

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES



**Investigation of Nutrient and Anti-nutrient Composition of
Dioscorea cayenensis-D.rotundata complex**

**By
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March 2011

Addis Ababa

**Investigation of Nutrient and Anti-nutrient Compositions of
Dioscorea cayenensis-D.rotundata complex**

**Research Paper 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 Biochemistry**

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ADDIS ABABA UNIVERSITY
FACULTY OF MEDICINE
DEPARTMENT OF BIOCHEMISTRY

The undersigned hereby certify that they have read and recommend to the Faculty of Medicine for acceptance a thesis entitled “Investigation of the nutrient and anti-nutrient composition of *Dioscorea cayenensis*-*Dioscorea rotundata* complex” by Winner Kucha in partial fulfillment of the requirements for the degree of Master of Science in Biochemistry.

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Declaration

This thesis is my original work and has not been presented for a degree in any other University, and that all sources of material used for the thesis have been acknowledged.

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LIST OF ABBREVIATIONS

AOAC = Association of Official Analytical Chemists

CF = crude fiber

CP = crude protein

DM = dry matter,

DW = Dry Weight

EE = ether extract

EHNRI = Ethiopian Health and Nutrition Research Institute

FAO = Food and Agriculture Organization

FW = Fresh Weight

ha = hectare

Kcal = kilo calori

WHO = World Health Organisation

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ABSTRACT

TITLE: Nutritional Evaluation of *Dioscorea cayenensis*-*D. rotundata* complex

BY

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Advisor: Dr. Yesehak Worku

Dioscorea species (Yams) rank as the world's fourth most important tuber crop in economic terms and make a significant contribution to diets in some rural areas of Ethiopia where Ethiopia is one of the major producers in East Africa. However, there is insufficient study on their nutritional value and anti-nutritional composition. This study was designed to evaluate the proximate, mineral and anti nutritional composition of yam tubers (*Dioscorea cayenensis*-*rotundata* complex) collected from Sheko wereda, Bench Maji zone, South Nations, Nationalities and people's Regional state. Effect of household processing methods namely boiling and frying on composition of *Dioscorea* was also investigated. To determine the proximate, mineral and anti nutritional composition chemical analysis were conducted using standard procedures. Data were analyzed using SPSS (SPSS Software for windows release 16.0; SPSS Inc., Chicago, IL, USA).

In raw yam tubers, moisture was high $69.56 \pm 0.2\%$ for the yellow and $73.44 \pm 0.5\%$ for the white tubers. Crude protein content was $1.85 \pm 0.04\%$ for the yellow and $2.03 \pm 0.03\%$ for the white tuber while ash was highest ($4.10 \pm 0.01\%$) in the yellow $4.62 \pm 0.01\%$ in the white tuber. Crude fat was low in both tubers $0.35 \pm 0.01\%$ for the yellow and $0.18 \pm 0.01\%$ for the white tuber while total carbohydrate was high in both tubers $20.95 \pm 0.1\%$ and $19 \pm 0.06\%$ for the yellow and white tubers respectively. The fiber contents were $3.19 \pm 0.05\%$ for the yellow and $2.68 \pm 0.03\%$ for the

white tubers. Calcium and potassium were the major minerals found in both tubers. calcium contents of 52.55 ± 0.01 and 60.52 ± 0.01 and potassium contents of 53.33 ± 0.33 and 45.73 ± 0.01 were found for the yellow and white tubers respectively. Phytate, tannin and oxalate contents were 38.2 ± 0.002 , 22.2 ± 0.001 and 0.006 ± 0.001 for the yellow tuber and 34.8 ± 0.002 , 28.8 ± 0.001 and 0.006 ± 0.001 for the white tuber. There were no significant difference ($p > 0.05$) in the proximate, mineral, phytate, tannin and oxalate contents of the raw tubers of the yellow and white varieties. Boiling and frying significantly reduced ($P < 0.05$) the level of phytate, tannins and oxalate in both tubers.

Both tubers are found to be a good source of crude fiber, carbohydrate and minerals. The anti nutritional factors (phytate and oxalate) found in both the investigated tubers were lower than previous reports. Both boiling and frying seem to be best for the removal / inactivation of anti-nutritional factors. In terms of energy, if consumed at 350g per day, none would meet the FAO recommended values. The investigated samples can not be consumed as the sole source of energy to humans. In order to derive full nutritional potential from the yellow and white yam, they should be consumed together with protein-and fat-giving foods.

Key words: *D.cayenensis*-*D.rotundata* complex, proximate composition, anti-nutritional factors

1. INTRODUCTION

Dioscorea (Yam) is a genus of over 600 species of flowering plants in the family Dioscoreaceae, native throughout the tropical and warm temperate regions of the world. According to Méige and Demesew (1997) the family includes about seven genera (Borderea Miége., Dioscorea L., Epietrum Phil., Testudinaria Salisb.ex Burch, Rajania L., Stenomeris Planch and Tamus L.) with the greatest diversity occurring in Central and South America, Indo-Malaysia, Micronesia and Madagascar. Representatives also occur in Eastern Europe and Africa, but here the diversity is relatively low. Of the seven genera recognized only one, Dioscorea, is represented in tropical Africa (Demissew et al., 2003). The vast majority of the species are tropical, with only a few species extending into temperate climates. The scientific name Dioscorea is named after the ancient Greek physician and botanist Dioscorides while the common name, Yam, derives from a West African word nyami. Yams in Ethiopia are known among many nations and nationalities by different vernacular names; Boye, by Kulo people; Kocho or Wocheno, Oromo; Bohe, Welayita; Kuso, yem; and Boina, Sidama. (Edossa, 1996)

Worldwide at least 50-60 species of Dioscorea of the more than 600 known (Govaerts & Wilkin, 2007) are recognized to be cultivated or wild-harvested, for food or pharmaceutical purposes (Craufurd et al., 2001). Many member of this genus are poisonous or require processing to remove toxins in order to make them safe for consumption.

Roots and tubers were critical components in the diet during the early evolution of mankind and were the most important food crops of very ancient origin in the tropics and sub tropics, associate with human existence, survival and socio-economic history (Asha and Nair, 2002).

Yams are important agricultural crops in tropical regions, grown for their large tubers and are particularly important in parts of Africa, Asia and Oceania. The tubers may be eaten with sauce direct after boiling, roasting, or frying in oil. The tubers may also be mashed or pounded into dough after boiling, processed into flour, or cooked into pottage with added protein sauce and oils.

Yams rank as the world's fourth most important tuber crop in economic terms (Mignouna et al, 2005), after potatoes (*Solanum tuberosum* L.), Cassava (*Manihot esculenta* Crantz), and Sweet potatoes (*Ipomoea batatas* (L.) Poir.). They are produced on 5 million hectares in about 47 countries in tropical and subtropical regions of the world (FAO, 2005). Yields are about 11 t/ha in the major producing countries of West Africa. According to Food and Agricultural Organization (FAO) statistics, 48.7 million tones of yams were produced worldwide in 2005, and 97% of this was in sub-Saharan Africa. West and Central Africa account for about 94% of world production. Nigeria is the leading producer with 34 million tones followed by Côte d'Ivoire (5 million tones), Ghana (3.9 million tones), and Bénin (2.1 million tones). Ethiopia (174 000 tones) and Sudan (137 000 tones) are the major producers in East Africa. Colombia (333 000 tones) leads the production in South America followed by Brazil (230 000 tones), while Japan (204 000 t) is the leader in Asia. Yams are also important in the Caribbean (e.g., Haiti with 197 000 t in 2005), and the South Pacific Islands. Ghana exports the largest quantity of yams (about 12 000 t) annually. Average yam consumption per capita per day is highest in Bénin (364 kcal) followed by Côte d'Ivoire (342 kcal), Ghana (296 kcal), and Nigeria (258 kcal) (FAO, 2005). See table 1.1 below for the mean annual production of yam for the period from 1990 to 2005.

Table 1.1. Mean annual production of yam for the period from 1990 to 2005 (source: FAO 2005)

	Area harvested (‘000’ha)	Yield (Kg/ha)	Total production (‘000’MT)
World	3,572	9,694	34,355
Africa	3,418	9,708	32,874
Africa(West)	3,149	10,088	31,388
Ethiopia	68	4,065	277

1.1. The genus *Dioscorea*

From the more than 600 species of *Dioscorea* (Govaerts and Wilkin, 2007), in Ethiopia and Erithrea 11 species are recognized so far (*D. quartiniana* A.Rich, *D. dumetorum* Pax., *D. cochleari-apiculata* De Wild., *D. gillettii* Milne-Redh., *D. bulbifera* L., *D. schimperiana* Kunth., *D. alata*, *D. abyssinica*, *D. cayenensis*-*D. rotundata* complex, *D. sagittifolia* Pax. and *D. praehensilis*) (Demissew et al 2003). One of the species, *D. gillettii*, is endemic occurring in Southeast Ethiopia and Northern Kenya bordering Ethiopia. The remaining species are widespread in sub-Saharan Africa. Some of the species, such as *D. cayenensis*-*D. rotundata* complex and *D. abyssinica* occur both in the wild and in cultivations, and others such as *D. quartiniana*, *D. dumetorum*, *D. cochleari-apiculata* and *D. schimperiana* occur only in the wild (Demissew et al., 2003).

Members of the genus *Dioscorea* have usually a thin twining stem which allows the plants to climb. The direction of the stem twining (clockwise or anticlockwise) is used as one of the major taxonomic characters, to classify the species within the genus into different sections (Table 1.2). The members of the *D. cayenensis* complex usually produce a single annual tuber, which

varies in size, shape and weight, depending on species/cultivar and growing conditions. The color of the tuber flesh also varies between species and cultivars.

Tuber morphology, stem twining direction, dioecy, and fruit/seed wing shape are among the most important characters in the systematic of Dioscorea. Some of the characteristics and representative examples of the major sections of the genus Dioscorea are presented in Table 1.2 (Alexander and Coursey, 1969). The section Enantiophyllum is the largest in terms of the number of species.

Table 1.2. The major sections of the genus Dioscorea based on morphological characteristics of the species (Alexander and Coursey, 1969).

Section	Major characteristics	Representative species
Enantiophyllum	Usually single tuber Twine to the right Winged stems Occasional bulbils	<i>D.alata</i> <i>D.cayenensis</i> (yellow Yam) <i>D.rotundata</i> (white yam) <i>D.preahensilis</i> <i>D.abysinnica</i>
Lasiphyton	Cluster of medium sized tubers Twine to the left Large thorns on stems	<i>D.hispida</i> .Dennst <i>D.dumetorum</i> <i>D.pentaphylla</i> L. <i>D.quartiniana</i>
Opsophyton	Aerial bulbils Twine to the left	<i>D.bulbifera</i>
Combilium	Large number of individually small tubers Twine to the right	<i>D.esculenta</i> (Lour.) Burkill
Macrourea	Small and very toxic bulbils twine to the right	<i>D.sansibarensis</i> .Pax
Macrgynodium	Small tubers twine to the left spineless stem	<i>D.trifida</i> L.f.

The African domesticates known as Guinea yams (*D.cayenensis* Lam.-*D.rotundata* Poir.

complex) are one of the most important, preferred and widely planted tuber crops in the tropics

(Mignouna and Dansi, 2003), although *D.alata* L., a cultigens of Asian origin, is also widely grown in Africa. They are the major source of carbohydrate in the “yam zone” of West Africa (Coursey, 1967). Elsewhere in Africa, there are pockets of extensive yam cultivation amid a widespread wild or semi-wild plants harvested as famine food when cereal crops run short or fail. Due to their importance in the diet of people in Africa, Guinea yams have been the subject of many studies by researchers in a number of disciplines including systematic, plant breeding, pathology and genetics. The primary goal of these researchers has been to look for the ancestral species from which the cultivated yams have been originated, both to understand the patterns of variation and processes of domestication, and to apply this knowledge to yam breeding and improvement. Guinea yams have been proposed to result from a process of domestication of wild yams of *Dioscorea* sect. *Enantiophyllum* Uline by African farmers (Mignouna and Dansi, 2003; Dumont et al., 2006).

However, there is still no agreement over the systematic or relationships within the species complex: the number of species and the names which should be applied to them have never been adequately clarified and determined. i.e., the delimitation of the *D.cayenensis*-*D.rotundata* complex and the relationships within the complex are still far from clear.

The taxonomy of the species *Dioscorea cayenensis* and *D.rotundata* has been the subject of much confusion (Coursey, 1967). They were first described as separate species: *D.cayenensis* by Lamark in 1789 based on specimens from French Guiana (hence the name Cayenne) and *D.rotundata* by Poiret in 1813 based on samples from Puerto Rico long before their African origin was established.

A numerical taxonomic analysis of 97 cultivars of Guinea yams (*D.rotundata* and *D.cayenensis* accessions), based on 75 morphological descriptors resulted in a phonetic tree with two main trunks interconnected with many anastomosing branches. The authors suggested that all the 97 cultivars investigated belong to a single highly evolved species (Martin and Rhodes, 1978). Using Restriction Fragment Length Polymorphism (RFLP) markers Terauchi et al., (1992) failed to evidently discriminate between the various species of Guinea yams. The authors proposed that species referred to Guinea yams are all closely related.

Yam cultivation systems in Ethiopia and East Africa have not been studied as well as their West African counter parts. A study conducted in South and Southwest Ethiopia have revealed that the local community has a strong tradition in cultivating and domesticating various species of yams with wide genetic bases (Demissew et al., 2003; Hilderbrand et al., 2002). However, this excellent knowledge of yam cultivation and domestication is beginning to deteriorate as farming practice in the area reorients towards cash crops and coffee plantation (Hildebrand et al., 2002).

The concept of *D.cayenensis* species complex, first proposed by Ayensu and Coursey (1972) was discussed in 1978 at a seminar on yams conducted in Cameroon. This concept was later supported by Hamon (1987) as a way of pooling all West African cultivated yams that are not bulbiferous and have entire leaves under the same name (Dumont et al., 2006).

According to Dumont et al. (2006), there has been considerable confusion regarding the yams *D.rotundata* and *D.cayenensis*. In general in English speaking West Africa, particularly Nigeria, they are known as white yams and yellow yams respectively, and pooled under the term Guinea yams. Farmers in French speaking Africa, on the other hand do not make a clear distinction between *D.rotundata* and *D.cayenensis* where the generic name is accordingly used for all the

cultivated yams. For the African farmer, a yam cultivar is identified by its common name, which often contains technical or historical information. Yam cultivars are best differentiated on the basis of their tuber traits like color or taste of the flesh. The characteristics of the vegetative organs are sometimes, but not always, also used as distinctive markers. Farmers, in general, define yam cultivars by sets of technical criteria consisting of agronomic requirements, harvesting time, cooking quality and storage life (Dumont et al., 2006).

D.cayenensis and *D.rotundata* are believed to be domesticated from wild African *Dioscorea* species of the section *Enantiophyllum*. The two taxa differ to some extent with respect to some traits. But none of the studies conducted so far have clearly established the identity of each taxon as a separate species (Dumont et al., 2006). It is difficult to differentiate the two taxa using morphology. This is because the original diagnoses are not complete enough to define them precisely and many forms are intermediate between the two (Meige and Sebsebe Demissew, 1997). However, *D.rotundata* could be described as a group of cultivated yams of African origin with a short annual vegetative cycle (6-8 months), tubers with a long dormancy period (3-5 months) with slightly pigmented to non-pigmented creamy or white flesh (Dumont et al., 2006). It is harvested twice a year, prefers a short rainy season, has ovate leaves and 4, 8 or 12 vascular bundles (Hamon and Toure, 1990). In *D.cayenensis* the vegetative cycle ranges from 8 to 12 months and the tuber flesh is usually yellow (Terauchi et al., 1992). It is harvested annually, prefers a long rainy season, has orbicular leaves, and 8 vascular bundles (Hamon and Toure, 1990).

Nutritional value is the main concern when a plant is considered as food source. However, endogenous toxic factors characteristic of plant material can also affect the content of nutrients. These toxic factors act as anti-nutrients and adversely affect the organism.

Anti-nutrients are chemicals which have been evolved by plants for their own defense, among other biological functions. They 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). Anti-nutrients are compounds that limit the digestion and absorption of nutrients and result in reduced bioavailability of nutrients and flatulence production (Brune *et al.* 1989). Anti-nutrients vary in chemical structures, ranging from amino acids to proteins; from simple amines to alkaloids, glycosides and many phenolic compounds. The biological effects of all these chemicals are diverse and complex.

This study paper was designed to investigate the nutritional and anti nutritional composition as well as the effect of household processing on the composition of *Dioscorea cayenensis*-*D.rotundata* complex (yellow yam and white yam) collected from Sheko.



Figure1.1.Tuber of yellow yam.



Figure1.2.Tuber of white yam.

The findings of nutritional and anti nutritional factors can help to understand the nutritional benefit and potential problems of consuming *Dioscorea* and proposing future direction for further study and/or promotion. Data on the effect of household processing of *Dioscorea* provide

important information to obtain the optimum food value from Dioscorea and eradicate or minimize unwanted effects.

1.2. Proximate Composition

1.2.1. MOISTURE

Moisture generally refers to the presence of water; often in trace amounts. The moisture content is the most important and widely used measurement in the processing and testing of foods. It is an important measure of storage quality to purchase the food in bulk and also to establish a dry weight basis for the analysis of the other nutrients. The safe limit for storage is 15% moisture and foods containing more than 15% moisture should not be stored as it may develop undesirable moulds and fungus and promote bacterial growth and results in rotting of the food over time.

1.2.2. ASH

Ash is the residue from burning any biological material in a furnace at 550^oc. It is composed of the inorganic residue in foods and includes both trace elements and macro elements. These elements may occur in the food naturally or be introduced by processing, production or transportation.

1.2.3. CRUDE PROTEIN

According to Weende's food analysis scheme, nitrogenous fraction of food may be divided into two portions; protein and non-protein. Protein is made up of dispensable amino acids-glutamic acid, aspartic acid, alanine, serine, proline, hydroxyl proline; semidispendable amino acids-arginine, glycine, histidine, cystine, tyrosine; indispensable amino acids-lysine, tryptophan,

leucine, phenylalanine, methionine, threonine, isoleucine and valine. Another part of non-protein nitrogen consists of urea, ammonia, uric acid, free amino acids, amines, etc. Crude protein is estimated by Kjeldahl method.

1.2.4. CRUDE FAT

Crude fat is prepared by ether extraction. This is a combination of simple fat-fatty acid esters, compound fat, neutral fat, and sterols. Fat is estimated by extraction with petroleum ether (boiling point 40-60°C) or by extraction with ether in a Soxhlet extractor. The gross energy yield of crude fat is 9.40 Kcal per gram of feed.

1.2.5. CRUDE FIBER

Crude fiber is the insoluble and combustible organic residue which remains after the sample has been treated with consecutive treatments of petroleum spirit to remove fat; boiling dilute sulfuric acid and boiling dilute sodium hydroxide and acetone to break down the protein and carbohydrates and to remove all soluble components. This treatment produces crude fiber consisting largely of cellulose and a proportion of lignin and hemicelluloses. The amount of each of these substances will vary with the conditions used for extraction. It is therefore imperative to adhere strictly to a standard procedure to obtain comparable results. Crude fiber analysis can be conducted either by boiling the sample with reagents and separating the residues by filtration through a Buchner funnel or by a more rapid semi automated method. Crude fiber gives an indication of bulkiness of a feed.

1.2.6. SOLUBLE CARBOHYDRATES

Sugars such as sucrose, glucose and fructose and polysaccharides such as starch and cellulose are the principal components of carbohydrates. Soluble carbohydrates consist of monosaccharides, oligosaccharides and polysaccharides. Analysis of carbohydrates tends to be complex because each sample is likely to contain a mixture of several types of carbohydrates. For the purpose of proximate analysis soluble carbohydrate content is often simply determined by difference.

$$\% \text{ Soluble carbohydrate} = \% \text{ DM} - (\% \text{ EE} + \% \text{ CP} + \% \text{ ash} + \% \text{ CF}).$$

Where: DM = dry matter, EE = ether extract or crude fat, CP = crude protein, CF = crude fiber

1.3. MINERALS

Of the mineral elements phosphorous, calcium, magnesium, potassium, sulfur, sodium and chlorine are sometimes called the “macro-nutrients”. The first five are essential to both plants and animals and the last two to animals alone. Of the “micro-nutrients” minerals, iron, copper, zinc and manganese are necessary to both plant and animal life. Boron, molybdenum, silicon, and possibly gallium are deemed necessary to plants but not to animals, while cobalt and iodine are necessary to animals but have never been proved to be essential to plants. Aluminum and fluorine, although found in both plants and animals, are of questionable necessity for either (Gilbert, 1984).

1.3.1. CALCIUM

Calcium is one of the most important of the biological mineral elements and is necessary to the growth of all animals and all green plants, with the exception only of some of the lower algae.

Calcium taken up by the plant is probably used for the neutralization and precipitation of the acids in the plant sap and germination of seed (Gilbert, 1984). In human beings calcium forms a vital part of bone and tooth structure, and is also important as a positive ion (Ca^{2+}) for blood clotting, muscle contraction, and nerve impulse transmission. It also participates in glycogen metabolism (Heydon, 1983, Tortora, 1997). Inadequate intake of calcium increases the risk of osteoporosis. While excess intake of calcium may cause kidney stones and reduces mineral absorption in general (Wardlaw, 1996).

1.3.2. COPPER

Copper is a catalyst for respiration and an activator of several enzymes. It is important for carbohydrate and protein synthesis. It may also play a role in carotene production. (Gilbert, 1984). Whereas, in animal it is used for helping the body utilize iron, reduce tissue damage caused by free radicals, maintain the health of bones and connective tissues, produce the pigment called melanin, keep the thyroid gland to function normally, and preserve the myelin sheath that surrounds and protects the nerves. Deficiency of copper causes low white blood cell count and poor growth. Excess intake of copper can cause vomiting, nervous system disorder and Wilson's diseases (Wardlaw, 1996).

1.3.3. ZINC

Zinc is an essential element found in the tissue of animals and plants 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,

1996). Zinc is the most ubiquitous of all trace elements involved in human metabolism. More than one hundred specific enzymes require zinc for their catalytic function. If zinc is removed from the catalytic site, activity is lost; replacement of zinc restores activity. Zinc participates in all major biochemical pathways and plays multiple roles in transcription of DNA, translation of RNA, and ultimately cell division. 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).

1.3.4. IRON

Iron is an essential constituent of plant and animal protoplasm. Plants require iron in larger amounts than any other micronutrient. It is used in chlorophyll synthesis; respiration; and as a constituent of various enzymes and proteins. It also serves as an activator for nitrogen fixation. In animals iron carries oxygen to the cells and is necessary for the production of energy, the synthesis of collagen, and the functioning of the immune system. Iron deficiency is common 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 (Tzonou, 1998, Hallayday, 1998). Iron absorption can be blocked by calcium, magnesium, manganese, zinc, anti-acids and tetracycline (Rebouche, 1999). Deficiency of iron results in anemia which is recognized by its symptom such as low blood iron level, small and fewer red blood cells and low blood hemoglobin values (Tortora, 1997). Iron

toxicity usually results from a genetic disorder called hemochromatosis. This disease causes over absorption and accumulations of iron, which can result in severe liver and heart damage (Wardlaw, 1996).

1.3.5. Phosphorus

Phosphorus is a key element in all known forms of life. Inorganic phosphorus in the form of the phosphate PO_4^{3-} plays a major role in biological molecules such as DNA and RNA where it forms part of the structural framework of these molecules. Living cells also use phosphate to transport cellular energy in the form of adenosine triphosphate (ATP). Nearly every cellular process that uses energy obtains it in the form of ATP. ATP is also important for phosphorylation, a key regulatory event in cells. Phospholipids are the main structural components of all cellular membranes. Calcium phosphate salts assist in stiffening bones (Lewis, 2006).

1.3.6. POTASSIUM

Potassium is the most abundant mineral found in the human body next to P and Ca. It is a major electrolyte of intracellular fluid. It is of great physiological importance, contributing to the transmission of nerve impulses, the control of skeletal muscle contractility, and the maintenance of normal blood pressure. It is obviously essential for plants and can not be entirely replaced by any other elements. Deficiency symptom includes irregular heart beat, loss of appetite and muscle cramps (Wardlaw, 1996).

1.4. Anti-nutritional Factors

1.4.1. Phytic acid

Phytic acid, inositol hexakisphosphate (IP₆), (see fig.1.3) is the principal storage form of phosphorus in many plant tissues (Hurrell, 2003) accounting for up to 80 per cent of the total phosphorus (Steiner *et al.*, 2007).

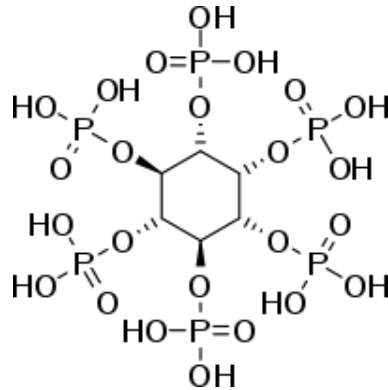


Fig.1.3. Structure of phytate (Adapted from Reddy, 2001).

Phosphorus in phytate form is, in general, not bioavailable to non-ruminant animals because they lack the digestive enzyme phytase, which is required to separate phosphorus from the inositol.

Phytic acid is a strong chelator of important minerals such as calcium, magnesium, iron and zinc, and can therefore contribute to mineral deficiencies in people whose diets rely on phytate containing foods for their mineral intake, such as those in developing countries (Hurrell, 2003).

Calcium ions interact with protein and phytate decreasing protein solubility (Frokiaer *et al.*, 2001). Phytate has been shown to inhibit the proteolysis of a number of enzymes such as trypsin, pepsin and amylases of the intestinal tract (Vaintraub and Blilimaga, 1991).

Phytic acid and starches are structurally capable of combining via phosphate linkages (Sirikka, 1997), hence phytic acids may also affect starch digestion. It also acts as an acid, chelating the

vitamin niacin, which is basic, causing pellagra (Anderson, 2005). In this way, it is an anti-nutrient. For people with a particularly low intake of essential minerals, especially young children and those in developing countries, this effect can be undesirable. However, in-home food preparation techniques can reduce the phytic acid in phytic acid containing foods. Simply cooking the food will reduce the phytic acid to some degree. Cooking has been reported to lower the phytate levels in yam (Bhandari and Kawabata, 2006).

1.4.2. OXALATE

Oxalate is a di anion with formula $C_2O_4^{2-}$ (see fig.1.4) that occurs widely in the plant kingdom. Many metal ions form insoluble precipitates with oxalate, a prominent example being calcium oxalate, the primary constituent of the most common kind of kidney stones.

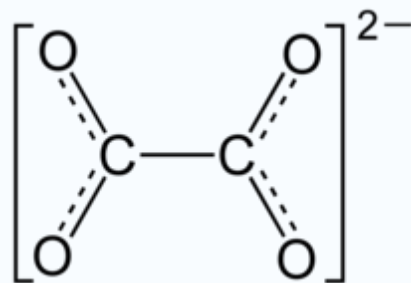


Fig.1.4. Structure of oxalate (Adapted from Wilhelm, 2002).

The affinity of divalent metal ions is sometimes reflected in their tendency to form insoluble precipitates. Thus in the body, oxalic acid also combines with metals ions such as Ca^{2+} , Fe^{2+} , and Mg^{2+} to deposit crystals of the corresponding oxalates, which irritate the gut and kidneys.

Because it binds vital nutrients such as calcium, long-term consumption of foods high in oxalic acid can be problematic. Healthy individuals can safely consume such foods in moderation, but those with kidney disorders, gout, rheumatoid arthritis or certain forms of chronic vulvar pain are

typically advised to avoid foods high in oxalic acid or oxalates. The calcium oxalate crystals or precipitate (better known as kidney stones) obstruct the kidney tubules. An estimated 80% of kidney stones are formed from calcium oxalate (Harold, 2004). Conversely, calcium supplements taken along with foods high in oxalic acid can cause calcium oxalate to precipitate out in the gut and drastically reduce the levels of oxalate absorbed by the body by 97% (Bate-Smith and Swain, 1962).

1.4.3. Tannins

Tannins are bitter plant polyphenols that bind and precipitate proteins and various other organic compounds including amino acids and alkaloids. The term is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. 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 (Coe FL, et al, 2005).

Tannins have molecular weights ranging from 500 to over 3,000 (Morozumi, 2006). Tannins are incompatible with alkalis, gelatin, heavy metals, iron, lime water, metallic salts, strong oxidizing agents and zinc sulfate, since they form complexes and precipitate in aqueous solution. Tannins are distributed in species throughout the plant kingdom. Tannins are found in leaf, bud, seed, root, and stem tissues.

Tannins are usually divided into two classes; hydrolysable tannins and condensed tannins (proanthocyanidins)

Hydrolysable tannins: At the center of a hydrolysable tannin molecule there is a carbohydrate (usually D-glucose) (see fig.1.5). The hydroxyl groups of the carbohydrate are partially or totally esterified with phenolic groups such as gallic acid (in gallotannins) or ellagic acid (in ellagitannins). Hydrolysable tannins are hydrolyzed by weak acids or weak bases to produce carbohydrate and phenolic acids. Examples of gallotannins are the gallic acid esters of glucose in tannic acid ($C_{76}H_{52}O_{46}$), found in the leaves and bark of many plant species.

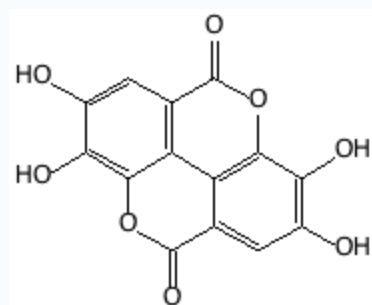
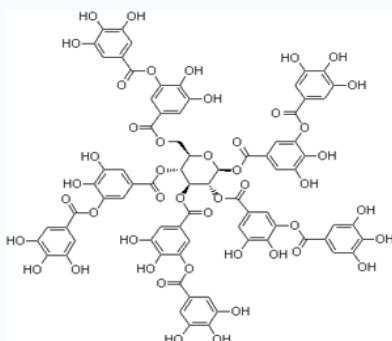


Fig.1.5. (A).Structure of tannic acid, gallotannin (B).Structure of ellagic acid

(Adapted from Branham, 1989).

Condensed tannins: Condensed tannins, also known as proanthocyanidins, are polymers of 2 to 50 (or more) flavonoid units that are joined by carbon-carbon bonds (see fig.1.6), which are not susceptible to being cleaved by hydrolysis. While hydrolysable tannins and most condensed tannins are water soluble, some very large condensed tannins are insoluble.

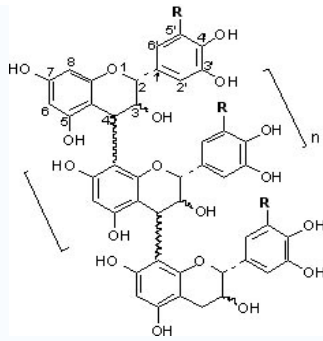


Fig. 1.6. Structure of condensed tannin molecule (Adapted from Branham, 1989).

If ingested in excessive quantities, tannins inhibit the absorption of minerals such as iron which may, if prolonged, lead to anemia (Brune, 1989). This is because tannins are metal ion chelators, and tannin-chelated metal ions are not bioavailable. However, condensed tannins do not interfere with iron absorption (Brune, 1989). Tannins have been shown to precipitate proteins, which inhibits in some ruminant animals the absorption of nutrients from high-tannin grains such as sorghum.

Approximately 600 Dioscorea species are eaten in various parts of the world. Yams, the edible starchy tubers, are of cultural, economic and nutritional importance in the tropical and subtropical regions of the world (Coursey, 1967). In fact they are one of the principal sources of food and nutrient energy for many people in the tropics. Study reports have also pointed out that a few yam species contain some toxic compounds and can impart serious health complications. Some species of wild yams, particularly wild forms, are toxic and/or unpalatable, taste bitter and cause vomiting and diarrhea when large amount are ingested without proper processing or if eaten raw. A toxic principle in some yam species has been reported as dioscorine (see fig.1.7), a toxic alkaloid (Bhandari and Kawabata, 2005).

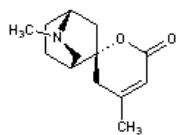


Fig.1.7. Structure of Dioscorine (Adapted from Webstre, 1984).

Dioscorine triggers the fatal paralysis of the nervous system when a fragment of the tuber weighing 100 g is ingested. Similarly, histamine was reported as the principal allergen, causing mild inflammation and itching, in some plants of Dioscoreaceae family. On the other hand, the bitter substances in some yam species have been reported as furanoid-norditerpene (see fig.1.8) groups of compounds (Bhandari and Kawabata, 2005).

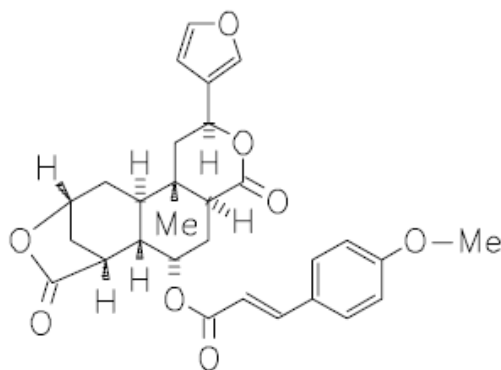


Fig.1.8. Structure of furanoid-norditerpene compound (Adapted from Wang, 2009).

The bitterness and toxicity of many yam species may be caused by high level of saponins (see fig.1.9). Such is the case with *Dioscorea tokoro* in Japan, in which saponin is responsible for its bitterness. The tubers were found with a high amount of protein, a good proportion of essential amino acids and appeared as a fairly good source of many dietary minerals. However, their wider utilization is limited due to the presence of some toxic and anti nutritional factors. Raw as well as

cooked tubers are sometimes bitter, and the bitterness may be strong enough to render the tuber unpalatable. Ingestion of large amount of wild tubers has also been reported to cause caustic effects, irritation to mouth, throat or intestinal tract and absorptive poisoning (Bhandari and Kawabata, 2005).

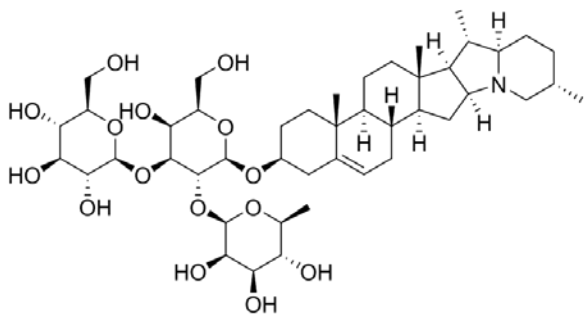


Fig. 1.9. structure of saponin (Adapted from Foerster,2006).

Dioscorea spp. also have medicinal properties as their tubers contain diosgenin (see fig.1.10), a biochemical precursor in the synthetic production of progesterone and other corticosteroids (Albrecht and McCarthy, 2006).

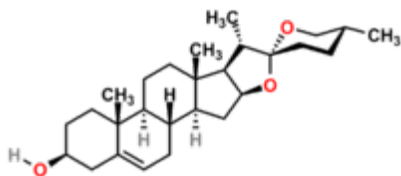


Fig.1.10. Structure of Diosgenin (Adapted from Wong, 2005).

Many different forms and cultivars of the edible yam species are available in different areas and it is likely that these differ in composition and nutritional values (Bhandari et al., 2003). Several factors influence the nutritional content of foods from plants. These include the genetic make-up

of the plant, the soil in which it is grown, use of fertilizer, weather condition, maturity at harvest, storage conditions and processing method (Morris et al., 2004).

According to FAO report on raw tuber of *Dioscorea* (unspecified species), the tuber has 119 Ca of food energy, 69.0 % moisture, 1.9 % protein, 0.2 % fat, 27.8 % carbohydrate, 0.8 % fiber, 1.1 % ash, 52 mg/100g calcium, 61 mg/100g phosphorus, 0.8 mg/100g iron, 10 µg/100g retinol, 0.11 mg/100g β-carotene equivalent, 0.02 mg/100g thiamine, 0.3 mg/100g riboflavin, 26 mg/100g niacin, 6 mg/100g tryptophan and 16 mg/100g ascorbic acid per 100 g of edible portion (FAO).

According to FAO report on composition of *D.cayenensis*-*D.rotundata* complex, the species have been reported to have 71ca energy value, 80.8 % moisture, 1.5 % protein, 0.1 % fat, 16.4 % carbohydrate, 0.6 % fiber, 1.2 % ash, 36 mg/100g calcium, 17 mg/100g phosphorus, and 5.2 mg/100g iron per 100 g of edible portion (FAO).

A study conducted in Nigeria (Ojwu and Ndu, 2006) on evaluation of phytonutrients, mineral and vitamin contents of some varieties of yam (*Dioscorea* species) revealed Mg, Ca, K, Na and phosphorus content of 0.73, 1.60, 0.75, 0.19 and 0.29 mg/100g for *D.cayenensis* and 0.85, 1.20, 0.39, 0.14 and 0.20 mg/100g for *D.rotundata* and tannin and phenol content of 0.0047 and 0.0024 mg/100g for *D.cayenensis* and 0.05 and 0.005 mg/100g for *D.rotundata*. This study also reported ascorbic acid, niacin, riboflavin and thimine content in mg/100g of 1.23, 0.13, 0.004, 0.007 for *D.cayenensis* and 0.97, 0.03, 0.006 and 0.008 for *D.rotundata*.

On a report done by Ethiopian Health and Nutrition Research Institute (EHNRI) (EHNRI, 1997) on raw *Dioscorea* (unspecified species and unspecified place of collection) they reported a value for food energy (in calories), moisture (in percent), nitrogen (in g/100g), protein (in g/100g), fat (in g/100g), carbohydrate (in g/100g), fiber (in g/100g), ash (in g/100g), Ca (in mg/100g), P (in

mg/100g), and Fe (in mg/100g) of 98.1, 74.5, 0.7, 3.1, 0.1, 21.2, 1.7, 1.1, 119,13.3 and 1.8 respectively.

In another report done by EHNRI (EHNRI,1997) on Dioscorea tubers (unspecified species) collected from Welita, Metekel and Sidama, after boiling of the Dioscorea tubers they reported a value for food energy (in calories), moisture (in percent), nitrogen (in g/100g), protein (in g/100g), fat (in g/100g), carbohydrate (in g/100g), fiber (in g/100g), ash (in g/100g), Ca (in mg/100g), P (in mg/100g), Fe (in mg/100g), Zn (in mg/100g), Cu (in mg/100g), Na (in mg/100g), K (in mg/100g), RE (in μ g/100g) , thiamine (in mg/100g) ,Riboflavin (in mg/100g), and ascorbic acid (in mg/100g) contents of 85, 77, 0.28, 1.80, 0.10, 19.9, 0.7, 1.2, 16, 56.8, 0.92, 2.2, 0.5, 7, 464, 5, 0.01, 0.04 and 2.12 for the Dioscorea collected from Welita; 166, 68.1, 0.18, 1.10, 0.10, 30.10, 2.5, 0.6, 8.6, 29.4, 1.3, 0.19, 0.14, 20.8, 211.8, 3.6, 0.03, 0.15, and 2.71 for the Dioscorea collected from Metekel; and 113, 69.2, 0.28, 1.8, 0.10, 28, 0.7, 0.9, 6, 68.1 ,0, 0.36, 0.57, 115, 310, 6, 0.01, 0.04 and 1.99 for the Dioscorea collected from Sidama.

Processing (cooking) can be beneficial or detrimental to nutrient composition of foods.

Processing techniques may decrease the food value of some nutrients (Nestares et al., 1996): for example, there is some leaching of nutrients into the cooking water during processing. The cooking water may or may not be discarded.

On the other hand, processing may enhance the nutritional quality of food by reducing or destroying the anti-nutrients, and also increasing the digestibility of proteins and starches (Siddhuraju and Becker, 2001). Elimination or inactivation of anti-nutrients is necessary to improve the nutritional quality and for effective utilization of foods.

Hence this study was mainly designed to investigate the nutritional and anti nutritional composition and the effect of boiling and frying on the nutritional and anti nutritional contents of yellow yam and white yam tubers collected from Sheko.

1.5. SIGNIFICANCE OF THE STUDY

This study will provide information on the proximate composition, some selected minerals and anti-nutrients found in *Dioscorea cayenensis*-*Dioscorea rotundata* complex and the best household processing method to obtain the optimum nutritional value and to minimize or eradicate the anti-nutrients.

1.6. HYPOTHESIS

There is no difference in the nutrient and anti nutrient composition of raw and processed yellow and white yam tubers.

1.7. OBJECTIVE

1.7.1. GENERAL OBJECTIVE:

The present study is mainly designed to investigate the nutritional and anti nutritional composition of yellow yam and white yam.

1.7.2. SPECIFIC OBJECTIVES:

1. To analyze the proximate, minerals, and anti nutritional factor composition of yellow yam and white yam.

2. To compare the proximate, minerals and anti nutritional composition between yellow yam and white yam.
3. To investigate the effect of household processing methods namely boiling and frying on the proximate, minerals and anti nutrient composition of yellow yam and white yam.
4. To determine the best method of processing to obtain the optimum nutritional value and to eliminate the anti nutritional factors.

2. METHODS AND MATERIALS

2.1. Sample Collection

Dioscorea tubers were collected from August 15-20, 2010; from South Nations Nationalities and Peoples Regional state; Bench Maji zone, Sheko woreda. Mature tubers were collected randomly from a farm, packed in a news paper and kept in a plastic bag and immediately transported to the laboratory to minimize loss of moisture.

2.2. Voucher Dioscorea specimen collection and identification

Voucher specimens of Dioscorea were collected by the investigator. The specimens were pressed, identified and authenticated by reconfirming at the National Herbarium of Addis Ababa University using Ethiopian flora volumes and checking against identified specimens in the herbarium. Voucher specimens of Dioscorea were numbered as 001/002 and deposited at the National Herbarium, Addis Ababa University, under the name of the principal investigator.

2.3. Sample Preparation

Tubers with no skin wounding were selected, washed, hand-peeled and cut into small pieces.

Samples were then divided into three groups:

- Samples to be analyzed unprocessed (referred as raw)
- Samples to be analyzed after boiling (referred as boiled) and
- Samples to be analyzed after frying (referred as fried).

The unprocessed samples were milled into a fine powder which was stored in a bottle until analysis. The boiled and fried samples were processed as described below.

2.4. Processing techniques

Dioscorea samples were subjected to the following processing techniques:

- Boiling: The samples were boiled in distilled water on a stove for 40 minutes, after which they were drained.
- Frying: The samples were fried in domestic cooking oil (100 per cent sunflower oil) for 10 minutes.

The processed samples were dried and then ground into a fine powder, which was stored in bottles until required for analysis. The experimental analysis was conducted at the Ethiopian Health and Nutrition Research Institute (EHNRI) food chemistry laboratory.

2.5. Moisture Determination

A. Principle:

The amount of water present in the sample is considered to be equal to the loss of weight after drying the sample to constant weight. The moisture is removed from the sample by heating the sample at 100 °c for 6 hours in an oven (see fig. 2.1). The percent moisture present in the sample is calculated by dividing the loss of weight of the sample by the weight of the wet sample and multiplying with 100.

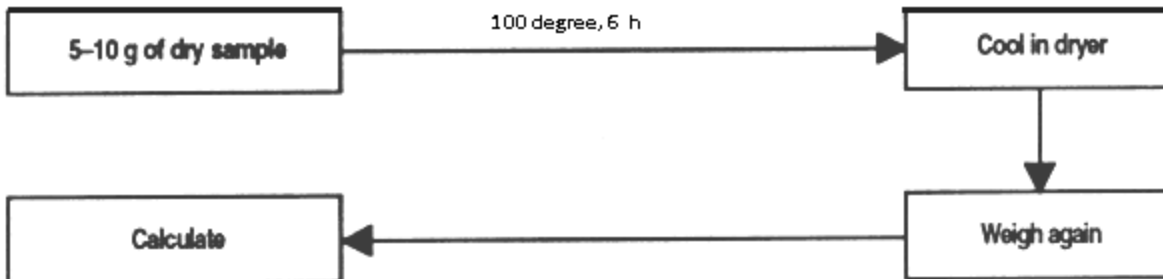


Figure2.1. Flow chart for determination of moisture content by dry oven method

B. Materials:

- Oven, 100-102°C.
- Nickel dishes.
- Desiccator. With granular silica gel (with a visual color change to indicate when regeneration is required).

C. Procedure:

1. Dry an empty dish and lid in the oven for 15 minutes and transfer to the desiccator to cool (10 minutes).
2. Weigh the empty dish and lid.
3. Mix the prepared sample thoroughly and transfer 5 g to the dish. Replace the lid and weigh the dish and the contents as rapidly as possible.
4. Remove the lid and place the dish and the lid in the oven, avoiding contact of the dish with the walls. Dry for 5 hours.
5. Remove the dish from the oven, replace the lid, cool in the desiccator, and reweigh when cooled.
6. Dry for further hour to ensure that the constant weight has been achieved (change in weight between successive dryings at 1 hour interval is < 5 mg).

D. Calculation:

$$\% \text{ Moisture} = (W_2/W_1) \times 100$$

$$\% \text{ Total solids} = (W_3/W_1) \times 100$$

Where: W_1 = weight of the sample before drying

W_3 = weight of the sample after drying

W_2 = loss of weight ($W_1 - W_3$)

2.6. Crude Protein Determination

A. Principle:

All nitrogen is converted to ammonia by digestion with a mixture of concentrated sulfuric acid and concentrated orthophosphoric acid containing potassium sulfate as a boiling point raising agent and selenium as a catalyst. The ammonia released after alkalization with sodium hydroxide is steam distilled into boric acid and titrated with sulfuric acid (see fig. 2.2). (Tecator manual, 1979)

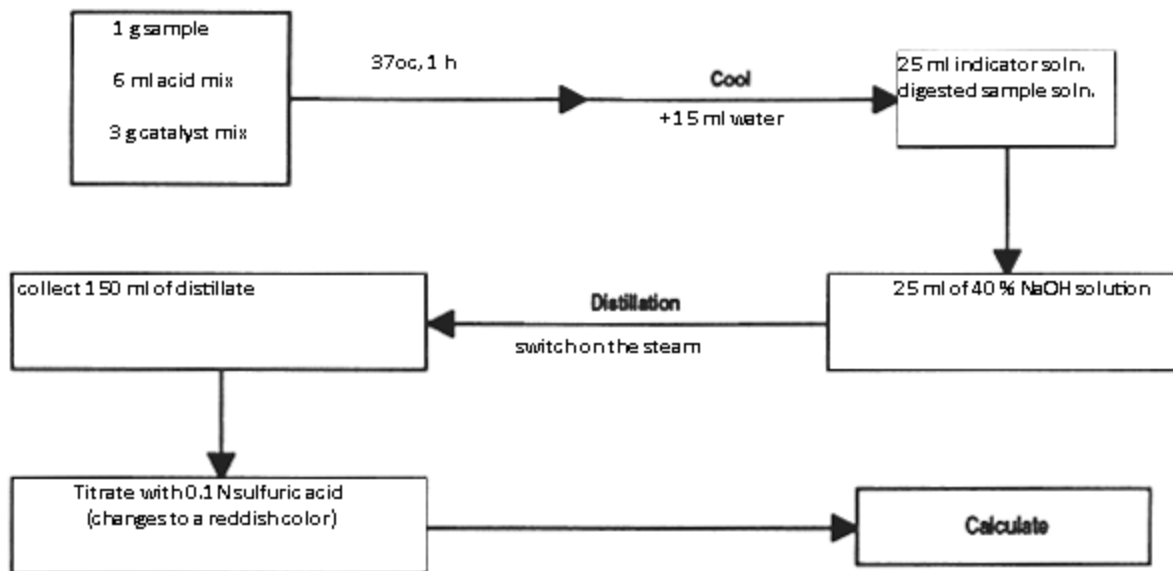


Figure 2.2. Flow chart for determination of crude protein by Kjeldahl method

B. Materials and Reagents:

- Digestion apparatus (aluminium block, tubes, etc).
- Tecator distillation apparatus.
- Acid mixture (5 parts of concentrated Orthophosphoric acid (85%) with 100 parts of concentrated Sulfuric acid (98%)).
- Selenium catalyst (0.5 g of selenium metal with 100 g of potassium sulfate).
- Hydrogen per oxide, 30 %.
- Sodium hydroxide 40 % (W/V). Dissolve 400 g NaOH in water and make up to 1 liter.
- Boric acid-indicator solution.
 - A. Dissolve 75 mg bromocresol green and 50 mg methyl red in 100 ml of 99 % ethanol.
 - B. Dissolve 50 g boric acid (H_3BO_3) in 1 liter of water (saturated solution).
 - C. To 25 ml of B add 10 drops of A.
- Sulfuric acid 0.1 N or 1 N.
- Standard ammonium chloride. Dissolve 4 g NH_4Cl in water and make up to 1 liter store in the refrigerator.

C. Procedure:

i. Digestion:



Digestion is accomplished by boiling a homogeneous sample in concentrated sulfuric acid. The end result is an ammonium sulfate solution.

1. Weigh 0.5-1.5 g dried sample in a Tecator tube.
2. Add 6 ml of the acid mixture from a pipette and mix.
3. Add step by step 3.5 ml of hydrogen peroxide. Watch out for violent reactions. As soon as the most violent reaction has ceased shake the tube a few times and put it back into the rack.
4. Add 3 g of the catalyst mixture. Let stand for 5-15 min before digestion.
5. With the temperature of the digester at 37°C lower the tubes (in the rack) into the digester. Locate the exhaust Manifold on top of the tubes and continue the digestion until a clear solution is obtained, about 1 hour during this step excessive foaming may occur.
6. Transfer the tubes in the rack into the fume hood for cooling.
7. Add 15 ml of water. Shake to avoid precipitation of sulfate in the solution.

ii. Distillation:



Excess base is added to the digestion product to convert NH_4 to NH_3 . The NH_3 is recovered by distilling the reaction product.

1. Place a 150 ml conical flask containing 25 ml of the boric acid-indicator solution under the condenser of the distiller with its tips immersed into the solution.
2. Transfer the digested and diluted solution into the sample compartment of the distiller.
Rinse the tubes with two portions of about 5 ml water and add the rinses.

3. Add 25 ml of the 40 % sodium hydroxide solution into the compartment and rinse it down with a small amount of water. Stopper and switch on the steam.
4. Distil 100 ml, and then lower the receiver so that the tip of the condenser is above the surface of the distillate. Continue the distillation until a total volume of 150 ml is collected. Rinse the tip with a few ml of water before the receiver is removed.

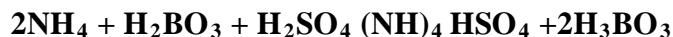
iii. Titration:

Titration quantifies the amount of ammonia in the receiving solution. The amount of nitrogen in a sample can be calculated from the quantified amount of ammonia ion in the receiving solution

In direct titration, boric acid is used as the receiving solution.



The boric acid captures the ammonia gas, forming an ammonium-borate complex. As the ammonia collects, the color of the receiving solutions changes.



D. Calculation:

$$\text{Mg nitrogen in the sample} = V \times N \times 14$$

$$\text{Mg nitrogen/100 g sample} = \text{mg of nitrogen} \times 100/\text{mg sample}$$

$$\text{Total nitrogen (\%)} = [(V_b - V_s) (N) (1.4007) / (W)]$$

$$\text{Crude protein (\%)} = \text{total nitrogen (\%)} \times 6.25$$

Where: V = volume of sulfuric acid consumed

14 = equivalent weight of nitrogen

V_b = mL titrant for the blank

V_s = mL titrant for the sample

N = Normality of the acid titrant (nominally 0.1)

1.4007 = a single factor that takes into account the molecular weight of nitrogen, the

conversion of the milli-equivalent result of $V*N$, and the conversion to %.

W = the weight of sample in grams

Note:

The factor 6.25(100/16) is based on the fact that protein contains 16% nitrogen.

2.7. Crude Fat Determination

A. Principle:

A dried, ground sample is extracted with diethyl ether (peroxide free) which dissolves fats, oils, pigments and other fat soluble substances in a Soxhlet apparatus. The ether is then evaporated from the fat solution. The resulting residue is weighed and referred to as crude fat (see fig. 2.3).

Low temperatures are used to evaporate the ether and remove residual moisture to prevent oxidation of the fat. The amount of fat is calculated from the difference in weight of the extraction flask before and after extraction. (Tecator manual, 1979).

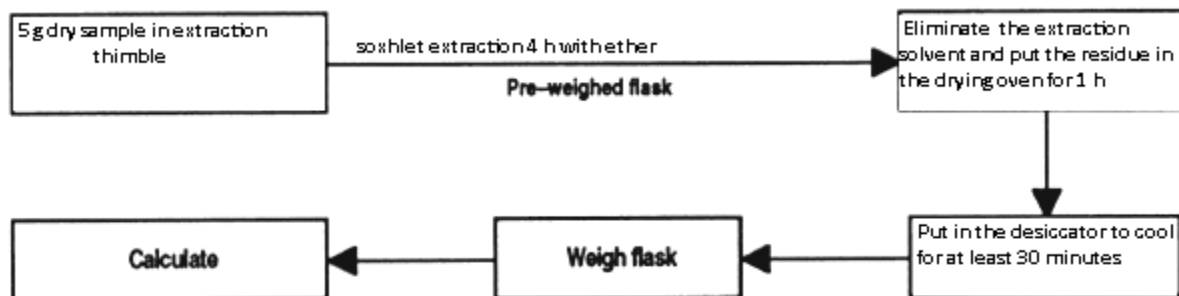


Figure 2.3. Flow chart for determination of crude fat by Soxhlet's method.

B. Materials and Reagents:

- Soxhlet extraction apparatus
- Extraction flask, Extraction thimble
- Beaker
- Desiccator
- Cotton (fat free)
- 0.5 M potassium iodide (KI). Dissolve 8.3 g KI in 100 ml water.
- Diethyl ether (Petroleum ether does not dissolve all of the plant lipid material, and therefore it cannot be substituted for diethyl ether).

Due to the risk of explosion, the ether must be checked for peroxides before use. Add 0.1-0.5 g of sodium bicarbonate and about 1 ml of 0.5 M KI and about 10 ml ether to a glass stoppered tube. Shake and wait a minute. In the presence of peroxides the I will be oxidized to I₂, which gives a yellow color. If peroxides are present they must be removed by shaking with ferrous sulfate. Store in the dark in an aluminum container.

Note: 40 g of 30 % ferrous sulfate solution in water is added to each liter of ether.

Caution: the reaction may be vigorous if the solvent contains a high concentration of peroxide

C. Procedure:

1. Weigh a clean extraction flask that has been dried at 92°C for at least an hour and then has been kept in the desiccator.
2. Cover the bottom of an extraction thimble with a 2 cm layer of fat free cotton.
3. Weigh in 5 g of the dried sample (WD) – obtained after determination of moisture – in the thimble and cover with a layer of fat free cotton.
4. Put the thimble into the extraction chamber.
5. Put on the cooling water.
6. Add 40 ml ether to the extraction flask through the condenser. Regulate the heating device so that the extraction container is emptied every 8th to 10th minute. Let the extraction go on for at least 4 hrs.
7. Remove the extraction flask when the extraction container is nearly full with ether. Tilt the extraction container and collect the ether in a waste beaker.
8. Reconnect the extraction flask and distill off as much ether as possible, and then disconnect the extraction flask and put it in the drying oven at 92°C for at least 1 hr.
9. Put it in the desiccator to cool for at least half an hour.
10. Weigh the extraction flask immediately after it is taken out of the desiccator.

D. Calculation:

$$W = W_2 - W_1$$

$$\text{Fat g/100 g fresh sample} = W \times (100 - \% \text{ moisture}) / \text{WD}$$

Where: W = weight of fat

W₂ = weight of extraction flask before extraction

W₁ = weight of extraction flask after extraction

WD = weight of dried sample

Remarks: During extraction some ether will disappear even if the cooling is fairly good. Check that there is enough ether during the whole run.

2.8. Crude fiber Determination

A. Principle:

A fat free or low fat content sample is treated with boiling sulfuric acid and subsequently with boiling potassium hydroxide or sodium hydroxide. The residue after subtraction of the ash is regarded as fiber (see fig.2.4) (Winton, 1947).

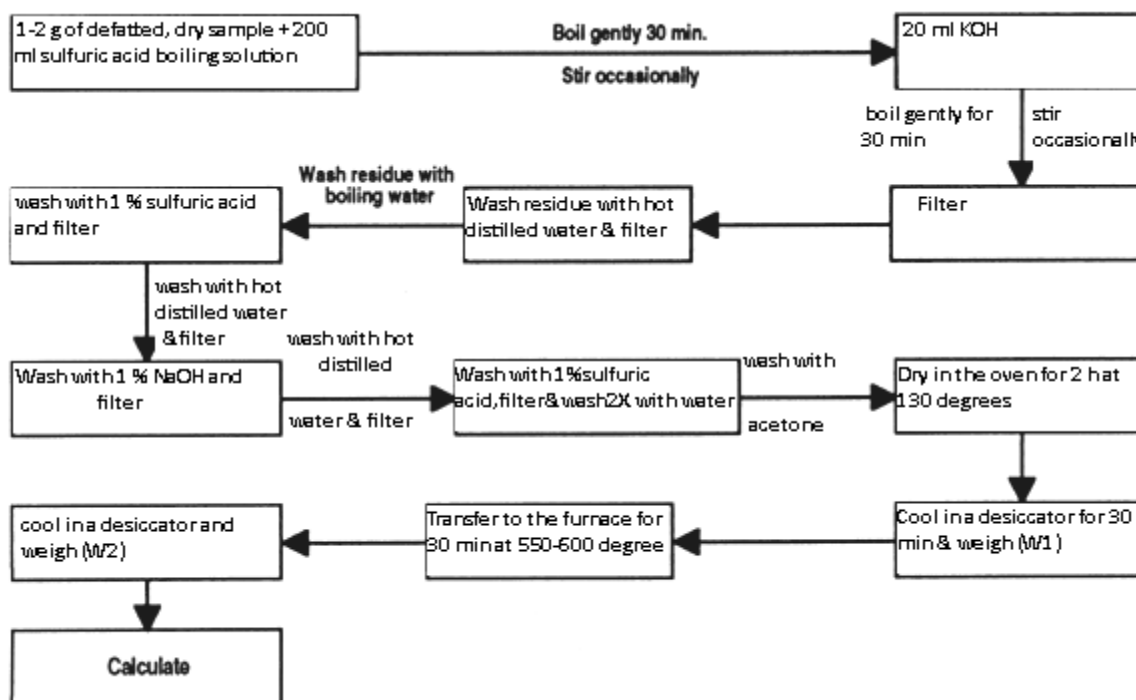


Figure 2.4. Flow chart for determination of crude fiber.

B. Materials and Reagents:

- Beaker, watch glass, stirrer
- Sintered glass crucible

- Vacuum pump
- Oven, desiccator
- Furnace
- 1.25 % sulfuric acid (13 g conc. H_2SO_4 (98%) weighed and made up to 1 liter)
- 1 % sulfuric acid
- 1 % NaOH
- 28 % KOH
- Anhydrous acetone

C. Procedure:

i. Boiling:

1. Weigh 1-2 g of dry sample into a 600 ml beaker.
2. Add 200 ml 1.25 % H_2SO_4 . Boil for 30 minutes placing a watch glass over the mouth of the beaker. Heat gently keeping the level constant with distilled water.
3. After 30 min, add 20 ml of 28 % KOH. Boil gently for a further 30 min, stirring occasionally.

ii. Filtration:

1. Cover the bottom of a sintered glass crucible with 10 mm sand and wet the layer of sand with a little distilled water.
2. Pour solution from beaker into sintered glass crucible and then turn on vacuum pump.
3. Rinse beaker walls with hot, distilled water several times, transferring washings to crucible. Filter.

iii. Washing:

1. Wash residue in crucible with hot distilled water. Filter.
2. Repeat as 1.
3. Wash with 1 % H₂SO₄. Filter.
4. Wash with hot distilled water. Filter.
5. Wash with 1 % NaOH. Filter.
6. Wash with hot, distilled water. Filter.
7. Wash with 1 % H₂SO₄. Filter.
8. Wash with twice more with hot, distilled water. Filter.
9. Wash residue with anhydrous acetone.

Note: do not switch vacuum pump on until all the washing liquid has been poured onto the residue.

iv. Drying and combustion:

1. Dry crucible for 2 hrs in the electric oven at 130°C.
2. Cool for 30 min in a desiccator and then weigh (W₁).

Note: Weigh immediately after taking out from the desiccators since the crude fiber is hygroscopic.

3. Transfer crucible to the furnace for 30 min at 550-600°C.
4. Cool in a desiccator and weigh (W₂).

D. Calculation:

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

Where: W₁ = crucible weight before drying

W_2 = crucible weight after drying

W_3 = sample dry weight

M = % moisture content of the sample

2.9. Soluble Carbohydrate Determination

Principle:

The percentage of soluble carbohydrate which consists of monosaccharides, oligosaccharides and polysaccharides is calculated by difference (Muller and Tobin, 1980).

%Soluble carbohydrate = % DM - (% EE + % CP + % ash + % CF).

Where: DM = dry matter

EE = ether extract or crude fat

CP = crude protein

CF = crude fiber

2.10. Ash Determination

A. Principle:

Organic matter is burned off at as low a temperature as possible and the inorganic material remaining is cooled and weighed. Heating is carried out in stages, first to drive the water, then to char the product thoroughly and finally to ash at 550°C in a muffle furnace (AOAC, 1990).

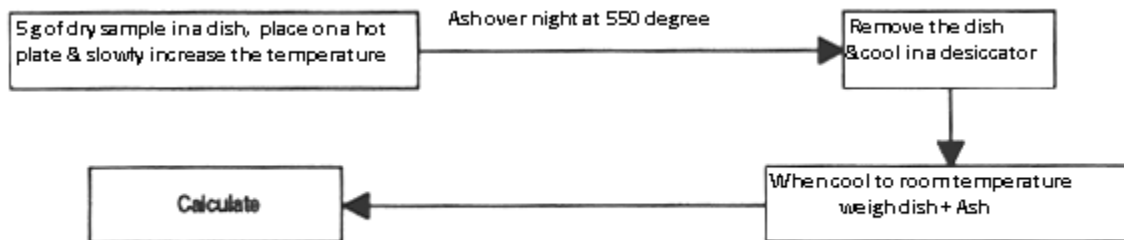


Figure 2.5. Flow chart for determination of ash content.

B. Materials:

- Muffle furnace. Thermostatically controlled at 550°C
- Electric hot plate. With thermostatic control
- Silica dishes. Diameter 8 cm, depth 2.5 cm (Note 1)
- Desiccators. With fresh silica gel desiccant.

C. Procedure:

1. Place the requisite number of silica dishes into a muffle furnace for 15 min or more.
2. Remove the dishes, cool in desiccator for at least 1 hr and, when cool to room temperature, weigh each dish.
3. Accurately weigh 5 g of material into each dish.
4. Place the dishes on a hot plate under a fume-hood and slowly increase the temperature (Note 2) until smoking ceases and the samples become thoroughly charred.
5. Place the dishes inside the muffle furnace, as near to the center as possible and ash overnight at 550°C.
6. Remove the dishes from the muffle and place in a desiccator for at least 1 hr to cool (the ash should be clean and white in appearance. If traces of carbon are still evident, cool the dish, add a few ml of water and stir with a glass rod to break up the ash. Dry on a steam bath and then return to the muffle furnace for 24 hrs).
7. When cool to room temperature reweigh each dish + ash.
8. Calculate the weight of ash.

D. Calculation:

$$\% \text{ Ash} = (W_2/W_1) \times 100$$

Where: W_1 = weight of sample

W_2 = weight of ash

2.11. Atomic absorption analysis of elements

A. Principle:

After removal of organic material by dry ashing or wet digestion the residue is dissolved in dilute acid. The solution is sprayed into the flame of Atomic Absorption Spectrophotometer (AAS) and the absorption or emission of the metal to be analyzed is measured at a specific wavelength (see fig. 2.6) (Issac and Johnson, 1975).

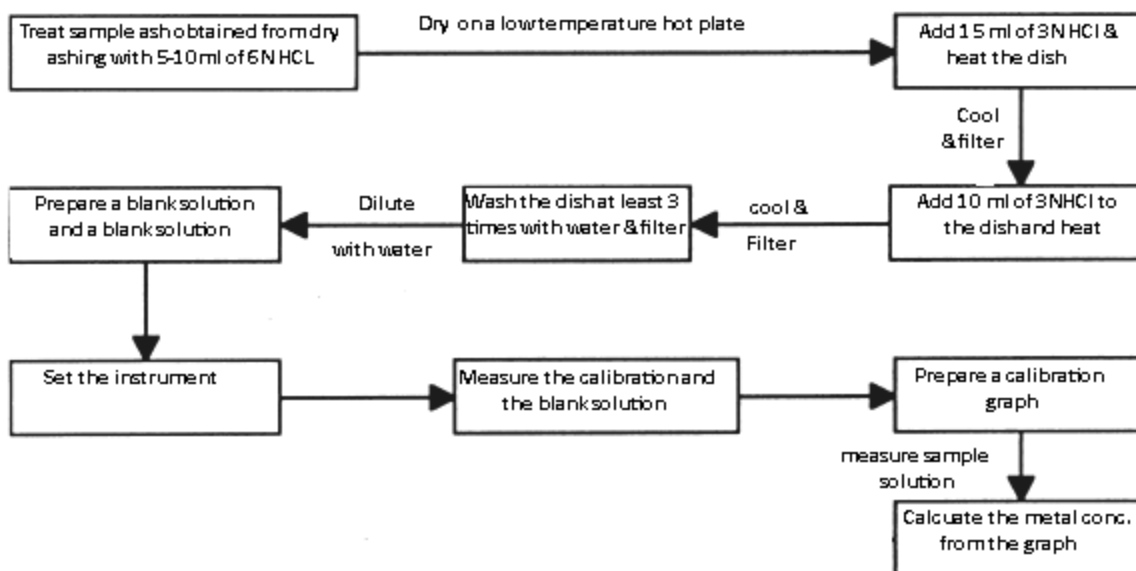


Figure 2.6. Flow chart for atomic absorption analysis of elements

B. Materials and Reagents:

- Atomic absorption spectrophotometer
- Glassware
- Hydrochloric acid: 6 N, 3 N, 0.3 N.
- Lanthanum chloride, 10 %.
- Distilled water. Good quality or deionized water.

- Filter paper. Wash the filter papers before use with 3N hydrochloric acid to remove trace of metals.
- Standard solutions. Dilute the stock standard solutions with 0.3 N HCl to concentrations that fall within the working range.

C. Procedure:

Ash obtained from dry ashing:

1. Treat the ash with 5-10 ml of 6N HCl to wet it completely, and carefully take to dryness on a low temperature hot plate.
2. Add 15 ml of 3N HCl and heat the dish on the hot plate until the solution just boils.
3. Cool and filter through a filter paper into a graduated flask retaining as much of the solids as possible in the dish.
4. Add 10 ml of 3N HCl to the dish and heat until the solution just boils.
5. Cool and filter into the graduated flask.
6. Wash the dish at least three times with water; filter the washings into the flask.
7. If calcium is to be determined add 5 ml of lanthanum chloride solution per 100 ml of solution.
8. Cool and dilute the contents of the flask to the mark with water.
9. Prepare a blank by taking the amount of reagents through the above instructions.

Calibration of the apparatus and measurement of the samples:

1. Set the apparatus according to the instruction of the instrument.
2. Measure the calibration solutions and the reagent blank solution.
3. While running the samples, periodically check that the calibration values remain constant.

4. For the metals required prepare a calibration curve by plotting the absorption or emission values against the metal concentration in $\mu\text{g/ml}$.

D. Calculation:

Read from the graph the metal concentration ($\mu\text{g/ml}$) that correspond to the absorption or emission values of the sample and blank.

$$\text{Metal content (mg/100 g)} = [(a - b) \times V] / 10 W$$

$$\text{Metal content (mg/kg)} = [(a - b) \times V] / W$$

Where: W = weight of samples in grams

V = volume of extract in ml

a = $\mu\text{g/ml}$ of metal in processed sample from calibration curve

b = $\mu\text{g/ml}$ reading of the blank solution from calibration curve

2.12. Flame Phometric method for potassium determination

A. Principle:

After wet digestion of the sample, the potassium content is measured by flame photometer (see fig. 2.7) (AOAC, 1984).

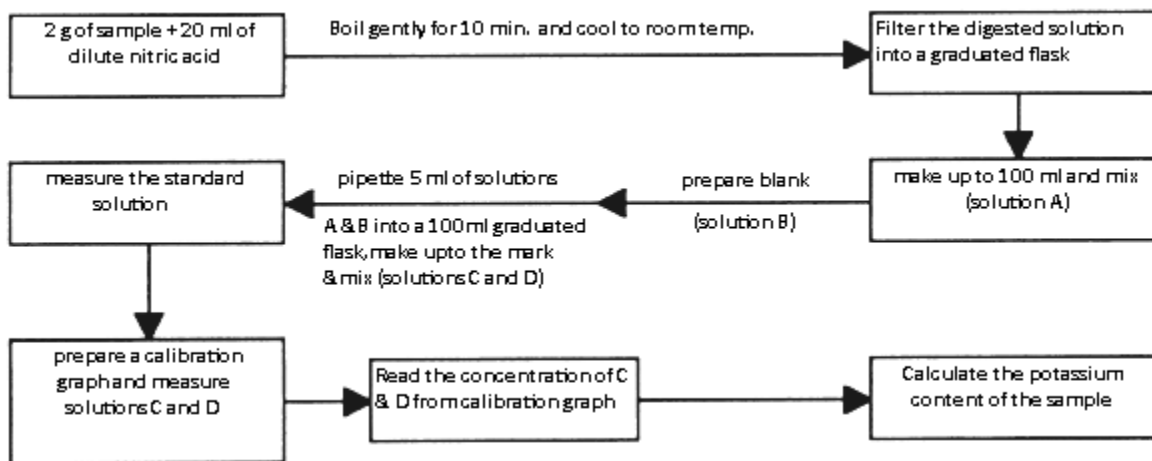


Figure 2.7. Flow chart for determination of potassium.

B. Materials and Reagents:

- Flame filter photometer.
- Glassware
- Filter paper
- Nitric acid. Dilute nitric acid (sp.gr. 1.42) 1:1 with water.
- Distilled water (or deionized water).
- Acid washing liquid for glass ware. Dilute nitric acid (sp.gr. 1.42) to 100 ml with distilled water.
- Potassium stock standard. 100 mg K/L. dry some potassium chloride at 105 °c for 2 hrs. weigh 190.7 mg of the dried product in water and make up to 1000 ml. keep this solution in a polyethylene bottle.
- Potassium diluting solution. Weigh 3.8g of KCl. Dissolve in water and dilute to 1 L.
- Working standard solutions. Pipette 0, 5, 10, 15, 20 ml of KCl stock solution into 100 ml graduated flasks.. Dilute to the marks. These solutions contain 0, 0.5, 1.0, 1.5, and 1 mg K/100 ml respectively.

C. Procedure:

i. Preparation of the sample:

1. Weigh 2 g of the sample on a filter paper.
2. Fold up the filter paper and transfer into a 250 ml Kjeldahl flask.
3. Add 20 ml of 1:1 dilute nitric acid.
4. Boil gently for about 10 min and cool to room temperature.
5. Filter the digested solution through a filter paper into a 100 ml graduated flask. Wash the kjeldahl flask and the filter paper 3 times each with a 100 ml of water.
6. Make up to 100 ml and mix (solution A).
7. Prepare a blank following the above instruction (solution B).

ii. Dilution of solutions:

Pipette 5 ml of solution A and B into a 100 ml graduated flask, make up to the mark and mix (solution C and D).

iii. Measurement:

1. Measure the standard solution.
2. Prepare a calibration graph.
3. Measure the solutions C and D.

D. Calculation:

Read the concentrations (mg/100 ml) of solutions C and D from the calibration graph.

Let: The concentrations (mg/100 ml) be c and d.

Weight (g) of the sample = w

Then: K content (mg/100 g product) = $[(c - d) \times 20 \times 100]/w$

Where: 20 = dilution factor (100/5).

100 = final volume

2.13. Phosphorous Determination

A. Principle:

The phosphorous is converted to phosphomolybdate, which is reduced to a blue molybdenum compound by aminonaphtholsulphonic acid to give a blue molybdenum compound. The amount of molybdenum blue formed in this reaction is proportional to the amount of phosphorous present in the sample and the absorbance is measured in a spectrophotometer at 660 nm against distilled water (see fig. 2.8) (AOAC, 1984)

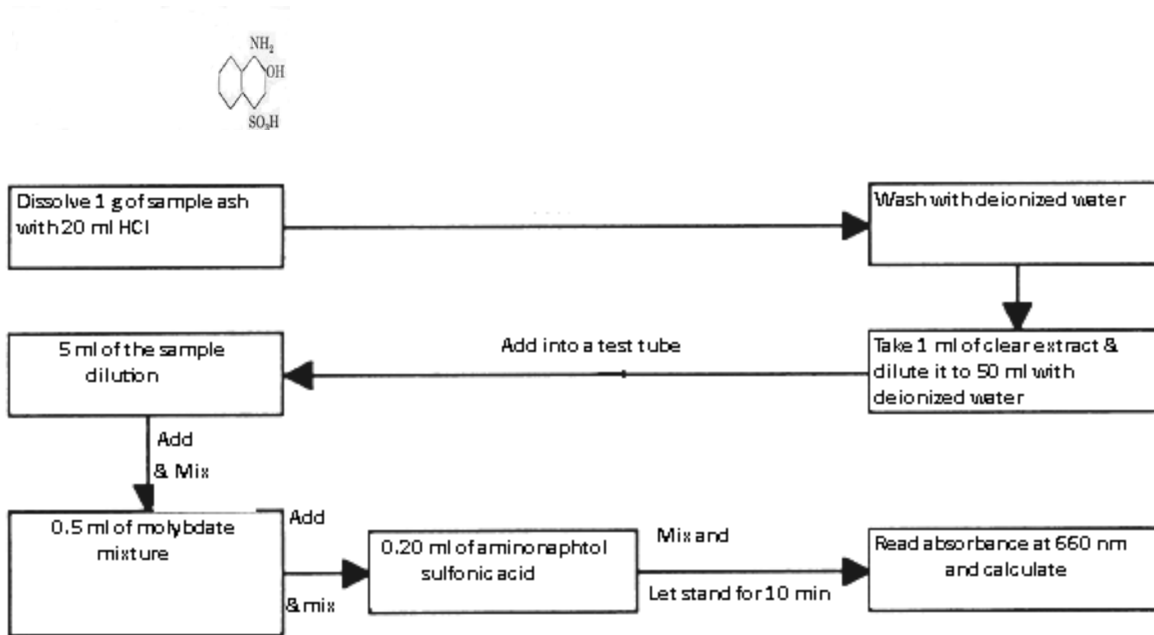
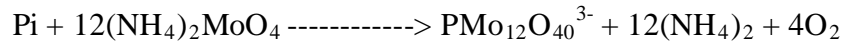


Figure 2.8. Flow chart for determination of phosphorus.

B. Materials and Reagents:

1. Spectrophotometer

2. volumetric flask
3. Sulfuric acid 10 N: dilute 25 ml conc.sulfuric acid to 90 ml with water.
4. Molybdate reagent: take 75 ml of 10 N H_2SO_4 in a 250 ml volumetric flask. Dissolve in a beaker 6.25 g of ammonium molybdate in about 50 ml water. Transfer this solution into the volumetric flask. Rinse the beaker with water and transfer the washings to the volumetric flask. Finally make the volume up to 250 ml with water.
5. Sodium sulphite, 20 %. Dissolve 2 g in 10 ml of water.
6. Sodiumbisulphite, 15 %. Dissolve 7.5 g in 50 ml of water.
7. Aminonaphtholsulphonic acid. In a 100 ml glass stoppered bottle weigh 100 mg of aminonaphtholsulfonic acid and 39 ml of sodium bisulphate solution. Then add 1 ml of sodium sulphite solution and shake again. If the solution is not clear, add an additional 0.2 ml of sodium sulphite solution and shake again. If necessary, this is repeated until a clear solution is obtained. Only 0.2 ml of sodium sulphite solution is added each time. This reagent is kept in the cold and is stable for four weeks only. A new solution must be prepared every four weeks.
8. Standard phosphorous solution: Dissolve 438.8 mg of KH_2PO_4 (water free) in some water in a 100 ml volumetric flask, add 1 ml conc. H_2SO_4 and dilute to the mark with water. This solution gives $1000\mu\text{g P/ml}$.

C. Procedure:

- a. Dissolve and digest the total ash of 1 gm of sample with 50 ml iron free HCl and wash it quantitatively with deionized water into a 100 ml volumetric flask.
- b. Take 1 ml of clear extract and dilute it to 100 ml with deionized water.
- c. Add into a test tube:

- a. 5 ml (duplicates) of the sample dilution
- b. 0.5 ml molybdate mixture
- c. 0.20 ml aminonaphtholsulphonic acid. Mix.
- d. Let stand for 10 min.
- e. Read absorbance (A) at 660 nm against distilled water.

With each analysis do simultaneously

1. Standard: As above but 5 ml of working standard in place of the sample dilution (reading A_S).
2. Blank: As above but put 5 ml deionized water in place of the sample dilution (reading A_B).

D. Calculation:

$$P \text{ mg/100g} = \frac{A_T - A_B}{\text{Slope}} \times \frac{50 \times 100 \times 100}{W \times 1000}$$

Where: A_T = absorbance of test

A_B = absorbance of blank

50 = ash dilution

100 = dilution factor

W = weight of sample

1000 = to convert μg into mg

2.14. Determination of Oxalate

A. Principle:

The extract of oxalate (as oxalic acid) is determined by titrating against 0.1 N KMnO_4 solution.

(See fig. 2.9) (AOAC, 1984).

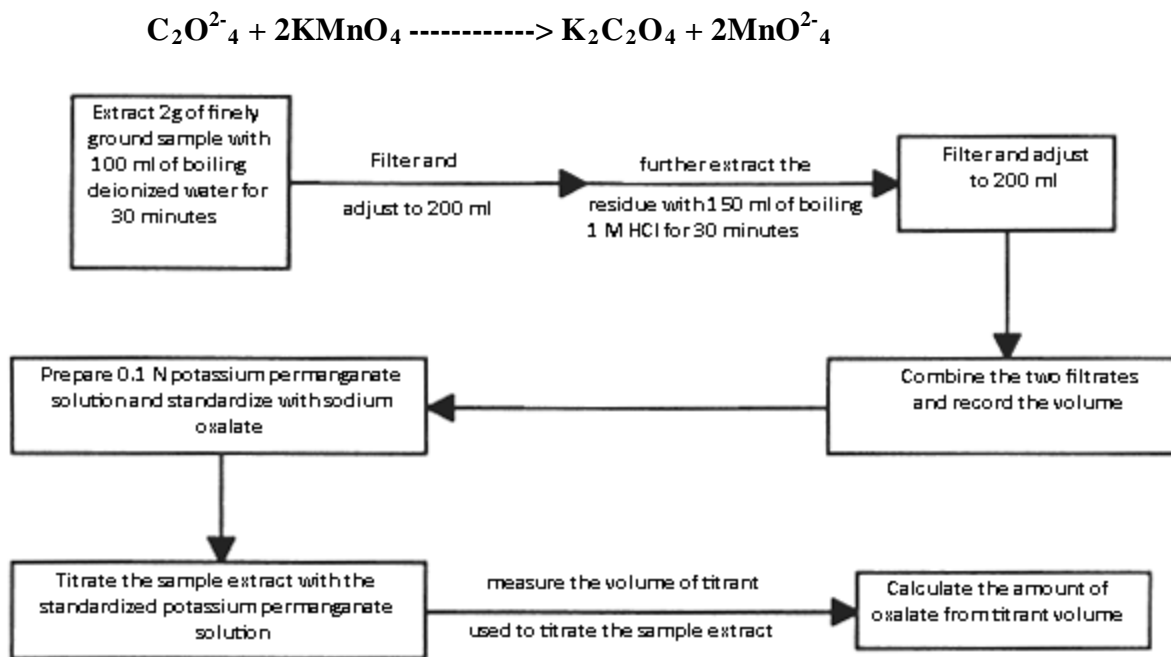


Figure 2.9. Flow chart for determination of oxalate by titration

B. Materials and Reagents:

- Analytical balance
- Thermometer
- Water bath
- Desiccator
- Tissue paper
- Filter paper

- Spatula
- Beaker
- Burette
- Analytical reagent grade potassium permanganate
- Analytical reagent grade sodium oxalate
- Sulfuric acid
- Deionized water

C. Procedure:

i. Extraction of Oxalate

1. Two grams of finely ground sample extract with 100 ml of boiling deionized water for 30 min.
2. Filter and adjust to 200 ml.
3. Further extract the residue with 150 ml of boiling 1 M HCl for 30 min.
4. Filter and adjust to 200 ml.
5. Combine the two filtrates.
6. Record the volume of the two extract.
7. The amount of oxalate will be determined by titration of the extract with potassium permanganate.

ii. Preparation of 0.1 N potassium permanganate

1. Weigh out about 3.2-3.25 g Analytical Reagent grade potassium permanganate on a watch glass. Transfer it into a 1500 ml beaker.

2. Add 1 liter deionized water. Cover the beaker with a clock glass. Heat the solution to boil gently for 15-20 min and allow the solution to cool to room temperature.
3. Filter the solution through a funnel.
4. Collect the filtrate in a vessel which has been cleaned with chromic acid mixture and then thoroughly washed with distilled water.
5. The filtered solution should be stored in a clean, glass stoppered bottle. Be kept in a bottle of dark or in diffuse light except when in use.
6. Alternatively, it may be kept in a bottle of dark brown colored glass.
7. Standardize the solution with sodium oxalate.

iii. Standardization of permanganate solutions with sodium oxalate

1. Dry some Analytical reagent grade sodium oxalate at 105-110°C for 2 hrs and allow it to cool in a covered vessel in a desiccator.
2. Weigh out accurately from a weighing bottle about 0.3 g of the dry sodium oxalate into a 600 ml beaker.
3. Add 240 ml of recently prepared distilled water and 12.5 ml of concentrated sulfuric acid (caution) or 250 ml of 2N sulfuric acid.
4. Cool to 25-30°C and stir until the oxalate has dissolved.
5. Put 0.1 N potassium permanganate into the titration burette.
6. Add 90-95 % of the required quantity of permanganate solution from a burette at a rate of 25-35 ml per minute while stirring slowly.
7. Heat to 55-60°C (use a thermometer as a stirring rod) and complete the titration by adding permanganate solution until a faint pink color persists for 30 seconds.

8. Add the last 0.5-1 ml drop wise, with particular care to allow each drop to become decolorized before the next is introduced.
9. Repeat the determination with two other similar quantities of sodium oxalate.

D. Calculation:

$$\% \text{ oxalate} = \frac{V_1 \times N \times \text{Eq.wt}}{V_2 \times W}$$

Where: V_1 = volume of permanganate

N = normality of permanganate

Eq.wt = equivalent weight of permanganate = 26.8

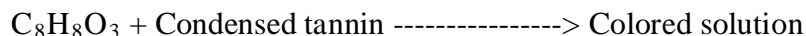
V_2 = extract volume of oxalate

W = weight of the sample

2.15. Condensed Tannin Determination

A. Principle:

Condensed tannin will be extracted from the sample and allowed to react with vanillin (4-Hydroxy-3-methoxybenzaldehyde, $C_8H_8O_3$) to form a colored solution which has absorption maxima at 500 nm; the concentration of the condensed tannin will be determined from a standard curve prepared from D-catechin (see fig. 2.10) (Burns, 1971).



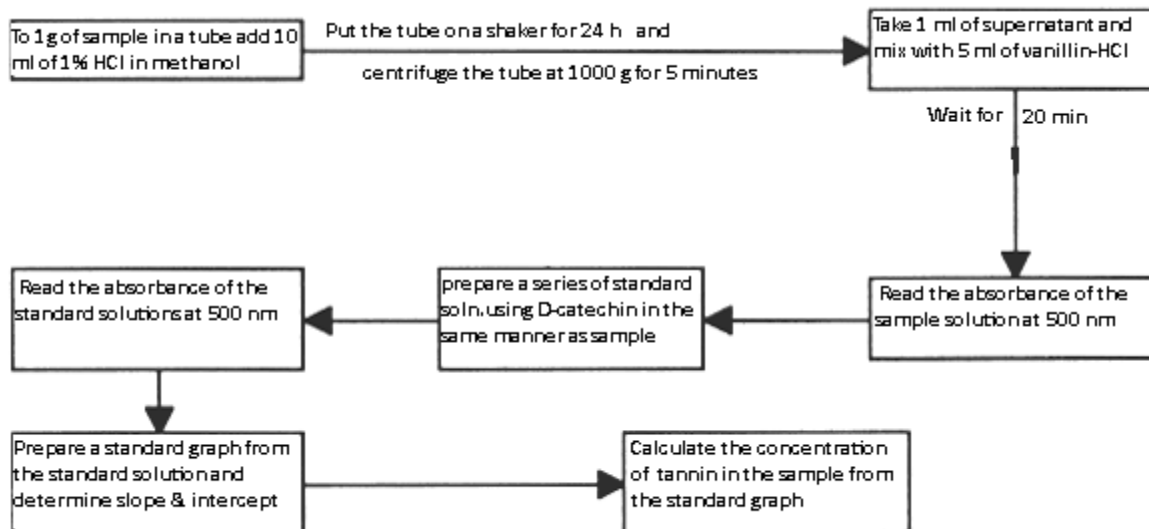


Figure 2.10. Flow chart for determination of tannin.

B. Materials and Reagents:

- Analytical balance
- Centrifuge
- Mechanical shaker
- Cuvette
- Micro pipette
- Spectrophotometer
- Tissue paper
- Test tube
- Spatula
- Hydrochloric acid
- Vanillin
- D-catechin
- Methanol

C. Procedure:

i. Extraction:

1. Weigh 1 g of sample in a screw cap test tube.
2. Add 10 ml of 1 % HCl in methanol to the tube containing sample.lid
3. Put the tube on mechanical shaker for 24 hr at room temperature.
4. Centrifuge the tube at 1000 g for 5 min.
5. Take 1 ml of supernatant and mix with 5 ml of vanillin-HCl reagent in another test tube.
6. Wait for 20 min to complete the reaction.
7. Read the absorbance at 500 nm.

ii. Standard solution preparation:

D-catechin is used as the standard value of tannin in mg D-catechin/ g of sample.

1. Weigh 40 mg D-Catechin and dissolve in 100 ml of 1 % HCl in methanol.
2. Take 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of stock solution in a test tube.
3. Adjust volume of each tube (NO.3) to 1 ml with 1% HCl in methanol.
4. Add 5 ml of vanillin-HCl reagent in each tube (4 % vanillin in methanol and 8% HCl in methanol in 1:1 proportion).
5. Wait for 20 min to complete the reaction.
6. Read the absorbance at 500 nm.
7. Reference curve is prepared from the series of standard solution.

Calculation:

Using the equation for a straight line, $y = mx + b$

Where: $y =$ absorbance

m = slope

x = concentration

b = intercept

The concentration (x) in mg/ml can be calculated as: $x = \frac{y - b}{m}$

Since the concentration is in mg/ml this concentration should be converted into mg/g, to convert this density of the sample-extraction solvent is included in the formula.

Therefore the tannin concentration in mg/g is calculated as:

$$\text{Tannin in mg/g} = \frac{\text{Absorbance} - \text{Intercept}}{\text{Slope} \times W} \times \frac{1}{\rho}$$

Where: ρ = density of the sample-extraction solvent mixture = 0.793g/ml

W = weight (given in grams) of the sample

2.16. Phytate Determination

A. Principle:

After extraction with HCl, phytate present in the sample reacts with Wade reagent and the absorbance of the resulting solution will be read on a spectrophotometer at 500 nm and the concentration of phytate present in the sample will be determined from phytic acid standard curve (see fig. 2.11) (Vaintraub and Lapteva, 1988).

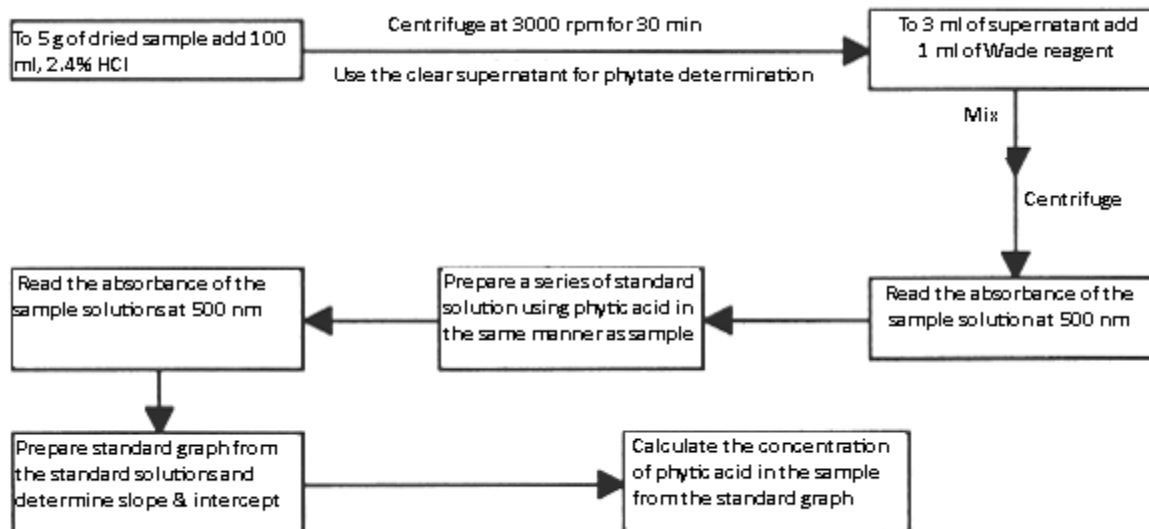


Figure 2.11. Flow chart for determination of phytate.

B. Materials and Reagents:

- spectrophotometer
- Analytical balance
- Centrifuge
- Vortex mixer
- Cuvette
- Pipette
- Water bath
- Hydrochloric acid
- Analytical reagent grade sodium salts of phytic acid
- $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$
- Sulfosalicylic acid

C. Procedure:

i. Extraction:

1. 0.5 gm of dried sample will be extracted with 10 ml 2.4 % HCl for 1 hr at an ambient temperature and centrifuge (3000rpm/30 min). The clear supernatant will be used for phytate determination.
2. 1 ml of Wade reagent (0.03 % solution of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ containing 0.3 % sulfosalicylic acid in water) will be added to 3 ml of the sample solution and the mixture will be centrifuged.
3. The absorbance at 500 nm will be measured using spectrophotometer.
4. The phytate concentration will be calculated from the difference between the absorbance of the control (3 ml of water + 1 ml of Wade reagent) and that of assayed sample.
5. The concentration will be calculated using phytic acid standard curve and the result will be expressed as phytic acid in mg/100 g fresh weight.

ii. Standard solution preparation

1. 3 ml of standard will be pipetted into 15 ml centrifuge tubes with 3 ml of water used as a zero level (blank).
2. Series of standard solution will be prepared containing 5-40 μg /ml phytic acid in 0.2 N HCl (Latta and eskin.1980).
3. 1 ml of the Wade reagent will be added to each tube and the solution will be mixed on a vortex mixer for 5 seconds.
4. The mixture will be centrifuged for 10 min and the absorbance of the supernatant will be read at 500 nm by using water to zero the spectrophotometer.

D. Calculation:

Using the equation for a straight line: $y = mx + b$,

Where: $y =$ absorbance

$m =$ slope

$x =$ concentration

$b =$ intercept

The concentration (x) in $\mu\text{g/ml}$ can be calculated as: $x = \frac{y - b}{m}$

Since the concentration is in $\mu\text{g/ml}$ this concentration should be converted into $\mu\text{g/g}$, to convert this density of the sample-extraction solvent is included in the formula.

Therefore, the phytic acid concentration in $\mu\text{g/g}$ will be calculated as:

$$\text{Phytic acid in } \mu\text{g/g} = \frac{\text{Absorbance} - \text{Intercept}}{\text{Slope} \times W} \times \frac{1 \times 10}{\rho \quad 3}$$

Where: $\rho =$ density of the sample-extraction solvent mixture = 1g/ml

$W =$ weight (given in gram) of the sample

$10 =$ volume of extraction solvent

$3 =$ volume of sample reacted with wade reagent

2.17. Statistical analysis

Each analysis was performed in duplicate and if the difference between the duplicate was greater than 0.5%, another analysis was performed and the result which was nearer to the third analysis was taken from the duplicate.

Data were analyzed using the statistical analysis system SPSS (SPSS Software for windows release 16.0; SPSS Inc., Chicago, IL, USA). Analysis of variance, T test and mean were calculated. Significance was set at 5% level (p-value = 0.05).

3. RESULT

The proximate composition of yellow and white Dioscorea tubers is presented in Table 3.1. The proximate composition reveals high content of moisture, ash and carbohydrate with a comparable similarity between the two tubers ($P>0.05$). There is also no significant difference in the fat, protein and fiber contents of the two tubers ($P>0.05$).

Table 3.1 Proximate composition of raw, boiled and fried yellow and white Dioscorea tubers.

Sample Type		Moisture %	Dry Matter%	Ash %	Fat %	Protein %	Fiber %	Carbohydrate %
Yellow Yam	Raw	69.56 ±0.2 c	30.44±0.1 c	4.10±0.01	0.35±0.01 c	1.85±0.04 c	3.19±0.05 b,c	20.95±0.1 b,c
	Boiled	71.49 ±0.4	31.51±0.1	2.49±0.02	0.35 ±0.01	1.70 ±0.01	16.1 ±0.01 b	10.87 ±0.01a,b
	Fried	5.83±0.2 c	94.17±0.5 c	2.47 ±0.01	9.61 ±0.01 c	4.77 ±0.2 c	25.9 ±0.02a,c	60.86±0.01 c
White Yam	Raw	73.44 ±0.5 c	26.56±0.2 c	4.62±0.01	0.18±0.01 c	2.03±0.03 c	2.68±0.03 b,c	17±0.06 c
	Boiled	74.92 ±0.4	30.86±0.1	2.64 ±0.01	0.15 ±0.01	2.88 ±0.02	5.27 ±0.01a,b	19.92±0.01 a
	Fried	3.59 ±0.2 c	96.41±0.4 c	2.64 ±0.02	9.78 ±0.02 c	5.36 ±0.1 c	8.51 ±0.02a,c	70.12±0.02 c

All values are means of duplicate determinations expressed in fresh weight basis. ± denotes standard error.

a. Significant difference ($P<0.05$) between the two varieties

b. significant difference ($p<0.05$) between raw and boiled samples of the same variety

c. significant difference ($p<0.05$) between raw and fried samples of the same variety

Table 3.2 shows the mineral composition of the investigated yam tubers.

Table 3.2 Mineral composition of raw, boiled and fried yellow and white Dioscorea tubers.

Sample Type		Calcium (mg/100g)	Phosphorous (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)	Copper (mg/100g)	Potassium (mg/100g)
Yellow Yam	Raw	52.55 ±0.01 b,c	12.89 ±0.02	1.95 ±0.01	1.01 ±0.01	0.29 ±0.01	53.33 ±0.02 c
	Boiled	90.22 ±0.02 a,b	7.11 ± 0.02	4.26 ± 0.01	0.79 ± 0.01	0.29 ± 0.01	44.30 ± 0.02
	Fried	77.63 ±0.02 c	7.08 ±0.01	4.59 ±0.01	0.63 ±0.02	0.27 ±0.01	145.55 ±0.03 c
White Yam	Raw	60.52 ±0.01 c	12.95 ±0.02	3.25 ±0.01	1.10 ±0.01	0.29 ±0.01	52.41 ±0.01 c
	Boiled	67.78 ±0.01 a	7.84 ±0.01	3.68 ±0.01	1.11 ±0.01	0.21 ±0.01	42.72 ±0.02
	Fried	85.78 ±0.03 c	11.83 ±0.02	4.12 ±0.01	0.68 ±0.01	0.25 ±0.01	145.26 ±0.02 c

All values are means of duplicate determinations expressed in fresh weight basis. ± denotes standard error.

a. Significant difference (P<0.05) between the two varieties

b. significant difference (p<0.05) between raw and boiled samples of the same variety

c. significant difference (p<0.05) between raw and fried samples of the same variety

Calcium and potassium are the major minerals found in both tubers. There were no significant (P>0.05) differences in the calcium, phosphorus, iron, zinc, copper and potassium composition of the two tubers.

The anti-nutritional factors phytate, tannins, and oxalate, are presented in Table 3.3.

Table 3.3 Anti nutrient composition of raw, boiled and fried yellow and white Dioscorea tubers.

Sample Type		Phytate (mg/100g)	Tannin (mg/100g)	Oxalate (mg/100g)
Yellow yam	Raw	38.2±0.002 b,c	22.2 ±0.001 b,c	0.006 ±0.001
	Boiled	21.2 ± 0.001 b	BDL b	BDL
	Fried	22.4 ±0.001 c	10.9 ±0.001 a,c	BDL
White yam	Raw	34.8 ±0.002 b,c	28.8 ±0.002 b,c	0.006 ±0.001
	Boiled	20.8 ±0.001 b	BDL b	BDL
	Fried	21.1 ±0.001 c	2.53 ±0.001 a,c	BDL

All values are means of duplicate determinations expressed in fresh weight basis. ± denotes standard error.

BDL= Below Detection Limit

a. Significant difference (P<0.05) between the two varieties

b. significant difference (p<0.05) between raw and boiled samples of the same variety

c. significant difference (p<0.05) between raw and fried samples of the same variety

There is no statistically significant difference ($p>0.05$) in the phytate, tannin and oxalate contents of the two tubers though the white yam contains relatively higher amounts of phytate and tannin.

The oxalate content in both tubers is very low and similar.

Many foods including the root crops cannot be digested in their natural state and hence requires cooking, which increases the palatability, the keeping qualities and the safety of the foods from potentially toxic substances (Bradbury and Holloway, 1988). The effect of boiling and frying on the proximate composition, mineral and anti nutritional factor content is presented in figures 3.1-3.6.

Effect of boiling and frying on the proximate composition of yellow and white Dioscorea tubers is presented in figures 3.1 and 3.2.

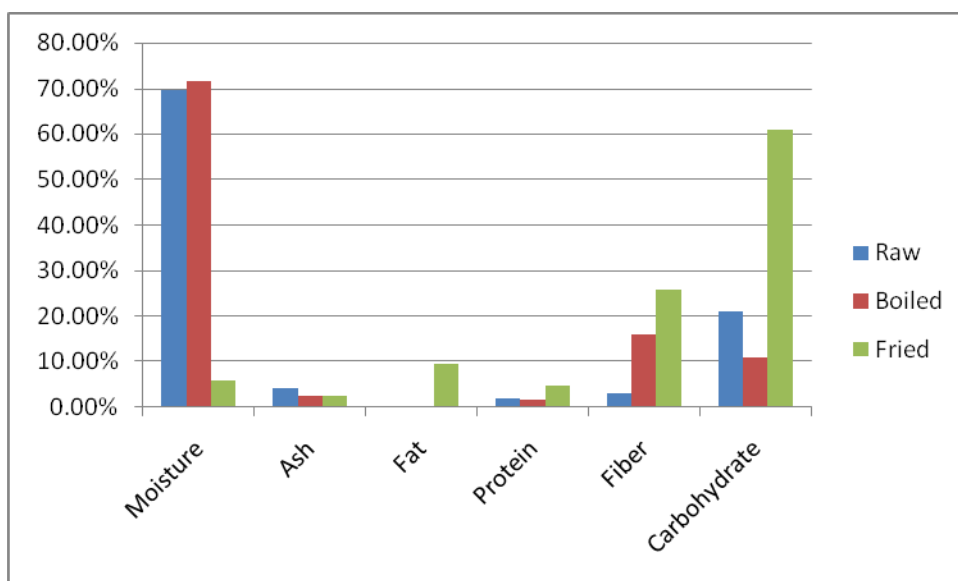


Fig 3.1. Effect of boiling and frying on the proximate composition of yellow yam

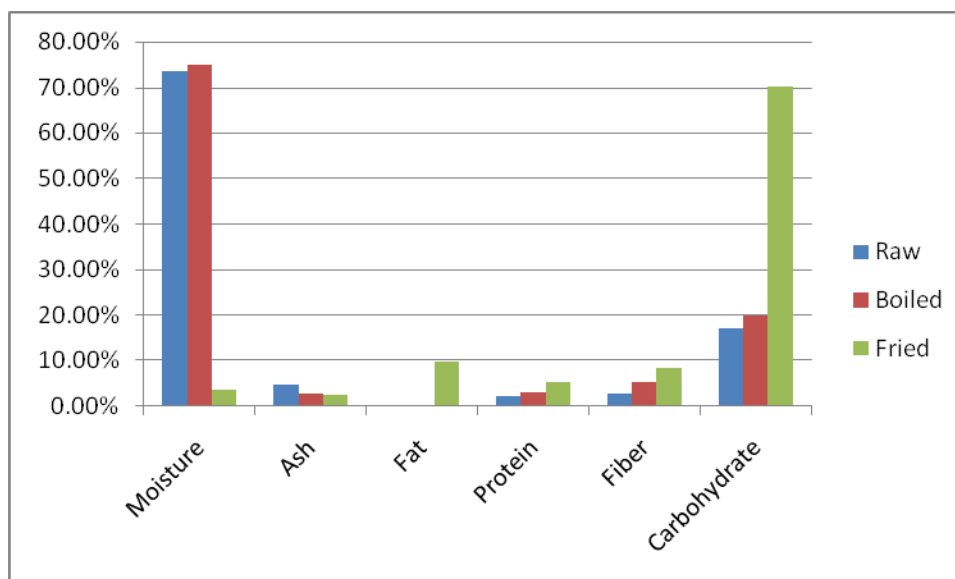


Fig 3.2. Effect of boiling and frying on the proximate composition of white yam

The moisture contents are increased whereas ash and fat contents are decreased by boiling but not statistically significant ($p > 0.05$) whereas the fiber content is significantly increased ($p < 0.05$)

in both tubers after boiling. The protein content is decreased in the yellow yam whereas it is increased in the white yam. However, in both cases it is not significant ($p>0.05$). There is insignificant ($p>0.05$) increase in the carbohydrate content in the white tuber and a significant decrease ($p<0.05$) is observed in the yellow tuber.

The moisture and ash contents of both tubers are significantly decreased ($p<0.05$) after frying.

The fat content is significantly increased ($p<0.05$) in both tubers.

Effect of boiling and frying on the mineral composition of yellow and white Dioscorea tubers is presented in figures 3.3 and 3.4.

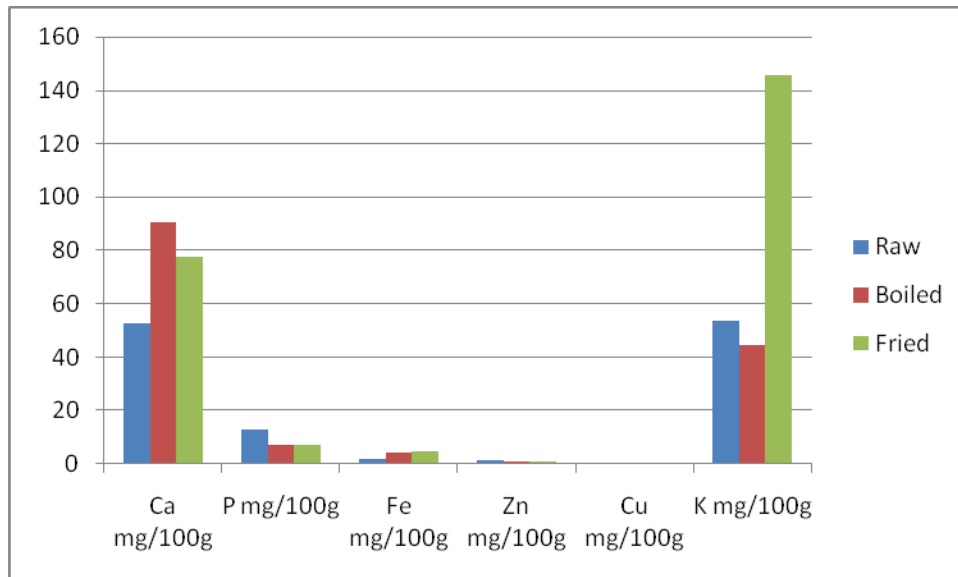


Fig 3.3. Effect of boiling and frying on the mineral composition of yellow yam

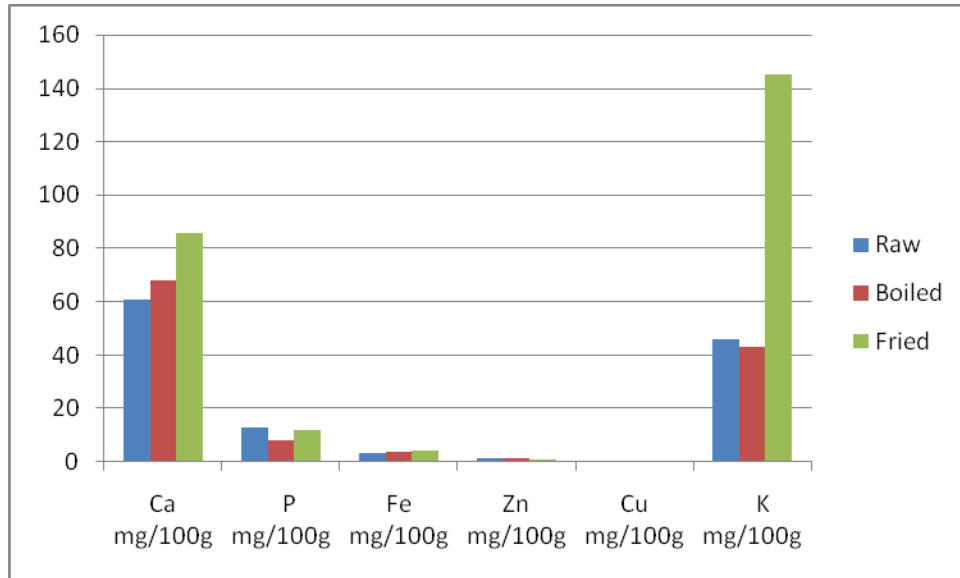


Fig 3.4. Effect of boiling and frying on the mineral composition of white yam

Boiling significantly increases ($p < 0.05$) the calcium content in the yellow tuber where as the contents of phosphorous and potassium decreased in both the tubers.

Frying significantly increases ($p < 0.05$) the calcium and potassium contents of both the tubers.

Effect of boiling and frying on the anti nutritional factors composition of yellow and white Dioscorea tubers is presented in figures 3.5 and 3.6.

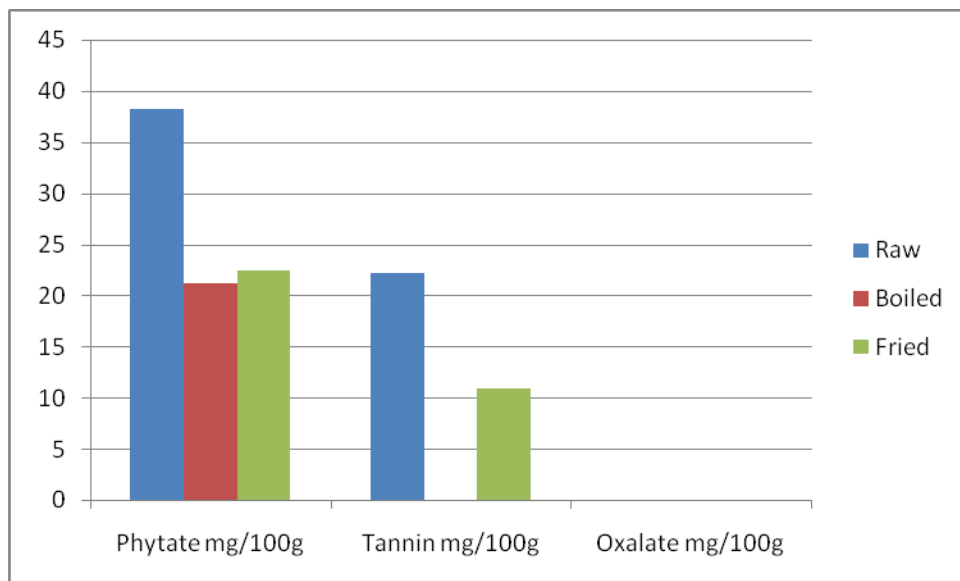


Fig 3.5. Effect of boiling and frying on anti nutritional composition of yellow yam

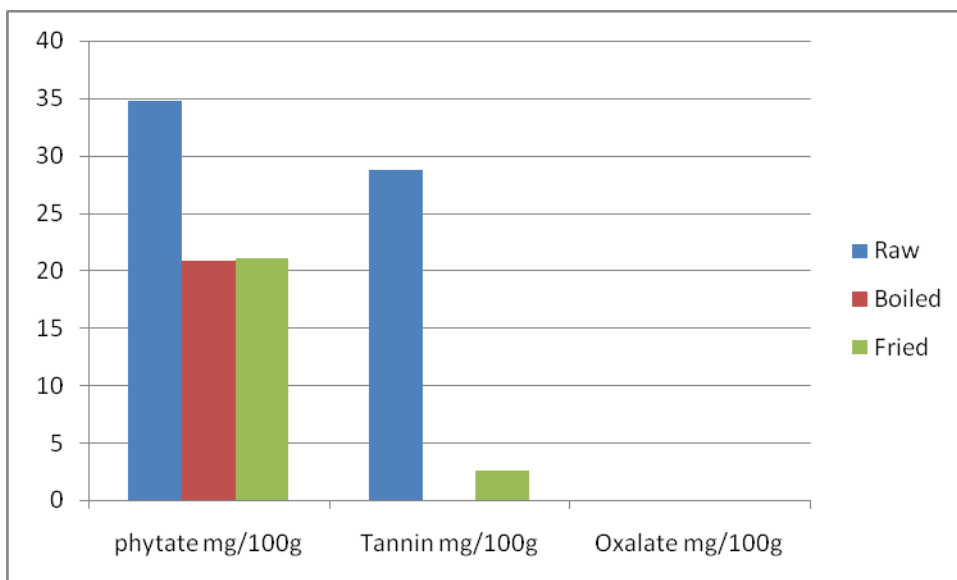


Fig 3.6. Effect of boiling and frying on the anti nutritional composition of white yam

Boiling significantly reduced ($P < 0.05$) the level of phytate, tannins and oxalate in both tubers.

Frying significantly reduced ($P < 0.05$) phytate, tannin and oxalate contents in both tubers. Tannin levels were below detection limit in both tubers after boiling. The levels of oxalate were below detection limits in both tubers after both types of treatments.

For the raw, boiled and fried yellow yam and for the raw, boiled and fried white yam tubers, the energy values are 94.39, 53.51, 349.01, 78.14, 92.59, and 389.94 Kcal per 100 g of fresh weight respectively. The relatively high energy values for the fried samples may be attributable to the oil which is used for the preparation of the fried samples. In contrast the low level of energy in the raw and boiled samples could be attributable to very low levels of fat and protein as well as high levels of moisture in the samples.

4. DISCUSSION

Moisture contents of the tubers were 69.56 ± 0.2 g/100g for yellow tuber and 73.44 ± 0.5 for white tuber. There were no statistically significant ($P > 0.05$) difference in the moisture contents between the two tubers. Comparable moisture contents (74.5 g/100g) have been reported for *Dioscorea* species (EHNRI, 1997). The observed high moisture levels of the tubers may influence the textural quality of yellow and white *Dioscorea* food products or keeping quality of the tubers. The moisture content of a food sample reflects the amount of solid matter in the sample. The higher the moisture content, the higher the rate of spoilage. Yam is, however, processed to flour and stored for various food products.

Ash content is a reflection of mineral status, even though contamination can indicate a high concentration in a sample. The amount of ash in a tuber depends on the type of soil from which it was harvested, the moisture content and the maturity of the crop (Osagie, A.U. 1992). Values were 4.1 ± 0.01 g/100g and 4.62 ± 0.01 g/100g on fresh weight basis for the yellow and white tubers respectively. The findings of this investigation is significantly different ($P < 0.05$) from previous reports of 1.1g/100g on fresh weight basis by EHNRI (EHNRI, 1997) for yam tubers. This difference may be attributed to the localities from which the tubers were collected and varietal effect.

Crude fat was generally low in the investigated tubers. The yellow tuber has 0.35 ± 0.01 g/100g and the white tuber has 0.18 ± 0.01 g/100g of fat on fresh weight basis. A comparably low level of fat (0.1g/100g on fresh weight basis) was reported by EHNRI (EHNRI, 1997) and FAO (0.1 and 0.2 g/100g on fresh weight basis). The result of this investigation is not significantly different ($p > 0.05$) the reports of EHNRI and FAO. The low level of crude fats in both tubers may be of

benefit to individuals suffering from hyperlipidaemia because of the role of fat in potentiating one's risk of developing certain kinds of cancer and heart disease.

Protein is essential in the human diet for growth. Protein content of the yellow and white Dioscorea tubers were 6.1 ± 0.12 g/100g and 7.64 ± 0.1 g/100g on dry weight basis (1.85 ± 0.04 and 2.03 ± 0.03 g/100g on fresh weight bases) respectively. There were no varietal differences in protein content of the test tubers. A similar low protein level (1.5 g/100g on fresh weight bases) was reported by FAO on *D.cayenensis*-*D.rotundata* complex. The tubers investigated in this study cannot contribute to the daily protein requirement of 23-56g for humans (National Research Council, 1974). The low levels of protein in these samples would therefore require dietary supplementation with animal proteins or complementary proteins from legumes especially in diets meant for children and pregnant women.

Fiber content of the tubers was 10.48 ± 0.1 % and 10.1 ± 0.11 % on dry weight basis (3.19 ± 0.05 % and 2.68 ± 0.03 % on fresh weight basis) for the yellow and white tuber respectively. Dietary fiber is an essential component in human and animal nutrition (Johansen., et al, 1993). A high intake of Dietary fiber is positively related to different physiological and metabolic effects. It contributes less to calories, and can bind and flush cholesterol, carcinogens and undesirable chemicals from the body. It provides bulk, regulates intestinal motility and thereby helps to prevent the development of diverticulosis and chronic diseases including coronary heart disease, colonal cancer and other disorders of the gastrointestinal lining (Topping, D.L. and Clifton, D.L. 2001). Cereal grains, particularly whole grains are the most important sources of Dietary fiber (Dongowski., et al, 2002). Whole oat flour typically has 14% Dietary fiber, whole wheat flour 12% and whole brown rice 5%. Even at these low levels, the fiber contents of whole-grain flours

would have a significant impact on performance (Best, D. 2005). In both tubers, Dietary fiber contents were as high as 10 %, higher than those reported in brown rice and more comparable to that of whole wheat flour. Woolfe (Woolfe, J.A. 1987) reported that yam flour has higher levels of fibre than potato flour, refined wheat flour, maize and rice. Intake of Dietary fiber could, therefore, be increased by the consumption of foods from yellow and white yam or by incorporating them into other sensorially accepted foods. The high Dietary fiber of yellow and white yams could also be of significance in the formulation of diets for diabetics and other health conscious individuals.

The carbohydrate contents were $20.96 \pm 0.1\%$ and $19.0 \pm 0.06\%$ for the yellow and white tubers respectively on fresh weight bases. A comparable result (21.2 g/100g on fresh weight bases) of carbohydrate on raw Dioscorea tubers was reported by EHNRI (EHNRI, 1997). The high levels of total carbohydrate in the investigated tubers indicate that they contribute significantly to the energy content of the food materials.

The mineral composition of yellow and white Dioscorea tubers is presented in figures 3.3 and 3.4.

Phosphorus is found in most foods because it is a critical component of all living organisms. About 800 mg of P is recommended for adults per day. A value of 12.89 ± 0.02 mg/100g and 12.95 ± 0.02 mg/100g on fresh weight basis was obtained for the yellow and white tubers respectively. Yam starches are reported to contain 3-4 times as much phosphorus as found in cassava and aroid ones (Moorthy, S.N. 1994). Moorthy and Nair (Moorthy, S.N. and Nair, S. G. 1989) reported 0.11 and 0.015% phosphorus in *D. rotundata* grown in India. The value being reported in this study (12.89 and 12.95 mg/100g) is significantly lower than the reports of

EHNRI, 56.8, 29.4 and 68.1 mg/100g on fresh weight basis for the yams collected from Welita, Metekel and Sidama respectively (EHNRI, 19997). This difference could be due to varietal and environmental differences.

Calcium contents were found to be 172.63 ± 0.03 mg/100g and 227.86 ± 0.06 mg/100g on dry weight basis (52.55 ± 0.01 and 60.52 ± 0.01 mg/100g on fresh weight basis) for the yellow and white yam respectively. There were no significant differences ($p > 0.05$) between the two tubers. In a similar study on yam, Dilworth et al. (Dilworth, et al., 2007) obtained 300 mg/kg calcium in cooked yellow yam and 410 mg/kg calcium in the uncooked. 14.3–46.9 mg/100 g on fresh weight basis has also been reported in wild yams (Bhandari, M.R et al 2003). The high calcium content found in this study could be due to environmental differences from which the tubers were collected. The RDA for calcium is about 800-1200 mg for adults. Mineral calcium is vital for the development of healthy bones and teeth. It is also needed for muscle contraction and regulation of the heartbeat, and is involved in the formation of blood clots. A long-term shortage of calcium can lead to osteoporosis, when the bones become brittle and break easily. Yellow and white Dioscorea, from this study, may contribute to calcium requirement to their consumers.

The yellow and white tubers had potassium content of about 175.21 ± 0.04 mg/100 g and 172.17 ± 0.05 mg/100 g on dry weight basis (53.3 ± 0.02 mg/100g and 52.41 ± 0.01 mg/100g on fresh weight basis). A significantly higher ($p < 0.05$) potassium content (250–560 mg/100 g on fresh weight bases) has been reported by Bhandari (Bhandari, M.R et al 2003). This difference could be due to environmental differences. Potassium is a mineral that helps the kidneys to function normally and control blood pressure. With this low content of K in the varieties, yellow

and white Dioscorea may not be recommended for people with high blood pressure (Osagie, A.U. 1992) but may be suitable for people with renal failure. The proper balance of potassium in the body depends on sodium. Therefore, an excessive use of sodium may deplete the body's stores of potassium. Daily intake of potassium (K) between 1875 and 5625 mg is considered adequate and safe, however, too much can be harmful.

The measured contents of Cu were 0.95 ± 0.01 mg/100g and 1.09 ± 0.01 mg/100g on dry weight bases (0.29 ± 0.01 mg/100g and 0.29 ± 0.01 mg/100g on fresh weight bases) for the yellow and white yam respectively. The values in this study seem to be comparable ($p > 0.05$) to what was obtained (0.10–0.21 mg/100 g fresh weight bases) in wild yams by Bhandari *et al.* (Bhandari, M.R et al 2003). Too much Cu can be harmful, however, 2 to 3 mg/day intake is considered adequate and safe. Good sources are liver, shellfish, whole grains, legumes and nuts; Cu is believed to help the body to absorb and use iron in making haemoglobin. It also helps the body to get energy from food.

Contents of Zn were 3.32 ± 0.01 mg/100g and 4.14 ± 0.01 mg/100g in dry weight bases (1.01 ± 0.01 mg/100g and 1.1 ± 0.01 mg/100g on fresh weight bases) for the yellow and white yam respectively. The amount recommended is 15 mg/day for adults and good sources are sea foods, meat, fish and whole grains. With the range of zinc content of yellow and white Dioscorea, the species could be recommended for diet formulations for hypertensive patients. Lower zinc contents (0.22–0.53 mg/100g on fresh weight bases) were reported by Bhandari *et al.* (Bhandari, M.R et al 2003) in wild yams. Zn helps to regulate many of the human body's processes. It is

essential for survival and deficiency has serious consequences for health (Brown, K. H. and Wuehler, S.E. 2000).

The iron contents of the yellow and white tubers were 6.4 ± 0.01 mg/100g and 12.24 ± 0.02 mg/100g on dry weight bases (1.95 ± 0.01 mg/100g and 3.25 ± 0.01 mg/100g on fresh weight bases) respectively. A comparable ($p > 0.05$) result was reported by Bhandari et al that reported a range of iron contents of 0.39–2.92 mg/100g on fresh weight bases (Bhandari, M.R et al 2003).

The phytate content of the yellow tuber is 125.49 ± 0.006 mg/100g and 131.02 ± 0.009 mg/100g for the white tuber. These values are relatively lower than that reported for *D. rotundata* and *D. esculenta* (Uka, 1985). The implication of the low values of phytate in these tubers is that the tubers will contain available minerals for absorption in the body. There is also current support for the supposition that reduced levels of phytate may enhance health through antioxidant and anti carcinogenic activity (Sirikka, 1997).

The tannin contents for the yellow and white tubers are 72.93 ± 0.002 mg/100g and 108.43 ± 0.002 mg/100g on dry weight basis respectively. The values are significantly higher ($p < 0.05$) than that reported for *D. rotundata* (20 mg/100g on dry weight basis) by Uka (1985), which implies that less protein may be available in the yellow and white yam varieties than in *D. rotundata* reported by Uka due to protein-tannin complex formation. However, it is important to note that boiling of yam tubers before consumption will eliminate or reduce the level of tannin in the food system thereby making the protein available.

The oxalate contents of both tubers are 0.02 ± 0.001 mg/100g on dry weight bases. This value is significantly lower ($p < 0.05$) than that reported for *D.caynensis*-*D.rotundata* complex (0.43-0.96 mg/100g on dry weight bases) by Nestor et al (Nestor., et al., 2010). When oxalate is consumed, it irritates the lining of the gut and can prove fatal in large doses. Oxalate is a common and wide-spread component of most plant families. However, the levels of oxalate in the investigated yam tubers are low, it is the high concentrations if consumed daily that are of concern.

INVESTIGATION OF THE EFFECT OF BOILING AND FRYING ON THE PROXIMATE COMPOSITION, MINERALS AND ANTI NUTRIENT CONTENTS OF YELLOW AND WHITE YAM TUBERS

For both tubers, boiling decreased moisture, ash and fat contents but the fiber content is increased in both tubers. The protein content is decreased in the yellow yam whereas it is increased in the white yam. The carbohydrate content is slightly increased in both tubers.

The effect of boiling on the protein contents of the two tubers is different. Afoakwa and Sefa-Dedeh (Afoakwa and Sefa-Dedeh, 2001) reported the differences in composition of *Dioscorea dumetorum* tubers after processing are due to various differences in other components of the investigated tubers. The different effect of boiling on the protein contents of the yellow and white tubers may be due to the slight differences in other components between the two tubers. Frying significantly increases the fat contents in both tubers.

Boiling decreased phosphorous, zinc, copper and potassium contents in both tubers. The calcium and iron contents are increased in the yellow tuber where as their contents are decreased in the white yam. The decrease of some of the minerals after boiling may be due to their great

solubility in water during hydrothermal treatment, similarly to those reported by Akin-Idowu et al (Nestor et al, 2010). Frying significantly decreased the mineral contents in both tubers. The decrease in the mineral contents may be attributable to the reduction in the ash contents and an increase in the fat contents after frying.

The anti nutrients occur in varying concentrations. The reduction of anti nutrients is expected to enhance the nutritional value of the investigated tubers. The anti nutritional factors, particularly phytate and tannin, though showing a high concentration in raw tubers, should not pose a problem in human consumption if the tubers are properly processed.

Levels of phytate in the investigated tubers were lower than the values reported for Dioscorea species (Bhandari and Kawabata, 2006). The phytate content of a plant could be determined by the availability of phosphorus in the environment (Raboy and Dickinson, 1993; Buerkert *et al.*, 1998). Environmental changes, locality, soil types, genetics, irrigation conditions, year and fertilizer treatment can also affect the phytate and phosphorus content (Dost and Tokul, 2006). Boiling significantly decreased the phytate content in both tubers. The significant reduction in phytic acid during boiling 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 (Siddhuraju and Becker, 2001). Cooking has been reported to lower the phytate levels in yam (Bhandari and Kawabata, 2006). Frying also significantly reduced the phytate contents in both tubers. A reduction of 74.1 % and 88.1 % were found for yellow and white yam tubers respectively.

Boiling significantly reduces tannin contents in both tubers. A reduction in the tannin contents during boiling may be due to heat degradation of the tannin molecules or to the formation of water-soluble complexes. Frying also significantly reduced the phytate contents in both tubers. When Oxalate containing foods are eaten, they impart an acrid taste or cause irritation (Holloway et al., 1989). Oxalates reduce calcium absorption and promoting the formation of kidney stones (Noonan and Savage, 1999), hence they can have a harmful effect on human nutrition and health. The levels of oxalates in the investigated tubers were very low and both boiling and frying was found to be effective in reducing the oxalate content of the tubers under investigation. Boiling may cause considerable cell rupture and facilitate the leakage of soluble oxalate into cooking water (Albihn and Savage, 2001). The oxalate contents found in the yellow and white yam tubers is significantly lower than that reported for some *Dioscorea* species (Bhandari and Kawabata, 2006).

However, in terms of energy, for instance, the consumption of up to 350 g per day per consumer of raw, boiled and fried samples of yellow and white yams would provide energy values of 330.37, 187.29, 1221.54, 273.49, 324.07 and 1364.8 kcal on fresh weight respectively. The daily energy requirement of 2500-3000 kcal has been reported for adults (Bingham, S., 1978). Again, the FAO recommended range was 800-1200 kcal (FAO, 1972). It therefore follows that none of the raw and processed samples would meet the daily energy need of an adult if consumed at 350g per day, and except for the fried samples none would also meet the FAO recommended values. These samples cannot therefore be consumed as the sole source of energy to humans.

5. CONCLUSION

Based on the nutritional evaluation studies on yellow and white Dioscorea tubers collected from Sheko, it can be concluded that

- Both tubers are found to be a good source of crude fiber, carbohydrate and minerals.
- The anti nutritional factors found in both the investigated tubers were lower than previous reports.
- Both boiling and frying seem to be best for the removal / inactivation of anti-nutritional factors.
- None of the raw and processed samples would meet the daily energy need of an adult if consumed at 350g per day, and except for the fried samples none would also meet the FAO recommended values. These samples cannot therefore be consumed as the sole source of energy to humans.

6. RECOMMENDATION

In order to derive full nutritional potential from the yellow and white yam, they should be consumed together with protein-and fat-giving foods since low energy may lead to low birth weight and increased infant mortality.

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