape very rapidly when grown in fertile soils. Growers know this from their long experience, and hence prefer to grow Anchote close to the home garden where a cattle pen can be put up, and rotated. This makes Cow dung available as organic manure. Other areas within the reach of the family can also be made suitable for Anchote through the use of other waster as organic manure in addition to that from Cow dung (Amare, 1973).

2.6. Social importance of Anchote

In Western parts of Ethiopia, Anchote is used for special occasions. The boiled after peeling, or boiled before peeling, and/or further cooked tuber with butter, and other ingredients are served for the Meskel Holiday in September, which commemorates the finding of the True Cross. It is also a current experience that 'Lanqaxaa', a finely prepared Anchote dish, is commonly served during ceremonies marking, weddings, circumcision, birthdays, and religious celebrations such as New Year, and Thanks giving day for the harvest as well as on other occasions (Ambecha, 2006).

The role played by Oromo Women in Anchote Culture is many folds. They work as: breeders by selecting the desirable quality of Anchote, and growing site; growers as they plan, plant, and weed the plants; harvesters as they determine the maturity stage that gives Anchote of the type they, and how to harvest properly; processors as they prepare, process, and taste for its quality both physically, and behavioral, and finally as 'marketers and distributors'. The existing experience is the Oromo Women can decide, or convince their Husbands in Family Forums, to reach decisions on the many affairs concerning Anchote. This includes site selection to decide on where to grow, what size of land area to use for Anchote, buying, and selling of Anchote, and its products, and for what purpose to use the money in return. What is encouraging, and should be appreciated is that, besides their right to decide on the many affairs of Anchote, Oromo Women have experience in exercising this right. They have stood on their own feet, decided freely, and fairly what they think should be done in cultivating, and using Anchote. It is this advantage that exceeds the monetary benefits, and as well as social importance (Ambecha, 2006).

2.7. Traditional processing methods of Anchote

The traditional food product refers to a product frequently consumed or associated to specific celebrations, wedding, birthday, and/or seasons, normally transmitted from one generation to another, made with care in a specific way according to the gastronomic heritage, with little or no processing/manipulation, that is distinguished, and known because of its sensory properties, and associated to a certain local area, region or country (Morris *et al.*, 2004). Food processing is any, and all processes to which food is subjected after harvesting for the purposes of improving its appearance, texture, palatability, nutritive value, keeping properties, ease of preparation, and for eliminating microorganisms, toxins, and other undesirable constituents (Vanhonacker *et al.*, 2008).

The method of processing of root and tuber crops range from simple boiling to elaborate fermentation, roasting, frying, drying, and grinding to make flour, depending on the varieties, and type of product to be produced. The basic purpose of these methods is to make it more palatable, digestible, and make them safe for human consumption. This processing also extends the storage shelf life of perishable product in their fresh condition. Like many other root, and tuber crops, Anchote is rarely eaten raw (Fufa and Urga, 1997). Traditionally, boiled after peeling or boiled before peeling and/ or further cooking are applied prior to consumption.

2.7.1. Boiled before peeling

The washed tuber was boiled in traditional Pot locally known as 'Tuwe' for about three to four hours, the excess water then drained off (Figure: 2.3), and peeled with knife to remove the outer skin of Anchote, and sliced into pieces. The boiled Anchote before peeling are consumed traditionally either alone or combined with ingredients such as Kochkocha (Datta) (Figure: 2.2), which is a fermented side-dish prepared from ground green pepper with green leafy varieties of spices like Coriander (*Coriandrum sativum*), Sweet Basil (*Ocimum basilium*), Ginger (*Zingiber officinale*), Garlic, Butter, and Salt. Also Anchote is prepared on festive occasions which usually served as stew Anchote locally called Ittoo Anchote, which needs further cooking the sliced boiled Anchote with sufficient Butter, and other ingredients like shallot , fenugreek, turmeric, and salt (Fufa and Urga, 1997) . Normally, the stew Anchote is served with Injera. Special Anchote, Shorba Anchote, and Anchote with Meat, and/or Yoghurt are served with many types of ingredients like garlic, meat, yoghurt, and ginger, which may require further cooking process.

and Anchote with Meat, and/or Yoghurt are served with many types of ingredients like Garlic, Meat, Yoghurt, and Ginger, which may require further cooking process.

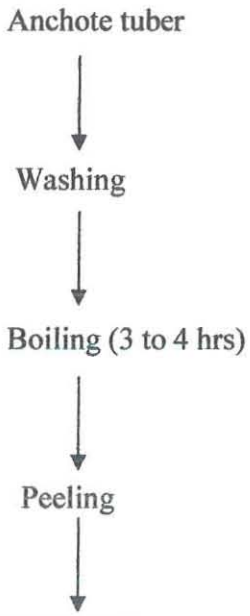

Figure: 2.2. Flow diagram for traditional processing methods of boiled before peeling Anchote tuber.

Figure: 2.3. Boiled before peeling Anchote tubers

2.7.2. Boiled after peeling

The washed tuber was peeled with knife, and boiled in traditional pot locally known as 'Tuwe' for about three to four hours, and the excess water then drained off (Figure: 2.5). The boiled Anchote after peeling are consumed traditionally either alone or combined with ingredients and other food groups mentioned under boiled Anchote before peeling (Figure: 2.4).

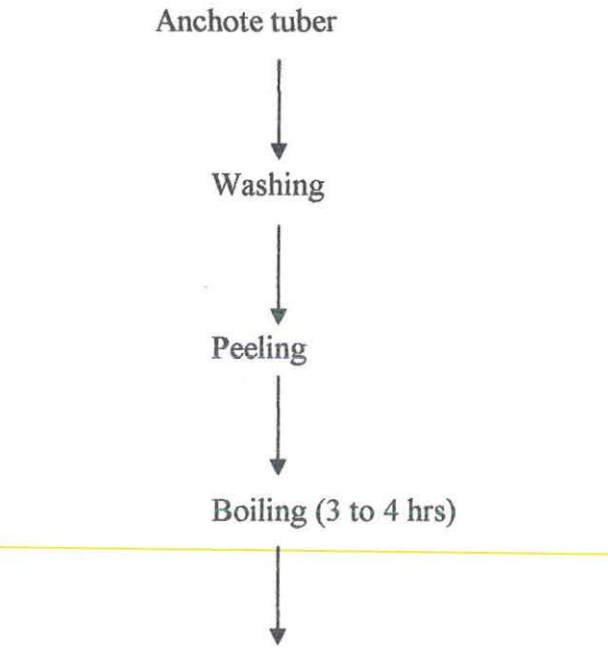

Figure: 2.4. Flow diagram for traditional processing methods of boiled after peeling Anchote tuber.

Figure: 2.5. Boiled after peeling Anchote tubers

2.8. Nutritional value and their use

Nutrition is defined as the science of food, and its relationship to health. It is a nourishing organic process by which an organism assimilates food, and uses it for growth, and maintenance. Good nutrition can help prevent disease, and promote health (Medical Encyclopaedia, 2007). Nutritional value is the main concern when a plant is considered as food source. They constitute an important source of Protein, Carbohydrates, Dietary Fiber, Vitamins, and Minerals. The aim of nutrition science is to give definitions to Metabolic and Physiological feedbacks from the body regarding sufficient diet. Additionally, nutritional science is developing into the study of metabolism, which seeks to separate diet, and health through the Prism of Biochemical process (Katina *et al.*, 2005).

Foods are complex substances that contain many chemical compounds (nutrients), which are required to nourish the body. The main compositions of foods are carbohydrates, proteins, water, fats, vitamins, and minerals. These nutrient classes can be categorized as either macronutrients (needed in relatively large amounts) or micronutrients (needed in smaller quantities). Macronutrients are the major sources of energy, and building materials for humans include carbohydrates, fats, protein, and water, while, micronutrients can be found in vitamins and minerals. They are only required in relatively small amounts to ensure proper functioning of all body cells. In addition, micronutrients, like water, do not provide energy (D'Mello and Duffus, 1991).

Food, therefore, is chemistry, and the mixture of chemicals that are represented, and divided into four basic categories: nutrients, non-nutritive (antinutritional factors and natural toxins), man-made contaminants, and additives. Nutrient accounts for more than 99.9% of the food contents. Some of these nutrients are considered to be essential while, others are considered to be non-essential. Essential nutrients are nutrients that cannot be synthesized by the human body. Therefore, they must be derived from food sources include vitamins, minerals, amino acids, fatty acids and some carbohydrates as a source of energy. Non-essential nutrients are nutrients which the body has the ability to synthesis from other compounds, as well as, from food sources. The majority of macronutrients are essential nutrients for life processes, produced by human body itself. Therefore, these essential nutrients can be received only from the food ingested. Most

importantly, macronutrients are constituent and indispensable ingredients of our diets, found in: carbohydrates, fat, protein, and water (Wilson, 2005).

Food is significant factor to the maintenance, development, functioning and reproduction of life. During life time an individual consumes 30 tons of food on average in seemingly endless dietary varieties. According to De Vries (1997), however, digestion splits all the foods found in all this variety of diets into the same basic nutrients. For the purpose of giving brief introduction on nutritional value, the review of this section focused only on some proximate composition (water, protein, lipids, and carbohydrate) and some minerals (Ca, Fe, Mg, Zn, and P).

2.8.1. Proximate Composition

2.8.1.1. Water

Water is the chemical substance with chemical formula H_2O: one molecule of water has two hydrogen atoms covalently bonded to a single oxygen Atom (Vaclavik *et al.*, 2007). Water is part of every living cell, and abundant in all living things, in almost all foods, unless steps have been taken to remove it. Two-thirds of our body weight consists of water. Water is essential for life and makes up the major part of living tissue, even though it contributes no calories to the diet (Van den Berg and Leniger, 1978). It is the medium for all metabolic changes (digestion, absorption and excretion) and transports the nutrients throughout the body, acts as a lubricant, and helps maintain our body temperature. Water is also important as a solvent or dispersing medium, dissolving small molecules to form True solutions, and dispersing larger molecules to form colloidal solutions. Acids and bases ionize in water; water also is necessary for many enzyme-catalyzed and chemical reactions to occur, including hydrolysis of compounds such as sugars. It is also important as a heating, and cooling medium and as a cleansing agent. Water is responsible for a countless number of bodily functions that are required for muscle growth, and recovery. Water also greatly affects the texture of foods, and tenderness of meat. For some food products, such as potato chips, salt, or sugar, lack of water is an important aspect of their quality, and keeping water out of such foods is important to maintain quality (Fennema, 1973).

Almost all food processing techniques involve the use of water or modification of water in some form: freezing, drying, emulsification, thickening and gels are a few examples (Leistner, 1987). Further, because Bacteria cannot grow without water, the water content has a significant effect on maintaining quality of the food. This explains why freezing, dehydration, or concentration of

foods increases shelf life, and inhibits bacterial growth. There are many available sources of water other than tap water, and bottled water (UNESCO, 2006). Some foods have high water content, including many fruits and vegetables. In addition, the body can make small amounts of water from various metabolic processes that result in molecules of water as a by-product. This, however, is by no means sufficient for the body's needs of water. It is generally recommended that people drink eight cups (or nearly 2 liters) of water a day to maintain an adequate supply (Roos, 1993).

The nature of hydrogen bonds allows water to bond with other water molecules as well as with sugar, starches, pectins, and proteins. Water absorbs energy as it changes from frozen to liquid to vapor state, and is an effective cooling medium. If water is easily extracted from foods by squeezing or pressing, it is known as free water. Inversely, water that is not easily removed from foods and that is not free to act as a solvent is known as bound water; water in foods imparts freshness. Water activity is the available free water for growth of microorganisms. A measure of water activity is the ratio of the vapor pressure of water in a solution to the vapor pressure of pure water. If water is unavailable for pathogenic or spoilage-causing bacteria to multiply, food is better preserved, and has a longer shelf life (James, 1995).

Water may exist in a bound form in a food due to the hydrogen bonds, ionic, and polar forces of attraction between the water molecules and food consequently. The moisture content of the food is often less satisfactory as a measure of the likely keeping qualities of the food than is water activity, aw. Water activity may range 0 to 1. Thus water activity is a measure of the energy status of the water in the food, and is a far better indicator of perishability than the water content. Most bacterial growth is affected above water activity 0.80. With the knowledge of moisture sorption isotherm that one can predict the maximum moisture that the food can be allowed to gain during storage (Decagon, 2004).

2.8.1.2. Protein

The term Protein, comes from the Greek Proteios meaning "First". It is important biological molecules that are made up of 'Building Blocks' called Amino Acids. Proteins are organic compounds made of amino acids arranged in a linear chain, and folded into a globular form. The amino acids in a polymer are joined together by the peptide bonds between the carboxyl and amino groups of adjacent amino acid residues (Ridley, 2006). They are a large

group of organic compounds consisting of Carbon, Hydrogen, Nitrogen and Oxygen atoms. Most proteins contain sulfur and some contain additional elements; for example, milk proteins contain phosphorus, hemoglobin and myoglobin contains Iron. Copper and Zinc also are constituents of some proteins. They are distinguished from fats and carbohydrates in being the only macromolecule in foods to contain Nitrogen. The presence of Nitrogen in Proteins is often used as the basis of the estimation of Protein in foods (Nelson and Cox, 2005).

Proteins are the most abundant molecules in cells, making up 50% or more of their dry weight. Every protein has a unique structure, and conformation or shape, which enables it to carry out a specific function in a living cell. Proteins comprise the complex muscle system, and the connective tissue network, and they are important as carriers in the blood system. All enzymes are proteins; enzymes are important as catalysts for many reactions (both desirable and undesirable) in foods (Dobson, 2000).

Proteins are very important in foods, both nutritionally, and as functional ingredients. The primary roles protein plays in the growth, and development of body building, repairing muscle, connective tissue, aiding immune system function, preserving muscle mass, building enzymes, and making hormones. They play an important role in determining the texture of a food. They are complex molecules, and it is important to have an understanding of the basics of protein structure to understand the behavior of many foods during processing. Protein is also provides the body with energy, and is needed for the manufacture of hormones, antibodies, enzymes, and tissues. It also helps maintain the proper acid-alkali balance in the body. Proteins provide amino-acids to build and support healthy body tissue (Voet, 2004).

Proteins are large molecules composed of one or more chains of amino acids. There are 20 different amino acids found in nature. They have different properties depending on their structure, and composition. Therefore, a body should be filled with all of them to function properly. Normally, the body produces twelve of these amino-acids; however, the other eight are the result of appropriate diet. Foods of animal origin such as milk or eggs often contain all these essential amino-acids, while a great number of plant products should be consumed in a certain combination to provide all these necessary protein components (Maton *et al.*, 1993).

Protein may be found in a variety of food sources. Proteins from animal sources (Meat, Poultry, Milk, and Fish) are considered to be of high biological value, because they contain all of the essential amino acids. Proteins from plant sources (Wheat, Corn, Rice, and Beans) are considered to be of low biological value, because an individual plant source does not contain all of the essential amino acids. Therefore, combinations of plant sources must be used to provide these nutrients (Nelson and Cox, 2005).

This excess intake of protein is not considered to be harmful for the average healthy individual. However, when protein intake is Inadequate, but total caloric intake is sufficient, a condition known as Kwashiorkor may occur. Symptoms of kwashiorkor include an enlarged stomach, loss of hair and hair color, and an enlarged Liver. Conversely, if protein, and caloric intake are both inadequate, a condition known as marasmus occurs. Marasmus presents with a stoppage of growth, extreme muscle loss, and weakness (Walker and Wilson, 2000).

2.8.1.3. Lipid

Lipids, which consist of fats and oils (fats are solid, and oils are liquid at room temperature), are high-energy yielding molecules composed mostly of carbon, hydrogen, and oxygen. Though lipids have a smaller number of oxygen molecules than carbohydrates have. This small number of oxygen molecules makes lipids insoluble in water, but soluble in certain organic solvents. The basic structure of lipids is a glycerol molecule consisting of three carbons, each attached to a fatty-acid chain. Collectively, this structure is known as a triglyceride. Triglycerides are the major form of energy storage (Nawar, 1996).

Fat is one of three nutrients used as energy sources by the body. The other ones are protein, and carbohydrates. The energy produced by fats is nine calories per gram. Protein and carbohydrates each provide four calories per gram. Fats that are in foods are combinations of Monounsaturated, Polyunsaturated, and Saturated Fatty Acids. The body needs only small amounts of fat. Excess intake of fat, especially saturated fat, can increase the risk of heart disease and stroke (Eskin, 1990).

The estimation of the fat content of food almost invariably involves the estimation not of the true fat content, but of the lipid fraction of the food. Those food constituents which are soluble in non-polar organic solvents such as petroleum ether or diethyl ether are known as lipids. This

fraction includes Phospholipids, Triglycerides, Sphingolipids, Waxes, Steroids, Terpenes, And Fat soluble vitamins (Simopoulos, 2001).

Fat provides energy and transport nutrients. There are two types of fatty Acids considered as essential for the human body: Omega-3 and Omega-6 fatty acids. These acids are required by the body to ensure normal functionality. They are received from cold-water fish, or fish oil, and any other components that comprise Omega-3 fatty acids, and black current seed oil, which comprise Omega-6 Fatty Acids. For example, the typical American diet often includes surplus of Omega-6 fatty acids and insufficient amount of Omega-3 fats. The increased consumptions of Omega-63 oils are highly recommended to decrease the risk of cardiovascular diseases, cancer etc. Fat and essential fatty acids in particular play many roles at the cellular level that are needed to build, and repair muscle tissue. Fat also plays an important role in the production of the hormones you need to grow muscle (Champe *et al.*, 2005).

2.8.1.4. Carbohydrate

Carbohydrates are organic compounds containing carbon, hydrogen, and oxygen. They may be simple or complex molecules including sugars, starch and cellulose. Historically, the term "Carbohydrate" has been used to classify all compounds with the general formula $C_n(H_2O)_n$; with a Hydrogen: Oxygen atom ratio of 2:1 (as in water). The arbohydrates (Saccharides) are divided into four chemical groupings: Monosaccharides, Disaccharides, Oligosaccharides, and Polysaccharides. In general, the Monosaccharides and Disaccharides, which are smaller (lower molecular weight) carbohydrates, are commonly referred to as sugars (Flitsch and Ulijn, 2003).

Important food carbohydrates include Sugars, Dextrins, Starches, Celluloses, Hemicelluloses, Pectins, and Gums. Carbohydrates hold a special place in human nutrition. They provide the largest single source of energy in the diet, and satisfy our instinctual desire for Sweetness. Glucose is the essential fuel for the brain, growing fetus, and is the main source of energy for the muscles during strenuous exercise. Carbohydrates may be used as sweeteners, thickeners, stabilizers, gelling agents, and fat replacers (WHO/FAO, 1998). Just like fuel in cars or cell phone battery, human body requires to be feed every day. Carbohydrate is the main form of energy necessary for human's body (Jegtyig, 2007).

Carbohydrates show a reciprocal relationship with fat in the diet, so that a high Carbohydrate diet is also a low Fat diet. Diets high in Carbohydrate are usually associated with lower prevalence of obesity, heart disease, non-insulin-dependent diabetes and some types of cancer. Available Carbohydrates may be defined as those which are susceptible to the endogenous enzymes of the upper digestive systems of humans, and are characterized as those Carbohydrates which produce energy in the human body (James, 1995).

Available carbohydrate = Total Carbohydrate minus Crude Fiber, which is sometimes known as Utilizable Carbohydrate.

Carbohydrates are the body's main source of energy, and should constitute the main ingredient of entire daily intake. Actually, there are two types of Carbohydrates: Simple Carbohydrates like Sugar, and Honey, and Complex Carbohydrates like Grains, Beans, Peas or Potatoes. Complex Carbohydrates are more nourishing, yet, have fewer calories per gram compared to fat, and cause fewer problems with over-nutrition than fat or sugar. Additionally, diabetics prefer Carbohydrates; since they allow better blood Glucose control. Foods high in Carbohydrate include Fruits, Sweets, Soft Drinks, Breads, Pastas, Beans, Potatoes, Bran, Rice, and Cereals. Carbohydrates are a common source of energy in living organisms; however, no carbohydrate is an essential nutrient in humans. Carbohydrates are not necessary building blocks of other molecules, and the body can obtain all its energy from protein and fats (Westman, 2001).

Based on the effects on risk of heart disease and obesity, the Institute of Medicine recommends that American and Canadian adults get between 45–65% of dietary energy from carbohydrates. The Food and Agriculture Organization and World Health Organization jointly recommend that national dietary guidelines set a goal of 55–75% of total energy from Carbohydrates, but only 10% directly from Sugars (their term for Simple Carbohydrates) (WHO/FAO, 2003).

2.8.2. Minerals

Throughout history, minerals were crucial to the growth and success of civilizations. Today, we are beginning to appreciate the importance of minerals to the growth, and health of the human body especially in light of so many new challenges to our health. As human beings, we are profoundly connected with our world. The elements of this earth become the minerals essential to every cell in the body. All form of living matter requires many minerals for their life

processes. The human body requires more than 22 mineral elements that can be supplied by an appropriate diet in varying amounts for proper growth, health maintenance, and general well-being (WHO, 1998). Plant-derived foods have the potential to serve as dietary sources for all human-essential minerals, and with a well-balanced diet that includes mixed sources of Grains, Fruits, Vegetables, Roots and Tuber crops. Plant foods can make a significant contribution to daily mineral needs at all stages of the life cycle (Dwyer, 1994).

Minerals represent about 5 to 6 percent of the total body weight in humans, and function in many different ways. Minerals are the primary inorganic component of the body that functions in conjunctions with enzymes, hormones, vitamins and other compounds. They are the left-over (ash) after cremation of a body, as they will not combust like most organic molecules or evaporate like water. They play important roles in nerve transmission, muscle contraction, blood formation and metabolism of macronutrients, and energy production. Some minerals such as Sodium, Potassium, and Chloride function as electrolytes, while other minerals, such as Copper, Zinc, Iron, Chromium, Selenium, and Manganese can be incorporated into enzyme molecules. Some minerals such as Calcium, Phosphorus, and Fluoride can play a vital structural role in strengthening bones and teeth. Some minerals can either block or enhance absorption of other nutrients, including other minerals and some vitamins. They are present in the skeletal system, and other hard tissues, and constitute approximately 4% of the body's weight (Heydon,1983).

The human body requires seven minerals in relatively large amounts such as Calcium, Sodium, Magnesium, Potassium, Phosphorous, Chlorine, and Sulfur. These are called major minerals. These important minerals participate in the majority of chemical reactions run in a body. Additionally, they are important to produce hormones. cobalt, copper, iodine, iron, manganese, molybdenum, and zinc are called minor minerals. For the purpose of giving a simple and brief introduction of minerals and human nutrition, the minerals can conveniently be classified into three main groups such as: those involved in the control of body fluid (e.g. water), those involved in the building of rigid structures to support the body (e.g. Ca, P, Mg) and those involved in chemical reactions in the body, and as chemical constituents of the body (Osborne and Voogt, 1978).

The minor minerals are not less important than the major ones; all are needed for good health. Deficiency in minerals, however, can have a major impact on health such as Anemia, and

Osteoporosis that commonly occur in both developed, and developing countries. Instead, deficiency depends on the natural availability of the mineral, if the mineral is found in lots of foods; it's unlikely the intake will be low. Example: Chromium, Copper, Iodine, Manganese and Phosphorus are found in a wide variety of foods, so deficiency is rare and sodium (salt) is the one mineral that we need to reduce in our diet (Rutherford, 2007)

A great number of vitamins and minerals act as antioxidants that protect our body against free radicals. As a consequence, these nutrients help us prevent cancer, and many other diseases, including heart disease, arthritis, cataracts, and alzheimer's disease. In this respect, antioxidants are special substances that protect cells against the adverse affects of free radicals. These molecules are produced when a body breaks down food. At that, the radicals can damage cells, and even cause Heart Disease, Cancer etc. Antioxidants include Beta-Carotene, Lutein, Lycopene, Selenium, and Vitamins A, C, E, and are found in Fruits, Vegetables, Nuts, Grains, Meats, and Fish (Medline Plus, 2007). The general roles played by some minerals like Calcium, Iron, Magnesium, Zinc, and Phosphorous in humans are briefly presented in the following section.

2.8.2.1. Calcium

Calcium (Ca) is one of an important mineral in which more than 99% of which is found in the bones, and teeth to keep them strong, and support their structure (Shils, 1999). The rest is stored in blood, muscles, and cells. It is obtained from the foods including: Milk, Cheese and Yogurt, Green Vegetables etc. Ca has a very important role in bone and tooth structure, in blood clotting, muscle contraction. Those of us who do not consume enough Ca should take Ca supplements. The exact amount of Ca depends on age and other factors; however, children, and teenagers need more Ca compared to adults (NIHODS, 2007). Aged women need Ca to prevent Osteoporosis, which weakens the bones that are likely to get broken. Half of women and men under 50 get their bones broken due to Osteoporosis. Therefore, a diet rich in Ca and vitamin D keep bones strong. Ca forms a vital part of bone and tooth structure, and is also important as a positive ion (Ca^{2+}) in blood clotting, muscle contraction, and nerve impulse transmission. It also participates in Glycogen metabolism (Heydon, 1983).

Inadequate intake of Ca increases the risk of Osteoporosis (bone loss with no apparent cause). Adequate Ca nutrition during childhood has important implications for bone growth, and

development, and is thought to reduce the incidence of Osteoporosis in later life. Excess intake of Ca may cause kidney stones and reduces mineral absorption in general (Wardlaw, and Insel, 1996). Phytic acids markedly decrease Ca bioavailability, and the Ca: Phytate molar ratio has been proposed as an indicator of Ca bioavailability. The critical molar ratio of Ca:Phy is reported to be 6:1 (Oladimeji *et al.*, 2000).

Ca is the outstanding single constituent of bones and teeth, and the most abundant mineral in the body with RDA for adults 1200 milligrams or approximately 1 g. Ca and PO_4^{3-} are properly utilized in the body by vitamin D (Anderson and smith, 2002). The best natural sources are Sea Vegetables, Low-Fat Yogurt, Skim Milk, Beans, Seeds, Nuts, Green Vegetables, etc. Intakes over 2000 milligrams per day may lead to Hypocalcaemia, Induce Constipation, and inhibit the Intestinal absorption of Iron, Zinc, and other essential minerals (Banerji, 2005).

2.8.2.2. Iron

Iron (Fe) is involved in many vital functions in the human body. First, Iron is important for Oxygen transport. Further, Iron is essential to Brain function, and development. Severe iron deficiency can cause retarded mental development, which may be irreversible. Iron has part in carrying of Oxygen as a critical component of the Hemoprotein, Hemoglobin, Myoglobin, and Cytochromes and it is also a Co-factor for some enzymes (Walker *et al.*, 2007).

Iron is a micronutrient that is most often deficient in developing countries, with children and women of reproductive age especially at risk of such deficiencies (Melaku *et al.*, 2005). Its deficiency causes anemia (Tortora, 1997). This deficiency is recognized by its symptom such as low blood iron level, small and red blood cells, and low blood hemoglobin values (Tortora, 1997). Iron deficiency is common only among children and pre-menopausal women. Great care must be taken not to take too much iron, as excess amounts are stored in the body's tissues, and adversely affect the body's Immune Function, Cell Growth and Heart Health. Iron toxicity usually results from a generic disorder called Hemochromatosis. This disease causes over Absorption and Accumulation of Iron, which can result in severe liver and heart damage (Wardlaw and Insel, 1996).

Low content and bioavailability of Iron in the typical cereal-based diet is a major cause of Iron deficiency (Sandberg, 2002). In developing countries Fe deficiency, due to poor

bioavailability, retards normal Brain development in Infants and affects the success of pregnancy by increasing premature deliveries, as well as morbidity of Mother and Child at or around child birth. It affects also working capacity, thus impairing socio-economic development as well (Malenganisho *et al.*, 2007). This is due to the high content of antinutrients in most cereals and other plant source staple foods and inadequate intake of animal foods in the diet . Iron absorption can be blocked by Calcium, Magnesium, Manganese, Zinc, Anti-acids, and Tetracycline (a common Antibiotic) (Rebouche *et al*,. 1999). Iron deficiency arises from the low bioavailability of Non-Haem Iron caused not only by Phytate, but also Tannins in the diet (Sandberg, 2002).

Iron nutrition is particularly important during the weaning period, when the infant is growing rapidly, and has a high demand for Iron. Cereal porridges are common complementary foods during the weaning period and often provide much of the dietary Iron intake, because the Iron contribution from human milk is low. Because of the high Phytate content of cereal porridges, Iron absorption of native Iron and fortification iron may be very low (Lorenz *et al.*, 2007). One mole of Phytic acid binds 6 mol Ferric Irons so that even relatively small quantities of residual Phytate are still strongly inhibitory. Studies indicated that adding 10 mg/100 g Phytic acid to bread rolls decreased Iron absorption by 20%, and that adding 20 mg/100 g decreased Iron absorption by 40%. Phytate: Iron molar ratios greater than 0.15 are regarded as indicative of poor Iron bioavailability. Absorption of iron from cereals can be increased by the degradation or removal of Phytic acid with a simple technology like fermentation (Hurrell *et al.*, 2003).

Iron is a constituent of hemoglobin. Body iron content is regulated by the amount absorbed. The absorption is influenced by body stores and by the amount and type of Iron in ingested foods. The RDA for adults is 15 milligrams. It is a vital component of many enzymes; it can promote resistance to disease and prevent fatigue. A deficiency can cause Anemia, resulting in impaired concentration, reduced physical performance, and work capacity, and decreased immune function. Ascorbic Acid is necessary for the proper assimilation of Iron. The best natural sources of Iron are Sea Vegetables, clams, Cockles, Mussels, Oysters, Yeast, Molasses, Beans, Nuts, Seeds, and Cereals. Tea, Coffee, Bran and Phytates decrease Iron absorption. There are no

reported cases of toxicity from foods but iron poisoning may occur from ingesting large amounts of medicinal Iron supplements (Morrison, 1965).

2.8.2.3. Magnesium

Magnesium (Mg) is essential to maintain both the acid-alkaline balance in the body, and healthy functioning of nerves, and muscles (including the heart), as well as to activate enzymes to metabolize Blood Sugars, Proteins and Carbohydrates (Rebouche *et al*,. 1999). A 2:1 ratio of Calcium to Magnesium is essential to maintain strong bones. Magnesium is the essential composition of Bone and Teeth with RDA for adults of 350 milligrams. It play important role in metabolism of Phosphorus, Starch, and Sugars. Many biochemical and physiological processes require Magnesium. It is necessary for Vitamin C and Calcium metabolism. It keeps Teeth healthy, brings relief from indigestion and can aid in fighting depression. More than 300 enzymes are known to be activated by Magnesium. Magnesium is a co-factor for many coenzymes, also affects metabolism of K and Ca (Banerji, 2005).

The best natural sources of Magnesium are whole Seeds, Nuts, Legumes, unmilled Grains, Green Vegetables, and Bananas. Phytate or Fiber may reduce Magnesium absorption. Alcohol acts as a Diuretic, causing vast quantities to be lost in the urine (Banerji, 2005). Its deficiency is related to High Blood Pressure, Pregnancy problems, and poor Heart function. In addition, indications of a Magnesium deficiency may be Muscle twitches, nervousness, Abnormal heart beat and Disorientation. Excess intake of Magnesium causes weakness in people with Kidney failure (Wardlaw and Insel, 1996).

2.8.2.4. Zinc

Zinc (Zn) is an essential trace mineral that is used for regulation of genetic activity, and balance of carbohydrate metabolism, and blood sugar (Adeyeye *et al.*, 2000). Zinc is the most ubiquitous of all trace elements involved in human metabolism. It is known to be essential component of over 200 enzymes, and necessary for normal collagen synthesis, and mineralization of bones. The metal has also been found to be involved in vital processes such as mitosis, synthesis of DNA, protein, gene expression, and activation (Walingo, 2009).

Zinc is also required for the action of both carbonic anhydrase, and super oxide dismutase. It is also vital for a variety of hormonal activities, including the Thymic Hormone, Glucagons,

Insulin, Growth Hormone, as well as the Sex Hormones. Furthermore, zinc has been found to be essential for normal Brain development, particularly concerning the Hippocampal function. In addition, zinc is also known for its Anti-viral, Anti-bacterial, Anti-fungal, and Anti-cancer properties, and has been found to protect animals against otherwise Lethal Irradiation by neutrons (Bryce-Smith, 1989). Zinc is an essential element found in the tissue of animals even at normal ambient concentrations. However, if plants and animals are exposed to large concentrations of bioavailable Zn, significant bioaccumulations can result, with possible toxic effects (Wardlaw and Insel, 1996).

Zinc deficiency is a public health problem, and is associated with poor growth, decreased immune function, increased susceptibility, severity of infections, adverse outcomes of pregnancy, and neurobehavioral abnormalities. Deficiency of Zn is highly prevalent in developing countries, but also in vulnerable groups with high requirements in industrialized countries, such as women of fertile Age, Infants and Adolescents (Sandberg, 2002). Approximately one third of children in low-income countries are stunted (Walingo, 2009). Zinc deficiency is presumed to be the underlying cause of stunting and delayed sexual maturation. In children zinc deficiency has been shown to Poor Growth, Impaired Immunity, increased Morbidity from common infectious disease, and increased mortality. Based on national food balance data, approximately 20.5% of the world's population is estimated to be at risk of inadequate Zn intake, with the percentage of individuals at risk highest in South East Asia (33.1%), Sub Saharan Africa (28.2%), South Asia (26.7%), Latin America and the Caribbean (24.8%) (Wuehler *et al.*, 2005).

If Zinc is removed from the catalytic site, activity is lost, replacement of Zinc restores activity. When the supply of dietary Zinc is insufficient to support these functions, biochemical abnormalities and clinical signs may develop. Studies in individuals with Acrodermatitis Enteropathica, a genetic disorder with Zinc mal-absorption resulting in severe deficiency, have provided much insight into the functional outcomes of Zinc deficiency. These include Impairments of Dermal, Gastrointestinal, Neurologic and Immunologic systems (ATSDR, 1995). Zinc deficiency arises to a large extent from impaired bioavailability of dietary zinc largely attributable to the high phytic acid content of diets. Zinc supplementation increases linear growth

in stunted children which suggests that these high rates of stunting may be due in part to Zinc deficiency (Walingo, 2009).

Zinc is present in most foods, but meat and fish provide the best sources, as bioavailability of zinc from animal products is considered to be far greater than from plant foods. Even though plant foods do contain Zinc, the bioavailability from them is relatively poor due to their high phytic acid, and fibre content. In fact, Zinc availability from vegetarian diets has been reported to be exceptionally low. Wheat germ is very rich in zinc, but unfortunately it is invariably discarded during food processing, which further depletes the vegetarian diet of Zinc. The RDA is 15 milligrams per day for Men and 12 milligrams per day for Women. Recent research suggests that Men have a higher need for Zinc than do women (Morris and Ellis, 1980).

Diets have been classified into high, medium and low-Zinc availability based on the phytate-Zinc molar ratio. Phytate: Zinc molar ratio is used to estimate the likely absorption of Zinc from a mixed diet. Diets with a Phytate-Zinc molar ratio greater than 15 have relatively mow Zinc bioavailability, those with Phytate-Zinc molar ratio between 5 and 15 have medium Zinc bioavailability and those with a Phytate-Zinc molar ratio less than 5 have relatively good Zinc bioavailability (Walingo, 2009). Phytate; Zinc molar ratio play a major role in inhibiting Zinc absorption such that Zinc absorption is typically less than 15% in high Phytate meals (Adeyeye *et al.*,2000).

2.8.2.5. Phosphorous

Phosphorous (P) is part of the phospholipids, an essential functional component of cell membranes. It is part of high energy phosphate compounds like e.g. Adenosine Triphosphate (ATP), and Creatine Phosphate, the biological energy conservation molecule which is essential to all vital processes. Phosphorus is also an essential component of Hydroxyapatite, the main structural bone mineral. Deficiency of phosphorus is common in malnourished children, and severe Hypophosphatemia is associated with increased mortality in Kwashiorkor (Manary *et al.*, 1998).

Phosphorus deficiency is also likely to cause Rickets-like bone changes in malnourished children. Phosphorous is likely to be a limiting nutrient in treatment of children. Absorption of dietary phosphorus is high (55-70%), relatively independent of dietary composition, and does not

appear to be up-regulated at low intakes. Dairy products, Meat, Poultry, Eggs, Fish, Nuts, and Legumes are generally good sources of highly available Phosphorus. However, the main form of Phosphorus from plant material is Phytate which is resistant to digestion unless enzymatically degraded by Phytase. Thus, Phosphorus from Phytate is only absorbed to a minor degree under normal conditions, and the Phytate fraction of Phosphorous should therefore be discounted from the calculations of the total Phosphorous requirements (Golden, 2009).

2.9. Anti-nutritional factors

Nature has endowed plants with the genetic capacity to synthesize substances that are toxic, and thus to ensure their survival against predators whether they be insects, fungi or animals including humans. Humans have learnt which foods are safe to eat or how such foods can be treated in order to destroy their toxicity use, and have developed suitable techniques for detoxifying the foods before consumption. One of these toxic materials are anti-nutritional factors (Shanthakumari *et al.*, 2008). Ant nutrients are chemicals which have been evolved by plants for their own defence, among other biological functions. Anti-nutrients reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals, thus preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value (Ugwu and Oranye, 2006).

One major factor limiting the wider food utilization of many tropical plants is the ubiquitous occurrence in them of a diverse range of natural compounds capable of precipitating deleterious effects in man, and animals compound which act to reduce nutrient utilization and/or food intake are often referred to as anti-nutritional factors. The biological effects of all these chemicals are diverse, and complex. When man ingests plant foods to meet nutritional needs, a wide variety of these non nutrient phytochemicals are ingested at the same time. Food crops regularly eaten have many beneficial nutrients but there are traces of ant nutritional factors components such as cyanides, oxalates, phytate, phenolics, tannin, protease inhibitors, heavy metals etc (Omoruyi *et al.*, 2007).

The remaining anti-nutrients can, however, be responsible for the development of serious Gastric Distress, and may interfere with digestion of nutrients, which inevitably results in chronic deficits in absorption of nutrients. According to Aletor (1993), there are several antinutritional factors that are very significant in plants used for human foods, and animal feeds. Several anti-

nutritional factors are present in root and tuber crops and are partially neutralized during ordinary cooking (Bhandari and Kawabata, 2004). Among Various antinutrients, and plant toxins, Phytate, Oxalate, Tannin, Cyanide and Trypsin inhibitors are found in root and tuber crops (Wanasundera and Ravindran, 1994). The review of this section focused on all these antinutrients separately except trypsin inhibitors.

2.9.1. Phytate

Phytate is the salt form of phytic acid. It is also known as Inositol hexakisphosphate (InsP6) (Figure 2.3), and a major component of plant storage organs such as seeds, roots, and tubers, where it serves as a phosphate source for germination and growth (Reddy, 2002). The phosphorus bound to phytate is not typically bio-available to any animal that is non-ruminant. Ruminant animals, such as cows and sheep, chew, swallow, and then regurgitate their food. This regurgitated food is known as *cud* and is chewed a second time. Due to an enzyme located in their first stomach chamber, the rumen, these animals are able to separate, and process the phosphorus in phytates. Humans and other non-ruminant animals are unable to do so (Cosgrove, 1980).

Phytate works in a broad pH-region as a highly negatively charged ion, and therefore its presence in the diet has a negative impact on the bioavailability of divalent, and trivalent mineral ions such as Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+}, and Cu^{2+} (Weaver, and Kannan, 2002). Whether or not high levels of consumption of phytate-containing foods will result in mineral deficiency will depend on what else is being consumed. In areas of the world where cereal proteins are a major and predominant dietary factor, the associated phytate intake is a cause for concern (IUFoST, 2008)

Besides, phytate has also been reported to form complexes with proteins at both low, and high pH values. These complex formations alter the protein structure, which may result in decreased protein solubility, enzymatic activity, and proteolytic digestibility. The phytate degrading enzyme, phytase, is in vogue for degradating phytate during food processing, and in the gastrointestinal tract. The major concern about the presence of phytate in the diet is its negative effect on mineral uptake (Greiner *et al.*, 2006). Phytate markedly decrease Ca bioavailability, and the Ca:Phy molar ratio has been proposed as an indicator of Ca bioavailability. The critical molar ratio of Ca: Phy is reported to be 6:1 (Oladimeji *et al.*, 2000). In human studies, Phy:Zn

molar ratios of 15:1 have been associated with reduced zinc bioavailability, and the molar ratio [Ca][Phy]/[Zn] is a better predictor of zinc availability, because calcium exacerbates phytate's effect on zinc absorption, and if the values were greater than 0.5 mol/kg, there would be interference with the availability of zinc (Davies and Warrington, 1986).

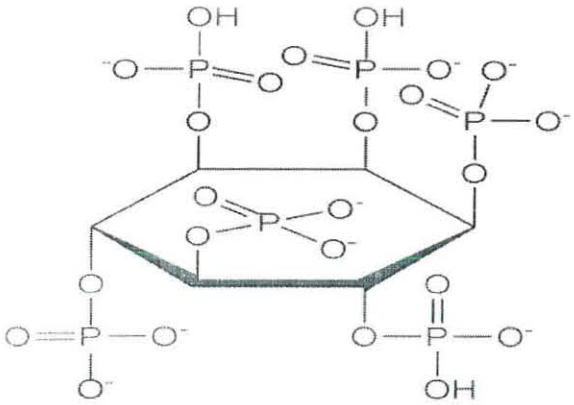

Figure: 2.6. Structure of Phytate ($Insp_6$)

At the same time, phytate may have beneficial roles as an Antioxidant, and Anticarcinogen (Jenab and Thompson, 2002). The outcome of surveillance of populations consuming vegetarian-type diets has shown lower incidence of Cancer, which suggests that phytate has an Anticarcinogen effect (Shamsuddin, 2002). Dietary phytate may have health benefits for Diabetes patients because it lowers the blood glucose response by reducing the rate of starch digestion and slowing gastric emptying. Likewise, phytate has also been shown to regulate Insulin secretion (Barker and Berggren, 1999). It is believed that phytate reduces Blood clots, Cholesterol, and Triglycerides, and thus prevents Heart diseases. It is also suggested that it prevents renal stone development. It is used as a complexing agent for removal of traces of heavy metal ions (Selvam, 2002).

Depending on the amount of plant-derived foods in the diet, and the grade of food processing, the daily intake of phytate can be as high as 4500 mg. On average, daily intake of phytate was estimated to be 2000–2600 mg for vegetarian diets as well as diets of inhabitants of rural areas in developing countries, and 150–1400 mg for mixed diets (Greiner *et al.*, 2006). Among the cooking treatments boiling appeared effective to reduce the phytate level, which could reduce as high as 20% of phytate (Bhandari and Kawabata, 2004).

2.9.2. Oxalate

A salt formed from oxalic acid (chemical formula HOOC-COOH) (Figure 2.4) is known as an Oxalate: for example, Calcium oxalate, which has been found to be widely distributed in plants (Liebman, 2002). Strong bonds are formed between oxalic acid, and various other minerals, such as Calcium, Magnesium, Sodium, and Potassium. This chemical combination results in the formation of oxalate salts. Some oxalate salts, such as sodium and potassium, are soluble, whereas calcium oxalate salts are basically insoluble. The insoluble calcium oxalate has the tendency to precipitate (or solidify) in the kidneys or in the urinary tract, thus forming sharp-edged calcium oxalate crystals when the levels are high enough. These crystals play a role to the formation of kidney stones formation in the urinary tract when the acid is excreted in the urine (Noonan and Savage, 1999).

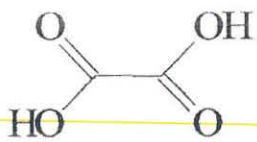

Figure: 2.7. Structure of oxalic acid

Higher content of oxalic acid can bind to calcium present in food, thereby rendering calcium unavailable for normal physiological, and biochemical role such as the maintenance of strong bone, teeth, cofactor in enzymatic reaction, nerve impulse transmission, and as clotting factor in the blood. The calcium oxalate, which is insoluble, may also precipitate around soft tissues such as the kidney, causing kidney stones. Though lost of calcium leads to degeneration of bones, teeth, and impairment of blood clotting process (Umaru *et al.*, 2007).

When oxalic acid is consumed, it irritates the lining of the gut, and can prove fatal in large doses. Currently, patients are advised to limit their intake of foods with a total intake of oxalate not exceeding 50–60 mg per day (Massey *et al.*, 2001). Oxalic acid is a common and wide-spread component of most plant families. While the levels of this acid in these plants are generally low, it is the high concentrations in the leaves, and conus of plants consumed daily that are of concern.

Oxalate is an anti-nutrient which under normal conditions is confined to separate compartments. However, when it is processed and/or digested, it comes into contact with the nutrients in the gastrointestinal tract (Kaushaiya *et al.*, 1988). When released, oxalic acid binds with nutrients, rendering them inaccessible to the body. If food with excessive amounts of oxalic acid is consumed regularly, nutritional deficiencies are likely to occur, as well as severe irritation to the lining of the gut. In ruminants oxalic acid is of only minor significance as an anti-nutritive factor since ruminal micro-flora can readily metabolize soluble oxalates, and to a lesser extent even insoluble Ca oxalate. While the importance of the anti-nutritive activity of oxalic acid has been recognized for over fifty years it may be a subject of interest to nutritionists in the future (Davies, 1979).

The values of oxalate changes as a result of processing. Soaking and cooking of foodstuffs high in oxalate will reduce the oxalate content by leaching. Boiling may cause considerable skin rupture, and facilitate the leakage of soluble oxalate into cooking water; this may be the possible reason to observed high reduction in oxalate level upon boiling (Bhandari and Kawabata, 2004). It is reported that boiling affects the highest reduction in oxalate (82.1% reduction after boiling for 40min as opposed to roasting and steeping those reduce 61.9% after 40-45 min and 43.3% after 24 years, respectively (Chinyere *et al.*, 1994).

Oxalic acid forms water soluble salts with Na^+, K^+, and NH_4^+ ions, it also binds with Ca^{2+}, Fe^{2+}, and Mg^{2+} rendering these minerals unavailable to animals. However Zn^{2+} appears to be relatively unaffected. In plants with a cell sap of approximately pH 2, such as some species of *Oxalis* and *rumex* oxalate exists as the acid oxalate (HC_2O_4), primarily as acid potassium oxalate. In plants with a cell sap of approximately pH 6, such as some plants of goosefoot family it exists as oxalate $(C_2O_4)^{2-}$ ion usually as soluble sodium oxalate and insoluble calcium and magnesium oxalates. Calcium oxalate is insoluble at a neutral or alkaline pH, but freely dissolves in acid. (Noonan and Savage, 1999).

Oxalate can be found as soluble and insoluble forms in plants. Soluble salts are formed when oxalate binds with potassium, sodium and magnesium (magnesium oxalate is less soluble than the potassium and sodium salts) while insoluble salts are produced when the oxalate binds with calcium and iron. Oxalate can also be found as free oxalic acid. Cooking can reduce the soluble

oxalate content of many common vegetables, but not the insoluble fraction, if the cooking water containing some of the leached soluble oxalate is discarded (Poeydomenge *et al.*, 2007).

Oxalate can be found in relatively small amounts in many plants and its distribution within plants is also uneven, in general oxalate content is highest in leaves followed by the seeds it is the lowest in the steams. Oxalic acid concentrations tend to be higher in plants than in meats, which can be considered oxalate free planning low oxalate diets. Although many plants have calcium oxalate crystals in their leaves, stems and roots, aroids are known to have these in their corms, rhizomes, etc. in extremely high concentrations (Horrocks *et al.*, 2008).

In view of this, the importance of the oxalate content of an individual plant product in limiting total dietary Ca availability is of significance only when the ratio, oxalate:Ca is greater than 1, since under these circumstances the oxalate has the potential to complex not only the Ca contained in the plant but also that derived from other food sources (Noonan and Savage, 1999).

2.9.3. Tannin

Tannin is an astringent, bitter plant polyphenolic compound that either binds or precipitates proteins and various other organic compounds including amino acids and alkaloids (Harold, 2004). The compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also in growth regulation. Tannins have traditionally been considered as antinutritional factors but it is now known that their beneficial or antinutritional properties depend upon their chemical structure and dosage and the total acceptable tannin daily intake for a man is 560 mg (Anonymous, 1973). The new technologies used to analyze molecular and chemical structures have shown that a division into condensed and hydrolyzable tannins is far too simplistic and readily form indigestible complexes with proteins and other macro-molecules under specific environmental conditions (Mole and Waterman, 1987)

Recent studies have demonstrated that products containing chestnut tannins included at low dosages (0.15-0.2 %) in the diet can be beneficial (Schiavone *et al.*, 2008). The most abundant polyphenols are the condensed tannins, found in virtually all families of plants, and comprising up to 50% of the dry weight of leaves. Condensed tannins inhibit herbivore digestion by binding to consumed plant proteins and making them more difficult for animals to digest, and by interfering with protein absorption and digestive enzymes. Tannins had been reported to affect

protein digestibility, adversely influencing the bioavailability of non-haem iron leading to poor iron and calcium absorption, also carbohydrate is affected leading to reduced energy value of a diet containing tannins (Adeparusi, 2001).

Bressani and Elias (1980) found tannins to be heat stable and that they decreased protein digestibility in animals and humans, probably by either making protein partially unavailable or inhibiting digestive enzymes and increasing fecal nitrogen. Tannins are known to be present in food products and to inhibit the activities of trypsin, chemotrypsin, amylase and lipase, decrease the protein quality of foods and interfere with dietary iron absorption (De Lumen and Salamat, 1980).

Tannins are known to be responsible for decreased feed intake, growth rate, feed efficiency and protein digestibility in experimental animals. Makkar *et al.*, (1988) reported that if tannin concentration in the diet becomes too high, microbial enzyme activities including cellulose and intestinal digestion may be depressed. Tannins also form insoluble complexes with proteins and the tannin-protein complexes may be responsible for the antinutritional effects of tannin containing foods (Mole and Waterman, 1987).

Widely distributed polyphenols in plants are not directly involved in any metabolic process and are therefore considered secondary metabolites. Some polyphenolic compounds have a role as defense chemicals, protecting the plant from predatory attacks of herbivores, pathogenic fungi and parasitic weeds. Polyphenols in the grains also prevent grain losses from premature germination and damage due to moulds (FAO, 1995).

Certain polyphenoles are able to bind Fe, which make the complex-bound Fe unavailable for absorption. The amount of Fe-binding phenol galloyl groups in foods roughly corresponds to the degree of inhibition of Fe absorption. All major types of food polyphenoles can strongly inhibit dietary non-haem iron absorption. The negative influence on Fe absorption is nutritionally the most important, especially in industrial products such as infant formulas, but more importantly in many developing countries where the diet is based on cereal and legume products. Cereals contain varying amounts of polyphones and generally the amounts are considered higher in the colored seeds (Sandberg, 2002).

2.9.4. Cyanide

Cyanides are organic or inorganic compounds which contain the $^{-}C{\equiv}N$ group (Health Canada, 1991). Cyanide is a naturally occurring chemical and is present in many food items such as Almonds, Maize, Apple Seeds, Millets, Bamboo, Mustard, Beans, Peas, Elderberry, Sorghum, Cassava Root, Sugar Cane, Lemon, Sweet Potato, Lima Beans, Wild Cherry, Lime, And Yam (Salkowski and Penney, 1994). It is also generated in fires and is one of many toxic components of tobacco smoke. The distribution and transformation of cyanides in air, on land or in water has been well described. Cyanide is a reactive compound which does not accumulate in the environment (USEPA, 1981). USEPA (1981), concluded that air, water and soil borne cyanide exposures to humans do not present significant risks to the general population when compared with potential exposure from naturally occurring sources, such as certain plants or plant products used as food (Hebert, 1993).

Cyanide is a potent and rapidly acting poison. The toxic effects of HCN and soluble inorganic cyanide salts are principally due to the propensity for the ^{-}CN ion to complex with certain metal ions. The respiratory enzyme cytochromec oxidase, which is necessary for intracellular utilization of oxygen, is especially sensitive to cyanide binding to the trivalent ion forming a stable coordination complex (USEPA, 1981). This complex subsequently inhibits cellular respiration and disables oxidative phosphorylation. Cyanide does not interfere with the transfer of oxygen to the tissues: rather it prevents cellular use of oxygen in energy production (Gordon and Amdur, 1991).

The cells are unable to utilize oxygen resulting is histotoxic hypoxia to which the CNS and the heart are particularly sensitive (Rieders, 1971). Cardiac irregularities are common, but death typically arises from respiratory arrest following CNS failure (Timbrell, 1994). It has been reported that higher intake of cyanides could result in the development of neurological disease in humans (Montgomery, 1980). The amounts of cyanide produced, only plants that accumulate more than 50 to 200 mg are considered to be dangerous (Kingsbury, 1964).

Cyanide is usually found joined with other chemicals to form compounds. Examples of simple cyanide compounds are hydrogen cyanide, sodium cyanide and potassium cyanide. Hydrogen cyanide is a colorless gas with a faint, bitter, almond-like odor. Sodium cyanide and potassium

cyanide are both white solids with a bitter, almond-like odor in damp air. Cyanide and hydrogen cyanide are used in electroplating, metallurgy, organic chemicals production, photographic developing, manufacture of plastics, fumigation of ships, and some mining processes (Hamilton and Hardy, 1974).

2.10. Effect of processing on nutrients and anti-nutrients

Several factors influence the nutritional content of food. These include the genetic make-up of the plant, the soil in which it is grown, use of fertilizer, prevailing weather, maturity at harvest, packaging, storage conditions and method utilized for processing. The primary purpose of processing is to render food palatable and develop its aroma, but it has inevitable consequences on the nutritional value of foods. Washing and peeling result in the loss of many water-soluble vitamins, since these are more concentrated in the peel and outer layers. With careful control of the processes, nutrient losses can be minimized without affecting palatability (Morris *et al.*, 2004).

The term "food processing" covers an enormous field, from simple boiling to the use of irradiation. The types of cooking methods differ in countries around the world and also vary with the ethnic background of the family. Processing (cooking) can be both beneficial and detrimental to nutrient composition of foods. It is known that processing techniques may decrease the food value of some nutrients: for example, there is some inevitable leaching of nutrients into the cooking water during processing. The cooking water may or may not be discarded, depending upon cultural and personal preference (Nestares *et al.*, 1996).

On the other hand, processing methods, such as peeling, boiling, baking, microwave and pressure cooking are may enhance the nutritional quality of food by reducing or destroying the anti-nutrients present in it, as well as increasing the digestibility of proteins and starches (Bhandari and Kawabata, 2006). Elimination or inactivation of anti-nutritional compounds is absolutely necessary to improve the nutritional quality, and effectively utilize human foods to their full potential. Processing generally inactivates heat-sensitive factors such as enzyme inhibitors, lectins and volatile compounds (El-Niely, 2007). Excessive heat processing, however, should be avoided, since it adversely affects the protein quality of foods. It is therefore important that processing is done within the recommended guidelines e.g. for heat, pH, as over processing will further destroy not only nutrient content but also taste and appearance (Morris *et al.*, 2004).

2.11. Sensory evaluation

Together with the nutritional and antinutritional analysis of a product it is important to conduct sensory evaluation as the sensory properties of a food product are important determinants of its acceptability, and preference among consumer. Sensory evaluation is defined as a scientific application used to evoke, measure, analyze, and interpret responses to food attributes or characteristics as they are perceived through a person's sense of sight, smell, hearing, touch, and taste in forming a food perception (Stone and Sidel, 1993).

Sensory methods were mainly developed for economical reasons because they allow to set up values of acceptance for any given food. Sensory analysis methods are used in quality control, product development, and research (compare defferent processing methods, effect of time and temperature on a given product) and development applications. The primary goal of sensory analysis is to conduct valid and reliable tests in producing data for which important, and sound decisions can be made (Meilgaard *et al.*, 1999). For product differentiation several sensory evaluation tests are also used. The two primary areas of sensory analysis are analytical and affective tests (Lawless and Heymann, 1999). Analytical tests are comprised of discrimination tests (paired-comparison, triangle, and duo-trio), threshold determinations, and descriptive analysis. Affective tests are classified under two categories: qualitative tests (focus groups, focus panels, and one-on-one interviews) and quantitative tests (preference tests and acceptance tests) (Meilgaard *et al.*, 1999).

The paired-comparison test is a discrimination test of two samples in which subject to determine differences or preferences between two samples that has higher desired characteristic by circling or selecting their choice using a scorecard or ballot. These differences may be directional or non-directional. Non-directional test requires the consumer to indicate which of the two coded samples preferred. Non directional tests are two-sided (two-tailed tests). The key advantage of using the paired-comparison test is its simple testing methodology; the test is easy to implement and organize, for the samples are served simultaneously and the subject can make a quick decision. However, a distinct drawback to using the paired-comparison test is the difficulty of specifying the difference or having the confidence in the subjects' understanding of the characteristic being evaluated (Stone and Sidel, 1993).

Chapter 3

Methods and Materials

3.1. Description of sampling sites

Anchote tuber samples were collected from Hara, Wayu kumba and Wayu kiltu kebeles found in Jimma Arjo district, Eastern Wollega Zone of Oromia Regional State (Figure 3.1). Jimma Arjo district is bordered on the southwest by the Didessa River which separates it from the Illubabor Zone, on the northwest by Diga Leka, on the northeast by Guto Wayu, and on the southeast by Nunu Kumba. Jimma arjo district is 378 km to the west of Addis Ababa and 48 km away from the zonal capital, Nekemte. The administrative city of the District is known as Arjo.

According to the census report of CSA (2005), the population of Jima Arjo is 93,459, with an estimated area of 741.41 square kilometers. The area is located at an altitudinal ranges from 1200-2816 meters above sea level. The area like most parts of the country has two periods of rainfall the "big" and "small" rains of June to September and March to April, respectively, receiving an average annual rainfall that ranges from 824-2616 mm. The average annual temperature ranges from 18-26 ^{0}C.

Agriculture (crop cultivation and rearing of livestock) is the leading economic activity in the district. The food crops cultivated in the district are cereals (sorghum, barley, teff, maize, and finger millet), pulses (peas, beans, and nug), root and tuber crops (Anchote, Oromo potatoe and sweet potatoe), fruits (lime, orange, mango, avocado, banana, papaya, and pumpkin), spices (cardamom, long pepper, chilies, and ginger), vegetables (cabbage and mustard seed). In addition, rearing of livestock such as cattle, sheep, chicken and donkeys, are common.

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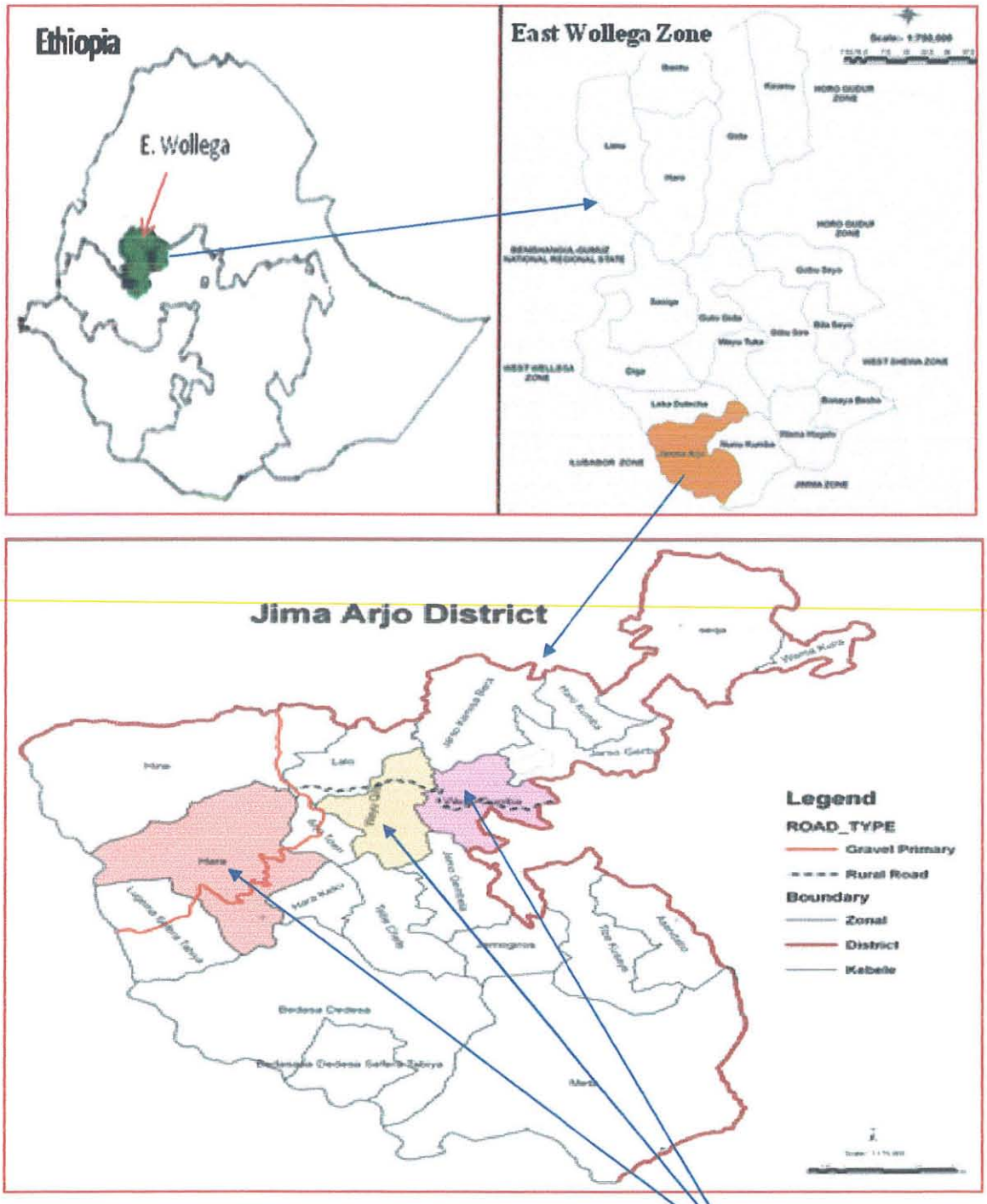

Source: Oromia Finance and Economic Development Bureau Sampling sites

Figure: 3.1. Map of Ethiopia and location of sampling sites.

3.2. Diagnostic survey for data collection

Semi-structured questionnaire was prepared and used to interview collect data from the respondents. Twenty two respondents were selected purposefully. Among them, twelve respondents were farmers who cultivate Anchote, six respondents were expertise who possesses knowledge in the production, marketing and consumption of Anchote, and four respondents were Anchote processors. The central theme of the interview was to gather prior information about production, handling, processing, and utilization of Anchote. The initial survey data lead the experimenter to have basic information as to where Anchote is produced and to fix sampling sites.

3.3. Selection of sampling sites

Preliminary survey was conducted to assess the production of Anchote in East Wollega Zone, prior to sampling. East Wollega Zone was chosen purposefully for reasons of accessibility and as an area potential Anchote production. Four districts namely Sibu Sire, Jima Arjo, Wayu Tuka and Limu are known as most producers and users of Anchote in the zone. Among these districts, Jima Arjo district was randomly selected for the survey study and Anchote sample collection area. In Jima Arjo district, twelve Anchote producing kebeles: Hinc, Lalo, Hara Keku, Hara, Tibbe Caffe, Tibe Kusaye, Wayu Kumba, Wayu Kiltu, Jamo Giros, Jarso Garbi, Asandabo, and Jamo Gambala were selected purposefully as major producers of Anchote. Among the tweleve Anchote producing kebeles, three kebeles were (Hara, Wayu Kumba and Wayu Kiltu) randomly selected. Forty seven (twelve, sixteen and nineteen in Hara, Wayu kiltu, and Wayu kumba kebeles, respectively) Anchote producing and marketing twice per week households were purposely selected from the 12 kebeles. Among the forty seven purposely selected Anchote producing farmers, 12 (three, four and five in Hara, Wayu kiltu, and Wayu kumba kebeles, respectively) were randomly selected and considered sampling units. About 12 kg Anchote samples were randomly collected from the sampling units (households).

3.4. Experimental study setting

A laboratory based experiment was conducted at the laboratories of Wollega University, AAU, EHNRI. Food Science and Nutrition Program and Chemistry Department of Science Faculty (AAU) hosted the experiment to determine the variation in gross composition and nutritional contents. The experiment was conducted on raw, boiled Anchote after peeling, and boiled

Anchote before peeling for analysis of nutritional composition and antinutritional factors of the tuber. Whereas, the sensory evaluation was conducted to determine preference of consumers for taste of boiled Anchote after peeling and boiled Anchote before peeling. Sample preparation, moisture content determination and sensory evaluation was conducted in the laboratory of Wollega University, in Food Science and Bio-processing Technology Institute. Anti-nutritional factors analysis was carried out in Ethiopian Health and Nutrition Research institute laboratory. All nutritional and antinutritional analysis were carried out in triplicate

3.5. Sample collection and preparation

A total of about 12 kilograms apparently uninfected Anchote were collected from the randomly selected households (1 kilogram per house hold) of study site (Hara, Wayu kumba and Wayu kiltu kebeles). The samples were packed in polyethylene bags, kept in an ice box (to prevent moisture loss), and transported to Food Science and Bioprocess Technology Institute Research laboratory of Wollega University within three hours. Once in the laboratory, samples were mixed for composite analysis of the study variables and washed by clean water all together. The washed tuber was grouped in to two sections of nine kilograms for the first section and three kilograms for the second section. The first section was used for nutritional and anti-nutritional analysis, whereas the second section was used for sensory analysis.

The first section was grouped into three lots of three kilograms each. The first lot was used for analysis as raw. The raw sample was sliced to uniform thickness 5 mm using a stainless steel knife. The second lot was used as boiled after peeling. The tuber was peeled (i.e. removal of the outer skin) with stainless steel knife, and boiled in traditional pot (locally known as Tuwe), containing tap water in the ratio of 1:2 (w/v) for about three to three and half hours until they became soft when felt between the fingers. Time of boiling was obtained average from interviewing Anchote processors, and excess water was drained off as is the usual household practice. After treatment the hot sample were exposed to the air for about 10 minutes to allow surface water to evaporate (Bhandari and Kawabata, 2004), the sample was sliced to uniform thickness 5 mm using a stainless steel knife.

The third lot was served as boiled before peeling. The washed tuber was boiled in traditional pot containing water in the ratio of 1:2 (w/v) , for about three to three and half hours until they became

soften and the excess water then drained off, and peeled with stainless steel knife. Then the sample was sliced to uniform thickness 5 mm using a stainless steel knife.

Moisture content of each lot was determined immediately after each lot was sliced into pieces. For other nutritional and antinutritional analyses, each of the three lot (control or raw, boiled after peeling, and boiled before peeling) of samples were dried at a time in oven (Gallenkamp Hotbox Oven, size 2, Gallenkamp, UK) at 60°C for 72 hours. Each dried samples were milled into fine powder using electric grinder (NIMA-8300Burman, Germany) until to pass through 0.425 mm sieve mesh size, and finally packed into airtight polyethylene plastic bags to minimize heat build-up, kept in ice box and transported to Addis Ababa University, and stored in the desiccator until required for analysis.

The second section of the samples used for sensory analysis was grouped into two lots of one and half kilograms each. The first lot was used for sensory analysis as boiled after peeling. The washed tuber was peeled (i.e. removal of the outer skin) with stainless steel knife, and boiled in traditional pot containing tap water in the ratio of 1:2 (w/v) for about three to three and half hours, and excess water was drained off as is the usual household practice. The boiled tuber was sliced to uniform thickness 50 cm using a stainless steel knife.

The second lot was served for sensory evaluation as boiled before peeling. The washed tuber was boiled in traditional pot containing tap water in the ratio of 1:2 (w/v), for about three to three and half hours and the excess water then drained off, and peeled with stainless steel knife. The boiled tuber was peeled and sliced to uniform thickness 50 cm using a stainless steel knife. Then, both Anchote tubers were evaluated by fifty consumers of the students and staffs of Food Sciences and Bioprocess Technology Institute at Wollega University.

3.6. Proximate analysis

3.6.1. Determination of moisture content

The moisture content of the Anchote samples was determined according to AOAC (2000) sub component 925.09 by oven drying method. The equipments used for moisture analysis are shown in Appendex I.

A clean empty aluminum dishes, and its lids (made of porcelain) were dried in drying oven (DHG- 9055A) at 100 ^{0}C for 1 hour, and cooled in desiccator (CSN-SIMAX) with fresh granular

silica gel desiccants for about 30 (min), and weighed. The samples prepared for each treatment (raw or control, boiled after peeling, and boiled before peeling) in triplicates were mixed thoroughly, and about 5.000g of Anchote samples were weighed in triplicate. The dishes and their contents were placed in the drying oven, and dried for 3 hr at 105 ^{0}C. After drying, the samples were cooled in a desiccators for 30min, and reweighed until constant weight obtained. The amount of water lost from the sample was considered to be directly proportional to the loss of weight due to drying of the sample. The amount of moisture was calculated by using the following formula, and the result was shown in Table 4.1.

$$\text{Moisture content (g/100g)} = \frac{W_1 - W_2}{W_1} \times 100$$

Where :

W_1 = Weight of fresh sample

W_2 = Weight of dry sample

3.6.2. Determination of crude protein content

The Protein content of the Anchote samples were determined according to AOAC (2000) sub component 979.09 by the Kjeldahl method in which digestion, distillation and titration was involved. The equipments used for moisture analysis are shown in Appendex I.

About 0.5000g of Anchote samples of each treatment in triplicates were taken in a Tecator tube, and 6ml of acid mixture of concentrated ortho-phosphoric acid, and concentrated sulfuric acid (5 parts of concentrated ortho-phosphoric acid, and 100 parts of concentrated sulfuric acid) was added and mixed thoroughly. And then, 3.5ml of 30% hydrogen peroxide was added step by step. As soon as the violet reaction had ceased, the tubes were shaken for a few minutes, and placed back into the rack. A 3.0000g of the catalyst mixture (ground 0.5000g of copper sulfate with 100 g of potassium sulfate) was added into each tube, and allowed to stand for about 10 min before digestion. The mixture was digested in the digester stove (HYP-1008 eight holes) at 370 ^{0}c for 4hrs. The digestion was continued for about 1 hr until a clear solution was obtained. The tubes in the rack was transferred into the fume hood for cooling, a 15ml of distilled water was added to dissolve the precipitate and to avoid further precipitation of sulfate in the solution.

A 250ml conical flask containing 25ml of the boric acid-indicator solution was placed under the condenser of the distiller (KDN-102F, nitrogen analyzer distillation device) with its tips immersed into the solution. The digested and diluted solution was transferred into the sample

compartment of the distiller. The tubes were rinsed with two portions of about 5ml distiller water, and the rinses were added into the solution. A 25ml of 40% sodium hydroxide solution was added into the compartment, and washed down with a small amount of water, and the steam switched on. A 100ml solution of the sample was distilled, and then the receiver was lowered so that the tip of the condenser is above the surface of the distillate. The distillation was continued until a total volume of 150ml is collected. The tip was rinsed with a few milliliter of distilled water before the receiver was removed.

The distillated solution was titrated with 0.1N hydrochloric acid to a reddish color, and the amount of hydrochloric acid was recorded. The amount of protein was calculated by using the following formula, and the result is shown in Table 4.1.

mg nitrogen in the sample = VxNx14

g nitrogen/100 g sample =mg of nitrogenx100/mg sample

Total nitrogen (g/100g) = $(V-V_b)$ xNx14/W

Crude protein (g/100g) = total nitrogen (g/100g) x6.25

Where: V: volume (ml) of hydrochloric acid solution required for the test sample; V_b: the volume (ml) of hydrochloric acid solution required for the blank test; N: normality of hydrochloric acid; 14: Equivalent weight of nitrogen; 6.25: conversion factor for tuber from total nitrogen to crude protein; W: weight of a sample.

3.6.3. Determination of total ash content

The Protein content of the Anchote samples was determined according to AOAC (2000) sub component 979.09 by oven drying method. The equipments used for moisture analysis are shown in Appendex I.

Total ash content Anchote samples were determined according to AOAC (2000) using sub component 923.03 by incineration of known weights of the samples in a muffle furnace (Carbolite CSF 1200) at 550^{0}C until a white ash was obtained. The Equipments and Analytical Reagent-Grade Chemicals used are shown in Appendix I.

About 2.000g of Anchote samples of each treatment (Raw or control, boiled after peeling and boiled before peeling) in triplicates were added into each dish. The dishes were placed on a hot plate under a fume hood, and the temperature was slowly increased until smoking ceases, and the samples become thoroughly charred. The charred samples were placed inside the Muffle Furnace (Carbolite CSF 1200), and ashed at 550^0C for 3 hrs. The charred samples were removed from a Muffle Furnace and cooled, seen to be clean and white in appearance. Few drops of de-ionized water and concentrated nitric acid were added, dried, and return to a Muffle Furnace. Then checked until traces of carbon are fully ashed. Finally taken out of the Muffle Furnace placing immediately in a desiccators till cooled to room temperature, and each dish plus ash was reweighed. Weight of total ash was calculated by difference, and expressed as percentage of sample. The amount of total ash was calculated by using the following formula, and the result is shown in Table 4.1.

$$\text{Total Ash (g/100g)} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where :

W_1 = Weight of the dish

W_2 = Weight of fresh sample and dish

W_3 = Weight of ash and dish

3.6.4. Determination of crude fiber content

Crude fiber content Anchote samples were determined according to AOAC (2000) using sub component 962.09 in which the steps of digestion, filtration, washing, drying and combustion were involved. The Equipments and Analytical Reagent-Grade Chemicals used are shown in Appendix I.

About 1.5000g of Anchote samples of each treatment (raw or control, boiled after peeling, and boiled before peeling) in triplicates were placed into a 600ml beaker, and about 200ml of 1.25% H_2SO_4 was added, and boiled gently exactly for 30 minutes placing a watch glass over the mouth of the beaker. During boiling, the level of the sample solution was kept constant with hot distilled water. After 30 minute boiling, 20ml of 28% KOH was added and boiled gently for a further 30 minute, with occasional stirring.

The bottom of a sintered glass crucible was covered with 10 mm sand layer, and wetted with a little distilled water. The solution was poured from beaker into sintered glass crucible, and then

the vacuum pump was turned on. The wall of the beaker was rinsed with hot distilled water several times; washings were transferred to crucible, and filtered

The residue in the crucible was washed with hot distilled water, and filtered (repeated twice). The residue was washed with 1% H_2SO_4 and filtered, and then washed with hot distilled Water, and filtered; and again washed with 1% NaOH and filtered. The residue was washed with hot distilled water and filtered and again washed with 1% H_2SO_4 and filtered. Finally the residue was washed with water- free acetone.

The crucible with its content was dried for 2 hours in an electric drying oven at 130 ^{0}C and cooled for 30 min in the Desiccator (with fresh granular silica gel dessicant), and then Weighed. The crucible was transferred to a Muffle Furnace (Gallenkamp, size 3) and incinerated for 30 min at 550^{0}C. Finally, it was cooled in the Desiccators, and re-weighed. The crude fiber content was determined by using the following formula, and the result is shown in Table 4.1.

$$\text{Crude fiber (g/100g)} = \frac{W_1 - W_2}{W_3} \times 100$$

Where :

W1 = weight of (crucible + sample) after drying

W2 = weight of (crucible + sample) after ashing

W3 = weight of sample

3.6.5. Determination of crude fat content

The crude fat content of Anchote samples were determined according to AOAC (2000) official using sub component 920.39 in a soxhlet extractor. The Equipments and Analytical Reagent-Grade Chemicals used are shown in Appendix I.

The cleaned extraction flasks with boiling chips were dried in Oven Drying (DHG-9055A) at 90^{0}C for 1hour, cooled in desiccators (with granular silica gel desiccants) for 30 minutes, and then weighed. The bottom of the extraction thimble was covered with about 2cm layer of fat free cotton. About 2.0000g of Anchote samples of each treatment (Raw or control, boiled after peeling, and boiled before peeling) in triplicates were added into the extraction thimbles, and then covered with about 2cm layer of fat free Cotton. The thimbles with the sample content were placed into Soxhlet (SHANGHAI QIANJIAN INSTRUMENT CO., LTD) extraction chamber. The cooling water was switched on, and a 50 ml of Diethyl Ether was added to the extraction flask through the condenser. The extraction was conducted for about 3 hrs. The extraction flasks

with their content were removed from the extraction chamber and placed in the drying oven at 90^{0}C for about 30min, cooled to room temperature in the Desiccator for about 30 minutes and re-weighed the flask with the extract. The amount of crude fat was calculated by using the following formula, and the result is shown in Table 4.1.

Weight of fat (W_f) = $W_a - W_b$

$$\text{Crude fat (g/100g)} = \frac{W_f}{W} \times 100$$

Where :
Wa = weight of extraction flask after extraction;
Wb = weight of extraction flask before extraction;
W = weight of sample.

3.6.6. Determination of utilizable carbohydrates

Utilizable Carbohydrate content was calculated by difference. The mathematical expression is as follow, and the result is shown in Table: 4.1.

Utilizable Carbohydrate (g/100g) = 100 – (% moisture + % crude protein + % crude fiber + % total ash+ % crude fat).

3.6.7. Determination of gross energy

The gross energy content was determined by calculation from fat, carbohydrate and protein contents using the Atwater's conversion factors; 16.7 kJ/g (4kcal/g) for protein, 37.4 kJ/g (9 kcal/g) for fat and 16.7 kJ/g (4 kcal/g) for carbohydrates and expressed in calories (Guyot *et al.*, 2007). The mathematical expression of Gross Energy is as follow, and the result is shown in Table 4.1.

Gross energy (Kcal/100g) = (9 * crude fat %) + (4 * crude protein %) + (4 * utilizable carbohydrate %).

3.7. Minerals Analysis

3.7.1. Determination of calcium, iron, magnesium and zinc

Calcium, iron, magnesium, and zinc were determined according to the standard method of AOAC (2000) using an Atomic Absorption Spectrophotometer (Varian Spectr AA. 20 plus). The equipments and analytical reagent-grade chemicals used are shown in Appendix I.

The washed silica dishes were placed in to Drying Oven at 90°c for15 min. The dishes were then removed, and cooled down in desiccators for about 30 minutes, when cooled to room temperature weighed. About 2.000 g of Anchote samples of each treatment (Raw or control, boiled after peeling, and boiled before peeling) (in triplicate) were weighed in to each dish, then placed on a hot plate under a fume-hood in slowly increasing temperature until smoking ceases. When the samples become thoroughly charred, the dishes then placed in a Muffle Furnace, as near to centre as possible and ashed at 550 ^{o}C. The dishes were removed from a muffle furnace, cooled, seen to be clean, and white in appearance. Few drops of de-ionized water and concentrated nitric acid were added, dried, and return to a Muffle Furnace. Then checked until traces of carbon are fully ashed. Finally taken out of the muffle furnace placing immediately in a desiccators till cooled to room temperature.

The ash each sample was digested with 5 ml of 6 M HCl to wet it completely and carefully dried on a low temperature hotplate. 7 ml of 3 M HCl were added and the dish was heated on a hot plate until the solution just boils. Then it has been cooled, and filtered through a Whatman no.1 filter paper in to a 50 ml volumetric flask retaining as much of the solids as possible in the dish. Again 7 ml 3 M HCl was added to the dishes, and heat until the solution just boils. Then, cooled and filtered in to the volumetric flask. The dishes were then washed with water, and filtered in to the volumetric flask. The filter paper was washed thoroughly and collected in the flask. Since calcium is to be determined 2.5 ml of 10 % Lanthanum chloride solution were added to the flask. Finally, diluted to the mark (50 ml) with freshly de-ionized water. The blank were prepared a blank by taking the same amount of reagents through all steps.

The stock standard solutions of minerals (zinc, calcium iron, and magnesium) were diluted with 0.3 N HCl to concentrations that fall within the working range (0.5, 1.0, 2.0, and 4.0 µg/ml for calcium analysis; 0.5, 1.0, 1.5, and 3.0 µg/ml for iron analysis; 0.25, 0.50, 0.75 and 1.50 µg/ml

for magnesium analysis; 0.10, 0.20, 0.40, and 0.80 µg/ml for zinc analysis). The Atomic Absorption Spectrophotometer used for mineral determination was calibrated using standard solutions and the reagent blank solution was run with the sample.

The apparatus were set according to the instructions, and a calibration curve was prepared by plotting the absorption values against the metal concentration in µg/ml (Appendex II, III, IV, and V). Reading was taken from the graph, which depicted the metal concentrations that correspond to the absorption values of the samples, and the blank. The metal contents were calculated by using the following formula, and the result is shown in Table 4.2.

$$\text{Metal content (mg/100g)} = \frac{(A - B)xV}{10W}$$

Where :
W = wight of the sample (g)
V = volume of the extract (ml)
A = concentration (μg/ml) of sample solution
B = concentration (μg/ml) of blank solution

3.7.2. Determination of phosphorus

Phosphorous was determined by the colorimetric method (Colorimeter SP 20, Bausch and Lamb) using Ammoniaum Molybdate (AOAC, 1984). It was converted to phosphomolybdate, which was reduced to a blue molybdenum compound by aminonaphtholsulphonic acid to give a blue molybdenum compound. The Equipments and Analytical Reagent-Grade Chemicals used are shown in Appendix I.

About 1 ml of the clear extract solution was taken from the sample solution prepared from mineral analysis (determination of Ca, Fe, Mg and Zn) and diluted to 100 ml with deionized water in a 100 ml volumetric flask. A 5ml (triplicates) of the sample dilution was added into test tubes. A 0.5ml of molybdate and a 0.20ml aminonaphtholsulphonic acid was added into the test tube (sample solution) and mixed thoroughly step by step. A 0.20ml aminonaphtholsulphonic acid was added into the test tube repeatedly each time until the solution becomes clear. The solution was allowed to stand for 10 minute. The absorbance of the solution was measured at 660 nm against distilled water. Simultaneously with sample phosphorous, standard and blank analysis were carried out. Standard and blank solutions were prepared as above but 5 ml of working standard (reading A) and 5 ml of deionized water (reading B) in place of the sample dilution were used respectively. A standard curve was made from absorbance versus

concentration (Appendex VI). The phosphorus contents were calculated by using the following formula, and the result is shown in Table 4.2.

$$\text{Phosphorus (mg/100g)} = \frac{(A - B) \times 50 \times 100}{\text{Slope} \times W_f \times 10}$$

Where :

A = reading of the sample solution

B = reading of the blank solution

W_f = weight of sample.

The molybdate reagent was prepared by taking 75ml of 10N H_2SO_4 in a 250 ml volumetric flask and dissolving 6.25g of ammonium molybdate in a beaker in about 50ml of water and then transferring the beaker solution into volumetric flask. The aminonaphtholsulphonic acid reagent was prepared by dissolving 100mg of 1, 2, 4 aminonaphtholsulphonic acid in 39 ml of sodium bisulphite solution, and then adding 1 ml of sodium sulphite solution. A 15% sodium bisulphite and 20% sodium sulphite solution was prepared by dissolving 7.5g sodium bisulphate in 50ml of water and 2g sodium sulphite in 10ml of water respectively. The standard phosphrus solution was prepared by dissolving 438.8 mg of KH_2PO_4 (water free) in some water in a 100ml volumetric flask, adding 1 ml concentrated H_2SO_4 and diluting to the mark with water. The standard curve was made by taking 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0ml of the standard solution and diluting to 100ml.

3.8. Analysis of antinutritional factors

3.8.1. Determination of phytate content

Phytate was determined by the method of Latta and Eskin (1980) and later modified by Vantraub and Lapteva (1988). The Equipments and Analytical Reagent-Grade Chemicals used are shown in Appendix I.

About 0.1000g of Anchote samples of each treatment (Raw or control, boiled after peeling, and boiled before peeling) were extracted with 10ml 2.4% HCl in a mechanical shaker (Eberbach) for 1hour at an ambient temperature and centrifuged at 3000rpm for 30 minute. The clear supernatant was used for phytate estimation. A 2ml of wade reagent (containing 0.03% solution of $FeC_{13}.6H_2O$ and 0.3% of sulfosalicilic acid in water) was added to 3ml of the sample solution (supernatant) and the mixture was mixed on a Vortex (Maxi Maxi II) for 5 seconds. The absorbance of the sample solutions were measured at 500 nm using UV- VIS spectrophotometer (Beckman DU-64- spectrophotometer, USA).

A series of standard solution were prepared containing 0, 4.5, 9, 18, 27 and 36 µg/ml of phytic acid (analytical grade sodium phytate) in 0.2N HCl. A 3ml of standard was added into 15ml of centrifuge tubes with 3ml of water which were used as a blank. A 1ml of the Wade reagent was added to each test tube and the solution was mixed on a Vortex mixer for 5 seconds. The mixtures were centrifuged for 10 minutes and the absorbances of the solutions (both the sample and standard) were measured at 500nm by using de ionized water as a blank. A standard curve was made from absorbance versus concentration (Appendex VII) and the slope and intercept were used for calculation. The phytate content was calculated by using the following formula, and the result is shown in Table 4.3.

Phytic acid (µg/100g) = [(absorbance - intercept)/ (slope * density * weight of sample)] * {10/3}

3.8.2. Determination of phytate phosphorus and non-phytate phosphorus content

Phytate and phosphorous were determined by the above methods. Phytate phosphorus was calculated by assuming 28.18% of phytate ($C_6P_6O_{24}H_{18}$) is phosphorus. The non-phytate phosphorus was determined from the difference between phytate phosphorus and total phosphorus, whereas, the proportion of phosphorous as phytate was calculated by phytate phosphorus devided by total phosphorus (Khetarpaul and Sharma, 1997). The phytate phosphorus, non-phytate phosphorus, and phosphorous as phytate content was calculated by using the following formula, and the result is shown in Table 4.4.

Phytate phosphorus (mg/100g) = phytate content (mg/100g) x 28.18%

Non-phytate phosphorus (mg/100g) = Phytate phosphorus (mg/100g) - Total phosphorus (mg/100g)

Phosphorous as phytate (%) = Phytate phosphorus (mg/100g)/ Total phosphorus (mg/100g)

3.8.3. Determination of oxalate content

Oxalate was analyzed using the method originally employed by Ukpabi and Ejidoh (1989) in which the procedures involve three steps: digestion, precipitation, and permanganate titration. The Equipments and Analytical Reagent-Grade Chemicals used are shown in Appendix I.

About 2.000 g of Anchote samples of each treatment (Raw or control, boiled after peeling, and boiled before peeling) in triplicates were suspended in 190 ml de-ionized water contained in a

250 ml volumetric flask; 10 ml of 6 M HCl was added and the suspension digested at the boiling pointof water for 1 h that followed by cooling. Then made up to 250 ml and filtered.

Duplicate portion of 125 ml of filtrate were measured in to a beaker and four drops of methyl red indicator added, followed by the addition of concentrated NH_4OH solution drop wise until the test solution changes from salmon pink color to faint yellow color (pH 4-4.5). Each portion was then heated to 90 ^{o}C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was then again heated to 90 ^{o}C and 10 ml of 5 % $CaCl_2$ solution was then added while being stirred constantly. After heating it was cooled and left overnight in refrigerator. The solution was then centrifuged at a speed of 2500 rpm for 5 min the supernatant was decanted and the precipitate completely dissolved in 10 ml of 20 % (v/v) H_2SO_4 solution.

At this point the total filtrate resulting from digestion of 2 g of flour was made up to 300 ml. aliquots of 125 ml of filtrate were heated until near boiling, and then titrated against 0.05 M standard $KMnO_4$ solution to a faint pink color which persists for 30 seconds. The oxalate content was calculated by using the following formula, and the result is shown in Table 4.3.

$$\text{Oxalate (mg/100g)} = [(T * V_1 * D_f * 10^5)] / (V_2) * W]$$

Where: T = Normality of Potassium permanganate; V_1= Volume of Potassium permanganate (titrant); D_f = the dilution factor which is 26.8; V_2 = Volume of extract oxalate (titrand) and W = Weight of sample (g).

After analysis of phytate and oxalate the molar ratio of phytate and oxalate to calcium, zinc and iron were calculated to evaluate the effect of elevated levels of phytate and oxalate in the bioavailability of dietary minerals. As the ratios are the better indicators of the bioavailability than the amounts of the mineral and the phytic acid in the diet (Omoruyi *et al.*, 2007).

3.8.4. Determination of condensed tannin content

Tannin content was determined by the method of Burns (1971) as modified by Maxson and Rooney (1972). The Equipments and Analytical Reagent-Grade Chemicals used are shown in Appendix I.

About 2.0000g of Anchote samples of each treatment (Raw or control, boiled after peeling, and boiled before peeling) in triplicates were weighed in a screw cap test tube and extracted with

10ml of 1% HCl in methanol for 24 hours at room temperature with mechanical shaking. After 24 hours shaking, the solution was centrifuged at 1000rpm for 5 minutes. A 1ml of supernatant was taken and mixed with 5 ml of vanillin-HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% Vanillin in methanol).

D-catechin was used as standard for condensed tannin determination. A 40mg of D- catechin was weighed and dissolved in 1000 ml of 1% HCl in methanol, which was used as stock solution. A 0, 12, 24, 36, 48 and 60 ml of stock solution was taken in test tube and the volume of each test tube was adjusted to 1ml with 1% HCl in methanol. A 5ml of vanillin-HCl reagent was added into each test tube. After 20 minutes, the absorbance of sample solutions and the standard solution were measured at 500nm by using water to zero the spectrophotometer, and the calibration curve was constructed from the series of standard solution. A standard curve was made from absorbance versus concentration and the slope (Appendex VIII) and intercept were used for calculation. The condensed tannin content was calculated by using the following formula, and the result is shown in Table 4.3.

$$\text{Condensed Tannin (mg/100g)} = \frac{(A_s - A_b) - \text{Intercept}}{\text{Slope} \times d \times W}$$

Where:
A_s = sample absorbance
A_b = blank absorbance
d = Density of solution (0.791g/ml)
W = Weight of sample in gram

3.8.5. Determination of cyanide content

Cyanide content of Anchote samples were determined according to the official standard method of AOAC (1984) by Silver Nitrate titrimetric methods, in which the steps of distillation and titration was involved. The Equipments and Analytical Reagent-Grade Chemicals used are shown in Appendix I.

About 10g of Anchote samples of each treatment (Raw or control, boiled after peeling, and boiled before peeling) in triplicates were weighed into a flask and soaked in 100ml of distilled water in separate 500 ml round bottom flask for 2hr. The Kjeldahl flask was adjusted before distilling the tip of delivery tube below surface of liquid and 100 ml distilled water were added. Thereafter, the mixtures in the flask were heated by steam distillation. The released cyanide was collected in a conical flask containing in 20 ml 0.01N $AgNO_3$ acidified with 1 ml concentrated HNO_3.

When the gas has passed over, the distillate was filtered through sintered glass crucible and rinsed the test tube with little water. The distillate was then titrated against excess $AgNO_3$ with 0.02N KSCN, using ferric alum indicator. At the end point of titration, the color of the indicator changed from red to purple color. Using the relationship1 ml of 0.01 N $AgNO_3$ = 0.27 mg of cyanide. The cyanide content was calculated by using the following formula, and the result is shown in Table 4.3.

$$\text{Cyanide (mg/100g)} = \frac{V_{AgNO3} * 0.27}{W} \times 100$$

Where:

V_{AgNO3}= Volume of silvernitrate=(20-(2* V_{KSCN}))

V_{KSCN} = Volume of potassium thiocyanate consumed

W = weight of sample.

3.9. Sensory analysis

Sensory evaluation was conducted in Food Science and Bioprocess Technology Institute research laboratory of Wollega University, Ethiopia. Boiled Anchote after peeling and boiled Anchote before peeling was evaluated by fifty consumers of 29(58%) males and 21(42%) females participants and 49 (98%) of the consumers were between the ages of 16-35 years. The consumers were recruited from staff and students of Food Science and Bioprocess Technology Institute at Wollega University, Ethiopia. The general demographic questions and frequency of consumption of consumers were completed before sensory evaluation of the products (Appendex IX). They were selected if they indicated that they consumed Anchote boiled before and after peeling at least once per month. Additional criteria used to screen consumers were: no food allergies and/or no frequent illness, nonsmoker, willing to evaluate Anchote and available to participate during the scheduled testing dates.

In the sensory evaluation session, the consumers were seated in an open well illuminated laboratory and about 20 grams of each two samples were presented to each consumer on a tray at ambient temperature (≈25°C) within 2 hrs after boiling. The consumers were asked to indicate which of the two coded samples taste is preferred on the score card (Appendex X). The non-directional paired comparison test, exactly a two-sided preference (a version of paired comparison test) according to the "forced choice" technique (ISO, 1983), with the question: "Of these two samples, which one do you prefer?" was carried out with fifty consumers. The samples were served with identical container coded with 3-digit random numbers, half of the consumers

were asked to taste one sample first, the others to taste it second. Necessary precautions were taken to prevent bias of tasting by ensuring that consumers rinsed their mouth with water before and after each tasting of sensory evaluation. Consumers expressed their preference taste of the boiled after peeling and boiled before peeling Anchote tubers using paired comparison test.

3.10. Statistical analysis

Nutritional and ant-nutritional analyses were followed one way analysis of variance (ANOVA). Means were compared using Duncan's multiple range test (Duncan, 1955). All the statistical analyses were performed on the results obtained using SPSS version 15.0 for windows. Also non-directional paired comparison t-test was used to analyze the responses of the consumers with regard to their preference taste for the sample (Roessler *et al.*, 1978). Significance difference was accepted at the 0.05 ($P<0.05$) level of probability (Steel and Torrie, 1980).

Chapter 4

Result and Discussion

In this section, the results of the study are presented and discussed in detail to address the objectives of the research. All data obtained from analysis of dry sample are presented on fresh weight basis.

4.1. Nutrient composition of raw and processed Anchote

Nutritional value is the main concern when a crop is considered as a food source. Anchote is endemic tuber crop used as a food source in parts of Western Ethiopia. The nutrient compositions of raw and processed Anchote tubers are presented in Table 4.1.

4.1.1. Moisture content

Moisture content determination is an integral part of the proximate composition analysis of food. The mean moisture content of the raw Anchote was 74.93 (g/100g), which is in agreement with the finding of EHNRI (1997) (74.50g/100g). In addition, this is in accordance with the finding of Fufa and Urga (1997) (73.00g/100g). The mean moisture content of Anchote tuber boiled after and before peeling had 81.74 (g/100g) and 76.73 (g/100g), respectively. The moisture content of Anchote boiled after peeling was significantly ($P<0.05$) higher than both boiled before peeling and raw Anchote tubers. Similarly, the mean moisture content of Anchote boiled before peeling was significantly ($P<0.05$) higher compared to mean raw Anchote. The moisture content was increased in boiled after peeling by 9.08% and in boiled before peeling 2.41% compared to raw tubers. The increased moisture content might be due to the water absorption capacity of fibers and other natural chemical components during heat treatment (Arias *et al.*, 2003).

4.1.2. Crude protein

The main functions of proteins are growth and replacement of lost tissues in the human body. It was observed that the mean raw Anchote tuber contain 3.25 g/100g of crude protein. The result in raw Anchote is in agreement with the finding of EHNRI (1997) (3.20 g/100g). Fufa and Urga (1997) reported the raw Anchote tuber contained 3.00 g/100g crude protein. The mean crude protein content of Anchote tuber boiled after and before peeling of Anchote tuber were 2.67 g/100g and 3.14 g/100g, respectively. The mean crude protein content of Anchote boiled after peeling was significantly ($P<0.05$) lower than both boiled before peeling and raw Anchote tubers. Neverthless, the mean crude protein content of Anchote boiled before peeling was non

significant (P>0.05) compared to mean raw Anchote. The crude protein content was decreased in boiled after peeling by 17.85% and in boiled before peeling by 3.38% compared to raw tubers. Such a reduction might have been due to protein denaturation during boiling. Consistent with this, Ekanayake *et al.*, (2000) stated that the reduction of crude protein during boiling may be attributed to leaching and denaturation of protein caused by boiling.

Table: 4.1. Mean (± SE) nutrient composition of raw and processed Anchote samples

Treatment	Moisture Content (g/100g)	Crude Protein (g/100g)	Total Ash (g/100g)	Crude Fiber (g/100g)	Crude Fat (g/100g)	Utilizable (g/100g)	Gross Energy (Kcal/100g)
RW	74.93 ± 0.345^{c}	3.25 ± 0.061^{a}	2.19 ± 0.014^{a}	2.58 ± 0.048^{b}	0.19 ± 0.020^{a}	16.86 ± 0.410^{a}	82.12 ± 1.300^{a}
BAP	81.74 ± 0.395^{a}	2.67 ± 0.145^{b}	1.33 ± 0.406^{b}	3.71 ± 0.135^{a}	0.13 ± 0.017^{b}	10.42 ± 0.310^{c}	53.48 ± 1.340^{c}
BBP	76.73 ± 0.465^{b}	3.14 ± 0.187^{a}	1.99 ± 0.168^{a}	2.77 ± 0.216^{b}	0.14 ± 0.010^{b}	15.23 ± 0.410^{b}	75.26 ± 2.390^{b}

Means not followed by the same superscript letters in the same column are significantly different (P<0.05).

NB. RW stands for Raw Anchote, BAP: for Boiled After Peeling and BBP: for Boiled before Peeling.

4.1.3. Total ash

The mean total ash content of raw, boiled after peeling and boiled before peeling were 2.19g/100g, 1.33g/100g and 1.99g/100g, respectively. The mean total ash content boiled after peeling was significantly (p<0.05) lower than both boiled before peeling and raw Anchote tubers. Total ash content is directily proportional with inorganic element content of Anchote. Hence the samples with high percentagesash contents are expected to have hogh concentrations of various mineral elements, which are advantage to speed up metabolic processes and improve growth and development (Bello *et al.*, 2008). The mean total ash content of raw Anchote was

comparable to the finding of Fufa and Urga (1997) (2.00g/100g). However, EHNRI (1997) reported a lesser value, which was 1.10g/100g. The slight differences in the total ash content might be related to the soil types, stage of maturity, and agronomic practices (Woolfe, 1987). In reference with the raw tubers, the total ash content of Anchote boiled after and before peeling decreased by 39.27% and 9.13%, respectively. The reduction of total ash may be due to leaching of the mineral compound and water absorption during boiling (Lewu *et al.*, 2009).

4.1.4. Crude fibre

The food fibres are defined as the sum of non starchy polysaccharides (cellulose, hemicelluloses, and pectic substances) and lignins, which are mainly components of plant cell walls. The mean crude fibre content of raw Anchote was 2.58 g/100g. The finding of Fufa and Urga (1997) and EHNRI (1997) in crude fiber content of raw Anchote is relatively lower values, which is 0.60 g/100g and 0.70 g/100g, respectively. These variations were probably due to extent time of storage and variations in the soils (Debre and Brindza, 1996).The mean crude fibre contents of boiled after and boiled before peeling of Anchote were 3.71 g/100g and 2.77 g/100g, respectively. The mean crude fiber content of Anchote boiled after peeling was significantly ($P<0.05$) higher than both boiled before peeling and raw Anchote tubers. The mean crude fiber content of Anchote boiled before peeling was non significant ($P>0.05$) compared to mean raw Anchote. Taking a raw Anchote tuber as a reference, the effect of traditional processing methods increased the crude fibre content by 43.79% and 7.36% in Anchote boiled after, and before peeling, respectively. These increases could be due to the fact that as samples were subjected to the boiling, and thus all soluble components might have lost in the process thereby increasing the crude fibre contents (Ahmed *et al*, 2010). Fibres exhibit beneficial physiological effects to human body, as they stimulat and accelerat intestinal contraction and transit, and increas feces volume (Ahmed *et al*, 2010). Therefore, the high levels of crude fiber observed in the boiled after and before peeling of Anchote could be an advantage of traditional processing as it might help in the treatment of diseases such as obesity, diabetes, cancer and gastrointestinal disorders (Saldanha, 1995) and in digestion and prevention of colon cancer (UICC/WHO, 2005).

4.1.5. Crude fat

Anchote is low crude fat content. The mean crude fat content of the raw Anchote was 0.19 g/100g, which is similar with the finding of Fufa and Urga (1997) (0.17g/100g) and EHNRI (1997) (0.1g/100g). The mean crude fat contents of boiled after peeling and boiled before peeling of Anchote tuber were 0.13 g/100g and 0.14 g/100g, respectively. The mean crude fat content of Anchote boiled after peeling and boiled before peeling was significantly ($P<0.05$) lower than raw Anchote tubers. The mean crude fat content of Anchote boiled before peeling was non significant ($P>0.05$) compared to mean boiled after peeling Anchote tuber. The crude fat content was decreased in boiled after peeling by 31.58% and in boiled before peeling by 26.32% compared to raw tubers. These decreases might be attributed to their diffusion into the boiling water (Ahmed *et al*, 2010).

4.1.6. Utilized carbohydrate

Utilizable carbohydrate content was determined by difference. The mean utilizable carbohydrate content of the raw Anchote was 16.86 g/100g. The mean utilizable carbohydrate content of boiled after peeling and boiled before peeling of Anchote tuber were 10.42 g/100g and 15.23g/100g, respectively. The mean utilizable carbohydrate content of Anchote boiled after peeling was significantly ($P<0.05$) lower than the mean of both boiled before peeling and raw Anchote tuber. Similarly, the mean utilizable carbohydrate content of Anchote boiled before peeling was significantly ($P<0.05$) lower compared to the mean of raw Anchote tuber. The result in raw Anchote was lower than the finding of Fufa and Urga (1997) (22.5 g/100g) and EHNRI (1997) (21.1 g/100g).The utilizable carbohydrate content was decreased in boiled after peeling by 38.19% and in boiled before peeling by 9.67% compared to raw tubers. Reduction in utilizable carbohydrate content during boiling might be due to leaching of soluble carbohydrates like sugers in to the cooking water (Esenwah and Ikenebomeh, 2008).

4.1.7. Gross energy

The gross energy was calculated by multiplying the mean values of crude proteins, crude fat and total carbohydrate by Atwater factors of 4, 9 and 4, respectively. The mean gross energy content of raw Anchote was 82.12 Kcal/100g. The mean gross energy contents of boiled after peeling and boiled before peeling of Anchote tuber were 53.48 Kcal/100g and 75.26 Kcal/100g, respectively. The mean gross energy content of Anchote boiled after peeling was significantly

($P<0.05$) lower than the mean of both boiled before peeling and raw Anchote tuber. Similarly, the mean gross energy content of Anchote boiled before peeling was significantly ($P<0.05$) lower compared to the mean raw Anchote tuber. The value in raw Anchote were found to be relatively low as compared to those reported by EHNRI (1997) (98.10 Kcal/100g) and Fufa and Urga (1997) (103.5 Kcal/100g). In reference with raw tubers, the gross energy content of raw Anchote after and before peeling decreased by 26.06% and 7.19%, respectively.

4.2. Mineral content of raw and processed Anchote

Minerals in the diet are responsible for several existing problems relating to human health (Milton, 2003). The human body requires more than twenty-two mineral elements that can be supplied by an appropriate diet in varying amounts for proper growth, health maintenance, and general well-being (WHO/FAO, 1998). Deficiency diseases could be prevented by sufficient intake of specific nutrients/minerals that are involved in many biochemical processes. Root and tuber crops are one of important sources of minerals that are linked to prevent deficiency diseases such as Anemia and Rickets and daily consumption of these foods is being encouraged (Leterme, 2002). The mineral content of raw and processed Anchote is presented in Table 4.2.

4.2.1. Calcium

Calcium is the major component of bone and assists in teeth development. Calcium concentrations are also necessary for blood coagulation and for the integrity of intracellular cement substances (Okaka and Okaka, 2001). The calcium content of the raw Anchote was 119.5mg/100g. The result was comparable with the finding of EHNRI (1997) (119 mg/100g). However, Fufa and Urga (1997) reported Very high calcium contents (344 mg/100g). The mean calcium contents of boiled after peeling and boiled before peeling of Anchote tuber was 115.70 mg/100g and 118.20 mg/100g, respectively. The mean calcium content of Anchote boiled after peeling was significantly ($P<0.05$) lower than both boiled before peeling and raw Anchote tubers. The mean calcium content of Anchote boiled before peeling was non significant ($P>0.05$) compared to mean raw Anchote. The calcium content was decreased in boiled after peeling by 3.18% and in boiled before peeling by 1.65% compared to raw tubers. The loss of calcium from boiling is not as such pronounced and this little reduction may be due to less leaching of the calcium to the boiling water (Brody, 1994).

4.2.2. Iron

The mean iron content of the raw, boiled after peeling and boiled before peeling Anchote were 5.49 mg/100g, 7.60mg/100g and 6.60g/100g, respectively. The mean iron content of Anchote boiled after peeling was significantly ($P<0.05$) higher than both boiled before peeling and raw Anchote tubers. The mean iron content of Anchote boiled before peeling was non significant ($P>0.05$) compared to mean raw Anchote. The result in raw Anchote was comparable with the finding of Fufa and Urga (1997) (5.5mg/100g). However, EHNRI (1997) reported lower iron contents (1.30mg/100g). The iron content was increased in boiled after peeling by 38.43% and in boiled before peeling by 20.22% compared to raw tubers. Increase in the iron content may be due to contamination of iron from the cooking utensils known as Tuwe (Omoruyi *et al.*, 2007). In addition, the increment could be due to peeling has been done in a knife made of stainless steel then leaching from its skin or cooking utensils (Akin-Idowu *et al.*, 2009).

Table: 4.2. Mean (± SE) mineral content of raw and processed Anchote samples

Treatment	Calcium (mg/100g)	Iron (mg/100g)	Magnesium (mg/100g)	Zinc (mg/100g)	Phosphorus (mg/100g)
RW	119.50±0.36^{a}	5.49±0.39^{a}	79.73±0.85^{a}	2.23±0.12^{a}	34.61±0.70^{a}
BAP	115.70±0.21^{b}	7.60±0.19^{b}	73.50±0.92^{c}	2.03±0.06^{b}	28.12±0.08^{b}
BBP	118.20±1.49^{a}	6.60±0.32^{a}	76.47±0.61^{b}	2.20±0.10^{ab}	25.45±0.25^{c}

Means not followed by the same superscript letters in the same column are significantly different ($P<0.05$).

NB. RW stands for Raw Anchote, BAP: for Boiled after peeling and BBP: for Boiled before peeling.

4.2.3. Magnesium

The mean magnesium content of the raw, boiled after peeling and boiled before peeling of Anchote tubers were 79.83 mg/100g, 73.50 mg/100g and 76.47 mg/100g, respectively. The magnisium content of Anchote boiled after peeling was significantly ($P<0.05$) lower than both boiled before peeling and raw Anchote tubers. Similarly, the mean magnisium content of Anchote boiled before peeling was significantly ($P<0.05$) different compared to mean raw Anchote. The mean value in raw Anchote was agreed with the finding of Fufa and Urga (1997) (80 mg/100g). The magnesium content was reduced in boiled after peeling by 7.93% and in boiled before peeling by 4.21% compared to raw tubers. The reduction of magnesium from

boiling might be due to magnesium oxalate is less soluble than the potassium and sodium salts (Poeydomenge *et al.*, 2007), this may be the possible reason to observed reduction in magnesium level upon boiling.

4.2.4. Zinc

The zinc content of raw Anchote tuber with a mean value was 2.23 mg/100g, which is inaccordance whith the finding of Fufa and Urga (1997) 1.8 mg/100gm. The mean zinc content of boiled after peeling and boiled before peeling of Anchote tuber was 2.03 mg/100g and 2.20 mg/100g, respectively. The zinc content of Anchote boiled after peeling was significantly ($P<0.05$) lower than raw Anchote tubers. The zinc content of Anchote boiled before peeling was non significant ($P>0.05$) compared to both mean boiled after peeling and raw Anchote. The mean zinc content was reduced in boiled after peeling by 8.97% and in boiled before peeling by 1.35% compared to raw tubers.

4.2.5. Phosphorus

The phosphorus content of the raw Anchote was 34.61 mg/100gm. The phosphorus content of boiled after peeling and boiled before peeling of Anchote tuber was 28.12 mg/100g and 25.45 mg/100g, respectively. The phosphorus content of Anchote boiled before peeling was significantly ($P<0.05$) lower than both boiled after peeling and raw Anchote tubers. In the same way, the mean phosphorus content of Anchote boiled after peeling was significantly ($P<0.05$) different compared to mean raw Anchote tubers. The mean phosphorus content was reduced in boiled after peeling by 18.75% and in boiled before peeling by 26.47% compared to raw tubers. The losses of phosphors content in tuber due to leaching on boiling might occur up to 25% (True *et al.*, 1979), this may be the possible reason to observed reduction in magnesium level in this study.

4.3. Anti-nutritional factors content of raw and processed Anchote

Anti-nutrients are known to reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals (Ugwu and Oranye, 2006). So that, the levels of anti-nutritional factors in the Anchote tubers are important in the assessment of its nutritional status. Some anti-nutritional factors (phytate, oxalate, tannin and cyanide) content of the raw and processed Anchote tuber is shown in Table 4.3.

4.3.1. Phytate

The raw Anchote tuber contained 389.30 mg/100g phytate.The phytate content of Anchote boiled after peeling and before peeling had 333.63 mg/100g and 334.74 mg/100g, respectively. The phytate content of Anchote boiled after peeling was significantly ($P<0.05$) lower than both boiled before peeling and raw Anchote tubers. Similarly, the mean phytate content of Anchote boiled before peeling was significantly ($P<0.05$) lower than raw Anchote tuber. The mean phytate content was reduced in boiled after peeling by 14.30% and in boiled before peeling by 14.01% compared to raw tubers. The evident reduction in phytate during cooking may be caused by leaching into the cooking medium, degeneration by heat or the formation of insoluble complexes between phytate and other components, such as phytate-protein and phytate-protein-mineral complexes (Sidhtraju and Becker, 2001). The reduction of phytate during processing Anchote tuber is expected to enhance the bioavailability of proteins and dietary minerals of the tubers and at the same time the lower level of phytate may have some health promotional activities. Currently there is evidence that dietary phytate at low level may have beneficial role as an antioxidant, anticarcinogens and likely play an important role in controlling hypercholesterolemia and atherosclerosis (Phillippy *et al.*, 2004). Because Anchote may provide a substantial portion of phytate, the nutritional consequences of phytate in Anchote should be investigated.

4.3.2. Oxalate

The raw Anchote tuber contained 8.26 mg/100g oxalate. The oxalate content of boiled after peeling and boiled before peeling of Anchote tuber had 4.23 mg/100g and 4.66 mg/100g, respectively. The oxalate content of Anchote boiled after peeling was significantly ($P<0.05$) lower than both boiled before peeling and raw Anchote tubers. Also the mean oxalate content of Anchote boiled before peeling was significantly ($P<0.05$) lower than raw Anchote tuber. The mean oxalate content was reduced in boiled after peeling by 48.79% and in boiled before peeling by 43.58% compared to raw Anchote tubers. The traditional processing methods were found effective methods to reduce the oxalate content in these tubers. Boiling may cause considerable cell rupture and facilitate the leakage of soluble oxalate into cooking water (Albihn and Savage, 2001), this may be the possible reason to observed high reduction in oxalate level upon boiling.

Table: 4.3. Mean (± SE) anti-nutritional factors content of raw and processed Anchote

Treatment	Phytate (mg/100g)	Oxalate (mg/100g)	Tannin (mg/100g)	Cyanide (mg/100g)
RW	389.30±0.39[a]	8.23±0.09[a]	173.55±0.35[a]	12.67±0.22[a]
BAP	333.63±0.29[c]	4.23±0.02[c]	102.36±0.46[c]	8.16±0.07[c]
BBP	334.74±0.42[b]	4.66±0.17[b]	121.21±0.11[b]	11.14±0.17[b]

Means not followed by the same superscript letters in the same column are significantly different (P<0.05).

NB. RW stands for Raw Anchote, BAP: for Boiled after peeling and BBP: for Boiled before peeling.

Oxalates can have a harmful effect on human nutrition and health, especially by reducing calcium absorption and aiding the formation of kidney stones (Noonan and Savage, 1999). High-oxalate diets can increase the risk of renal calcium oxalate formation in certain groups of people (Libert and Franceschi, 1987). The majority of urinary stones formed in humans are calcium oxalate stones (Hodgkinson, 1977). Currently, patients are advised to limit their intake of foods with a total intake of oxalate not exceeding 50–60 mg per day (Massey *et al.*, 2001). The traditionally processed Anchote tubers analyzed in this study are low compared to the recommendations for patients with calcium oxalate kidney stones. Under these guidelines, processed Anchote tubers analyzed could be recommended not only for normal healthy people but also consumption for patients with a history of calcium oxalate kidney stones, assume about 1 kg of Anchote would be necessary for consumption per day. Therefore, the reduced oxalate content resulting from traditionally processed Anchote tubers could have a positive impact on the health of consumers to enhance the bioavailability of essential dietary minerals of the tubers, as well as reduce the risk of kidney stones occurring among consumers. Hence, boiling the tuber would reduce the nutritional problems that the high levels of oxalates could cause.

4.3.3. Tannin

The tannin content of raw Anchote tuber was 173.55 mg/100g. The tannin content of boiled after peeling and boiled before peeling of Anchote tuber had 102.36 mg/100g and 121.21 mg/100g, respectively. The tannin content of Anchote boiled after peeling was significantly (P<0.05) lower than both boiled before peeling and raw Anchote tubers. Similarly, the mean tannin content of Anchote boiled before peeling was significantly (P<0.05) lower than raw Anchote tubers. The

mean tannin content was reduced in boiled after peeling by 41.87% and in boiled before peeling by 30.12% compared to raw tubers. The decrease in the levels of tannin during heat treatment might be due to thermal degradation and denaturation of the antinutrients as well as the formation of insoluble complexes (Kataria *et al.*, 1989), this may be the possible reason observed in this study. Tannin content of most food is usually reduced by processing and this has been reported to enhance the bioavailability of iron. The toxicity effects of the tannin may not be significant since the total acceptable tannic acid daily intake for a man is 560 mg (Anonymous, 1973). Since the tannin content of raw Anchote tuber is very low compared to its critical toxicity effect and further reduced during traditional processing, its antinutritional effect may be insignificant in both raw and processed tuber.

4.3.4. Cyanide

Cyanide, either in synthetic inorganic forms as in KCN or NaCN, or organic forms as in cyanogenic glucosides, is a potent specific inhibitor of several enzyme-catalyzed processes (Aletor, 1993). The results of the present study showed that cyanide in raw, boiled after peeling and boiled before peeling Anchote tuber were 12.67 mg/100g, 8.16 mg/100g, and 11.14 mg/100g, respectively. The cyanide content of Anchote boiled after peeling was significantly ($P<0.05$) lower than both boiled before peeling and raw Anchote tubers. The mean cyanide content of Anchote boiled before peeling was also significantly ($P<0.05$) lower compared to mean raw Anchote tuber. The mean cyanide content was reduced in boiled after peeling by 35.59% and in boiled before peeling by 12.08% compared to raw tubers. It has been reported that higher intake of cyanides could result in the development of neurological disease in humans (Montgomery, 1980). The amounts of cyanide produced, only plants that accumulate more than 50 to 200 mg are considered to be dangerous (Kingsbury, 1964). However, smaller amount of cyanides could have several long-term adverse effects on human health (Bhandari and Kawabata, 2004). The results obtained showed that the processed tuber could be considered safe with regard to cyanide poisoning due to the fact that the cyanide levels were far below the detrimental levels of 50 to 200 mg (Kingsbury, 1964). However, the amount remaining cyanide content might be slightly toxic to people who consume high quantities of Anchote tubers and need to be further study.

4.4. Phytate phosphorus and non-phytate phosphorus

The phytate phosphorus and non-phytate phosphorus content of raw and processed Anchote is shown in Table 4.4. The phytate phosphorus content of raw, boiled after peeling and boiled before peeling Anchote tubers were 109.71 mg/100g, 94.02 mg/100g, and 94.22 mg/100g, respectively. The phytate phosphorus content of Anchote boiled after peeling and boiled before peeling was significantly ($P<0.05$) lower than raw Anchote tubers. The mean phytate phosphorus content of Anchote boiled before peeling was non significant ($P>0.05$) compared to mean boiled after peeling Anchote tuber. The mean phytate phosphorus content was reduced in boiled after peeling by 14.31% and in boiled before peeling by 14.12% compared to raw tubers.

Table: 4.4. Mean (± SE) phytate phosphorus and non-phytate phosphorus Contents of raw and processed Anchote samples

Treatment	[1]Phytate phosphorus (mg/100g)	[2]Non-phytate phosphorus(mg/100g)	[3]Proportion of phosphorous as phytate (%)
RW	109.71±0.12^{a}	75.10±0.82^{a}	3.17±0.07^{c}
BAP	94.02±0.08^{b}	65.89±0.01^{c}	3.34±0.01^{b}
BBP	94.22±0.29^{b}	68.77±0.09^{b}	3.71±0.03^{a}

Means not followed by the same superscript letters in the same column are significantly different ($P<0.05$).

[1] Phytate phosphorus was calculated by phytate times 28.18%.

[2] Non-phytate phosphorus was the difference between phytate phosphorus and total phosphorus.

[3]Proportion of phosphorous as phytate was calculated by phytate phosphorus devided by total phosphorus.

NB. RW stands for Raw Anchote, BAP: for Boiled after peeling and BBP: for Boiled before peeling.

The non phytate phosphorus content of raw, boiled after peeling and boiled before peeling Anchote were 75.10 mg/100g, 65.89 mg/100g, and 68.77 mg/100g, respectively. The mean non phytate phosphorus content of Anchote boiled after peeling was significantly ($P<0.05$) lower than both boiled before peeling and raw Anchote tubers. Similarly, the mean non phytate phosphorus content of Anchote boiled before peeling was significantly ($P<0.05$) lower compared to mean raw Anchote tubers. The proportion of phosphros as phytate content of raw, boiled after peeling and boiled before peeling Anchote tubers were 3.17%, 3.34%, and 3.71%, respectively. The percentage of phytate phosphorus to total phosphorus is very important since the phytate

phosphorus cannot be utilized by human beings. The effect of phytate on phosphrus absorption in the presence of high phytate intakes has led to the suggestion that the proportion of phosphorus as phytate may be a better index of phosphrus bioavailability (Melaku *et al.*, 2005), in which the diets with proportion of phosphorus as phytate (%) ≤ 50 % in foods from roots and tubers are regarded as being adequate in bioavailable phosphate. The values in this study were lower than the reported critical proportion of phosphors as phytate, which implies the Anchote tubers are adequately bioavailable the phosphors element. Therefore, consumptions of Anchote tuber may help to ameliorate prevalent mineral deficiencies caused by their limited bioavailability and may lead to better mineral status of phosphorous.

4.5. Molar ratios of Ca: Phy, Ox: Ca, Phy: Zn, Phy: Fe and [Ca] [Phy]/[Zn]

The molar ratios for oxalate, calcium, zinc, Iron and phytate were calculated to evaluate the effects of elevated levels of oxalate and phytate in the bioavailability of dietary minerals. Bioavailability is the ability of the body to digest and absorb the mineral in the food consumed. The calculated values are also compared with the reported critical toxicity values for these ratios. The calculated Ca: Phy, Ox: Ca, Phy: Zn, Phy: Fe and [Ca] [Phy]/ [Zn] molar ratios of raw and processed Anchote is shown in Table 4.5.

4.5.1. [Calcium]/ [Phytate] molar ratios

The molar ratios of Ca:Phy in raw, boiled after peeled and boiled before peeled Anchote were 5.05, 5.71, and 5.78, respectively. The mean Ca:Phy molar ratio of Anchote boiled before peeling and boiled after peeling was significantly ($P<0.05$) higher than raw Anchote tubers. The mean Ca:Phy molar ratio of Anchote boiled after peeling was significantly ($P<0.05$) lower than boiled before peeling Anchote tubers. Phytic acids markedly decrease Ca bioavailability and the Ca:Phy molar ratio has been proposed as an indicator of Ca bioavailability. The Ca: Phy molar ratios >6, indicative of poor calcium bioavailability (Oladimeji *et al.*, 2000). The values in the present study were lower than the reported critical molar ratio of Ca:Phy, indicating that absorption of calcium not adversely affected by phytate in these tubers.

4.5.2. [Oxalate]/ [Ca] molar ratios

The molar ratios of Ox:Ca obtained in raw, boiled after peeled and boiled before peeled Anchote were 0.03, 0.02, and 0.02, respectively. The Ox:Ca molar ratios of Anchote boiled after peeling and boiled before peeling was significantly ($P<0.05$) lower than raw Anchote tubers. The Ox:Ca

molar ratios of Anchote boiled before peeling was non significant (P>0.05) compared to mean boiled after peeling Anchote tuber. The importance of oxalate contents of an individual plant product in limiting total dietary Ca availability is of significance only when the ratio of Ox:Ca is greater than one (Frontela *et al.*, 2009). Under this circumstance, the oxalate has potential to complex, not only the Ca contained in the plant, but also that derived from other food sources (Davis, 1979). Consumption of oxalates may result in kidney disease and a high ratio of Ox:Ca in the diet also may cause chronic calcium deficiency (Hassan *et al.*, 2007). From the result, it was observed that, Anchote tubers had Ox:Ca values are lower than the reported critical value (1.0), which implies that a low level of oxalate could have no adverse effects on bioavailability of dietary calcium in these tubers.

Table: 4.5. Mean (± SE) calculated Ca:Phy, Ox:Ca, Phy:Zn, Phy:Fe and [Ca][Phy]/[Zn] molar ratios of raw and processed Anchote samples.

Treatment	Ca:Phy (molar ratio)[1]	Ox:Ca (molar ratio)[2]	Phy:Zn (molar ratio)[3]	Phy:Fe (molar ratio)[4]	[Ca][phy]/[Zn] (mol/kg)[5]
RW	5.05±0.02^{c}	0.03±0.001^{a}	17.35±0.91^{a}	5.58±0.75^{a}	0.52±0.02^{a}
BAP	5.66±0.01^{b}	0.02±0.001^{b}	14.82±0.54^{b}	3.92±0.19^{b}	0.47±0.01^{b}
BBP	5.78±0.07^{a}	0.02±0.001^{b}	14.83±0.23^{b}	4.28±0.15^{b}	0.44±0.02^{b}

Means not followed by the same superscript letters in the same column are significantly different (P<0.05)

[1] mg of Calcium/molecular weight of Calcium: mg of phytate/molecular weight of phytate.

[2]mg of oxalate/molecular weight of oxalate: mg of calcium /molecular weight of calcium.

[3]mg of phytate/molecular weight of phytate: mg of zink/molecular weight of zink.

[4]mg of phytate/molecular weight of phytate: mg of iron/molecular weight of iron.

[5](mg of Calcium/molecular weight of Calcium) (mg of phytate/molecular weight of phytate)/ (mg of zink/molecular weight of zink) devided by 100.

NB. RW stands for Raw Anchote, BAP: for Boiled after peeling and BBP: for Boiled before peeling.

4.5.3. [Phytate]/ [Zinc] molar ratios

The Phy:Zn molar ratios of Raw, boiled after peeled and boiled before peeled Anchote were 17.35, 14.86 and 14.98, respectively. The Phy:Zn molar ratios of Anchote boiled after peeling and boiled before peeling was significantly (P<0.05) lower than raw Anchote tubers. The Phy:Zn molar ratios of Anchote boiled before peeling was non significant (P>0.05) compared to mean

boiled after peeling Anchote tuber. The importance of foodstuffs as a source of dietary zinc depends on both the total zinc content and the level of other constituents in the diet that affect zinc bioavailability. Phytate may reduce the bioavailabity of dietary zinc by forming insoluble mineral chelates at a physiological pH (Oberleas, 1983). Zinc deficiency has been shown to be the cause of dwarfism and hypogonadism among adolescents (Prasad, 1984). Zinc has been described as the essential mineral most adversely affected by phytate and the phytate-to-zinc molar ratio has been proposed as an indicator of zinc bioavailability (Sirkka, 1997). Phytate: zinc molar ratios >15, indicative of poor zinc bioavailability (Morris and Ellis, 1989). The values of boiled after peeling and boiled before peeling Anchote were lower than the critical molar ratios of Phy:Zn, which indicates the availability of zinc good due to traditional processed Anchote tuber.

4.5.4. [Phytate]/[Iron] molar ratios

The phytate:iron molar ratios of raw, boiled after peeling and boiled before peeling Anchote tuber was 5.58, 3.92 and 4.28, respectively. The phytate:iron molar ratios of Anchote boiled after peeling and boiled before peeling was significantly ($P<0.05$) lower than raw Anchote tubers. The phytate:iron molar ratios of Anchote boiled before peeling was non significant ($P>0.05$) compared to mean boiled after peeling Anchote tuber. Phytate begins to lose its inhibitory effect on iron absorption when phytate:iron molar ratios are less than 1.0, although even ratios as low as 0.2 exert some negative effect (Hurrell *et al.*, 2003). The phytate:iron molar ratios greater than 0.15 regarded as indicative of poor iron bioavailability (Siegenberg *et al.*, 1991). This result indicated that, the phytate:iron molar ratios raw and processed tubers are greater than the critical value, which implies the absorption of iron from raw and processed Anchote inhibited by phytate and as a result the bioavailability of iron is poor.

4.5.5. [Calcium][Phytate]/[Zinc] molar ratios

The values of [Ca][phytate]/[Zn] millimolar ratio for raw, boiled after peeling and boiled before peeling Anchote had 0.52 mol/kg, 0.47 mol/kg, and 0.35 mol/kg, respectively. The [Ca][phytate]/[Zn] millimolar ratios of Anchote boiled after peeling and boiled before peeling was significantly ($P<0.05$) lower than raw Anchote tubers. The [Ca][phytate]/[Zn] millimolar ratios of Anchote boiled before peeling was non significant ($P>0.05$) compared to mean boiled after peeling Anchote tuber. The potentiating effect of calcium on zinc absorption in the presence

of high phytate intakes has led to the suggestion that the [Phy][Ca]/[Zn] millimolar ratio may be a better index of zinc bioavailability than the [Phy]/[Zn] molar ratio alone (Obah and Amusan, 2009). High calcium levels in foods can promote the phytate-induced decrease in zinc bioavailability when the [Ca][phytate]/[Zn] millimolar ratio exceeds 0.5 mol/kg (Gibson, 1994). In this study, except the raw tuber, the values of [Ca][Phy]/ [Zn] millimolar ratios of both processed tubers were found less than the critical level. Therefore, traditional processing methods would appear that the possible contribution to increase zinc availability.

4.6. Nutritional and antinutritional factors of raw Anchote and other roots and tubers

The nutritional and anti-nutritional content of the raw Anchote, sweet potato, potato, yam, taro and cassava are shown in Table 4.6. Like other root and tuber crops, Anchote have high moisture content. It is evident from the data that the raw Anchote contains 2.5, 2.03, and 6.1 times higher crude protein compared to sweet potato, potato and cassava, respectively. Fufa and Urga (1997) reported whole Anchote contains 2 times higher than the values reported for potato and sweet potato, which is inaccordance with this study. These tubers could be regarded as potential source of proteins. Utilized carbohydrate in raw Anchote tuber was at least three times higher than the sweet potato, cassava, and potatos. The raw Anchote contains low crude fat which is similar to other root and tuber crops. This finding confirms the reports of Fufa and Urga (1997), that Anchote exhibits a low crude fat content. The crude fibre in Anchote was atleast 2, 6, 2 times higher than that in sweet potato, potato and cassava, respectively. Raw Anchote tubers have higher average energy content (389.3 Kcal/100g) compared to sweet potato (136 Kcal/100g), and potato (97 Kcal/100g). Finally, with regard to the supply of nutrients, Anchote tubers may be considered a good source of crude protein, crude fiber, and carbohydrate content.

The total ash content in Anchote was at least two times higher than in above mentioned other root and tuber crops. This implies, Anchote is as a potential source of minerals than other mentioned crops. For instance, the Calcium content of Anchote is 2, 12, 3, 3, and 6 times higher than those of sweet potato, potato, yam, taro and cassava tubers, respectively. Fufa and Urga (1997) reported traditionally, it is believed that Anchote heals broken or fractured bones, helps sick people to recuperate and makes lactating mothers healthier and stronger, this may be due to the high calcium contents of the tuber.

Table: 4.6. Comparison of nutritional and antinutritional factors of raw Anchote and other recorded raw roots and tubers.

	Anchote	Sw. potato[1]	Potato[2]	Yam[3]	Taro[4]	Cassava[5]
Moisture (g/100g)	74.93	67.40	74.7	NF[6]	NF[6]	62.8
Cru. Pro (g/100g)	3.25	1.30	1.6	NF[6]	NF[6]	0.53
Uti. CHO (g/100g)	92.11	28.20	22.6	NF[6]	NF[6]	31.0
Cru. Fat (g/100g)	0.19	2.00	0.1	NF[6]	NF[6]	0.17
Tot. Ash (g/100g)	2.19	1.10	0.6	NF[6]	NF[6]	0.84
Cru. Fiber (g/100g)	2.58	1.10	0.4	NF[6]	NF[6]	1.48
Gro.Energy (Kcal/100g)	382.78	136.00	97	NF[6]	NF[6]	NF[6]
Ca (mg/100g)	119.5	52.00	10	41-53	41-45	20
Fe (mg/100g)	5.49	3.40	6.7	1.9-3.2	1.9-3.2	0.23
P (mg/100g)	34.61	34.00	40	NF[6]	NF[6]	46
Mg (mg/100g)	79.73	NF[6]	NF[6]	7.3-7.5	7.3-7.5	30
Zn (mg/100g)	2.23	NF[6]	NF[6]	42-49	42-49	NF[6]
Phy.(mg/100g)	389.3	NF[6]	NF[6]	27.8–62.2	94.1-106	NF[6]
Oxa. (mg/100g)	8.23	NF[6]	NF[6]	86.2 – 162	189-261	NF[6]
Tan.(mg/100g)	174.55	NF[6]	NF[6]	NF[6]	NF[6]	NF[6]
Cya. mg/100g)	12.67	NF[6]	NF[6]	NF[6]	NF[6]	NF[6]

[1]EHNRI, (1997); [2] USDA, (2002); [3,4] Esayas Ayele, (2009); [5]Bradbury and Holloway,(1988); [6]NF=Not Found.

The iron and magnisium are also higher than those of potato, sweet potato, yam, cassava and taro. This indicates that the Anchote tubers may be another cheap source of important minerals like Ca, Fe, and Mg. Other mineral like zinc and posphorus was also found in appreciable amounts, but were significantly lower than those present in other tubers.

It has been reported that several anti-nutritional factors are present in root and tuber crops (Bhandari and Kawabata, 2004). The levels of anti-nutritional factors of Anchote are important in the assessment of its nutritional status. In this investigation, like other root and tuber crops the Anchote tuber was found to contain phytates, oxalates, tannin, and cyanide. Raw Anchote tubers were shown to contain fairly low levels of oxalate (8.23 mg/100g) whereas, the phytate content (389.30 mg/100g) in the present study was found to be higher than the values reported in yam (27.8– 62.2mg/100g) and taro (94.1-106mg/100g).

4.7. Sensory preference of traditional processed Anchote

Anchote tubers boiled after peeling and boiled before peeling were presented to fifty consumers panels to preference taste. Among them 33 (66%) consumers preferred tubers boiled before peeling whereas 17 (34%) consumers preferred boiled after peeling Anchote (Figure 4.1). The results were evaluated according to statical t-test table of paired compareson test (Appendex XI), at $p<0.05$ level of significance; one sample must be selected at least 33 times out of fifty consumers to be a significantly different. The Anchote boiled before peeling was selected 33 times out of fifty consumers, which meets the critical value of the table to be a significantly difference. As a result, there is a significant ($P<0.05$) preference of taste of Anchote boiled before peeling to Anchote tubers boiled after peeling.

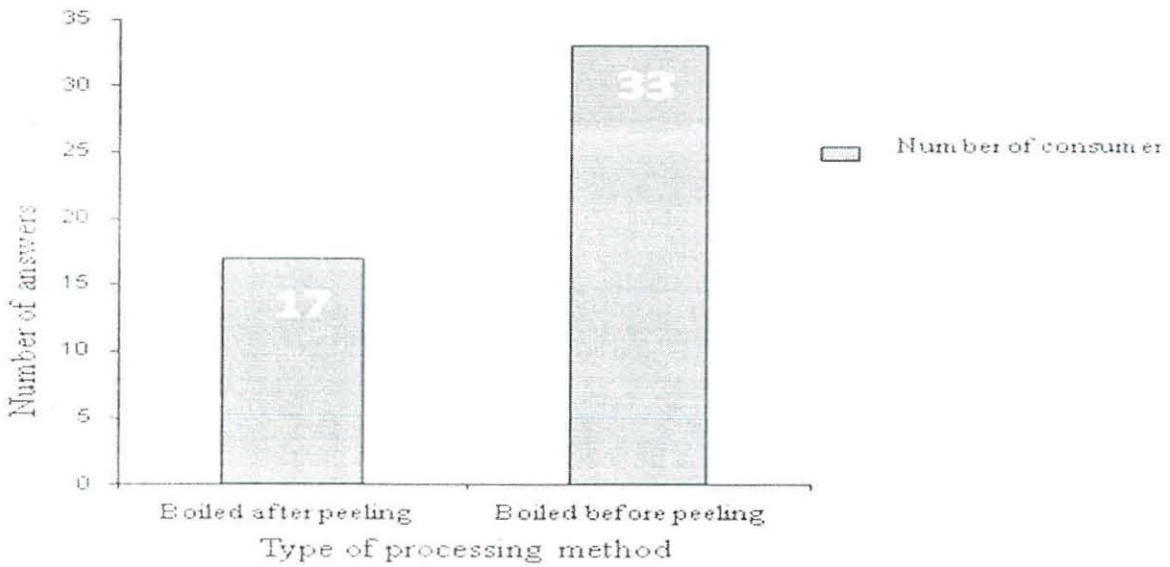

Figure: 4.1. Results of the consumer preference taste of processed Anchote.

Chapter 5

Conclusion and Recommendation

5.1. Conclusion

The present finding uncovered information on the nutritional composition (crude fiber, crude fat, crude protein, total ash, moisture content, utilized carbohydrate, gross energy, Zinc, Iron, Calcium, Sodium, Magnesium and Phosphorus) and antinutritional factors (Phytate, Oxalate, Tannin and Cyanide) of raw and processed Anchote tubers from western Ethiopia. Sensory preference taste of Anchote boiled after peeling and boiled before peeling was also reported. In addition, the relative bioavailability of the minerals was assessed by calculating molar ratios of antinutrient to the contained minerals.

The results of this study showed that raw Anchote contains appreciable quantity of carbohydrate, crude Protein, crude fiber, calcium, magnesium, iron and low levels of antinutrients (Oxalate, tannin, and cynide) except phytate, when compared to other reported raw roots and tubers.

As shown in this study the traditional processing methods of Anchote were very important because that increased in crude fibre content and improved the bioavailability of zinc contained in the Anchote tubers. This study also indicated that traditional processing methods decreased the crude protein, total ash, calcium, iron, zinc content of the tubers. Among the traditionl processing methods, boiled before peeling proved to be better in some nutrient and mineral contents considered in this investigation.

The levels of anti-nutritional factors in Anchote are important in the assessment of its nutritional status. In this study, the raw Anchote tubers were found to contain low antinutritional factors, except phytate. Moreover, there were further reductions of the antinutritional factors during traditional processing (Figure 5.1). This implies, except phytate which might hinder iron bioavailability, traditional processing enables that the antinutritional factors in the Anchote couldn't hamper its nutritional value. Therefore, both methods of traditional preparation of Anchote were effective to significantly reduce the levels of antinutritional factors, thereby improving the bioavailability of minerals such as zinc and calcium known to be affected by these anti-nutrients.

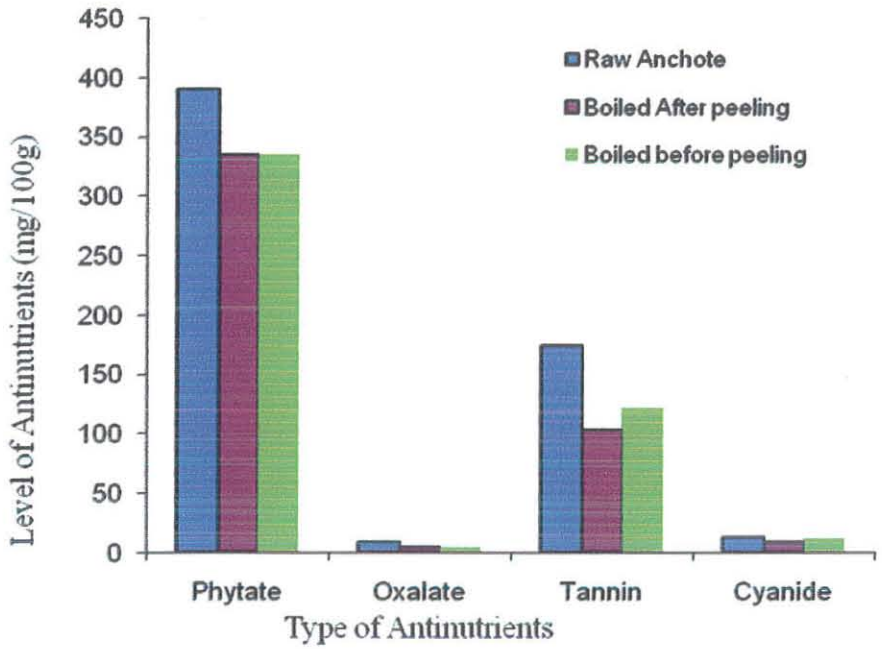

Figure: 5.1. Effect of processing treatments on antinutritional factors of Anchote tubers

This study also indicated that consumer panels preferred the taste of Anchote boiled before peeling. Therefore traditional processing method of Anchote boiled before peeling is also effective technique and need to be encouraged in terms of consumers preference of Anchote taste

5.2. Recommendation

The following recommendations are made based on the findings.

- Increased cultivation of Anchote need to be enouraged in all part of the western part of the country, because of its high nutrient contents like protein, crude fiber, Ca, Zn, Mg and low antinutritional factors.

- Both methods of food preparation were effective in reducing the levels of anti-nutritional factors and increase zinc bioavailability. However, boiling of Anchote before peeling is recommended for Anchote preparation in households and restaurants not only due to minimal processing losses in some nutrient compositions but also in terms of consumer preference taste.

- The iron content in the Anchote tuber increased during traditional processing. On the other hand, the absorption of iron from processed Anchote tubers were significantly inhibited by phytate. Consequently, the bioavailability of iron is presumed to be poor. Therefore, Anchote tubers should either be supplemented with other iron reach foods or fortified with iron elements to combat Iron deficiency.

- Future works need to focus on identifcation and quantification of other nutrients, and unaddressed antinutrients in Anchote tubers.

- Studies should be undertaken for new product development using Anchote tubers.

- Moreover, the effect of boiling periods, optimal boiling temperature, and storage times, soil types, varieties, and maturity time on the nutritional and antinutritional factors of Anchote should be studied.

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Appendices

Apendex I. The equipments and chemicals used for the analysis

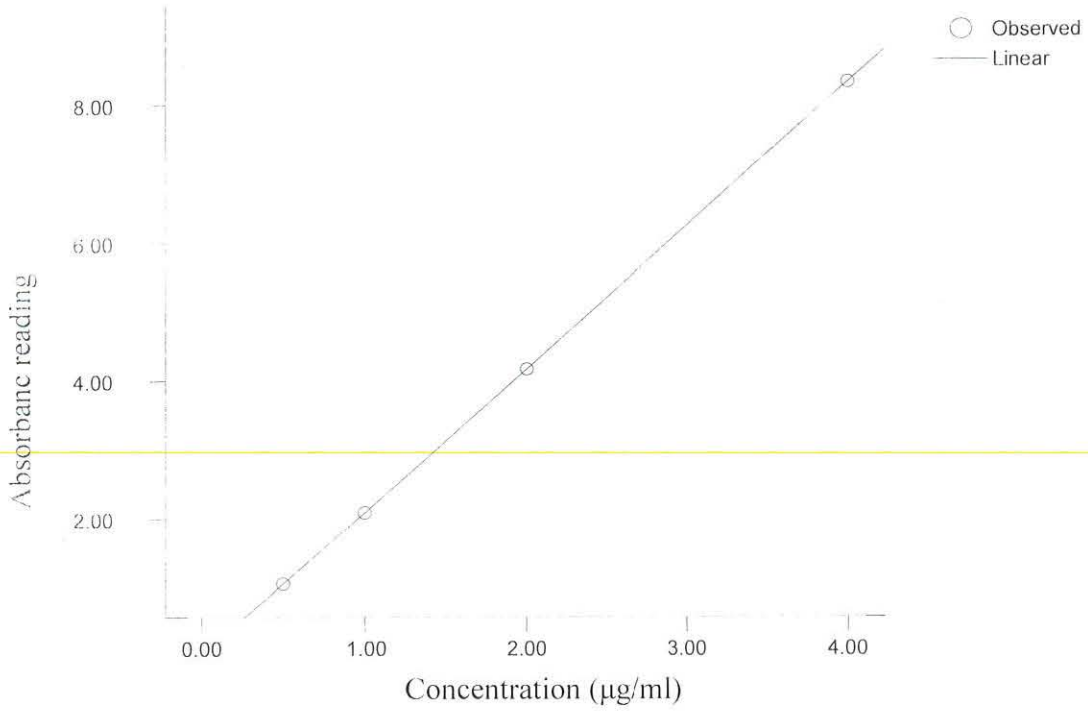
Ana.	Equipments and Analytical grade Chemicals used for the Analysis of Nutritional value, Minerals and Anti-nutritional factors contents of Raw and Processed Anchote tuber.	
Moi.	Equ.	Drying Oven (DHG- 9055A), Analytical Balance (ARZ 140, N315, SNR, USA), Desiccator (CSN-SIMAX), Tong, Aluminum Dishes.
Crude Protein	Equ.	Drying Oven (DHG- 9055A), Digester Stove (HYP-1008 eight holes), Analytical Balance (ARZ 140, N315, SNR, 1203290469, USA), Distiller (KDN-102F, nitrogen analyzer distillation device), Tong, Tecator Tube, Hood (NORDIA, LONDON E17 6AB), Crucibles, Desiccator (CSN-SIMAX), 25 ml, 100 ml, 250 ml Volumetric Flasks.
	Che.	Concentrated Sulfuric Acid (Uni-Chem. Sulfaric acid S867472-4N), 0.1N Hydrochloric Acid (37 % Riedel-Dehaen, sigma-ALDRICH chemicals Gmbh, Germany), 30% Hydrogen Peroxide (Uni-Chem. Hydrogen Peroxide H47055 4J), 40% Sodium Hydroxide (Uni-Chem. Sodium Hydroxide Pellets S41298-3I), Copper Sulfate (Uni-Chem. Cuppor Sulfatecryst, gran C53200-4I), Potassium Sulfate (L.R LABMAR Potassium Sulfate PVT. LTD), Boric Acid (Uni-Chem. Boric Acid B29890-4I), Distilled Water.
Total Ash	Equ.	Drying Oven (DHG- 9055A, Memment Germany), Muffle Furnace (Carbolite CSF 1200), Dessicator (CSN-SIMAX), Analytical Balance (LA 204, Measure tech.), Hot Plate (wadtech, UK Hotplate SH3), Hood (NORDIA, LONDON E17 6AB), Tong, Crucible.
	Che.	Concentrated Nitric Acid (about 69 % LR. Eurostar scientific LTD. Unit 113 century buildings summers road Liverpool, UK), De-ionized Water.
Crude Fiber	Equ	Muffle Furnace (Gallenkamp, size 3), 600ml Beaker, Drying Oven, Desiccator, Cruciable Whatman no. 1 filter paper, Hot Plate.
	Che.	1.25% and 1% Sulfuric Acid, 28% Potassium Hydroxide, 1% Sodium Hydro oxide, Acetone, Distilled Water

Crude Fat	Equ.	Analytical Balance (LA 204, Measure Tech.) Soxlet (SHANGHAI QIANJIAN INSTRUMENT CO., LTD), Thimbles (Whatman International LTD Maidstone England), Drying Oven (DHG-9055A), Desiccator (CSN-SIMAX), Boiling Chips (LABMARK Boiling Stone (India) PVT. LTD.), Extraction Flask, Cotton.
	Che	Diethyl Ether (Laboratory Reagent Diethyl Ether ISLAMPUR (F-14) 425 409).
Zn, Ca, Fe, Mg	Equ.	Muffle Furnace (carbolite Astonlane,hope, Sheffield S30 2RR England serial No 2/00/362 type OAF/1111), Hot Plate (wadtech, UK Hotplate SH3), Analytical Balance (item ARZ140 N315: Max. capacity 210 g C20379705, reliability 0.0001 g SNR 1203290469, ohaus corp. pineBrook, NS USA). Atomic Absorption Spectrometer (Varian Spectr AA. 20 plus), Hood (NORDIA), Crucibles, Desiccators, 50 ml, 100 ml, and 200 ml volumetric Flasks, Whatman No 1 filter paper.
	Che.	Hydrochloric Acid (37 % Riedel-Dehaen, sigma- ALDRICH chemicals Gmbh, Germany), Concentrated Nitric Acid (about 69 % LR. Eurostar scientific LTD. Unit 113 century buildings summers road Liverpool, UK), Lanthanum Chloride, De-ionized Water.
Phosphorus	Equ	Muffle Furnace (carbolite Astonlane,hope, Sheffield S30 2RR England serial No 2/00/362 type OAF/1111), Hot Plate (wadtech, UK Hotplate SH3), Analytical Balance (item ARZ140 N315: Max. capacity 210 g C20379705, reliability 0.0001 g SNR 1203290469, ohaus corp. pineBrook, NS USA), Atomic Absorption Spectrophotometer (Buck scientific AAS model 210VGP), Hood (NORDIA), Crucible, Desiccator, 50 ml, 100 ml, and 200 ml Volumetric Flasks and Whatman No. 1 filter paper
	Che.	Hydrochloric Acid, Conc. Nitric Acid, Aminonaphtholsulphonic Acid, Conc. Sulfuric Ammonium Molybdate, Sodium Bisulphite, Sodium Sulphite, Aminonaphtholsulphonic Acid, Potasium Biphoshhate, De-ionized Water.
Phytate	Equ.	Analytical Balance (ARZ140 N315: Max. capacity 210 gm C20379705, reliability 0.0001gm SNR 1203290469, ohaus corp. pine Brook, NSUSA), Test Tubes (screwed), Mechanical Shaker, 5 and 10mls Graduated Pipettes, Micropipettes, Vortex Mixer, Water Bath, UV- VIS Spectrophotometer (Beckman DU-64- spectrophotometer, USA), Spatula, Centrifuge (DYNAC II centrifuge, clay adams, division of Becton dikinson and company, made in USA).
	Che.	Hydrochloric Acid (37 % Riedel-Dehaen, sigma- ALDRICH chemicals Gmbh), Wade Reagent, Sulfosalisalic Acid, $FeCl_3.6H_2O$ (Iron (III) chloride cryst. Pure MERCK), Sodium Phytate Salt (phytic acid dodeca sodium salt hydrate water 10-15 % product of USA, ALDRICH), Fresh De-ionized Water.

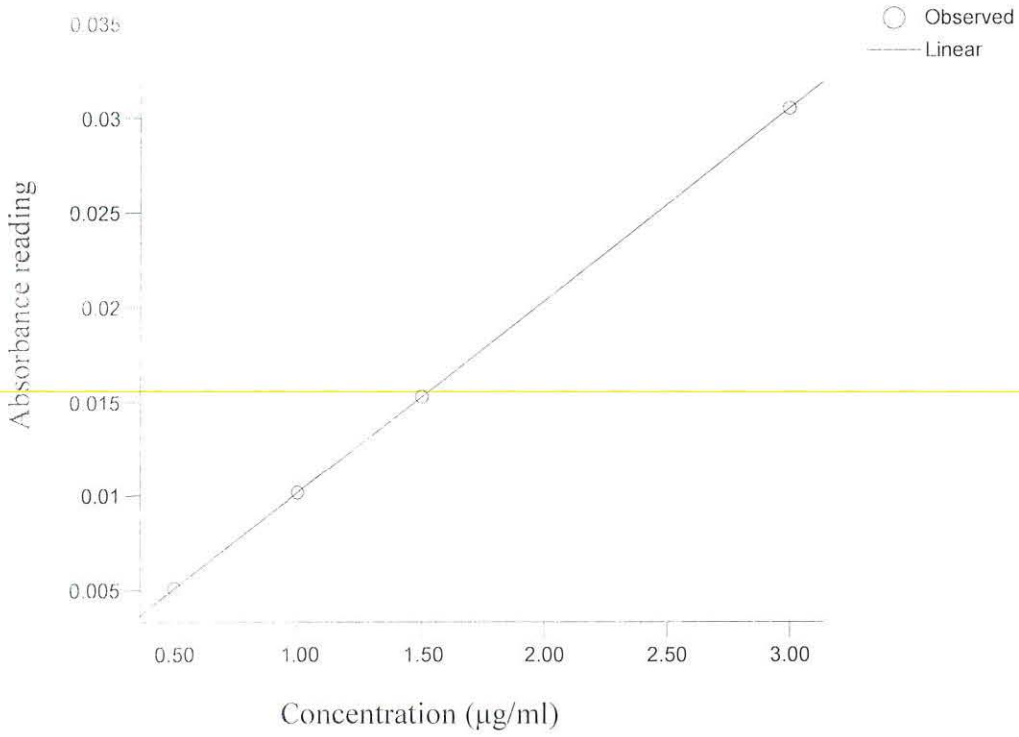
Tannin	Equ.	Analytical balance. Centrifuge, Mechanical Shaker. UV-Vis spectrometer. Tissue Paper, Test Tube. Spatula, Volumetric flask. Micropipette. Beaker. Measuring cylinder.
	Che.	Hydrochloric acid. Vanillin-HCl reagent. D-catechin. Methanol. vanillin.
Oxalate	Equ.	Pyrex 250 ml Volumetric flask with Stopper. Analytical balance, Graduated measuring Cylinders up to 200 ml , 250 ml Pyrex Beakers.Water Bath, Centrifuge, Titration Burette. Deseccator, Thermometer, Filter Paper. Spatula. Hotplate (Wagtech UK hot plate SH3).
	Che.	Methyl-red indicator 0.1 %, Concentrated Ammonia (about 33% w/w AR Euro star scientific LTD. Liverpool UK), $CaCl_2$. $2H_2O$ (calcium chloride -2- hydrate cryst. GR. MERCK). H_2SO_4 (RIEDEL-DEHAEN AG. SEELZE-AANNOVER, Germany), Potassium Permanganate (1.09935.titrisol, 3.161$KMnO_4$ for 1000 ml 0.02 mol/lit. Merck KGaA, 64271 Darmstadt, Germany), Fresh De-ionized Water.
Cyanide	Equu.	Analtical Balance, Kjeldahl Flask, Round Bottom Flask, Steam Distillation, Conical Flask, Sintered Glass Crucible.
	Che.	Silver Nitrate. Concentrated Nitric acid (about 69 % LR. Eurostar scientific LTD. Unit 113 century buildings summers road Liverpool, UK), Potassium Thiocyanate, Distilled Water.

Where: An. stands for type of Analysis; Mio. for Moisture contents; Equ. for Equipments; Che. for Chemicals.

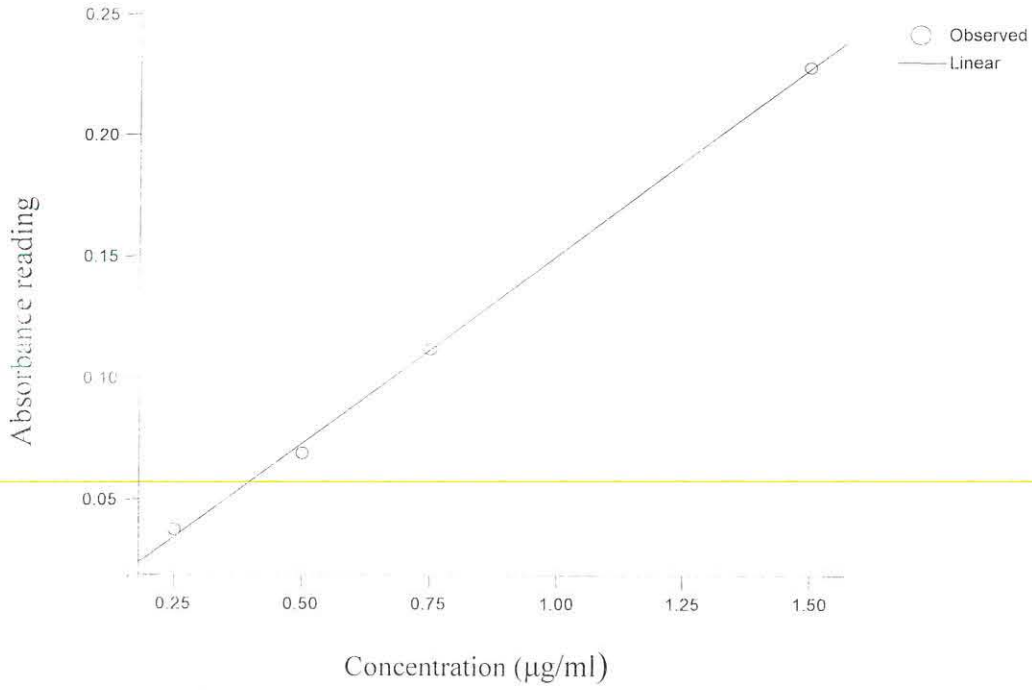
Appendex II. Absorbance reading of Calcium Standard curve (R^2=0.997)



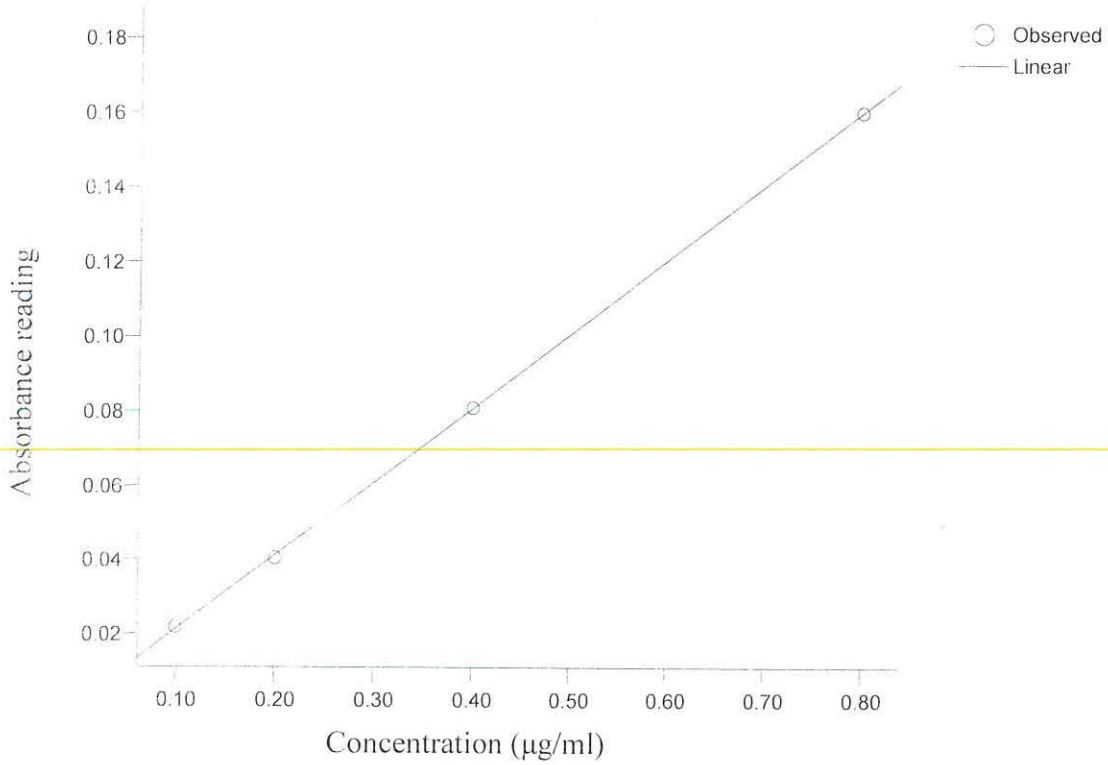
Appendex III. Absorbance reading of Iron Standard curve (R^2=0.999)



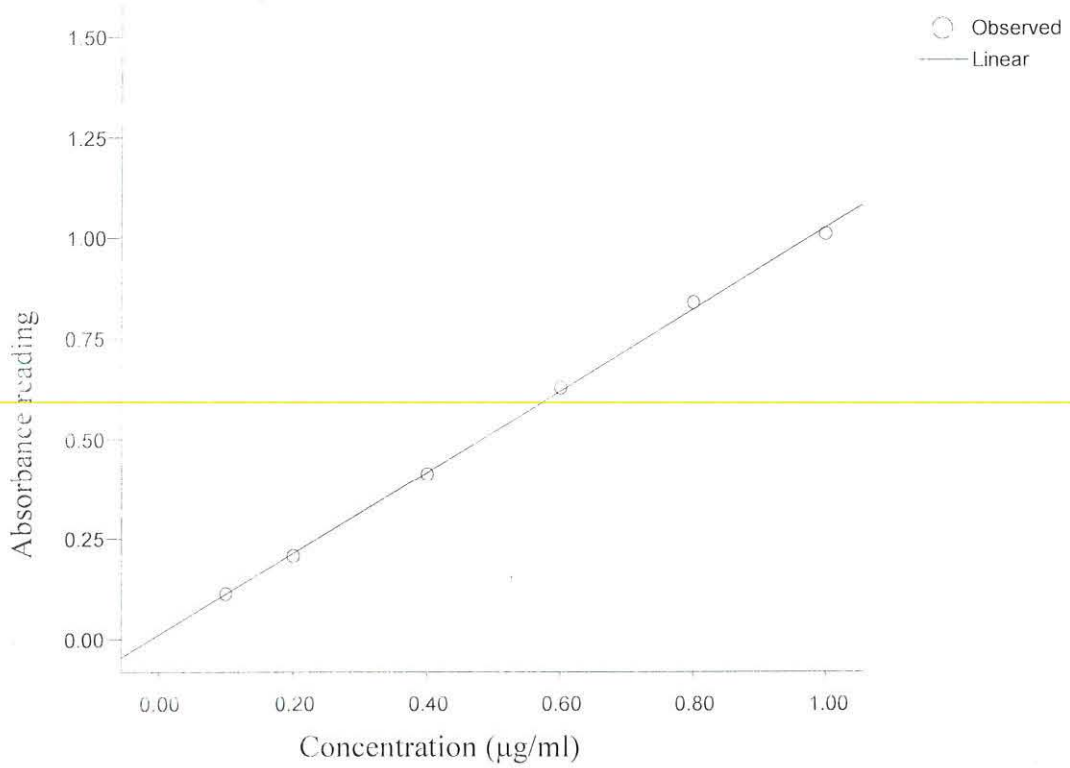
Appendex IV. Absorbance reading of Magnisium Standard curve (R^2=0.999)



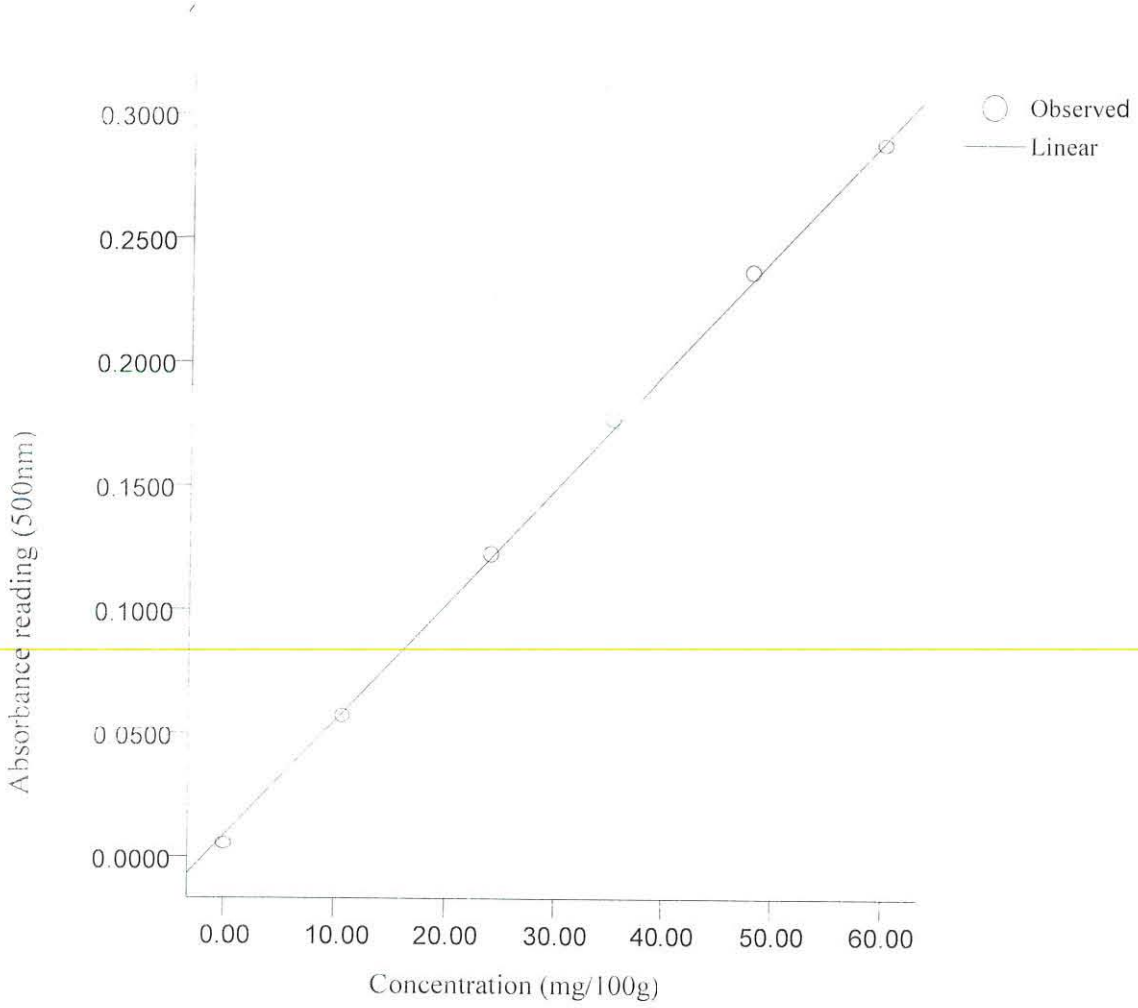
Appendix V. Absorbance reading of Zinc Standard curve (R^2=0.999)



Appendex VI. Absorbance reading of Phosphorus Standard curve (R^2=0.998)



Appendix VIII. Standard curve for the determination of Tannin concentration (R^2=0.999)



Appendix IX. Panelist questionnaire screener

Please check the appropriate answer for the following demographic information:

1. Sex _____male _____female

2. Age _____16-25 yrs, _____26-35yrs, _____> 35yrs.

3. Employment_____

Please answer the following questions. We want to know about you and what you think. Please ask if you have any questions!

4. Do you have any food allergies to Anchote? ____yes _____no

5. Do you smoke cigarette? ____yes _____no

6. Is this the first time you have ever seen or heard Anchote or its products? _____yes _____no

7. Have ever consumed Anchote products? _____yes _____no

8. If you consumed Anchote, which products did you consumed? Check all that apply:

_____ Boiled anchote after peeled without addition of ingredients

_____ Boiled Anchote before peeled without addition of ingridients

_____ Further cooked Anchote with additions of the ingridients

9. How often did you consumed boiled after peeling and/ or boiled before peeling Anchote without additions of ingridients?

_____ Occasionally

_____ At least once per month

_____ At least 2-3 times per month

At least once per week

_____ Two to three times per week

_____ Four or more times per week

Declaration

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other University, and that all sources of materials used for the thesis have been duly acknowledged.

Name: Habtamu Fekadu

Signature: _____

Place: Addis Ababa, Ethiopia

Date of submission: _____

This thesis has been submitted for examination with my approval as a supervisor.

Name: Professor Fekadu Beyene

Signature: _____

Date: _____

Name: Dr. Gullelat Dessie

Signature: _____

Date: _____