

JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF CHEMISTRY



MSc THESIS ON

**PROXIMATE AND SELECTED METALS ANALYSIS OF YAM
(*Dioscorea alata*) COMMERCIALIZED IN LOCAL MARKETS OF
JIMMA CITY, ETHIOPIA**

APRIL, 2022

JIMMA, ETHIOPIA

JIMMA UNIVERSITY
COLLEGE OF NATURAL SCIENCES
DEPARTMENT OF CHEMISTRY

PROXIMATE AND SELECTED METALS ANALYSIS OF YAM
(*Dioscorea alata*) COMMERCIALIZED IN LOCAL MARKETS OF
JIMMA CITY, ETHIOPIA

BY: EBSA TERFA

ADVISORS: ABERA GURE (PhD, Assoc. Prof)

MR. KASIM KEDIR (MSc. Assist. Prof)

THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES
OF JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF MASTER OF
SCIENCE IN CHEMISTRY

Advisor's Approval Sheet

We approve that Ebsa Terfa has carried out his MSc research work entitled as “*Proximate and Selected metals analysis of Yam (Dioscorea alata) commercialized in Jimma city, Ethiopia*” under our supervision. In our judgment, the candidate has completed his research work, and therefore he is ready to proceed to the defense.

Advisors

Signature

Date

Abera Gure (PhD)

Kassim Kedir (MSc, Assit Prof)

ACKNOWLEDGMENTS

First, I thank God who by his absolute love and protection helped me to perform this work. Next, I would like to thank my advisors Dr.Abera Gure and Kassim Kedir for their valuable scientific comment and guidance starting from the beginning. Last but not the least I wish to thank my family and friends for their moral support, consistent encouragement and valuable advice.

TABLE OF CONTENTS

ACKNOWLEDGMENTS	I
LIST OF TABLES	IV
LIST OF FIGURES	V
ABSTRACT.....	VI
1. INTRODUCTION	1
1.1. Background of the Study.....	1
1.2. Statements of Problems.....	3
1.3 Objectives of the study.....	4
1.3.1 General objective	4
1.4. Significances of the study	5
2. LITERATURE REVIEW	6
3. MATERIAL AND METHODS	9
3.1. Chemical and Reagents	9
3.2 Instruments and Apparatus.....	9
3.3. Study Area	9
3.4. Sample Collection and Preparation.....	10
3.5. Nutritional Content Analysis	11
3.5.1. Determination of moisture content.....	11
3.5.2. Determination of crude protein contents.....	11
3.5.3. Determination of crude fat contents.....	12
3.5.6 Determination of carbohydrates.....	14
3.5.7 Determination of gross energy value	14
3.6 Analysis of ant-nutritional factors.....	14
3.6.1 Determination of oxalate contents	14
3.6.2 Determination of phytate content.....	15
3.7 Determination of minerals	15
3.8. Method Validation for Metal Analysis	16
3.8.1. Instrument calibration	16
3.8.2. Limit of detection.....	16
3.8.3. Limit of Quantification	16

3.8.4. Precision and accuracy.....	17
3.9. Statistical Analysis.....	17
4. RESULTS AND DISCUSSION	18
4.1. Nutrient Composition of Yam Tubers	18
4.1.1. Moisture content	18
4.1.2. Crude protein content.....	19
4.1.3. Crude fat content	19
4.1.4. Ash content	20
4.1.5. Crude fiber content	20
4.2. Anti-nutritional Factor Content of Yam.....	21
4.3. Mineral Contents of Yam Tubers.....	22
4.3.1 Analytical performance study	22
4.3.2. Metal concentration of yam	23
4.3.2.1. Iron contents.....	24
4.3.2.2. Calcium content	24
4.3.2.3. Magnesium content	25
4.3.2.4. Zink content	25
5. CONCLUSION AND RECOMMENDATION	27
5.2. Recommendations	27
Appendix: Calibration curves	35

LIST OF TABLES

Table 1 The nutritional compositions of red and white yams	18
Table 2. Anti-nutritional factor content (Mean \pm SD, n =3) of Yam roots sample.	21
Table 3: Analytical performance of the method	23
Table 4: Mineral content (Mean \pm SD, mg/100 g) of yam samples	24

LIST OF FIGURES

Figure 1:Picture (a) red and (b) white <i>Dioscorea alata</i> (Source: taken from internet)	9
Figure 2:Map of Jimma City	10

ABSTRACT

In this study, nutritional, anti-nutritional, and mineral compositions of yam tubers (Dioscorea alata) commercialized in the local markets of Jimma City, Ethiopia were investigated. The study was on red and white yam varieties, which are commonly commercialized in the city. For each variety 1kg sample was separately collected in plastic bags. Their nutritional compositions such as moisture, crude protein, total ash, crude fiber, crude fat, total carbohydrates and gross energy were determined using the standard, Association of Official Analytical Chemists (AOAC) method. The obtained results for the red and white yam samples, respectively, were (59 and 60 mg/100g) moisture content; (2.42 and 6.12 mg/100 g) crude protein; (3.23 and 3.74 mg/100 g) total ash; (1.36 and 1.31 mg/100 g) crude fiber; (0.16 and 0.09 mg/100 g) crude fat; (33.83 and 28.74 mg/100 g) carbohydrate content; (146.44 and 140.25 mg/100 g) gross energy. The anti-nutritional components, i.e., oxalate and phytate components were determined by titrimetric method. The obtained values were 0.47 and 0.34mg/100 phytate and 1.80and 1.76 mg/100 g oxalate, respectively. Mineral compositions such as Ca, Mg, Fe and Zn were determined using a flame atomic absorption spectroscopy. The mineral contents of the yam samples were Fe (6.96 and 16.52 mg/100g), Ca (121.26 and 221.73 mg/100g), Mg (104.54 and 118.37 mg/100g) and Zn (44.44 and 56.30 mg/100g) in red and white yam samples, respectively. Student t-test ($p < 0.05$) demonstrated that there was significant difference between the two yams (red and white) in the studied nutritional, anti-nutritional, and metal compositions.

Keywords: Nutritional, Anti-nutritional, Mineral compositions, yam (dioscorea alata) tubers

1. INTRODUCTION

1.1. Background of the Study

Yam (*Dioscorea alata*) is mainly grown for its tuber. It is an important source of carbohydrates and nutrients for many people in tropical countries including East and West Africa, the Caribbean, South Africa, India and Southeast Asia [1, 2]. Ethiopia is the fifth largest yam producing country in Africa and its annual production is estimated to be around 1,191,809 tons [3]. Harvesting plays an important role in local livelihoods, particularly in densely populated areas in the south, southwest and west of the country [4, 5]. *Dioscorea spp.* is tubers consisting of about 600 species; of which there are about 50-60 species are cultivated and used as food and medicinal plants [6]. About nine species of yams are grown in Ethiopia, indicating the diversity of species in the country [7]. Some species have been used domestically due to their unique color, texture, and flavor compared to those of sweet potato, potato and cassava tubers.

It grows in the elevation range of 1140 to 2200 and on a variety of soils, primarily in clay, clay-loam, sand and sand-loam types. It is planted in October (in most parts of southern Ethiopia), November and December (in the south-west and west of the country) [8]. Yams have become a major cash crop in most areas. Yams are also served during the traditional festival that coincides with the main harvest season, allowing farmers to take advantage of the market.

On the other hand, yams growing in different environments are expected to contain different nutrient, anti-nutrient and mineral compositions. So far much attention has not been paid to the nutrient, anti-nutrient and mineral compositions of yams grown in various areas of the country. Therefore, the main objective of this study was to determine the nutritional, anti-nutritional and

mineral compositions of yam (*Dioscorea alata*) tubers commercialized in local market of in Jimma city, Ethiopia.

1.2. Statements of Problems

Yam (*Dioscorea alata*) is commonly grown in West and Southwest Oromia Regional States such as Jimma, Illu Ababa Bora, West and East Wollaga, Horo Guduru Wollega and Kellem Wollega zones. Despite being one of the preferred crops in these areas, very limited researches have been conducted on its nutrient components. It is also known that the nutrient compositions of crops can vary with varieties; environmental conditions and soil where they are cultivated. Therefore, in this study, the proximate and selected metals compositions of red and white *Dioscorea alata* were investigated to answer the following basic research questions.

- Do the proximate and selected metals compositions of red and white *Dioscorea alata* varieties are varied or the same?
- Do the nutritional proximate and selected metals compositions of red and white *Dioscorea alata* varieties are similar or different from the reported values?
- Do the proximate and selected metals compositions of red and white *Dioscorea alata* varieties are similar or different from other tubers such as sweet potato, potato and cassava crops?

1.3 Objectives of the study

1.3.1 General objective

The main objective of this study is to determine proximate and selected metals compositions of *Dioscorea alata* commercialized in local market of Jimma City, Ethiopia.

1.3.2 Specific objectives

This study has the following specific objectives.

- ❖ to determine the approximate compositions such as *moisture, crude protein, total ash, crude fiber, crude fat, total carbohydrates and gross energy* of red and white *Dioscorea alata* commercialized in Jimma City local markets
- ❖ To analyze of the *anti-nutritional, i.e., oxalate and phytate components* of red and white *Dioscorea alata* commercialized in Jimma City local markets.
- ❖ To investigate the level of selected metals in red and white *Dioscorea alata* commercialized in Jimma City local markets.
- ❖ To compare *nutritional, anti-nutritional, and mineral compositions of the Dioscorea alata* tubers with other tubers such as sweet potato, potato and cassava plants.

1.4. Significances of the study

The findings of this study could have the following significances:

- It could help to know the proximate and mineral contents of red and white *Dioscorea alata* varieties commercialized in Jimma City. It could be used to as baseline information for other researchers who want to conduct similar studies on tubers or other crops.
- It could be used as a literature for other researchers on the *nutritional, anti-nutritional, and mineral compositions* red and white *Dioscorea alata* commercialized in Jimma City.

2. LITERATURE REVIEW

Yam (*Dioscorea alata*) is the most important food crop since the time of immemorial in the tropics and sub tropics [9]. It is highly linked with the human existence, endurance, and the socio economic history [10]. It is cultivated to a greater extent to combat the food security threats of the increasing population in the world [11]. It is the third most important root crop in West Africa, after cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* (L.), Poir)[10, 12]. From the nutritional standpoint, it is better than cassava on its higher vitamin C (40-120 mg/g edible portion) and crude protein content (40-140 g/kg dry matter) [13]. Moreover, during the off seasons, some people prefer using yams to solve their seasonal food shortage rather than cassava and sweet potato [14, 15]. Yams have been domesticated and cultivated by over 60 million of people in tropical and sub-tropical regions [16-17]. In these regions, yams are well integrated into the social and cultural life style of the people who cultivate and consume them and have significant contribution for food security, medicine and commercial value particularly in rural areas, where they are freely available [10,18,19]. Apart from providing basic food security and income source, yam is a rich source of carbohydrate, vitamins and minerals, especially where it is consumed in large amounts. The crop is estimated to provide more than 285 dietary calories per person per day for 300 million people in sub-Saharan Africa [20-22]. It is mainly grown for human consumption in Africa, South America, and South Pacific [21]. In the Philippines, South-East Asia, the well-known species cultivated are *Dioscorea alata* and *Dioscorea esculenta* [23].

Yam has bioactive compounds include steroidal saponins called diosgenin and dioscin, storage protein dioscorin, and anthocyanins [24–25]. The Chinese yams, namely, *D. opposita* Thunb, *D.*

D. alata L., *D. fordii* Prain ET Burkill, and *D. persimilis* Prainet Burkill of 25 germplasms are a good source of nutrient and bioactive compounds for medicinal use [25]. However, the diversity of nutrients and bioactive compounds quantitatively varied within the same species [26, 25]. Some species have been used domestically due to its unique color, texture, and flavor compared to those of sweet potato and cassava tubers [27].

In Ethiopia, yam is a highly valued crop, which provides food for household consumption and improves many livelihoods through the sale of harvested tubers [28]. Wild types of yam are also consumed by some farming communities in South and Southwest Ethiopia to overcome hunger and make a significant contribution in the diets of the people [29]. The tubers were found with a high amount of carbohydrates, fibers, and low level fats and protein, a good proportion of essential amino acids which make them a good dietary source and could be eaten as cooked vegetable, boiled yam, steamed, baked or fried in oil [30,31]. Conversely, the wider utilization of yam in Ethiopia is limited; due to information on the biochemical composition of yam is meager. Besides, yam in itself is not a balanced food and malnutrition occurs when yam is consumed alone as staple food [32]. Studies of nutritional composition on yam have considerable significance since it may help to identify long forgotten food resource [33].

In this regards, few attempt was made to understand the proximate composition and anti-nutritional factors of the underutilized tubers of Yams have been domesticated and cultivated by over 60 million of people in tropical and sub-tropical regions [34-35]. In these regions, yams are well integrated into the social and cultural lifestyle of the people who cultivate and consume them and have significant contribution for food security, medicine and commercial value particularly in rural areas, where they are freely available [10,18,19]. Apart from providing basic

food security and income source, yam is a rich source of carbohydrate, vitamins and minerals, especially where it is consumed in large amounts. The crop is estimated to provide more than 285 dietary calories per person per day for 300 million people in sub Saharan Africa [20-22].

Yam to make edible tubers as the safe food sources for mass consumption [34]. Moreover, many different forms and landraces of the edible yam species are available in different areas with variable in composition and nutritional values. In contrast to cultivated tubers, little is known about the proximate composition and reasons to expect that some of the species differ in composition from common agricultural varieties [35]. Furthermore, several species of yams also have medicinal properties and the tuber contains some pharmacologically active substances including dioscorine, saponin and saponin [36].

In spite of its food security and medicinal importance, to the best of our knowledge, there are no efforts so far done in the nutritional composition and medicinal value on Ethiopian yams and information on the biochemical composition of the landraces is scarce. Furthermore, the culinary attributes of the existing landraces have never been assessed and the nutritional importance of yam at country level is still unknown; which hinders the wider utilization and researchers to access the biochemical composition indigenous yam genetic resources in the country. Thus, exhaustive imagery of landraces based on biochemical composition and medicinal values in connection with farmers' indigenous knowledge and use have tremendous impact to make the genetic enhancement and sustainable use of yam genetic resource in Ethiopia. Consequently, the present study was designed to assess the biochemical composition of yams collected from Southwest Ethiopia for breeding and conservation. The Figure 1 shows picture of red and white *Dioscorea alata*.



(a) Red *Dioscorea alata*



(b) White *Dioscorea alata*

Figure 1: Picture (a) red and (b) white *Dioscorea alata* (Source: taken from internet)

3. MATERIAL AND METHODS

3.1. Chemical and Reagents

All chemicals and reagents used in this study were analytical grades. These chemicals and reagents include (conc. hydrochloric acid (HCl, 37%), sulfuric acid (H₂SO₄, 98%)), boric acid (H₂BO₃, 4%)), (hydrogen peroxide (H₂O₂, 30%)), sodium hydroxide (NaOH), nitric acid (HNO₃, 70%)), potassium permanganate (KMnO₄), ammonium thiocyanate (NH₄SCN) and ferric chloride (FeCl₃). Deionized water was for solution preparation and dilution.

3.2 Instruments and Apparatus

Kjeldahl flask, Flame Atomic Absorption Spectroscopy(Buck Scientific Model 280FS, Malaysia), Oven (DHG-9070A), Quartz Digestion Tube, Centrifuge, Muffle Furnace (D-6072 Driesch, West Germany), Mortar and pestle as well as separating funnels were used during the experimental works.

3.3. Study Area

Yam samples were collected from local markets in Jimma city, Jimma, Ethiopia. Jimma City located in the southwestern part of the Oromia Regional State of the country. It is the capital of Jimma Zone. The city is situated at latitude: 740'43'N; Longitude: 3650'18'E and at, 352 km distance from Addis Ababa, Ethiopia. The altitude of the area is about 1700 m above sea level. The area's annual rainfall ranges from 100 mm to 1500 mm with a mean annual temperature of 24 °C to 28 °C. Figure 2 shows map of Jimma city.

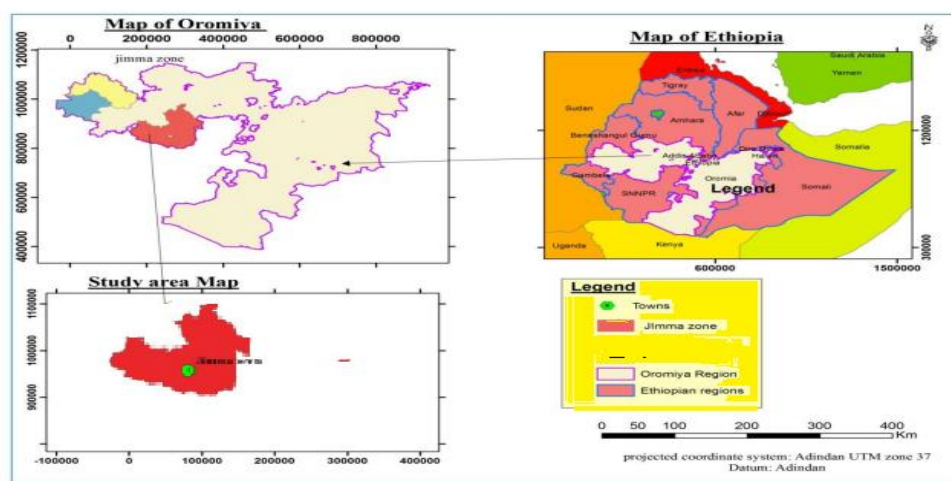


Figure 2: Map of Jimma City

3.4. Sample Collection and Preparation

Two types of *Dioscorea alata* (yam), i.e., red and white yams, were considered in the study. For each variety, 1 kg sample was separately collected from local markets of Jimma city, Ethiopia. The samples were collected in new and pre-cleaned polyethylene bags and then transported to the Jimma University Analytical Chemistry Laboratory for analysis. Before, analysis, the samples were washed with tap water and their covers were peeled by hands. They were then chopped into small pieces using stainless steel. Their moisture content was determined immediately after cutting into pieces. Prior to the determinations of nutrient, anti-nutrient and mineral content, samples were dried in open air by spreading on plastic pan. The dried samples were then ground into a fine powder using a grinding machine. Finally, the samples were stored in dry glass bottles for further analytical studies.

3.5. Nutritional Content Analysis

3.5.1. Determination of moisture content

The moisture content was determined by AOAC Sub-component 925.09, oven drying method [37]. Accordingly, 10 g well-mixed sample was weighed into a dried crucible (W_1). The crucible was placed in an oven at 105°C for about 6 h until constant weight was reached. Then the crucible with the dried sample was taken out of the oven and placed in the desiccator for 30 min until it was cooled. Finally, the sample was weighed (W_2) to determine the percent moisture content of the product using the following formula:

$$\% \text{ Moisture} = \frac{W_1 - W_2}{\text{Sample weight}} \times 100 \dots\dots\dots (1)$$

Where, W_1 and W_2 is initial and final weight of the crucible containing the sample, respectively.

3.5.2. Determination of crude protein contents

The protein content of the yam samples was determined according to AOAC sub-component 979.09 by the Kjeldahl method involving digestion, distillation and titration [38]. Accordingly, approximately 1 g dried yam sample was taken into the digestion flask. Then, 20 mL conc. H_2SO_4 , 7 g K_2SO_4 , and 0.2 g $CuSO_4$ were added. The flask was swirled to mix the contents thoroughly and then placed on a heater to begin digestion until the mixture it gets clear (until all color was removed), which took about 1:30 h. The digest was cooled and transferred to a 100 mL volumetric flask and made up to the mark with distilled water. Afterwards, 10 mL of the digest was transferred to the distillation tube and then 50 mL NaOH (40 %, w/v) was added. Distillation was continued for about 10 min and the produced NH_3 was collected as NH_4OH in an

Erlenmeyer flask containing 20 mL boric acid (4%, w/v) with a few drops of methyl red indicator. A yellowish color was observed during distillation due to NH_4OH . The distillate was then titrated against 0.2N HCl until a pink color was observed. A blank sample was also prepared by the same procedure. The percentage (%) crude protein content of the sample was calculated using the following formula:

$$\begin{aligned} \text{\%Crude protein} &= 6.25 \times \text{\%N} \\ \text{\%N} &= \frac{(S-B) \times 0.014 \times D \times 100}{\text{Wt.sample}} \dots\dots\dots (2) \end{aligned}$$

Where 0.014 is milli-equivalent weight of nitrogen, S is the sample titration reading, B is the blank titration reading, N is the normality of HCl, D is the dilution of the sample after digestion, and V is the volume withdrawn for distillation.

During the digestion step: $\text{Protein (-N)} + \text{H}_2\text{SO}_4 \longrightarrow (\text{NH}_4)_2\text{SO}_4 + \text{CO}_2 + \text{H}_2\text{O}$

During the distillation step: $(\text{NH}_4)_2\text{SO}_4 + 2\text{NaOH} \longrightarrow 2\text{NH}_3(\text{g}) + \text{Na}_2\text{SO}_4 + 2\text{H}_2\text{O}$

3.5.3. Determination of crude fat contents

The crude fat content yam samples was determined by AOAC sub-component 2003.06; hexane extraction method [37]. Therefore, 2 g of dry sample was placed in an extraction thimble and the thimble was then placed in a previously weighed beaker containing hexane. Fat extraction was performed for 4 h. Become water by heating on the water bath. After fat extraction, the beaker was taken out, dried in a hot air oven at about 105°C for 2 h, cooled in a desiccator, and weighed. The weight gain of the cups was calculated as the fat of the yam sample.

$$\text{Fat (\%)} = \frac{\text{weight of hexane extracted}}{\text{sample wight}} \times 100 \dots\dots\dots (3)$$

3.5.4. Determination of total ash content

The total ash content of the samples was determined according to AOAC, a sub-component of the drying oven method by burning known weights of the samples in a muffle furnace at 550°C until a white ash was obtained [37]. About 2 g yarn sample (W_s) was placed in the previously dried and weighed crucible (W_1). The sample was placed in the muffle furnace and ignited at 550°C for 3 h. The ignited sample, which is white in color, was removed from the oven and immediately placed in a desiccator until it cooled to room temperature. Finally, the crucible was weighed with the ashes (W_2). The %ash of the sample was calculated as follows.

$$\% \text{Ash} = \frac{W_2 - W_1}{W_s} \times 100 \dots\dots\dots (4)$$

Where W_1 , W_2 and W_s are weight of the crucible, weight of the crucible containing the sample after drying and weight of the sample before drying, respectively.

3.5.5 Determination of crude fiber contents

The crude fiber content of the yarn samples was determined by the AOAC method, subcomponent 962.09, which includes pulping, filtration, washing, drying and burning [38]. Therefore, 0.15 g of dried yarn sample (W_o) was weighed and transferred to a crucible and 150 mL hot H_2SO_4 (1.25%, v/v) was added. It was then boiled under condensers for 45 min. The acid was drained by vacuum suction and the sample was washed with distilled water. The precipitates were again boiled with hot 150 mL NaOH (1.25%) for 45 min. The alkali was removed by vacuum suction and the sample was washed with distilled water. It was dried at 105°C to constant weight and recorded as the weight after drying (W_1). Ashing was taken place in a muffle furnace at 550°C for 3 h. The crucibles were cooled in desiccators and the weight

recorded as weight after ash (W_2). The crude fiber was then calculated using the following formula.

$$\% \text{ crude fiber} = \frac{W_1 - W_2}{W_o} \times 100 \dots\dots\dots (5)$$

3.5.6 Determination of carbohydrates

The carbohydrate content was calculated based on the values of other proximate [39]. Which is mathematical calculated as follows:

$$\% \text{ Carbs} = 100 - (\% \text{ Moisture} + \% \text{ Ash} + \% \text{ Crude fat} + \% \text{ Crude fiber} + \% \text{ Crude Protein}) \dots\dots\dots (6)$$

3.5.7 Determination of gross energy value

The gross energy value of the yam sample was estimated using the water factors for protein (4), fat (9), and carbohydrate (4) [39]. The equation is:

$$\% \text{ Gross energy} = (\% \text{ Crude protein} \times 4) + (\% \text{ Fat} \times 9) + (\% \text{ Carbohydrates} \times 4) \dots\dots\dots (7)$$

3.6 Analysis of anti-nutritional factors

3.6.1 Determination of oxalate contents

Oxalate was analyzed using a titrimetric method that includes sample digestion, filtration and titration. Oxalates were extracted with acid followed by titrimetric analysis. Accordingly, 1 g yam sample was added to 75 mL of 1.5 N H_2SO_4 . The resulting mixture was gently stirred for 1 h, followed by filtration. The filtrate (25 mL) was then warmed and titrated against 0.1 M

KMnO₄ solution until a faint pink color was observed, representing the endpoint of the titration.

The oxalate content was then estimated using Equation:

$$\text{Oxalate} = (\text{Titer value} \times 0.9004) \text{ mg/100g} \dots\dots\dots 8$$

3.6.2 Determination of phytate content

To determine the phytate content, 100 mL of 2% HCl was added to 2.5 g of ground yam sample [41]. The mixture was shaken for 3 h. After filtration, 25 mL was removed and 5 mL of 0.3% NH₄SCN was added. Then 50 mL of distilled water was added to obtain the desired acid. The resulting solution was titrated against a 0.00195 g/mL FeCl₃ solution until a persistent brownish-yellow color was observed. The %phytate content was calculated as:

$$\% \text{Phytate} = t \times 0.1635 \dots\dots\dots (9)$$

Where, t is titrate value

3.7 Determination of minerals

The standard method of AOAC sub-component 923.03 [37] was used for the determination of Fe, Ca, Mg and Zn using FAAS. Accordingly, 10 g yam sample was weighed into a crucible and placed in a muffle furnace at 550°C for 3 h. The sample was removed from the oven and immediately placed in a desiccator until cooled to room temperature. Then 1 g ash sample was placed in the beaker and 12 mL 1:3 (HNO₃: HCl) was added to completely dissolve the sample, followed by gentle drying on a low temperature hot plate. After adding 5 mL of H₂O₂, the sample was reheated on a hot plate until just boiling, and then it was cooled and filtered through Whitman filter paper into a 50 mL volumetric flask. Then the beaker was washed with water

added to the sample in the volumetric flask. Finally, the volume was marked with deionized water and analyzed by FAAS. A blank was also prepared according to the procedure with the same amount.

3.8. Method Validation for Metal Analysis

3.8.1. Instrument calibration

Calibration curves were constructed for each metal (Fe, Ca, Mg and Zn) using five series of solutions from 1 to 5 mg/L, which prepared from 1000 mg/L stock solutions. Immediately after constructing calibration curve for each element, the digest was analyzed and the response is recorded.

3.8.2. Limit of detection

The limit of detection (LOD) is the minimum analyte concentration that can be detected but not necessarily quantified with an acceptable uncertainty. The LOD for each metal was determined from five replicates of the blank samples. The blank sample was digested using the same digestion method used for the actual samples. LOD was determined as three times the standard deviation of the blank samples, i.e., $3SD_b$.

3.8.3. Limit of Quantification

The limit of quantification (LOQ) is the lowest concentration of an analyte in a sample that can be quantified with an acceptable level of uncertainty. The blank sample was digested using the same digestion method as the actual samples. LOQ was determined as $10SD_b$.

3.8.4. Precision and accuracy

Precision and accuracy of the results were evaluated by determining the repeatability and percent recovery (%R) of the spiked sample of known concentration of the analytes. The samples were spiked with known concentration of each metal (i.e. close to the middle of the calibration curve). The spiked samples were digested and analyzed following similar procedure. Precision was expressed as the SD of the replicative analysis. The accuracy of the method was reported as %R calculated as follows:

$$\%R = \frac{\text{conc.in spiked sample}-\text{conc.unspiked sample}}{\text{actual spiked conc.}} \times 100 \dots\dots\dots (9)$$

3.9. Statistical Analysis

In the present study, student t-test ($p < 0.05$) was used to assess the variation of nutritional, anti-nutritional and the studied metals contents of the two yam samples: red and white yams.

4. RESULTS AND DISCUSSION

4.1. Nutrient Composition of Yam Tubers

Nutritional value is the primary concern when considering a plant as a food source. Yam is an endemic bulbous plant used as a food source in parts of southwestern Ethiopia. Table 1 Nutritional compositions (Mean \pm SD, mg/100g) and total energy (kcal) in the studied samples. The findings of study showed that yams have good nutritional compositions to be used as food.

Table 1: The nutritional compositions of red and white yams

Parameter	Varieties		Reported [26]		
	Red	White	Sweet potato	Potato	Cassava
Moisture	59.00 \pm 2.000	60.00 \pm 1.000	67.43	74.70	62.86
Protein	2.53 \pm 0.291	6.39 \pm 1.041	1.30	1.60	0.53
Fat	0.16 \pm 0.015	0.09 \pm 0.013	2.00	0.10	0.17
Ash	3.23 \pm 0.130	3.74 \pm 0.405	1.10	0.60	0.84
Fiber	1.37 \pm 0.130	1.31 \pm 0.002	1.10	0.40	1.48
Total carbohydrate	33.83 \pm 2.260	28.74 \pm 2.020	28.20	22.60	31.0
Total energy	146.44 \pm 9.530	140.25 \pm 10.610	136.00	97.00	NF

NF: not found

4.1.1. Moisture content

Generally, this study revealed that presence of high moisture content in yam species. The presence of high content of moisture in yam is an indication of existence of good source of minerals [42]. In this study, the moisture content of the Red and White Yam samples were 59 mg/100 g and 60 mg/100 g, respectively (Table 1). Higher moisture content was observed in white yam. This variation could be attributed to the variation in environmental conditions and the

variability of the collected sample. Moisture content affects the physical, chemical aspects of food, which relates to the freshness and stability for long-term storage of the food. Moisture-rich foods are easily susceptible to microbial attack and become spoiled and damaged. Foods with low moisture content usually show slower growth of microorganisms. The studied yam samples have comparable moisture contents to other tubers plants such as sweet potato, potato and cassava [26].

4.1.2. Crude protein content

Proteins are used for the growth and replacement of lost tissues in the human body. As can be seen from Table 1, the white yam sample has greater protein content (6.39 mg/100 g) than the red one which contained (2.53 mg/100g). The higher protein content observed in the white yam indicates the presence of higher nitrogenous substances in the sample. The presence of these differences in total protein content could be related to soil nitrogen content as well as the variation of yam types. The t- test $t_{\text{calculated}} > t_{\text{critical}}$ also indicated the presence of significant difference in crude protein content between the white and red yam samples. On the other hand, the studied yam samples have higher crude protein contents than sweet potato, potato and cassava [26].

4.1.3. Crude fat content

In the current study, the crude fat contents of the examined yam samples were 0.16mg/100 g for red and 0.09 mg/100 g for white yam samples, which were similar to literature values [42, 43]. From the two the red yam has relatively higher crude fat content than white yam samples. The $t_{\text{calculated}} > t_{\text{critical}}$ indicated the presence of significant difference in crude fat content between

the studied two yam samples. These variations could be related to soil types, maturity stage, and farming practices. The two yam samples have comparable crude fat content to potato and cassava, but have lower than that of sweet potato [26].

4.1.4. Ash content

The observed %ash contents of the two yams were comparable: 3.23% (for red) and 3.74% (for White) yam samples. (Table 1), Ash content is directly proportional to the inorganic element content of the yam. Therefore, samples that have high ash contents are expected to have high concentrations of various mineral elements, which are beneficial to accelerate metabolic processes and enhance growth and development of the consumers. The t -calculated < t -critical showed that there was no significant difference in the ash content of the yam samples examined. However, the studied yam samples have relatively ash content and protein contents than other tubers including sweet potato, potato and cassava [26].

4.1.5. Crude fiber content

Dietary fiber is defined as the sum of non-starch polysaccharides (cellulose, hemicelluloses and pectic substances) and lignins, which are mainly components of plant cell walls. The fiber content of the red and white yam samples were 1.37 and 1.31 g/100g, respectively. The obtained fiber contents were higher than the reported values [42, 43]. The red yam sample showed relatively higher crude fiber content than the white yam sample. These variations were probably related to storage time, scale and variations in the soils on which they were grown. Dietary fiber shows positive physiological effects on the human body as it stimulates and accelerates intestinal contraction and transit and increases fecal volume [44].

4.5.6. Total carbohydrate

Carbohydrates are the main source of energy for our body. Table 1 shows the carbohydrate content of the yams. The carbohydrate contents were 33.83 g/100g for red and 28.74 g/100 g for white yam samples. The slight differences in total carbohydrate content could be related to the degree of ripeness. The t- calculated < t-critical showed that there was no significant difference in the carbohydrate content of the yam samples examined. The result of this research implies that yam is rich in carbohydrates as sweet potato, potato and cassava [26]. Therefore, consumption of yam as a food can help to give more energy [47].

4.5.7. Total energy

The observed total energy levels were 146.44 kcal/100g for the red and 140.25 kcal/100g for white yam samples. The two varieties demonstrated higher total energy contents than potato, but comparable to sweet potato [26]. The obtained total energy is similar to the previously reported values [43].

4.2. Anti-nutritional Factor Content of Yam

Anti- nutrients are known to reduce the maximum utilization of nutrients, especially proteins, vitamins and minerals [40]. In this study the contents anti-nutritional factors: phytate and oxalate of red and white yams were studied and the obtained results are given in Table 2.

Table 2. Anti-nutritional factor content (Mean \pm SD, n =3) of Yam roots sample.

Parameter	Varieties	
	Red Yam	White Yam
Oxalate content	1.80 \pm 0.27	1.76 \pm 0.12
Phytate content	0.47 \pm 0.05	0.34 \pm 0.01

In this study, the observed oxalate levels were 1.80 for the red and 1.76 mg/100 g for the white yam tuber samples. Likewise, the phytate contents were 0.47 for the red and 0.34 mg/100 g for the white yam samples. Oxalic acid inhibits the uptake of calcium by forming insoluble calcium oxalate [48]. However, there is also the possibility of bacterial degradation in the intestine, which makes the calcium available from calcium oxalate [49]. Phytate is a salt form of phytic acid. Phytic acid is powerful in the formation of protein and mineral phytic acid complex chelates; resulting in net reduction of bioavailable protein and mineral [51].

Anti-nutritional factors are those substances or chemical compounds found in fruits and foods in general that are toxic to humans or in some way limit nutrient availability to the body. Anti-nutritional factors are present in different food substances in varying amounts depending on the type of food, the way it is propagated, the chemicals used in growing the plants, and the chemicals used in storing and preserving the food substances. Starchy roots and tubers like potatoes and sweet potatoes contain anti-nutritional factors like tannin, trypsin inhibitors, phytic acid and oxalic acid. The presence of anti-nutritional factors in the food (raw or cooked products) reduces the bioavailability of nutrients and also the food qualities [40].

4.3. Mineral Contents of Yam Tubers

4.3.1 Analytical performance study

Prior to determination of mineral content including Ca, Mg, Fe and Zn in the yam samples, calibration curves were constructed using five concentration points ranging from 1 to 5 mg/L. The linear dynamic range (LDR), coefficient of determination (R^2), LOD and LOQ curves obtained are shown in Table 3.

Table 3: Analytical performance of the method

Metal	LDR	LOD	LOQ	R²	%R
Fe	1.0 – 5.0	0.02	0.62	0.9989	93.40
Ca	1.0 – 5.0	0.03	0.25	0.9988	83.00
Mg	1.0 – 5.0	0.02	0.19	0.9977	86.30
Zn	1.0 – 5.0	0.04	0.09	0.9981	89.30

As can be seen, the calibration curve of each element showed a satisfactory R^2 , i.e., 0.998 or better, a low LOD for metals analysis.

For the recovery study, 3 mg/L of each metal was spiked and the %R obtained were ranging from 83 to 93.4% (Table 3) The %R were within the acceptable range for metals analysis, indicating good accuracy of the method.

4.3.2. Metal concentration of yam

The mineral contents of the red and white samples are presented in Table 4. Presences of minerals in foods are responsible for several to human health benefits. The human body requires minerals, in varying amounts from the diet, in order to grow properly, maintain health and promote general well-being [39]. Root and tuber crops are important sources of minerals associated with the prevention of deficiency diseases such as anemia and rickets, and daily consumption of these foods is encouraged [45].

Table 4: Mineral content (Mean \pm SD, mg/100 g) of yam samples

Metal	Varieties		Reported [26]		
	Red yam	White yam	Sweet potato	Potato	Cassava
Fe	6.96 \pm 1.25	16.52 \pm 0.38	52.00	6.70	0.23
Ca	121.26 \pm 0.78	221.73 \pm 0.01	NF	10.00	20.00
Mg	104.54 \pm 2.53	118.37 \pm 1.38	3.40	NF	30.00
Zn	44.44 \pm 0.25	56.30 \pm 0.01	NF	NF	NF

4.3.2.1. Iron contents

In this study, the determined Fe levels were 6.96 in red and 16.52 mg/100 g in white yam samples. White yam contained higher Fe than the red one. The t-test ($p < 0.05$) also showed the presence of difference between the two samples. The Fe content detected in the red yam was comparable to the reported literature [42]. The red yam has comparable Fe content to potato, but, contained higher than cassava [26]. Fe is essential element. It is primarily used to carry oxygen to the blood cells and therefore about two-thirds of the iron in the body is found in hemoglobin. It is also used to regulate the proper growth of the human body to maintain robust health and produce red blood cells. However, at very high concentrations, it causes vomiting, abdominal pain and liver enlargement.

4.3.2.2. Calcium content

Ca is the main component of bones and teeth. It is also necessary for blood coagulation and for the integrity of intracellular cement substances [46]. The obtained Ca levels were 121.26 for red and 221.73 mg/100 g for white yams. From the two higher concentration of Ca was detected in white yam sample. Statistical t-test ($p < 0.05$) also showed the presence of significant difference

in the levels of Ca in the two studied samples. These differences could be related to soil pH, availability of Ca-bearing minerals in soil and water and/or because of yam types,

4.3.2.3. Magnesium content

The obtained Mg contents of the red and white yams were 104.54 and 118.37 mg/100 g, respectively. The white yam contained relatively higher Mg concentration than the red yam sample. The recommended daily Mg intake in most countries is 128 mg for children 1 to 2 years of age, 280 mg for women and 350 mg for men [47]. The two varieties have significantly different Mg contents ($p < 0.05$). The two varieties could have different Mg levels may be due to the soil type on which they grow and variation of its uptake by the two plants. Mg is very mobile in plant tissue and is transferred from old plant tissue to new plant tissue.

4.3.2.4. Zink content

Zn is an essential element for humans, animals and certain plant species. It is necessary for a healthy immune system, cell division and the synthesis of protein and collagen, which is great for wound healing and healthy skin. However, a higher amount of it can cause anemia, damage to the pancreas and the production of high-density lipoprotein cholesterol [50]. In this study, the Zn contents of the two yams were 44.44 for red and 56.30 mg/100 g for white yam samples. The Zn contents of red and white yams were significantly different at $p < 0.05$. Relatively, higher Zn content was found in the White Yam sample. However, the obtained Zn levels in both varieties were higher than the reported values [42]. This variation could be related to the nature of the soil since Zn is readily available in acidic soils. The recommended daily dose for Zn in most countries is 3.9 mg for infants and 7.4 mg for children [47].

General, the findings of this study demonstrated that compared to other tuber crops such as sweet potato, potato and cassava, both red and white yams are rich in Fe, Ca, Mg and Zn , indicating their high potential for food security [26].

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

In the present study, the nutritional, anti-nutritional and mineral compositions of red and white yams commercialized in Jimma city were investigated. The finding showed that from the two varieties of the studied yams: red yam contained relatively higher concentrations of phytate and oxalate, crude fiber, fat, carbohydrates, and total energy than white yam. However, relatively higher levels of moisture, ash, protein, Fe, Ca, Mg and Zn than the red variety. Compared to other tropical root and tuber crops such as sweet potato, potato and cassava the studied red and white yams are rich nutrient and mineral contents. In general, the finding revealed that the yams, contained significant amounts of nutrients such as crude protein, calcium, iron and zinc. Both yam samples have also low oxalate and phytate (anti-nutritional) contents. Therefore, increasing its production and consumption is helpful to secure food security of the country.

5.2. Recommendations

Based on the findings, the researchers would like to share the following recommendations:

- ✓ In order to obtain detailed scientific evidence on the variation of nutrient, ant and mineral contents of yam, further studies involving the soils and maturity of the yam tubers are needed.
- ✓ It could be good if cultivation and consumption of white yam is encouraged since it has high nutritional content such as protein, ash, Fe, Ca, Zn, Mg and low anti-nutritional factors.

6. REFERENCES

1. Yang, D.J., Lu, T.J. and Huang, L.S. (2009) Effect of Endogenous Glycosidase on Stability of Steroidal Saponins in Taiwanese Yam (*Dioscorea pseudojaponica* Yamamoto) during Drying Processes. Food Chemistry, 113, 155-159.
<https://doi.org/10.1016/j.foodchem.2008.07.060>
2. Food and Agriculture Organization (1991) Food Outlook. Food and Agriculture Organization, Rome.
3. Food and Agriculture Organization (2015) FAOSTAT Agriculture Database, Agricultural Production, Crops Primary. Yams. Food and Agriculture Organization, Rome.
<http://www.fao.org/faostat/en/#data/QC> .
4. Hildebrand, E. (2003) Motives and Opportunities for Domestication: Anthropological Study in Southwest Ethiopia. Journal of Anthropological Archeology, 22, 358-375.
[https://doi.org/10.1016/S0278-4165\(03\)00031-X](https://doi.org/10.1016/S0278-4165(03)00031-X).
5. Muluneh, T. (2006) Assessing Diversity in Yams (*Dioscorea* spp.) from Ethiopia Based on, Morphology, AFLP Markers and Tuber Quality, and Farmers Management of Landraces. Culvillier Verlag, Göttingen
6. Coursey, D. G. 1967. Yams: An account of the nature, origins, cultivation and utilisation of the useful members of the *Dioscorea spp.*ceae. Tropical Agriculture Series. London: Longmans, Green and Co. 230 p.
7. Westpal, E. (1975) Agricultural System in Ethiopia. Center for Agricultural Publishing and Documentation, Wageningen.

8. Miège, J. and Sebsebe, D. (1997) Dioscoreaceae. In: Edwards, S., Sebsebe, D. and Hedberg, I., Eds., Flora of Ethiopia and Eritrea, National Herbarium, Biology Department, AAU, Ethiopia, Department of Systematic Botany, Uppsala University, Sweden.
9. Asiedu R, Fatokun CA, Mignouna JHD, NG SYC, Quin FM (1999) under researched tropical food crops: Cowpea, banana, plantain and yams. In: Hohn T, Leisinger KM (eds) Biotechnology of food crops in developing countries. Springer Wien, NewYork, pp: 187-216.
10. Kambaska K, Trinanth M, Santilata S, Aratibala P (2009) Biochemical quantification of protein, fat, starch, crude fibre, ash and dry matter content in different collection of greater yam (*Dioscorea alata* L.) found in Orissa. J Nat Sci 7: 24-32.
11. Asiedu R, Sartie A (2010) Crops that feed the World 1 Yams: Yams for income and food security. Food Sci 2: 305-315.
12. Philip, Taylor D, Sanni LO, Dixon AGO (2004) Nigeria's Cassava Industry: Statistical Handbook. International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria.
13. Baah FD, Maziya DB, Asiedu R, Oduro I, Ellis WO (2009) Nutritional and biochemical composition of *D. alata* (*Dioscorea* spp.) tubers. J Food Agric Environ 7: 373-378.
14. Opara LU (1999) Yam storage In: Bakker Arekema (eds) CIGR Handbook of Agricultural Engineering 4: 182-214.
15. Norman PE, Tongoon P, Danson J, Shanahan PE (2012) Molecular characterization of some cultivated yam (*Dioscorea* spp.) genotypes in Sierra Leone using simple sequence repeats. Inter J Agron Plant Prod 3: 265-273.

16. Lebot V (2009) Tropical root and tuber crops: Cassava, sweet potato, yams and aroids. Crop Production Science in Horticulture Series. 17th edn. CABI Publishing, Wallingford, UK.
17. Sesay L, Norman PE, Massaquoi A, Gboku ML, Fomba SN (2013) Assessment of farmers' indigenous knowledge and selection criteria of yam in Sierra Leone. *Sky J Agric Res* 2: 1-6.
18. Megh RB, Takanori K, Jun K (2003) Nutritional evaluation of wild yam (*Dioscorea* spp.) tubers of Nepal. *Food Chem* 82: 619-623.
19. Dansi A, Dantsey B, Vodouhè R (2013) Production constraints and farmers' cultivar preference criteria of cultivated yams (*Dioscorea cayenensis*/ *Dioscorea rotundata* complex) in Togo. *Inter J Biol* 4: 191-199.
20. Degras L (1983) the yam. A Tropical Root Crop. 2nd edn. Macmillan Press, London, pp: 137-138.
21. R. N. Cavalcanti, D. T. Santos, and M. A. A. Meireles, "Nonthermal stabilization mechanisms of anthocyanins in model and food systems – An overview," *Food Research International*, vol. 44, no. 2, pp. 499–509, 2011.
22. Adejumo BA, Okundare B, Balogun SA (2013) Quality attributes of yam flour (Elubo) as affected by blanching water temperature and soaking time. *Int J Eng Sci* 2: 216-221.
23. D. F. Cornago, R. G. O. Rumbaoa, and I. M. Geronimo, "Philippine yam (*Dioscorea* spp.) tubers phenolic content and antioxidant capacity," *Philippine Journal of Science*, vol. 140, no. 2, pp. 145–152, 2011.

24. M. Araghiniknam, S. Chung, T. Nelson-White, C. Eskelson, and R. R. Watson, "Antioxidant activity of Dioscorea and dehydroepiandrosterone (DHEA) in older humans," *Life Sciences*, vol. 59, no. 11, pp. PL147–PL157, 1996.
25. Z.-G. Wu, W. Jiang, M. Nitin, X.-Q. Bao, S.-L. Chen, and Z.-M. Tao, "Characterizing diversity based on nutritional and bioactive compositions of yam germplasm (*Dioscorea*spp.) Commonly cultivated in China," *Journal of Food and Drug Analysis*, vol. 24, no. 2, pp. 367–375, 2016.
26. Zinash, D. (2008). Minim izing Post Harvest Losses in Yam (*Dioscorea* spp.). In: *Using Food Science and Technology to Improve Nutrition and Promote National Development*, Robertson, G. L. & Lupien, J. R. (Eds), International Union of Food Science & Technology.
27. D. F. Cornago, R. G. O. Rumbaoa, and I. M. Geronimo, "Philippine yam (*Dioscorea* spp.) tubers phenolic content and antioxidant capacity," *Philippine Journal of Science*, vol. 140, no. 2, pp. 145–152, 2011.
28. Mulualem T (2012) *Production and post-harvest utilization system of yam (Dioscorea spp.)*. Lambert Academic Publishing, Germany.
29. Tamiru M, Heiko C, Brigitte L (2007) Genetic Diversity in yam germplasm from Ethiopia and their relatedness to the main cultivated *Dioscorea* species assessed by AFLP markers. *J Crop Sci* 47: 1744-1753.
30. Osman H (1990) Dietary fiber composition of common vegetables and fruits in Malaysia. *Food Chem* 37: 21-26.

31. Nashriyah M, Nurathiqah MY, Syahril H, Norhayati N, Mohamad AW (2011) Ethnobotany and distribution of wild edible tubers in Pulau Redang and Nearby Islands of Terengganu, Malaysia. *Inter J Biol Biomol Agric Food Biotechnol Engg* 5: 911-914.
32. Shanthakumari S, Mohan VR, Debritto A (2008) Nutritional evaluation and elimination of toxic principles in wild yam (*Dioscorea* spp.). *J Trop Subtrop Agro Ecosys* 8: 313-319.
33. Alozie Y, Akpanabiatu MI, Eyong EU, Umoh LB, Alozie G (2009) Amino acid composition of *Dioscorea dumetorum* varieties. *Pakistan J Nutr* 8: 103-105.
34. Arinathan V, Mohan VR, Maruthupandian A (2009) Nutritional and ant nutritional attributes of some underutilized tubers. *J Trop Subtrop Agro ecosys* 10: 273-278.
35. Schoeninger MJ, Bunn HT, Murray SS, Marlette JA (2000) Composition of tubers used by Hadza foragers of Tanzania. *J Food Compos Anal* 13: 1-11.
36. Jaleel CA, Gopi R, Manivannan P, Kishorekumar A, Gomathinayagam M, et al. (2007) Changes in biochemical constituents and induction of early sprouting by triadimefon treatment in white yam (*Dioscorea rotundata* Poir.) tubers during storage. *J Zhejiang Uni Sci* 8: 283-288.
37. AOAC. Association of Official Analytical Chemists. Official methods of Analysis (Vol. II 17th edition) of AOAC International. Washington, DC, USA 2010. Official methods 925.09, 923.03, 979.09, 962.09, 4.5.01 and 923.05.
38. AOAC (Association of Official Analytical Chemists),(2015) Official methods of Analysis Washington, DC, USA. Official methods 925.09, 923.03, 979.09, 962.09, 4.5.01 and 923.05.

39. WHO/FAO (1998). The role of carbohydrates in nutrition, chapter 1. Carbohydrates in human nutrition. Maria C. Linder. Nutritional biochemistry and metabolism with clinical applications. Second edition. 1991:194.
40. Liener IE, Kakade ML. Protease inhibitors in Toxic constituents of plant foodstuffs, ed Liener I.E. Academic Press, New York 1969:8-69.
41. Aina VO, Binta Sambo, Amina Zakk2ari, et al. Determination of Nutritional and Antinutrient Content of *Vitis vinifera* (Grapes) Grown in Bomo (Area C) Zaria, Nigeria. *Advance Journal of Food Science and Technology*. 2013; 4(6):445–448.
42. Opara, L. (1999). Yam storage. In: CIGR Handbook of Agricultural Engineering Volume IV Agro Processing. The American Society of Agricultural Engineers, St. Joseph, MI.
43. Vavilov, N.I (1951). The Origin, Variation, Immunity and Breeding of Cultivated Plants.
44. Harlan, J. (1969). Ethiopia: A centre of diversity. *Economic botany*, 23.
45. Leterme P (2002) Recommendations by health organizations for pulse consumption. *Br J Nutr* 88 Suppl 3: S239-242.
46. Okaka JC, Okaka ANO (2001) Food composition, spoilage and shelf life extension. *ocjarc`* o Academic Publishers, Enugu, Nigeria, P: 54-56.
47. Maria C. Linder. Nutritional biochemistry and metabolism with clinical applications. Second ed. 1991:194.
48. Davidson S, Passmore R. Human Nutrition and Dietetics. Eighth Edition. Churchill Livingstone. Edinburgh London Melbourne and New York. 1986:108, 134, 233.
49. Brune H, Bredehon. The physiology of bacterial degradation of calcium oxalate and the ability to utilize calcium from calcium oxalate in the pig. *Chem Abstr* 1962; 56: 5190.

50. Farag, A.M.; May, T.; Marty, G.D.; Easton, M. and Harper, D.D. The effect of chronic chromium exposure on the health of Chinook salmon (*Oncorhynchus tshawytscha*). *Journals of Aquatic and Toxicology*, 2006, 76, 246-257.
51. Khare, S.K. (2000). Application of immobilized enzymes in soybean processing .The Third International Soybean Processing and Utilization Conference (ISPCRC III): 2000 of the 29 Innovation Era for mycotoxins. In: 15-20, October, 2000, Tsukuba, Ibaraka, Japan, pp: 381-382

Appendix: Calibration curves

